Invasion of the signal crayfish, *Pacifastacus leniusculus*, in England: implications for the conservation of the white-clawed crayfish, *Austropotamobius pallipes*

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I, Daniel David Adrian Chadwick, confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

Abstract

The spread of invasive species is a key driver of UK native biodiversity loss. The UK's native white-clawed crayfish, *Austropotamobius pallipes*, is in severe decline. The primary contemporary cause of this decline is the invasive non-native signal crayfish, *Pacifastacus leniusculus*, and associated 'crayfish plague' *Aphanomyces astaci*. In this thesis, I provide an updated distribution map of crayfish in England. This work shows that *A. pallipes* continues to significantly decline within England, whilst *P. leniusculus* continues to spread. Special Areas of Conservation were also analysed in the context of localised threats. At a regional scale, I explored the impacts of *P. leniusculus* on native ecological communities in headwaters, using both *A. pallipes* and crayfish-free rivers as controls. At the highest observed Catch-Per-Unit-Effort, populations of *P. leniusculus* severely depleted both invertebrate abundance and richness. I considered *P. leniusculus* population density and structure to be paramount in understanding its invasion ecology, but the literature was often based on biased sampling methods or semi-quantitative data. A novel technique, referred to as a 'triple drawdown', was developed and tested along a high density invaded river, with the intention of defining an exhaustive method of surveying *P. leniusculus*. Densities in excess of 110 m^{-2} crayfish dominated by young-of-year and juvenile cohorts were recorded. The conservation significance of these findings are considered. Finally, the impact of dense *P. leniusculus* populations was explored, using Gut Contents Analysis (GCA) and Stable Isotope Analysis (SIA). *P. leniusculus* exhibited high levels of cannibalism in both low and high density sites. Both SIA and GCA showed a diversification to include other invertebrate groups under high density pressure. As a whole, the thesis shows the importance of understanding the fundamental information of distribution, structure and density of *P. leniusculus* populations, when attempting to manage this highly damaging invasive species, and conserve *A. pallipes*.

Statement of originality with regards to post-graduate collaborations

Collaborative fieldwork with postgraduate students was undertaken during the 2015 and 2016 field seasons. To ensure safety standards in the field, a minimum of two operatives were required at all times to operate the electrofishing equipment. The electrofishing data gathered in 2015 by myself and Lawrence Eagle comprised the core of his MSc thesis at UCL, however both parties agreed to share and use the raw data. All analysis of fish data presented in this thesis was performed solely by the author (Chapter 3), and all discursive ideas presented are the authors own.

Collaborative field work was conducted in 2016 with Eleri Pritchard, who assisted with the drawdown method, and utilised the data in support of her MSc thesis at UCL. The drawdown method was acknowledged within her thesis as having been developed by myself and Paul Bradley. All analysis and discussion of ideas pertaining to the drawdown within the present thesis are the authors own (Chapter 4).

Acknowledgements

The author is grateful to NERC, who funded this work through the London NERC Doctoral Training Partnership and through a grant at the NERC East Kilbride isotope facility, Glasgow. The author is grateful to Dr Carl Sayer (UCL), Dr Michael Chadwick (KCL) and Dr Jan Axmacher (UCL) for their roles in supervising this PhD. Finally, I would like to extend my thanks to the lab staff at both UCL and KCL for their expertise and assistance.

I would like to thank my wonderful girlfriend Eleri, for all her help, support, and patience during this PhD. Being there with a kind word or a coffee has been a great help and comfort. I would like to thank my mum and dad, Susan and Paul, for encouraging me to develop a childhood fascination with my pond and wildlife into (hopefully!) a passionate career in ecology. I would like to thank my sister Ella for our sibling solidarity, and wish her luck finishing her PhD. And finally, I would like to thank Paul Bradley, for being a continued source of inspiration and sage advice.

UCL Thesis Impact Statement

This thesis has developed a novel field sampling method capable of obtaining in-situ data on signal crayfish population size and structure, leading to density estimates far in excess of those previously recognised (Chapter 4). This method, which involves three successive de-waterings of the channel (a so-called "triple drawdown") and subsequent crayfish collection, is applicable to small streams and is of immediate interest to aquatic practitioners concerned with invasive crayfish populations and threats to the native white-clawed crayfish. Importantly, application of the triple drawdown approach highlights current flaws in contemporary methods of sampling crayfish (especially trapping) and has paved the way forward to new crayfish sampling approaches. The data derived from a drawdown can be of wider use to persons modelling systems containing crayfish, as size classes and growth functions can be derived from it. In particular results from this work have been shared with the Environment Agency and Natural England, the governmental bodies responsible for licensing crayfish work and for implementing legislation. The drawdown method is currently under further study (through a London NERC DTP studentship to Eleri Pritchard) and has recently been published as a case study in the 2018 textbook: *Freshwater Ecology and Conservation: Approaches and Techniques*, edited by Jocelyne M. R. Hughes, by Oxford University Press.

Through a collaborative internship with the Environment Agency and Natural England, the author has generated the most recent and comprehensive distribution dataset and associated set of maps of both native white-clawed crayfish and invasive signal crayfish in England. This output has been directly used for UK reporting to Europe under Article 17 of the EC Habitats Directive. Reporting population trends for both species on a European platform facilitates delivery of key conservation messages at a scale much larger than usually attainable by a doctoral study. A major impact here is highlighting the unfavourable status of native crayfish within England, which will hopefully stimulate better white-clawed crayfish conservation prioritisation.

Finally, the database produced through the digitisation and collation of the Environment Agency and Natural England records, will form a central resource for the new 'National Crayfish Strategy' document for the Environment Agency in England, set to be implemented in January 2019. The strategy will outline how the Environment Agency will approach conservation of the white-clawed crayfish in the short to medium term.

Contents

Chapter 1 – Introduction, theories and concepts

Invasive species in freshwater ecosystems

In the UK freshwater systems have undergone drastic changes due to anthropogenic influences and management, especially nutrient enrichment induced by agricultural intensification, major drainage schemes and river habitat degradation. Despite a history of habitat degradation and pollution, UK freshwaters support a wealth of native biodiversity. Of the 3,148 UK species assessed in the State of Nature report, however, 60% are in decline, of which 31% are strongly in decline (Burns *et al.*, 2013). Species abundance as a whole is a dynamic picture, however species exhibiting specialised adaptations and narrow environmental niches are mostly faring worse than generalists. Increasingly there is a call to both protect existing biodiversity, and promote native species and habitats, often in the form of increased funding (Buglife, 2014), management strategies (e.g. Sayer, 2014), or legislative stringency (e.g. Reynolds, 1998). However, a major and growing threat to remaining biodiversity are invasive species.

Invasive species are a major and direct driver of global biodiversity loss (Millennium Ecosystem Assessment, 2005), which disrupt both species conservation efforts and ecosystem services (e.g. Molnar *et al.*, 2008; Vilà *et al.*, 2009). Invasive species are estimated to cost $120 billion year^{-1} in the US alone (Pimentel *et al.*, 2005), with figures

of £1.7 billion year^{-1} estimated for the UK (Williams *et al.*, 2010). These species remain as significant threats due to their continued spread through anthropogenic activities (Hulme, 2009). Inland water systems have been estimated to comprise 20% of the entire value of the global biosphere ($6.6 trillion year^{-1} of $33 trillion, in Costanza *et al.*, 1997), but have also been identified as particularly vulnerable to invasion due to the high connectivity which is often inherent within aquatic landscapes, combined with the distinct evolutionary lineages that have developed due to geographical isolation of systems (Gherardi, 2007). Freshwater systems are known to support incredible levels of biodiversity (Dudgeon et al., 2006; Reid et al., 2018) whilst also providing important socioeconomic, ecological, and broader cultural and aesthetic benefits.

Invasive success of crayfish species

Crayfish are a diverse group of freshwater decapod crustaceans with over 590 species described, and with a global distribution centred around temperate latitudes and a notable absence of crayfish native to Africa and India (Richman *et al.*, 2015). The majority of this diversity occurs in North America, where an estimated 415 species occur naturally, primarily from the family *Cambaridae* (Richman *et al.*, 2015). However, in excess of 30% of global crayfish species are threatened, estimated in some instances to be as many as 50% of the local crayfish species (Taylor *et al.*, 2007). Indeed, extinctions rates of crayfish are at risk of increasing to an order of magnitude greater than extinction rates of freshwater fishes and amphibians (Ricciardi and Rasmussen, 1999), with invasive species identified as a major threat to crayfish species. However, there is a lack of data on basic crayfish ecology of many species, with 21% being described as data deficient (Richman *et al.*, 2015).

Crayfish species have a much higher invasive success rate than might be expected (Holdich *et al.*, 1999). A general theory proposed for invasion biology is the ‘tens’ rule

(Williamson, 1996; Williamson and Fitter, 1996). This rule theorises that, at each stage of the invasion process, namely species transitioning from being imported to introduced, from being introduced to becoming established, and ultimately from being established to causing negative social, ecological and economic impacts, 10% of the species will succeed. Crayfish are adept and successful invaders, with 28 species currently established globally outside of their native range (Holdich *et al.*, 2014), of which 7 are described as 'invasive' (Gherardi, 2010). This is due to increased trade and movement of these species for aquaria purposes over the last few decades, and the shared ecological traits that make certain species both suitable for aquaculture (e.g. being robust and fecund) and invasive (Pimentel *et al.*, 2005; Holdich & Sibley, 2009). The red-swamp crayfish *Procambarus clarkii* (Girard), for example, has successfully established populations and has caused ecological damage in 86% of the countries globally that it has been introduced into (García-Berthou et al., 2005). Indeed Holdich *et al.* (2009) reported that, of the 10 species of crayfish introduced into Europe, 9 have established self-sustaining populations. Within the UK there are 7 non-native species of crayfish with breeding population(s) confirmed in the wild (Table 1). Species distribution varies from a few isolated populations to UK wide. These non-native crayfish are currently exerting significant pressure on the UK's only native species, the white-clawed crayfish *Austropotamobius pallipes* (Lereboullet). The signal crayfish *Pacifastacus leniusculus* (Dana), spiny-cheeked crayfish *Orconectes limosus* (Rafinesque), red swamp crayfish *P. clarkii* and virile crayfish *Orconectes virilis* (Hagen) all act as vectors of a fungal-like pathogen termed 'crayfish plague' (Table 1; Ahern *et al.*, 2008). Crayfish plague is caused by the oomycete, *Aphanomyces astaci* (Shikora), to which non-European crayfish, and thus *P. leniusculus*, are immune to but function as vectors. However the noble crayfish *Astacus astacus* (L.), turkish crayfish

Astacus leptodactylus (Eschscholtz) and white-clawed crayfish *A. pallipes* are all highly susceptible to crayfish plague (Holdich & Rogers, 1997).

The most widely distributed and prevalent of the invasive crayfish present in the UK is the signal crayfish (hereafter *P. leniusculus*), a globally invasive crayfish which has had major negative impacts on *A. pallipes* in the UK (Holdich and Reeve, 1991; Holdich *et al.*, 2009, 2014). Indeed, in the UK and Europe, native populations of crayfish are thought to be particularly at risk from invasive crayfish species (Holdich *et al.*, 2014), with this threat exacerbated by additional environmental pressures such as water quality issues (Richman *et al.*, 2015).

Table 1 – The 7 confirmed non-native crayfish species present in the UK.

Common name	Latin name	Native range	Locations in the UK	Literature reporting first population
Signal crayfish	*Pacifastacus leniusculus* (Dana 1852)	Northern USA	England, Wales, Scotland,	e.g. Holdich & Reeve, 1991
Virile crayfish	*Orconectes virilis* (Hagen 1870)	North-Central USA	London	Ahern *et al.*, 2008
Spiny-cheeked crayfish	*Orconectes limosus* (Rafinesque 1817)	Eastern USA	Warwickshire, East England, London	Holdich & Black, 2007
Noble crayfish	*Astacus astacus* (L.)	Europe	South West England	Holdich *et al.*, 1995
Red swamp crayfish	*Procambarus clarkii* (Girard 1852)	Southern USA and Mexico	London	Holdich *et al.*, 1999
Turkish crayfish	*Astacus leptodactylus* (Eschscholtz 1823)	Ponto Caspian Basin	Southeast England, isolated populations in England & Wales	Goddard & Hogger, 1986
White river crayfish	*Procambarus acutus acutus* (Girard 1852)	Southern USA	Single lentic introduction site, Windsor	NNSS, 2011

Ecology of *A. pallipes* in the UK

The white-clawed crayfish (hereafter *A. pallipes*) is the UKs largest native freshwater invertebrate, and only native crayfish species (Holdich & Reeve, 1991). *A. pallipes* is a freshwater species, inhabiting rivers, ponds and lakes across the country. *A. pallipes* is a slow growing species, achieving a maximum carapace length (CL) of >60 mm, however adult sizes of 45 mm CL are more typical (Reynolds, 1998). Individuals can live for as long as 12 years, and reach sexual maturity at around 22-26 mm CL, approximately 3-4 years of age (Thomas & Ingle, 1987; Woodlock & Reynolds, 1988; Smith *et al.*, 1996; Reynolds, 1998). However, growth potential and growth rates are known to vary according to temperature and habitat suitability. Key habitat characteristics that facilitate abundant and recruiting populations of *A. pallipes* within lotic and lentic systems are vertical undercut banks (Holdich and Rogers, 2000) along with tree roots (Benvenuto *et al.*, 2008), and suitably sized stones and cobbles (Foster, 1993). These habitats provide shelter and refuge from predators and hydrological extremes (Smith *et al.*, 1996). Habitat use is related to the age and thus size of individual animals (Benvenuto *et al.*, 2008), as smaller individuals are most at risk of predation from aquatic predators and thus utilise shallow riffles, and larger individuals are most at risk from terrestrial predators and thus utilise deeper pools, respectively (Englund & Krupa, 2000). Habitat complexity is therefore important for supporting a healthy cohort structure within *A. pallipes* populations (Hutchings, 2009).

Like many crayfish species, *A. pallipes* are sexually dimorphic, with males having larger and broader claws, but a narrower abdomen, and females having smaller claws but a wider flatter abdomen (Rhodes & Holdich, 1979). Following mating in late Autumn, the now ‘berried’ females carry the fertilised eggs through the winter months, using the wide abdomen to secure and shelter the eggs over winter (Ingle and Thomas, 1974; Gledhill *et al.*, 1993). The eggs hatch during May and June the following year

(Villanelli & Gherardi, 1998). *A. pallipes* are smaller and less fecund than many invasive crayfish species, with brood sizes typically varying from approximately 40 to 80 eggs (Brewis & Bowler, 1985; Reynolds, 1998), with an average of 50% of the eggs surviving. The brood number is, however, related to female size, and may range from as few as 20 eggs to over 220 (Carral *et al.*, 1994). Juvenile *A. pallipes* undergo multiple successive moults in their first year (Smith *et al.*, 1996), with the frequency of moults reducing to a single moult per year in fully matured adults (Reynolds, 1998). *A. pallipes* require calcium to harden their exoskeleton following moulting, and as such calcium availability is important in supporting healthy populations of *A. pallipes* (Jay & Holdich, 1981). Values of 5 mg L^{-1} are often stated to be the minimum required concentration for *A. pallipes* populations (e.g. Gledhill *et al.*, 1993; Holdich and Rogers, 2000; Haddaway *et al.*, 2015), however there is some evidence to suggest that lower calcium concentrations can be tolerated (see Trouilhé *et al.*, 2003). *A. pallipes* is often attributed in the literature with requiring consistent excellent water quality to thrive, with a general intolerance to anoxia (e.g. Holdich & Reeve, 1991; Haddaway *et al.*, 2015). Benvenuto *et al.* (2008) describe *A. pallipes* as stenoecious, highlighting both the sensitivity and narrow environmental niche and tolerance of the species. In Ireland, where *A. pallipes* populations display a more natural distribution due to the absence of invasive crayfish species (Reynolds & Demers, 2006), *A. pallipes* is found in waters of good quality, often over limestone substrates and rarely in acidic or polluted catchments (Lucey & McGarrigle, 1987). *A. pallipes* is considered to be associated with easily weathered base-rich substrata (Jay & Holdich, 1981) which typically support soils and waters in a range of neutral to alkaline pH (Haddaway *et al.*, 2015), offering a sufficient supply of inorganic ions (Goddard & Hogger, 1986; Lucey & McGarrigle, 1987).

A. pallipes will consume organic detritus, thus providing an important conduit for energy transfer through food webs (Lorman & Magnuson, 1978). Macrophytes and algae are also grazed by *A. pallipes*, which has even been known to feed on terrestrial vegetation (Gledhill *et al.*, 1993). Whilst *A. pallipes* is both a detritivore and a herbivore, it also occupies the role of an active predator, with invertebrate, amphibian and fish matter thought to have been previously understated as diet components (Momot, 1995).

Ecology of *P. leniusculus* in the UK

The invasive crayfish species with the greatest impact on *A. pallipes* in the UK is *P. leniusculus* due largely to is wide distribution in UK waters. From its native range in North Western America, *P. leniusculus* has been spread to California, onwards to Scandinavia and into the UK via Europe (Lewis, 2002; Souty-Grosset *et al.*, 2006; Holdich *et al.*, 2009). The reason for this long-range anthropogenic spread of *P. leniusculus* was primarily the aquaculture industry which was struggling due to failing stocks of native crayfish species (Abrahamsson and Goldman, 1970). Somewhat ironically but unbeknownst at the time, native European crayfish (including *A. pallipes*) were declining due to the spread of invasive species such as *P. leniusculus* and associated disease.

In its native range, *P. leniusculus* inhabits both lentic and lotic systems, and is also tolerant of a wide range of aquatic conditions and habitats within its invasive ranges such as ponds and ditches (Lewis, 2002). The habitat requirements of *P. leniusculus* are similar to *A. pallipes*, however, with a notable exception of the burrowing behaviour of *P. leniusculus*. In its native range, *P. leniusculus* is not reported to burrow (Lewis, 2002); however, *P. leniusculus* extensively and prolifically burrows in the UK, such that it causes bank destabilisation and reduces water quality through bioturbation (Harvey *et*

al., 2011). *P. leniusculus* are highly fecund, with larger females having larger brood sizes (Fig. 1), which are often in excess of 200-400 eggs (McGriff, 1983). *P. leniusculus* are a polytrophic, omnivorous crayfish species, capable of feeding on organic detrital matter (Ercoli *et al.*, 2014), macrophytes (Nyström *et al.*, 1999), macroinvertebrates (Mathers *et al.*, 2016), and fish (Guan and Wiles, 1997). Due to the direct and indirect impacts that this polytrophic feeding can have on biodiversity (e.g. Momot, 1995) and trophic functioning (Crawford *et al.*, 2006), *P. leniusculus* have been the subject of several studies (see Ibbotson and Furse, 1995; Holdich *et al.*, 2009). One major impact that *P. leniusculus* has had for crayfish species native to Europe is due to the crayfish plague. *A. pallipes* lack the immunity to the plague spores (Unestam, 1972, in Schrimpf *et al.*, 2012), which enter through the cuticle, affecting the nervous system and releasing neurotoxic compounds. A loss of mobility and control is observed in the animals, as well as animals appearing during daylight. This behaviour goes against their nocturnal nature, and exposes them to increased predation pressure. Individual *A. pallipes* may die as soon as 7 days after exposure, occasionally taking several months to die; the disease however is always fatal (Alderman *et al.*, 1987). *A. astaci* is capable of wiping out entire populations (Kemp *et al.*, 2003, in Dunn *et al.*, 2008), such as in the River Lathkill (Derbyshire) in 1993 (Rogers, 1998). There is no cure for the crayfish plague. In the absence of plague, populations of *P. leniusculus* are ecologically dominant, and cause local extinctions through competitive exclusion of *A. pallipes* populations, usually within 6-7 years of *P. leniusculus* being recorded (Bubb *et al.*, 2005).

Morphological differences and identifying features of *A. pallipes* and *P. leniusculus*

A. pallipes is distinguishable from the most common invasive crayfish species in the UK, *P. leniusculus*, through several morphological differences. Adult *A. pallipes* are smaller than *P. leniusculus*, and *A. pallipes* is less colourful, being olive, brown or beige

in colour (with the notable exception of the rare blue colour morph of *A. pallipes*, Fig. 2a and b). The claws (chela) are pitted in appearance, with a white to pink tinge underneath (Fig. 2c), which is not to be confused with the obviously red underside of *P. leniusculus* claws (Fig. 2d). *A. pallipes* also do not possess the white 'signal' mark on the upper surface of the chela as seen in *P. leniusculus* (Fig. 2d). The ability to distinguish a native and a non-native crayfish species from one another is a crucial skill, for example in mixed populations, when discovering previously unknown populations of any species, and for accurate reporting for biosecurity. The carapace of *A. pallipes* sports spines protruding from the shoulders which are absent in the smooth bodied *P. leniusculus* (Fig. 2e). The rostrum converges into a pronounced point, and is flanked on either side by post-orbital ridges. For further morphometric detail and how to distinguish between species see Grandjean *et al.* (1997) and Gledhill *et al.* (1993), respectively.

Figure 1 – Large female *P. leniusculus* with recently hatched out juveniles still attached.

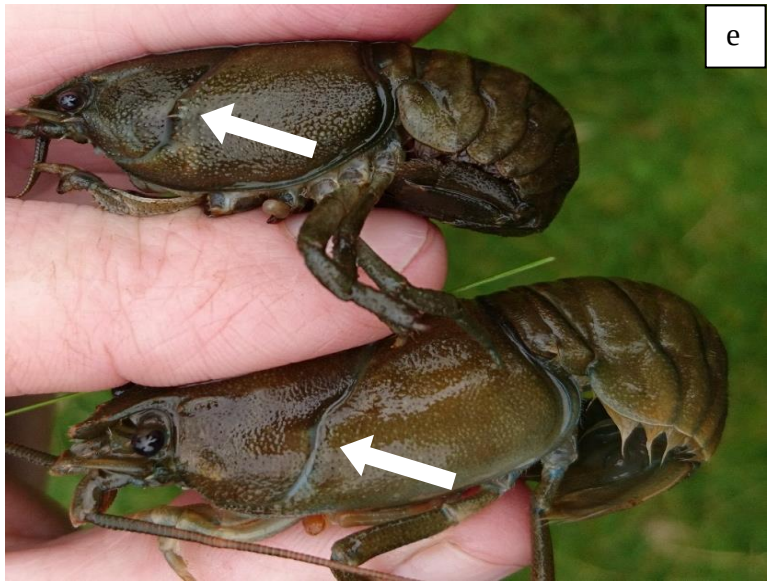


Figure 2 – Dorsal view of an adult *A. pallipes* (a) with the blue colour morph (b), and a ventral view of *A. pallipes* (c), showing pale chela (claws). Ventral view of *P. leniusculus* showing bright red chela (d), and the recent moult (d, right) showing white marks at the chela joints. ID feature of the presence of spines in *A. pallipes* and not in *P. leniusculus*, shown at the cervical groove (e), in animals missing chela.

Historical context of *A. pallipes* and *P. leniusculus* in the UK

A. pallipes were historically widespread across England, Wales and Northern Ireland (Rogers and Watson, 2011). During the mid-1900s, populations began to decline due to dredging, bank realignments, and the increase of pollutants entering waterways (Goddard and Hogger, 1986). The subsequent reduction of both habitat quality and quantity through physical management of rivers, and a combination of pollution incidents causing chronic environmental stress and acute die offs resulted in widespread declines in populations of *A. pallipes*.

Crayfish plague was first recorded in Europe (Italy) in 1860 (Unestam, 1972, in Goddard & Hogger, 1986). Multiple outbreaks have been and continue to be recorded in European stocks (e.g. Goddard & Hogger, 1986; Kozubíková *et al.*, 2008; Pârvulescu *et al.*, 2012). When native species stocks declined across Europe following the introduction of the plague, *P. leniusculus* were stocked in some instances, to replenish crayfish fisheries. Newly introduced non-native populations provided repositories for *A. astaci*, further threatening native stocks through increased exposure to the plague and competitive interactions (Holdich *et al.*, 2009).

During the 1970s, England began to stock populations of *P. leniusculus* into discrete water bodies and farm them, with encouragement of the Government (Rogers and Watson, 2011). Stocking continued until the early 1990s, when the evidence of ecological damage to native crayfish through the outbreak of plague incidents (Fig. 3), and the threats posed by *P. leniusculus* to the broader ecological communities drove the Government to introduce a suite of crayfish byelaws, aimed at restricting the spread of *P. leniusculus* in England and Wales. However, *A. pallipes* have continued to undergo extreme population declines since the 1970s (Sibley, 2003), with the remaining UK populations of *A. pallipes* becoming fragmented and reduced, whilst *P. leniusculus* has

increased its distribution almost exponentially. Despite this, the British populations of *A. pallipes* form the strongest in Europe (Holdich, 2003). There are strong populations of *A. pallipes* in Ireland (Lucey and McGarrigle, 1987), where it is the only crayfish species to have populations established in the wild. As such, the Irish stocks of *A. pallipes* can be considered a key conservation resource (Nightingale *et al.*, 2017). However, and despite the continued absence of invasive crayfish species in Ireland, plague outbreaks have occurred (e.g. Holdich and Reeve, 1991), with Ireland unfortunately currently experiencing plague epidemics across multiple catchments (P. Bradley, pers. comms., 2018).

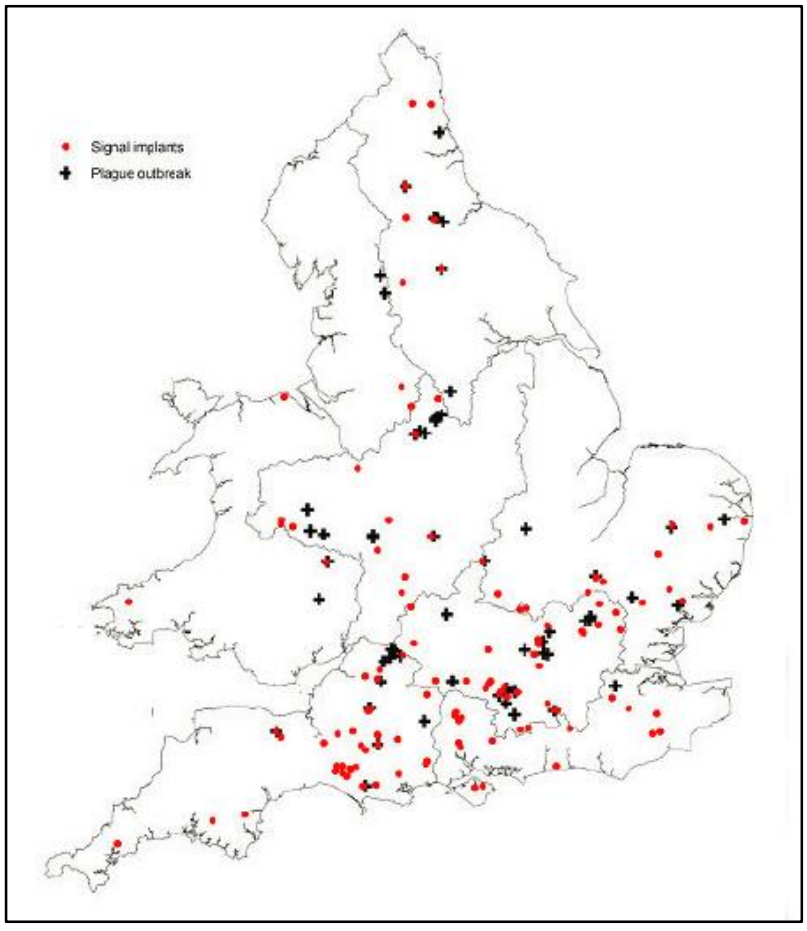


Figure 3 – Distribution of early *P. leniusculus* farms and implants (dots) and the first recorded plague outbreaks (crosses) in England and Wales. Adapted from Rogers and Watson (2011).

Conservation challenges and opportunities for *A. pallipes* in the face of invasive crayfish

The two main contemporary threats to *A. pallipes* are from invasive crayfish species and the associated pathogen *A. astaci*. Disease has been a major driver in the decline of *A. pallipes* populations, and minimising the risks associated with biosecurity of the extant populations are key to conservation efforts. The rapid and unchecked spread of *P. leniusculus* in England has made maintaining biosecurity standards exceptionally difficult. The plague and thus *P. leniusculus* as vectors pose severe biosecurity problems, due to the 100% mortality that *A. astaci* inflicts on native populations. The range of methods of transport for a plague spore, whether it be on an infected invasive crayfish species, contained in infected water, or harboured in non-disinfected equipment, makes recognising and mitigating for biosecurity risks an incredibly difficult and involving task. Limiting the spread of *A. astaci* through initiatives such as 'Check Clean Dry' (NNSS, 2006), aimed at reducing the spread of *A. astaci* and invasive species, are key. A further confounding factor in maintaining biosecurity is that there is no regulated monitoring for either *P. leniusculus* or *A. pallipes* in the UK, and as such the current understanding of the distribution of either species is inadequate. The failure to consider the biosecurity risks of *P. leniusculus* and *A. astaci* through a lack of spatial data is a key threat to *A. pallipes* conservation. The threat to *A. pallipes* is further exacerbated by the current inability to remove populations of *P. leniusculus* through management once established in a waterbody or watercourse (Stebbing *et al.*, 2014). Control of crayfish populations falls under six broad categories, being mechanical (e.g. trapping), physical (e.g. barriers), biological (e.g. predatory fish), biocidal (e.g. toxins), autocidal (e.g. male sterilisation) and legislative (following Gherardi *et al.*, 2011). All of the above control techniques are well reviewed by Stebbing *et al.* (2014), and while an integrated approach to invasive crayfish control appears most promising in mitigating

the impact on the ecological communities, there remains no fully effective eradication techniques (Freeman *et al.*, 2010).

The establishment of new biosecure populations of *A. pallipes,* termed ark sites, is a relatively new conservation strategy becoming increasingly common since the late 2000s (Nightingale *et al.*, 2017). Ark sites require isolated habitat such as headwaters, lotic systems that drain directly into the sea, or offline lentic systems. Ark sites should facilitate the establishment of self-sustaining bio-secure *A. pallipes* populations, isolated from *P. leniusculus,* in which to ensure the continued recruitment of individuals for species continuity. Holdich (2009) stated the need for strategic conservation through ark sites in light of the increased urgency of the situation, for example through the loss of three of the four most abundant populations of *A. pallipes* in South-West England between 2006-2009 (Kindemba and Whitehouse, 2009). Captive breeding initiatives have been established to attempt to provide stock for reintroductions and relocations (e.g. Bristol Zoo, UK). Improvements to the science behind these techniques has enabled artificial stocks to breed successive generations (Kozák et al., 2011), and thus when coupled with the considered establishment of ark sites, may provide a robust conservation tool for the continuation of *A. pallipes* in the UK.

Following mass mortalities, populations held in 'natural' ark sites such as headwaters and tributaries are often able to recolonise main channels (Holdich & Reeve, 1991; Holdich & Rogers, 1997). Low order streams typically offer high water quality and undisturbed natural habitats, upstream of major anthropogenic threats and often invasive crayfish populations. Artificial barriers such as mills and weirs can provide a crucial divide between invasive crayfish populations downstream and *A. pallipes* populations upstream (Kerby *et al.*, 2005). Actions undertaken to increase longitudinal connectivity of systems to improve fish passage and thus access to spawning habitat can be in

conflict with this objective (e.g. Roni *et al.*, 2008), however, as the removal of barriers may directly increase the biosecurity risk to *A. pallipes* populations. Given the severe negative impacts that *P. leniusculus* can have on fish recruitment (Findlay *et al.*, 2015) and the oxygenation of spawning gravels (Johnson *et al.*, 2010), increased connectivity may therefore directly jeopardise not only populations of *A. pallipes*, but the broader ecological community in these important habitats. It is therefore of great importance to understand the role headwater systems play in the biosecurity and persistence of *A. pallipes* populations, and the impact that *P. leniusculus* can have in these systems. The comparative impact of *A. pallipes* and *P. leniusculus* on native ecological communities in headwaters also requires research in order to feedback empirical data as to the consequences of such actions.

Overall thesis aims

Before the future of *A. pallipes* and the threats posed by *P. leniusculus* in England can be understood, there are fundamental questions that must be considered with respect to the ecology and distribution of both species. Firstly, where are the current populations of both *A. pallipes* and *P. leniusculus* in England? The lack of monitoring of both species risks unintentional violations of biosecurity, and also fails to deliver a representative account of the status of *A. pallipes* conservation in England, hindering strategic management. Given this research need, this thesis updates the distribution of both *A. pallipes* and *P. leniusculus* in England, with a focus on improving the reporting metrics for both species. Through new data collected from the Environment Agency and Natural England, the distribution of *A. pallipes* and *P. leniusculus* is presented using established and novel reporting methods (Chapter 2).

Secondly, what impact does *P. leniusculus* have? An important consideration for freshwater biodiversity in England is if the ecological impacts of *P. leniusculus* are

limited to the functional replacement of *A. pallipes,* or if there are concerns for the wider ecological community. This question is addressed in this thesis at a population level, through comparative study of headwater streams containing either *A. pallipes*, *P. leniusculus* or no crayfish (Chapter 3). Additionally, at an individual scale, the impacts of *P. leniusculus* on an ecosystem are explored through Stable Isotope Analysis (SIA) and Gut Content Analysis (GCA), focussing on density-dependent dietary preferences amongst *P. leniusculus* cohorts (Chapter 5).

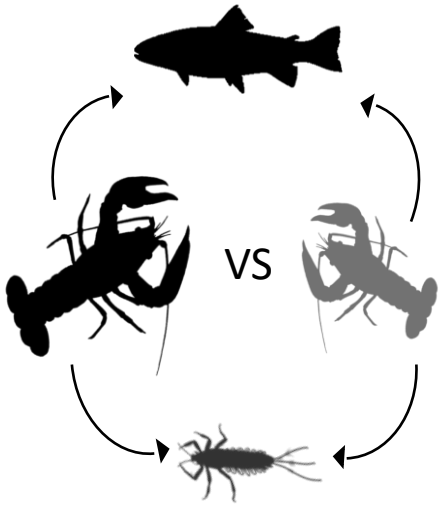
Thirdly, what is the structure and density of invasive populations of *P. leniusculus* in England? It would stand to reason that the scale of the impacts of *P. leniusculus* in England should be related to the abundance and demographics of a population. However, as a result of the lack of monitoring, and in part due to limitations of current sampling methodologies, little is known about *P. leniusculus* population structure and density in its invasive range in England. In this thesis novel sampling methodologies are explored to determine the structure and density of invasive *P. leniusculus* populations in the field. Through the development of a novel method for sampling *P. leniusculus* populations, which relies on dewatering small study sites and removing all substrate and crayfish, the best in-situ population density and structure estimates to date for *P. leniusculus* are provided (Chapter 4). **As a whole, this thesis aims to provide a foundation of core geographical and ecological information, from which to better inform the conservation and management of both *A. pallipes* and *P. leniusculus* in England (Fig. 4)**

Chapter 2.

A. pallipes and *P. leniusculus* distribution in England

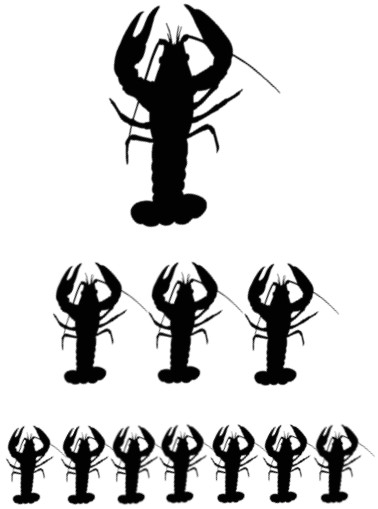
Chapter 3.

A. pallipes and *P. leniusculus* impacts on headwater stream communities



Chapter 4.

Novel sampling technique: population density and structure of *P. leniusculus*



Chapter 5.

Diet of *P. leniusculus* using stable isotope and gut content analysis

Figure 4 – Conceptual diagram highlighting the key themes of each empirical chapter in this thesis, starting from a broad resolution analysis of *A. pallipes* and *P. leniusculus* distribution in England, and focusing progressively from community level impacts of *P. leniusculus* and *A. pallipes* on headwater streams, to details of *P. leniusculus* population structure and density, to direct and isotopic analysis of the diet of an invasive population of *P. leniusculus*

Chapter 2 – Status and distribution of crayfish species in England

Introduction

English populations of *Austropotamobius pallipes* were in decline throughout the 1950s-70s due to a combination of anthropogenically driven physical habitat degradation and the chemical pollution of waterways (Goddard and Hogger, 1986). Following introductions of the invasive signal crayfish *Pacifastacus leniusculus* in the early 1970s, and, in consequence, the introduction of the associated crayfish plague in the early 1980s, the remaining stocks of native *A. pallipes* began to severely decline. Holdich *et al.* (1999) described a major loss of populations in the south and central regions of the UK between the 70s and late 90s, attributed to the direct and indirect effects of the spread of *P. leniusculus* populations. Estimates of *A. pallipes* population losses were as severe as >95% in some instances, such as in the Thames catchment and in Hampshire (Füreder *et al.*, 2010). When considered on a regional scale, the status and distribution of *A. pallipes* was suspected to be in a critical condition, and regional extinctions were expected. Due to this concentrated loss of the southern populations of *A. pallipes*, remaining populations were concentrated in central and northern regions England (Souty-Grosset *et al.*, 2006). These regions supported the highest population densities of *A. pallipes* in Europe (Holdich, 2003), and remain of international significance for the conservation of *A. pallipes* (Dunn *et al.*, 2008).

Early efforts to describe the distribution of crayfish species in the UK were successful in tracing population trends of all key species (Goddard and Hogger, 1986; Sibley, 2003a). Sibley (2003b) reported both a steady linear decline in the number of 10 km grid squares containing populations of *A. pallipes* in the UK, and a polynomial increase in 10 km grid squares containing invasive crayfish species, between 1976 and 2003. Sibley (2003b) extrapolated these trends and predicted that *A. pallipes* could be 'virtually extinct' within 30 years in mainland Britain, and, perhaps of equal concern, that the distribution of invasive crayfish would double over the subsequent 15 years.

At approximately the same time as Sibley's early 2003 work on crayfish distributions, the Joint Nature Conservation Committee (JNCC) was formed (1990) as a result of the Environmental Protection Act (EPA, 1990), and reconstituted in 2006 under the Natural Environment and Rural Communities Act (NERC, 2006) that also underpinned the re-naming of English Nature into Natural England. The purpose of the JNCC was primarily to advise the UK government and corresponding bodies on nature conservation, and facilitate the incorporation of European laws into practice within the UK. One such major legislative piece is the EU Directive on the Conservation of Habitats, Flora and Fauna (92/43/EEC) or Habitats Directive (1992). The Habitats Directive aimed to maintain and promote biodiversity of European plants and animal species, along with the key habitats that support them, primarily through the establishment of a network of protected sites, termed the Natura 2000 network. The specific implementation of the Directive varied with each member state within Europe, to accommodate local and regional requirements when delivering the targeted actions of supporting and promoting biodiversity. In England, the implementation of the Directive was realised through achieving and maintaining 'favourable conservation status' of protected species, principally through Special Areas of Conservation (SACs) and other appropriate conservation measures, with monitoring and subsequent reports (detailed in Article 11 and 17 respectively) providing periodic assessments. Both *A. pallipes* and its habitat are listed in Annex II of the Habitats Directive. Through the legal framework, the UK as a member state entered into an obligation to monitor and report on the distribution of *A. pallipes*. Three previous reports have therefore been compiled, covering the time periods 1994-2000, 2001-2006, and 2007-2012. These 6-year reporting cycles provide a framework through which a regular review of the status of all listed habitats and species is required, including whether conservation targets were being met through current measures, and if further legislative change is required.

In this chapter, the previous Article 17 reports were summarised and reviewed, to provide the contextual understanding of crayfish distribution reporting in the UK. Following this, a database was compiled using novel records (see Methods), and the distribution of *A. pallipes* and *P. leniusculus* were mapped for their 2018 distribution in England, not least in order to fulfil European requirements for the fourth Article 17 report. Finally, alternative methods for mapping the distribution of both species in England were explored, to provide a greater contextual understanding of the status of their populations with regards to the future conservation and management of crayfish in England.

Review of past crayfish distribution in the UK through the Habitats Directive

Establishment of the SAC network over the 1994-2000 Article 17 reporting period in the UK

The first report on the implementation of the Habitats Directive and its success in protecting listed species was provided in 2001. It focused on identifying areas that contain habitats or populations of species that are of conservation interest at a national scale. Distribution and abundance or extent data were gathered for each Annex II species and Annex I habitat, which informed the site selection process. Of the 623 Annex II species listed for Europe, 51 were recorded in the UK, with 41 forming extant resident populations (DEFRA, 2001). Appendix IV of the report detailed the number of candidate SACs (cSACs) presented within the UK for consideration, requiring considerable presence of the named species (Table 1). In addition, management plans were created to describe and promote the keeping of the cSACs in favourable condition. As a result of this, *A. pallipes* (European species code S1092) populations formed a basis for the designation of a total of 10 cSACs out of a total of 479 cSACs put forward by the UK to Europe, while a Species Action Plan (SAP) was also prepared as part of a UK Biodiversity Action Plan (BAP) for this native crayfish species. Of these 10 cSACs,

8 were located in England, with one further cSAC located in Wales and Northern Ireland, respectively (Fig. 1). Primary designations were for outstanding (Grade A) or excellent (Grade B) examples of a given feature in a European context. An additional 2 cSACs in England contained populations of *A. pallipes* of at least national importance (Table 2), but these were not features of sufficient importance to satisfy a primary designation with the species in mind – instead, they were included as a secondary interest feature (Grade C) in the designations. All cSACs that were proposed for *A. pallipes* were accepted by the European Commission (EC) and became full SACs, and as such are referred to as SACs for the sake of clarity forthwith. SACs are a key way to satisfy the requirements of Article 3 of the Habitats Directive, namely the establishment of a coherent protected network of European sites. SACs that contain populations of *A. pallipes* that are not of national importance are categorised as Grade D, and are only required to list the species as present, but populations are not awarded European protection or specific conservation targets within the relevant SAC. A further 5 sites considered for SAC designation in England contained Grade D populations of *A. pallipes*, and were not incorporated in the review of the 2000 report.

Table 1 - Key cSAC assessment criteria, adapted from DEFRA (2001)

Species	General
v. Proportion of UK population	ix. Priority/non-Priority Status
vi. Conservation of features important for species survival	x. Rarity
vii. Isolation of species populations	xi. Geographical range
viii. Global assessment	xii. Special UK responsibilities
	xiii. Multiple interest

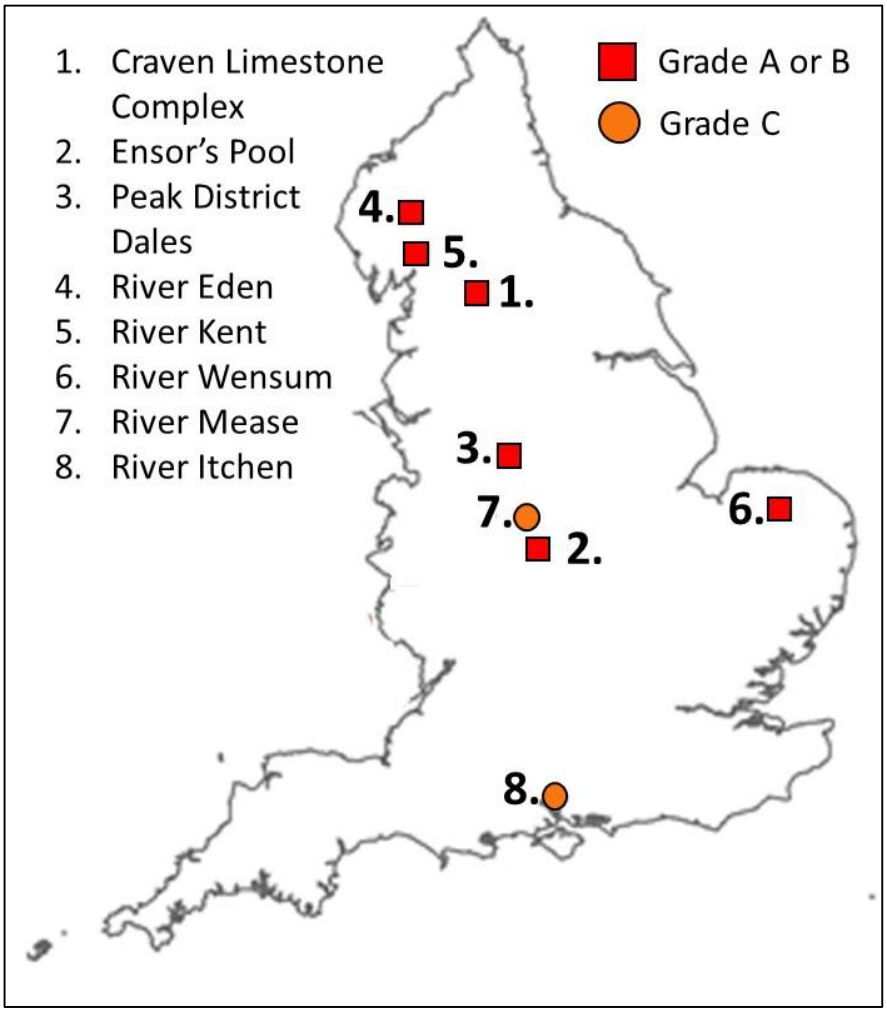


Figure 1 - Distribution of SACs in England which contain *A. pallipes* as a primary or secondary designation feature.

Table 2 - Summary of English SACs proposed in the 1994-2000 Article 17 Report. Italicised SACs indicate that *A. pallipes* were present, but that this was only a secondary reason for designation. Adapted from DEFRA (2001).

SAC Name	Local Authority	Abundance **(C = common, R = rare, V = very rare, P = present)**	Data Quality **(G = good, M = moderate, P = poor, DD = data deficient)**	Global Habitat Grade	Population	Conservation Status	Isolation
Craven Limestone Complex	North Yorkshire	P	DD	B	B	A	C
Ensor`s Pool	Herefordshire, Worcestershire & Warwickshire	-	G	A	C	A	C
Peak District Dales	Derbyshire & Nottinghamshire, Shropshire & Staffordshire	C	DD	B	C	B	C
River Eden	Cumbria, North Yorkshire, Northumberland & Tyne & Wear	-	M	A	C	B	B
River Kent	Cumbria	C	DD	A	C	A	B
River Wensum	East Anglia	C	DD	B	C	B	B
River Itchen	Hampshire & Isle of Wight (IoW)	-	M	C	C	C	B
River Mease	Derbs. & Notts., Leic., Rutland & Northamptonshire, Shrop. & Staff.	C	DD	C	C	B	C

Summary of the general assessment for all species under the 2001-2006 Article 17 reporting period in the UK

The summary report for the UK under the second reporting round (JNCC, 2008) subsequently provided a broad picture of the conservation status of the named protected species since the implementation of the Habitats Directive (Table 3). The summary of the general assessment for all species between 2001-2006 is provided both to aide in the interpretation of the metrics used in the species specific assessment of *A. pallipes*, but also to provide a broader context with which to compare the status of *A. pallipes* in England against all other named species on the Directive. Article 17 reporting requires that the species distributions to be provided in a standard occupied 10 x 10 km grid square unit, so as to maintain a common unit across taxa for the purposes of broader summaries, and to allow for temporal comparisons to be drawn without the inconsistency of units. The JNCC make the point that the high number of species failing assessments is not to be unexpected, as the reason these species are being protected is due to concern over their current conservation status. Additionally, appropriate changes in conservation practice may take time to improve the conservation status of a species, and thus, while work has been ongoing during the reporting period, and the Directive has been implemented, these changes may not yet be reflected in the monitored status of the species for a subsequent reporting round. Four parameters were recorded for each species in the Article 17 assessment, namely range, population, habitat, and future prospects. These four parameters were then combined to provide a single 'Overall Assessment' value. How each parameter was determined was outlined in the evaluation matrix Annex C (JNCC, 2007a, Table 2.3, page 18) in the second JNCC Article 17 report. There were in essence four broad categories, with an example of their implementation provided in Table 4. Directional trends could also be added to both the 'Unfavourable – Inadequate' and' Unfavourable– Bad' classifications, these being

either improving or deteriorating, thus affording further detail to status assessments of species. The UK as a member state made the decision to assess ‘future prospects’ of all UK species on a 12-year cycle, equivalent to two reporting rounds.

In general terms, the Favourable Reference Population (FRP), a key benchmark from which to assess temporal trends in named species, was determined in the UK to be one of two values. In the first instance, FRPs could be set at a value greater than, but not in excess of, 125% of the 1994 population level for a species, providing that the 1994 values were deemed suitable to satisfy the aims of the FRP, namely to ensure the continued and successful viability of a species in perpetuity. Some species had a FRP set in excess of 125% of the 1994 population values, and this in part helped to determine if the species’ subsequent status was judged as ‘Inadequate’ or ‘Bad’. This decision was largely based on trend data from the first reporting round, with increasing or stable population trends indicating the 1994 value to be suitable, and decreasing trends indicating a value greater than the 1994 value to be required. The reference categorisation of ‘Favourable’ was heavily reliant on the quality of available data, with trends of >1% annual population loss designating a species as ‘Bad’, and <1% per year as ‘Inadequate’. Favourable Reference Range (FRR) was taken into consideration too, with a species falling >10% under the FRR value also being considered to be in a ‘Bad’ conservation status.

Table 3 - Summary statistics for all species reported on for 2001-2006 round. Numbers in brackets denote % of species within a category considered 'improving' (JNCC, 2008)

Rating Category	***Favourable***	***Unknown***	***Unfavourable - Inadequate***	***Unfavourable – Bad***
Range	78%	9%	7% (3)	7% (3)
Population	34%	31%	17% (4)	18% (6)
Habitat	22%	45%	28% (10)	4%
Future prospects	44%	24%	26% (9)	7%
Overall Assessment	26%	26%	30% (12)	18% (6)

Table 4 – Example of the criteria required for the assessment of conservation status (e.g. population) as stated per the Article 17 Annex C reporting matrix. Adapted from JNCC (2007a).

Parameter	**Conservation Status**			
	Favourable ('Green')	**Unfavourable – Inadequate ('Amber')**	**Unfavourable – Bad ('Red')**	**Unknown**
Population	The population (s) is(are) above Favourable Reference Range (FRR) AND Reproduction, mortality and age structure is not deviating from normal (if data is available to test this)	Any other combination	Large decline: Equivalent to a loss of more than 1% per year (the member state (MS) may deviate from this indicative value if duly justified) AND Below 'FRR' OR More than 25% below 'FRR' OR Reproduction, mortality and age structure strongly deviating from normal (if data is available to test this)	No information, or insufficient information to make an assessme

General assessment of A. pallipes during the 2001-2006 Article 17 reporting period in England

The overall range for *A. pallipes* was described to be 'declining' in the 2006 report which estimated this trend over the period of 1994 to 2006. This decrease was attributed to a combination of direct and indirect anthropogenic influences, as well as driven by the spread of non-native species. As estimated from data between 2000-2003 in the second Article 17 report, the population cell count for *A. pallipes* was at 166 in the UK, using occupied 10 x 10 km grid squares as a common population unit. Of these, 137 cells were located within England (Fig. 2). The data was partly extrapolated from survey data, and as such was afforded less confidence than Environment Agency-gathered data. This population estimate supported a general decreasing trend of unknown magnitude since the 1994 reference value, again attributed to the direct and indirect impacts of anthropogenic activities and invasive species. Additional pressures and threats were noted as water quality and connectivity, and the introduction of disease, closely linked to the spread of invasive crayfish. At the end of the 2006 reporting round, *A. pallipes* were concluded to be in an 'Unfavourable – Bad' condition for range, population, future prospects, and overall assessment, with habitat falling under 'Unfavourable – Inadequate' due to the lack of data, with all categories additionally assigned 'and deteriorating' status. Definitions of an overall assessment of a species as 'Unfavourable – Bad', as stated by the European Commission Guidance are "where the…species is in serious danger of becoming extinct (at least locally)" (JNCCa, 2007, p. 16), with 'Unfavourable – Bad' prospects indicating that a species' "long term viability [is] at risk" (JNCCa, 2007, p. 17).

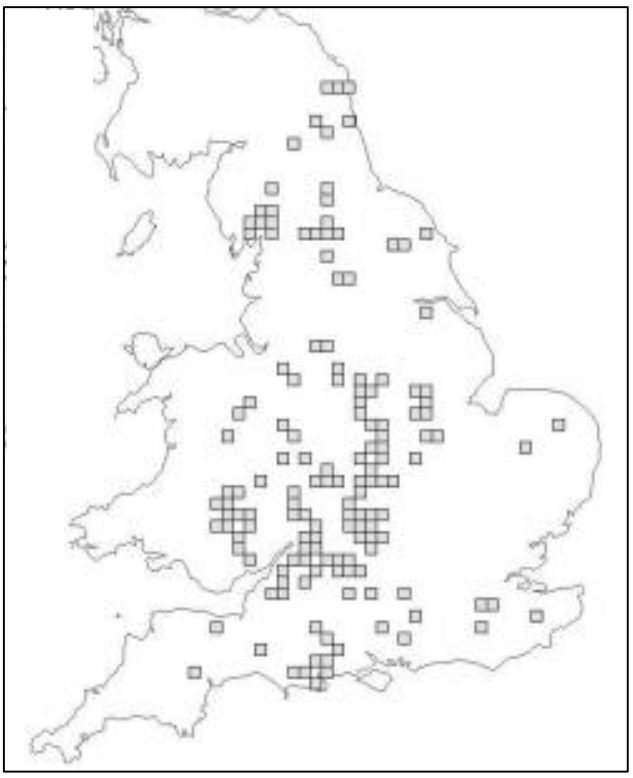


Figure 2 – *A. pallipes* populations in England, showing the 137 10 x 10 km grid squares occupied between 2000-2003 (adapted from JNCC, 2007b).

These results put *A. pallipes* amongst the most threatened species in the UK, with the continued deterioration of all aspects of their conservation placing *A. pallipes* in the bottom 4% for range, 12% for population, 7% for current and 12% for overall conservation status. The population extent reported for the period 1970-2003 was 464 10 x 10 km grid squares (Fig. 3). The range reported in 2006 therefore represented a 60% loss between 1970 and 2003, providing data accuracy and comparability (JNCC, 2007c). As shown by the historic data, numbers of grid squares occupied by *A. pallipes* had declined at a rate greater than 1% per year. Therefore, according to the EC definitions under the general species assessments, the populations status of *A. pallipes* was reported as 'Inadequate – Bad', and it was deemed necessary to set the Favourable Reference Population for *A. pallipes* at a value in excess of 125% of the 1994 value of 464 i.e. 580 occupied grid squares.

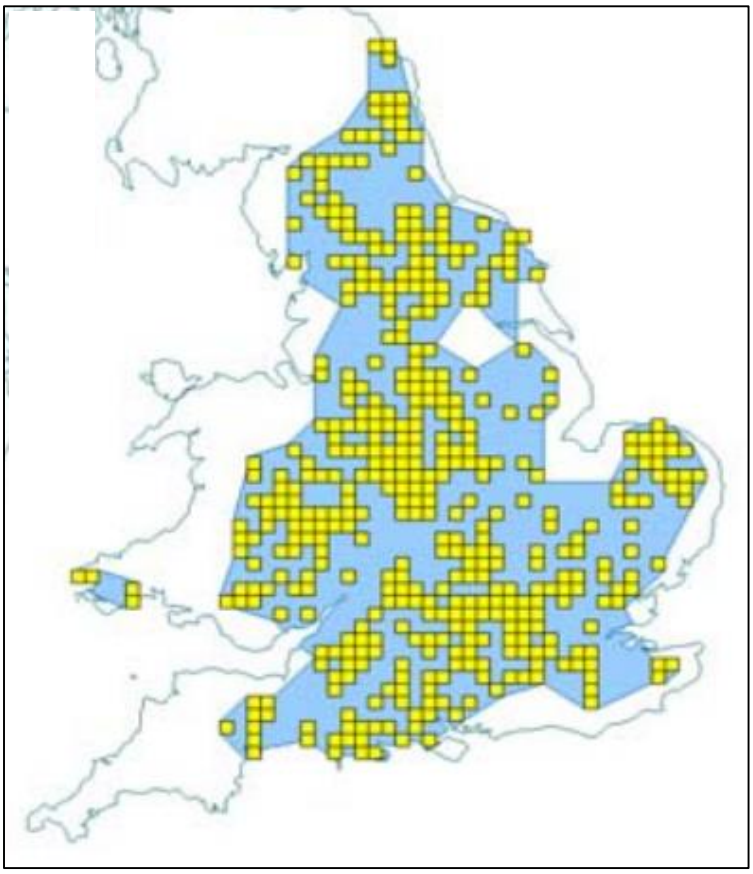


Figure 3 – Historic population extent of *A. pallipes* in the UK, showing 464 occupied 10 x 10 km grid squares between 1970-2003 (N. Ireland not shown but included in count, adapted from JNCCc, 2007).

Referenced within the Audit trail for the second report (JNCC 2007c) was a 2002 report from the UK BAP programme. This estimated that 260 10 x 10 km grid squares were occupied by *A. pallipes* within the UK, with a second estimate from 2005 reporting 241 occupied grid squares to be occupied between 2003 and 2005. Whilst slightly more encouraging and likely realistic, this loss still equated to >1% per year. Whilst the data remained of poor spatial coverage, the common trends of severe and sustained declines for *A. pallipes* were present within all Article 17 data sets. However, the grid square unit should be interpreted with caution, as it represents the presence or absence of *A. pallipes* within a geographical unit area, and not the relative frequency and abundance of discrete populations of *A. pallipes.* As such, a significant thinning of populations

could have occurred within each grid cell, whilst no corresponding change was recorded under the grid cell recording metric.

Summary of the second period of Article 17 assessment for A. pallipes in England, covering the 2007-2012 reporting cycle

The 2012 reporting round confirmed that *A. pallipes* still showed an 'Unfavourable – Bad' range, population, future prospects, and overall assessment. Habitat was re-assessed, and found to be 'Favourable', drawing upon findings that habitat and water chemistry in UK rivers is of moderate quality in areas suitable for crayfish, with quality commonly 'increasing'. The 2012 report (JNCC, 2013a) suggested that this conclusion may not be accurate, as *P. leniusculus* occupied much of the 'favourable' habitat that *A. pallipes* would otherwise occupy, but has been excluded from by its superior competitor. Invasive non-native species were ranked as one of only two 'High' importance pressures, and one of only two 'High' level threats to *A. pallipes*. The spread of *P. leniusculus* and the associated crayfish plague were specified as the drivers behind these designations (JNCC, 2013b). All other categories characterising the status and trends in native crayfish were assigned an 'and deteriorating' status. The occupied grid square estimate for *A. pallipes* in 2012, reported as 147 grid squares, was based on records generated during the reporting round duration, i.e. 2007-2012 (Fig. 4), and was interpreted as a 58% loss of populations' distributions since 1989. Additionally, the 2012 report included, for the first time, the number of population units contained within the Natura 2000 network, principally SACs. An estimate of 41-54 occupied 10 x 10 km grid squares was provided for the network in the main report (JNCC, 2013a). Supporting information in the JNCC (2013b) report stated that only 24 of the occupied grid squares were not directly linked to populations of *P. leniusculus*, and only 3 SACs, namely the River Kent, River Eden and Ensor's Pool (see Fig. 1), were considered to be

in favourable condition (however, see discussion of the SAC network, this chapter). The results of this report maintained the position of *A. pallipes* as amongst the most threatened species within the UK, with the continued deterioration of all aspects of their conservation putting them in the bottom 8% of species in the UK in terms of overall conservations status.

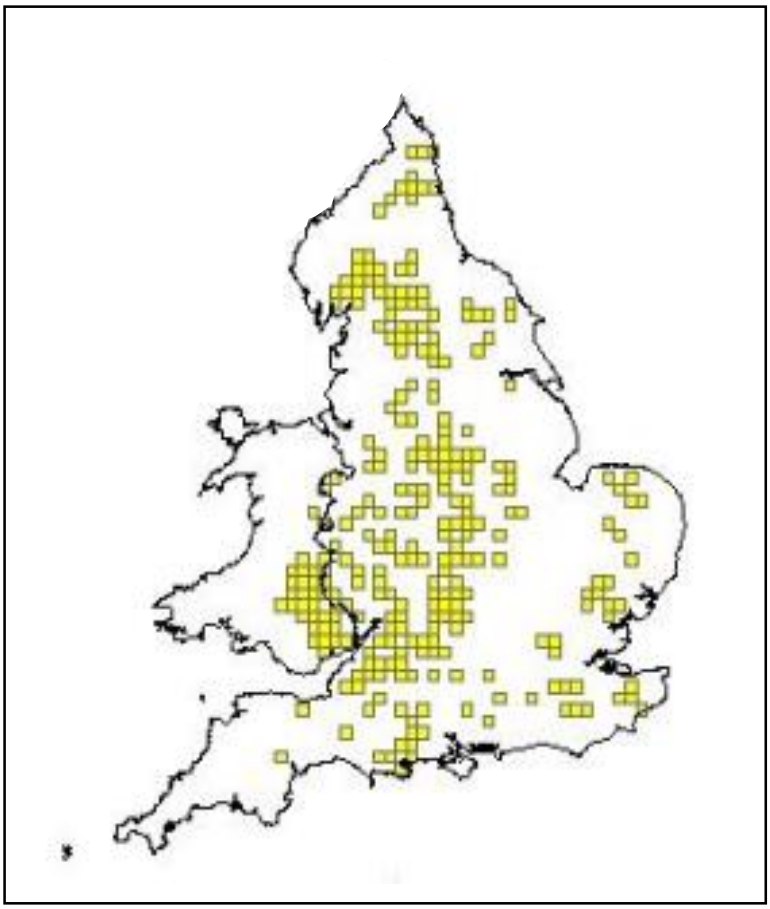


Figure 4 – Distribution of *A. pallipes* within England during 2007-2012 reporting round. Adapted from JNCCa, 2013.

Implementation of the sub-catchment metric for population level analysis of crayfish species in England

The quality of the spatial data is a main limitation for methods attempting to show higher resolution when describing crayfish populations, and whilst no centralised project has been established to record crayfish presence within the UK, broader scale analyses must be used. In addition to the prescribed 10 km grid square population count, another key metric can and has been usefully employed to describe distributions of *A.*

pallipes and *P. leniusculus* populations within England and the UK. Analysis on a sub-catchment scale offers an appealing mix of coarser spatial resolution to allow for patchiness in the data, whilst still providing a meaningful and measurable method for displaying and monitoring crayfish distribution through time and space. The sub-catchment boundaries operate off pre-Water Framework Directive (WFD) units, and as such do not fully match the Integrated Hydrological Units that the EA currently use. The sub-catchments were, however, used for previous iterations of crayfish distribution mapping, and for the sake of continuity have been requested to remain for the purpose of Article 17 commissioned reporting.

In a study of crayfish distribution by Rogers and Watson (2011), sub-catchment maps of past distributions of both *A. pallipes* and *P. leniusculus* in the UK were created. These most recent 'past distribution' maps (2002-2010) correlated with the third instalment in the Article 17 reporting cycle, and were commissioned by the Department for Environment, Food and Rural Affairs (DEFRA) for the purpose of reporting conservation status. The past maps presented data as coloured cells, with each colour representing a different population status (Fig. 5).

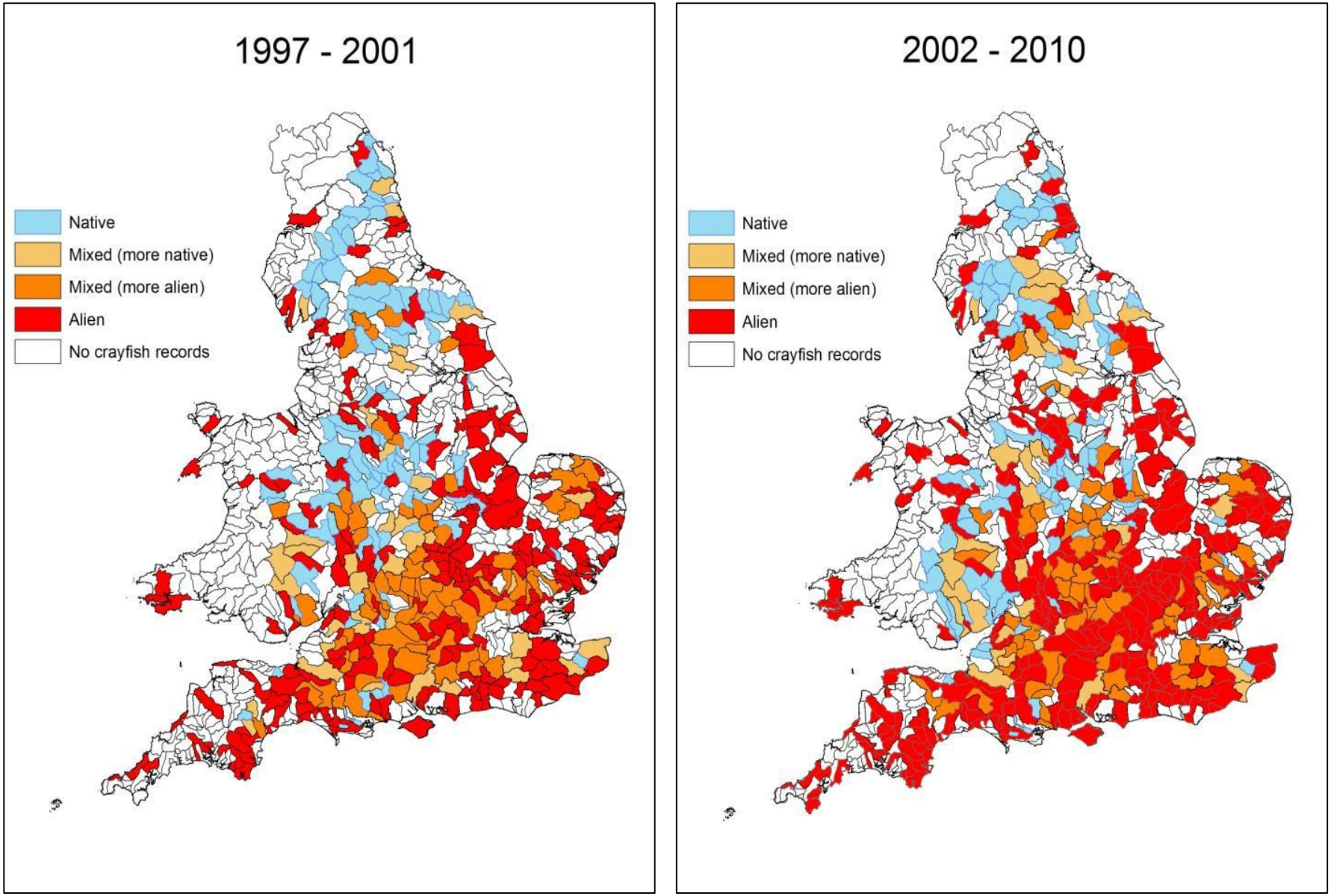


Figure 5 – Adaption of data presented by Rogers and Watson (2011) indicating distribution status of *A. pallipes* and *P. leniusculus* during reporting periods for the second and third Article 17 round.

The sub-catchment polygon maps showed the steady retraction of *A. pallipes* territories and concomitant expansion of *P. leniusculus* territories. From pre-1990 to 2010 cells containing *A. pallipes* were reported to decrease from 187 to 81, and cells containing invasive *P. leniusculus* populations increased from 96 to 390, 115 of which were mixed (Rogers and Watson, 2011; JNCCb, 2013). The sub-catchment Favourable Reference Population (FRP) for *A. pallipes* was re-calculated as 350 cells as per the 1989 UK mapped distribution (D. Heaver pers. comms. 2018, based on Holdich and Reeve, 1991).

Chapter aims

These maps are now outdated, and the method and data from which they were constructed was no longer available. However, they do provide the best baseline from which to construct maps for the 2018 reporting round, and more generally from which to assess the status of crayfish species in England. The aim of this chapter therefore was firstly to prepare a standardised occupied grid count, and distribution map of *A. pallipes* populations within the SAC network, as per the European reporting requirements for England in 2018. Secondly, following the reconstruction of the Rogers and Watson sub-catchment layer map, an updated sub-catchment map for 2018 was produced for England. It was hypothesised that the 2018 sub-catchment map would show the continued expansion of *P. leniusculus* at rates in excess of 1% per year, and the continued retraction of *A. pallipes* in England at 1% per year, with respect to the 2010 distribution. It was also hypothesised that significant populations of *P. leniusculus* exist within the current SAC network for *A. pallipes*, as determined through comparative counts of occupied 10 x 10 km grid squares.

Methods

Consolidation of the Environment Agency dataset of crayfish point data

A dataset of 1567 *P. leniusculus* records and 1372 *A. pallipes* records was compiled and provided by Ian Marshall, the Environment Agency Crayfish Lead. This dataset ran from 2005 to 2018, and contained records from the EASIMAP server, the EA data storage system. There is no strict or organised form of recording crayfish data, and records were mainly entered following crayfish sightings and by-catch made during electrofishing or macroinvertebrate surveys. There is no official requirement nor centralised training offered to EA personnel to be able to identify crayfish to species level in the field. However, multiple records are often available for a given site, increasing the reliability of the data.

The point data for both species were transferred using a Spatial Join algorithm onto the sub-catchment GIS layer, and I subsequently re-created a map based on this data in the style of Rogers and Watson (2011). This new layered map was checked for discrepancies between the previous distribution map, and any differences were added to generate a single unified layer, representative of both data from the EA and collated by Rogers and Watson (2011).

Unfortunately, no data was forthcoming from Natural Resource Wales, and as such no comparisons with previous Welsh distributions for either species was possible. Welsh cells were therefore removed from the analyses, and data is being presented for England, only.

Revision of the sub-catchment methodology

To further the process of updating the Rogers and Watson (2011) sub-catchment map, several assumptions were made. Firstly, it was agreed upon during a meeting of the Environment Agency, Natural England, and myself, to remove the ‘orange’ category

from the sub-catchment polygon map. It was argued that the data was not of sufficient quality to accurately state whether a polygon contained mostly native or mostly invasive populations, and was at best adding little to the broader picture, and at worst misleading. Having decided to remove the 'orange' layer, a decision was reached on how to classify these cells. It was argued that once a sub-catchment polygon (termed cell henceforth) was confirmed to have populations of invasive crayfish present, it would never lose these populations, and should be considered for all intents and purposes as a 'red' cell. Since there is still no contemporary method to eradicate *P. leniusculus* once a population is established, the habitat is permanently unavailable to be recolonised by *A. pallipes*. In truly mixed populations, *P. leniusculus* would outcompete (or infect) and quickly eradicate the native populations (Bubb *et al.*, 2005), leading to a 'red' cell. Alternatively, *A. pallipes* may be found in an isolated population within the cell, in which case the cell was never truly mixed, and should be considered 'red' with isolated native populations contained within an 'exceptions layer'. This approach may better reflect the true extent of the issues posed by *P. leniusculus*, as a more pertinent and severe measure of population retraction than a 10 km grid square. The above rationale was agreed between NE, EA and myself to have merits, and a new baseline map was therefore produced displaying a simplified set of categories: red (*P. leniusculus*), blue (*A. pallipes*) and white (no crayfish records).

Natural England Crayfish Licence Returns

As an additional source of point data for *A. pallipes*, a 3 month 'internship' was conducted at Natural England. The aim of this internship was to access and review all crayfish licensing data from 2012 to 2018. There are currently three grades of licence in effect for work pertaining to *A. pallipes* within the UK framework. The CL11, CL23 and personal licence principally differ in what work can be carried out and when. The standard monitoring season for *A. pallipes* is 1st July through to 30th September. This is

to avoid disturbing females when they are berried (carrying young), as this is a delicate time in the reproductive cycle, and disturbances can result in brood loss. The CL11 license allows for an operative to "hand search", and is issued for all home counties, providing the licensee with the ability to survey for *A. pallipes*. Licensed methods are manual searches and hand netting during the standard monitoring timeframe across the UK. Trapping for animals for survey purposes is permitted as well, providing Environment Agency authorisation through a CR1 crayfish trapping form has been acquired (NB. Landowner consent is still required).

The CL23 is a low impact works licence and grants the additional allowances of removal and movement of animals, and is typically granted in cases where industrial actions are being conducted, such as repairs to infrastructure (e.g. bridges, walls) which may impact *A. pallipes* populations. This licence allows for operatives to relocate the animals, for example upstream of in-channel works. The CL23 is also restricted to the standard monitoring timeframe of July to September, and the low impact nature of the works. For example, works under a CL23 cannot impact more than 20 m of bankside and if the works are in excess of this, a personal licence must be applied for to cover the larger scale of works.

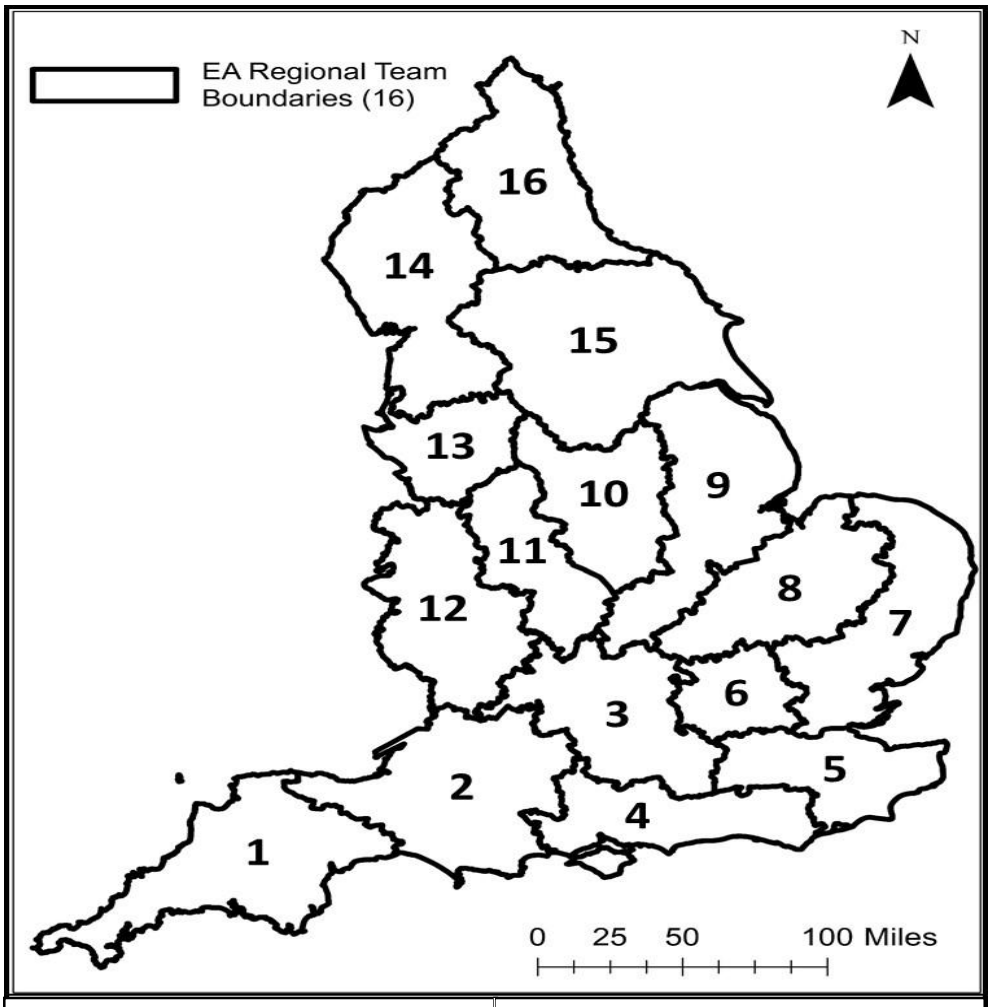
The final licence class is a personal class licence, and is a bespoke permit that allows for activities that do not fall under the aforementioned approved activities. Reasons for requiring a bespoke licence could be due to the scale of the proposed project, or because the work may have to fall outside of the approved seasonal timings. Natural England, as part of CL23 and personal licenses, stipulate that catch returns are to be reported following the cessation of projects. CL11 catch returns are reported on an annual basis, and records are sent to the Ecological Records Centre (ERC), not Natural England (NE). CL11 catch returns are sent to the ERC, and as such form part of the Environment

Agency EASIMAP database that was already acquired. CL23 and personal licence data are only stored at NE, and as such required collating.

The NE CL23 and bespoke licence records, whilst stored digitally, were not organised or compiled in their present state, and came in no standard format due to the inherently varied nature of crayfish works. In total 161 records were standardised and digitised. Whilst some caveats exist with this data, such as a lack of clarity on whether works were actually undertaken, and catch returns not being completed, an additional 68 point data records were added to the crayfish distribution dataset compiled here.

Review round with Regional Environment Agency Offices

Having compiled data from Environment Agency and Natural England license returns, and following the replacement of orange cells by red, the sub-catchment distribution map of *A. pallipes* and *P. leniusculus* was correct as of 2010. To better represent our knowledge of the species' current distribution, the map was divided up, as defined by the 16 current Environment Agency working regions (Fig. 6). These regional maps were then distributed to the respective Environment Agency team leads for each area, who were also provided with a standardised reporting form and tasked to report any changes from the current distribution using staff knowledge. This approach has the benefit of utilising local expert knowledge of many trained Agency staff, and of reducing errors and problems associated with the current reporting system, such as records not being logged despite presence of crayfish being known (a common occurrence for long-standing populations) or after the recent and sudden loss of native populations. In addition, EA officers were asked to confirm if the distribution they were presented with was accurate, to control for any previous errors being continued forward.



1. Devon & Cornwall
2. Wessex
3. West Thames
4. Solent & South Downs
5. Kent & South London
6. Hertsforshire & North London
7. Essex, Norfolk, & Suffolk
8. Cambridgeshire & Bedforshire
9. Lincolnshire & Northamptonshire
10. Derbyshire, Nottinghamshire, & Leicestershire
11. Staffordshire, Warwickshire, & West Midlands
12. Shropshire, Herefordshire, Worcestershire, & Gloucestershire
13. Greater Manchester, Merseyside, & Cheshire
14. Cumbria & Lancashire
15. Yorkshire
16. Northumberland, Durham, & Tees

Figure 6 – Boundaries delimiting the 16 Environment Agency regions, for purpose of regional reviews.

For the purposes of clarity and brevity, sub-catchments containing populations of *P. leniusculus* are in the following sections referred to as ‘red cells’, sub-catchments containing only *A. pallipes* as ‘blue cells’, and sub-catchments that had records for

neither species as ‘white cells’. In addition to the above, two new cell types are presented. A yellow cell with black diagonal hatchings denotes where the last remaining *A. pallipes* populations within a cell have been lost since the previous iteration of the Rogers and Watson (2011) map (often due to plague outbreaks), but where there had been no records for *P. leniusculus* populations. Thus, this cell contains no crayfish, but is differentiated from the aforementioned white cells as it had relevant records pertaining to the cell, and so is a ‘lost’ cell rather than a ‘data deficient’ one. Similarly, a red cell with black diagonal hatchings is a cell that previously only contained *A. pallipes* populations (blue cell), but has recently become invaded by *P. leniusculus*. The hatchings were provided to distinguish between simpler cases of new *P. leniusculus* populations being recorded on previously white cells (i.e. a newly red cell that never previously contained crayfish), or where there had been an incursion of *P. leniusculus* into a blue cell and thus the replacement of native *A. pallipes* populations (i.e. a red hatched cell). Red hatching cells were only shown in maps indicating change between the reporting rounds, and presented as normal red cells for the final contemporary distribution maps. No losses of *P. leniusculus* and thus of red cells were envisaged. Additionally, an online version of this map was created (https://arcg.is/0n0Lez) to utilise scaling basemaps with a river network overlay that would not be possible to display effectively in a static map at this level of detail.

Standardised 10 x 10 km grid square reporting metric

Since Article 17 reporting requires that species distributions be provided in a standard occupied 10 x 10 km grid square unit, an occupied grid square map for *A. pallipes* was therefore created in addition to the sub-catchment map. The data covered 2005-2018 rather than relying solely on records from the reporting period (2013-2018), as the author felt this more fairly represented the present distribution of *A. pallipes* in England. Records provided through the review round by EA officers were used in this analysis.

Analysis of distribution of A. pallipes and P. leniusculus in and around the SAC network

Data from 2005 to 2018 were used to report the occupied grid squares of *A. pallipes*. The current SAC network was mapped, and the *A. pallipes* point data overlaid using a Spatial Join algorithm to determine the current number of 10 km grid squares within the SAC network. SAC data was accessed from the open source dataset through https://naturalengland-defra.opendata.arcgis.com/datasets/ , and is available to be publicly downloaded. Both the full SAC network (Grade D and above), and the SACs where *A. pallipes* are a named feature (Grade C and above) were mapped. To account for the threat and impact of local populations of *P. leniusculus*, an additional 'heat map' style analysis was performed. Known populations of *P. leniusculus* were overlaid onto the current Grade C+ SAC network, again using a 10 x 10 km grid square unit. These populations were presented as either High threat (Red), Medium threat (Orange) or Low threat (Yellow). These categories were based on the spatial proximity of the populations to the SAC network alone, and do not constitute assessments of population density, abundance, or connectivity. Populations were considered High threat if they fell within the same 10 km grid squares as the SAC network, Medium if they fell within the adjacent 10 km grid square, and Low threat if they fell within 2 adjacent grid squares.

Results

Reconstruction of the Rogers and Watson 2010 sub-catchment distribution map

The Environment Agency EASIMAP dataset contained a total of 2939 individual point data records for *A. pallipes* and *P. leniusculus* (Fig. 7), which yielded a sub-catchment count for England and Wales of 243 red cells, 111 orange cells, 101 blue cells and 420 white cells (Fig. 8a). Rogers and Watson reported an additional 73 red cells and 35 blue cells using data absent from the EA database (Fig. 8b). Following the amalgamation of both map resources, the occupied sub-catchment cell count was reported as 316 red cells, 111 orange cells, 136 blue cells and 312 white cells for both England and Wales (Fig. 9a), with 301 red cells, 110 orange cells, 137 blue cells and 264 white cells reported solely for England (Fig. 9b). All intermediate map stages culminated in Figure 10, which presented a distribution of 411 red cells, 137 blue cells, and 264 white cells for England as of 2012.

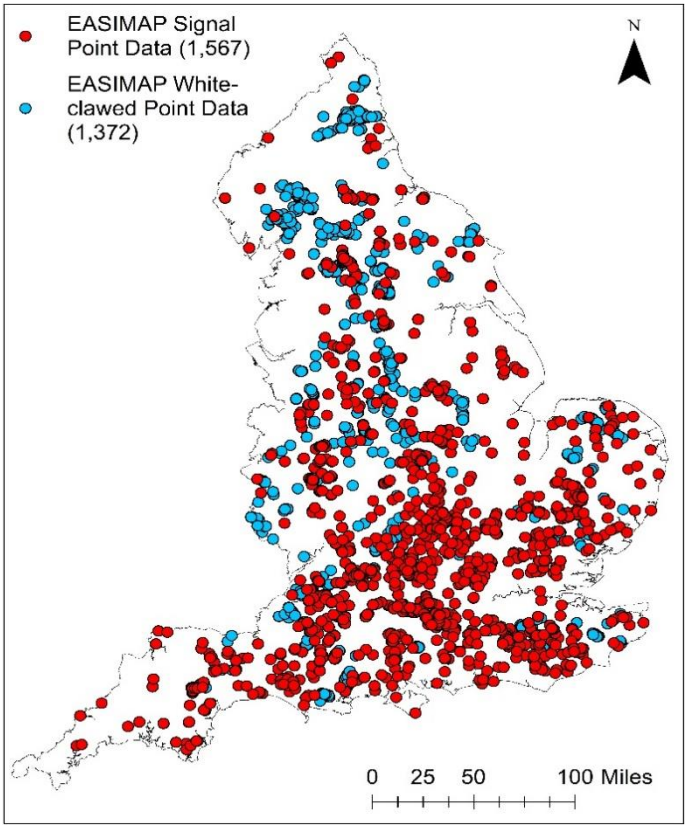


Figure 7 – Point data from EASIMAP EA database for native *A. pallipes* (blue) and *P. leniusculus* (red). Supplied by Ian Marshall, EA 2018.

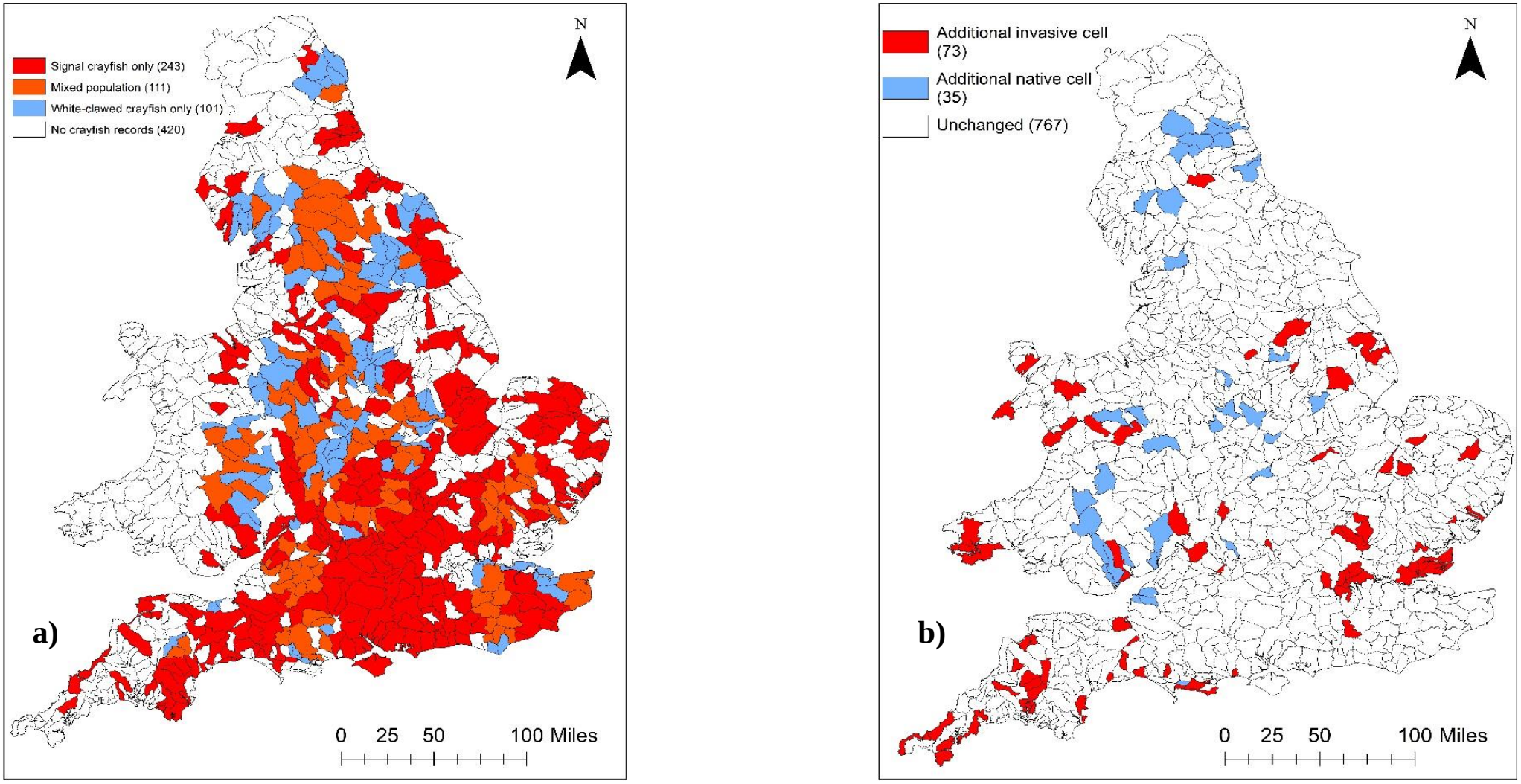


Figure 8 – a) Sub-catchment polygon layer using Environment Agency point data (Spatial Join, ArcGIS 10.4), and b) differences between the Environment Agency database and the 2010 sub-catchment map.

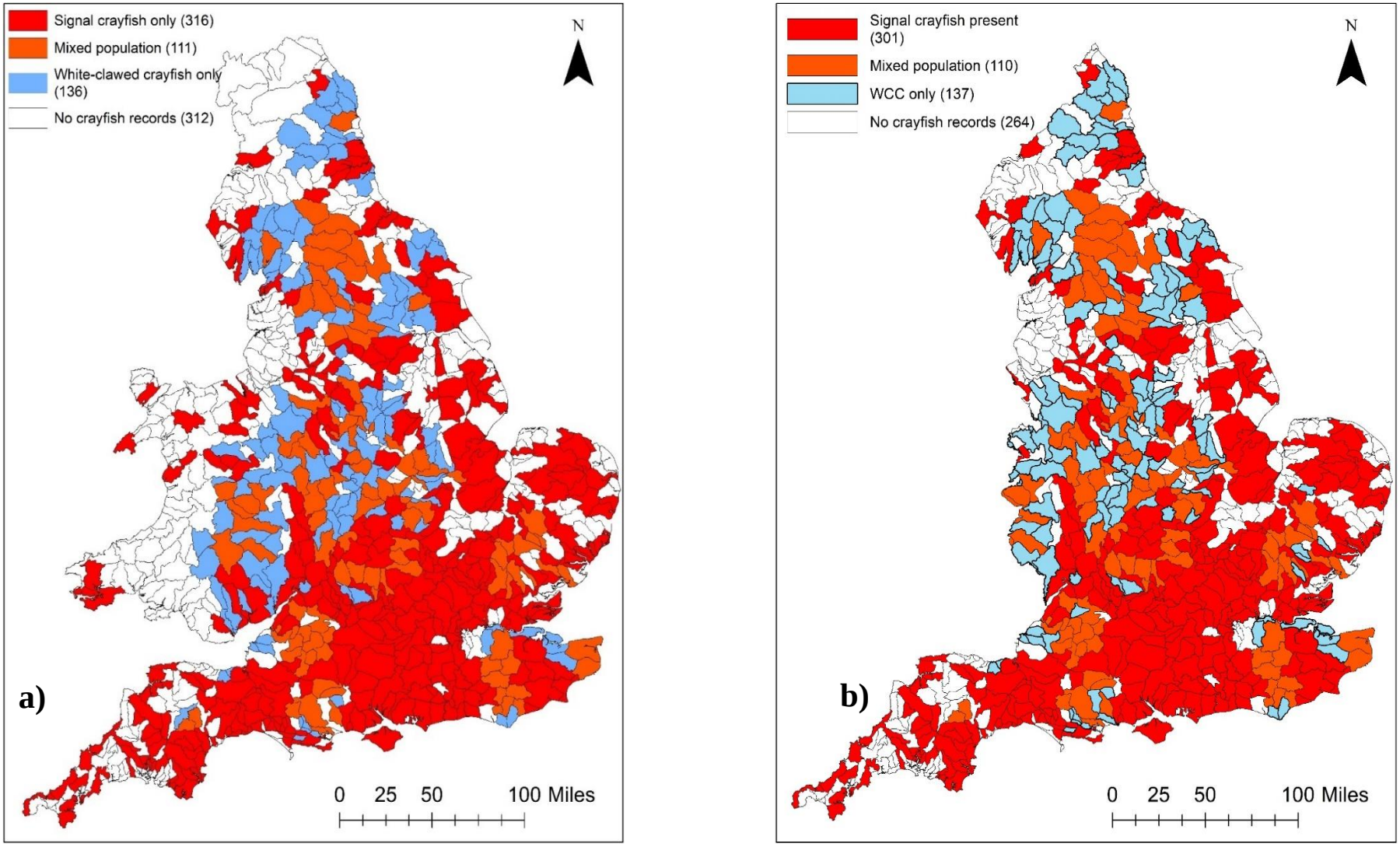


Figure 9 –Unified sub-catchment polygon map, presenting a) both EA data and Rogers and Watson's 2010 data, and thus including Wales, and b) with Wales now removed to account for the respective lack of updated regional data.

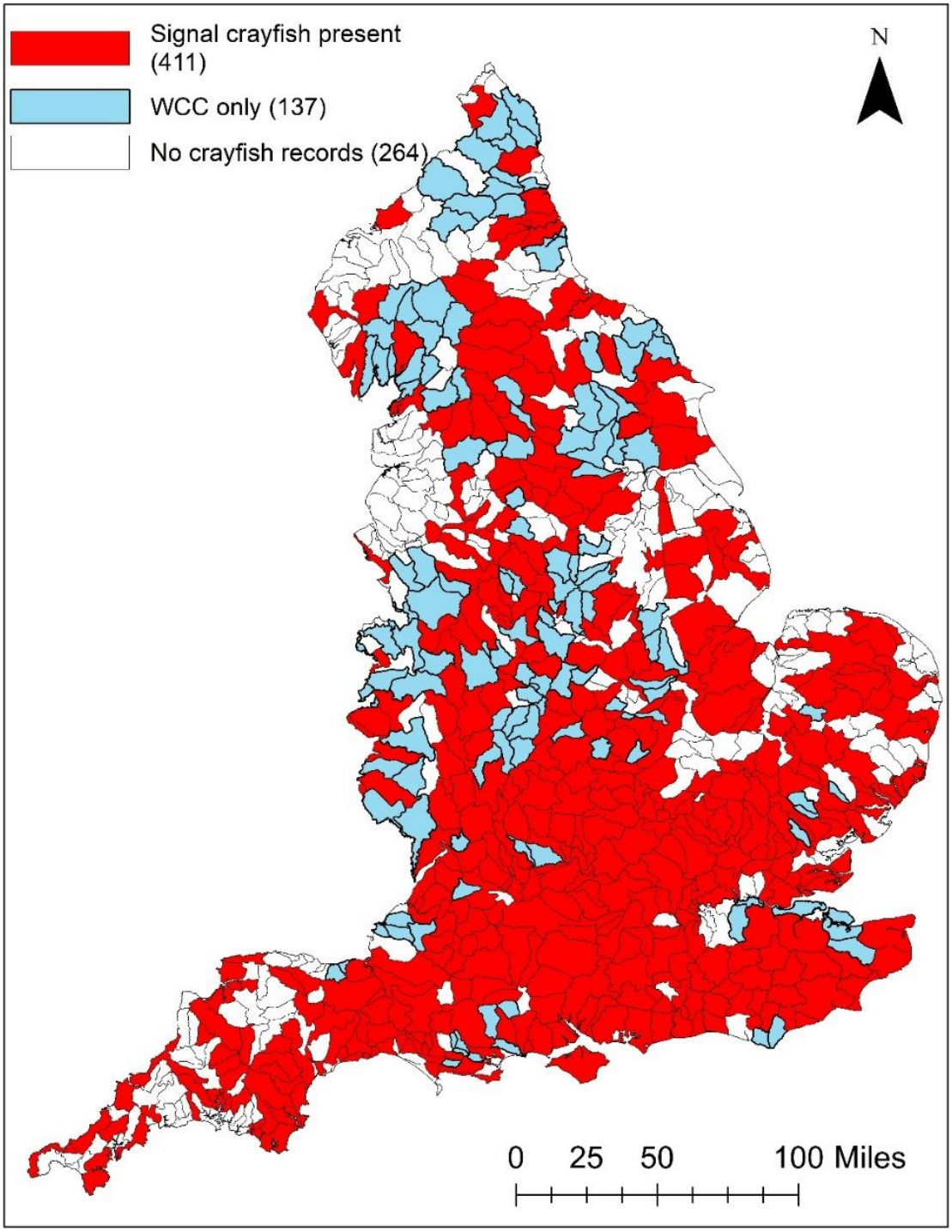


Figure 10 – Baseline sub-catchment polygon map with EA, NE and Rogers and Watson 2010 data, and 'orange' cells replaced by 'red'.

Following the consultation round with both the regional teams of the Environment Agency (Appendix 1), and experts within the UK field, the general trends of the distributions of both *P. leniusculus* and *A. pallipes* in England were collated (Table 5 and 6 respectively). Where regions contained no blue cells in both 2010 and 2018 (Table 6), percentage change is not provided, to avoid the impression of population stability, rather than the consistent local absence of *A. pallipes*. Out of 812 sub-catchments within EA operational boundaries for England, the 2010 map presented 411 red cells, 110 of which were previously considered 'mixed', 137 blue cells, and 264 white cells. These figures are higher than both the 390 cells for *P. leniusculus* and 81 cells for *A. pallipes* reported by Rogers and Watson (2011) for 2010, but were supplemented by the Environment Agency dataset. A consistent increase in the distribution of *P. leniusculus* occurred in England during 2010-2018, with another 54 sub-catchments reported to contain populations. Collating the regional changes (Table 5 and 6), the 2018 map reported a summary for England of 44 cells that were no longer blue during this time period (32% loss for *A. pallipes*), alongside an increase of 54 red cells (13% gain for *P. leniusculus*; Fig. 11). Now reported for 2018 are 465 red cells, 93 blue cells, 231 white cells and 23 yellow hatched cells (Fig. 12). This represents a yearly rate of spread in *P. leniusculus* of 1.6%, and a yearly rate of decline for *A. pallipes* of 4% between 2010 and 2018, if all reported changes in population distributions are considered to have occurred during this reporting cycle. On average, *P. leniusculus* occupied 60% of each region (range 28-92%), and *A. pallipes* solely occupied just 12% (range 0-30%).

Table 5 – Changes to *P. leniusculus* distribution following EA regional consultations

Signal crayfish - *P. leniusculus*						
Region	2010 Count	New (replaced) cells	Lost cells	2018 Count	Percentage Change	Percentage of region occupied
Cambridgeshire & Bedfordshire	47	6 (0)	-	53	+13%	67%
Cumbria & Lancashire	14	10 (4)	-	24	+71%	32%
Devon & Cornwall	47	3 (0)	-	50	+6%	54%
Derbs, Notts & Leicestershire	33	1(0)	-	34	+3%	41%
Essex, Norfolk & Suffolk	49	8 (4)	-	57	+17%	78%
GMMC	11	2 (1)	-	13	+18%	28%
Hertfordshire & North London	37	0 (0)	-	37	0%	90%
Kent & South London	25	-	-	25	-	71%
Lincolnshire & Northamptonshire	37	0 (0)	-	37	0%	47%
Northumberland, Durham & Tees	12	8 (4)	-	20	+67%	38%
SHWG	31	10 (9)	-	41	+32%	67%
Solent & South Downs	43	-	-	43	-	88%
SWWM	32	0 (0)	-	32	0%	56%
Wessex	69	3 (2)	-	72	+4%	69%
West Thames	68	1 (1)	-	69	+2%	92%
Yorkshire	36	3 (1)	-	39	+8%	45%

Table 6 – Changes to *A. pallipes* distribution following EA regional consultations.

White-clawed crayfish - *A. pallipes*						
Region	2010 Count	New cells	Removed (replaced)cells	2018 Count	Percentage Change	Percentage of region occupied
Cambridgeshire & Bedfordshire	1	0	1 (0)	0	-100%	0%
Cumbria & Lancashire	19	0	4 (4)	15	-21%	20%
Devon & Cornwall	0	0	0 (0)	0	N/A	0%
Derbs, Notts & Leicestershire	25	0	0 (0)	25	0%	30%
Essex, Norfolk & Suffolk	4	1	4 (4)	1	-75%	1%
GMMC	5	2	2 (1)	5	0%	11%
Hertfordshire & North London	0	0	0 (0)	0	N/A	0%
Kent & South London	5	-	-	5	-	14%
Lincolnshire & Northamptonshire	10	0	5 (0)	5	-50%	6%
Northumberland, Durham & Tees	13	0	4 (4)	9	-31%	17%
SHWG	23	1	9 (9)	15	-35%	25%
Solent & South Downs	3	-	-	3	-	6%
SWWM	22	0	12 (0)	10	-46%	18%
Wessex	15	0	4 (2)	11	-27%	11%
West Thames	3	0	2 (1)	1	-66%	1%
Yorkshire	25	2	1 (1)	26	+4%	30%

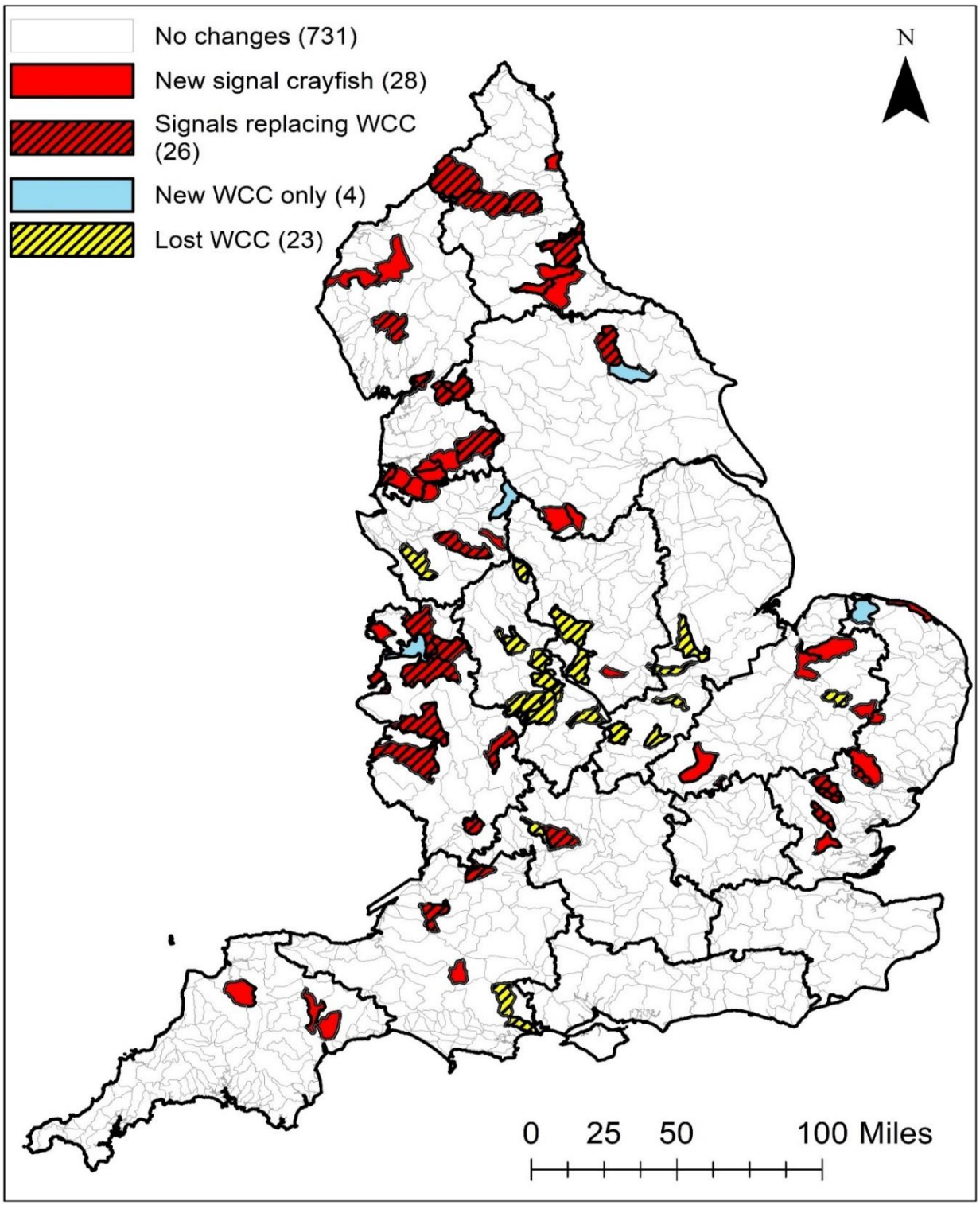


Figure 11 – Changes from the 2010 to the 2018 Article 17 sub-catchment map, showing distribution of *A. pallipes* and *P. leniusculus* in England by EA region

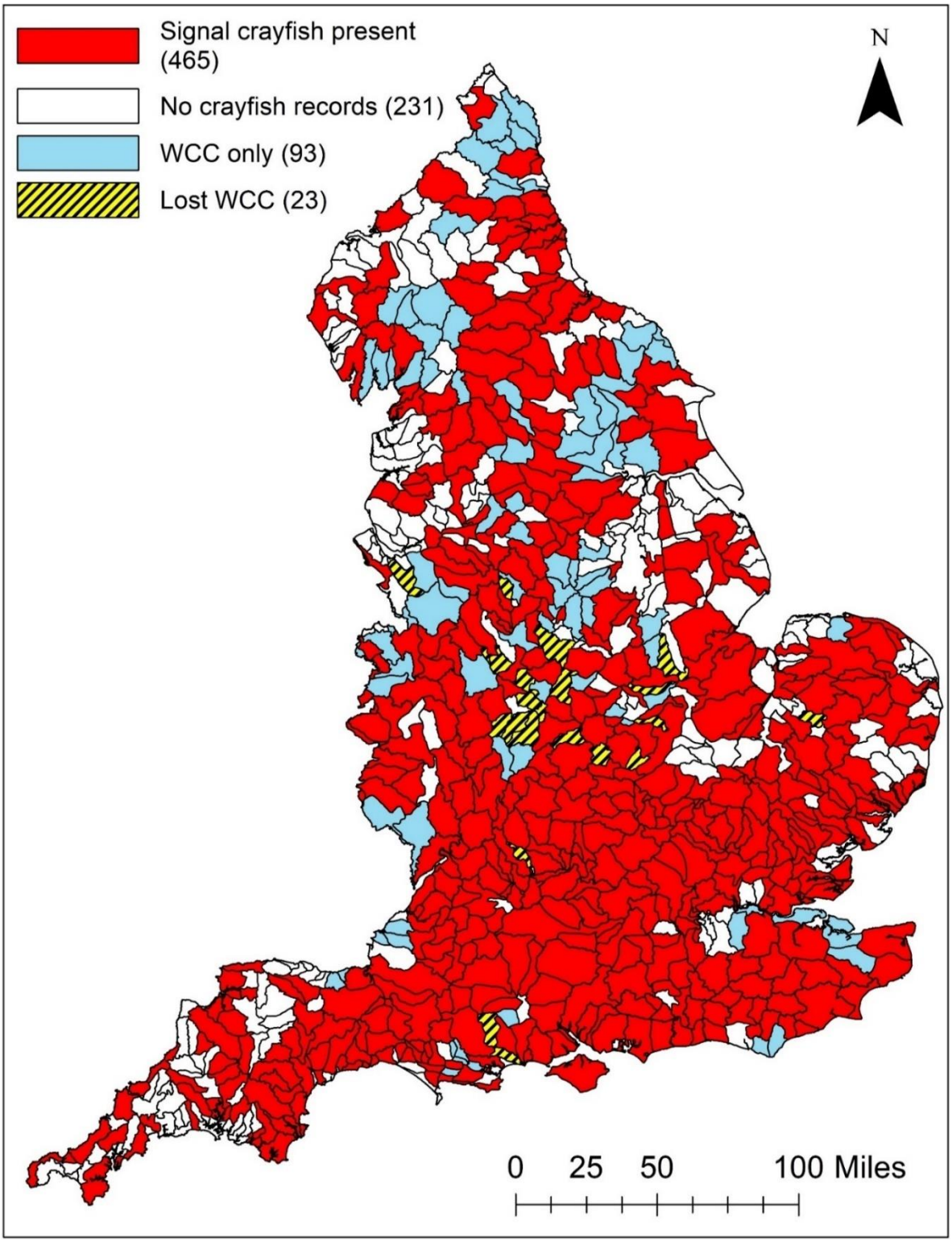


Figure 12 – Complete 2018 Article 17 sub-catchment map, showing distribution of *A. pallipes* and *P. leniusculus* in England

Standardised 10 x 10 km population grid squares for* A. pallipes *in England

As required by the JNCC, population distribution was reported in terms of number of 10 x 10 km grid squares that were occupied by *A. pallipes* (Fig. 13). Occupied 10 x 10 km grid square counts for *A. pallipes* have declined from a baseline of 464 occupied grid squares in the UK between 1970-2002, to between 241-260 in 2002-2005 (JNCC, 2007b), of which 137 were in England (JNCC, 2007b), to 147 in 2012 (JNCC 2013a). In 2018, using data compiled from 2005-2018 inclusive, 239 grid squares were reported to be occupied by *A. pallipes* in England. This figure is in keeping with previous broader counts in 2002-2005 for the UK, and is still significantly less than the UK baseline value of 464.

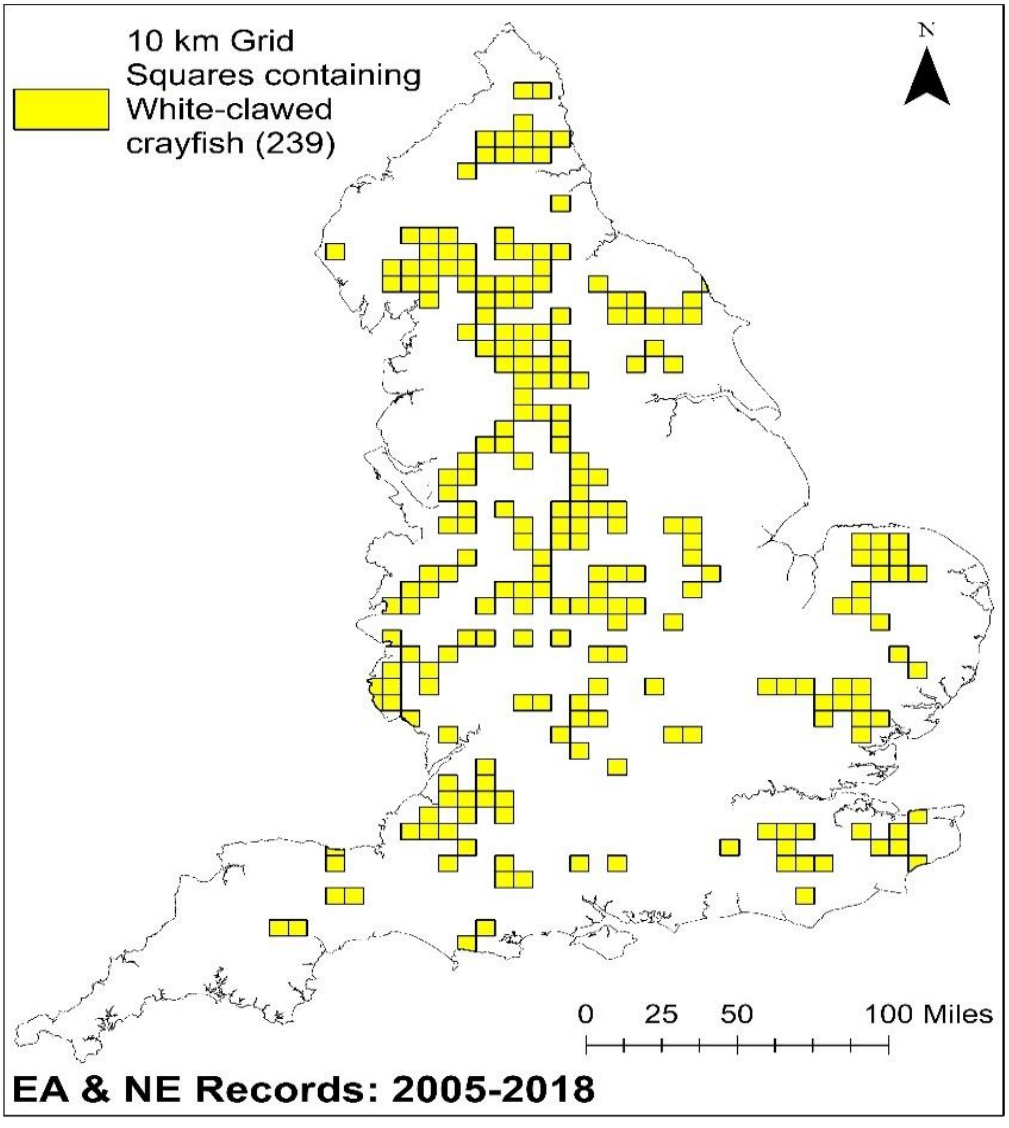


Figure 13– *A. pallipes* distribution through occupied 10 x 10 km grid squares in 2018

A. pallipes and P. leniusculus populations within the SAC network

The reported number of 10 x 10 km grid squares occupied by *A. pallipes* within the total SAC network for the 2018 report was 40 (Fig. 14); this figure included all Grade D SAC's. When considering only populations of national or European importance (Grade C or above), the number of occupied 10 x 10 km grid squares in 2018 was reported as 28 (Fig. 15). The 28 presently occupied grid squares within the Grade C+ SAC network for *A. pallipes* represents approximately 11.7% of the total occupied grid squares for the species (2005-2018 count). The occupied total SAC coverage was just outside of the reported range of occupied grid squares for the 2012 report, of 41-54 (JNCC, 2013b), however this figure was for the UK and not just England. Additionally, the 2012 report stated that only 24 of the occupied 10 x 10 km grid squares within the SAC network were not hydrologically connected to a population of *P. leniusculus*. A heat map-style analysis was performed to display the distribution of 10 x 10 km grid squares containing populations of *P. leniusculus*, with respect to the current distribution of *A. pallipes* populations within the Grade C+ SAC network (Fig. 16). *P. leniusculus* populations were present within every SAC with an *A. pallipes* designation, with 9 'High' threat populations, 33 'Medium' threat and 41 'Low' threat populations being reported, totalling 83 occupied grid squares immediately in or around current *A. pallipes* populations within SACs. Therefore, 22.5% of the occupied grid squares for *A. pallipes* within the most ecologically important populations in England were within 10 km or less of established populations of *P. leniusculus*.

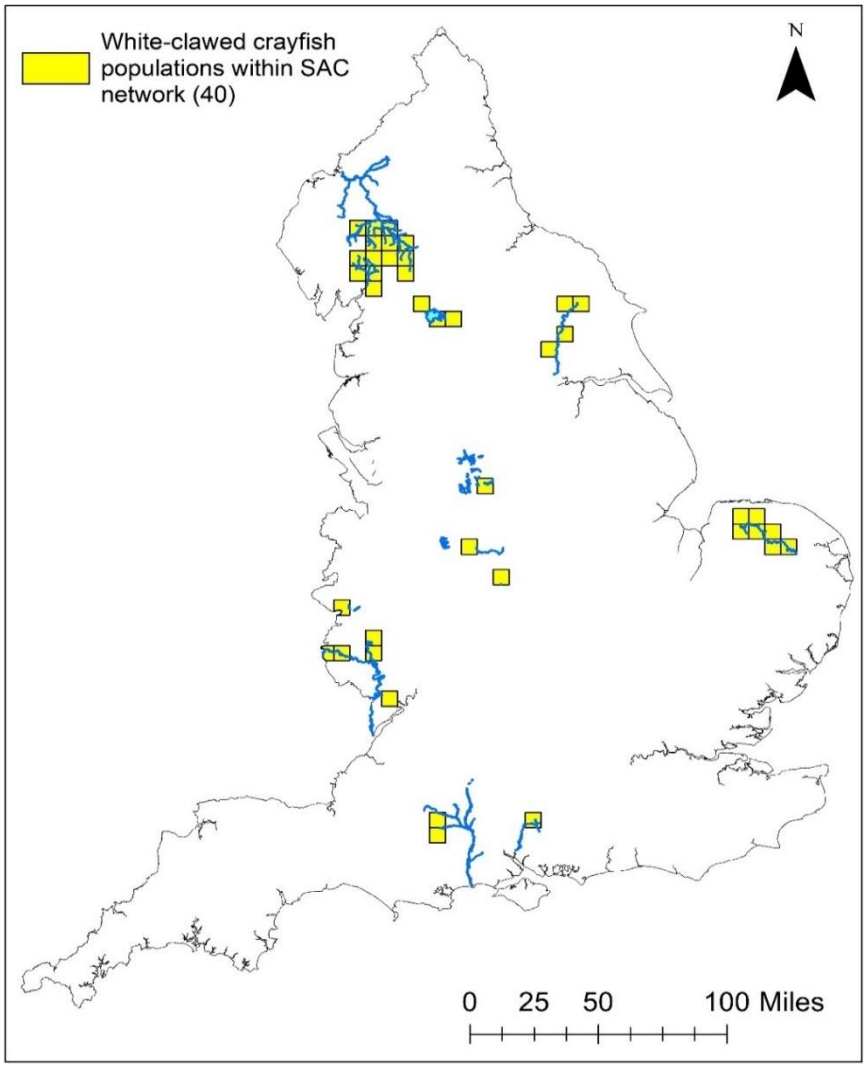


Figure 14 – Occupied grid squares from all SACs containing *A. pallipes* (Grade D and above) for the 2018 Article 17 report.

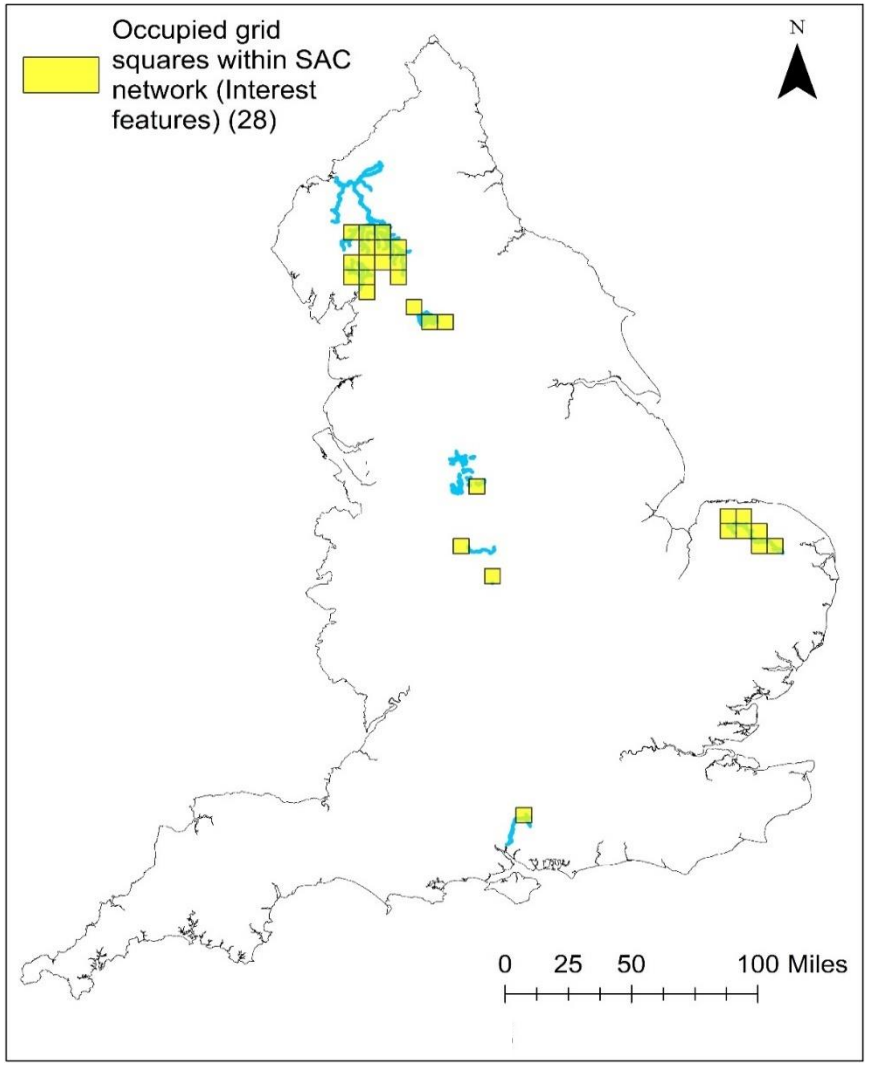


Figure 15 – Number of occupied grid squares containing *A. pallipes* within the Grade C or above SAC network.

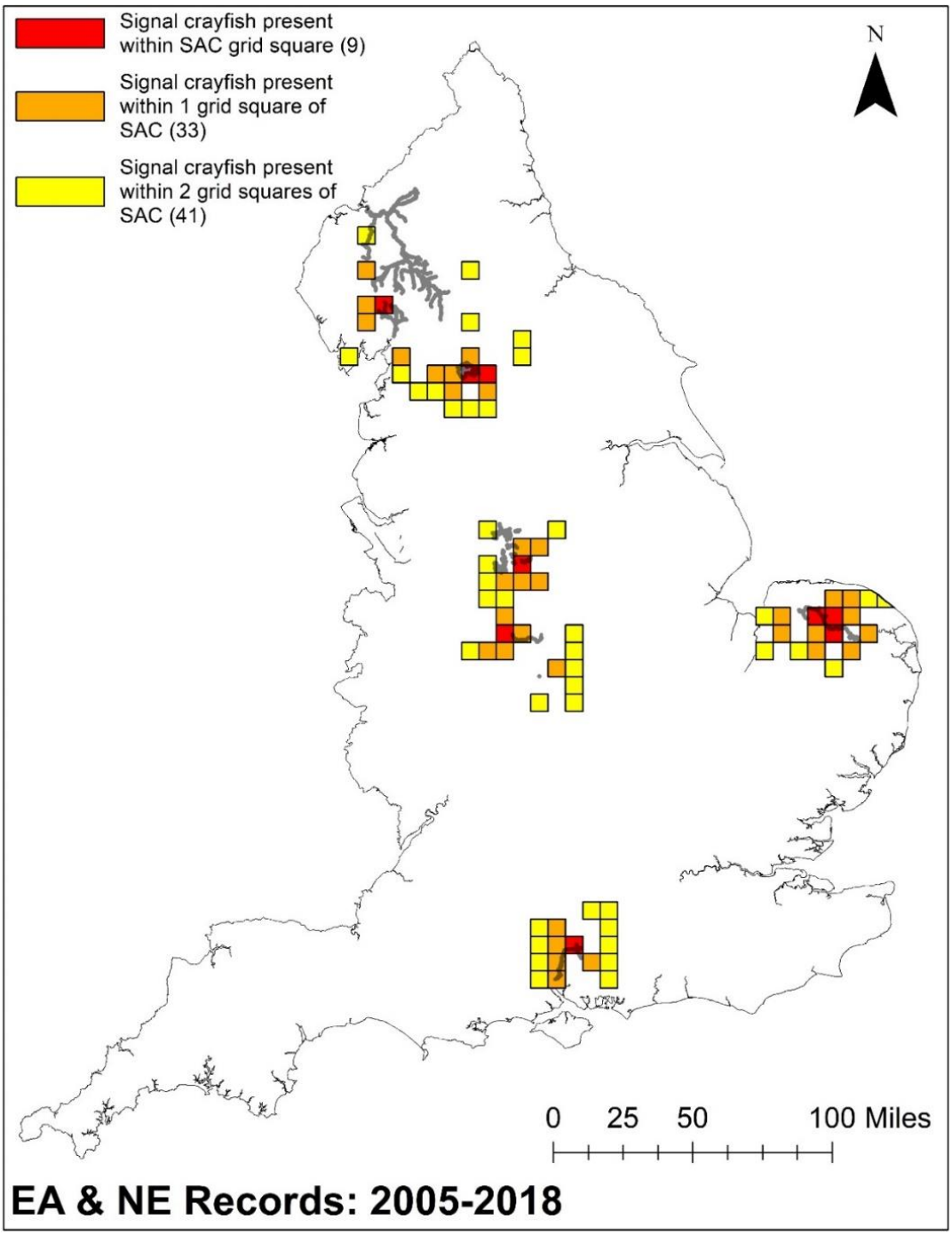


Figure 16 – 'Heat map' style presentation of invaded 10 x 10 km grid squares in and around the SAC network (Grade C or above). Data from Environment Agency and Natural England, covering 2005-2018.

Discussion

Distribution of occupied sub-catchments cells within England

The relative changes in the distribution of both *P. leniusculus* (increasing) and *A. pallipes* (decreasing) in England between the 2010 and 2018 sub-catchment maps were reported as in excess of 1% per year, and thus confirming the proposed hypotheses. However, this interpretation may fail to account for potential biases in the reporting of the crayfish in England at present. While these losses and gains in most instances represent sub-catchments rather than discrete populations, this coarser resolution trend is consistent with previous data (Rogers and Watson, 2011) and cause for concern. However, several factors influence the relative rates of change in the distribution of both species. For example, much of the South of England was already invaded by *P. leniusculus* in 2010, and as such the rates of newly invaded sub-catchments are likely to decline over time, as the regional distribution of *P. leniusculus* becomes increasingly ubiquitous. Likewise, where there are few populations of *A. pallipes* present, as populations are lost the absolute change in the distribution of *A. pallipes* will be small, but will represent a large relative loss to the remaining *A. pallipes* populations regionally. When reporting the status and distribution of crayfish populations, careful consideration should therefore be given to the appropriate reporting metric, as for example the loss of a single population may be 100% of the remaining *A. pallipes* stock of an area.

The sub-catchment mapping method changes the colour category of a corresponding cell in response to the confirmed occupation by a single population of *A. pallipes* or *P. leniusculus*. Whilst the continued spread and consolidation of *P. leniusculus* populations within existing red cells is likely, there would fail therefore to be a corresponding change in the number of occupied sub-catchments for *P. leniusculus* (assuming natural dispersion did not span sub-catchment boundaries). However, a previously white or blue

cell would be turned red in response to occupation by a single confirmed population of *P. leniusculus*. As such, many of the new records for sub-catchments invaded by *P. leniusculus* since 2010 are therefore established in regions containing relatively fewer previous populations of *P. leniusculus*, and indeed often in the few remaining *A. pallipes* strongholds. Whilst the sub-catchment reporting metric therefore does represent a useful combination of the distribution data for both species, it does not fully account for population level changes to *A. pallipes* and *P. leniusculus* distribution, and as such may over-represent the dominance of *P. leniusculus* in England. In the absence of population level data for either species in England at present, the continued use of the sub-catchment map is advocated, due to the competitive dominance of *P. leniusculus* over *A. pallipes*. Indeed, Rogers and Watson (2011) suggested that in sub-catchments with mixed populations of *A. pallipes* and *P. leniusculus*, it was simply a matter of time before native populations were outcompeted and lost. This finding was supported by Bubb *et al.* (2005), who reported *A. pallipes* driven to localised extinction in the absence of plague over a 6-7 year period, through being outcompeted by *P. leniusculus*. In order to make effective use of this sub-catchment mapping resource, consistency should be established within the method. Work is now needed going forward to curate the sub-catchment map effectively, to maintain the temporal and spatial accuracy of records, and to form a repository for records collected going forwards. Collaboration is a key aspect of management of crayfish in England, and should be central to this endeavour.

Standardised reporting of population units within the Article 17 framework

While the sub-catchment mapping method offers information on both *A. pallipes* and *P. leniusculus* on a coarser resolution in England, the current standard unit for reporting *A. pallipes* distribution under Article 17 is the 10 x 10 km grid square. The reporting of the grid square population unit is, however, inherently flawed for crayfish, due to the way

crayfish records are generated and recorded in England. This is due to the aforementioned issues regarding ad-hoc sampling for crayfish in England, and the biases associated with reporting only data from within a single reporting window. As such, despite populations not having been re-sampled for several years, positive records from outside of the reporting round were included, resulting in a potential overestimate of extant populations; I viewed this as an acceptable alternative to assuming all un-surveyed populations are extinct. To explain this viewpoint, the data from the 2012 Article 17 population estimates are discussed (JNCC, 2013a). Three numbers were provided from the supporting document using the occupied 10 km grid squares standardised units, namely a current estimate of population, a short term trend, and a long term trend (JNCC, 2013b). The occupied grid square estimate for 2012, reported as 147, was based on records generated during the reporting round duration, i.e. 2007-2012 (Fig. 4), and was presented as a 58% loss of population since 1989. In contrast to the general trends of *A. pallipes*, this was also an increase from the 137 occupied grid squares reported in 2006 for England. The main species report (JNCC, 2013a, p. 1) reported a range of 192 to 223 occupied grid squares, citing them as "based on species records which are considered to be representative of the range within the current reporting period". The short term trend was then reported at 214 grid squares, utilising data generated from 2001-2012. Both the current and short term figures show strong declines in numbers at >1% per year, and so justifying 'Bad' classification under the EC guidelines. The final trend data was long term data, and reported a figure of some 295 occupied 10 x 10 km grid squares to be occupied during 1989-2012, with an accompanying decrease of <1% of population per year.

The temporal range from which records are selected has a clear and important impact on the values reported for *A. pallipes*, and must be given careful consideration going forward. Whilst the process of selecting crayfish records pertaining to the same time

period as the Article 17 report (i.e. 6 yearly cycles) is logical, this method does not function in the current framework of crayfish sampling in England and the UK. Given the strict guidelines the JNCC have provided for classifying species trends, there exists a disconnect between the emphasis on high quality distribution data as required by Europe, and the current provision of such data in England. Either these guidelines must be relaxed or become accommodating of the current sampling efforts within England, or fundamental changes must be implemented to facilitate the monitoring of crayfish species in England.

Status of the SAC network with reference to distributions of both A. pallipes and P. leniusculus

Presenting and discussing the current extant population range of *A. pallipes* within the SAC network, without also acknowledging the current extent of *P. leniusculus,* fails to address the key contemporary driver of the reductions and exclusions of historical native range. Populations of *P. leniusculus* overlap strongly with the current distribution of *A. pallipes* within the SAC network, and as such presenting only *A. pallipes* data can provide a misleading or overly optimistic account of the efficacy of the SAC network. The inclusion of the proximity of *P. leniusculus* populations to populations of *A. pallipes* within the SAC network provides key contextual information on the current threat levels and thus status of the SAC network for *A. pallipes*, and raises important questions as to the management of the network regarding biosecurity and the spread of invasive crayfish populations. Ensor's Pool and the River Wensum have both experienced outbreaks of crayfish plague resulting in catastrophic or total loss of native crayfish stocks, and the River Mease, River Itchen, River Eden and Peak District Dales have populations of *P. leniusculus* expanding within the catchments. These findings are supported in sub-catchment maps, where significant breaches in biosecurity, characterised by the loss of *A. pallipes* populations due to plague outbreaks without the

subsequent discovery of localised *P. leniusculus* populations (or appropriate vector species), accounted for a large number of *A. pallipes* sub-catchment losses as compared to direct invasion by *P. leniusculus* populations. The SAC network is under huge pressure from *P. leniusculus* and crayfish plague, and is failing at present to protect and safeguard populations of *A. pallipes* to ensure the long term viability of the species.

General trends and future directions for conservation and management of crayfish in England

The contemporary metrics for measuring change in distribution over time for crayfish in England do not function fully under the current reporting framework. Data deficiency is a key issue, as is the provision of a relative excess of data through uneven sampling efforts. Increases or decreases in the reported distributions of *A. pallipes* in England have in part been attributed to changes in survey effort (e.g. JNCC 2007c), such as the increase of occupied grid squares between the second and third report, despite the general trend being acknowledged as 'Bad and deteriorating'. Therefore, until a centralised monitoring effort is established, all available data should be considered, and all metrics that are available for presenting distribution should be used. What is consistent, however, between reporting methods is the continued decline of *A. pallipes* and incursion of *P. leniusculus* across England. The process of establishing and implementing a monitoring program for both *A. pallipes* and *P. leniusculus* is discussed further in Chapter 6, as it forms a key component of the future of astacology in England.

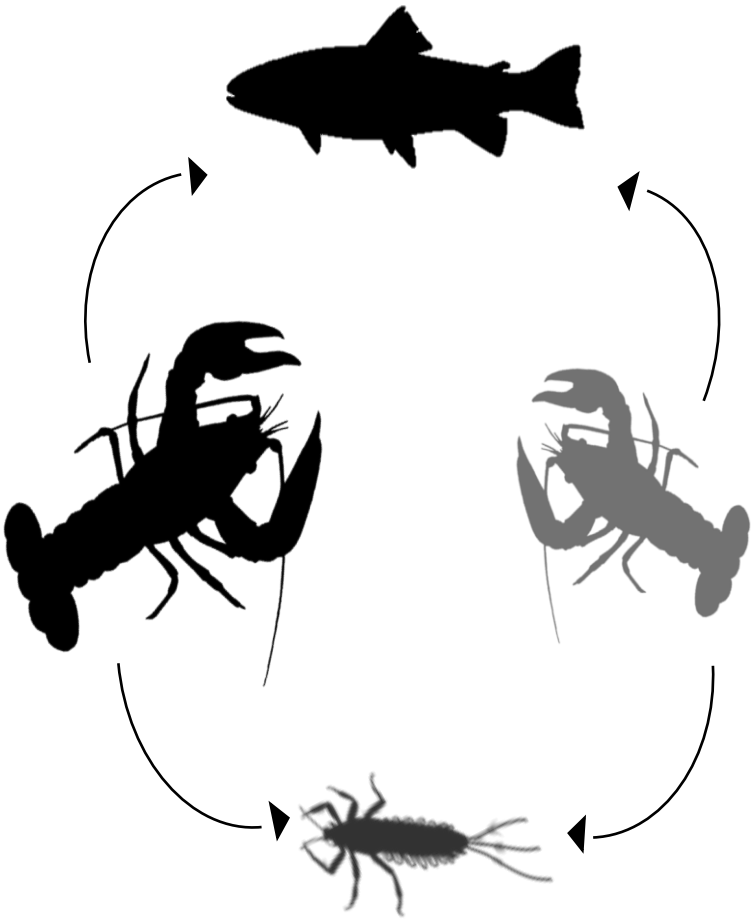
The database and distribution maps created in this chapter form a key central resource for management and conservation. Management decisions can be facilitated through understanding where populations of both native and invasive crayfish occur, and thus where to invest the finite resources available to maximise impact. Additional overlays that were included in the online version of the sub-catchment map, such as river networks and automatically scaling basemaps, can further assist targeted localised

conservation and management efforts. Issues such as biosecurity, especially of protected sites such as the SAC network, can now be viewed both in terms of presence of *A. pallipes* populations, but also of the proximity to populations of *P. leniusculus* (Rogers and Watson, 2011).

Conclusion

A. pallipes continued to decline in England, and *P. leniusculus* continued to expand its invasive range. The inclusion of biotic data into habitat calculations is not currently part of the habitat assessment but should be considered, given the relevant and important impact invasive species can have. Fundamentally, in order to successfully protect and restore a species to favourable conservation status, the main aim of the Directive, there must be in place an understanding of the species stock at present. Where this is not the case, it must follow that determining ways to collect this data, and then expending the resources to do so, is a management priority. Understanding crayfish distribution from local to national scale is key to effective management, and to understanding the ecological impacts associated with the loss of *A. pallipes* and introduction of *P. leniusculus*.

Chapter 3. Effects of native and invasive crayfish species in headwater streams of Northern England



Introduction

Freshwater systems represent only 0.01% of the water in the world, but account for almost 6% of all described species (Dudgeon *et al.*, 2006; Reid *et al.*, 2018). However, freshwaters are under huge pressures, resulting in accelerated rates of freshwater biodiversity loss (Ricciardi and Rasmussen, 1999), with the Living Planet Report 2018 estimating an 83% loss of freshwater invertebrate populations since 1970 (WWF, 2018). Invasive species are identified as one of the five major drivers of biodiversity loss in freshwaters (Dudgeon et al., 2006; Reid et al., 2018). Freshwaters have been identified as particularly vulnerable to invasion, due to the ease of dispersal for species within systems, and the niche evolutionary lineages facilitated by the geographical isolation of freshwater systems from each other (Gherardi, 2007). Crayfish in particular can be accomplished invasive species, having a much higher invasive success rate than might be predicted through invasion theory (Holdich *et al.,* 1999). Indeed, Holdich *et al.* (2009) reported that, of the 10 species of crayfish introduced into Europe, 9 had established self-sustaining populations by 2009.

Of particular note is the role of headwater systems in supporting freshwater biodiversity. Headwaters can support highly diverse ecological communities including bacteria, macrophytes, macroinvertebrates, and fishes (see Meyer *et al.*, 2007). In addition, headwater habitats may be utilised intermittently for specific ecological needs, such as for spawning in salmonids or as fish refuges during peak flow events (Fig. 1). The support and protection of headwater habitats has been identified as a conservation priority, as for example they often provide a final refuge to native biota following the spread of invasive species elsewhere within a catchment (Saunders *et al.*, 2002). Changes to headwaters, whether biotic or abiotic, can have far reaching consequences for the entire length of the lotic system, such as altering downstream productivity and eutrophication (see Freeman *et al.*, 2007).

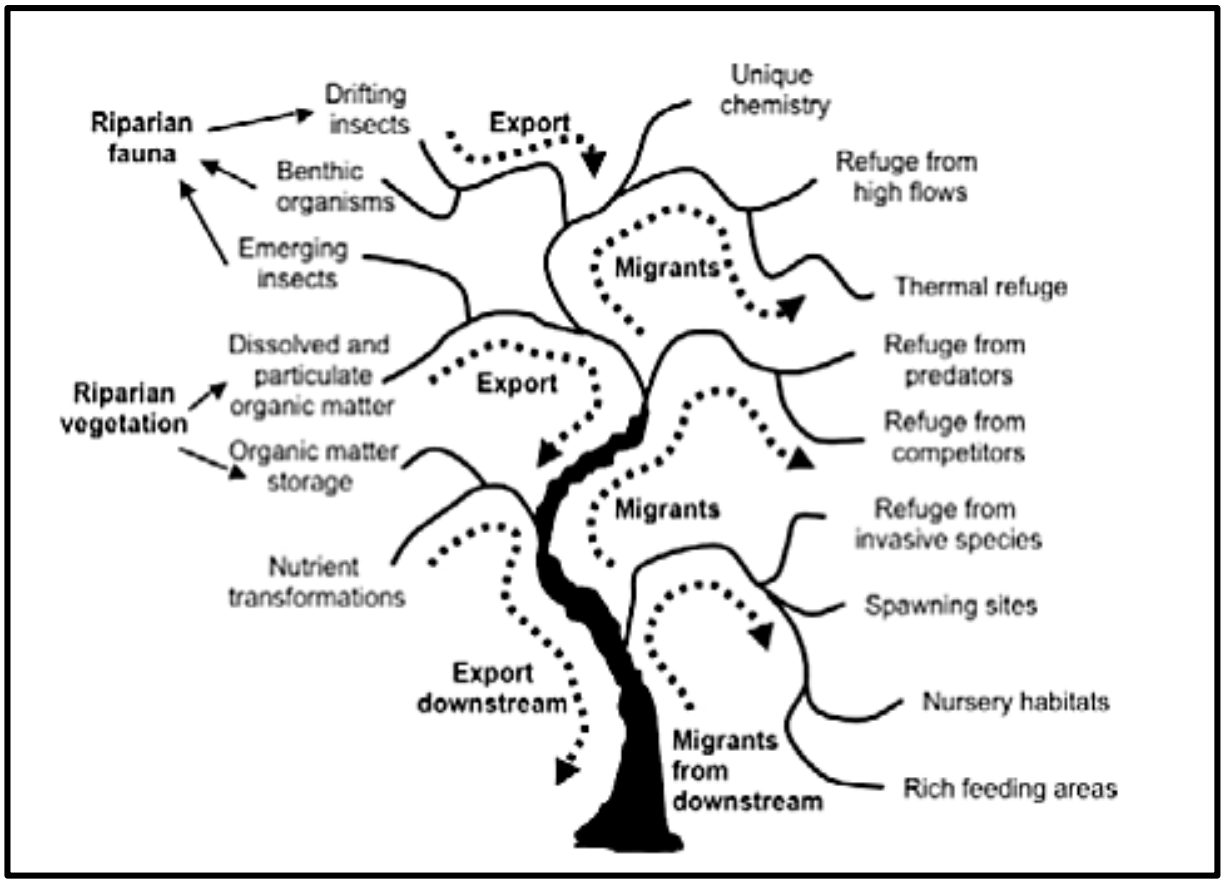


Figure 1 – Conceptual diagram of the ways headwaters support biodiversity within river networks. Factors on the left denote contributions of headwaters to downstream processes and ecosystems, and factors on the right denote benefits headwaters provide to aquatic biota. Adapted from Meyer *et al.* (2007).

The white-clawed crayfish (*Austropotamobius pallipes*, Lereboullet) is the UKs largest native invertebrate, and only native crayfish species (Holdich & Reeve, 1991). As demonstrated in Chapter 2, populations of *A. pallipes* continue to decline in England. The remaining populations of *A. pallipes* are concentrated in the North of England due to continued concentrated losses of southern populations (Souty-Grosset *et al.*, 2006). These northern regions support the densest populations of *A. pallipes* in Europe (Holdich, 2003), and are of international significance to *A. pallipes* conservation (Dunn *et al.*, 2008). Headwaters offer highly suitable habitat that facilitate abundant and recruiting populations of *A. pallipes*, such as vertical undercut banks (Holdich & Rogers, 2000) tree roots (Benvenuto *et al.*, 2008), and suitably sized stones and cobbles

(Foster, 1993). These habitats provide shelter and refuge from predators and hydrological extremes (Smith *et al.*, 1996). Many crayfish species are associated closely with headwater systems (e.g. Meyer *et al.*, 2007), where they can also dominate macroinvertebrate biomass (Haggerty *et al.*, 2002). It has been suggested in the literature, however, that *A. pallipes* are more suitably adapted to colonising the headwaters and upper reaches of riverine systems than invasive crayfish species in Europe (e.g. Gil-Sánchez and Alba-Tercedor, 2002). This is important as headwaters may provide refuge from invasive crayfish through abiotic barriers such as high flow and low water temperature, as well as via physical barriers such as weirs or waterfalls (Gil-Sánchez and Alba-Tercedor, 2002). The retraction of *A. pallipes'* range within a system can be caused by localised pollution events or outbreaks of an invasive fungal-like pathogen termed crayfish plague (*Aphanomyces astaci* Schikora), an ever present threat which can eliminate an entire *A. pallipes* population (Holdich & Rogers, 1997). Following mass mortalities of *A. pallipes*, populations held in headwaters and tributaries are often able to recolonise main channels (Holdich & Reeve, 1991; Holdich & Rogers, 1997). Low order streams typically offer high water quality and undisturbed natural habitats (Haddaway *et al.*, 2015), that can often be upstream of major anthropogenic threats, and as such both headwaters and the populations of *A. pallipes* that they support can be of great conservation value.

There is comparatively little published with respect to the feeding preferences and thus functional role of *A. pallipes*, and if this role can change as a function of either the size of the animal or the population. Gladman *et al.* (2009) suggested that *A. pallipes* do not obviously negatively impact native biota, however this area remains largely untested and is questioned in some instances (James *et al.*, 2014). Omnivory is common in crayfish species (e.g. rusty crayfish, Lodge *et al.*, 1994; Northern koura *Paranephrops*

planifrons White, Parkyn *et al.*, 2001; red swamp crayfish, Alcorlo *et al.*, 2004), and has been shown in both *A. pallipes* and signal crayfish, *Pacifastacus leniusculus* Dana (Gherardi *et al.*, 2004 and Bondar *et al.*, 2005, respectively). Omnivory has a strong influence on the range of potential impacts crayfish can exert on ecological communities, and can be a key regulatory factor in stabilising ecosystems (Lodge *et al.*, 1994).

A. pallipes will consume detritus, thus providing an important conduit for energy transfer through the food web (Lorman & Magnuson, 1978). Macrophytes and algae are also grazed by *A. pallipes*, which has even been known to feed on terrestrial vegetation (Gledhill *et al.*, 1993). Whilst *A. pallipes* is both a detritivore and a herbivore, it also occupies the role of an active predator, with invertebrates, amphibians and fish thought to have been previously understated as dietary components (Momot, 1995). *A. pallipes* form an important prey species, present in the diet of bird, fish and mammalian predators (e.g. Stein, 1977; Englund & Krupa, 2000), especially the European otter (*Lutra lutra* L.) (Almeida *et al.*, 2012). As such, the conversion of allochthonous materials into biomass available to higher trophic levels increases the energy available in the system. Following the loss of *A. pallipes* populations from several Irish lakes, Matthews and Reynolds (1992) reported a substantial increase in the stand size and growth rates of the dominant macrophyte Bearded Stonewort (*Chara curta*). Additionally, invertebrate abundance within the lakes significantly increased following the loss of *A. pallipes*, driven in part by taxa specific increases in *Planorbidae* (Ramshorn snail) and Trichoptera (caddisfly) populations. This was likely a consequence of both a direct release from the predation pressure of *A. pallipes*, and an indirect facilitation of invertebrate communities through the then absent grazing pressure of *A. pallipes* on *C. curta*, which forms both a habitat and a food source for the invertebrate communities. Gherardi *et al.* (2004) showed through laboratory trials on

food selection that *A. pallipes* can exhibit preferential selection of food types, and that while *A. pallipes* are primarily detritivores, plant, aquatic invertebrate and fish material were all consumed. Reynolds and O'Keeffe (2005) further supported this hypothesis, and reported opportunistic feeding in *A. pallipes*, another trait common in omnivorous crayfish, through analysis of the diets of stream and lake based populations in Ireland. *A. pallipes* were also shown to exhibit different feeding strategies between the lotic and lentic populations, reflecting the different food types and thus opportunities available to them. In addition, Scalici and Gibertini (2007) described an ontogenetic and gender related shift in the diet of *A. pallipes* in a brook population in central Italy, with male *A. pallipes* showing decreased consumption of aquatic invertebrates upon reaching maturity, and female *A. pallipes* maintaining levels of invertebrate consumption comparative to juvenile *A. pallipes* sampled.

P. leniusculus is a globally successful invasive crayfish species (Souty-Grosset *et al.*, 2006; Larson *et al.*, 2016), and populations of *P. leniusculus* are well established across much of England (see Chapter 2; Holdich *et al.*, 2014), The impacts of *P. leniusculus* on *A. pallipes* have been well documented (see Ibbotson and Furse, 1995; Holdich *et al.*, 2009), driving localised extinctions through the spread of the crayfish plague and also through the dominance of *P. leniusculus* over *A. pallipes* in terms of size, fecundity and voracity. *P. leniusculus* have also been shown to negatively impact macroinvertebrate community abundance (Ercoli *et al.*, 2015), richness (Mathers *et al.*, 2016) and diversity (Crawford *et al.*, 2006). In addition, *P. leniusculus* can also reduce macrophyte abundance through grazing submerged macrophytes species such as *Elodea* and *Chara* (Nyström *et al.*, 1999), as well as floating-leaved macrophyte species (Usio *et al.*, 2009), leading to microhabitat loss, altered flow regimes and reduced water quality (Carpenter and Lodge, 1986). There is also evidence that *P. leniusculus* can impact the recruitment of fish species, through predation of eggs and larval stages, as shown for

salmonids (Findlay *et al.*, 2015), and whitefish (Karjalainen *et al.*, 2015). This is of particular interest with respect to the impacts of *P. leniusculus* in headwaters, as headwaters commonly function as spawning and nursery grounds for many fish species (e.g. Peay *et al.*, 2009; Fig. 1).

Both *P. leniusculus* and *A. pallipes* are thought to be keystone species and ecosystem engineers in freshwater habitats, being capable of disproportionately structuring the communities around them through direct and indirect ecological pathways. The question arises, therefore, as to whether *A. pallipes* and *P. leniusculus* have different influences on the biology in headwater systems, given the many overlapping traits expressed by both species. It is important to understand if the threats posed by *P. leniusculus* are limited to the functional replacement of one crayfish species by another, or if they have wider implications for native ecosystems. The aims of this study were to therefore assess whether the presence of a crayfish in headwater streams impacted resident fish and aquatic invertebrate communities, and if so, whether these impacts were different between *A. pallipes* or *P. leniusculus*. It was hypothesised that there would be significant differences in the aquatic invertebrate communities between sites containing *A. pallipes* and *P. leniusculus*, and that *P. leniusculus* would significantly reduce macroinvertebrate abundance, biomass and diversity, whereas *A. pallipes* would have little impact on aquatic invertebrates. Additionally, it was hypothesised that fish abundance and biomass would be significantly lower at sites containing populations of *P. leniusculus* than at sites containing *A. pallipes*.

Methods

Project design

Headwater streams were selected for study in North Yorkshire and Cumbria (Fig. 2 and 3). Sites were selected based on past survey data such that they contained no crayfish (control), or supported populations of *A. pallipes* or of *P. leniusculus*. A total of 14 sites were sampled within this study designed to provide an even spread of headwaters containing *P. leniusculus* (n = 6), *A. pallipes* (n = 4) and no crayfish (n = 4; Fig. 3). All sites were low order rocky streams, with underlying limestone geologies, and had similar land use, this being unimproved and semi-improved agricultural land (Fig. 2). Sites containing *P. leniusculus* were concentrated around the Wharfe catchment, with site selection informed by the well documented invasion and spread of *P. leniusculus* shortly after its introduction into Kilnsey trout farm in 1983 (Bubb, 2004). Cumbrian headwaters were included in order to provide additional sites that had strong populations of *A. pallipes*, comparable to the dense populations of *P. leniusculus* found in North Yorkshire.

Figure 2. Example of a rocky headwater stream (Cowside Beck) in North Yorkshire. Photo credit: Lawrence Eagle.

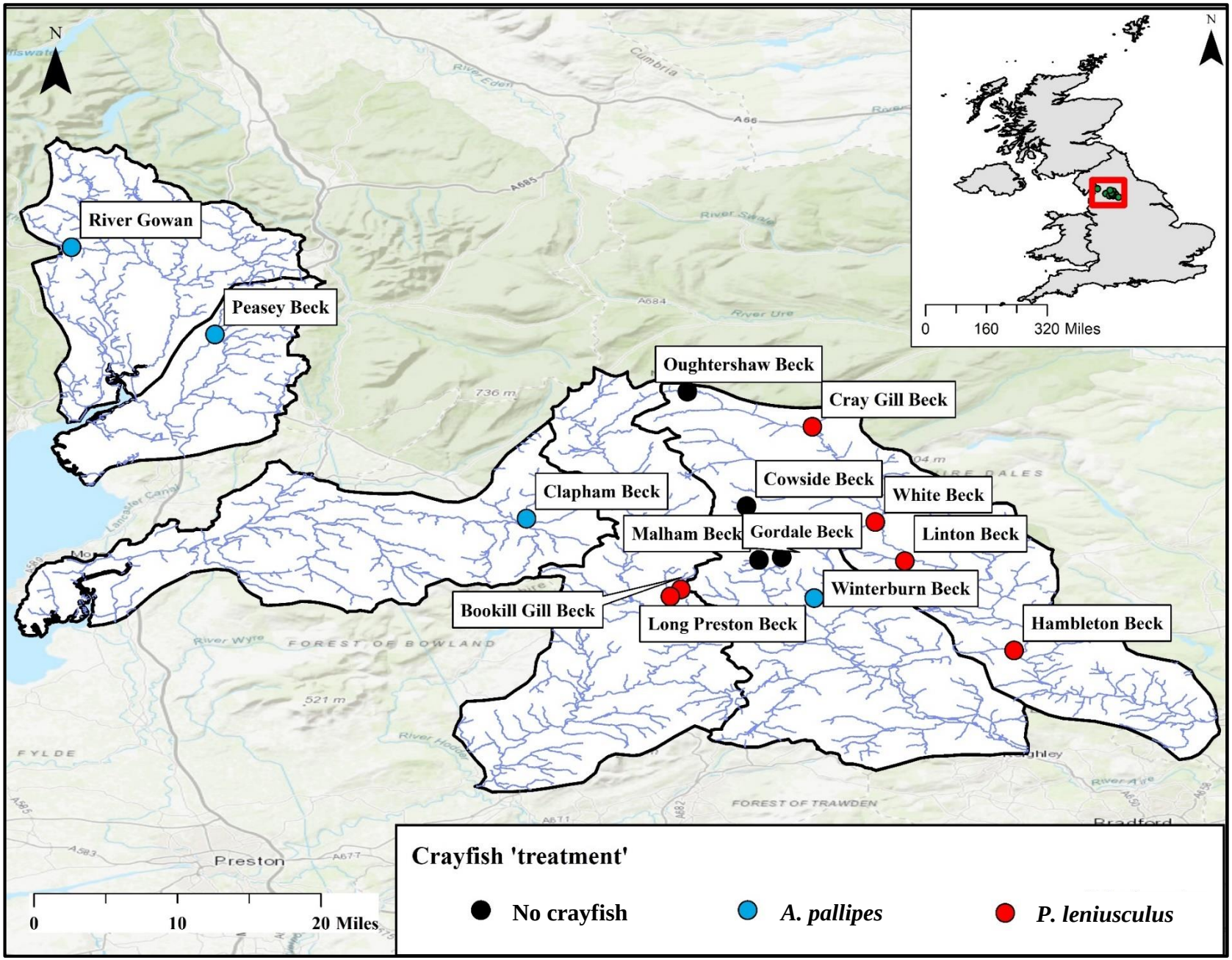


Figure 3 – Catchment maps of all headwater sites in Northern England, from left to right Kent, Bela, Lune, Ribble, Aire, and Wharfe.

Invertebrate analysis

The sampling, identification and analysis of aquatic invertebrate specimens provides an insight into the long term ecological quality and 'health' of a river system. Surber sampling (dimensions 25 cm x 25 cm x 25 cm) of the invertebrate communities was undertaken at 10 replicate locations at each site. Surber sampling is a highly suitable technique to use in headwater streams, as it provides quantitative abundance data unlike the semi-quantitative D-net style kick sampling. Furthermore, surber sampling is a highly effective sampling method in small, shallow (<30 cm) waters with moderate flows (Cummins, 1962). Sampling was randomised using a random number function to generate a number between 0 to 4 inclusive (width) and 0 to 50 inclusive (length), which were paired to form a set of 10 coordinates for each headwater stream. This removed any sampling bias attributed to users selecting preferential habitats. In the rare eventuality of a coordinate pair being in a location that was not possible to sample, sampling was undertaken at nearest adjacent wetted area to the right of the initial sampling location.

Invertebrate samples were live picked and then preserved in industrial grade methylated spirit (IMS) with the ratio of 2 parts IMS to 1 part sample in order to ensure high quality preservation of the sample and identifying features. From this, a dataset comprising 11,001 individual invertebrate specimens was compiled, detailing species (where possible), abundance and length for each individual. Identification of invertebrates was aided by the use of dichotomous keys, and quality assured against the National Biodiversity Network Gateway (https://data.nbn.org.uk/) data, and by an experienced colleague. NBN Gateway, now Atlas, draws upon almost 900 environmental datasets totalling over 113 million species records to provide species distributional data across the UK, as well as providing a useful method for checking and maintaining consistent up-to-date nomenclature. Where invertebrates were not routinely taken to species level

due to the level of expertise required (e.g. for *Chironomidae*, *Physella* sp., *Pisidium* sp.) the most appropriate level of identification was performed (typically family or genus level), with *Chironomidae* taken to tribe.

Total abundance, expressed as number of animals m^{-2}, biomass, expressed as g/m^2 of Ash-Free Dry Weight (AFDW), and measures of species diversity (Simpsons Diversity Index (1 - D), Shannon-Weiner, β-diversity) were calculated through the R package 'Vegan' (Oksanen *et al.*, 2018) for all sites, as common but complementary indices of richness and evenness (see Tuomisto, 2010). β-diversity was calculated from the equation:

$$\beta = \frac{S}{\bar{\alpha} - 1}$$

where $\bar{\alpha}$ is the average richness per one sample, and S is the total number of species from all samples (Tuomisto, 2010). Biomass was calculated for each individual invertebrate specimen by using length-weight regression data available in the literature (see Benke *et al.*, 1999). Power regressions for leeches (Edwards *et al.*, 2009), aquatic beetles (Smock, 1980), larval *Elmid* beetles (Stagliano and Whiles, 2008), *Hydroptila* caddisflies (Baumgärtner and Rothhaupt, 2003), *Physellid* snails (Vaughn *et al.*, 1993), and oligochaetes (Greiner *et al.*, 2010) were used to supplement data where regressions were not available from Benke *et al.* (1999).

Functional groups allow for broader analysis of the functional roles that invertebrates fulfil within stream ecosystems (Vannote *et al.*, 1980), and were used in this study to explore changes to the relative proportions of the functional groups with respect to the presence or absence of crayfish in headwaters. Shredders, such as many Plecoptera species, feed on the Coarse Particulate Organic Matter (CPOM), which in turn allows for Collectors such as larval Dipterans to feed on the created Fine Particulate Organic

Matter (FPOM) created. Grazers and Predators make up the remaining two categories, feeding on algal biofilms on cobble substrates and other aquatic invertebrates, respectively. The relative proportions of functional groups for each site were calculated using abundance data, and compared using the invertebrate feeding guilds as above (following Cummins, 1973, and Tachet *et al.* 2000).

Quality assurance measures were implemented to ensure that collected samples fairly represented each study site. Species accumulation curves are a common and useful tool when assessing local diversity (Colwell and Coddington, 1994), and return cumulative species counts as a function of sampling effort, to determine if a sample is approaching 'completeness' as the curve begins to plateau. The R package 'Vegan' was used to construct species accumulation curves for each site (Oksanen *et al.*, 2018), following Kindt's exact method. One key limitation of species accumulation curves, as discussed by Colwell and Coddington (1994), is the order in which samples are entered into the analysis. To address this, species accumulation curves were run using a randomised load order and averaged over 1000 iterations (Appendix 2). Another key underlying assumption of species accumulation curves, namely that no habitats and thus species were targeted through sampling, was met through randomly generating the surber sample coordinates.

Fish communities

To survey fish populations in each 50 m stream reach, an Electrofishing E-Fish 500W electric fishing backpack system was run on a pulsed direct current, with a duty cycle of 10% at 400W. The voltage was altered between sites in response to changing conductivity, as too high a voltage results in fish mortality, and too low a voltage results in less than optimal fishing efficiency. A 3-sweep depletion method was used over each river reach at each site, to provide abundance data on the fish species present. Depletion

calculations were run in R (3.4.2.), using the 'Carle-Strub method' (Carle and Strub, 1978) function in the Fish Stock Assessment (FSA) package created by Ogle (2018). A data set totalling 2127fish was produced, with data on species, length (1 mm) and weight (0.1 g) for each individual fish. Fork length was recorded for pelagic species, and total length was used for benthic species, due to the minimal fork in these species' tails making for difficult readings. Biomass was additionally calculated, and expressed as g / m^{-2}, using a 2-D area estimate of the survey site where length was the 50 m survey reach, and width was the average width of the survey site.

Water chemistry

Water chemistry can impact the population viability of *A. pallipes* (e.g. Holdich and Rogers, 2000; Haddaway *et al.*, 2015), and can vary across and along catchments. As such, water chemistry variables that relate to crayfish population viability were measured at each headwater site. A Hach Lange HQD outdoor meter and probe was used to determine temperature (^oC), dissolved oxygen (recorded as mg/L), pH and conductivity (µS/cm) at each site, and a Hach Lange field titration kit Model AL-DT was used to determine alkalinity (mg $CaCO_3$). Water samples were collected in acid washed 500 ml polyethylene bottles and filtered in the field, where required, using Whatman GF/F filters (as in Sayer *et al.*, 2010). Soluble reactive phosphorous (SRP) and Nitrate-Nitrogen (NO_3-N) were determined for filtered water samples, following the molybdenum blue procedure (Murphy and Riley, 1962) and procedures detailed in Wetzel and Likens (1991), respectively. Total phosphorous (TP), which measures both dissolved and particulate phosphorous following microwave digestion of the sample, was calculated using unfiltered water samples (Johnes and Heathwaite, 1992). Known standards were included in sample runs to produce calibration curves, to ensure reliability of the results was maintained. The above laboratory techniques were selected

ahead of the more common ‘powder pillow’ techniques due to the greater accuracy of the results, and the increased control the user has over the quality of the reagents.

Physical habitat characteristics

Basic physical channel characteristics were measured in the field, to confirm that the sites were similar and to help explain any variation in the fish and invertebrate community data. Channel wetted width (to nearest 1 cm) was recorded at each site through 10 measurements at 5 m intervals. Flow velocity was measured using a Valeport Electromagnetic Flow Meter (Model 801), producing an average of 30 readings taken once per second. Flow was read at both margins and in the centre of the stream at 10 m intervals. Water depth (to nearest 1 cm) and in-channel substrate type (silt/sand, gravel, cobble) were recorded at 5 m intervals at both margins and in the centre of the stream, with substrate estimated visually to the nearest 5% using a quadrat.

Crayfish

Swedish style “trappie” traps were used to sample the crayfish. Traps were modified with a 5 mm mesh in order to increase capture and retention of sub-35 mm carapace length (CL) specimens. Traps were left submerged in the study sites overnight for 15 hours, baited with fresh mackerel. A total of 10 traps were used per site, with a second separate set of 10 traps being used for *A. pallipes* sites to avoid biosecurity risks. Carapace length (CL, tip of rostrum to posteriomedial edge of the cephalothorax, Vernier callipers, 1 mm), weight (0.1 g), gender, and cheliped condition were recorded for each crayfish caught. Catch-Per-Unit-Effort (CPUE) was used as a relative measure of density, and was calculated as the average number of crayfish per trap. False negatives occurred at Linton Beck (*P. leniusculus*) and Cray Gill Beck (*P. leniusculus*), so all *P. leniusculus* sites were trapped for a second time, with the greatest individual CPUE used to describe relative densities. No crayfish had been removed or destroyed prior to re-trapping, and the highest CPUE was recorded for each site to avoid issues

with re-sampling the same animal twice. ‘No crayfish’ sites were trapped in order to support the pre-assertion of crayfish absence, and additional hand searches (circa. 100 stone turns per site) were conducted to check for false negatives. No crayfish were found during either trapping or hand searches at any ‘no crayfish’ sites. The inclusion of sites containing *A. pallipes* into the experimental design provides an important distinction between the impacts of a native crayfish species and an invasive one, rather than simply the potential difference of including a crayfish or not.

Biosecurity

Biosecurity is an incredibly important aspect of *A. pallipes* conservation, and indeed conservation as a whole. The industry standard for agricultural disinfectants is Virkon S Aquatic, a disinfectant formulation that has been proven to kill crayfish plague at a working concentration of 100 g in 10 L of water, as well as many other viruses and infective agents. In addition, adhering to the Non-Native Species Secretariat ‘Check-Clean-Dry’ campaign (NNSS, 2006) is a key step in ensuring proper biosecurity, as UV rays and desiccation are incredibly effective at killing aquatic invasive species. A full biosecurity protocol was in operation during all field work, which incorporated all of the above stages.

Statistical analysis

Data were tested for normality (Kolmogorov-Smirnov test) and compared using Students t-tests, one-way ANOVAs, Kruskal-Wallis tests and chi-squared (χ^2) tests where appropriate (SPSS 24). Post-hoc analysis of χ^2 tests used adjusted alpha values to account for the increased likelihood of Type-1 errors. Ordinations were conducted in Primer-E (v.6.1.13) to compare the studied headwater sites, using methods outlined in Clarke (1993), following Field *et al.* (1982). A Principal Component Analysis (PCA) was run to compare dissimilarities between all sites, based on data from major suspected influences such as water quality, habitat diversity, and ecological parameters

of fish, crayfish and other invertebrates (e.g. biomass, abundance). A non-metric multidimensional scaling (NMDS) analysis was run, to compare the aquatic macroinvertebrate community composition between sites, using the Bray-Curtis dissimilarity index (Bray and Curtis, 1957). Prior to NMDS ordination analysis, the data were log(X+1) transformed to reduce the impact of rarer taxa. Further analysis of the macroinvertebrate communities was conducted through analysis of similarities (ANOSIM). SIMPER (similarity of percentages) analyses were run (Clarke, 1993) to compare the relative percentage contributions of individual taxa to the similarity both within the crayfish 'treatments' and between them.

Results

Basic physiological site descriptors

Calibration curves for SRP, TP and NO_3-N all had R^2 values of >0.995, and all samples fell well within the limits of the standards. Sites containing either crayfish species showed similar water chemistry to the control sites and to each other (Fig. 4), with low levels of TP, SRP and NO_3-N shown. All nutrient values were within expected ranges of high altitude calcareous rivers, with all sites exhibiting high or good quality (see UKTAG, 2008). All sites had high pH, alkalinity, DO, with comparable conductivity, thus consistent with supporting crayfish (Table 1). All sites were dominated by cobble and gravel substrates, and had average widths between 2-4.5 m (Table 2).

The relative proportions of in-channel substrates, mean depth, mean flow, and mean width were all normally distributed, and did not differ significantly between treatments ($p > 0.05$). TP, NO_3-N, pH and alkalinity data were normally distributed and did not differ significantly between treatments ($p > 0.05$). SRP and conductivity data were non-normally distributed, but also did not differ significantly between treatments ($p > 0.05$).

Crayfish

Trapping confirmed the presence of *P. leniusculus* and *A. pallipes* at all expected sites, and no crayfish were recorded through a combination of trapping and hand searches at the control sites. Basic information on the catches of both *A. pallipes* and *P. leniusculus* are presented in Table 3. In total, 89 crayfish were caught (n = 29 *A. pallipes*, n = 60 *P. leniusculus*), with all captured crayfish being of a typical size for traps (~35 mm CL, e.g. Peay *et al.*, 2009; Almeida *et al.*, 2013). Total catches ranged from a single individual (*A. pallipes*, Clapham Beck) to 25 crayfish (*P. leniusculus*, Bookill Gill Beck) in a single nights trapping (n = 10 traps).

Fish

In total, 2127 fish from 8 species were captured across all sites (Table 4). The most abundant and widespread species caught were bullhead (*Cottus gobio* L.) and brown trout (*Salmo trutta* L.), which were both present at 12 sites, accounting for 69.7% and 24.1% of the total catch from all sites, respectively. Atlantic salmon (*Salmo salar*) were the next most abundant fish species, accounting for 3.2% of the total catch, and were present at 3 sites. All other fish species accounted for less than 1.2% of the total catch, respectively. Both sites containing *A. pallipes* (Peasey Beck) and *P. leniusculus* (Long Preston Beck) were associated with diverse fish communities (n = 6 unique species), however only one site was associated with no fish community, namely Bookill Gill Beck, which supported the highest comparative CPUE of *P. leniusculus* in this study.

Table 1 - Basic water chemistry and temperature for all sites

Treatment and Site		pH	Alkalinity (mg/L $CaCO_3$)	Conductivity (µS/cm)	Dissolved Oxygen (mg/L)	Temperature (ºC)
No crayfish	Malham Beck	8.1	165	371	10.8	10.0
	Gordale Beck	7.6	172	362	10.7	11.4
	Cowside Beck	8.0	145	386	12.4	9.7
	Oughtershaw Beck	7.4	88	217	10.6	14.2
P. leniusculus	White Beck	8.3	171	384	12.7	14.6
	Linton Beck	7.8	159	419	9.9	16.3
	Hambleton Beck	8.0	139	388	11.4	13.9
	Cray Gill Beck	8.1	134	314	10.2	13.2
	Long Preston Beck	8.2	140	301	9.6	14.2
	Bookill Gill Beck	7.5	128	314	10.4	12.5
A. pallipes	Peasey Beck	7.7	70	232	9.9	14.4
	River Gowan	7.0	59	166	9.9	14.7
	Clapham Beck	8.3	162	354	10.4	13.7
	Winterburn Beck	7.8	146	327	9.9	12.8

Table 2 - Physical characteristics for all sites

Treatment and Site		Mean width (m, ±St. Dev.)	Mean depth (cm)	Mean flow (m/s)	In-channel substrate (Silt/Sand, Gravel, Cobble, % cover)		
No crayfish	Malham Beck	3.5 ±1.2	13.4	0.2	0.3	2.7	96.8
	Gordale Beck	3.3 ±0.6	15.6	0.2	1.9	24.9	73.2
	Cowside Beck	3.4 ±0.7	15.3	0.2	2.2	22.8	75.0
	Oughtershaw Beck	2.6 ±0.8	16.2	0.2	2.6	9.9	87.5
P. leniusculus	White Beck	2.7 ±0.4	13.3	0.4	12.7	41.4	45.9
	Linton Beck	3.2 ±0.5	18.4	0.3	7.6	17.3	75.5
	Hambleton Beck	3.0 ±0.3	15.6	0.2	17.3	19.1	63.6
	Cray Gill Beck	3.5 ±01.3	27.5	0.1	2.1	5.0	92.9
	Long Preston Beck	3.3 ±0.5	16.4	0.2	0.9	7.1	92.0
	Bookill Gill Beck	2.1 ±0.5	9.4	0.1	0.8	31.2	68.0
A. pallipes	Peasey Beck	3.6 ±0.4	13.7	0.3	9.9	13.8	76.4
	River Gowan	2.6 ±0.7	13.9	0.4	3.3	38.3	58.3
	Clapham Beck	4.2 ±0.7	15.9	0.2	0.5	29.7	69.8
	Winterburn Beck	4.3 ±0.9	18.3	0.2	0.3	14.4	85.3

Table 3 – Crayfish population information and relative CPUEs per site (CL = carapace length).

Treatment and Site		CPUE	Gender ratio (M:F)	Average CL (mm ±St. Dev.)	Biomass (g)
P. leniusculus	White Beck	0.7	1:6	47.3 ±6.2	214.5
	Linton Beck	1.2	2:10	50.8 ±7.4	441.8
	Hambleton Beck	0.8	1:7	40.4 ±6.0	187.8
	Cray Gill Beck	0.4	4:0	39.2 ±3.4	103.2
	Long Preston Beck	0.4	2:2	38.5 ±3.5	87.2
	Bookill Gill Beck	2.5	15:10	34.5 ±5.1	295.7
A. pallipes	Peasey Beck	0.2	2:0	35.6 ±5.8	28
	River Gowan	2.1	19:2	34.7 ±3.8	275.9
	Clapham Beck	0.1	1:0	33.3	15.3
	Winterburn Beck	0.5	4:1	40.3 ±1.4	113.5

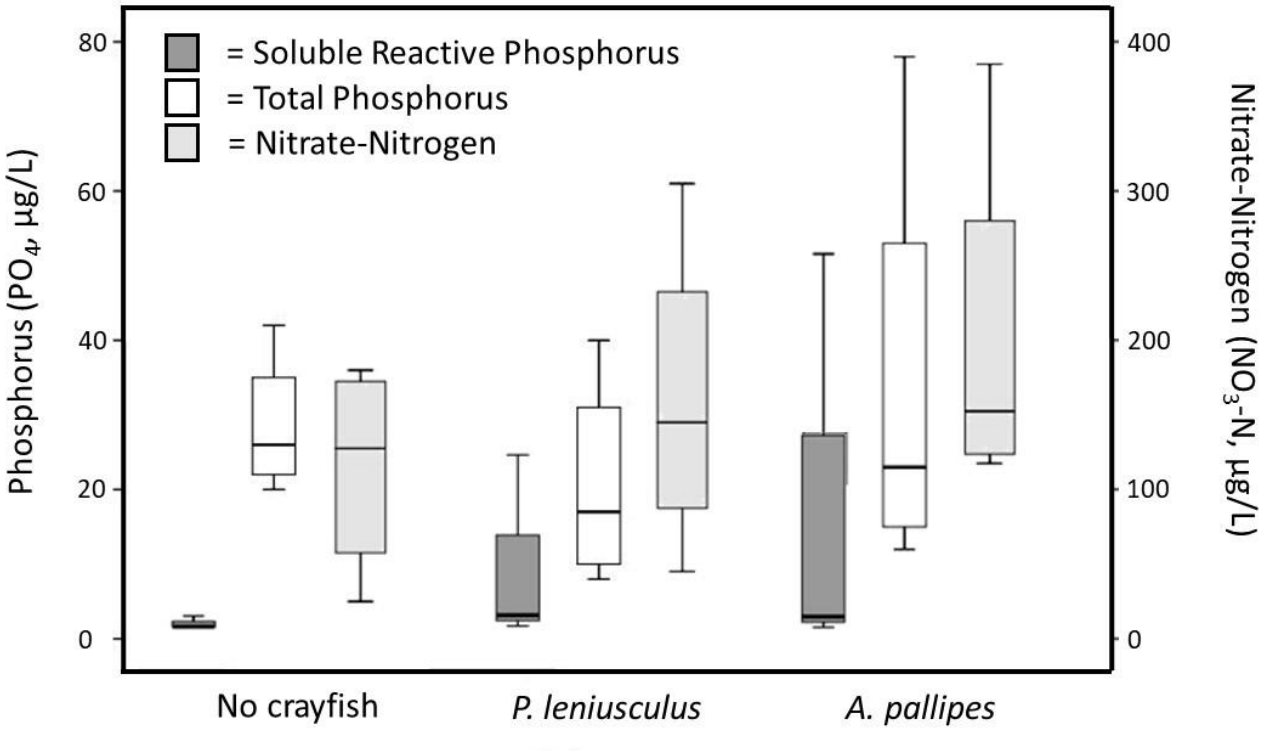


Figure 4 - Soluble reactive phosphorous, total phosphorous and Nitrate-Nitrogen concentrations for all sites with standards

Table 4 – Fish population data by site (3-sweep depletion).		**Bullhead**	**Brown trout**	**Atlantic Salmon**	**Minnow**	**Stone Loach**	**Eel**	**Lamprey sp.**	**3-spined Stickleback**	**Total count *(site)***	**Total species**
Treatment	**Site**	*Cottus gobio (BH)*	*Salmo trutta (BT)*	*Salmo salar (S)*	*Phoxinus phoxinus (M)*	*Barbatula barbatula (SL)*	*Anguilla anguilla (E)*	*Lampetra* sp. *(L)*	*Gasterosteus aculeatus (SB)*		
No crayfish	Malham Beck	300	3	0	0	0	0	0	0	303	2
	Gordale Beck	229	0	0	0	0	0	0	0	229	1
	Cowside Beck	0	14	0	0	0	0	0	0	14	1
	Oughtershaw Beck	55	5	0	0	0	0	0	0	60	2
P. leniusculus	White Beck	29	43	0	0	0	0	0	0	72	2
	Linton Beck	35	33	0	0	0	0	4	0	72	3
	Hambleton Beck	24	22	0	21	11	0	0	3	81	5
	Bookill Gill Beck	0	0	0	0	0	0	0	0	0	0
	Cray Gill Beck	64	31	0	0	1	0	0	0	96	3
	Long Preston Beck	284	29	13	4	4	1	0	0	335	6
A. pallipes	Clapham Beck	203	138	43	0	4	3	0	0	391	5
	Winterburn Beck	115	24	0	0	0	0	0	0	139	2
	Peasey Beck	127	36	11	0	0	6	3	1	184	6
	River Gowan	17	134	0	0	0	0	0	0	151	2
Total count (*species*)		1482	512	67	25	20	10	7	4	**2127**	**8**

Invertebrates

A total of 11,001 individual aquatic invertebrates (other than crayfish) were identified, spanning a total of 69 taxa. Intra- and inter-site invertebrate abundances from the surber sampling varied greatly (Table 5). However, there were no significant differences in the species/taxa richness ($p > 0.05$) or invertebrate abundance ($p > 0.05$) between the different crayfish treatments. Diversity data were normally distributed (Kolmogorov-Smirnov test, $p > 0.05$), and values did not differ significantly between no crayfish, *A. pallipes* and *P. leniusculus* sites for Shannon-Weiner ($F = 0.247$, $df = 2$, $p > 0.05$), Simpsons Diversity ($F = 0.550$, $df = 2$, $p > 0.05$), and β-diversity ($F = 1.740$, $df = 2$, $p > 0.05$; Fig. 6-8). However, one site in the *P. leniusculus* treatment, namely Bookill Gill Beck, was notably reduced in both invertebrate taxa richness ($n = 12$) and abundance ($n = 318$ m^{-2}) as compared to all other sites. Separate biomass estimates for crayfish, fish and invertebrates for each site differed significantly ($\chi^2 = 16.760$, $df = 2$, $p = 0.002$). Following post-hoc tests (adjusted $\alpha = 0.0056$), there was significantly more crayfish biomass and significantly less fish biomass at *P. leniusculus* sites, and significantly more fish biomass at *A. pallipes* sites. All other values did not differ significantly between the treatments. Functional feeding group proportions were significantly different between sites ($\chi^2 = 566.534$, $df = 6$, $p < 0.001$). Post hoc analysis (adjusted $\alpha = 0.0042$) revealed that *A. pallipes* sites had significantly more collectors than no crayfish and *P. leniusculus* sites, while *P. leniusculus* sites had significantly less shredders than no crayfish and *A. pallipes* sites (Fig. 5). *A. pallipes* sites had significantly less scrapers than both no crayfish and *P. leniusculus* sites. Finally, no crayfish sites had significantly less predators than both *A. pallipes* and *P. leniusculus* sites.

Table 5. Summary table of invertebrate community composition and diversity by site

Treatment	Site	Species/taxa richness	Invertebrate abundance *(n/m^2)*	Invertebrate biomass *(g/m^2, AFDW)*	Shannon-Weiner	Simpson Diversity	β-diversity
No crayfish	Malham Beck	32	998	1.936	1.972	0.789	1.576
	Gordale Beck	38	1717	2.674	1.930	0.740	1.485
	Cowside Beck	29	1765	2.196	1.846	0.772	1.411
	Oughtershaw Beck	34	702	0.761	2.060	0.838	2.136
P. leniusculus	White Beck	33	1738	2.185	1.929	0.766	1.147
	Linton Beck	37	1038	1.188	2.147	0.822	1.789
	Hambleton Beck	29	677	0.873	2.256	0.853	1.077
	Bookill Gill Beck	12	318	0.154	1.481	0.728	1.241
	Cray Gill Beck	38	2571	1.47	1.972	0.758	1.5
	Long Preston Beck	31	2363	2.225	2.383	0.866	0.875
A. pallipes	Clapham Beck	29	936	0.744	2.260	0.870	1.230
	Winterburn Beck	32	1912	0.973	2.030	0.810	1.41
	Peasey Beck	31	526	0.923	1.907	0.777	1.832
	River Gowan	26	690	1.552	2.062	0.821	1.25

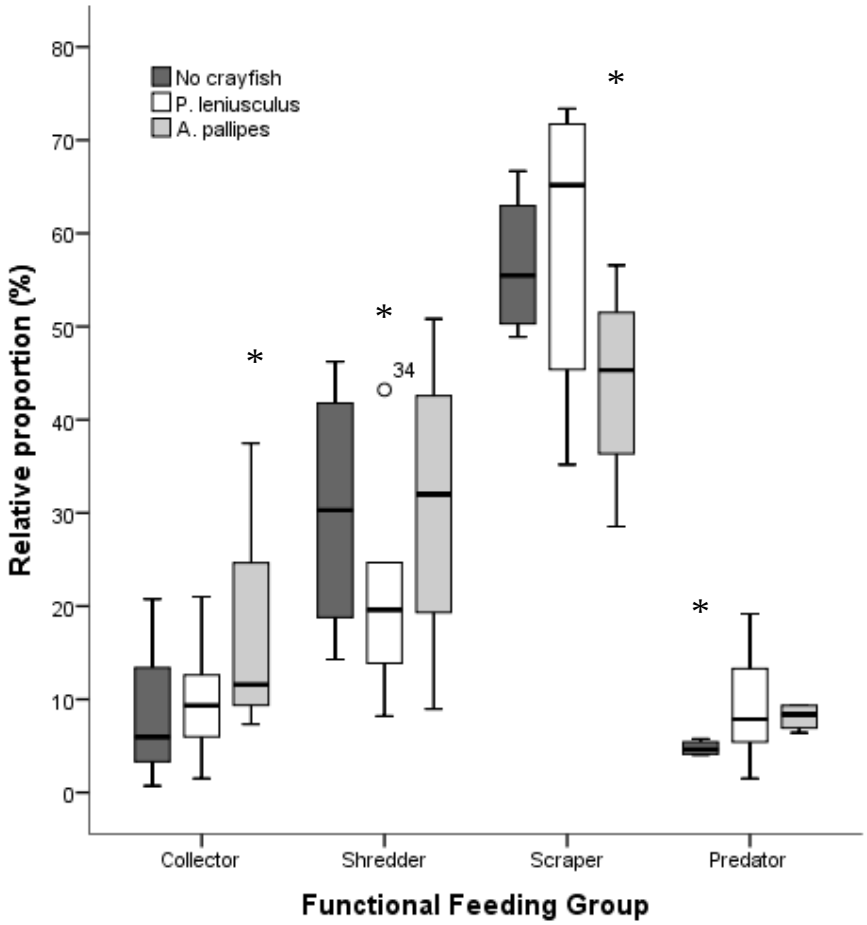


Figure 5 – The relative proportions by count of functional feeding group assemblages at each site. * denotes significant departure from the remaining two groups at p = 0.05 level.

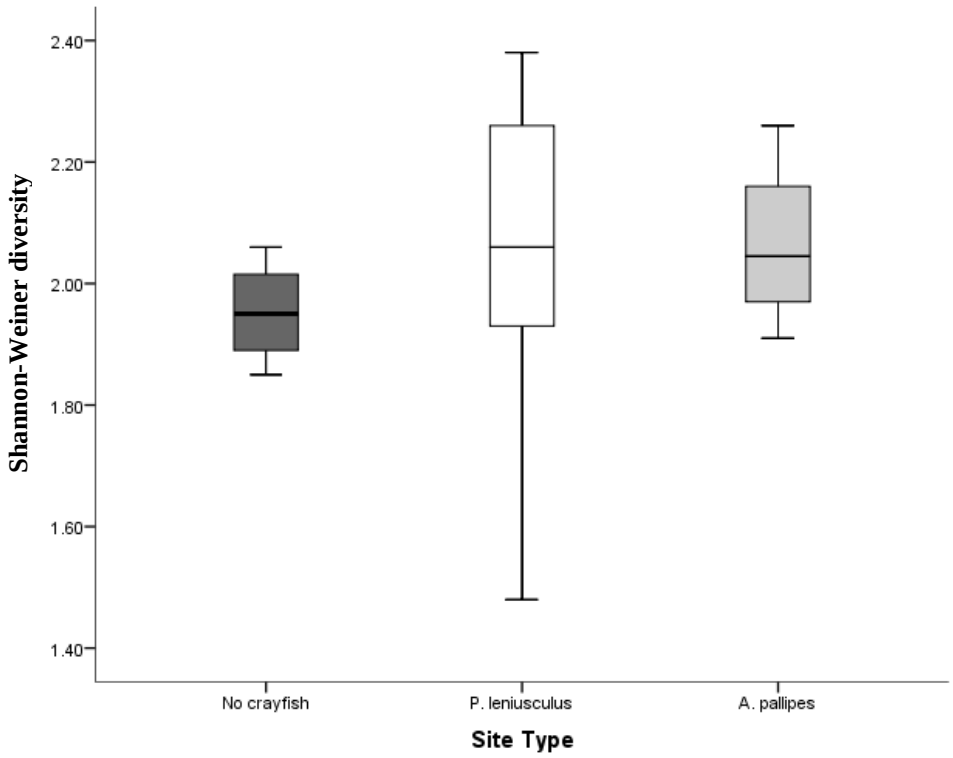


Figure 6 – Shannon-Weiner diversity of the invertebrate communities for no crayfish, *P. leniusculus* and *A. pallipes* sites

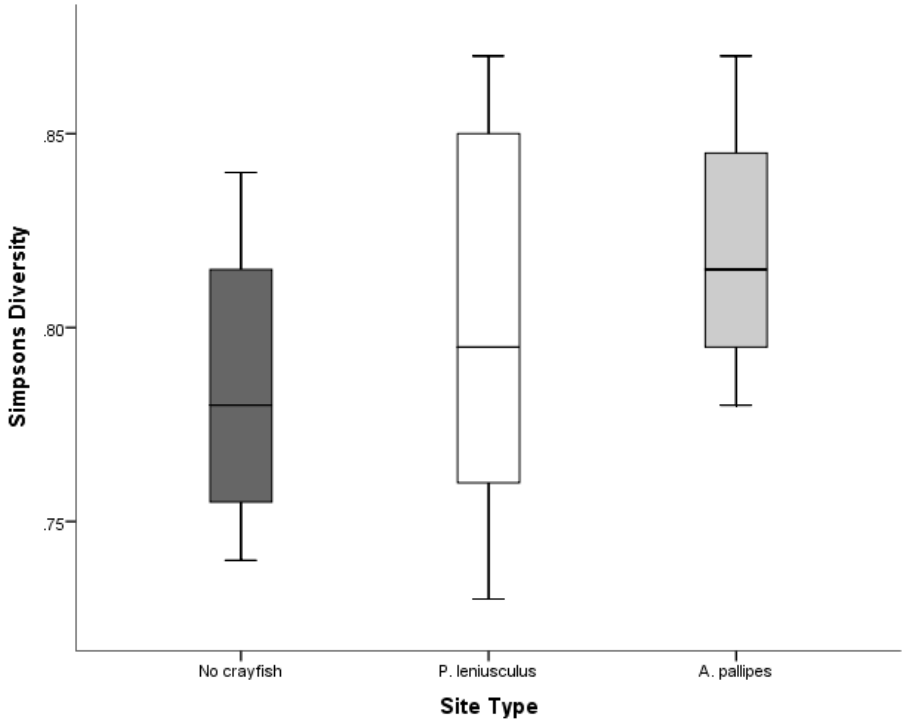


Figure 7 - Simpsons Index of Diversity of the invertebrate communities for no crayfish, *P. leniusculus*, and *A. pallipes* sites

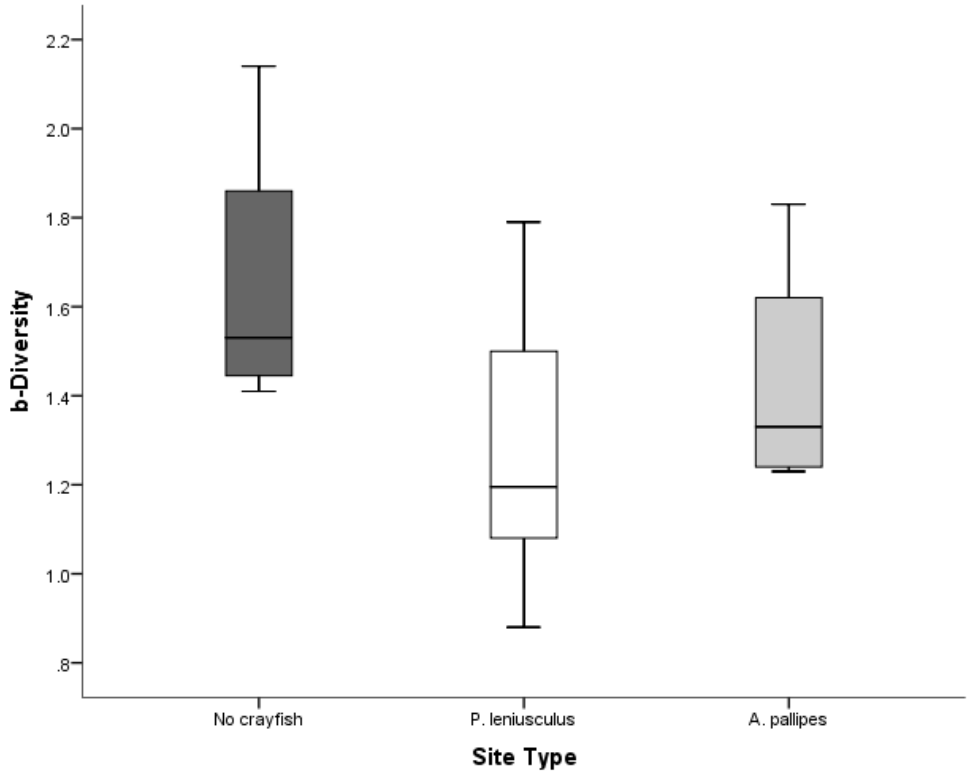


Figure 8 – β-diversity of surber samples for no crayfish, *P. leniusculus*, and *A. pallipes* sites

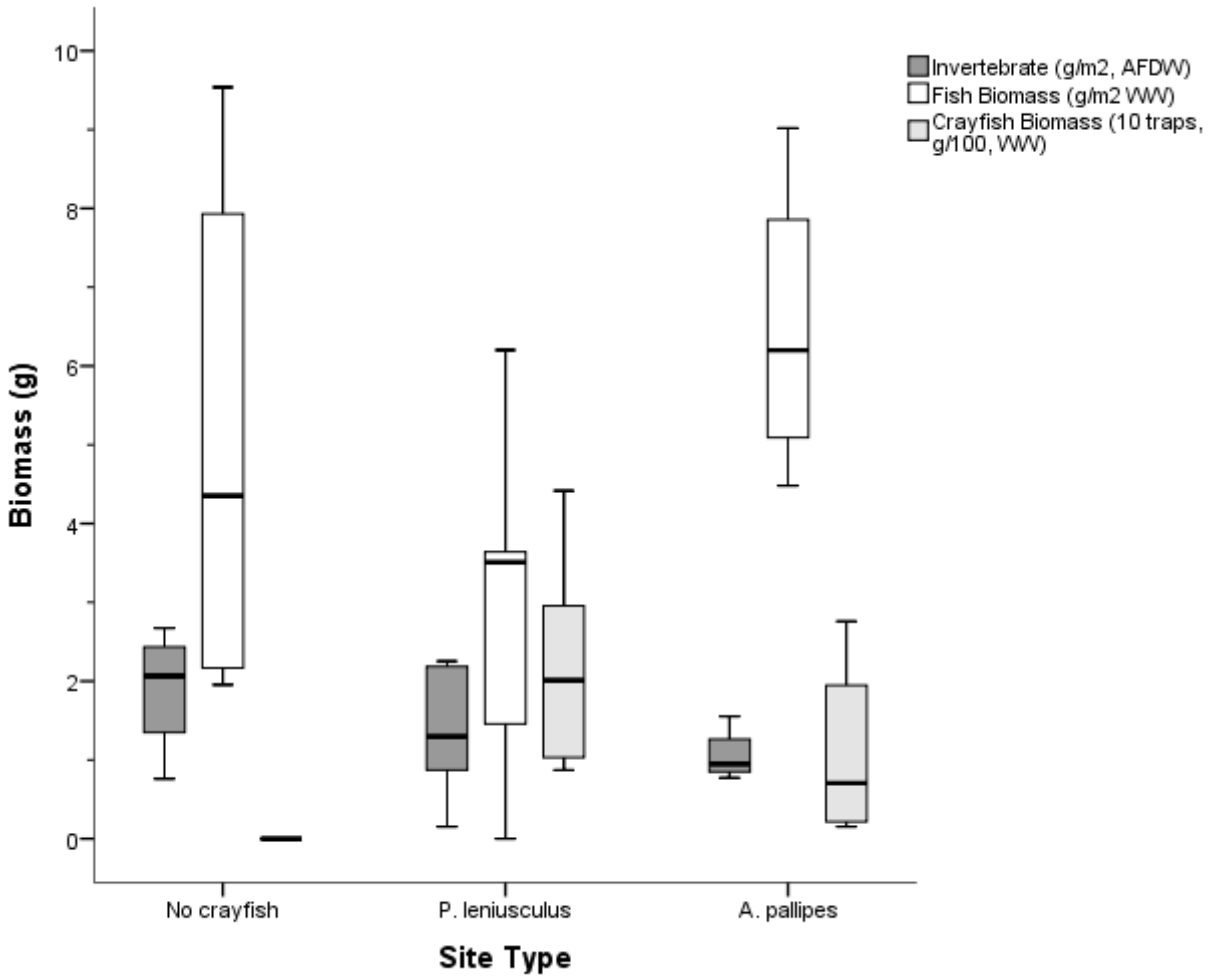


Figure 9 – Biomass estimates for no crayfish, *P. leniusculus* and *A. pallipes* sites. For the purpose of increased clarity of this figure, crayfish biomass (g wet weight) was divided by 100 to scale it to fish (g wet weight m^{-2}) and invertebrate biomass (AFDW m^{-2}).

The patterns in the grouping of environmental variables between sites were explored through PCA (Fig. 10). Z scores were used to standardise the data, and many variables were similarly weighted and showed weak scores, due to the high levels of correlation between biological and environmental variables. As such a threshold was set of ±0.05 by which to remove variables from the PCA that did not contribute strongly to the explanatory power of the model. Following stepwise regression, a model was produced that explained 91.5% of the variance between sites with PCA axes 1 and 2 (Table 6). Macroinvertebrate biomass and fish abundance were positively loaded on PCA axis 1, and crayfish biomass, macroinvertebrate abundance, conductivity, alkalinity, and relative percentages of cobble and invertebrate scrapers, respectively, all loaded

negatively (Table 7). Macroinvertebrate abundance was strongly negatively loaded on PCA axis 2, whilst all other variables were positively loaded. The sites containing both no crayfish and populations of *A. pallipes* scored relatively similarly to each other, both within and between treatments (Fig. 10). Sites containing populations of *P. leniusculus*, however, were much more variable, showing close associations with both no crayfish sites (White and Long Preston) and *A. pallipes* sites (Hambleton, Linton and Cray Gill). One site, Bookill Gill Beck, was a clear outlier, scoring highly negatively for both invertebrate biomass and abundance, but positively for crayfish biomass.

Table 6 – Results from the PCA and stepwise regression

Axis	PCA		
	Eigenvalue	**PCA Variance (%)**	**Cum. Variance (%)**
1	151	52.3	52.3
2	113	39.2	91.5
3	17.9	6.2	97.7
4	4.83	1.7	99.4
5	1.01	0.3	99.8

Table 7 – Eigenvectors for variables that, following stepwise regression, were strongly associated with dissimilarities between sites.

Variable	***Eigenvectors***	
	PCA1	***PCA2***
Macroinvertebrate biomass (mg/m^{-2} AFDW)	0.901	0.035
Crayfish biomass (g)	-0.271	0.341
Conductivity (µS/cm)	-0.259	0.242
Macroinvertebrate abundance (n/m^{-2})	-0.154	-0.900
Alkalinity (mg/L $CaCO_3$)	-0.110	0.079
Fish abundance (n)	0.064	0.010
Relative % scraper	-0.059	0.057
Relative % cobble	-0.051	0.043

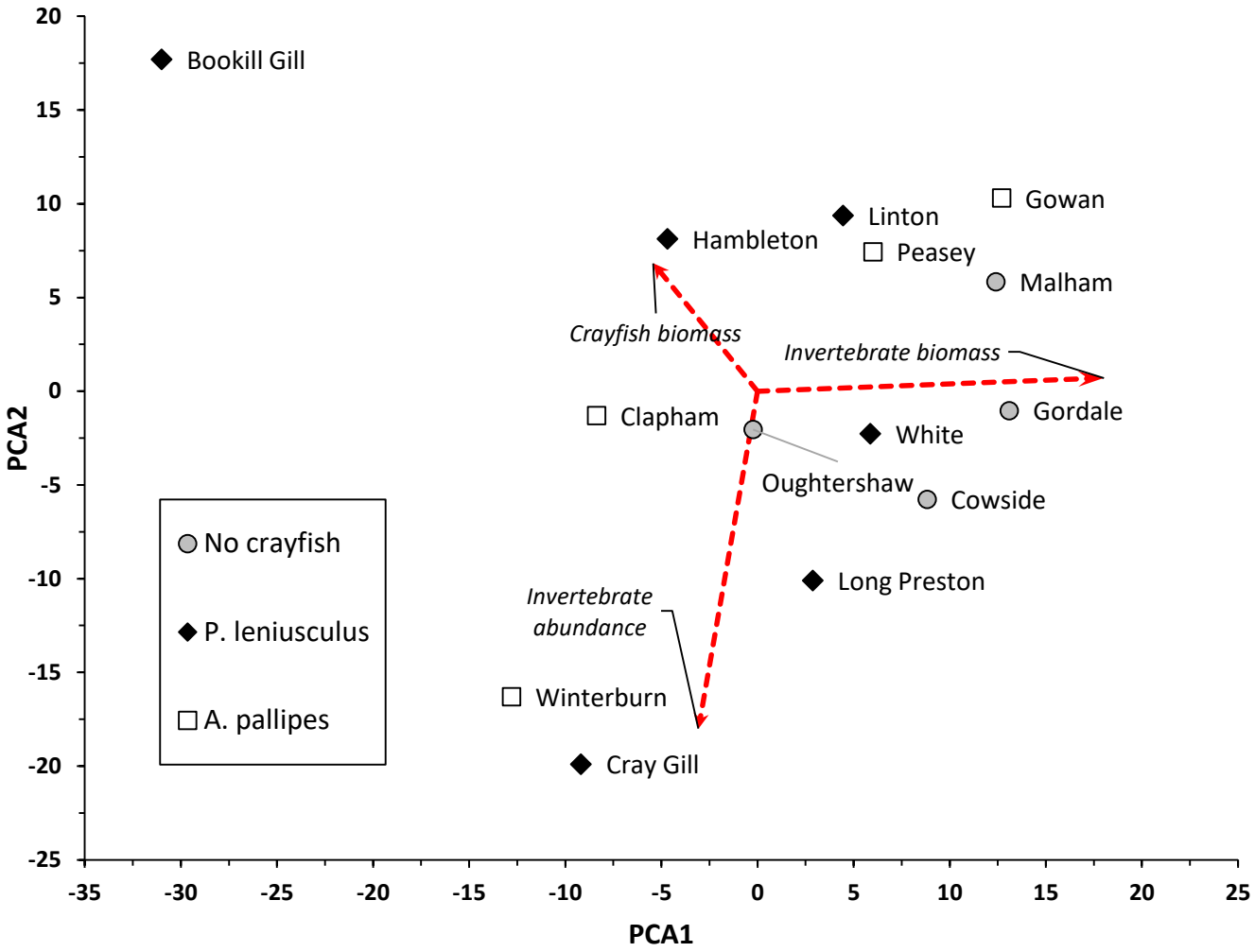


Figure 10 – PCA plot showing grouping of sites based on populations of *P. leniusculus*, *A. pallipes*, or no crayfish respectively. Variables that significantly contribute to explaining variance are plotted (italicised), along with their direction.

Patterns in invertebrate community structure were explored through a non-metric multidimensional scaling (NMDS) analysis, which showed there to be grouping of sites by invertebrate community (Fig. 11; 2D stress = 0.09). ANOSIM analysis revealed that sites containing no crayfish, and sites containing populations of *A. pallipes*, were both strongly grouped and dissimilar to one another (Pairwise R = 0.813, p = 0.029). The variation in *P. leniusculus* sites was much greater than in either of the other two treatments. Despite greater spread amongst the *P. leniusculus* sites, however, a strong separation was shown between Bookill Gill Beck and all other sites on NMDS axis 1, and between Long Preston Beck, and to a lesser extent Cray Gill Beck, and all other sites on NMDS axis 2. SIMPER analysis revealed that no crayfish sites had an average

aquatic invertebrate community similarity of 60.8%, with 4 dominant taxa contributing to almost 40% of this, namely *Baetis rhodani* (11%), *Serratella ignita* (10.6%), *Gammarus pulex* (9.1%) and *Leuctra inermis* (7.8%). The invertebrate communities present at *A. pallipes* sites had an average similarity of 63.3%, of which the primary contributions were by *Serratella ignita* (10.9%), *Leuctra hippopus* (9%), and *Baetis rhodani* (7%). Invertebrate communities differed to a greater extent for the *P. leniusculus* sites than for both the no crayfish and *A. pallipes* sites, showing only 43.1% similarity, with primary contributions from *Baetis rhodani* (12.1%), *Leuctra hippopus* (8.8%) and *Serratella ignita* (6%). Differences in the invertebrate community structure between the no crayfish and *A. pallipes* sites (50.1%), no crayfish and *P. leniusculus* sites (53.7%), and *A. pallipes* and *P. leniusculus* sites (48.3%) were small, with all taxa contributing less than 5.2% each to the cumulative difference. Headwater streams that were more separated from the central cluster were associated with proportionally rarer invertebrate taxa, such as *Radix peregra* at Long Preston Beck, and *Athripsodes cinereus* at Hambleton Beck. The absence of the core community species *Serratella ignita* at Bookill Gill Beck and Long Preston Beck is likely to have contributed to the strong separation of these sites from all other headwater streams in this study. However, where Long Preston Beck supported a diverse invertebrate community despite the absence of *Serratella ignita*, Bookill Gill Beck had a strongly reduced community in terms of abundance and diversity. The invertebrate community at Bookill Gill Beck was likely further separated due to reduced numbers of other common taxa such as *Gammarus pulex*, and an association with larger numbers of relatively rarer taxa, such as *Habrophlebia fusca*.

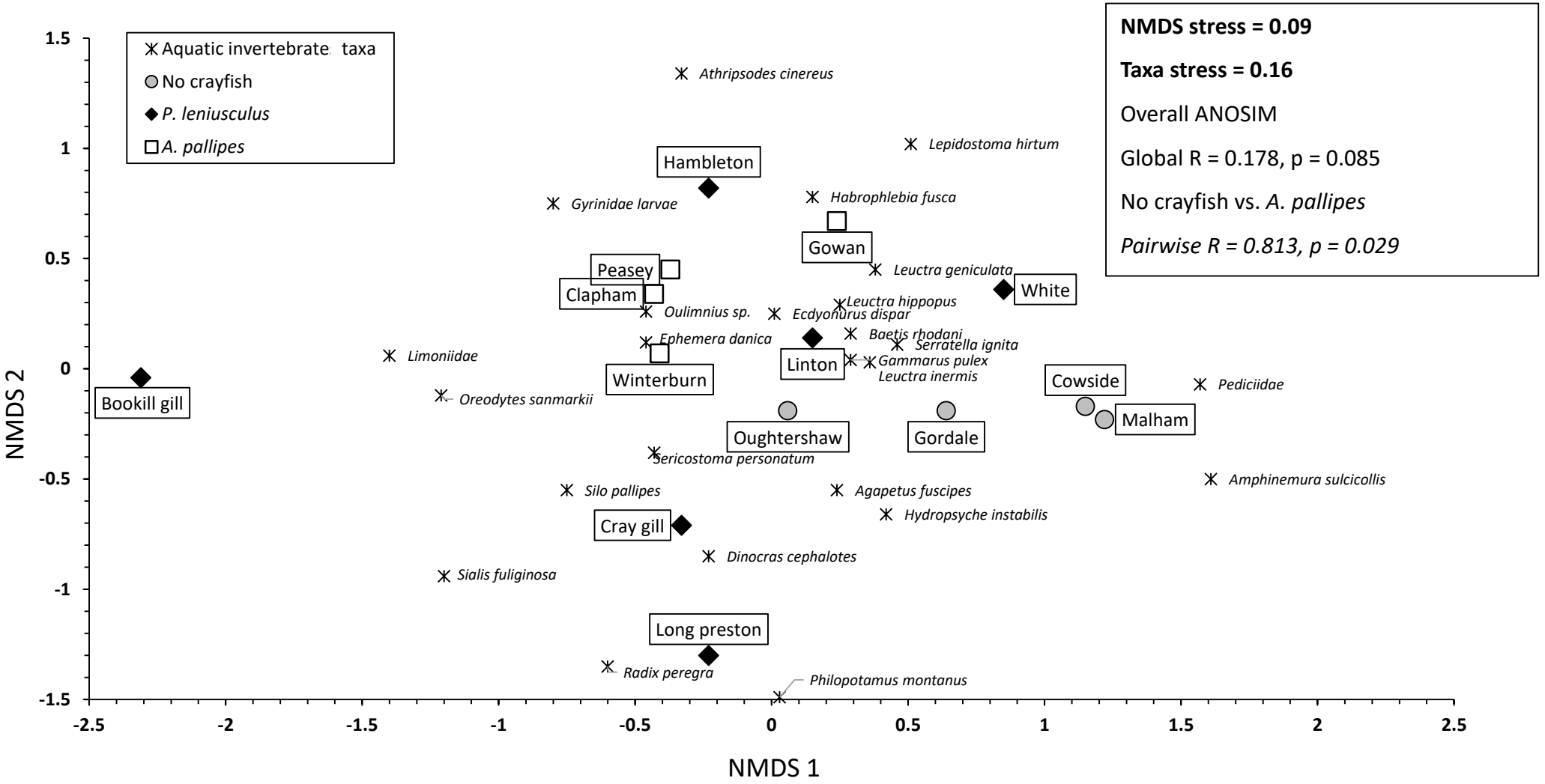


Figure 11 – NMDS of no crayfish, *A. pallipes*, and *P. leniusculus* sites (by name) distributed by patterns in their aquatic invertebrate community structure. Only common and influential taxa are plotted.

Discussion

The fact that some of the studied headwater streams have retained strong populations of *A. pallipes* indicates that these habitats are highly suitable for *A. pallipes*, which would have been present at many more sites were it not for the spread of *P. leniusculus* and crayfish plague throughout England (Bubb *et al.*, 2008). Headwaters can therefore provide invaluable in-catchment *A. pallipes* refuges, as well as affording important stocks from which to repopulate downstream areas. This pattern of populations of native crayfish becoming restricted to headwaters has been seen before (e.g. Gil-Sánchez and Alba-Tercedor, 2002; Almeida *et al.*, 2014), and further strengthens the argument that headwater habitats should be prioritised for the conservation of *A. pallipes* in England.

Fish community structure in the presence of differing crayfish species

Headwaters containing populations of *P. leniusculus* were associated with significantly lower fish biomass than headwaters containing either no crayfish or populations of *A. pallipes*, whilst headwaters sites containing *A. pallipes* were associated with significantly higher fish biomass than both *P. leniusculus* and no crayfish sites. Of interest in this respect are the sites Bookill Gill and Long Preston Beck, which are hydrologically joined by a downstream confluence (approximately 1.8 km downstream of the Bookill Gill Beck study site, and 150 m downstream of the Long Preston Beck study site), despite having highly differing fish communities. While Long Preston Beck (LPB) supported the second highest fish abundance and joint highest fish diversity, the neighbouring Bookill Gill Beck (BGB) had no fish present at the time of sampling (2015). BGB historically had strong, diverse and healthy fish communities (Peay *et al.*, 2009). More recent surveys (Pritchard, 2016) suggest a gradient of fish abundance along BGB, whereby very few fish are supported in the upper reaches, with fish returning downstream towards the confluence with LPB. Why then, given the great abundance and diversity of fish species in the neighbouring LPB, has BGB lost its fish community

at the studied site? While Catch-Per-Unit-Effort cannot be relied on for exact quantitative density estimates (addressed in detail in Chapter 4), comparative densities can be used to explore if numbers of invasive *P. leniusculus* are driving losses of fish at BGB. The CPUE for BGB was the highest of any site, at 2.5 crayfish per trap. *P. leniusculus* have been shown to directly predate benthic and juvenile fish (Guan and Wiles, 1997), as well as fish eggs (Findlay *et al.*, 2015; Karjalainen *et al.*, 2015). Furthermore, *P. leniusculus* can have a wide range of indirect impacts on fish communities, through competition for shelters and prey (Reynolds, 2011). One indirect impact of *P. leniusculus*, which is particularly pertinent to headwaters is the bioturbation effect of *P. leniusculus* (Harvey *et al.*, 2011), whereby fine sediments from the bank enter the channel through crayfish burrowing, and are re-suspended through foraging behaviours, reducing oxygenation of the hyporheic zone. Additionally, *P. leniusculus* can also reduce the natural consolidation and structuring of gravel substrates through walking and foraging behaviours (Johnson *et al.*, 2010), decreasing the retention of gravel substrates in upland streams. The geomorphological restructuring of sediment and substrates in headwater habitats has serious implications for the spawning habitat quality, and thus recruitment potential for salmonids. It is likely that the high density of *P. leniusculus* present in BGB was a key driver of the localised loss of fish communities.

Given the shared ecological traits of both *P. leniusculus* and *A. pallipes*, the highest 'density' *A. pallipes* population, namely the River Gowan, was also examined with respect to the impacts of high density crayfish populations on the ecological community. Whilst *A. pallipes* has been shown as capable of predating fish, fish are unlikely to comprise a significant dietary component (Gherardi *et al.*, 2004). This is supported through the River Gowan site having the highest CPUE of *A. pallipes* in this study (2.1), whilst also supporting an abundant fish community (Table 4). Additionally,

fish species such as brown trout and bullhead have been shown to predate *A. pallipes* (Foster and Slater, 1990, in Robinson *et al.*, 2000), suggesting that *A. pallipes* may in fact promote fish biomass through providing an important food source. Thus, data support the observations of this study, in that populations of *A. pallipes* and fish can co-exist in headwater streams without negatively impacting one another, and frequently do so. Conversely, populations of *P. leniusculus* do not have predictable associations with fish populations in headwaters, showing a greater variability in the amount of fish biomass present. In the most extreme of cases at BGB, *P. leniusculus* are associated with the localised extinction of resident fish populations. Further studies, therefore, are needed on the temporal aspect of fish community structure following invasion by *P. leniusculus*, to provide more direct empirical evidence of both direct predation and indirect suppression of fish populations by *P. leniusculus*. The use of stable isotope analysis and gut content analysis could further support these conclusions.

Invertebrate community structure in the presence of differing crayfish species

No significant differences were found in the abundance, biomass or species/taxa richness for aquatic invertebrates between the different crayfish treatments. Additionally, no significant differences were found for diversity indices (Simpson and Shannon-Weiner), and β-diversity. However, the relative proportions of the functional feeding groups varied significantly between treatments. The lack of a substantial difference between aquatic invertebrate community abundance and diversity in the studied headwaters was surprising, given the attention that the relatively severe negative impacts of *P. leniusculus* has received (e.g. Ibbotson and Furse, 1995; Crawford *et al.*, 2006; Holdich *et al.*, 2014; Mathers *et al.*, 2016). However, there is evidence within the literature that the impacts of invasive crayfish can be restricted to the equivalent native crayfish species, with a like-for-like functional replacement occurring (Usio *et al.*, 2006; Ercoli *et al.*, 2014), with only minimal impact on the broader ecological community. In

a comparative study of *P. leniusculus* and the endemic Japanese crayfish (*Cambaroides japonicas* De Haan), Usio *et al.* (2006) argued that both species fulfilled a similar functional role, providing evidence of a similar rate of processing and turnover of organic detritus, and comparable impacts on a local *Gammarid* shrimp species' abundance. In a study exploring the functional redundancy between a European native crayfish species (noble crayfish, *Astacus astacus* L.) and three invasive American crayfish species, Dunoyer *et al.* (2013) found varying degrees of functional overlap between species. The native *A. astacus* and the invasive spiny cheek crayfish (*Orconectes limosus*, Rafinesque) did not increase litter breakdown rates, however the presence of both *P. leniusculus* and red swamp crayfish (*Procambarus clarkii* Girard) resulted in elevated breakdown rates. Dunoyer *et al.* (2013) therefore stressed the importance of acknowledging potential species specific impacts, and not generalising between invasive crayfish species. This final point is salient, as despite *P. leniusculus* being the most widespread of the invasive American crayfish in England, there are established populations of *P. clarkii*, *O. limosus*, the virile crayfish *Orconectes virilis* Hagen. In addition, in terms of broader impacts, it is important to recognise that all of these invasive American crayfish species carry crayfish plague and pose a threat to native *A. pallipes* populations.

Changes in proportion of the invertebrate community functional feeding groups

The increase in the proportion of collectors at *A. pallipes* sites is likely due to the important functional role played by *A. pallipes*. *A. pallipes* are large-bodied, omnivorous invertebrates, capable of consuming large amounts of organic detritus such as leaf litter, and as such can proportionally dominate the shredder biomass within a system. In this study, collector abundance increased at *A. pallipes* sites, likely as a direct result of large amounts of FPOM generated from the shredding of CPOM by *A. pallipes*. That populations of *A. pallipes* were associated with reduced proportions of

scrapers is surprising, as there is evidence to suggest that *A. pallipes* readily co-inhabit with and may even be associated with an increased diversity in *Ephemeroptera* spp. (Trouilhé *et al.*, 2012), the dominant scrapers in this study. One theory could be that *A. pallipes* preferentially feed on these scrapers. Invertebrate tissues are an important component of the diet of *A. pallipes* (e.g. Scalici and Gibertini, 2007), however scrapers still comprised almost 50% of the invertebrate community by count in *A. pallipes* sites, and as such were not under significant predation pressure from *A. pallipes*. A reduction in algal biofilms may also cause a decrease in scrapers, and could be due to sedimentation or direct consumption by *A. pallipes*. However, an increase in sedimentation is unlikely too, as *A. pallipes* do not tolerate siltation and low water quality (Haddaway *et al.*, 2015), and rarely burrow in rocky streams. It would appear that the reduction in relative scraper abundance in *A. pallipes* headwater sites is not controlled by a crayfish related top-down process, which are suggested to be over-emphasised in modern literature regarding trophic releases and species assemblages (Woodward *et al.*, 2008). However, salmonids such as brown trout and Atlantic salmon are known to heavily predate the two most common scrapers families in this study, *Baetidae* and *Ephemerellidae* (e.g. Grey, 2001), and as such this may be a reflection of the significantly larger fish biomass present at headwater sites containing *A. pallipes. P. leniusculus* headwater communities were associated with proportionately less shredders and collectors, but supported an increased proportional abundance of scrapers. *P. leniusculus* has been shown to consume large numbers of aquatic macroinvertebrates (e.g. Ercoli *et al.*, 2015; Mathers *et al.*, 2016), to be a significant bioturbator (Harvey *et al.*, 2011), and the species is known to consume large amounts of organic detritus (Guan and Wiles, 1998). Therefore, *P. leniusculus* can impact aquatic macroinvertebrate communities both directly and indirectly, and at more than one trophic level. Through the selective feeding of *P. leniusculus* on large bodied shredders, such as Trichoptera

and Plecoptera species, a corresponding decrease in both shredder and collector proportional abundance would be seen. Since *P. leniusculus* can fulfil the functional role of a shredder in the macroinvertebrate community, organic detritus would still be consumed at a system level and converted from CPOM to FPOM. However, if populations of *P. leniusculus* were also exerting a significant predation pressure on collectors, this increase in FPOM would not result in a corresponding proportional increase in collector abundance. As such, the system would begin to become less supported by allochthonous carbon, and instead rely more heavily on autochthonous sources of carbon. This shift would be seen in an increase in scrapers, feeding on the increasingly dominant algal biofilms, and potentially represents a trophic cascade, due to a crayfish-mediated shift from one state to another (Pace *et al.*, 1999).

Different biological communities of individual headwater sites

Sites associated with *A. pallipes* were consistently associated with a distinct invertebrate community, categorised by a greater dominance of collectors and comparatively fewer scrapers than the other headwater sites. However, there were no significant losses or gains in invertebrate abundance or biomass between the two treatments. Headwater sites containing populations of *P. leniusculus* showed very little grouping, being associated with *A. pallipes* sites (e.g. Hambleton Beck), sites containing no crayfish (e.g. Linton Beck), or being distinct to either treatment (e.g. Bookill Gill Beck and Long Preston Beck). As supported through the PCA, the NMDS analysis showed the most distinct differences in invertebrate communities to be between the Bookill Gill Beck (BGB) and Long Preston Beck (LPB) headwater sites (Fig. 11). LPB had the second greatest abundance and greatest biomass of aquatic invertebrates out of all headwater sites in this study, and BGB supported the lowest abundance, biomass and diversity of aquatic invertebrates, despite the total absence of fish species, and thus potential invertebrate predators, at the study site. The strong positive association with *P. leniusculus* biomass

shown by BGB suggests a link between the largest reported CPUE in this study (2.5 at BGB), and the collapse of a localised native ecosystem. The second largest CPUE for *P. leniusculus* reported in this study was 1.2 at Linton Beck, under half that of BGB, with Linton Beck supporting a diverse and healthy invertebrate community (Table 5). These data suggest that a density dependent effect of the BGB population of *P. leniusculus* may have driven localised biodiversity loss. However, an abundant invertebrate and fish community was supported at the River Gowan site, the only headwater stream in this study to contain *A. pallipes* populations of comparable CPUE (2.1). One potential explanation of this is that despite both *A. pallipes* and *P. leniusculus* being large-bodied potential predators, the feeding efficiency and voracity can differ between native and invasive crayfish species. For example, in their study of *A. pallipes* and *P. clarkii* feeding behaviour on European anuran larvae (tadpoles), Gherardi *et al.* (2001) found that, whilst both species were capable of predating the tadpoles, the invasive crayfish *P. clarkii* had a quicker predatory response. Likewise, Nyström *et al.* (1999) reported that whilst both European native crayfish *A. astacus* and invasive *P. leniusculus* were able to impact macrophyte and macroinvertebrate biomass within their mesocosm study, *P. leniusculus* were associated with a greater reduction in biomass than *A. astacus*. Both biotic and abiotic factors, such as how biodiverse a native community is and the extent of habitat modification, can dictate how vulnerable a system is to invasion (Marufu *et al.*, 2018). Since invasive crayfish species including *P. leniusculus* are capable of modifying both the biotic and abiotic components of their habitats, headwater streams and the communities within them may be particularly susceptible to becoming dominated by *P. leniusculus*.

Limiting factors and research priorities

A key limiting factor in the strength of inferences drawn from this study is due to the sampling methodology for crayfish, namely trapping. Trapping for crayfish was and

remains the most commonly utilised sampling methodology (Parkyn, 2015), and so was an appropriate methodology to employ at the time of this study. However, the traps themselves are based upon commercial designs for the sustainable harvest of a commercial crop (Moorhouse and Macdonald, 2011a), and as such are biased towards the capture of large specimens. There is also uncertainty associated with trapping in relation to sampling efficiency, such as effective range of the bait, retention success, and the risk of false negatives as was seen at both Linton and Cray Gill Beck (e.g. Gladman *et al.*, 2010). As such, trapping cannot provide quantitative data on the density of crayfish present in a headwater, and the CPUEs provided through trapping may be subject to bias, thus reducing certainty regarding comparative density of the sampled crayfish populations. Whist in broad terms, there was little difference between the invertebrate communities in the headwaters containing no crayfish, *P. leniusculus* or *A. pallipes*, there was a greater level of variation in fish and invertebrate abundance, biomass and diversity when explored at an individual site level, in particular at Bookill Gill Beck. Future research should focus on identifying and sampling *P. leniusculus* populations where they have become the dominant component of the ecological communities, to attempt to understand the relative frequency of this scenario. In order to attempt to understand the mechanisms behind these differences, quantitative density data on the crayfish populations should be sought. In this respect, therefore, there is a clear need to develop and trial new quantitative sampling methodologies for crayfish in the field.

This study was limited to a single sampling effort for each headwater site with respect to electrofishing, crayfish and invertebrate sampling. Due to temporal restrictions on sampling of both the legal open season (June-September inclusive) and the PhD's timeframe, this study instead opted for a space-for-time approach, including multiple replicates in each treatment. As such, the invertebrate and fish communities prior to the

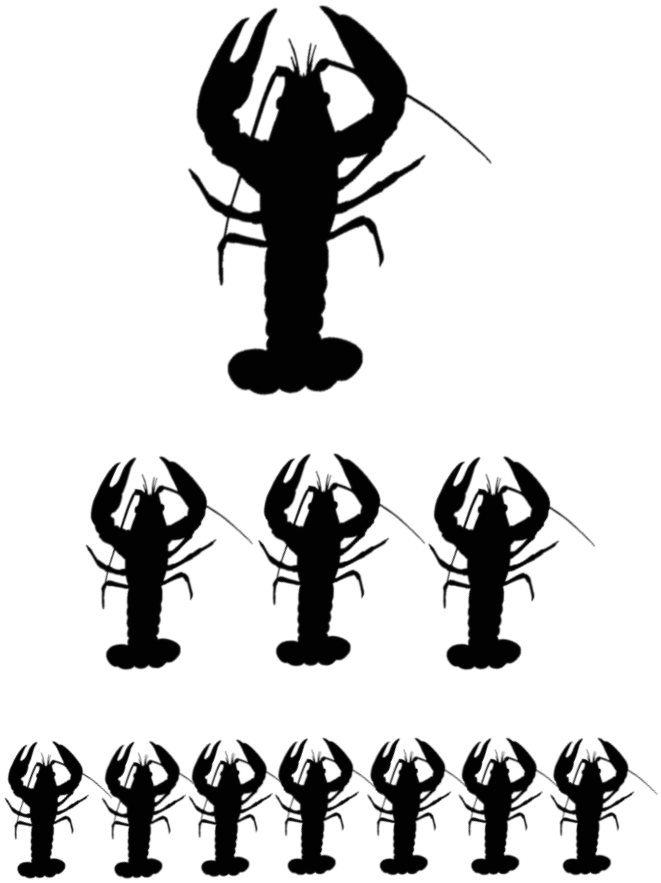
establishment of *P. leniusculus* populations were unknown, and the processes by which they have changed in the presence of either *A. pallipes* or *P. leniusculus* can only be inferred. To address this, research should attempt to isolate the processes associated with community level restructuring following the arrival of a crayfish species by collecting temporal data before a population becomes established. There are two methods potentially available to attain this data, under the assumption that purposefully introducing *P. leniusculus* into the wild is unacceptable in England. Firstly, identification of expanding *P. leniusculus* populations through survey works can determine the invasion front of a population. Study sites can then be established ahead of this front, and the communities therein monitored over time to assess the impact of an arriving invasive crayfish population. For *A. pallipes*, however, the establishment of ark sites, an increasingly common conservation method (e.g. Nightingale *et al.*, 2017), offers a unique opportunity to monitor the changes in invertebrate (and fish, if present) communities following the introduction of *A. pallipes*.

Conclusion

Populations of both *A. pallipes* and *P. leniusculus* were able to be survive in headwater systems in England, and co-exist with equally diverse aquatic macroinvertebrate assemblages and abundant fish communities. *A. pallipes* consistently altered the macroinvertebrate assemblages present in headwaters, but did not decrease macroinvertebrate abundance, biomass or diversity. Populations of *P. leniusculus* were associated with a greater variety of macroinvertebrate communities, and a reduced biomass of fish. Of note, is that the highest comparative density headwater site, Bookill Gill Beck, was highly ecologically degraded with reduced macroinvertebrate abundance and diversity, and a lack of fish. The loss of a highly diverse and abundant native headwater community to the invasive crayfish *P. leniusculus* is of particular concern,

with implications for the future management of salmonids and benthic fish species in headwater streams.

Chapter 4 - Use of a novel sampling methodology to determine invasive crayfish population density and structure



Introduction

Invasive populations of signal crayfish *Pacifastacus leniusculus* have spread across much of England (Chapter 2), and through disease and being a superior competitor, have triggered corresponding losses in populations of the white-clawed crayfish *Austropotamobius pallipes*. The overall impacts of *P. leniusculus* on native fauna are reported to be severe and negative in most cases (e.g. Ibbotson and Furse, 1995; Mathers *et al.*, 2016). However, some instances have occurred where the presence of *P. leniusculus* has had an apparent negligible impact on aquatic biodiversity (Chapter 3), or even benefited aspects of native biodiversity, for example increasing growth rates of large European chub (*Squalius cephalus* L.), a predatory fish which fed on *P. leniusculus* in Wood *et al.*'s (2017) study of four English lowland rivers. *P. leniusculus* undergo ontogenetic shifts in behaviour and resource utilisation as they grow (Bondar and Richardson, 2009; Usio *et al.*, 2009). Specimens in different life stages will therefore have different interactions with other species and thus ecological effects on the aquatic ecosystem. To understand these potential impacts of an invasive population of *P. leniusculus*, both the structure and density of a population needs to be known in detail. When sampling for crayfish, a combination of techniques has often been advocated for greater detection and capture efficiency (Gladman *et al.*, 2010). Trapping is the most commonly utilised method of sampling crayfish (Fig. 1; Parkyn, 2015), and is usually reported in terms of a semi-quantitative catch-per-unit-effort (CPUE). However, many other methods are also utilised, such as artificial refuge traps, hand netting and direct observation through torching and snorkelling, often dependent on the environmental conditions present at a site.

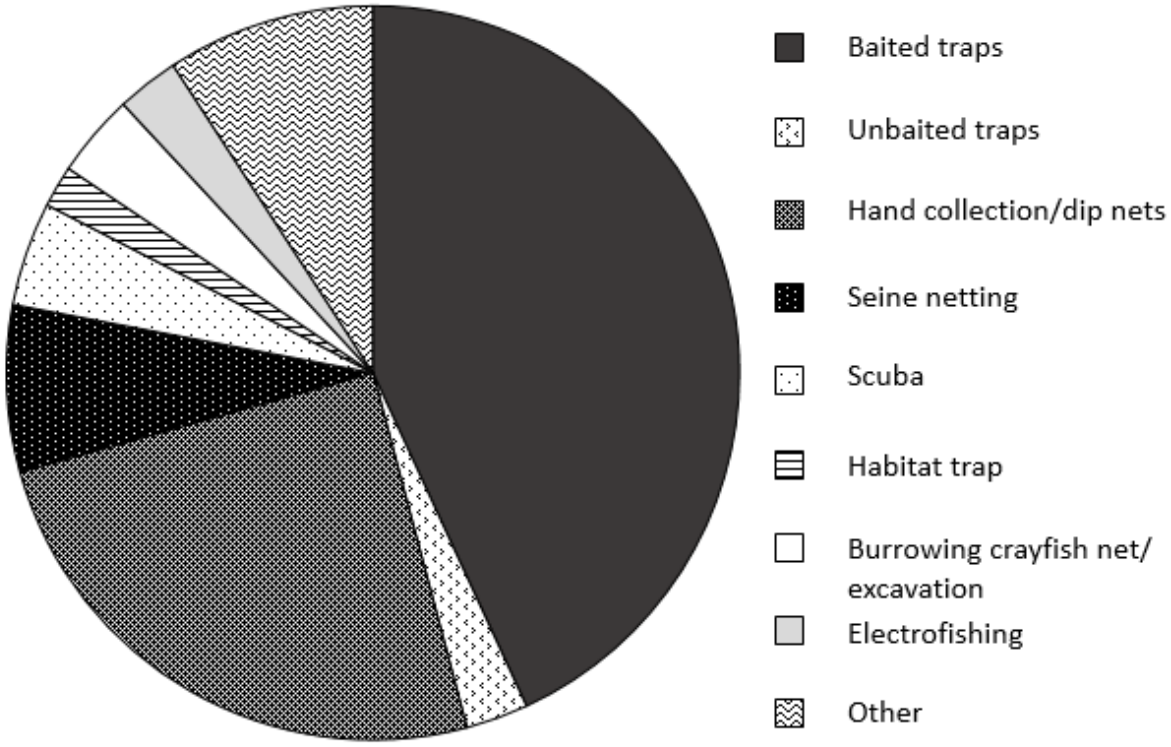


Figure 1 – A review of methods used to sample crayfish from published literature (n = 109 from 68 papers, 2006-2013; adapted from Parkyn, 2015)

Trapping studies often form the basis for density estimates of *P. leniusculus* (e.g. Westman *et al.*, 1999), with the resulting estimated densities commonly being very low (<1 individual m^{-2}) and based on a variety of assumptions (Ibbotson and Furse, 1995). For example, due to the bait attracting individuals to baited traps, the effective sampling area is often unknown and can be at best estimated, with different types of bait also likely varying in their attraction to crayfish. Trapping also generally targets larger and more active individuals, often specifically sampling males with carapace lengths of >35 mm (Moorhouse and Macdonald; 2011 Almeida *et al.*, 2013). Young-of-year and smaller crayfish in contrast commonly fail to be sampled and thus reported in trapping studies, which is a major flaw since in some crayfish populations, these early life stages can represent a very substantial component (e.g. DiStefano *et al.*, 2003). Brown and Brewis (1978) suggested that mark re-capture methods based on trapping alone therefore underestimate a population by a factor of three. Uncertainties are further

highlighted by Byrne *et al.* (1999), where a 95% confidence interval of 39%-221% was applied to the estimated population of the 3588 specimens of the native *A. pallipes* reported in a mark-recapture study of population densities in small rocky streams in Ireland. As such, whilst comparative CPUE can be provided by standardised trapping approaches, specific density estimates based on this technique are not considered robust.

Electrofishing has been utilised in the past to some success, (e.g. Alonso, 2001). However, electrofishing, and the depletion estimates derived from multiple passes rely on the assumption that capture probability is constant between animals, and that a depletion is observed between each consecutive sweep. This is often not the case in practice (e.g. Hedger *et al.*, 2013), due to abiotic factors such as conductivity and biotic factors such as body shape and behavioural responses (see Zalewski, 1983).

Due to the varied efficiencies of these techniques, and all other currently widely utilised methods on recording crayfish in different life stages (Rabeni *et al.*, 1997), past population estimates therefore crucially lacked reliability, and are therefore of limited use to management.

In this chapter, I am introducing a novel technique to record crayfish referred to as a 'triple drawdown', with the intention of providing a thorough description of invasive *P. leniusculus* population structure, density and demographics that has remained unattainable through conventional sampling means. The approach involves de-watering a small section of a river and removing all substrate and crayfish within, as a suitable approach for small headwater streams. The objectives of my respective study were to 1) trial the drawdown method in the field, 2) quantify if the method can provide a realistic picture of the overall crayfish population as confirmed by depletion estimates, and 3) fully describe the populations of *P. leniusculus* present in the de-watered stretch of river.

Methods

The methods of this study fall into two distinct sections; 1) a detailed description of the novel triple drawdown sampling technique (termed simply 'drawdown' henceforth) and how it is employed in the field, and 2) the broader methods of the empirical chapter, including descriptions of the study site and analyses presented within this study. In order for the method to be repeated satisfactorily, in terms of accurate replication, biosecurity and effectiveness, the drawdown methodology is described in detail.

1. Drawdown equipment and general procedure

The drawdown method involves dewatering a short section of stream and removing all accessible in-channel substrate in order to thoroughly sample a measured area of the river benthos. The method utilises a range of equipment including small fuel-based pumps that draw water in through a rigid intake pipe and pump it through a lay-flat pipe, which allows water to be diverted around a dammed, closed off section of the river. The method is limited physically by several factors, including river flow, depth, width and gradient. The process of the method under favourable conditions is described below, as it needs to be recognised that the limits of the method will vary depending on the user's requirements and resources.

Site preparation

The first step is to define the area to be dewatered by identifying and defining clear upstream and downstream limits; within this study, physical conditions limited this to sites <20 m in length and <4 m in width. The site should then be closed off to any immigration or emigration of crayfish specimens. Stop nets are appropriate for this purpose, providing the net aperture is small enough to prevent crayfish from passing through. In this respect, coarse stop nets (e.g. 10 x 10 mm aperture) can be placed across

the wetted width, and finer nets should be used where the central channel forms to prevent crayfish hatchlings (5mm CL) escaping (see below).

At the upstream limit, a watertight dam should be built up using sandbags. A sump is then dug immediately upstream of the dam. Together the dam and sump create a pool where the end of a rigid intake pipe is positioned (Fig. 2a). The pump's layflat pipe should run parallel to the river channel and allow water to re-enter the stream below the downstream limit of the site (Fig. 2b).

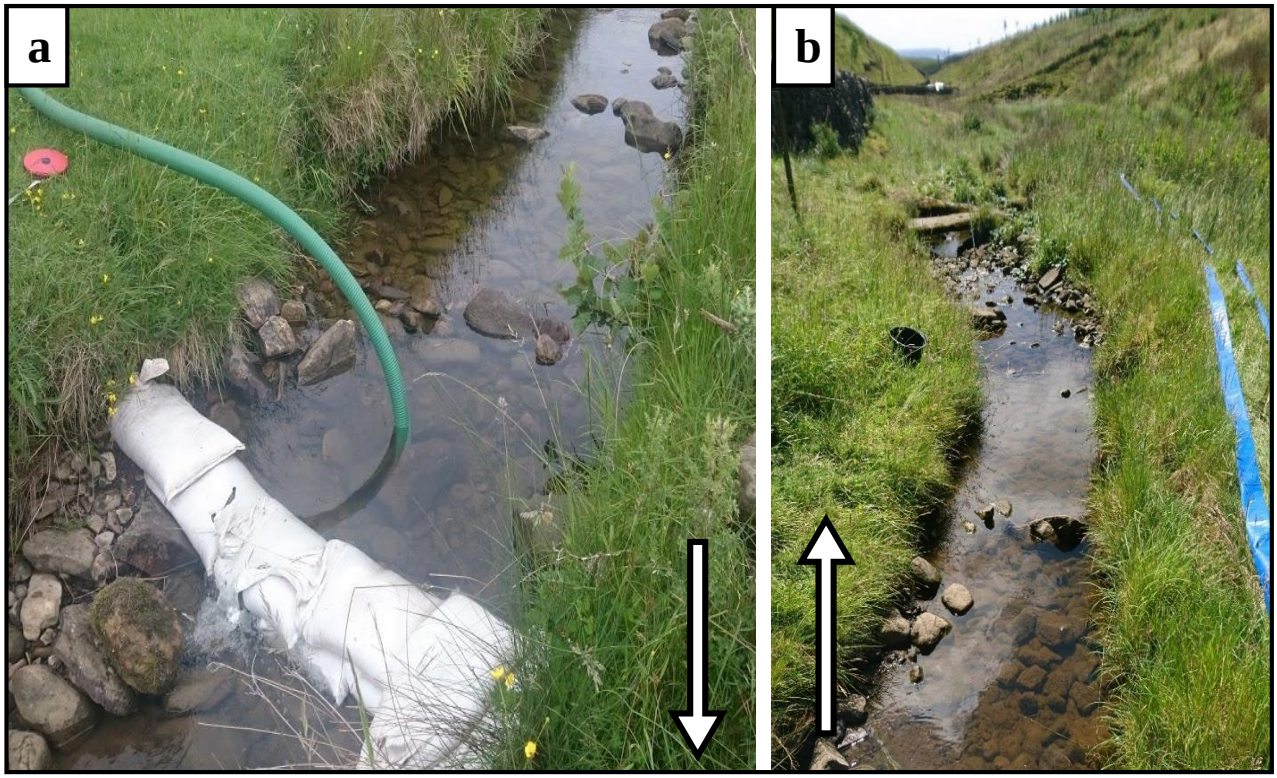


Figure 2 – Position of rigid intake pipe secured in the deepest part of the sump and the sandbag dam upstream of the working site (a), and layflat pipes parallel to the working site, which pump water around and back into the channel below the drawdown area (b). Arrows indicate direction of flow.

De-watering

When the pump is first switched on, the volume of water being pumped should be in excess of the incoming flow in order to remove the standing water in the sump area. Once this is removed, the pump power should be adjusted to match the incoming flow to allow the site to drain. The pump is left running as work is undertaken. As the water drains from the site, any suitable crayfish refugia (cobbles, woody debris, etc.) should be removed from the river bed and placed onto the river bank to reveal the bare channel bed. It is important to work in a methodical manner for the purpose of health and safety of the operatives, but also to minimise the risk of crushing animals residing beneath refugia underfoot. It is best practice, therefore, to firstly remove substrate from the river margins and gradually work inwards towards the central channel. Within the context of this study, team size should be between 4-5 individuals, and should scale accordingly with larger sites. Exposed crayfish should be collected by hand and identified to species level as they are encountered. Invasive species should be stored in buckets of cool, well-oxygenated water on the bankside during the de-watering, whereas, if any native crayfish are found, they should be processed (gender, carapace length, weight, claw damage) on site as soon as practicable and released into a pool a safe distance upstream of the study area. Small pools of water may remain in the site depending on the channel gradient and river bed bathymetry. Digging a narrow channel with a spade or trowel assists in draining these last remaining wetted areas (Fig. 3a & b).

Figure 3 – Stop-net for young-of-year and juvenile crayfish (a), positioned in the central channel (b) to catch animals following the drained water flow. Arrows indicate direction of flow.

It is not always possible to fully de-water the entire site and crayfish may become localised in the remaining pools. It is then recommended that small aquarium handnets are used to sweep through the pools to collect any remaining young-of-year and juvenile crayfish. Hand-searches in the bank area may also prove successful in finding remaining larger individuals and berried females. When all operatives cease to find any more crayfish, the pump is switched off, and the first 'sweep' is complete (Fig. 4).

Re-wetting and consecutive sweeps

With the pump switched off, water begins to flow over the dam and re-wets the site. In some instances, operatives may wish to use sandbags to create a dam at the downstream limit of the site, to hold water in the site area to allow sufficient re-wetting. Re-wetting of the channel is important as it usually proves successful in luring remaining hidden crayfish out into the main river channel. After sufficient re-wetting (15-20 minutes), the second sweep may commence. The pump is switched back on, and newly exposed crayfish should be captured by hand or net as the site drains. This process is repeated for a third sweep. It is expected that less crayfish will be captured with each sweep, creating a depletion curve. However, if a depletion is not observed after three sweeps, additional

consecutive sweeps may be required. Once the collection of crayfish has finished, the pump is switched off and all removed substrate is returned to the channel. The upstream dam and stop nets should be removed from the site and care should be taken to return the river to as near to its original state as possible. All non-native invasive crayfish collected should be humanely destroyed and stored appropriately. For the purposes of future studies of for example stable isotopes, gut contents, or eDNA, the authors recommend storing crayfish on ice and then freezing where facilities permit rather than other long term storage options (e.g. Industrial Methylated Spirits (IMS)). Placing crayfish on ice at site reduces incidences of intraspecific aggression, and reduces the time taken to complete drawdowns in the field; crayfish can be processed later once defrosted.

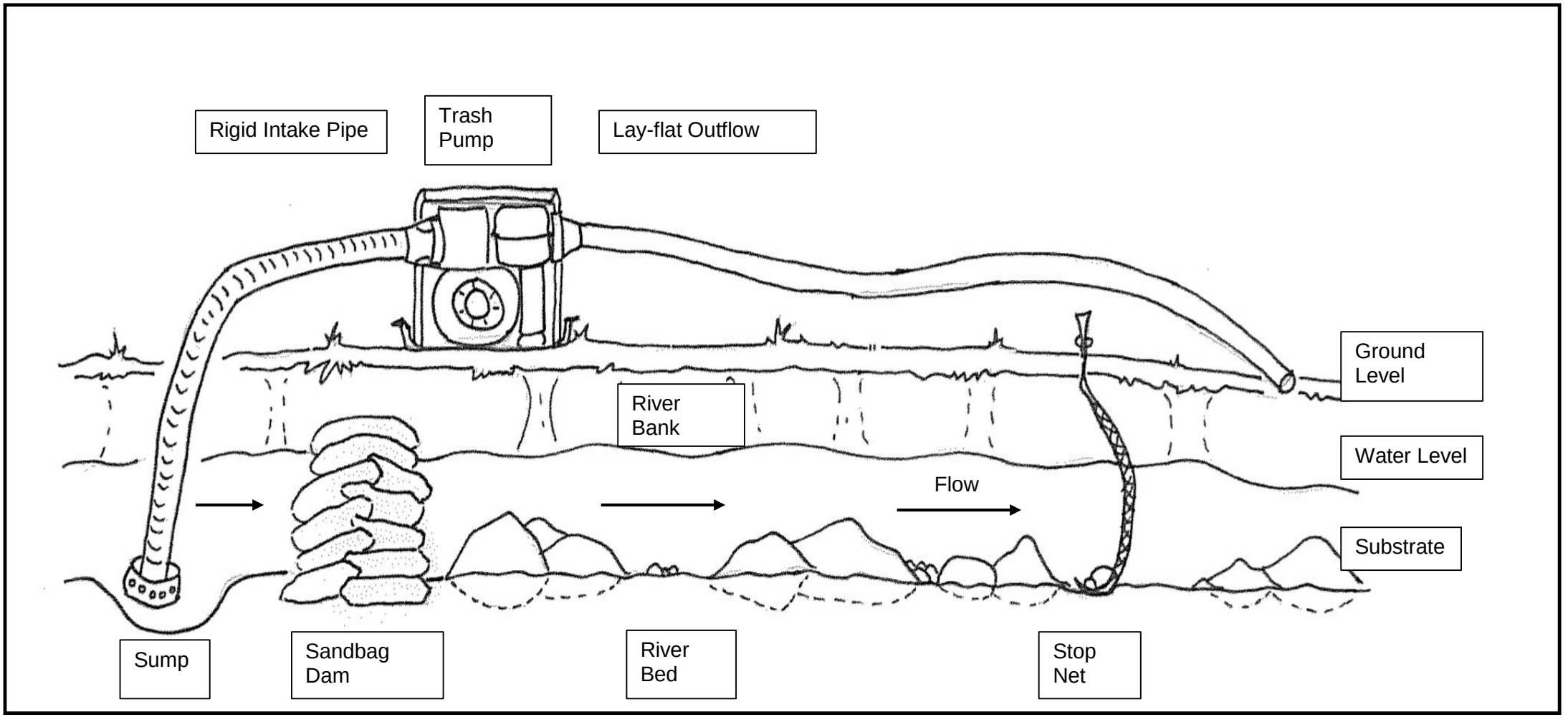


Figure 4 - A conceptual diagram prepared by Pritchard (unpublished) to summarise the process of drawing down a river for the purpose of sampling crayfish populations.

2. Study area

The study sites were located along Bookill Gill Beck, a rocky limestone headwater stream in the upland area of the Yorkshire Dales, England. Bookill Gill Beck (henceforth BGB) is a steep (1:28 average gradient, Peay *et al.*, 2009), fast-flowing tributary of Long Preston Beck in the River Ribble Catchment (Fig. 5). BGB runs approximately 5.1 km from source to its confluence with Scaleber Beck, with BGB increasing in width from an average 0.7 m at the top, to 1.9 m at the confluence (Peay *et al.*, 2009). BGB is situated in a farmed sub-catchment of unimproved or semi-improved grazed pasture but no farmyards, sheep-dips or domestic buildings are present, and as such the threat to water quality is low.

Historically, BGB supported substantial populations of native *A. pallipes* and diverse fish communities, including salmon *Salmo salar*, brown trout *Salmo trutta*, bullhead *Cottus gobio* and eel *Anguilla anguilla* (Peay *et al.*, 2009). However, a stream survey by local experts in 2002 revealed a mixed population of *P. leniusculus* and *A. pallipes* (P. Bradley, pers, comm., 2018). Local experts state that the initial introduction occurred ~1995, when a small number (4-12) of *P. leniusculus* were illegally released approximately 2.3 km downstream from the source of BGB. Since this time *P. leniusculus* have become established in BGB and have spread in both upstream and downstream directions. The established range of *P. leniusculus* now (2018) extends the entire length of BGB, and beyond the confluence into Long Preston Beck.

BGB provides unique opportunities to investigate well-established *P. leniusculus* populations that have drastically affected the native ecosystem in recent decades. BGB was therefore selected as the study area to trial the triple drawdown method. Three sites, namely Double Gate Bridge (DGB), Paddock (PAD) and Confluence (CON) were selected for study, to represent a continuum along the invasive population range, and

due in part to ease of access for equipment (Fig. 5). DGB was sampled in 2016 and again at the same site in 2017, resulting in a total of 4 drawdown sampling events, namely DGB2016, CON2016, DGB2017, and PAD2017. All crayfish caught were removed and humanely disposed of during the DGB2016 sampling event, and as such no repeated measures design was required, and assumptions of the statistical independence of observations were not violated. All drawdown sampling was undertaken during the summer months (June-September inclusive) in 2016 and 2017, to fit in with the standard sampling window for crayfish. Additionally, each drawdown was executed only following several prior days of consistent low summer flows; as such, an opportunistic approach was taken to sampling timings. Hours of labour varied due to weather, amount of rock and substrate moved, and number of crayfish caught, but all drawdowns were conducted over a single sampling effort and within 10 hours. Physical parameters (, depth, width, river substrate) were recorded at each site (Table 1), flow was recorded using a Valeport Electromagnetic Flow Meter (as in Chapter 3), and river substrate was estimated every 5 m at each margin and the centre using a quadrat.

Once all the available crayfish had been caught, they were processed and either frozen (for isotopic analysis) or preserved in IMS. Species, carapace length (CL, tip of rostrum to posteriomedial edge of the cephalothorax) measured using Vernier callipers (1 mm), thawed wet weight (0.1 g), gender, and cheliped condition were recorded for crayfish. No *A. pallipes* were encountered during the study. For the purpose of statistical analysis, cheliped condition was reported as a crayfish exhibiting damaged or non-damaged chelipeds, with damage referring to evidence of mutilation, regeneration or total loss of either or both chelipeds. Gender was initially categorised into male, female, or juvenile for animals of <=12 mm CL, as these animals cannot reliably be sexed due to undergoing insufficient moults to begin displaying sexual appendages; all animals

>12 mm CL were successfully sexed. Cheliped condition was reported for animals >12 mm CL, as all crayfish often received extensive contact during the sampling and capture procedures at drawdown sites and the subsequent freezing process and individuals <=12 mm CL were regarded as too delicate to reliably determine if cheliped damage was present prior to or as a result of sampling. Therefore, these smaller animals may have represented a sampling bias rather than a true reflection of the incidence of cheliped damage within the population. Juvenile crayfish were recorded in abundance counts. However, cheliped condition, length, and weight were not recorded individually for each juvenile crayfish. Length and weight of juvenile crayfish were averaged from counts of 100 animals, with these average values being applied to hatchlings (5 mm CL, 0.05g) and juveniles (12 mm CL. 0.3 g) respectively.

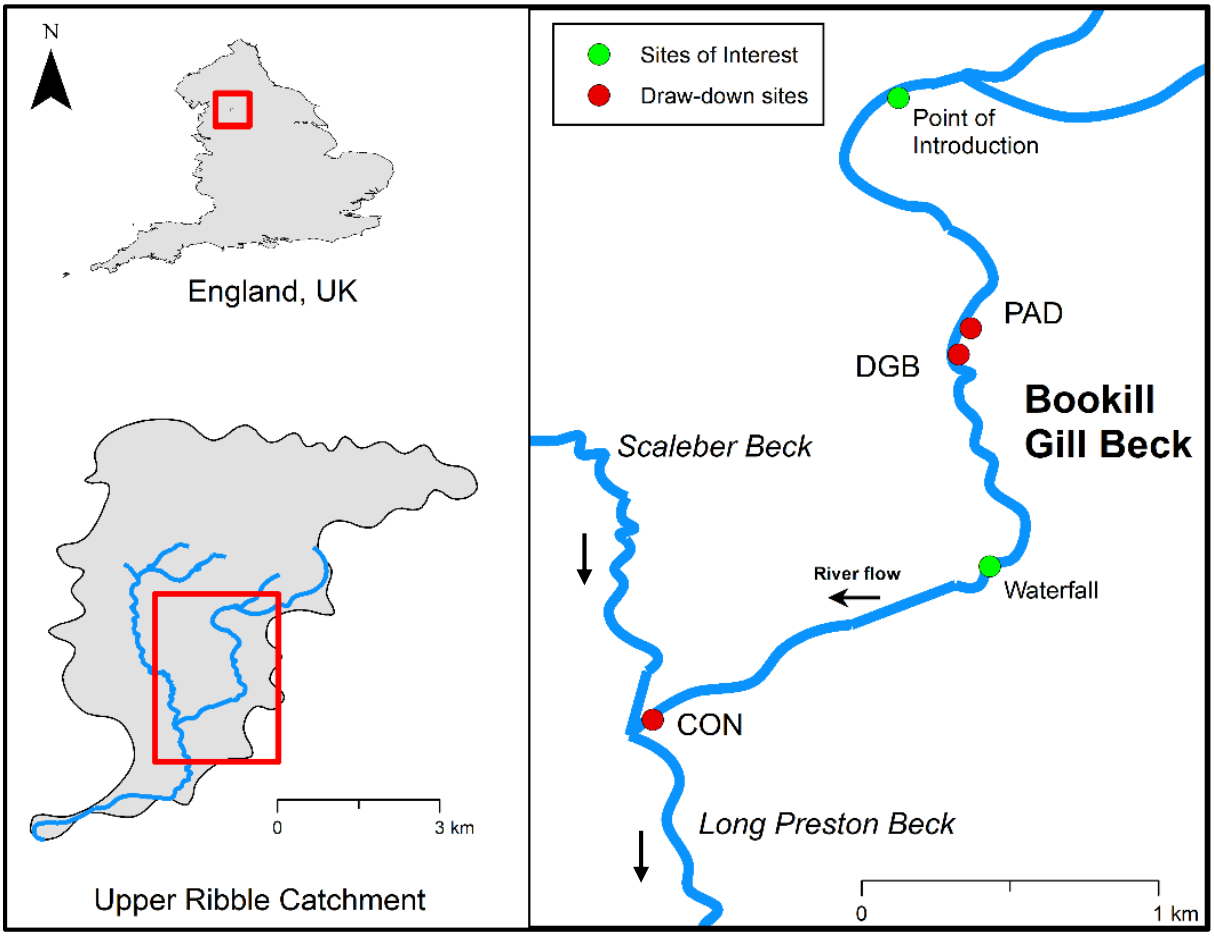


Figure 5 - Location of Bookill Gill Beck within the Ribble catchment in Northern England showing location of the 3 discrete sampling sites for the 4 drawdown sampling events.

Table 1 - Environmental variables for each drawdown site, with site locations as in Figure 5.

Physical parameter	Site name		
	DGB	CON	PAD
Sample reach length (m)	10	15	20
Average wetted width (m)	2.0	1.9	1.5
Average water depth (cm)	7.6	8.3	11.4
Flow (m/s, 30 second average)	1.5	3.7	1.0
In-channel substrate (%Silt/Sand, %Gravel, %Cobble)	8, 12, 80	2, 21, 77	6, 5, 89
pH	8.2	8.2	8.0
DO (mg/L)	9.6	8.9	9.3
Water temperature (^{o}C)	15.4	21.9	15.4
Conductivity (µs/cm)	293	293	292

Statistical analyses

Depletion calculations were made in R (3.4.2.), using the 'Carle-Strub method' (Carle and Strub, 1978) function in the Fish Stock Assessment (FSA) package created by Ogle (2018). Depletion data were then used to calculate the population estimates. This is a common and long-standing method for estimating fish populations from three-sweep electrofishing depletion data (Carle and Strub, 1978). Non-parametric analyses of the cheliped condition between populations was conducted using Kruskal-Wallis tests, with post-hoc pairwise conducted using Mann-Whitney U tests. Non-parametric comparisons of gender ratios were conducted using chi-squared (χ^2) tests (SPSS 24). When

comparing differences in the proportions of males and females across the cohorts, size classes were combined into larger groups where expected counts violated chi-squared assumptions. New alpha significance values were calculated for post-hoc chi squared analyses, following (MacDonald and Gardner, 2000). Crayfish size categories were defined as juvenile crayfish (CL <=12 mm) and adult crayfish (CL >12 mm), following Alonso (2001). The smallest berried female in this study was 26 mm CL, and all crayfish above this length were hence classified as sexually mature. Crayfish >35 mm CL were classified as catchable through conventional trapping (Almeida *et al.*, 2013).

Two multiple regression analyses were conducted in SPSS 24, to model the effects of crayfish demographics and fish presence on crayfish length and weight. Statistical assumptions of normality and sufficient group sizes (χ^2) were checked for violations, and Durbin-Watson values were within acceptable ranges (>1.5, <3.5). Density was categorised as Low at <50 individuals m^{-2}, and High at >50 individuals m^{-2}. Sites were further categorised by the presence (CON2016) or absence (DGB2016-17, PAD2017) of fish.

Biosecurity and ethics

Biosecurity was crucial, and all equipment and Personal Protective Equipment (PPE) such as waders, were cleaned using Virkon S Aquatic or Iodophore at working concentrations prior to and after use. Drying and disinfection via sunlight was also used. All invasive crayfish were handled carefully and humanely disposed of initially via freezing. All fish were moved upon contact to safe wetted areas. Recent surveys have shown that *A. pallipes* have been completely displaced throughout BGB and fish communities have become severely diminished, with no fish being recorded upstream of a small waterfall located in the lower reaches of the beck (Pritchard 2016, MSc). As such, fish were absent from drawdown sites upstream of this feature (PAD and DGB),

and present only at the downstream drawdown site (CON, see Fig. 5). Where fish were present, animal welfare was carefully considered, with efforts made to relocate fish quickly and safely; no fish mortalities were recorded during the CON2016 drawdown.

Results

Relative gender proportions within P. leniusculus populations

Juvenile crayfish were numerically dominant at all sites, on average comprising 55% of the total population (Fig. 6), despite varying significantly between 36 and 72% in terms of relative proportion of animals found across the different sites ($\chi^2 = 245.402$, df = 6, $p < 0.001$, Table 2). Juvenile crayfish were discounted from further gender and inferential analysis to further examine the Male:Female (M:F) proportions in isolation (Fig. 7). This analysis revealed that M:F proportions showed limited variation between the three sites ($\chi^2 = 0.342$, df = 6, $p = 0.933$).

Population demographics

Densities of *P. leniusculus* were very high in this study, averaging 66.2 m^{-2} (range 20.5-110.4 m^{-2}; Table 2). These densities represent conservative values, as they were based on the actual raw catches, rather than on estimates of a total population or any extrapolation. Both carapace length and individual wet weight were non-normally distributed ($p < 0.001$), and median carapace length (CL) and wet weight varied significantly between sites ($\chi^2 = 279.39$, df = 3, $p < 0.001$ and $\chi^2 = 284.862$, df = 3,$p < 0.001$, respectively). Post-hoc analysis (adjusted $\alpha = 0.0083$) revealed that the median CL was significantly lower at the DGB2016 drawdown site compared to all other sites ($Z = -13.687$, $Z = -12.404$, $Z = -12.225$, $p < 0.001$ in all instances) due to large numbers of freshly hatched crayfish caught in this survey (Table 2). The median CL of the CON2016 drawdown was significantly higher than DGB2017 ($Z = -5.125$, $p < 0.001$) and the PAD2017 drawdown ($Z = -3.699$, $p < 0001$). The median CL of crayfish in

DGB2017 and PAD2017 did not significantly differ. Post-hoc analysis (adjusted α = 0.0083) revealed that median individual wet weight was significantly lower at the DGB2016 drawdown site compared to all other sites ($Z = -13.948$, $Z = -12.483$, $Z = -12.321$, $p < 0.001$ in all instances). The median individual wet weight of the CON2016 drawdown was significantly higher than DGB2017 ($Z = -5.074$, $p < 0.001$) and the PAD2017 drawdown ($Z = -3.596$, $p < 0.001$). The median individual wet weight of crayfish in DGB2017 and PAD2017 did not significantly differ. Crayfish abundance was lower at the CON2016 drawdown site than at all others, with this reflected in the decreased biomass as compared to the other drawdowns, despite having the largest median individual wet weight for crayfish inhabiting the site. CON2016 is the only site to contain fish, and had the lowest density of crayfish (20.5 m^{-2}, Table 2). PAD2017 had the largest total weight of caught crayfish, but this was partly due to a larger drawdown area being sampled; once corrected for grams/m^{-2}, the biomass estimates were similar to both the DGB drawdowns. Population structure and size class distribution were calculated for each site (Figure 8).

Table 2 – Key population demographic for drawdown sites.

Parameter	DGB2016	CON2016	DGB2017	PAD2017
Median carapace length (1 mm)	5	14	12	12
Median weight (0.1 g)	0.1	0.6	0.3	0.3
Total crayfish abundance (n)	1656	538	1290	1319
Total crayfish weight (0.1 g)	1393.1	1046.7	1871.7	3070.0
Density (m^{-2})	110.4	20.5	86.0	44.0
Biomass (g/m^2)	92.9	46.5	124.8	102.3

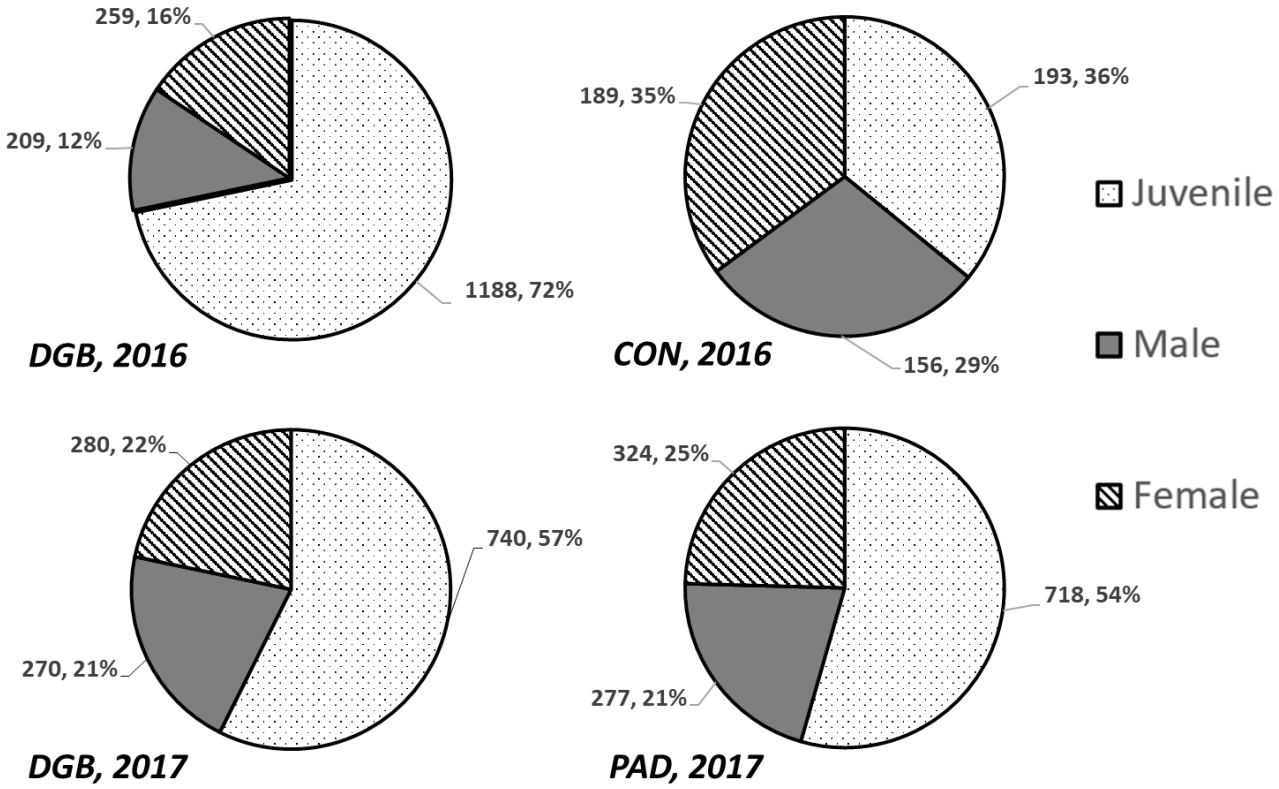


Figure 6 – Demographics of each crayfish population split by gender ratios (juvenile, male & female), with total number of captured crayfish and percentage of population shown.

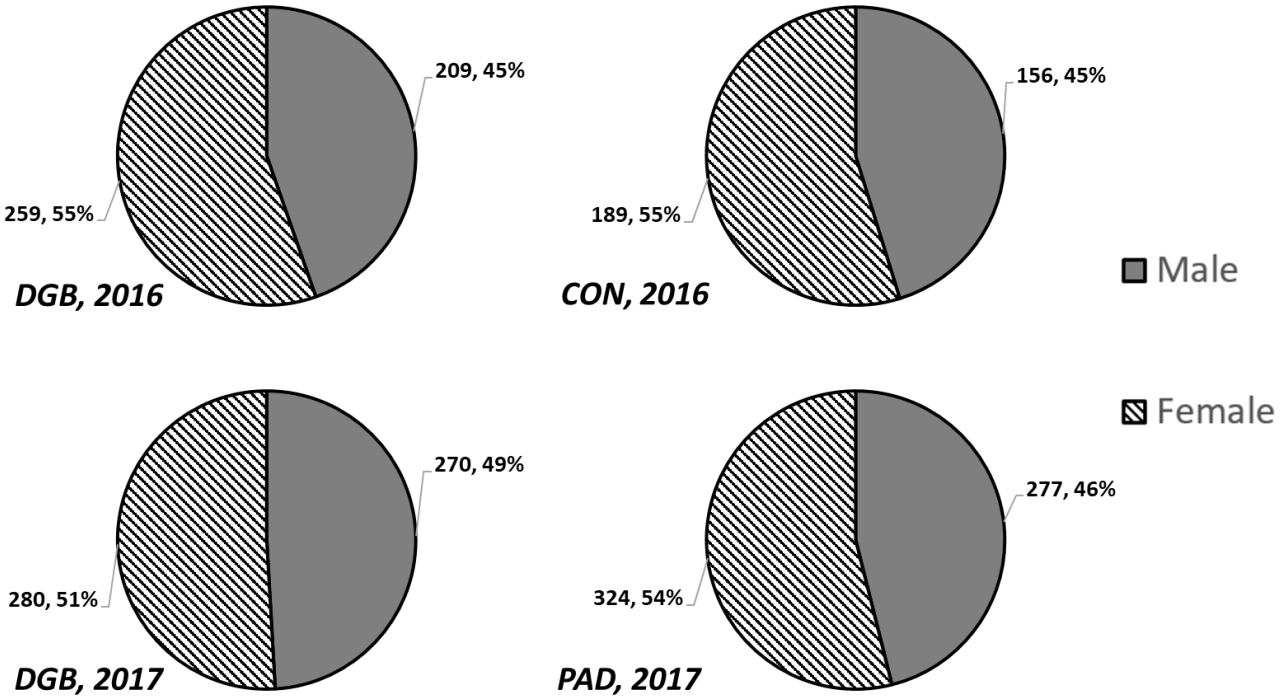


Figure 7 – Demographics of crayfish populations in each drawdown split by gender ratios, after removing the juvenile (J) class (showing only M & F) with total number of captured crayfish and percentage of the population shown.

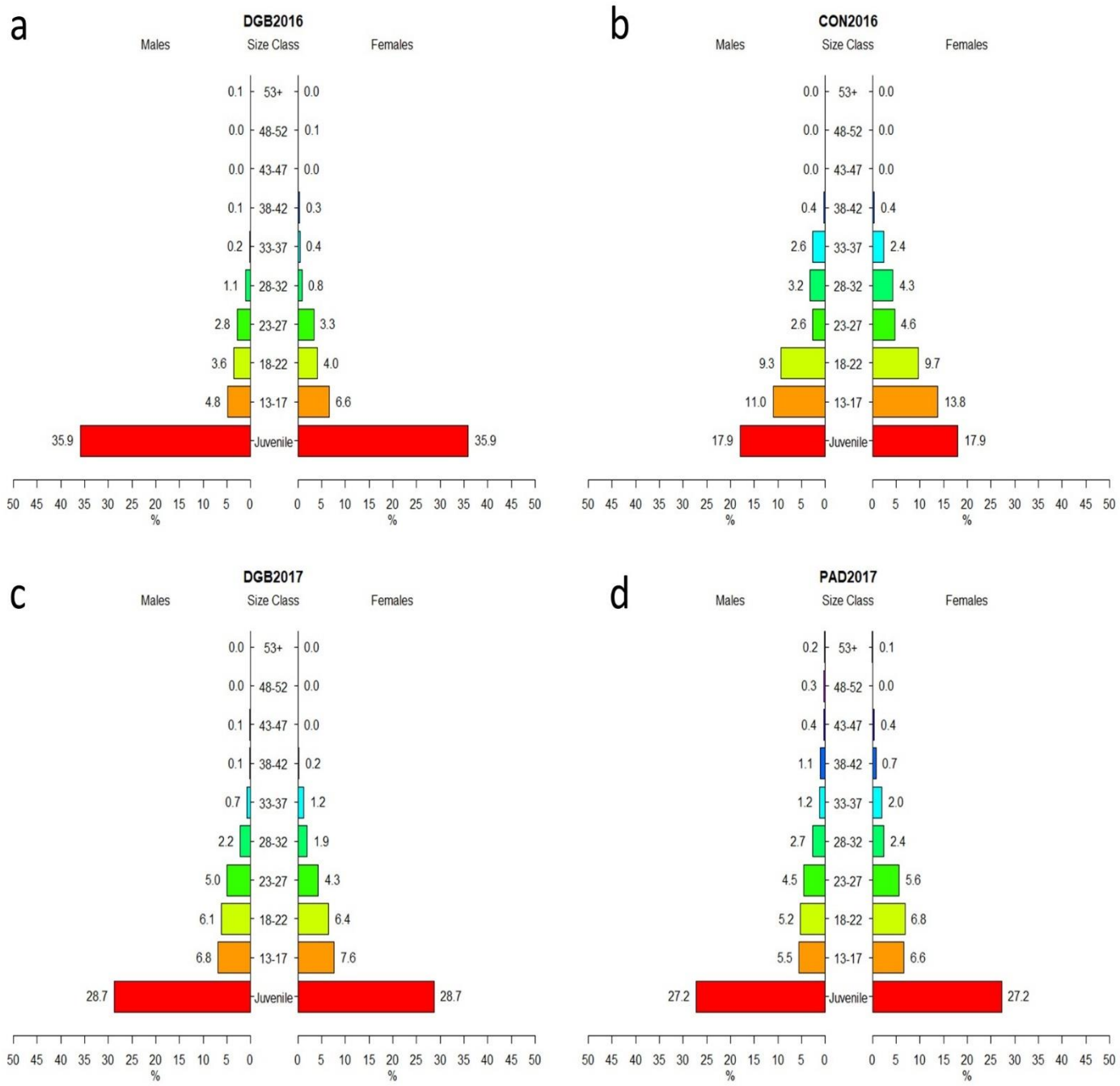


Figure 8 – Population demographics for all 4 drawdowns presented as population pyramids. Bin widths are 5 mm increments, except for juvenile class (<=12 mm) which was split evenly between the M and F for data presentation.

All sites showed a steady decrease in crayfish abundance as size increased, seen in both males and females. Demographic data is presented below (Table 3), in accordance with size categories specified in the methods. Size classes were collapsed into 4 groups (Table 3), and the proportions of size classes differed significantly between drawdown populations ($\chi^2 = 307.7$, df = 9, $p < 0.001$). Post-hoc comparisons (adjusted $\alpha = 0.003$)

showed DGB2016 to have significantly more juveniles and less of all other size classes, CON2016 to have significantly less juveniles and more sub-adult and sexually viable animals, DGB2017 to have more trappable adults, and PAD2017 to have significantly less juveniles and more sexually viable and trappable adults; all other proportions did not differ significantly.

The smallest berried female found was from the DGB2016 drawdown (26 mm CL), and had a brood size of 37 hatched young and 5 unviable eggs attached at time of capture. The largest berried female found was also from the DGB2016 drawdown (46 mm CL), and had a brood size of 189 hatched young, and 6 unviable eggs attached at time of capture. The proportion of the sexually viable population from each drawdown that was of trappable size (>= 35 mm CL) was 14.3% in DGB2016, 21.7% in CON2016, 11.8% in DGB2017, and 33.2% in PAD2017.

Table 3 – Demographics of crayfish across drawdown event (juvenile, sub-adult, sexually mature and trappable categories).

Size Class (CL, mm)	**DGB2016 (n = 1656)**	**CON2016 (n = 538)**	**DGB2017 (n = 1290)**	**PAD2017 (n = 1319)**
Juvenile (<=12)	1188	193	740	718
Sub-adult (13-25)	385	262	431	402
Sexually viable (26-34)	71	65	105	133
Trappable Adult (>=35)	12	18	14	66

Cheliped Condition

The overall incidence of cheliped damage was calculated for each site. Crayfish >12 mm CL were considered large enough to reliably sex, and thus had gender and cheliped condition recorded. Of the 1656 crayfish captured in the DGB2016 drawdown, 386

were >12 mm CL, and 33.2% (n = 128) showed clear signs of cheliped damage. Of the 538 crayfish in the CON2016 drawdown, 242 were >12 mm CL, and 42.2% (n = 102) of these had signs of damaged chelipeds. Of the 1290 crayfish in the DGB2017 drawdown, 550 were >12 mm CL, and 40.2% (n = 221) of these showed signs of cheliped damage. Finally, of the 1319 crayfish in the PAD2017 drawdown, 601 were >12 mm CL, and 42.4% (n = 255) of these had damaged chelipeds. The incidence of cheliped damage differed significantly by site ($\chi^2 = 9.421$, p = 0.024). Post-hoc analysis revealed significant pairwise differences between DGB2016 and all other sites (Z = -2.274, p = 0.023 Site 1:2, Z = -2.186, p = 0.029 Site 1:3, Z = -2.915, p = 0.004, Site 1:4 respectively), with DGD2016 having a significantly lower incidence of cheliped damage than all other drawdown populations. All other pairwise interactions were non-significant (p > 0.05).

Carle-Strub depletion

Depletions were strong across all drawdowns (Fig. 9 and 10), with high capture efficiency observed (Fig. 11), with the exception of CON2016, where the third sweep had a greater catch than the second sweep. The DGB2016 drawdown had a raw abundance of 1656 crayfish, with 1339, 227, and 88 crayfish captured during sweeps 1, 2 and 3 respectively. Capture efficiency was estimated at 78.6% (Standard Error (SE) 0.01), with a true population value of 1670 (SE4.74) and lower and upper intervals of 1661 (SE0.77) and 1680 (SE0.81). The CON2016 drawdown had a raw abundance of 538 crayfish, with 294, 95 and 148 crayfish captured during sweeps 1, 2 and 3 respectively. Despite CON2016 failing to achieve depletion between the second and third sweep, Carle-Strub estimates could be calculated, as a strong depletion was observed between the first and second, and first and third sweep, respectively. Capture efficiency was estimated at 34.8% (SE0.04), with a true population value of 742 (SE50.1) and lower and upper intervals of 644 (SE0.28) and 840 (SE0.42). The

DGB2017 drawdown had a raw abundance of 1290 crayfish, with 1121, 99 and 70 crayfish captured during sweeps 1, 2 and 3 respectively. Capture efficiency was estimated at 84% (SE0.01), with a true population value of 1295 (SE2.64) and lower and upper intervals of 1290 (SE0.82) and 1300 (SE0.86). The PAD2017 drawdown had a raw abundance of 1319 crayfish, with 912, 320 and 86 crayfish captured during sweeps 1, 2 and 3 respectively. Capture efficiency was estimated at 68% (SE0.01), with a true population value of 1363 (SE9.35) and lower and upper intervals of 1345 (SE0.65) and 1381 (SE0.71).

Further Carle-Strub depletion analysis of separate size classes was run for both juvenile crayfish (CL <=12 mm) and adult crayfish (CL > 12 mm) from each drawdown (as in Alonso, 2001; Table 4), to determine if size of crayfish influenced the catchability (Fig. 12). Juvenile crayfish from the CON2016 depletion were unable to have a Carle-Strub depletion estimate performed, as consecutive sweeps failed 'deplete' with respect to sweep 1, and thus data failed to conform to the requirements of the method. The number of crayfish caught in each subsequent sweep was strongly linearly associated with the sum of the previous sweeps ($R^2 = 0.99$) in all drawdowns apart from CON2016, which had a weaker linear relationship ($R^2 = 0.77$). Similarly, the estimated total percentage of the population captured for adult and juvenile crayfish from each drawdown was very high (average 98.2% ±1.5% St. Dev.; Table 4), and a value could not be calculated for the juvenile crayfish at the CON2016 drawdown.

Figure 9 – Catch from the DGB2016 drawdown, showing strong depletions between a) sweep 1, n = 1339, b) sweep 2, n = 227 and c) sweep 3, n = 88.

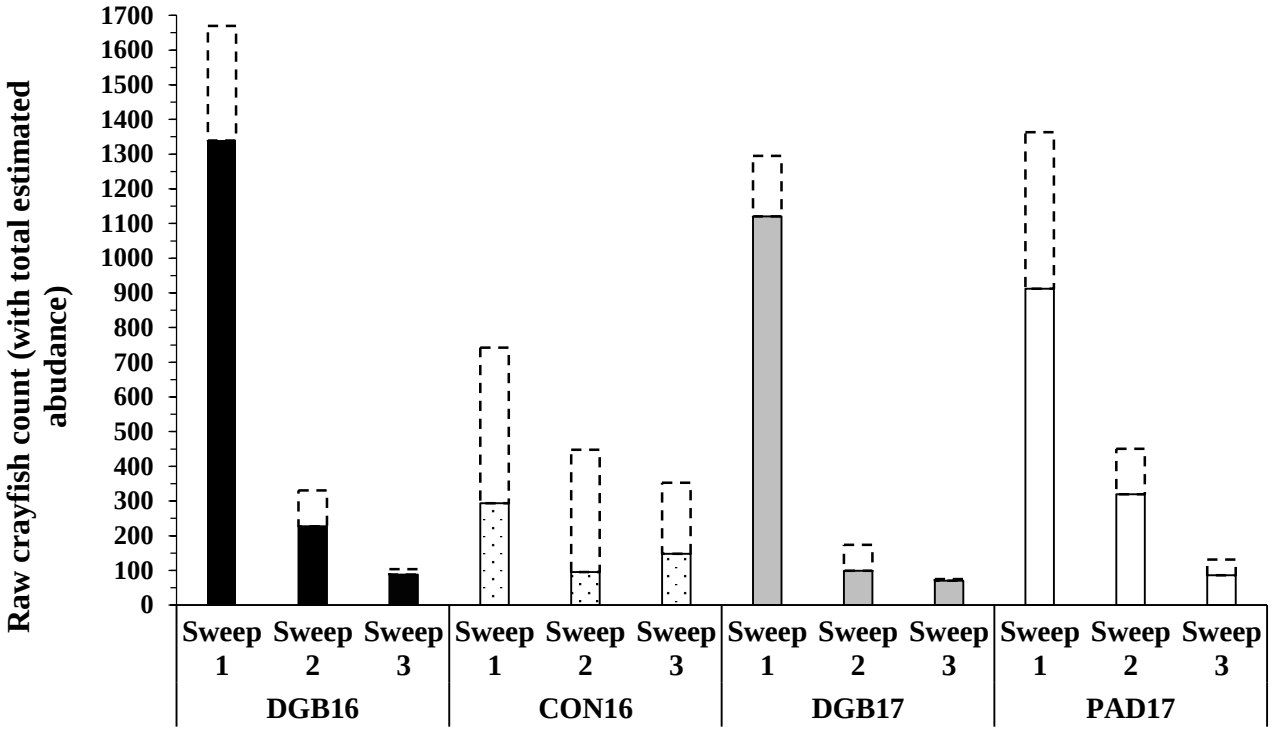


Figure 10 – Three-sweep depletion per drawdown, with solid lines indicating total catch, and dotted lines the Carle-Strub estimated true population available to be caught.

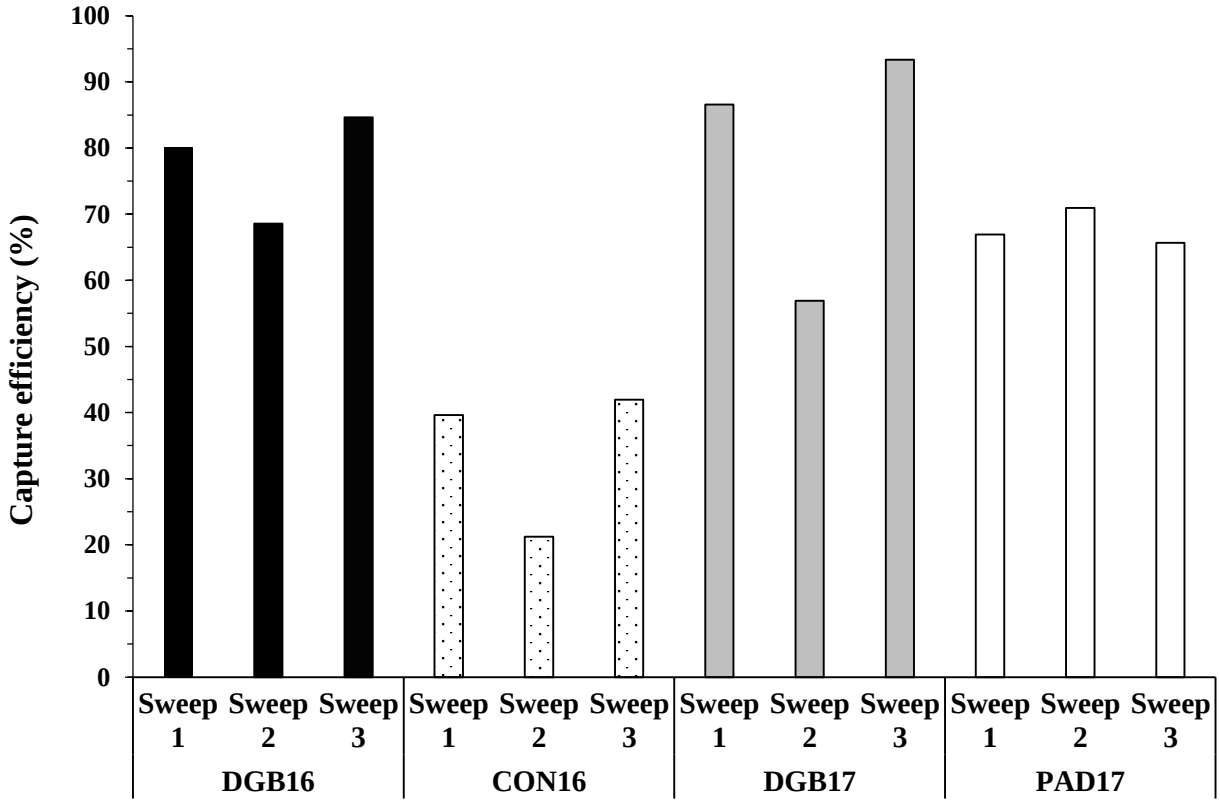


Figure 11 – Capture efficiency (Carle-Strub) of each sweep by drawdown.

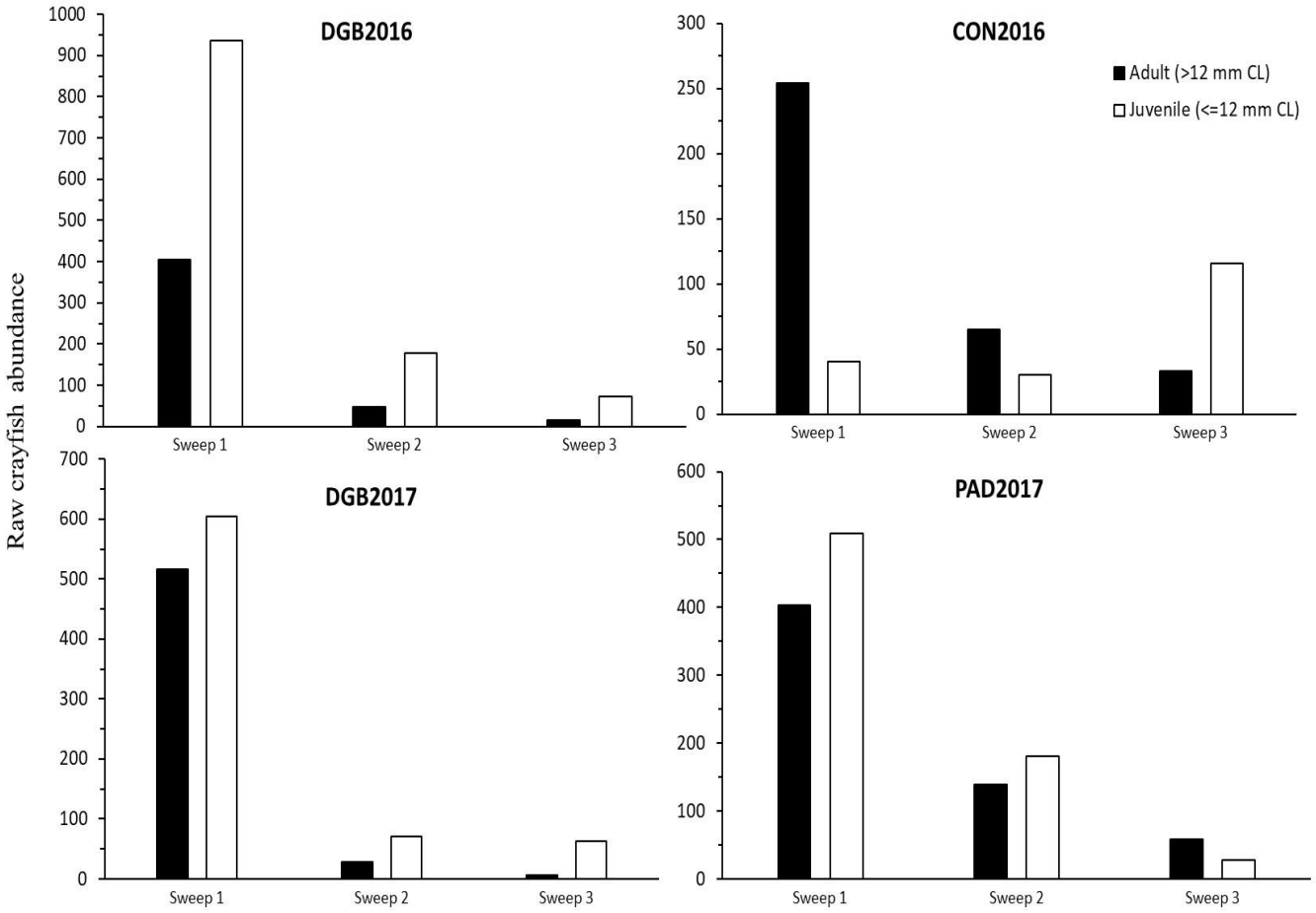


Figure 12 – Abundance of crayfish caught at each drawdown per sweep, split by size category (juvenile or adult).

Table 4 – Carle-Strub depletion estimates with upper and lower confidence intervals for drawdowns split by size class of animal (juvenile <=12 mm CL, adult >12 mm CL).

		Capture efficiency (0-1)	True population estimate *(LCI-UCI, 95%)*	Total percentage of population captured (%)
DGB2016	Adult	0.850	470 (*467-473)*	99.8
	Juvenile	0.762	1203 (*1194-1213*)	98.7
CON2016	Adult	0.682	363 (*354-372*)	97.0
	Juvenile	N/A	N/A	N/A
DGB2017	Adult	0.930	552 (*551-553*)	100.0
	Juvenile	0.767	747 (*740-754*)	98.8
PAD2017	Adult	0.635	631 (*615-647*)	95.3
	Juvenile	0.714	735 (*724-746*)	97.7

Explaining key population characteristics in crayfish populations

For the crayfish individual carapace length model, cheliped condition, presence of fish, gender, individual wet weight and density were added as predictor variables, with carapace length as the dependent variable. All predictor variables were entered into the model simultaneously. The model was found to significantly predict carapace length of crayfish ($F = 649.07$, $df=5,1773$, $p < 0.001$), and accounted for 65% of the variance in length ($R^2 = 0.65$). Density, presence of fish, and individual wet weight all significantly predicted variance in carapace length (Table 5). Cheliped condition was marginally non-significant ($p = 0.057$).

For the crayfish individual wet weight model, cheliped condition, presence of fish, gender, individual carapace length and density were added as predictor variables, with weight used as dependent variable. All predictor variables were entered into the model simultaneously. The model was found to significantly predict individual wet weight of crayfish ($F = 631.12$, $df=5,1773$, $p < 0.001$), accounting for 64% of the variance in weight ($R^2 = 0.64$). Presence of fish and carapace length both significantly predicted variance in individual wet weight (Table 6). Similar to the carapace length model, cheliped condition was marginally non-significant ($p = 0.071$).

Table 5 - Multiple regression model predicting carapace length ($R^2 = 0.65$).

Predictor	B (unstandardized beta)	SE B	β (standardised beta)	t	p values
Gender	.193	.195	.014	0.992	.321
Weight	1.134	.021	.788	54.837	<.001
Fish presence	-1.614	.313	-.080	-5.157	<.001
Density	-1.189	.218	-.086	-5.457	<.001
Cheliped Condition	.379	.199	.027	1.905	.057

Table 6 – Multiple regression model predicting individual wet weight ($R^2 = 0.64$).

Predictor	B (unstandardized beta)	SE B	β (standardised beta)	t	p values
Gender	-.215	.136	-.023	-1.579	.115
Length	.555	-.010	.800	54.837	<.001
Fish presence	.532	.220	.038	2.416	.016
Density	-.010	.154	-.001	-.065	.948
Cheliped Condition	-.252	.139	-.026	-1.807	.071

Discussion

The high catch efficiency of the triple drawdown method at the study sites enabled the detailed examination of demographic data for all size classes of *P. leniusculus* present. Due to the acknowledged limitations of survey data from contemporary methodologies such as trapping, similar data on the structure of crayfish populations has not previously been able to be presented. The triple drawdown methodology therefore provides very important and fundamental ecological information that can crucially inform monitoring, management and potentially eventually also control of invasive crayfish.

Relative proportions of males, females and juveniles within P. leniusculus populations

The ratio of males to females in this study is consistent with the available literature for *P. leniusculus* (see Almeida *et al.,* 2013), and indeed other crayfish species (e.g. Streissl and Hödl, 2002), being in support of an approximately 50:50 ratio. The relative proportions of males, females and juveniles within a population, however, became significantly different between sites once the juvenile class was included in the analysis. The inclusion of the juvenile class influenced whether males and females were under- or over-represented in the sample. The varying proportions of juveniles within each drawdown sample could indicate several limitations or biases associated with the efficiency of the drawdown method for capturing animals of differing sizes. The

potential reasons as to why juvenile abundances varied between drawdowns is therefore an important consideration, and one that is likely related to seasonality and mortality rates. Juveniles hatch from their eggs in early June, and undergo several initial moults to develop whilst still attached to the mother (Mason, 1978, in Ibbotson and Furse, 1995). After several weeks, they begin to become independent of the mother, dispersing to feed and seek out refuges. DGB2016 currently supports no resident populations of fishes, so mortality through predation would be through terrestrial predators (e.g. European otter *Lutra lutra* or grey heron *Ardea cinerea*) and cannibalism, only. Additionally, the DGB2016 drawdown was conducted in mid-June, and as such captured females with hatchlings still attached to the mothers, with some hatchlings in the pre-hatchling 'berried' stage. Considered in a broader context, this proportion of juveniles within a population is likely to be towards the upper range of any given population, as the hatchlings present in this sample would have minimal time to have undergone mortality and independent dispersion, thus maximising their chances of survival and subsequent capture. Conversely, the same factors of mortality through predation and seasonality were likely negatively influencing the proportion of juvenile *P. leniusculus* at the CON2016 drawdown site. As juveniles disperse from the relative safety of the mother and seek refuges and food on their own, significant mortality can be expected to occur through predation and starvation, thus decreasing the base population. Following dispersal, the use of refugia will further decrease the likelihood of the remaining juveniles being sampled. It is likely therefore that the juvenile densities seen in CON2016 were low due to a combination of both predation effects through fish presence (brown trout *Salmo trutta*, salmon *Salmo salar* and bullhead *Cottus gobio*), and sampling being conducted later in the season (August 2016).

Population demographics

It should be noted that the densities reported in this study were categorised relative to one another, and should be considered in the context of new data obtained through the use of the drawdown technique. The sites sampled during this study are considered to support *P. leniusculus* populations at an extremely high density (20.5-110.4 m^{-2}) according to field estimates from the literature (e.g. <1-8 m^{-2}, Ibbotson and Furse, 1995). However, a body of the literature regarding growth, antagonistic interactions such as cannibalism, and population densities is derived from aquaculture, where stocking densities can be far in excess of this (e.g. 1200 m^{-2} juvenile crayfish, Ulikowski *et al., 2006).* As such, comparisons must consider the context from which these crayfish population data are reported.

What is clear from all sites is the large number and overall dominance of juveniles in all the populations. The populations of juveniles at CON2016 (2.88 km downstream of introduction point) are further from the original source of the invasive population, and are subject to many more selection pressures (e.g. fish presence) than the other populations, as well as a smaller adult population from which to be recruited initially. As such, the proportion of juveniles captured through the drawdown is likely to be well below the reproductive potential of the population. The populations at DGB and PAD, however, have been established for almost 20 years (initial population introduced 1.10 km upstream of DGB, 0.84 km upstream of PAD in 1995), and as such do not represent invasive populations undergoing an initial 'boom' to reach or even temporarily exceed the carrying capacity of a site. These populations should therefore to be considered a true reflection of both the reproductive capacity of these sites for *P. leniusculus* and the potential population structure they can achieve given favourable conditions.

In addition to the number of animals in each length-based size class, cohorts can be considered in terms of the functional role they play within the population. Adults that

are below the conventional size to be caught within commercial traps (generally <35 mm CL), but that were found to be sexually mature (>26 mm CL in this study) formed on average 8.7% (range 4.3-12.1%) of the total population (Table 3). In addition, and of particular interest, is the proportion of the total sexually viable demographic that can be removed by trapping (i.e. of the animals >=26 mm CL how many are >=35 mm CL); this value was only on average 20.3% (range 11.8-33.2%). Therefore, almost 80% (by abundance) of the reproductive potential of the *P. leniusculus* populations sampled within this study would fail to be captured, and thus recorded or removed, through conventional trapping. However, it should be noted that as *P. leniusculus* mature and increase in size, the reproductive potential of an individual female increases (McGriff, 1983), as supported in this study. Therefore, whilst the proportion of individuals that are sexually mature but not of trappable size are numerically dominant as compared to the trappable proportion, this may not reflect a dominance in terms of the reproductive potential of this demographic. Future work should focus on what factors influence and control the onset of sexual maturity in populations of *P. leniusculus* across their invasive range, and the comparative reproductive potential of female *P. leniusculus* as they mature, utilising the novel population demographic data revealed by the drawdown technique. Knowledge of the timing, requirements and controls of breeding success in *P. leniusculus* will help implement targeted management to reduce invasive populations.

Cheliped condition

The incidence of cheliped damage was high across this study (33.2-42.4%). Cheliped condition was only reported for animals >12 mm CL, and as such may represent elevated rates of damage when compared to the total population. Westman *et al.* (1999) reported the incidence of cheliped damage to be 7.5-16.5% for *P. leniusculus* using conventional traps in a lake population in Finland. It must be noted that these authors

used trap data and focussed on larger-bodied animals, and were examining a commercial population being established to create a fishery. As such, competition for food and shelter resources would have been minimal, resulting in reduced incidences of cheliped damage through intraspecific antagonistic interactions. Westman *et al.* (1999) found that gender did not influence the probability of damage to occur, which is in agreement with the model results in this chapter. Hudina *et al.* (2012) found this similarity in cheliped damage across the sexes to be maintained throughout the full annual cycle in *P. leniusculus*, with 22% of females and 29% of males displaying damage to chelipeds (35% and 38% displaying any physical damage to the body, respectively).

The majority of studies addressing juvenile crayfish cheliped damage are from stocking and growth experiments for the purposes of aquaculture (e.g. Jones and Ruscoe, 2001; Ahvenharju *et al.*, 2005; Ulikowski *et al.*, 2006). The information provided by these studies, while far removed from the dynamic in-situ systems studied in this chapter, can be used to contextualise current findings. For example, Figiel and Miller (1995) reported 13.1% of juvenile red swamp crayfish (*Procambarus clarkii*, Girard) to have received damage to at least one cheliped. It should be noted that this figure was for individuals who survived the rearing process, and the incidence may well have been higher with injured animals succumbing to cannibalism by conspecifics (e.g. Taugbøl and Skurdal, 1990). No difference in cheliped damage was observed between genders again in Figiel and Miller's (1995) study, but sustaining a cheliped injury did significantly reduce the length of animals.

Density dependent incidence of cheliped damage

When populations of *P. leniusculus* are present at extreme densities, the prevalence of cheliped damage can increase to elevated levels, as shown by Kouba *et al.* (2011), who

reported values approaching 50% of the population in a study of juvenile *P. leniusculus* stocked at densities of over 1000 m^{-2}. Abrahamsson (1966) reported damage rates of 11-32% to the chelipeds of noble crayfish (*Astacus astacus*, Linnaeus), and attributed the variation to the size of the ponds from which they were sampled, with higher density populations from smaller ponds leading to increased antagonistic interactions. Taugbøl and Skurdal (1990) reported mortality rates of 68-90% in 4 month old *A. astacus*, with corresponding rates of cheliped damage at 29-70%, stocked at densities of 40 and 86 individuals m^{-2}. There was no difference between the low and high stocking density treatment for mortality or cheliped condition, however, Taugbøl and Skurdal (1990) acknowledged that the high cheliped damage rates and correlated mortality of the two treatments were likely the cause of the apparent lack of difference, effectively reducing both treatments down to low density through intensive cannibalism.

In this study, cheliped damage was significantly lower at DGB2016 as compared to all other sites, while crayfish density was the highest (110.4 crayfish m^2) at this site. DGB2016 was the earliest of the four drawdowns, occurring in early June, with the subsequent drawdowns occurring in July-August. DGB2016 was the only drawdown where female crayfish were caught that still had hatchling crayfish attached. When female crayfish are harbouring hatchlings, they exhibit behavioural differences in their activity, hiding in refuges to protect their young. Once these berried females have released young, typically two weeks after hatching, the females emerge from refugia and increase their foraging activity to replenish lost reserves. It seems likely, therefore, that this behaviourally driven reduction in the number of large bodied, feeding crayfish contributed to the apparent reduction in antagonistic interactions resulting in cheliped loss. Whilst males are anecdotally the more aggressive gender, evidence suggests that female crayfish are equally as likely to engage in antagonistic interactions (Söderbäck, 1991). DGB2017 was conducted at the same site as DGB2016, but in July rather than

June, and cheliped damage was recorded at 40.2%, supporting the hypothesis of seasonality impacting damage rates.

Abundance of refugia in reducing competition and increasing population density

The presence of adequate refugia is also linked to antagonistic interactions. If shelter is a limiting factor, individuals will compete for space, and thus the carrying capacity of the site will be decreased. The aquaculture literature has considered the provision and abundance of refuges, in the context of increased survivorship and reduced confrontations leading to a greater crop yield. In a study of an Australian commercial crayfish species, the Australian redclaw crayfish (*Cherax quadricarinatus* Von martens), Jones and Ruscoe (2001) found the provision of shelter to significantly increase survival, with shelters offering the greatest heterogeneity best increasing survival and growth. Olsson and Nyström (2009) stocked juvenile *P. leniusculus* at 88 individuals m^{-2} under two experimental refuge densities, 20% and 40% cobble coverage. Both survival and growth rate were significantly higher in the high refugia density treatment, which the authors attributed to a combination of reduced direct and indirect intraspecific interactions, such as increased moulting success due to habitat complexity, and decreased wasted energy expenditure through antagonistic interactions. Thus, the availability and quality of habitat, particularly cobble substrates, can be key determinants in the overall survival and growth of crayfish populations.

Given the abundance of >40 mm cobbles within all sites in this study (77-89%), habitat availability and quality can both be considered to be high. The heterogeneous and complex three-dimensional structure of this cobble habitat could be a key driving force in reducing intraspecific antagonistic interactions and competition, leading to an inflated carrying capacity and ultimately the support of the very high densities of crayfish observed within this study. The suitability of other habitat types or river types should therefore be considered when attempting to understand potential impacts of *P.*

leniusculus, given the potential of the species to achieve extreme densities under highly favourable conditions.

Influence of piscine predators on P. leniusculus populations

Only one site in this study, CON2016, had fish communities present. Brown trout, salmon, bullhead, and European eel were all present at this site, and all these species are known to directly predate crayfish (e.g. Dahl, 1998; Freeman *et al.*, 2010; Reynolds, 2011), as well as indirectly compete with crayfish for food resources. The presence of fish in this study had a significant negative impact on crayfish carapace length ($p < 0.001$), but a significant positive impact on crayfish weight ($p = 0.016$). In the presence of fish predators, crayfish were on average 1.61 mm shorter and 0.05 g heavier. CON2016 had the lowest density of crayfish (20.5 individuals m^{-2}) and crayfish biomass (46.5g m^{-2}) of all sites, as well as the smallest relative percentage of juvenile and smaller crayfish (Fig. 6; Table 2). It seems likely, therefore, that fish presence strongly influences crayfish populations. In some cases, fish predation may even override habitat quality as an influence on crayfish population dynamics, as shown in Nyström *et al.* (2006). The mechanisms by which habitat and fish individually and interactively influence crayfish populations are not fully understood. For the present study, bullhead occupy a similar habitat niche to *P. leniusculus*, being a bottom-dwelling benthivorous species. Large adult bullhead could certainly consume recently hatched or moulting crayfish, but are also themselves predated on by larger crayfish. The efficacy of European eel as predators of crayfish is believed in part to be due to their long cylindrical shape, allowing eels to enter burrows and refuges when hunting crayfish. Indeed, evidence from the eels captured from the study sites indicated eels were predating *P. leniusculus* (Appendix 4). Reynolds (2011) reviewed the interactions between fish and crayfish, describing both the direct and indirect impacts that crayfish can have on fish, but also the impacts fish populations can have on crayfish, for

example competition for prey, competition for habitat, and direct predation on multiple life stages. To add further complexity to the various interactions between fish and crayfish, ontogenetic shifts in habitat usage, feeding strategy and behaviour expressed by crayfish will also alter these interactions depending on the life stages present (e.g. Guan and Wiles, 1998; Usio *et al.*, 2009; Wood *et al.*, 2017), as will the relative demographic proportions of these populations. As such, the pathways by which fish populations interacted with crayfish in the drawdown sites were beyond the scope of the current study, and were not further addressed. However, the development and use of techniques that provide both highly accurate in-situ density estimates and insight into population structure of both fish and crayfish are key to understanding the importance of these interactions. In this respect, the drawdown technique described in this study forms a key potential future part of the crayfish scientific toolkit.

Depletion estimates and capture efficiency

The crayfish capture efficiency of the drawdown method as a whole was generally high (average 66.4%), and two sites achieved a very high capture efficiency (DGB2016 and DGB2017, 78.6% and 84% respectively). When considered in isolation, capture efficiencies of adults and juveniles (excluding CON2016 juveniles) were 76.7% (range 63.5-93%) and 74.8% (range 71.4-76.7%), respectively. There is scarce data available on depletion sampling methods for crayfish, as depletion techniques are typically used for sampling fish communities. As such, to aid in the contextual comparison of sampling techniques, values from the fish literature are used to compare to that of the drawdown for crayfish. The crayfish-derived drawdown values are an improvement on typical values obtained from three-sweep electrofishing for fishes, such as 40-52% for salmonids in cobbled-dominated lotic systems in Norway (Hedger *et al.*, 2013), and 20-57% for trout species in forested streams in Idaho obtained by Peterson *et al.* (2004). Greater capture efficiencies have been reported (e.g. 72% in salmonids, Hanks *et al.*,

2018), but are suggested to be overestimates due to overreliance on a single sampling methodology (Peterson *et al.*, 2004). Interestingly, Peterson *et al.* (2004) also stated that for every 10% increase in cobble substrate cover, a corresponding 37% decrease in capture efficiency was seen.

For crayfish, electrofishing can be effective at determining presence, but provides poor populations estimates. In a comparative study of sampling techniques in streams in Ontario, Reid and Devlin (2014) reported mean capture efficiencies of 30% for electrofishing, alongside 31% for handsearches, for the rusty crayfish (*Orconectes rusticus* Girard), a successful invasive crayfish in many ways analogous to *P. leniusculus*. These poor capture efficiencies were attributed in part to behavioural responses of the crayfish, which resided in refugia on the first sweep, and after being initially shocked became more exposed on subsequent sweeps.

Alonso (2001) reported a capture efficiency of 60% using electrofishing as a survey method for *A. pallipes*, in three gravel dominated headwater streams in Central Spain. A high level of cheliped loss (26%) was observed, and animals of <=13 mm CL comprised only ~17% of the captured animals, compared to animals <=12 mm CL comprising on average 55% of the drawdown catch in the present study. Whilst this may be due to true differences in the population structures between the studies, capture probability of the animal increased with CL in Alonso's study, and the electrofishing method is recognised to be less effective on smaller bodied animals (see Beaumont, 2016).

CON2016 failed to achieve depletion on the third sweep, and only achieved a capture efficiency of 38.4% (Table 4, Fig. 11). As such, CON2016 had wider confidence intervals for the Carle-Strub population estimates. The estimated population was between 644-840, and the number caught during the drawdown was 538. Whilst the drawdown still caught a large number of the individuals, it is important to attempt an

understanding of why this site failed to achieve depletion. Determining accurate crayfish counts in a given sweep in the field was impractical, due to the large number of animals being captured. Given the intensity of resources already required to complete a drawdown within a day, a much larger team would be required to process crayfish on site to confirm numbers, further increasing the resource demand of this method. Weighing animals *en masse* would not solve this issue, as the smaller lighter animals far outnumbered the larger bodied individuals in the populations studied here. As a precautionary approach to incorporate into future attempts at the method, a fourth or even fifth sweep should be trialled where deemed appropriate. Even though the drawdown method is the best in-situ method to sample crayfish populations, 100% capture efficiency will most likely not be achieved, so a reasonable compromise must be sought between capture efficiency and the resource cost of sampling effort.

Methodological limitations of the drawdown technique

The triple drawdown technique provides the best in-situ estimate of *P. leniusculus* population density and population demographics to date. However, the authors acknowledge that the method is not without limitations and therefore do not recommend the total replacement of contemporary sampling methods (i.e. trapping and manual handsearches) with the drawdown method.

As discussed in the previous section, CON2016 failed to achieve depletion for juvenile crayfish, likely related to the general inconspicuousness of these smaller animals. When turning a rock, for example, an operative is drawn to larger animals, as they are more instantly recognisable and often more aggressive, waving their bright red chelipeds in the air in deterrence. The smaller animals were often much more cryptic, as their colouration is closer to that of the substrate, and they are physically smaller and thus more able to hide within the substrate and more likely to be initially overlooked. Additionally, they are less physically active than larger crayfish when exposed. As such,

many of the juvenile crayfish were caught using handnets, either in the main central channel or in small pools that form during the drawdown during the latter sweeps once the majority of the immediately available crayfish had been collected. The issue of an operative shifting focus from larger to smaller animals, and thus invalidating the assumptions of the Carle-Strub method have often been noted (e.g. Hedger *et al.*, 2013). However, with the exception of the juveniles in CON2016, all other sites and size classes achieved strong depletions (Fig. 12; Table 4.). To develop this method further, and protect against the occurrence of increasing juvenile catches towards latter sweeps, operatives should consider expending a fixed handnetting effort within the remaining wetted areas towards the end of each sweep, to attempt to capture significant numbers of juveniles in all sweeps.

The principle physical and logistical considerations in undertaking drawdown sampling relates to resource labour intensiveness. A team of personnel with sufficient training and expertise is essential and the method required a substantial suite of equipment. Good vehicular access to the study site is required to transport heavy pieces of equipment such as pumps (in this case 60 kg each). Each drawdown conducted in this study took a full day on site to complete and several hours in the laboratory to process the catch. In addition, biosecurity was paramount and all equipment had to be disinfected and dried before and after each use, with this significantly increasing the amount of time required per drawdown; the disinfection process often required a day either side of a drawdown event.

The success of a drawdown depends largely on the ability of the pump(s) to overcome the flow of water entering the site and as such, the pumping capacity of the equipment was the main limit to the size and scope of the drawdown. Therefore, the summer months provide the best opportunity for undertaking drawdowns, whilst flows are typically lower. The watercourse used in this study is defined by DEFRA and the EA as

not being a main watercourse, and as such did not require a flood risk assessment or environmental permit to temporarily dewater it. However, larger watercourses, whilst being harder to drawdown due to the volume of water, are further complicated by this licence requirement, at least in England.

Additional considerations for drawing down watercourses are the presence of fish or protected species, which require careful planning to maintain animal welfare, or could necessitate the need for further permits (e.g. for disturbing populations of the European water vole *Arvicola amphibious* L.). High macrophyte cover can also be an issue, increasing the time required to effectively clear or search through the substrate, which also reduces the capture efficiency of the technique. A final consideration is the highly impactful and potential destructive nature of the sampling. Whilst the sample area is kept relatively small and contained, and efforts are made to maintain the welfare of animals in the site and restore the site to conditions as close to before sampling occurred, dewatering a section of a river and removing all of the refugia, however temporary, has a clear negative impact on the local ecosystem.

As a result of all these factors, drawdowns cannot be undertaken in all river systems, and have higher practical and economical costs that other methods. In comparison, the contemporary method of trapping is easy, cost effective and requires relatively little training. Importantly, however, trapping is unable to yield the important population level information, such as density and size class distribution that a drawdown is able to generate. This highlights the need for an intermediate method and future research should prioritise the development of a method that incorporates both the ease and cost efficiency of trapping with the high data quality of the drawdown technique.

Comparison of reported densities

Momot *et al.* (1978) reviewed a range of 24 early studies of crayfish densities (1936-1977), from a range of habitats and species, of which 8 were lotic systems. Densities were generally low (<10 m^{-2}), with the 3 studies concerning *P. leniusculus* reporting values of <1 m^{-2}. None of these studies, however, were from their invasive range in England. In Bubb *et al.* (2004), using a modified surber sampler, it was estimated that there were 20 *P. leniusculus* m^{-2} in the main River Wharfe, a neighbouring catchment to the Ribble used in this study. Unfortunately, neither the data nor the details of the method were reported, and remain unpublished. Guan and Wiles (1997) reported densities of 3-20 m^{-2} in the Great River Ouse (eastern England), a lowland river within the invasive range of *P. leniusculus*, again using a modified netted surber sampler, while Wooster *et al.* (2012) found *P. leniusculus* to attain densities of 15 m^{-2} in a large river in their native range of the Umatilla basin, with 58% of the catch being young-of-year animals. The sampling method for this was a quantified kick net, whereby substrate upstream of the collection bag was disturbed for a fixed period to collect the crayfish. Limitations of this method are clear, in that the evasion potential of larger animals is much greater than that of young-of-year crayfish due to the more developed swimming tail muscles. However, the results presented by Wooster *et al.* (2012) are broadly in agreement with the population structure presented in this study.

The reported density of 110.4 m^{-2} achieved by an invasive population of *P. leniusculus* in this study is concerning, being far in excess of previous estimates. Despite this, the reported density values in this study are still conservative underestimates of the true population size, being based on raw abundance data. Whilst the aforementioned studies of other rivers provide a range of densities covering a range of habitats, this study

provides data on an established population of *P. leniusculus* in its invasive range, in highly suitable habitat, under minimal predation pressure and thus mortality. Therefore, this population density should not necessarily be considered as a baseline population within England, and instead should be viewed as a highly successful population thriving under optimal conditions. Despite this, the evidence that *P. leniusculus* can achieve and maintain such high densities in the wild in England is concerning. Determining the impact of these highly dense populations, along with accurately reporting the densities of other invasive populations, should therefore be at the forefront of management and research.

Conclusion

Knowledge of the structure and density of *P. leniusculus* populations throughout its invasive range are fundamental to both conservation of native species, and to managing this potentially highly damaging invasive species. Contemporary sampling methodologies, in particular trapping, have failed to describe densities of populations in invaded rivers on the scale reported in this study, and as such may miss key aspects of either invasive population structure or density that drive interactions between crayfish and native biota. Established methods of physical crayfish control, such as trapping, are effective at targeting larger individuals. However, the triple drawdown methodology has shown the relative importance of smaller individuals within a population with nearly 80% of the sexually mature, and thus actively recruiting, individuals in this study being too small to be captured by conventional traps. This point is particularly salient with respect to the potential for trapping as a control method for *P. leniusculus* populations, due to the likelihood of failure in removing individuals from the population before they attain sexual maturity.

Whilst this study provides insight into the demographics of juveniles and the broader size classes of larger crayfish, there is a great deal to be further researched in terms of recruitment and mortality of juvenile crayfish both within a single reproductive season, and on a longer temporal scale, as a population establishes, matures and stabilises. There exists an urgent need for new sampling methodologies to be trialled, which can harness the sampling success of the drawdown technique with the technical and economic ease of methods such as trapping.

Chapter 5 – Density dependent shifts in diet and cannibalism rates in the invasive signal crayfish *Pacifastacus leniusculus*

Introduction

Crayfish act as keystone species as defined by Paine (1980), disproportionally impacting other taxa through direct interspecific interactions (see Holdich *et al.*, 2014). Crayfish can also function as ecosystem engineers in freshwater ecosystems (Jones *et al.*, 1994), altering the habitats available within the system through their activities, and functioning as key components of energy recycling and transfer within food webs (Alcorlo *et al.*, 2004). Despite the acknowledgement that crayfish can be integral to ecosystem processes at multiple levels (Twardochleb *et al.*, 2013), the literature provides differing evidence for impacts of crayfish on ecosystem function (e.g. James *et al.*, 2014; Wood *et al.*, 2017), and has notably changed over time due to new findings through the utilisation of complementary dietary study techniques (e.g. views expressed in Momot *et al.*, 1978; Momot, 1995). Omnivory is a key strategy by which crayfish can regulate energy and nutrient transfer within freshwater systems (e.g. Lodge *et al.*, 1994), and understanding how their diet changes under different conditions is key to understanding their impacts within ecosystems (Singer and Bernays, 2003). Crayfish behaviour can change as a function of geography, and thus impacts can vary on whether crayfish are in their native or invasive range. For example, in a study of signal crayfish *Pacifastacus leniusculus* by Bondar and Richardson (2009), there was no ontogenetic difference or density mediated impact of *P. leniusculus* on invertebrate communities in its native range. In contrast, populations of *P. leniusculus* in its invasive range in England are known to severely negatively impact many aspects of native ecological communities, including through direct predation on fish and invertebrates (Guan and Wiles, 1997; Mathers *et al.*, 2016).

The polytrophic feeding of crayfish can facilitate indirect impacts on a system, as well as direct impacts of consumption. For example, decimation of macrophyte stands by crayfish (as in Lodge and Lorman, 1987) can not only directly impact macrophyte

biomass, but also remove physical habitat for invertebrates that inhabit macrophyte stands, whilst also impacting abiotic features such as flow dynamics in rivers. However, in a study by Creed (1994), grazing by the North clearwater crayfish (*Oronoctes propinquus* Girard) on the macroalga *Cladophora* resulted in a 2-3 fold increase in two grazing invertebrate species, indicative of an indirect facilitation of aquatic invertebrate communities by the crayfish. As such, the complex interactions that crayfish form can limit and challenge our understanding of the role of crayfish in ecosystems (Reynolds *et al.*, 2013).

To attempt to understand these interactions, multiple complementary methods are often advocated in studies of crayfish diet (e.g. Rudnick and Resh, 2005; Olsson *et al.*, 2008), and thus Gut Content Analysis (GCA) and Stable Isotope Analysis (SIA) have often been employed to attempt to adequately understand the diet of *P. leniusculus* in situ (e.g. Bondar *et al.*, 2005; Nyström *et al.*, 2006). Contradictory results which fail to explain diet can occur when using analytical methods for diet in isolation, particularly those relying solely on either direct GCA quantification or SIA (Parkyn *et al.*, 2001; Stites *et al.*, 2017).

SIA has been used in freshwater ecology to great effect in answering questions on the transfer of energy within a system (Post, 2002), and between trophic levels (Peterson and Fry, 1987). Such an approach provides a longer term record of the assimilated prey items, with isotopic signatures taking weeks to months to typically form (Stenroth *et al.*, 2006). The isotopic relationship between a predator and prey suggests on average a 2-3‰ enrichment of heavy nitrogen ($\delta^{15}N$) between trophic levels (Minagawa and Wada, 1984), with an enrichment in heavy carbon ($\delta^{13}C$) of 0-1 ‰ indicating the food item is a likely source of carbon for an animal (Finlay and Kendall, 2008; Bašić *et al.*, 2015). GCA has long been used to begin to determine feeding habits of individuals to populations, including crayfish (e.g. Frost, 1954; Hollows *et al.*, 2002). While GCA

provides a snap-shot view of the prey items that are consumed it can be biased against items that are easily assimilated, such as the soft body tissues of invertebrates (Marufu *et al.*, 2018). Crayfish heavily masticate their prey items as they enter the foregut, due to their chitinous grinding mill. As such, whilst numerical methods such as direct counts of prey items can be used, and are fast and relatively easy to employ, they can overestimate highly abundant smaller prey types (Hyslop, 1980).

Changes in diet as a function of density and ontogeny in crayfish

The density and life stage of populations can often determine the impacts invasive species exert (Catford *et al.*, 2012; Ruokonen *et al.*, 2014; Wood *et al.*, 2017). Early reports on *P. leniusculus* diet concluded them to be functional recyclers of detritus and plant material (Momot *et al.*, 1978), facilitating the transfer of energy to higher tropic levels (D'Abramo and Robinson, 1989). Many crayfish species, including *P. leniusculus,* have been reported to undergo ontogenetic shifts in feeding patterns, relying on invertebrate tissues at smaller sizes then switching to a diet dominated by plant and detrital matter at larger carapace lengths (Guan and Wiles, 1998). Whilst *P. leniusculus* has been proven to readily consume plant and detrital material in aquatic systems (Guan and Wiles, 1998), the proportion of animal protein in their diet is believed to have been underestimated by former studies (Momot, 1995). Although omnivorous, crayfish may preferentially predate invertebrates at all sizes, as invertebrate tissues are an optimal source of dietary protein for growth in *P. leniusculus* (Bondar *et al.*, 2005), significantly increasing growth rates over detrital and plant based energy sources. In addition, in response to high densities of conspecifics, crayfish can alter their feeding strategies through preferentially feeding on select prey (Nilsson *et al.*, 2000), potentially increasing their trophic niche width (Olsson, 2008). The incidence of cannibalism may also be linked to crayfish population density, as suggested by Houghton *et al.* (2017) in their study of invasive populations of *P. leniusculus* in a

lowland rivers in Scotland. The relative abundance of conspecifics approaching half the size of the cannibal was reported as a key determinate of cannibalism rates in the studied *P. leniusculus* population. Ecosystems can be regulated through omnivory by both top predators and intermediate consumers (Pace *et al.,* 1999), of which crayfish can function as both. Additionally, habitat structure and diversity can buffer the impacts of omnivorous predators (e.g. Diehl, 1992). Whilst omnivory is a stabilising mechanism in ecosystems, another potential stabilising mechanism in crayfish is cannibalism, which is thought to operate when crayfish occur at high densities, leading to intraspecific competition for resources (Bondar *et al.*, 2005; Kouba *et al.,* 2011). *P. leniusculus* are purported to exhibit size-mediated, density-dependent cannibalism (e.g. Guan and Wiles, 1998) but this behaviour is often confounded through data derived in the literature from aquaculture scenarios, which are over-stocked and lack habitat complexity when compared to natural systems. Therefore, in order to begin to understand the processes by which *P. leniusculus* achieve and maintain extreme densities in their invasive range in England, there exists a need to better understand dietary strategies in the field, supported through the now available high quality demographic data for *P. leniusculus* populations (Chapter 4).

Chapter aims

The aims of this study were firstly to identify, through the use of Gut Content Analysis, the frequency of occurrence and dietary importance of cannibalism within two well-established populations of *P. leniusculus* in their invasive range in England. A further aim was to determine if diet changed as a function of the size of an individual *P. leniusculus* specimen, or due to increasing population density. It was hypothesised that larger *P. leniusculus* specimens would have a higher trophic positioning, and that cannibalism would be more likely to occur in these larger animals than in smaller conspecifics. Secondly, it was hypothesised that diet would differ between populations

densities, with higher densities increasing sub-optimal foraging, resulting in a broader niche width for high density populations of *P. leniusculus* (Chapter 4).

Methods

Site description and density estimates

This study utilised crayfish sampled during the drawdown study of Bookill Gill Beck (BGB). BGB is a site where high densities of invasive *P. leniusculus* are present. With densities in excess of 110 m^{-2}, at a biomass of almost 96 g m^{-2} (Chapter 4), significant energy sources must be utilised to maintain this standing crop. In addition to this, much of the previous faunal diversity has been lost, resulting in a degraded ecosystem depleted in both variety and abundance of prey items (Chapter 3). No fish are present in the upper reaches of BGB and macroinvertebrate communities are reduced in terms of both biomass and biodiversity (see Chapter 3). Predatory fish can reduce the number of trophic levels that crayfish can feed on and also the diversity of crayfish diet (Jackson *et al.*, 2012), and as such their exclusion from the study (through absence) helps strengthen the analysis.

By using the data derived from the drawdown technique, two sites on BGB were chosen to compare the diet and occurrence of cannibalism between, namely sites PAD2017 and DGB2017 (see Fig. 5 of Chapter 4). These sites had the advantage of being sampled in the same season, being within the same stretch of BGB, and having similar environmental characteristics (Table 1), but with DGB2017 having double the population density of *P. leniusculus* than PAD2017 (86 m^{-2} and 44 m^{-2}, respectively). Invertebrate community data for DGB2017 and PAD2017 was derived from surber samples (n = 20), providing estimates of the relative abundance of invertebrate families in the sampled river reaches, namely *Leuctridae* (60%), *Heptageniidae* (35%), *Chironomidae* (3%) and *Ephemeridae* (2%).

Table 1 – Physical descriptors of both study sections, namely DGB2017 and PAD2017, in Bookill Gill Beck. In-channel substrate reported as nearest 5%.

Physical parameter	DGB2017	PAD2017
Average wetted width (m)	2.0	1.5
Average water depth (cm)	7.6	11.4
Flow (m/s, 30 second average)	1.5	1.0
Substrate (%Silt/Sand, %Gravel, %Cobble)	10, 10, 80	5, 5, 90
pH	8.2	8.0
DO (mg/L)	9.6	9.3
Water temperature (^{o}C)	15.4	15.4
Conductivity (µs/cm)	293	292

For the sake of ease of the reader, and consistency within this chapter, the two sites (DGB2017 and PAD2017) are hereafter referred to by their comparative density, being either 'high' or 'low' respectively, and therefore justified within the context of comparison within this study. It should be noted that whilst PAD2017 is considered low as compared to DGB2017 (44 to 86 individuals m^{-2}), both of these values are far in excess of what is often reported within the literature (e.g. <1-8 m^{-2}, Ibbotson and Furse, 1995, see Chapter 4 for further commentary). Following capture in the drawdown studies, all crayfish were put onto ice for transportation back to the laboratory, then frozen. Freezing arrests digestion and preserves both the gut and gut contents for analysis. *P. leniusculus* were selected for diet analysis on the basis of being the largest individuals caught at either site, with efforts made to equally represent both males and females (n = 14, M:F 8:6 at 'High Density', n = 15, M:F 9:6 at 'Low Density'). In addition, Young-of-Year (5 mm CL) and yearling (12 mm CL) *P. leniusculus* were selected from each site for stable isotope analysis.

Gut content analysis

The foreguts of all 29 adult signal crayfish were dissected out (Fig. 1), and full and empty gut weights were obtained for each crayfish (to an accuracy of 0.0001 g), with the difference forming the gut content weight. Gut contents were separated using a 1000 µm and 300µm sieve stack. Food items smaller than 300 µm were deemed too small to identify. Macrofragments (retained in the 1000 µm sieve) were counted and then air dried and weighed, and microfragments were counted.

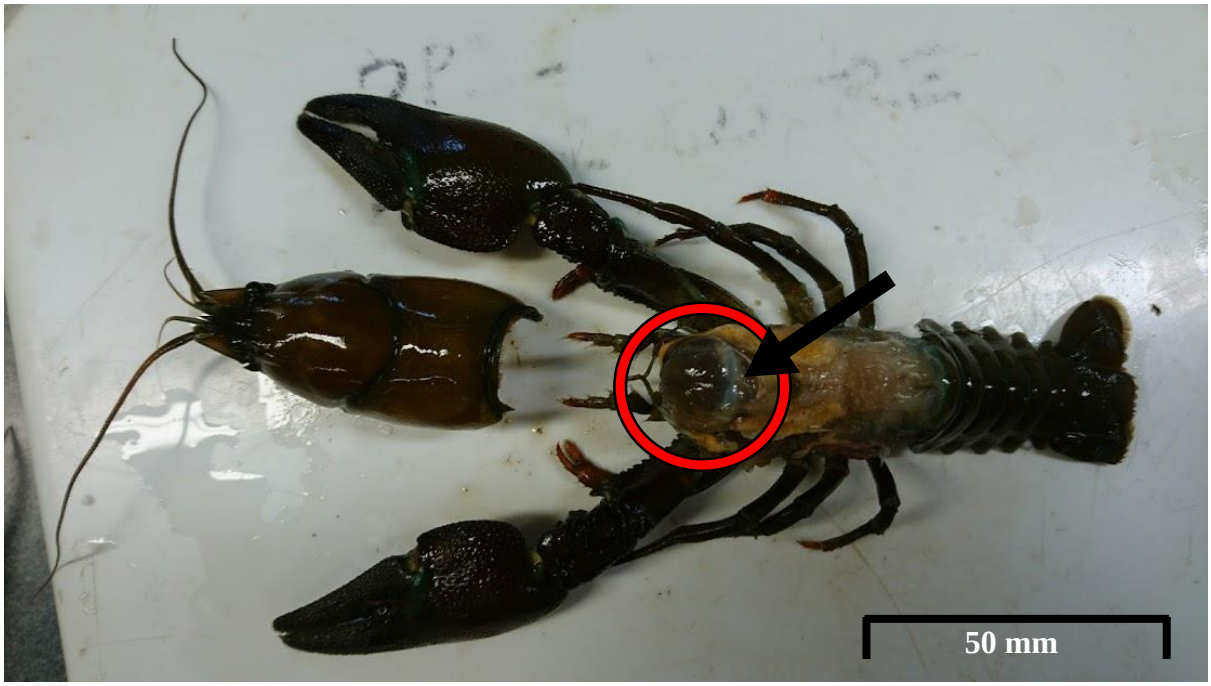


Figure 1 – Location of the foregut in *P. leniusculus*, shown circled in a large adult male.

Food sources were grouped into one of 7 categories; crayfish, Coarse Particulate Organic Matter (CPOM), amorphous organic detritus, or the invertebrate families *Ephemeridae*, *Leuctridae*, *Heptageniidae* and *Chironomidae*. Inorganics (n=11 individual items) are not required for digestion in crayfish due to the masticating plates and grinding mill in the foregut, and as such were viewed as being in there by chance rather than intentional consumption and thus omitted from analysis. The frequency of

occurrence (F_P) of prey items between guts was calculated as in Marufu *et al.* (2018), using the formula:

$$F_P = NS_j \, x \, \frac{100}{NS}$$

where NS is the number of stomachs, and j is the specific food item (Hyslop, 1980). The relative frequency of prey items by count within the guts were also compared between low and high density crayfish populations. Comparative gravimetric analysis was conducted on the dry weights of both CPOM and crayfish material within the crayfish guts, as determined by separating out both components and air drying until a constant weight was achieved. Large errors can be associated with increased water retention of small food items (Hyslop, 1980), which are common within crayfish guts due to the grinding feeding action. Therefore, both wet weight and volumetric analyses of the comparative amounts of CPOM and crayfish components in the guts were not attempted.

The wet weight of gut contents was determined for each animal, and compared against the total wet weight of the animal to determine a Fullness Index (F_i), which is a measure of feeding intensity, calculated as:

$$F_i = \frac{Total\ wet\ weight\ gut\ content}{Total\ wet\ weight\ animal} \, x \, 100$$

The Fullness Index (F_i) is a measure that is therefore relative to the size of the animal, which is a more useful measure when comparing the gut contents of differently sized animals (Hyslop, 1980), as larger animals have larger guts and thus a larger maximum potential content. No empty guts were recorded, indicative of highly voracious feeding (e.g. Marufu *et al.*, 2018).

The electivity of crayfish for each invertebrate family was calculated using Jacob's electivity index (Jacobs, 1974), with 0 to -1 indicating negative selection, and 0 to 1 indicating positive selection. Values for Jacob's electivity index were compared against the relative proportions of *Leuctridae, Ephemeridae, Heptageniidae*, and *Chironomidae* found in the respective stream site.

Stable Isotope Analysis

P. leniusculus were frozen upon capture, as freezing and defrosting processes are not thought to impact either the $\delta^{13}C$ or $\delta^{15}N$ in tissues as preservative agents such as IMS do. Tail muscle tissue was chosen for isotopic analysis, as is common practice in crayfish (e.g. Bondar *et al.*, 2005; Jackson *et al.*, 2014), because whole body values can provide alternative or misleading isotopic results (Stenroth *et al.*, 2006). A total of 29 adult *P. leniusculus* (average 43.2 mm ± 7.3), 15 yearlings (12 mm CL, 8:7 High:Low density), and 18 composite hatchling samples (5 mm CL, 10:8 High:Low density) were processed.

Crayfish gut tracts were removed from the tail to prevent cross-contamination of the isotopic signature of the muscle tissue with the processed gut waste (Fig. 2). Muscle tissue was then freeze dried, and ground into a fine power in a pestle and mortar. Composite samples were used for hatchlings, consisting of 5 individuals, due to minimal material being left after freeze drying; all other samples represent a single discrete animal. Samples of 0.7 mg dried ground tissue were weighed out into 5 x 7 mm tin capsules, and processed at the Life Sciences Mass Spectrometry Facility (LSMSF), a NERC facility in East Kilbride. Samples were analysed using an Elementar Pyrocube elemental analyser and a Thermo Fisher Scientific Delta Plus XP mass spectrometer.

Figure 2 – Preparation of tail muscle tissue in a young-of-year (Y-o-Y) *P. leniusculus*, showing a) the full juvenile, b) the separation of the tail, and c) the removal of the gut tract facilitated by removal of the telson, the middle plate of the tail, which is connected to the gut tract.

The delta notation δ was used to express isotopic ratios, reported as per mil (‰), with positive values indicating an enrichment in the heavy isotope, using the formula:

$$\delta(‰) = \left(\frac{R_{sample}}{R_{standard}} - 1\right) x\ 1000$$

with R being the ratio of heavy-to-light isotope for $^{13}C/^{12}C$ or $^{15}N/^{14}N$. Internal working lab standards GEL (gelatine), ALAGEL (alanine) and GLYGEL (glycine), and USGS40 (L-Glutamic acid) with known $\delta^{13}C$ and $\delta^{15}N$ were used (Sigma-Aldrich Company Ltd, Gillingham, UK), and calibrated to the international standards Vienna Pee Dee Belemnite (VPDB) for carbon and atmospheric nitrogen (Air), respectively. Internal standards were run every 10 samples to check and account for drift over the analytical run.

Lipid extraction for stable isotope analysis

As well as different tissues having different ratios of heavy isotopes, the relative lipid content of the same tissues can affect the enrichment of heavy carbon and nitrogen (Stenroth *et al.*, 2006). As such, both tissue selection and tissue preparation can be important in accurately determining isotopic values. Lipid extraction was conducted on

a subset of animals from all size classes and densities, following methods adapted from Sweeting *et al.* (2006) and Joyce and Pirozzi, (2016), originally from Folch *et al.* (1957). One male, one female, and two juveniles were analysed from each of the two studied crayfish populations. A hatchling composite sample was also analysed from the low density population, but it was not possible to analyse a hatchling sample from the high density population due to insufficient material being retrieved from the lipid stripping process. Lipid extracted samples were then included in the isotopic analysis runs to test for changes in $\delta^{13}C$ and $\delta^{15}N$ ratios in treated tissues.

Statistical analysis

Pearsons chi-squared tests were used to compare GCA count data between both crayfish populations and the environment. Macroinvertebrates violated the assumptions of chi-squared by having over 20% of the expected counts at <5 due to their relative scarcity, and so were pooled to strengthen analysis. *P. leniusculus*, CPOM, amorphous and 'macroinvertebrate' prey categories were therefore used to compare both dietary prevalence and abundance by counts, with relative proportions of individual macroinvertebrate families being presented graphically. Only crayfish that had consumed other invertebrates were used to determine relative macroinvertebrate abundances in the guts and dietary electivity. Two multiple regression analyses were conducted (SPSS 24), to model the effects of crayfish size, density and either $\delta^{13}C$ or $\delta^{15}N$ on the ratio of the other heavy isotope, respectively. Statistical assumptions of normality and collinearity (MLR) were checked, and Durbin-Watson values were within acceptable ranges (>1.5, <3.5). Where assumptions were violated due to over-correlation of the predictor variables, models were run with each violating predictor variable, and the greatest R^2 value was used to determine the most suitable predictor variable. Any and all outliers were corrected for by adding one unit to the second largest value of the dataset. This was undertaken to retain the outlier as the largest value for

that group, but to prevent the outlier from over-influencing results of analysis. This method of correcting outliers is preferable to replacing the outlier with the mean, or deleting the value, due to the impact on the distribution of the dataset of these methods.

Results

Gut content by sex, size, and site

Male and female *P. leniusculus* did not have significantly different carapace lengths, weights, items by count, full gut weights or empty gut weights between the high and low density sites ($p > 0.05$ for all). However, *P. leniusculus* specimens in the low density site were significantly larger than the high density site ($t = -8.804$, $df = 27$, $p < 0.001$, mean 36.7 and 49.2 mm CL, respectively), and had significantly higher total gut weights ($t = -7.693$, $df = 27$, $p < 0.001$) and empty gut weights ($t = -6.823$, $df = 27$ $p < 0.001$). The (F_I) revealed one anomalous animal from the high density site (Fig. 3), this being a male crayfish (36.0 mm CL and 15.1 g) with a F_I of 3.20, with the next fullest animal in the high density site having a F_I of 2.01. When corrected for size of animal, and the one anomalous crayfish from the high density site, density significantly affected fullness ($t = -2.183$, $df = 27$, $p = 0.038$), with crayfish in the low density sites having fuller guts ($F_I = 1.96$) than in the high density site ($F_I = 1.29$).

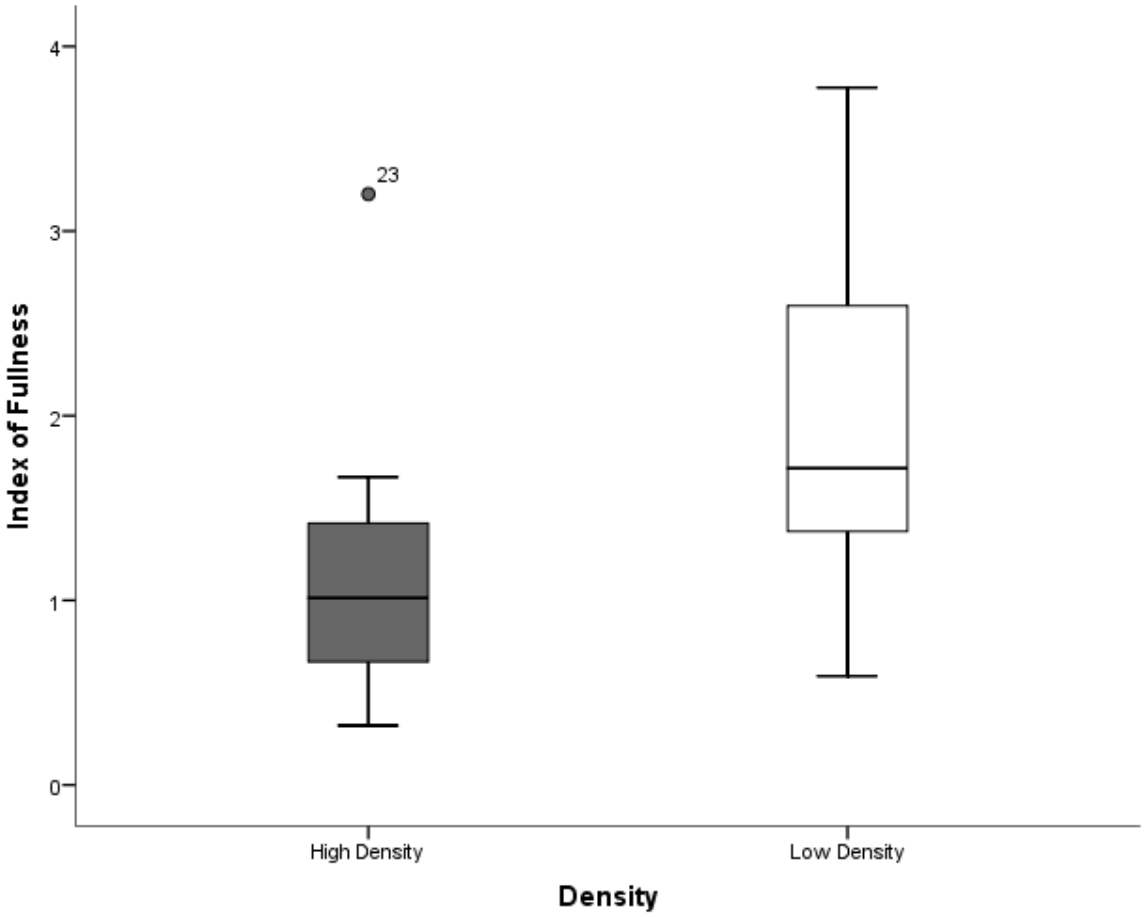


Figure 3 – Index of Fullness (F_I) for low and density site, with one anomalous data point

Descriptive analysis of gut contents

The crayfish guts from both the high and low density sites contained all 7 categories of food items (Fig. 4 and 5). Crayfish remains and CPOM occurred in 100% of the guts in the high density site, and 87% and 93% of the crayfish guts in the low density site respectively (Fig. 6). Crayfish material made up significantly more of the diet by count in the low density site than in the high density site ($\chi = 255.044$, df = 3, $p < 0.001$), on average 47% and 24.7% respectively; counts of CPOM and amorphous materials did not differ significantly between densities ($p > 0.05$).

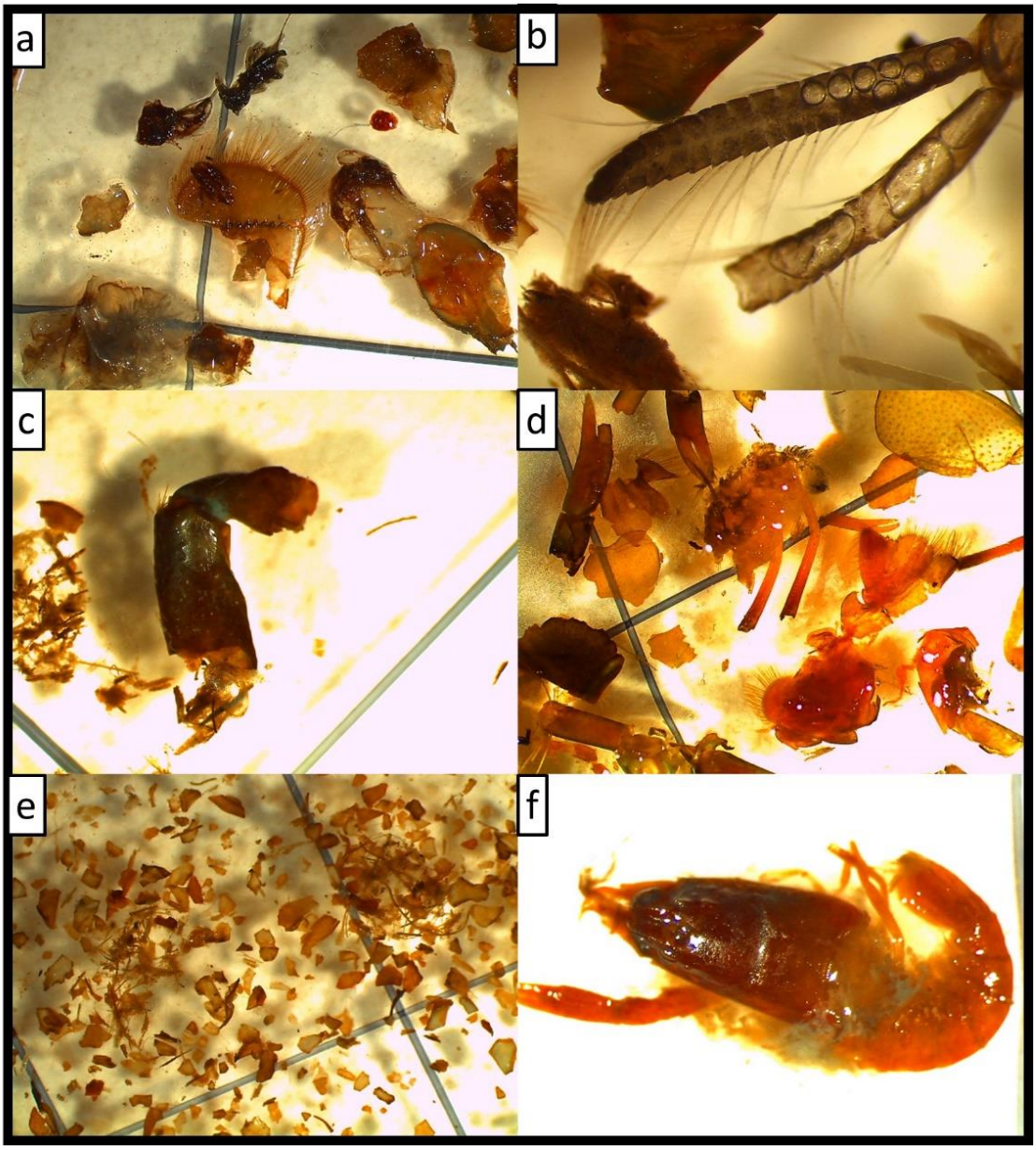


Figure 4 – Remains of consumed crayfish, showing a) a telson (middle piece of tail), b) a maxilliped (feeding apparatus), c) a chitinous joint, d) assorted uropod (tail piece), cheliped (claw), and carapace (shell) pieces of several crayfish, e) multiple smaller carapace fragments (square is 1 x 1 mm), and f) entire Y-o-Y crayfish that was consumed whole.

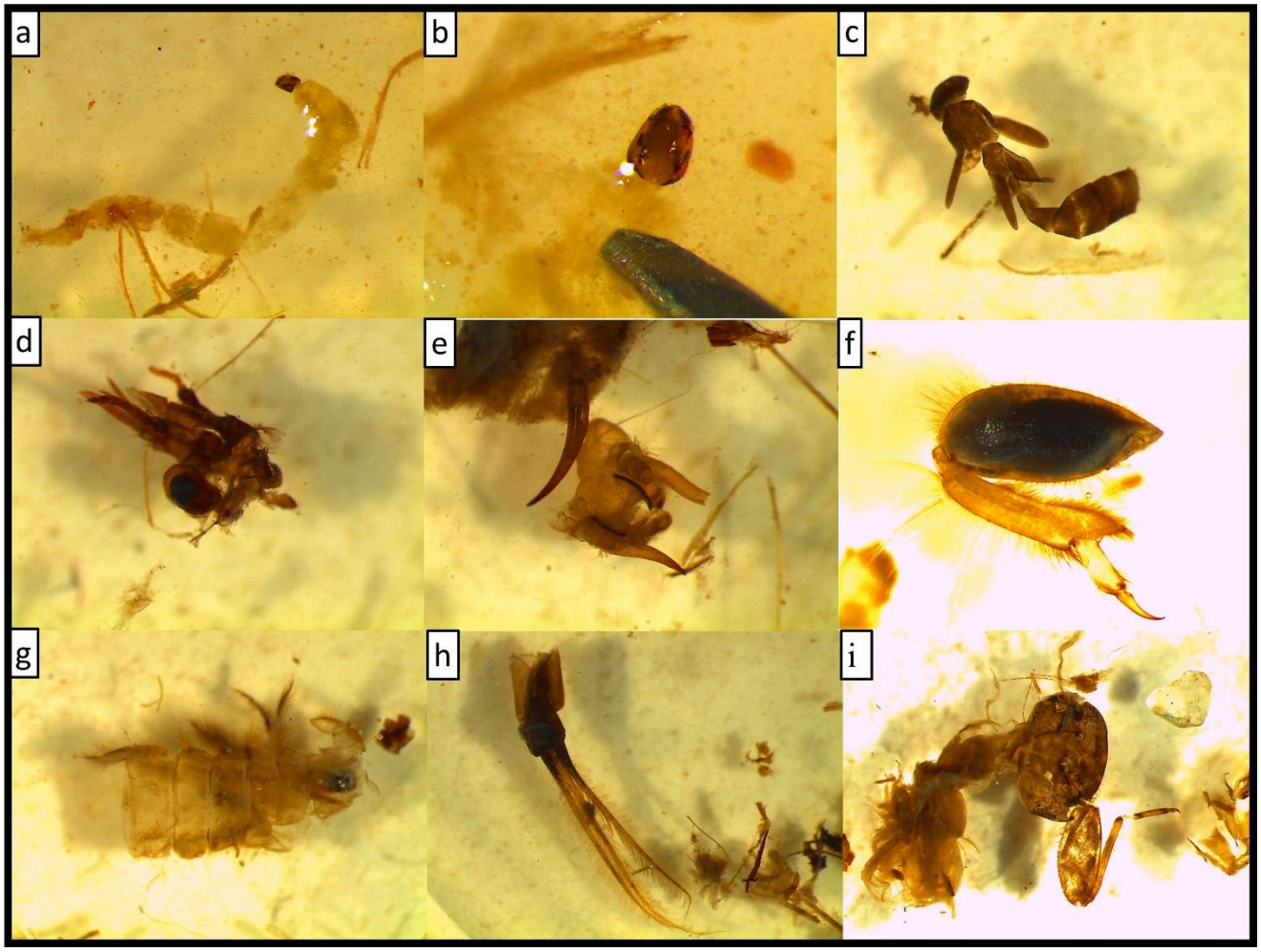


Figure 5 – Invertebrate remains found in guts of *P. leniusculus*, including *Chironomidae* (a-b), *Leuctridae* (c), the head, burrowing tusks, leg, abdomen and gills, and tail of *Ephemeridae* (d-h, respectively) and a *Heptageniidae* (i).

Evidence of *Leuctridae* within the guts could not facilitate an identification to species level, as both *L. inermis* and *L. hippopus* were present at BGB; this was often the case for both *Chironomidae* and *Heptageniidae* remains, too. Only one species of *Ephemeridae* was present at BGB, namely *Ephemera danica*, an easily identifiable large bodied burrowing mayfly (Fig. 5 d-h). When pooled into a single 'macroinvertebrate' prey category to satisfy the assumptions of chi-squared analysis, other invertebrates were not significantly more likely to be found in the guts of crayfish from either the high or the low density site ($p > 0.05$; Fig. 6). Other invertebrates were, however, consumed significantly more in the high density site ($\chi = 255.044$, $df = 3$, $p < 0.001$) comprising on average 8.8% of crayfish gut contents by count, as opposed to 0.9% in the low density site (Fig. 7).

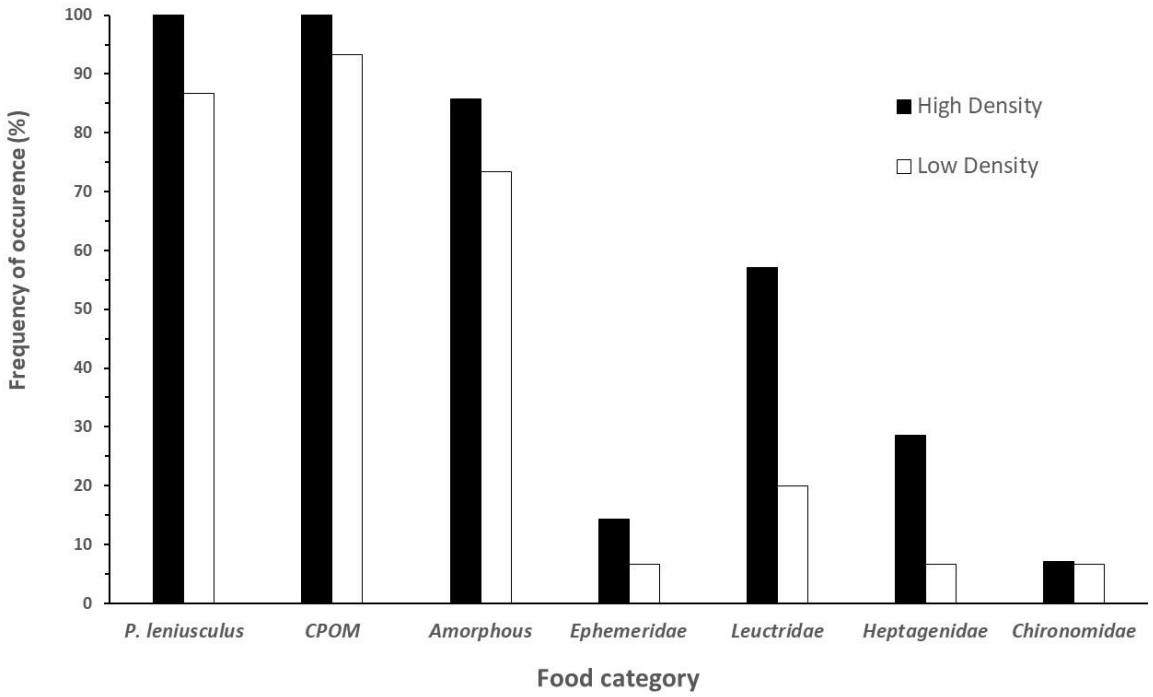


Figure 6 – Frequency of occurrence (F_P) of 7 food groups in the GCA of crayfish at the high density (dark bars) and low density (light bars) study sites.

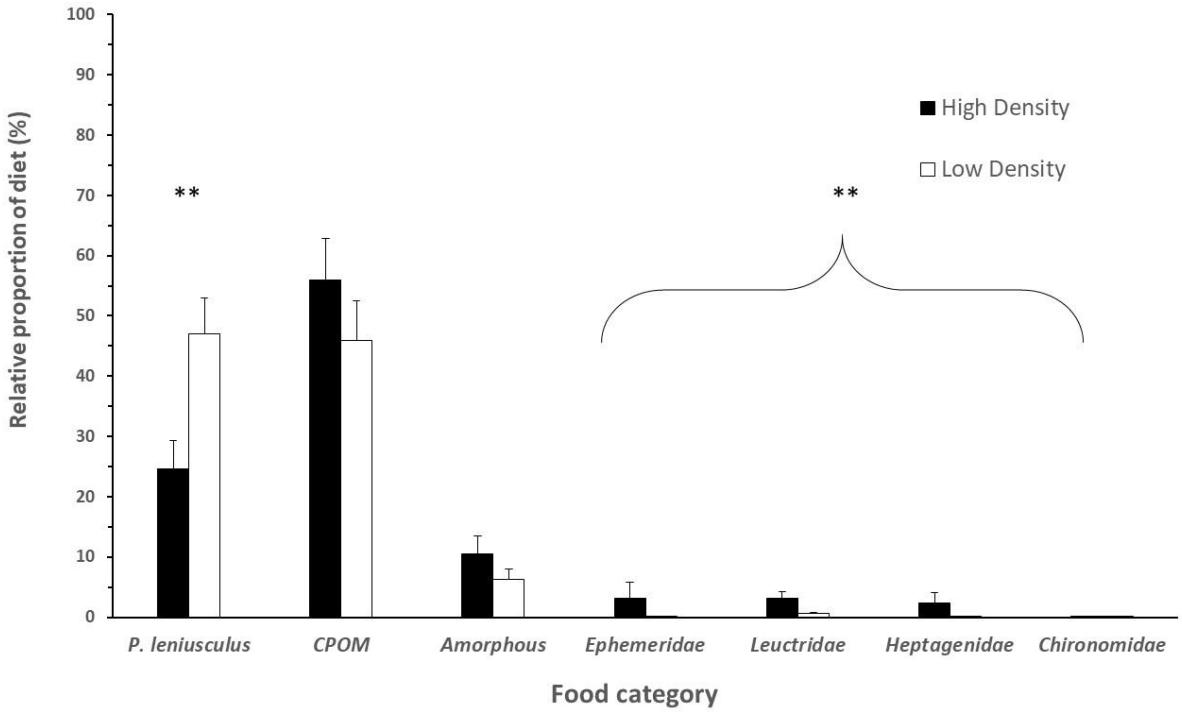


Figure 7 – Relative proportions (%) of the 7 food categories found in the guts of *P. leniusculus* at the high (dark bar) and low (light bar) density sites (error bars = SEM). ** indicates couplet significantly differing at the p = 0.05 significance threshold.

The community composition of the crayfish guts differed when compared to the community composition in the stream environment (Fig. 8 all values are averages). *Leuctridae* were the most commonly found invertebrate item in the environment (60 %), followed by *Heptageniidae* (35%), *Chironomidae* (3 %), and *Ephemeridae* (2 %). The contents of crayfish guts by count differed significantly from the environmental surber sample data ($\chi^2 = 127.110$, df = 3, $p < 0.001$). The relative proportion of *Ephemeridae* found by count in the guts of both the low (16.7 %) and high (21.5 %) density crayfish was significantly higher than in the environment (1.6 %), and the relative proportions of *Heptageniidae* by counts in the low (11.1 %) and high (20.7 %) density populations were significantly lower than that of the environment (35.3 %). Finally, *Chironomidae* were proportionally more abundant in the guts of *P. leniusculus* in the low density population (11.1 %) than in the environment (2.9 %). No other macroinvertebrates differed significantly from the environment in GCA counts.

Crayfish from both density sites exhibited strong prey electivity, as shown through Jacob's electivity Index (D_J, Fig. 9). *Chironomidae* were strongly positively selected for in the low density population (0.6), and negatively selected for in the high density population (-0.24). *Leuctridae* were not selected for in either density population (-0.08 to 0.02). *Heptageniidae* were negatively selected for in both low and high density populations (-0.63 and -0.35 respectively), while E*phemeridae* were strongly positively selected for in both the low (0.85) and high (0.89) density sites, respectively.

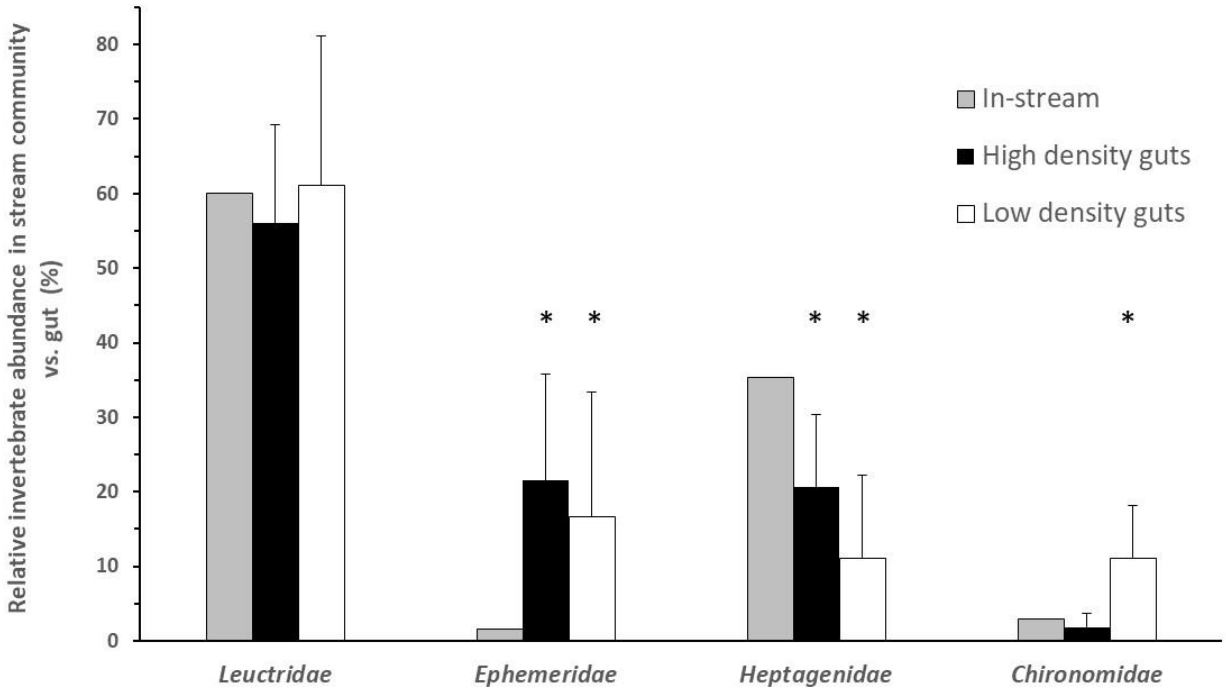


Figure 8 – Comparison of invertebrate composition (family level) in the environment (grey bars), and in the high (dark bars) and low (light bars) density crayfish guts (error bars = SEM; * indicates significance at the p = 0.05 level).

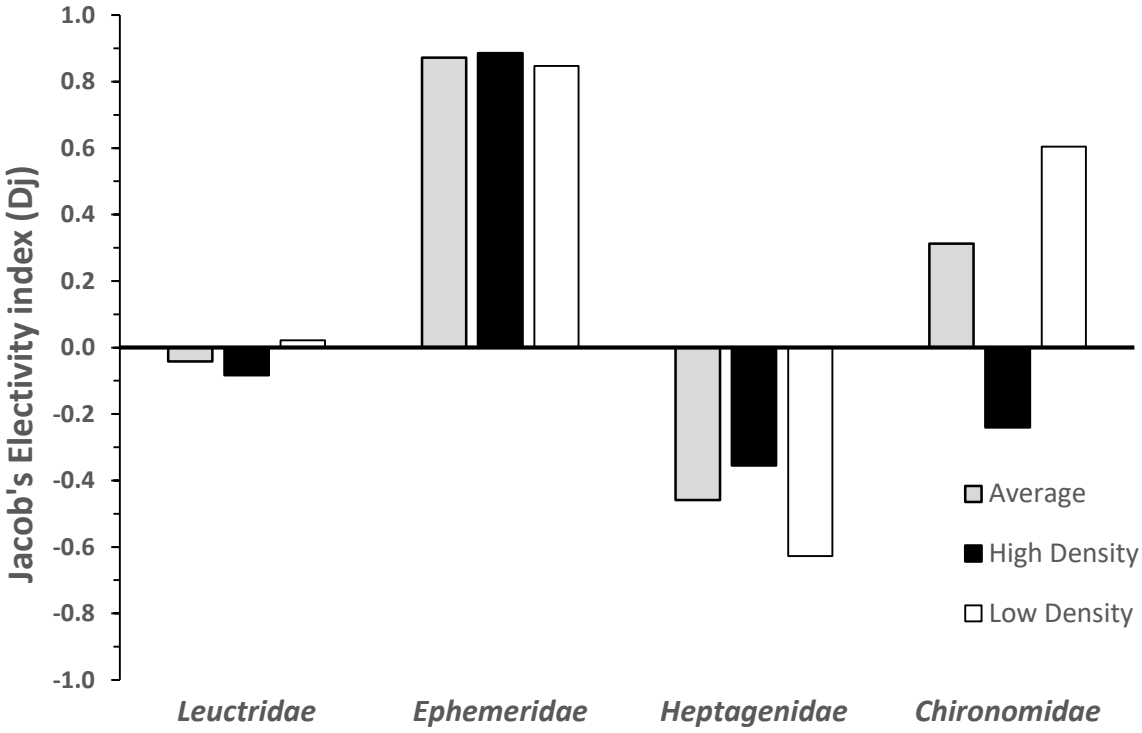


Figure 9 – Jacob's Electivity Index (D_j) for each invertebrate family group, indicating positive (>0) or negative (<0) selection, by counts from crayfish guts.

Lipid extraction

Lipids were extracted to test whether $\delta^{13}C$ or $\delta^{15}N$ values differed for stripped tissues. Lipid concentrations of tail muscle tissue for both high and low density populations were high (average 57.5%), and did not differ significantly between populations ($p > 0.05$).

Tissue samples became significantly enriched for heavy nitrogen ($t = 2.710$, $df=16$, $p = 0.015$), with an average increase of 0.45‰ (Fig. 10a). Samples did not change in the mean value or distribution of carbon (Fig. 10b; mean difference = - 0.04‰, $p > 0.05$). Due to the consistent average enrichment of $\delta^{15}N$ at both the high and low density crayfish sites and in the tissues of all size classes of *P. leniusculus*, lipid extracted values were not used, thus avoiding applying unnecessary errors when calibrating using a sub-set of lipid extracted tissue values.

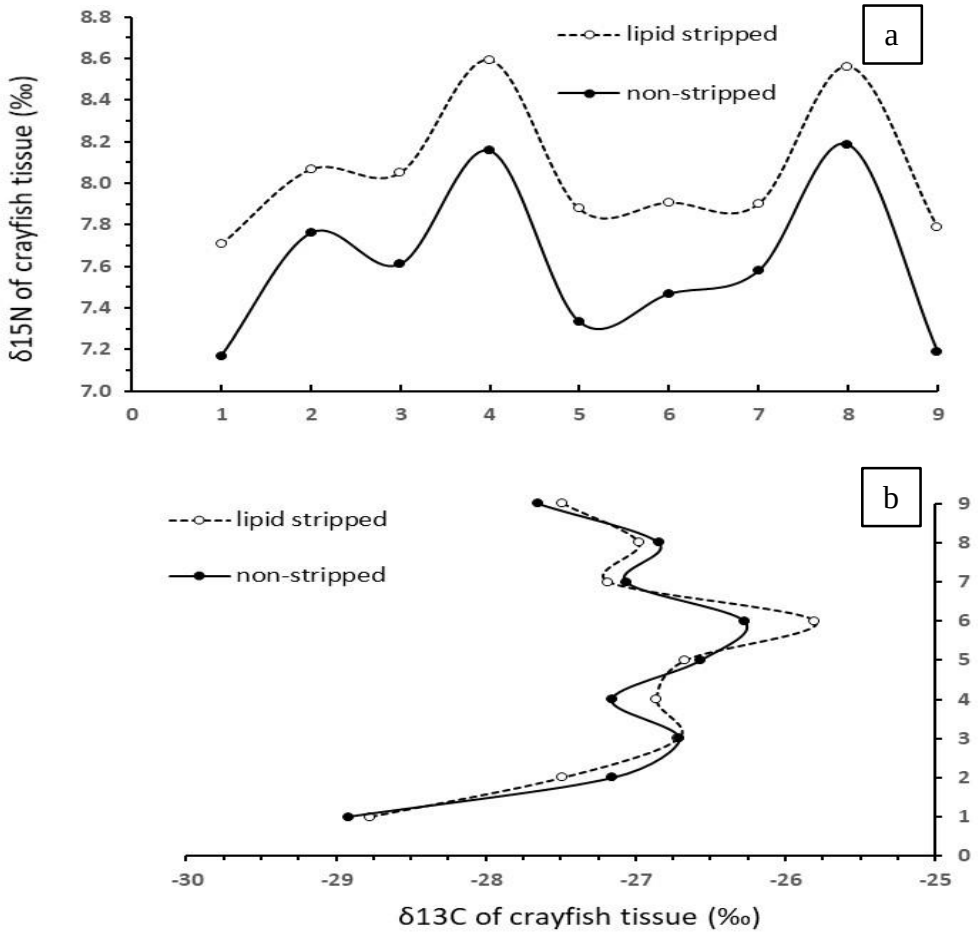


Figure 10 – Heavy isotope values for non-treated tissue samples versus lipid extracted samples for $\delta^{15}N$ (a) and $\delta^{13}C$ (b).

Stable Isotope Analysis

Firstly, adult *P. leniusculus* were examined through the use of a Multiple Linear Regression, to determine those factors influencing heavy isotope ratios. When modelled with carapace length, weight, gender, population density and with either $\delta^{13}C$ or $\delta^{15}N$ predicting the other, respectively, models for each isotope were significant, but no individual predictor was significant. High levels of collinearity were observed between carapace length and weight (Variance Inflation Factors (VIF) of >15.4 when predicting $\delta^{13}C$, and >15.6 when predicting $\delta^{15}N$, respectively), with values of tolerance values falling below the recommended 0.2 threshold (Hair *et al.*, 2010). VIF values were also high between both carapace length and weight, and population density (due to the largest crayfish being found at the low density population site), and as such models excluding each variable in turn were run, with the highest R^2 value used to select the preferred predictor variable for the model. Carapace length as a predictor of $\delta^{13}C$ had an R^2 value of 0.466, and population density had an R^2 value of 0.487, and weight had an R^2 value of 0.451, thus population density was used (Table 2). Carapace length as a predictor of $\delta^{15}N$ had an R^2 value of 0.604, population density had an R^2 value of 0.470, and weight had an R^2 value of 0.654, thus weight was used (Table 3).

In the carbon model, gender, $\delta^{15}N$, and population density were added as predictor variables, with $\delta^{13}C$ as the outcome. All predictor variables were entered into the model simultaneously. The model was found to significantly predict $\delta^{13}C$ ($F = 7.904$, df=3,28, $p = 0.001$), and accounted for 48.7% of the variance in $\delta^{13}C$ ($R^2 = 0.487$). Population density uniquely significantly predicted variance in $\delta^{13}C$ (Table 2).

In the nitrogen model, gender, $\delta^{13}C$, and weight were added as predictor variables, with $\delta^{15}N$ as the outcome. All predictor variables were entered into the model simultaneously. The model was found to significantly predict $\delta^{15}N$ ($F = 15.761$, df=3,28, $p < 0.001$), and accounted for 65.4% of the variance in $\delta^{15}N$ ($R^2 = 0.654$).

Weight uniquely significantly predicted variance in $\delta^{15}N$ (Table 3). Heavier adult animals showed increasing enrichment in $\delta^{15}N$, and a clear division based on population density was seen in the $\delta^{13}C$ values (Fig. 11). The $\delta^{13}C$ and $\delta^{15}N$ of all size classes (hatchling, juvenile yearlings and both adult male and females) showed crayfish to be occupying few trophic levels, with trophic level increasing as body size increased (Table 4). Adult *P. leniusculus* in the high density population occupied a wider trophic niche as a function of utilising a broader range of carbon sources. Juvenile *P. leniusculus* from both populations occupied a very similar trophic niche, and were slightly enriched in $\delta^{15}N$ with respect to adults from the high density population, but on a similar trophic level to adults from the low density population.

Table 2 - Multiple regression model predicting $\delta^{13}C$.

Predictor	**B (unstandardized beta)**	**SE B**	**β (standardised beta)**	**t**	**p values**
Gender	0.186	0.262	0.103	0.708	0.485
$\delta^{15}N$	0.626	0.458	0.259	1.365	0.185
Population density	0.912	0.340	0.506	2.683	0.013*

*significant at p = 0.05 threshold.

Table 3 - Multiple regression model predicting $\delta^{15}N$.

Predictor	**B (unstandardized beta)**	**SE B**	**β (standardised beta)**	**t**	**p values**
Gender	0.035	0.094	0.047	0.375	0.711
$\delta^{13}C$	0.035	0.065	0.085	0.536	0.597
Weight	0.020	0.004	0.762	4.665	< 0.001*

*significant at p = 0.05 threshold.

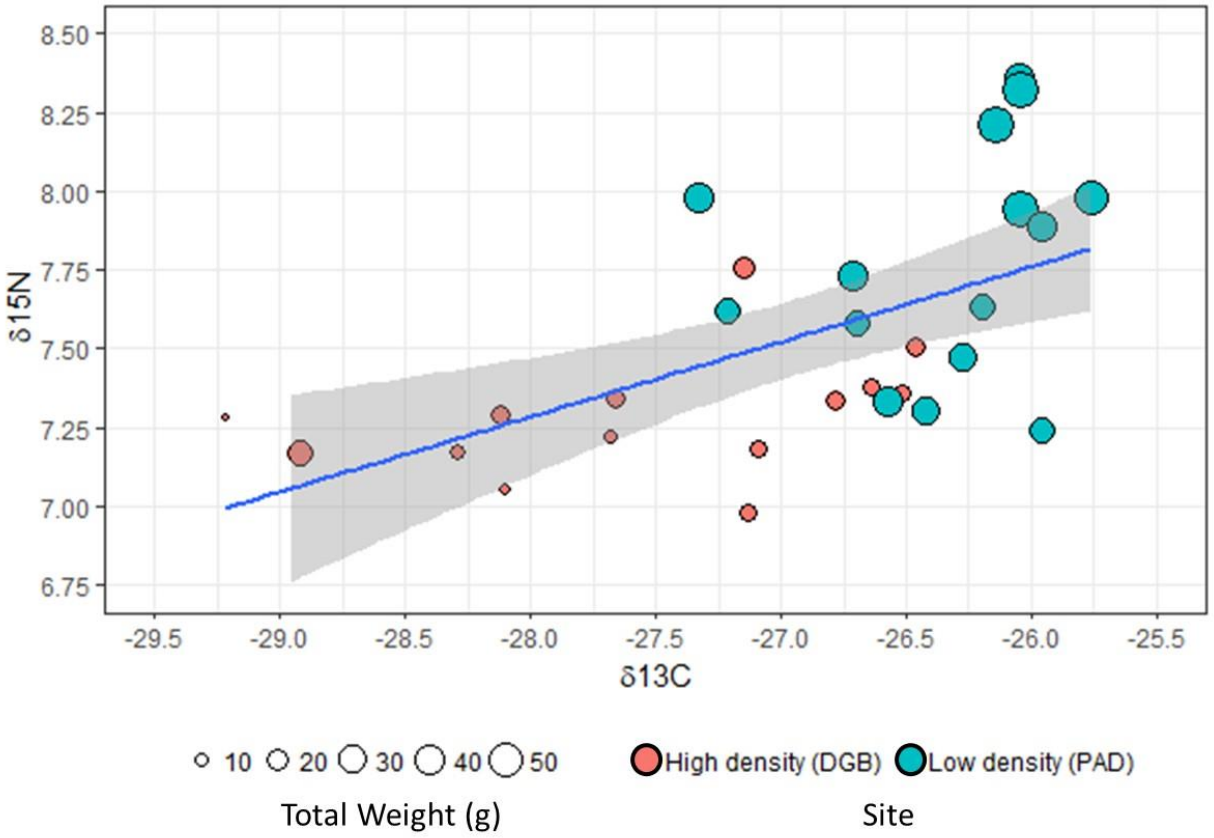


Figure 11 – $\delta^{13}C$ and $\delta^{15}N$ for adult animals from the high (DGB, pink) and low density (PAD, blue) populations, with trend line showing general enrichment of heavy nitrogen at more depleted heavy carbon values. Size of plotted data indicates weight of crayfish (g).

Table 4 - $\delta^{13}C$ and $\delta^{15}N$ of adult male, adult female, juvenile and hatchling crayfish from both low and high density populations.

Site and gender		**$\delta^{13}C$ (‰) (±St. Dev.)**	**$\delta^{15}N$ (‰) (±St. Dev.)**
High density (DGB2017)	Male	-27.93 ±0.91	7.27 ±0.11
	Female	-27.95 ±0.58	7.31 ±0.28
	Juvenile	-27.51 ±0.52	7.68 ±0.26
	Hatchling	-27.85 ±0.11	6.41 ±0.13
Low density (PAD2017)	Male	-26.33 ±0.48	7.86 ±0.36
	Female	-26.40 ±0.47	7.64 ±0.36
	Juvenile	-27.49 ±0.47	7.61 ±0.28
	Hatchling	-27.74 ±0.15	7.29 ±0.19

Juvenile *P. leniusculus* from both populations (high density $\delta^{15}N$ 7.68‰, low density $\delta^{15}N$ 7.61‰) were on a similar trophic level as adult male and female *P. leniusculus* from the low density population (7.86‰ and 7.64‰ respectively), and enriched in $\delta^{15}N$ as compared to adult male and female crayfish from the high density site (7.27‰ and 7.31‰ respectively). The largest animals from the study were from the low density site and had the highest $\delta^{15}N$ values, being males of 50mm CL ($\delta^{15}N$ 8.36‰) and 56 mm CL ($\delta^{15}N$ 8.21‰), and a female of 58mm CL ($\delta^{15}N$ 8.32‰).

Hatchling *P. leniusculus* from the low density population also occupied a similar trophic position to the high density adult *P. leniusculus*, but showed a restricted use of carbon sources. Hatchlings from the high density population were depleted in $\delta^{15}N$ as compared to all other samples, but appeared to utilise the same carbon source as low density hatchlings.

Discussion

Feeding intensity, prey selection and dietary electivity

When controlling for the size of the animal, *P. leniusculus* from the low density population had significantly fuller guts, suggesting that food may be limiting in the higher density population. It is likely that the larger bodied animals are more aggressive and control the better habitat, forcing the smaller animals into sub-optimal habitat with sub-optimal feeding opportunities. However, it should be noted that the 'low' density population in this study was still >40 individuals m^{-2}, which is a substantial abundance of invasive crayfish to be supported in a small rocky headwater system.

P. leniusculus living at a low population density did not favour other aquatic invertebrate protein, and instead relied on CPOM and crayfish tissue. In the high density population dietary diversification occurred, with proportionally more invertebrate prey items included in the diet, and at relative abundances. Protein is an important energy

source, as well as promoting the optimal growth of crayfish. Crayfish protein requirements are generally >30% of diet (D'Abramo and Robinson, 1989), and can be met through a combination of dietary animal and plant proteins. There is evidence for the general theory that when intraspecific competition increases at a population level, individuals can respond by diversifying their diet to include sub-optimal prey items not selected for either presently or by conspecifics to satisfy their protein requirements (Svanback and Bolnick, 2007). This diversification therefore increases the niche width of both the individual and the population, as evident in this study. It could be hypothesised therefore that there was a deficit of animal protein in the high density population, and that this deficit was in some means satisfied through the increased consumption of invertebrate families. What is not explained by this hypothesis is why a lower rate of cannibalism would be observed in populations containing greater densities of conspecifics, and thus potential food items.

Therefore, an alternative hypothesis for this change in diet could be put forward, related to the body size of the animals. Individuals of *P. leniusculus* at the low density site were significantly larger than their conspecifics at the high density site ($p < 0.001$), and as chelipeds continue to grow with the animals, with this particularly the case for large adult male *P. leniusculus* (e.g. Capurro *et al.*, 2015). With this growth of the chelae comes a loss of handling precision. As such, it may be a factor of dexterity, and as such a physical driver, rather than a behaviourally mediated shift, that influences the predation rates of other macroinvertebrates shown here between low and high density populations. Hollows *et al.* (2002), however, reported no size-related difference in the number of aquatic invertebrates consumed by crayfish in their study of the Southern koura (*Paranephrops zealandicus* White). Equally, gut fullness in populations of *P. leniusculus* in their native range was not reported to alter with respect to either density or size of animals (Bondar *et al.*, 2005), suggesting that either populations in the native

range fail to reach similar densities to that of the invasive range, or that native systems where *P. leniusculus* are found naturally are more productive.

P. leniusculus were exhibiting selective preference in terms of the invertebrate prey items in both high and low density populations, preferentially feeding on the large bodied, slow moving, burrowing mayfly *Ephemera danica*, in the family *Ephemeridae*, rather than the flattened mayfly in the family *Heptageniidae*. *E. danica* are associated with benthic habitats, and would be often encountered during the foraging behaviour of *P. leniusculus*, and represent a highly profitable energetic resource which might be gained at a low energetic cost (Schoener, 1971; Krebs, 1978). Only 4 remains of *Chironomidae* were found in the guts of crayfish, and as such conclusions regarding the electivity of crayfish for this prey item are hard to draw. *Heptageniidae*, however, are much faster moving, more cryptic, and are physiologically adapted to cling to cobbles due to their flattened body shape, making them harder to catch, and potentially energetically less appealing. In support of this idea, there is some evidence that the presence of crayfish can benefit populations of Heptageniids. For example, in a study of rocky headwater streams in the USA (Creed and Reed 2004) supporting the Apalachian brook crayfish (*Cambarus bartonii* Frabricius), a large crayfish with similar abiotic requirements to *P. leniusculus*, *Heptageniidae* were the only taxa to increase in abundance when *C. bartonii* was present. Creed and Reed (2004) suggested indirect facilitation of *Heptageniidae* by *C. bartonii* through both an increase of Fine Particulate Organic Matter (FPOM) created through the crayfish feedings activities, and hence suitable food for *Heptageniidae*, and via crayfish activity increasing habitat quality for *Heptageniidae* by removing Fine Particulate Matter (FPM) from cobble refuges. Further work is required on *P. leniusculus* influences on organic matter and sediment transport regimes (e.g. Harvey *et al.*, 2011). However, it is clear that the impacts of *P. leniusculus*

on invertebrate communities are not as simple as direct unidirectional predation of one species on the other.

Crayfish often target slow moving, large bodied prey such as snails (e.g. Hollows *et al.* 2002), and as such these preferential invertebrate prey will be depleted in the environment first. Active and fast moving prey items such as genera of the *Baetidae* family are often not impacted, or can even benefit from the presence of crayfish through trophic release (Nyström *et al.*, 1999). It is likely therefore that invertebrate communities in this study were already depleted of many preferentially selected prey items, due to the high density and well established populations of *P. leniusculus* present. The ability of *P. leniusculus* to select for individual prey items, and thus directly and indirectly influence aquatic invertebrate community structure, remains an important driver of aquatic invertebrate community structure and function even at extreme crayfish densities.

Stable Isotope Ratios of $\delta^{13}C$ and $\delta^{15}N$ in P. leniusculus

Stable isotope analysis indicated that high and low density populations were using different sources of carbon, which was supported by the results of the MLRs, with the population a crayfish was from being the sole significant predictor of $\delta^{13}C$. Both SIA and the MLRs also supported the hypothesis that the size of the animal was main driver of trophic position, as $\delta^{15}N$ was uniquely predicted by weight, and increased across adult crayfish from both populations as weight increased. The *P. leniusculus* specimens in this study had $\delta^{13}C$ and $\delta^{15}N$ values consistent with values from other invasive populations, such as a -26.9‰ depletion in $\delta^{13}C$ and an 8.9‰ enrichment in $\delta^{15}N$ in Ercoli *et al.*'s (2015) study of *P. leniusculus* populations in Finnish lakes, and an approximate -25‰ depletion in $\delta^{13}C$ and approximate 8‰ enrichment in $\delta^{15}N$ in Larson *et al.*'s (2016) study of lake populations in Seattle, USA. Gut Content Analysis provided further evidence for the hypothesis that density was driving changes in diet, as *P.*

leniusculus from the high density site incorporated more invertebrate matter into diet than the low density site. Both GCA and SIA support the hypothesis that *P. leniusculus* can diversify its diet and feeding habits, dependent on life stage, to include a range of energy sources. There was, however, a large degree of niche overlap seen in the adults from the low density population, therefore there is a tolerance to a certain threshold of competition. Niche partitioning of crayfish has been shown to facilitate increased densities, as reduced trophic overlap decreases competition for resources (Jackson *et al.*, 2014). Therefore, it is likely that this omnivorous, ontogenetically-dependent feeding strategy is a facilitative mechanism by which invasive populations of *P. leniusculus* achieved the extreme densities reported in Chapter 4.

The assimilation efficiencies of animal matter and protein are far greater than that of plant and detrital materials (Whitledge and Rabeni, 1997), and as such the use of direct counts and particularly volumetric analysis of gut contents should be considered with caution (Marufu *et al.*, 2018), as numerically infrequent prey items can actually account for the most energy for growth (Parkyn *et al.*, 2001).

High levels of omnivory can decrease the difference in $\delta^{15}N$ between trophic levels (Nyström *et al.*, 1999), and therefore increase uncertainty when interpreting plots of $\delta^{13}C$ and $\delta^{15}N$. It may therefore be the case that the adults of the low density population and both groups of juveniles, and the adults of the high density population, are in fact feeding at two separate trophic levels, with the typical difference of ~2‰ in enrichment of $\delta^{15}N$ being reduced through high dietary omnivory. Additionally, through feeding heavily on conspecifics, the difference in ‰ enrichment of $\delta^{15}N$ between trophic levels might further be truncated, through assimilating tissues of a small prey pool of similarly enriched crayfish. The inclusion of aquatic invertebrate isotopic signatures in the model would elucidate the trophic position of this prey type, and help explain the positioning of adult *P. leniusculus* from the high density site in isotopic space.

Lipid content

Lipid extraction showed no difference for $\delta^{13}C$ values, and resulted in a consistent enrichment of 0.45‰ $\delta^{15}N$. These results are similar to that of Sweeting *et al.* (2006), where a 0.77‰ increase in $\delta^{15}N$ was reported for lipid extracted liver and muscle tissue from European sea bass (*Dicentrarchus labrax* L.), and no change in $\delta^{13}C$. However, Stenroth *et al.* (2006) reported no change in the $\delta^{15}N$ values for *P. leniusculus* following lipid extraction in their study, and a 0.8‰ ±0.4‰ increase in $\delta^{13}C$. However, these values were for whole body samples, and as such represent a combination of tissues with varying lipid contents, and thus should be considered with caution. The proportion of lipids in the muscle tissue of crayfish in both density populations was high (57.5 %), and could potentially have been affected by consistent methodological error as typical lipid contents of the protein-rich tail muscle tissues in crayfish are <10% (e.g. Seemann *et al.*, 2015) . However, excess food availability and protein intake can result in changes to feeding regimes, and increased lipid content of tissues (D'Abramo and Robinson, 1989). Therefore, the high prevalence of cannibalism reported in this study (86.7-100%) and densities of crayfish present (44-88 m^{-2}, Chapter 4), may have led to increased lipid contents in the tissues. To explore this, the relative proportion of lipids within tail muscle, hepatopancreas and whole body samples should be explored further in animals from high density sites, to ascertain the impact of varying degrees of lipid storage in different tissues on isotopic ratios of $\delta^{13}C$ and $\delta^{15}N$.

Contextual discussion of theoretical isotope placements of P. leniusculus

Gut Content Analysis provides a snapshot of what was ingested, and remained in the gut, when an animal was sampled. It is therefore an imperfect method with respect to items that either fragment greatly when consumed (as these become vulnerable to overestimations), items that have fast assimilation and thus low retention times in the gut, or items that are seasonally consumed (Hyslop, 1980; Momot, 1995). Stable isotope

analysis on the other hand provides a longer term interpretation of an animal's diet, with dietary items being incorporated into the isotopic signature over a period of months (Whitledge and Rabeni, 1997). Whilst both SIA and GCA are useful tools for understanding food webs and the interactions within, they can therefore provide conflicting results (e.g. Rudnick and Resh, 2005). In my study, there was evidence for cannibalism from both GCA and SIA, and evidence showing that larger bodied crayfish were feeding at a higher trophic level than smaller individuals. To strengthen this argument, GCA should be applied to juvenile and hatchling crayfish in further work, and be complemented by SIA of basal food groups present within the system. My study focussed on understanding how the underlying mechanisms of omnivory and cannibalism sustain a highly degraded and invaded system. Building a more holistic model of the food web was therefore not the immediate focus of this study, however, the inclusion of additional SIA and GCA would allow for much greater insight into the interactions and thus impacts *P. leniusculus* can have on a wider range of ecosystems.

An obvious limitation of this study is the lack of isotopic values for the basal food sources within the food web, such as invertebrates and detritus. Whilst the grant money that supported the project was gratefully received, funding limited the study to analysing only crayfish samples. Available published resources that provide values of $\delta^{13}C$ and $\delta^{15}N$ for typical basal food types in aquatic systems should be approached with a certain degree of caution, as isotopic signatures of detritus, invertebrates and algae can vary considerably between sites, for example in the $\delta^{13}C$ values reported for *Chironomidae* of -26.5‰ by Bondar *et al.* (2005), and of -39‰ by Stenroth *et al.* (2006). To develop the understanding of how high density populations of *P. leniusculus* transfer energy through food webs, future studies should seek to determine high quality in-situ density estimates, and fully sample all potential prey sources within a system.

Implications of cannibalism in P. leniusculus; structure controls function

P. leniusculus were clearly the dominant component of invertebrate and benthic biomass (see Chapter 4) at both sites, achieving significant abundance and biomass. Whilst crayfish material clearly formed a large component of diet in both the low and high density populations, either direct counts, comparative masses, or relative proportions of carapace fragments in the gut do not fully account for the importance of crayfish material in a cannibal's diet. The consumed material consisted of both indigestible carapace fragments, which is what was observed in the gut, and ingestible muscle tissues, which were readily assimilated into the crayfish. For example, the body of a chironomid (e.g. Fig 5a and b) would degrade and be ingested quickly, whereas the chitinous head capsule would remain; this would not impact abundance counts, but would impact comparative volumetric analysis. As such, whatever analysis is conducted on the gut content, can only reliably describe the indigestible element of consumed crayfish material, and hence does not directly relate to nutrition, energy flow, volumes or importance of material assimilated (Hyslop, 1980).

In a study of *P. leniusculus* in its native American range, Bondar *et al.* (2005) found that cannibalism occurred more frequently in larger bodied animals, but was not related to stocking density. However, the densities described in their study were 1, 2 or 3 adult animals in a 1 m^2 enclosure, and as such represent a limited density gradient, far lower than seen in this study. In Bondar *et al.* (2005), woody debris, leaves and macrophyte debris were the most common food items of Y-o-Y, juvenile and adult *P. leniusculus*, however this was likely due to the forested streams sampled, and therefore high relative abundance of these resources; the study sites in this study were open, with limited allochthonous input. However, approximately 34% of the diets adult *P. leniusculus* by count was other *P. leniusculus* remains and moults. These values are in agreement with cannibalism rates in this study.

Eubanks and Denno (2000) found that having an abundance of an alternative, high quality prey item can mediate the effects of omnivorous predators supressing prey items, thus allowing prey to survive at low densities. Therefore, the cannibalistic tendencies of the crayfish in this study site, in which conspecifics are the high quality abundant alternative prey item, is a potential mediator of further declines to other aquatic invertebrate populations, allowing invertebrate communities to persist in the environment. This hypothesis needs further investigation, however if true, it could pose important questions for the control of invasive crayfish. If larger animals are cannibalising conspecifics and consuming proportionally less other aquatic invertebrates, removing them through for example trapping would release the smaller size classes of *P. leniusculus* from conspecific predation pressure, increasing the number of invertebrate prey items consumed and thus indirectly further destabilising native aquatic invertebrate communities.

Knowledge of the size class distribution of *P. leniusculus* at a site is therefore critical in understanding the impacts of the species, but often not known or understood (see Chapter 4). The relative proportions of smaller animals that preferentially feed on invertebrates, versus the relative proportion of larger cannibalistic animals, will determine in part the impact that *P. leniusculus* exerts on the ecological community. There is work to be done here on understanding the relative impact the different life stages of *P. leniusculus* can have on aspects of the ecological community, for example shredders, and quantifying this impact using high resolution density data. In essence, the structure of the population will have important implications for the ecological function of the population. For example, questions arise around the relative impact of a single large adult *P. leniusculus* as compared to several juveniles or many hatchlings, which could be explored through the use of functional response and feeding trials, and how this impact might change under different size-class ratios. Additionally, the impact of

predatory fish on the structure and thus function of *P. leniusculus* populations should be further explored, again using high resolution density data, as fish may re-structure the population by predating and thus removing smaller individuals.

Conclusion

This study demonstrated cannibalism to be prevalent feeding strategy within *P. leniusculus* in a headwater stream of Northern England. Cannibalism provided a major proportion of the diet and thus protein requirement of *P. leniusculus*, and evidence from SIA suggests that animals as young as 1-year-old engage in regular cannibalism. *P. leniusculus* likely fed across several trophic levels, and consumed a range of carbon sources; as such, *P. leniusculus* structured the energy flow through the system through niche separation of different life stages. In addition, *P. leniusculus* exhibited preferential feeding on invertebrate taxa, selectively predating *Ephemera danica*, and thus driving further restructuring of the already depleted invertebrate community present in the study site. The occupation of multiple functional and trophic niches by a single invasive species, along with the high rates of cannibalism and omnivory, suggest mechanistic pathways by which *P. leniusculus* can sustain itself in a degraded system, forming a 'resilient invasion'.

Chapter 6 – Final synthesis

Through a combination of collaborative field, laboratory and desktop study, this thesis has generated a range of novel data that informs many aspects of the continued conservation of *A. pallipes* in England. **Chapter 2** updated the distribution of both *A. pallipes* and *P. leniusculus* in England (2018). **T**he specific findings of **Chapter 2** are given individual attention in the context of the ongoing challenges posed to monitoring *A. pallipes* in England. However, there is value in the broader discussion surrounding the provision of accurate and up-to-date distribution information for any conservation effort. Identifying areas that support strong networks of *A. pallipes*, and areas that are at immediate or future risk from the threats posed by *P. leniusculus*, is crucial to effectively utilising the resources available to conservation practitioners. A proactive rather than reactive approach to conserving *A. pallipes* populations can be employed if the spatial knowledge is available to allow horizon-scanning of threats and opportunities, and efforts should be made to maintain these databases.

Chapter 3 presented a case study of multiple sites containing no crayfish, *A. pallipes*, or *P. leniusculus*, and explored the broader consequences for ecosystem structure around these crayfish populations. Catch-Per-Unit-Effort data suggested the population of *P. leniusculus* at Bookill Gill Beck was the highest density crayfish population out of all the sampled headwaters. The populations of fish and native *A. pallipes* that previously inhabited Bookill Gill Beck (Peay *et al.*, 2009) were completely lost,

following the invasion of *P. leniusculus.* The macroinvertebrate communities sampled in Bookill Gill Beck were severely depleted both in terms of abundance and richness. The presence of invasive *P. leniusculus* populations within headwater streams can have unpredictable and severe ecological consequences, and research should focus on determining the pathways and mechanisms by which *P. leniusculus* can establish dominance over native biota.

Chapter 4 sought to develop and test a novel sampling methodology, designed to provide the best in-situ estimate of *P. leniusculus* density, population structure and demographics to date. Through the use of the novel triple drawdown technique, Bookill Gill Beck was shown to support populations of *P. leniusculus* at densities of over 110 m^{-2}, far in excess of previous estimates from the literature. Additionally, the structure of the population was revealed for the first time in the field, showing hatchling and juvenile crayfish of <12mm CL to be the dominant cohorts in a population. Conversely, the trappable proportion of the population was only 2.3% of the total population caught through the drawdowns. **Chapter 4** therefore presented key novel data on the demographics of invasive *P. leniusculus* populations in England. In doing so, **Chapter 4** advances the field by supporting the development and use of models for population assessments of *P. leniusculus*, and brings into question the efficacy of contemporary sampling techniques for crayfish in England.

Both stable isotope analysis and gut contents analysis in **Chapter 5** confirmed that cannibalism was a highly prevalent and energetically important process in invasive populations of *P. leniusculus* in Bookill Gill Beck. The gut contents of the largest *P. leniusculus* specimens captured contained the greatest abundance of *P. leniusculus* remains, and the smaller specimens of *P. leniusculus* contained a significantly greater proportion of other aquatic macroinvertebrate remains. These findings suggest that

research furthering our understanding of the conditions controlling cannibalism in *P. leniusculus* are of priority when choosing and applying management techniques.

These key findings, and the management recommendations that are proposed as a result of them, are discussed in greater detail within the relevant sections forthwith. Specifically, this thesis provides an empirical platform from which to more broadly discuss two key areas of the continued conservation of *A. pallipes*, namely to challenge commercial and public crayfish trapping in England, and the ongoing challenges for monitoring *A. pallipes* in England.

The futility and direct risks of trapping activities in England

The use of commercial style crayfish traps to capture non-native crayfish, primarily *P. leniusculus*, is still currently permitted in England. Trappers can be licensed to either harvest *P. leniusculus* commercially or for control, catch them for personal consumption, or for science and research. No prior training is required in either the identification of native and non-native crayfish species, or in the awareness and best practice of biosecurity. Additionally, there is currently no cost to obtaining a crayfish licence, unlike elver licenses (£85 per licence), adult European eel licenses (£60 to £580 depending on number requested) and smelt fishing licences (£85 per licence). The current restrictions on where trapping is legal are the presence of local designated sites, such as Sites of Special Scientific Interest (SSSIs), or the presence of populations of native *A. pallipes*. Additionally, following the advice of the Prohibition of Keeping of Live Fish (Crayfish) Order (1996) which operates using the antiquated 'postcode system', there is no requirement for a licence in order to keep live *P. leniusculus* providing the enclosure is secure and there are well-established feral populations present in the area (for example Plymouth, Birmingham, London, Norwich, Gloucester).

Whilst the Environment Agency (EA) does not have an official stance on trapping, the EA has on occasion spoken publicly in favour of trapping (Fig. 1a), inspiring both local politicians and the public to engage with and promote trapping for *P. leniusculus* (Fig. 1c & d). At the same time, other regional EA teams are outspoken in their promotion of good biosecurity and prevention of the spread of non-native invasive species (Fig. 1b), recognising the risks that trapping can pose and thus not supporting the broader engagement with and application of trapping.

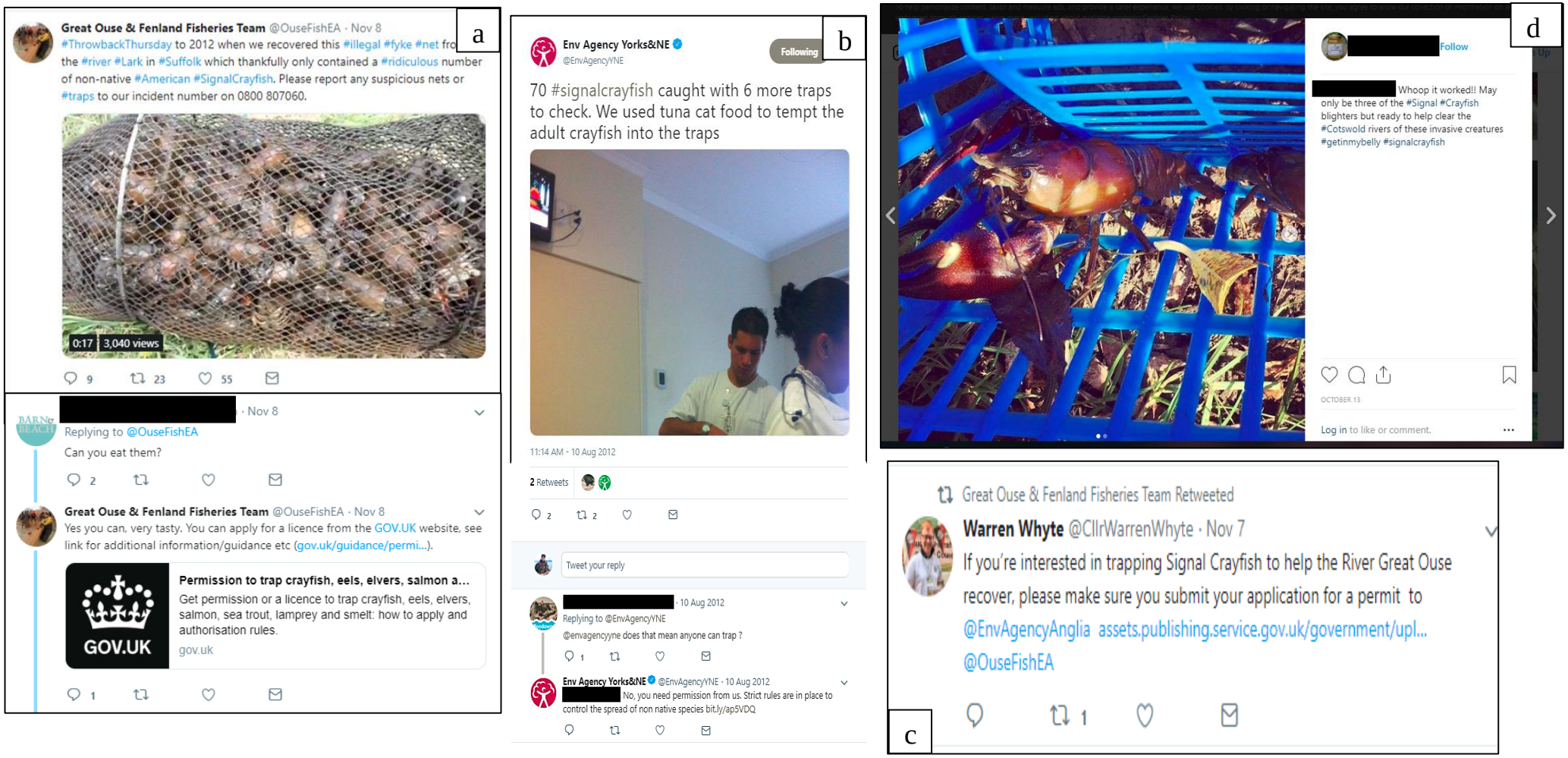


Figure 1 – Collection of publicly shared statements regarding trapping for *P. leniusculus* in England, from the Environment Agency (a & b), local politicians (c) and the public (d).

In contrast to some of the more general sentiments of the above regarding the public trapping of *P. leniusculus*, there is growing recognition that trapping is a major pathway of the spread of both the highly damaging *P. leniusculus* and the highly virulent crayfish plague. One of the clear forerunners in this respect is Scotland. Both *P. leniusculus* and *A. pallipes* are not native to Scotland, and several damaging populations of *P. leniusculus* are established in the wild (e.g. Gladman *et al.*, 2010). The Scottish Environmental Protection Agency (SEPA) and Scottish Natural Heritage (SNH) were instrumental in blocking a petition to government to legalise commercial trapping of *P. leniusculus* in Scotland in 2015 (Petition PE01558: American signal crayfish, 2015). The petition was blocked on the premise of a weight of evidence approach, and referenced published literature pertaining to *P. leniusculus* trapping and harvesting in Spain and Sweden, and the negative implications of the spread of *P. leniusculus* for native ecosystems. Legalising trapping for *P. leniusculus* had not prevented illegal trapping from still occurring in these countries, and had also failed to stop the incidence of new invasive populations of *P. leniusculus* from being established.

Most recently, Baroness Kennedy of Cradley asked Lord Gardiner of Kimble, the Parliamentary Under-Secretary for the State of Environment, Food and Rural Affairs

"[Her Majesty's Government] what action they are taking to combat the threat to biodiversity from biological invaders" (House of Lords Deb 11526 cW, 28/11/2018).

Lord Gardiners reply was in line with EU Regulation No. 1143 (2014), on which he stated

"*a core provision of which is the creation of a list of species subject to strict restrictions. These species cannot be imported, kept, bred, transported, sold, used or exchanged, allowed to reproduce, grown or cultivated, or released into the environment*".

P. leniusculus are on this list. If we are to take Lord Gardiners words at face value, then the more stringent, although arguably warranted, stance of Scotland changes in light of this statement. Scotland's stance towards *P. leniusculus* becomes less of a precautionary approach, and instead is simply adhering to European legislation and law. This is in stark contrast with the weaker and conflicting stance provided at present in England. The current position of facilitating the trapping and harvest of *P. leniusculus* in England, clearly runs the risk of violating the interpretation of EU Regulation 1143/2014 that Lord Gardiner has presented. Specifically, commercial trapping contravenes the Regulation through the keeping, transportation and sale of live *P. leniusculus*. It comes as little surprise, therefore, that there is confusion from stakeholders and the public as to whether trapping for *P. leniusculus* is encouraged or not, on either its economic or ecological merit, and is indeed even legal. This thesis, through the combination of evidence provided by each empirical chapter, takes a holistic approach to addressing why the practice of trapping for *P. leniusculus* in England should be banned.

Through the work of **Chapter 2**, populations of *A. pallipes* were shown to have continued to decline since 2012, including the notable loss of the population at Ensors Pool SAC. If it were just the case that losses of *A. pallipes* were being recorded without the corresponding establishment of new *P. leniusculus* populations, then the argument may focus on biosecurity alone, and ensuring best practice is followed with regards to the 'Check-Clean-Dry' protocol (NNSS, 2006). However, populations of *P. leniusculus* were shown to have further increased since 2012, occupying sub-catchments that were not hydrologically linked to previous invasive populations in both the Midlands and North of England. As such, the spread of *P. leniusculus* is likely not through 'natural' means, such as expansion of populations through a waterbody, and is suspected to be driven through anthropogenic capture and release of live *P. leniusculus* into new

waterbodies (as supported by Petition PE01558: American signal crayfish, 2015). Many hundreds of trapping licenses for *P. leniusculus* are consented every year by the EA (P. Bradley pers. comms., 2018), and as previously stated the retention of live *P. leniusculus* specimens is not prohibited in much of England. As such, the trapping of *P. leniusculus* in England is likely to be driving both the spread of the species to new waterbodies, and the consequent spread of crayfish plague and corresponding losses of local populations of *A. pallipes*. **Chapter 3** addressed the consequences of the replacement of the native crayfish *A. pallipes* with the invasive crayfish *P. leniusculus*, focusing on headwater systems in the North of England to determine any impacts on the ecological communities present. This chapter aimed to explore the ramifications for ecosystem services, such as biodiversity or ecosystem function, of the loss of *A. pallipes* and introduction of *P. leniusculus* in native ecosystems. If these were limited to impacts on *A. pallipes* alone, then the immediate concerns may be contained to addressing the future of *A. pallipes* in England, rather than the wider ecological communities. Populations of *P. leniusculus* were associated with a broad range of ecological communities, but, one site in particular stood out, namely Bookill Gill Beck. It stood to reason at the time (2015), therefore, that Bookill Gill Beck represented the worst case scenario for an invasion of *P. leniusculus*, whereby the highest comparative density of *P. leniusculus* resulted in the greatest negative impact on the local ecosystem. However, based on the standard trapping approach used in this study, the true density of *P. leniusculus* remained unknown, and as suspected at the time and now understood based on the data from **Chapter 4**, data from trapping can be unreliable in estimating populations of *P. leniusculus*.

Through the use of the novel triple drawdown technique, **Chapter 4** revealed that populations of *P. leniusculus* in Bookill Gill Beck exceeded densities of over 110 m^{-2}, dominated by young-of-year and juvenile animals, with 'trappable' adults making up

less than 3%. This has important implications for both the scientific utility of trapping as a sampling technique, and the validity of any management or control strategies that relies solely on trapping as a means of deriving baseline and ecological impact data. For example, determining the success of a removal program for a population of *P. leniusculus*, whilst potentially failing to sample up to 97% of the population, has clear and serious implications.

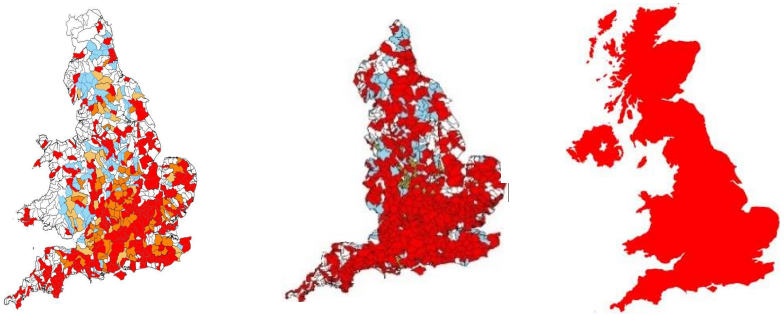
A final consideration of trapping is the impact that selectively removing only the largest *P. leniusculus* specimens has on the population. The largest and most physically dominant *P. leniusculus* specimens within Bookill Gill Beck are exerting a top-down pressure on the crayfish population, through cannibalising smaller conspecifics. These same dominant large bodied animals are the ones most likely to be caught and thus removed through conventional trapping. When trapping removes these large animals, it also removes with them this top-down pressure, thus increasing the survival and abundance of these smaller cohorts. Smaller cohorts consume proportionally greater numbers of other aquatic invertebrates. Thus, following the removal of larger *P. leniusculus* specimens and consequent reductions in predation pressure, a cascade effect could occur as a direct consequence of trapping, resulting in the subsequent proliferation of the smaller cohorts, and a potentially much increased pressure on remaining aquatic macroinvertebrates. The population density of *P. leniusculus* is likely a key factor in the severity of the impact *P. leniusculus* exerts on ecological communities (**Chapter 3**), and as such the indirect proliferation of *P. leniusculus* populations through trapping poses a considerable threat to native biota.

Trapping as an activity poses a biosecurity risk both to *A. pallipes* and wider ecological communities, through the spread of *P. leniusculus* and the crayfish plague. There is relatively little scientific merit in using trapping as a sampling tool beyond confirming presence through positive CPUE, due to the substantial proportion of a population that

is below trappable size. Trapping to control a population will promote survival and growth of the population, further harming local biodiversity. Taken as a weight of evidence, this thesis provides arguments and empirical data in agreement with SEPA and SNH. The negative impacts of trapping (within England) warrant the effective and immediate ban of the authorisation and practice of public and commercial trapping England (Fig. 2).

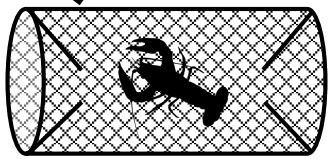
Figure 2. Discussion Synthesis Trapping

Populations of *P. leniusculus* continue to spread, whilst *A. pallipes* continues to decline (Ch. 2)



Biodiversity & ecosystem function

Biosecurity & Spread



Sampling

Control

Key findings

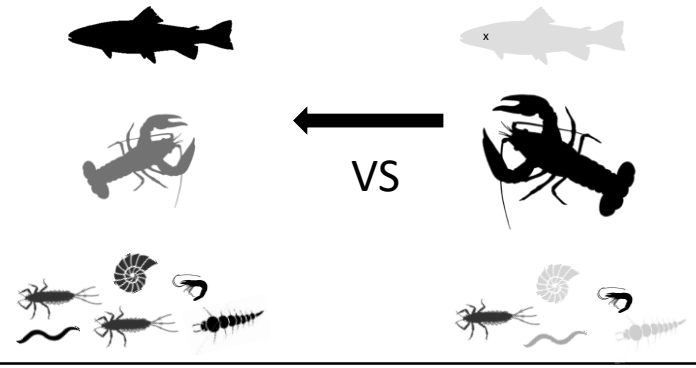
Trapping as an activity

1. High biosecurity risk
2. Increased network of spread

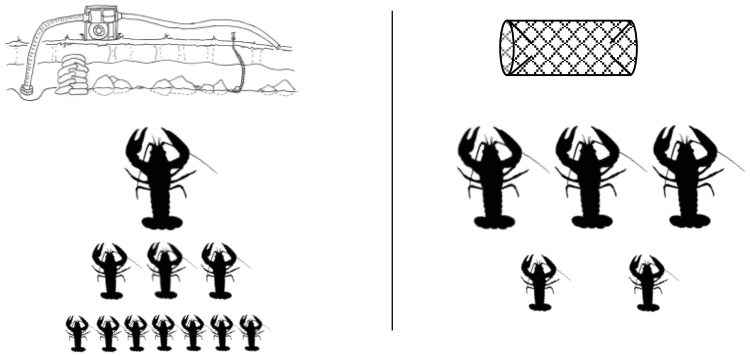
Trapping for conservation & management

1. Inefficient sampling technique
2. Futile and risky control method

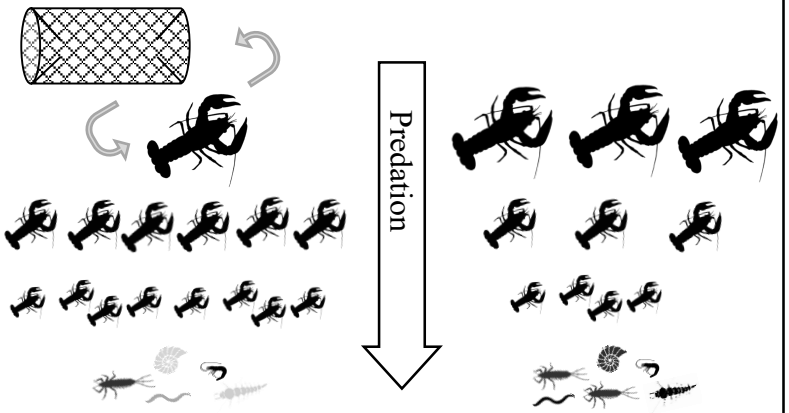
***P. leniusculus* has the potential to severely negatively impact native fish, crayfish and invertebrate communities** (Ch. 3)



Novel sampling shows *P. leniusculus* populations to reach high densities, dominated by sub-trappable YoY and juvenile crayfish (Ch. 4)



Adult *P. leniusculus* are highly cannibalistic of smaller cohorts, which in turn predate other aquatic invertebrates (Ch. 5)



Challenges in monitoring and reporting *A. pallipes* populations in England

Under Article 17 of the Habitats Directive, and as a current member state of Europe (2018), England has a legal obligation to report on the conservation status of *A. pallipes* to Europe every 6 years. England, and the bodies working therein to provide and report this information, are amongst the leading members in this respect. As part of this obligation, there is an expectation that sufficient monitoring of a suitable quality will have been undertaken, such that the current status of *A. pallipes* can be accurately represented. This thesis highlights three major areas in which England can further improve in order to deliver on this expectation, these being:

1. The creation of a centralised and standardised monitoring framework for crayfish species
2. The revision and development of the metrics and methodologies currently employed to describe the distribution of *A. pallipes*
3. The need to fully recognise and incorporate the threats posed by invasive crayfish species when reporting on the status of *A. pallipes*

Firstly, the reporting framework in effect at present in England relies on ad-hoc records, generated from multiple sources, to populate *A. pallipes* distribution databases. In effect, no routine monitoring is in place for *A. pallipes*, and as such there is no consistency in sampling frequency, location and quality. The monitoring infrastructure that would enable this sampling does exist already; the Environment Agency (EA) sample every river in England on a minimum of a 4 yearly basis for fish communities, and conduct routine aquatic invertebrate samples annually through the analysis and reporting team. For sites on main rivers, this routine sampling is indeed an accepted method for generating both *P. leniusculus* and *A. pallipes* records, which are stored on both the EA database and by the National Biodiversity Network (NBN). An additional source of *A. pallipes* records is through the catch returns of CL11, CL23 and bespoke

licence holders, who are authorised through Natural England (NE) to work with *A. pallipes*. Within the legal Directive framework, Natural England is responsible for assessing monitoring needs, and the Secretary of State is responsible for implementing these needs. However, no specific requirements are detailed within the Directive as to the frequency or quality of such surveillance. Further, the collection of new data is not necessarily required for each reporting round under the current directive. A collaborative approach towards the establishment of a centralised monitoring scheme, based between the EA and NE, would be the most appealing and likely successful endeavour. However, to attempt to address and improve on this lack of monitoring, the limitations and constraints must be acknowledged. Funding is likely the main preventative factor in improving monitoring of *A. pallipes* in England. Solutions must be sought to either support monitoring through finding more funding, or through finding ways to survey and monitor *A. pallipes* that cost less. In terms of the latter, eDNA analysis may offer cost effective alternatives to more time consuming and expensive field surveys, and could form part of an initial suite of sampling methodologies for *A. pallipes*. However, the current risks associated with eDNA analysis, such a false negatives and false positives, should be considered when using this emerging technique in the field.

The use of high resolution and quality distribution data, such as that provided by the EA officers in Chapter 2, can form a key management tool that can be utilised in decision making processes surrounding monitoring of *A. pallipes*. For example, focusing survey efforts on 'conflict zones' where blue and red cells touch may help to prioritise finite resources, and ensure that crayfish distribution records are up-to-date. Equally, when considering the management of hydrological connectivity at a catchment scale, such as the removal of weirs, the knowledge of crayfish distributions can help inform biosecurity protocols and protect populations of native crayfish. Ultimately, the creation

of a centralised monitoring strategy, that is underpinned by legislation, is the key first requirement in continuing conservation efforts for *A. pallipes* in England.

The second key challenge surrounding the monitoring and reporting of *A. pallipes* in England is the manner in which the data that we do have is presented and interpreted. In the absence of a centralised monitoring strategy for *A. pallipes* in England, the survey data generated will always be subject to biases, especially due to differences in the relative survey effort for a given region in a given year. For example, running the scenario that records generated from bycatch in EA electrofishing surveys were to reduce by 30%, or private sector reports on crayfish rescues at bridge repairs might rise by 40% in a given year, should one believe that populations of *A. pallipes* did so to? Evidence in support of this idea is found in the 2012 Article 17 report (JNCC, 2013b), which acknowledged that the increased number of occupied *A. pallipes* grid squares between the 2006 and 2012 report was likely due to increased survey effort, and not in fact true population recovery. This increased survey effort is attributed to increased conservation attention regarding the plight of *A. pallipes* garnering greater monitoring interest. There exists a disconnect, therefore, between the strict guidance and emphasis placed on the monitoring and reporting of species population trends under the Habitats Directive (Articles 1, 2, and 17), and the reliability of distribution data currently available in England for *A. pallipes*.

While the issue of survey effort is closely related to improving the monitoring situation for *A. pallipes* in England, there are other ways of improving the accuracy and utility of our current distribution data that do not require increased surveys. Both methods for displaying the distribution of *A. pallipes* in England under Article 17 of the Habitats Directive, namely the standardised 10 x 10 km grid square approach and the sub-catchment polygon map, are generated for each report using records pertaining to that reporting round; equivalent to 6 years. This process is likely, therefore, to produce

distribution estimates less accurately reflect the status of *A. pallipes* at a given time, due to the disconnect between reporting and monitoring cycles. However, the JNCC do allow the standard 6 year record window to be adjusted, provided there is a reasoned argument to do so. Therefore, it is suggested that this window be changed to a minimum of two reporting rounds (12 years) of data, with the option to adjust the window again in the future, following improvements to the monitoring strategy for *A. pallipes* in England. However, simply adjusting the window to include more records runs the risk of overcompensating for the lack of up-to-date monitoring, and reporting more populations of *A. pallipes* that are truly present. Attaching some measure of uncertainty to the survey records, therefore, is prudent, with a potential mechanism being through a time-related scale, which increases the uncertainty attributed to a population remaining extant as the age of the record it is based on increases.

The final, and potentially most important, consideration for the monitoring and reporting of the status of *A. pallipes* in England is centred around the recording of *P. leniusculus*. Article 17 reporting provides perhaps the most useful benchmark from which to measure the capability of the Special Area of Conservation (SAC) network to provide continued in-situ conservation for *A. pallipes*. It is expected that the network will provide a best case scenario for the conservation of *A. pallipes* in England. At present, however, the SAC network is not meeting its objectives of safeguarding significant populations of *A. pallipes*, as evidenced by the continued decline observed in populations of *A. pallipes* within the SAC network. This loss of *A. pallipes* populations is driven by the direct and indirect biosecurity risk posed by the continued advancement of *P. leniusculus* into and around SAC territories. For example, newly established populations of *P. leniusculus* in the North of England have increased the vulnerability of the SAC network to invasion and breaches in biosecurity, while outbreaks of crayfish plague in the South and in the Midlands have further weakened the SAC networks

resilience. A notable example of this is the loss of the Ensor's Pool SAC population (Nuneaton, England), which contained one of the best *A. pallipes* populations in England. Distribution data of both *A. pallipes* and *P. leniusculus*, when plotted together, present a clearer idea of what is likely to have happened at Ensor's Pool (Fig. 3).

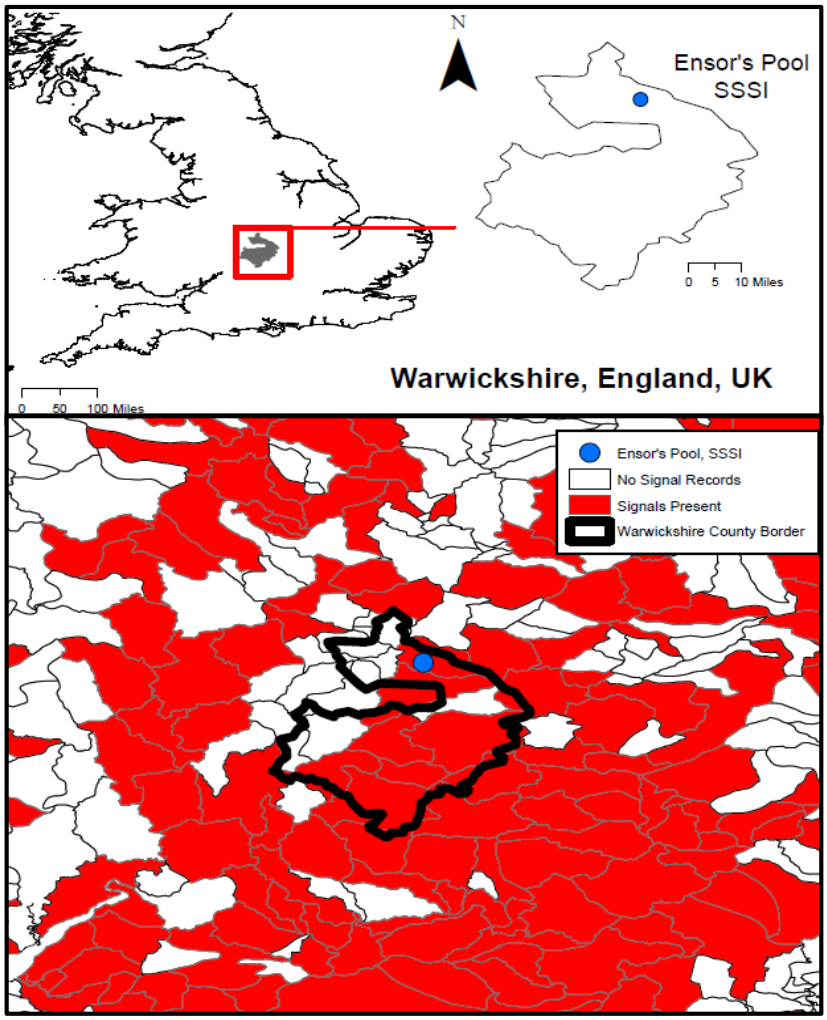


Figure 3 – Distribution of *P. leniusculus* populations surrounding Ensor's Pool SAC, on the sub-catchment polygon map.

It would seem likely, therefore, that Ensor's Pool, a hydrologically isolated SAC containing an internationally important population of *A. pallipes*, was compromised by the biosecurity risk posed by local populations of *P. leniusculus*. Failing then to consider the threats posed by *P. leniusculus* (and crayfish plague) is therefore failing to report the true status of *A. pallipes* populations in a given area or time. Despite this, when reporting distribution data for *A. pallipes* under Article 17, through both the hectad and SAC maps, the presence of *P. leniusculus* is not considered or shown.

Therefore, the presence and thus threat of *P. leniusculus* populations should be considered at all levels of *A. pallipes* conservation, including importantly the monitoring and reporting of status of *A. pallipes* populations. For example, the 'heat-style' map produced in this thesis (Chapter 2), which assigned a level of risk associated to the proximity of *P. leniusculus* to *A. pallipes* populations within the SAC network, would be a useful addition to the reporting of SAC status.

Future research directions

This thesis has shown the legacy that a single invasive species can have, from a network of populations right down to an individual animal. Building on the findings of this thesis, there are several areas of research that offer advancement in astacology and freshwater ecology alike.

There is opportunity to expand on the work done in Chapter 5 on the trophic positioning and feeding habitats of a high density population of *P. leniusculus*, to include the isotopic analysis of basal resources such as algal biofilms and detritus, and invertebrate taxa. Gaining further understanding on the structure and function of a highly degraded food web, dominated by an invasive omnivorous crayfish such as *P. leniusculus*, would provide key ecological information on the ontogenetic positions that *P. leniusculus* occupy within these systems. Understanding the different functional roles *P. leniusculus* can perform, and how they may vary with age or population density, will assist in predicting the impacts that *P. leniusculus* might have on ecosystems, and aid management in deciding how we might protect against or mitigate these impacts.

Whilst a drawdown is the best performing method to date for sampling crayfish, it is unrealistic to expect drawdowns to be incorporated as a standard methodology on a larger scale. There exists a critical need for a revision of crayfish sampling methods, to reflect both the scientific evidence of the ecological and scientific risks of trapping, and

with which to equip practitioners with adequate demographic information to attempt to successfully manage *P. leniusculus* and its impacts. These novel methods should prioritise biosecurity and reducing the risk of bycatch. In addition, the collection of quantitative data on *P. leniusculus* population structure and density should be central to the design. The costs and logistical considerations of drawdowns are a major obstacle in the broader uptake of this method, and as such any new sampling methods should also aim to be economically accessible to as wide a range of trained users as possible, and retain the physical ease of use that a trap offers. Whilst this may seem a slightly farfetched and idealistic list, there is current ongoing research into quantitative sampling methods for crayfish which has delivered on many of the above points (Pritchard, unpublished). One should consider that due to the relative convenience of trapping, few alternative methods have been explored for sampling crayfish. There exists a great many opportunities to develop the field of crayfish monitoring and surveying, which are likely to be to the benefit of academics and applied practitioners alike.

The data generated from the drawdown technique offers new opportunities in modelling populations of *P. leniusculus.* Key demographic data on the structure and density of *P. leniusculus* cohorts can contribute towards estimates of productivity, the building of life tables, and modelling population changes over time. Ultimately, modelling is a cost effective primary method to explore management and control options, and may in some cases be preferable to multiple expensive and time consuming field trials. The data underpinning these models could therefore provide an important resource, informing the allocation of funds and management effort. It is likely that models that predict the effects of control techniques on populations of *P. leniusculus* would become more efficient through the incorporation of demographic data generated through drawdowns. As the efficacy of models increases, the reliance on the provision of high resolution

demographic data becomes increasingly important, and as such methods to generate and test population data for *P. leniusculus* should be prioritised.

Finally, while much attention has been focussed on *P. leniusculus*, the potential future threats posed by other invasive crayfish species in England should also be considered. The rapid spread of *P. leniusculus* throughout England due to farming and harvesting of the species is unlikely to be repeated for future invasive crayfish species, given our current awareness of the negative impacts *P. leniusculus* has exerted on our native biota. However, an additional 6 invasive crayfish species are established in England, all of which have the potential to spread further than their current invasive distribution. While the prevention of *P. leniusculus* population spread is an ongoing management challenge, the scale of the problem for the remaining invasive crayfish species in England is substantially smaller. Efforts to understand the current distribution of these species in England are often even more limited than those of *P. leniusculus* and *A. pallipes*, and little is known of their potential impacts in English systems. Research into the distribution, ecological consequences, and management options for these crayfish species should be prioritised, whilst the opportunity remains to implement comparatively small-scale management at an early stage of invasion. Indeed, prevention in the first instance is the most ecologically and cost effective control method, and should be central to mitigating the threats posed by invasive crayfish in England.

Final remarks

As a final comment, I feel it is extremely important I acknowledge the fundamental role that collaboration across institutes has had on both my thesis and the PhD as a whole. The link between academia and applied industry has been highly influential in shaping the direction of my work. There is great value in the collaborative relationships between

academia and applied practitioners, which inform good science and help focus the work to bring an evidence based approach to applied conversation.

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Appendices

Appendix 1- Regional consultation with the Environment Agency (EA) for crayfish distribution in England (Chapter 2).

Cambridgeshire and Bedfordshire

Denoted as region 8 on the National scale map (Fig. 6 in Ch. 2), Cambridgeshire and Bedfordshire covers an area of 8195 mi^{-2}, and contains 79 sub-catchment cells. According to the most accurate previous records presented in the 2010 map, the Cambridgeshire and Bedfordshire region contained 47 red cells, 1 blue cell and 31 white cells.

Following the consultation round, several changes were notified for the region (Fig.1). Firstly, new *P. leniusculus* populations were added for the Cutoff and Renew Channel (213) which had not been recorded through official sampling. Further new *P. leniusculus* records were added for the River Ivel (397), where they were reported as being prevalent throughout the catchment, the Little Ouse (440) where multiple dead *P. leniusculus* were observed from a pollution incident and for the River Nar (504) and the Ouse Beds (547) where the presence of *P. leniusculus* had been confirmed from ongoing trapping work. Additionally, the River Brett (154), which shares a boundary with the Essex, Suffolk and Norfolk region, was updated to include a population of *P. leniusculus*. Finally, the loss of the only blue cell in the region was recorded for the Little Ouse (442), attributed to an outbreak of crayfish plague. No *P. leniusculus* populations were reported for this cell; thus it has been designated as a lost catchment (yellow with black hatchings).

The updated regional map has 0 native blue cells. This is a huge loss, both in relative and actual terms. The updated regional figures stand at 52 red cells, 0 blue cells, 26 white cells, and one lost population yellow cell (Fig 2).

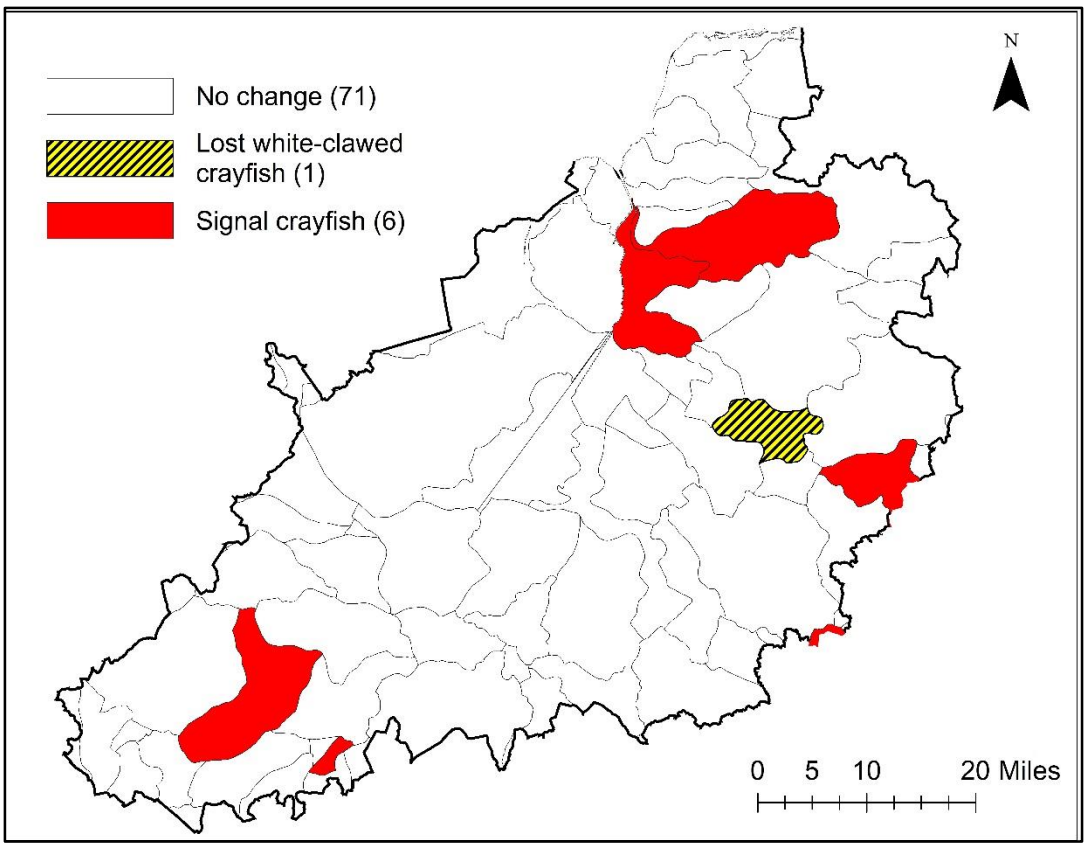


Figure 1 - Changes to the Cambridgeshire and Bedfordshire map between 2012 and 2018

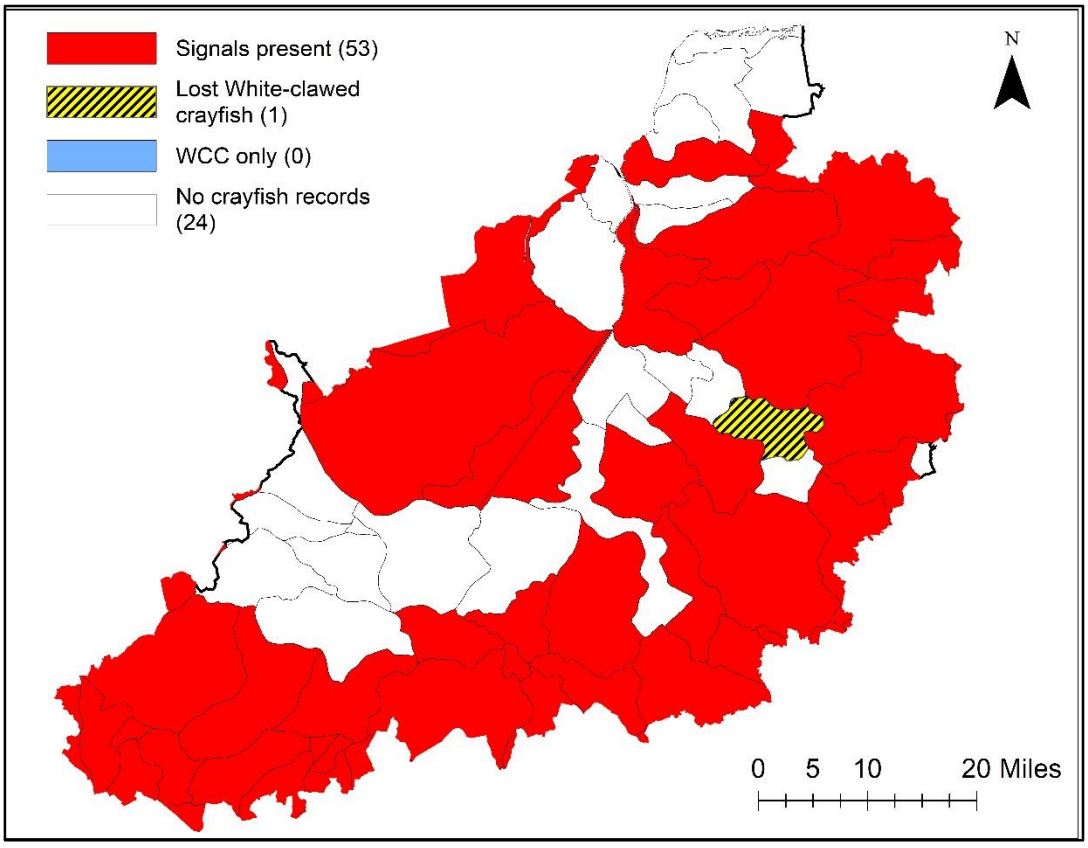


Figure 2 - A17 map for the Cambridgeshire and Bedfordshire region for the 2018 report

Cumbria and Lancashire

Denoted as region 14 on the National scale map (Fig 6 in Ch. 2), Cumbria and Lancashire covers an area of 4035 mi^{-2}, and contains 75 sub-catchment cells. According to the most accurate previous records presented in the 2010 map, the Cumbria and Lancashire region contained 14 red cells, 19 blue cell and 42 white cells. In practice, the region is operated as two sub-regions, namely North Cumbria, and South Cumbria and Lancashire, but the region was addressed in whole for the purpose of data presentation.

The Caldew (108), Lower Derwent in Cumbria (262), Crossens (205), Yarrow (895), Douglas (231), and Darwen (166) had *P. leniusculus* populations reported (Fig 3). In addition, the Calder in Lancashire (104), Brathay (151), Keer (402) and Wenning (839) sub-catchments that were previously uninvaded and contained only *A. pallipes*, now reported *P. leniusculus* populations to be present. No new *A. pallipes* populations were reported.

The updated regional figures are 24 red cells, 4 of which are red hatched replacement cells, 15 blue cells, and 36 white cells (Fig 4). The steady incursion of invasive crayfish into a region considered to be one of the last strongholds for native crayfish is of major concern.

Due to the historically large range of the species, populations of *A. pallipes* still occurred (albeit fragmented and reduced) across much of England. As such, unlike Annex II species with restricted ranges, many of the current populations do not fall within the Natura 2000 network of SACs. As is the case with many similarly distributed Annex II species, *A. pallipes* management consists, therefore, of a complimentary mix of on-site (SAC) management and off-site conservation plans.

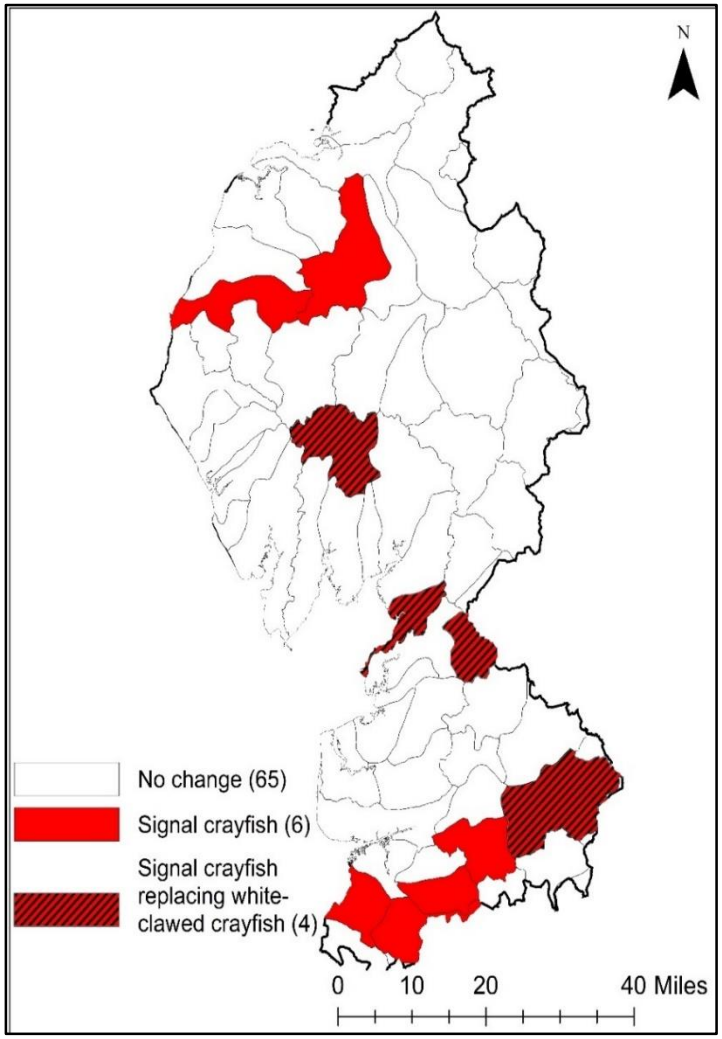


Figure 3 - Changes to the Cumbria and Lancashire region between 2012 and 2018

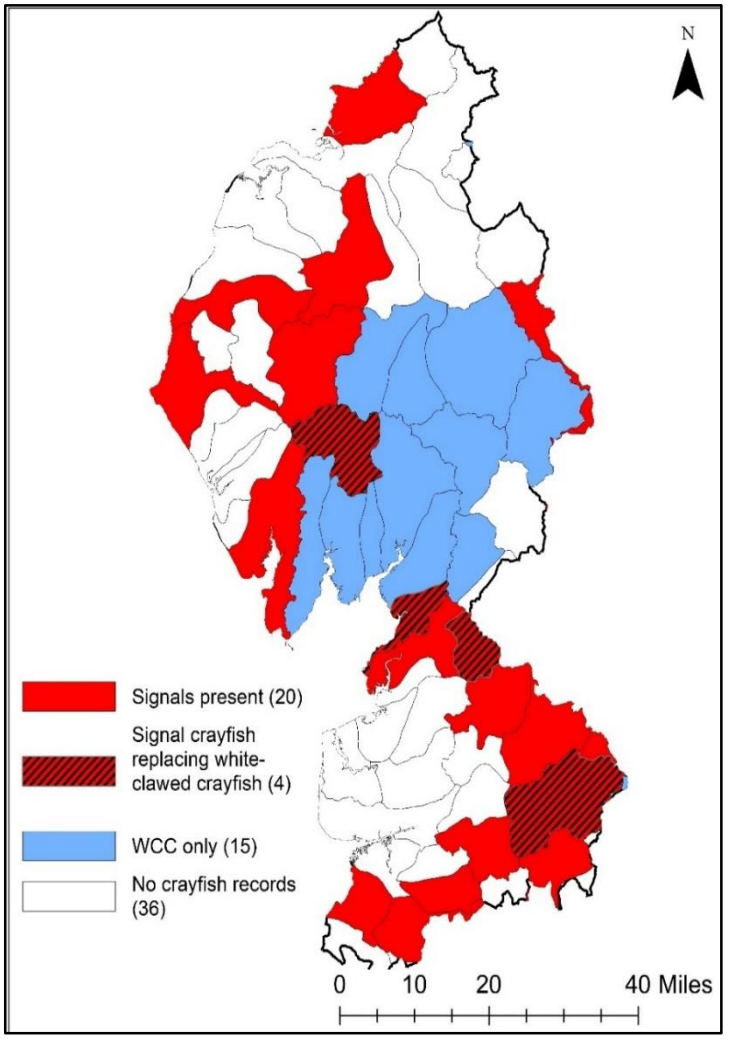


Figure 4 - A17 map for the Cumbria and Lancashire region for 2018 report

Devon and Cornwall

Denoted as region 1 on the National scale map (Fig 6 in Ch. 2), Devon and Cornwall covers an area of 4042 mi^{-2}, and contains 92 sub-catchment cells. According to the most accurate previous records presented in the 2010 map, the Devon and Cornwall region contained 47 red cells, 0 blue cells and 45 white cells.

The Clyst (140), Exe Lower (308), and Torridge Middle (803) have been updated to reflect *P. leniusculus* populations being present; these populations are believed to have been established for a number of years (Fig 5). The Yeo Devon and Dalch (900) was wrongly identified as containing *A. pallipes* in the previous map iteration, with records instead referring to the neighbouring Yeo Devon (899) sub-catchment. This has been rectified, but not recorded as a 'loss' of a cell. In addition, a population of *A. pallipes* was reported in the Culm (212), however, due to the previously confirmed presence of *P. leniusculus*, this cell remained red.

The updated figures for the region now stand at 50 red cells, 0 blue cells and 42 white cells. Perhaps somewhat in opposition to this map (Fig. 6), Devon and Cornwall has arguably been the most industrious region in active preservation and conservation of native *A. pallipes* stocks, principally through the use of ark sites and the work of the South West Crayfish Partnership. Whilst 16 ark sites have been established in the South West by the SWCP (Nightingale *et al*., 2017), dramatically increasing both the distribution and biosecurity of remaining native stocks, the extent of the spread of *P. leniusculus* is clear, and many cells remain absent of crayfish records; there remains the potential for populations of either species to be established across this region.

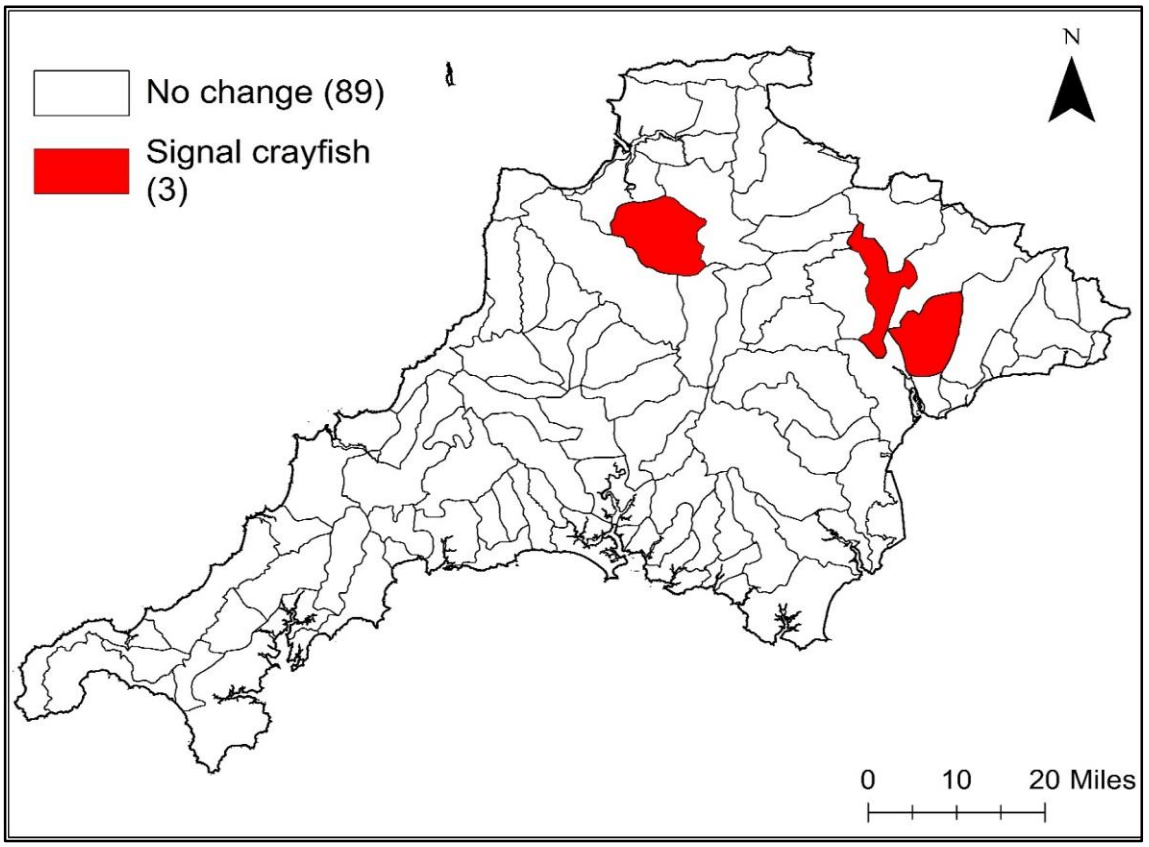


Figure 5 - Changes to the Devon and Cornwall region between 2012 and 2018

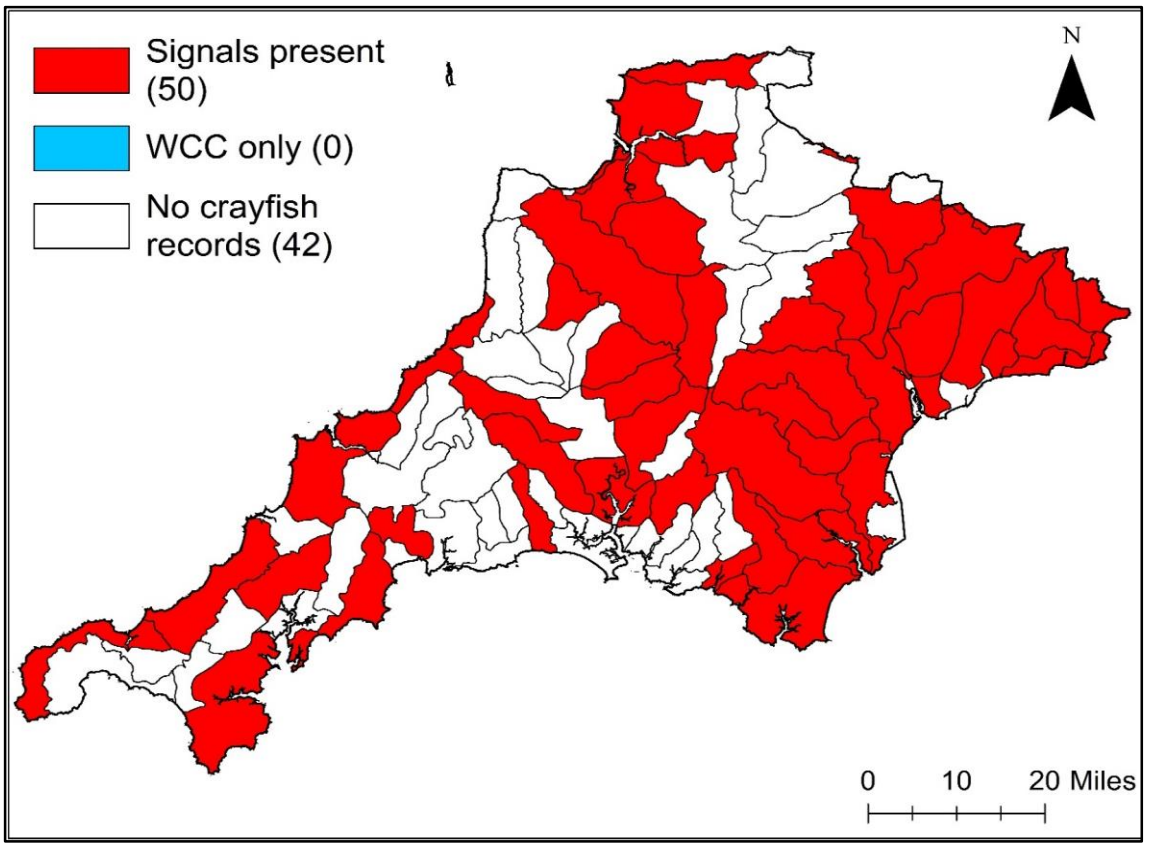


Figure 6 - A17 map for the Devon and Cornwall region for 2018 report

Derbyshire Nottinghamshire and Leicestershire

Denoted as region 10 on the National scale map (Fig 6 in Ch. 2), Derbyshire, Nottinghamshire and Leicestershire cover an area of 2679 mi^{-2}, and contains 83 sub-catchment cells. According to the most accurate previous records presented in the 2010 map, the Derbyshire, Nottinghamshire and Leicestershire region contained 33 red cells, 25 blue cells and 25 white cells.

Following consultation, there was a single new cell containing *P. leniusculus* populations reported in the Leicester Soar (614) (Fig. 7).

The updated figures for the region now stand at 34 red cells, 25 blue cells and 24 white cells (Fig. 8). Of concern in this region are the many historical records and populations not receiving regular status updates through sampling. The distribution presented here could be far worse if *P. leniusculus* have indeed spread, but without further monitoring data it is much harder to interpret the regional distribution trends. Many blue cells are bordering red cells, and these 'conflict zones' are where management efforts should be focussed initially to attempt to determine the advancing edge of the *P. leniusculus* invasion.

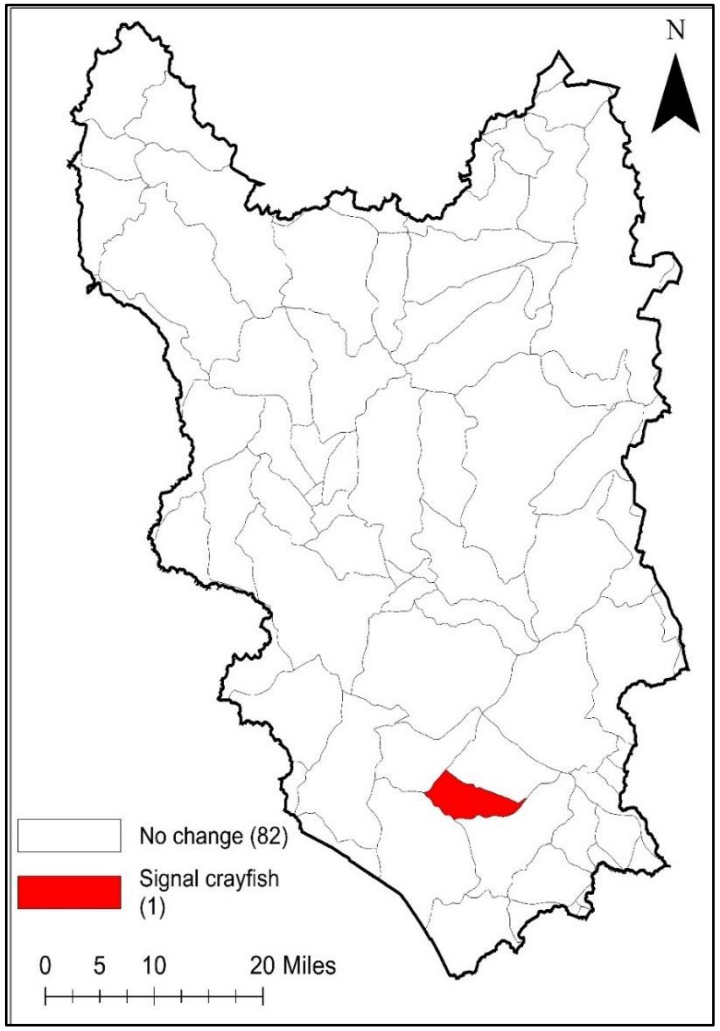


Figure 7 - Changes to the Derbyshire, Nottinghamshire and Leicestershire region between 2012 and 2018

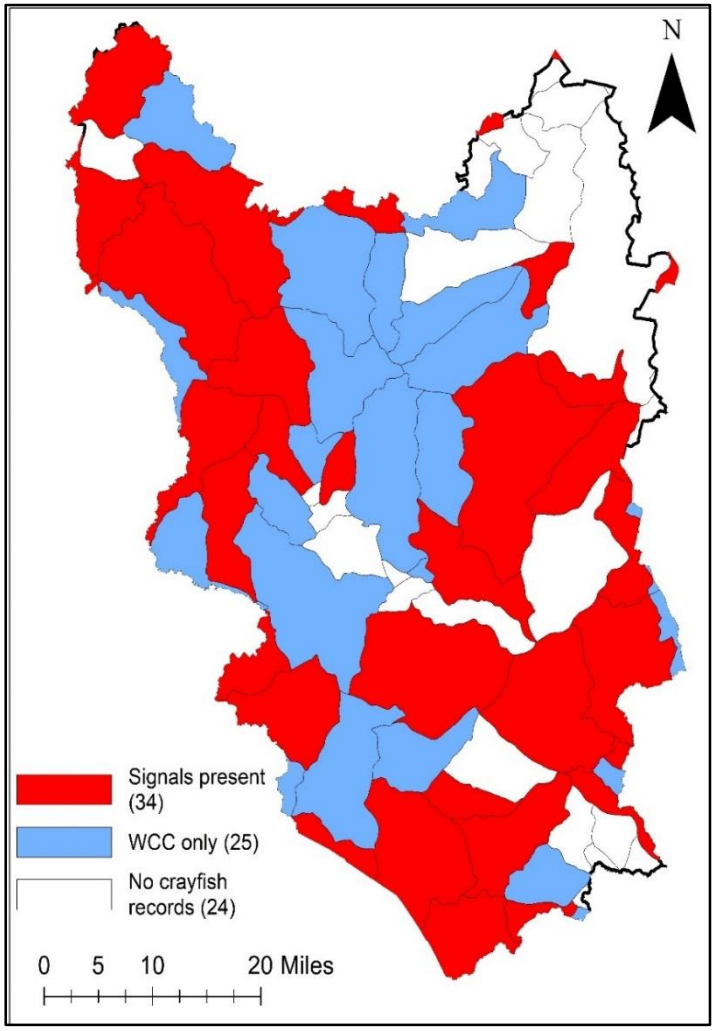


Figure 8 - A17 map for the Derbyshire, Nottinghamshire and Leicestershire region for 2018 report

Essex Norfolk and Suffolk

Denoted as region 7 on the National scale map (Fig 6 in Ch. 2), Essex Suffolk and Norfolk cover an area of 3504 mi^{-2}, and contain 73 sub-catchment cells. According to the most accurate previous records presented in the 2012 map, the Essex Suffolk and Norfolk region contained 49 red cells, 4 blue cells and 20 white cells.

The Brett (154), Sandon Brook (636), the Mun and Coast (502) the Little Ouse (440) which overlaps with neighbouring region Cambridgeshire and Bedfordshire, were updated to reflect well established *P. leniusculus* populations being present (Fig. 9). In addition, the Box (145), Bourne Brook in Essex (54), the Ter (754) and the Upper Colne near Essex (184), 4 sub-catchments that were previously uninvaded and contained only *A. pallipes*, now reported *P. leniusculus* populations to be present, represented by red cell with black hatchings. One new *A. pallipes* sub-catchment was also included in this review, for the Stiffkey (699), where several small populations are present. Also of note, is a population of invasive Turkish crayfish (*Astacus leptodactylus*) in the Waveney South Tidal (823) sub-catchment; this population is isolated, and not addressed within this series of maps.

The regional figures for Essex, Norfolk and Suffolk now stand at 57 red cells, 4 of which are replacements of previously blue cells, 1 blue cell, and 15 white cells (Fig. 10). The loss of blue cells and expansion of red cells is indicative of much of England over the previous 6-year period, but also highlights some of the issues in the monitoring and reporting process for crayfish within the UK. For example, the Stiffkey had held a small population of *A. pallipes* for several years, but had not been officially recorded. There exists a likely disconnect between establishment dates and recording dates of crayfish populations within England.

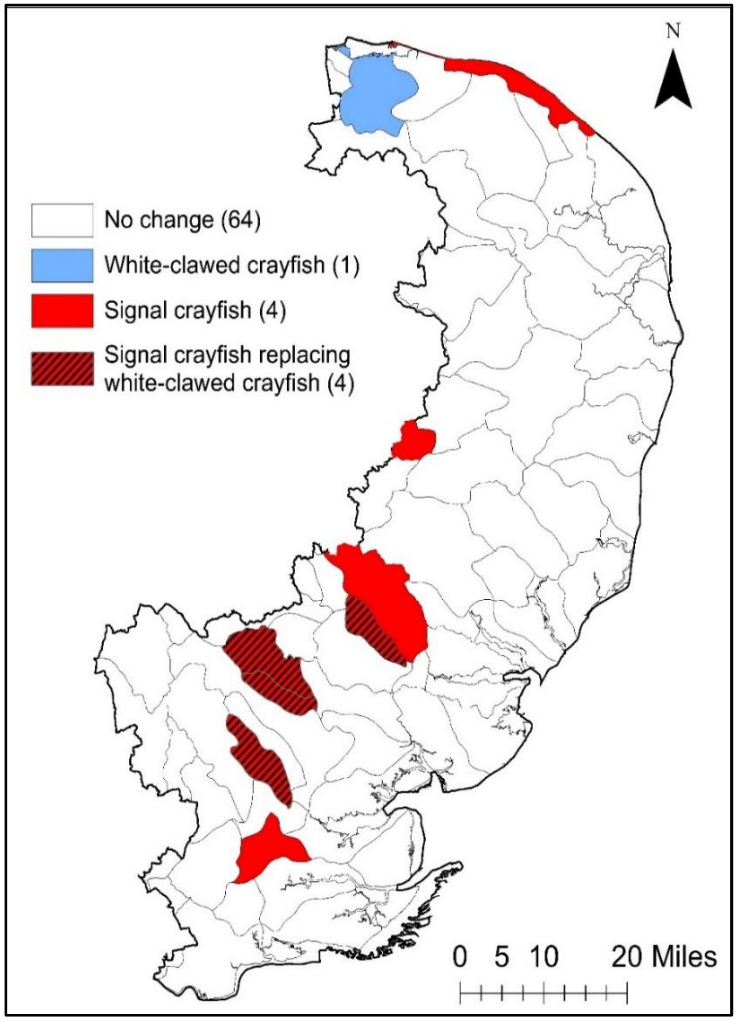


Figure 9 – Changes to the Essex Suffolk Norfolk A17 map between 2012 and 2018

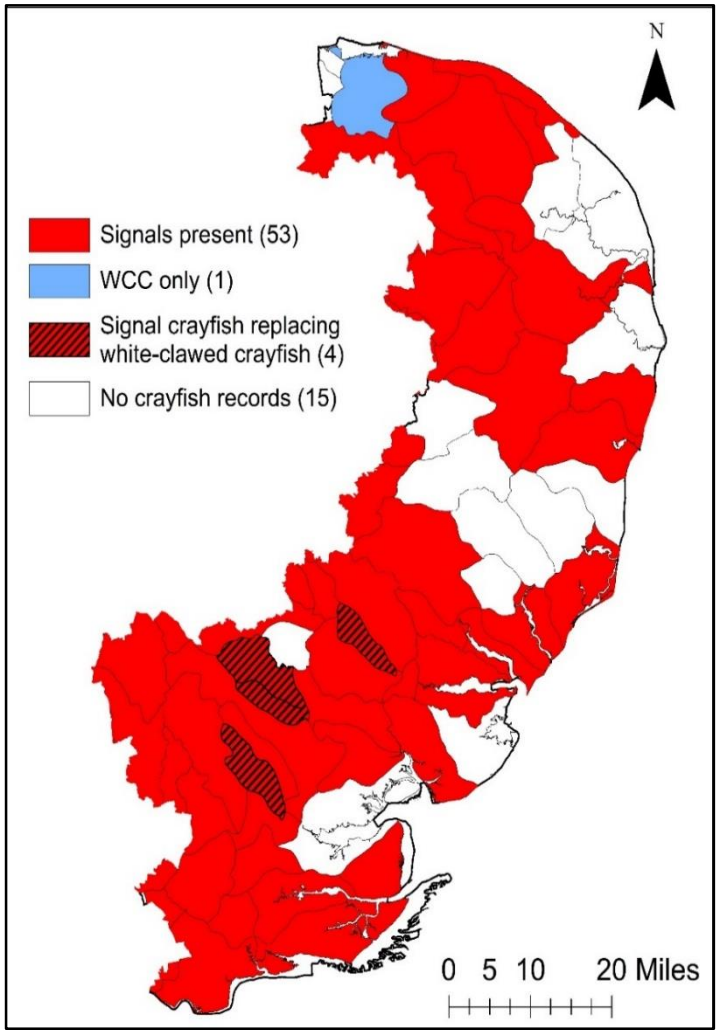


Figure 10 - A17 map for the Essex Suffolk Norfolk region for 2018 report

Greater Manchester Merseyside and Cheshire

Denoted as region 13 on the National scale map (Fig 6 in Ch. 2), Greater Manchester, Merseyside and Cheshire cover an area of 1727 mi^{-2}, and contain 47 sub-catchment cells. According to the most accurate previous records presented in the 2012 map, the Greater Manchester Merseyside and Cheshire region contained 11 red cells, 5 blue cells and 31 white cells.

A previously unrecorded population of *P. leniusculus* was reported in the Dean (168), and a previously unrecorded population of *A. pallipes* was reported in the Tame (678) (Fig. 11). The Peover Eye (565) sub-catchment has been updated to reflect records of both *P. leniusculus* and *A. pallipes* populations being present; whilst newly discovered populations of *A. pallipes* are well received, the presence of *P. leniusculus* forced a mixed sub-catchment and thus red category. In addition, the *A. pallipes* from the Gowy (294) had been lost.

The regional figures for Greater Manchester, Merseyside and Cheshire now stand at 13 red cells, 5 blue cells, 1 yellow hatched cell denoting a straight loss of native populations, and 28 white cells (Fig. 12). The steady spread of signals to the east of the region displays a clear strengthening of the invasive foothold in the region, and particular interest should be shown to sub-catchment cells currently coloured white bordering this territory. Indeed, there may be populations of native and invasive crayfish yet to be officially reported, as 28 of 47 cells remain with no crayfish records, which does not necessarily confer an absence of crayfish populations.

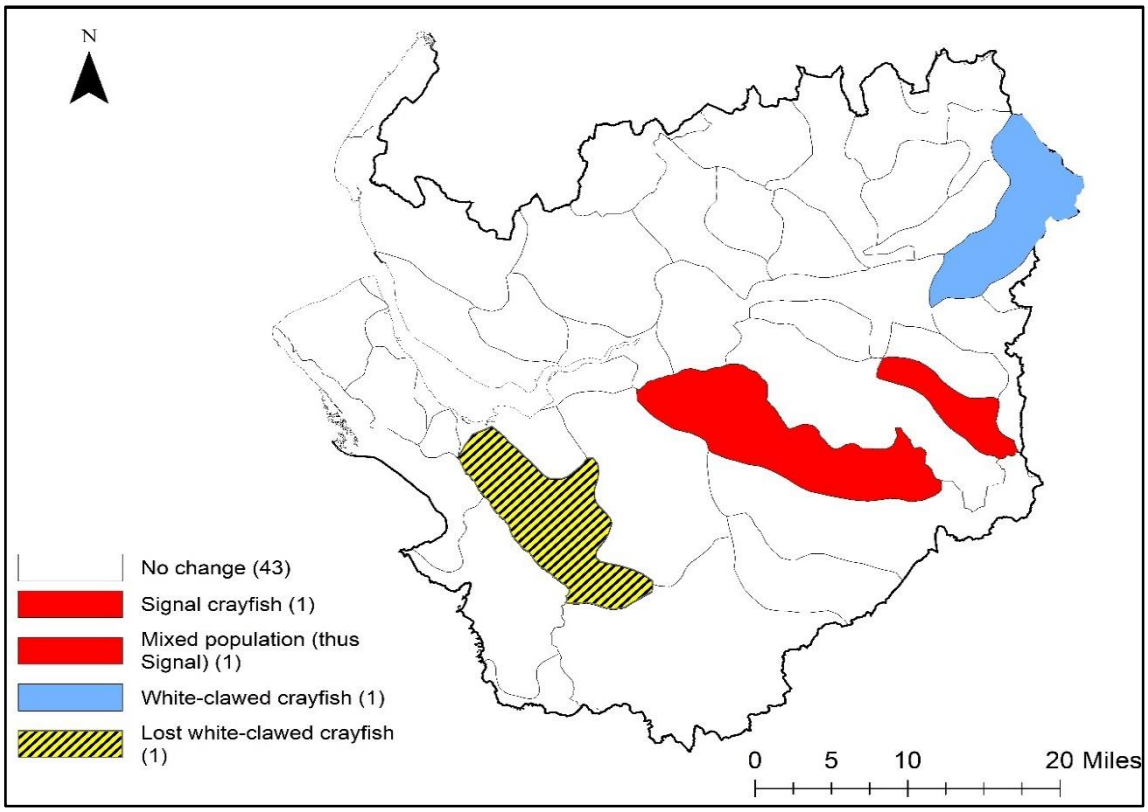


Figure 11 - Changes to the Greater Manchester, Merseyside and Cheshire region between 2012 and 2018

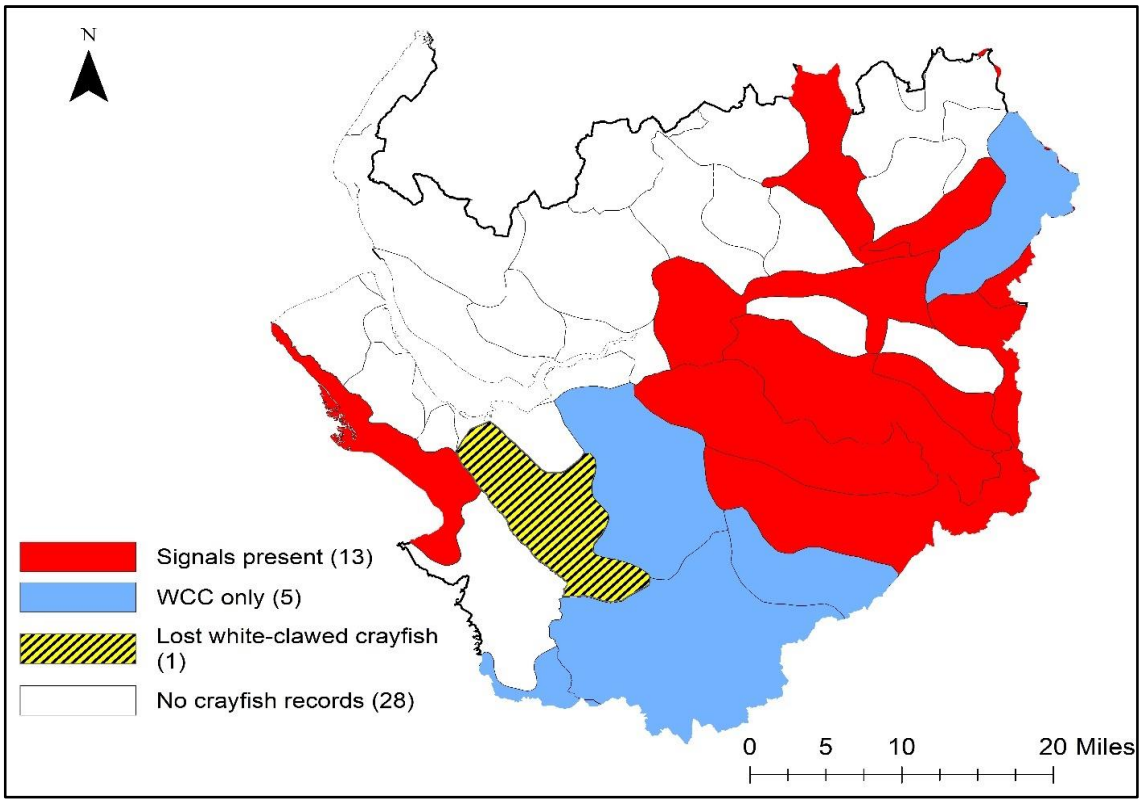


Figure 12 - A17 map for the Greater Manchester, Merseyside and Cheshire region for 2018 report

Hertfordshire and North London

Denoted as region 6 on the National scale map (Fig 6 in Ch. 2), Hertfordshire and North London covers an area of 1369 mi^{-2}, and contain 41 sub-catchment cells. According to the most accurate previous records presented in the 2012 map, the Hertfordshire and North London region contained 37 red cells, 0 blue cells and 4 white cells.

The Hertfordshire and North London reported no changes to the current status of their native *A. pallipes* populations (Fig. 13). Additionally, no changes were reported to the *P. leniusculus* territories within this region. Only 4 white cells, and 0 blue cells, remain. Given the widespread and established nature of the invasive populations within this region, little benefit is to be gained from conservation efforts relating to crayfish here, until a reliable solution to tackling invasive populations is implemented.

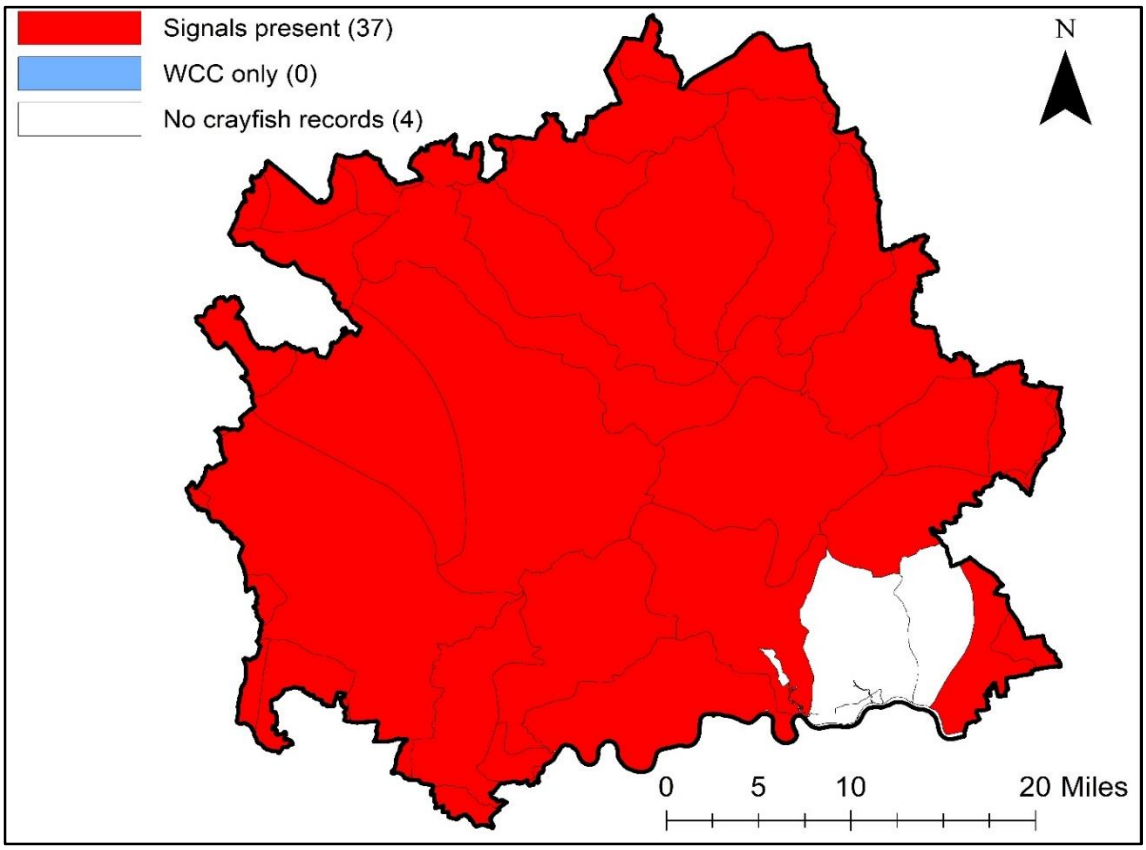


Figure 13 - A17 map for the Hertfordshire and North London region for 2018 report (*please note that this map represents the status for 2010, since no newer data was available)

Kent and South London

Denoted as region 5 on the Nation Scale map (Fig. 6 in Ch. 2), Kent and South London contain 35 sub catchment cells. No return was provided for this region, and so no updated figures could be provided. The previous count for the Kent and South London region was 25 red cells, 5 blue cells, and 5 white cells (Fig. 14).

Given the spread of *P. leniusculus* in the region, and surrounding regions, the remaining blue and white cells are likely to be under invasive pressure, if they have not already been invaded.

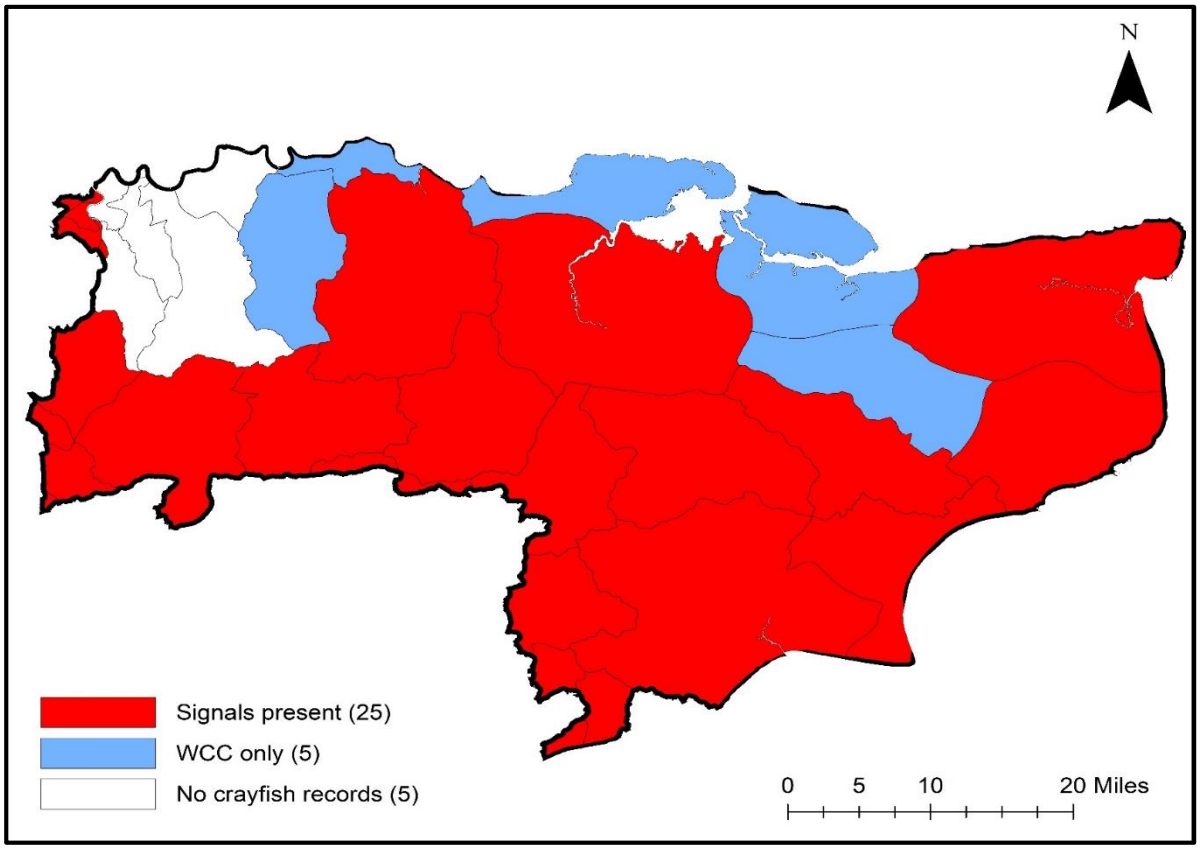


Figure 14 - A17 map for the Kent and South London region for the 2018* report (*please note that this map represents the status for 2010, since no newer data was available)

Lincolnshire and Northamptonshire

Denoted as region 9 on the National scale map (Fig 6 in Ch. 2), Lincolnshire and Northamptonshire covers an area of 3972 mi^{-2}, and contains 79 sub-catchment cells. According to the most accurate previous records presented in the 2012 map, the Lincolnshire and Northamptonshire region contained 37 red cells, 10 blue cell and 32 white cells.

Following the consultation round, a noticeable trend was reported (Fig. 15). Populations of native *A. pallipes* were lost from the Chater (112), Harpers Brook (378), the Jordan (400), the Nene (511 & 512), and the West Glen (842). No *P. leniusculus* populations were reported within these cells; thus they were designated as lost catchments (yellow with black hatchings).

The updated regional figures now stand at 37 red cells, 5 blue cells, and 32 white cells, and 5 yellow hatched cells (Fig. 16). Whilst a corresponding expansion of *P. leniusculus* territories has not been reported in conjunction with these losses, thus retaining the previously recorded 32 red cells, it is likely given the location and context of these losses that *P. leniusculus* populations have indeed spread and remain as yet undetected.

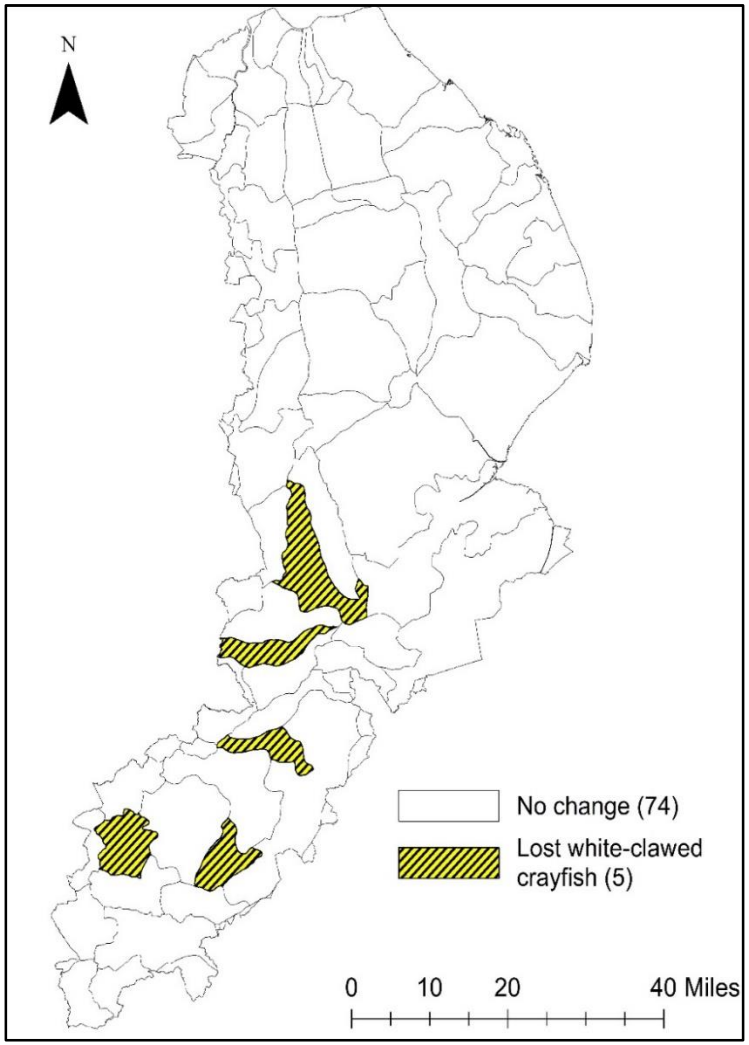


Figure 15 - Changes to the Lincolnshire and Northamptonshire region between 2012 and 2018

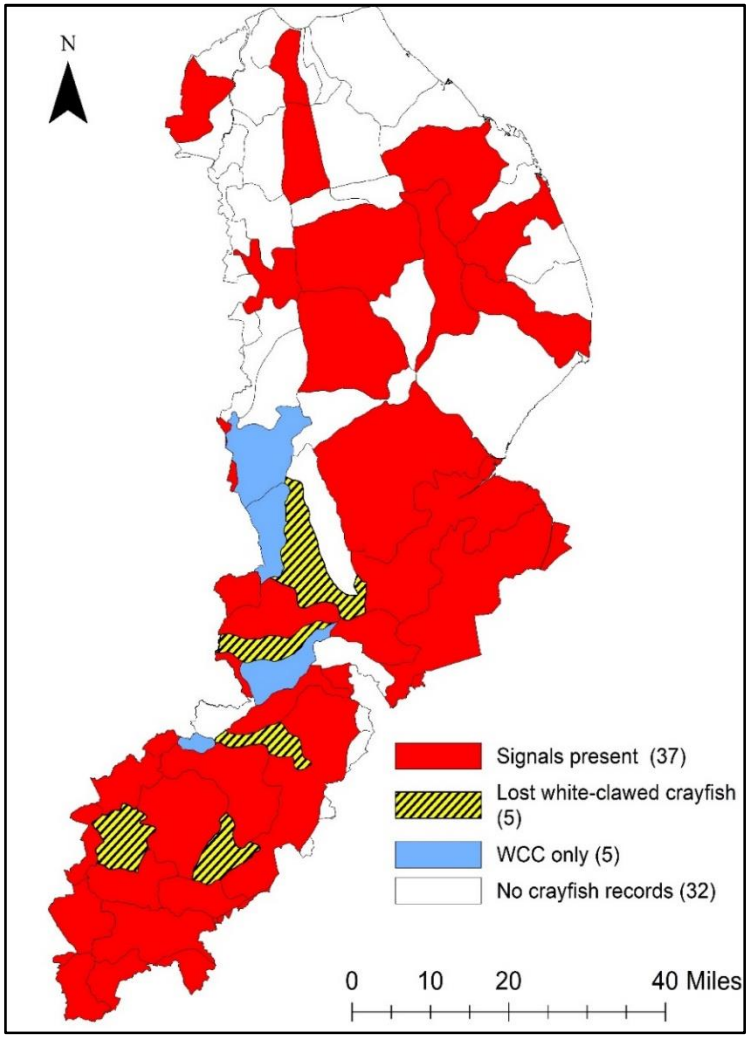


Figure 16 - A17 map for the Lincolnshire and Northamptonshire region for 2018 report

Northumberland, Durham and Tees

Denoted as region 16 on the National scale map (Fig 6 in Ch. 2), Northumberland Durham and Tees cover an area of 3350 mi^{-2}, and contain 52 sub-catchment cells. According to the most accurate previous records presented in the 2012 map, the Northumberland Durham and Tees region contained 12 red cells, 13 blue cells and 27 white cells.

Following consultation with the regional team, several changes are noted (Fig. 17). Firstly, new *P. leniusculus* populations have been reported in the previously uninvaded areas of Skerne (609), Lower Wear (825), Upper Wear (829), and Druridge Bay Coastal Area (240). These populations have been confirmed with high confidence through local Environment Agency officers conducting standardised Analysis and Reporting surveys, and input from the local Rivers Trust. In addition, *P. leniusculus* have now been reported in four sub-catchments previously solely colonised by *A. pallipes*, namely Pont (577), North Tyne (480 and 481) and the Lower Wear (826).

Following these changes, the region now contains 20 red cells, 4 of which were previously blue, 9 blue cells and 23 white cells (Fig. 18). This region is a Northern stronghold for populations of *A. pallipes*. The steady expansion of *P. leniusculus* territories in this region, along with the large number of white cells bordered by signal populations, gives reason for concern.

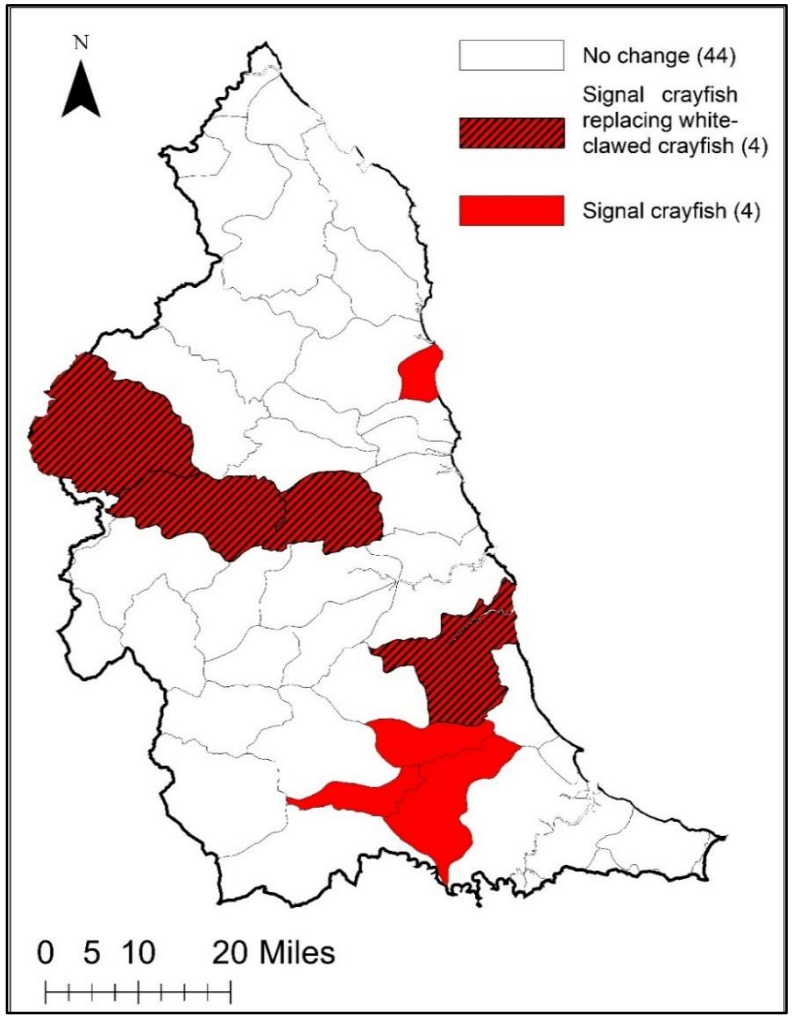


Figure 17 - Changes to the Northumberland Durham and Tees region between 2012 and 2018

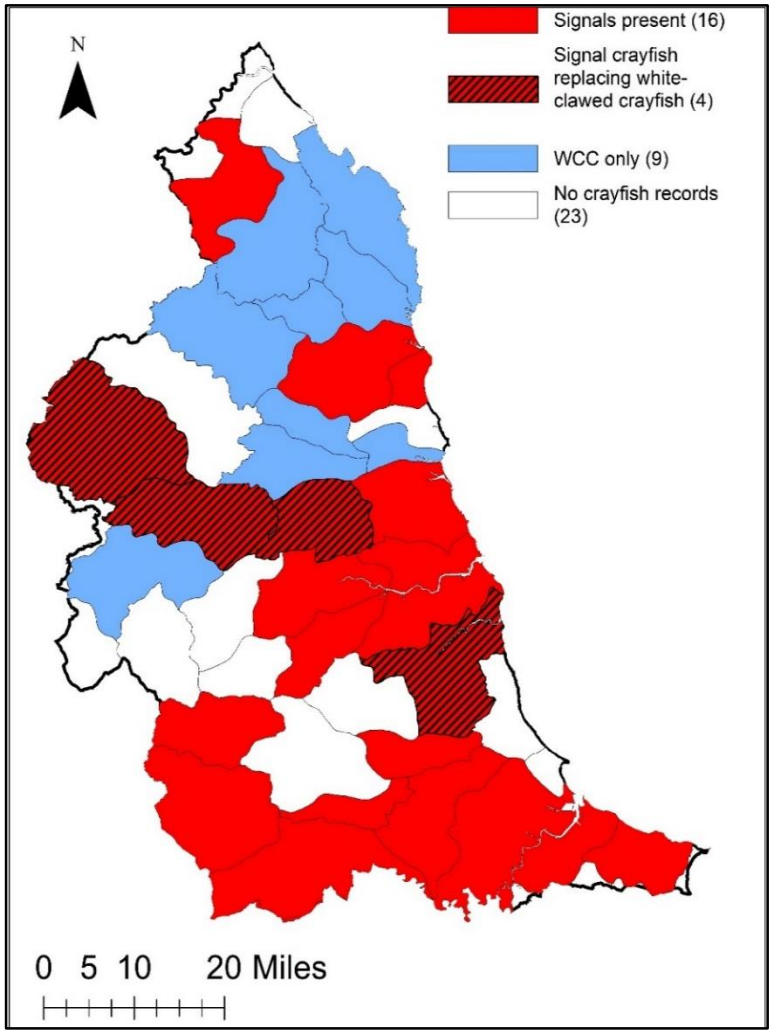


Figure 18 - A17 map for the Northumberland Durham and Tees region for 2018 report

Shropshire Herefordshire Worcestershire and Gloucestershire

Denoted as region 12 on the National scale map (Fig 6 in Ch. 2), Shropshire, Herefordshire, Worcestershire and Gloucestershire cover an area of 3455 mi^{-2}, and contain 61 sub-catchment cells. According to the most accurate previous records presented in the 2012 map, the Northumberland Durham and Tees region contained 31 red cells, 23 blue cells and 7 white cells.

Several changes were notified for the region (Fig. 19). The Arrow (8), Bow Brook (144), the Lugg (448), the Lower Severn (651), the Upper Severn (601, 603 & 654), the Roden (618) and the Teme (748) all now have *P. leniusculus* populations, where in the previous map iteration they were solely occupied by native *A. pallipes*. An unreported *P. leniusculus* population was reported in the Upper Severn (598), and *A. pallipes* were reported in the Upper Severn (654).

Following these changes, the region now holds 41 red cells, 9 of which were previously blue, 15 blue cells, and 5 white cells (Fig. 20). It is unclear if the high levels of *P. leniusculus* spread into native areas reflects true increased dispersal, or an increase in reporting or sampling efforts. What is clear is that *P. leniusculus* now have a strong connected network of populations throughout the centre of the region, putting the remaining *A. pallipes* populations under increasing pressure.

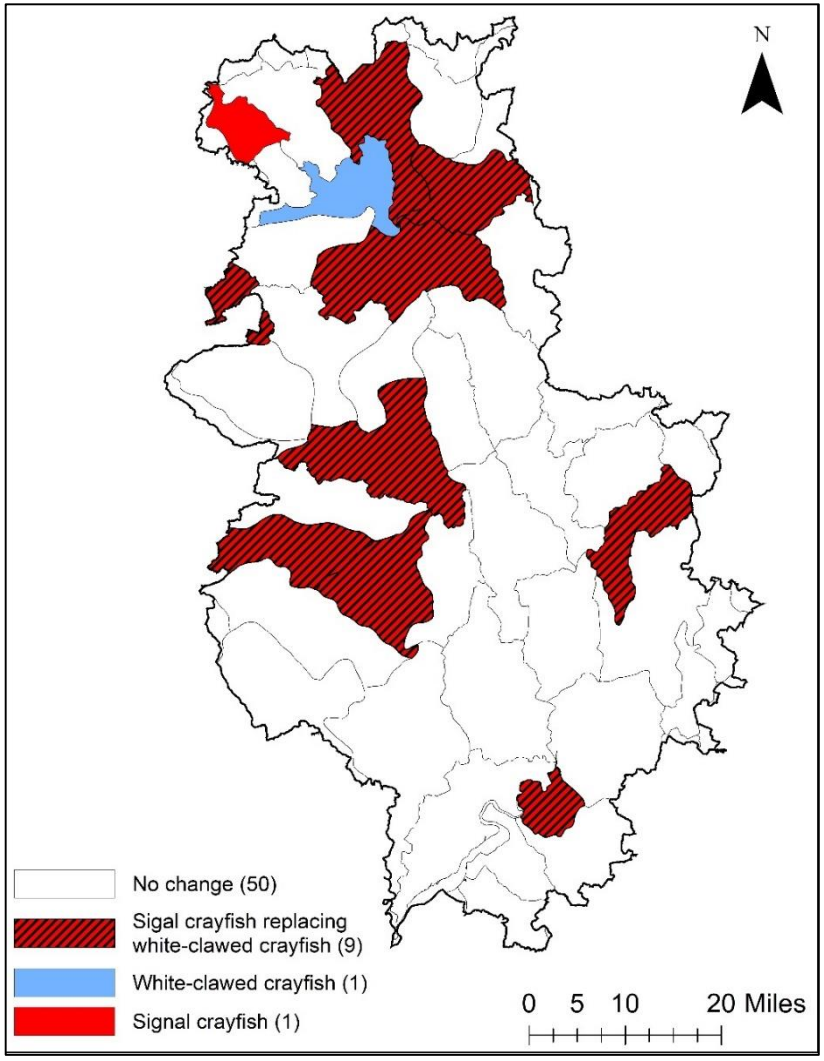


Figure 19 - Changes to the Shropshire, Herefordshire, Worcestershire and Gloucestershire region between 2012 and 2018

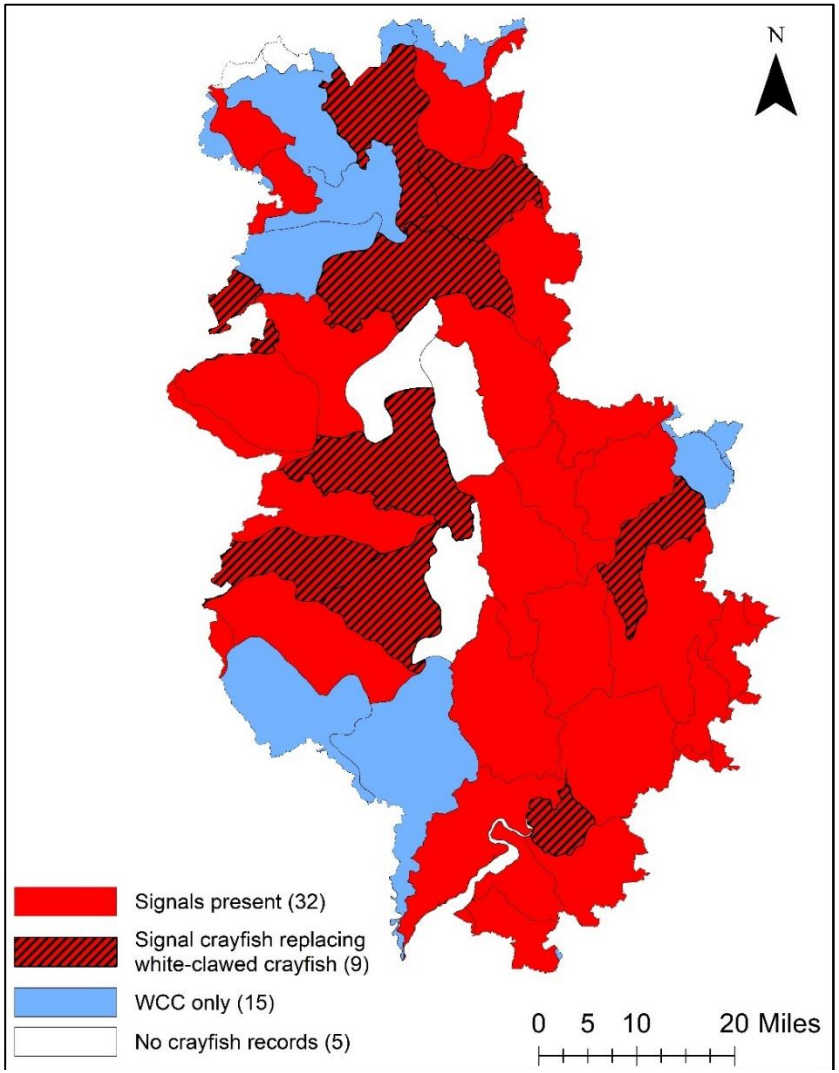


Figure 20 - A17 map for the Shropshire, Herefordshire, Worcestershire and Gloucestershire region for 2018 report

Solent and South Downs

Denoted as region 4 in the National Scale map (Fig. 6 in Ch. 2), Solent and South Downs contain 49 sub catchment cells. No return was provided for this region, and so no updated figures could be provided. The previous count for the Solent and South Downs region was 43 red cells, 3 blue cells, and 3 white cells (Fig. 21).

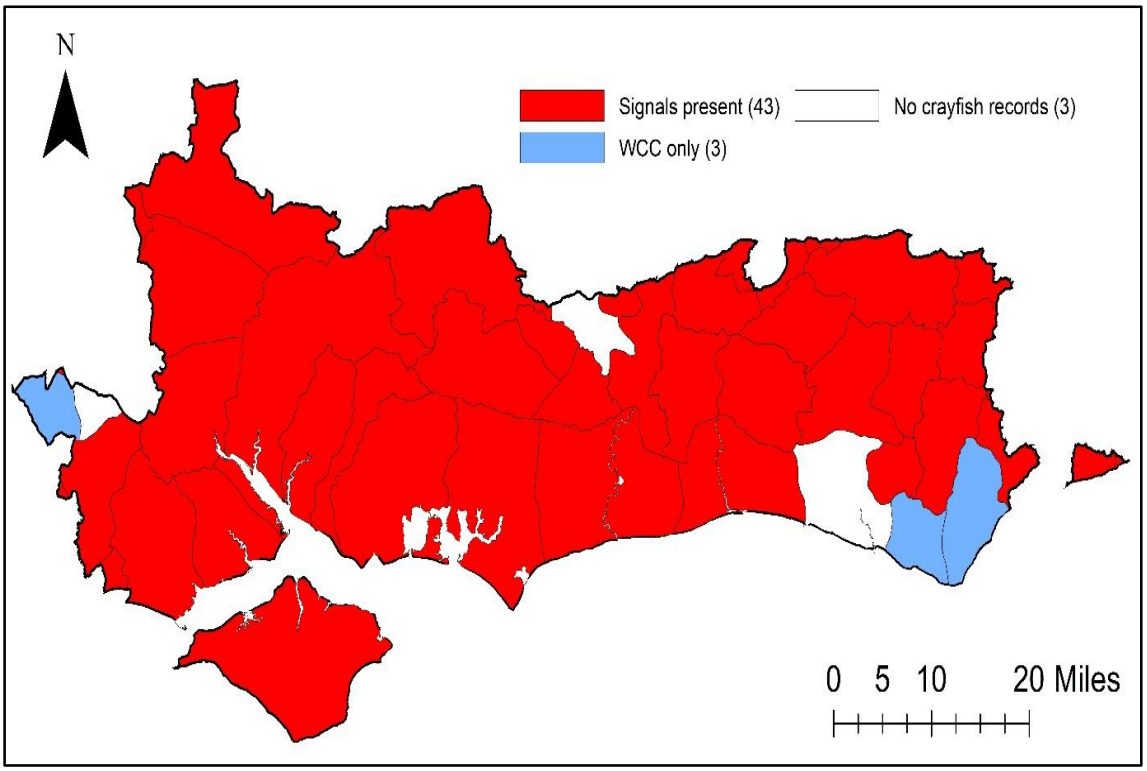


Figure 21 - A17 map for the Solent and South Downs region, recreated as per the 2012 JNCC report (*please note that this map represents the status for 2010, since no newer data was available).

Staffordshire Warwickshire and West Midlands

Denoted as region 11 on the National scale map (Fig 6 in Ch. 2), Staffordshire, Warwickshire and West Midlands cover an area of 2161 mi^{-2}, and contain 57 sub-catchment cells. According to the most accurate previous records presented in the 2012 map, the Staffordshire, Warwickshire and West Midlands region contained 32 red cells, 22 blue cell and 3 white cells.

Following consultation with the regional team, a rather bleak update was reported. All populations of *A. pallipes* in the Blythe (47), the Bourne (52), the Upper Avon (74), the Cole (181), the Upper Manifold (462), the Rea (589), the Tame (679) and lower Tame (681), the Lower Sow (691), the Tame and Bourne Brook (729), and Trent confluence (765) were lost since the last map iteration (Fig. 22).

The previously reported 3 white cells remain at present. The loss of a number of important *A. pallipes* populations is of immediate and full concern, as the regional figures drop from 22 to just 10 blue cells, a significant decline in just 6 years (Fig. 23). Whilst again a corresponding expansion of *P. leniusculus* territories has not been reported in conjunction with these losses as is the case in the Lincolnshire and Northamptonshire region, thus retaining the previously recorded 32 red cells, it is suspected that *P. leniusculus* have spread, and populations exist at limits below current survey efforts.

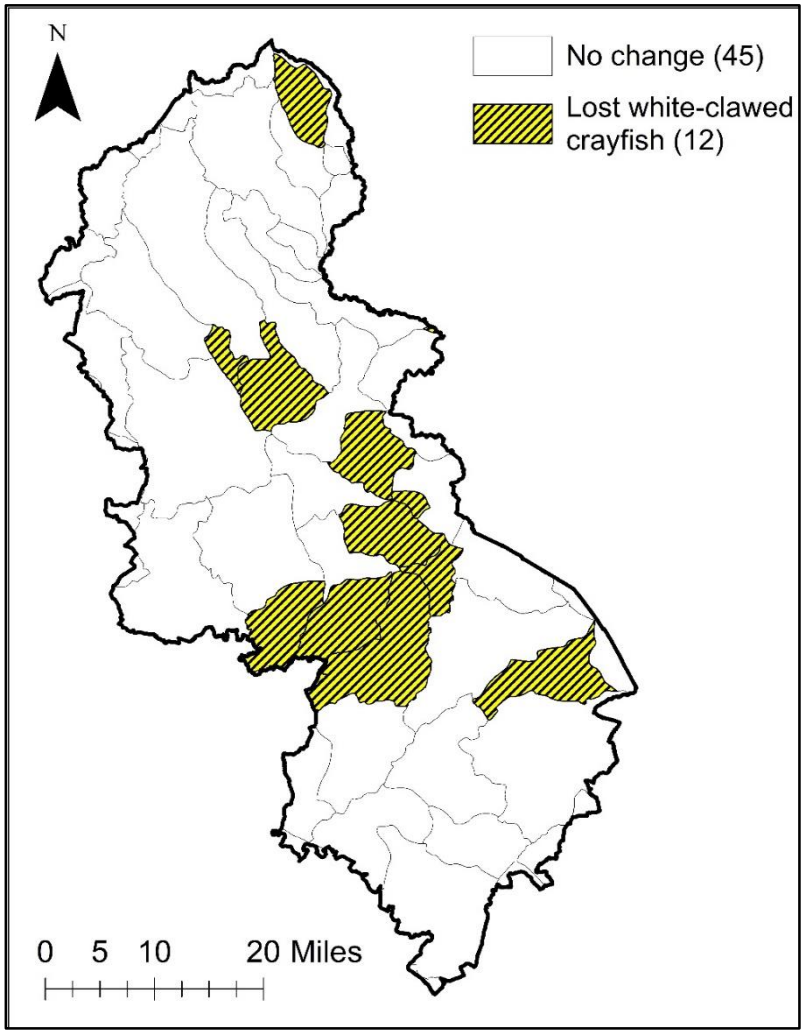


Figure 22 - Changes to the Staffordshire, Warwickshire and West Midlands region between 2012 and 2018

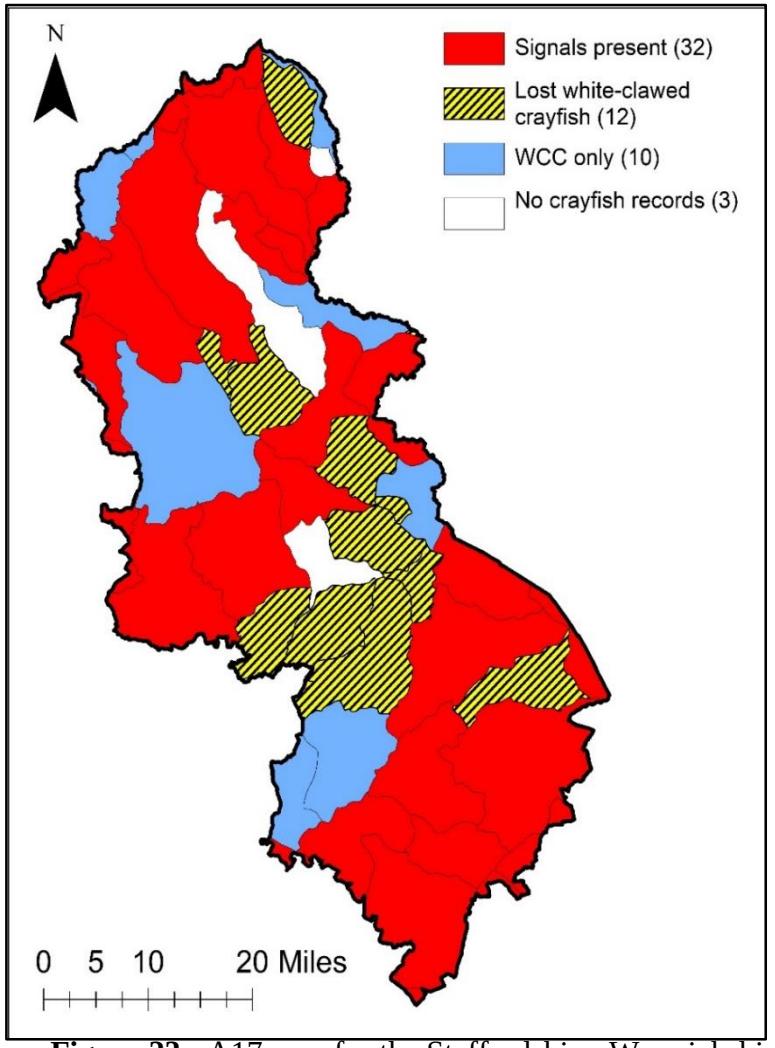


Figure 23 - A17 map for the Staffordshire, Warwickshire and West Midlands region for 2018 report

Wessex

Denoted as region 2 on the National scale map (Fig 6 in Ch. 2), Wessex covers an area of 4239 mi^{-2}, and contain 105 sub-catchment cells. According to the most accurate previous records presented in the 2012 map, the Wessex region contained 69 red cells, 15 blue cell and 21 white cells.

The Cale (126) was updated to include a *P. leniusculus* population. Both the Chew (119) and Sherston Avon (605) have lost *A. pallipes* populations that have subsequently been replaced by *P. leniusculus* populations. The populations of *A. pallipes* in the Stour Dorset Lower (701) and Allen Dorset (29) were both lost due to a crayfish plague outbreak; however, *P. leniusculus* were not believed to be present (Fig. 24).

As such, the region now holds 72 red cells, of which 2 are replacements of previously blue cells, 11 blue cells, 20 white cells and 2 cells where *A. pallipes* have been lost from but not replaced by invasive crayfish (denoted by yellow hatchings; Fig. 25). As previously addressed, given the biogeographical context of the lost populations, it is likely that *P. leniusculus* are present and have yet to be discovered in these two sub-catchments.

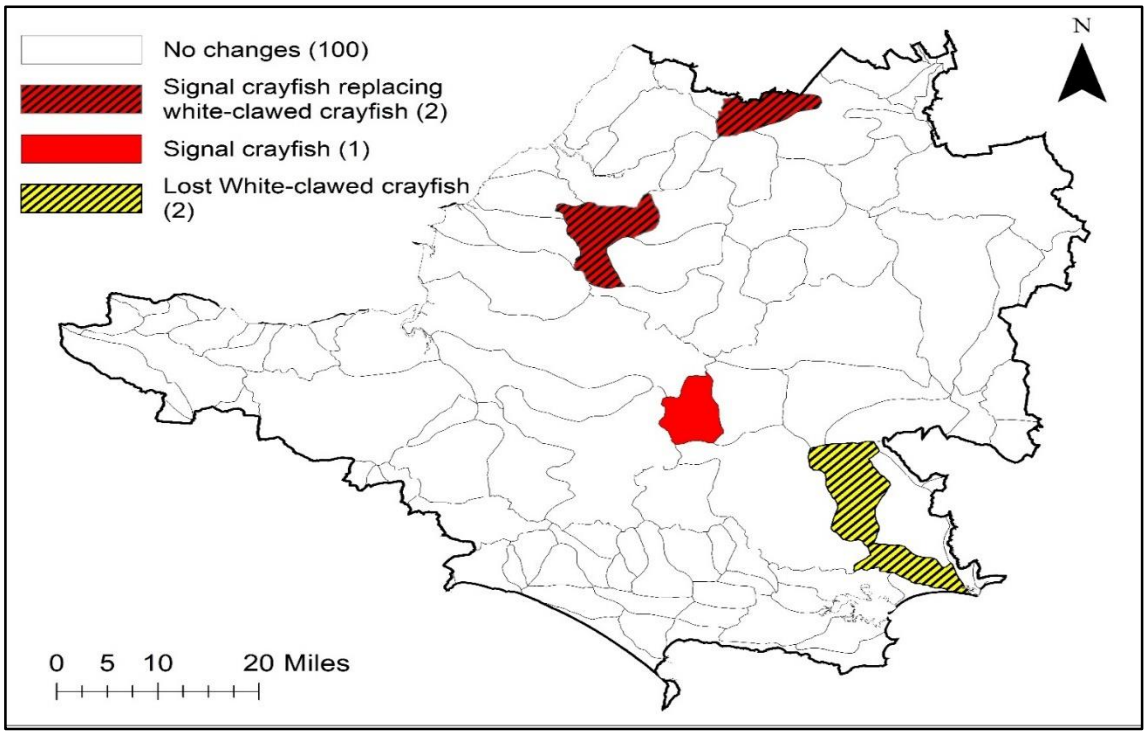


Figure 24 – Changes to the Wessex A17 map between 2012 and 2018

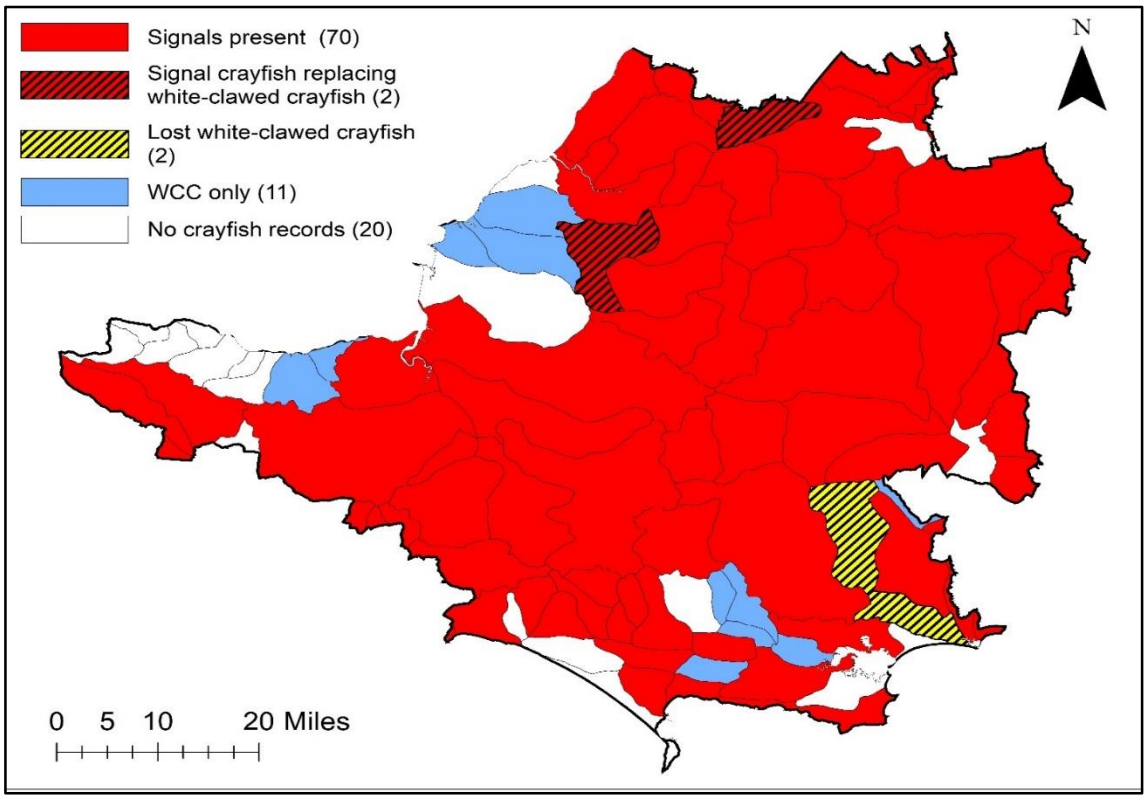


Figure 25 - A17 map for the Wessex region for 2018 report

West Thames

Denoted as region 3 on the National scale map (Fig 6 in Ch. 2), West Thames covers an area of 3236 mi^{-2}, and contain 75 sub-catchment cells. According to the most accurate previous records presented in the 2010 map, the West Thames region contained 68 red cells, 3 blue cell and 4 white cells.

The records in the Leach (422) were believed to no longer be accurate (Fig. 26). The original record dated from 1999, and no populations of *A. pallipes* have been reported for the Leach since then. This absence was consistent with data from the high resolution local records provided by the Environment Agency officers. As such, this sub-catchment was considered lost. The neighbouring cell in the Thames (709) had seen the incursion of *P. leniusculus* into the previously only *A. pallipes* sub-catchment. Given the high number of *P. leniusculus* cells surrounding this area, it was likely that native populations had been lost, and replacement by *P. leniusculus* was ongoing.

The West Thames region now holds 69 red cells, one of which is a replacement of a previously blue cell, one yellow hatched cell denoting lost *A. pallipes*, one blue cell, and 4 white cells (Fig. 27). This region was heavily invaded during the 70s, being one of the first areas in the UK to harvest *P. leniusculus*; the near ubiquitous spread of *P. leniusculus* in the West Thames area is testament to this legacy.

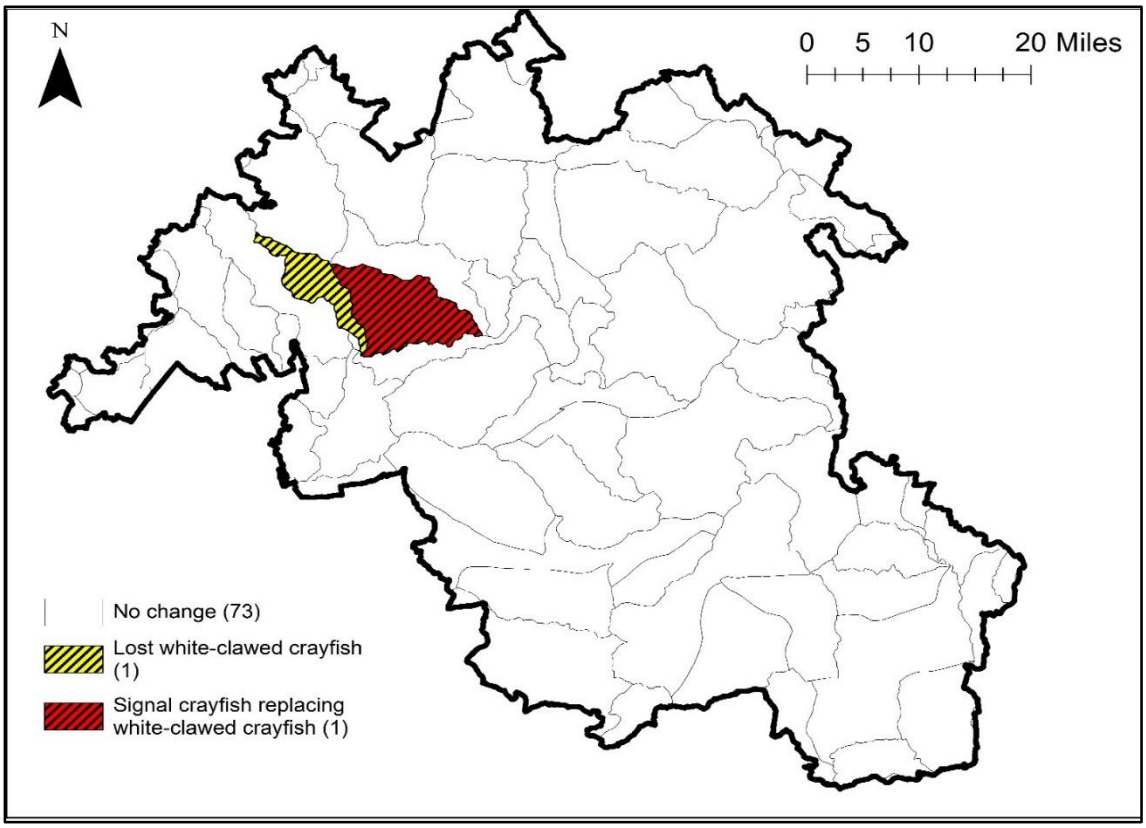


Figure 26 – Changes to the West Thames A17 map between 2012 and 2018

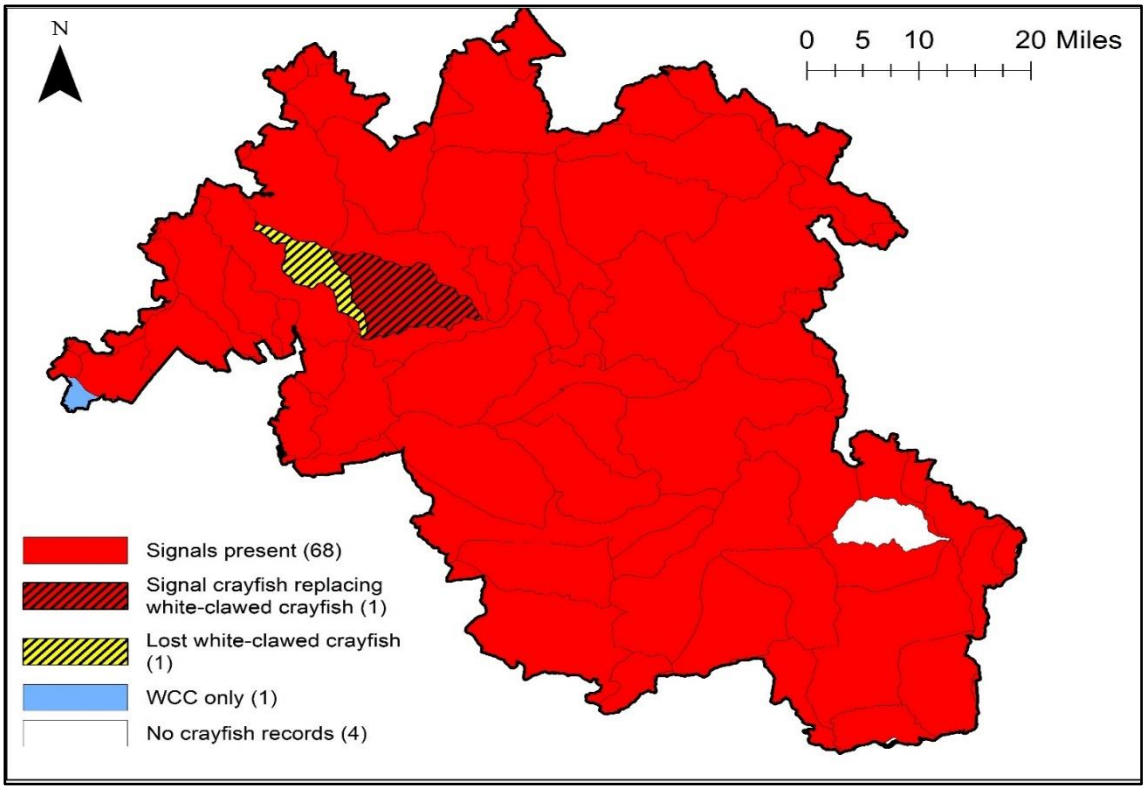


Figure 27 - A17 map for the West Thames region for 2018 report

Yorkshire

Denoted as region 15 on the National scale map (Fig 6 in Ch. 2), Yorkshire covers an area 5593 mi^{-2}, and contains 87 sub-catchment cells. According to the most accurate previous records presented in the 2010 map, the Yorkshire region contained 36 red cells, 25 blue cells and 26 white cells.

Following the consultation round, several changes were notified for the region (Fig. 28). Populations of *P. leniusculus* were reported for the Upper Don (227 & 228) for which no crayfish were noted in the 2012 report, as well as in the Rye (645), which previously was solely occupied by *A. pallipes*. *A. pallipes* were reported in the Rye (629), which was believed to have been missed in the previous map iteration. A second new native cell was reported for the Tame in Greater Manchester (678) due to the overlapping boundary with the GMMC region.

The Yorkshire region now holds 39 red cells, of which 1 is a replacement of a previous blue cell, 26 blue cells, and 22 white cells (Fig. 29). The region retains strong populations of *A. pallipes*, and shows limited spread of *P. leniusculus*. This makes Yorkshire on of the few remaining strongholds for *A. pallipes* populations in England, and an important conservation resource for the species. Many cells remain white however, and thus the regional map has the potential to change quite dramatically should additional populations of either species be discovered.

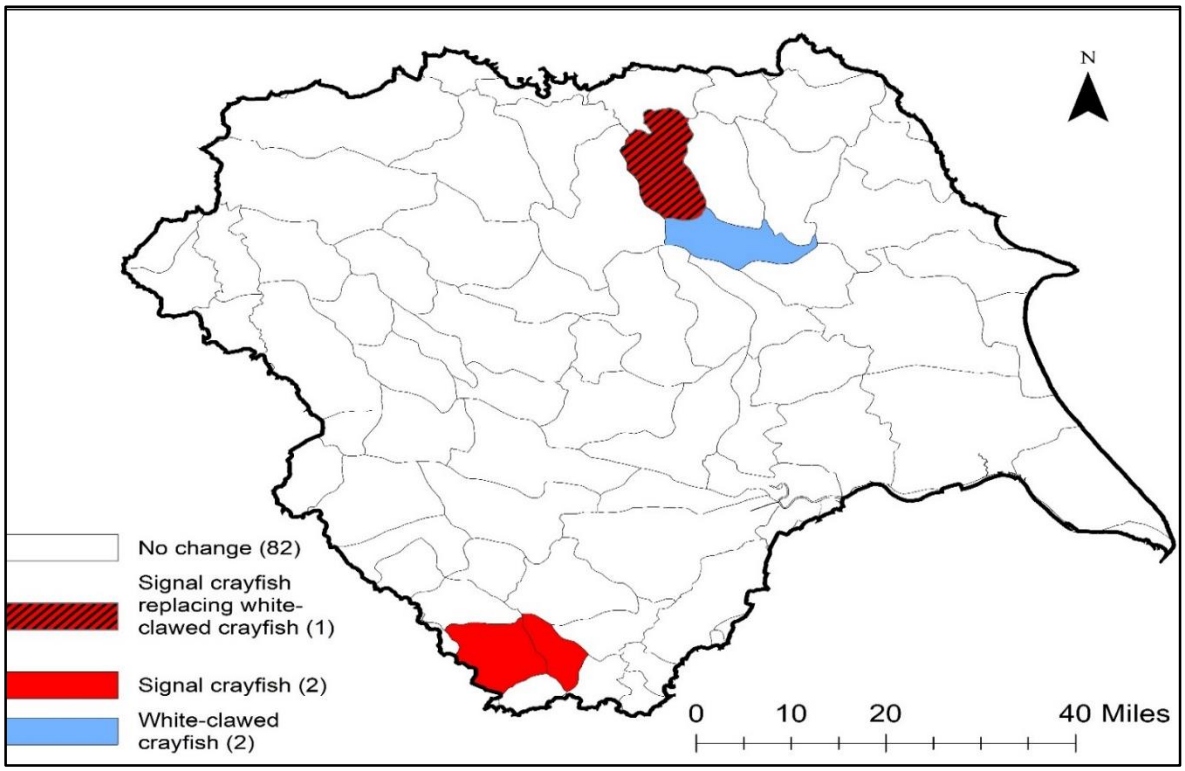


Figure 28 – Changes to the Yorkshire A17 map between 2012 and 2018

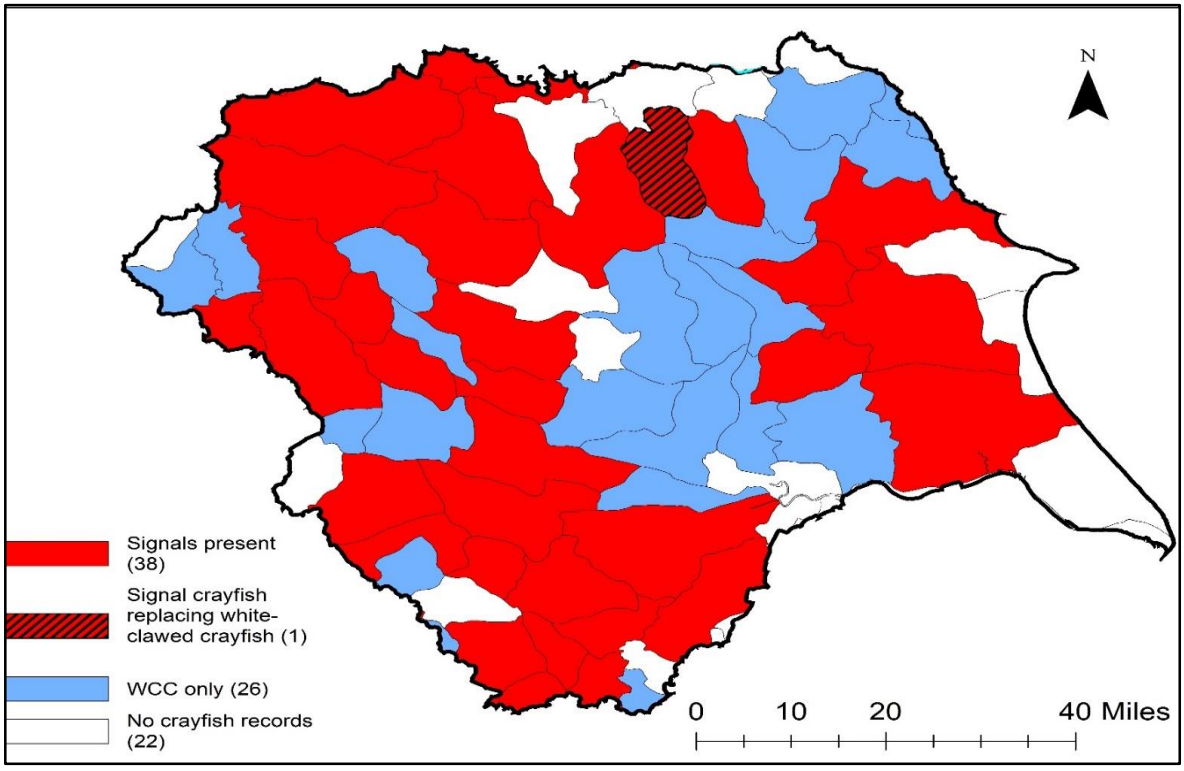
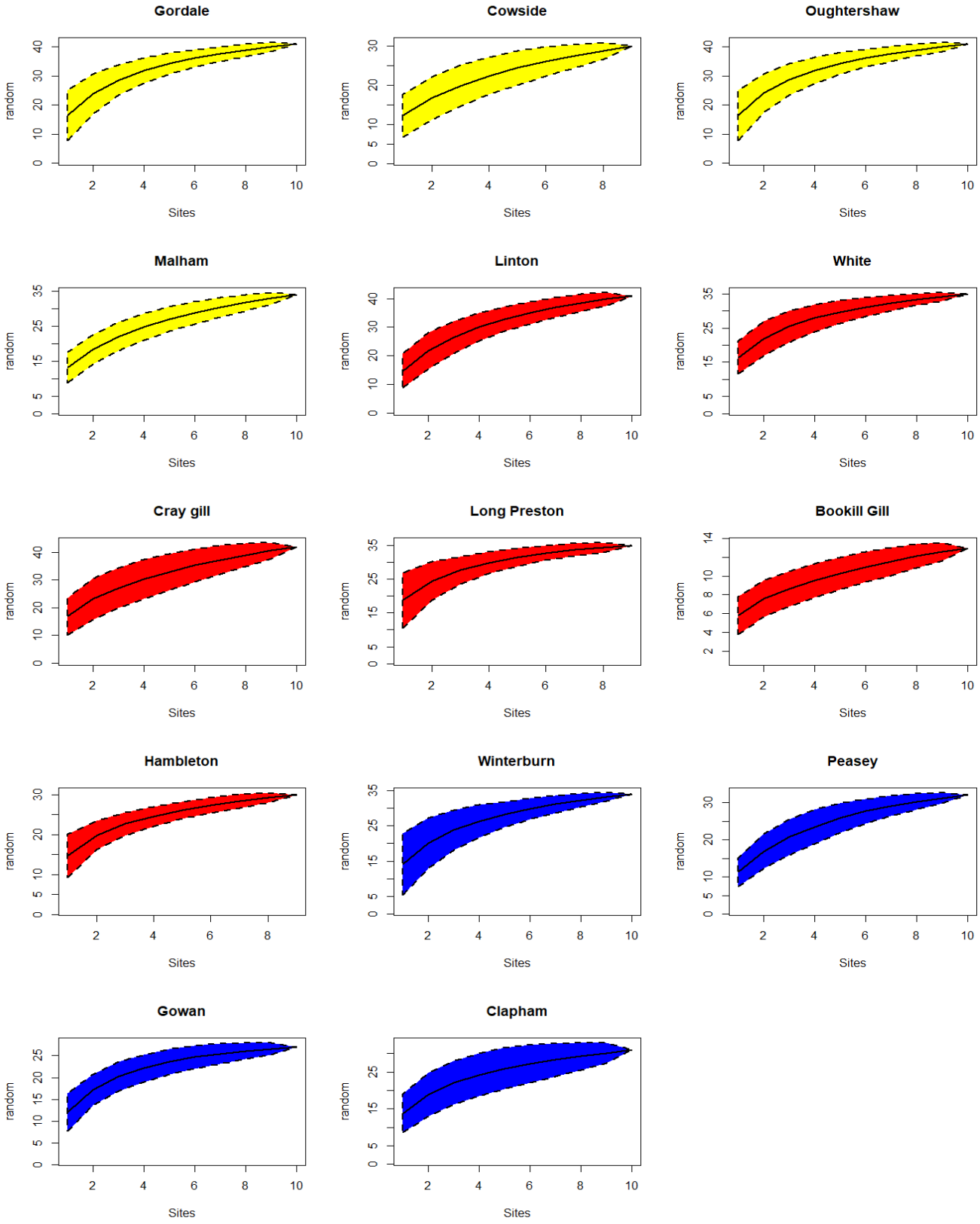


Figure 29 – A17 map for the Yorkshire region for 2018 report

Appendix 2

Species Accumulation figures, derived from 1000 randomised iterations of surber data per site using R 'Vegan'package, by Oksanen et al. (2018). Polygon boundaries indicate 95% LCI and UCI. (Chapter 3).



Appendix 3

Full species list of invertebrate abundance by site (Chapter 3).

	Cowside	*Gordale*	*Oughtershaw*	*Malham*	*Bookill gill*	*Linton*	*White*	*Hambleton*	*Cray gill*	*Long preston*	*Winterburn*	*Peasey*	*Gowan*	*Clapham*
Agapetus fuscipes	45	41	2	35	0	2	164	0	0	0	0	0	0	0
Amphinemura sulcicollis	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Ancylus fluviatilis	2	1	0	2	0	1	23	0	0	0	1	1	6	2
Antocha	0	0	0	0	0	0	0	36	0	0	0	0	0	0
Asellus aquaticus	0	0	0	0	0	0	1	0	0	0	0	0	0	0
Athripsodes cinereus	0	0	0	0	0	1	0	0	0	0	0	0	2	1
Baetis rhodani	98	70	109	170	51	133	21	15	118	134	222	8	20	75
Caenis rivulorum	0	0	55	0	1	11	0	26	12	1	26	7	6	23
Centroptilum luteolum	0	0	0	0	6	0	0	0	0	0	0	0	0	1
Ceratopogonidae	1	7	1	0	0	1	1	1	0	1	1	1	1	1
Chironomidae	0	27	0	0	0	7	3	1	7	0	282	0	3	0
Chloroperla torrentium	0	17	8	0	0	2	1	0	14	13	0	1	0	0
Chloroperla tripunctata	0	5	0	0	1	4	0	0	4	12	0	0	0	0
Clinocerinae	0	0	2	0	0	0	0	0	0	0	0	0	0	0
Collembola	0	0	0	0	0	0	0	0	0	0	1	0	0	0
Dicranota	4	6	12	3	0	3	17	0	11	38	7	1	5	1
Dinocras cephalotes	1	10	0	1	0	0	0	0	2	0	0	0	0	0
Drusus annulatus	91	11	2	5	0	0	0	0	1	0	0	2	0	0
Dytiscidae larvae	0	0	0	0	0	0	0	0	3	1	0	0	0	0
Ecdyonurus dispar	1	10	25	3	20	2	0	1	72	180	5	12	3	30
Elmis aenea	4	1	4	5	0	2	1	0	12	5	11	0	0	0
Ephemera danica	0	1	2	1	0	2	0	10	1	12	0	0	0	0
Erpobdella octoculata	0	0	0	0	0	0	0	0	0	0	0	0	2	0
Gammarus pulex	357	45	22	142	1	24	107	7	6	6	2	31	104	5
Gerridae	0	0	0	0	0	0	0	0	0	0	2	0	0	0
Glossiphoniidae	0	1	1	0	0	0	0	0	0	0	0	2	0	0
Gyrinidae larvae	0	0	0	0	0	0	0	0	0	68	0	0	0	2
Habrophlebia fusca	0	0	8	0	67	0	0	1	1	0	11	6	0	4
Halesus radiatus	10	0	1	44	0	1	0	0	1	5	0	1	4	0
Hydracarina	9	11	2	13	0	25	6	0	0	7	1	2	0	8
Hydraena sp.	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Hydropsyche instabilis	0	1	0	26	0	0	0	2	1	0	2	0	0	0
Hydropsyche siltalai	0	0	0	0	0	7	4	2	0	30	8	7	20	3
Hydroptila	0	0	3	0	0	2	0	0	0	3	0	0	0	0
Isoperla grammatica	1	25	1	11	0	6	1	0	1	3	0	0	0	0
L. Elmis aenea	15	7	1	2	0	3	4	7	46	153	22	1	3	0
L. L. volckmari	0	0	5	0	0	3	0	3	43	134	6	6	0	1
L. Oulimnius sp.	0	1	0	1	0	1	0	0	41	147	0	0	0	0

Lepidostoma hirtum	0	0	0	0	0	0	0	0	0	0	3	4	0	7
Leuctra geniculata	0	0	0	0	0	30	2	32	3	0	24	7	0	55
Leuctra hippopus	0	21	0	0	45	13	9	8	642	14	67	47	22	114
Leuctra inermis	82	114	18	18	0	10	11	7	0	0	4	3	11	6
Limnius volckmari	2	0	1	3	0	2	2	0	16	8	0	0	0	1
Limoniidae	0	0	0	0	0	0	0	0	0	6	0	0	0	0
Limoniidae sp.	0	0	0	0	0	0	0	2	0	0	0	0	0	0
Odontocerum albicorne	0	0	0	0	0	0	0	0	6	2	0	0	0	0
Oligochaete	6	30	27	1	0	12	110	13	3	16	4	2	12	16
Oreodytes sanmarkii	0	0	2	0	0	0	0	0	1	1	0	0	0	0
Orthocladinae	10	12	28	2	0	46	12	7	136	151	34	5	38	52
Oulimnius sp.	1	0	0	0	0	10	0	1	39	34	0	0	0	0
Paraleptophlebia submarginata	0	0	1	0	2	3	0	0	0	54	0	2	0	7
Pediciidae	1	0	0	0	0	0	0	0	0	0	1	0	0	0
Philopotamus montanus	0	0	0	5	0	0	0	0	0	0	0	0	0	0
Physella sp.	0	0	0	0	0	0	15	0	0	0	0	0	0	0
Pisicola geometra	0	0	0	0	0	0	1	0	0	0	0	0	0	0
Pisidium sp.	0	0	1	0	0	0	0	0	0	0	0	0	0	0
Polycelis nigra/tenuis	33	0	2	22	0	0	137	0	3	0	5	0	0	0
Polycentropus flavomaculatus	3	0	9	1	0	29	0	18	4	24	7	10	25	18
Potamopyrgus antipodarum	0	5	0	0	0	3	6	2	0	0	0	0	71	9
Protonemura meyeri	0	7	0	6	0	0	5	0	1	0	0	0	0	0
Psychodidae	0	0	0	1	0	0	2	0	3	0	0	0	0	0
Psychomyia pusilla	0	0	3	1	0	6	2	2	0	0	2	3	0	3
Pyralidae	0	0	0	0	0	0	0	0	1	0	0	0	0	0
Radix peregera	0	3	0	0	0	0	0	0	0	0	0	0	0	0
Rhithrogena semicolorata	3	12	19	5	2	18	8	1	7	0	3	1	1	2
Rhyacophila dorsalis	1	4	1	4	1	19	10	17	2	4	1	1	5	0
Sericostoma personatum	0	2	0	3	0	0	1	0	2	9	0	2	0	0
Serratella ignita	208	515	50	81	0	194	476	96	197	0	210	140	50	97
Sialis fuliginosa	0	0	0	0	0	0	0	0	1	0	0	0	0	0
Silo pallipes	0	3	0	0	0	2	0	0	0	0	0	0	2	0
Simuliidae	0	2	1	1	0	1	4	9	71	49	95	6	1	18
Tanypodinae	1	9	7	3	2	2	8	37	71	0	84	7	7	22
Tanytarsini	1	3	3	2	0	6	6	17	1	4	30	0	7	1

Appendix 4

Remains of *P. leniusculus* (indicated with arrows) that were ejected from an eel (*A. anguilla*) stomach following capture in Bookill Gill Beck, 2017 (Chapter 3).