Native and Invasive Freshwater Decapods in the UK: Conservation and Impacts

Paula Joy Rosewarne

Submitted in accordance with the requirements for the degree of Doctor of Philosophy

The University of Leeds

School of Biology

September 2013

The candidate confirms that the work submitted is her own, except where work which has formed part of jointly authored publications has been included. The contribution of the candidate and the other authors to this work has been explicitly indicated below. The candidate confirms that appropriate credit has been given within the thesis where reference has been made to the work of others.

Chapter Four is based on a jointly authored publication: **Rosewarne, P.J., Mortimer, R.J.G. & Dunn, A. M. (2013) Size-dependent impacts of the endangered white-clawed crayfish (*Austropotamobius pallipes*) (Lereboullet) on the littoral community, *Knowledge and Management of Aquatic Ecosystems*, 409, 06, p.1-10**

P. Rosewarne formulated the idea, conducted the experiment, analysed the data and wrote the manuscript. A. Dunn and R. Mortimer supervised the research and contributed to writing the manuscript.

Chapter Five is based on a jointly authored publication: **Rosewarne, P., Mortimer, R. & Dunn, A. (2012) Branchiobdellidan infestation on endangered white-clawed crayfish (*Austropotamobius pallipes*) in the UK. *Parasitology*, 139, p.774-780.**

P. Rosewarne collected the data, analysed the data and co-wrote the manuscript. A. Dunn formulated the idea, provided research supervision, advised on data analysis and co-wrote the manuscript. R. Mortimer provided research supervision and contributed to writing the manuscript.

Chapter Six is based on a jointly authored publication: **Rosewarne, P., Svendsen, J. C., Mortimer, R. & Dunn, A. (in press) Muddied waters: suspended sediment impacts on gill structure and aerobic scope in an endangered native and an invasive freshwater crayfish. *Hydrobiologia*.**

P. Rosewarne formulated the idea, collected the data, analysed the data and wrote the manuscript. J. C Svendsen advised on study design, data collection, and data analysis, and commented on initial drafts of the manuscript. A. Dunn and R. Mortimer supervised the research and contributed to writing the manuscript.

Professional Acknowledgments

I sincerely thank my many collaborators who have always been generous with their time, knowledge and equipment. Research supervision for all the work presented in this thesis was provided by Dr Alison Dunn (AD) and Professor Robert Mortimer (RM). Additional supervision for Chapter Three was provided by Bobbie Millar. The specific contributions of others to each chapter are summarised below:

Chapter Two: AD and RM provided feedback on initial drafts

Chapter Three: Bobbie Millar, AD and RM assisted in idea formulation and provided feedback on initial drafts. Case partners Lafarge-Tarmac provided research support and background information. Graeme Smart (GSL Ecological Consultants) carried out the restoration work, crayfish translocation and post-translocation crayfish trapping. Emma Pickering, Neal Haddaway, Lucy Anderson, Nigel Taylor and Adam Piper assisted with fieldwork. Samuel Allshorn provided technical assistance.

Chapter Four: AD and RM provided feedback on initial drafts. Emma Pickering, Chris Wright, Neal Haddaway, Fiona Reynolds, and Adam Piper assisted with fieldwork. Samuel Allshorn provided technical assistance.

Chapter Five: AD formulated the idea, advised on data analysis and advised on writing. RM provided feedback on initial drafts.

Chapter Six: Jon Svendsen provided loan of the respirometry equipment, advised on data collection methods and analyses, and provided feedback on initial drafts. AD and RM advised on experimental design and provided feedback on initial drafts.

Chapter Seven: Chris Wing and Chris Grocock assisted with data collection. AD advised on experimental design, data analysis and writing. Rachel Paterson advised on analysis of functional response data. RM and Robert Newton provided advice and assistance with stable isotope work. Samuel Allshorn provided technical assistance.

Chapter Eight: Adam Piper designed the telemetry antennas and assisted with fieldwork. Ros Wright provided research support. AD and RM advised on experimental design. AD provided feedback on initial drafts.

I thank the Natural Environment Research Council and Case-partners Lafarge Tarmac for funding this work. I also thank Lafarge Tarmac, in particular Nick Beale and the staff at

Swinden Quarry, for their practical help and enthusiastic support. Thanks also to Dan Hayter and Ben Norrington at the Environment Agency for assistance with field sampling.

Personal Acknowledgements

I thank Alison Dunn for providing the bulk of my research supervision and for generously inputting your knowledge and time over the course of my PhD; your positivity, realism and sense of humour are hugely appreciated. I also thank my co-supervisor Rob Mortimer for his sound scientific and common sense advice; and for being immensely efficient at answering emails - it really helps.

To all the Dunn lab group members, past and present, you have helped make my time in Leeds sociable, fun, productive and rewarding. I owe particular thanks to Katie Arundell, Freya (Asa) Johannesen, Lucy Anderson, and Neal Haddaway, with whom I have shared much of this journey.

I thank all my family and friends for their part in helping me reach this goal. Particular thanks to Carol Rosewarne for sharing her home and lovely cooking, and to Vicki Stratton and Vicky Lacey for assistance with proof-reading. Finally, a huge ‘thank you’ to Adam Piper for his practical help and advice and, most importantly, his unwavering support and encouragement throughout our ‘PhD Years’.

Abstract

Crayfish species have been translocated by humans, with both positive and negative consequences. Conservation-led translocations of the endangered white-clawed crayfish (*Austropotamobius pallipes*) to safe ‘Ark sites’ where threats are minimised are underway. Restored quarries may represent ideal Ark sites and several questions pertaining to their suitability were addressed, along with a 3-year case study of Ark site creation in a limestone quarry. Quarries contain large quantities of unconsolidated sediment and crayfish experienced gill fouling and reduced aerobic scope after exposure to suspended solids concentrations of 62 mg L^{-1} and above. A novel parasite detected in the gills of *A. pallipes* caused gill pathology, highlighting the need for health assessment of donor stock prior to translocation. Most Ark sites do not historically hold crayfish and their introduction may cause strong effects on existing freshwater communities. In a mesocosm study, juvenile and adult *A. pallipes* reduced the abundances of grazers and shredders; however consumption of the gastropod *Physa fontinalis* and detritus varied between life-stages. The American signal crayfish (*Pacifastacus leniusculus*) is rapidly invading catchments and replacing *A. pallipes*. In a field-based telemetry study, a flow-gauging weir reduced upstream movements of *P. leniusculus* by 45%, highlighting the need to balance invasion risk with EU targets for improved riverine connectivity. *P. leniusculus* co-occurs with another invasive decapod, the Chinese mitten crab (*Eriocheir sinensis*), and their feeding behaviour was compared. Both consumed a range of prey items, including fish eggs. Juvenile *E. sinensis* had a higher per capita intake of the keystone shredder *Gammarus pulex* than native *A. pallipes* and showed greater preference for this prey item than did *P. leniusculus.* Overall, *E. sinensis* is likely to have an equal, if not higher, per capita impact on prey species than *P. leniusculus.* Recommendations for quarry Ark site creation and management of invasive decapods were formulated.

Table of Contents

Abstract vii
List of Figures xiii
List of Tables xvii
Chapter 1 General Introduction 1
  1.1. Freshwater Crayfish 1
    1.1.1 Morphology and Life History 2
    1.1.2. Crayfish as components of freshwater ecosystems 5
    1.1.3 Crayfish and humans 8
  1.2. The white-clawed crayfish, a species in decline 8
  1.3 Translocation for conservation 10
  1.4 Ark sites for white-clawed crayfish 12
    1.4.1. Ark site selection 12
    1.4.2 Former quarries as Ark sites 13
    1.4.3. Habitat suitability 14
    1.4.4. Taxonomic considerations 18
    1.4.5. Pathogens, parasites and disease 18
    1.4.6. Post-translocation monitoring 20
  1.5. Translocation and invasion 22
    1.5.1 Aquatic invaders 23
    1.5.2 The signal crayfish 25
    1.5.3 The Chinese mitten crab 27
  1.6 Research aims and thesis plan 29
Chapter 2 Habitat use by the endangered white-clawed crayfish *Austropotamobius* species complex: a systematic review 33
  2.1 Summary 33
  2.2 Introduction 33
  2.3 Methods 36
  2.4 Results and Discussion 37
    2.4.1 Water chemistry parameters 42
    2.4.2 Channel substrate parameters 42
    2.4.3 Vegetation parameters 43

2.4.4 Hydrodynamic parameters 43

2.4.5 Bank structure 44

2.4.6 Land-use 44

2.4.7 Macro-invertebrates 45

2.4.8 Habitat partitioning 45

2.5 Conclusion 45

Chapter 3 Ark site introductions and quarries: a case study 49

3.1 Summary 49

3.2 Introduction 49

3.3 Threshfield Quarry Ark site 51

3.3.1 Restoration 51

3.3.2 Donor population 53

3.3.3 The translocation 53

3.4 Study rationale and aims 55

3.5 Methods 56

3.5.1 Water chemistry monitoring 56

3.5.2 Biodiversity monitoring 56

3.5.3 Crayfish monitoring 58

3.6 Results 60

3.6.1 Water chemistry monitoring 60

3.6.2 Biodiversity monitoring 63

3.6.3 Crayfish monitoring 67

3.7 Discussion 68

3.7.1 Water chemistry 68

3.7.2 Macro-invertebrates 71

3.7.3 Zooplankton 73

3.7.4 Crayfish 73

Chapter 4 Size-dependent impacts of the endangered white-clawed crayfish (*Austropotamobius pallipes*, Lereboullet) on the littoral community 77

4.1 Summary 77

4.2 Introduction 77

4.3 Materials and methods 79

4.3.1 Mesocosm set-up 79

4.3.2 Experimental design......80
4.3.3 Statistical analysis......81
4.4 Results......82
4.4.1 Water chemistry......82
4.4.2 Crayfish......84
4.4.3 Macrophytes and macro-invertebrates......84
4.5 Discussion......87
Chapter 5 Branchiobdellidan infestation on endangered white-clawed crayfish (*Austropotamobius pallipes*) in the UK......89
5.1 Summary......89
5.2 Introduction......89
5.3 Materials and methods......91
5.3.1 Phylogenetic analysis......93
5.3.2 Data analysis......93
5.4 Results......93
5.5 Discussion......97
Chapter 6 Impacts of suspended solids on gill structure and aerobic scope in an endangered native and an invasive freshwater crayfish......101
6.1 Summary......101
6.2 Introduction......101
6.3 Methods......104
6.3.1 Collection of animals......104
6.3.2 Suspended solids exposure......104
6.3.3 Gill examination......106
6.3.4 Respirometry......107
6.3.5 Data analysis......108
6.4 Results......110
6.4.1 SS treatments......110
6.4.2 Survival......110
6.4.3 Sediment accumulation in gills......111
6.4.4 Standard metabolic rate (SMR)......114
6.4.5 Aerobic scope......114
6.4.6 Aerobic scope after SS exposure......116

6.5 Discussion 116
Chapter 7 Feeding behaviour and predatory functional responses of two invasive freshwater decapods 121
7.1 Summary 121
7.2 Introduction 121
7.3 Methods 125
7.3.1 Prey choice, functional response and mesocosm studies 125
7.3.2 Stable isotope analysis and gut contents analysis (GCA) 134
7.4 Results 138
7.4.1 Prey choice experiments 138
7.4.2 Predatory functional response experiment 140
7.4.3 Outdoor mesocosm experiment 142
7.4.4 Stable isotope analysis 148
7.4.5 Gut contents analysis 151
7.5 Discussion 153
Chapter 8 Do low-head riverine structures hinder the spread of invasive crayfish? Case study of signal crayfish (*Pacifastacus leniusculus*) movements at a flow gauging weir ... 159
8.1 Summary 159
8.2 Introduction 159
8.3 Methods 162
8.3.1 Study site 162
8.3.2 Telemetry configuration 163
8.3.3 Capture and tagging protocol 164
8.3.4 Environmental variables 165
8.3.5 Data analysis 165
8.4 Results 166
8.4.1 Crayfish Activity 166
8.4.2 Environmental factors 167
8.4.3 All passages through site 167
8.4.4 Weir passage 169
8.4.5 Control passage 169
8.5 Discussion 169
Chapter 9 General Discussion 173

9.1 Translocation for conservation: key findings and management recommendations ..173
9.2 Crayfish as invasive species: key findings and management recommendations 181
9.3 Concluding remarks 184
References 185
Appendix I...226

List of Figures

Figure 1.1 Anatomy of male crayfish *Astacus astacus* (adapted from Vogt, 2002).............. 3

Figure 1.2 Lateral view of left branchial chamber of crayfish *Astacus astacus* showing direction of water flow up through the chamber and over the gills (Vogt, 2002) 4

Figure 1.3 Summary of recommendations for conservation translocations formulated by the IUCN 11

Figure 1.4 Summary of recommended water quality guidelines for *Austropotamobius pallipes* extracted from the literature by Haddaway (2010). Total range (light grey) corresponds to the widest range of variable levels cited for *A. pallipes*, the outer buffer zone (dark grey) and inner buffer zone (black) refer to ranges within 5 and 2.5 standard errors of means reported in the literature (reproduced with permission). 15

Figure 1.5 Framework for biological invasions indicating the stages of an invasion, potential management interventions and the point at which species are considered invasive (simplified from Blackburn *et al.*, 2011).. 23

Figure 2.1 Number of primary research studies assessing the importance of 18 habitat variables for *Austropotamobius pallipes* and *Austropotamobius italicus* at the catchment scale (i.e. multiple watercourse). 41

Figure 2.2 Number of primary research studies assessing the importance of 13 habitat variables for *Austropotamobius pallipes* and *Austropotamobius italicus* at the reach scale (i.e. in one watercourse). 41

Figure 3.1 Threshfield Ark site comprising ponds 1, 2 and 3 (photo taken May 2012; facing south-east).. 52

Figure 3.2 Plan of Threshfield Ark site showing position of waterbodies (dotted lines represent prepared planting beds), water depths and location of baited cameras during trial. Distances and depths are approximate.. 52

Figure 3.3 Hester dendy (a), during deployment at Threshfield (b). 57

Figure 3.4 Schematic of underwater baited camera set-up for crayfish monitoring............ 59

Figure 3.5 Baited-camera station (a), and during deployment at Threshfield (b). 60

Figure 3.6 Concentrations of calcium (a), sodium (b), magnesium (c), potassium (d), nitrate (e) and phosphate (f) in Ponds 1, 2 & 3 at the Threshfield Ark site, and in the donor site Eller Beck (where measured) over the period September 2009 to October 2012. Dark and light grey shading denotes values within 2.5 and 5 standard errors, respectively, of mean values where *A. pallipes* occurs, extracted from the literature by Haddaway (2010). 61

Figure 3.7 Concentrations of sulphate (a) and chloride (b), and conductivity level (c) in Ponds 1, 2 & 3 at the Threshfield Ark site, and in the donor site Eller Beck (where measured) over the period September 2009 to October 2012. Dark and light grey shading denotes values within 2.5 and 5 standard errors, respectively, of mean values where *A. pallipes* occurs, extracted from the literature by Haddaway (2010). 62

Figure 3.8 Smooth newt (*Lissotriton vulgaris*) recorded by a baited camera deployed in Pond 1 at Threshfield Ark site, October 2012...68

Figure 4.1 Percentage change (± S.D.) in mass of allochthonous detritus over 5 weeks in mesocosms under 3 crayfish treatments: juveniles; mixed (juveniles and adults), and adults only, and with no crayfish (control). * indicates treatment is significantly different from at least one other, or control...85

Figure 4.2 Percentage change (± S.D.) in abundance of grazers (gastropods *Physa fontinalis* and *Planorbarius corneus*), shredders (*Asellus aquaticus* and *Gammarus pulex*) and filterers (chironomid larvae) over 5 weeks in mesocosms under 3 crayfish treatments: juveniles; mixed (juveniles and adults), and adults only, and with no crayfish (control). * indicates treatment is significantly different from at least one other, or control..................86

Figure 5.1 Portion of podobranch from crayfish *Austropotamobius pallipes* showing gill filaments with melanisation spots (a), melanised tips (b), and attachment of *Branchiobdella astaci* cocoons (c)...92

Figure 5.2 Relationship between size (carapace length, mm) of crayfish host *Austropotamobius pallipes* and the total number of viable cocoons of *Branchiobdella astaci* found in branchial chamber (b = 0.0614, $r^2 = 0.40$, $p < 0.001$, n =34).................................94

Figure 5.3 Phylogeny of six European *Branchiobdella* species, including *B. astaci,* based on mitochondrial cytochrome *c* oxidase I (CO-I) sequences with outgroup *H. medicinalis*. Numbers at branch points indicate neighbour joining bootstraps (1000 replicates, K2P model)...96

Figure 6.1An experimental chamber 106

Figure 6.2 Mean actual suspended solids concentration as a function of time since water replacement in the experimental chamber, determined by filtration for four treatments (control, 250 mg L^{-1}, 500mg L^{-1} and 1000 mg L^{-1}) with four replicates in each. Error bars denote standard deviation..110

Figure 6.3 Stepped survival plots for crayfish *Pacifastacus leniusculus* and *Austropotamobius pallipes* during 45 day exposure to four suspended solids (SS) concentrations: control (0 mg L^{-1}); low (250 mg L^{-1}); medium (500 mg L^{-1}) and high (1000 mg L^{-1})...111

Figure 6.4 Mean (± S.E) gill area (%)of crayfish *Pacifastacus leniusculus* and *Austropotamobius pallipes* visibly affected by sediment accumulation after 45 days exposure to 4 suspended solids (SS) concentrations (control, low, medium and high). 113

Figure 6.5 Podobranchs of crayfish *Austropotamobius pallipes* after exposure to suspended solids (1000 mg L^{-1} experimental treatment yielding time-averaged concentration of 133 mg L^{-1}) for 45 days showing heavy sediment accumulation (a), cocoons of *Branchiobdella astaci* (b), *Branchiobdella astaci* (c), gill filament with melanisation spot (d), and base of podobranchs free from sediment (e). 113

Figure 6.6 Aerobic scope (mg O2 kg^{-1} h^{-1}) for crayfish *P. leniusculus* and *A. pallipes* corrected to 5 g individual, as a function of time to exhaust (sec) (b = -0.61, r^2 = 0.17,p = 0.01, n = 38).. 115

Figure 7.1 UK distributions of a) *Pacifastacus leniusculus* (Global Biodiversity Invasions Facility, 2013) and b) *Eriocheir sinensis* (Mitten crab recording project, 2013). 124

Figure 7.2 Prey items used within mobile prey choice experiment, from left to right: chironomid larva; *Gammarus pulex; Radix peregra,* and *Asellus aquaticus* (photo courtesy of Chris Wing) 127

Figure 7.3 Relationship between the number of prey eaten (*Ne*) and density of prey supplied (*N*) for the three types of functional response (I, II and III) (adapted from Murdoch, 1972). 131

Figure 7.4 Field collection sites a) Flatford, where *Eriocheir sinensis* and a range of potential food sources were collected, and b) Wixoe, where *Pacifastacus leniusculus* and 135

Figure 7.5 Consumption of 4 prey items: *Asellus aquaticus* (isopod), chironomid larvae, *Gammarus pulex* (amphipod) and *Radix peregra* (gastropod), within treatments with decapod predators *Pacifastacus leniusculus* and *Eriocheir sinensis,* and in the control (no decapod). Plots show medians (thick line), interquartile ranges (boxes) and data range (whiskers). 139

Figure 7.6 Consumption data of three decapod predators *Eriocheir sinensis, Pacifastacus leniusculus* and *Austropotamobius pallipes* preying upon *Gammarus pulex.* Circles denote mean number of prey items consumed at each initial density (4 replicates). Solid curves indicate mean fit of functional response curves (Roger’s equation) for each species, dashed curves indicate 95% confidence intervals generated by bootstrapping (n = 1500). 141

Figure 7.7 Coefficients of attack rate (a) and handling time (h) for 3 decapod predators: E. sinensis, P. leniusculus and A. pallipes derived from Rogers random predator equation. Error bars denote upper and lower 95% confidence limits obtained by bootstrapping (n = 1500), * denotes significant difference between groups (0.05 significance level)............ 142

Figure 7.8 Remaining periphytic algae (measured as total chlorophyll per ceramic tile (µg)) and mean percentage change (± S.D.) in mass of allochthonous detritus, filamentous algae (*Cladophora* sp.) and two macrophyte species (*Ceratophyllum demersum* and *Potamogeton perfoliatus*) after four weeks in mesocosm pools (n = 16) within the treatments: *E. sinensis*, *P. leniusculus*, both *E. sinensis* and *P. leniusculus*, and no decapod control......................143

Figure 7.9 Mean percentage change (± S.D.) in abundance of *Gammarus pulex, Asellus aquaticus* and Trichoptera larvae after four weeks in mesocosm pools (n = 16) within the treatments: *E. sinensis, P. leniusculus*, both *E. sinensis* and *P. leniusculus*, and no decapod control..145

Figure 7.10 Mean percentage change (± S.D.) in abundance of Gastropoda and Chironomidae larvae after four weeks in mesocosm pools (n = 16) within the treatments: *E. sinensis*, *P. leniusculus*, both *E. sinensis* and *P. leniusculus*, and no decapod control. 146

Figure 7.11 Stable isotope bi-plots for a) *E. sinensis* and b) *P. leniusculus* (open circles), and potential food sources in the lower River Stour, UK. Squares indicate mean values adjusted for trophic enrichment factors, bars indicate range of values. 149

Figure 7.12 Boxplots indicating range of contribution of each potential food source to the diet of a) *E. sinensis* and b) *P. leniusculus* as determined by concentration-dependent mixing models using mean isotopic values for consumers. Boxed areas represent 95, 75 and 50% confidence intervals. 150

Figure 7.13 Gut contents of *E. sinensis* (n= 5) expressed as mean (± S.D.) volumetric % of total contents. 152

Figure 7.14 Gut contents of *P. leniusculus* (n = 10) expressed as mean (± S.D.) volumetric % of total contents..152

Figure 8.1 Glemsford flow gauging weir, a trapezoidal flume of concrete construction, under low flow conditions (a), and drawn in 3-D with arrow denoting the direction of flow (b). 162

Figure 8.2 Study site showing the location of control and weir stretches, 3 PIT antennas, data logging receivers and release sites of tagged crayfish...163

Figure 8.3 Number of detection events per month during the two study years (1 and 2) expressed as a function of the cumulative number of tagged crayfish released, excluding detections within the first 24 hrs post-release. Absence of detection events during September in year 2 reflects a period of downtime in the telemetry system.......................167

Figure 8.4 Summary of passages by crayfish through the weir and control stretches in both up- and downstream directions during year 1 and year 2. * indicates a significant difference at the 0.05 level, Chi-sq test, 1 d.f...168

List of Tables

Table 1.1 Proposed criteria for initial selection of Ark sites for *Austropotamobius pallipes* in the UK (derived from Peay 2009) 13

Table 2.1 Predefined criteria for selection of literature returned by systematic search for full review 36

Table 2.2 Summary of data extracted from 12 studies examining habitat use by *Austropotamobius pallipes* and *Austropotamobius italicus* at the catchment scale (i.e. multiple watercourses)........ 39

Table 2.3 Summary of data extracted from 7 studies examining habitat use by *Austropotamobius pallipes* and *Austropotamobius italicus* at the reach scale (i.e. in one watercourse)........ 40

Table 3.1 Macro-invertebrate taxa found in Pond 1, Threshfield Quarry, November 2009 to October 2012 64

Table 3.2 Macro-invertebrate taxa found in Pond 2, Threshfield Quarry, April 2010 to October 2012 65

Table 3.3 Macro-invertebrate taxa found in Pond 3, Threshfield Quarry, April 2010 to October 2012 66

Table 3.4 Zooplankton found in Ponds 1, 2 and 3 at Threshfield Ark site April 2010 to October 2012 67

Table 3.5 BMWP score categories (BMWP, 1978) 72

Table 3.6 Summary of key findings and management recommendations for Threshfield Quarry ark site, Yorkshire 76

Table 4.1 Mean (± S.D.) water chemistry parameters of pools after 5 weeks under 3 crayfish treatments (juveniles only (J); Adults only (A); and mix of juveniles and adults (M), and control (C) with no crayfish, and results of one-way ANOVA or Kruskal Wallis tests for differences between treatments. 83

Table 4.2 One way ANOVA and Tukey's HSD post-hoc test comparing the biomass of macrophytes and abundances of invertebrate taxa remaining in pools after 5 weeks under 3 crayfish treatments (juveniles only (J); Adults only (A); and mix of juveniles and adults (M), and control (C) with no crayfish........ 84

Table 7.1 Summary of field samples collected autumn 2012 and analysed for stable isotope ratios of $^{13}C/^{12}C$ and $^{15}N/^{14}N$. 136

Table 7.2 Pairwise linear comparisons between prey types consumed within *E. sinensis* and *P. leniusculus* treatment groups........ 139

Table 7.3 One way ANOVA and Tukey’s HSD post-hoc tests (or Kruskal Wallis and Nemenyi-Damico-Wolfe-Dunn post-hoc tests where data could not be normalised) comparing the biomass of macrophytes, mass of detritus and abundances of invertebrate taxa remaining in pools (n = 16) after four weeks within the treatments: *E. sinensis* (CMC), *P. leniusculus* (SC), both *E. sinensis* and *P. leniusculus*, and no decapod control. 144

Table 7.4 Mean (± S.D.) water chemistry parameters of mesocosm pools at the end of the experiment, with 4 pools in each of the following treatments: *E. sinensis*, *P. leniusculus*, and both *E. sinensis* and *P. leniusculus;* along with no decapod control; and results of one-way ANOVA to test for differences between treatments. 147

Table 8.1 Summary of total passages by crayfish through the weir and control reaches over both years 168

Table 9.1 Summary of general management recommendations for Ark sites formulated through this thesis...180

Chapter 1 General Introduction

Freshwater ecosystems, heavily relied upon for irrigation, potable water, transportation and power generation, are among the most anthropogenically impacted with over half of all accessible freshwater in use by humans (Vitousek *et al.*, 1997b; Vörösmarty *et al.*, 2010). Consequences include habitat fragmentation (Fischer & Lindenmayer, 2007), pollution and disrupted flow regimes (Poff *et al.*, 1997). One quarter of freshwater taxa are considered 'endangered' or 'threatened' (IUCN, 2011), with projected extinction rates up to fivefold higher than terrestrial or marine ecosystems (Ricciardi & Rasmussen, 1999; Revenga *et al.*, 2005; Xenopoulos *et al.*, 2005). Of the many pressures impacting freshwater ecosystems, human-mediated introduction of invasive species is one of the most widespread and deleterious (Dudgeon *et al.*, 2006 Leprieur *et al.*, 2009; Ricciardi & MacIsaac, 2011). Introduced species are a global problem, causing a range of direct and indirect effects on native communities and considerable economic burden due to the loss of ecosystem services (Simberloff *et al.*, 2013). In the case of species favoured for human consumption, there exists a strong economic motivation to facilitate establishment beyond native ranges. Crayfish are one such group, and many species have been widely translocated as a consequence (Holdich *et al.*, 1999a). Indeed, it is in part due to such introductions, that crayfish are also one of the most imperilled freshwater taxa (Usio *et al.*, 2007); of the 640 ca. species globally, 26% are classified 'vulnerable' or 'endangered' (IUCN, 2011). This thesis addresses questions concerning both the positive and negative aspects of human-mediated crayfish translocation. The following introduction outlines the background and scope of this work. First, a general overview of crayfish in freshwater systems is provided, followed by a more detailed review of literature relevant to the two main themes of the thesis: translocation of crayfish for the purpose of conservation; and translocated crayfish as invasive species.

1.1. Freshwater Crayfish

Taxonomically, freshwater crayfish comprise three superfamiles: Astacidae; Cambaridae and Parastacidae, all lying within the order Decapoda, class Crustacea, and phylum Arthropoda. The large and diverse order Decapoda also includes crabs, lobsters and shrimp, which in common with crayfish, have ten legs usually comprising four pairs of walking legs and a fifth pair terminating in large chelae or 'claws'. There are over 640 described crayfish species worldwide, with two key centres of diversity: the southern

Appalachian mountains of the United States, and South-Eastern Australia (Crandall & Buhay, 2008). The European continent is relatively species poor compared to North America and Australia; Western Europe has just five native species within 2 genera (Holdich, 2002b).

Crayfish are highly adaptable, both physiologically and behaviourally, and occupy a range of habitats including streams, lakes, subterranean caves and brackish water; with a few Australian species occupying almost entirely terrestrial habitats (Holdich, 2002a). This thesis considers species that occur exclusively within the first two habitat types: streams and lakes.

1.1.1 Morphology and Life History

The crayfish body comprises two distinct regions; the anterior cephalothorax which contains the main organs, and the posterior jointed abdomen or tail which terminates in a ‘fan’ composed of the telson and uropods (Figure 1.1). The body is covered in a thick calcified exoskeleton which is periodically shed to enable growth. The chelae, which are larger in males, have a dual function of food capture and defence or posturing during agonistic interactions. The first two pairs of walking legs (pereopods) also end in small ‘claws’ which are used to collect food and pass it forwards towards the mouthparts. Crayfish possess a gastric mill in which coarse food particles are ground before passing into the posterior part of the stomach (Holdich, 2002a). The antenna and antennuals host many chemoreceptors which are important for sexual reproduction and navigation to food sources (Ameyaw-Akumfi & Hazlett, 1975; Giri & Dunham, 1999). The circulatory system is open and comprises the heart, a secondary pump called the *cor frontale*, seven main arteries, and the sinuses. The blood flows through these open tissue spaces before returning to the heart (Vogt, 2002).

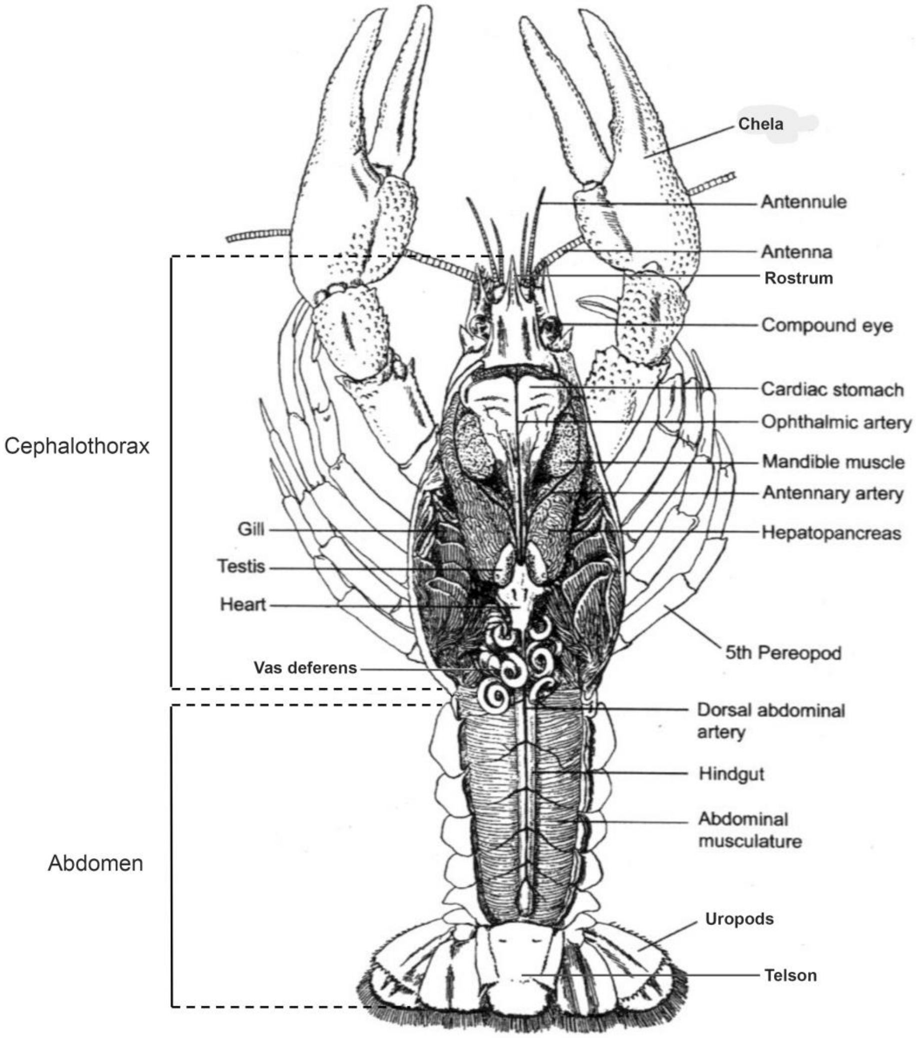


Figure 1.1 Anatomy of male crayfish *Astacus astacus* (adapted from Vogt, 2002)

The respiratory system of crayfish is open and the gills are the main site of gaseous exchange, though they also have an important role in the excretion of ammonia and for osmoregulation (Vogt, 2002). The gill 'branches' called the podobranchs, pleurobranchs and arthrobranchs are attached to the bases of the thoracic appendages, between the body wall and the appendages, and on the epimeral wall lining the branchial chamber, respectively (Vogt, 2002). The gills terminate in flat lamellae and the epithelium is modified to form numerous gill filaments through which the haemolymph passes very close to the surface, thereby providing a large surface area for gaseous exchange (Fisher, 1972). Movement of water over the gills is maintained by the beating action of the scaphognathite which forces water out below the antenna creating negative pressure within the branchial chamber causing water to be drawn in at the bases of the limbs and up over the gills (Burggren *et al.*, 1974) (Figure 1.2). Crayfish possess several mechanisms, both physiological and behavioural, for cleaning the gills to prevent the accumulation of epibionts and abiotic debris (Bauer, 1998). A detailed account of these is provided in Chapter Six that describes a study which investigated the impacts of suspended solids on gill function and aerobic scope in a native and invasive crayfish species.

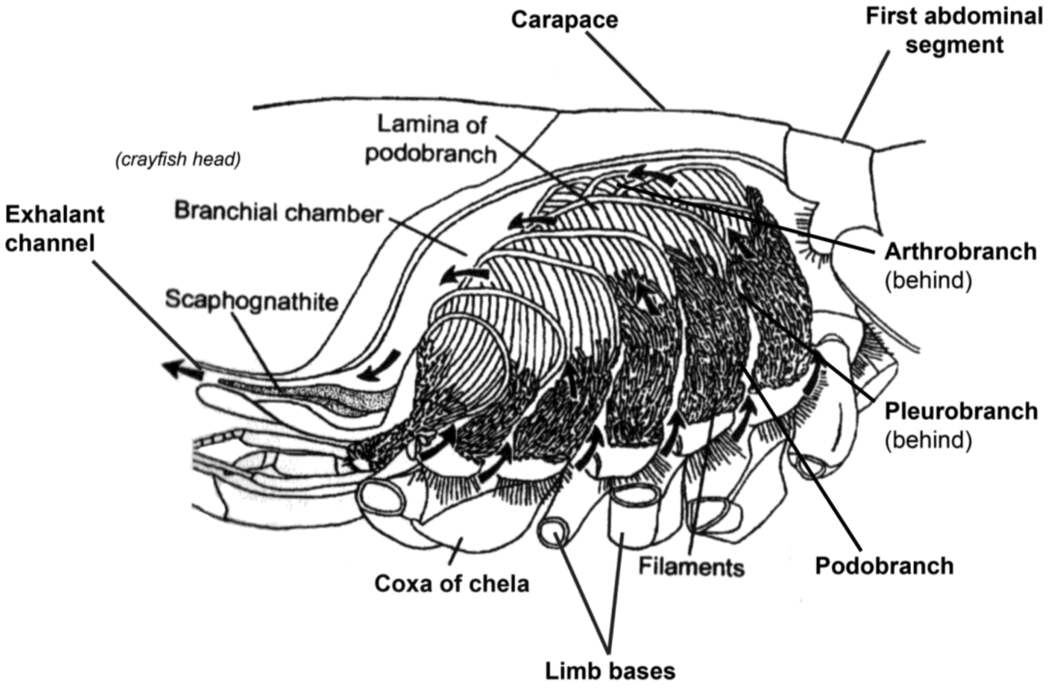


Figure 1.2 Lateral view of left branchial chamber of crayfish *Astacus astacus* showing direction of water flow up through the chamber and over the gills (Vogt, 2002)

Crayfish are highly mobile, able to walk forwards, sideways and backwards both underwater and on land, and are also able to swim rapidly backwards by tail-flipping (Holdich, 2002a). Walking is the predominant mode of movement and the energy-expensive tail propulsion is generally reserved to escape predators (Baldwin *et al.*, 1999). Crayfish are predominantly nocturnal and exhibit circadian rhythms with peak activity and most foraging occurring during the three hours following dusk (Robinson *et al.*, 2000; Styrishave *et al.*, 2007). Daylight hours are spent avoiding predators within refugia which may be under rocks, among tree roots, or within purposefully constructed burrows (Bubb *et al.*, 2006). Crayfish are ectothermic; hence activity is strongly related to ambient temperature and little movement occurs during the winter months (Bubb *et al.*, 2002a).

European crayfish take two years or longer to reach sexual maturity. During this main juvenile growth phase ecdysis may occur as many as eight times per year (Pratten, 1980), reducing to twice annually as adults (Brewis & Bowler, 1982). The lifespan of crayfish may extend to over 15 years, though is shorter among warm water species (Holdich, 2002a). Crayfish may reproduce multiple times during their adult life-stage, and all species besides one (Marmorkrebs, (*Procambarus fallax f. virginalis*)) reproduce sexually (Scholtz *et al.*, 2003). Decreasing water temperature is the predominant environmental cue for mating, which in the Northern hemisphere occurs from late September to November (Brewis & Bowler, 1985; Westin & Gydemo, 1986). In common with many aquatic organisms, chemical as well as visual cues are used in mate selection (Brönmark & Hansson, 2000; Acquistapace *et al.*, 2002). During mating the male deposits spermatophores on the ventral side of the female's abdomen, which remain there until egg-laying occurs. Female fecundity varies greatly between species, ranging from approximately 60‒160 eggs in *Austropotamobius pallipes* (Rhodes & Holdich, 1982) to in excess of 500 in warm water species (Holdich, 2002a). The eggs remain attached to the abdomen for the duration of incubation (ca. 6 months) and the female is largely inactive during this time (Berrill & Arsenault, 1982). Crayfish are fully formed when hatched, but remain closely associated to the female for the first two (Astacidae) or three (Cambaridae) juvenile stages until they disperse two to four weeks later (Ingle, 1997).

1.1.2. Crayfish as components of freshwater ecosystems

Crayfish are a functionally important component of both lotic and lentic freshwater ecosystems, and constitute keystone species in many instances (Creed, 1994). As the largest freshwater invertebrates, crayfish may comprise up to 85% of total invertebrate biomass in a system (Neveu, 2009). Recognition of their functional importance coupled with growing concern about the potential impacts of invasive crayfish has led to much research examining the role of crayfish in freshwater ecosystems. They exert a strong influence through trophic interactions (Nystrom *et al.*, 1996; Dorn & Trexler, 2007), but also as ecosystem engineers moving substantial amounts of material during burrowing (Statzner *et al.*, 2000; Brown & Lawson, 2010), and modifying detrital processing rates and the distribution of fine particulates (Creed & Reed, 2009).

Crayfish as a group are omnivorous, consuming a wide range of food sources from multiple trophic levels (Correia, 2002; Bondar *et al.*, 2005; Gherardi & Acquistapace, 2007). Cannibalism is also reported for many species (Taugbøl & Skurdal, 1992; Englund & Krupa, 2000), particularly when food is limiting (Abrahamsson, 1966; Elgar & Crespi, 1992). Palatability and preference tests suggest that crayfish dietary choices are driven by nutritional content (Adams *et al.*, 2005), past experience (Gherardi & Barbaresi, 2007) and ease of capture (Brown, 1998), whereas in the field, behavioural studies suggest foraging patterns reflect a complex balance between food preference and competition (Gherardi *et al.*, 2001). There is also evidence of diet-shifting based on food availability (Correia, 2002), and life-stage (Guan & Wiles, 1998; Olsson & Nystrom, 2009).

At the lowest trophic level, crayfish directly consume detritus, periphyton and macrophytes, and may indirectly structure macrophyte and algal communities through predation of grazers and burrowing activity which disturbs sediments, increases turbidity and impairs plant growth (Lodge, 1991; Lodge *et al.*, 1994; Nystrom *et al.*, 1996; Dorn & Wojdak, 2004). Allochthonous detritus is often the predominant basal resource in crayfish food webs, followed by aquatic macrophytes and algae (Whitledge & Rabeni, 1997b; Rudnick & Resh, 2005; Reid *et al.*, 2008); though direct consumption of living terrestrial plants has been demonstrated for *P. clarkii* when aquatic resources become limiting (Grey & Jackson, 2012).

Although dietary preferences and consumption rates vary among crayfish species, it is generally considered that they exert strong negative impacts on aquatic macrophyte biomass and species richness (Nystrom, 1999; Rodríguez *et al.*, 2005; Roth *et al.*, 2007). Submerged macrophyte species such as *Chara*, *Potamogeton* and *Nuphar* (Chambers *et al.*, 1990; Matthews *et al.*, 1993; Matsuzaki *et al.*, 2009) are particularly vulnerable to crayfish due to their structure, digestibility and, in the case of *Chara*, high nutritional content (Nystrom, 1999); nevertheless, emergent species are also frequently consumed whilst at the seedling stage (Nystrom & Strand, 1996).

More difficult to predict are indirect interactions between crayfish and other herbivores. For example, male virile crayfish (*Orconectes virilis*) reduced the biomass of four macrophyte species even at relatively low densities (5 and 18 g m^{-2}), but presence of female crayfish indirectly stimulated macrophyte growth through consumption of herbivorous snails (Chambers *et al.*, 1990). Similarly, crayfish do graze directly upon periphytic algae, though are inefficient consumers of this resource compared to aquatic snails which are heavily preyed upon by crayfish (Crowl & Schnell, 1990; Lodge *et al.*, 1994; Wilson *et al.*, 2004)); hence crayfish presence is generally, but not always (e.g. Bobeldyk & Lamberti, 2008), associated with an increase in periphyton due to reduced grazing pressure by snails (Lodge *et al.*, 1994; Charlebois & Lamberti, 1996). Crayfish also directly consume microalgae such as *Cladophora* sp. (Guan & Wiles, 1998) and may exert a strong influence on biomass (Wallace & Webster, 1996), to the point of excluding it in deeper microhabitats (Creed, 1994).

Macro-invertebrates are frequently the second most commonly occurring food types in crayfish gut contents (Whitledge & Rabeni, 1997b; Guan & Wiles, 1998; Correia, 2003; Bondar *et al.*, 2005). As generalist predators of the benthos, crayfish consume a wide range of taxa from all functional feeding groups including predators (e.g. Odonata, Trichoptera), shredders (e.g. Trichoptera, Amphipoda), gatherers (e.g. Chironomidae) and grazers (most notably, Gastropoda) (Momot, 1995; Gherardi *et al.*, 2004; Reynolds & O'Keeffe, 2005). Concordant with optimal foraging theory that prey is consumed in order of decreasing ease of capture and nutritional value (Krebs, 1978), slow-moving isopods tend to be most favoured by crayfish, followed by snails and then swimming species (Haddaway *et al.*, 2012b). Indeed, several authors report a community shift towards burrowing and swimming species in lakes where crayfish are present (Abrahamsson, 1966; Nystrom *et al.*, 1996). Of the snails, thin-shelled species are the most favoured (Alexander & Covich, 1991; Brown, 1998), presumed as a function of their ease of handling. Crayfish also consume the eggs, and to a lesser extent, the swimming larvae of larger aquatic species such as amphibians (Axelsson *et al.*, 1997) and fish (Guan & Wiles, 1997; Edmonds *et al.*, 2011). There is generally a paucity of literature on the population level impacts of crayfish on aquatic vertebrates, though there is much concern about the impact of introduced crayfish on commercially important fish (Griffiths *et al.*, 2004; Degerman *et al.*, 2007) and threatened amphibian species (Cruz *et al.*, 2008).

Crayfish themselves are important prey for higher predators including otters and mink, fish such as perch, eels, and bass (Stein, 1977; Taylor *et al.*, 1988; Blake & Hart, 1995), as well as avian predators (Correia, 2001). As generalist feeders of detritus, periphyton, macrophytes and benthic invertebrates, crayfish convert energy from lower trophic levels into a form readily exploited by their predators i.e. increase the efficiency of the food web (Rabeni, 1992; Foster & Slater, 1996; Dorn & Mittelbach, 1999). This polytrophic role as a key energy transformer between trophic levels is likely to be most important in oligotrophic lakes, where crayfish contribute to the stability of such communities (Momot *et al.*, 1978; Statzner *et al.*, 2000; Matsuzaki *et al.*, 2009) and facilitate energy transfer between the littoral and profundal zones (Ruokonen *et al.*, 2012).

1.1.3 Crayfish and humans

The absence of a larval life-stage among crayfishes makes them comparatively easier to culture than other commercially exploited decapods such as lobsters or prawns; hence several crayfish species are commercially farmed for the table market (Holdich, 2002a). Species of the genus *Cherax* originating from Australia, and *Procambarus clarkii* which originates from the south-eastern US and Mexico are particularly favoured due to their productivity (Holdich, 1993). Large-scale exploitation of wild stocks also occurs in the US, China and Australia (Holdich, 1993). Global production was in the region of 550000 tonnes per annum in 2011 (FAO 2013), with over 90% of harvest derived from non-indigenous species (Lodge *et al.*, 2012). The UK alone consumes over 1000 tonnes of crayfish meat per year, largely comprised of *P. clarkii* imported from Asia, and it is generally considered a gourmet food item. Elsewhere there are strong cultural traditions surrounding crayfish consumption. For example, in Scandinavia crayfish parties are held during August to coincide with the traditional harvest period of wild stocks; in the US, ‘crawfish boils’ are a much celebrated feature of the summer social calendar across Louisiana and southern Texas.

The decline of many indigenous crayfish species, coupled with a growing body of scientific literature highlighting their importance in aquatic ecosystems, has fostered interest about crayfish generally and concern for their conservation (Souty-Grosset *et al.*, 2006b). The recent IUCN ‘endangered’ status of several crayfish species has raised their profile (Kozák *et al.,* 2011), and conservationists frequently advocate their use as flagship species to increase public awareness and highlight the need for restoration efforts for the recovery of freshwaters generally (Fuereder & Reynolds, 2003; Kozák *et al.*, 2011). Considering that the greatest threat to indigenous crayfish is introduced species, raising awareness among stakeholders and the public at large is considered crucial to reduce the spread of invasive crayfish to new areas, either intentionally or unintentionally; provide an early warning system of new introductions, and to encourage the shared responsibility necessary to underpin intervention measures and effectively manage remaining native crayfish populations (Gherardi *et al.*, 2011).

1.2. The white-clawed crayfish, a species in decline

Britain has only one native crayfish species, the white-clawed crayfish (*Austropotamobius pallipes*), and represents both the most Northerly and Westerly limits of its distribution (Holdich, 2002c). Although range reduction of *A. pallipes* due to exploitation and habitat destruction is thought to have begun as early as the 1860’s

(Souty-Grosset *et al.*, 2006a), declines rapidly accelerated following introduction of the signal crayfish (*Pacifastacus leniusculus*) in 1976 (Holdich & Gherardi, 1999). *P. leniusculus* originates from North America and had already been cultured in Sweden since the 1960s to supplement wild stocks of the native noble crayfish (*Astacus astacus*), which were declining due to crayfish plague caused by the Chromista *Aphanomyces astaci (Henttonen & Huner, 1999)*. This obligate parasite originates from North America (Unestam, 1972) and crayfish species from the region may carry the infective zoospores of this parasite with no fitness impacts, but European species have no resistance and once infected experience progressive paralysis and ultimately death (Unestam & Weiss, 1970). The resistance of *P. leniusculus* to crayfish plague was appealing from an aquaculture perspective and numerous crayfish farms for *P. leniusculus* were started in England, often using financial support from Government, with a view to supply the Scandinavian market and develop a similar market in the UK. However, the comparatively long lag time before harvest (4 years) and competition from cheaper Asian imports meant such ventures soon became uneconomical and farms were closed down or re-developed; though frequently without adequate containment of existing stock. *P. leniusculus* has consequently spread substantially since its introduction and the associated spread of *A. astaci* has caused the extirpation of many native crayfish populations. As of 2010, 83% of sub-catchments in England and Wales contained *P. leniusculus* whereas just 42% of sub-catchments contained *A. pallipes*, either in combination with *P. leniusculus* or alone (Rogers & Watson, 2011).

Although plague has undoubtedly had the greatest impact on *A. pallipes*, other mechanisms are in operation. In contrast to stenotopic *A. pallipes*, which has low ecological resistance and resilience (Benvenuto *et al.*, 2008), *P. leniusculus* grows faster, is more fecund and has wider environmental tolerances (Laurent, 1988; Firkins & Holdich, 1993), enabling them substantial competitive advantages. Evidence from mixed populations indicates that white-clawed crayfish are excluded from both prime food sources (apparent from their small size relative to non-mixed populations) (Dunn *et al.*, 2009) and refugia, rendering them more susceptible to predation (Bubb *et al.*, 2006). Even in the absence of crayfish plague, the white-clawed crayfish is competitively excluded from mixed populations within four to five years (Peay & Rogers, 1998).

A. pallipes is afforded protection under both EU and UK legislation. Within Europe it is listed under annexes II and V of the EU Habitats Directive (92/43/EEC) and Appendix II of the Bern Convention. In the UK it is protected under Schedule 5 of the Wildlife and Countryside Act (1981), a status requiring management plans be put in place to conserve both the species and its habitat. Further, as a priority species under the UK Biodiversity Action Plan (Departement of the Environment, 1995), nine Special Areas of Conservation (SACs) have been designated solely on the basis of their native crayfish populations (JNCC, 2011). Despite this legislative framework, *A. pallipes* is still declining and is predicted to become virtually extinct except in isolated pockets, within 20 years (Sibley, 2003). Of the nine SACs set up for *A. pallipes*, only two remain free of *P. leniusculus*, and some criticise the legislation as unenforceable and ineffective (Anonymous); perhaps a justifiable view considering the small number of prosecutions that have occurred t under this law.

Much work concerning control and eradication measures for *P. leniusculus* is underway; yet in the absence of a treatment for plague, or satisfactory means to control the spread of invasive crayfish, translocation of imminently threatened populations of natives to sites where such threats are reduced is advocated by many (Kemp *et al.*, 2003; Whitehouse *et al.*, 2009). These aptly named “Ark sites” have become a key component of crayfish conservation strategy in Europe (Schulz *et al.*, 2002) and are hoped to provide long-term strongholds for native populations in invaded catchments, as well as re-stocking material if invader-free watercourses can be secured in the future.

1.3 Translocation for conservation

Human-mediated translocation of species has occurred for millennia; as long as humans have overcome geographical barriers to explore and populate new areas (Di Castri, 1989). More recently, intentional translocations are being used for conservation purposes, for example, to restock after a pollution incident, re-introduce animals to their former range, or reinforce a dwindling population. Islands have been a particular focus for conservation translocation efforts, not least because their taxa are disproportionately threatened, but also because their isolation frequently offers benefits in terms of managing the threat (e.g. exotic predators) (Armstrong & Seddon, 2008). Some high profile examples of successful translocations in the UK are the red kite (*Milvus milvus*) which was formerly globally threatened, but after initial re-introduction to two sites in the UK, quickly formed breeding pairs (Carter & Newbery, 2004); reinforcement of otter (*Lutra lutra*) populations in East Anglia (Jefferies *et al.*, 1986) and several reintroductions of the sea eagle (*Haliaeetus albicilla*) to Scotland commencing in 1975, which by 2004 had established 30 breeding pairs (Evans *et al.*, 2009). Despite the many high profile success stories, systematic reviews collating the outcomes of reintroductions have revealed generally low success rates (Armstrong & Seddon, 2008). Of the many cited reasons for failure, lack of habitat suitability and spread of disease are among the most prominent (Wolf *et al.*, 1996; Hodder & Bullock, 1997).

In recognition of the increasing use of translocation for conservation and improved knowledge of the processes underlying successes and failures of such interventions, the IUCN recently published revised guidelines of best practise for conservation translocations (IUCN, 2012). These provide guidance for each stage of the translocation process from project justification through to post-translocation monitoring and dissemination of information, the salient points of which are summarised in Figure 1.3. A risk-based decision making process is advocated, emphasising the need to minimise undesirable outcomes for both the donor population and recipient site. In a review of 454 projects, Seddon *et al.* (2007) identified careful project planning, multidisciplinary collaboration between project partners, and thorough evaluation and reporting of outcomes as key areas for improvement in conservation translocations.

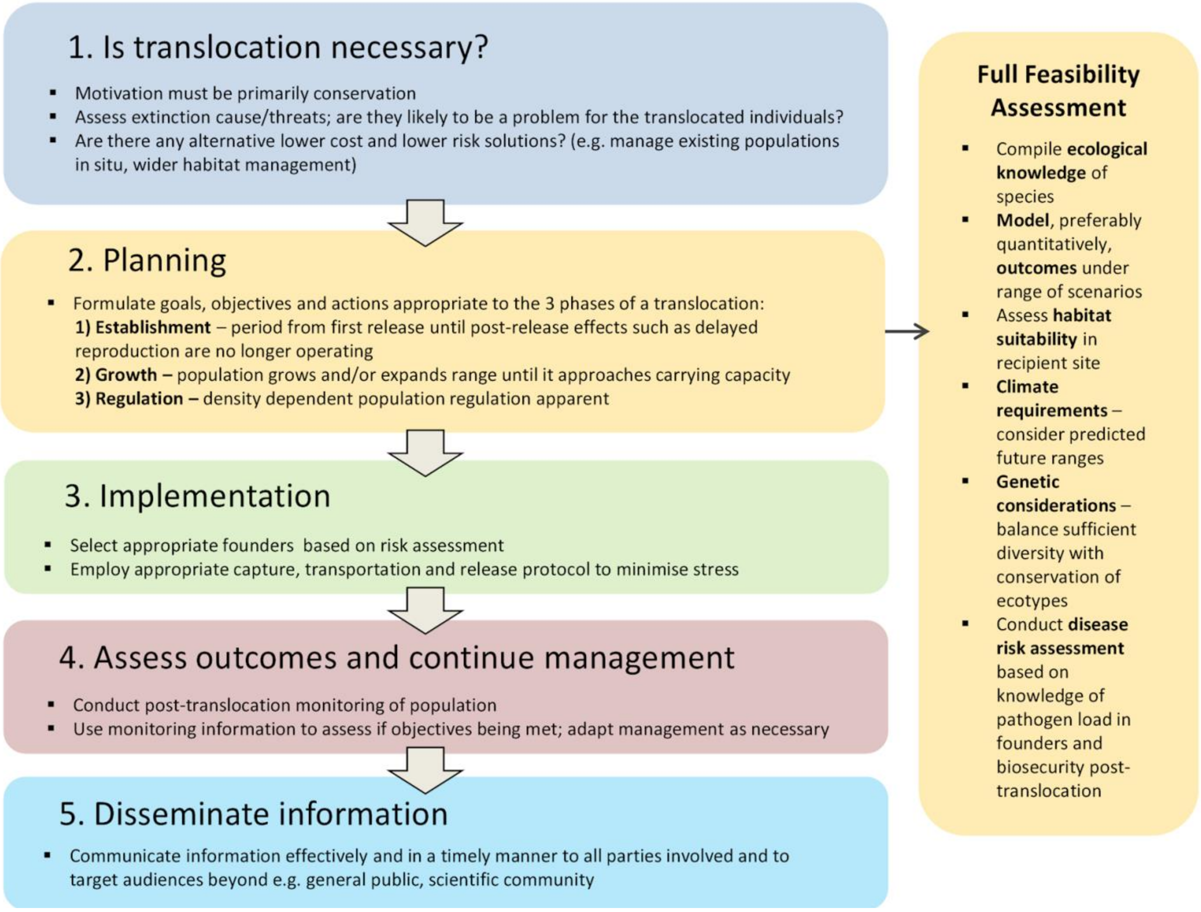


Figure 1.3 Summary of recommendations for conservation translocations formulated by the IUCN

1.4 Ark sites for white-clawed crayfish

Crayfish Ark sites are waterbodies free of invasive crayfish and with low risk of future invasion where imminently threatened native populations may be translocated with the aim to facilitate long-term survival. Many successful crayfish re-introductions to sites where they had been extirpated, and introductions to sites where crayfish were previously unknown have, already taken place within Europe for both the noble crayfish (*Astacus astacus*) (Schulz *et al.*, 2002; Sint & Fureder, 2004) and *A. pallipes* (Gil-Sanchez & Alba-Tercedor, 2002; Gil-Sanchez & Alba-Tercedor, 2006). In the UK, the first record of a crayfish re-introduction following an outbreak of crayfish plague occurred in 1987 to five sites on the Tetbury Avon, Wiltshire (Rogers, 2000), and the first authorised introduction solely for the purposes of conservation was carried out in 2006 in the Bristol Avon catchment (Teixeira *et al.*, 2007). These early successes have focussed efforts on creating further Ark sites across Europe; however records of high and very rapid post-translocation mortality of crayfish, over 50% in one study (Reynolds *et al.*, 2000; Rogers & Watson, 2007), highlight the importance of both the appropriate selection of suitable sites, and the development of effective translocation protocols.

1.4.1. Ark site selection

Proposals of potential Ark sites may derive from strategic conservation planning on the part of national and regional bodies such as the Environment Agency, Natural England and the Rivers Trusts. An alternative bottom-up approach is driven by local stakeholders including conservation groups focussed on ‘saving’ a particular crayfish population, or landowners with potential sites looking for restoration opportunities.

A simple set of selection criteria for the assessment of potential Ark sites is provided by Peay (2009) who advocates a risk-based approach. An initial selection stage is used to determine how likely the site is to become invaded in the future, what potential vectors of crayfish plague may be present and consider any other adverse factors. The second stage examines the site in relation to nine specific selection criteria which generate an overall rating of suitability (Table 1.1).

Table 1.1 Proposed criteria for initial selection of Ark sites for *Austropotamobius pallipes* in the UK (derived from Peay 2009)

Importance (1=most)	**Criterion**
1	Degree of enclosure
2	Terrestrial barriers
3	Aquatic barriers
4	Water quality and quantity
5	Local status of non-indigenous crayfish and crayfish plague
6	Angling presence
7	Usage and risks from access
8	Ownership
9	Physical habitat

These criteria are indicative of the emphasis placed on assessing the risk of failure due to either invasion of non-native crayfish or transferral of crayfish plague by other means. Understandably, this is a major concern as the use of resources to create an Ark site that will not be safe in the medium to long-term would be false economy, however, little consideration is given at this stage to the actual conditions of the site in terms of the ecological requirements of the species. Once the potential sites have passed the initial stages, it is recommended that a full feasibility study be undertaken examining in detail issues such as the likely impact of introducing crayfish on other fauna, securing resources and identifying factors for “improvement” to meet the habitat requirements of the target species (Peay, 2009).

1.4.2 Former quarries as Ark sites

Former mineral extraction sites such as stone quarries and gravel pits, for which restoration is often a legal obligation, may present opportunities as Ark sites (Whitehouse *et al.*, 2009). The remote and isolated nature of many sites, combined with limited or no connectivity with the surrounding catchment, greatly reduces the risk of accidental or deliberate translocation of non-native crayfish and crayfish plague. Further, the physical, biological and chemical habitat remaining at the termination of mineral extraction may require comparatively little restoration and cost to optimise its potential as suitable habitat for *A. pallipes*. From the perspective of the mineral industry, the creation of Ark sites is highly compatible with wider requirements for post-extraction restoration to provide diverse habitats and improve biodiversity; it may be used to off-set against expansion of operations elsewhere, and enhances a ‘greener’ image of the industry through community involvement and raising awareness. In addition, Ark site creation contributes to the fulfilment of environmental requirements within Permitted Development agreements, and there is scope to finance such projects through the redirection of funds raised via the Government's Aggregate Levy.

The UK invertebrate conservation charity Buglife strongly promotes the use of former mineral extraction sites as Ark sites. In a GIS-based scoping study centred on the South-West, Kindemba *et al.* (2009) identified 39 potential sites from 231 former quarries using coarse scale filters such as proximity to non-native crayfish and geology. They advocate extending this approach nationally to identify and prioritise potential locations, ultimately creating numerous ark sites throughout the UK. A case study of Ark site creation in one such site, a former limestone quarry in the Yorkshire Dales, is presented in Chapter Three.

1.4.3. Habitat suitability

1.4.3.1. Water chemistry

The remaining strongholds of *A. pallipes* largely exist in pristine headwaters (Grandjean *et al.*, 2000; Collas *et al.*, 2007), contributing to its assumed status as a bio-indicator of good water quality (Füreder & Reynolds, 2003; Füreder *et al.*, 2003); however, this has been reassessed following studies that indicate *A. pallipes* can persist in watercourses of sub-optimal water quality (Broquet *et al.*, 2002; Demers *et al.*, 2006). With the exception of pH (Haddaway *et al.*, 2013) and oxygen (Demers *et al.*, 2006), there is an absence of controlled laboratory studies investigating the tolerance of *A. pallipes* to water chemistry parameters. Consequently, tolerances may generally only be inferred from the many studies correlating presence/absence data for the species with concurrent water chemistry variables (Troschel, 1997; Broquet *et al.*, 2002; Trouilhe *et al.*, 2003). Often only weak correlations are reported which may indicate that crayfish will occur as long as threshold levels are met, and the population only significantly affected if parameters fall outside acceptable ranges (Lodge & Hill, 1994). A full review and meta-analysis on this subject is provided by Haddaway (2010) and is not repeated here, though a summary of key findings is presented in Figure 1.4.

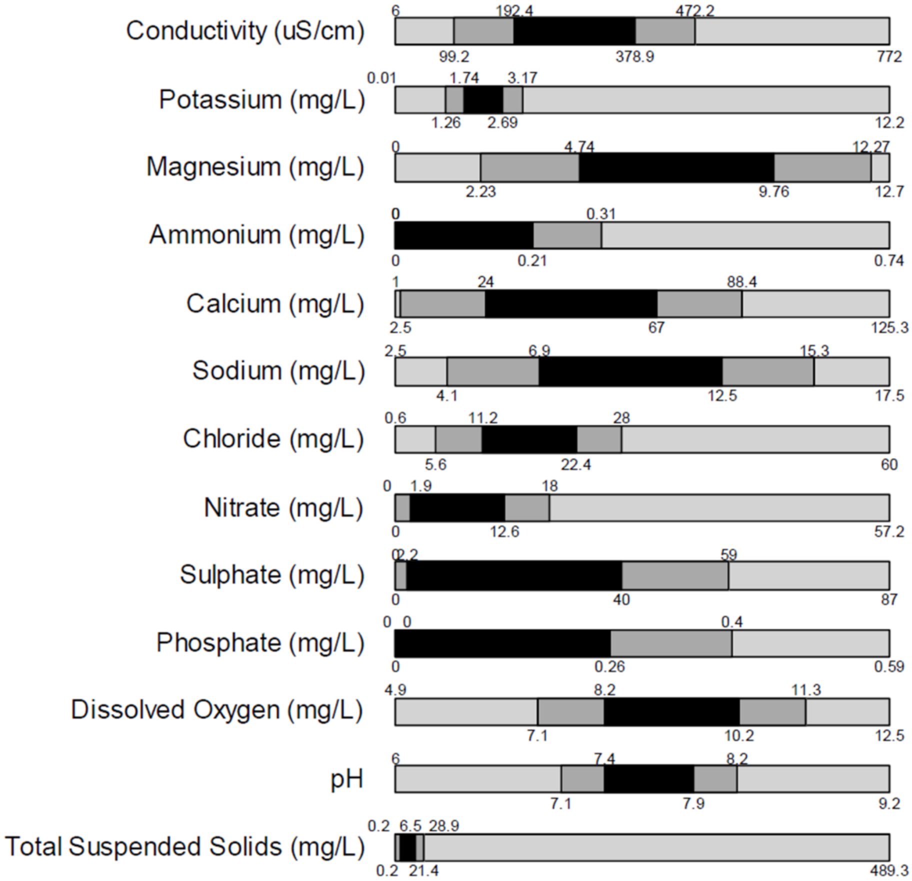


Figure 1.4 Summary of recommended water quality guidelines for *Austropotamobius pallipes* extracted from the literature by Haddaway (2010). Total range (light grey) corresponds to the widest range of variable levels cited for *A. pallipes*, the outer buffer zone (dark grey) and inner buffer zone (black) refer to ranges within 5 and 2.5 standard errors of means reported in the literature (reproduced with permission).

For many water chemistry parameters, the range of concentrations over which *A. pallipes* occurs is high. For example, populations have been found at chloride concentrations ranging from 0.6 mg $^{-}$L, to 100 times higher at 60 mg L^{-1}; and in calcium concentrations ranging from 1 to 125 mg L^{-1} (Haddaway, 2010). For parameters such as calcium, magnesium, pH and potassium, where the mean ± 2.5 S.E. buffer zone occurs near the centre of the maximum range, it may be inferred that moderate concentrations of these ions are beneficial, and in the case of calcium and magnesium, essential for crayfish survival (Huner *et al.*, 1976; Rukke, 2002). The location of the means for ammonium, sulphate, phosphate, nitrate and suspended solids towards the lower end of the tolerance range indicates that they may be detrimental to the survival of *A. pallipes*, and indeed all are common pollutants in freshwaters (Cooper, 1993).

Despite recognition of suspended solids as a key water quality parameter (Gilliom *et al.*, 1995), and one that has increased over the past century due to agricultural intensification (Foster *et al.*, 2011) becoming a major environmental stressor in freshwaters (Bilotta &

Brazier, 2008; Kemp *et al.*, 2011), the level of suspended solids is rarely measured in studies relating occurrence of *A. pallipes* to water chemistry parameters. In the few studies that do, *A. pallipes* is generally found to occur in waters with relatively low total suspended solids levels (< 30 mg L^{-1}) (Broquet *et al.*, 2002; Trouilhe *et al.*, 2003); though it is not apparent whether this relationship reflects an intolerance of *A. pallipes* to suspended solids, or that suspended solids co-varies with other parameters which directly impact *A. pallipes*. Chapter Six details a controlled laboratory-based study which investigated the impacts of chronic exposure to suspended solids on gill structure and function in *A. pallipes*, and compared impacts with the invasive crayfish *P. leniusculus*.

1.4.3.2. Physical habitat

A. pallipes inhabits a wide variety of environments, both lotic and lentic, including rivers, streams, canals and reservoirs; though rarely occurs in smaller ponds (Jay & Holdich, 1981). When water chemistry parameters are within acceptable ranges, predation is the key determinant of crayfish population size (Lodge & Hill, 1994) and habitat quality is an important determinant of predation risk (Kershner & Lodge, 1995). In avoidance of predation, crayfish seek shelter in refugia such as under cobbles, within tree roots or undercut banks during inactive daylight hours (Barbaresi & Gherardi, 2001), and the availability of such refugia is considered a strong limiting factor to crayfish numbers (Lodge & Hill, 1994; Holdich, 2003).

A general review of the habitat factors affecting cool water crayfish species was carried out in 1994 which dealt mainly with species of commercial importance such as those of genus *Orconectes* or *Astacus*, though did identify a preference of *A. pallipes* for rocky, lotic environments (Lodge & Hill, 1994). Within another review based on four studies, Holdich and Rogers (2000) compiled a broad list of habitat attributes deemed to be desirable for the species including rocks, rubble and tree roots, stable banks, and emergent and submerged vegetation; however the authors themselves noted that *A. pallipes* certainly occurs in locations where many of those habitat attributes are not present. Conversely, it is often absent from watercourses where conditions appear optimal, with no previous pollution or invasive crayfish issues, and no apparent dispersal limitations (Nardi *et al.*, 2005). Understanding habitat requirements is a key part of the conservation of declining species generally, but particularly when reintroductions are planned as part of the recovery strategy (Seddon *et al.*, 2007; Sutherland, 2008). Chapter Two presents a systematic review of studies primarily concerned with habitat use by *A.*

pallipes species complex, including more recent studies that investigated microhabitat use in the wild.

1.4.3.3. Food resources

Sufficient food resources to sustain the projected crayfish population is of obvious importance in a prospective Ark site. As omnivores, crayfish are able to utilise a range of resources such as plant detritus, macrophytes, invertebrates, and even small fish (Momot, 1967; Nystrom & Perez, 1998; Nystrom *et al.*, 1999; Reynolds & Donohoe, 2001; Reynolds & O'Keeffe, 2005), partly reflecting the availability of such food sources at the particular site. On a small scale, foraging patterns and diet choice studies reveal a complex balance between dietary preferences and competition. In a study of foraging activity of *A. pallipes* in a stream pool, crayfish undertook foraging excursions of up to one hour, systematically searching for food using a “transecting strategy” seeking resource patches of highest carbon and nitrogen content; in this case plant detritus (Gherardi *et al.*, 2001). However, intra-specific competition likely exerts a stronger influence on foraging patterns than nutritional content (Abrahamsson, 1966; Momot, 1993; Gherardi *et al.*, 2001). In lake situations where range expansion is not possible, less competitively advantaged juvenile and female crayfish may be excluded by larger male crayfish from optimal foraging habitat resulting in slow growth rates, even in fairly productive systems (Nystrom, 2002 and references therein). Quantitative assessment of the availability of food resources at all potential Ark sites is likely to be prohibitively challenging and expensive, however post-translocation monitoring of crayfish growth rates, condition and fitness indicators such as chelae size would provide early indication if food resources had become limiting.

Particularly in the case of still-water Ark sites, the differences in diet choice that may occur as a population moves from a lotic to lentic environment should be considered. In the only comparison of diet composition of stream dwelling and lake dwelling *A. pallipes*, Reynolds & O’Keefe (2005) found that within the stream community more plant fragments, insects and crayfish were consumed, whereas the lake dweller’s diet contained more fish, molluscs, small Crustacea and charaphytes. As no attempt was made to quantify the availability of food resources in the two environments one cannot know if this reflects actual changes in food preference or is driven by bottom-up processes such as relative availability. Several studies indicate that crayfish undergo ontogenic shifts in diet (Mason, 1975; Nystrom *et al.*, 1996; Nystrom & Strand, 1996; Nystrom, 2002; Reynolds & O'Keeffe, 2005); hence patterns of exploitation of food resources in Ark sites may reflect crayfish population structure. In *A. pallipes* this has only been investigated indirectly though gut content analyses, with adults found to preferentially consume vegetal items such as macrophytes, woody fragments and aufwuchs (Nystrom *et al.*, 1996; Nystrom & Strand, 1996; Reynolds & O'Keeffe, 2005), whereas juveniles more frequently act as predators, with micro-crustaceans comprising a greater proportion of the diet (Mason, 1975; Nystrom, 2002). Differences may be attributed to variation in energy requirements between life-stages (Whitledge & Rabeni, 1997b), or reflect their ability to handle certain prey types (Reynolds & O'Keeffe, 2005). Ontogenic differences in the trophic interactions of *A. pallipes* were further investigated experimentally using a mesocosm study described in Chapter Four.

1.4.4. Taxonomic considerations

IUCN guidelines for re-introductions recommend that the donor stock should be related at closely as possible to the original population. The taxonomy of *Austropotamobius*, particularly *A. pallipes* species complex, is controversial. The historic classification, based on 16S rRNA which defined *A. pallipes* complex as two genetically distinct lineages (*A. italicus* and *A. pallipes*) (Grandjean & Souty-Grosset, 2000; Grandjean *et al.*, 2002; Fratini *et al.*, 2005), has recently been robustly challenged by a combined analyses of CO-I gene fragments and AFLP fingerprinting which indicated that *A. pallipes* and *A. italicus* are one species (Chiesa *et al.*, 2011). Only *A. pallipes* occurs in the UK, with populations closely related to those of Western France, most likely the descendents of a cluster surviving the last ice age in an Atlantic refugium (Gouin *et al.*, 2001). The genetic structure of Irish stocks suggests an historic human-mediated introduction from French populations (Reynolds *et al.*, 2002; Gouin *et al.*, 2003). Current protocol states that where possible, a donor population should be sought from within the same watercourse, then catchment, then river basin (Kemp *et al.*, 2003); however, considering the apparent genetic conformity of *A. pallipes* in the UK, inter-catchment translocations would be acceptable (Grandjean *et al.*, 1997; Kemp *et al.*, 2003).

1.4.5. Pathogens, parasites and disease

During translocations, communities of parasitic organisms may also be transported unintentionally along with their hosts, with potential implications for both the target species and for other organisms in the recipient site (Cunningham, 1996; Van Oosterhout *et al.*, 2007). The process of translocation is inherently stressful and animals are likely to suffer increased susceptibility to pathogenic organisms as a result (Teixeira *et al.*, 2007); indeed, disease is often cited as a key factor in unsuccessful translocations

(Viggers *et al.*, 1993). Current crayfish translocation protocols recommend surveillance of the donor stock prior to transport (Kemp *et al.*, 2003); however routine health screening is not commonly conducted.

The main disease concern for crayfish translocation is of course crayfish plague and risk of introduction of *A. astaci* is a major consideration in the selection of appropriate Ark sites (Souty-Grosset & Reynolds, 2009). Besides plague, *A. pallipes* potentially suffers chronic losses and fitness impacts due to a number of parasites and diseases including fungal and bacterial ‘burn spot disease’ which damages the cuticle making the crayfish more susceptible to secondary infections (Evans & Edgerton, 2002), and the microsporidian parasite *Thelohania contejeani*, the causative agent of thelohaniasis or ‘porcelain disease’ (Vogt, 1999; Longshaw, 2011). *T. contejeani* commonly infects 1-2% of individuals in wild populations, though prevalence up to 50% has been reported (Imhoff *et al.*, 2012). This microsporidian infects muscle tissue causing it to appear opaque, and though the fitness consequences of infection are not well-documented (Imhoff, 2010), it may cause mortality after one to two years (Skurdal *et al.*, 1990).

Crayfish may also be host to branchiobdellidans, or crayfish worms. These clitellate annelids complete their entire lifecycle on crayfish and several species may be found living sympatrically on the same host (Gelder *et al.*, 1994; Klobucar *et al.*, 2006). Most species are considered commensals, for example *Branchiobdella parasita*, *Branchiobdella balanica* and *Branchiobdella pentodonta*, which largely reside on the carapace or mouthparts and consume epibionts, diatoms and other detritus on the exterior of the crayfish (Vogt, 1999). The similarly carapace-dwelling branchiobdellidan species found on the crayfish *Cambaroide similis* has been shown to offer fitness benefits to the host by consuming epibionts from the carapace, thereby reducing fouling (Lee *et al.*, 2009). Other species such as *B. hexodonta* dwell almost entirely within the branchial chambers and appear to operate as parasites by directly consuming gill tissue (Grabda & Wierzbicka, 1969). The occurrence of branchiobdellidans in the UK is very low compared to mainland Europe (Gelder *et al.*, 1999a), with only two reported sightings (Leeke & Price, 1965; Rogers *et al.*, 2003). Chapter Five describes the discovery of *Branchiobdella astaci* in two *A. pallipes* populations in Yorkshire and investigates the impacts of this gill-dwelling epibiont on the host crayfish, including associated pathology of gill tissues.

1.4.6. Post-translocation monitoring

Post-translocation monitoring should be an important consideration in the planning of all translocations for conservation (IUCN, 2012), yet is overlooked in many programmes (Armstrong & Seddon, 2008). Sutherland *et al.* (2010) recently called for a standardised method of monitoring for bird populations to ensure that organisations plan and commit to a monitoring regime prior to translocation, and to facilitate comparison between projects providing greater data-gathering potential and knowledge to improve translocation success. Monitoring crayfish populations quantitatively in lotic or lentic systems presents a challenge as individuals are often naturally clustered (Momot, 1967; Rabeni, 1985). Mindful of this, Di Stefano *et al.* (2003) developed the only fully quantitative survey method for crayfish in lotic systems, using a 1 m^2 quadrat within a sampling design stratified by habitat at two levels: primary and marginal, and five microhabitats within. Lentic environments present quite different sampling challenges, largely due to water depth (crayfish have been found at depths up to 100 m (Barefoot, 2008)), potential turbidity and dense macrophyte growth.

For quantitative sampling in stillwaters there are two options: Catch Per Unit Effort (CPUE) and mark recapture, with crayfish capture normally carried out using baited traps to overcome the problem of depth. CPUE denotes the number of individuals per trap and can be used to compare crayfish density between trapping areas, or repeat data at the same site. However, variance between traps is high, and other factors such as temperature can affect trap returns year on year, so it is only directly comparable if returning to the same site multiple times (Edsman & Soderback, 1999). The biases associated with trapping are widely reported, with a tendency to capture larger size classes (>40 mm carapace length), particularly males which tend to be the most active components of the population (Matthews & Reynolds, 1992; Demers *et al.*, 2003; Reynolds & O'Keeffe, 2005; Moorhouse & MacDonald 2011a,b and c). A trapping study in a canal which was subsequently de-watered showed that only less than 1% of the potentially trappable population at the site were sampled by trapping (Peay, 2004). Clearly, under-sampling generally, and particularly of juvenile age classes is a key problem with this approach. CPUE can also be applied to hand-search by SCUBA divers in a similar way to streams, using sampling stratification on the basis of habitat to reduce variance between sessions (Stucki & Romer, 2001). The choice of sample locations in a waterbody is important as populations may be segregated according to substrate type (Stucki & Romer, 2001), depth (Abrahamsson, 1966) or temperature (Skurdal *et al.*, 1988).

Mark-recapture can also provide a means of estimating population size; and crayfish should be an ideal study species using this method due to high local densities, high site fidelity and considerable longevity (Nowicki *et al.*, 2008). Nowicki *et al.* (2008) conducted a mark-recapture study on a stream population of *A. pallipes* using hand-search and trapping, but found very high variability between capture sessions, highlighting the need for multiple sampling at a single site. Furthermore, if trapping is the only capture method used, the sex bias towards males could preclude inclusion of females in the dataset (Matthews & Reynolds, 1995). A less biased approach appears to be the use of quadrat sampling with the aid of SCUBA to overcome the depth and enable hand-search within the quadrat. Pilotto *et al.* (2008) found quadrat sampling yielded a density of 6.4 individuals m^{-2}, whereas multiple mark-recapture using SCUBA and hand-search yielded 16.7 individuals m^{-2}, even with large underestimation of juveniles.

Other approaches such as seine netting have been used with success in the Marl lakes of the U.S. to capture *Orconectes virilis* down to depth of 8.5m; however, danger of snagging on rocky substrates (where crayfish are most likely to occur), and high labour requirements for this method most likely render it inapplicable for Ark sites in the UK. Electric fishing or night viewing along the shore may have some applicability; however both are ineffective at depth.

Consideration of the genetics of translocated populations should also be given at the planning stage, and can provide valuable measures of project success (Weeks *et al.*, 2011). For example, use of genetic markers can provide an alternate means of measuring effective population size, and can also be applied to estimate the degree of inbreeding in a population; a valuable indicator of fitness (Wang 2005). Such methods have been applied within the context of conservation translocations for a range of taxa, including butterflies (Schmitt *et al.* 2005), mammals (Ottewell *et al.*, 2014) and marsupials (Houlden *et al.*, 1996); though, not to the author's knowledge, for translocated crayfish populations.

A general review of monitoring methods from a UK perspective is provided by Peay (2004). This evaluation is mainly cost-based, recognising the needs of statutory bodies to meet legislative monitoring requirements in the most cost effective way, which almost always means the least labour-intensive option. A major challenge for Ark site monitoring is trying to capture crayfish from a fairly small population which could be dispersed over a large area. In Chapter Three, a trial was conducted of a novel and inexpensive underwater camera set-up to monitor crayfish populations in deep Ark sites, such as former quarries.

1.5. Translocation and invasion

Many human-mediated translocations are intentional, such as plant and animal species valued for food; yet many are unintentional. The black rat (*Rattus rattus*) is a classic example and now has a global distribution since being inadvertently transported to the New World via European ships (Harris, 2009). The increasing rate of human movements and globalised trading patterns is accelerating the rate of species introductions (Cohen & Carlton, 1998; Mack *et al.*, 2000). Estuaries are particularly vulnerable because the release of ballast water during shipping activities is the most important invasion vector globally (Ruiz *et al.*, 1997). In the Thames catchment, the invasion rate has increased rapidly since 1800 reaching a total of 96 non-indigenous species, a trend concurrent with increases in both local shipping and human population density (Jackson & Grey, 2012).

Not all translocated species will establish viable populations outside their native range (Zenni & Nuñez, 2013). Numerous interacting factors influence establishment success including propagule pressure, abiotic and biotic characteristics (Catford *et al.*, 2009). Of those species that do establish viable populations, some will become ‘invasive’ that is: ‘proliferate, spread and persist to the detriment of the environment’ (Mack *et al.*, 2000). Invasion biologists widely view invasion as a series of stages: transport, introduction, establishment and spread, each with barriers, including geographical, reproductive or dispersal barriers, that a species must overcome to become invasive (Blackburn *et al.*, 2011). The ‘tens rule’ approximates that one in ten species will persist after each stage (Williamson & Fitter, 1996a).

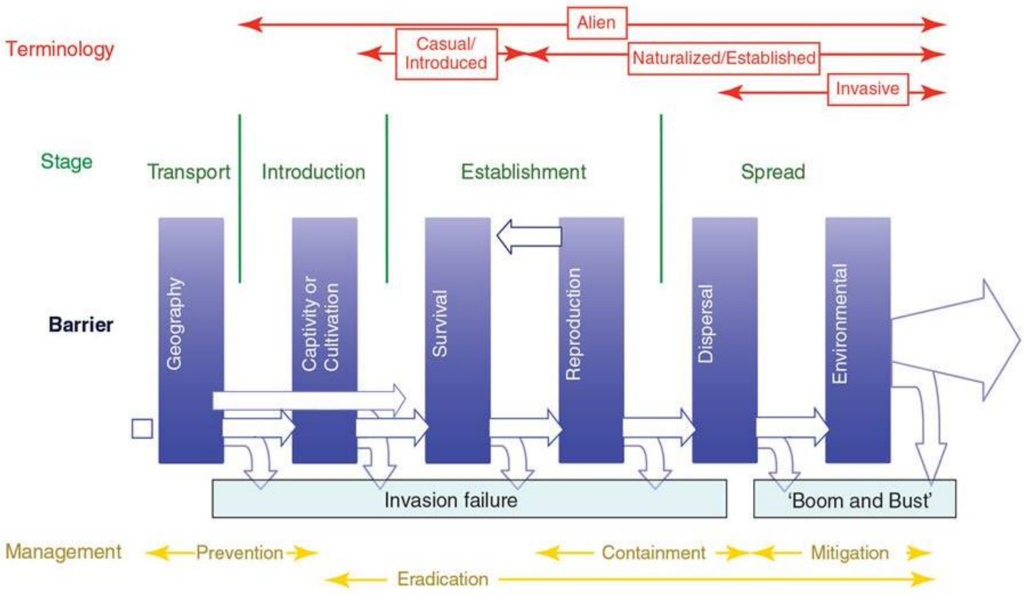


Figure 1.5 Framework for biological invasions indicating the stages of an invasion, potential management interventions and the point at which species are considered invasive (simplified from Blackburn *et al.*, 2011)

1.5.1 Aquatic invaders

Invasive species can have major impacts on species and ecosystems in their introduced range (Parker *et al.*, 1999; Simberloff, 2011) and biological invasions are recognised as a significant component of global environmental change (Vilà *et al.*, 2011). Aquatic ecosystems are particularly vulnerable to invasions due to their physical connectivity and high levels of human disturbance (Dudgeon *et al.*, 2006), and have been significantly impacted by non-indigenous species (; Leprieur *et al.*, 2009; Ricciardi & MacIsaac, 2011). In the heavily exploited River Rhine, invasive species comprise up to 80% of biomass and dominate benthic communities (Tockner *et al.*, 2010). Impacts of biological invasions include loss or reduction of native species, disruption of ecosystem processes and loss of ecosystem services (Strayer, 2010); though direct causality is not always discernible as systems are frequently subject to multiple human-induced stressors (Didham *et al.*, 2005). Climate change, habitat fragmentation and increasing urbanisation are major causes of biodiversity loss and ecosystem impacts (Sala *et al.*, 2000). The interactions between invasive species and these other drivers of global change are often complex and species specific. For example, the zebra mussel (*Dreissena polymorpha*) is expected to expand its range by 15-20% by 2050 as a result of climate warming, though the signal crayfish is expected to undergo range contraction; reducing overlap with endangered crayfish species such as *A. pallipes* (Gallardo & Aldridge, 2013). The invasion process tends to favour ecological traits which promote adaptability and tolerance to environmental stress (Lee, 2002). On this basis, it may be predicted that the other drivers of environmental change will interact synergistically with invasive species by facilitating invasion (Bauer, 2012), and in combination will modify ecosystems both structurally through species loss and replacement, and functionally through disruption of fundamental processes including nutrient cycling (Strayer, 2010; Gutiérrez *et al.*, 2014). Examination of long-term datasets, which afford greater understanding of the relative contribution of each stressor, therefore provide a valuable resource to study environmental change through time (Magurran *et al.*, 2010).

Extirpation or reduction in the abundance of native species by invaders may occur directly due to competitive displacement (Bøhn *et al.*, 2008), for example the red shiner (*Cyprinella lutrensis*) fish which excludes the native cyprinid Spikedace (*Meda fulgeda*) from optimal habitat, forcing it into faster flow conditions (Douglas *et al.*1994); predation, as was the case with Nile perch (*Lates niloticus*) which devastated the native cichlids of Lake Victoria; or by some other means such as the invader acting as a reservoir or vector of pathogens (Prenter *et al.*, 2004). The crayfish plague pathogen carried by North American crayfish is a good example of this and has, as previously mentioned, resulted in the extirpation of many European crayfish populations (Holdich *et al.*, 2003).

Invasive species can also indirectly affect other species in the ecosystem and/or the physical environment. Freshwater food webs are often complex with high connectance (Polis & Strong, 1996; Woodward *et al.*, 2005); hence reduction of one consumer can cause indirect effects at other trophic levels via trophic cascade (Polis *et al.*, 2000). In a top-down example, brown trout (*Salmo trutta*) introduced to New Zealand streams excluded or reduced the native galaxiids and exerted a stronger predatory pressure on grazers causing an overall increase in algal biomass (Townsend, 1996). If they are ecosystem engineers, invaders may also directly influence the physical environment and nutrient cycling, with bottom-up effects for the rest of the community (Crooks, 2002). The zebra mussel filters large quantities of plankton, increases light penetration thereby encouraging macrophyte growth, and creates shell-bed habitat for macro- and meiofauna, which also feed on wastes excreted by the mussels. The net result is a redirection of nutrients from open water toward the benthos, particularly littoral zones, which has several cascading effects including increased algal biomass on shorelines (Hecky *et al.*, 2004). Through their multiple impacts on ecosystems and the services those ecosystems provide to humans, invaders are a huge economic problem (Vilà *et al.*, 2009). A recent estimate for Great Britain alone indicated that non-native species cost the economy £1.7 billion per annum (Williams *et al.*, 2010).

The mechanisms by which invaders influence ecosystems are frequently complex and interlinked with other stressors, and vary greatly between species; therefore assessing invader impacts is often difficult (Ruiz *et al.*, 1999; Simberloff *et al.*, 2013). Further, multiple invaders in an ecosystem may operate synergistically, modifying ecological and abiotic components so as to facilitate further invasion, thereby causing an 'invasional meltdown' (Simberloff & Von Holle, 1999; Ricciardi, 2001). Nevertheless, predicting invader impacts and making comparisons between species is crucial for effectively targeting the limited resources available for management and control (Keller *et al.*, 2011). The notion of a standardised index of impact incorporating three key elements: an invader's range, abundance, and 'effect' is compelling, but data on the 'effect' of invaders at multiple levels of organisation, from individual through to ecosystem level, are lacking for most species (Parker *et al.*, 1999). Further, it is important to consider both the structural and functional ecosystem consequences of invaders (e.g. Townsend 2003; Hladyz *et al.*, 2011; Jackson *et al.*, 2014). Other considerations for managers are the relative feasibility of control measures, i.e. it is easiest to contain and eradicate species in early stages of an invasion (Mehta *et al.*, 2007); and the need to balance multiple, and perhaps conflicting, management demands. For example, there are currently strong legislative drivers in Europe to increase habitat connectivity for migratory fish species; however this must be balanced against the risk of facilitating the spread of invasive species through the removal of physical invasion barriers. An experiment described in Chapter Nine quantitatively assessed the influence of a flow gauging weir on the movements of signal crayfish with a view to predicting the outcomes of weir removal or modification on the rate of spread of this invasive species.

1.5.2 The signal crayfish

Many crayfish species have been introduced outside their native ranges, primarily for aquaculture, and have subsequently spread to develop widespread and dense wild populations (Lodge *et al.*, 2012). As the rate of biological invasions accelerates, freshwater systems are increasingly home to multiple invaders; including those that exhibit similar trophic habits. When invaders are functionally equivalent in a trophic sense, i.e. have a high degree of niche overlap, they may be predicted to undergo increased interspecific competition during co-occurrence, thereby causing a reduction in fitness, and even complete exclusion (Sale, 1974; Facon *et al.*, 2006). Conversely, competing invaders may adjust their feeding habits, thereby widening or shifting their trophic niche to enable coexistence; with additive ecosystem impacts (Preston *et al.*, 2012). Many argue the latter scenario is more likely in the context of invasion biology because successful invaders generally possess plastic life traits and high adaptability making them more able to undergo trophic shifts (Mooney & Cleland, 2001; Sakai *et al.*, 2001).

Several decapod invaders may be present in the same watercourse. In a UK example, invasive *P. clarkii* and *P. leniusculus* were recently found to co-occur in London's Regents Canal, with Turkish crayfish *Astacus leptodactylus* and another decapod invader, the Chinese mitten crab (*Eriocheir sinensis*) also detected within 6 km (Ellis *et al.*, 2012; Mitten Crab Recording Project, 2013). *E. sinensis* is native to eastern Asia, but has been introduced to Europe, and North America during the last century and was first detected in the UK in the River Thames in 1935 (Gilbey *et al.*, 2008). Populations are now established in all the other major east coast rivers including the Humber, Trent, Great Ouse and Aire (Mitten Crab Recording Project, 2013). Transportation via ships' ballast water has been attributed as the main vector of introduction (Dittel & Epifanio, 2009), although recent spread has at least in part been blamed on deliberate releases for commercial exploitation (Herborg *et al.*, 2003). The crabs are a favoured food source in Asia, and other uses include fish meal production, as bait for eel fishing, and agricultural fertilizer (Herborg *et al.*, 2005).

The signal crayfish (*P. leniusculus*), one such species, is currently invading Europe, Japan and parts of North America. Its role in the extirpation of native European crayfish populations through spreading the crayfish plague pathogen *A. astaci* is of primary concern; however wider impacts on freshwater ecosystems are less well understood.

One theory as to why invasive species do better than their native counterparts is that they tend to have r-selected characteristics such as high fecundity, short time to maturity and more aggressive behaviours that enable them to better exploit resources and competitively dominate in new ranges (Sakai *et al.*, 2001). The high incidence of plastic life traits among invaders further increases adaptability and can accelerate invasion rates once in the spread phase of an invasion (Phillips *et al.*, 2006). Signal crayfish are more fecund than *A. pallipes* with 200-400 eggs per individual, compared to 60–160 eggs in *A. pallipes* (Rhodes & Holdich, 1982; Savolainen *et al.*, 1997); and reach similar, if not higher, densities with 0.9 to 20 m^{-2} individual m^{-2} (Abrahamsson & Goldman, 1970; Goldman & Rundquist, 1977; Bubb *et al.*, 2004).

P .leniusculus has replaced native crayfish species across much of western Europe, but does it perform the same functional role in freshwater ecosystems? Trophically, *P. leniusculus* exhibits similar feeding preferences to native European species, consuming a wide range of diet items including periphyton, detritus, macrophytes, aquatic invertebrates and fish (Axelsson *et al.*, 1997; Guan & Wiles, 1998). However, to assess its potential impacts on freshwater food webs we need to fully understand its prey preferences and measure how its per capita intake rate of key prey items compares with native crayfish species. Measuring a predator's functional response is one means of quantitatively assessing potential impacts on prey and comparing predation levels between predator species (Bollache *et al.*, 2008; Dick *et al.*, 2012). Haddaway (2012b) recently showed that adult *P. leniusculus* had a higher predatory functional response towards a key prey item, *Gammarus pulex*, than *A. pallipes* with a 10% higher prey intake rate, inferring greater predatory strength. In mesocosms, *P. leniusculus* similarly exerted stronger impacts on grazers and macrophyte biomass than native *A. astacus* (Nystrom *et al.*, 1999). In a comparison of the predatory impact of *P. leniusculus* and *A. pallipes* on the eggs and emerging fry of Atlantic salmon (*Salmo salar*), neither crayfish species consumed eggs, but both preyed on emerging fry with *P. leniusculus* killing 5.5% compared to 0.5% for *A. pallipes* (Edmonds *et al.*, 2011). Similar comparisons for other crayfish life-stages, prey types and ecosystem roles (e.g. relative bioturbation rates) are necessary to enable reliable predictions of the ecosystem impacts of signal crayfish; the first two of which are addressed in this thesis.

1.5.3 The Chinese mitten crab

As the rate of biological invasions accelerates, freshwater systems are increasingly home to multiple invaders; including those that exhibit similar trophic habits. When invaders are functionally equivalent in a trophic sense, i.e. have a high degree of niche overlap, they may be predicted to undergo increased interspecific competition during co-occurrence, thereby causing a reduction in fitness, and even complete exclusion (Sale, 1974; Facon *et al.*, 2006). Conversely, competing invaders may adjust their feeding habits, thereby widening or shifting their trophic niche to enable coexistence; with additive ecosystem impacts (Preston *et al.*, 2012). Many argue the latter scenario is more likely in the context of invasion biology because successful invaders generally possess plastic life traits and high adaptability making them more able to undergo trophic shifts (Mooney & Cleland, 2001; Sakai *et al.*, 2001).

Several decapod invaders may be present in the same watercourse. In a UK example, invasive *P. clarkii* and *P. leniusculus* were recently found to co-occur in London's Regents Canal, with Turkish crayfish *Astacus leptodactylus* and another decapod invader, the Chinese mitten crab (*Eriocheir sinensis*) also detected within 6 km (Ellis *et al.*, 2012; Mitten Crab Recording Project, 2013). *E. sinensis* is native to eastern Asia, but has been introduced to Europe, and North America during the last century and was first detected in the UK in the River Thames in 1935 (Gilbey *et al.*, 2008). Populations are now established in all the other major east coast rivers including the Humber, Trent, Great Ouse and Aire (Mitten Crab Recording Project, 2013). Transportation via ships' ballast water has been attributed as the main vector of introduction (Dittel & Epifanio, 2009), although recent spread has at least in part been blamed on deliberate releases for commercial exploitation (Herborg *et al.*, 2003). The crabs are a favoured food source in Asia, and other uses include fish meal production, as bait for eel fishing, and agricultural fertilizer (Herborg *et al.*, 2005).

Unlike *P. leniusculus* which completes its lifecycle entirely within freshwater, *E. sinensis* is catadromous. Reproduction occurs in estuarine waters and the larvae develop through five zoeal stages before metamorphosis into juvenile crabs. These migrate into freshwater during spring to undergo the main growth phase (~3 years in Europe), before returning to the estuary as sexually mature adults (34–100 mm carapace width) to breed in autumn (Panning, 1939; Veldhuizen, 2001; Gilbey *et al.*, 2008). Inland migration distances may be large, up to 1400 km in its native China, and in the UK *E. sinensis* has been recorded some 100 km upstream of the River Thames tidal limit (Panning, 1939; Mitten Crab Recording Project, 2013). Due to this catadromy, *E. sinensis* has the potential to impact both marine and freshwater ecosystems. In brackish and freshwater, adult crabs cause significant damage through burrowing into banks, creating tightly packed and often interconnected tunnels up to 0.5 m in length (Dutton & Conroy, 1998; Rudnick *et al.*, 2000). Banks and levees that are eroded and undermined in this way are prone to collapse, which is a major concern and potential cost in areas where 'naturalised' flood defences are in use (Dutton & Conroy, 1998). Other impacts are particularly apparent during the autumn breeding migration when adults clog fishing gear and enter nets, consuming bait and reducing catches of target species (Veldhuizen & Stansih, 2002; Gilbey *et al.*, 2008). In German waters alone, total economic costs of *E. sinensis* are estimated at 80 million Euros since its introduction in 1912 (Gollasch, 2006).

E. sinensis co-occurs with various native and invasive crayfish in freshwaters and occupies similar microhabitats such as macrophyte margins, crevices underneath rocks, roots and debris (Rudnick *et al.*, 2000; Veldhuizen & Stansih, 2002; Rudnick & Resh, 2005). In common with *P. leniusculus*, *E. sinensis* is described as an opportunistic omnivore and consumes a range of items including detritus, macro-invertebrates, small fish and algae (Rudnick & Resh, 2005; Dittel & Epifanio, 2009; Czerniejewski *et al.*, 2010); though no study has directly compared the feeding ecology of the two species. *E. sinensis* co-occurs with *P. clarkii* in the freshwater tributaries of San Francisco Bay and comparison of their feeding ecology using stable isotope analyses revealed *E. sinensis* was more strongly aligned with algae and algal-associated invertebrates than *P. clarkii*, which showed a stronger relationship with terrestrially derived detritus (Rudnick & Resh, 2005). In the same study, examination of gut contents analysis suggested diets dominated by plant material for both species, however, mesocosm results indicated *E. sinensis* preferentially fed on surface-dwelling invertebrates. There is a paucity of research on many aspects of the biology of *E. sinensis*, in particular its population dynamics and feeding ecology. To disentangle the effects of multiple invaders in an ecosystem it is necessary quantify impacts (e.g. predation) for each species separately, but also in combination in order to detect potential niche shifts, behavioural plasticity and wider functional impacts.

1.6 Research aims and thesis plan

The creation of Ark sites for *A. pallipes*, where populations will persist in the absence of invasive crayfish and crayfish plague, is considered necessary if we are to conserve this endangered species into the long-term. This strategy is still in its infancy and success will depend upon the use of sites with suitable physical conditions where threats are minimised.

Potential Ark sites should be subject to a full feasibility study to assess their suitability prior to translocation (Kemp *et al.*, 2003; IUCN, 2012). A thorough understanding of the habitat requirements of *A. pallipes* is therefore necessary to first, facilitate assessment of the current suitability of potential Ark sites, and second, direct restoration or habitat improvement interventions where necessary. Research into *A. pallipes* habitat use has not been collated since Holdich and Rogers' (2000) review and there have been a number of subsequent studies. A systematic review carried out in Chapter Two used a semi-quantitative approach to identify habitat variables important for *A. pallipes* with the aim to inform Ark site selection and habitat restoration.

Fundamental to the success of the Ark site strategy is the acquisition of isolated sites, where the threats associated with invasive crayfish are minimised. Former mineral extraction sites offer several advantages as Ark sites. They are often isolated and enclosed with permanent waterbodies, and habitat restoration may be a legal requirement. Although translocation of *A. pallipes* to Ark sites has been occurring across Europe for over 20 years (Schulz *et al.*, 2002), few are documented in the literature and none specifically refer to quarry sites. This is symptomatic of the wider lack of documentation and appraisal of animal relocations, particularly when they are unsuccessful, hindering advancement of the process through ‘lessons learned’ and subsequent refinement of protocols (Fischer & Lindenmayer, 2000). Chapter Three presents a case-study of one of the first quarry Ark sites in the UK, from creation to post-translocation monitoring, with the aim to provide baseline data for the site and inform future crayfish translocations in quarry sites.

Ark site translocations commonly represent introductions, i.e. attempts to establish a species outside its recorded distribution. The IUCN state that such introductions are a feasible conservation tool, though assessment of likely impacts on the existing ecosystem is necessary to avoid unforeseen ecological consequences (IUCN, 2012). As an omnivore, *A. pallipes* has the potential to impact a range of taxa through trophic interactions, both direct and indirect. Only one previous study has tested the influence of *A. pallipes* on the littoral community (Matthews *et al.*, 1993); however no consideration was made of the size structure of the crayfish population. Evidence from gut contents analyses suggests that *A. pallipes* undergoes ontogenic diet shift (Reynolds & O'Keeffe, 2005); hence impacts on freshwater communities would be predicted to reflect crayfish population structure. In Chapter Four a field-based mesocosm experiment was used to assess the short-term impacts of *A. pallipes* introduction on the benthic invertebrate community, and to test the hypothesis that impacts differ between juvenile and adult life-stages of crayfish.

Animal translocations can have unintended consequences, for example, communities of parasitic organisms may be transported unintentionally along with their hosts (Cunningham, 1996; Van Oosterhout *et al.*, 2007) and disease is often cited as a key factor in unsuccessful translocations (Viggers *et al.*, 1993). Chapter Five evaluated the prevalence and associated pathology of a novel crayfish parasite *Branchiobdella astaci* detected in two local populations of *A. pallipes*, and considered the potential consequences of translocation of this parasite along with donor stock.

Suspended solids (SS) loadings in freshwater streams and lakes have increased substantially over the last century and SS is now a significant environmental stressor (Bilotta & Brazier, 2008; Palmer-Felgate *et al.*, 2009; Kemp *et al.*, 2011). The negative effects of SS on freshwater fish are well documented (Bilotta & Brazier, 2008; Kemp *et al.*, 2011). Although SS been implicated in declines of *A. pallipes* (Füreder *et al.*, 2006; Environment Agency, 2011), no study has assessed its impacts on crayfish. Further, a thorough understanding of the environmental tolerances of *A. pallipes* is a necessary prerequisite for assessing the habitat suitability of potential Ark sites. Former quarries in particular may be subject to elevated SS concentrations due to unconsolidated material. The impact of chronic exposure to SS on native *A. pallipes* and its invasive competitor *P. leniusculus* was assessed in Chapter Six, leading to management recommendations for acceptable SS levels in Ark sites. It was also investigated whether infection by two crayfish parasites modified the effects of SS on *A. pallipes,* with the aim to further inform on the potential consequences of inadvertent translocation of parasites with donor stock.

Chapters Seven and Eight concern invasive *P. leniusculus* which now occupies much of the former range of *A. pallipes*. Trophic interactions are a key mechanism by which invaders influence community structure, though impacts are particularly difficult to predict for omnivores such as crayfish. Further, the accelerating rate of biological invasions means that invaders increasingly co-occur; hence many ecosystems already support several sympatric invasive species. In Chapter Seven a variety of methods were employed to investigate the dietary preferences, predatory strength and potential community impacts of *P. leniusculus*, both alone and in combination with the co-occurring decapod invader *E. sinensis*. This study provided the first measure of potential relative impacts of these two decapods with a view to informing management strategies for invasive species.

Increasing legislative drivers demand the removal or modification of riverine barriers to enhance habitat connectivity for fish; however there is also concern that greater connectivity will hasten the spread of aquatic invaders such as *P. leniusculus*. In Chapter Eight, the potential of riverine barriers to hinder the spread of signal crayfish was investigated using a case-study at a flow gauging weir, a common type of river structure, the findings of which will inform managers balancing the need to enhance fish migration with the threat of invasive species.

In Chapter Nine, the main findings of the research outlined above are summarised and discussed in the context of current knowledge, and potential management outcomes are highlighted. Though the chapters of this thesis are intrinsically linked, the data chapters (Two to Eight) are presented as standalone manuscripts.

Chapter 2 Habitat use by the endangered white-clawed crayfish *Austropotamobius* species complex: a systematic review

2.1 Summary

Understanding habitat requirements is a key part of conserving declining species, particularly when reintroductions are planned as part of the recovery strategy. The white-clawed crayfish (*Austropotamobius pallipes*) has undergone severe declines across its range and is now IUCN classified as 'endangered'. Translocation of threatened *A. pallipes* populations to isolated 'Ark sites' where threats are minimised is an increasingly used conservation tool. A full feasibility assessment of potential Ark sites, including an assessment of habitat suitability is recommended within translocation guidelines developed by the IUCN. This literature review employed a systematic search and 'vote counting' approach to identify and assess the relative importance of habitat requirements of *A. pallipes*. Many habitat features were positively associated with crayfish presence, in particular: boulder substrate, trees/ shading, woody debris, exposed roots, and undercut banks. Ark sites should incorporate heterogeneous habitat, with as many of these features as possible, to provide both ample refugia and rich foraging areas. Consideration should also be given to variations in habitat use patterns among crayfish sexes and size classes.

2.2 Introduction

Range reduction of the white-clawed crayfish (*Austropotamobius pallipes*), one of only five crayfish species indigenous to Europe began as early as the 1860's (Souty-Grosset *et al.*, 2006a), although declines rapidly accelerated following introduction of invasive non-indigenous crayfish species, such as *Pacifastacus leniusculus* and *Procambarus clarkii* from North America in the 1970s (Holdich & Gherardi, 1999; Gil-Sanchez & Alba-Tercedor, 2002). The spread of crayfish plague *Aphanomyces astaci*, an oomycete pathogen carried by North American crayfish, but fatal to European crayfish, has had the greatest impact on populations (Alderman *et al.*, 1990; Holdich *et al.*, 2003); although pollution (Fureder *et al.*, 2003) and habitat modification (Hogger, 1986), loss and degradation (Holdich & Reeve, 1991) have all been implicated in the decline. Despite legislative protection at the European scale, with *A. pallipes* listed under annexes II and V of the EU Habitats Directive (92/43/EEC) and Appendix II of the Bern Convention, it has declined in Europe by 50-80% over the last decade (Souty-Grosset & Reynolds, 2009).

While a widely applicable and cost-effective means to control invasive crayfish remains elusive, translocation of threatened populations has become a key component of crayfish conservation strategy in Europe (Schulz *et al.*, 2002). In addition to restocking areas of dwindling population and reintroduction of *A .pallipes* to watercourses where it has been extirpated, introductions to isolated stillwaters free from invasive crayfish and aptly named 'Ark sites' are widely being carried out (Holdich *et al.*, 2004; Souty-Grosset & Reynolds, 2009). With a significantly lower invasion threat, these off-line waterbodies are hoped to provide long-term strongholds for native populations in invaded catchments, and ultimately re-stocking material if invader-free watercourses can be secured in the future (Schulz *et al.*, 2002).

Understanding habitat requirements is a key part of the conservation of declining species generally, but particularly when reintroductions are planned as part of the recovery strategy (Seddon *et al.*, 2007; Sutherland, 2008). The IUCN highlights that habitat assessment of the recipient site as a key stage in the process of conservation translocation (IUCN, 2012); however some argue that this is rarely carried out to an adequate level (Osborne & Seddon, 2012). In the UK, best practise guidelines have been formulated for translocations of *A. pallipes* and suitable habitat is highlighted as a key criterion in the selection of recipient Ark sites (Kemp *et al.*, 2003; Peay, 2009). Large-scale GIS-mapping and interrogation of variables, including habitat parameters, on a national or regional scale is advocated as a valuable starting point in the identification of possible recipient sites (Kindemba *et al.*, 2009). Once potential sites have been identified, the next stage involves more detailed assessment of habitat against pre-defined criteria compiled using expert opinion, against which the site is subjectively rated on a scale of 1-4 based on the description of a range of habitat variables (Buglife, 2009).

Within translocation guidelines, the habitat features considered important for *A. pallipes* may be broadly summarised as suitable water chemistry, stable flow regimes, ample physical habitat structure to provide refugia, presence of in-stream vegetation and low pollution/disturbance risk (Kemp *et al.*, 2003). There have been several reviews of the literature, including a general review of the habitat factors affecting cool water crayfish species by Lodge & Hill (1994), which dealt mainly with species of commercial importance such as *Orconectes* or *Astacus* spp., though did identify a preference of *A.*

pallipes for rocky, lotic environments. Within another review based on four studies, Holdich & Rogers (2000) compiled a broad list of attributes deemed to denote desirable habitat for the species, however the authors themselves note that *A. pallipes* does occur in locations where many of those habitat attributes are not present. Conversely, it is often absent from watercourses where conditions appear optimal, with no previous pollution or invasive crayfish, and no apparent dispersal limitations (Nardi *et al.*, 2005). Concurrent with increasing recognition of the plight of the endangered white-clawed crayfish and of the many knowledge gaps concerning this species, much work has been carried out over the last decade. Habitat has been one research focus, addressing species distribution patterns and habitat use at range of temporal and spatial scales. Novel approaches and advancements in technology (e.g. radio telemetry) have for the first time enabled study at the microhabitat level in the wild, revealing much about the resource use and spatial ecology of *A. pallipes*. In recognition of recent advancements, this review aims to collate and synthesise all published work relating to *A. pallipes* and physical habitat. The data will be explored for patterns of presence/absence and microhabitat use with respect to a series of habitat variables including channel substrate, vegetation and hydrodynamic parameters. Further, a systematic search methodology, data selection and extraction protocol was used to provide transparency and reduce bias.

The traditional ‘narrative review’ is still the standard approach in conservation and environmental management disciplines (Gates, 2002), and has been widely criticised for providing only qualitative assessment, a tendency to introduce biases, and lack of transparency in search methodology and assessment of the relevance of studies and quality of the original experiments (Roberts *et al.*, 2006). In recognition of these shortcomings, systematic review protocols were developed for ecological applications, principally to assess the effectiveness of a management intervention or conservation strategy (Sutherland *et al.*, 2004; Pullin & Stewart, 2006). A review protocol developed within those guidelines was employed here to ask ‘what are the habitat requirements of *A. pallipes*?, with the aim to provide an up to date assessment, incorporating a degree of transparency and repeatability not available in previous narrative reviews on the subject.

2.3 Methods

The ecological literature was searched for studies that reported habitat use or associations for genus *Austropotamobius*. Searches were performed 12th November 2010, and updated 10th June 2013 to include studies published in the interim period. Web of Knowledge and Google Scholar search engines were searched using the search string: '*Austropotamobius* AND habitat OR microhabitat OR distribution' and the records returned from each search engine were imported into reference management software (Endnote X2, The Thomson Corporation, 2008). Despite the very large number of hits returned by Google Scholar, pilot searches showed that relevance declined rapidly after the initial 60 or so records, therefore only the first 140 records were exported. Duplicate articles were subsequently eliminated and the remainder underwent screening using predefined retention criteria (Table 2.1). The selection criteria were designed to retain only those articles that were relevant to the aims of this review i.e. that quantitatively assessed the relationship between crayfish occurrence/density and habitat variables. It was decided to exclude studies that focussed solely on water chemistry parameters as these have been previously reviewed quantitatively elsewhere (Trouilhe *et al.*, 2007; Haddaway, 2010).

Table 2.1 Predefined criteria for selection of literature returned by systematic search for full review

Criterion	**Priority within screening process**
Considers *Austropotamobius pallipes* or *A. italicus*	1
Quantitatively assesses relationship between habitat features and crayfish occurrence*	2
Is primary literature published in peer reviewed journals, or conference proceedings	3
Full text available in English	4

*Some aspect of physical habitat structure must be addressed; not solely water chemistry data. Where a study had multiple aims, only data relating directly to habitat-use were considered.

It was decided to include studies addressing both *A. pallipes* and *A. italicus,* despite the controversial taxonomy of this group. The historic classification of *A. pallipes* and *A. italicus* as two distinct species based on 16S rRNA (Grandjean & Souty-Grosset, 2000; Grandjean *et al.*, 2002; Fratini *et al.*, 2005) has recently been robustly challenged by a combined analyses of CO-I gene fragments and AFLP fingerprinting which indicated that *A. pallipes* and *A. italicus* are one species (Chiesa *et al.*, 2011).

The articles retained after screening were interrogated to extract data and populate a database. The fields of enquiry included: 1) species of interest; 2) location; 3) number of catchments, watercourses and sites studied; 4) crayfish sampling method; 5) crayfish sampling time i.e. day or night; 6) habitat variables measured, and presence and direction of any quantitative relationships identified statistically between crayfish occurrence/density and each measured habitat variable.

2.4 Results and Discussion

A total of 278 unique records were retrieved from the searches and 19 were retained for full review after the screening process. The retained studies originated from the UK (37%), Italy (37%), Spain (16%) and France (11%) with publication dates ranging from 1995 to 2011. Studies pre-dating 1995 were retrieved by the searches, but were excluded on the basis that they did not statistically test quantitative relationships between *A. pallipes* and habitat variables. All studies that met the selection criteria addressed only lotic crayfish populations. Catchment-scale studies examining crayfish patterns at several sites on multiple watercourses comprised 63% of studies, whereas reach scale studies examining just one watercourse comprised 37%. All but one of the catchment-scale studies used presence-absence crayfish data derived from a combination of methods: hand-search during the day; night viewing, or trapping (Table 2.2). No studies at the reach scale used presence-absence data; instead they investigated crayfish abundance or finer scale movement patterns (Table 2.3). Reach-scale studies employed similar methods to those used in catchment studies, with the addition of radio-tracking and Surber sampling.

Data concerning 17 habitat variables were extracted from the studies, where available (Tables 2.2 and 2.3). The abundance and diversity of macro-invertebrate communities was also investigated in 5 studies and although not a physical habitat feature was included for completeness.

Within the catchment scale studies, channel substrate parameters were the most investigated of all the habitat variables, addressed in 92% of studies, and land-use was the least (17%). A significant relationship was found between crayfish presence/abundance and 15 of the variables measured. Flow velocity, flow type and silt coverage were not related to crayfish presence/abundance at the catchment scale. Taking into account the number of studies that investigated each variable, relationships were most frequently recorded for land-use (100% of studies); water temperature (60%), boulder/cobble coverage (45%), and the amount of trees/shading (45%) (Table 2.2) (Figure 2.1).

Fewer habitat variables (13 in total) were investigated within the 7 studies conducted at the reach scale (Table 2.3), and all besides bedrock coverage were significantly related to crayfish habitat use. Again, channel substrate parameters were the most investigated of all the variables, addressed in 86% of studies. Relationships were most frequently recorded for to the presence of woody debris and roots (100% of studies where measured), flow type (100%) and bank structure (100%) (Figure 2.2).

Table 2.2 Summary of data extracted from 12 studies examining habitat use by *Austropotamobius pallipes* and *Austropotamobius italicus* at the catchment scale (i.e. multiple watercourses).

	Study	Species	Day (d) / night (n)	Sampling method (no.crayfish)	No. watercourses (no. sites)	Habitat variables																	Macro-invertebrates
							Channel substrate parameters					Vegetation parameters				Hydro-dynamic parameters							
						Water chemistry parameters	Boulders / cobbles	Pebbles	Gravel & sand	Silt / mud	Bedrock	In-stream vegetation	Woody debris &	Detritus	Trees/ shading	Water depth	Flow velocity	Water Temperatur	Flow type e.g. riffle, glide	Bank structure	Land-use	Altitude	
Catchment-scale studies	Arce & Alonso (2011)	*A. pallipes*	d,n	HS, NV, T (p/a)	? (91)	•	•	•	•	•	•	•			•	•		-	•	•	+	+	
	Barbaresi *et. al.* (2007)	*A. italicus*	n	NV (p/a)*	16(16)	+	+	+	+	•	•		•		•	•		+		•			+
	Brusconi *et al.* (2008)	*A. italicus*	d	HS (1237)	19(19)	•	+/-	•	•	•	•		•	+	+	•			•				•
	Favaro *et al.* (2011)	*A. pallipes*	d,n	HS, T (p/a)	? (175)	+	•	•	•	•	+				•		•	-				+	
	Foster (1995)	*A. pallipes*	d	HS,KS	?(94)	•	+	•	-	•	-	+	+	•	+	+			•	+		•	
	Gallagher *et al.* (2005)	*A. pallipes*	d,n	T, KS (p/a)	19(40)	+	•	•	•	•	+	+	•	•	•	•	•	•	•	•		•	•
	Martinez *et al.* (2003)	*A. italicus*	d,n	HS, NV, T (p/a)	?(262)		•	•	•	•	•	•	•	•	+				•	+	•	•	
	Nardi *et al.* (2005)	*A. pallipes*	d,n	HS, T (p/a)	?(235)	•	+	+	•	•	•	•		•	•	•	•			•	•	•	+
	Naura &Robinson (1998)	*A. pallipes*	d	HR (p/a)	?(150)		-	•	•	•	•	+/-	+/-	•	+				•	+/-		•	
	Renai *et al.* (2006)	*A. pallipes & A. italicus*	d,n	HS,NV, T (p/a)	19(19)	•	•	•	•	•	•		•	•	•	•	•			•			•
	Smith *et al.* (1996)	*A. pallipes*	d	HS,KS (616)	43(62)	•	•	•	•	•	•	•	+	•	+	•		•	•	+	•	•	
	Souty-Grosset *et al.* (2010)	*A. pallipes*	d,n	HS,NV, T (p/a)	1 (38)																+/-		

NV= night-viewing; HS= hand search; T= trapping; KS=kick-sampling; HR=historic records; p/a denotes presence absence data. '•'denotes variable measured '+' and '-', denote positive and negative, respectively statistical correlation between parameter and presence/abundance; . The presence of both signs indicates different relationship for different parts of population, or different aspects of the parameter measured.

Table 2.3 Summary of data extracted from 7 studies examining habitat use by *Austropotamobius pallipes* and *Austropotamobius italicus* at the reach scale (i.e. in one watercourse).

	Study	Species	Day (d) / night (n)	Sampling method (no.crayfish)	No. watercourses (no. sites)	Habitat variables																	Macro-invertebrates
							Channel substrate parameters					Vegetation parameters				Hydro-dynamic parameters							
						Water chemistry parameters	Boulders / cobbles	Pebbles	Gravel & sand	Silt / mud	Bedrock	In-stream vegetation	Woody debris & roots	Detritus	Trees/ shading	Water depth	Flow velocity	Water Temperature	Flow type e.g. riffle, glide	Bank structure	Land-use	Altitude	
Reach scale studies	**Armitage (2000)**	***A. pallipes***	**n**	**RT (12)**	**1(2)**		+	•	•	•	•	+		+					+				
	Benvenuto *et al.* (2008)	***A. pallipes***	**n**	**NV (265)**	**1(1)**		•	•	•	•	•	•	+	•	•	•	+/-		+/-				
	Broquet *et al.* (2002)	***A. pallipes***	**d**	**HS (271)**	**1(1)**		+						+			•	•			+			
	Bubb *et al.* (2006)	***A. pallipes & P. leniusculus***	**d**	**RT (20)**	**1(1)**		+	•	•	•	•				+	+	-						
	Clavero *et al.* (2009)	***A. pallipes***	**n**	**NV (435)**	**1(15)**		-	•	+	+	•		+	•		+/-							
	Gherardi *et al.* (2001)	***A. pallipes***	**n**	**NV (60)**	**1(1)**		•	•	•	•	•	+		+									
	Ream *et al.* (2009)	***A. pallipes***	**d**	**SS (75)**	**1(1)**		•	+	•	•		•			•	•	•						

NV= night-viewing; HS= hand search; T= trapping; KS=kick-sampling; HR=historic records; RT=radio-tracking; SS= Surber sampler. '•'denotes variable measured '+' and '-', denote positive and negative, respectively statistical correlation between parameter and presence/abundance. The presence of both signs indicates different relationship for different parts of population, or different aspects of the parameter measured.

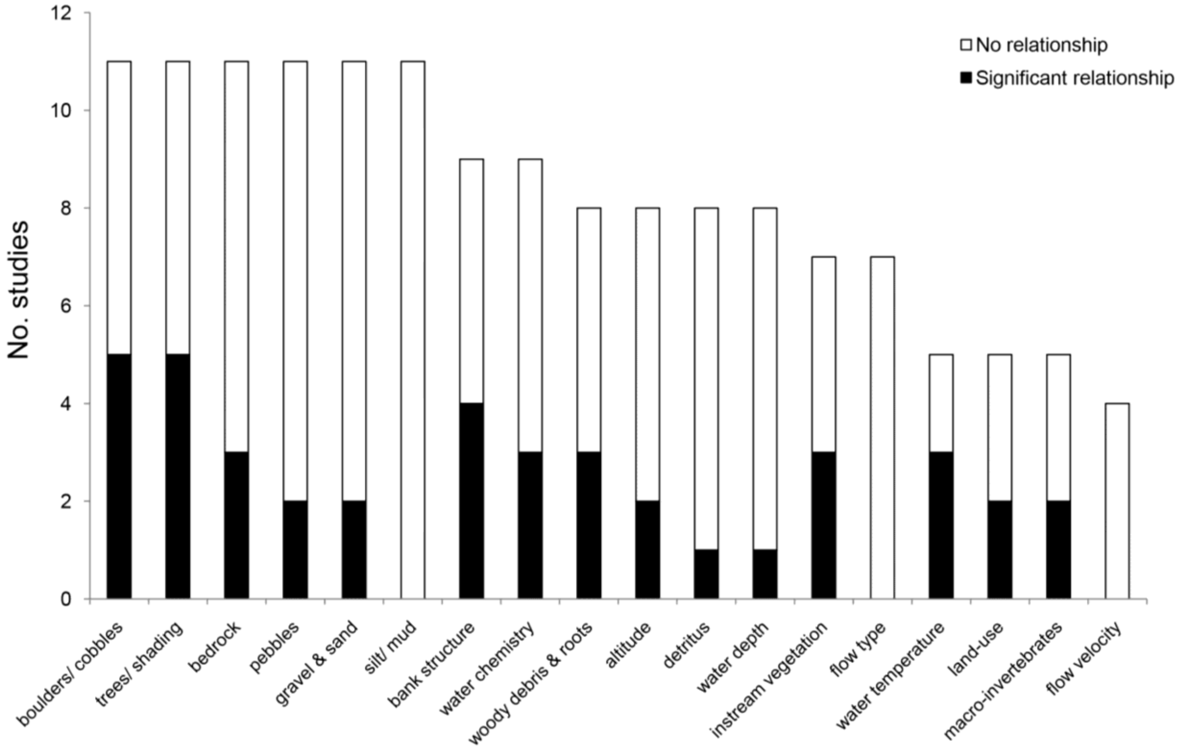


Figure 2.1 Number of primary research studies assessing the importance of 18 habitat variables for *Austropotamobius pallipes* and *Austropotamobius italicus* at the catchment scale (i.e. multiple watercourse).

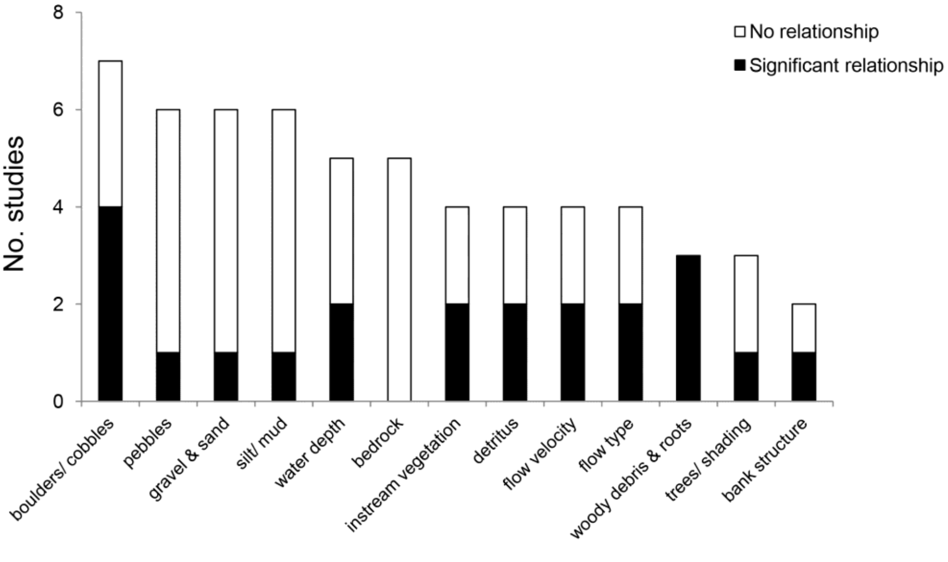


Figure 2.2 Number of primary research studies assessing the importance of 13 habitat variables for *Austropotamobius pallipes* and *Austropotamobius italicus* at the reach scale (i.e. in one watercourse).

2.4.1 Water chemistry parameters

Water chemistry parameters were identified as significant predictors of crayfish occurrence in 3 out of 9 studies at the catchment scale. The concentration of calcium was particularly important with a positive relationship between crayfish occurrence and concentration (Gallagher *et al.*, 2006; Barbaresi *et al.*, 2007; Favaro *et al.*, 2011), as has been previously highlighted in numerous studies (e.g. Trouilhe *et al.*, 2007; Favaro *et al.*, 2010). Crayfish have a strong dependence on calcium for maintenance of the exoskeleton (Greenaway, 1985) and generally do not persist in locations where calcium concentration is below 5 mg L^{-1} (Holdich, 2003). Six of the 9 studies did not identify a relationship with water chemistry parameters, which likely reflects the lack of variation in such parameters over the scales studied i.e. within catchment, rather than importance more generally. Suitable water chemistry is known to be a crucial determinant of whether crayfish will be present in a catchment or not (Jay & Holdich, 1981), but is clearly less indicative of occurrence over smaller scales.

2.4.2 Channel substrate parameters

The importance of channel substrate parameters for crayfish varied with substrate size. The coverage of boulders and pebbles was most important, with relationships identified in 45% of relevant studies at both the catchment and reach scales. Generally a positive relationship was found between the occurrence of crayfish and prevalence of boulder substrates; however Brusconi *et al.* (2008) identified varying relationship based on size classes whereby juvenile crayfish preferentially selected cobbles, though adults tended to avoid them. Naura & Robinson (1998) differentiated between boulders and cobbles and found a positive relationship with regards boulders, but a negative one for cobbles. Larger rocks such as boulders and cobbles provide refugia as crayfish hide underneath them; the largest and flattest rocks representing the most desirable dimensions (Watson & Rogers, 2003). Predation, and to a lesser extent cannibalism cause crayfish to seek shelter in refugia during inactive daylight hours (Barbaresi & Gherardi, 2001). Indeed, disputes over shelter are one of the main reasons for agonistic interactions (Gherardi *et al.*, 2002; Gherardi & Cioni, 2004), and the availability of such refugia is a strong limiting factor to crayfish numbers (Lodge & Hill, 1994; Holdich, 2003) and growth rates (Olsson & Nystrom, 2009).

One study showed equal association of crayfish with finer substrates such as pebbles, gravel and sand (Barbaresi *et al.*, 2007), and another inferred that crayfish actually *avoided* cobble and boulder substrates, positively selecting fine substrate and more exposed microhabitats (Clavero *et al.*, 2009). Both studies were conducted at night and therefore indicate habitat use during active foraging periods rather than during the day when crayfish remain in refugia. The preference for finer substrates during foraging may reflect an association between fine particulate and detritus, an important food resource for crayfish, with high nutritional content (Gherardi *et al.*, 2001).

2.4.3 Vegetation parameters

All four of the vegetation parameters investigated were significant in 13 to 100% of studies at both the catchment and reach scales, though their relative importance varied with scale. The presence of canopy cover was most important at the catchment scale, with a positive relationship identified in all studies. Woody debris and roots were most important at the reach scale, again always positively associated with crayfish. The positive relationship between detritus and crayfish was more apparent at the reach scale, though was also identified in one catchment scale study. In-stream vegetation generally positively reflected crayfish occurrence, though a single study differentiated between vegetation and found a negative relationship between crayfish and liverworts, but a positive relationship with amphibious vegetation (Naura & Robinson, 1998).

Riparian vegetation provides two key functions with regards to crayfish: as a direct and indirect food source, and as physical habitat. Crayfish directly consume macrophytes, allochthonous detritus and mosses and these primary producers form the basis of freshwater food webs; this may underpin the observed positive associations between crayfish and in-stream vegetation, detritus and tree presence. The less expected negative association found with liverworts may reflect covariance between variables as liverworts tend to be found in areas of acidic geology which are chemically less suitable for crayfish (Jay & Holdich, 1981). Trees, in addition to providing leaf inputs, supply cover and shade, thereby likely reducing predation risk, and maintain cool water temperatures; associated with higher dissolved oxygen concentration. The woody debris and roots associated with trees are important refugia, particularly for juveniles (Neveu, 2009) and where flow regimes are “flashy” (Smith *et al.*, 1996).

2.4.4 Hydrodynamic parameters

Overall, hydrodynamic parameters emerged as less important in crayfish habitat use than vegetation parameters, with 29% of the potential associations investigated found to be significant compared to 42% for vegetation variables, but more important than channel substrate whereby only 26% investigated associations were significant. Water temperature was significant at the catchment scale (Table 2.2), and flow velocity and type were significant at the reach scale (Table 2.3). In general, crayfish were associated with lower water temperatures, which always co-varied with altitude, indicating that *A. pallipes* tended to occur in higher and cooler sites. It is unclear whether this relationship reflects a true habitat preference among *A. pallipes*, or distribution patterns resulting from the spread of invasive crayfish which generally expand their range upstream from the lower reaches of watercourses, with the remaining native populations persisting in headwaters (Collas *et al.*, 2007; Weinländer & Füreder, 2012). Further research aimed to fill this knowledge gap would be highly beneficial for formulating habitat parameters for ark sites. Two studies in the review found a negative association with flow rate (Bubb *et al.*, 2006; Benvenuto *et al.*, 2008), and one a negative association with riffles (Benvenuto *et al.*, 2008). It has been previously recognised that although *A. pallipes* is largely restricted to running waters, it is absent from those with very high flow rates (Foster, 1995). In a study with co-occurring signal crayfish, white-clawed crayfish individuals were found to inhabit slower water than signals, perhaps reflecting a lower tolerance of high velocities (Bubb *et al.*, 2006). Similarly, Clavero *et al.* (2009) found *A. pallipes* to preferentially use the deeper, slower water of in-stream pools, with larger crayfish found in the deepest areas, perhaps excluding smaller individuals from this prime foraging habitat. Deeper habitats also offer a reduced risk of predation (Nystrom, 2002).

2.4.5 Bank structure

Bank structure was significant at the catchment scale, with a positive relationship reported in 4 of the 9 studies (44%) that included this variable; one of which also found negative association with poached or reinforced banks (Naura & Robinson, 1998). Only one study considered bank structure at the reach scale, though it also identified a positive relationship (Broquet *et al.*, 2002). Undercutting is a key feature of bank structure which creates refugia readily exploited by crayfish (Brusconi *et al.*, 2008); particularly where the abundance of boulder and cobbles substrates is low (Smith *et al.*, 1996).

2.4.6 Land-use

Land-use within a catchment is informative of physical habitat characteristics and potential pressures as the result of human activities. Certain land-uses favour crayfish such as wooded areas (Souty-Grosset *et al.*, 2010), which provide leaf inputs and habitat complexity as previously discussed. In general, there is a negative relationship between crayfish presence and human population/influence which is likely due to localised human mediated introductions of invasive crayfish, and perhaps also reflects a wider degradation of water courses making them less suitable for *A. pallipes* (Füreder *et al.*, 2002).

2.4.7 Macro-invertebrates

Macro-invertebrates are an important food source for *A. pallipes* (Reynolds & O'Keeffe, 2005), but in the context of the studies examined, the diversity and abundances of macro-invertebrates were used to calculate indices representative of water quality. In two of the five studies that recorded macro-invertebrates, crayfish presence was positively associated with greater index scores, inferring that crayfish tend to prefer sites with better water quality; though this apparent relationship may be confounded by preferential predation by crayfish on low-scoring sediment-dwelling taxa, thereby inflating water quality indices. Work is currently being carried out by the Environment Agency to investigate this. The absence of a relationship in the other three studies offer support to the recent supposition that *A. pallipes* is not a reliable bio-indicator (Fureder & Reynolds, 2003). *A. pallipes* can indeed persist in watercourses of sub-optimal water quality (Broquet *et al.*, 2002; Demers & Reynolds, 2002) and there is also evidence that it can survive low oxygen, at least for short periods. In a summer study, oxygen concentrations dropped as low as 4.93mg L^{-1} with no apparent detriment to the resident crayfish (Trouilhe *et al.*, 2007).

2.4.8 Habitat partitioning

The use of presence-absence crayfish data precluded consideration of potential variation in habitat use due to crayfish size class or sex in the majority of studies. Where this was investigated (6 studies), clear patterns of habitat partitioning were apparent. For example, Clavero *et al.* (2009) identified a clear preference for deeper habitat among adult size classes that was lacking in juveniles. Two further studies similarly found that juveniles made greater use of the shallow channel margins than adults, and were presumed to be exploiting refugia within exposed roots (Smith *et al.*, 1996; Benvenuto *et al.*, 2008). Variations in habitat use due to different foraging patterns between the sexes were highlighted in another study (Gherardi *et al.*, 2001). Such patterns may be mediated by actual variations in habitat preferences, through intra-specific competition whereby larger, male crayfish exclude younger year classes or subordinate females from prime refugia (Gherardi *et al.*, 2001); or through habitat-specific and size selective predation (Garvey *et al.*, 2003).

2.5 Conclusion

Studies examining habitat use of *A. pallipes* comprised two clear groups: those incorporating habitat variables measured in several watercourses and related to *A. pallipes*, usually by presence absence data (catchment scale); and those examining microhabitat use within just one watercourse (reach scale). Vegetation parameters were most commonly identified as important for *A. pallipes*; in particular, trees/ shading at the catchment scale and woody debris and roots at the reach scale were positively associated with crayfish. Channel substrate parameters were second most important, with larger substrates such as cobbles and boulders positively associated with crayfish presence, though finer substrates such as silt were used during foraging.

The reviewed studies originated from four Western European countries and therefore addressed populations over a wide geographical range. Species may vary in their environmental tolerances, and therefore habitat requirements, through genetic and phenotypic plasticity (e.g. Ayrinhac *et al.*, 2004; Sassi *et al.*, 2007). In a study of 19 populations of *A. pallipes* across the UK, Haddaway *et al.*, (2012a) found morphological variation in branchial volume between crayfish from lotic and lentic sites. A strong bias towards the study of crayfish from lotic rather than lentic systems was apparent in the literature retrieved and only lotic studies were reviewed. While most wild populations of *A. pallipes* occur in lotic environments; the majority of suitable ark sites are likely to be lentic waterbodies due to their low connectivity and associated low risk of invasion (Peay, 2009; Whitehouse *et al.*, 2009). The applicability of some findings from this review to lentic ark sites is therefore limited due to differences in the structure and ecosystem functioning between lotic and lentic systems. For example, hydrodynamic parameters such as flow velocity, flow type and, to some extent, water depth in lotic environments do not correspond appropriately to habitat characteristics in a lentic sites. Similarly, aspects of bank structure such undercutting, which emerged as one of the most important variables at the catchment scale, cannot be directly related to waterbodies without flow. Nevertheless, the behavioural mechanisms that underlie the habitat preferences of *A. pallipes* will likely persist in both. For bank structure, the tendency for crayfish to seek refuge from predators during the day is thought to underlie the apparent preference for undercut banks (Naura & Robinson 1998; Smith *et al.*, 1996). This is also the case for the preference for larger substrate classes, though the occurrence and nature of such substrates will vary markedly between lotic and lentic systems. Water chemistry is directly comparable between lotic and lentic sites, as are the preferences relating to the availability of food resources e.g. detritus. Therefore, in the absence of statistically robust habitat studies addressing lentic sites, the current review nevertheless represents a useful resource to inform Ark site creation. On the basis of findings it is recommended that Ark sites incorporate habitat features that provide refugia during daytime (e.g. boulders, undercut banks, exposed roots), and enhance night-time foraging (e.g. overhanging trees, ample detritus, silt beds and instream vegetation). Clear patterns were lacking for many of the variables examined, in part due to habitat partitioning among size classes and sexes. To reduce intra-specific competition in Ark sites it is desirable to meet the key requirements for refugia and foraging through the provision of ample heterogeneous habitat with a range of features beneficial to crayfish, thereby facilitating habitat partitioning.

Chapter 3 Ark site introductions and quarries: a case study

3.1 Summary

Restoration of quarries post-production presents an opportunity to increase biodiversity by creating wildlife rich habitats. One potential after-use is the creation of Ark sites for the endangered white-clawed crayfish (*Austropotamobius pallipes*). This, the only native crayfish species in the UK, has undergone severe declines across its range due to habitat destruction and spread of the invasive signal crayfish (*Pacifastacus leniusculus*) from North America. Translocation of threatened crayfish populations to 'Ark sites' free from signal crayfish is a key part of current conservation strategy for the species. Quarries offer several advantages as Ark sites, most importantly they are often isolated, enclosed and in remote locations so there is low risk of invasion by non-native crayfish and other potential bio-security risks can be easily managed. This case study reports on the creation and establishment of a crayfish Ark site at Threshfield Quarry, a former limestone quarry in the Yorkshire Dales. The initial crayfish donor stock (n = 17) were translocated in August 2010. Monitoring during the period November 2009 to October 2012 revealed high water quality of the Ark site, with all measured chemical parameters within the tolerance limits of *A. pallipes*. Macro-invertebrates and zooplankton were found at relatively low densities and many taxa, including Gastropoda and Odonata, were completely absent. The communities present indicate that the waterbodies, particularly ponds 2 and 3, are in the early stages of species colonisation and diversity is expected to increase over the next 3 to 5 years. Crayfish monitoring showed that *A. pallipes* persisted at the site for at least one year after translocation. In an initial trial in one pond, a novel baited camera system showed potential to make a valuable contribution to future crayfish monitoring efforts. It is recommended that further crayfish translocations are a management priority and that continued monitoring of wider biodiversity at the site will be beneficial both to inform future Ark site projects, and help gauge success of the restoration for wider nature conservation goals.

3.2 Introduction

Quarries have existed for as long as humans have demanded stone for construction, metals and precious gemstones; hence they are historic and ubiquitous features of our landscape. In the UK alone there are approximately 2313 active sites, with sand, gravel and limestone the principal commodities (British Geological Survey, 2010). The lifetime over which a quarry is economically viable varies from tens of years to several hundred and the fate of quarries at the end of this production period is a much discussed, and often contentious, issue with a diverse array of stakeholders. The wide range of quarry after-uses includes recreation, for example angling, walking, climbing, diving and other watersports; nature conservation; redevelopment, for example shopping centres; residential or business centres, or as industrial storage and landfill. With careful management, several after-uses may be accommodated at one site. The physical nature of aggregate extraction and the natural succession that occurs during disuse means that quarries harbour unique habitats such as sandstone cliffs, calcareous grassland, and meagre meadows (Ratcliffe, 1974; Cullen *et al.*, 1998; Cooke & Johnson, 2002; Tropek *et al.*, 2010). Over 700 UK Sites of Special Scientific Interest (SSSI) are located in former quarries (Natural England, 2013), and Biodiversity Action Plans are in place at many more (Tarmac, 2012). The Government-endorsed Nature After Minerals Programme aims to heighten the contribution of former quarries to priority habitats and species within the England Biodiversity Strategy (Nature After Minerals, 2013). Mineral extraction frequently occurs below the water table; hence many quarry sites naturally and permanently fill with water. Waterbodies provide high amenity value for sports, but also aesthetic and nature conservation value. The invertebrate conservation charity Buglife is currently leading an initiative to restore former quarry sites for conservation of the endangered white-clawed crayfish (*Austropotamobius pallipes*).

White-clawed crayfish are afforded protection under EU and UK legislation, with measures in place to conserve them such as Biodiversity Action Plans (BAPs) and Special Areas of Conservation (SACs). A key component of UK, and indeed European, conservation policy for *A. pallipes* involves the creation of ‘Ark sites’. These are waterbodies free of invasive crayfish and with low risk of future invasion, where imminently threatened A. *pallipes* may be translocated with the aim to establish a breeding population. Former quarries present great opportunity as potential Ark sites and can make a valuable contribution to regional conservation strategies (Kindemba *et al.*, 2009; Whitehouse *et al.*, 2009). The remote and isolated nature of many quarry sites greatly reduces the risk of introduction of non-native crayfish and crayfish plague, and the physical, biological and chemical habitat remaining at the termination of mineral extraction may require comparatively little restoration to optimise its potential for *A. pallipes*. Further, there will be a succession of potential sites becoming available in the future as more quarries reach the end of their operational lifetime (Kindemba *et al.*, 2009).

3.3 Threshfield Quarry Ark site

Threshfield Quarry lies within the Yorkshire Dales National Park (54° 4'57.71"N, 2° 2'42.10"W). The site, now covering 29.38 hectares, was first commercially exploited for limestone in 1902. Under its owners Tarmac, production peaked at 1,000,000 tonnes per year before production declined in the mid-nineties (Johnson & Martlew, 2008). In 2006 Tarmac proposed an offset agreement to transfer the remaining extraction license of 24 million tonnes to nearby Swinden Quarry and restore the Threshfield site for the purposes of nature conservation. After consultation with the Yorkshire Dales National Park Authority and local communities, the application was accepted and restoration works began in 2009.

3.3.1 Restoration

A key and early stage in the restoration of Threshfield entailed the creation of an Ark site for white-clawed crayfish. The isolated location of the quarry, its existing large and permanent groundwater-fed waterbody, and proximity to the nearby threatened Eller Beck crayfish population made it an ideal potential Ark site under the risk-based criteria suggested by Peay (2009). After approval from the BAP Crayfish Steering Group in 2008, works began at the site including the demolition of derelict buildings, creation of two more ponds adjacent to the large existing water body, and grading of steep pond margins using smaller substrate already present onsite. This latter measure further increased the availability of potential refugia for crayfish, an important habitat requirement for *A. pallipes* (Holdich & Rogers, 2000).

The largest pond (Pond 1, approximately 800 m^2) was already present at the start of the restoration project as it functioned as a settling pond during extraction activities. Pond 2 (approximately 440 m^2) and Pond 3 (approximately 145 m^2) were created in March 2010 and naturally filled with groundwater over subsequent months (Figures 3.1 and 3.2).

Figure 3.1 Threshfield Ark site comprising ponds 1, 2 and 3 (photo taken May 2012; facing south-east).

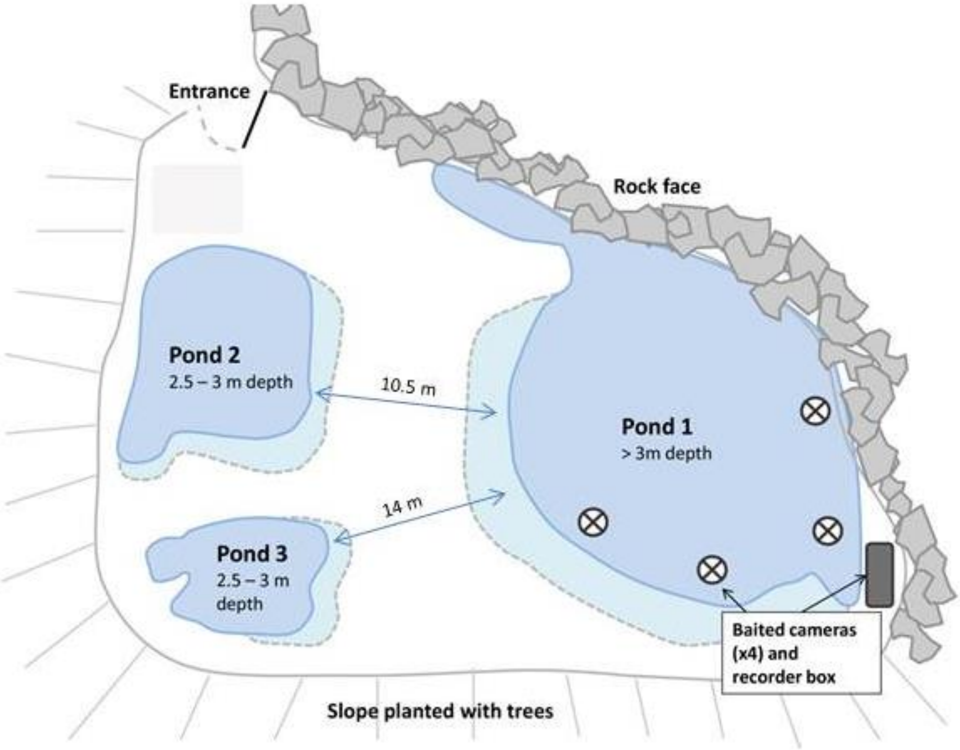


Figure 3.2 Plan of Threshfield Ark site showing position of waterbodies (dotted lines represent prepared planting beds), water depths and location of baited cameras during trial. Distances and depths are approximate.

In spring 2010 all accessible shorelines of the 3 ponds were levelled and covered with a layer of top soil in preparation for planting of submerged and marginal macrophytes in June 2010. All plants were native species sourced from locations free of invasive crayfish, thereby reducing the risk of inadvertently introducing the pathogen *Aphanomyces astaci*, the cause of crayfish plague (Alderman *et al.*, 1984). Submerged macrophyte species were weighted with stem clips and distributed within 2 m of pond margins. Unfortunately, water levels in all 3 ponds unexpectedly dropped by approximately 1 m between May and June 2010 so it was not feasible to plant marginal macrophytes in many of the pre-made planting beds; they were consequently restricted to the few areas in each pond with ample substrate and sufficiently high water level.

3.3.2 Donor population

Eller Beck is a tributary of the River Wharfe, Yorkshire and runs approximately 7 km west to east before its confluence near Grassington (54° 3'56.42"N, 2° 0'2.17"W). The white-clawed crayfish of Eller Beck are one of the last remaining populations in the entire Wharfe catchment because signal crayfish have spread rapidly through it since they escaped from culturing facilities in 1987 (Peay & Rogers, 1998). Due to their rapid advancement towards the upper reaches of Eller Beck (Bubb *et al.*, 2005), translocation was considered the only option for protecting the remaining white-clawed crayfish persisting there.

A preliminary survey by hand-search and night-viewing was carried out in Eller Beck in July 2009 by GSL consultants (under license from Natural England) and indicated a healthy and comparatively dense population of *A. pallipes* still residing there. Translocation of donor stock was scheduled to take place in August 2010 with the intention of moving 610 adult *A. pallipes*. Juvenile crayfish (<25 mm carapace length) were considered unsuitable for translocation due to the difficulty in distinguishing between *A. pallipes* and *P. leniusculus* at this life-stage, and therefore increased risk of introducing *P. leniusculus* to the Ark site. The anticipated numbers of crayfish to be introduced to each of the 3 ponds were: Pond 1, 330; Pond 2, 160; and Pond 3, 120, based on a density of 1 individual m^{-2} littoral habitat (Graeme Smart, 2010, pers. comm.).

3.3.3 The translocation

Trapping and night-viewing in Eller Beck in August 2010 revealed that *P. leniusculus* had spread throughout the donor reach and that the population of *A .pallipes* had undergone severe decline. Only 4 *A. p*allipes were captured and translocated to Pond 1 of the Ark site. Capture efforts were subsequently extended upstream of the original donor reach incorporating an area where *P. leniusculus* was not detected. This stretch yielded 10 *A.*

pallipes (3 males and 7 females) which were translocated to Pond 2 of the Ark site. A further 3 crayfish were captured from the donor reach and translocated to Pond 1, giving a total of 6 males and 11 females within the two ponds. It was considered a necessary precaution to separate the crayfish captured from stretches with and without *P. leniusculus* in case *A. pallipes* from the mixed population had been exposed to the plague pathogen *A. astaci*, of which *P. leniusculus* is a key vector. Although this would discourage intermixing of the two populations, it would not necessarily prevent it as crayfish will leave the water and move across land, as shown for *Orconectes virilis* (Dorn & Wojdak, 2004)3.3.4 Post-translocation monitoring

Understanding the fate of translocated individuals and the longer-term viability of the (re)introduced population is a key recommendation within IUCN best practice guidelines for conservation translocations (IUCN, 2012). Further, planned monitoring is a prerequisite for obtaining the necessary license from Natural England for crayfish translocations such as the one at Threshfield. Monitoring crayfish populations quantitatively in lotic or lentic systems presents a challenge as individuals are often naturally clustered (DiStefano *et al.*, 2003). A wide variety of techniques and equipment have been employed to sample crayfish in different habitats, including baited traps, throw traps, hand-search, kick sampling, Surber sampling and electric fishing, with mixed outcomes. While a fully quantitative survey method based on hand-search has been established for shallow lotic systems (DiStefano *et al.*, 2003), lentic environments present quite different sampling challenges; predominantly due to water depth, turbidity and dense macrophyte growth. Indeed, crayfish have been found at depths up to 100 m (Barefoot, 2008). This poses a major challenge for the conservation managers of Ark sites because re-introduced crayfish are notoriously “hard to find again” after release (Rogers & Watson, 2007), making success difficult to gauge.

A widely used survey approach in stillwaters is baited trapping with cylindrical traps fitted with funnel entrances. These are usually deployed on weighted lines from boats, with a distance between traps of 3-10 m (Edsman & Soderback, 1999; Reynolds, 2006). The sampling biases associated with trapping are widely reported, with a tendency to capture larger size classes (>40 mm carapace length), and particularly males which tend to be the most active components of the population (Matthews & Reynolds, 1992; Demers *et al.*, 2003; Reynolds & O'Keeffe, 2005; Moorhouse & MacDonald 2011 b&c). An alternative approach, seine netting, has been used with success in the Marl lakes of the U.S. to capture *Orconectes virilis* down to depth of 8.5 m; however the danger of snagging on rocky substrates (where crayfish are most likely to occur) and high labour requirements for this method most likely render it inapplicable for Ark sites in the UK. Electric fishing or night-viewing along the shore may have some applicability; however both are ineffective at depth. Further methods that involve personnel entering the water such as scuba or snorkelling are likely to be unworkable in most instances due to health and safety considerations.

Planned post-translocation monitoring for Threshfield Ark site comprised a trapping survey within a two month period following the translocation, then annual trapping (1 night) during Autumn in each of the ponds for a period of 5 years. With the aim to supplement the existing monitoring programme and overcome some of the biases associated with trapping, a novel baited camera system was constructed and trialled at Threshfield. This approach is widely used for marine surveys (e.g. Priede *et al.*, 1994; Farnsworth *et al.*, 2007; Stoner *et al.*, 2008), though has rarely been applied to freshwater situations (but see Frezza *et al.*, 2003). Deployment simply entails the lowering of a bait station into the water which is monitored by either a continuous video or motion-detection camera, thereby providing a record of all species that visit the bait. Unlike trapping which relies on crayfish remaining in the trap until they are retrieved, baited cameras record any individual that is attracted into the field of view, leading to the prediction that they will more effectively sample smaller size classes. Baited cameras provide a cost-effective means of monitoring species at depth and have been shown particularly useful for detecting animals at very low densities in marine situations (Cappo *et al.*, 2006).

3.4 Study rationale and aims

The use of Ark sites as a conservation tool for white-clawed crayfish is relatively new; the first documented UK example is from 2006 in the Bristol Avon catchment (Holdich *et al.*, 1995b). Preliminary best practise guidelines for Ark site selection and crayfish translocations have recently been developed by conservation practitioners (Buglife, 2009). Foremost, these highlight the importance of assessing the risk of invasion by non-native crayfish or transferral of crayfish plague by other means. Beyond this, a potential Ark site should have appropriate crayfish habitat and water quality must be within acceptable ranges for *A. pallipes*, which although tolerant of moderate pollution levels (Demers & Reynolds, 2002) is generally associated with high water quality (Fureder *et al.*, 2003). The availability of ample food resources to sustain the projected crayfish population is also of obvious importance. As omnivores, crayfish are able to utilise the range of resources such as plant detritus, macrophytes, invertebrates, and even small fish (Momot, 1967; Nystrom & Perez, 1998; Nystrom *et al.*, 1999; Reynolds & Donohoe, 2001; Reynolds & O'Keeffe, 2005). It is therefore desirable to conduct a full feasibility study of potential crayfish Ark sites, incorporating assessments of water chemistry, physical habitat, and potential food resources. Further, where Ark site creation is integral to a larger restoration scheme, as at Threshfield, longer term monitoring of non-target taxa also supported by the Ark site is beneficial for assessment of the project against wider biodiversity targets.

The current study had three aims: first, to provide baseline data regarding the water chemistry, macro-invertebrate and zooplankton communities present in the Threshfield Ark site prior to translocation; second, to provide a longer-term (~3 year) record of water chemistry and macro-invertebrate diversity after translocation; and third, to trial a novel baited camera tool for detecting crayfish in deep waterbodies, with a view to supplementing post-translocation monitoring in Ark sites.

3.5 Methods

3.5.1 Water chemistry monitoring

Sub-surface (~10 cm) water samples (0.05 L) were collected from the Threshfield ponds at minimum intervals of two months during the period September 2009 to October 2012. Two water samples were also taken from Eller Beck in September 2009 to enable comparison of water chemistry between donor and recipient sites. Samples were frozen (-20 ˚C) and analysed in batches using ion chromatography on a Dionex (ICS-90) instrument at the University of Leeds. The water chemistry parameters measured were: calcium; sodium; magnesium; potassium; nitrate; phosphate; sulphate and chloride. *In situ* surface measurements of water temperature, conductivity, dissolved oxygen and pH were also taken on each sampling occasion using field probes (Hanna Instruments). Total suspended solids concentration was measured in ponds 1, 2 and 3 on 24th August 2010 and 4th October 2011; the latter followed five days of heavy rain in the region. Water samples (2 L) were filtered and suspended solids concentration (mg L^{-1}) was determined by change in mass (± 0.0001g) of the oven dried filter paper (0.45 μm, cellulose nitrate membrane, Whatman).

3.5.2 Biodiversity monitoring

3.5.2.1 Macro-invertebrates

The diversity of aquatic macro-invertebrates in the Threshfield Ark site was monitored over the period September 2009 to October 2012 using a combination of sweep netting, hand-search and passive invertebrate samplers or ‘hester dendies’ (Figure 3.3). On sampling occasions, which were separated by intervals of at least 2 months, sweep netting from the margins was carried out for 3 minutes in each pond. Due to the rocky nature of the site it was necessary to cease sweep netting while moving between different locations around the margin; however total time netting time was always maintained at 3 minutes. A further minute was spent hand-searching under rocks and in vegetation. Any invertebrates captured were transported live to University of Leeds for species identification within two days of collection. Due to the difficulty associated with sampling the deeper habitats, hester dendies (0.16 m^2 surface area) suspended at depths of approximately 1.5 m below the water surface were deployed in spring 2010, 2011 and 2012 and collected each autumn. Two were deployed from the margin in Pond 1, and one each in ponds 2 and 3. After collection, the hester dendies were deconstructed to collect invertebrates sheltering between the plates.

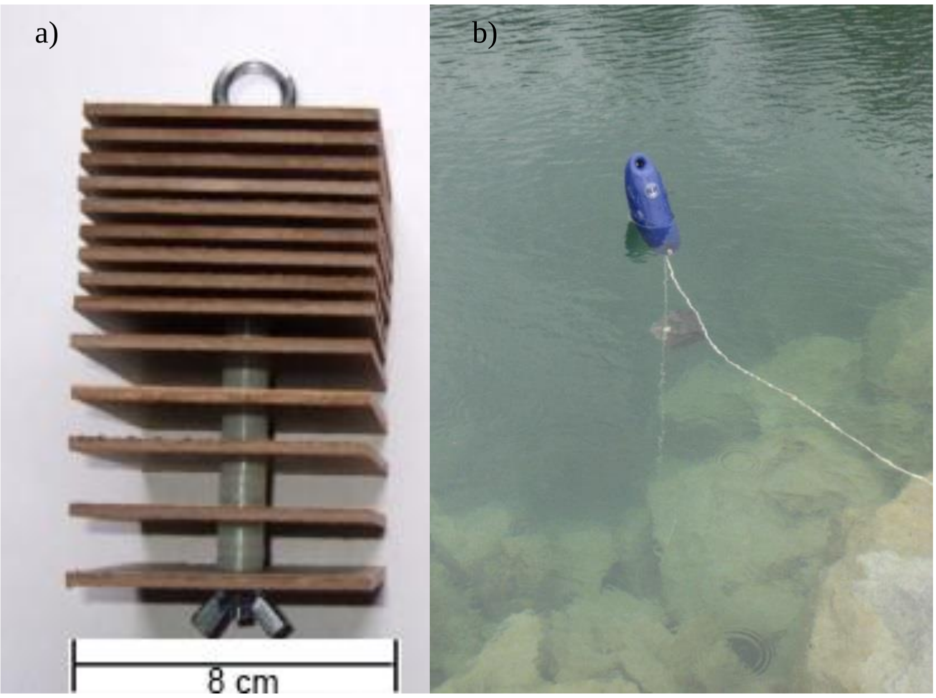


Figure 3.3 Hester dendy (a), during deployment at Threshfield (b).

Identification of macro-invertebrates was carried out at to a minimum of family level for most groups and class level for oligochaetes. The PSYM (Predictive System for Multimetrics) standardised method for monitoring the quality of stillwaters is used by regulatory bodies such as the Environment Agency and takes into account both plant and macro-invertebrate assemblages to assess water quality and potential degradation (Environment Agency & Pond Conservation, 2002). Under this method three metrics are calculated from invertebrate samples: 1) average score per taxon (ASPT) derived from the sum of BMWP (Biological Monitoring Working Party) scores divided by number of scoring taxa; 2) number of dragonfly (Odonata) and alderfly families (Megaloptera); and 3) number of beetle (Coleoptera) families. Due to difficulties and safety concerns associated with sampling at the Threshfield Ark site, it was not feasible to follow closely the PSYM sampling protocols; however, PSYM metrics were nevertheless calculated to facilitate comparison of invertebrate assemblages between ponds and provide a baseline against which to compare future data.

3.5.2.2 Zooplankton

Zooplankton samples were taken from each pond on the same sampling occasions as for macro-invertebrates. A weighted conical net (250 µM retention mesh) was thrown into the water and allowed to sink to a depth of 2 m before being pulled through the water column to the surface, representing a sample volume of 98.2 L. Three samples were taken per pond and each separate sample was preserved in 50% ethanol until examination. All the zooplankton in each sample were identified to a minimum of class level and then counted using a Bogorov tray (Gannon, 1971).

3.5.3 Crayfish monitoring

A crayfish monitoring survey was carried out on Oct 6th to 7th 2010 by GSL consultants. A total of 40 traps baited with sardine were set in Pond 1, at an approximate density of 1 trap per 4 m of bank. Ten traps were set in shallow water areas (up to 1m deep) in the margins and the other 10 were thrown out into deeper water areas. Eighteen traps were set around the perimeter of Pond 2 at an approximate density of 1 trap per 5m of bank. . A visual search of the littoral zones from the bankside using torches (Clulite CB2, 1 million candle power) was also conducted in ponds 1 and 2 over a period of 1.5 h commencing 1 h after sunset. Annual monitoring using the same methodology was carried out by GSL consultants in October 2011 and 2012.

In October 2012 a baited camera system was trialled in Pond 1 on the night preceding the annual monitoring survey. The set-up comprised 4 underwater bullet cameras with inbuilt infra-red LEDs (Sony CCD, Model 37CSHR-IR), each mounted 40 cm above a weighted wooden platform and connected to a digital recorder unit (Everfocus, Model EDR810H). One gel cell leisure battery (125 Ah, 12 V) was used to power all 4 cameras and the recorder unit (Figure 3.4). The platforms were covered in corrugated plastic board to increase surface roughness and thereby facilitate crayfish climbing onto them. The board was painted with a black and white checkerboard pattern to provide greater visual contrast between crayfish and the background, and a scale bar was drawn on to enable measurement (Figure 3.5a).

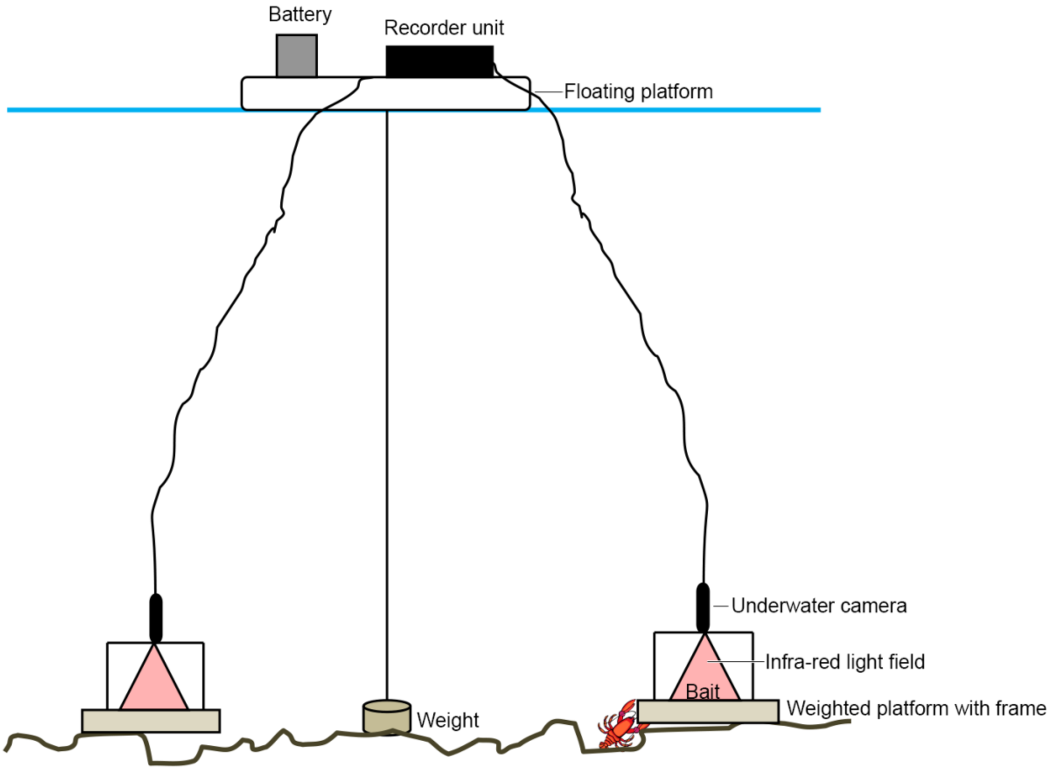


Figure 3.4 Schematic of underwater baited camera set-up for crayfish monitoring

Bait (sardine) was secured to each camera platform within a mesh bag to ensure it remained within the field of view of the camera. The camera platforms were deployed within 2 m of the shoreline, along the north and eastern sides of Pond 1 and at water depths ranging 1.5 to 4 m (Figure 3.5b). Due to the proximity of the camera platforms to the shoreline it was not necessary to float the recorder unit and battery; instead they were secured within a waterproof box next to the water's edge. The system was powered on at 17:15 and video images from all 4 cameras were continuously recorded (12 fps) until 08:30 when the camera platforms were retrieved. All video footage was watched back (4X speed) to detect crayfish presence.

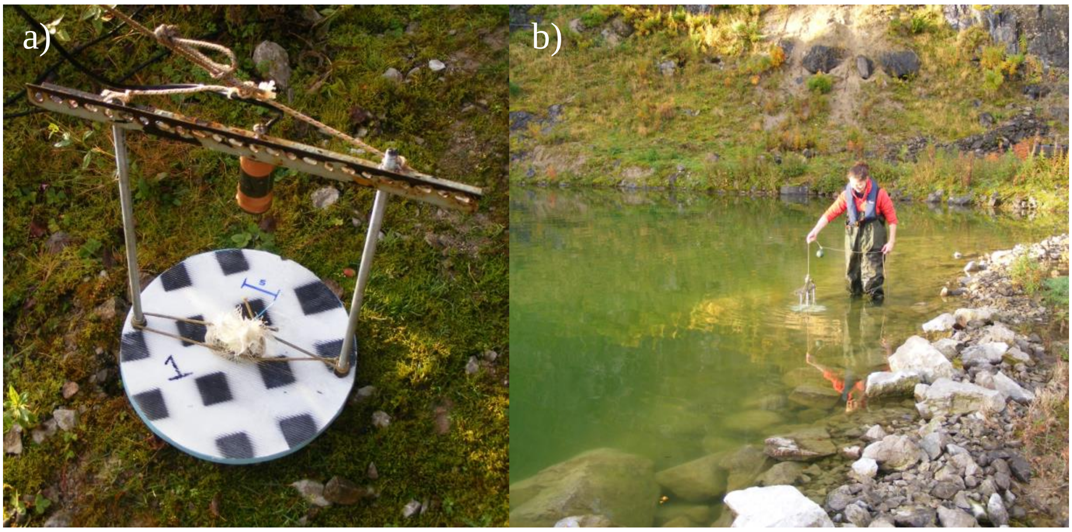


Figure 3.5 Baited-camera station (a), and during deployment at Threshfield (b).

3.6 Results

3.6.1 Water chemistry monitoring

Temperature across all three ponds at sampling times in spring through to autumn ranged from 14.5 to 16.3 ˚C, pH ranged from 7.7 to 7.9 and dissolved oxygen ranged from 9.7 to 10.83. Suspended solids concentration ranged from 33.3 to 35.3 mg L^{-1}; 21.9 to 40.0 mg L^{-1}, and 53.1 to 60.0 mg L^{-1} in Ponds 1, 2 and 3, respectively. The measured water chemistry parameters were similar for Ponds 1, 2 and 3, though nitrate and sulphate were consistently lower in Pond 1 than Ponds 2 and 3. Besides potassium, which was consistently lower, the concentrations of all other water chemistry parameters measured in the Ark site mainly fell within 5 S.E. of mean values extracted from the literature concerning sites with *A. pallipes* (Haddaway, 2010). The water chemistry of Eller Beck in 2009 was characterised by lower concentrations of calcium, sodium, magnesium, nitrate, sulphate and chloride than the Ark site ponds 2009 to 2012; potassium and phosphate concentrations were similar (Figures 3.6 and 3.7).

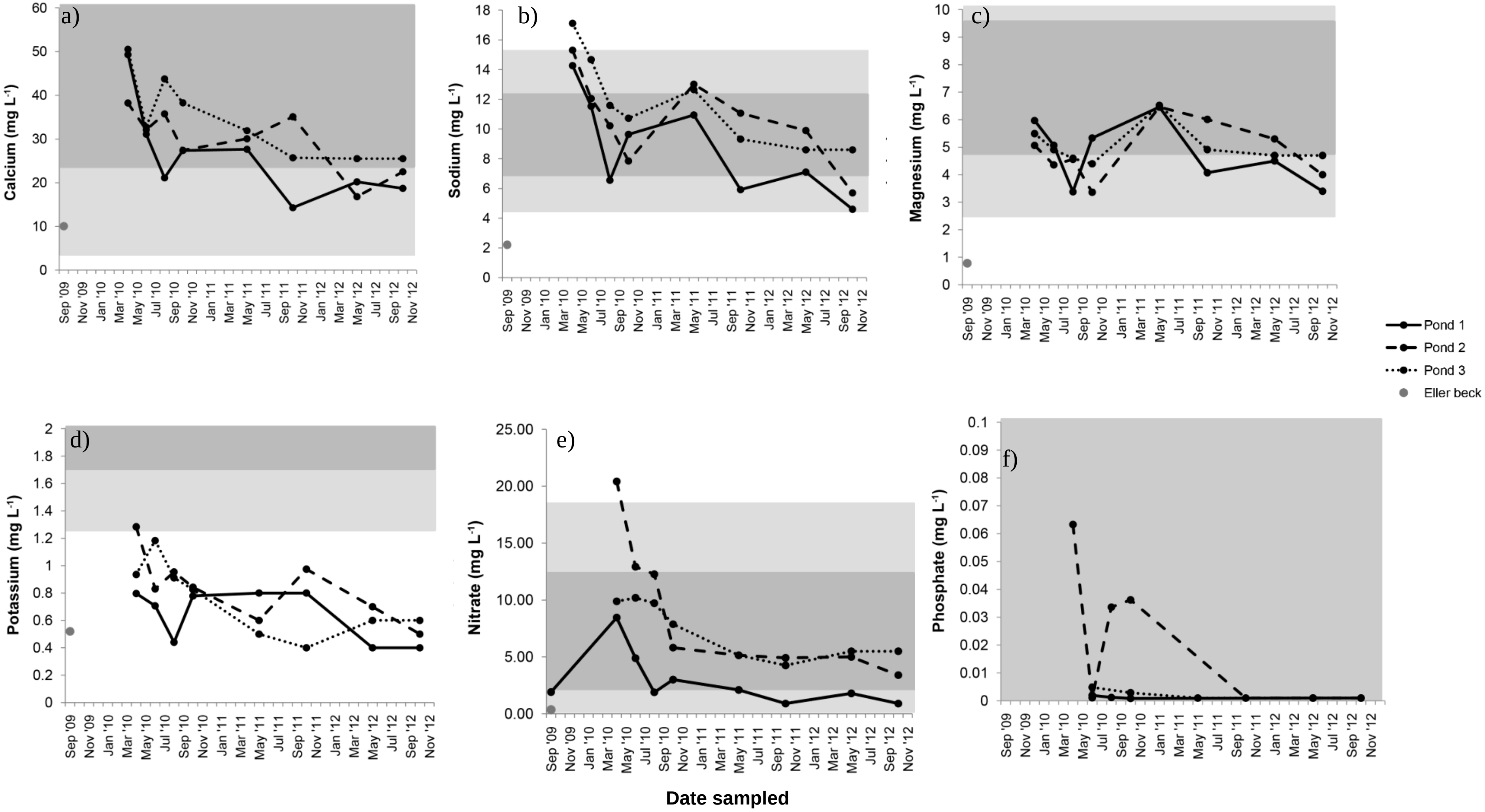


Figure 3.6 Concentrations of calcium (a), sodium (b), magnesium (c), potassium (d), nitrate (e) and phosphate (f) in Ponds 1, 2 & 3 at the Threshfield Ark site, and in the donor site Eller Beck (where measured) over the period September 2009 to October 2012. Dark and light grey shading denotes values within 2.5 and 5 standard errors, respectively, of mean values where *A. pallipes* occurs, extracted from the literature by Haddaway (2010).

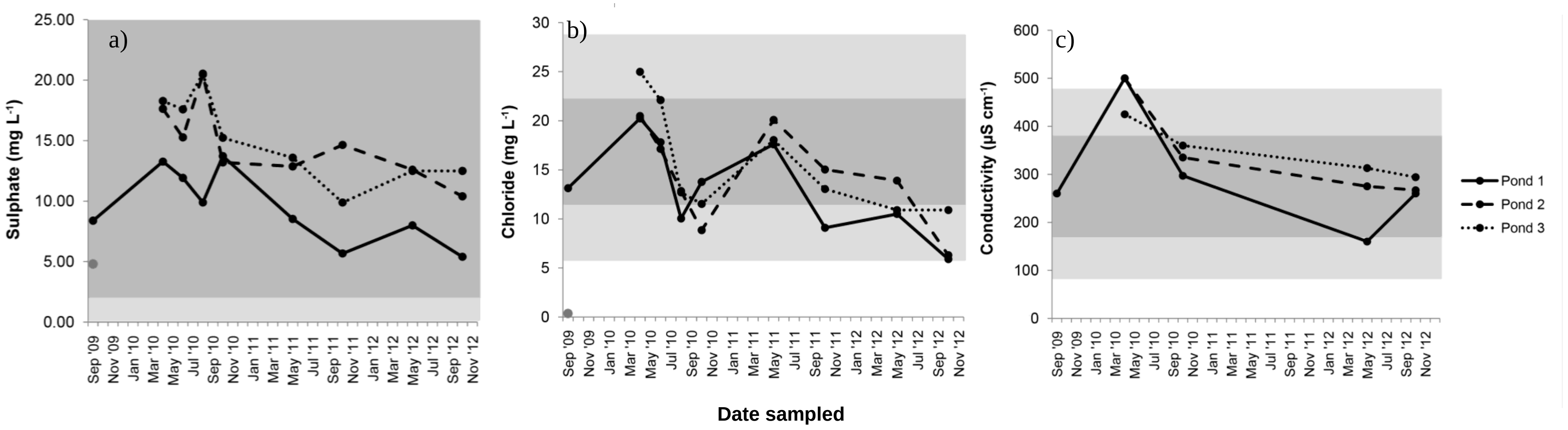


Figure 3.7 Concentrations of sulphate (a) and chloride (b), and conductivity level (c) in Ponds 1, 2 & 3 at the Threshfield Ark site, and in the donor site Eller Beck (where measured) over the period September 2009 to October 2012. Dark and light grey shading denotes values within 2.5 and 5 standard errors, respectively, of mean values where *A. pallipes* occurs, extracted from the literature by Haddaway (2010).

3.6.2 Biodiversity monitoring

3.6.2.1 Macro-invertebrates

Macro-invertebrates from 21 families were found in the Threshfield Ark site over the study period (Tables 3.1, 3.2 and 3.3). Pond 1 exhibited the highest diversity, followed by Pond 3. The total BMWP score of Pond 1 declined from approximately 50 in 2009 and spring 2010 to 33 in Oct 2010, and remained at 30 or below into 2012. Average scores per taxon ranged from 3.3 to 5.5. No species were recorded in Ponds 2 and 3 in April 2010, one month after their creation; however several species had colonised by August of the same year. Pond 2 exhibited the lowest diversity overall, with only 5 families recorded throughout the entire study period and a maximum BMWP score of 12 (Table 3.2). A total of 11 families were detected in Pond 3, including high scoring mayflies and caddis flies. The maximum BMWP score was 21, however species abundance was very low with only one or two individuals found from each family (Table 3.3). A full list of species recorded by the author at the Threshfield Ark site is provided in Appendix 1.

Table 3.1 Macro-invertebrate taxa found in Pond 1, Threshfield Quarry, November 2009 to October 2012

Date sampled	Class/Family (BMWP score) Abundance per sample																			BMWP score	No. taxa	Average score per taxon
	Asellidae (3)	Baetidae (4)	Caenidae (7)	Chironomidae (2)	Corixidae (5)	Crangonyctidae (0)	Culcidae (0)	Dytiscidae (5)	Elmidae (5)	Erpobdellidae (3)	Glossiphoniidae (3)	Haliplidae (5)	Hydracarina (0)	Leptoceridae (10)	Limnephillidae (7)	Notonectidae (5)	Oligochaeta (2)	Polycentropidae (7)	Tipulidae (5)			
03/11/2009		17	139	72	26			9			7	1	20		35	1	7		2	50	12	4.2
29/04/2010			58	89	29			13		2	9		33	3	3		12	3		51	11	4.6
05/10/2010			3	2	5			4							2			9		33	6	5.5
04/10/2011			3	30														6		16	3	5.3
01/05/2012		1			2		3								12					16	4	4
05/10/2012	22			29	47	19		5	1	1			50					9		30	9	3.3

Table 3.2 Macro-invertebrate taxa found in Pond 2, Threshfield Quarry, April 2010 to October 2012

Date sampled	**Class/Family (BMWP score)** Abundance per sample					**BMWP score**	**No. taxa**	**Average score per taxon**
	Asellidae (3)	Baetidae (4)	Chironomidae (2)	Corixidae (5)	Notonectidae (5)			
29/04/2010						0	0	0
24/08/2010					1	5	1	5
05/10/2010			3			2	1	2
04/10/2011			23			2	1	2
01/05/2012						0	0	0
05/10/2012	6	1		2		12	3	4

Table 3.3 Macro-invertebrate taxa found in Pond 3, Threshfield Quarry, April 2010 to October 2012

Date sampled	**Class/Family (BMWP score)** Abundance per sample												**BMWP score**	**No. taxa**	**Average score per taxon**
	Asellidae (3)	Baetidae (4)	Caenidae (7)	Chironomidae (2)	Corixidae (5)	Crangonyctidae (0)	Culcidae (0)	Dytiscidae (5)	Gyrinidae (5)	Polycentropidae (7)	Sialidae (4)	Tipulidae (5)			
29/04/2010													0	0	0
24/08/2010		1			1		1	1	1				19	5	3.8
05/10/2010			2	2						1		1	21	4	5.3
04/10/2011				1					1		1		11	3	3.7
01/05/2012													0	0	0
05/10/2012	2					1							3	2	1.5

3.6.2.2 Zooplankton

Zooplankton from 3 classes were recorded in the Threshfield Ark site, with the communities of all 3 ponds dominated by Ostracods (Table 3.4). Plankton density ranged 0 to 1.3 individuals m^{-3} across all samples. The results indicate that zooplankton abundance declined within all 3 ponds over the study period, with only 1 individual found in the two sampling periods in 2012 (Table 3.4).

Table 3.4 Zooplankton found in Ponds 1, 2 and 3 at Threshfield Ark site April 2010 to October 2012

	Pond 1		**Pond 2**		**Pond 3**	
Date sampled	**Zooplankton class (order)**	**Abundance**	**Zooplankton class (order)**	**Abundance**	**Zooplankton class (order)**	**Abundance**
29/04/2010	Ostracoda Copepoda	112 2	Ostracoda	12	Ostracoda	1
25/06/2010	Ostracoda Branchiopoda	2 2	Ostracoda Copepoda Branchiopoda	99 12 19	Ostracoda Copepoda Branchiopoda	33 5 9
17/05/2011	Ostracoda Branchiopoda	1 2	Ostracoda Copepoda Branchiopoda	1 3 2	Ostracoda Copepoda Branchiopoda	1 1 1
01/05/2012	NONE		NONE		NONE	
05/10/2012	NONE		NONE		Copepoda	1

3.6.3 Crayfish monitoring

The trapping and night-viewing survey carried out in October 2010 did not detect crayfish in ponds 1 and 2, however the annual survey carried out in October 2011 trapped 1 male *A. pallipes* in the north-west corner of Pond 1. The October 2012 annual trapping and night-view survey did not detect crayfish by either method. The baited camera trial carried out in October 2012 did not detect any crayfish, however, a number of other taxa were recorded including a smooth newt (*Lissotriton vulgaris*) (Figure 3.8), aquatic beetles, cased caddisfly larvae and lesser water boatmen.

Figure 3.8 Smooth newt (*Lissotriton vulgaris*) recorded by a baited camera deployed in Pond 1 at Threshfield Ark site, October 2012.

3.7 Discussion

3.7.1 Water chemistry

The water chemistry of all ponds in the Threshfield Ark site varied quite markedly from a spot sample taken at the donor site, Eller Beck. Although the ponds are still stabilising, measured concentrations of water chemistry parameters were within the tolerance levels compiled for *A. pallipes* (Haddaway, 2010).

Calcium is a component of the exoskeleton of Crustacea and if not present in sufficient concentration will result in stunted growth and reduced survival (Rukke, 2002). Considering the limestone geology of the study site it is unsurprising that calcium concentrations in the ponds were high, and certainly ample to sustain *A. pallipes* (Holdich & Reeve, 1991). Concentrations in two ponds exceeded 50 ml L^{-1} in spring 2010 which likely reflected a peak following pond creation and modification during which exposed rock surfaces and fine grained debris equilibrated with the waterbodies, then concentrations declined over time. Magnesium is also an essential element in Crustacean exoskeletons, though *A. pallipes* have been found at very low concentrations (<0.01 mg L^{-1}) (Trouilhe *et al.*, 2007). Magnesium is also present in limestone and concentrations in the Ark site were between 3 and 6 mg L^{-1} and therefore not limiting for crayfish. Water of pH 7.1 and lower is similarly associated with reduced survival and loss of exoskeleton integrity in *A. pallipes*, compared to higher pH levels (Haddaway *et al.*, 2013). Water in the Ark site was found to be slightly alkaline, also reflecting the calcareous geology, and within the 7.5 to 8.5 range most commonly associated with *A. pallipes* occurrence (Haddaway *et al.*, 2013).

Elevated levels of sodium and chloride may indicate water pollution, particularly road runoff, though both are also products of halite (NaCl) weathering and maritime rainfall (Feth, 1981). Limestone commonly contains small quantities of halite (El–Rahim & Khallaf, 2011) which dissolves from fresh surfaces, such as those created during groundworks in spring 2010. Concentrations in the Ark site declined over time reflecting gradual equilibration of the disturbed surfaces with water in the ponds.. Despite elevated levels of these ions in the Ark site relative to the donor site, the concentrations are within guidelines recommended by Haddaway (2010) and are therefore unlikely to present a problem for the translocated crayfish.

Potassium ions are essential for many physiological processes, in particular, healthy nerve function (Fenn, 1940). Although potassium concentrations in the Ark site ponds were approximately threefold lower than the mean concentration extracted from the literature, this is not cause for concern because values were similar to Eller Beck where *A. pallipes* have persisted for many years, and populations have been found at concentration lower than 0.001 mg L^{-1}, with no reported adverse implications (Rallo & Garcia-Arberas, 2002).

Phosphate and nitrate loadings in freshwaters are primarily derived from agricultural or industrial processes, delivered via surface runoff, and are considered pollutants, with high levels causing eutrophication (Carpenter *et al.*, 1998). The Environment Agency classes concentrations of phosphate greater than 0.1 mg L^{-1} and of nitrate greater than 30 mg L^{-1} as 'high' (Environment Agency, 2013). Nitrate levels in the Ark site ponds never exceeded 25 mg L^{-1} and declined over the study period indicating good water quality with little pollution. Similarly, phosphate concentration never exceeded 0.07 mg L^{-1} and declined over time. Sulphate concentration, another potential indicator of pollution, declined in the Ark site over the study period to below 15 mg L^{-1}, which is considered suitable for *A. pallipes* (Haddaway, 2010). The high water quality indicated by these results likely reflects the remote location of Threshfield Ark site in a catchment with little urbanisation and where the predominant land-use is low intensity sheep farming.

Conductivity reflects the amount of dissolved ions present in water and is affected by a range of factors including geology, nutrient loading and salinity. Conductivities in the Ark site were below the upper threshold of 700 µS cm^{-1} for *A. pallipes* as suggested by Haddaway (2010), again indicating suitable water chemistry with low nutrient loadings. Dissolved oxygen levels at the near-surface sampling points were above the EU guideline concentration of 9 mg L^{-1} for waters that are suitable for salmon and trout (EU Freshwater Fish Directive (78/659/EEC)); and more than double the 5 mg L^{-1} concentration below which effects on biological communities are expected (Davis, 1975; European Environment Agency, 2000). These results are again consistent with high water quality in the Ark site, with little or no organic pollution; however for oxygen especially, the infrequent 'spot check' nature of sampling did not capture likely fluctuations in concentration due to both dial and seasonal variation i.e. rising oxygen during the day followed by declines at night, with highest fluctuations during summer (Christensen *et al.*, 2013), Hence, it is not possible to conclude that these parameters were always above the 5 mg L-1 baseline

The maximum suspended solids (SS) concentration in any pond was 60 mg L^{-1}, a value which likely represented short-term elevation due to the suspension of unconsolidated dust during rain periods. SS is considered a stressor in freshwaters, causing biodiversity loss and ecosystem change (Bilotta & Brazier, 2008). Documented organism level impacts include gill damage, reduced feeding rates, metabolic changes and mortality (Lake & Hinch, 1999; Donohue & Irvine, 2003; Bilotta & Brazier, 2008). Ark site SS concentrations exceeded the current recommended limit of 25 mg L^{-1} for fish (EU Freshwater Fish Directive (78/659/EC); however there is no current recommendation for other taxa including crayfish. *A. pallipes* has been found in locations where SS exceeded 489 mg L^{-1}, but it is not known whether the population continued to persist under those conditions (Trouilhe *et al.*, 2007). Research presented in Chapter Six of this thesis investigated the tolerance of *A. pallipes* to SS. *A. pallipes* exhibited gill fouling in 45% of gill area and a 17% reduction in aerobic scope when exposed to a SS time-averaged concentration of 62 mg L^{-1} over a 45 day period. Although SS concentrations at Threshfield approached this value, time-averaged concentrations are likely to be much lower. However, because this parameter was only measured two occasions, it would be necessary to sample intensively and over a range of weather conditions to gain a full understanding of the range of SS concentrations in the quarry waterbodies . Generally, SS concentration would be expected to decline over time as marginal vegetation develops and binds unconsolidated material, thereby reducing sediment inputs to the ponds via runoff. Due to its deleterious effect on *A. pallipes*, SS should be minimised in Ark sites as much as is feasible. The detected SS levels at Threshfield are not cause for concern, however in other quarry sites substrate remediation may be necessary to facilitate plant growth and reduce high sediment input during the initial stages of restoration.

3.7.2 Macro-invertebrates

The diversity and abundance of macro-invertebrates in the Ark site was generally low, with all ponds falling within the ‘poor’ category as defined by the BMWP (1978) (Table 3.5). This is to be expected of recently created or restored waterbodies that are still in the colonisation phase and is therefore not indicative of poor water quality or insufficient habitat. Colonisation rate is dependent on several factors, most importantly the proximity and quality of the nearest ‘source’ waterbodies from which species may disperse (Gore, 1982). In a study of 30 newly dug small ponds, Jeffries (2011) found species accumulation did not slow until 6 years after pond creation. Pond 1 held the most diverse invertebrate assemblage of all the ponds, likely reflecting its longer time of establishment. The number of taxa and average score per taxon in Pond 1 fell in late 2010. The causes of this are unknown; one possible explanation is that re-grading of the margins in spring 2010 caused habitat disturbance and negatively impacted on biodiversity in the margins, where most sampling was conducted.

Alternatively, it may be a consequence of the crayfish introduction in July 2010. It was not possible to determine the community impacts of crayfish in the Threshfield Ark site because replicated ponds of equal age were not available; however, despite very low crayfish densities in the Ark site (only 0.05 individuals m^{-2} of littoral habitat compared to reported densities of 0.25 to 1.74 individuals m^{-2} in other lake populations (O'Keeffe, 1986; Matthews *et al.*, 1993)), macro-invertebrates were also at low abundance prior to translocation, so it is possible that the low invertebrate scores could reflect the impact of crayfish. Crayfish are key predators of the benthos and exert strong direct and indirect impacts on freshwater communities, predominantly through trophic interactions (Nystrom *et al.*, 1996; Dorn & Wojdak, 2004). Individual adult *A. pallipes* have been shown to consume over 50 freshwater shrimps (*Gammarus pulex*) in a 24 h period (Haddaway *et al.*, 2012) and in lake enclosures caused reductions in the biomass of *Chara* sp., *Gammarus* sp. and Chironomidae larvae when stocked at densities of 2 crayfish m^{-2} and higher. Research presented in Chapter Four of this thesis investigated experimentally the impacts of *A. pallipes* on the littoral community, with crayfish influence apparent across all the macro-invertebrate taxa included, particularly slow moving isopods and gastropods.

Table 3.5 BMWP score categories (BMWP, 1978)

BMWP Score	Category	Interpretation
0 – 10	Very poor	Heavily polluted
11 – 40	Poor	Polluted or impacted
41 – 70	Moderate	Moderately impacted
71 – 100	Good	Clean but slightly impacted
> 100	Very good	Unpolluted / unimpacted

Of the 3 key orders identified within the PSYM method, only Coleoptera (beetles) (predominantly adults, and therefore able to fly) were detected in all 3 ponds. Adult beetles, along with true bugs (Heteroptera) tend to be fairly early colonisers of new waterbodies whereas the larger dragonflies and damselflies (Odonata) arrive later (Danell & Sjoberg, 1982). The presence of high scoring taxa such as caddisfly and mayfly larvae concurs with the water chemistry findings as these species are indicative of good water quality. It is not clear why colonisation appears to be occurring at a slower rate in Pond 2, than Pond 3 as the ponds are equidistant to the more species-rich Pond 1, a probable source of colonisers. Crayfish are present in Pond 2 and not Pond 3. Again, they are only at very low density, but the potential influence of crayfish on colonisation patterns cannot be discounted. The abundances of macro-invertebrates in both the newly created ponds were very low so it may be that these differences just reflect a lack of chance encounters with taxa during sampling in Pond 2. Molluscs were absent from all the ponds but would be expected to colonise over time because calcium levels are adequate to support them (Jay & Holdich, 1981). In a study of a newly created manmade lake in Sweden, Danell & Sjoberg (1982) did not detect snails until the 6th year, after which they increased rapidly. Pioneer pond communities comprise Ostracoda, Copepoda, Coleoptera and Diptera, followed by Ephemeroptera and Heteroptera, then taxa such as Amphipoda, Isopoda and Gastropoda (Danell & Sjoberg, 1982; Jeffries, 2011).

The non-native amphipod *Crangonyx pseudogracilis* was detected in 2 of the ponds whereas the native, and functionally equivalent, amphipod *Gammarus pulex* was absent from all ponds. *C. pseudogracilis* originates from North America and is now widespread across the UK, though potential impacts on invaded communities and habitats are low (GB Non-Native Species Secretariat, 2011) and it does coexist with native *G. pulex* (MacNeil *et al.*, 1999). Likely vectors of introduction are the macrophytes planted within the restoration scheme or birds; *Crangonyx* sp. are frequently carried in bird feathers (Swanson, 1984).

People may also act as vectors; however biosecurity measures at the Ark site such as compulsory boot-washing and restricted public access render this unlikely.

3.7.3 Zooplankton

Ostracods are commonly found in abundance in freshwaters and dominated the metazoic zooplankton community at Threshfield. Cladocerans and cyclapoid copepods were also found in all the ponds and are often among the early colonists of newly created waterbodies due to their high dispersal capability via wind and animal vectors (Rundle *et al.*, 2002; Louette & De Meester, 2005; Frisch & Green, 2007; Frisch *et al.*, 2012). Zooplankton densities were relatively low, which likely reflects both the young age of the ponds and low nutrient status. Zooplankton would be expected to increase in diversity and abundance over time as macrophyte and phytoplankton communities develop and allochthonous inputs increase.

3.7.4 Crayfish

The low stocking densities of the Threshfield Ark site present a particular challenge for post-translocation monitoring of the crayfish population. It is encouraging that one adult was trapped in Pond 1 a year after translocation as this provides an indication of potential suitability of conditions at the site for *A. pallipes*. Assuming that all donor crayfish were persisting in the Ark site on all monitoring occasions, trapping results to date indicate a trap rate of 1.19×10^{-3} % of trappable population per trap night for Pond 1 and 0% for Pond 2, lower than the 3×10^{-3} % of trappable population per trap night reported by Peay (2004) in a canal study. This difference likely reflects the very low population density in the Threshfield Ark site, with few individuals potentially spread over large areas (up to 330 m^2 per pond) of suitable habitat and very low trapping frequency (once per annum), thereby not allowing crayfish to acclimate to the baited area, or the bait. The baited camera did not detect crayfish; however water clarity was sufficiently good to enable observation had any been drawn to the bait, hence this lack of detection is again likely to have resulted from the low population densities. The system was nevertheless useful in detecting other species such as Amphibia, and was easy to deploy in the margins from the bankside, though deeper deployment would require a boat. Further trials of the system in a range of conditions, including sites with greater macrophyte growth and variable water clarity, are necessary to fully assess the value of this method for monitoring crayfish in deep water sites.

The establishment of new populations from a small donor stock, as in the case of Ark sites, brings inherent risk of negative allee effects associated with low genetic variation, such as reduced fitness, increased susceptibility to parasites and disease, and reduced ability to respond to long-term environmental change (Frankham, 2002; Hale & Briskie, 2007). The current size of the donor population comprising 17 individuals is clearly undesirably small, particularly as crayfish are split between the two ponds. Their potential distribution over large areas reduces the likelihood of encounters, and therefore mating opportunities. Even if the current donor population does successfully breed, the likelihood of an inbreeding depression is high with such small population sizes (Hedrick & Kalinowski, 2000). Predicting likely outcomes for an introduced population, preferably through quantitative modelling, is recommended within IUCN guidelines (IUCN, 2012). Population Viability Analysis (PVA) uses stochastic simulation models to predict likely outcomes based on population structure and a series of life history parameters such as mortality rates and fecundity (Shaffer *et al.*, 2002). The population consequences of a range of scenarios, e.g. drought and disease, can also be modelled to assess longer term vulnerability of a population to environmental change (Possingham *et al.*, 1993). In a PVA of a noble crayfish (*Astacus astacus*) population in a German stream, Meyer *et al.* (2007) identified spawning probability, followed by juvenile and adult mortality rates, as the most important parameters for population survival. Translocation of further donor stock should therefore be a management priority.

3.8 Conclusion

Monitoring of the Threshfield Quarry ark site during the first 3 years of creation revealed substantial flutuations in water chemistry and macro-invertebrate diversity and abundances in the three waterbodies present. The last sampling event in Autumn 2012 indicated that the ponds had not yet reached chemical steady state or biological equilibrium, and that there has been only limited macrophyte establishment. Water chemistry of the ponds was within the tolerance limits of *A. pallipes* and the physical habitat and remote location of the quarry offer represent conditions that will favour ark site success. The restoration approach taken at Threshfield Quarry has entailed minimal intervention; instead, favouring natural colonisation processes. However, considering the short time scales of the project i.e. pond creation/restoration and crayfish translocation within the same year, greater management interventions are considered necessary to enhance the natural restoration. Findings suggest that a longer restoration phase is necessary before crayfish translocation. In view of this, greater efforts should be undertaken to identify potential future quarry ark sites as soon as possible, so that restoration can begin at least 4 years before translocation of donor stock.

A summary of findings and management recommendations arising from the current study are presented in Table 3.6. It is hoped that introducing more donor stock , optimising crayfish habitat and continuing monitoring into the long-term will heighten the chances of establishing a successful breeding population at the site, facilitate the conservation of other imminently threatened crayfish populations, and contribute to realising the potential of this newly restored site for the wider goal of nature conservation.

Table 3.6 Summary of key findings and management recommendations for Threshfield Quarry ark site, Yorkshire

	Findings	**Recommendations**
Water chemistry		
	• All measured parameters were within recommended tolerance limits for white-clawed crayfish • Concentrations fluctuated markedly over measurement period (2009-2012), reflecting stabilisation of the newly created water bodies	• Waterbodies have not yet reached chemical steady state, therefore it would be prudent to delay translocation if feasible • Biannual monitoring should continue into the future as part of a long-term monitoring strategy
Physical habitat structure		
	• Rocky substrate in all three ponds provides plentiful refugia for crayfish • Establishment of macrophyte beds has been slow, limiting habitat heterogeneity	• Macrophyte colonisation and establishment should be supplemented by additional planting if translocation occurs within 3 years of ark site creation.
Crayfish food resources		
	• Diversity and abundance of macro-invertebrates were generally low and fluctuating, indicating that ponds have not yet reached biological equilibrium • The taxa present indicate ponds are still in the early stages of colonisation; later colonisers such as snails were not detected • The paucity of mature trees at the site limits allochthonous inputs • Productivity is low; likely reflecting nutrient limitation	• Crayfish may be food limited so supplementation of existing resources by the addition of leaf packs into ponds (both in the margins and deeper areas) is advisable; particularly if further donor stock are to be translocated in the short-term.
Crayfish population		
	• Crayfish abundance is currently very low due to limited availability of donor stock. This renders the population vulnerable to environmental change and reduced breeding success • Annual post-translocation monitoring using baited trapping detected one crayfish in the year following translocation. A baited camera system detected no crayfish but represents a feasible method to supplement trapping, with the potential to sample smaller size-classes	• Further translocation of donor stock should be a management priority; however habitat at the site should be optimised before this occurs to maximise chances of a establishing a self-sustaining population • Post-translocation monitoring is the only means to measure project success and therefore should continue beyond the five year period required by license. Greater sampling effort is advisable considering the current low crayfish numbers.

Chapter 4 Size-dependent impacts of the endangered white-clawed crayfish (*Austropotamobius pallipes,* Lereboullet) on the littoral community

4.1 Summary

The decline of Britain's only native crayfish (*Austropotamobius pallipes* (Lereboullet)) has prompted conservation-led translocations to safe isolated stillwaters (Ark sites). Many translocations represent introductions rather than re-stocking; hence it is important to ensure that there will not be a detrimental impact on communities already present. Current knowledge is insufficient to predict likely effects, although gut contents analyses suggest that *A. pallipes* is omnivorous and exhibits ontogenic diet shifts. A mesocosm study was conducted with the aim to first, determine community impacts of introducing *A. pallipes* on the benthic invertebrate community, and second investigate if impacts vary with crayfish life-stage. All crayfish life-stages strongly reduced shredder and grazer abundance, particularly thin-shelled *Lymnaea* snails. The small snail *Physa fontinalis* was preferentially consumed by juveniles, perhaps reflecting different prey-handling ability. Adults showed greater reliance on terrestrially derived detritus compared to juveniles. There were limited effects on other trophic levels, although by reducing shredders that process basal resources, crayfish impacts may be felt through the community in the long-term.

4.2 Introduction

Translocation of animals, whether it be movement of imminently threatened populations or reintroduction to restored habitats, is a widely used tool for the conservation of both terrestrial and aquatic species (Seddon *et al.*, 2007; Armstrong & Seddon, 2008). The endangered white-clawed crayfish (*Austropotamobius pallipes* (Lereboullet)) has declined across its range since the introduction of the invasive signal crayfish (*Pacifasticus leniusculus*) to Europe in the 1960s (Lewis & Holdich, 2002). *P. leniusculus* outcompetes *A. pallipes* for refugia (Dunn *et al.*, 2009) and is a vector of *Aphanomyces astaci*, the cause of 'crayfish plague', which is fatal to *A. pallipes* (Alderman *et al.*, 1990; Peay & Rogers, 1998). In the absence of effective means to control the spread of invasive crayfish or plague, translocation of imminently threatened populations of *A. pallipes* to isolated locations (Ark sites) has become a key part of current conservation strategy for the species (Kozák *et al.*, 2011).

Two of the five most important criteria for the selection of recipient Ark sites are absence of non-native crayfish and low risk of future invasion (Peay, 2009). Isolated waterbodies, such as natural lakes and restored quarries or gravel pits, represent the most biosecure sites available and the majority do not hold existing crayfish populations (Kindemba *et al.*, 2009). Translocations to Ark sites therefore commonly represent introductions, i.e. attempts to establish a species outside its recorded distribution. Recommendations from the International Union for Conservation of Nature (IUCN) state that such introductions are a feasible conservation tool, though assessment of likely impacts on the existing ecosystem is necessary to avoid unforeseen ecological consequences (IUCN, 2012).

Introduced species have potential to impact the existing ecology, for example through predation, habitat modification or release of pathogens (Conant, 1988; Hodder & Bullock, 1997), and the need to exercise caution during translocations is frequently voiced (e.g. Hodder & Bullock, 1997; Carter & Newbery, 2004). Crayfish are opportunistic omnivores, yet selective consumers; hence community impacts are predominantly mediated through dietary choices, causing direct and indirect effects at a range of trophic levels (Nystrom *et al.*, 1999; Usio & Townsend, 2002; Dorn & Wojdak, 2004). Presence of crayfish of *Astacus* sp. has been associated with reduction in submerged macrophytes (Nystrom & Strand, 1996; Nystrom *et al.*, 1999), reduced biomass of grazers, particularly gastropods, and shifts in community composition towards predatory invertebrates (Nystrom *et al.*, 1999). Some crayfish species are also significant bioturbators, modifying sediment transport and increasing turbidity (Harvey *et al.*, 2011; Johnson *et al.*, 2011), with indirect effects on algae and macrophyte cover (Dorn & Wojdak, 2004; Usio *et al.*, 2009). Palatability and preference tests suggest dietary choices of crayfish are dependent on nutritional content (Adams *et al.*, 2005), past experience (Gherardi & Barbaresi, 2007), and ease of capture (Brown, 1998); while field-based foraging patterns reflect a complex balance between food preference and competition (Gherardi *et al.*, 2001).

The diet of *A. pallipes*, examined indirectly through gut content analyses of field-caught specimens or through laboratory preference and predation tests, indicates generalist omnivory with a wide range of food types including terrestrially derived detritus, algae, molluscs, isopods, fish and fish eggs, amphipods and insect larvae (Gherardi *et al.*, 2001; Reynolds & O'Keeffe, 2005; Scalici & Gibertini, 2007). Gut analysis also provides evidence of an ontogenic diet shift in *A. pallipes*, as evidenced for other crayfish species e.g. *P. leniusculus* (Guan & Wiles, 1998) and *Procambarus clarkii* (Correia, 2003), with juvenile diet comprising a greater proportion of micro-crustaceans, whereas adults consume more vegetal items such as macrophytes, woody fragments and aufwuchs

(Reynolds & O'Keeffe, 2005). In view of this, it may be predicted that crayfish impacts on existing communities will vary with population structure. The influence of *A. pallipes* on the littoral community has been previously investigated by Matthews *et al.* (1993) who found a reduction in *Chara*, Chironomidae, *Gammarus* and Hemiptera biomass when *A. pallipes* were reintroduced to enclosures in a mesotrophic marl lake system; though the size range of crayfish studied was not indicated. A field-based mesocosm experiment was used to assess the short-term impacts of *A. pallipes* introduction on the benthic invertebrate community, and to test the hypothesis that impacts differ between juvenile and adult life-stages of crayfish.

4.3 Materials and methods

The experiment was conducted August-September 2010 using outdoor mesocosm pools (n = 12) to mimic lentic Ark sites. Mesocosms overcome some of the limitations of gut contents analyses such as providing only a snapshot of diet at the moment of capture, and bias due to over representation of less digestible diet items (Correia, 2003). Pools were seeded with identical communities of plants, detritus and invertebrates to reflect flora and fauna which are widespread in the UK. Individuals of *A. pallipes* were introduced to the pools at the start of the experiment within three treatments: juvenile crayfish, adult crayfish, mixed juvenile and adult crayfish and a no crayfish control, using a randomised-block design with 3 replicates in each. The experiment lasted 5 weeks after which all the remaining invertebrates in each pool were recovered and counted, and the macrophytes and detritus were weighed.

4.3.1 Mesocosm set-up

Plastic pools (0.78 m^2, 1 m diameter, 0.65 m depth) were sunk into a meadow at the University of Leeds Field Research Unit, UK. The rim of each pool was positioned 15 cm above ground level to prevent accidental drowning by insects and small mammals. Pools were filled to 35 cm depth with groundwater and seeded with additional lake water (3 L) to facilitate the development of phytoplankton and zooplankton communities. Lids were placed over the pools, each with a central circular opening (50% area) to enable light penetration yet limit extreme temperature fluctuations. Netting (20 mm mesh) was secured over the opening to prevent both crayfish escaping and predation by birds, and to reduce leaf litter inputs.

Macrophytes, *Ceratophyllum demersum* (65 g wet mass), *Elodea nuttallii* (65 g) and *Potamogeton natans* (25 g) were added to the pools on 5th August. Stems were secured within 12 shallow circular trays (300 mm diameter, 40 mm depth) filled with 60:40 mix of pure sand and dried loam soil. These were placed in the centre of each pool and covered with a layer of gravel. A slope of gravel extended approximately 10 cm beyond the margin of the tray to the floor of the pool, thereby enabling crayfish access to the plants. Allochthonous detritus (100 g wet mass), as leaves of beech (~80%) and alder (~20%) soaked for over 2 months, was also added to each pool.

Pools were left to stabilise for 3 weeks before stocking the invertebrates and crayfish. The following macro-invertebrates were added to each pool: 6 *Lymnaea stagnalis* (20 – 30 mm, total length (TL)), 10 *Planorbarius corneus* (15 – 22 mm, TL), 60 *Physa fontinalis* (6 – 9 mm, TL), 8 *Bithynia tentaculata* (9 – 14 mm, TL); 57 *Gammarus pulex* (9 –16 mm, TL), 52 *Asellus aquaticus* (10 –14 mm, TL) and 80 chironomid larvae. All were collected from still or slow-flowing waters nearby and densities within the pools approximately reflected those found in the source sites.

Crayfish were collected from the Aire catchment, Yorkshire (under license from Natural England), held within the laboratory for at least one week under 16:8 light/dark regime at 16°C and fed crab pellets (Hinari) *ad libitum*, before being placed in the pools. All crayfish were numbered on the cephalothorax using non-toxic correction fluid to enable recognition of individuals, then weighed (wet weight) and measured (carapace length, CL). Eight sections of PVC pipe (2 x 50 mm diameter, 120 mm length; 6 x 25 mm diameter, 80 mm length) were also added to serve as refugia, with a view to reducing aggressive interactions and post-moult cannibalism.

4.3.2 Experimental design

Crayfish were assigned to the three treatments based primarily on mass, with the aim to reduce variation in total mass between treatments. Juvenile treatment comprised 6 juveniles (2.1 – 5.5 g, 16.1 – 24.2 mm CL), adult treatment comprised 2 adults (12 – 22.7 g, 31.6 – 38.9 mm CL), and mixed treatment comprised 1 adult (12.1 – 16.1 g, 33.2 – 36.1 mm CL) and 4 juveniles (1.87 – 2.78 g, 17.5-20.4 mm CL). Crayfish densities in the pools ranged from 2.56 to 9 individuals m^{-2}, within the density range 0.1 to 10 individuals m^{-2} previously reported for lake populations (Mees, 1983; Reynolds & Matthews, 1993; Reynolds *et al.*, 2000). Mean total mass of crayfish did not vary significantly between pools within the same treatment (23.4 ± 3.8 g S.D., F= 2.8, p= 0.09 (juveniles); 33.7 ± 3.5 g S.D., F= 0.223, p=0.81 (adults); 21.8 ± 1.9 g S.D., F= 2.24, p=0.98 (mixed), ANOVA). The limited availability of study animals meant sex ratios could not be fully balanced between treatments and that due to the size range of individuals collected it was not possible to fully balance mass between treatments. The anticipated higher consumption level among adults compared to juveniles meant it was not appropriate to balance densities; however crayfish encounters is likely to be more frequent in the higher density treatments, potentially leading to interference competition (Gherardi & Cioni, 2004), which was not controlled for in the experimental design. With regards sex ratios, while there is some evidence for sex-dependent feeding patterns for crayfish in the wild (Gherardi *et al.*, 2004), most studies report no dietary differences due to sex (e.g. Renai & Gherardi, 2004; Reynolds & O'Keeffe, 2005; Stenroth *et al.*, 2008).

Pools were checked weekly for crayfish mortalities and evidence of moult. In the event of mortality, crayfish were replaced with equivalent sized individuals. In the event of ecdysis, moults were removed and identification numbers repainted on post-moult individuals once the carapace had hardened. Water temperature and dissolved oxygen were measured in each pool at weekly intervals during mid-afternoon and sub-surface water samples were also collected at the end of the experiment for subsequent laboratory analyses of nitrate, phosphate, ammonium, calcium, potassium, magnesium and sulphate.

Pools were emptied at the end of the experiment. Macrophytes were removed, blotted dry and reweighed, and all water and sediment were passed through a net of mesh 1 mm to recover remaining invertebrates and detritus. Invertebrates and detrital fragments were separated from the gravel by hand sorting and then identified to species and counted. Detrital fragments exceeding approximately 4 mm diameter were collected and weighed.

4.3.3 Statistical analysis

An independent samples median test was used to test for a treatment effect on the overall change in crayfish mass, and for a difference in percentage mass change between juveniles and adults. Data of measured water chemistry parameters were tested for normality (Shapiro-Wilk test) and one way ANOVA was used to test for differences between treatments for each parameter separately. Kruskal-Wallis independent samples test with .05 significance level were used for data where the assumptions of one way ANOVA were not met. Treatment effects on the remaining biomass of macrophytes and absolute abundances of macro-invertebrate taxa were tested using one way ANOVA with treatment as a factor. All data were tested for normality using a Shapiro-Wilk test and were log10+1 transformed where necessary. Levene’s test was used to determine compliance with the assumption of homogeneity of variance between groups. Tukey’s HSD post-hoc test was use to identify differences between groups if there was a significant overall treatment effect. The Shannon diversity (H) and evenness (E_H) indices were calculated for the macro-invertebrate communities present in the pools after 5 weeks and compared across treatments using Kruskal-Wallis independent samples test with 0.05 significance level. All analyses were performed using PASW Statistics 18 (IBM).

4.4 Results

4.4.1 Water chemistry

Water temperature in the pools varied from 14 to 17˚C over the study period and there was no significant temperature difference between treatments ($F_{3,56} = 0.48$, $p = 0.70$). Dissolved oxygen saturation ranged from 89 to 116 % at the weekly checks, and did not differ significantly between treatments ($F_{3,56} = 0.11$, $p = 0.96$). Treatments did not differ for any other water chemistry parameters measured (Table 4.1).

Table 4.1 Mean (± S.D.) water chemistry parameters of pools after 5 weeks under 3 crayfish treatments (juveniles only (J); Adults only (A); and mix of juveniles and adults (M), and control (C) with no crayfish, and results of one-way ANOVA or Kruskal Wallis tests for differences between treatments.

Treatment	**Nitrate (mg L^{-1})**	**Sulphate (mg L^{-1})**	**Phosphate (mg L^{-1})**	**Sodium (mg L^{-1})**	**Potassium (mg L^{-1})**	**Chloride (mg L^{-1})**	**Magnesium (mg L^{-1})**	**Calcium (mg L^{-1})**
Juveniles (J)	50.72 (±2.69)	64.15 (±2.83)	0.031 (±0.020)	13.07 (±0.30)	1.66 (±1.22)	37.18 (±1.42)	23.43 (±2.10)	52.95 (±3.42)
Adults (A)	43.84 (±6.60)	59.97 (±6.82)	0.025 (±0.005)	12.29 (±3.22)	1.64 (±0.46)	33.37 (±5.70)	21.77 (±2.96)	45.23 (±8.39)
Mixed (M)	48.05 (±15.76)	59.59 (±11.23)	0.025 (±0.013)	11.02 (±3.70)	1.60 (±1.05)	32.51 (±9.89)	22.54 (±6.35)	47.57 (±8.43)
Control (C)	42.7 (±8.20)	59.57 (±2.83)	0.01 (±0.007)	12.78 (±1.70)	1.74 (±0.63)	33.92 (±3.14)	22.30 (±1.84)	47.51 (±10.22)
ANOVA /Kruskal Wallis results	$F_{3,8}$=0.45 p = 0.73	H_3= 1.51 p = 0.68	$F_{3,8}$=1.4 p = 0.31	H_3=0.74 p = 0.86	H_3= 0.53 p = 0.91	H_3= 1.51 p = 0.68	$F_{3,8}$=0.10 p = 0.96	H_3= 1.56 p = 0.67

4.4.2 Crayfish

Two juvenile crayfish were found dead on day 28 of the experiment, both in small treatment pools, and one was partially consumed. Both were removed and replaced with crayfish of similar mass. Four individuals, all juveniles, successfully moulted during the 35 day study period. Change in total crayfish biomass was highly variable between pools within the same treatment. Mean total mass increase per pool was 5.51 ± 7.8 g (20.4%), 1.21 ± 1.3 g (5.8%) and 2.34 ± 1.5 g (7.3%) for the juvenile, mixed and adult treatments respectively, with no treatment effect (T_2=4.56, p=0.10, Independent samples median test); however juvenile crayfish experienced a significantly higher percentage change in mass compared to adults, irrespective of treatment (T_1=7.26, p<0.05, Independent samples median test).

4.4.3 Macrophytes and macro-invertebrates

There was no treatment effect on the biomass of the 3 macrophyte species, however the mass of detrital leaves was significantly reduced relative to control in both treatments where adults were present (mean overall reduction of 70 and 74% in adult and mixed treatments, respectively), though not when only juveniles were present (mean 63% reduction) (F3,8 = 10.78, p<0.01) (Table 4.2). Detrital leaves in the control treatment were reduced on average by 53% (Figure 4.1).

Table 4.2 One way ANOVA and Tukey's HSD post-hoc test comparing the biomass of macrophytes and abundances of invertebrate taxa remaining in pools after 5 weeks under 3 crayfish treatments (juveniles only (J); Adults only (A); and mix of juveniles and adults (M), and control (C) with no crayfish

	Treatment		**J vs. A**	**J vs.**	**A vs.**	**J vs. C**	**M vs.**	**A vs. C**
	$F_{3,8}$	p	p	p	p	p	p	p
Macrophyte biomass								
Potamogeton natans	2.23	0.16	-	-	-	-	-	-
Ceratophyllum demersum	1.52	0.28	-	-	-	-	-	-
Elodea nuttallii	3.87	0.06	-	-	-	-	-	-
Detrital leaves	10.78	**<0.01**	0.30	0.09	0.80	0.14	**<0.01**	**<0.01**
Grazers								
Bithynia tentaculata	2.149	0.17	-	-	-	-	-	-
Physa fontinalis	20.03	**<0.01**	**<0.05**	0.74	**<0.01**	**<0.01**	**<0.01**	0.50
Planorbarius corneus	17.37	**<0.01**	0.11	0.59	0.58	**<0.05**	**<0.01**	**<0.01**
Lymnaea sp.	11.62	**<0.01**	0.78	0.89	0.40	**<0.01**	**<0.05**	**<0.01**
Shredders								
Gammarus pulex	4.22	**<0.05**	0.97	0.79	0.95	0.16	**<0.05**	0.09
Asellus aquaticus	32.24	**<0.01**	0.96	0.98	0.99	**<0.01**	**<0.01**	**<0.01**
Filterers/collectors								
Chironomid larvae	10.18	**<0.01**	0.99	0.72	0.55	**<0.05**	**<0.01**	**<0.05**

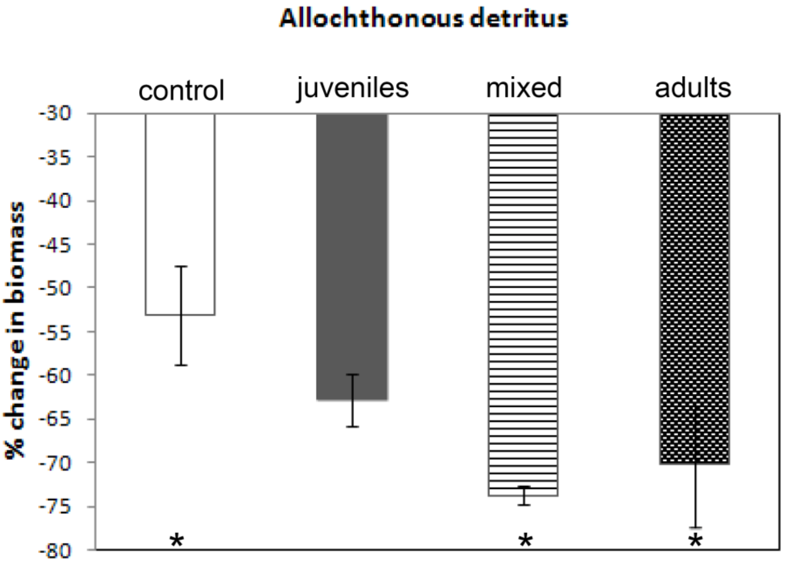


Figure 4.1 Percentage change (± S.D.) in mass of allochthonous detritus over 5 weeks in mesocosms under 3 crayfish treatments: juveniles; mixed (juveniles and adults), and adults only, and with no crayfish (control). * indicates treatment is significantly different from at least one other, or control

With the exception of the gastropod *B. tentaculata*, there was a treatment effect on the abundances of all benthic invertebrates at the end of the 35 day period (Table 4.2). Of the grazers, abundance of the small gastropod *P. fontinalis* was significantly lower in the juvenile and mixed treatments, relative to the adult treatment ($p<0.05$ and $p<0.01$, respectively), but there was no difference in abundance between adult treatment and control ($p = 0.50$) (Table 4.2) (Figure 4.2). Mean abundance of *B. tentaculata* increased in all treatments by an average of 40%; these were all comprised of juveniles. For the other gastropod species (*P. corneus* and *Lymnaea* sp.) there was no difference in remaining abundances between the different crayfish treatments (Table 4.2).

The shredder *Asellus aquaticus* was significantly reduced in all treatments relative to control ($F_{3,8} = 32.24$, $p <0.01$), whereas *Gammarus pulex* was only reduced in the mixed treatment relative to control ($F_{3,8} = 4.2$, $p < 0.05$) (Figure 4.2). There was no difference in shredder abundances between crayfish treatments (Table 4.2). The abundance of chironomid larvae was significantly reduced in all crayfish treatments relative to the control ($F_{3,8} = 10.18$, $p < 0.01$), with no differences between crayfish treatments (Table 4.2) (Figure 4.2).

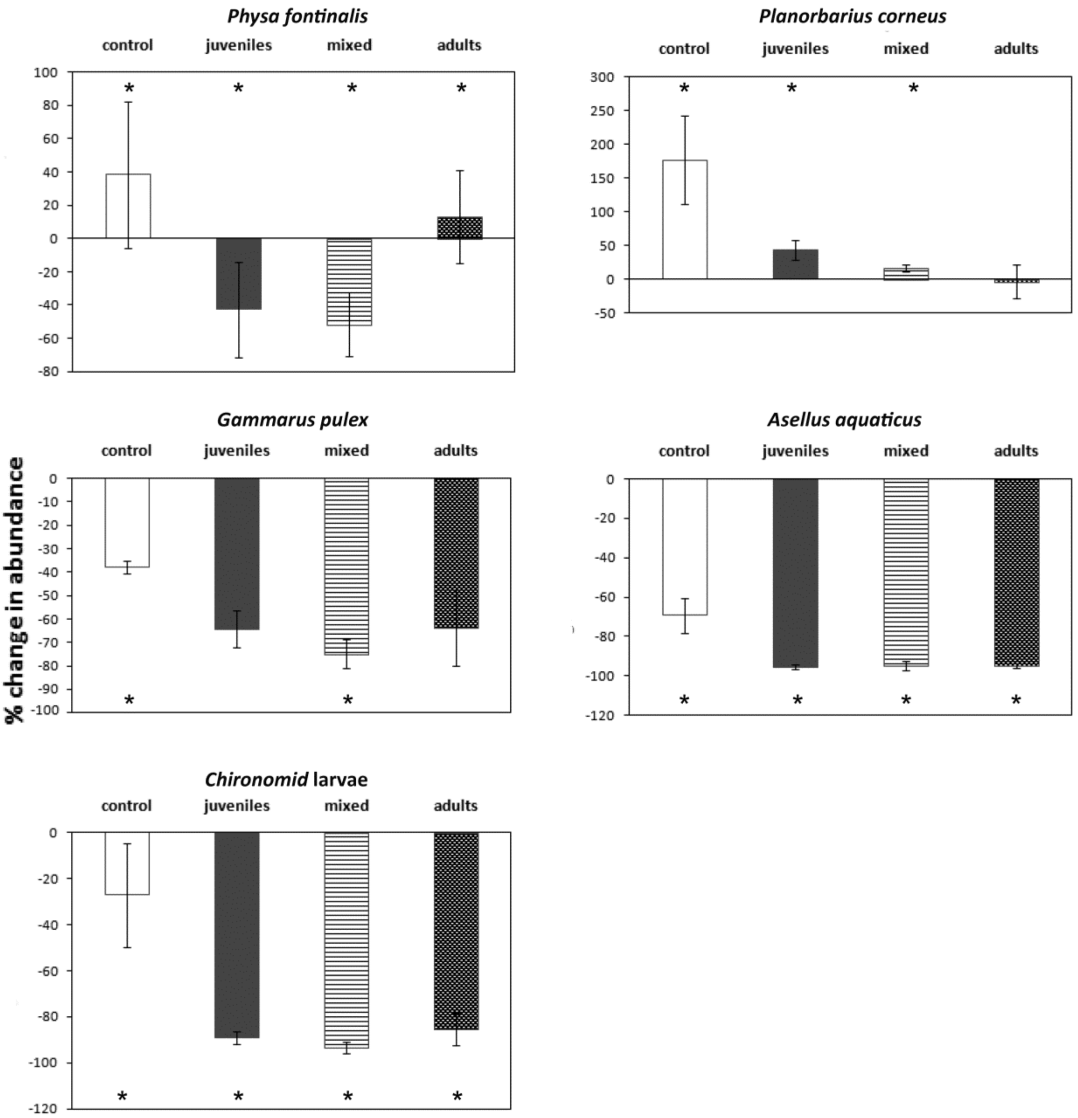


Figure 4.2 Percentage change (± S.D.) in abundance of grazers (gastropods *Physa fontinalis* and *Planorbarius corneus*), shredders (*Asellus aquaticus* and *Gammarus pulex*) and filterers (chironomid larvae) over 5 weeks in mesocosms under 3 crayfish treatments: juveniles; mixed (juveniles and adults), and adults only, and with no crayfish (control). * indicates treatment is significantly different from at least one other, or control

Several other species colonised the pools during the experiment including *Callicorixa praeusta* and damselfly larvae *(Coenagrion* sp.*)*; however they occurred only at low abundances with less than 4 individuals per pool.

The Shannon diversity index in each pool ranged from 0.87 to 1.44, and did not vary between treatments ($H_{3,12}$ = 7.78, p = 0.05). Shannon evenness ranged from 0.24 to 0.49, and again did not vary between treatments ($H_{3,12}$ =6.08, p = 0.11).

4.5 Discussion

Presence of the crayfish *A. pallipes* had a strong influence on the abundances of benthic invertebrates and on the biomass of detritus in the littoral community over the short-term. However, their presence did not have an influence on the macrophytes. Ontogenic differences were apparent in a species shift among grazers.

The three macrophyte species present within the pools did not appear to be important food items for either adults or juveniles of *A. pallipes,* although terrestrially derived detritus was reduced in the presence of adult crayfish. Both *G. pulex* and *A. aquaticus* function as shredders processing these basal resources within freshwater systems; *A. aquaticus* was significantly reduced in all crayfish treatments, and *G. pulex* in the mixed treatment, which infers that reduction in detrital leaves was predominantly due to direct consumption by crayfish. Through removal of the shredders and direct consumption of this basal resource, the crayfish potentially increase the efficiency of energy transfer of the food web (Wetzel, 1995; Nilsson *et al.*, 2012). The size of this effect depends on how much of this energy is incorporated into crayfish body tissue; work on another crayfish *Paranephrops planifrons* suggests detritus contributes little to crayfish growth (Parkyn *et al.*, 2001). Previous results of gut content analysis of *A. pallipes* similarly infer greater consumption of detritus among adults (Reynolds & O'Keeffe, 2005) which may be explained by lower energy demands for growth within the adult life-stage compared to juveniles (Whitledge & Rabeni, 1997b); indeed, juvenile crayfish showed a threefold higher increase in mass than adults during the current study. However, adults have been shown to adopt a more protein rich diet comprising a greater proportion of macro-invertebrates during times of high energy demand (e.g. breeding) (Stenroth *et al.,* 2008).

In a study of *P. leniusculus,* Nyström *et al.* (1996) concluded that the activity of a prey type is the most important determinant on their consumption by crayfish, with a shift towards a community dominated by sediment dwelling taxa in the presence of crayfish. There was a general trend towards greater reduction of *A. aquaticus* than *G. pulex* in crayfish treatments, which may reflects the ease of capturing this slow moving isopod (Nystrom *et al.*, 1996). However, *A. aquaticus* was also greatly reduced in the controls, which may be a consequence of predation by other invertebrates such as *G. pulex* (MacNeil *et al.*, 1997). The reduction of *G. pulex* in the control pools, though to a lesser degree than *A. aquaticus*, may reflect post-moult cannibalism (Dick, 1995), or reduced survival due to unfavourable conditions (e.g. food or shelter). The high predation level of sediment-dwelling chironomid larvae in the crayfish treatments (81 to 87%) is likely to be higher than in natural environments due to the relatively shallow depth of sediment in the pools, and therefore less opportunity to escape predation.

As slow moving species, snails are highly vulnerable to crayfish predation (Lodge *et al.*, 1994; Nystrom *et al.*, 1996). This was apparent in the current study, with the exception of *B. tentaculata* which increased in abundance, presumably as a consequence of reproduction by adults, or perhaps hatching of eggs present on plants at the start of the experiment. It is suggested that the presence of the operculum and thicker shell of this species makes it less preferable to crayfish compared to the thinner-shelled species such as *Lymnaea* spp. (Brown, 1998; Nystrom, 1999). Predation upon *P. fontinalis* was dependent upon crayfish size, with greater consumption among juveniles. One possible explanation for this may be the differential ability of adults and juveniles to handle these small snails. Reynolds and O' Keeffe (2005) supposed that the size of *A. pallipes* is inversely related to motility of its prey, yet the current study suggests that physical size of the prey is also important. This finding highlights that once crayfish are introduced to an Ark site, the influence on specific species such as the shift towards predation resistant gastropods, is likely to change as the crayfish population structure changes from that dominated by the adult donor stock (Souty-Grosset & Reynolds, 2009), to greater representation by the smallest size classes.

Over the 5 week period of study, a large reduction in the abundance of shredders was observed. Although it is acknowledged that the use of mesocosms may lead to overestimation of predation rates due to more homogenous habitat than in the wild (Nilsson *et al.*, 2008), relatively high predator densities in the first instance and reduced chance of colonisation from nearby habitats, the changes observed, even if at lower magnitude, are likely to have consequences for the structure and functioning of the ecosystem. The predatory impact on shredders may lead to a change in the processing of basal resources, with implications for nutrient cycling and therefore availability to primary producers (Vanni, 2002). However, the current study also highlighted the role of crayfish, particularly adults, in processing basal resources, which may compensate for the loss of shredders in this regard. This could potential increase the efficiency of energy transfer to higher levels by reducing the number of steps in the food chain (Wetzel, 1995); although a reduction in smaller shredders will likely have knock on effects on other higher order predators that compete with crayfish, for example, fish.

Chapter 5 Branchiobdellidan infestation on endangered white-clawed crayfish (*Austropotamobius pallipes*) in the UK

5.1 Summary

Branchiobdellidans or crayfish worms are clitellate annelids and ectosymbionts of freshwater crayfish. An investigation of branchiobdellidan infestation was undertaken in a population of endangered white-clawed crayfish (*Austropotamobius pallipes*) in the River Aire, UK. Thirty two percent of animals were infested either by the adult parasite or their cocoons (n = 107). Parasite burden increased with host size, but did not differ with sex. Observations of crayfish gill tissue revealed a strong positive relationship between melanisation of filaments and parasite prevalence and burden. Taxonomic identification revealed that one species of branchiobdellidan was present, *Branchiobdella astaci*. The first sequences were generated for this species and phylogenetically analysed alongside published sequences for 5 other branchiobdellidan species in Europe. The position of *B. astaci* within the genus *Branchiobdella* was confirmed, and it was found to cluster as a sister group to *B. parasita*.

5.2 Introduction

Movement of threatened and endangered species for the purpose of recolonisation of native habitats can have unintended consequences. In particular, communities of parasitic organisms may also be transported unintentionally along with their hosts (Cunningham, 1996; Van Oosterhout *et al.*, 2007). Threatened populations of the endangered white clawed crayfish *Austropotamobius pallipes* are currently being translocated within Europe to establish new protected populations of the species (Schulz *et al.*, 2002). Branchiobdellid parasites were recently detected in a population of *A. pallipes* in the UK. To evaluate the infestation and potential impact of branchiobdellidan worms on *A. pallipes*, the prevalence, intensity and associated pathology was assessed within a UK population.

Branchiobdellidans are clitellate annelids that live ectosymbiotically, either on the outer carapace or in the branchial chamber, of freshwater crustaceans; almost always astacoid crayfishes (Gelder & Brinkhurst, 1990). They are widespread with over 150 species within 21 genera from three continents (Gelder, 1996). Despite numerous studies of branchiobdellidan occurrence in continental Europe (Gelder *et al.*, 1994; Mori *et al.*, 2001; Klobucar *et al.*, 2006; Fureder *et al.*, 2009), there exist only two previous reports for the UK and no study of prevalence or impacts on the host (Leeke & Price, 1965; Rogers *et al.*, 2003).

All endemic European branchiobdellidans are of the genus *Branchiobdella* (Vogt, 1999). Sympatry is widely reported, with up to 6 species on an individual host, and variable host specificity (Holt, 1976). For example, *B. balanica* has only been found associated with noble crayfish *Astacus astacus* whereas *B. pentodonta* has been found on *A. astacus*, *Austropotamobius torrentium* and *A. pallipes* (Klobucar *et al.*, 2006; Fureder *et al.*, 2009). A study from Croatia found very high diversity (*B. italica, B. parasita, B. astaci, B. hexodonta, B. pentodonta* and *B. balanica*), and frequency with worms present in 58.75% of *Austropotamobius pallipes, Austropotamobius torrentium* and *Astacus astacus* populations studied (Klobucar *et al.,* 2006).

In the UK only one native crayfish species, the white-clawed crayfish, *Austropotamobius pallipes* Lereboullet, is present. In 1964 populations of *A. pallipes* in the River Kennet and Holy Brook were found infested with *Branchiobdella astaci* Odier (Leeke & Price, 1965). The next report was 33 years later when a single worm was discovered on a white-clawed crayfish in the River Ouse, Yorkshire (Rogers *et al.*, 2003). There are no other reports of branchiobdellidans in the UK.

Branchiobdellidans attach themselves to the host using duo-gland attachment organs on the anterior and posterior segments (Brinkhurst, 1999). Impacts of infestation on the host are little studied and appear to vary between branchiobdellid species. They are generally considered commensals grazing on epibionts on the crayfish exoskeleton (Jennings & Gelder, 1979); however there is evidence of mutualism and parasitism in some species. Brown *et al.* (2002) found that the presence of branchiobdellidans, *Cambarincola* spp., increased growth and survival in crayfish host *Cambarus chasmodactylus,* purporting a possible cleaning symbiosis. Conversely, tracer experiments with the gill infesting species *Branchiobdella hexodonta* showed that the worm ingests host tissue (Grabda & Wierzbicka, 1969), and there is documented gill damage in the case of heavy infestations of both *B. hexodonta* and *B. astaci*, suggestive of a parasitic effect (Vogt, 1999).

Populations of *A. pallipes* have severely declined across its range since the 1980s, and it is now IUCN red-listed ‘endangered’ and a UK BAP species. Further to pollution and habitat loss, its greatest threat is the spread of fatal ‘crayfish plague’ caused by *Aphanomyces astaci* (Holdich & Reeve, 1991). The invasive signal crayfish *Pacifastacus leniusculus* is a carrier of this oomycete, and since its introduction to Europe in the 1960s has spread rapidly. Besides plague, *A. pallipes* potentially suffers chronic losses and fitness impacts due to a number of parasites and diseases including *Psorospermium haeckeli,* fungal and bacterial 'burn spot disease', and the microsporidian parasite *Thelohania contejeani,* the causative agent of porcelain disease (Imhoff *et al.*, 2009; Longshaw, 2011).

In 2009 the author noticed a live juvenile branchiobdellidan on the cephalothorax of an *A. pallipes* host from the Aire catchment, Yorkshire, an area where white-clawed crayfish remain abundant. Branchiobdellidan prevalence and intensity was evaluated in this population in relation to sex and size of crayfish hosts. The potential presence of pathology in the gills of the host was also investigated. Worms were identified on the basis of morphological characteristics as no molecular sequence data were available for the species found. The first molecular data for the species was generated and a phylogeny was reconstructed based on mitochondrial CO-I sequences to determine its position within the genus *Branchiobdella*.

5.3 Materials and methods

A total of 107 specimens of *A. pallipes* were examined from collections made in 2009 and 2010 from Wyke Beck (NGR SE34133636, 53° 49' 20.93"N, 1° 28' 58.73"W), a 1st order stream within the Aire catchment, Yorkshire. This tributary contains only native crayfish, although the population is imminently threatened by signal crayfish which are present in two locations in the main river (West Yorkshire Ecological Records). The animals were primarily harvested under license from Natural England for other research which necessitated sacrifice and subsequent dissection; however this concurrently enabled detailed examination of individuals for the presence of branchiobdellidans. Crayfish were captured from a 160m stretch of river in September 2009 and October 2010 using hand-search during daylight hours with two people wading upstream and searching under all sizeable cobbles. Modified kick sampling and a drift net were employed to collect juveniles within root bundles. In 2011 a second population of *A. pallipes* within Adel beck, a watercourse 12km to the west (SE280400, 53°51'20.80"N, 1°34'29.91"W) was examined for branchiobdellidans using non-lethal methods. Seven specimens of *A. pallipes* were collected and immediately submerged in 1:1 solution of stream and carbonated water for 2 minutes (Gelder *et al.*, 1994). Immobilised branchiobdellidans on the carapace, along with those that had fallen into the sample pot, were then collected and the crayfish returned to the stream after a short recovery period.

Crayfish carapace length (CL) was measured in all individuals from the tip of the rostrum to the distal edge of the carapace, and sex and visible signs of injury or disease, including porcelain disease (thelohaniasis) caused by *Thelohania contejeani* recorded (Imhoff *et al.*, 2009). Crayfish were either killed and immediately dissected, or individually bagged and frozen for dissection at a later date. The carapace was inspected externally for branchiobdellidans before full examination of the branchial cavity. Branchiobdellidans found in crayfish that had not been frozen were usually alive and still attached to the host, thus enabling determination of their exact location on the host. Where no adult worms were found, the presence of cocoons (eggs and encapsulated larvae) (Figure 5.1) demonstrated that adult worms had been present (Gelder *et al.*, 2009). Branchiobdellidan lifecycles are poorly understood; however transmission of adult worms is thought to occur via host to host. Cocoons are subsequently laid on the surfaces of crayfish tissue, followed by the in situ development of juveniles (Longshaw, 2011). The number of worms and cocoons per host was counted and the percentage of melanised gill tissue within each podobranch visually estimated on a 5 point scale: 0= no visible sign of melanisation; 1 = <1%; 2=1-5%, 3=6-25%, 4=26-50%; 5= >50%. Branchiobdellidans were preserved in 95% ethanol and later mounted for identification using the taxonomic key by Gelder *et al.* (1994).

Figure 5.1 Portion of podobranch from crayfish *Austropotamobius pallipes* showing gill filaments with melanisation spots (a), melanised tips (b), and attachment of *Branchiobdella astaci* cocoons (c).

Genomic DNA was extracted from 9 adult branchiobdellid specimens, 7 from Wyke Beck and 2 from Adel Beck, using chelex 100 resin (50-100 mesh) (Sigma) and proteinase K method (Yue & Orban, 2005). Mitochondrial cytochrome *c* oxidase I (CO-I) sequences (560bp) were amplified from purified genomic DNA using the universal primers LCO1490: 5'-GGTCAACAAATCATAAAGATATTGG and HCO2198: 5'-TAAACTTCAGGGTGACCAAAAAATCA-3') (Folmer *et al.*, 1994) and a protocol modified from that of Gelder and Sidall (2001). Amplification reaction mixtures 31.5μL, comprised 10μL 5X Buffer,2.5mM Mg_2Cl, 0.28mM of each DNTP, 7μL of each primer (10μM), 2.5units GoTaq DNA Polymerase (Promega) and 3μL template DNA (50ng μL^{-1}) in a 50-μL total volume. The reaction mixtures were heated to 95˚C for 2 min and then cycled for 35 cycles at 94˚C for 20 s, 50˚C for 30 s and 68˚C for 60 s, with a final extension 72˚C for 60 s. PCR products were purified using QIAquick PCR Purification kit protocol (Qiagen). Automated sequencing of PCR products was carried out by GATC Biotech (Konstanz, Germany).

5.3.1 Phylogenetic analysis

Mitochondrial CO-I sequences from study specimens were analysed alongside all *Branchiobdella* sequences available via Genbank and 2 sequences for the outgroup *Hirudo medicinalis*. Sequences were aligned using ClustalX 2.1 (http://www.clustal.org.html) before construction of a neighbour joining tree using the K2P model of nucleotide substitution within PHYLIP (http://evolution.genetics.washington.edu/phylip.html); and boot strapped (1000 replicates) to test robustness.

5.3.2 Data analysis

Branchiobdellidan presence versus host size was analysed with logistic regression. General linear models were used to test for a relationship between cocoon number and host size, and gill melanisation. The former were log10 transformed to obtain normality prior to analysis. All analyses were performed using SPSS Version18 (SPSS Inc.).

5.4 Results

A total of 34 specimens (32%) of *A. pallipes* in the Wyke Beck population were found either supporting adult branchiobdellidan worms or cocoons. With the exception of 3 worms found on the exterior surface of the carapace, all were found within the branchial chamber of the host; many directly attached to host gill filaments. Multiple cocoons tended to be clustered in groups and generally located towards the anterior or ventral edges of the branchial chamber. Of the 7 crayfish examined from Adel Beck, worms were present on 2 individuals.

Prevalence did not vary with sex of crayfish, though was positively related to crayfish size (W=0.42, b= 0.427, p<0.01). The number of viable cocoons per host ranged from 1 to a maximum of 116 distributed across both branchial chambers, with a mean count of 26.4 ± 29.7 (S.D.). A strong positive relationship was found between host size (CL) and total number of viable cocoons present (Log_{10} transformed) (r^2=0.40, p<0.001, n=34) (Figure 5.2). The total number of adult worms found on a single host ranged from 1 to 6, and presence of worms was not always associated with cocoons. No relationship was found between number of adult worms and size of host (r^2= 0.09, p <0.97, n = 26). Similarly, mean number of adult worms (t = 1.018, p = 0.326, df =19) and cocoons (t = 1.141, p = 0.265, df = 24) did not differ significantly with host sex.

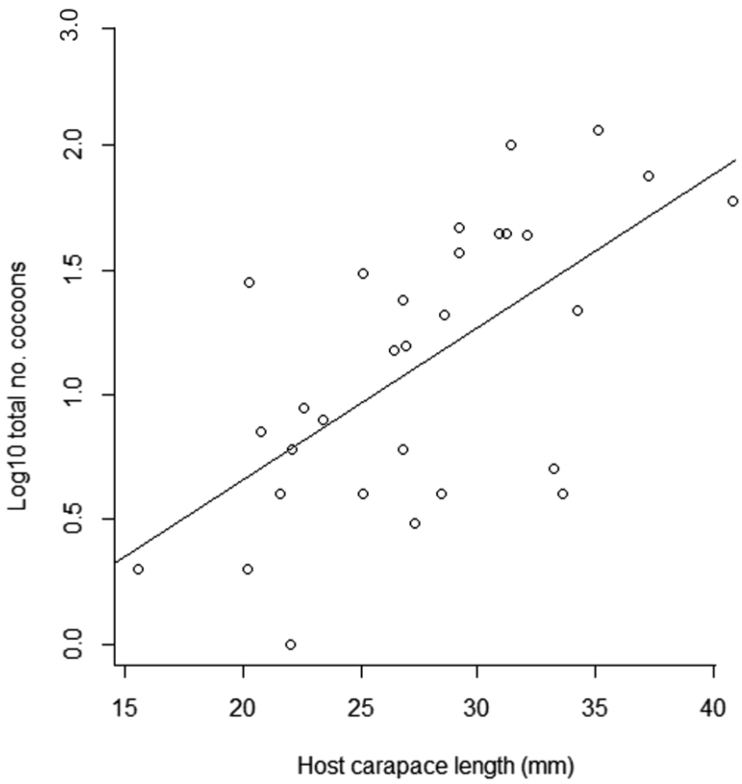


Figure 5.2 Relationship between size (carapace length, mm) of crayfish host *Austropotamobius pallipes* and the total number of viable cocoons of *Branchiobdella astaci* found in branchial chamber (b = 0.0614, r^2 = 0.40, p <0.001, n =34).

Melanisation of the gill filaments, ranging from small patches on the stem of the filament to melanisation of the entire filament tip (Figure 5.1), was observed in 92% of infested individuals, significantly higher than in uninfested (67%) (X^2 = 31.56, p <0.001, n = 107). Mean melanisation score per host was also significantly higher for infested individuals (1.90 ± 0.99 S.D.) relative to uninfested individuals (0.65 ± 0.65 S.D.) (t = 7.765, p <0.001, df =105); and was positively associated with number of cocoons per host (r^2 = 0.40,

$p<0.001$, n = 107). Visible signs of infection with *Thelohania contejeani* (porcelain disease) were present in 29% of all crayfish specimens examined; however, no relationship was found between occurrence of porcelain disease and infestation by *B. astaci* ($X^2 = 0.046$, $p = 0.153$, n = 96).

The species *Branchiobdella astaci* can be distinguished from others by a triangular jaw shape, in particular the large size of the dorsal jaw relative to the ventral jaw (Gelder *et al.*, 1994). The 21 mature branchiobdellidans identified using morphological characteristics all keyed out as *B. astaci*. Identification was later verified by a taxonomic expert (S. Gelder, pers. comm.). Genbank accession numbers for the partial CO-I sequences from 9 *B. astaci* specimens are as follows: JN204263, JN204264, JN204265, JN204266, JN204267, JN204268, JN204269, JN204270 and JN204271. Phylogenetic analysis placed *B. astaci* as a sister group to *B. parasita*, but with only with moderate bootstrap support (59) (Figure 5.3). A few sequences from species *B. hexodonta*, *B. pentodonta* and *B. balanica* do not lie with their conspecifics; however, re-analysis of the phylogeny with these sequences omitted did not change the outcome position for *B. astaci* or improve bootstrap values.

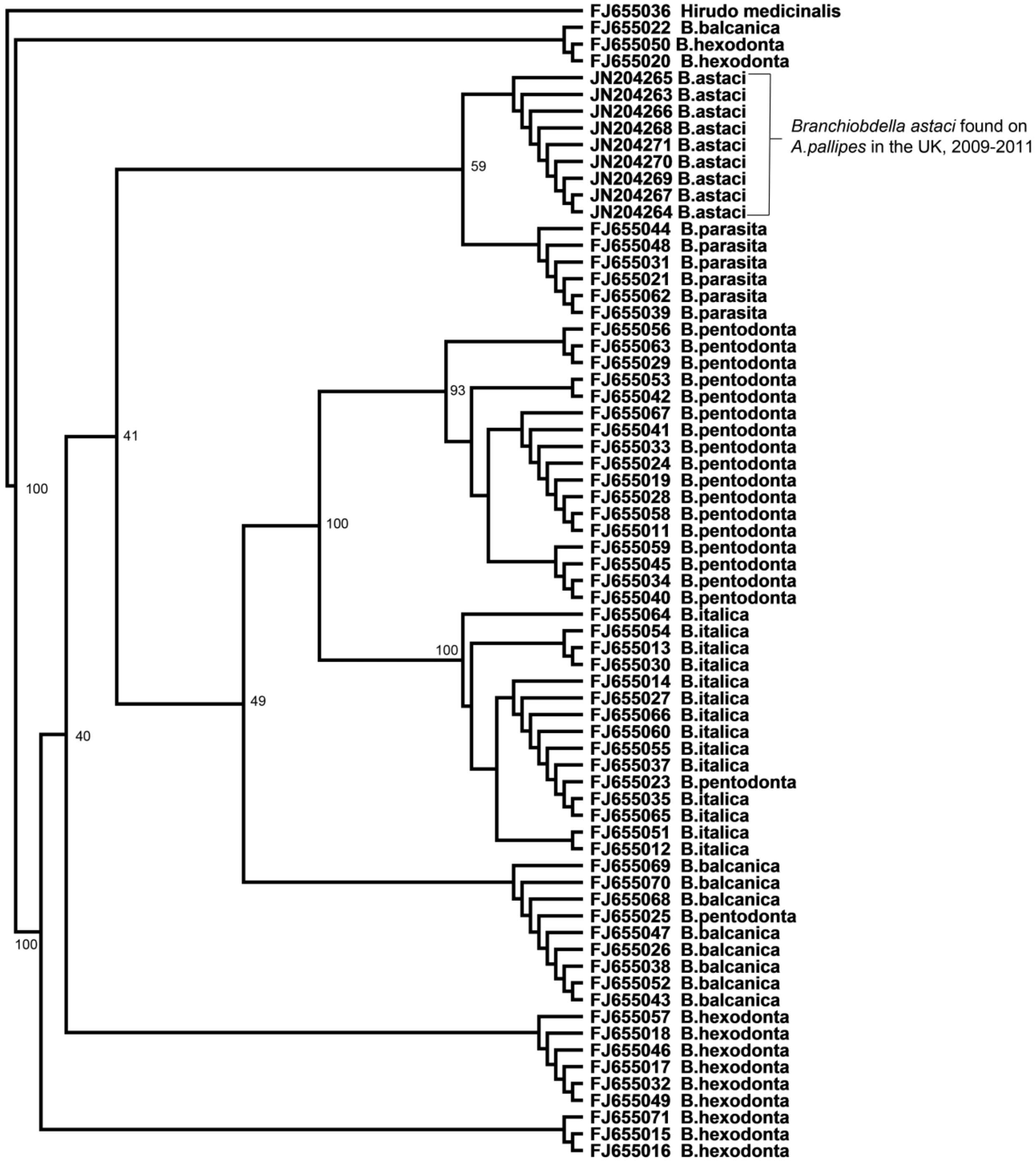


Figure 5.3 Phylogeny of six European *Branchiobdella* species, including *B. astaci,* based on mitochondrial cytochrome *c* oxidase I (CO-I) sequences with outgroup *H. medicinalis*. Numbers at branch points indicate neighbour joining bootstraps (1000 replicates, K2P model).

The jaw and tooth arrangement observed in *B. astaci* specimens would indeed appear to support its proximity to *B. parasita* as both species share the same triangular jaw shape with one dominant central tooth. They can only be differentiated by jaw size as in *B. astaci* the dorsal jaw is larger than the ventral jaw, where as in *B. parasita* the jaws are similarly sized

(Gelder *et al.*, 1994). Mean sequence similarity index between *B. astaci* and *B. parasita* was 84±0.4% (S.D.). The 9 *B. astaci* haplotypes were tightly linked and did not reflect differentiation between the two geographical locations.

5.5 Discussion

The prevalence and intensity of *Branchiobdella astaci* increased with crayfish size reaching up to 116 cocoons per host. As was observed for *Branchiobdella italica* on *A. pallipes* in Italy, there was no association with host sex (Mori *et al.*, 2001). Previous studies of branchiobdellidan prevalence in crayfish populations commonly use the non-lethal submersion method (e.g. Oberkofler *et al.*, 2002) which is likely to strongly underestimate prevalence of gill-dwelling worms. Presence of cocoons in the branchial chamber cannot be detected by this method, and although worms residing in the branchial chamber are anaesthetised they may not always fall out of the animal. In contrast, the current study provides accurate measures of *B. astaci* prevalence in a crayfish population.

Mature worms and cocoons were frequently found directly attached to host gill filaments. The positive relationship between *B. astaci* burden and melanisation of gill filaments, and lower damage level in uninfested crayfish, is consistent with the hypothesis that *B. astaci* is a causative agent of the observed pathology, as suggested by previous authors (Vogt, 1999). This may reflect damage through the attachment of cocoons as well as consumption of host tissue by the mobile juvenile and adult life-stages (Grabda & Wierzbicka, 1969). Melanisation of tissue is a generic, localised immune response among Crustacea to injury, parasites or pathogens, and impairs the function of affected tissue (Alderman & Polglase, 1988). The potential impacts of branchiobdellidans on gill function have not yet been evaluated.

Using morphological characteristics, *B. astaci* from the gills of *A. pallipes* was identified and corresponding molecular data was generated to compare it to other branchiobdellidan species. This is the only sequence data for *B. astaci,* enabling for the first time determination of this species' position within the phylogeny of the *Branchiobdella* genus (Fureder *et al.*, 2009). The location of *B. astaci* as a sister group to *B. parasita* is noteworthy. Both species have previously been found on *Austropotamobius pallipes*, *Astacus astacus* and *Austropotamobius torrentium* crayfish in Europe, but whereas *B. astaci* is largely gill-dwelling, *B. parasita* is commonly found on the outer carapace of the host (Gelder *et al.*, 1994; Mori *et al.*, 2001). The phylogenetic tree presented here is solely based on CO-I sequences, and as such is inherently weaker than analyses incorporating morphological data, although the structure of the tree concurs closely with that of Fureder *et* *al.* (2009). Jaw width and number and height of teeth have previously been used to differentiate *Branchiobdella* species and support the molecular phylogeny (Gelder *et al.*, 1994; Fureder *et al.*, 2009).

A major branchiobdellidan infestation on endangered white-clawed crayfish in the UK was detected. It is notable that whilst only two river drainages were sampled, previous examinations of crayfish from this and a neighbouring catchment in the 1970's recovered no worms (Gelder *et al.*, 1999b), raising questions as to whether these are new introductions or just previously undetected symbionts. The branchiobdellidans recorded on the Kennet and Holy Brook *A. pallipes* populations in 1964 were also *B. astaci* and although the worms found in the Ouse in 2003 were not identified, it is likely that only this single branchiobdellidan species is present on *A. pallipes* in the UK. Genetic studies suggest that all UK *A. pallipes* populations are in fact derived from one or several recolonisations or introductions from France pre-1500s (Gouin *et al.*, 2001; Souty-Grosset *et al.*, 2003). Low branchiobdellidan diversity in the UK is likely reflective of this population bottleneck; parasite species may have been lost as a result of sub-sampling of hosts from the source populations, or through selection pressures experienced during translocation and establishment (e.g. Dunn, 2009; Tompkins *et al.*, 2011).

Parasites are frequently transported to new regions through introduction of the host (Prenter *et al.*, 2004; Tompkins *et al.*, 2011). Extensive introductions of commercially important crayfish species *P. leniusculus* (signal crayfish) and *Procambarus clarkii* (red swamp crayfish), native to North and Central America respectively brought exotic branchiobdellidans *Xironogiton instabilis, X. victoriensis* and *Cambarincola mesochoreus* to Europe (Gelder *et al.*, 1994; Gelder *et al.*, 1999b). Whilst in Italy native *Branchiobdella* spp. have switched host from *A. pallipes* to the exotic *P. clarkii*, there is no evidence of exotic branchiobdellidans switching onto native European crayfish (Gelder *et al.*, 1999b). *P. leniusculus* was first reported in the study catchment in 1986. Whilst there are no records of *B. astaci* on this species in either its native North American or non-native ranges, there have been no systematic studies of branchiobdellidans on signal crayfish in the UK and its potential to act as a reservoir of this parasite is of concern.

Current distributional limits of branchiobdellidan species generally are likely to be expanded as new endemic sites are found, and as a result of the importation of exotic crayfishes for aquaculture, sport fishing and the pet industry (Gelder *et al.*, 2009). The current translocation strategy of moving imminently threatened populations of *A. pallipes* to safe sites within the same, or adjacent, catchment is likely to lead to the redistribution of parasites along with their host; and perhaps extend the range of branchiobdellidans in the UK.

Disease is often cited as a key factor in unsuccessful translocations (Viggers *et al.*, 1993). This study highlights the importance of investigating parasites present in the donor population (particularly those not easily detectable by eye) prior to translocation; as well as the need to further investigate the effect of branchiobdellidans on host growth and survival. Routine health screening of a statistically useful sample from the donor population would make managers aware of what else will be translocated with the crayfish, and thus enable them to make informed, risk-based decisions (Alberts *et al.*, 1998; Armstrong & Seddon, 2008). In short, considering the vulnerability of many crayfish populations and ongoing recolonisation strategies, understanding what symbionts could be spread is of fundamental importance.

Chapter 6 Impacts of suspended solids on gill structure and aerobic scope in an endangered native and an invasive freshwater crayfish

6.1 Summary

Suspended solids (SS) loadings in freshwater habitats have increased over the past century and SS is now a significant environmental stressor. Greater tolerance to environmental stressors has been proposed as a factor in the success of aquatic invasive species. Further, parasites may interact with environmental stressors to increase host susceptibility to loss of fitness and mortality. The effects of SS exposure on the gill structure and aerobic scope of the endangered white-clawed crayfish (*Austropotamobius pallipes*), and the invasive signal crayfish (*Pacifastacus leniusculus*), were compared and assessed in relation to parasite burden. SS caused gill fouling and reduction in aerobic scope in both species, though *A. pallipes* was more susceptible than invasive *P. leniusculus*. The parasite *Branchiobdella astaci*, a crayfish worm that infests the gills, interacted with the sediment to affect gill structure whereas infection with the microsporidian parasite *Thelohania contejeani* had no effect on crayfish response to SS. Juvenile *P. leniusculus* had a higher standard metabolic rate than *A. pallipes*, which may be linked to competitive advantages such as higher growth rate and behavioural dominance. Conservation of *A. pallipes* often involves relocation of threatened populations to isolated stillwaters; our findings suggest that SS concentrations should be assessed before relocation.

6.2 Introduction

Freshwater fauna are proportionately more threatened by environmental change than terrestrial or marine species (Jenkins, 2003; Assessment, 2005); with projected extinction rates up to five fold higher (Ricciardi & Rasmussen, 1999; Revenga *et al.*, 2005; Xenopoulos *et al.*, 2005). The multiple threats to freshwater systems include loss of aquatic habitats; stream fragmentation and flow regulation by dams; channelisation; pollution; and the spread of invasive species (Poff *et al.*, 1997; Nilsson *et al.*, 2005; Dudgeon *et al.*, 2006). Suspended solids (SS) loadings in freshwater streams and lakes have increased substantially over the last century, primarily due to the intensification of agriculture (Foster *et al.*, 2011), and SS is now a significant environmental stressor causing biodiversity loss and ecosystem change (Bilotta & Brazier, 2008; Kemp *et al.*, 2011). Parasites may interact with multiple environmental stressors to increase host susceptibility to loss of fitness and mortality

(Lafferty & Kuris, 1999; Marcogliese & Pietrock, 2011). Crayfish are a functionally important component of lotic and lentic freshwater ecosystems where they feed at multiple trophic levels, modify community structure and transfer energy from primary producers to top predators (Momot, 1995; Nystrom *et al.*, 1996; Usio & Townsend, 2002). Several crayfish species, usually the invasive ones including *Pacifastacus leniusculus* and *Procambarus clarkii*, are also significant bioturbators, operating as ecosystem engineers causing physical changes including increased sediment transport and turbidity due to burrowing (Harvey *et al.*, 2011; Johnson *et al.*, 2011; Lodge *et al.*, 2012).

Of the ca. 640 crayfish species globally, 26% are classified 'vulnerable' or 'endangered', largely due to invasive species, disease, and habitat degradation (IUCN, 2011). The white clawed crayfish (*Austropotamobius pallipes*, Lereboullet), the UK's only native crayfish species, is IUCN red-listed as 'endangered', principally due to population decline since introduction of the invasive signal crayfish *P. leniusculus*). *P. leniusculus* originates from North America and was initially introduced to Europe in the 1960s for the table market. Traits such as fast growth rate, high fecundity and large size made it more suitable for aquaculture than native crayfish species (Holdich & Gherardi, 1999; Gil-Sanchez & Alba-Tercedor, 2002). Where *P. leniusculus* and *A. pallipes* co-occur, the invader will typically outcompete the native within 4 to 5 years (Peay & Rogers, 1998), and is the main vector of *Aphanomyces astaci*, the cause of 'crayfish plague, which is fatal to *A. pallipes* (Alderman *et al.*, 1984). Understanding the mechanisms underpinning the success of an invader is key for predicting future invasions and devising effective control measures (Davis, 2009).

. UK populations of *A. pallipes* are infected by a number of parasites, including the widespread microsporidian *Thelohania contejeani* which reduces function of muscle tissue and commonly infects 0–10% of crayfish in a population (Cossins & Bowler, 1974). The fitness impacts and associated mechansims of this parasite are poorly understood; though it is thought to cause death within 4-5 years of infection (Alderman & Polglase, 1988; Imhoff *et al.*, 2009).As described in Chapter five, A. *pallipes* may also be host to the annelid worm *Branchiobdella astaci* which infests the gills of its host and causes pathology, most likely through the consumption of host tissue (Grabda & Wierzbicka, 1969; Vogt, 1999).

The negative effects of SS on freshwater fish are well documented and include abrasion of the gills and hyperplasia, reduction in feeding rates, and increased susceptibility to disease (Martens & Servizi, 1993; Metzeling *et al.*, 1995; Lake & Hinch, 1999). There is a mean threshold target of 25 mg L^{-1} for SS within the EU Freshwater Fish Directive (78/659/EC), and although no specific targets for SS are stated within the EU Water Framework Directive

(EC 2000), mitigation for diffuse sediment pollution is considered critical for achieving ‘good ecological status’ by 2015 (Collins & Anthony, 2008; Defra, 2012).

Impacts of SS on invertebrates such as zooplankton, chironomids and freshwater mussels, range from reduced feeding rates, metabolic changes, and clogged gills, to mortality (Donohue & Irvine, 2003 and references within; Bilotta & Brazier, 2008). The effect of increased sediment loadings on crayfish has received comparably little attention, though has been implicated in declines of some species e.g. *Astacus astacus* (Fureder *et al.*, 2006; Environment Agency, 2011). It is generally invasive crayfish that are associated with generating SS through burrowing activities (Statzner *et al.*, 2003; Johnson *et al.*, 2011). As greater tolerance to environmental stressors such as fluctuating temperatures and degraded water quality has been proposed as a factor in the success of aquatic invasive species (Karatayev *et al.*, 2009; Crooks *et al.*, 2011; Weir & Salice, 2012), it may be predicted that invasive crayfish will be less sensitive to SS than native species.

In the absence of quantified impacts, a conservative threshold SS target of 25 mg L^{-1} (based on the target for salmon) for *A. pallipes* was suggested on the basis that sediment ‘blocks gills’ (Smith *et al.*, 2003). Crayfish gills are indeed vulnerable to fouling by both particulate matter and epibionts (Holdich, 2003), which may lead to gill pathology (Bauer, 1998), although this has not previously been examined or quantified in relation to SS concentrations. Investigation of the impact of suspended solids on *A. pallipes* is therefore important to determine acceptable ranges of SS; and also to inform conservation strategy for this endangered species which currently involves relocation of threatened populations to isolated sites without *P. leniusculus* (Schulz *et al.*, 2002; Kemp *et al.*, 2003). The selection of suitable receptor sites is dependent on a thorough understanding of the habitat requirements of *A. pallipes*, including tolerance of water quality parameters such as SS (Kemp *et al.*, 2003).

Environmental factors fundamentally influence animal activity through metabolism (Fry, 1947; Claireaux & Lefrançois, 2007). Aerobic scope denotes the maximum amount of oxygen available to an ectotherm at a particular temperature and is the difference between maximum oxygen uptake, such as that reached after exercise to exhaustion (maximum metabolic rate, MMR); and oxygen uptake at complete rest, in an unfed state (standard metabolic rate, SMR) (Brett, 1972). An individual must function within the confines of its aerobic scope, so a reduction in scope limits the energy which may be allocated to activities beyond basic survival and maintenance (e.g. growth, reproduction, foraging and predator avoidance), thereby causing loss of performance, and potentially overall fitness (Fry, 1947;

Boddington, 1978; Claireaux & Lefrançois, 2007). For example, gill morphological changes induced by exposure to aluminium have been shown to reduce aerobic scope in rainbow trout *Oncorhynchus mykiss* as a consequence of reduced gill surface area (Wilson *et al.*, 1994). SS is known to foul the gills of crayfish *Procambarus clarkii* (Bauer, 1998), and therefore has the potential to reduce aerobic scope by modifying gill structure and limiting the area available for gas exchange.

To compare the impact of chronic exposure to SS on native *A. pallipes* and its invasive competitor *P. leniusculus*, and to investigate the influence of parasitism on the host responses to SS: 1) the effect of chronic periodic exposure to high SS on gill structure and aerobic scope of native and invasive juvenile crayfish was measured, and 2) it was tested whether infection by *T. contejeani* or *B. astaci* modified the effects of SS on *A. pallipes.*

6.3 Methods

The effect of the SS treatment on infected and uninfected individuals was investigated by: 1) comparing individual aerobic scope before and after the treatment; 2) measuring mortality in the different treatment groups, and 3) at the end of the experiment, the gills were dissected and examined for evidence of sediment accumulation and associated pathology.

6.3.1 Collection of animals

Juvenile *A. pallipes* (19 – 30 mm cephalothorax length, CL) representing ages of 2 – 3 yrs (Brewis and Bowler, 1982) were collected from Wyke Beck, UK (53° 49' 20.93" N, -1° 28' 58.73"E) using hand-search, under license from Natural England (20103521). Infection with *T. contejeani* (thelohaniasis) was determined by visual examination (Imhoff *et al.*, 2012). Juvenile *P. leniusculus* (20 – 31mm CL) were collected from the River Pant, UK (51°55'28.14"N, 0°31'16.59"E), using hand-search. Crayfish were maintained in the laboratory in de-chlorinated tap water (16°C, 16:8 light, dark regime) and fed crab pellets (Hinari) every 3 days for a minimum of 3 weeks before experiments.

6.3.2 Suspended solids exposure

Juvenile crayfish were allocated to four SS concentrations (0, 250, 500 and 1000 mg L^{-1}) for 45 days. Since a large proportion of the added SS does not remain in suspension, even under moderate turbulence, this range of elevated SS concentrations was chosen to ensure high, but realistic concentrations of measurable SS in the experiment based on pilot studies informed by the literature (Au *et al.*, 2004).

SS treatments were prepared using dried sediment re-suspended in de-chlorinated tap water. Sediment was collected from a limestone quarry in the Yorkshire Dales, UK (54° 4'30.36"N, 2° 2'18.29"W). The location was chosen because it is already used as an Ark site for the conservation of *A. pallipes.* After collection, sediment was re-suspended in 5 L of water and the water and particles still suspended after 60 s were decanted. Sediment was allowed to settle out 7 days before subsequent air drying at room temperature. Mineralogy of the resultant particles was determined using X-ray diffraction. To minimise potential variation in water chemistry between treatments caused by addition of the sediment, tap water was equilibrated during dechlorination by placing a block of limestone in the water container. Test solutions were fully replaced and the test containers rinsed with water every 3 days to both prevent the build-up of wastes, and to account for settling out of particles over time. To measure actual SS concentrations in the treatments over the 3 days settling period, water samples (50 ml) were taken from randomly selected experimental chambers at 5, 30, 240, and 960 min, and then every 8 hours thereafter from the time water was replaced, four replicates for each treatment at each time. SS concentration (mg L^{-1}) in each sample was determined by change in dry mass (± 0.0001g) of pre-dried filter papers (0.45 μm, cellulose nitrate membrane, Whatman) after filtration.

Treatments comprised *P. leniusculus* and *A. pallipes,* with 8 individuals per treatment at each SS concentration, and *A. pallipes* visibly infected with *T. contejeani*, with 6 individuals per treatment at each SS concentration. The sex ratio was 1:1 in all treatments. As the presence of *B. astaci* cannot be reliably determined in live animals, it was not possible to assign crayfish to treatments based on infestation with *B. astaci,* thereby presenting the risk of potentially unbalanced treatments. All *A. pallipes* were examined for *B. astaci* at the end of the exposure period.

Crayfish were weighed (wet mass, g) numbered on the carapace using non-toxic correction fluid and transferred to aerated experimental containers with two individuals per container. The set-up comprised upturned funnels (300 mm diameter, 1 L vol.), and central platform on which the crayfish were housed. Air flow was provided via an air stone secured in the base of each container; the flow of bubbles also served to maintain the sediment in suspension. The platform was composed of aluminium insect screen (mesh size 1.5mm) which whilst solid enough to support the crayfish, permitted water flow throughout the experimental chamber. To reduce stress and aggressive interactions, crayfish were provided refugia in the form of PVC pipe sections (5cm x 3cm diameter), one per animal. Crayfish were fed every 2 days with Hinari crab pellets, 2 pellets per crayfish. Any uneaten food was removed after approximately 3 hours to prevent fouling of the water (Figure 6.1).

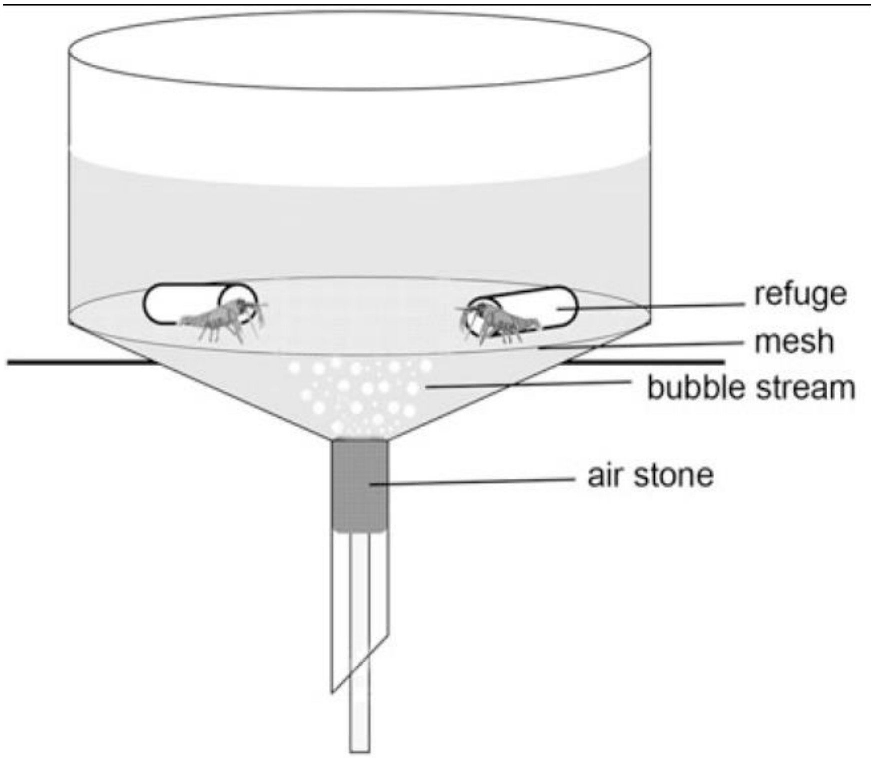


Figure 6.1An experimental chamber

6.3.3 Gill examination

To investigate the impact of SS on the gills, and to screen for *B. astaci* infection, at the end of the experiment all crayfish were euthanized by freezing and the branchiostegites removed to enable *in situ* examination of the podobranchs. Individuals that did not survive to day 45 were examined as close as possible to the time of death (<10 h). For infected individuals, *B. astaci* burden was recorded as the number of cocoons (egg and embryo stage) attached to the gill filaments. The presence of sediment particles was visually assessed for each of the 12 podobranchs as percentage of total area affected. A photograph was taken of each side of the animal under a dissecting microscope, and a grid superimposed over the photo (Adobe Photoshop CS6). The number of grid squares where sediment particles were visible in > 50% of the square was counted, relative to the total number of squares containing gill tissue, and was used to calculate % total gill area affected. The area of melanised tissue, denoted by dark brown coloration (see Chapter Five) was assessed using the same method. Melanisation is a generic immune response among Crustacea and therefore used as an indicator of gill damage (Cerenius & Söderhäll, 2004). ,

6.3.4 Respirometry

Oxygen consumption rates (MO_2) were measured using intermittent flow respirometry in which the respirometer operates as a closed system during measurement, but is periodically flushed through to fully replace water in the chamber before the next measurement period. This method overcomes the problem of metabolites building up over long measurement periods during closed respirometry, without the difficulties of achieving steady state required by flow-through respirometry (Steffensen, 1989). Single channel Loligo Autoresp respirometry equipment and software (LoligoSystems, ApS, Tjele, Denmark) were used. The glass respirometer chamber (4.5cm inner diameter, 10cm length) and mixing pump were submerged in 50 L dechlorinated tap water, aerated using air stones and maintained at 16 ± 0.1˚C by means of a cooling coil and temperature regulation unit. The experimental temperature was chosen to correspond to summer temperatures in lotic systems (Webb & Walling, 2009), a time when crayfish are most likely to be subject to oxygen limitation and therefore the effects of impacted gill function would be most apparent. Water in the chamber was re-circulated through tubing connected at each end. Total respirometric volume was 0.25 L. Oxygen partial pressure of the circulating water was recorded each second using a fibre optic oxygen sensor dipping probe (Fibox 3, Presens, Regensburg, Germany) mounted into the tubing via a Y-connector. Water in the respirometer was fully replaced in the flush period; ensuring oxygen did not fall below 20 K Pa. The coefficient of determination (R^2) associated with each MO_2 measurement was >0.9 (Behrens & Steffensen, 2007). Blank runs with no animal present were made each week to determine background respiration..

SMR was measured in a subset of individuals of *P. leniusculus* (n=6), *A. pallipes* infected with *T. contejeani* (n=6) and uninfected *A. pallipes* (n=6) (1:1 males to females) prior to the start of the experiment. Crayfish were starved for 24 h before being transferred to the respirometer and MO_2 measured at least every 13 min for 24 h, 3pm to 3pm the following day. A refuge in the form of a section of PVC pipe (5cm length, 2cm diameter) was placed in the respirometer to minimise stress and activity in the crayfish. The respirometer and tank were loosely covered with a lid to limit visual disturbance to the crayfish, but gaps around the edge permitted entrance of light to denote photoperiod. Animals that were suspected of being immediately pre-moult, or had moulted in last 3 days were excluded from measurements because ecdysis increases oxygen consumption rate in crayfish (Rice & Armitage, 1974).

MMR was initially determined in all crayfish at commencement of the study, and again on day 45. Prior to measurement, each crayfish was placed in a shallow tray of dechlorinated water (40 cm x 25 cm, 4cm water depth) and exhausted using a two stage protocol. First, the crayfish was induced to tail flip by gently touching the telson with a blunt pencil. This escape behaviour is common among crayfish whereby the abdomen is rapidly contracted propelling the animal backwards. The tail musculature has a limited capacity for aerobic contraction and on depletion of arginine phosphate reserves in the tail the animal will adopt stationary threat posture (England & Baldwin, 1983). Once tail flipping ceased, the second stage entailed repeatedly turning the crayfish onto its back until it could no longer right itself. At this point the crayfish was immediately transferred to the respirometer.

Both MMR and SMR represent measurements that cannot be self-regulated (Priede, 1985), and are therefore repeatable within individuals over time (Norin & Malte, 2011). Results from a pilot study conducted prior to the experiment conformed to this assertion.

6.3.5 Data analysis

The survival of crayfish across the four sediment treatments was analysed using cox proportional hazard survival models (Therneau & Lumley, 2011) in R v 2.14.0 (R Development Core Team 2011). In the first instance time to death was modelled as a function of SS concentration, species and crayfish mass on day 1. As only *A. pallipes* were parasitised in this study, a second analysis was undertaken. Time to death for *A. pallipes* only was modelled as a function of SS concentration, infection with *T. contejeani*, infection with *B. astaci*, and first order interactions between the two parasites, and between each parasite and SS concentration. The assumption of constant hazard in all models was tested using function cox.zph.

All MO_2 measurements were corrected for background respiration (i.e. oxygen consumed by bacteria) prior to analysis. Using raw MO_2 data, SMR can be derived in a number of ways. As there is no apparent consensus in the literature as to the optimum method, in this study, SMR was calculated for each crayfish using two common methods. First, a frequency distribution was fit to the set of MO_2 values collected during the entire 24 h period, typically revealing a bi-modal normal curve. The distribution reflects a short ‘adaptation phase’ when oxygen consumption is high reflecting stress in response to handling, then a longer period of lower, settled values. The peak of the second curve of lower values was taken to be SMR (see Svendsen *et al.*, 2012). Curve-fitting was carried out in Tablecurve2D v.5.01 (Systat software Inc., California). In the second method, values in the ‘adaptation phase' were disregarded and lowest 10% of values in the following settled period, excluding outliers, were averaged to arrive at SMR (see Herrmann & Enders, 2000).

To enable comparison of MO_2 values between crayfish of different mass, SMR and MMR were corrected to a body mass of 5g using the equation MO_2 (5 g) $=MO_{2(BM)}x(BM/5)^{(1-A)}$, where $MO_{2(BM)}$ is oxygen consumption of animal with body mass BM and A is the mass exponent describing the relationship between metabolic rate and oxygen consumption (Steffensen *et al.*, 1994). A value of 0.71 for the mass exponent determined for crayfish species *Orconectes rusticus* using field metabolic rate in 137 individuals of different populations (McFeeters *et al.*, 2011) was used within this study because no published empirically-derived mass exponents were available for either of the study species.

Prior to all analyses, data were tested for normality using Shapiro-Wilk test. Generalised linear models (GLMs) were used to investigate predictors of sediment accumulated in the gills of *A. pallipes* (%) at day 45. All models were fitted with quasibinomial error distributions due to overdispersion of the data (Ver Hoef & Boveng, 2007). Variables included in the maximal model were SS concentration, infection with *T. contejeani*, burden of *B. astaci* and first order interaction terms. GLMs with quasibinomial error distributions were also used to investigate predictors of melanisation levels in *P. leniusculus* and *A. pallipes*. Variables included in the maximal model for *P. leniusculus* were: sediment accumulation in gills at time of death (%), sediment concentration, and days in experiment. For *A. pallipes*, variables were sediment accumulation in gills at time of death (%), sediment concentration, days in experiment, and *B. astaci* burden. Non-significant terms were stepwise deleted from the maximal model and model fit assessed by examination of residual plots and tests for normality of residuals.

Independent samples t-tests were used to test differences in mean SMR and aerobic scope between groups. General linear models (LM) were used to determine significant predictors of aerobic scope on day 1. Aerobic scope (mg O_2 kg^{-1} h^{-1}) on day 1 was modelled as a function of species, sex, start mass, number of tail contractions before exhaustion, and time to exhaust. Linear models were also used to explore change in aerobic scope between day 1 and day 45 for the same individuals. In the maximal model, change in aerobic scope (mg 0_2 kg^{-1} h^{-1}) was modelled as a function of sediment treatment, gill area affected by sediment on day 45 (%), gill area affected by melanisation on day 45 (%), species, difference in number of tail flips during exhaustion protocol between day 1 and day 45, and difference in time to exhaustion between day 1 and day 45. Non-significant variables were excluded stepwise and all models were carried out in R v 2.14.0 (R Development Core Team, 2011).

6.4 Results

6.4.1 SS treatments

Composition of the sediment was 53.7% calcite, 43.6% magnesium calcite and 2.7% quartz silica. Actual time-averaged SS concentrations calculated from determination of settlement rates between water replacement (Figure 11.2) were 2.5, 42, 65, 133 mg L^{-1}, for control, 250 mg, 500 mg and 1000 mg treatments, respectively.

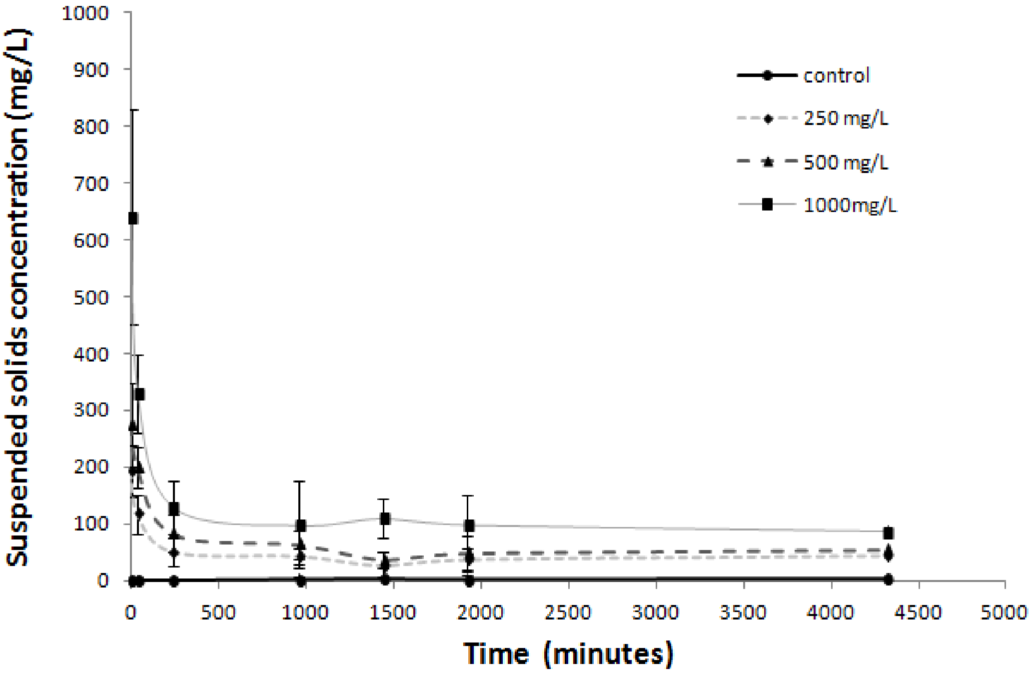


Figure 6.2 **Mean actual suspended solids concentration as a function of time since water replacement in the experimental chamber, determined by filtration for four treatments (control, 250 mg L^{-1}, 500mg L^{-1} and 1000 mg L^{-1}) with four replicates in each. Error bars denote standard deviation.**

6.4.2 Survival

SS concentration was not a significant predictor of crayfish survival, for either species, through the 45 day period (*z* = 0.94, n = 92, p= 0.35) (Figure 6.3). Species was the only significant predictor of survival (z = 1.92, n= 92, p = 0.05) with higher survival for *P. leniusculus* than *A. pallipes* across all treatments (Figure 6.3). Adult *B. astaci* or cocoons were found in 18 *A. pallipes* individuals (32%), with a maximum of 6 adult worms and 80 cocoons per individual. The number of individuals parasitised with *B. astaci* did not vary significantly between the 4 SS concentrations (X^2= 5, p = 0.17, 3 d.f.). Survival of *A. pallipes* was not affected by infection with *T. contejeani*, burden of *B. astaci* (no. cocoons), or the interaction between them (*z* = -0.81, n = 57, p = 0.42, *z* = -0.33, n = 57, p= 0.74, and *z* = 1.29, n = 57, p = 0.64, respectively).

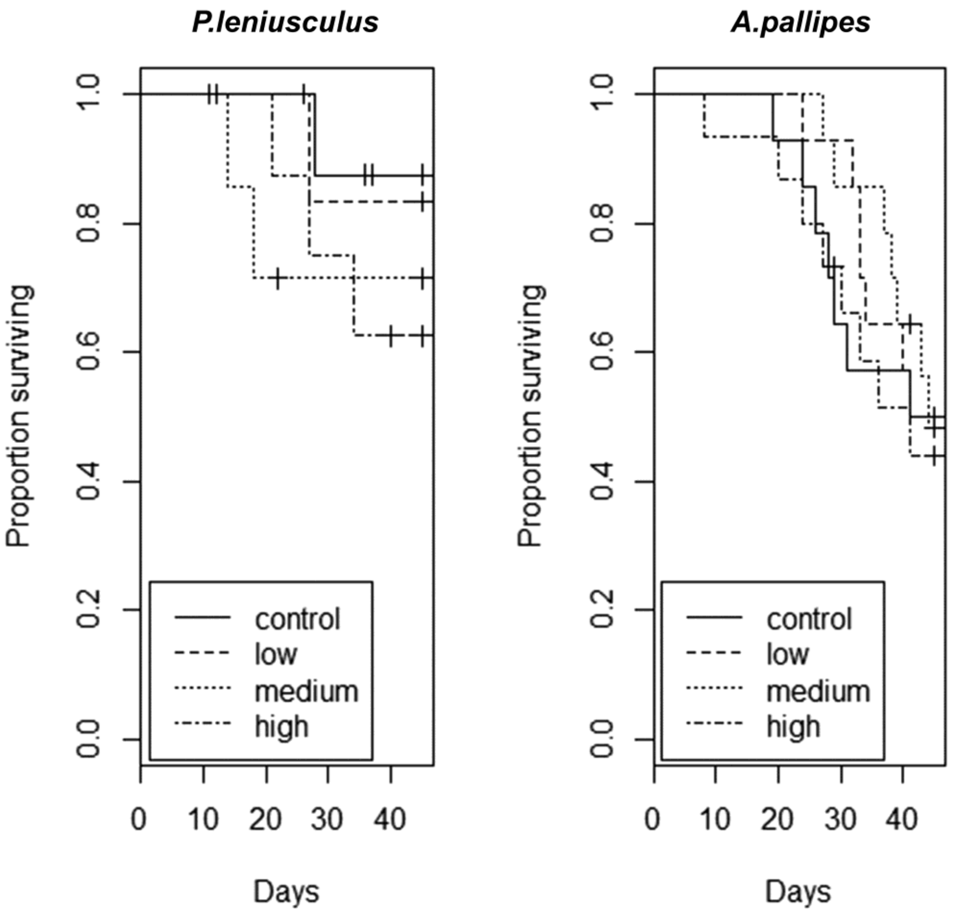


Figure 6.3 Stepped survival plots for crayfish *Pacifastacus leniusculus* and *Austropotamobius pallipes* during 45 day exposure to four suspended solids (SS) concentrations: control (0 mg L^{-1}); low (250 mg L^{-1}); medium (500 mg L^{-1}) and high (1000 mg L^{-1}).

6.4.3 Sediment accumulation in gills

Sediment particles were evident within the podobranchs of all crayfish exposed to the medium and highest SS concentrations and in 92% and 56 % of *A. pallipes* and *P. leniusculus* exposed to the low concentration, respectively. *A. pallipes* accumulated particles significantly more readily than *P. leniusculus* over the 45 day period (23% residual deviance, p <0.01, 1 & 43 d.f.; Figure 6.3). Sediment accumulation in *A. pallipes* was not affected by the *B. astaci* burden, or infection with *T. contejeani*; hence SS concentration was the only significant predictor of sediment accumulation in the gills (51% residual deviance, p <0.01, 3 & 20 d.f.). In some animals, despite heavy sediment accumulation elsewhere in the gills, there was a clear band at the posterior edge of the podobranchs, near the joints of the thoracic appendages (Figure 6.5).

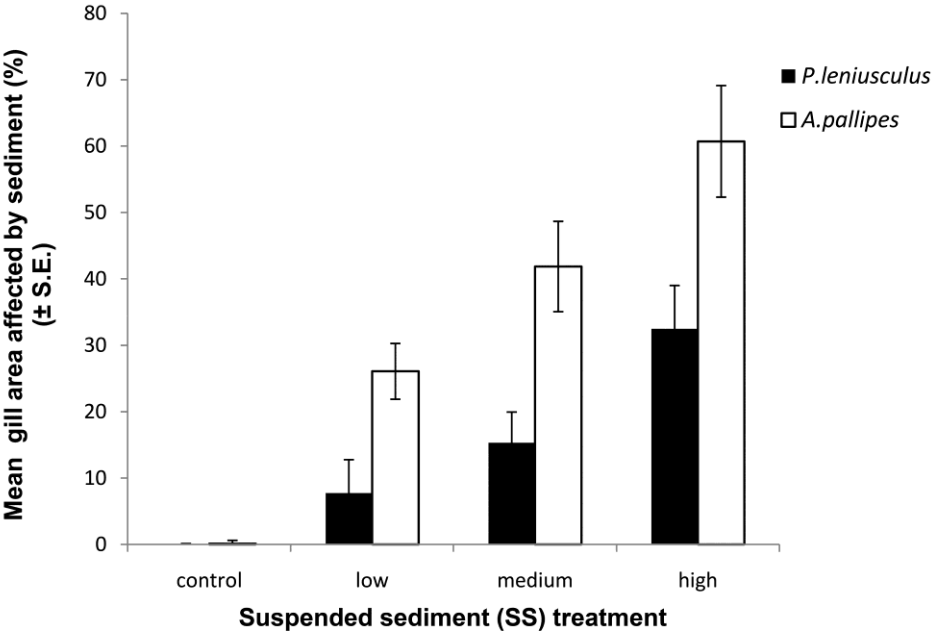


Figure 6.4 Mean (± S.E) gill area (%)of crayfish *Pacifastacus leniusculus* and *Austropotamobius pallipes* visibly affected by sediment accumulation after 45 days exposure to 4 suspended solids (SS) concentrations (control, low, medium and high).

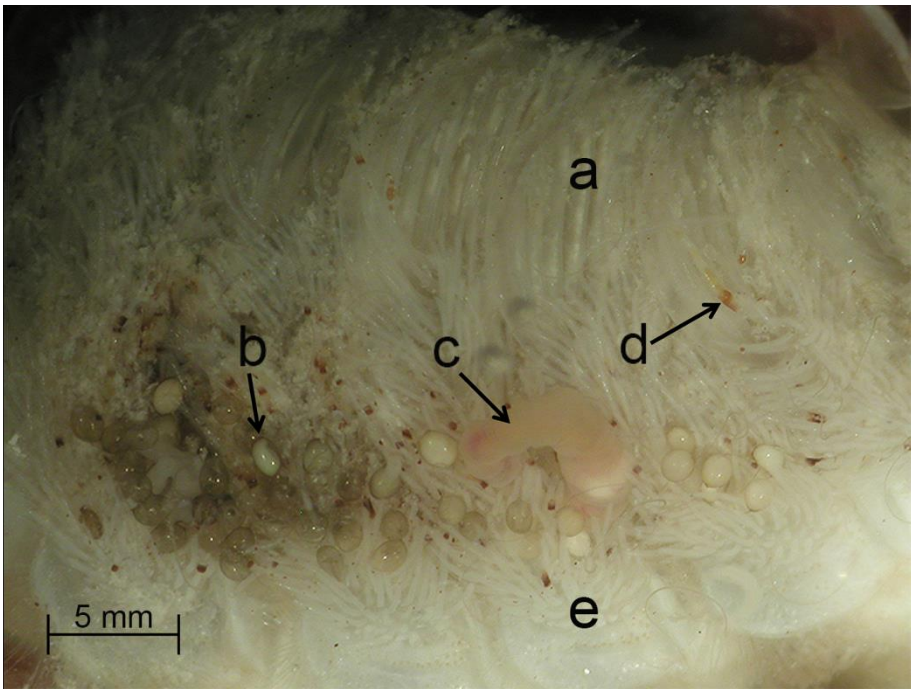


Figure 6.5 Podobranchs of crayfish *Austropotamobius pallipes* after exposure to suspended solids (1000 mg L^{-1} experimental treatment yielding time-averaged concentration of 133 mg L^{-1}) for 45 days showing heavy sediment accumulation (a), cocoons of *Branchiobdella astaci* (b), *Branchiobdella astaci* (c), gill filament with melanisation spot (d), and base of podobranchs free from sediment (e).

Melanisation as a percentage of total gill area ranged from 0 to 18% in *P. leniusculus* and 0 to 22 % in A. pallipes. For *P. leniusculus*, melanisation did not significantly reflect SS concentration, or accumulated sediment concentration in the gills (7% residual deviance, 23 d.f., and <1% residual deviance, 22 d.f. respectively). In *A. pallipes*, burden of *B. astaci* was a strong predictor of melanisation levels, irrespective of time spent in the experiment (27% residual deviance, $p < 0.01$, 1 & 48 d.f.), and there was a significant interaction between *B. astaci* burden and accumulated sediment in the gills, explaining 10% of residual deviance, 1& 46 d.f., $p<0.01$). This result indicated a potential additive effect between *B. astaci* and SS to cause melanisation of gill tissue.

6.4.4 Standard metabolic rate (SMR)

Estimates of SMR generated using the curve-fitting method (Svendsen *et al.*, 2012), and using 10% of lowest MO_2 values (Herrmann & Enders, 2000), differed by 10-15%. Curve-fitting estimates were always higher and are the only values used hereafter. For juveniles, mean (± S.E.) size-corrected (5g) estimates of SMR were significantly higher in *P. leniusculus* (86.2 ± 3 mg O_2 $kg^{-1}hr^{-1}$ than *A. pallipes* (infected and uninfected combined) (70.2 ± 3 mg O_2 $kg^{-1}hr^{-1}$.)($t = 3.35$,$p = 0.005$, 14 d.f.). Within *A. pallipes,* mean SMR was not affected by infection with *T. contejeani* ($t=1.09$, $p=0.30$, 8 df). It was determined during subsequent dissection that *A. pallipes* infested with *B. astaci* were not present in this subset of animals for which SMR was measured.

6.4.5 Aerobic scope

Individual aerobic scope was determined for juveniles for which SMR had been previously determined. Mean (± S.E.) aerobic scope did not differ significantly between *A. pallipes* individuals with and without thelohaniasis ($t = 0.47$, $p = 0.65$, 8 d.f.), or between *P. leniusculus* (251.5 ± 18 mg O_2 kg^{-1} h^{-1}) and *A. pallipes* (infected and uninfected combined) (206.6 ± 15 mg O_2 kg^{-1} h^{-1}) ($t = 1.80$, $p = 0.09$, 14 d.f.). Factorial scope, the ratio of MMR to SMR, ranged 3.3 to 5.7 among all subjects, with mean value 3.77 ± 0.16 (± S.E.). For all individuals, MMR was at least 2.8 fold higher than the highest MO_2 values recorded during routine phase in SMR measurements as a result of spontaneous activity.

On day 1 and 45 of the SS exposure experiment aerobic scope was determined for all juvenile crayfish (>3g) using individual MMR and, in the absence of estimates of SMR for each individual, mean SMR for the species. Mean scope on day 1 was 221.67 ± 14 mg O_2 kg^{-1} h^{-1} (± S.E.) for *P. leniusculus* and 223.4 ± 12 mg O_2 kg^{-1} h^{-1} (± S.E.) for *A. pallipes* (infected and uninfected combined), with no significant difference between the species (t =

0.09, p = 0.93, 41 d.f.). Mean scope on day 1 was not significantly different between crayfish infected with *T. contejeani* and those without (t=1.9, p=0.29, 19 d.f., equal variances not assumed). Burden of *B. astaci* (no. cocoons) did not affect aerobic scope on day 1.

During the exhaustion protocol *P. leniusculus* on average performed more tail contractions before exhaustion than *A. pallipes* with means 57.2 ± 3 and 48.0 ± 3 (±S.E.), respectively though the difference was not significant (t = 1.72, $p < 0.09$, 36 d.f.). There was no significant difference in the mean number of tail flips performed by *A. pallipes* with and without thelohaniasis (49.1 ± 4.5 and 46.0 ± 4.2 (±S.E) flips respectively, t = 0.469, p = 0.65. 17 d.f.). Number of tail flips and time to exhaustion were positively correlated (b = 0.2, $r^2 = 0.47$, n = 36). In the best fitting linear model describing variation in scope at day 1, time to exhaustion was the only significant predictor of scope (b = -0.61, $r^2 = 0.17$, p = 0.01, n = 38) (Figure 6.6).

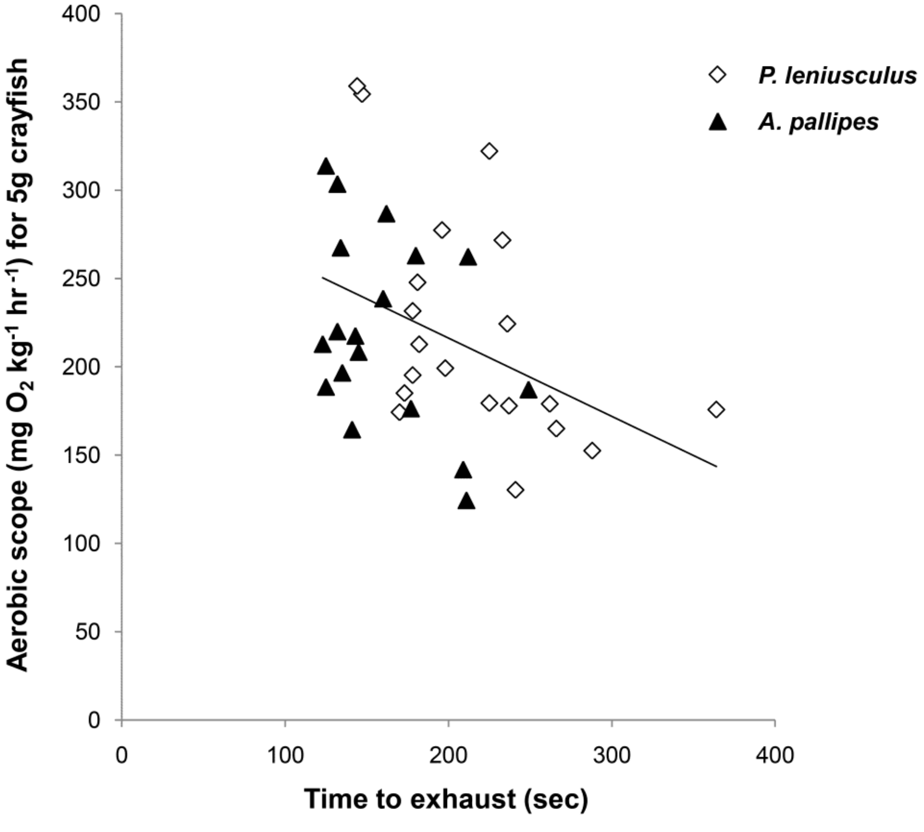


Figure 6.6 Aerobic scope (mg O2 kg^{-1} h^{-1}) for crayfish *P. leniusculus* and *A. pallipes* corrected to 5 g individual, as a function of time to exhaust (sec) (b = -0.61, r^2 = 0.17,p = 0.01, n = 38).

6.4.6 Aerobic scope after SS exposure

For all individuals besides two, aerobic scope on day 45 was lower than on day 1 across all treatments. The minimum adequate model describing 40% of variation in change in aerobic scope over the exposure period identified three significant predictors. Reduction in scope was greater in *A. pallipes* than *P. leniusculus* ($b = 98.17$, $t = 2.62$, $p = 0.02$), and was positively related to both sediment concentration ($b=0.15$, $t = 2.90$, $p < 0.01$), and accumulated sediment in the gills on day 45 ($b = -2.79$, $t = -2.85$, $p = 0.01$) ($r2 = 0.4$, $n = 23$). Scope was reduced by 17 and 28 % in the two highest SS concentrations, respectively, relative to control. Melanisation in the gills, change in time taken to reach exhaustion, and change in number of tail flips performed before exhaustion were not significant predictors of change in scope. All interaction terms were non-significant.

6.5 Discussion

Understanding a species' habitat requirements and range of environmental tolerances is important for conservation planning, particularly if conservation is to be facilitated through relocation, as is the case for the endangered white-clawed crayfish (Kemp *et al.*, 2003; Armstrong & Seddon, 2008; IUCN, 2012). This study demonstrated a reduction in aerobic scope of crayfish after medium term (45 day) exposure to SS, a common environmental stressor in freshwater systems, and that the native *A. pallipes* crayfish was more susceptible to the SS than invasive *P. leniusculus*. Juveniles of the invasive crayfish were also found to have a higher standard metabolic rate than the native.

The highest time-averaged SS concentration in this study (133 mg L^{-1}) exceeded the highest mean concentration that *A. pallipes* has been found to persist at in the wild (34 mg L^{-1}) (Trouilhe *et al.*, 2007) and exceeded the current recommended concentrations of 25 mg L^{-1}. Survival did not reflect SS treatment for either species; however there was mortality in all treatments and the control, particularly for *A. pallipes.* Therefore, a factor besides SS was responsible for the lower overall survivorship in this study than would be expected for crayfish maintained in aquarium facilities (Sáez-Royuela *et al.*, 2002; Gonzalez *et al.*, 2009). One explanation for this may be that the bubble stream within the experimental chambers impaired successful moulting as several crayfish were found dead during their moult, which for the juvenile life-stage used in this study, occurs 4 to 6 times per year (Pratten, 1980). Ecdysis is naturally associated with heightened risk of mortality in crayfish, both through the physical difficulties of leaving the hardened cuticle and increased risk of cannibalism in the wild (Pratten, 1980; Olsson & Nystrom, 2009). Further investigation, ideally over a longer time period than the 45 days used in this study, is therefore required to elucidate on the implications of SS for crayfish survival.

Crayfish exposed to SS showed signs of sediment accumulation, or fouling, in their gills. Highly fouled individuals showed common patterns with clear bands towards the base of the podobranchs and highest sediment load towards the tips of podobranchs, which likely reflects higher branchial flow velocities near water intake points at the bases of the thoracic joints. Crayfish possess several mechanisms to reduce fouling of the gill filaments by particulates and epibionts. Most important are the setae on the epipods, setobranchs, scaphogthathites and inner surface of the branchiostegites, which are entwined around the gill filaments and furnished with scale setules that scrape over the gills (Bauer, 1998; Batang & Suzuki, 2000). ‘Limb rocking’ behaviour whereby crayfish move limbs apparently to jostle the setae to aid cleaning has also been observed in *Procambarus clarkii* (Bauer, 1998), a species associated with extensive burrowing and turbid waters (Rodríguez *et al.*, 2003), and *Cherax quadricarinatus* (Batang & Suzuki, 2000),. It was not feasible to make observations of limb rocking in this study. Periodic reversal of direction of branchial flow is another mechanism used to clear debris on the gills of decapods (Arudpragasam & Naylor, 1966). However, cleaning mechanisms were apparently unable to prevent widespread sediment accumulation in the gills under all SS concentrations. The generally lower accumulation in *P. leniusculus* compared to *A. pallipes* may reflect more efficient gill cleaning, though there is to date no comparison of cleaning mechanisms.

SS alone did not cause melanisation of gill tissue; however there was an additive effect between SS accumulation and burden with *B. astaci.* As a mainly gill-dwelling parasite, the impacts of *B. astaci* would be expected to be most apparent in the gills. Adult worms reside mainly in the branchial chamber and the cocoons are deposited among, and attached to, the gill filaments. The presence of melanised gill tissue in *A. pallipes* strongly reflected burden with *B. astaci*, as has been shown in previous studies (Quaglio *et al.*, 2006), most likely the result of consumption of gill tissue by the adult worms. Melanisation results from release and activation of the enzyme phenoloxidase, induced by the presence of microbial products or tissue damage by mechanical wounding, and also possibly due to enzymes released from pathogens (Cerenius & Söderhäll, 2004; Cerenius *et al.*, 2008). The increased presence of melanisation observed in parasitised individuals exposed to high SS concentrations, may result from sediment particles causing mechanical injury to gill tissues which were already compromised by the action of the parasite (Cerenius & Söderhäll, 2004).

Crayfish that accumulated high levels of sediment within their gills showed greater decline in aerobic scope compared to unaffected crayfish. Sediment particles coating the filaments presumably reduced the gill surface area effective for gas exchange, and thus limited the maximum oxygen uptake capacity of individuals with heavy fouling. Scope reduction did not reflect the amount of melanised gill tissue in the present study. A similar study, in which crayfish were exposed to lead, showed impairment of oxygen uptake in *P. clarkii* due to gill damage (Torreblanca *et al.*, 1987). Oxygen uptake capacity in decapods is dependent on several factors including ventilation rate, gill area, diffusion distance, and haemolymph capitance (Harrison & Humes, 1992). Inefficient or damaged gills may induce functional hypoxia to which crayfish are able, up to a point, to compensate for using a variety of measures such as increased ventilation rate, increased diffusive conductance of the gill (Wheatly & Taylor, 1981), and also bradycardia in some cases (Reiber & McMahon, 1998). However, the impacts of gill fouling were evident when crayfish were under increased oxygen demands, as is the case during intense activity.

The overall fitness consequences of reduced scope reflect limitations of energy available for different activities. An individual's maximum scope may only be required at times of extreme oxygen demand such as during rapid bursts of activity to escape from predators, or to compete with compatriots during challenges (Brown *et al.*, 2004). However, if reduced enough it will affect all aspects of the individual's performance as less energy can be devoted to feeding, growth and reproduction (Fry, 1947; Nilsson *et al.*, 2009). In salmonids it has been suggested that at least 60-75% of the aerobic scope is required to avoid daily metabolic constraints and increased risk of mortality (Priede, 1977; Priede, 1985; Evans, 2007). Crayfish are most active at night, coinciding with oxygen declines during the diurnal cycle in freshwater systems when respiration outweighs photosynthesis (Staehr *et al.*, 2012; Christensen *et al.*, 2013). Further, as poikilotherms, crayfish activity is strongly positively related to temperature (see Chapter Eight), which again coincides with seasonally low oxygen concentrations (Rösner *et al.*, 2012; Bouffard *et al.*, 2013). Crayfish may therefore suffer oxygen limitation during peaks of activity; a situation that would be exacerbated by reduced scope through gill fouling. This is likely to be most severe in lentic systems because, as indicated by recent morphological evidence of phenotypic plasticity, the branchial chambers of *A. pallipes* from lentic waterbodies have a larger volume than those from lotic systems, an inferred adaptation to environmental oxygen constraints (Haddaway *et al.*, 2012a).

There was no clear evidence of parasites exacerbating the negative impact of SS on crayfish fitness or mortality. This would be expected when parasites increase the host's susceptibility to a stressor, thereby causing disproportionate increases in mortality or loss of fitness (Lafferty & Kuris, 1999). Parasites may interact with environmental stressors in a variety ways, including the converse relationship whereby a stressor reduces the immunological capabilities of hosts making them more susceptible to parasitism, as shown for bivalve molluscs (McDowell *et al.*, 1999). Recognition of the cumulative effects of multiple stressors on organism health, and ecosystems more generally, is increasing (Marcogliese & Pietrock, 2011), though this topic remains largely unaddressed for crayfish.

The mean factorial scopes in this study (3.3 to 5.7) corresponded to the range quoted for the majority of crustaceans, i.e. 3 to 5 (Adamczewska & Morris, 1994). Values were lower than the only known previous study of crayfish aerobic scope that recorded a mean factorial scope of 12.4 (at 15 ˚C) for adult *P. leniusculus* (Rutledge & Pritchard, 1981; Wheatly & Taylor, 1981), which is comparable to aerobic scopes reported for the most active of salmonid fish, for example, 12.5 for sockeye salmon (*Oncorhynchus nerka*) (Brett, 1964), and 7 – 8.5 for pink salmon (*Oncorhynchus gorbuscha*) (Clark *et al.*, 2011). Crayfish, in contrast to fish, have an open circulatory system with generally less efficient gill arrangement and lower oxygen carrying capacity (Rutledge & Pritchard, 1981). Crayfish exhaustion protocols differed between the current and previous study; Rutledge & Pritchard (1981) employed a forced swimming method and measured oxygen consumption concurrently for longer periods, which was not applicable to the current study because it did not identify the point of exhaustion. However, they found that consumption rate peaked within the first 5 min of forced activity, which is comparable with the range of times taken to reach exhaustion in the current study

Although crayfish in this study accumulated sediment in time averaged concentrations at 42 mg L^{-1} and above, it is not clear whether this fouling would persist between moults. Moulting has been hypothesised as a means to escape gill fouling in crayfish (Bauer, 1998), so the impacts on aerobic scope may only persist until the next moult; however, in habitats where SS inputs are chronic and prolonged, lowered scope could be an almost constant state. Further work is required to elucidate on the role of ecdysis for escaping fouling in crayfish.

Estimates of SMR indicated that juvenile *P. leniusculus* has higher basal energy requirements than *A. pallipes*. SMR denotes the minimum energy requirements for life, excluding all non-essential activity and the specific dynamic action of digestion, and can be indicative of several life history traits and behaviours (Sih, 2004). Although indicative of higher energy costs, higher SMR is commonly associated with faster growth rates and dominance (Metcalfe *et al.*, 1995; Biro & Stamps, 2010; Burton *et al.*, 2011). For example, Brown *et al.* (2003) found resting metabolic rate a key determinant of outcomes of aggressive interactions between prawns. Higher SMR in *P. leniusculus* compared to *A. pallipes* is indeed consistent with known differences in life history traits between the species such as higher growth rates in *P. leniusculus* (Guan & Wiles, 1999), higher predatory functional response (Haddaway *et al.*, 2012), and dominance during interspecific interactions (Holdich *et al.*, 1995a). Such life-history traits are also frequently cited explanations for the invasive capabilities of *P. leniusculus*, and other invasive crayfish (Gherardi *et al.*, 2002; Gherardi & Daniels, 2004; Pintor & Sih, 2009).

The current study suggests that although the crayfish species investigated are reasonably tolerant of periodic chronic exposure to suspended solids, the structure and function of gills is impaired at high SS concentrations. Water quality decline with respect to sediment loadings is likely to further exacerbate the effects of the invasive species on the decline of the white clawed crayfish, which tends to occur in less turbid waters (Trouilhe *et al.*, 2003). When formulating appropriate relocation strategies for white-clawed crayfish, the findings of the current study suggest that SS concentration within potential receptor sites should be considered before selection and a concentration not exceeding 62 mg $^{-}$L is recommended. This is particularly important for lentic, as opposed to lotic, Ark sites because crayfish are likely to be subject to greater oxygen constraints in these systems. The presence of high and chronic SS inputs may negatively affect population fitness, though sites with periodic and low SS inputs may still be considered suitable.

Chapter 7 Feeding behaviour and predatory functional responses of two invasive freshwater decapods

7.1 Summary

The invasive signal crayfish (*Pacifastacus leniusculus*) and Chinese mitten crab (*Eriocheir sinensis*) are large benthic decapods that increasingly co-occur in freshwater systems. The feeding ecology of these two decapods was investigated using a combination of laboratory prey choice experiments, gut analyses and stable isotope analyses of field caught individuals. Predatory strength was also compared with a native crayfish species using predatory functional responses, and a mesocosm experiment was used to investigate community impacts. *P. leniusculus* and *E. sinensis* consumed a similar range food items, including the eggs of two coarse fish species. Juvenile *E. sinensis* had a greater predatory strength than the native crayfish *Austropotamobius pallipes* on the keystone shredder *Gammarus pulex*, and also displayed a greater preference for this prey item than did *P. leniusculus*. In mesocosms, the abundance of amphipods, isopods, chironomids and gastropods declined in the presence of decapods, though a decapod>gastropod>periphyton trophic cascade was only detected when both species were present. *E. sinensis* impacted a wider range of animal taxa than *P. leniusculus*, and gut contents and stable isotope analysis of adults highlighted the importance of shredders in its diet. In contrast, *P. leniusculus* was more reliant on allochthonous detritus and algae. *E. sinensis* is likely to exert a greater per capita impact on the macro-invertebrate communities in invaded systems than *P. leniusculus,* with potential indirect effects on productivity and energy flow through the community.

7.2 Introduction

Human-mediated introduction of invasive species is one of the most widespread and deleterious threats to freshwaters (Sala *et al.*, 2000; Leprieur *et al.*, 2009). Invaders exert multiple impacts on ecosystems, ranging from individual effects, such as reduced growth or mortality of native species, to profound changes in community structure and ultimately ecosystem functioning (Parker *et al.*, 1999). Trophic interactions are a key mechanism by which invaders influence communities. Invaders may compete with native species for food resources and are often more successful competitors due to r-selected traits such as boldness, faster growth rate and higher fecundity (Williamson & Fitter, 1996b; Karatayev *et* *al.*, 2009). Invaders can reduce the abundances of native species by direct predation and through a variety of mechanisms may exert a stronger predation pressure than functionally equivalent native predators (Noonburg & Byers, 2005; Salo *et al.*, 2007). Invaders may exert stronger predation pressure if they occur at higher densities, and may exert a stronger per capita effect on prey than native species. For example, naïve native prey may not exhibit effective predator avoidance strategies for the exotic predator as they would for a native predator with which they have co-evolved (Diamond *et al.*, 1989). Further, invasive predators may capture or handle prey more effectively, potentially resulting in stronger impacts on prey abundance (Haddaway *et al.*, 2012b).

Freshwater food webs are often complex with high connectance (Polis & Strong, 1996; Woodward *et al.*, 2005), and predation by invaders at one trophic level can cause cascading effects. In a top-down example, brown trout (*Salmo trutta*) introduced to New Zealand streams excluded or reduced the native galaxiid fish and exerted a stronger predatory pressure on grazers causing an overall increase in algal biomass (Townsend, 1996). In addition to structural ecosystem effects such as changes to species abundances and community composition, invaders may fundamentally affect ecosystem processes including energy flow and nutrient cycling (Crooks, 2002). Ecosystem engineers mediate such changes through modifying their physical environment. For example, invasion of lake systems by the zebra mussel (*Dreissena polymorpha*) results in a net redirection of nutrients from open water toward the benthos (Hecky *et al.*, 2004). This occurs because the mussels filter large quantities of plankton from the water column causing increased light penetration and therefore associated increases in macrophyte growth; and may cause functional changes through forcing zooplankton to rely more heavily on allochthonous basal sources (Maguire & Grey, 2006). Concurrently, the mussels create a shell-bed habitat for the macro- and meiofauna feeding on their excreted wastes

The invasive signal crayfish (*Pacifastacus leniusculus*) and Chinese mitten crab (*Eriocheir sinensis*) are large benthic decapods which have developed widespread introduced distributions and are both listed within the top 100 worst invaders (Lowe *et al.*, 2000). *P. leniusculus* has spread rapidly through Europe since its introduction for aquaculture, largely extirpating and replacing native crayfish populations through its role as a vector of *Aphanomyces astaci*, the cause of crayfish plague which is lethal to European crayfish (Alderman *et al.*, 1990). As an omnivorous keystone consumer and ecosystem engineer, *P. leniusculus* has the potential to modify communities through trophic interactions (Nystrom *et al.*, 1996; Crawford *et al.*, 2006) and physical changes such as increased sediment transport and turbidity due to burrowing (Harvey *et al.*, 2011; Johnson *et al.*, 2011). Studies of invaded systems indicate that *P. leniusculus* causes a reduction in the biomass and species richness of macrophyte and macro-invertebrate communities (Stenroth & Nystrom, 2003; Crawford *et al.*, 2006), with an accompanying shift towards predation resistant (e.g. sediment-dwelling) taxa (Nystrom *et al.*, 1999). Other impacts include predation on the emerging fry of commercially important fish (Edmonds *et al.*, 2011), and on the eggs and tadpoles of amphibians (Axelsson *et al.*, 1997).

E. sinensis is native to eastern Asia, though has spread to Europe and more recently North America. Unlike *P. leniusculus* which completes its lifecycle entirely within freshwater, *E. sinensis* is catadromous whereby reproduction and larval development occurs in estuarine waters, with the main growth phase (~3 years in Europe) in freshwater (Panning, 1939; Gilbey *et al.*, 2008). Due to its larval estuarine phase, ships' ballast is considered the main vector of introduction (Dittel & Epifanio, 2009), although recent spread has in part been blamed on deliberate releases for commercial exploitation (Cohen & Carlton, 1997). Global concerns regarding *E. sinensis* derive from its burrowing activities which undermine river banks and flood defences causing huge economic cost (ca. € 80 million since 1912 in Germany alone) (Gollasch, 2006), and also from interference with commercial fishing operations due to bait interference and clogging of fishing gear (Veldhuizen & Stansih, 2002). Few studies have addressed the ecological impacts of *E. sinensis* either through its potential role as an ecosystem engineer, or through trophic interactions. In common with *P. leniusculus*, *E. sinensis* is described as an opportunistic omnivore and consumes a range of items including macrophytes, algae, detritus, aquatic invertebrates and small fish (Rogers, 2000; Rudnick & Resh, 2005; Dittel & Epifanio, 2009; Czerniejewski *et al.*, 2010). In a study of *E. sinensis* in the freshwater tributaries of San Francisco Bay, diet was found to be dominated by plant material, predominantly algae, though macro-invertebrates, particularly gastropods, were heavily preyed upon in mesocosms which suggests a strong preference for this prey type when available (Rudnick & Resh, 2005).

The mechanisms by which invaders influence ecosystems are frequently complex and interlinked with other stressors, and vary greatly between species; therefore assessing invader impacts is often difficult (Ruiz *et al.*, 1999; Simberloff *et al.*, 2013). The accelerating rate of biological invasions means invaders increasingly co-occur; hence many ecosystems already support several sympatric invasive species, with potentially complex interactions (Simberloff & Von Holle, 1999; Jackson & Grey, 2013). Classic competition theory supposes that where resources are limited, sympatric invaders with dietary overlap will be subject to interspecific competition and suffer associated reductions in fitness and/or competitive exclusion (Sale, 1974; Facon *et al.*, 2006). Others argue that the plastic life traits and high adaptability that make invaders so successful will limit competitive interactions, thereby enabling them to coexist with resultant additive impacts on the existing community (Preston *et al.*, 2012). Despite such complexities, predicting invader impacts and making quantitative comparisons between species is crucial for effectively targeting the limited resources available for management and control (Parker *et al.*, 1999; Keller *et al.*, 2011). To disentangle the effects of multiple invaders in an ecosystem it is necessary to quantify impacts (e.g. predation) for each species separately, but also in combination with other potential invasive competitors to detect potential niche shifts and behavioural plasticity.

Although their life histories are quite different, *P .leniusculus* and *E. sinensis* increasingly overlap as they undergo range expansion. This is the case in the UK where advancement of *E. sinensis* inland has created three known overlap zones with *P. leniusculus*, which presently occurs in 83 % of sub-catchments in England and Wales (Rogers & Watson, 2011) (Figure 7.1). Further, the extent of overlap is likely to be far greater than this due to underreporting of *E. sinensis* occurrence (Mitten Crab Recording Project, 2013). No study has yet compared the feeding ecology of the two species, and for *E. sinensis* we currently lack any quantitative data on feeding rates, both of which hinder the reliable assessment of potential impacts (Ojaveer *et al.*, 2007).

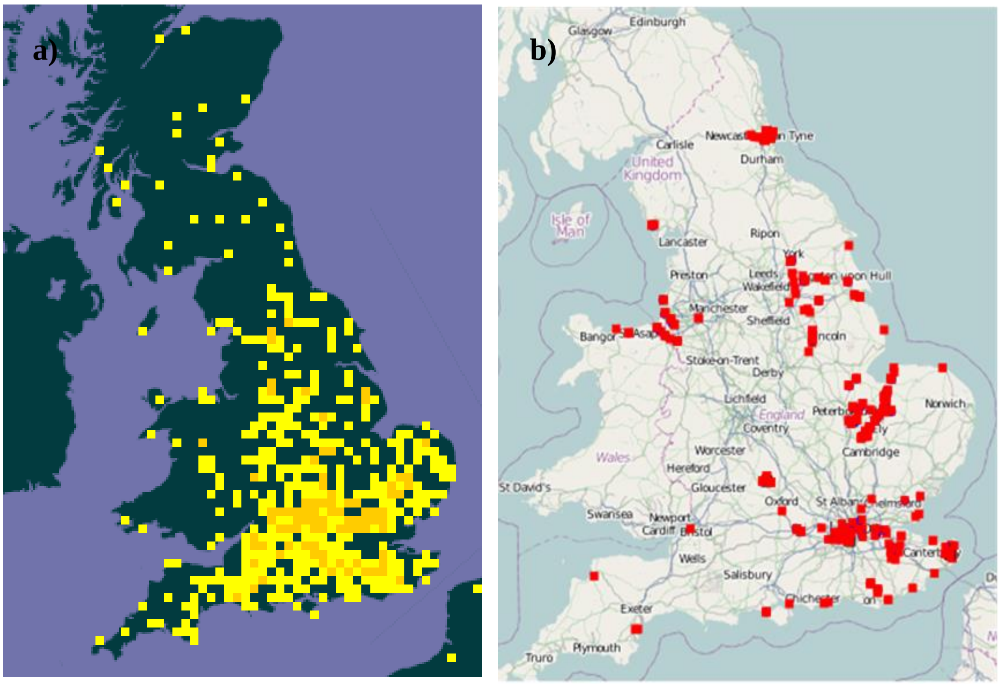


Figure 7.1 UK distributions of a) *Pacifastacus leniusculus* (Global Biodiversity Invasions Facility, 2013) and b) *Eriocheir sinensis* (Mitten crab recording project, 2013).

This study had 3 main aims. First, to compare the dietary preferences and feeding habits of *P. leniusculus* and *E. sinensis* using a combination of laboratory prey choice experiments, along with gut analyses and stable isotope analysis of field samples, which provides a longer term indication of food sources than the snapshot provided by gut analyses of animals at the time of capture. Second, to quantify the predatory impact of *P. leniusculus* and *E. sinensis* on a key prey species using predatory functional responses, i.e. the relationship between prey density and prey consumption by a predator (Holling, 1959; Bollache *et al.*, 2008) determined through laboratory experiments, and compare with that of the native crayfish species *A. pallipes*. Third, to examine the effects of *P. leniusculus* and *E. sinensis* on freshwater communities using a field mesocosm experiment. Mesocosms provide a more realistic representation to the natural environment than laboratory experiments, but still with a level of control and replication difficult to obtain in the field. This combination of approaches was used with a view to reduce the overall bias in results created by the inherent limitations of each, and therefore strengthen interpretation.

7.3 Methods

7.3.1 Prey choice, functional response and mesocosm studies

7.3.1.1 Study organisms

Juvenile invasive *E. sinensis* and *P. leniusculus* were collected from various locations in south-eastern UK and combined to form a laboratory stock used in all laboratory and mesocosm experiments. *E. sinensis* were collected from both the River Thames at Chiswick Eyot (51°29'13.97"N, 0°14'44.81"W) located 17.2 km downstream of the tidal limit, using hand-search at low tide; and from the tidal limit of the river Blackwater at Beeleigh (51°44'34.31"N, 0°39'41.85"E) as by-catch within an elver trap used for monitoring. *P. leniusculus* were collected from freshwater reaches of the River Pant (51°55'28.14"N, 0°31'16.59"E), and the nearby River Glem (52° 5'33.44"N, 0°41'36.69"E) using hand-search. Juvenile native *A. pallipes* were collected under license from Natural England (20122661) from Adel Beck, UK (53° 51' 20.80", -1° 34' 29.91") using hand-search, and were returned to the collection site after completion of the study. The size of *E. sinensis* ranged 20.4 to 30.5 mm carapace width, 3.6 to 10.82 g wet mass (WM), which corresponds to sexually immature juveniles of less than 2 years age (Dittel & Epifanio, 2009). *P. leniusculus* ranged from 19.2 to 32.7 carapace length, 3.4 to 10.6 g (WM), corresponding to sexually immature crayfish of less than 2 years (Guan & Wiles, 1999). *A. pallipes* ranged from 25.2 to 28.3 carapace length, 5.1 to 6.8 g (WM), corresponding to 2-3 year old juveniles (Pratten, 1980).

After collection, animals were transferred to aquaria (38 L) filled with dechlorinated tap water and maintained in the laboratory (17˚C, 16 h light: 8 h dark) on a combination diet of crab pellets (Hinari) and algal wafers (King) for a minimum of 3 weeks prior to the start of experiments. Species were maintained separately, with up to 8 animals per tank. Shelters in the form of PVC pipe sections were provided to reduce aggressive interactions and risk of injury. To ensure animals had experience of encountering all the freshwater prey types to be offered in experiments, a kick sample collected from Meanwood Beck, UK (53° 49' 51.60", -1° 34' 37.19") was added to each tank weekly. Allochthonous detritus (20 g) as leaves of beech (~80%) and alder (~20%) soaked for over 2 months, and fresh algae (*Cladophora* sp.) (5g) were also added to each tank every week.

7.3.1.2 Collection of prey items

Macro-invertebrates were collected from local streams and ponds using a combination of kick sampling, hand-searching and sweep netting, with the exception of chironomid larvae which were purchased live from a pet retailer. After collection, invertebrates were transported to the laboratory and sorted into taxa which were maintained separately in aerated aquaria (8 L) until required.

The eggs of two common UK freshwater coarse fish species, roach (*Rutilus rutilus*) and common bream (*Abramis brama*), were obtained from Calverton Fish Farm, UK, which is operated by the Environment Agency for restocking purposes. Eggs were collected on the day following fertilisation and were retained on the spawning medium (Matala filter mat, CA, USA) in aerated water (17˚C) until use (<3 days).

7.3.1.3 Prey choice experiments

The prey choices of *E. sinensis* and *P. leniusculus* were compared using four mobile prey items widespread in UK freshwaters: *Gammarus pulex* (amphipod), *Asellus aquaticus* (isopod), *Radix peregra* (gastropod) and bloodworm (unidentified chironomid larvae) (Figure 7.2).

Figure 7.2 Prey items used within mobile prey choice experiment, from left to right: chironomid larva; *Gammarus pulex; Radix peregra,* and *Asellus aquaticus* (photo courtesy of Chris Wing)

Size matched juvenile *E. sinensis* and *P. leniusculus* (5 ± 0.4 g, WM) were isolated in individual aquaria (8L) filled with 2 L water (50 mm depth), aerated via an air stone and maintained at 17˚C, 16 h light: 8 h dark. The sides of the aquaria were covered in black plastic to reduce stress and promote foraging. Decapods were starved for 24 h prior to the start of the experiment at which point 20 individuals of each prey type were added to each aquarium, with one prey type per corner of the tank. To reduce the possibility of total prey depletion, the experiment lasted 4 hours in light conditions to limit the activity of both decapod species as they are predominantly nocturnal feeders (Guan & Wiles, 1998; Jin *et al*, 2001). At the end of the experiment, the decapod was removed from each aquarium and the remaining prey items counted. A total of 10 replicates were carried out per treatment group (*E. sinensis* and *P. leniusculus*), along with 5 controls with no decapod present. Each decapod was used only once.

In a second experiment, predation by *E. sinensis* and *P. leniusculus* on the eggs of two species of coarse fish was investigated by means of a simple pairwise choice. *E. sinensis* and *P. leniusculus* (9.5 ± 1.5 g, WM) were isolated and starved as described previously, then 50 eggs of *Rutilus rutilus* and 100 eggs of *Abramis brama* were introduced to aquaria. Twice as many *A. brama* eggs were used because they were approximately half the size of the *R. rutilus* eggs. Due to the fragile and sticky nature of the eggs it was not feasible to remove them from the spawning medium, instead, this was cut into small squares (~6 cm^2), ensuring the appropriate number of eggs were present on each. The experiment ran for 23 h after which the remaining eggs were retrieved and counted. A total of 7 replicates were carried out per treatment group (*E. sinensis* and *P. leniusculus*), along with 7 controls with no decapod present. Each decapod was used only once.

Statistical analysis

For the first experiment, the total number of prey items (all prey types combined) consumed per trial was calculated and the data tested for normality using a Shapiro-Wilk test ($W = 0.97$, $p = 0.67$). One way ANOVA was used to test for differences in total prey consumption between treatment groups, with Tukey's HSD post-hoc test to identify pairwise differences. In a second analysis, data from *E. sinensis* and *P. leniusculus* treatment groups were analysed separately. Number of prey consumed (dependant variable) was modelled as a function of prey type using GLMs with quasibinomial error distribution (lme4 package; Bates *et al.*, 2013), due to the over-dispersion of the data (dispersion parameter 1.93 for *E. sinensis* and 2.47 for *P. leniusculus*). Where significant differences between prey types were detected, all pairwise comparisons were made using Tukey contrasts with single-step adjustment of p values (Multcomp package; Hothorn *et al.*, 2008).

For the second experiment, generalised linear models with binomial error distributions were used to test for a treatment effect (*E. sinensis* or *P. leniusculus*) on the proportion of eggs of each fish species consumed per trial. Plots of standardised residuals against square root of the fitted values and quantile-quantile normal plots were used to assess model fit. Statistical analyses were performed using R v3.0 (R Core Team, 2013)

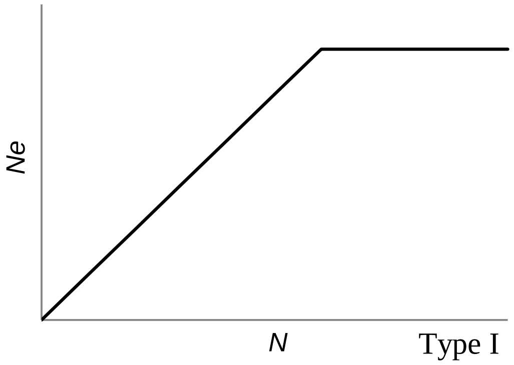
7.3.1.4 Predatory functional response experiments

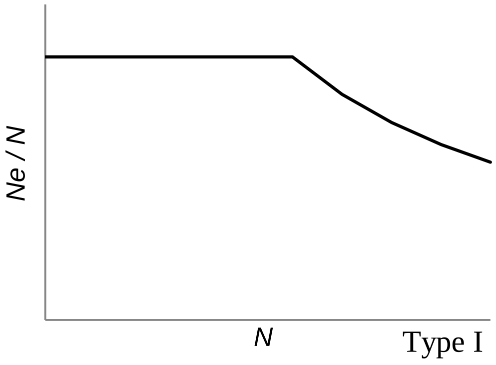
The invasive decapods *E. sinensis* and *P. leniusculus* and the native *A. pallipes* were tested for differences in predatory functional response. Size matched decapods (6 ± 1 g) were isolated in individual aquaria and starved for 24 h, as previously described, before prey was added at approximately 1700 h. A section of plastic pipe (50 mm diameter, 120 mm length) was placed in each aquarium to provide refuge. The prey item chosen was *G. pulex,* a freshwater amphipod widely distributed in both lotic and lentic waterbodies, and the same prey as used in the only previous study investigating predatory functional response in crayfish (Haddaway *et al.*, 2012b). The amphipods were size matched (12 ± 1 mm, TL) to both standardise biomass between trials and reduce cannibalism among *G. pulex* (Dick, 1995). Prey was introduced at 10 different densities (5, 10, 16, 20, 30, 40, 60, 80, 120, and 160), corresponding to densities of 120, 240, 385, 480, 721, 962, 1442, 1923 and 2885 individuals m^{-2}, per treatment group (*E. sinensis* and *P. leniusculus* and *A. pallipes*), with 4 replicates of each, yielding a total of 120 trials. Each trial lasted 24 hours, after which the decapod was removed and the number of intact remaining prey items counted. Five control trials without any decapods were also conducted for each density treatment to assess natural mortality and cannibalism among the prey. In between trials, the decapods were returned to the communal aquaria (38 L) after being marked on the carapace with non-toxic correction fluid to enable identification of individuals. Trials were conducted in a randomised order and each decapod was used between 3 and 5 times for different prey densities; it was necessary to replace animals as they grew beyond the permitted mass range. The mean mass of decapods used in the three treatment groups was 5.62 ± 0.7, 6.14 ± 0.7 and 6.10 ± 0.7 (± S.D) for *E. sinensis*, *P. leniusculus* and *A. pallipes*, respectively, and did not vary between groups (Linear Mixed Effect model; $\chi^2 = 0.67$, $p = 0.41$). Data from individuals that moulted within the 3 days following the experiment were excluded because crabs and crayfish reduce or desist from feeding prior to ecdysis (Zhou *et al.*, 1998; Reynolds & O'Keeffe, 2005).

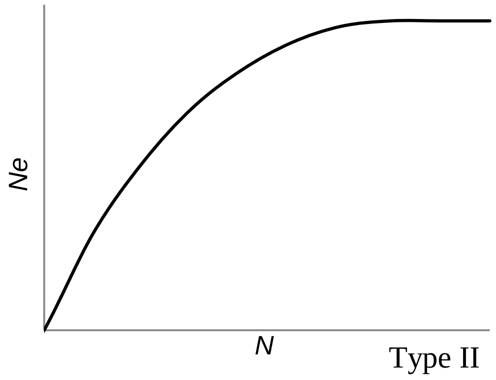
Statistical analysis

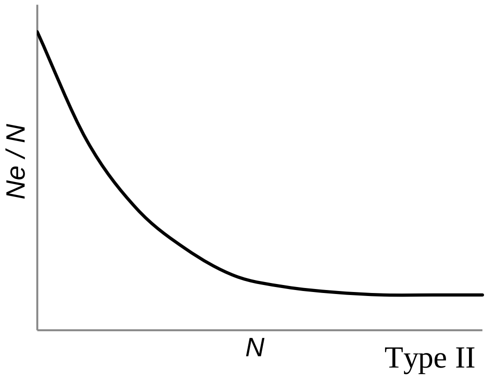
Differences in decapod mass across species groups were tested using linear mixed effects models (LMEs) with identification number as a random factor because individuals were used multiple times across densities. A chi-squared test was used to detect a significant difference in log likelihoods between models with and without species as an explanatory variable.

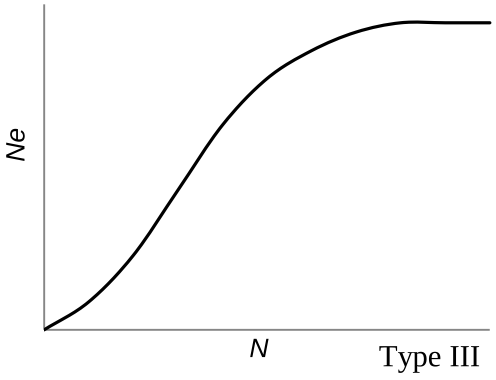
To compare quantitatively the functional responses between predators it was first necessary to determine whether consumption data for each species conformed to a type I, type II or type III functional response. GLMs with binomial error distributions were used to identify the relationship between the proportion of prey eaten and prey density supplied, for the 3 decapod species separately. A Type I functional response is characterised by a linear increase in consumption as prey density increases, up to a point beyond which consumption rate remains constant, therefore the initial linear coefficient (β_1) in the model may be positive or negative and will not be significantly different from zero. In a Type II response the consumption rate decelerates with increasing prey density, therefore β_1 will be significantly negative. In a Type III response, at low prey densities consumption rate increases more than density increases i.e. accelerates, up to a point at which it decelerates with increasing prey density, therefore β_1 will be significantly positive (Figure 7.3). All 3 decapod species were found to exhibit a Type II functional response.


Ne
N
Type I


Ne / N
N
Type I


Ne
N
Type II


Ne / N
N
Type II


Ne
N
Type III

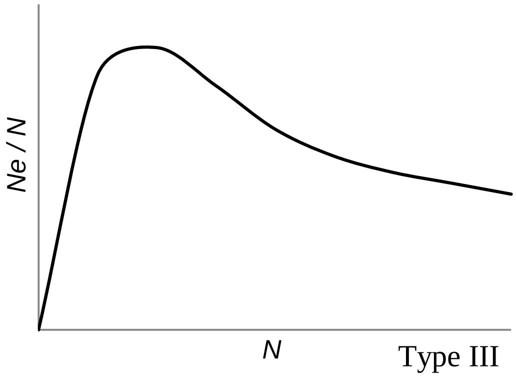

Ne / N
N
Type III

Figure 7.3 Relationship between the number of prey eaten (*Ne*) and density of prey supplied (*N*) for the three types of functional response (I, II and III) (adapted from Murdoch, 1972).

Functional response data for each species were then modelled to obtain coefficients of a (attack rate) and h (handling time) using Rogers random predator equation (Rogers, 1972), modified with the Lambert W function:

$$\boldsymbol{N = N_0 - \left(\frac{W(ahN_o e^{-a(-hN_o)}}{ah}\right)}$$

Where N is the number of prey eaten, N_0 is the number of prey supplied, a is attack rate, h is handling time and W is the Lambert W function (Bolker, 2008). This model incorporates decreasing prey density during the period of each trial as prey are depleted and not replaced. Data were bootstrapped (n = 1500) and 95% confidence intervals for a and h calculated using R package Frair (Pritchard *et al*, 2013).

7.3.1.5 Outdoor mesocosm experiment

To compare the impact of *E. sinensis* and *P. leniusculus* on the littoral community an outdoor mesocosm experiment was conducted between 26th June and 24th July 2012 using circular plastic pools (0.78 m^2, , 0.65 m depth) (n = 16) sunken into a meadow at the University of Leeds Field Research Unit, UK. Biosecurity measures such as weed suppression and newt fencing around the ponds were implemented following consultation with Cefas. Pools were tightly lined with polyester netting (0.9 mm mesh), to aid recovery of invertebrates at the end of the experiment, and the bases were covered with a mix of pure sand and dried loam soil (60:40) with a sporadic covering of stones (~20 – 30 mm, long axis) to provide both a substrate for the macrophytes and increase habitat complexity. Pools were then filled with groundwater to a depth of 0.5 m (0.39 m^3) and 1 L aliquot of lake water was added to each pool to seed the zooplankton community.

Macrophytes, *Ceratophyllum demersum* (55 g wet mass), *Callitriche stagnalis (*15 g), *Potamogeton perfoliatus* (25 g) were planted in the pools on 17th June. Filamentous algae (*Cladophora* sp., 8 g), allochthonous vascular detritus (100 g, 80:20 beech and alder, soaked for over 2 months) and periphytic algae colonised for six weeks on two ceramic tiles (16 cm^2 surface area each) were also added to each pool to test the effects of *P. leniusculus* and *E. sinensis* on basal resources. All macrophytes, algae, detritus and ceramic tiles were hand cleaned of invertebrates prior to adding them to the pools.

Macro-invertebrates representing a range of functional feeding groups were added to each pool in abundance ratios approximating those witnessed during collection. The community added to each pool comprised: 27 Mollusca (8 *Radix peregra* (7 of size ~11mm, longest axis, and 1 of size ~16 mm, longest axis); 2 *Lymnaea stagnalis* (~23 mm, longest axis); 17 *Physa fontinalis* (12 of size ~6 mm, longest axis, and 5 of size ~3.5 mm longest axis)); 5 Trichoptera (Limnephilid sp.); 56 Isopoda (*Asellus aquaticus*); 135 Amphipoda (*Gammarus pulex*), and 500 bloodworm (unidentified chironomid larvae). Macro-invertebrates were added four days prior to start of the experiment to allow them to acclimate to conditions in the pools in the absence of decapod predators.

Decapods were added to the pools within three treatments: *E. sinensis*, *P. leniusculus*, and both *E. sinensis* and *P. leniusculus*, in addition to a no decapod control. Pools were assigned using a randomised-block design with 4 replicates in each treatment group and control. Four juvenile individual decapods were assigned to each treatment pool; the mixed treatment comprised two individuals of each species. *E. sinensis* ranged from 13 to 22 mm in carapace width (19.14 ± 1.84; mean ± SD) and *P. leniusculus* ranged from 19 and 26 mm in carapace length (23.11±1.20; mean ± SD). Sex ratios were 50:50 in all pools. Total decapod biomass ranged from 19.5 to 22.9 g across all treatment pools and did not vary between treatments ($F_{2,9} = 0.75$, $p = 0.50$). Eight sections of PVC pipe (2 x 50 mm diameter, 120 mm length; 6 x 25 mm diameter, 80 mm length) were added to each pool to serve as refugia with a view to reducing aggressive interactions and post-moult cannibalism. After addition of the decapods, the lining nets were closed using cable ties and pools were covered with Enviromesh^{R} (Agralan, Swindon, UK) secured with shock cord to prevent animals escaping and disturbance by birds.

The experiment lasted four weeks. Pools were checked after two weeks for decapod mortalities and evidence of moult. Moults were removed if found, and water temperature was also measured. Sub-surface water samples were collected at the end of the experiment for subsequent laboratory analyses of nitrate, sulphate, phosphorus, sodium, potassium, chloride, magnesium and calcium.

At the end of the experiment the ceramic tiles were removed and placed in black polythene bags to be frozen for later analysis. The decapods were collected and the net linings were removed from each pool and transported to the laboratory in plastic bags. Macrophytes and algae were isolated and hand cleaned of macro-invertebrates. The remaining sediment and gravel in each bag was repeatedly washed to rinse off any macro-invertebrates, algae, detritus and macrophyte fragments, which were recovered using a net (1 mm mesh size).

Sediment, gravel, lining nets and polythene bags were then carefully inspected to recover any remaining macro-invertebrates and detrital fragments (fragments exceeding ~4 mm were collected). The macro-invertebrates were counted and the macrophytes, algae and detritus were blotted dry and weighed.

Total chlorophyll was used as a proxy measure for the remaining biomass of periphytic algae on the ceramic tiles. Each tile was placed in a sealed, lidded petri dish containing 90% ethanol (20 ml) and soaked overnight to extract the chlorophyll from any algal cells present. The ethanol from each dish was then transferred to a centrifuge tube (50 ml) and the dish rinsed with a further 3 ml of 90% ethanol which was also added to the centrifuge tube. Samples were centrifuged at 4 x 10^4 rpm for 20 minutes to separate the chlorophyll sample from any remaining artefacts (e.g. micro-fragments of detritus or sediment). Samples were first visually checked for clarity, then analysed using a spectrophotometer (Biochrom WPA Biowave II) to measure absorbance at 750nm, 664nm, 647nm and 630nm wavelengths (1cm path length). Total chlorophyll (µg) per tile was calculated as the sum of chlorophyll-a, b and c determined by equations 1, 2 and 3 respectively:

$$Chl-a=\frac{[12.12(\mathrm{D664}-\mathrm{D750})-1.58(\mathrm{D647}-\mathrm{D750})-0.08(\mathrm{D630}-\mathrm{D750})]}{\delta} \tag{1}$$

$$Chl-b=\frac{[-5.55(\mathrm{D664}-\mathrm{D750})+21.51(\mathrm{D647}-\mathrm{D750})-2.72(\mathrm{D630}-\mathrm{D750})]}{\delta} \tag{2}$$

$$Chl-c=\frac{[-1.71(\mathrm{D664}-\mathrm{D750})-7.77(\mathrm{D647}-\mathrm{D750})+25.08(\mathrm{D630}-\mathrm{D750})]}{\delta} \tag{3}$$

where D630, D647, D664 and D750 are the absorbance at the wavelengths of 630, 647 , 664 and 750 nm, respectively, and δ is the optical path length of the cuvette (cm) (Huang & Cong, 2007).

Statistical analysis

Data of measured water chemistry parameters were tested for normality (Shapiro-Wilk test) and normalised using log10+1 transformation where necessary. One way ANOVA was used to test for differences between treatments for each parameter separately.

Data on the remaining biomass of macrophytes, chlorophyll concentration (averaged from 2 tiles) and absolute abundances of macro-invertebrate taxa, excluding *Lymnaea* snails as there were too few individuals for analysis, were tested for normality using Shapiro-Wilk test and were log10+1 transformed where necessary. Levene's test was used to determine compliance with the assumption of homogeneity of variance between groups. One way ANOVA with treatment as a factor and Tukey's HSD post-hoc test was used to detect and identify differences between treatment groups. Kruskal-Wallis with post-hoc Nemenyi-Damico-Wolfe-Dunn tests were used where data could not be normalised.

Four *E. sinensis* individuals moulted during the course of the study, and two *P. leniusculus* individuals in separate pools in the *P. leniusculus* only treatment were missing at the end of the experiment. There was no evidence that the animals had climbed out of the tanks or broken through the netting, so it was assumed that these animals had died (perhaps during moult) and been consumed by the other decapods and detritivores. It was decided not to exclude these pools from the dataset because checks on day 14 revealed that all decapods were still present, hence pools had their intended decapod biomass for at least half the duration of the experiment. Further, preliminary analyses of the data revealed that macro-invertebrate abundance (all species) and macrophyte biomass (all species) of the two pools in question did not differ significantly from other pools within the same treatment.

7.3.2 Stable isotope analysis and gut contents analysis (GCA)

Adult specimens of *E. sinensis* and *P. leniusculus* were collected from two sites on the River Stour, Suffolk, UK, during October and November 2012. The sites were similar in terms of land-use, channel morphometry and riparian vegetation (Figure 7.4). Although both species have long been present in this lowland watercourse (minimum of 9 and 17 years, respectively), they have not yet been encountered at the same location, but have been observed at locations within 22 km (Adam Piper, Environment Agency, pers. comm.). *E. sinensis* were collected immediately upstream of the tidal limit (51°57'17.59"N, 1° 1'32.31"E) and *P. leniusculus* were collected 62 km further upstream (52° 3'31.55"N, 0°29'32.58"E).

Figure 7.4 Field collection sites a) Flatford, where *Eriocheir sinensis* and a range of potential food sources were collected, and b) Wixoe, where *Pacifastacus leniusculus* and a range of potential food sources were collected.

E. sinensis and *P. leniusculus* were captured in Fladen traps baited with a mixture of leaves, bloodworm and sardine in oil, all contained within a nylon mesh (1 mm) and further encased in a box (5 cm^2) constructed of aluminium mosquito mesh to ensure that captured animals could not consume the bait. Traps were checked each morning and any decapods were immediately frozen for stable isotope and gut contents analysis. Collections of potential diet items were made during the same period using a combination of kick sample in the shallows, dredge trawling in deeper water and hand collection of riparian vegetation. All macro-invertebrates were maintained live in distilled water for 24 h to clear their gut contents before being frozen. Plant material was carefully rinsed in distilled water prior to freezing.

7.3.2.1 Stable isotope analysis

To prepare tissue for analysis of isotopes ^{13}C and ^{15}N, a claw from each individual of *E. sinensis* and *P. leniusculus* was freeze dried and the muscle tissue extracted, weighed and placed in tin capsules for combustion. Decapod sample weights ranged from 0.03 to 0.11mg for carbon analysis and from 0.71 to 1.04 mg for nitrogen analysis. All macro-invertebrates were separated into genera, freeze dried and combusted whole, with the exception of gastropods for which only the muscle tissue of the foot was used. Fish were dissected to isolate muscle tissue which was subsequently freeze dried and weighed. All plant material was freeze dried, then immersed in liquid nitrogen and ground to a fine homogenous powder using a pestle and mortar before weighing. Samples of 23 potential diet items were analysed in addition to *E. sinensis* and *P. leniusculus* (Table 7.1).

Table 7.1 Summary of field samples collected autumn 2012 and analysed for stable isotope ratios of ^{13}C/^{12}C and ^{15}N/^{14}N.

Group	Species/details	Flatford *	Wixoe *
Decapods	*E. sinensis*	5	
	P. leniusculus		4
Sediments	Organic-rich sediment	3 (3 locations)	3 (3 locations)
Terrestrial producers	Terrestrial leaves (decaying)	2 (5 leaves, various sp.)	2 (8 leaves, various sp.)
Submerged/ floating producers	*Nuphar lutea*	1 (3 leaves, 1 tuber)	
	Elodea canadensis	1 (2 sprigs)	
	Cladophora sp.	1 (multiple strands)	1 (multiple strands)
	Lemna minor		1 (> 20 thalli)
Marginal producers	*Phalaris arundinacea*		1 (2 stems)
	Sparganium erectum (decaying)	2 (3 stems)	1 (3 stems)
	Rorippa nasturtium-aquaticum		2 (~5 leaves each)
	Myosotis scorpioides	1 (4 leaves)	
Moss	*Fontinalis antipyretica*		1 (4 shoots)
Grazers	*Lymnaea sp.*		3
	Theodoxus fluviatilis	1 (4 individuals)	
Collectors	chironomid larvae	1 (~10 individuals)	1 (~23 individuals)
	Ephemeroptera	1	
Shredders	*Gammarus pulex*	1	1
	Asellus aquaticus	1 (2 individuals)	1 (3 individuals)
	Limnephilidae		1
Predatory invertebrates	Notonectidae	1 (3 individuals)	1 (2 individuals)
	Odonata	1	1 (2 individuals)
Fish	*Rutilus rutilus*	1	
	Perca fluviatilis		1
	Gobio gobio		1

*** Numbers outside of parenthesis denote number of samples analysed for stable isotope ratios, numbers inside parenthesis denote composition of samples in the case of composite samples**

Samples were analysed on an Isoprime continuous flow mass spectrometer coupled to an Elementar Pyrocube elemental analyser. The sample masses for plants and animals were selected to ensure peaks within the 1–10 nA range for carbon, and 2–10 nA for nitrogen. C and N were analysed on separate runs. Stable isotope ratios were expressed in conventional notation as parts per thousand (‰), according to the following equation:

$$\delta X = \left[\left(\frac{R_{sample}}{R_{standard}}\right) - 1\right] x1000$$

where *X* is ^{15}N or ^{13}C and R is the corresponding ratio ^{15}N/^{14}N or ^{13}C/^{12}C. The standards for ^{15}N and ^{13}C are atmospheric nitrogen (VAIR) and Vienna Pee Dee Belemnite (VPDB), respectively. International standards of ammonium sulphate USGS-25 (−30.1‰) and USGS-26 (+53.7‰) for Nitrogen; and ANU-sucrose (-10.47‰) and IAEA-CH-7 (polyethylene film, -31.83‰) for carbon, were interspersed every 8–12 samples to calibrate the system and compensate for any drift over time. Repeats of in-house yeast and sucrose standards produced standard deviations of 0.13 and 0.24‰ for nitrogen and carbon respectively, from 88 analyses.

7.3.2.2 Gut Content Analysis

Frozen decapods were allowed to defrost, and then sex, carapace width/length and wet mass were recorded for each before the foregut was carefully removed by dissection. Foreguts were cut open and the contents were emptied into a gridded Petri dish (~24 squares (25 mm^2), sub-divided into smaller squares (1 mm^2). Any remaining contents were rinsed out into the dish using 70% ethanol (10 ml). The gut contents were then spread out and examined under a dissecting microscope. First, the number of small squares (1 mm^2) with material present were recorded as a percentage of each larger square (25 mm^2). Second, the material in each small square was assigned to one of 7 categories: inorganic; algae; macrophyte; moss; leaves (terrestrial); unidentifiable plant matter and macro-invertebrates. Where possible, the macro-invertebrate fragments were identified to order, and occasionally genera. In total, 5 adult *E. sinensis* (54–87 mm CW), and 10 adult *P. leniusculus* (37–52 mm CL) were examined for gut content analysis.

Statistical analysis

Bayesian stable isotope mixing models (SIAR; Parnell *et al.*, 2008) were used to estimate the relative contributions of the potential food sources sampled to the diet of *E. sinensis* and *P. leniusculus* at the two sites separately. Assumed fractionation factors of 2.4 ± 0.18 ‰ for δ^{15}N and 0.5 ± 0.17 ‰ for δ^{13}C, based on a meta-analysis of studies using non-acidified samples (McCutchan *et al.*, 2003), were used to adjust the isotopic values of food sources. Elemental concentrations of C and N within each of the food sources were also incorporated in the model to account for concentration-dependent variation in fractionation (Phillips & Koch, 2002).

7.4 Results

7.4.1 Prey choice experiments

7.4.1.1 Mobile prey

The total number of prey consumed per trial differed significantly between treatments ($F_{2,22}$= 15.93, $p<0.01$). Prey abundance was reduced significantly more in the *E. sinensis* treatment (mean 41.2 ± 9.1 S.D.) compared to *P. leniusculus* (mean 30.9 ± 9.3 S.D., $p<0.05$). Consumption in the control was less than half the consumption in the presence of the decapods (mean 15.2 ± 3.1 S.D., $p<0.01$), inferring that predation by the decapods was the main source of prey reduction in the *E. sinensis* and *P. leniusculus* treatment groups.

Chironomid larvae were the most consumed prey type in all treatments though all prey types were consumed by the decapods to some extent (Figure 7.5).Due to high consumption of chironomids in the control, it is unclear whether decapods preferred this prey type and consumed large numbers before switching to alternate prey, or that *G. pulex* and *A. aquaticus* consumed up to 75% of the chironomids before they themselves were preyed upon by the decapods (Table 7.2), Not accounting for inter-prey consumption, pairwise comparisons of consumption between prey types in the same treatment group revealed a slightly different hierarchy of prey preference among *E. sinensis* and *P. leniusculus* (Table 7.2). *E. sinensis* consumed prey in the following hierarchy: chironomid larvae> *Gammarus pulex*> *Asellus aquaticus* > *R. peregra*, whereas *P. leniusculus* showed greater preference for the isopod *Asellus aquaticus*, and consumed prey in the following hierarchy: chironomid larvae > *Asellus aquaticus* > *Gammarus pulex*> *R. peregra*.

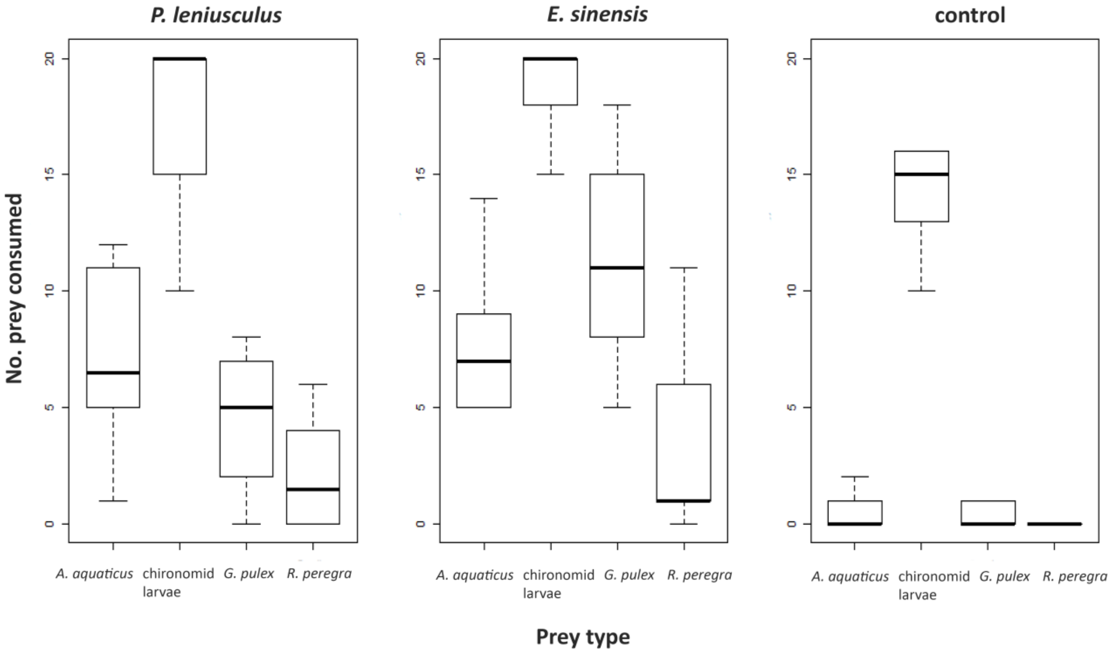


Figure 7.5 Consumption of 4 prey items: *Asellus aquaticus* (isopod), chironomid larvae, *Gammarus pulex* (amphipod) and *Radix peregra* (gastropod), within treatments with decapod predators *Pacifastacus leniusculus* and *Eriocheir sinensis*, and in the control (no decapod). Plots show medians (thick line), interquartile ranges (boxes) and data range (whiskers).

Table 7.2 Pairwise linear comparisons between prey types consumed within *E. sinensis* and *P. leniusculus* treatment groups.

Comparison	***E. sinensis***		***P. leniusculus***	
	Z value	**p**	**Z value**	**p**
chironomid larvae ~ *A. aquaticus*	4.71	**<0.001**	4.748	**<0.001**
G. pulex ~ *A. aquaticus*	1.845	0.2435	-1.604	0.363
R. peregra ~ *A. aquaticus*	-2.895	**0.0186**	-3.355	**0.0041**
G. pulex ~ chironomid larvae	-3.075	**0.011**	-5.86	**<0.001**
R. peregra ~ chironomid larvae	-6.575	**<0.001**	-6.565	**<0.001**
R. peregra ~ *G. pulex*	-4.422	**<0.001**	-1.988	0.183

7.4.1.2 Fish egg predation

There was no difference in consumption between decapod species for either *R. rutilus* or *A. brama* eggs (Z = 0.30, p = 0.76 and Z = -0.72, p = 0.47, respectively). *E. sinensis* consumed between 72 and 100% of *R. rutilus* eggs and between 95 and 100% of *A. brama* eggs in each trial. *P. leniusculus* consumed between 88 and 100% of *R. rutilus* eggs and between 60 and 98% of *A. brama* eggs in each trial. The number of eggs retrieved at the end of trial in the control, varied from the start of the trial by a maximum of ± 2 eggs.

7.4.2 Predatory functional response experiment

7.4.2.1 Functional response

The predatory functional response curve of *E. sinensis* was higher than *A. pallipes*, with a mean asymptote of 44 prey items consumed compared to 28 for *A. pallipes*. The functional response curve of *P. leniusculus* was intermediate between *E. sinensis* and *A. pallipes* with a mean asymptote of 35 prey items. However, the upper 95% confidence interval of the *P. leniusculus* curve overlapped slightly with the lower 95% confidence interval of the *E. sinensis* curve indicating no significant difference between the two species. The lower 95% confidence interval also overlapped with the upper *A. pallipes* confidence interval, similarly indicating no difference in consumption between the species (Figure 7.6).

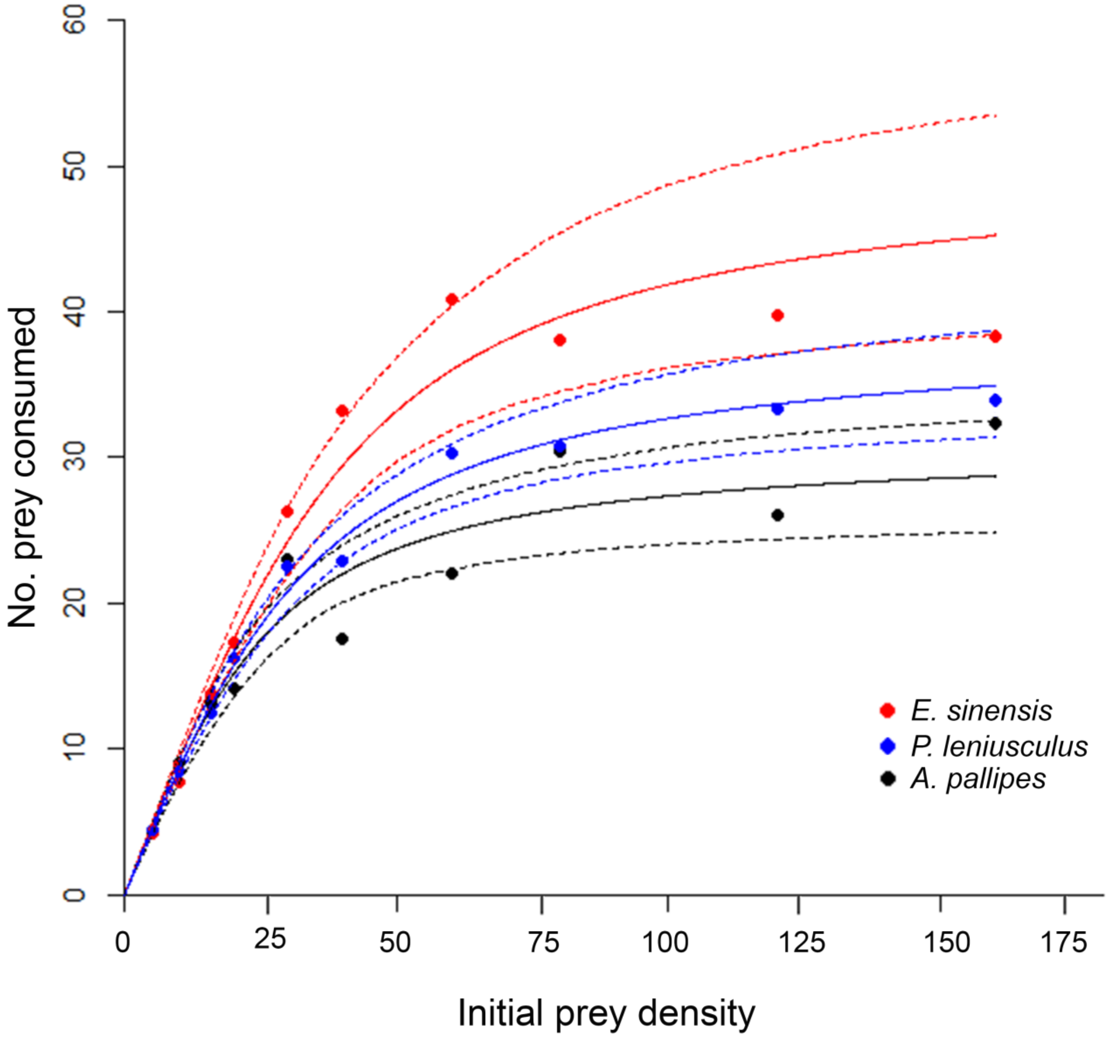


Figure 7.6 Consumption data of three decapod predators *Eriocheir sinensis, Pacifastacus leniusculus* and *Austropotamobius pallipes* preying upon *Gammarus pulex.* Circles denote mean number of prey items consumed at each initial density (4 replicates). Solid curves indicate mean fit of functional response curves (Roger's equation) for each species, dashed curves indicate 95% confidence intervals generated by bootstrapping (n = 1500).

The attack rate did not vary between decapod species, but handling time did. *E. sinensis* exhibited a faster handling time than *A. pallipes*, but handling time by *P. leniusculus* did not differ from the other two decapod species (Figure 7.7).

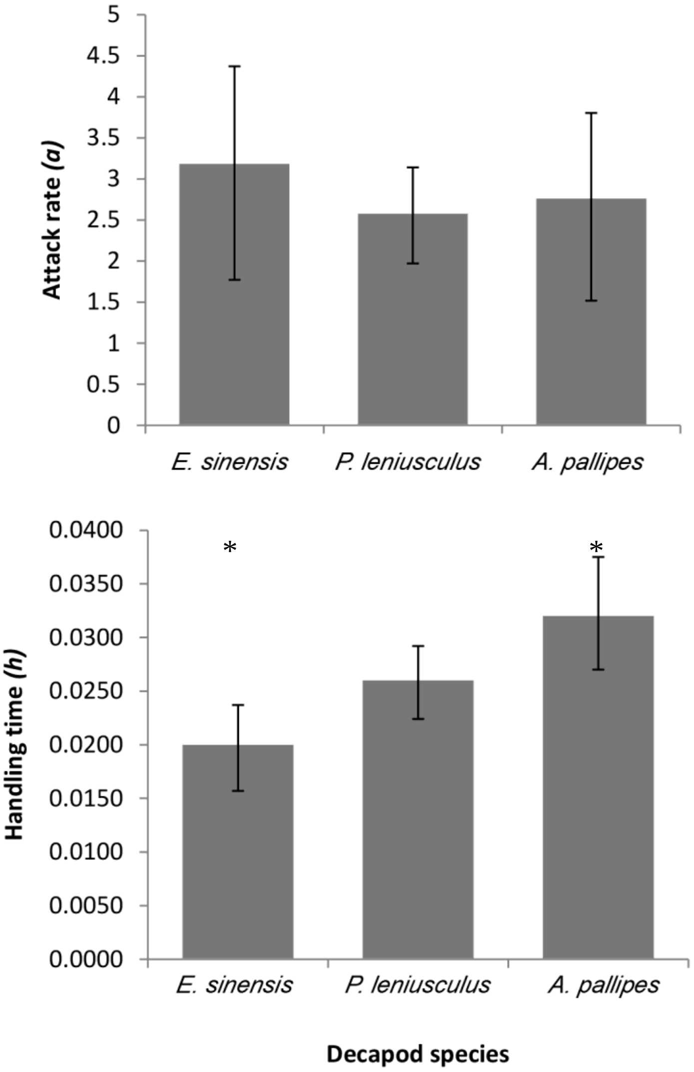


Figure 7.7 Coefficients of attack rate (a) and handling time (h) for 3 decapod predators: E. sinensis, P. leniusculus and A. pallipes derived from Rogers random predator equation. Error bars denote upper and lower 95% confidence limits obtained by bootstrapping (n = 1500), * denotes significant difference between groups (0.05 significance level).

7.4.3 Outdoor mesocosm experiment

7.4.3.1 Basal resources

The mass of allochthonous detritus was reduced by between 16 and 68% in decapod treatments, and between 52 and 74% in the control (Figure 7.8); there was no significant difference in detrital mass among treatments at the end of the experiment (Table 7.3). Change in biomass of filamentous algae (*Cladophora* sp.) varied greatly between pools within treatments (Figure 7.8), with no significant difference between treatments (Table 7.3). Periphyton chlorophyll at the end of the experiment was on average 48% higher in the combined *E. sinensis* and *P. leniusculus* treatment relative to control, but did not differ between other treatments (Table 7.3). There was a general reduction in the biomass of all three macrophyte species during the experiment. *Callitriche stagnalis* was absent from all except four pools (which were within a range of treatments), and so was omitted from further analyses. Remaining macrophyte biomass did not vary between treatments (Table 7.3).

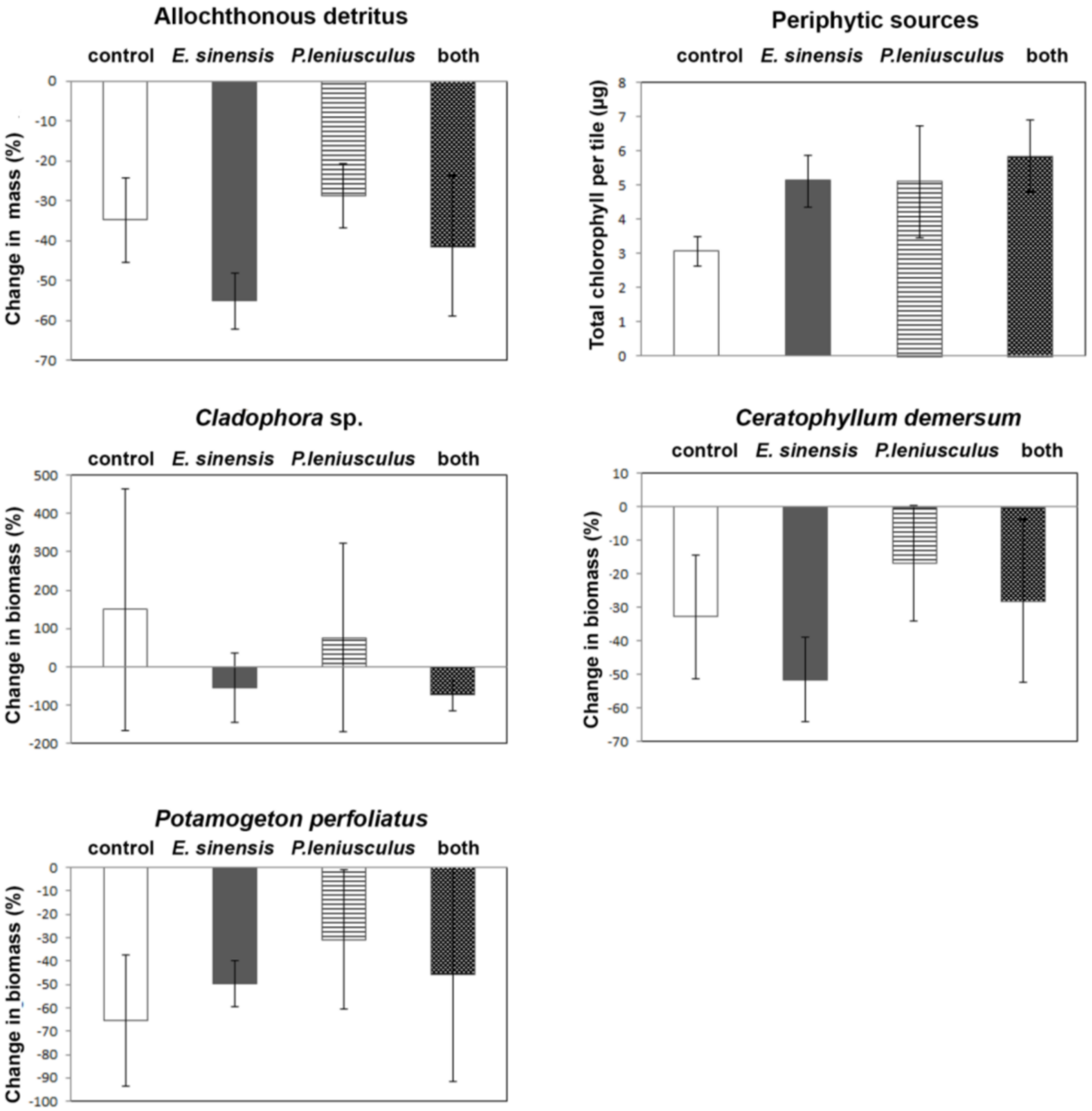


Figure 7.8 Remaining periphytic algae (measured as total chlorophyll per ceramic tile (µg)) and mean percentage change (± S.D.) in mass of allochthonous detritus, filamentous algae (*Cladophora* sp.) and two macrophyte species (*Ceratophyllum demersum* and *Potamogeton perfoliatus*) after four weeks in mesocosm pools (n = 16) within the treatments: *E. sinensis*, *P. leniusculus*, both *E. sinensis* and *P. leniusculus*, and no decapod control.

Table 7.3 One way ANOVA and Tukey's HSD post-hoc tests (or Kruskal Wallis and Nemenyi-Damico-Wolfe-Dunn post-hoc tests where data could not be normalised) comparing the biomass of macrophytes, mass of detritus and abundances of invertebrate taxa remaining in pools (n = 16) after four weeks within the treatments: *E. sinensis* (CMC), *P. leniusculus* (SC), both *E. sinensis* and *P. leniusculus*, and no decapod control.

	Treatment effect test statistic		**Pairwise comparisons**					
			CMC v control	**SC v control**	**both v control**	**CMC v SC**	**CMC v both**	**SC v both**
	F/H$_{3,12}$	**p**	p	p	p	p	p	p
Basal resources								
Potamogeton perfoliatus	0.845	0.495	-	-	-	-	-	-
Ceratophyllum demersum	2.424	0.1163	-	-	-	-	-	-
Cladophora sp.	1.675	0.225	-	-	-	-	-	-
Detrital leaves	0.857	0.49	-	-	-	-	-	-
Periphyton	4.251	**0.0171**	0.0754	0.0795	**0.013**	0.999	0.7683	0.7513
Grazers								
All	8.571	**0.0025**	**0.0018**	**0.0420**	**0.0161**	0.3167	0.6029	0.9456
Radix peregra	12.91(H)	**0.0048**	**<0.05**	**<0.05**	**<0.05**	>0.05	>0.05	>0.05
Physa fontinalis	4.37	**0.0268**	**0.0218**	0.2593	0.0807	0.4711	0.8730	0.8799
Lymnaea stagnalis	n/a							
Shredders								
Gammarus pulex	13.56	**0.0003**	**0.0002**	0.0747	**0.0064**	**0.0208**	0.2201	0.5182
Asellus aquaticus	9.56(H)	**0.0226**	**<0.05**	**<0.05**	**<0.05**	>0.05	>0.05	>0.05
Trichoptera *sp.*	11.76(H)	**0.0082**	**<0.05**	>0.05	**<0.05**	>0.05	>0.05	>0.05
Filterers/collectors								
Chironomid larvae	8.99(H)	**0.029**	**<0.05**	**<0.05**	**<0.05**	>0.05	>0.05	>0.05

7.4.3.2 Shredders

The abundance of *G. pulex* was reduced in both treatments containing *E. sinensis*, relative to the control, not in the *P. leniusculus* only treatment relative to control. The other shredders, *Asellus aquaticus* and larvae of the order Trichoptera (Limnephilid sp.) were substantially reduced in all the decapod treatments relative to the control (Figure 7.9) (Table7.3).

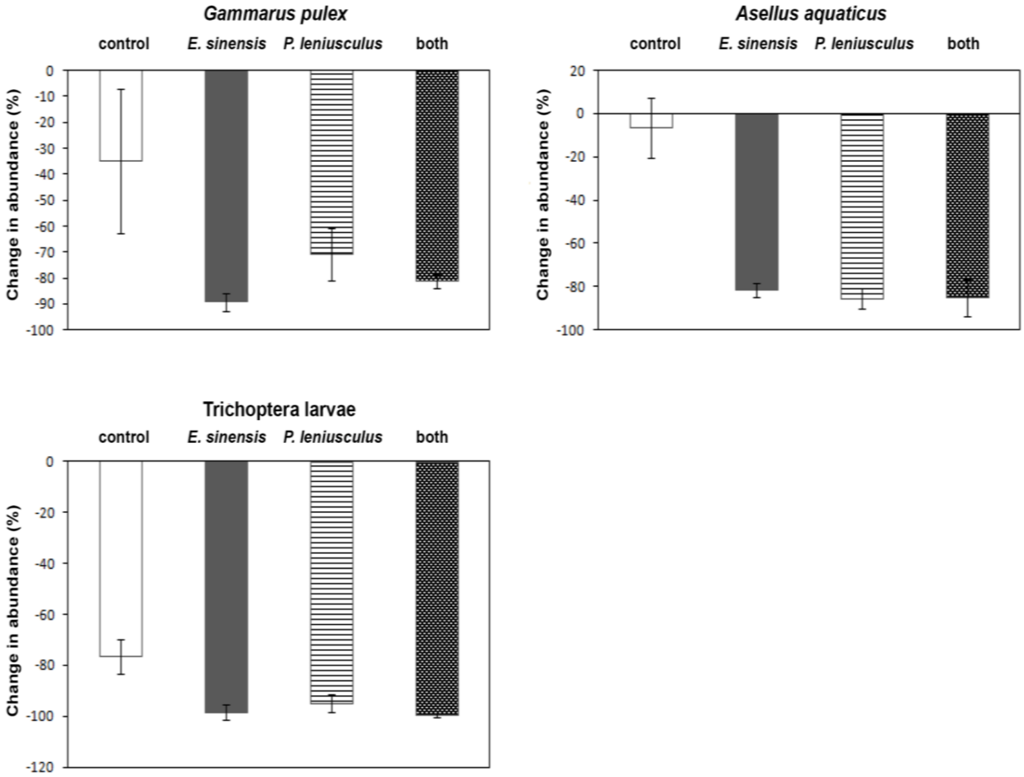


Figure 7.9 Mean percentage change (± S.D.) in abundance of *Gammarus pulex*, *Asellus aquaticus* and Trichoptera larvae after four weeks in mesocosm pools (n = 16) within the treatments: *E. sinensis*, *P. leniusculus*, both *E. sinensis* and *P. leniusculus*, and no decapod control.

7.4.3.3 Grazers and collectors

The abundance of Gastropoda increased in the controls during the experiment, but was significantly reduced in all the decapod treatments with no differences among them (Figure 7.10) (Table 7.3). The abundance of chironomid larvae was similarly reduced in all decapod treatments relative to the control, but did not vary among the three decapod treatments (Figure 7.10) (Table 7.3).

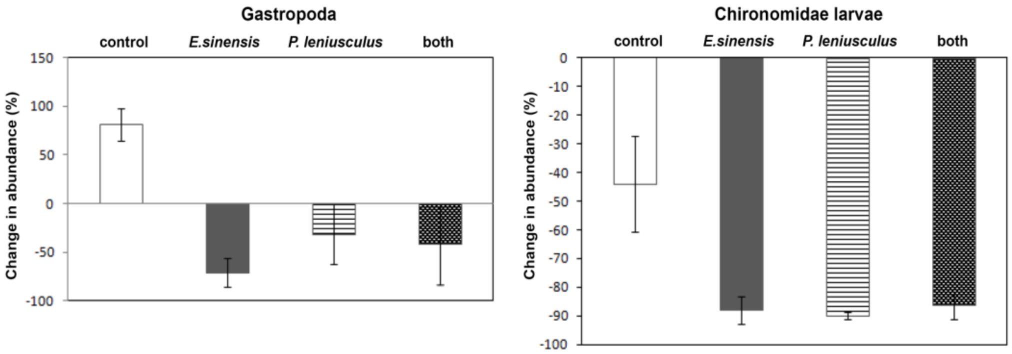


Figure 7.10 Mean percentage change (± S.D.) in abundance of Gastropoda and Chironomidae larvae after four weeks in mesocosm pools (n = 16) within the treatments: *E. sinensis*, *P. leniusculus*, both *E. sinensis* and *P. leniusculus*, and no decapod control.

The Shannon diversity index in each pool at the end of the experiment ranged from 0.44 to 1.44 and did not vary between treatments ($F_{3,12}$= 1.43, p = 0.283). Shannon evenness ranged from 0.28 to 0.86 and also did not vary between treatments ($F_{3,12}$= 0.28, p = 0.839).

Water temperature and the water chemistry parameters measured did not differ significantly between treatments (Table 7.4).

Table 7.4 Mean (± S.D.) water chemistry parameters of mesocosm pools at the end of the experiment, with 4 pools in each of the following treatments: *E. sinensis*, *P. leniusculus*, and both *E. sinensis* and *P. leniusculus;* along with no decapod control; and results of one-way ANOVA to test for differences between treatments.

Treatment	**Temperature (°C)**	**Nitrate (mg L^{-1})**	**Sulphate (mg L^{-1})**	**Phosphate (uM)**	**Sodium (mg L^{-1})**	**Potassium (mg L^{-1})**	**Chloride (mg L^{-1})**	**Magnesium (mg L^{-1})**	**Calcium (mg L^{-1})**
E. sinensis	17.7 (±0.57)	15.2 (±4.7)	30.4 (±9.2)	1.33 (±0.5)	5.2 (±0.9)	0.8 (±0.1)	13.3 (±1.4)	13.5 (±3.1)	13.8 (±8.7)
P. leniusculus	17.9 (±0.46)	24.8 (±13.8)	46.0 (±27.7)	1.9 (±0.9)	9.3(±5.7)	1.3 (±1.0)	22.4 (±13.1)	21.6 (± 11.7)	19.0 (±9.3)
E. sinensis* & *P. leniusculus	18.25 (±0.62)	24.9 (±10.8)	39.2 (±12.4)	2.3 (±2.4)	8.0 (±2.3)	1.2 (±0.4)	20.8 (±6.5)	20.6 (±8.1)	13.8 (±3.3)
Control	17.95 (±0.45)	17.1 (±17.6)	42.7 (±15.1)	2.39 (±1.4)	8.8 (±3.8)	1.8 (±0.9)	20.8 (±12.1)	19.0 (±14.0)	16.2 (±1.8)
ANOVA results	$F_{3,12}$=0.73 p= 0.55	$F_{3,12}$=1.47 p = 0.27	$F_{3,12}$=1.49 p=0.27	$F_{3,12}$=0.56 p=0.65	$F_{3,12}$=3.04 p=0.07	$F_{3,12}$=1.98 p=0.17	$F_{3,12}$=2.79 p= 0.08	$F_{3,12}$=1.42 p= 0.29	$F_{3,12}$=1.33 p= 0.31

7.4.4 Stable isotope analysis

Mean (±S.D.) stable isotope values of *E. sinensis* (-29.9 ± 0.48 ‰ for δ^{13}C and 17.04 ± 0.91 ‰ for δ^{15}N) showed greater variation between individuals than for *P. leniusculus* (-28.9 ± 0.33 ‰ for δ^{13}C and 17.40 ± 0.49 ‰ for δ^{15}N), but were very similar for the two species (Figure 7.11a & Figure 7.12a). Isotopic signatures of equivalent sources were similar between the two collection sites in most cases, but carbon isotope values of Gastropoda and organic rich sediment were markedly lower at the *E. sinensis* site. *E. sinensis* was closely aligned with shredders and the mixing models estimated these to comprise the main proportion of diet (mean 51%), followed by marginal vegetation, terrestrial detritus, sediments and filamentous algae; though there was high uncertainty about the relative proportions of each of these latter sources (Figure 7.11b). *P. leniusculus* was aligned with a number of sources, many of which overlapped in their isotopic signatures (Figure 7.12a). Accordingly, no marked differences were apparent in the proportion of diet contributed by each source as estimated by mixing models (Figure 7.12b). In general, plant and detrital material were estimated to comprise the majority of *P. leniusculus* diet, with a lower reliance on macro-invertebrates than *E. sinensis*. The modelled high contribution of organic-rich sediments to the diet of *P. leniusculus* is a consequence of the high δ^{15}N value for this source; however this result should be treated with caution. Nitrogen enrichment likely reflects the highly eutrophic nature of the sample site; however much of this nitrogen may be bound to sediments and therefore not available to crayfish, so the model likely overestimates the contribution of this source to diet.

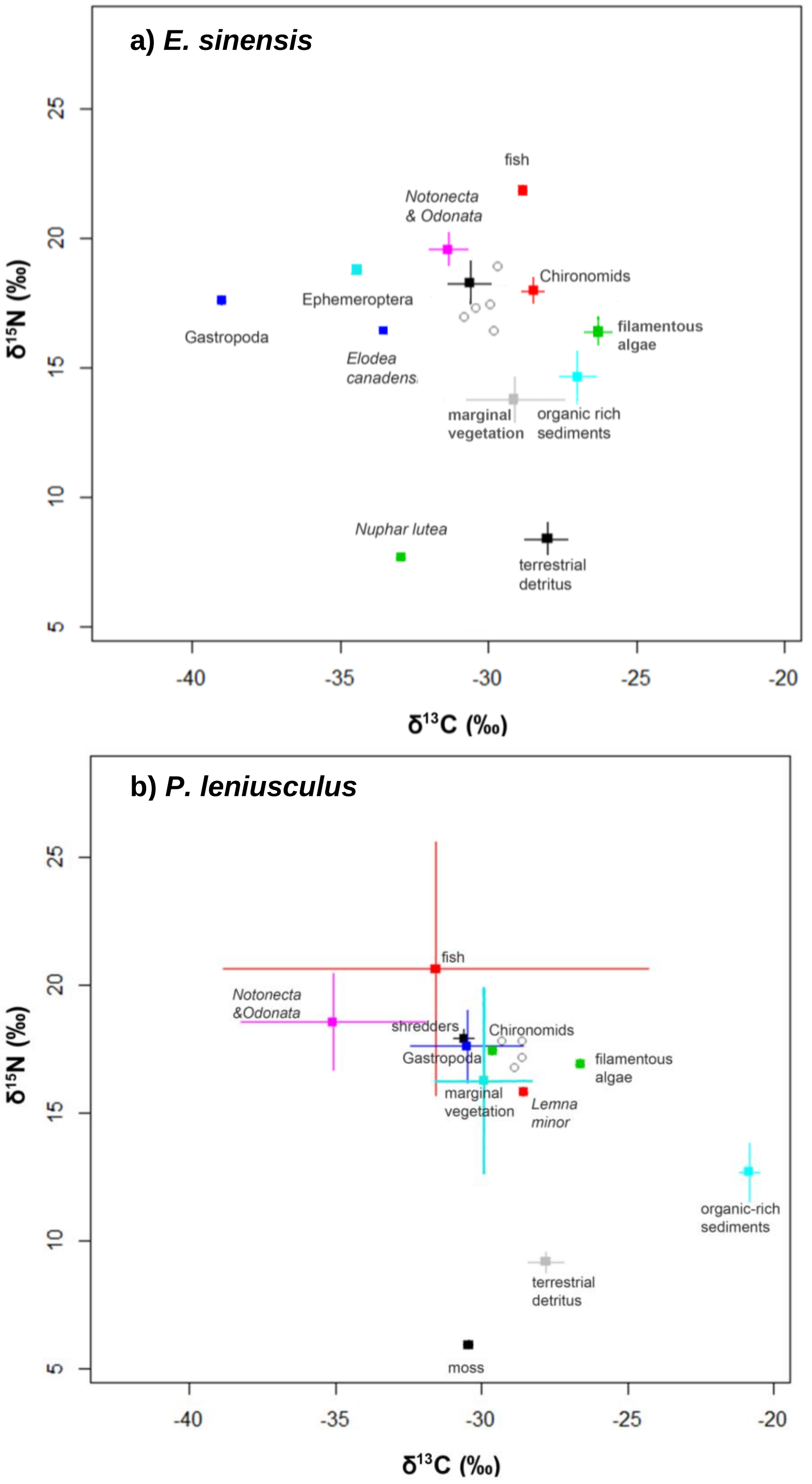


Figure 7.11 Stable isotope bi-plots for a) *E. sinensis* and b) *P. leniusculus* (open circles), and potential food sources in the lower River Stour, UK. Squares indicate mean values adjusted for trophic enrichment factors, bars indicate range of values.

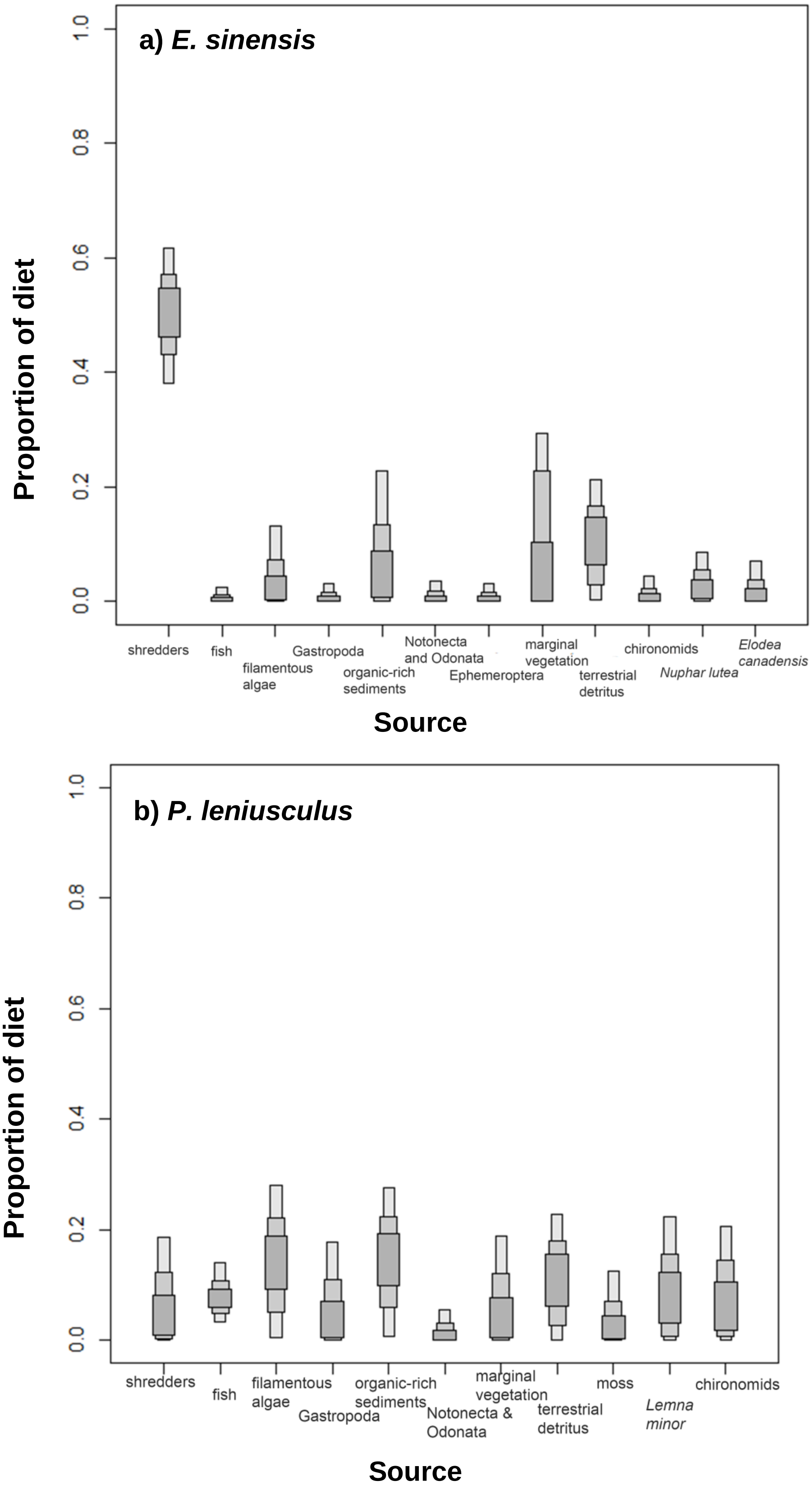


Figure 7.12 Boxplots indicating range of contribution of each potential food source to the diet of a) *E. sinensis* and b) *P. leniusculus* as determined by concentration-dependent mixing models using mean isotopic values for consumers. Boxed areas represent 95, 75 and 50% confidence intervals.

7.4.5 Gut contents analysis

Invertebrate material comprised the largest proportion of *E. sinensis* gut contents, followed by macrophytes and algae. Terrestrial detritus was the least detected category, comprising on average only 6.5%. The invertebrate taxa detected in *E. sinensis* gut content included Trichoptera, Coleoptera, Ephemeroptera and Gastropoda, with Trichoptera the most commonly encountered (Figure 7.13). The gut content of *P. leniusculus* was dominated by terrestrial detritus. Algae was second most common, followed by invertebrates which included the taxa Gastropoda, Isopoda and Trichoptera. Fish scales were also detected in 3 *P. leniusculus* individuals (Figure 7.14).

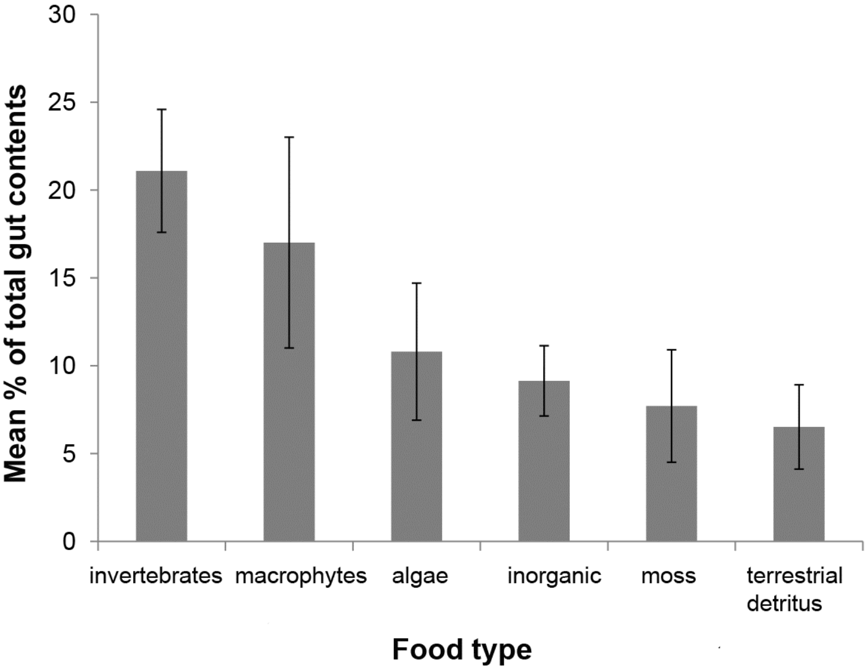


Figure 7.13 Gut contents of *E. sinensis* (n= 5) expressed as mean (± S.D.) volumetric % of total contents.

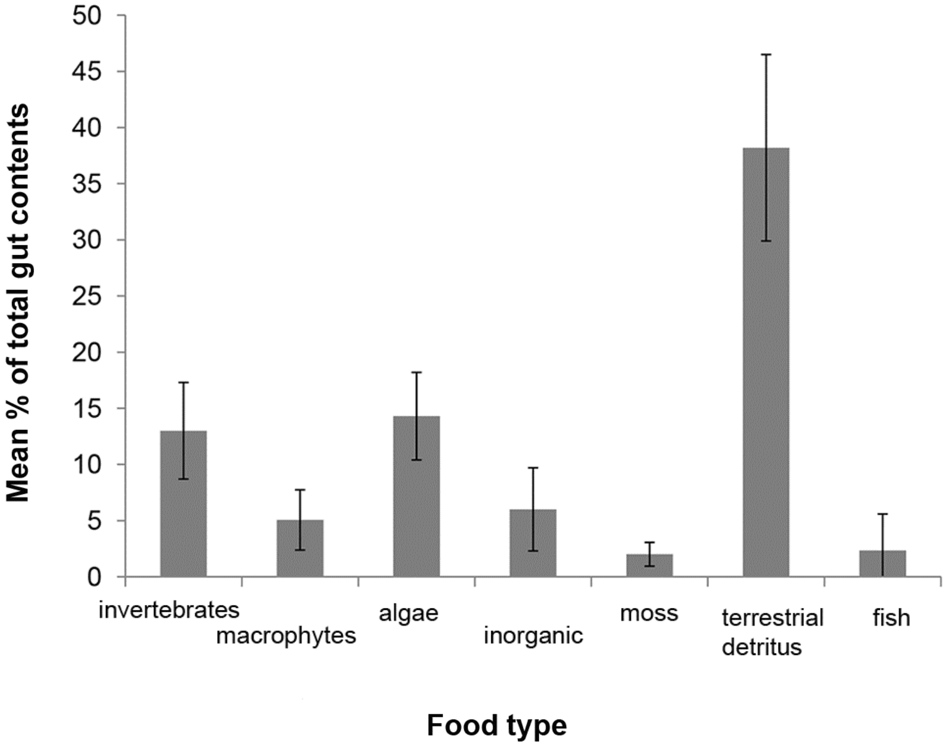


Figure 7.14 Gut contents of *P. leniusculus* (n = 10) expressed as mean (± S.D.) volumetric % of total contents.

7.5 Discussion

Three elements of invader impact are considered important: area invaded; abundance, and per capita effects (Parker *et al.* 1999). The current study indicated that *E. sinensis* had a higher per capita consumption rate on a keystone freshwater shredder *G. pulex*, whereas predatory functional response of the other invasive decapod *P. leniusculus* did not differ from either. Both invaders consumed a wide range of prey types, though differences in prey preferences and in the diets of wild caught specimens were apparent with *E. sinensis* showing greater reliance on invertebrate food sources, particularly shredders. Community impacts of the decapods investigated through a mesocosm experiment were similar with marked declines in all animal taxa. The presence of both caused a trophic cascade resulting in elevated periphyton levels, presumably mediated by reduced grazing pressure by gastropods. Invasive *E. sinensis* displayed a greater predatory strength on *G. pulex* than the native crayfish *A. pallipes*, and there was a trend to suggest that *E. sinensis* may also be a stronger predator than *P. leniusculus* for this prey type. The latter is supported by prey choice trials in which *E. sinensis* consumed more prey items overall and displayed a greater preference for *G. pulex* than did *P. leniusculus*, and also by the mesocosm experiment in which the abundance of this prey was reduced more in the presence of *E. sinensis* than *P. leniusculus*. A stronger predatory functional response among invaders compared to natives has been demonstrated previously for crayfish (Haddaway *et al.*, 2012b); amphipods (Bollache *et al.*, 2008), and gambusias (Rehage *et al.*, 2005). Haddaway *et al.* (2012b) showed that adult *P. leniusculus* preyed at a 10% higher rate than *A. pallipes* and although this trend was also apparent in the current study with juveniles of the same species, no clear species difference was apparent due to high intra-specific variability. From the 36% higher predation rate by *E. sinensis* than the native *A. pallipes*, mediated through a faster ability to handle this prey item, it may be inferred that *E. sinensis* is likely to negatively impact native prey species as it invades freshwater systems; however, caution should be applied when upscaling these findings to freshwater systems. In the highest prey density treatments there was evidence of wasteful killing whereby prey were killed and only part consumed, leading to a potential overestimation of predation strength. Further, data concerning the population densities of *E. sinensis* are required to inform quantitative predictions of invader impacts in the wild.

E. sinensis and *P. leniusculus* have both previously been described as ‘generalist omnivores’ which consume a wide variety of food items (Guan & Wiles, 1998; Stenroth *et al.*, 2006; Czerniejewski *et al.*, 2010) and this was also the case in the current study, though clear preferences for certain prey types were detected. When not accounting for inter-prey predation, the prey choice hierarchy of both decapods generally reflected a decline in preference based on ease of capture and handling, with chironomids most preferred and the gastropod species least preferred. However, preference for chironomids is likely to be over exaggerated because inter-prey predation was apparent in the control, presumably by *G. pulex* which is known to prey upon chironomids and *Asellus* sp. (MacNeil *et al.*, 1997). As *G. pulex* was also consumed by the decapods, inter-prey predation would have been reduced in the decapod treatments relative to control, so it was decided not to correct values for predation in the control prior to analysis as has been done in previous studies (Haddaway, 2012b).

All prey items used in laboratory experiments and field mesocosms were from sites without *P. leniusculus*, *E. sinensis* or *A. pallipes* and were therefore naïve to these predators. Prey may exhibit diverse and often complex predator-avoidance behaviours (Sih *et al.*, 2011) which may be evolved over time and passed to successive generations genetically, or reflect localised behavioural plasticity (Alvarez & Nicieza, 2003). Previous work has identified strong predator-avoidance strategies among gastropods in the presence of predators including crayfish (Cotton *et al.*, 2004); crawling up to the waterline in response to chemical cues is commonly reported (e.g. Covich *et al.*, 1994). The potential implication of using naïve prey in the current study is an overestimation of predation rates. Further, variation in the effectiveness of such strategies between prey types would contribute to species-specific patterns of predation that could not be detected when using naïve prey. Greater understanding of predator avoidance strategies among the prey types investigated is necessary to quantify this.

Both *E. sinensis* and *P. leniusculus* also preyed heavily upon the eggs of coarse fish, with no apparent preference between species. In many trials the decapods consumed all the eggs that were accessible to them i.e. not deeply embedded in the spawning medium, suggesting that they would have consumed more eggs had they been provided. *R. rutilus* and *A. brama* are both highly fecund, producing up to ca. 32 000 and 460 000 eggs per female (Jobling *et al.*, 2002; Adámek *et al.*, 2004), with the associated trade-offs of small egg size and low investment in egg concealment and brood protection (Flegler-Balon, 1989; Elgar, 1990). Consequently, eggs are highly predated on by a range of native species including eel (*Anguilla anguilla*) and chub (*Squalius cephalus*). This is the first evidence that *E. sinensis* predates on the eggs of these common fish species which spawn in the lower reaches of rivers; areas where *E. sinensis* are likely to reach their highest densities in freshwater habitats (Rudnick *et al.*, 2003). In the current study, fish were not identified as an important food source in the diet of *E. sinensis* sampled from the field; however it is not possible to determine the importance of fish eggs in wild diet through these data because sampling occurred during Autumn when fish eggs would not have been an available food source. .. Previous studies have highlighted predation by *P. leniusculus* on fish eggs and emerging fry (Harlioğlu, 1996; Edmonds *et al.*, 2011), and localised reductions in salmonid recruitment in the presence of *P. leniusculus* (Peay *et al.*, 2009); however no overall impact on fish populations was detected in a larger scale comparison of invaded and non-invaded streams (Degerman *et al.*, 2007). Future comparison of the functional responses of *E. sinensis*, *P. leniusculus* and a range of native species preying on fish eggs would be valuable step towards assessing potential impacts.

The effects of decapod presence on mesocosm communities were generally similar for *E. sinensis* and *P. leniusculus*, with a strong decline in the abundances of all animal taxa. The stronger impact of *E. sinensis* on *G. pulex,* compared with *P. leniusculus,* is consistent with its demonstrated preference and tendency towards a higher functional response for this prey item. This was also consistent with the feeding patterns detected for *E. sinensis* in the wild, providing strong evidence that invasion by this decapod is likely to reduce shredder abundance in freshwater systems to a greater extent than would be the case where only crayfish (native or invasive) are present. This could have cascading effects whereby reduced shredder abundance results in a dramatic decline in detrital processing, as shown for the predatory impacts of bullhead (*Cottus gobio*) on *G. pulex* in a chalkstream (Woodward *et al.*, 2008). Conversely, macro-consumers of detritus such as crayfish may decouple such a cascade thereby still creating availability of nutrients to pass to higher levels (Usio, 2000; Vanni, 2002). Combined evidence from the mesocosms, gut contents and stable isotopes in the current study indicated that *E. sinensis* is also a significant consumer of allochthonous detritus so, depending on comparative processing rates and the degree to which shredder populations are reduced, this invader could similarly decouple such a cascade.

There was only weak evidence of additive community impacts in the combined presence of both decapods. Periphyton biomass did not differ from the control in either of the single decapod treatments; however it increased significantly in the presence of both. Although *E. sinensis* and *P. leniusculus* may directly graze on periphyton, they are inefficient consumers compared to gastropod grazers (Nystrom, 1999), therefore the observed increase in periphyton is likely a consequence of a top-down cascade created by the decapods feeding on gastropods, and thereby reducing algal grazing pressure, as has been reported for the crayfish *Orconectes rusticus* (Weber & Lodge, 1990; Charlebois & Lamberti, 1996). *E. sinensis* had a stronger impact than *P. leniusculus* on the abundance of the gastropod *Physa fontinalis* in mesocosms; however impacts on the other grazer *R. peregra* were similar, so it is unclear why *E. sinensis* in isolation did not also cause a trophic cascade. A reduction in periphyton may also have been due to light limitation in the additive treatment. Both decapod species are considered ecosystem engineers due to their burrowing activities (Wallentinus & Nyberg, 2007; Harvey *et al.*, 2011); unfortunately water turbidity was not measured in the current study, though this would be an interesting avenue for future research. The likelihood of such a cascade occurring in natural systems depends upon both the predation pressure of the decapods, the importance of gastropods in the food web, and potentially water turbidity. Gastropods did not appear to be an important part of the diet of *E. sinensis* in the River Stour, inferring that the mesocosms may have overestimated gastropod predation by this invader. However, it must be taken into account that only a very small sample of the *E. sinensis* population was captured and analysed, and only adult wild specimens were sampled so there be undetected ontogenic differences in the diet of this invader. *P. leniusculus* had a strong negative impact on animal taxa in the mesocosms and no impact on primary producers, whereas the field study suggested strong dietary dependence on detritus, algae and macrophytes. Again, the mesocosms are likely to have overestimated predation rates due to lack of habitat complexity (Nystrom *et al.*, 1999); however this disparity may in part reflect ontogenic diet shift in *P. leniusculus*. Juvenile decapods were used in all laboratory experiments whereas only adults were captured in the field, so all gut contents and stable isotope analyses are based only on adults. Some studies report a greater reliance on invertebrates among juvenile crayfish, with adults consuming more detritus (Guan & Wiles, 1998; and see Chapter Four); though some authors report the reverse. For example, Stenroth *et al.* (2008) found *P. leniusculus* in a lake system shifted to become more predatory with increasing crayfish size. Previous studies have to date provided no evidence of ontogenic diet shift among *E. sinensis,* (Rudnick *et al.*, 2000) and the apparent consistency between mesocosm and field results in the current study is in accord with this.

Ontogenic differences are just one potential source of variation in dietary patterns. Seasonal diet shifts, usually reflecting changes in the availability of resources, occur among many taxa, including crayfish (Whitledge & Rabeni, 1997a; Grey & Jackson, 2012). Dietary variation between individuals of the same species is also commonly reported (Usio & Townsend, 2002; Ahvenharju, 2007) and can provide useful indications of behavioural plasticity and niche width among invaders (Bearhop *et al.*, 2004; Grey, 2006; Jackson *et al.*, 2012). Comparison of isotopic signatures between individuals provides a measure of niche variation within a population and therefore diet diversity (Bolnick *et al.*, 2002). In a recent study, the spread of red swamp crayfish (*Procambarus clarkii*) individuals through isospace declined in response to an increase in common carp (*Cyprinus carpio*) abundance; indicating that the crayfish were less able to exploit diverse resources, probably as a consequence of habitat alteration by the carp (Jackson *et al.*, 2012). Due to limitations in sample collection and analysis, the stable isotope and gut content analyses presented here provide only an initial indication of dietary patterns of *E. sinensis* and *P. leniusculus* in the wild. Further sampling, ideally including locations where both species can be captured in both juvenile and adult life-stages in multiple seasons, are required to elucidate on trophic interactions of these two invaders in their freshwater ranges.

Chapter 8 Do low-head riverine structures hinder the spread of invasive crayfish? Case study of signal crayfish (*Pacifastacus leniusculus*) movements at a flow gauging weir

8.1 Summary

Increasing legislative drivers demand the removal or modification of riverine barriers to enhance habitat connectivity for aquatic biota including fish; however there is also concern that greater connectivity will hasten the spread of aquatic invaders such as the signal crayfish (*Pacifastacus leniusculus*). Passive Integrated Transponder (PIT) telemetry was used to assess passage of signal crayfish (n = 392) over a typical low-head riverine structure, a flow gauging weir, during a 17 month period. Sixty percent of tagged crayfish were detected, with greatest crayfish activity associated with high water temperatures and long day lengths. The weir reduced upstream crayfish movements, with 45% less passages than in the control stretch with no weir, but there was no difference in the downstream direction. Crayfish movement patterns varied according to sex and size, with male crayfish more likely to successfully ascend the weir, and larger crayfish to descend the weir. Although increased fluvial connectivity will benefit migratory fish species, results suggest that the removal or modification of even quite minor, low-head structures could hasten the upstream spread of signal crayfish.

8.2 Introduction

A biological invasion may conceptually be considered as a series of stages, each with inherent barriers that a species must be overcome for the invasion to progress (Williamson & Fitter, 1996a; Blackburn *et al.*, 2011). After transport of the potential invader to a new location and initial introduction, there follows a stage of establishment in which viable breeding populations are founded, and then a fourth stage of spread in which the species disperses and becomes invasive (Blackburn *et al.*, 2011). The rate and pattern of dispersal in this spread stage, whether natural or human-mediated, is an important influence on the extent of impacts on invaded systems, but also on the feasibility of potential control measures (Mack *et al.*, 2000).

The signal crayfish (*Pacifastacus leniusculus*) is an invasive freshwater decapod that originates from north-western North America but was introduced to California and then to Europe in the 1960-70s for aquaculture. It subsequently escaped or was released from culturing facilities (Rogers & Watson, 2011), has substantially extended its invasive range over the last 4 decades, and now occurs in 13 European countries (Global Biodiversity Invasions Facility, 2013*)*. There is currently no effective large-scale means of controlling signal crayfish populations, though localised extirpation has been reported after treatment with biocide (Peay *et al.*, 2006), and reduced abundances through exhaustive trapping (Rogers *et al.*, 1997). Repeated trapping reduces crayfish movement distances, suggesting potential to slow advancement at invasion fronts (Moorhouse & Macdonald, 2011a); however, this method predominantly removes the larger components of the population and enhances body condition of the remaining individuals due to reduced interference competition (Moorhouse & Macdonald, 2011c). Largely unabated, *P. leniusculus* continues to spread through freshwater systems causing multiple deleterious impacts such as loss of species richness and restructuring of benthic invertebrate communities (Nystrom *et al.*, 2001; Crawford *et al.*, 2006); predation upon fish and emerging fry (Guan & Wiles, 1998; Edmonds *et al.*, 2011); loss of river bank integrity due to burrowing (Holdich *et al.*, 1999b), and decimation of native crayfish species through its role as a vector of *Aphanomyces astaci*; the cause of crayfish plague (Alderman *et al.*, 1990; Edgerton *et al.*, 2004).

Understanding and predicting the future spread of *P. leniusculus* in freshwater systems is a crucial step in targeting limited resources for management to protect remaining strongholds of native crayfish species and highly sensitive watercourses (e.g. important fisheries) (Gherardi *et al.*, 2011). Much work has been conducted to this end, ranging from empirical calculations of invasion rates (Peay & Rogers, 1998; Hudina *et al.*, 2009) and individual dispersal ability (Bubb *et al.*, 2006), to continent-scale modelling (Capinha *et al.*, 2013). In their spread through freshwaters, crayfish may encounter a range of structures of both natural (e.g. waterfalls, rock ramps, rapids) and manmade (e.g. dams, weirs, sluices) origin. Although such barriers have the potential to hinder spread, their role is rarely considered in distribution analyses of invasive crayfish. In a notable exception, Kerby *et al.* (2005) found in a survey of six streams in southern California that barriers, both natural and manmade, restricted the movements of the invasive red swamp crayfish (*Procambarus clarkii*), contributing to a general downstream pattern of spread after colonisation. Also in California, Light (2003) similarly found that *P. leniusculus* did not occur in the sites upstream of cross-channel structures.

From a European perspective, riverine barriers are particularly topical due to recent legislative drivers such as the EU Water Framework Directive (WFD), Eel Regulations and, in the UK, forthcoming Fish Passage Regulations, which all require water managers to improve habitat connectivity for fish. Riverine structures throughout Europe are being assessed for necessity and for porosity to fish. Those deemed redundant will be removed and mitigation may be necessary for others, likely in the form of fish-friendly modifications (e.g. reducing head drop, changing surface material); or by installing fish passes. Even relatively minor structures such as culverts and low-head weirs cause habitat fragmentation for fish species (Gibson *et al.*, 2005; McLaughlin *et al.*, 2006), and increasing evidence suggests that the physical impediment or altered flow regimes created by such barriers also influence the dispersal patterns of invasive species (Kerby *et al.*, 2005; Foster & Keller, 2011; MacNeil & Platvoet, 2013). Accordingly, there is concern that the removal or modification of structures may hasten invasion rates. For *P. leniusculus*, enhanced upstream movement is of particular concern as invasive crayfish tend to occur lower down in catchments with the remaining native populations largely forced into the headwaters (Collas *et al.*, 2007; Weinländer & Füreder, 2012). A greater understanding of how river structure types affect crayfish dispersal is therefore required to formulate an integrated management approach to both facilitate fish migration and protect remaining native crayfish populations.

Conversely, there is interest around purposefully installing structures that function as ‘migration barriers’ to prevent the spread of invasive species into highly sensitive areas such as remaining strongholds of natives (Rahel *et al.*, 2008). This approach has been implemented in Southern Spain where field trials revealed that series of three dams effectively prevented the spread of *P. clarkii* into headwaters over a four year period (Dana *et al.*, 2011). Further, Frings *et al.* (2013) recently proposed design metrics for a fish-passable crayfish barrier based on flume trials. Increasing our understanding of how structures hinder or prevent the spread of crayfish in the field is fundamental to this management approach.

This study investigated the influence of a very common riverine structure, a flow gauging weir, on the spread of signal crayfish. Movements of signal crayfish past the weir were investigated over a 17 month period with the aim to determine if the structure presented a barrier to crayfish movement, and how any ‘barrier effect’ was influenced by environmental conditions such as water temperature and flow.

8.3 Methods

A Passive Integrated Transponder (PIT) telemetry array was used to monitor signal crayfish movements over a flow gauging weir between July 2011 and November 2012. A total of 392 crayfish were captured up- and downstream of the weir, uniquely tagged and released each July. Crayfish movements across the weir were compared with movements across a control stretch of river of equivalent length and analysed in relation to environmental variables (flow, water temperature, day length and moon phase).

8.3.1 Study site

The study was conducted at a concrete trapezoidal flume (weir hereafter) used for flow gauging in the River Glem, Suffolk, UK (52° 5'33.14"N, 0°41'34.65"E) (Figure 8.1a, b). Low-head structures such as this one are estimated 2–4 orders of magnitude more numerous than high-head dams (Lucas *et al.*, 2009), with over 700 similar weirs in the UK alone (CEH, 2010). The River Glem is a tributary of the River Stour, with mean discharge 0.48 $m^3 s^{-1}$ (10 year average). The study reach has a low gradient and a mainly silt substrate with loose cobbles and boulders. Immediately downstream of the weir, the river widens into a pool (Figure 8.2). Signal crayfish are well established at the study site, both up and downstream of the weir, and are likely to have invaded upstream from the confluence of the two rivers (EECOS, 2008); though the possibility of human-mediated introduction(s) upstream of the weir cannot be dismissed.

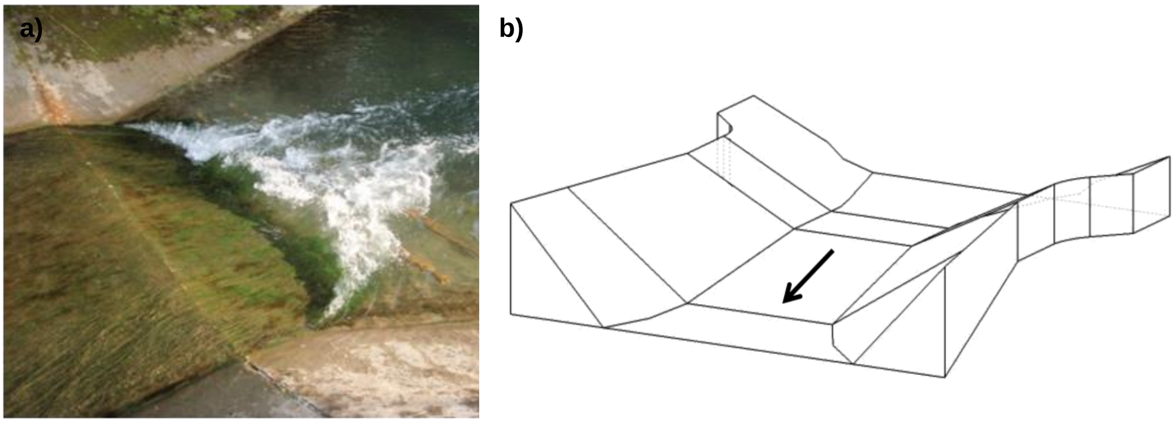


Figure 8.1 Glemsford flow gauging weir, a trapezoidal flume of concrete construction, under low flow conditions (a), and drawn in 3-D with arrow denoting the direction of flow (b).

8.3.2 Telemetry configuration

A half duplex PIT telemetry system (134.2 kHz, 100 ms scan cycle, Wyre Micro Design, Lancashire, UK) comprising an array of 3 equidistantly-spaced antennas and 2 data-logging receivers was employed to track crayfish through the study reach which comprised a 'weir stretch' and an unobstructed 'control stretch' (Figure 8.2). Antenna 1 was a flatbed pass-over rectangular loop (6.1 m long, 0.5 m wide), attached to weir face 2.8 m downstream from the crest and aligned perpendicular to streamwise flow. The loop extended 1.3 m up the sloping weir sides to ensure it would remain within the wetted perimeter under high flows. Antenna 2, a rectangular pass-though loop (6.77 m long, 0.5 m wide) located immediately upstream of the weir structure was extended up the banks and stretched across the river above the waterline by means of a taut rope. Antenna 3, another pass-over loop (6.8 m long, 0.5 m wide), was placed 8 m upstream of antenna 2 and affixed flat onto the channel bed. Time-stamped detection data of tagged crayfish at antennas 1 and 2 were used to establish successful ascent or descent of the weir structure. Similarly, detections of individuals at antennas 2 and 3 were used to deduce directionality of movements through the control stretch (Figure 8.2).

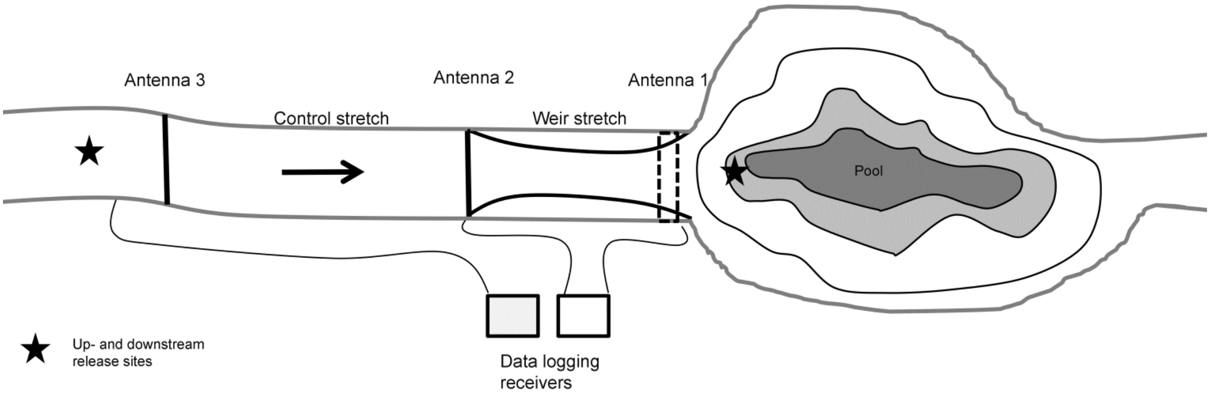


Figure 8.2 Study site showing the location of control and weir stretches, 3 PIT antennas, data logging receivers and release sites of tagged crayfish.

Antennas 1 and 2, and antenna 3 were connected to two separate receiver and logger systems housed in the onsite gauging hut. As crayfish are relatively slow moving animals, a filter was used to reduce the amount of data logged if an individual remained within the detection zone for an extended period. Individuals remaining within a detection zone were logged only once every 25 s; however movements in and out of the detection zone were always logged. Monthly range testing of the antennas demonstrated consistently greater than 97% detection efficiency at maximum read ranges of 0.45, 0.60 and 0.45 m for antennas 1, 2 and 3 respectively.

8.3.3 Capture and tagging protocol

Crayfish were collected from a stretch extending 100 m upstream to 100 m downstream of the weir using a combination of hand-search during daylight and baited traps at night. After capture, crayfish were held in aerated tanks of river water (500 L) for a maximum of 2 h prior to tagging. Three separate collections were made in each study year (13th, 18th and 29th July 2011 and 10th, 11th and 19th July 2012). A total of 214 and 178 tagged crayfish were released in year 1 and 2, respectively. Tagged crayfish ranged from 25.9 to 76.9 mm carapace length (CL), measured from tip of the rostrum to posterior end of the cephalothorax, and from 6.0 to 119.2 g wet mass. A total of 74 males and 140 females were tagged and released in year 1, and 92 males and 86 females in year 2. Males and females did not differ in CL ($t = 0.07$, $p=0.94$, 389 d.f.) or mass ($t = 1.72$, $p = 0.09$, 259 d.f., equal variances not assumed). Only crayfish exceeding 25 mm CL, corresponding to an age of 2 years and older (Guan & Wiles, 1999), were tagged as this is considered the minimum size suitable for PIT tagging (Bubb *et al.*, 2002b).

A PIT tag (Oregon RFID 12 mm length, 2.1 mm dia., 0.1 g mass in air) was surgically implanted into each crayfish. A 3 mm incision was made on the ventral surface of the crayfish, towards the posterior of the cephalothorax and directly behind the fifth pereopod (fourth walking leg) using a hypodermic needle. The glass encapsulated PIT tag was inserted through the incision, pushing it anteriorally to rest in line with the body and underneath the digestive glad (after Bubb *et al.*, 2008). After insertion, the incision was sealed with protective powder (Orahesive^R, ConvaTec,UK) and the crayfish was measured (CL, mm), sexed and the individual tag identification number recorded. Crayfish were transferred to an in-river holding cage for a minimum of 8 hours to recover post-tagging and acclimate prior to release. Low mortality was experienced at this stage (0.06% over both years combined). After recovery, crayfish were released either up- or downstream of the weir, corresponding to the location of original capture. Release sites were located 5 m down from and 5 m up from antenna 3 and 1 respectively (Figure 8.2). Releases took place in the evening (20:00 h) to minimise predation on post-release individuals.

To assess long-term post tagging survival, a group of 40 individuals were captured, tagged and measured. Individuals were transferred to a 2000 L recirculation holding tank with filter, provided with PVC pipe refugia, and maintained for 6 months on a diet of crab pellets

(Hinari) and soaked beech and alder leaves. Overall mortality rate at the end of the holding period was 5%.

8.3.4 Environmental variables

Flow data ($m^3 s^{-1}$) recorded at 15 minute intervals were obtained from the Environment Agency for the entire study period (13th July 2011–20th November 2012). Water temperature data were collected every hour using a data logger (Tinytag Aquatic T-2100, Gemini Data Loggers, Chichester, UK) secured near the channel bed immediately upstream of the weir. Moon phase predictions (as % illuminated) were obtained for each day (NASA/GSFC, 2012), along with day lengths (Time and Date AS, 2013).

8.3.5 Data analysis

Crayfish frequently remained in the vicinity of an antenna for long periods (> 6 hrs) producing a stream of near-continuous detection records. As movements between antennas were of primary interest, each individual's detection record was broken down into a series of 'detection events' to enable extraction of these larger scale movement patterns from small scale movements in the vicinity of antennas. A 'detection event' was defined as a continuous stream of detections either on a single antenna or two consecutive antennas, where consecutive detections were separated by time intervals of less than 20 min. However, only one movement between consecutive antennas was included in each detection event. If, for example, a crayfish moved from antenna 1 to antenna 2, and then back to antenna 1, the moment when it was detected at antenna 1 for the second time was considered as the start of a new detection event. Detection events within the first 24 hrs after a crayfish was released were considered to denote flight responses rather than typical movement (Barbaresi *et al.* 2004) and were removed from datasets (47% of detection events).

To determine predictors of daily activity (excluding a period of downtime in the telemetry system, see below), the total number of detection events per 24 hr period was modelled as function of mean daily flow, mean daily temperature, moon phase (% illumination), number of daylight hours and all first order interactions. Due to the zero-inflated negative binomial distribution of the data, a two-part 'hurdle' model with zero-altered negative binomial error distribution was used. First, a maximum model with all terms was fitted, and then terms were stepwise-deleted to reach the Minimum Adequate Model (MAM) according to the outcomes of likelihood ratio chi-squared tests between alternate models. Model fit was assessed used plots of Pearson residuals against fitted values and comparison of observed and fitted values using linear regression (Zuur, 2009). Extended hurdle models were fitted with the pscl library (Jackman, 2012).

Generalised linear mixed models (GLMMs) with binomial error distributions and random factor (individual crayfish ID) were used to investigate the effect of environmental factors (mean daily flow and mean daily temperature) and morphometric variables (CL, mass, sex) on successful passage of crayfish across the weir and the control stretches. The dataset was split into four and modelled separately: 1) upstream movements in the weir stretch; 2) upstream movements in the control stretch; 3) downstream movements in the weir stretch, and 4) downstream movements in the control stretch. For the first dataset, the outcome of movements of crayfish detected at antenna 1 at the start of each detection period were modelled in terms of binary response i.e. 1 = successful passage over the weir by the end of the detection period, or 0 = no weir passage. Similarly, for the second dataset, the outcome of movements of crayfish detected at downstream end of the control stretch (antenna 2) at the beginning of a detection period was modelled in terms of binary response i.e. 1 = successful passage through the control stretch to antenna 3 by the end of the detection period, or 0 = no passage. Model fit and simplification was performed as previously described; the MAM was arrived at as the most parsimonious model with lowest AIC value. Binomial GLMMs were fitted using lmer in the lme4 library (Bates *et al.* 2008) and all statistical analyses were performed in R v3.0.0 (R Core Team, 2013).

8.4 Results

8.4.1 Crayfish Activity

The overall detection rate for the study was 57.9%, with 227 crayfish detected at least once over the two year period. Of the crayfish released in year 1, 22 (10%) were also detected in year 2. Detection rate did not vary between males and females (59.7% and 55.4% respectively, X^2=0.16, p=0 .69, 1 d.f.). The total number of detection events per crayfish, which may be considered as a surrogate measure of activity, ranged from 1 to 72, with a median of 2 events. A total of 81 crayfish (21%) were associated with just one detection event. The duration of detection events ranged from 1 second to 21 h 44 min 20 sec, with median 4 min 8 sec.

Activity was highest in July, followed by August (Figure 8.3). An absence of detection events during September in year 2 reflects a period of downtime in the telemetry system due to a large piece of woody debris lodged in antenna 1 and damage to the wires of antenna 2 during groundworks at the site. The system was fully restored by October 4th.

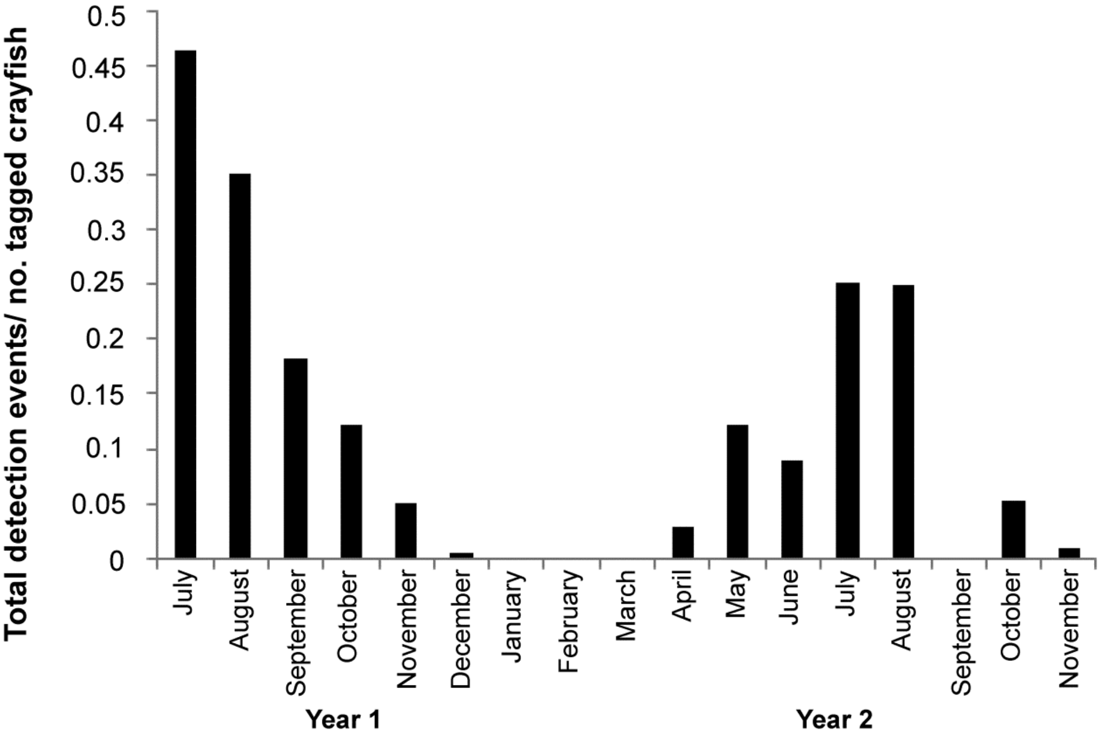


Figure 8.3 Number of detection events per month during the two study years (1 and 2) expressed as a function of the cumulative number of tagged crayfish released, excluding detections within the first 24 hrs post-release. Absence of detection events during September in year 2 reflects a period of downtime in the telemetry system.

8.4.2 Environmental factors

Mean daily water temperature ranged during the study period from a low of 2.4 ˚C in February 2012 to a high of 16.6˚C in August 2012. Flow ranged from 0.05 to 5.53 m^3 s^{-1}, with median 0.15 m^3 s^{-1}; equating to water depths of 0.05 to 1.06 m and a velocity range of 0.29 to 1.17 m s^{-1} at the weir crest.

Mean daily water temperature (X^2 = 69.9, p<0.01, 2 d.f.) and day length (X^2 = 6.25, p<0.05, 2 d.f.) were identified as significant predictors of daily activity level (no. of detections per day) in the MAM. In this two part model, the occurrence of a detection event within a 24 h period (zero hurdle part of model) was most likely when temperatures were highest (estimate = 0.50, 0.06 S.E.). In the count part of the model, most activity was associated with long day lengths (estimate = 0.12, 0.06 S.E.).

8.4.3 All passages through site

There were significantly more weir passages in the downstream direction than the upstream direction for both years 1 and 2 (X^2 = 4.67, p = 0.03, 1 d.f. and X^2 = 4.41, p= 0.036, 1 d.f., respectively). Conversely, for the control reach, there was no significant difference in the number of upstream and downstream passages in year 1 (X^2 = 0.10, p=0.75, 1 d.f.) or year 2 (X^2 = 0.05, p=0.83, 1 d.f.) (Figure 8.4). Overall, there were 45% fewer passages across the weir than the control reach in the upstream direction (X^2 = 8.49, p<0.01, 1 d.f.), but there was no difference for the downstream direction (X^2 = 0.20, p=0.65, 1 d.f.). A summary of crayfish passage through the site is presented in Table 8.1.

Table 8.1 Summary of total passages by crayfish through the weir and control reaches over both years

Parameter	Weir reach	Control reach
Total number of passages	100	124
Passages in downstream direction	65	60
Passages in upstream direction	35	64
Maximum number passages made by single crayfish	7	14

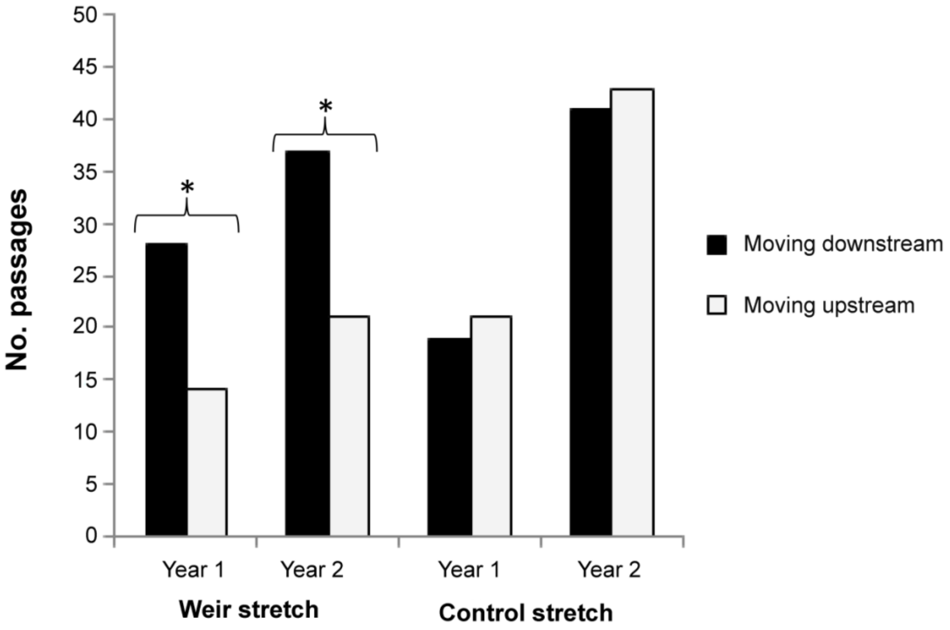


Figure 8.4 Summary of passages by crayfish through the weir and control stretches in both up- and downstream directions during year 1 and year 2. * indicates a significant difference at the 0.05 level, Chi-sq test, 1 d.f.

8.4.4 Weir passage

Of the 215 detection events that commenced on the lower weir (antenna 1), 16% resulted in successful ascent of the weir. Crayfish sex was the only significant predictor of weir ascent with 22% success rate for males compared to 12% for females (estimate = 0.80, 0.37 S.E., X^2=4.6, 1 d.f., p= 0.03). For crayfish moving in a downstream direction, 24% of the 272 detection events that commenced at the upper weir (antenna 2) resulted in successful descent of the weir. There were two significant predictors of downstream passage; both higher water temperature (estimate = 0.13, 0.11 S.E., X^2=8.3, 1d.f., p< 0.01) and greater crayfish mass (estimate = 0.03,0.01 S.E., X^2=71.2, 1 d.f., p< 0.01) increased the probability of weir descent.

8.4.5 Control passage

Passage through the control stretch in the upstream direction occurred during 20% of detection events that commenced at the upper weir (antenna 2). Temperature was the only significant predictor of passage with greatest probability of passing upstream when water temperatures were high (estimate = 0.41, 0.12 S.E., X^2 = 14.7, 1 d.f., p< 0.01). In the downstream direction, successful passage through the control stretch occurred during 39% of detection events that commenced at antenna 3. None of the measured variables were significant predictors of passage through the control reach in the downstream direction.

8.5 Discussion

This study demonstrated that the presence of a low head weir reduced crayfish upstream movements by 45% compared to an unobstructed reach, suggesting that the low head weir is a partial barrier to invasive *P. leniusculus*. The structure did not reduce downstream movements. Surprisingly, the porosity of the weir 'barrier' for crayfish did not vary with flow. In contrast to fish, crayfish can both walk and swim, hence they may pass a barrier in walking mode where water velocities exceed critical swimming velocity and vice versa where steep slopes prevent walking (Frings *et al.*, 2013). An effective invasion barrier must therefore combine both flows above the critical swimming velocity and steep slopes (> 25˚). The gradient of the downstream weir face was 28˚ which, based on flume trials (Frings *et al.*, 2013), would be expected to be largely impassable at flow velocities above 0.5 m s^{-}1 (54% of the study period). An alternative means by which crayfish may have passed the weir during higher flow periods is by leaving the water and traversing the weir sides; thereby decoupling the expected relationship between flow and barrier passability. Unfortunately, this behaviour could not be detected with the telemetry set-up, hence it was not possible to measure the degree to which this occurred, or determine concurrent flow conditions.

More generally, there was no evidence that flow influenced activity or crayfish movements in either direction throughout the study area. In contrast, Kerby (2005) identified flow as an important predictor of crayfish spread in streams in the Santa Monica mountains; however, mountainous regions are characterised by ‘flashy’ flow regimes with rapid rises in water level and high flows, whereas the lowland location of the present study meant changes in flow occurred more gradually with lower peaks. Indeed, flow in the study site exceeded 1 $m^3 s^{-1}$ for only 6% of the time, much of which occurred during the winter months when crayfish were largely inactive in response to short day lengths and low water temperatures. Water temperature was found to be a much stronger predictor of both general activity levels and passage across the weir than flow. Movement distances of *P. leniusculus* have previously been found to be positively related to temperature (Bubb *et al.*, 2004), likely reflecting higher metabolic rate at higher temperatures (Armitage & Wall, 1982).

There was evidence for differences in crayfish movement patterns based on sex and size. Male crayfish were more likely to successfully pass the weir in the upstream direction and larger crayfish to move over the weir in the downstream direction. No such patterns were evident in the control reach. In mark-recapture studies, Moorhouse and Macdonald (2011a; 2011b) found migration distances increased with crayfish size, though they found no relationship with sex. Our results may reflect a greater physical capability among larger male crayfish to pass the weir structure, or common behavioural traits that favoured passage. There has been much recent research interest surrounding the role of individual traits within invasion dynamics (Holway & Suarez, 1999; Duckworth & Badyaev, 2007; Sih *et al.*, 2012). One salient hypothesis is that individuals at an invasion front will display behavioural characteristics that facilitate colonisation (Cote *et al.*, 2010), as was demonstrated for western bluebirds, with the most aggressive individuals associated with range expansion and displacement of native conspecifics (Duckworth & Badyaev, 2007). In crayfish, large males tend to be bolder and more aggressive than other components of the population (Bovbjerg, 1956), and exhibit larger nearest-neighbour distances than less dominant crayfish (Fero & Moore, 2008). If aggressiveness and/or size are similarly favourable traits for crayfish with regards to range expansion, one may expect, (as was observed), this portion of the population to be more likely to move past a ‘barrier’, potentially expanding into new areas. Based on this finding, it may be hypothesised that obstructions such as weirs will modify crayfish population structure in the immediately vicinity causing, in the case of upstream population expansion, an accumulation of juveniles and females below the weir and larger male crayfish above it. This may have implications for potential control methods to prevent or slow population expansion. For example, trapping is biased towards the capture of larger males (Matthews and Reynolds, 1992; Demers *et al.*, 2003), and crayfish removal reduces the movement distances of remaining individuals; potentially limiting dispersal and further invasion (Moorhouse and MacDonald, 2011a). Therefore, in addition to trapping at the invasion front (Moorhouse and MacDonald, 2011a), trapping immediately upstream of structures such as flow gauging weirs may prove the most efficient means of applying this control method.

While legislation is driving increased habitat connectivity for fish, invasive species are recognised as a significant pressure that may cause waterbodies to fail to achieve 'good ecological status' by 2015 as required under the WFD (Cardoso & Free, 2008). This study demonstrated that even the relatively minor structure investigated hindered upstream movement of invasive crayfish, thus highlighting the potential conflict between barrier removal or modification to enhance habitat connectivity, and invasive species management. Only a single crayfish population and weir were studied, therefore further investigation of invasive species movements and colonisation rates at a range of weir types, gradients and flow regimes would provide the porosity metrics necessary to make informed risk-based decisions. Further, due to limitations of the telemetry set-up, the weir and control reaches were adjacent and therefore not truly independent. Therefore, it would be beneficial to conduct further research at replicated barriers, but also replicated, independent control reaches, to obtain more accurate indications of crayfish movement in unobstructed areas. The information derived from such studies could be integrated within the standard regulatory planning and consent process for the installation or modification of riverine structures. Further, the apparent efficacy of any barrier in limiting crayfish spread must also be evaluated against the risk of human-mediated introductions of invasive crayfish within and between catchments.

Chapter 9 General Discussion

The research presented in this thesis followed two key themes: translocation of crayfish for the purpose of conservation; and translocated crayfish as invasive species. Several questions pertaining to the conservation of the white-clawed crayfish (*Austropotamobius pallipes*) by translocation were addressed, with a particular focus on the use of former quarries as Ark sites. Arising from these studies are a number of recommendations for Ark site conservation. Invasion by the signal crayfish (*Pacifastacus leniusculus*) has occurred as a result of translocation outside its range and subsequent escape and spread. *P. leniusculus* is rapidly replacing *A. pallipes* in the UK and work was conducted examining its effect on native biota compared to native *A. pallipes* and another invasive decapod the Chinese mitten crab (*Eriocheir sinensis*), and on the influence of riverine barriers on rates of *P. leniusculus* spread through lotic systems. Arising from this research are predictions of the impact of these invasive decapods on freshwater communities and recommendations for managing further spread of *P. leniusculus*.

9.1 Translocation for conservation: key findings and management recommendations

In a systematic review of the literature on habitat use by *A. pallipes* (Chapter Two), vegetation parameters were most commonly identified as important for the occurrence of *A. pallipes*; in particular, the presence of trees/shading, woody debris and roots. Channel substrate parameters were second most important, with larger substrates such as cobbles and boulders generally positively associated with crayfish presence, though finer substrates such as silt were favoured during foraging. Channel morphometry, in particular undercut banks, was also positively associated with crayfish presence. Findings varied markedly between studies for several of the habitat variables examined, in part reflecting more complex patterns of habitat use such as habitat partitioning among crayfish size classes and sexes. These results strengthen the current translocation guidelines for *A. pallipes* based on expert knowledge, which principally emphasise the need for ample refugia and for suitable water quality (Whitehouse *et al.*, 2009, Peay, 2002). The review similarly identified the presence of refugia, whether in the form of boulders, woody debris, tree roots or undercut banks as a key habitat requirement and provision of such features should therefore be a priority in Ark sites. In addition, habitat features that enhance crayfish foraging, for example, overhanging trees, silt beds and in-stream vegetation will be beneficial by increasing food availability. Based on the implication that habitat partitioning frequently occurs within crayfish populations, it is recommended that Ark sites be enhanced where necessary to create heterogeneous habitat with a range of features beneficial to crayfish, thereby providing key requirements for refugia and foraging, and also facilitating habitat partitioning to reduce intraspecific competition.

Despite recognition of the importance of suitable water chemistry in Ark sites (Peay, 2002; Whitehouse *et al.*, 2009), few studies have investigated the tolerance of *A. pallipes* to water chemistry parameters experimentally. In a laboratory study (Chapter Six), suspended solids was shown to negatively impact *A. pallipes* at time-averaged concentrations of 62 mg L^{-1} and above during a 45 day period, causing gill fouling and a reduction in aerobic scope. The waterbodies within former quarries may be expected to have relatively high SS concentrations due to the presence of unconsolidated material on site; at least in the early stages of restoration before widespread plant growth consolidates loose sediments. The measurement of SS concentrations in the ponds at Threshfield Ark site of up to 60 mg L^{-1} (Chapter Three) indicated that SS periodically approached a level that could negatively impact *A. pallipes*. Although this concentration likely reflected high sediment inputs due to heavy rainfall and associated runoff, the findings indicate that periodic measurement of SS in all Ark sites, particular those in quarries, is advisable. If SS concentrations are found to be persistently above 62 mg L^{-1}, remedial measures such as the application of sediment binding products or hydroseeding to accelerate plant growth could be applied to limit chronic deleterious effects on resident *A. pallipes* populations.

Disease can be a major influence on the success or failure of conservation translocations (Mathews *et al.*, 2006). The discovery of *Branchiobdella astaci*, a gill dwelling crayfish worm (Chapter Five), in a population of *A. pallipes* highlights the possibility of inadvertently transferring organisms besides the target organism to the recipient site during translocation. Examination of host gill tissue revealed a strong correlation between the burden of *B. astaci* and melanisation of gill tissue, a generic immune response among Crustacea, thereby providing the clearest evidence to date that this branchiobdellidan operates as a parasite. The other gill-dwelling species in this genus, *B. hexodonta* is also parasitic, having been shown to directly consume gill tissue (Grabda and Wierzbicka, 1969); however all other *Branchiobdella* species, which tend to dwell on the exoskeleton or near the hosts mouthparts, are considered ectosymbionts (Gelder *et al.*, 1994, Vogt, 1999). No evidence was found to suggest that infection with this parasite compromised the aerobic scope of *A. pallipes*. Parasitised crayfish were more susceptible to melanisation when exposed to high suspended solids levels; however no relationship between melanisation and aerobic scope was found, so the overall fitness consequences of this interaction are uncertain. Aerobic scope is just one potential indicator of host fitness and further investigation into the fitness consequences of this parasite, both alone and in combination with other stressors such as pollutants, is required.

A key recommendation arising from this work is that a health check on a statistically meaningful number of each *A. pallipes* donor population should be carried out prior to translocation to assess the likelihood of other, potentially damaging, organisms being inadvertently translocated; particularly for pathogens that are not easily detected by eye. Translocation is a stressful process and may compromise the immune system of the donor stock, so any pathogens already present are likely to have greater impacts in the recipient site than in the donor site (Teixeira *et al.*, 2007). From a different perspective, one might argue that parasites are a component of biodiversity and are functionally important within ecosystems (Poulin, 1999; Hudson *et al.*, 2006). Further, parasites are likely to decline along with their host; hence they may also be threatened and should perhaps be conserved in their own right. A degree of pragmatism is essential for any conservation intervention; however routine health screening would provide an important basis on which to assess the likely risk of pathogens to the success of the translocation project, and therefore facilitate informed decision-making in this regard.

The three year case study of Ark site creation in a former limestone quarry provides one of the first long-term studies of this relatively new conservation strategy in a real world situation (Chapter Three). Former mineral extraction sites are advocated as Ark sites for a number of reasons, most importantly for their isolation with low risk of invasion by non-native crayfish and/or crayfish plague. Conditions in Threshfield Quarry were generally found to be favourable for *A. pallipes* and known survival of the donor stock into the second year supports this indication of its suitability as an Ark site. Water quality was high, with all measured water chemistry parameters within the tolerance limits reported for *A. pallipes* (Haddaway, 2010), and the rocky substrate in all three ponds provided plentiful refugia. The establishment of macrophytes has unfortunately been slow due to fluctuations in water levels following pond creation, which has resulted in low habitat heterogeneity and presumably contributed to the low macro-invertebrate densities. In light of the finding that vegetation is an important component of crayfish habitat, conditions within the quarry may be considered sub-optimal in this respect. Vegetation cover will increase over time; however it would be beneficial to supplement the current planting if funds allowed, particularly if translocation of additional crayfish occurs within the next year or so.

Macro-invertebrates will increase in diversity and abundance as colonisation proceeds and considering the small size of the current donor population, crayfish are unlikely to be food limited; however, it is recommended that a re-evaluation of the potential food resources be carried out when additional crayfish are added in the future. Food could be supplemented by the addition of leaf packs. The mesocosm study (Chapter Four) showed the importance of allochthonous detritus in the diet of *A. pallipes*, yet leaf inputs into the Threshfield Ark site over the study period were minor. Several hundred trees have been planted on the slopes bounding the Ark site, but these will take in excess of five years to reach sufficient size to provide significant allochthonous inputs. The restoration approach used at Threshfield has been ‘minimalist’ i.e. with minimal engineering and using materials onsite as much as possible. This is an admirable approach and one borne out of increasing evidence that ‘natural restoration’ produces greater biodiversity benefit. In a study comparing technical reclaimed limestone quarry sites, i.e. carried out by adding topsoil, planting trees, and sowing seeds, to sites that spontaneously colonised with minimal intervention, the latter sites were found to harbour more rare species of plants and arthropods because vegetation remained longer in the earliest stages of succession (Tropek *et al.*, 2010). Also key to this, however, is the quality of the surrounding habitats as this is the source for colonising species (Novák and Konvička, 2006). Large-scale restoration projects will likely incorporate several management aims, particularly so in quarries due to the range of stakeholders and diversity of possible after uses for such sites; hence prioritising those goals is a management challenge. For example, if crayfish conservation is a key management target and they are to be translocated at an early stage in the restoration process, (particularly as populations may be threatened and therefore speed is of the essence), it may be necessary to artificially accelerate processes such as plant establishment and the development of detritus. The prioritisation of such targets will rest with project managers but there should ideally be a clear hierarchy of goals to facilitate decision making.

Periodic monitoring of macro-invertebrate diversity and abundance over the three year study period provided a valuable indication of colonisation rates in the recently restored ponds. Long-term biodiversity monitoring over the course of restoration projects facilitates assessment of whether the wider aims of the project for nature conservation are being met; it would therefore be beneficial to continue this bi-annual monitoring over the coming years. Further, the current monitoring protocol could be supplemented by a full survey, including plants, according to PYSM methods. This could be carried out 5 years after Ark site creation, which also marks the end of the committed monitoring period for crayfish and culmination of the project. Although, this will incur additional costs, this will likely represent only a small percentage of the overall project costs.

The Ark-site strategy for crayfish is relatively new and while the available guidelines (Kemp *et al.*, 2003; Whitehouse *et al.*, 2009) for such projects all agree that post-translocation monitoring is necessary, the objectives of monitoring and, hence, the level to which a population should be monitored is as yet un-prescribed. In light of findings from the Threshfield Quarry case study, the effort and frequency of monitoring should exceed the single annual survey required under the Natural England project licence. Post-translocation monitoring is the most important indicator of project success and the allocation of funds should reflect this. Ideally, intensive monitoring should be conducted over several days, preferably in late summer when crayfish will most active (Peay, 2004). A methodology for monitoring should ideally be developed based on methods known to work and taking into account the particular conditions of the site. The founder population in Threshfield Ark site was unfortunately smaller than hoped; however even if the planned 610 crayfish had been translocated, detecting individuals in ponds that total 1385 m^2 is a challenging task. Largely due to its ease and low cost, baited trapping has become the mainstay of Ark site post-monitoring (Schulz *et al.*, 2002), despite its widely reported biases towards large, active males (Matthews and Reynolds, 1992, Demers *et al.*, 2003; Reynolds and O'Keeffe, 2005; Moorhouse & MacDonald, 2011a,b&c). Trapping may also be unsuitable in certain sites due to the presence of Amphibia such as the protected great crested newt (*Triturus cristatus*) which are attracted to the bait and vulnerable to drowning due to entanglement in the mesh. Annual trapping at the site captured just one crayfish over three years and the baited camera system did not successfully detect any crayfish during the single night trial. Further trials of the baited camera system are necessary to evaluate its efficacy, however trials conducted by the author in another former limestone quarry with a large population of *A. pallipes* revealed it successfully detected juvenile crayfish of 14 mm CL, compared to a minimum size of 28 mm CL sampled by baited trapping at the same site (unpublished data). Preliminary findings from this work suggest both that this tool has potential application for monitoring in quarry sites, and also may be more effective than trapping for sampling juvenile size classes. A key indicator of success in conservation translocations is the establishment of a breeding population, so for crayfish translocations where usually only adults are translocated, the reaching of this goal would be assured by detection of the smallest size classes (yrs 1 and 2). Due to the capture biases of baited traps they are unlikely to detect these size classes, therefore, it is advocated that trapping programmes be supplemented with night viewing (as is already happening at Threshfield) and the baited camera system developed within this thesis.

Translocations to Ark sites are often introductions whereby crayfish donor stock is added to a community that has not previously included crayfish. Previous research on various crayfish species has demonstrated that crayfish can exert strong community effects both through direct predation and indirect interactions. At Threshfield quarry it was not feasible to measure the effects of crayfish introduction on the existing community due to insufficient replicate ponds, but a mesocosm study (Chapter Four) showed that *A. pallipes* caused a reduction in the abundance of chironomid larvae, gastropods, isopods and gammarids. Although crayfish of all size classes preyed heavily upon snails, juvenile crayfish preyed more heavily than adults on the smallest snail species *Physa fontinalis*. This finding indicates that the macro-invertebrate communities of ark sites will likely change in species composition over time as the exclusively adult crayfish donor stock is supplemented by the recruitment of juvenile offspring. A further consideration is that crayfish could have negative impacts on taxa which are protected in their own right (e.g. great crested newt), so it is therefore advisable to conduct a biodiversity survey of the site to assess potential conflicts with other biodiversity goals, before crayfish are introduced.

Ark site translocations are frequently initiated to ‘rescue’ a declining population of *A. pallipes* which is under imminent threat of extirpation. It is therefore desirable to progress the project so crayfish can be translocated as quickly as feasible so as to optimise outcomes. The rapid decline of the donor population in the Threshfield case study during restoration of the Ark site clearly illustrates the potential risk of any delay, irrespective of its cause. However, this need for rapid action must be balanced against the precautionary measures that should be taken prior to translocation, for example, surveys to ensure that the recipient site is suitable to receive the crayfish and that the crayfish have a high chance of survival after translocation e.g. are in good health, as previously discussed. The development of clear guidelines for project managers and landowners, ideally with a recommended sequence of steps in chronological order with expected timeframes, would facilitate the process of Ark site creation. Further, adequate monitoring and reporting of projects as they occur will facilitate knowledge transfer and the refinement of best practice measures, as highlighted in IUCN guidelines for translocation (2012). The research carried out at Threshfield will contribute to the currently limited body of knowledge concerning crayfish translocations for conservation, particularly for quarry sites.

It is hoped that the Threshfield Ark site will persist into the long-term; a key requirement of this is supplementing the existing population with additional donor stock in order to increase the likelihood of developing a breeding population and reduce the negative allee effects associated with very small founder populations (Deredec & Courchamp, 2007). Second to this, biosecurity will be an important determinant of long-term success. Rigorous biosecurity measures at the site include the prohibition of public vehicles into Ark site ponds, no angling, and boot washing facilities for personnel entering the ponds (e.g. contractors). A public awareness programme is underway, including information boards highlighting the problem of crayfish plague, and it is hoped that this will prevent transmission by visitors to Threshfield Quarry; particularly important because a population of *P. leniusculus* is located less than 2 km from the Ark site. A summary of general management recommendations for *A. pallipes* ark sites arising from work presented in this thesis is provided in Table 9.1.

Table 9.1 Summary of general management recommendations for Ark sites formulated through this thesis

Habitat
• The availability of suitable habitat for *A. pallipes* is fundamental for Ark site success • Most of our knowledge of *A. pallipes* habitat requirements is derived from studies in rivers and streams rather than still waterbodies • Key requirements are: 1) water chemistry within the species' tolerance limits; 2) adequate refugia to avoid predation; and 3) sufficient food resources (see section below) • General water chemistry guidelines are provided by Haddaway (2010) and water chemistry testing should be undertaken prior to translocation and repeated at least bi-annually post-translocation. More frequent monitoring (e.g. diurnal oxygen measurements) should be undertaken in sites undergoing restoration • Elevated concentrations of suspended solids (> 62 mg L^{-1}) clog crayfish gills and reduce oxygen uptake capacity; therefore measures should be implemented to limit sediment runoff in high risk sites e.g. quarries • Boulder substrate, woody debris, tree roots and undercut banks provide optimum habitat for refugia and their availability should be maximised. • Planting of trees is essential in nutrient-poor and restored sites such as quarries, both for habitat provision and as a source of leaf litter
Food resources
• *A. pallipes* is omnivorous, consuming a wide range of food items including macro-invertebrates, macrophytes and leaf detritus • In newly created Ark sites colonisation by macro-invertebrates should be accelerated by planting schemes and the addition of leaf packs if crayfish are to be translocated within five years.
Health of donor stock
• An evaluation of disease risks should be carried out at the early stages of translocation planning • *A. pallipes* may be host to a range of pathogens, most notably *Aphanomyces astaci*, the cause of crayfish plague, but also chronic infections such as porcelain disease and crayfish worms which inhabit the gill chambers. • A statistically useful sample of the donor population (30 individuals at least) should be screened for pathogens and the results used to inform a disease risk assessment prior to translocation
Post-translocation monitoring
• Monitoring of the translocated population is a crucial indicator of project success and funds should be allocated to ensure sufficient monitoring effort into the long-term (10 years) • Baited trapping provides a relatively low-cost monitoring method; however due to under sampling of juveniles and females this should be supplemented with alternate approaches e.g. night viewing and baited camera traps • Monitoring should be conducted over several nights in late summer when crayfish are most active

9.2 Crayfish as invasive species: key findings and management recommendations

Invasive signal crayfish currently occupy more catchments in the UK than the native *A. pallipes* and as a consequence of both historic introduction patterns and variation in environmental tolerances between the species, native crayfish mainly persist in the upper reaches of watercourses whereas invaders are found lower down catchments (Collas *et al.*, 2007). In such circumstances, preventing the spread of *P. leniusculus* upstream is understandably a management priority in order to protect remaining pockets of *A. pallipes*. In a telemetry study it was shown that a flow gauging weir reduced the number of upstream movements by *P. leniusculus* past the weir by 45% compared to a stretch with no weir; though no 'barrier effect' was apparent for downstream movements. It may therefore be concluded that such weirs do not act as a complete upstream barrier to *P. leniusculus*, but may hinder their upstream spread. Where such structures are present, fewer crayfish will move into the upstream reaches, potentially slowing the rate of increase of population density in these reaches and consequently upstream advancement. A previous study in the River Wharfe, Yorkshire, indicated that *P. leniusculus* advanced to new reaches by means of 'peristaltic spread', that is, by colonising a new stretch and increasing in population density until all habitat refuges were occupied, then advancing relatively rapidly to the next stretch containing suitable habitat (Peay and Rogers, 1999). It may be inferred from the telemetry study that the presence of gauging weirs will likely increase the time taken for the population to reach a level that all available habitat is occupied, thereby slowing advancement of the invasion front.

This research provides timely and valuable empirical knowledge for managers that are increasingly required to balance improvements in fish passage driven by legislation such as WFD, yet safeguard remaining populations of *A. pallipes*. As a consequence of concerns over this issue, the Environment Agency recently adopted a precautionary approach to stop barrier removal and bypass works in catchments where *A. pallipes* populations are located upstream of a barrier, and *P. leniusculus* below (Ros Wright, Environment Agency, pers. comm.). This has major implications for rivers which are currently failing to meet 'good ecological status' due to a low fish index rating. For example, Hauxton Mill on the River Cam is an assumed migration barrier between the *P. leniusculus* population immediately downstream of the mill and a residual population of *A. pallipes* approximately 15 km upstream. The mill structure is also a major barrier to fish migration and the provision of a bypass would provide a key step towards improving fish passage in the catchment. In another example, a project to modify a flow gauging weir on the River Nar was recently halted due to concerns that this would hasten the advancement of *P. leniusculus* upstream towards a population of *A. pallipes* in the headwaters (Ros Wright, Environment Agency, pers. comm.). There is currently a dearth of knowledge on which to base such management decisions. The findings of this research provide useful quantification of ‘barrier effect’ for the weir type investigated, though further investigation of different barrier types and scenarios is needed to inform the decision-making process.

The potential conflict between the drive to improve connectivity for fish and contain the spread of invasive species, highlighted in this study, raises important issues concerning how apparently opposing objectives are prioritised by managers. Environmental management decisions are increasingly cost-led; founded on an economic model that assigns monetary value to the components of ecosystems through the ecosystem services they provide to humans (Fisher *et al.*, 2009; Redford & Adams, 2009). Examples of this ‘natural capital’ include forests which contribute to climate stabilisation, salt marshes which provide flood defence protection, and the pollinators which underpin agricultural productivity (Turner & Daily, 2008). Fisheries in both freshwater and marine systems are a major source of natural capital. In the UK alone, calculations based on a ‘willingness to pay’ model estimated the value of inland fisheries at £350 million annually (Environment Agency, 2007). This is realised through both direct value (e.g. harvest for consumption, angling and associated tourism), but also utility value (e.g. enjoyment, health benefits). Assigning comparative costs to invasive species impacts is difficult, with direct economic costs quantified for just 13% of Europe’s alien species (Vilà *et al.*, 2009). There are often complex interactions between invaders and the ecosystems they invade; hence they may impair the delivery of several ecosystem services concurrently (Lodge *et al.*, 2012). For example, *P. clarkii* impacts on nine different ecosystem services (Vilà *et al.*, 2009). It should be noted, however, that the economic consequences of invaders are not exclusively negative. They may directly provide a food harvest, facilitate habitat restoration (Rodriguez, 2006); and even benefit conservation efforts (Schlaepfer *et al.*, 2011). Considering the huge economic value of freshwater fisheries, particular for salmonids (O’Reilly & Mawle, 2008; Butler *et al.*, 2009), it is unlikely in the context of the present study that riverine connectivity will be forfeited to limit invasive species spread. However, the balance may tip in particular cases, for example, if signal crayfish directly threaten those fisheries such as through direct consumption of eggs (Edmonds *et al.*, 2011).

Even where *A. pallipes* are not present in a catchment, growing evidence of the impacts of *P. leniusculus* on freshwater communities means that preventing, or at least slowing, their spread is desirable in all cases. Investigation of the feeding behaviour of *P. leniusculus* indicated consumption of a wide range of invertebrate taxa, as well as allochthonous detritus and algae. The predatory strength of juvenile *P. leniusculus*, as quantified by predatory functional response, did not differ from native *A. pallipes*, although a difference has been previously shown for adult crayfish whereby *P. leniusculus* consumed 10% more of the same prey species *Gammarus pulex* than did *A. pallipes* (Haddaway *et al.*, 2012b).

In addition to *P. leniusculus*, another invasive decapod the Chinese mitten crab (*E. sinensis*) is spreading through freshwater systems in the UK. The structural impact of these crabs on river banks and sea defences by burrowing is well known, but little attention has been given to its potential biotic impact. In the current study, juvenile *E. sinensis*, exhibited the greatest predatory strength on *G. pulex*, with a 57% higher per capita intake than native *A. pallipes*, inferring that this invader could cause strong reductions in this keystone shredder. This is supported by other findings that *E. sinensis* had a preference for this prey species over gastropods and isopods, and preliminary stable isotope data from wild populations suggested that shredders comprise a large proportion of the diet of adult crabs.

This work provides the first quantitative comparison of potential impacts of *P. leniusculus* and *E. sinensis* on the communities they invade; however caution should be applied when extrapolating these findings to real world situations. The community impacts of an invader depend on an array of interlinked factors including its population dynamics and the availability of prey (Parker, 1999). Further, as shown for *A. pallipes*, decapods may exhibit ontogenic diet shift so further investigation of juvenile *P. leniusculus* and *E. sinensis* diet in the wild is required to elucidate on this. Mindful of these caveats, comparisons of invader impacts are crucial for managers assessing where best to target limited resources for invasive species control. The most widely reported impacts of *E. sinensis* are for estuarine environments where it causes substantial bank erosion (Dittel & Epifanio, 2009). The current research suggests that the spread of *E. sinensis* into freshwaters is also cause for concern due to ecological impacts on existing communities; particularly so as it is likely that this invader will have an equal, if not higher, per capita impact on prey species than *P. leniusculus*. Similarly, in addition to their direct trophic interactions with communities, there is increasing evidence that *P. leniusculus* have significant impacts on sediment transport in freshwaters through activities such as burrowing and foraging (Harvey *et al.*, 2011; Johnson *et al.*, 2011).

9.3 Concluding remarks

Biological invasions are a major cause of global environmental change (Sala *et al.*, 2000) and aquatic ecosystems are particularly vulnerable to this stressor (Dudgeon *et al.*, 2006). In the absence of effective controls, invasive crayfish such as *P. leniusculus* continue to proliferate in their introduced range, causing the extirpation of native crayfish populations and wider community impacts. While research continues to seek effective methods for invasive crayfish eradication and the control of crayfish plague, Ark sites may represent the best means to conserve populations of native crayfish such as *A. pallipes*. The success of the Ark site strategy relies on firstly finding suitable sites secure from invasive crayfish and plague in the medium to long-term, and secondly, managing those sites in a way to provide the highest chance of establishing self-sustaining populations. It is a strategy very much in its infancy and close monitoring in these early stages will be crucial for its success and future development. In a wider context, crayfish conservation will pave the way for restoration of marginal sites such as former mineral workings, and there is hope that public engagement with such projects will offer trickle-down benefits for the protection of freshwaters in general.

References

Abrahamsson, S.A.A. (1966) Dynamics of an isolated population of crayfish *Astacus astacus* Linne. *Oikos,* **17**, 96-&.

Abrahamsson, S.a.A. & Goldman, C.R. (1970) Distribution, density and production of crayfish *Pacifastacus leniusculus* dana in Lake Tahoe, California Nevada. *Oikos,* **21**, 83-&.

Acquistapace, P., Aquiloni, L., Hazlett, B.A. & Gherardi, F. (2002) Multimodal communication in crayfish: sex recognition during mate search by male *Austropotamobius pallipes*. *Canadian Journal of Zoology-Revue Canadienne De Zoologie,* **80**, 2041-2045.

Adamczewska, A. & Morris, S. (1994) Exercise in the terrestrial christmas island red crab *Gecarcoidea natalis*-energetics of locomotion. *Journal of Experimental Biology,* **188**, 18.

Adámek, Z., Andreji, J. & Henshaw, A. (2004) Stripping fecundity of common bream (*Abramis brama* L.) from the rivers Trent and Sow (Nottinghamshire, UK). *Aquaculture International,* **12**, 133-137.

Adams, J.A., Tuchman, N.C. & Moore, P.A. (2005) Effects of CO2-altered detritus on growth and chemically mediated decisions in crayfish (*Procambarus clarkii*). *Journal of the North American Benthological Society,* **24**, 330-345.

Ahvenharju, T. (2007) Food intake, growth and social interactions of signal crayfish, *Pacifastacus leniusculus* (Dana).

Alberts, A.C., Oliva, M.L., Worley, M.B., Telford, S.R., Morris, P.J. & Janssen, D.L. (1998) The need for pre-release health screening in animal translocations: a case study of the Cuban iguana (*Cyclura nubila*). *Animal Conservation,* **1**, 165-172.

Alderman, D.J., Holdich, D. & Reeve, I. (1990) Signal crayfish as vectors in crayfish plague in Britain. *Aquaculture,* **86**, 3-6.

Alderman, D.J. & Polglase, J.L. (1988) Pathogens, parasites and commensals In: *Freshwater Crayfish Biology Management and Exploitation.* (Eds D. Holdich & R.S. Lowery), pp. 167-212. Timber Press, Portland, Oregon.

Alderman, D.J., Polglase, J.L., Frayling, M. & Hogger, J. (1984) Crayfish plague in Britain. *Journal of Fish Diseases,* **7**, 401-405.

Alexander, J.E. & Covich, A.P. (1991) Predation risk and avoidance behavior in two freshwater snails. *The Biological Bulletin,* **180**, 387-393.

Alvarez, D. & Nicieza, A. (2003) Predator avoidance behaviour in wild and hatchery-reared brown trout: the role of experience and domestication. *Journal of Fish Biology,* **63**, 1565-1577.

Ameyaw-Akumfi, C. & Hazlett, B.A. (1975) Sex recognition in the crayfish Procambarus clarkii. *Science,* **190**, 1225-1226.

Armitage, K.B. & Wall, T.J. (1982) The effects of body size, starvation and temperature-acclimation on oxygen-consumption of the crayfish *Orconectes nais*. *Comparative Biochemistry And Physiology A-Physiology,* **73**, 63-68.

Armstrong, D.P. & Seddon, P.J. (2008) Directions in reintroduction biology. *Trends in Ecology & Evolution,* **23**, 20-25.

Arudpragasam, K. & Naylor, E. (1966) Patterns of gill ventilation in some decapod Crustacea. *Journal of Zoology,* **150**, 401-411.

Au, D.W.T., Pollino, C.A., Wu, R.S.S., Shin, P.K.S., Lau, S.T.F., & Tang, J.Y.M. (2004) Chronic effects of suspended solids on gill structure, osmoregulation, growth, and triiodothyronine in juvenile green grouper *Epinephelus coioides*. *Marine Ecology-Progress Series,* **266**, 255-264.

Axelsson, E., Nystrom, P., Sidenmark, J. & Bronmark, C. (1997) Crayfish predation on amphibian eggs and larvae. *Amphibia-Reptilia,* **18**, 217-228.

Ayrinhac, A., Debat, V., Gibert, P., Kister, A. G., Legout, H., Moreteau, B., Vergilino, R. & David, J. (2004) Cold adaptation in geographical populations of Drosophila melanogaster: phenotypic plasticity is more important than genetic variability. *Functional Ecology,* **18**, 700-706.

Baldwin, J., Gupta, A. & Iglesias, X. (1999) Scaling of anaerobic energy metabolism during tail flipping behaviour in the freshwater crayfish, *Cherax destructor*. *Marine and Freshwater Research,* **50**, 183-187.

Barbaresi, S., Cannicci, S., Vannini, M. & Fratini, S. (2007) Environmental correlates of two macro-decapods distribution in Central Italy: Multi-dimensional ecological knowledge as a tool for conservation of endangered species. *Biological Conservation,* **136**, 431-441.

Barbaresi, S. & Gherardi, F. (2001) Daily activity of the white-clawed crayfish, *Austropotamobius pallipes* (Lereboullet): a comparison between field and laboratory studies. *Journal of Natural History,* **35**, 1861-1871.

Barefoot, I. (2008) Stoney Cove- Past Present and Future. p. 39. Nautical Archaeology Society.

Batang, Z.B. & Suzuki, H. (2000) Gill structure and gill-cleaning mechanisms of the redclaw crayfish *Cherax quadricarinatus* (Decapoda, Astacidea, Parastacidae). *Journal of Crustacean Biology,* **20**, 699-714.

Bates, D., Maechler, M., Bolker, B. (2013) Linear mixed-effects models using S4 classes, R package lme4

Bauer, R.T. (1998) Gill-cleaning mechanisms of the crayfish Procambarus clarkii (Astacidea : Cambaridae): experimental testing of setobranch function. *Invertebrate Biology,* **117**, 129-143.

Bauer, J.T.(2012) Invasive species:"back-seat drivers" of ecosystem change? *Biological Invasions,* **14**, 1295-1304.

Bearhop, S., Adams, C.E., Waldron, S., Fuller, R.A. & Macleod, H. (2004) Determining trophic niche width: a novel approach using stable isotope analysis. *Journal of Animal Ecology,* **73**, 1007-1012.

Behrens, J.W. & Steffensen, J.F. (2007) The effect of hypoxia on behavioural and physiological aspects of lesser sandeel, *Ammodytes tobianus* (Linnaeus, 1785). *Marine Biology,* **150**, 1365-1377.

Benvenuto, C., Gherardi, F. & Ilheu, M. (2008) Microhabitat use by the white-clawed crayfish in a Tuscan stream. *Journal of Natural History,* **42**, 21-33.

Berrill, M. & Arsenault, M. (1982) Spring breeding of a northern temperate crayfish, *Orconectes rusticus*. *Canadian Journal of Zoology,* **60**, 2641-2645.

Bilotta, G.S. & Brazier, R.E. (2008) Understanding the influence of suspended solids on water quality and aquatic biota. *Water Research,* **42**, 2849-2861.

Biro, P.A. & Stamps, J.A. (2010) Do consistent individual differences in metabolic rate promote consistent individual differences in behavior? *Trends in Ecology & Evolution,* **25**, 653-659.

Blackburn, T.M., Pyšek, P., Bacher, S., Carlton, J.T., Duncan, R.P., Jarošík, V., Wilson, J.R.U. & Richardson, D.M. (2011) A proposed unified framework for biological invasions. *Trends in Ecology & Evolution,* **26**, 333-339.

Blake, M.A. & Hart, P.J.B. (1995) The vulnerability of juvenile signal crayfish to perch and eel predation. *Freshwater Biology,* **33**, 233-244.

Bobeldyk, A.M. & Lamberti, G.A. (2008) A decade after invasion: evaluating the continuing effects of rusty crayfish on a Michigan river. *Journal of Great Lakes Research,* **34**, 265-275.

Boddington, M. (1978) An absolute metabolic scope for activity. *Journal of Theoretical Biology,* **75**, 443-449.

Bøhn, T., Amundsen, P.-A. & Sparrow, A. (2008) Competitive exclusion after invasion? *Biological Invasions,* **10**, 359-368.

Bollache, L., Dick, J.T.A., Farnsworth, K.D. & Montgomery, W.I. (2008) Comparison of the functional responses of invasive and native amphipods. *Biology Letters,* **4**, 166-169.

Bolker, B. M. 2008. *Ecological Models and Data in R,* Princeton University Press.

Bolnick, D.I., Yang, L.H., Fordyce, J.A., Davis, J.M. & Svanbäck, R. (2002) Measuring individual-level resource specialization. *Ecology,* 83**,** 2936-2941.

Bondar, C.A., Bottriell, K., Zeron, K. & Richardson, J.S. (2005) Does trophic position of the omnivorous signal crayfish (*Pacifastacus leniusculus*) in a stream food web vary with life history stage or density? *Canadian Journal of Fisheries and Aquatic Sciences*, **62**, 2632-2639.

Bovbjerg, R.V. (1956) Some factors affecting aggressive behavior in crayfish. *Physiological Zoology*, **29**, 127-136.

Brett, J. (1964) The respiratory metabolism and swimming performance of young sockeye salmon. *Journal of the Fisheries Board of Canada*, **21**, 1183-1226.

Brett, J. (1972) The metabolic demand for oxygen in fish, particularly salmonids, and a comparison with other vertebrates. *Respiration Physiology*, **14**, 151-170.

Brewis, J.M. & Bowler, K. (1982) The growth of the freshwater crayfish *Austropotamobius pallipes* in Northumbria. *Freshwater Biology*, **12**, 187-200.

Brewis, J.M. & Bowler, K. (1985) A study of reproductive females of the freshwater crayfish *Austropotamobius pallipes*. *Hydrobiologia*, **121**, 145-149.

British Geological Survey (2010) Directory of Mines and Quarries 2010 (ninth edition), Keyworth, Nottingham.

Brinkhurst, R.O. (1999) Lumbriculids, branchiobdellidans and leeches: an overview of recent progress in phylogenetic research on clitellates. *Hydrobiologia*, **406**, 281-290.

Brönmark, C. & Hansson, L.A. (2000) Chemical communication in aquatic systems: an introduction. *Oikos*, **88**, 103-109.

Broquet, T., Thibault, M. & Neveu, A. (2002) Distribution and habitat requirements of the white-clawed crayfish, *Austropotamobius pallipes*, in a stream from the Pays de Loire region, France: An experimental and descriptive study. *Bulletin Francais de la Peche et de la Pisciculture*, 717-728.

Brown, B.L., Creed, R.P. & Dobson, W.E. (2002) Branchiobdellid annelids and their crayfish hosts: are they engaged in a cleaning symbiosis? *Oecologia*, **132**, 250-255.

Brown, B.L. & Lawson, R.L. (2010) Habitat heterogeneity and activity of an omnivorous ecosystem engineer control stream community dynamics. *Ecology*, **91**, 1799-1810.

Brown, J.H., Gillooly, J.F., Allen, A.P., Savage, V.M. & West, G.B. (2004) Toward a metabolic theory of ecology. *Ecology*, **85**, 1771-1789.

Brown, J.H., Ross, B., Mccauley, S., Dance, S., Taylor, A.C. & Huntingford, F.A. (2003) Resting metabolic rate and social status in juvenile giant freshwater prawns, *Macrobrachium rosenbergii*. *Marine and Freshwater Behaviour and Physiology*, **36**, 31-40.

Brown, K.M. (1998) The role of shell strength in selective foraging by crayfish for gastropod prey. *Freshwater Biology*, **40**, 255-260.

Brusconi, S., Bertocchi, S., Renai, B., Scalici, M., Souty-Grosset, C. & Gherardi, F. (2008) Conserving indigenous crayfish: stock assessment and habitat requirements in the threatened *Austropotamobius italicus*. *Aquatic Conservation-Marine and Freshwater Ecosystems*, **18**, 1227-1239.

Bubb, D.H., Lucas, M.C. & Thom, T.J. (2002a) Winter movements and activity of signal crayfish *Pacifastacus leniusculus* in an upland river, determined by radio telemetry. *Hydrobiologia*, **483**, 111-119.

Bubb, D.H., Lucas, M.C., Thom, T.J. & Rycroft, P. (2002b) The potential use of PIT telemetry for identifying and tracking crayfish in their natural environment. *Hydrobiologia,* **483**, 225-230.

Bubb, D.H., Thom, T.J. & Lucas, M.C. (2004) Movement and dispersal of the invasive signal crayfish *Pacifastacus leniusculus* in upland rivers. *Freshwater Biology*, **49**, 357-368.

Bubb, D.H., Thom, T.J. & Lucas, M.C. (2005) The within-catchment invasion of the non-indigenous signal crayfish *Pacifastacus leniusculus* (Dana), in upland rivers. *Bulletin Francais de la Peche et de la Pisciculture*, 665-673.

Bubb, D.H., Thom, T.J. & Lucas, M.C. (2006) Movement, dispersal and refuge use of co-occurring introduced and native crayfish. *Freshwater Biology*, **51**, 1359-1368.

Bubb, D.H., Thom, T.J. & Lucas, M.C. (2008) Spatial ecology of the white-clawed crayfish in an upland stream and implications for the conservation of this endangered species. *Aquatic Conservation-Marine and Freshwater Ecosystems*, **18**, 647-657.

Buglife (2009) Criteria for selection of Ark sites for white-clawed crayfish- MS EXcel tool. Draft 1. Vol. Version: 5th April 2009, draft final for consultation. Buglife – The Invertebrate Conservation Trust, Peterborough.

Burggren, W.W., Mcmahon, B.R. & Costerton, J.W. (1974) Branchial water and bloodlfow patterns and the structure of the gill of the crayfish *Procambarus clarkii*. *Canadian Journal of Zoology*, **52**, 7.

Burton, T., Killen, S., Armstrong, J. & Metcalfe, N. (2011) What causes intraspecific variation in resting metabolic rate and what are its ecological consequences? *Proceedings of the Royal Society B: Biological Sciences*, **278**, 3465-3473.

Butler, J.R., Radford, A., Riddington, G. & Laughton, R. (2009) Evaluating an ecosystem service provided by Atlantic salmon, sea trout and other fish species in the River Spey, Scotland: the economic impact of recreational rod fisheries. Fisheries Research, **96**, 259-266.

Capinha, C., Larson, E.R., Tricarico, E., Olden, J.D. & Gherardi, F. (2013) Effects of Climate Change, Invasive Species, and Disease on the Distribution of Native European Crayfishes. *Conservation Biology*.

Cappo, M., Harvey, E. & Shortis, M. (2006) Counting and measuring fish with baited video techniques-an overview. In: AFSB conference and workshop "Cutting-Edge Technologies in Fish and Fisheries Science", 28- 29 August 2006, Hobart, Tasmania.

Cardoso, A.C. & Free, G. (2008) Incorporating invasive alien species into ecological assessment in the context of the Water Framework Directive. *Aquatic Invasions,* **3**, 361-366.

Carpenter,S. R., Caraco, N. F., Correll, D. L., Howarth, R. W., Sharpley, A. N. & Smith, V. H. 1998. Nonpoint pollution of surface waters with phosphorus and nitrogen. *Ecological Applications,* **8**, 559-568.

Carter, I. & Newbery, P. (2004) Reintroduction as a tool for population recovery of farmland birds. *Ibis,* **146**, 221-229.

Catford, J.A., Jansson, R. & Nilsson, C. (2009) Reducing redundancy in invasion ecology by integrating hypotheses into a single theoretical framework. *Diversity and Distributions,* **15**, 22-40.

CEH (2010) Centre for Ecology and Hydrology (CEH). http://www.ceh.ac.uk [accessed 14th May, 2013].

Cerenius, L., Lee, B.L. & Söderhäll, K. (2008) The proPO-system: pros and cons for its role in invertebrate immunity. *Trends in Immunology,* **29**, 263-271.

Cerenius, L. & Söderhäll, K. (2004) The prophenoloxidase-activating system in invertebrates. *Immunological Reviews,* **198**, 116-126.

Chambers, P.A., Hanson, J., Burke, J.M. & Prepas, E.E. (1990) The impact of the crayfish *Orconectes virilis* on aquatic macrophytes. *Freshwater Biology,* **24**, 81-91.

Charlebois, P.M. & Lamberti, G.A. (1996) Invading crayfish in a Michigan stream: direct and indirect effects on periphyton and macro-invertebrates. *Journal of the North American Benthological Society,* 551-563.

Chiesa, S., Maldini, M., Scalici, M., Marzano, F.N. & Gibertini, G. (2011) Assessment of genetic differentiation in Italian populations of *Austropotamobius pallipes* species complex: taxonomic and management implications. *Molecular Phylogenetics and Evolution,* **61**, 1-11.

Christensen, J.P.A., Sand-Jensen, K. and Staehr, P. A. (2013) Fluctuating water levels control water chemistry and metabolism of a charophyte-dominated pond. *Freshwater Biology*, **58**, 1353-1365.Claireaux, G. & Lefrançois, C. (2007) Linking environmental variability and fish performance: integration through the concept of scope for activity. *Philosophical Transactions of the Royal Society B: Biological Sciences,* **362**, 2031-2041.

Clark, T.D., Jeffries, K.M., Hinch, S.G. & Farrell, A.P. (2011) Exceptional aerobic scope and cardiovascular performance of pink salmon (*Oncorhynchus gorbuscha*) may underlie resilience in a warming climate. *The Journal of Experimental Biology*, **214**, 3074-3081.

Clavero, M., Benejam, L. & Seglar, A. (2009) Microhabitat use by foraging white-clawed crayfish (*Austropotamobius pallipes*) in stream pools in the NE Iberian Peninsula. *Ecological Research,* **24**, 771-779.

Cohen, A.N. & Carlton, J.T. (1997) Transoceanic transport mechanisms: The introduction of the Chinese mitten crab *Eriocheir sinensis* to California. *Pacific Science,* **51**.

Cohen, A.N. & Carlton, J.T. (1998) Accelerating invasion rate in a highly invaded estuary. *Science,* **279**, 555-558.

Collas, M., Julien, C. & Monnier, D. (2007) La situation des écrevisses en France résultats des enquêtes nationales réalisées entre 1977 et 2006 par le conseil supérieur de la pêche. *Bulletin Francais de la Peche et de la Pisciculture*, 1-38.

Collins, A.L. & Anthony, S.G. (2008) Assessing the likelihood of catchments across England and Wales meeting 'good ecological status' due to sediment contributions from agricultural sources. *Environmental Science and Policy,* **11**, 163-170.

Conant, S. (1988) Saving endangered species by translocation: are we tinkering with evolution? *Bioscience*, 254-257.

Cooke, J. & Johnson, M. (2002) Ecological restoration of land with particular reference to the mining of metals and industrial minerals: A review of theory and practice. *Environmental Reviews,* **10**, 41-71.

Cooper, C. (1993) Biological effects of agriculturally derived surface water pollutants on aquatic systems—a review. *Journal of Environmental Quality,* **22**, 402-408.

Correia, A.M. (2001) Seasonal and interspecific evaluation of predation by mammals and birds on the introduced red swamp crayfish Procambarus clarkii (Crustacea, Cambaridae) in a freshwater marsh (Portugal). *Journal of Zoology,* **255**, 533-541.

Correia, A.M. (2002) Niche breadth and trophic diversity: feeding behaviour of the red swamp crayfish (*Procambarus clarkii*) towards environmental availability of aquatic macro-invertebrates in a rice field (Portugal). *Acta Oecologica-International Journal of Ecology,* **23**, 421-429.

Correia, A.M. (2003) Food choice by the introduced crayfish *Procambarus clarkii*. *Annales Zoologici Fennici,* **40**, 517-528.

Cossins, A. & 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.

Cote, J., Fogarty, S., Weinersmith, K., Brodin, T. & Sih, A. (2010) Personality traits and dispersal tendency in the invasive mosquitofish (*Gambusia affinis*). *Proceedings of the Royal Society B: Biological Sciences,* **277**, 1571-1579.

Cotton, P.A., Rundle, S.D. & Smith, K.E. (2004) Trait compensation in marine gastropods: shell shape, avoidance behavior, and susceptibility to predation. *Ecology,* **85**, 1581-1584.

Covich, A.P., Crowl, T.A., Alexander JR, J.E. & Vaughn, C.C. (1994) Predator-avoidance responses in freshwater decapod-gastropod interactions mediated by chemical stimuli. *Journal of the North American Benthological Society,* 283-290.

Crandall, K.A. & Buhay, J.E. (2008) Global diversity of crayfish (Astacidae, Cambaridae, and Parastacidae—Decapoda) in freshwater. *Hydrobiologia,* **595**, 295-301.

Crawford, L., Yeomans, W.E. & Adams, C.E. (2006) The impact of introduced signal crayfish *Pacifastacus leniusculus* on stream invertebrate communities. *Aquatic Conservation: Marine and Freshwater Ecosystems,* **16**, 611-621.

Creed, R.P., Jr. (1994) Direct and Indirect Effects of Crayfish Grazing in a Stream Community. *Ecology,* **75**, 2091-2103.

Creed, R.P. & Reed, J.M. (2009) Ecosystem engineering by crayfish in a headwater stream community.

Crooks, J.A. (2002) Characterizing ecosystem-level consequences of biological invasions: the role of ecosystem engineers. *Oikos,* **97**, 153-166.

Crooks, J.A., Chang, A.L. & Ruiz, G.M. (2011) Aquatic pollution increases the relative success of invasive species. *Biological Invasions,* **13**, 165-176.

Crowl, T.A. & Schnell, G.D. (1990) Factors determining population-density and size distribution of a freshwater snail in streams - effects of spatial scale. *Oikos,* **59**, 359-367.

Cruz, M., Segurado, P., Sousa, M. & Rebelo, R. (2008) Collapse of the amphibian community of the Paul do Boquilobo Natural Reserve (central Portugal) after the arrival of the exotic American crayfish *Procambarus clarkii*. *The Herpetological Journal,* **18**, 197-204.

Cullen, W.R., Wheater, C.P. & Dunleavy, P.J. (1998) Establishment of species-rich vegetation on reclaimed limestone quarry faces in Derbyshire, UK. *Biological Conservation,* **84**, 25-33.

Cunningham, A.A. (1996) Disease risks of wildlife translocations. *Conservation Biology,* **10**, 349-353.

Czerniejewski, P., Rybczyk, A. & Wawrzyniak, W. (2010) Diet of the Chinese mitten crab, *Eriocheir sinensis* H. Milne Edwards, 1853, and potential effects of the crab on the aquatic community in the River Odra/Oder estuary (N.-W. Poland). *Crustaceana,* **83**, 195-205.

Dana, E.D., García-De-Lomas, J., González, R. & Ortega, F. (2011) Effectiveness of dam construction to contain the invasive crayfish *Procambarus clarkii* in a Mediterranean mountain stream. *Ecological Engineering,* **37**, 1607-1613.

Danell, K. & Sjoberg, K. (1982) Successional patterns of plants, invertebrates and ducks in a man-made lake. *Journal of Applied Ecology*, 395-409.

Davis, J.C. (1975) Minimal dissolved oxygen requirements of aquatic life with emphasis on Canadian species: a review. *Journal of the Fisheries Board of Canada,* **32**, 2295-2332.

Davis, M.A. (2009) *Invasion biology,* Oxford University Press, USA.

Defra (2012), Department for Environment, Food and Rural Affairs, www.defra.gov.uk [accessed:14th June.]

Degerman, E., Nilsson, P.A., Nystrom, P., Nilsson, E. & Olsson, K. (2007) Are fish populations in temperate streams affected by crayfish? - A field survey and prospects. *Environmental Biology of Fishes,* **78**, 231-239.

Demers, A. & Reynolds, J.D. (2002) A survey of the white-clawed crayfish, *Austropotamobius pallipes* (Lereboullet), and of water quality in two catchments of eastern Ireland. *Bulletin Francais de la Peche et de la Pisciculture*, 729-740.

Demers, A., Reynolds, J.D. & Cioni, A. (2003) Habitat preference of different size classes of *Austropotamobius pallipes* in an Irish river. *Bulletin Francais de la Peche et de la Pisciculture,* 127-137.

Demers, A., Souty-Grosset, C., Trouilhe, M.C., Fureder, L., Renai, B. & Gherardi, F. (2006) Tolerance of three European native species of crayfish to hypoxia. *Hydrobiologia,* **560**, 425-432.

Department of the Environment. (1995) UK Biodiversity Action Plan.

Di Castri, F. (1989) History of biological invasions with special emphasis on the Old World. *Biological invasions: a global perspective*, 1-30.

Diamond, J.M., Ashmole, N. & Purves, P. (1989) The present, past and future of human-caused extinctions [and discussion]. *Philosophical Transactions of the Royal Society of London. B, Biological Sciences,* **325**, 469-477.

Dick, J.T.A. (1995) The cannibalistic behaviour of two *Gammarus* species (Crustacea: Amphipoda). *Journal of Zoology,* **236**, 697-706.

Dick, J.T.A., Gallagher, K., Avlijas, S., Clarke, H.C., Lewis, S.E., Leung, S., Minchin, D., Caffrey, J., Alexander, M.E. & Maguire, C. (2012) Ecological impacts of an invasive predator explained and predicted by comparative functional responses. *Biological Invasions*, 1-10.

Didham, R.K., Tylianakis, J.M., Hutchison, M.A., Ewers, R.M. & Gemmell, N.J. (2005) Are invasive species the drivers of ecological change? *Trends in Ecology & Evolution,* **20**, 470-474.

Distefano, R.J., Gale, C.M., Wagner, B.A. & Zweifel, R.D. (2003) A sampling method to assess lotic crayfish communities. *Journal of Crustacean Biology,* **23**, 678-690.

Dittel, A.I. & Epifanio, C.E. (2009) Invasion biology of the Chinese mitten crab *Eriochier sinensis*: A brief review. *Journal of Experimental Marine Biology and Ecology,* **374**, 79-92.

Donohue, I. & Irvine, K. (2003) Effects of sediment particle size composition on survivorship of benthic invertebrates from Lake Tanganyika, Africa. *Archiv fur Hydrobiologie,* **157**, 131-144.

Douglas, M. E., Marsh, P. C. & Minckley, W. (1994) Indigenous fishes of western North America and the hypothesis of competitive displacement: *Meda fulgida* (Cyprinidae) as a case study. Copeia, 9-19.

Dorn, N.J. & Mittelbach, G.G. (1999) More than predator and prey: A review of interactions between fish and crayfish. *Vie Et Milieu-Life and Environment,* **49**, 229-237.

Dorn, N.J. & Trexler, J.C. (2007) Crayfish assemblage shifts in a large drought-prone wetland: the roles of hydrology and competition. *Freshwater Biology,* **52**, 2399-2411.

Dorn, N.J. & Wojdak, J.M. (2004) The role of omnivorous crayfish in littoral communities. *Oecologia*, **140**, 150-159.

Duckworth, R.A. & Badyaev, A.V. (2007) Coupling of dispersal and aggression facilitates the rapid range expansion of a passerine bird. *Proceedings of the National Academy of Sciences,* **104**, 15017-15022.

Dudgeon, D., Arthington, A.H., Gessner, M.O., Kawabata, Z.I., Knowler, D.J., Lévêque, C., Naiman, R.J., Prieur-Richard, A.H., Soto, D. & Stiassny, M.L.J. (2006) Freshwater biodiversity: importance, threats, status and conservation challenges. *Biological Reviews*, **81**, 163-182.

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

Dunn, J.C., Mcclymont, H.E., Christmas, M. & 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.

Dutton, C. & Conroy, C. (1998) Effects of burrowing Chinese mitten crabs (*Eriocheir sinensis*) on the Thames tideway. *Environment Agency, London, UK.*

Edgerton, B.F., Henttonen, P., Jussila, J., Mannonen, A.R.I., Paasonen, P., Taugbøl, T., Edsman, L. & Souty-Grosset, C. (2004) Understanding the Causes of Disease in European Freshwater Crayfish. *Conservation Biology,* **18**, 1466-1474.

Edmonds, N.J., Riley, W.D. & Maxwell, D.L. (2011) Predation by *Pacifastacus leniusculus* on the intra-gravel embryos and emerging fry of *Salmo salar*. *Fisheries Management and Ecology,* **18**, 521-524.

Edsman, L. & Soderback, B. (1999) Standardised sampling methodology for crayfish- the Swedish protocol. *Freshwater Crayfish,* **12**, 10.

EECOS (2008) The River Stour Crayfish Survey 2007. Essex Ecology Service Ltd, EECOS, 50 pp.

El–Rahim, S.A. & Khallaf, M. (2011) Deterioration and Treatment Study of Archaeological Limestone Statues, Sakkara, Egypt. *Life Science Journal,* **8**.

Elgar, M.A. (1990) Evolutionary compromise between a few large and many small eggs: comparative evidence in teleost fish. *Oikos*, 283-287.

Elgar, M.A. & Crespi, B.J. (1992) *Cannibalism: ecology and evolution among diverse taxa*, Oxford University Press, USA.

Ellis, A., Jackson, M., Jennings, I., England, J. & Phillips, R. (2012) Present distribution and future spread of louisiana red swamp crayfish *Procambarus clarkii* (Crustacea, Decapoda, Astacida, Cambaridae) in Britain: Implications for conservation of native species and habitats. *Knowledge and Management of Aquatic Ecosystems,* 05.

England, W. & Baldwin, J. (1983) Anaerobic energy metabolism in the tail musculature of the Australian yabby *Cherax destructor* (Crustacea, Decapoda, Parastacidae): role of phosphagens and anaerobic glycolysis during escape behavior. *Physiological Zoology*, 614-622.

Englund, G. & Krupa, J.J. (2000) Habitat use by crayfish in stream pools: influence of predators, depth and body size. *Freshwater Biology,* **43**, 75-83.

Environment Agency, 2013 http://www.environment-agency.gov.uk/research/planning/34383.aspx [accessed 14th June 2013].

Environment Agency and Pond Conservation (2002) A guide to monitoring the ecological quality of ponds and canals using PSYM. 15 pp.

European Environment Agency (2000). Total oxygen in river stations by river size. Report no. YIR01WQ2.

Evans, D.O. (2007) Effects of hypoxia on scope-for-activity and power capacity of lake trout (*Salvelinus namaycush*). *Canadian Journal of Fisheries and Aquatic Sciences,* **64**, 345-361.

Evans, L.H. & Edgerton, B.F. (2002) Pathogens, Parasites and Commensals. In: *Biology of freshwater crayfish.* (Ed. D. Holdich), pp. 377-438. Blackwell Science, Oxford.

Evans, R.J., Wilson, J.D., Amar, A., Douse, A., Maclennan, A., Ratcliffe, N. & Whitfield, D.P. (2009) Growth and demography of a re-introduced population of White-tailed Eagles *Haliaeetus albicilla*. *Ibis,* **151**, 244-254.

Facon, B., Genton, B.J., Shykoff, J., Jarne, P., Estoup, A. & David, P. (2006) A general eco-evolutionary framework for understanding bioinvasions. *Trends in Ecology & Evolution,* **21**, 130-135.

FAO (2013) Food and Agriculture Organisation of the United Nations (URL: http://www.fao.org/fishery/statistics/global-production/en) [accessed June 3, 2011]

Farnsworth, K.D., Thygesen, U.H., Ditlevsen, S. & King, N.J. (2007) How to estimate scavenger fish abundance using baited camera data. *Marine Ecology Progress Series,* **350**, 223-234.

Favaro, L., Tirelli, T. & Pessani, D. (2010) The role of water chemistry in the distribution of *Austropotamobius pallipes* (Crustacea Decapoda Astacidae) in Piedmont (Italy). *Comptes Rendus Biologies,* **333**, 68-75.

Favaro, L., Tirelli, T. & Pessani, D. (2011) Modelling habitat requirements of white-clawed crayfish (*Austropotamobius pallipes*) using support vector machines. *Knowledge and Management of Aquatic Ecosystems*, 21.

Fenn, W. (1940) The role of potassium in physiological processes. *Physiological Reviews,* **20**, 377-415.

Fero, K. & Moore, P.A. (2008) Social spacing of crayfish in natural habitats: what role does dominance play? *Behavioral Ecology and Sociobiology,* **62**, 1119-1125.

Feth, J.H. (1981) Chloride in natural continental water: a review. *United States. Geological survey. Water-supply paper*.

Firkins, I. & Holdich, D. (1993) Thermal studies with three species of freshwater crayfish. *Freshwater Crayfish,* **9**, 7.

Fischer, J. & Lindenmayer, D.B. (2000) An assessment of the published results of animal relocations. *Biological Conservation,* **96**, 1-11.

Fischer, J. & Lindenmayer, D.B. (2007) Landscape modification and habitat fragmentation: a synthesis. *Global Ecology and Biogeography,* **16**, 265-280.

Fisher, J.M. (1972) Fine-structural observations on gill filaments of freshwater crayfish, *Astacus pallipes* Lereboullet. *Tissue & Cell,* **4**, 287-&.

Fisher, B, Turner, R.K. & Morling, P. (2009) Defining and classifying ecosystem services for decision making. Ecological economics, **68**, 643-653.

Flegler-Balon, C. (1989) Direct and indirect development in fishes—examples of alternative life-history styles. In: *Alternative life-history styles of animals.* (Ed^Eds, pp. 71-100. Springer.

Folmer, O., Black, M., Hoeh, W., Lutz, R. & 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.

Foster, H.R. & Keller, T.A. (2011) Flow in culverts as a potential mechanism of stream fragmentation for native and nonindigenous crayfish species. *Journal of the North American Benthological Society,* **30**, 1129-1137.

Foster, I.D.L., Collins, A., Naden, P., Sear, D., Jones, J. & Zhang, Y. (2011) The potential for paleolimnology to determine historic sediment delivery to rivers. *Journal of Paleolimnology,* **45**, 287-306.

Foster, J. (1995) Factors influencing the distribution and abundance of crayfish *Austropotamobius pallipes* in Wales and the Marches. *Freshwater Crayfish,* **8**, 78-93.

Foster, J. & Slater, F.M. (1996) A global review of crayfish predation with particular reference to *Austropotamobius* in the Welsh Wye in relation to plague. *Freshwater Crayfish,* **10**.

Frankham, R. (2002) *Introduction to conservation genetics,* Cambridge University Press.

Fratini, S., Zaccara, S., Barbaresi, S., Grandjean, F., Souty-Grosset, C., Crosa, G. & Gherardi, F. (2005) Phylogeography of the threatened crayfish (genus *Austropotamobius*) in Italy: implications for its taxonomy and conservation. *Heredity,* **94**, 108-118.

Frezza, T., Carl, L. & Reid, S. (2003) Evaluation of a portable underwater video camera to study fish communities in two Lake Ontario tributaries. *Journal of Freshwater Ecology,* **18**, 269-276.

Frings, R.M., Vaeßen, S.C., Groß, H., Roger, S., Schüttrumpf, H. & Hollert, H. (2013) A fish-passable barrier to stop the invasion of non-indigenous crayfish. *Biological Conservation,* **159**, 521-529.

Frisch, D., Cottenie, K., Badosa, A. & Green, A.J. (2012) Strong Spatial Influence on Colonization Rates in a Pioneer Zooplankton Metacommunity. *PLoS One,* **7**, e40205.

Frisch, D. & Green, A.J. (2007) Copepods come in first: rapid colonization of new temporary ponds. *Fundamental and Applied Limnology / Archiv fur Hydrobiologie*, **168**, 289-297.

Fry, F.E.J. (1947) *Effects of the environment on animal activity,* Toronto, University of Toronto Press.

Fuereder, L. & Reynolds, J.D. (2003) Is *Austropotamobius pallipes* a good bioindicator? *Bulletin Francais de la Peche et de la Pisciculture*, 157-163.

Füreder, L., Edsman, L., Holdich, D., Kozák, P., Machino, Y., Pockl, M., Renai, B., Reynolds, J., Schulz, R. & Schulz, H. (2006) Indigenous crayfish. Habitat and threats. In: *Atlas of crayfish in Europe* , pp. 25-47. Publications Scientifiques du Muséum National d'Histoire Naturelle (= Collection patrimoines naturels Vol. 64), Paris.

Fureder, L., Ettinger, R., Boggero, A., Thaler, B. & Thies, H. (2006) Macroinvertebrate diversity in alpine lakes: Effects of altitude and catchment properties. *Hydrobiologia,* **562**, 123-144.

Fureder, L., Oberkofler, B., Hanel, R., Leiter, J. & Thaler, B. (2003) The freshwater crayfish *Austropotamobius pallipes* in South Tyrol: Heritage species and bioindicator. *Bulletin Francais de la Peche et de la Pisciculture*, 81-95.

Füreder, L., Oberkofler, B., Hanel, R., Leiter, J. & Thaler, B. (2003) The freshwater crayfish *Austropotamobius pallipes* in south Tyrol: Heritage species and bioindicator. *Bulletin Francais de la Peche et de la Pisciculture,* **370**, 79-95.

Fureder, L., Oberkofler, B., Hanel, R. & Machino, Y. (2002) Freshwater crayfish in South Tyrol (Italy): Distribution and protection measures of endangered *Austropotamobius pallipes*. *Bulletin Francais de la Peche et de la Pisciculture*, 651-662.

Fureder, L. & Reynolds, J.D. (2003) Is *Austropotamobius pallipes* a good bioindicator? *Bulletin Francais de la Peche et de la Pisciculture*, 157-163.

Fureder, L., Summerer, M. & Brandstatter, A. (2009) Phylogeny and species composition of five European species of *Branchiobdella* (Annelida: Clitellata: Branchiobdellida) reflect the biogeographic history of three endangered crayfish species. *Journal of Zoology,* **279**, 164-172.

Gallagher, M.B., Dick, J.T.A. & Elwood, R.W. (2006) Riverine habitat requirements of the white-clawed crayfish, *Austropotamobius pallipes*. *Biology and Environment,* **106B**, 1-8.

Gallardo, B. & Aldridge, D. C. (2013) Evaluating the combined threat of climate change and biological invasions on endangered species. *Biological Conservation,* 160**,** 225-233.

Gannon, J.E. (1971) Two counting cells for the enumeration of zooplankton micro-crustacea. *Transactions of the American Microscopical Society*, 486-490.

Garvey, J.E., Rettig, J.E., Stein, R.A., Lodge, D.M. & Klosiewski, S.P. (2003) Scale-dependent associations among fish predation, littoral habitat, and distributions of crayfish species. *Ecology*, **84**, 3339-3348.

Gates, S. (2002) Review of methodology of quantitative reviews using meta-analysis in ecology. *Journal of Animal Ecology,* **71**, 547-557.

GBIF, 2013 Global Biodiversity Invasions Facility (GBIF). http://www.data.gbif.org [accessed 24th April, 2013].

GB Non-native Species Secretariat (2011) https://secure.fera.defra.gov.uk/nonnativespecies/factsheet/factsheet.cfm?speciesId=1010 [accessed 13th June 2013].

Gelder, S. (1996) A review of the taxonomic nomenclature and a checklist of the species of the Branchiobdellae (Annelida: Clitellata). *Proceedings of the Biological Society Of Washington,* **109**, 653-663.

Gelder, S.R. & Brinkhurst, R.O. (1990) An assessment of the phylogeny of the branchiobdellida (annelida, clitellata), using PAUP. *Canadian Journal of Zoology-Revue Canadienne De Zoologie,* **68**, 1318-1326.

Gelder, S.R., Delmastro, G.B. & Ferraguti, M. (1994) A report on branchiobdellidans (annelida, clitellata) and a taxonomic key to the species in northern Italy, including the 1st record of *Cambarincola mesochoreus* on the introduced American red swamp crayfish. *Bollettino di Zoologia*, **61**, 179-183.

Gelder, S.R., Delmastro, G.B. & Rayburn, J.N. (1999a) Distribution of native and exotic branchiobdellidans (Annelida: Clitellala) on their respective crayfish hosts in northern Italy, with the first record of native *Branchiobdella* species on an exotic North American crayfish. *Journal of Limnology,* **58**, 20-24.

Gelder, S.R., Delmastro, G.B. & Rayburn, J.N. (1999b) Distribution of native and exotic branchiobdellidans (Annelida: Clitellata) on their respective crayfish hosts in northern Italy, with the first record of native *Branchiobdella* species on an exotic North American crayfish. *Journal of Limnology,* **58**, 20-24.

Gelder, S.R., Mccurry, L. & Mcalpine, D.F. (2009) Distribution and First Records of Branchiobdellida (Annelida: Clitellata) from Crayfishes (Crustacea: Decapoda) in the Maritime Provinces of Canada. *Northeastern Naturalist,* **16**, 45-52.

Gelder, S.R. & Siddall, M.E. (2001) Phylogenetic assessment of the Branchiobdellidae (Annelida, Clitellata) using 18S rDNA, mitochondrial cytochrome c oxidase subunit I and morphological characters. *Zoologica Scripta,* **30**, 215-222.

Gherardi, F. & Acquistapace, P. (2007) Invasive crayfish in Europe: the impact of *Procambarus clarkii* on the littoral community of a Mediterranean lake. *Freshwater Biology,* **52**, 1249-1259.

Gherardi, F., Acquistapace, P. & Santini, G. (2001) Foraging by a threatened species - the white-clawed crayfish, *Austropotamobius pallipes*. *Archiv Fur Hydrobiologie,* **152**, 339-351.

Gherardi, F., Acquistapace, P. & Santini, G. (2004) Food selection in freshwater omnivores: a case study of crayfish *Austropotamobius pallipes*. *Archiv fur Hydrobiologie,* **159**, 357-376.

Gherardi, F., Aquiloni, L., Diéguez-Uribeondo, J. & Tricarico, E. (2011) Managing invasive crayfish: is there a hope? *Aquatic sciences,* **73**, 185-200.

Gherardi, F. & Barbaresi, S. (2007) Feeding preferences of the invasive crayfish, *Procambarus clarkii*. *Bfpp-Connaissance Et Gestion Du Patrimoine Aquatique*, 7-20.

Gherardi, F. & Cioni, A. (2004) Agonism and interference competition in freshwater decapods. *Behaviour,* **141**, 1297-1324.

Gherardi, F. & Daniels, W.H. (2004) Agonism and shelter competition between invasive and indigenous crayfish species. *Canadian Journal of Zoology-Revue Canadienne De Zoologie,* **82**, 1923-1932.

Gherardi, F., Smietana, P. & Laurent, P. (2002) Interactions between non-indigenous and indigenous crayfish species. *Bulletin Francais de la Peche et de la Pisciculture,* **367**, 899-907.

Gibson, R.J., Haedrich, R.L. & Wernerheim, C.M. (2005) Loss of fish habitat as a consequence of inappropriately constructed stream crossings. *Fisheries,* **30**, 10-17.

Gil-Sanchez, J. & 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.

Gil-Sanchez, J.M. & Alba-Tercedor, J. (2002) Ecology of the native and introduced crayfishes Austropotamobius pallipes and *Procambarus clarkii* in southern Spain and implications for conservation of the native species. *Biological Conservation*, **105**, 75-80.

Gilbey, V., Attrill, M.J. & Coleman, R.A. (2008) Juvenile Chinese mitten crabs (*Eriocheir sinensis*) in the Thames estuary: distribution, movement and possible interactions with the native crab *Carcinus maenas*. *Biological Invasions*, **10**, 67-77.

Gilliom, R.J., Alley, W.M. & Gurtz, M.E. (1995) *Design of National Water-Quality Assessment Program*, US Government Printing Office.

Giri, T. & Dunham, D. (1999) Use of the inner antennule ramus in the localisation of distant food odours by Procambarus clarkii (Girard, 1852) (Decapoda, Cambaridae). *Crustaceana*, **72**, 123-127.

Goldman, C. & Rundquist, J. (1977) A comparative ecological study of the California crayfish, *Pacifastacus leniusculus* (Dana), from two subalpine lakes (Lake Tahoe and Lake Donner). *Freshwater Crayfish*, **3**, 51-80.

Gollasch, S. (2006). NOBANIS – invasive alien species fact sheet – *Eriocheir sinensis*. From: Online Database of the North European and Baltic Network on Invasive Alien Species, OBANIS www.nobanis.org.

Gonzalez, R., Celada, J.D., Garcia, V., Gonzalez, A., Carral, J.M. & Saez-Royuela, M. (2009) The artificial incubation of crayfish eggs: review and report from an experimental study concerning the effects of offspring origin (maternal or artificial incubation) on the survival and growth of juvenile signal crayfish (*Pacifastacus leniusculus*, Astacidae). *Reviews in Fish Biology and Fisheries*, **19**, 167-176.

Gore, J.A. (1982) Benthic invertebrate colonization: source distance effects on community composition. *Hydrobiologia*, **94**, 183-193.

Gouin, N., Grandjean, F., Bouchon, D., Reynolds, J.D. & Souty-Grosset, C. (2001) Population genetic structure of the endangered freshwater crayfish *Austropotamobius pallipes*, assessed using RAPD markers. *Heredity*, **87**, 80-87.

Gouin, N., Grandjean, F., Pain, S., Souty-Grosset, C. & Reynolds, J. (2003) Origin and colonization history of the white-clawed crayfish, *Austropotamobius pallipes*, in Ireland. *Heredity*, **91**, 70-77.

Grabda, E. & Wierzbicka, J. (1969) The problem of parasitism of the species of the genus *Branchiobdella* Odier, 1823. *Polskie Archiwum Hydrobiologii*, **16**, 93-104.

Grandjean, F., Cornuault, B., Archambault, S., Bramard, M. & Otrebsky, G. (2000) Life history and population biology of the white-clawed crayfish, *Austropotamobius pallipes* pallipes, in a brook from the Poitou-Charentes region (France). *Bulletin Francais de la Peche et de la Pisciculture*, 55-70.

Grandjean, F., Frelon-Raimond, M. & Souty-Grosset, C. (2002) Compilation of molecular data for the phylogeny of the genus *Austropotamobius*: One species or several? *Bulletin Francais de la Peche et de la Pisciculture*, 671-680.

Grandjean, F. & Souty-Grosset, C. (2000) Mitochondrial DNA variation and population genetic structure of the white-clawed crayfish, *Austropotamobius pallipes pallipes*. *Conservation Genetics,* **1**, 309-319.

Grandjean, F., Soutygrosset, C. & Holdich, D.M. (1997) Mitochondrial DNA variation in four British populations of the white-clawed crayfish, *Austropotamobius pallipes*: Implications for management. *Aquatic Living Resources,* **10**, 121-126.

Greenaway, P. (1985) Calcium balance and moulting in the Crustacea. *Biological Reviews,* **60**, 425-454.

Grey, J. (2006) The use of stable isotope analyses in freshwater ecology: current awareness. *Polish Journal of Ecology,* **54**, 563-584.

Grey, J. & Jackson, M.C. (2012) 'Leaves and Eats Shoots': Direct Terrestrial Feeding Can Supplement Invasive Red Swamp Crayfish in Times of Need. *PLoS One,* **7**, e42575.

Griffiths, S.W., Collen, P. & Armstrong, J.D. (2004) Competition for shelter among over-wintering signal crayfish and juvenile Atlantic salmon. *Journal of Fish Biology,* **65**, 436-447.

Guan, R.Z. & Wiles, P.R. (1997) Ecological impact of introduced crayfish on benthic fishes in a British lowland river. *Conservation Biology,* **11**, 641-647.

Guan, R.Z. & Wiles, P.R. (1998) Feeding ecology of the signal crayfish *Pacifastacus leniusculus* in a British lowland river. *Aquaculture,* **169**, 177-193.

Guan, R.Z. & Wiles, P.R. (1999) Growth and reproduction of the introduced crayfish *Pacifastacus leniusculus* in a British lowland river. *Fisheries Research,* **42**, 245-259.

Gutiérrez, J.L., Jones, C.G. & Sousa, R. (2014) Toward an integrated ecosystem perspective of invasive species impacts. *Acta Oecologica,* **54**, 131-138.

Haddaway, N.R. (2010) *Conservation of British white-clawed crayfish (Austropotamobius pallipes) using Ark sites*, University of Leeds.

Haddaway, N.R., Mortimer, R.J., Christmas, M., Grahame, J.W., Dunn, A.M. (2012a) Morphological diversity and phenotypic plasticity in the threatened British white-clawed crayfish (*Austropotamobius pallipes*). *Aquatic Conservation: Marine and Freshwater Ecosystems,* **22**, 220-231.

Haddaway, N.R., Wilcox, R.H., Heptonstall, R.E.A., Griffiths, H.M., Mortimer, R.J.G., Christmas, M. & Dunn, A.M. (2012b) Predatory Functional Response and Prey Choice Identify Predation Differences between Native/Invasive and Parasitised/Unparasitised Crayfish. *PLoS One,* **7**, e32229.

Haddaway, N.R., Mortimer, R.J., Christmas, M. & Dunn, A.M. (2013) Effect of pH on Growth and Survival in the Freshwater Crayfish *Austropotamobius pallipes*. *Freshwater Crayfish*, **19**, 53-62.

Hale, K. & Briskie, J. (2007) Decreased immunocompetence in a severely bottlenecked population of an endemic New Zealand bird. *Animal Conservation,* **10**, 2-10.

Harlioğlu, M.M. (1996) *Comparative biology of the signal crayfish, Pacifastacus leniusculus (Dana), and the narrow-clawed crayfish, Astacus leptodactylus Eschscholtz,* University of Nottingham.

Harris, D.B. (2009) Review of negative effects of introduced rodents on small mammals on islands. *Biological Invasions,* **11**, 1611-1630.

Harrison, F. & Humes, A. (1992) *Microscopic anatomy of invertebrates: Decapod, Crustacea,* Wiley-Liss, New York.

Harvey, G.L., Moorhouse, T.P., Clifford, N.J., Henshaw, A.J., Johnson, M.F., Macdonald, D.W., Reid, I. & Rice, S.P. (2011) Evaluating the role of invasive aquatic species as drivers of fine sediment-related river management problems: The case of the signal crayfish (*Pacifastacus leniusculus*). *Progress in Physical Geography,* **35**, 517-533.

Hecky, R., Smith, R.E., Barton, D., Guildford, S., Taylor, W., Charlton, M. & Howell, T. (2004) The nearshore phosphorus shunt: a consequence of ecosystem engineering by dreissenids in the Laurentian Great Lakes. *Canadian Journal of Fisheries and Aquatic Sciences,* **61**, 1285-1293.

Hedrick, P.W. & Kalinowski, S.T. (2000) Inbreeding depression in conservation biology. *Annual Review of Ecology and Systematics*, 139-162.

Henttonen, P. & Huner, J. (1999) The introduction of alien species of crayfish in Europe: A historical introduction. *Crustacean Issues*, **11**, 13-22.

Herborg, L.M., Rushton, S.P., Clare, A.S. & Bentley, M.G. (2003) Spread of the Chinese mitten crab (*Eriocheir sinensis* H. Milne Edwards) in Continental Europe: analysis of a historical data set. *Hydrobiologia,* **503**, 21-28.

Herborg, L.M., Rushton, S.P., Clare, A.S. & Bentley, M.G. (2005) The invasion of the Chinese mitten crab (*Eriocheir sinensis*) in the United Kingdom and its comparison to continental Europe. *Biological Invasions,* **7**, 959-968.

Herrmann, J.P. & Enders, E.C. (2000) Effect of body size on the standard metabolism of horse mackerel. *Journal of Fish Biology,* **57**, 746-760.

Hladyz, S., Åbjörnsson, K., Giller, P.S. & Woodward, G. (2011) Impacts of an aggressive riparian invader on community structure and ecosystem functioning in stream food webs. *Journal of Applied Ecology,* **48**, 443-452.

Hodder, K.H. & Bullock, J.M. (1997) Translocations of native species in the UK: implications for biodiversity. *Journal of Applied Ecology*, 547-565.

Hogger, J.B. (1986) Aspects of the introduction of signal crayfish *Pacifastacus leniusculus* (Dana), into the southern United Kingdom .1. Growth and survival. *Aquaculture,* **58**, 27-44.

Holdich, D. (2002a) Background and functional morphology. In: *Biology of freshwater crayfish.* (Ed. D. Holdich), pp. 3-29. Blackwell Science Ltd, Oxford.

Holdich, D. (2002b) Distribution of crayfish in Europe and some adjoining countries. *Bulletin Francais de la Peche et de la Pisciculture*, 611-650.

Holdich, D., Reader, J., Rogers, W. & Harlioglu, M. (1995a) Interactions between three species of crayfish (*Austropotamobius pallipes*, *Astacus leptodactylus* and *Pacifastacus leniusculus*). *Freshwater Crayfish,* **10**, 46-56.

Holdich, D. & Rogers, D. (2000) Habitat requirements of the white-clawed crayfish, *Austropotamobius pallipes*. In: *Crayfish Conference* (Eds, p. 97.

Holdich, D., Sibley, P.J. & Peay, S. (2004) The white-clawed crayfish- a decade on. *British Wildlife,* **15**, 11.

Holdich, D.M. (1993) A review of astaciculture: freshwater crayfish farming. *Aquatic Living Resources,* **6**, 307.

Holdich, D.M. (2002c) Distribution of crayfish in Europe and some adjoining countries. *Bulletin Francais de la Peche et de la Pisciculture*, 611-650.

Holdich, D.M. (2003) Ecology of the White-clawed Crayfish. In: *Conserving Natura 2000 Rivers Ecology Series No.1.* English Nature, Peterborough.

Holdich, D.M., Ackefors, H., Gherardi, F., Rogers, W.D. & Skurdal, J. (1999a) Native and alien crayfish in Europe: Some conclusions. *Crayfish in Europe as Alien Species,* **11**, 281-292.

Holdich, D.M. & Gherardi, F. (1999) Native and alien crayfish in Europe: An introduction. In: *Crayfish in Europe as Alien Species: Species: How to Make the Best of a Bad Situation? .* pp. 3-9. CRC Press.

Holdich, D.M., Holdich, D. & Sibley, P. (2003) Crayfish in Europe–an overview of taxonomy, legislation, distribution, and crayfish plague outbreaks. In: *Proceedings of a conference held on 7th November 2002 at the Nottingham Forest Football Club, Notthingham, UK.* pp. 15-34.

Holdich, D.M. & Reeve, I.D. (1991) Distribution of freshwater crayfish in the British Isles, with particular reference to crayfish plague, alien introductions and water quality. *Aquatic Conservation-Marine and Freshwater Ecosystems,* **1**, 139-158.

Holdich, D.M., Rogers, W. & Reader, J. (1995b) *Crayfish conservation,* National Rivers Authority.

Holdich, D.M., Rogers, W.D. & Reynolds, J.D. (1999b) Native and alien crayfish in the British Isles. *Crayfish in Europe as Alien Species,* **11**, 221-235.

Holling, C.S. (1959) Some characteristics of simple types of predation and parasitism. *The Canadian Entomologist,* **91**, 385-398.

Holt, P. (1976) The branchiobdellid (Annelida: Clitellata) associates of astacoidean crawfishes. *Freshwater Crayfish,* **2**, 9.

Holway, D.A. & Suarez, A.V. (1999) Animal behavior: an essential component of invasion biology. *Trends in Ecology & Evolution,* **14**, 328-330.

Hothorn, T., Bretz, F. & Westfall, P. (2008) Simultaneous Inference in General Parametric Models. *Biometrical Journal,* **50**, 346-363.

Houlden, B., England, P., Taylor, S., Greville, W. & Sherwin, W. (1996) Low genetic variability of the koala *Phascolarctos cinereus* in south-eastern Australia following a severe population bottleneck. *Molecular Ecology,* 5**,** 269-281.

Huang, T.-L. & Cong, H.-B. (2007) A new method for determination of chlorophylls in freshwater algae. *Environmental Monitoring and Assessment,* **129**, 1-7.

Hudina, S., Faller, M., Lucić, A., Klobučar, G. & Maguire, I. (2009) Distribution and dispersal of two invasive crayfish species in the Drava River basin, Croatia. *Knowledge and Management of Aquatic Ecosystems*, 09.

Hudson, P. J., Dobson, A. P. & Lafferty, K. D. 2006. Is a healthy ecosystem one that is rich in parasites? *Trends in Ecology & Evolution,* **21**, 381-385.

Huner, J.V., Kowalczuk, J.G. & Avault, J.W. (1976) Calcium and magnesium levels in the intermolt carapaces of three species of freshwater crawfish (Cambaridae: Decapoda). *Comparative Biochemistry and Physiology Part A: Physiology,* **55**, 183-185.

Imhoff, E.M. (2010) *The impact of water chemistry and parasitism by Thelohania contejeani (Microspora) on native (Austropotamobius pallipes) and invasive (Pacifastacus leniusculus) crayfish and their interactions.* PhD, University of Leeds, Leeds.

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

Imhoff, E.M., Mortimer, R.J.G., Christmas, M. & Dunn, A.M. (2012) Horizontal transmission of Thelohania contejeani in the endangered white-clawed (*Austropotamobius pallipes*) and the invasive signal crayfish (*Pacifastacus leniusculus*). *Parasitology,* **1**, 1-7.

Ingle, R. (1997) *Crayfishes, lobsters, and crabs of Europe: an illustrated guide to common and traded species,* Springer.

IUCN (2011) IUCN Red List of Threatened Species. Version 2011.2. . Vol. 22 May

IUCN (2012) IUCN Guidelines for Reintroductions and Other Conservation Translocations.

Jackman, S (2012) pscl: Classes and Methods for R Developed in the Political Science Computational Laboratory, Stanford University. Department of Political Science, Stanford University. Stanford, California. R package version 1.04.4., http://pscl.stanford.edu/

Jackson, M.C., Donohue, I., Jackson, A.L., Britton, J.R., Harper, D.M. & Grey, J. (2012) Population-Level Metrics of Trophic Structure Based on Stable Isotopes and Their Application to Invasion Ecology. *PLoS One,* **7**, e31757.

Jackson, M.C. & Grey, J. (2012) Accelerating rates of freshwater invasions in the catchment of the River Thames. *Biological Invasions*, 1-7.

Jackson, M.C., Jones, T., Milligan, M., Sheath, D., Taylor, J., Ellis, A., England, J. & Grey, J. (2014). Niche differentiation among invasive crayfish and their impacts on ecosystem structure and functioning. *Freshwater Biology,* DOI: 10.1111/fwb.12333Jay, D. & Holdich, D.M. (1981) The distribution of the crayfish, *Austropotamobius pallipes,* in British waters. *Freshwater Biology,* **11**, 121-129.

Jefferies, D.J., Wayre, P., Jessop, R.M. & Mitchelljones, A.J. (1986) Reinforcing the native otter *Lutra lutra* population in East Anglia **-** an analysis of the behavior and range development of the 1st release group. *Mammal Review,* **16**, 65-79.

Jeffries, M.J. (2011) The temporal dynamics of temporary pond macroinvertebrate communities over a 10-year period. *Hydrobiologia,* **661**, 391-405.

Jenkins, M. (2003) Prospects for biodiversity. *Science,* **302**, 1175-1177.

Jennings, J.B. & Gelder, S.R. (1979) Gut structure, feeding and digestion in the branchiobdellid oligochaete *Cambarincola macrodonta* Ellis 1912, an ectosymbiote of the freshwater crayfish *Procambarus clarkii*. *Biological Bulletin,* **156**, 300-314.

JNCC (2011) Joint Nature Conservation Committee (URL: http://jncc.defra.gov.uk) [accessed February 23 2011]

Jobling, S., Coey, S., Whitmore, J., Kime, D., Van Look, K., Mcallister, B., Beresford, N., Henshaw, A., Brighty, G. & Tyler, C. (2002) Wild intersex roach (*Rutilus rutilus*) have reduced fertility. *Biology of Reproduction,* **67**, 515-524.

Johnson, D. & Martlew, R. (2008) Threshfield Quarry: Industrial Hertitage in the Yorkshire Dales. Y.D.L.R. Trust).

Johnson, M.F., Rice, S.P. & Reid, I. (2011) Increase in coarse sediment transport associated with disturbance of gravel river beds by signal crayfish (*Pacifastacus leniusculus*). *Earth Surface Processes and Landforms,* **36**, 1680-1692.

Karatayev, A., Burlakova, L., Padilla, D., Mastitsky, S. & Olenin, S. (2009) Invaders are not a random selection of species. *Biological Invasions,* **11**, 2009-2019.

Keller, R.P., Geist, J., Jeschke, J.M. & Kühn, I. (2011) Invasive species in Europe: ecology, status, and policy. *Environmental Sciences Europe,* **23**, 23.

Kemp, E., Birkinshaw, N., Peay, S. & Hiley, P.D. (2003) *Reintroducing the White-clawed Crayfish Austropotamobius pallipes*. . In: *Conserving Natura 2000 Rivers Conservation Techniques Series No. 1*. English Nature, Peterborough.

Kemp, P., Sear, D., Collins, A., Naden, P. & Jones, I. (2011) The impacts of fine sediment on riverine fish. *Hydrological Processes,* **25**, 1800-1821.

Kerby, J.L., Riley, S.P.D., Kats, L.B. & Wilson, P. (2005) Barriers and flow as limiting factors in the spread of an invasive crayfish (*Procambarus clarkii*) in southern California streams. *Biological Conservation,* **126**, 402-409.

Kershner, M.W. & Lodge, D.M. (1995) Effects of littoral habitat and fish predation on the distribution of an exotic crayfish, *Orconectes rusticus*. *Journal of the North American Benthological Society,* **14**, 414-422.

Kindemba, V., Whitehouse, A. & Peay, S. (2009) Using GIS to prioritise and identify regional Ark sites for White-clawed crayfish: South West aggregate and mineral extraction sites. *Buglife–The Invertebrate Conservation Trust, Peterborough*.

Klobucar, G.I.V., Maguire, I., Gottstein, S. & Gelder, S.R. (2006) Occurrence of Branchiobdellida (Annelida : Clitellata) on freshwater crayfish in Croatia. *Annales De Limnologie-International Journal of Limnology,* **42**, 251-260.

Kozák, P., Füreder, L., Kouba, A., Reynolds, J. & Souty-Grosset, C. (2011) Current conservation strategies for European crayfish. *Knowledge and Management of Aquatic Ecosystems,* **401**, 8.

Krebs, J.R. (1978) Optimal foraging: decision rules for predators. *Behavioural ecology: an evolutionary approach*, 23-63.

Lafferty, K.D. & Kuris, A.M. (1999) How environmental stress affects the impacts of parasites. *Limnology and Oceanography,* **44**, 925-931.

Lake, R.G. & Hinch, S.G. (1999) Acute effects of suspended solids angularity on juvenile coho salmon (*Oncorhynchus kisutch*). *Canadian Journal of Fisheries and Aquatic Sciences,* **56**, 862-867.

Laurent, P. (1988) *Austropotamobius pallipes* and *A.torrentium*, with observations on their interactions with other species in Europe. In: *Freshwater Crayfish.* (D.M. Holdich & R.S. Lowery), pp. 341-364. Chapman & Hall, London.

Lee, C.E. (2002) Evolutionary genetics of invasive species. *Trends in Ecology & Evolution,* **17**, 386-391.

Lee, J.H., Kim, T.W. & Choe, J.C. (2009) Commensalism or mutualism: conditional outcomes in a branchiobdellid-crayfish symbiosis. *Oecologia,* **159**, 217-224.

Leeke, C.J. & Price, A. (1965) *Branchiobdella astaci* in Reading. *Reading Naturalist,* **17**, 18-20.

Leprieur, F., Brosse, S., Garcia-Berthou, E., Oberdorff, T., Olden, J.D. & Townsend, C.R. (2009) Scientific uncertainty and the assessment of risks posed by non-native freshwater fishes. *Fish and Fisheries,* **10**, 88-97.

Lewis, S. & Holdich, D. (2002) *Pacifastacus*. *Biology of freshwater crayfish*, 511-540.

Light, T. (2003) Success and failure in a lotic crayfish invasion: the roles of hydrologic variability and habitat alteration. *Freshwater Biology,* **48**, 1886-1897.

Lodge, D.M. (1991) Herbivory on freshwater macrophytes. *Aquatic Botany,* **41**, 195-224.

Lodge, D.M., Deines, A., Gherardi, F., Yeo, D.C.J., Arcella, T., Baldridge, A.K., Barnes, M.A., Chadderton, W.L., Feder, J.L., Gantz, C.A., Howard, G.W., Jerde, C.L., Peters, B.W., Peters, J.A., Sargent, L.W., Turner, C.R., Wittmann, M.E. & Zeng, Y. (2012) Global Introductions of Crayfishes: Evaluating the Impact of Species Invasions on Ecosystem Services. *Annual Review of Ecology, Evolution, and Systematics,* **43**, 449-472.

Lodge, D.M. & Hill, A.M. (1994) Factors governing species composition, population size, and productivity of cool-water crayfishes. *Nordic Journal of Freshwater Research,* **0**, 111-136.

Lodge, D.M., Kershner, M.W., Aloi, J.E. & Covich, A.P. (1994) Effects of an omnivorous crayfish (*Orconectes rusticus*) on a fresh-water littoral food-web. *Ecology,* **75**, 1265-1281.

Longshaw, M. (2011) Diseases of crayfish: A review. *Journal of Invertebrate Pathology,* **106**, 54-70.

Louette, G. & De Meester, L. (2005) High dispersal capacity of cladoceran zooplankton in newly founded communities. *Ecology,* **86**, 353-359.

Lowe, S., Browne, M., Boudjelas, S. & De Poorter, M. (2000) *100 of the world's worst invasive alien species: a selection from the global invasive species database,* Invasive Species Specialist Group Auckland, New Zealand.

Lucas, M.C., Bubb, D.H., Damian H, Jang, M., Ha, K. & Masters, J.E.G. (2009) Availability of and access to critical habitats in regulated rivers: effects of low-head barriers on threatened lampreys. *Freshwater Biology,* **54**, 621-634.

Mack, R.N., Simberloff, D., Mark Lonsdale, W., Evans, H., Clout, M. & Bazzaz, F.A. (2000) Biotic invasions: causes, epidemiology, global consequences, and control. *Ecological Applications,* **10**, 689-710.

MacNeil, C., Dick, J.T.A. & Elwood, R.W. (1997) The trophic ecology of freshwater *Gammarus* spp. (Crustacea:amphipoda): problems and perspectives concerning the functional feeding group concept. *Biological Reviews,* **72**, 349-364.

MacNeil, C., Elwood, R.W. & Dick, J.T. (1999) Differential microdistributions and interspecific interactions in coexisting *Gammarus* and *Crangonyx* amphipods. *Ecography,* **22**, 415-423.

MacNeil, C. & Platvoet, D. (2013) Could artificial structures such as fish passes facilitate the establishment and spread of the ‘killer shrimp’ *Dikerogammarus villosus* (Crustacea: Amphipoda) in river systems? *Aquatic Conservation: Marine and Freshwater Ecosystems*, DOI: 10.1002/aqc.2337.

Maguire, C. M. & Grey, J. (2006) Determination of zooplankton dietary shift following a zebra mussel invasion, as indicated by stable isotope analysis. *Freshwater Biology,* **51**, 1310-1319.Marcogliese, D.J. & Pietrock, M. (2011) Combined effects of parasites and contaminants on animal health: parasites do matter. *Trends in Parasitology,* **27**, 123-130.

Martens, D.W. & Servizi, J.A. (1993) Suspended solids particles inside gills and spleens of juvenile Pacific salmon (*Oncorhynchus* spp.). *Canadian Journal of Fisheries and Aquatic Sciences,* **50**, 586-590.

Mason, J.C. (1975) Crayfish production in a small woodland stream. *Freshwater Crayfish,* **2**, 30.

Mathews, F., Moro, D., Strachan, R., Gelling, M. & Buller, N. (2006) Health surveillance in wildlife reintroductions. *Biological Conservation,* **131**, 338-347.

Matsuzaki, S.S., Usio, N., Takamura, N. & Washitani, I. (2009) Contrasting impacts of invasive engineers on freshwater ecosystems: an experiment and meta-analysis. *Oecologia,* **158**, 673-686.

Matthews, M., Reynolds, J. & Keatinge, M. (1993) Macrophyte reduction and benthic community alteration by the crayfish *Austropotamobius pallipes* (Lereboullet). *Freshwater Crayfish,* **9**, 289-299.

Matthews, M. & Reynolds, J.D. (1992) Ecological impact of crayfish plague in Ireland. *Hydrobiologia,* **234**, 1-6.

Matthews, M.A. & Reynolds, J.D. (1995) A population study of the white-clawed crayfish *Austropotamobius pallipes* (Lereboullet) in an Irish reservoir. *Biology and Environment-Proceedings of the Royal Irish Academy,* **95B**, 99-109.

Mccutchan, J.H., Lewis, W.M., Kendall, C. & Mcgrath, C.C. (2003) Variation in trophic shift for stable isotope ratios of carbon, nitrogen, and sulfur. *Oikos,* **102**, 378-390.

McDowell, J. E., B. A. Lancaster, D. F. Leavitt, P. Rantamaki, & B. Ripley, 1999. The effects of lipophilic organic contaminants on reproductive physiology and disease processes in marine bivalve molluscs. Limnology and Oceanography 44: 903-909.

Mcfeeters, B.J., Xenopoulos, M.A., Spooner, D.E., Wagner, N.D. & Frost, P.C. (2011) Intraspecific mass-scaling of field metabolic rates of a freshwater crayfish varies with stream land cover. *Ecosphere,* **2** (2)

Mclaughlin, R.L., Porto, L., Noakes, D.L., Baylis, J.R., Carl, L.M., Dodd, H.R., Goldstein, J.D., Hayes, D.B. & Randall, R.G. (2006) Effects of low-head barriers on stream fishes:

taxonomic affiliations and morphological correlates of sensitive species. *Canadian Journal of Fisheries and Aquatic Sciences,* **63**, 766-779.

Mees, C.C. (1983) *Ecological and pollution studies of the British crayfish.* University of Nottingham.

Mehta, S.V., Haight, R.G., Homans, F.R., Polasky, S. & Venette, R.C. (2007) Optimal detection and control strategies for invasive species management. *Ecological Economics,* **61**, 237-245.

Metcalfe, N.B., Taylor, A.C. & Thorpe, J.E. (1995) Metabolic rate, social status and life-history strategies in Atlantic salmon. *Animal Behaviour,* **49**, 431-436.

Metzeling, L., Doeg, T. & O'connor, W. (1995) The impact of salinization and sedimentation on aquatic biota. *Conserving biodiversity: threats and solutions,* 126-136.

Meyer, K.M., Gimpel, K. & Brandl, R. (2007) Viability analysis of endangered crayfish populations. *Journal of Zoology,* **273**, 364-371.

Millenium Ecosystems Assessment (2005) Ecosystems and Human Well-being: Biodiversity Synthesis. In: *World Resources Institute.* Washington DC.

Mitten Crab Recording Project, 2013 (URL: http://mittencrabs.org.uk/) [accessed July 20 2013]

Momot, W. (1993) The role of exploitation in altering the processes regulating crayfish populations. *Freshwater Crayfish,* **9**, 101-117.

Momot, W.T. (1967) Population dynamics and productivity of crayfish *Orconectes virilis* in a marl lake. *American Midland Naturalist,* **78**, 55-&.

Momot, W.T. (1995) Redefining the role of crayfish in aquatic ecosystems. *Reviews in Fisheries Science,* **3**, 33-63.

Momot, W.T., Gowing, H. & Jones, P.D. (1978) Dynamics of crayfish and their role in ecosystems. *American Midland Naturalist,* **99**, 10-35.

Mooney, H.A. & Cleland, E. (2001) The evolutionary impact of invasive species. *Proceedings of the National Academy of Sciences,* **98**, 5446-5451.

Moorhouse, T.P. & Macdonald, D.W. (2011a) The effect of manual removal on movement distances in populations of signal crayfish (*Pacifastacus leniusculus*). *Freshwater Biology,* **56**, 2370-2377.

Moorhouse, T.P. & Macdonald, D.W. (2011b) Immigration rates of signal crayfish (*Pacifastacus leniusculus*) in response to manual control measures. *Freshwater Biology,* **56**, 993-1001.

Moorhouse, T.P. & Macdonald, D.W. (2011c) The effect of removal by trapping on body condition in populations of signal crayfish. *Biological Conservation,* **144,** 1826-1831Mori, M., Pretoni, Y., Salvidio, S. & Balduzzi, A. (2001) Branchiobdellid size-crayfish size: a possible relationship. *Journal of Limnology,* **60**, 208-210.

Murdoch, W. (1972) The functional response of predators. *Biological Control,* **15**, 237-240.

Nardi, P., Bernini, F., Bo, T., Bonardi, A., Fea, G., Ghia, D., Negri, A., Razzetti, E., Rossi, S. & Spairani, M. (2005) Status of *Austropotamobius pallipes* complex in the watercourses of the Alessandria province (NW Italy). *Bulletin Francais de la Peche et de la Pisciculture,* 585-598.

NASA/GSFC, 2012 Moon Phase Predictions by Fred Espenak, (http://eclipse.gsfc.nasa.gov/phase/phasecat.html) [accessed 12th February 2013]

Natural England (2013) (URL: www.naturalengland.org.uk) [accessed 3 July 2013]

Nature After Minerals (2013) (URL: www.afterminerals.com) [accessed 3 July 2013]

Naura, M. & Robinson, M. (1998) Principles of using River Habitat Survey to predict the distribution of aquatic species: an example applied to the native white-clawed crayfish *Austropotamobius pallipes*. *Aquatic Conservation-Marine and Freshwater Ecosystems,* **8**, 515-527.

Neveu, A. (2009) A functional approach to patch suitability using biomass dynamics: application to a residual population of the white-clawed crayfish. *Fundamental and Applied Limnology,* **175**, 185-202.

Nilsson, C., Reidy, C.A., Dynesius, M. & Revenga, C. (2005) Fragmentation and Flow Regulation of the World's Large River Systems. *Science,* **308**, 405-408.

Nilsson, E., Olsson, K., Persson, A., Nystrom, P., Svensson, G. & Nilsson, U. (2008) Effects of stream predator richness on the prey community and ecosystem attributes. *Oecologia,* **157**, 641-651.

Nilsson, G.E., Crawley, N., Lunde, I.G. & Munday, P.L. (2009) Elevated temperature reduces the respiratory scope of coral reef fishes. *Global Change Biology,* **15**, 1405-1412.

Nilsson, E., Solomon, C.T., Wilson, K.A., Willis, T.V., Larget, B. & Vander Zanden, M.J. (2012) Effects of an invasive crayfish on trophic relationships in north-temperate lake food webs. *Freshwater Biology,* **57**, 10-23.

Noonburg, E.G. & Byers, J.E. (2005) More harm than good: when invader vulnerability to predators enhances impact on native species. *Ecology,* **86**, 2555-2560.

Norin, T. & Malte, H. (2011) Repeatability of standard metabolic rate, active metabolic rate and aerobic scope in young brown trout during a period of moderate food availability. *The Journal of Experimental Biology,* **214**, 1668.

Nowicki, P., Tirelli, T., Mussat Sartor, R., Bona, F. & Pessani, D. (2008) Monitoring crayfish using a mark-recapture method: potentials, recommendations, and limitations. *Biodiversity and Conservation,* **17**, 3513-3530.

Nystrom, P. (1999) Ecological impact of introduced and native crayfish on freshwater communities: European perspectives. In: *Crayfish in Europe as Alien Species.* (Ed^Eds, pp. 63-85.

Nystrom, P. (2002) Ecology. In: *Biology of freshwater crayfish.* (Ed, D.M. Holdich), pp. 192-235. Blackwell Science Ltd, Oxford.

Nystrom, P., Bronmak, C. & Graneli, W. (1999) Influence of an exotic and a native crayfish species on a littoral benthic community. *Oikos,* **85**, 545-553.

Nystrom, P., Bronmark, C. & Graneli, W. (1996) Patterns in benthic food webs: A role for omnivorous crayfish? *Freshwater Biology,* **36**, 631-646.

Nystrom, P. & Perez, J.R. (1998) Crayfish predation on the common pond snail (Lymnaea stagnalis): the effect of habitat complexity and snail size on foraging efficiency. *Hydrobiologia,* **368**, 201-208.

Nystrom, P. & Strand, J.A. (1996) Grazing by a native and an exotic crayfish on aquatic macrophytes. *Freshwater Biology,* **36**, 673-682.

Nystrom, P., Svensson, O., Lardner, B., Bronmark, C. & Graneli, W. (2001) The influence of multiple introduced predators on a littoral pond community. *Ecology,* **82**, 1023-1039.

O'Keeffe, C. (1986) *The ecology of two populations of the freshwater crayfish Austropotamobius pallipes (Lereboullet) in Ireland*, Trinity College Dublin.

Oberkofler, B., Quaglio, F., Fureder, L., Fioravanti, M.L., Giannetto, S., Morolli, C. & Minelli, G. (2002) Species of Branchiobdellidae (Annelida) on freshwater crayfish in South Tyrol (Northern Italy). *Bulletin Francais de la Peche et de la Pisciculture*, 777-784.

Ojaveer, H., Gollasch, S., Jaanus, A., Kotta, J., Laine, A.O., Minde, A., Normant, M. & Panov, V.E. (2007) Chinese mitten crab *Eriocheir sinensis* in the Baltic Sea - a supply-side invader? *Biological Invasions,* **9**, 409-418.

Olsson, K. & Nystrom, P. (2009) Non-interactive effects of habitat complexity and adult crayfish on survival and growth of juvenile crayfish (*Pacifastacus leniusculus*). *Freshwater Biology,* **54**, 35-46.

Ottewell, K., Dunlop, J., Thomas, N., Morris, K., Coates, D. & Byrne, M. (2014) Evaluating success of translocations in maintaining genetic diversity in a threatened mammal. *Biological Conservation,* **171**, 209-219.

Osborne, P.E. & Seddon, P.J. (2012) Selecting suitable habitats for reintroductions: variation, change and the role of species distribution modelling. *Reintroduction biology: integrating science and management*, 73-104.

Palmer-Felgate, E.J., Jarvie, H.P., Withers, P.J.A., Mortimer, R.J.G. & Krom, M.D. (2009) Stream-bed phosphorus in paired catchments with different agricultural land use intensity. *Agriculture, Ecosystems & Environment,* **134**, 53-66.

Panning, A. (1939) The Chinese mitten crab. *Smithsonian Annual Report,* **1938**.

Parker, I., Simberloff, D., Lonsdale, W., Goodell, K., Wonham, M., Kareiva, P., Williamson, M., Von Holle, B., Moyle, P. & Byers, J. (1999) Impact: toward a framework for understanding the ecological effects of invaders. *Biological Invasions,* **1**, 3-19.

Parkyn, S.M., Collier, K., & Hicks, B.J. (2001) New Zealand stream crayfish: functional omnivores but trophic predators? *Freshwater Biology,* **46**, 641-652.

Parnell, A., Inger, R., Bearhop, S. & Jackson, A. (2008) SIAR: stable isotope analysis in R. (http://cran. r-project. org/web/packages/siar/index. Html).

Peay, S. (2004) Keynote - A cost-led evaluation of survey methods and monitoring for white-clawed crayfish - Lessons from the UK. *Bulletin Francais de la Peche et de la Pisciculture,* 335-352.

Peay, S. (2009) Selection criteria for "Ark sites" for white-clawed crayfish. *Crayfish Conservation in the British Isles*, 63.

Peay, S., Guthrie, N., Spees, J., Nilsson, E. & Bradley, P. (2009) The impact of signal crayfish (*Pacifastacus leniusculus*) on the recruitment of salmonid fish in a headwater stream in Yorkshire, England. *Knowledge and Management of Aquatic Ecosystems*.

Peay, S., Hiley, P.D., Collen, P. & Martin, I. (2006) Biocide treatment of ponds in Scotland to eradicate signal crayfish. *Bulletin Francais de la Peche et de la Pisciculture*, 1363-1379.

Peay, S. & Rogers, D. (1998) The peristaltic spread of signal crayfish (*Pacifastacus leniusculus*) in the River Wharfe, Yorkshire, England. *Freshwater Crayfish,* **12**, 665-676.

Phillips, B.L., Brown, G.P., Webb, J.K. & Shine, R. (2006) Invasion and the evolution of speed in toads. *Nature,* **439**, 803-803.

Phillips, D.L. & Koch, P.L. (2002) Incorporating concentration dependence in stable isotope mixing models. *Oecologia,* **130**, 114-125.

Pilotto, F., Free, G., Crosa, G., Sena, F., Ghiani, M. & Cardoso, A.C. (2008) The invasive crayfish *Orconectes limosus* in lake varese: estimating abundance and population size structure in the context of habitat and methodological constraints. *Journal of Crustacean Biology,* **28**, 633-640.

Pintor, L.M. & Sih, A. (2009) Differences in growth and foraging behavior of native and introduced populations of an invasive crayfish. *Biological Invasions,* **11**, 1895-1902.

Poff, N.L.R., Allan, J.D., Bain, M.B., Karr, J.R., Prestegaard, K.L., Richter, B.D., Sparks, R.E. & Stromberg, J.C. (1997) The natural flow regime. *Bioscience,* **47**, 769-784.

Polis, G.A., Sears, A.L.W., Huxel, G.R., Strong, D.R. & Maron, J. (2000) When is a trophic cascade a trophic cascade? *Trends in Ecology & Evolution,* **15**, 473-475.

Polis, G.A. & Strong, D.R. (1996) Food web complexity and community dynamics. *American Naturalist,* 813-846.

Possingham, H., Lindenmayer, D. & Norton, T. (1993) A framework for the improved management of threatened species based on population viability analysis (PVA). *Pacific Conservation Biology,* **1**, 39.

Poulin,1999. The functional importance of parasites in animal communities: many roles at many levels? *International Journal for Parasitology,* **29**, 903-914.

Pratten, D.J. (1980) Growth in the crayfish *Austropotamobius pallipes* (Crustacea, Astacidae). *Freshwater Biology,* **10**, 401-412.

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

Preston, D.L., Henderson, J.S. & Johnson, P.T. (2012) Community ecology of invasions: direct and indirect effects of multiple invasive species on aquatic communities. *Ecology,* **93**, 1254-1261.

Priede, I. (1977) Natural selection for energetic efficiency and the relationship between activity level and mortality. *Nature,* **267**, 610-611.

Priede, I. (1985) Metabolic scope in fishes. *Fish energetics: new perspectives*, 33-64.

Priede, I., Bagley, P., Smith, A., Creasey, S. & Merrett, N. (1994) Scavenging deep demersal fishes of the Porcupine Seabight, North-east Atlantic: observations by baited camera, trap and trawl. *Journal of the Marine Biological Association of the United Kingdom,* **74**, 481-498.

Pritchard (2013) Functional response analysis in R, R package Friar.

Pullin, A.S. & Stewart, G.B. (2006) Guidelines for systematic review in conservation and environmental management. *Conservation Biology,* **20**, 1647-1656.

Quaglio, F., Morolli, C., Galuppi, R., Bonoli, C., Marcer, F., Nobile, L., De Luise, G. & Tampieri, M. (2006) Preliminary investigations of disease-causing organisms in the white-clawed crayfish *Austropotamobius pallipes* complex from streams of northern Italy. *Bull. Fr. Pêche Piscic,* **380**, 1271-1290.

R Development Core Team (2011) R: A language and environment for statistical computing. R Foundation for Statistical computing, Vienna, Austria. (URL: www.R-project.org/)

R Development Core Team (2012) R: A language and environment for statistical computing. R Foundation for Statistical computing, Vienna, Austria. (URL: www.R-project.org/)

R Development Core Team (2013) R: A language and environment for statistical computing. R Foundation for Statistical computing, Vienna, Austria. (URL: www.R-project.org/)

Rabeni, C.F. (1985) Resource partitioning by stream-dwelling crayfish: the influence of body size. *American Midland Naturalist,* 20-29.

Rabeni, C.F. (1992) Trophic linkage between stream centrarchids and their crayfish prey. *Canadian Journal of Fisheries and Aquatic Sciences,* **49**, 1714-1721.

Rahel, F.J., Bierwagen, B. & Taniguchi, Y. (2008) Managing Aquatic Species of Conservation Concern in the Face of Climate Change and Invasive Species, *Conservation Biology,* **22**, 551-561.

Rallo, A. & Garcia-Arberas, L. (2002) Differences in abiotic water conditions between fluvial reaches and crayfish fauna in some northern rivers of the Iberian Peninsula. *Aquatic Living Resources*, **15**, 119-128.

Ratcliffe, D. (1974) Ecological effects of mineral exploitation in the United Kingdom and their significance to nature conservation. *Proceedings of the Royal Society of London. A. Mathematical and Physical Sciences,* **339**, 355-372.

Redford, K.H. & Adams, W.M. (2009) Payment for ecosystem services and the challenge of saving nature. *Conservation Biology,* **23**, 785-787.

Rehage, J., Barnett, B. & Sih, A. (2005) Foraging behaviour and invasiveness: do invasive *Gambusia* exhibit higher feeding rates and broader diets than their noninvasive relatives? *Ecology of Freshwater Fish,* **14**, 352-360.

Reiber, C. & Mcmahon, B. (1998) The effects of progressive hypoxia on the crustacean cardiovascular system: a comparison of the freshwater crayfish,(*Procambarus clarkii*), and the lobster (*Homarus americanus*). *Journal of Comparative Physiology B: Biochemical, Systemic, and Environmental Physiology,* **168**, 168-176.

Reid, D.J., Quinn, G.P., Lake, P. & Reich, P. (2008) Terrestrial detritus supports the food webs in lowland intermittent streams of south-eastern Australia: a stable isotope study. *Freshwater Biology,* **53**, 2036-2050.

O'Reilly, P. & Mawle, G. (2008) An appreciation of the social and economic values of sea trout in England and Wales. *Sea Trout: Biology, Conservation and Management*, Harris, G & Milner, N (eds), p.457-465.

Renai, B. & Gherardi, F. (2004) Predatory efficiency of crayfish: comparison between indigenous and non-indigenous species. *Biological Invasions,* **6**, 89-99.

Revenga, C., Campbell, I., Abell, R., De Villiers, P. & Bryer, M. (2005) Prospects for monitoring freshwater ecosystems towards the 2010 targets. *Philosophical Transactions of the Royal Society B: Biological Sciences*, **360**, 397-413.

Reynolds, J. (2006) Manual for monitoring Irish Lake stocks of white-clawed crayfish, *Austropotamobius pallipes* (Lereboullet).

Reynolds, J., Souty-Grosset, C., Gouin, N., Devaney, S. & Grandjean, F. (2000) Experimental restocking of native crayfish in White Lake , Co. Westmeath, Ireland. In: *Crayfish Conference.* (Eds D. Rogers & J.H. Brickland), pp. 123-130. Environment Agency.

Reynolds, J.D., Demers, A. & Marnell, F. (2002) Managing an abundant crayfish resource for conservation - *A. pallipes* in Ireland. *Bulletin Francais de la Peche et de la Pisciculture*, 823-832.

Reynolds, J.D. & Donohoe, R. (2001) Crayfish predation experiments on the introduced zebra mussel, *Dreissena polymorpha*, in Ireland, and their potential for biocontrol. *Bulletin Francais de la Peche et de la Pisciculture*, 669-681.

Reynolds, J.D. & O'keeffe, C. (2005) Dietary patterns in stream- and lake-dwelling populations of *Austropotamobius pallipes*. *Bulletin Francais de la Peche et de la Pisciculture*, 715-730.

Rhodes, C.P. & Holdich, D.M. (1982) Observations on the fecundity of the freshwater crayfish, *Austropotamobius pallipes* (Lereboullet) in the British Isles. *Hydrobiologia,* **89**, 231-236.

Ricciardi, A. & Rasmussen, J.B. (1999) Extinction Rates of North American Freshwater Fauna Tasas de Extinción de Fauna de Agua Dulce en Norteamérica. *Conservation Biology,* **13**, 1220-1222.

Ricciardi, A. (2001) Facilitative interactions among aquatic invaders: is an" invasional meltdown" occurring in the Great Lakes? *Canadian Journal of Fisheries and Aquatic Sciences,* **58**, 2513-2525.

Ricciardi, A. & MacIsaac, H. J. 2011. Impacts of biological invasions on freshwater ecosystems. *Fifty years of invasion ecology: the legacy of Charles Elton***,** 211-224.

Rice, P.R. & Armitage, K.B. (1974) The effect of photoperiod on oxygen consumption of the crayfish *Orconectes nais*. *Comparative Biochemistry and Physiology A,* **47**,1261-1270.

Roberts, P.D., Stewart, G.B. & Pullin, A.S. (2006) Are review articles a reliable source of evidence to support conservation and environmental management? A comparison with medicine. *Biological Conservation,* **132**, 409-423.

Robinson, C.A., Thom, T.J. & Lucas, M.C. (2000) Ranging behaviour of a large freshwater invertebrate, the white-clawed crayfish *Austropotamobius pallipes*. *Freshwater Biology,* **44**, 509-521.

Rodríguez, C.F., Bécares, E. & Fernández-Aláez, M. (2003) Shift from clear to turbid phase in Lake Chozas (NW Spain) due to the introduction of American red swamp crayfish (Procambarus clarkii). *Hydrobiologia,* 506**,** 421-426.

Rodríguez, C.F., Bécares, E., Fernández-Aláez, M. & Fernández-Aláez, C. (2005) Loss of diversity and degradation of wetlands as a result of introducing exotic crayfish. *Biological Invasions,* **7**, 75-85.

Rodriguez, L. F. 2006. Can invasive species facilitate native species? Evidence of how, when, and why these impacts occur. *Biological Invasions,* **8**, 927-939

Rogers, D. (1972) Random search and insect population models. *The Journal of Animal Ecology*, 369-383.

Rogers, D., Hoffmann, R. & Oidtmann, B. (2003) Diseases in selected *Austropotamobius pallipes* populations in England. In: *Management & Conservation of Crayfish.* (Eds, pp. 169-174. Environment Agency, Bristol.

Rogers, D. & Watson, E. (2007) Increasing the chances of successful reintroduction of white-clawed crayfish (*Austropotamobius pallipes*) in the Peak District National Park, UK. *International Journal of Biodiversity Science & Management,* **3**, 209-216.

Rogers, D. & Watson, E. (2011) Distribution database for crayfish in England and Wales. In: *Species Survival: Securing white-clawed crayfish in a changing environment. Proceedings of a conference held on 16th and 17th November 2010 in Bristol, UK.* (Eds M. Rees & J. Nightingale & D.M. Holdich), pp. 14-22.

Rogers, L. (2000) The Feeding Ecology of the Invasive Chinese Mitten Crab, *Eriocheir sinensis*: Implications for California's Freshwater Communities. Senior Research Seminar. *Environmental Science Group Major. University of California at Berkeley, Berkeley, CA.*

Rogers, W., Holdich, D. & Carter, E. (1997) Crayfish eradication. *Report for English Nature, Peterborough*.

Rösner, R., Müller-Navarra, D.C. & Zorita, E. (2012) Trend analysis of weekly temperatures and oxygen concentrations during summer stratification on Lake Plußsee: A long-term study. *Limnology and Oceanography,* **57**, 1479.

Roth, B., Tetzlaff, J., Alexander, M. & Kitchell, J. (2007) Reciprocal Relationships Between Exotic Rusty Crayfish, Macrophytes, and Lepomis Species in Northern Wisconsin Lakes. *Ecosystems,* **10**, 75-86.

Rudnick, D. & Resh, V. (2005) Stable isotopes, mesocosms and gut content analysis demonstrate trophic differences in two invasive decapod crustacea. *Freshwater Biology,* **50**, 1323-1336.

Rudnick, D.A., Halat, K.M. & Resh, V.H. (2000) *Distribution, ecology and potential impacts of the Chinese mitten crab (Eriocheir sinensis) in San Francisco Bay*.

Rudnick, D.A., Hieb, K., Grimmer, K.F. & Resh, V.H. (2003) Patterns and processes of biological invasion: the Chinese mitten crab in San Francisco Bay. *Basic and Applied Ecology,* **4**, 249-262.

Ruiz, G.M., Carlton, J.T., Grosholz, E.D. & Hines, A.H. (1997) Global invasions of marine and estuarine habitats by non-indigenous species: mechanisms, extent, and consequences. *American Zoologist,* **37**, 621-632.

Ruiz, G.M., Fofonoff, P., Hines, A.H. & Grosholz, E.D. (1999) Non-indigenous species as stressors in estuarine and marine communities: assessing invasion impacts and interactions. *Limnology and Oceanography,* **44**, 950-972.

Rukke, N. (2002) Effects of low calcium concentrations on two common freshwater crustaceans, *Gammarus lacustris* and *Astacus astacus*. *Functional Ecology,* **16**, 357-366.

Rundle, S. D., Foggo, A., Choiseul, V. & Bilton, D. T. (2002) Are distribution patterns linked to dispersal mechanism? An investigation using pond invertebrate assemblages. *Freshwater Biology,* **47**, 1571-1581.

Ruokonen, T.J., Kiljunen, M., Karjalainen, J. & Hämäläinen, H. (2012) Invasive crayfish increase habitat connectivity: a case study in a large boreal lake. *Knowledge and Management of Aquatic Ecosystems*, 08.

Rutledge, P. & Pritchard, A. (1981) Scope for activity in the crayfish *Pacifastacus leniusculus*. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology,* **240**, R87-R92.

Sáez-Royuela, M., Carral, J.M., Celada, J.D. & Pérez, J.R. (2002) Effects of shelter type and food supply frequency on survival and growth of stage-2 juvenile white-clawed crayfish (*Austropotamobius pallipes* Lereboullet) under laboratory conditions. *Aquaculture International,* **9**, 489-497.

Sakai, A.K., Allendorf, F.W., Holt, J.S., Lodge, D.M., Molofsky, J., With, K.A., Baughman, S., Cabin, R.J., Cohen, J.E. & Ellstrand, N.C. (2001) The population biology of invasive species. *Annual Review of Ecology and Systematics*, 305-332.

Sala, O.E., Chapin, F.S., Armesto, J.J., Berlow, E., Bloomfield, J., Dirzo, R., Huber-Sanwald, E., Huenneke, L.F., Jackson, R.B. & Kinzig, A. (2000) Global biodiversity scenarios for the year 2100. *Science,* **287**, 1770-1774.

Sale, P.F. (1974) Overlap in resource use, and interspecific competition. *Oecologia,* **17**, 245-256.

Salo, P., Korpimäki, E., Banks, P.B., Nordström, M. & Dickman, C.R. (2007) Alien predators are more dangerous than native predators to prey populations. *Proceedings of the Royal Society B: Biological Sciences,* **274**, 1237-1243.

Sassi, P.L., Borghi, C.E. & Bozinovic, F. (2007) Spatial and seasonal plasticity in digestive morphology of cavies (*Microcavia australis*) inhabiting habitats with different plant qualities. *Journal of Mammalogy,* **88**, 165-172.

Savolainen, R., Westman, K. & Pursiainen, M. (1997) Fecundity of Finnish noble crayfish, Astacus astacus L., and signal crayfish, *Pacifastacus leniusculus*, in various natural habitats and in culture. *Freshwater Crayfish,* **11**, 319-338.

Scalici, M. & Gibertini, G. (2007) Feeding habits of the crayfish *Austropotamobius pallipes* (Decapoda, Astacidae) in a brook in Latium (central Italy). *Italian Journal of Zoology,* **74**, 157-168.

Schlaepfer, M.A., Sax, D.F. & Olden, J.D.(2011) The Potential Conservation Value of Non-Native Species. *Conservation Biology,* **25**, 428-437.

Schmitt, T., Cizek, O. & Konvicka, M. (2005) Genetics of a butterfly relocation: large, small and introduced populations of the mountain endemic Erebia epiphron silesiana. *Biological Conservation,* 123**,** 11-18.

Scholtz, G., Braband, A., Tolley, L., Reimann, A., Mittmann, B., Lukhaup, C., Steuerwald, F. & Vogt, G. (2003) Ecology: parthenogenesis in an outsider crayfish. *Nature,* **421**, 806-806.

Schulz, R., Stucki, T. & Souty-Grosset, C. (2002) Management: Reintroductions and restocking. *Bulletin Francais de la Peche et de la Pisciculture,* **367**, 917-922.

Seddon, P.J., Armstrong, D.P. & Maloney, R.F. (2007) Developing the science of reintroduction biology. *Conservation Biology,* **21**, 303-312.

Shaffer, M., Watchman, L.H., Snape Iii, W.J. & Latchis, I.K. (2002) Population viability analysis and conservation policy. *Population viability analysis. The University of Chicago Press, Chicago*, 123-142.

Sibley, P.J. (2003) Conservation management and legislation the UK experience. *Bulletin Francais de la Peche et de la Pisciculture*, 209-217.

Sih, A. (2004) Behavioral syndromes: an ecological and evolutionary overview. *Trends in Ecology & Evolution,* **19**, 373-378.

Sih, A., Bolnick, D.I., Luttbeg, B., Orrock, J.L., Peacor, S.D., Pintor, L.M., Preisser, E., Rehage, J.S. & Vonesh, J.R. (2010) Predator–prey naïveté, antipredator behavior, and the ecology of predator invasions. *Oikos,* **119**, 610-621.

Sih, A., Cote, J., Evans, M., Fogarty, S. & Pruitt, J. (2012) Ecological implications of behavioural syndromes. *Ecology Letters,* **15**, 278-289.

Simberloff, D. (2011) How common are invasion-induced ecosystem impacts? *Biological Invasions,* **13**, 1255-1268.

Simberloff, D., Martin, J.-L., Genovesi, P., Maris, V., Wardle, D.A., Aronson, J., Courchamp, F., Galil, B., García-Berthou, E., Pascal, M., Pyšek, P., Sousa, R., Tabacchi, E. & Vilà, M. (2013) Impacts of biological invasions: what's what and the way forward. *Trends in Ecology & Evolution,* **28**, 58-66.

Simberloff, D. & Von Holle, B. (1999) Positive interactions of nonindigenous species: invasional meltdown? *Biological Invasions,* **1**, 21-32.

Sint, D. & Fureder, L. (2004) Reintroduction of *Astacus astacus* L. in East Tyrol, Austria. *Bulletin Francais de la Peche et de la Pisciculture*, 301-314.

Skurdal, J., Fjeld, E., Hessen, D.O., Taugbol, T. & Dehli, E. (1988) Depth distribution habitat segregation and feeding of the crayfish *Astacus astacus* in Lake Steinsfjorden southeastern Norway. *Nordic Journal of Freshwater Research*, 113-119.

Skurdal, J., Qvenild, T., Taugbol, T. & Fjeld, E. (1990) A 6-year study of *Thelohania contejeani* parasitism of the noble crayfish, *Astacus astacus* L, in Lake Steinsfjorden, SE Norway. *Journal of Fish Diseases,* **13**, 411-415.

Smith, B., Naden, P. & Cooper, D. (2003) Siltation in Rivers. 3: Integrated Assessment Procedure. Conserving Natura 2000 Rivers Conservation Techniques Series. *English Nature, Peterborough*.

Smith, G.R.T., Learner, M.A., Slater, F.M. & Foster, J. (1996) Habitat features important for the conservation of the native crayfish *Austropotamobius pallipes* in Britain. *Biological Conservation,* **75**, 239-246.

Souty-Grosset, C., Grandjean, F. & Gouin, N. (2003) Involvement of genetics in knowledge, stock management and conservation of *Austropotamobius pallipes* in Europe. *Bulletin Francais de la Peche et de la Pisciculture*, 167-179.

Souty-Grosset, C., Hardy, V., Raimond, R. & Ollivier, L. (2010) Land-use in headwaters and distribution of the native white-clawed crayfish *Austropotamobius pallipes* (Lereboullet) in a stream from the Poitou-Charentes region. *Freshwater Crayfish,* **17**, 129-134.

Souty-Grosset, C., Holdich, D., Noel, P., Reynolds, J. & Haffner, P. (2006a) *Atlas of Crayfish in Europe,* Museum National d'Histoire Naturelle (Patrimoines naturels; 64).

Souty-Grosset, C., Holdich, D.M., Noël, P.Y., Reynolds, J. & Haffner, P. (2006b) *Atlas of crayfish in Europe,* Muséum national d'Histoire naturelle.

Souty-Grosset, C. & Reynolds, J. (2009) Current ideas on methodological approaches in European crayfish conservation and restocking procedures. *Knowledge and Management of Aquatic Ecosystems*, 394-395.

Statzner, B., Fievet, E., Champagne, J.Y., Morel, R. & Herouin, E. (2000) Crayfish as geomorphic agents and ecosystem engineers: Biological behavior affects sand and gravel erosion in experimental streams. *Limnology and Oceanography,* **45**, 1030-1040.

Statzner, B., Peltret, O. & Tomanova, S. (2003) Crayfish as geomorphic agents and ecosystem engineers: effect of a biomass gradient on baseflow and flood-induced transport of gravel and sand in experimental streams. *Freshwater Biology,* **48**, 147-163.

Staehr, P.A., Testa, J.M., Kemp, W.M., Cole, J.J., Sand-Jensen, K. & Smith, S.V. (2012) The metabolism of aquatic ecosystems: history, applications, and future challenges. *Aquatic sciences*, **74**, 15-29.

Stefani F, Zaccara S, Delmastro G, Buscarino M. (2011) The endangered white-clawed crayfish Austropotamobius pallipes (Decapoda, Astacidae) east and west of the Maritime Alps: a result of human translocation? *Conservation Genetics,* **12**: 51-60.

Steffensen, J.F. (1989) Some errors in respirometry of aquatic breathers - how to avoid and correct for them. *Fish Physiology and Biochemistry,* **6**, 49-59.

Steffensen, J.F., Schurmann, H. & Bushnell, P.G. (1994) Oxygen consumption in 4 species of teleosts from Greenland - no evidence of metabolic cold adaptation. *Polar Biology,* **14**, 49-54.

Stein, R.A. (1977) Selective predation, optimal foraging, and the predator-prey interaction between fish and crayfish. *Ecology,* 1237-1253.

Stenroth, P., Holmqvist, N., Nystrom, P., Berglund, O., Larsson, P. & Graneli, W. (2008) The influence of productivity and width of littoral zone on the trophic position of a large-bodied omnivore. *Oecologia,* **156**, 681-690.

Stenroth, P. & Nystrom, P. (2003) Exotic crayfish in a brown water stream: effects on juvenile trout, invertebrates and algae. *Freshwater Biology,* **48**, 466-475.

Stenroth, P., Holmqvist, N., Nystrom, P., Berglund, O., Larsson, P. & Graneli, W. (2006) Stable isotopes as an indicator of diet in omnivorous crayfish (Pacifastacus leniusculus): the influence of tissue, sample treatment, and season. *Canadian Journal of Fisheries and Aquatic Sciences,* **63**, 821-831.

Stoner, A.W., Laurel, B.J. & Hurst, T.P. (2008) Using a baited camera to assess relative abundance of juvenile Pacific cod: Field and laboratory trials. *Journal of Experimental Marine Biology and Ecology,* **354**, 202-211.

Strayer, D.L. (2010) Alien species in fresh waters: ecological effects, interactions with other stressors, and prospects for the future. *Freshwater Biology,* **55**, 152-174.

Stucki, T.P. & Romer, J. (2001) Will *Astacus leptodactylus* displace *Astacus astacus* and *Austropotamobius torrentium* in Lake Ageri, Switzerland? *Aquatic sciences,* **63**, 477-489.

Styrishave, B., Bojsen, B.H., Witthofft, H. & Andersen, O. (2007) Diurnal variations in physiology and behaviour of the noble crayfish *Astacus astacus* and the signal crayfish *Pacifastacus leniusculus*. *Marine and Freshwater Behaviour and Physiology,* **40**, 63-77.

Sutherland, W.J. (2008) *The conservation handbook: research, management and policy,* Wiley. com.

Sutherland, W.J., Armstrong, D., Butchart, S.H., Earnhardt, J.M., Ewen, J., Jamieson, I., Jones, C.G., Lee, R., Newbery, P. & Nichols, J.D. (2010) Standards for documenting and monitoring bird reintroduction projects. *Conservation Letters,* **3**, 229-235.

Sutherland, W.J., Pullin, A.S., Dolman, P.M. & Knight, T.M. (2004) The need for evidence-based conservation. *Trends in Ecology & Evolution,* **19**, 305-308.

Svendsen, J.C., Steffensen, J.F., Aarestrup, K., Frisk, M., Etzerodt, A. & Jyde, M. (2012) Excess posthypoxic oxygen consumption in rainbow trout (*Oncorhynchus mykiss*): recovery in normoxia and hypoxia. *Canadian Journal of Zoology,* **90**, 1-11.

Swanson, G.A. (1984) Dissemination of amphipods by waterfowl. *The Journal of wildlife management,* **48**, 988-991.

Tarmac (2012) (URL: www.tarmac.co.uk) [accessed 3 July 2013)

Taugbøl, T. & Skurdal, J. (1992) Growth, mortality and moulting rate of noble crayfish, *Astacus astacus* L., juveniles in aquaculture experiments. *Aquaculture Research,* **23**, 411-420.

Taylor, I., Jeffries, M., Abbott, S., Hulbert, I. & Virdee, S. (1988) Distribution, habitat and diet of the otter *Lutra lutra* in the Drina catchment, Yugoslavia. *Biological Conservation,* **45**, 109-119.

Teixeira, C.P., De Azevedo, C.S., Mendl, M., Cipreste, C.F. & Young, R.J. (2007) Revisiting translocation and reintroduction programmes: the importance of considering stress. *Animal Behaviour*, **73**, 1-13.

Therneau, T. & Lumley, T. (2011) Survival: Survival analysis, including penalised likelihood. R package version 2.36-5. http://cran.r-project.org/package=survival.

Time and Date AS (2013) [http://www.timeanddate.com].

Tockner, K., Pusch, M., Borchardt, D. & Lorang, M.S. (2010) Multiple stressors in coupled river–floodplain ecosystems. *Freshwater Biology,* **55**, 135-151.

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

Torreblanca, A., Diazmayans, J., Delramo, J. & Nunez, A. (1987) Oxygen-uptake and gill morphological alterations in *Procambarus clarkii* (Girard) after sub-lethal exposure to lead. *Comparative Biochemistry and Physiology C-Pharmacology Toxicology & Endocrinology,* **86**, 219-224.

Townsend, C.R. (1996) Invasion biology and ecological impacts of brown trout *Salmo trutta* in New Zealand. *Biological Conservation,* **78**, 13-22.

Townsend, C.R. (2003) Individual, Population, Community, and Ecosystem Consequences of a Fish Invader in New Zealand Streams Consecuencias de un Pez Invasor sobre Individuos, Poblaciones, Comunidades y Ecosistema en Arroyos de Nueva Zelanda. *Conservation Biology*, 17**,** 38-47.

Tropek, R., Kadlec, T., Karesova, P., Spitzer, L., Kocarek, P., Malenovsky, I., Banar, P., Tuf, I.H., Hejda, M. & Konvicka, M. (2010) Spontaneous succession in limestone quarries as an effective restoration tool for endangered arthropods and plants. *Journal of Applied Ecology*, **47**, 139-147.

Troschel, H.J. (1997) Distribution and ecology of *Austropotamobius pallipes* in Germany. *Bulletin Francais de la Peche et de la Pisciculture*, 639-647.

Trouilhe, M.C., Ricard, F., Parinet, B., Grandjean, F. & Souty-Grosset, C. (2003) Management of the white-clawed crayfish (*Austropotamobius pallipes*) in Western France: Abiotic and biotic factors study. *Bulletin Francais de la Peche et de la Pisciculture*, 97-114.

Trouilhe, M.C., Souty-Grosset, C., Grandjean, F. & Parinet, B. (2007) Physical and chemical water requirements of the white-clawed crayfish (*Austropotamobius pallipes*) in western France. *Aquatic Conservation-Marine and Freshwater Ecosystems,* **17**, 520-538.

Turner, R. & Daily, G. (2008) The ecosystem services framework and natural capital conservation. *Environmental and Resource Economics,* 39**,** 25-35.

Unestam, T. (1972) On the host range and origin of the crayfish plague fungus. *Rep Inst Freshw Res Drottningholm,* **52**, 192-198.

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

Usio, N. (2000) Effects of crayfish on leaf processing and invertebrate colonisation of leaves in a headwater stream: decoupling of a trophic cascade. *Oecologia,* **124**, 608-614.

Usio, N., Kamiyama, R., Saji, A. & Takamura, N. (2009) Size-dependent impacts of invasive alien crayfish on a littoral marsh community. *Biological Conservation,* **142**, 1480-1490.

Usio, N., Nakata, K., Kawai, T. & Kitano, S. (2007) Distribution and control status of the invasive signal crayfish (*Pacifastacus leniusculus*) in Japan. *Japanese Journal of Limnology,* **68**, 471-482.

Usio, N. & Townsend, C.R. (2002) Functional significance of crayfish in stream food webs: roles of omnivory, substrate heterogeneity and sex. *Oikos,* **98**, 512-522.

Ver Hoef, J.M. & Boveng, P.L. (2007) Quasi-Poisson vs. negative binomial regression: how should we model overdispersed count data? *Ecology,* **88**, 2766-2772.

Van Oosterhout, C., Smith, A.M., Hänfling, B., Ramnarine, I.W., Mohammed, R.S. & Cable, J. (2007) The guppy as a conservation model: implications of parasitism and inbreeding for reintroduction success. *Conservation Biology,* **21**, 1573-1583.

Vanni, M.J. (2002) Nutrient cycling by animals in freshwater ecosystems. *Annual Review of Ecology and Systematics*, 341-370.

Veldhuizen, T.C. (2001) Life history, distribution, and impacts of the Chinese mitten crab, *Eriocheir sinensis*. *Aquat Invaders,* **12**, 1-9.

Veldhuizen, T.C. & Stansih, S. (2002) Overview of the life history, distribution, abundance and impacts of the Chinese mitten crab, *Eriocheir sinensis*. *A Draft National Management Plan For the Genus Eriochheir*, 37.

Viggers, K., Lindenmayer, D. & Spratt, D. (1993) The importance of disease in reintroduction programmes. *Wildlife Research,* **20**, 687-698.

Vilà, M., Basnou, C., Pyšek, P., Josefsson, M., Genovesi, P., Gollasch, S., Nentwig, W., Olenin, S., Roques, A. & Roy, D. (2009) How well do we understand the impacts of alien species on ecosystem services? A pan-European, cross-taxa assessment. *Frontiers in Ecology and the Environment,* **8**, 135-144.

Vilà, M., Espinar, J.L., Hejda, M., Hulme, P.E., Jarošík, V., Maron, J.L., Pergl, J., Schaffner, U., Sun, Y. & Pyšek, P. (2011) Ecological impacts of invasive alien plants: a meta-analysis of their effects on species, communities and ecosystems. *Ecology Letters*, **14**, 702-708.

Vitousek, P.M., D'antonio, C.M., Loope, L.L., Rejmanek, M. & Westbrooks, R. (1997a) Introduced species: a significant component of human-caused global change. *New Zealand Journal of Ecology*, **21**, 1-16.

Vitousek, P.M., Mooney, H.A., Lubchenco, J. & Melillo, J.M. (1997b) Human domination of Earth's ecosystems. *Science*, **277**, 494-499.

Vogt, G. (1999) Diseases of European freshwater crayfish, with particular emphasis on interspecific transmission of pathogens. In: *Crayfish in Europe as alien species. How to make the best of a bad situation.* (Eds. F. Gherardi & D. Holdich), pp. 87-103. CRC Press

Vogt, G. (2002) Functional anatomy. In: *Biology of freshwater crayfish.* (Ed. D. Holdich), pp. 53-151. Blackwell Science, Oxford.

Vörösmarty, C.J., McIntyre, P., Gessner, M.O., Dudgeon, D., Prusevich, A., Green, p., Glidden, S., Bunn, S.E., Sullivan, C.A. & Liermann, C.R.(2010). Global threats to human water security and river biodiversity. *Nature,* 467**,** 555-561.

Wallace, J.B. & Webster, J.R. (1996) The role of macro-invertebrates in stream ecosystem function. *Annual Review of Entomology*, **41**, 115-139.

Wang, J. (2005) Estimation of effective population sizes from data on genetic markers. *Philosophical Transactions of the Royal Society B: Biological Sciences*, **360**, 1395-1409.

Wardle, D. A., Bardgett, R.D., Callaway, R.M. & Van der Putten, W.H. (2011) Terrestrial Ecosystem Responses to Species Gains and Losses. *Science,* 332**,** 1273-1277.

Watson, E. & Rogers, D. (2003) A model for the selection of refugia for white-clawed crayfish. *Management & Conservation of Crayfish*, 121.

Webb, B. & Walling, D. (1992) Long term water temperature behaviour and trends in a Devon, UK, river system. *Hydrological sciences journal*, **37**, 567-580.

Weber, L.M. & Lodge, D.M. (1990) Periphytic food and predatory crayfish - relative roles in determining snail distribution. *Oecologia*, **82**, 33-39.

Weeks, A.R., Sgro, C.M., Young, A.G., Frankham, R., Mitchell, N.J., Miller, K.A., Byrne, M., Coates, D.J., Eldridge, M.D.B., Sunnucks, P., Breed, M.F., James, E.A. & Hoffmann, A.A. (2011) Assessing the benefits and risks of translocations in changing environments: a genetic perspective. *Evolutionary Applications,* 4**,** 709-725.

Weinländer, M. & Füreder, L. (2012) Associations between stream habitat characteristics and native and alien crayfish occurrence. *Hydrobiologia*, **693**, 237-249.

Weir, S. & Salice, C. (2012) High tolerance to abiotic stressors and invasion success of the slow growing freshwater snail (*Melanoides tuberculatus*). *Biological Invasions,* **14**, 385-394.

Wetzel, R.G. (1995) Death, detritus, and energy flow in aquatic ecosystems. *Freshwater Biology,* 33**,** 83-89.

Westin, L. & Gydemo, R. (1986) Influence of light and temperature on reproduction and moulting frequency of the crayfish, Astacus astacus L. *Aquaculture*, **52**, 43-50.

Wheatly, M.G. & Taylor, E.W. (1981) The effect of progressive hypoxia on heart-rate, ventilation, respiratory gas-exchange and acid-base status in the crayfish *Austropotamobius pallipes*. *Journal of Experimental Biology,* **92**, 125-141.

Whitehouse, A.T., Peay, S. & Kindemba, V. (2009) Ark sites for White-clawed crayfish - guidance for the aggregates industry. Buglife - The Invertebrate Conservation Trust, Peterborough.

Whitledge, G.W. & Rabeni, C.F. (1997a) Diel and seasonal variation in the food habits of crayfishes in a Missouri Ozark stream. *Freshwater Crayfish 11: A Journal of Astacology,* 159-169.

Whitledge, G.W. & Rabeni, C.F. (1997b) Energy sources and ecological role of crayfishes in an Ozark stream: insights from stable isotopes and gut analysis. *Canadian Journal of Fisheries and Aquatic Sciences,* **54**, 2555-2563.

Williams, F., Eschen, R., Harris, A., Djeddour, D., Pratt, C., Shaw, R., Varia, S., Lamontagne-Godwin, J., Thomas, S. & Murphy, S. (2010) The economic cost of invasive non-native species on Great Britain. *CABI Project No. VM10066. CABI Europe-UK.*

Williamson, M. & Fitter, A. (1996a) The varying success of invaders. *Ecology,* **77**, 1661-1666.

Williamson, M.H. & Fitter, A. (1996b) The characters of successful invaders. *Biological Conservation,* **78**, 163-170.

Wilson, K.A., Magnuson, J.J., Lodge, D.M., Hill, A.M., Kratz, T.K., Perry, W.L. & Willis, T.V. (2004) A long-term rusty crayfish (*Orconectes rusticus*) invasion: dispersal patterns and community change in a north temperate lake. *Canadian Journal of Fisheries and Aquatic Sciences,* **61**, 2255-2266.

Wilson, R.W., Bergman, H.L. & Wood, C.M. (1994) Metabolic costs and physiological consequences of acclimation to aluminum in juvenile rainbow trout (*Oncorhynchus mykiss*). 2: Gill morphology, swimming performance, and aerobic scope. *Canadian Journal of Fisheries and Aquatic Sciences,* **51**, 536-544.

Wolf, C.M., Griffith, B., Reed, C. & Temple, S.A. (1996) Avian and mammalian translocations: update and reanalysis of 1987 survey data. *Conservation Biology,* **10**, 1142-1154.

Woodward, G., Papantoniou, G., Edwards, F. & Lauridsen, R.B. (2008) Trophic trickles and cascades in a complex food web: impacts of a keystone predator on stream community structure and ecosystem processes. *Oikos,* **117**, 683-692.

Woodward, G., Speirs, D.C. & Hildrew, A.G. (2005) Quantification and Resolution of a Complex, Size-Structured Food Web. In: *Advances in Ecological Research.* (Ed^Eds C. Hal), pp. 85-135. Academic Press.

Xenopoulos, M.A., Lodge, D.M., Alcamo, J., Märker, M., Schulze, K. & Van Vuuren, D.P. (2005) Scenarios of freshwater fish extinctions from climate change and water withdrawal. *Global Change Biology,* **11**, 1557-1564.

Yue, G.H. & Orban, L. (2005) A simple and affordable method for high-throughput DNA extraction from animal tissues for polymerase chain reaction. *Electrophoresis,* **26**, 3081-3083.

Zenni, R.D. & Nuñez, M.A. (2013) The elephant in the room: the role of failed invasions in understanding invasion biology. *Oikos,* no-no.

Zhou, S., Shirley, T.C. & Kruse, G.H. (1998) Feeding and growth of the red king crab *Paralithodes camtschaticus* under laboratory conditions. *Journal of Crustacean Biology*, 337-345.

Zuur, A.F. (2009) *Mixed effects models and extensions in ecology with R,* Springer.

Appendix I

Threshfield Ark Site – Species recorded September 2009 to October 2012

Aquatic macroinvertebrates

Beetles

Hydroporus palustris

Gyrinius suffriani

Stictotarsus duodecimpustulatus

Oulimnius tuberculatus

Nebrioporus elegans

Leeches

Helobdella stagnalis

Greater water boatmen

Notonecta maculata

Lesser water boatmen

Glaenocorisa propinqua

Sigara lateralis

Sigara distincta

Mayflies

Caenis luctuosa

Baetidae

Procloeon bifidum

Caddisflies

Polycentropus flavomaculatus

Athripsodes sp.

Limnephilid sp.

Holocentropus picicornis

True-flies

Culicidae sp.

Chironomidae

Sialis sp.

Tipulidae

Birds

Oyster catcher (*Haematopus ostralegus*)

Mallard duck (*Anas platyrhynchos)*

Carrion crow (*Corvus corone*)

Amphibia

Common toad (*Bufo bufo*)

Smooth newt (*Lissotriton vulgaris*)