New approaches for exploring signal crayfish invasion biology and ecological impacts in headwater streams

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Declaration

I, Eleri Gwenllian Pritchard 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.

Signature:

Eleri G. Pritchard.
Date: 22/09/2021

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Abstract

The global spread of invasive alien species has had severe ecological, social and economic implications, with freshwater systems proving particularly vulnerable to invasion. Freshwater crayfish are exceptionally successful invaders, and 90% of species introduced to Europe have become established in the wild. As ecosystem engineers, crayfish present a significant threat to aquatic ecosystems. However, methodological constraints have thus far limited our understanding of invasive crayfish ecology and environmental impacts.

This project aims to better our understanding of the spatio-temporal ecological impact invasive crayfish have on native biota, using Bookill Gill Beck and Long Preston Beck in the Yorkshire Dales as a case study. A novel method was developed to produce quantitative data on crayfish populations along an invasion gradient from well-established sites to the invasion front, where native crayfish still persist. The method was rigorously tested to evaluate efficiency and optimal deployment time for both crayfish and benthic fish. Following proof-of-concept, the method was then used to explore the invasion ecology and ecological impacts of signal crayfish within the headwater system. Population demographics of signal crayfish were investigated along the invasion gradient over three subsequent field seasons (2018 - 2020), focussing on population density, size structure and biomass, including relationships with substrate composition. Subsequently, density-dependent impacts of signal crayfish on macroinvertebrate and fish communities were explored, comparing sites along the invasion gradient and temporal changes at individual sites over the three-year timeframe. Notable changes in macroinvertebrate community composition and severe declines of native fish were observed, with European bullhead the most affected fish species. The results of this research can be used to inform conservation and management decisions by greatly enhancing our understanding of the invasion biology and ecological impacts of invasive crayfish, whilst also offering a novel method to be used in quantitative population assessments in future research and monitoring.

Impact Statement

This thesis sought to advance our understanding of signal crayfish invasion biology and their ecological impacts through the development and testing of novel survey techniques, namely the ‘Pritchard Trap’ (PT), in intensive field surveys (Ch. 3). PTs afforded, for the first time, logistically simple, truly quantitative insights into crayfish population demographics for headwater streams (Ch. 4). This highlights the ability of PTs to facilitate access to large-scale crayfish density and demographic data for practitioners involved in conservation and management of freshwater crayfish. Uptake of the PT could therefore allow significant impact for advancing our understanding of crayfish distribution, invasion biology, density-dependent impacts, and relationships between crayfish populations and their environment. Furthermore, the method could be used to inform management, by allowing efficacy evaluations of crayfish control strategies through ‘before’ and ‘after’ population assessments. The proven ability of PTs to sample benthic fish species (Ch. 3) further broadens the scope of their applications.

The work presented in this thesis on the development of the PT as a novel quantitative survey method for crayfish has already been published open access in June 2021 (Pritchard *et al.* 2021). This allows a wide dissemination of my findings to scientists and practitioners who may benefit from using this method and the data it generates, further encouraging a wide uptake of the PT. The paper has been well received by other researchers, and interest in using the PT method for crayfish distribution surveys is already developing (R. Battarbee 2021, pers. comms). A manuscript detailing the use of PTs for benthic fish sampling, which also highlighted the inefficiency of the traditional method of electrofishing, has also been published open access in October 2021 (Pritchard *et al.* 2021b).

The work presented in this thesis also provides novel information on signal crayfish invasion biology, including population dynamics and dispersal (Ch. 4). This enabled the exploration of crayfish density as a driver of ecological impacts (Ch. 5 & 6). Severe impacts on native white-clawed crayfish, macroinvertebrate assemblages and fish communities present a worrying finding and is of great importance to freshwater conservation. The overall approach used in this thesis and detailed case study in North Yorkshire, provides a template for future investigation of invasive species and their impacts across a wider geographical scale. The evaluation of quantitative population demographic data of an invasive species, coupled with environmental surveys of native ecosystems, provides a foundation to test invasion science theories and advance our understanding of biological invasions.

The findings of this thesis have been, and continue to be, disseminated to a range of audiences, including scientists, practitioners, students and the general public. The channels by which I expect the work to have an impact are publications, education and collaboration. The overall findings of this thesis also formed the basis of a Natural Environment Research Council (NERC) Standard grant with the aim of continuing this important research. Further details of the ‘Routes to Impact’ are discussed in Chapter 7 of this thesis – Final Synthesis and Future Directions.

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Chapter 1. General Introduction

1.1 Invasive species in freshwater systems

Freshwater systems represent extremely valuable natural resources with ecological, socio-economic and cultural benefits. While freshwater only constitutes 0.01% of global water, freshwater systems support over 100,000 different species - approximately 6% of all described species in the world (Dudgeon *et al.* 2006; Reid *et al.* 2019). Worryingly, freshwater ecosystems are experiencing biodiversity loss at a far greater rate than many terrestrial systems (Ricciardi and Rasmussen 1999; Jenkins 2003; WWF 2020). The Living Planet Index (2020), identified an average decline in the abundance of freshwater species' populations by 84% between 1970 and 2020 (WWF 2020). Freshwater systems are subject to a plethora of anthropogenic activities which can be related to six overarching threats: habitat loss and degradation, climate change, over-exploitation, hydrological alteration, water pollution and the introduction of invasive alien species (IAS), hereafter termed 'invasive species' (Nunes *et al.* 2015). In many instances, these threats work in synergy, exerting a high pressure on freshwater ecosystems. For example, climate change and habitat degradation can accelerate the spread of invasive species, pathogens and diseases (Fisher *et al.* 2012). In some cases, it is difficult to disentangle the confounding effects of individual specific pressures. Invasive species, however, are widely recognised as direct drivers of biodiversity loss (Sala *et al.* 2000; Pyšek *et al.* 2012), and they have been reported to represent a major cause of species extinctions worldwide (MEA (Millenium Ecosystem Assessment) 2005; IPBES 2019).

The International Union for Conservation of Nature (IUCN) defines an invasive species as "animals, plants or other organisms introduced by man into places out of their natural range of distribution, where they become established and disperse, generating a negative impact on the local ecosystem and species" (IUCN 2019). Globalisation has greatly facilitated the spread of invasive species worldwide, and the global number of introduced species is currently increasing exponentially with little sign of abating (Jackson and Grey 2013; Nunes *et al.* 2015). The method by which a species is transported outside of its natural geographic range is known as an introduction pathway. Improved global transport links mean the number of introduction pathways has proliferated in recent decades (Hulme 2009). The top three most common introduction pathways are horticulture, trade (industry, live food, pet and aquarium trade) and intentional release (hunting, sports fishing, research, smuggling or medicinal purposes), respectively (Turbelin, Malamud and Francis 2017). The global costs associated with aquatic invasive species alone has been estimated at approximately £250 billion, with the majority attributed to invertebrates (62%), followed by vertebrates and then plants (Cuthbert *et al.* 2021). Despite the substantial sum, this value is highly conservative, with actual costs considerably higher due to underreporting and the complexity of impacts involved. Furthermore, the cost of invasive species' damages and management will continue to rise with increasing numbers of invasions (Cuthbert *et al.* 2021). For example, the number of invasive species in Europe has increased by 76% between 1970 and 2007 (Butchart *et al.* 2010), putting native ecosystems under constant and increasing pressure.

1.2 Invasion process and theory

Invasive species are agents of change that commonly have the ability to disrupt ecosystem processes - which can have severe ecological, social and economic implications (Simberloff *et al.* 2013; Ricciardi *et al.* 2021). The invasion process involves three main stages; the **introduction** of a non-native species into the wild; the **establishment** of a self-sustaining population that is reproductively viable; and subsequent **population growth** and species dispersal (invasion transition) (García-Berthou *et al.* 2005; Williamson and Fitter 1996). A theory known as the "tens rule", proposed by Williamson *et al.* states that approximately 10% (5% - 20%) of species succeed in each of the three stages, which means that of all species transported outside of its natural range, only 0.1% will become an established invasive species (Williamson 1996; Williamson and Fitter 1996). However, García-Berthou and colleagues (2005) argue that freshwater systems are an exception to the "tens rule", as many aquatic invasive species are intentionally introduced. Species that are deliberately selected and introduced into a system are more likely to successfully establish than species being accidentally released. A study of 123 aquatic species introductions into six European countries found that 63% of species became established in the wild, far exceeding Williamson's 5 - 20% threshold (García-Berthou *et al.* 2005).

The ability to understand the circumstances by which an introduced non-native species may establish as an invasive species with negative consequences for the recipient ecosystem has long been a challenge in invasion science. Whilst substantial progress has been made in understanding invasion pathways and the establishment of self-sustaining populations (Catford, Jansson and Nilsson 2009; Hulme 2009; Turbelin, Malamud and Francis 2017), there have been fewer developments in predicting when and where precisely introduced species will negatively impact invaded ecosystems (Ricciardi *et al.* 2013; Ricciardi *et al.* 2021). Yet, there is an urgent need to forecast which non-native species are likely to cause negative impacts, and what this impact will look like, so that preventative action can be taken (Roy *et al.* 2014; Roy *et al.* 2019). This process of examining and prioritising potential threats and opportunities for invasive species is known as 'horizon scanning' (Roy *et al.* 2014; Roy *et al.* 2019). One tool utilised in horizon scanning for invasive species is the Parker-Lonsdale equation, which proposes that the total impact of an invader includes three fundamental dimensions: range, abundance and specific per-capita or per-biomass effects (Parker *et al.* 1999). Understanding these elements enables forecasts of where, when and how invasive species will impact recipient ecosystems – including in locations currently not invaded, but where invasion appears almost inevitable due e.g. to the presence of an invasive species within the local area / catchment and a lack of dispersal barriers preventing its further expansion into the neighbouring, uninvaded ecosystems. The ability to predict the ecological impacts of biological invasions under rapid environmental change, and associated simultaneous disturbances like climate change-related extreme weather events, is therefore a main priority in future invasion research (Ricciardi *et al.* 2021).

A long-standing theory in invasion science is that an ecosystem's diversity is positively linked to its resilience to biological invasions (Elton 1958). The underlying proposed mechanism is that, as species accumulate, there is higher competition and less available resource space for new colonists (Elton 1958; Stachowicz *et al.* 2002). This may explain why freshwater systems are particularly vulnerable to invasion, as they have been subject to extensive disturbance and modification through anthropogenic activities, resulting in decreased biodiversity and reduced competition pressure (Convention on Biological Diversity 2018). However, there is still much debate over the validity of this theory, and although often supported through theoretical models, results of empirical studies are decidedly mixed (Levine and D'Antonio 1999; Hughes 2010). The natural connectivity of inland watercourses presents an alternative explanation of the vulnerability of freshwaters to invasions, as the resulting connected landscape aids the rapid dispersal of a species following an initial introduction into freshwater systems, causing widespread establishment (Gherardi 2007). For centuries, freshwater systems have therefore encountered more extensive invasions than terrestrial systems (Sala *et al.* 2000; Karatayev *et al.* 2007).

1.3 Freshwater crayfish

Freshwater crayfish (Astacidea) represent one of the most widely introduced freshwater taxa (Twardochleb *et al.* 2013). Introductions of non-native crayfish are often attributed to aquaculture for human consumption, enhancement of both recreational and commercial fisheries and intentional release of unwanted aquarium pets (Lodge *et al.* 2000; Gherardi 2007; Taylor *et al.* 2007). Freshwater crayfish are regarded as ecosystem engineers (Edwards, Jackson and Somers 2009) and keystone species (Dorn and Wojdak 2004), due to their ability to create and modify habitat around them by changing the morphology and the availability of resources to other species (Creed and Reed 2004). They are able to dominate benthic biomass through their dietary plasticity (Wood *et al.* 2017), for example influencing detrital decomposition rates (Schofield *et al.* 2001) and bioturbation of fine and coarse sediments (Statzner *et al.* 2000). As ecosystem engineers, non-native freshwater crayfish are particularly adept in becoming invasive with ecosystem-wide impacts. The robust nature of many invasive crayfish species with regards to water quality, coupled with various life history traits such as fast growth rates, large size, high fecundity and omnivorous diet, allow them to quickly become established within a system (Lindqvist and Huner 1999). As such, the invasive success rate for introduced crayfish species in Europe reaches up to 90%, far exceeding the average for other aquatic taxa or the aforementioned “tens rule” (García-Berthou *et al.* 2005; Holdich *et al.* 2009).

The introduction of numerous non-native crayfish species, coupled with anthropogenic activities such as habitat modification and water pollution (Holdich 2002), has in turn caused irreversible changes to the distribution patterns of native crayfish (Holdich *et al.* 2009; Kouba, Petrusek and Kozák 2014). An evaluation of the world’s 590 crayfish species using International Union for Conservation of Nature (IUCN) criteria found 32% of species to be threatened with extinction and a further 21% classified as ‘Data Deficient’ (Richman *et al.* 2015). As keystone species, any changes in crayfish populations, such as colonisation of crayfish-free habitats, loss of native crayfish populations, or replacement of native crayfish species by non-native crayfish species, are likely to have substantial effects on freshwater biodiversity and associated ecosystem services (Kouba, Petrusek and Kozák 2014).

1.4 Signal crayfish

The signal crayfish, *Pacifastacus leniusculus* (Figure 1.1; Dana, 1852), is the most widespread invasive crayfish species in Europe (Kouba, Petrusek and Kozák 2014). It is listed as a “species of union concern” in the EU Invasive Alien Species (IAS) Regulation (1143/2014, European Commission 2016), further highlighting its status as one of the most problematic invasive species on the continent (Kouba *et al.* 2022).

Figure 1.1 Adult signal crayfish (taken 2016 – 2020, North Yorkshire, UK).

Signal crayfish were originally introduced to Sweden in 1959 from Lake Tahoe in California (Abrahamsson 1973 in Holdich *et al.* 2009). Many further introductions derived from this initial population, including the introduction in the UK in the 1970s, in an effort to establish a new aquaculture industry (Reynolds and Souty-Grosset 2011; Holdich *et al.* 2014). Subsequent intentional and accidental introductions have facilitated the spread of signal crayfish in the wild, and signal crayfish are now widespread across the UK, with the notable exception of Northern Ireland (Figure 1.2).

Figure 1.2 Distribution of signal crayfish across the UK (9,299 records on NBN Atlas 2021).

In its native range, the signal crayfish thrives in both lentic and lotic environments, including sub-alpine lakes, headwater streams and lowland rivers (Holdich *et al.* 2014). Accordingly, signal crayfish have been successfully introduced to a vast range of habitats including canals, ponds and wetland drainage ditches. They are extremely tolerant to a wide range of environmental conditions like brackish water, high water temperatures and low pH (McMahon 2002). Signal crayfish are omnivorous and can alter their generalist diet according to resource availability (Olsson *et al.* 2009; Holdich *et al.* 2014). They are believed to exhibit a general ontogenetic shift in feeding behaviour, with the juvenile diet typically comprised of freshwater invertebrates, whereas the adult diet is dominated by plant material (Lewis 2002b). Their broad diet is known to include aquatic plants, detritus, macroinvertebrates, fish eggs and fish (Guan and Wiles 1998; Nyström and Strand 1996; Nystrom, Bronmark and Graneli 1999; Lewis 2002a; Findlay, Riley and Lucas 2015). Signal crayfish are also known to exhibit cannibalistic tendencies (Guan and Wiles 1997; Houghton, Wood and Lambin 2017).

Signal crayfish are generally nocturnal (Abrahamsson 1983), potentially in response to predatory pressure from diurnal predators such as fish. During the day, they seek refuge under cobbles, boulders and tree roots. Signal crayfish are not documented as a burrowing species in their native range (Lewis 2002b). However, extensive burrowing behaviour has been reported throughout their invasive range (Statzner *et al.* 2000). Signal crayfish tend to reach sexual maturity between two and three years of age, although this can occur as early as one year old (Holdich *et al.* 2014). In the vast majority of populations in Europe, mating occurs in September - October, and egg incubation can take between 166 and 280 days. Brood sizes vary depending on the size of the female, but typically comprise between 200 - 400 eggs (Figure 1.3). Hatching occurs between March and late July, depending on the environmental conditions such as water temperature. Biotic and abiotic factors then strongly influence the successful recruitment of hatchlings, with estimates of survivorship to 2 years varying between 10% and 52% (Holdich *et al.* 2014). Juveniles tend to moult up to 11 times during their first year, depending on their growth rate, but this reduces to two moults per year at age three, and to one moult per year at ages 4 and above (Lewis 2002b). Females grow up to 12 cm in total body length, whereas males can reach much larger sizes of up to 16 cm. It has been estimated that under ideal conditions, signal crayfish can live for at least 16 years (Belchier *et al.* 1998).

The life history traits of signal crayfish, including its tolerance to a wide range of environmental conditions, generalist diet and high fecundity, all contribute to its success as an invasive species. It has been suggested that signal crayfish can attain higher population densities than native counterparts, yet density estimates are rarely reported in the literature. Signal crayfish population density and structure is discussed in more detail in Chapter 4 (Crayfish population dynamics through space and time).

Figure 1.3 Berried female signal crayfish (taken 2016, North Yorkshire, UK).

1.5 Challenges of signal crayfish invasions

1.5.1 Environmental impacts

Impacts of signal crayfish invasions range from ecological and geomorphological to economic and cultural (Lodge *et al.* 2012). Most notably, signal crayfish commonly displace native crayfish species through direct competition and transmission of disease, primarily *Aphanomyces astaci* (Schikora)*,* commonly known as ‘crayfish plague’ (Lodge *et al.* 2000; Holdich *et al.* 2009; Richman *et al.* 2015). Signal crayfish act as a vector of crayfish plague, and many European crayfish species are susceptible and show 100% mortality (Holdich 2002).

There is only one species of crayfish considered native to the UK, the white-clawed crayfish (Figure 1.4) *Austropotamobius pallipes* (Lereboullet). The indigenous status of white-clawed crayfish has come under investigation, but it was concluded that there was sufficient evidence of establishment in England and Wales pre-1500 AD, and white-clawed crayfish should be considered indigenous, especially for the purpose of assessing its conservation status (Holdich *et al.* 2009). The introduction of signal crayfish has been a major driver of decline in white-clawed crayfish populations, through the transmission of crayfish plague and competitive exclusion (Holdich *et al.* 2009). A 95% reduction in white-clawed crayfish populations has been recorded in some areas, and it has even been predicted that the native species will be extinct in the wild in the UK by 2030 (Sibley 2003). Simultaneous declines across its range in mainland Europe have led to the Conservation Status 'Endangered' being assigned to white-clawed crayfish on the IUCN global red list (Füreder *et al.* 2010). Conservation efforts include the creation of 'ark sites', whereby self-sustaining populations of white-clawed crayfish can persist in areas with appropriate water quality and where they are safely isolated from non-native crayfish and crayfish plague (Nightingale *et al.* 2017).

Figure 1.4 Adult white-clawed crayfish (taken 2018 in North Yorkshire, UK).

Interactions between crayfish and surrounding ecosystems are highly complex due to a variety of direct interactions such as predation and competition for resources, and indirect interactions, such as modification to habitat and trophic cascades (Figure 1.5). As a result, non-native crayfish invasions generally have strong, complex consequences for freshwater biodiversity and ecosystem services (Kouba *et al.* 2014). Extensive burrowing behaviour can cause bioturbation, increased sediment load, habitat degradation and bank destabilisation (Harvey *et al.* 2011; Sanders, Rice and Wood 2021). Furthermore, common ecological impacts of signal crayfish invasions include a reduced abundance and diversity of macrophyte, macroinvertebrate and fish communities (Nyström and Strand 1996; Crawford, Yeomans and Adams 2006; Peay *et al.* 2009; Reynolds 2011; Mathers *et al.* 2016; Galib, Findlay and Lucas 2021). Whilst the interactions between signal crayfish and fish have been explored (Peay *et al.* 2009; Bubb *et al.* 2009), current survey methods present a limitation on quantifying the level of impact. For example, electrofishing is a common fish survey technique, but benthic fish are often underestimated in samples (see Chapter 3). Therefore, the lack of quantitative fish survey methods also constrains our ability to quantitatively assess the ecological impacts of invasive crayfish. The impact of signal crayfish on fish and macroinvertebrates are discussed in more detail in Chapters 5 and 6 of this thesis, respectively.

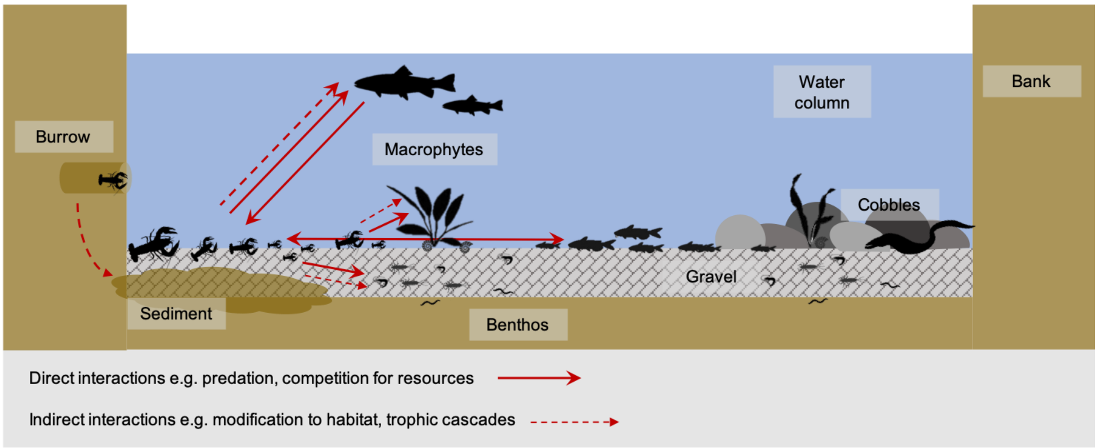


Figure 1.5 Examples of crayfish interactions with native ecosystems.

1.5.2 Crayfish survey

The nature and strength of invasion impacts are furthermore closely linked to the population density of invasive crayfish (Parker *et al.* 1999; Bubb *et al.* 2009). Good knowledge of the distribution and demographics of both native and non-native freshwater crayfish populations is therefore critical to understand their impact on the structure and functioning of aquatic ecosystems. However, limitations of conventional crayfish survey methods have prevented the quantitative assessment of crayfish populations *in situ.* Baited funnel trapping is the most commonly employed survey method (Parkyn 2015). However, the method provides semi-quantitative catch-per-unit-effort (CPUE) values and is generally selective for large, male individuals (Almeida *et al.* 2013). A range of other survey techniques are used for crayfish sampling, including manual handsearches, Artificial Refuge Traps (ARTs) and environmental DNA (eDNA) as discussed in more detail in Chapter 3 (section 3.2.1). Each survey method presents a host of advantages and limitations as summarised in Table 1.1.

Such limitations have presented a major obstacle in understanding the invasion biology of signal crayfish, and to quantifying their associated ecological impacts. Recent work sought to address this, with the development of a novel sampling technique for crayfish, the Triple Drawdown (Appendix 1; Chadwick, **Pritchard** *et al.* 2021).

The Triple Drawdown (TDD) method generated robust population demographic data of signal crayfish within an invaded headwater stream in North Yorkshire, UK. Recorded densities of 20 – 110 signal crayfish m^{-2} far exceeded estimates in the literature and highlighted the importance of such data to better understand the invasion biology of signal crayfish (Chadwick, Pritchard, *et al.* 2021). However, the TDD requires a suite of equipment and a team of trained operatives, which make it an expensive undertaking and may limit accessibility to many users. It is further limited by logistics and site requirements, including vehicle access to transport the equipment and river flows to be low enough to overcome with pumps. These factors will limit the wide-scale application of the TDD and highlights a need for an intermediate survey technique that is more user-friendly and cost-effective. Crayfish survey techniques, including the TDD are discussed in more detail in Chapter 3 (Novel methods) of this thesis.

Table 1.1 Summary of crayfish survey methods and associated advantages and limitations including cost, sampling effort, type of data produced, size selectivity, level of skill required.

Sampling method	**Advantages**	**Limitation**
Trapping	Cost-effective (~£15 per trap) Low skill threshold Can be used in a range of habitats including deep water	Semi quantitative (catch-per-unit-effort) High risk to non-target organisms (bycatch), Biosecurity, Bait attractancy,

	Short deployment/retrieval time	Ghost fishing/loss of traps, Two site visits required, Size and sex bias
Manual hand searches	Cost-effective Broad range of size classes Single visit required No risk to non-target organisms	Semi quantitative (catch-per-unit-effort), Limited to specific site requirements (depth and flow), Medium skill threshold Biosecurity Time taken per survey
Environmental DNA	No training required Low skill threshold Limited equipment required Reduced biosecurity risk (do not need to enter watercourse) Single and short site visit	Presence/Absence Expensive per sample Spatial resolution No population demographic data Limit of detection (uncertainty)
Artificial Refuge Traps (ARTs)	Cost-effective Low skill threshold Broad range of size classes Low risk to non-target organisms	Long deployment time (weeks – months) Semi-quantitative (catch-per-unit-effort) Two site visits (deployment and retrieval)
Triple Drawdowns	Fully quantitative Produces density estimates	Expensive Resource and labour intensive Biosecurity

	Can use depletion analyses for total population estimate Catch efficiency recorded Full range of size classes Population demographic data	High skill threshold Risk to non-target organisms Additional mitigation measures may be required e.g. fish rescues Site limitations (access and size of site)

1.5.3 Control and management

A further pertinent challenge following signal crayfish invasion is their management and control. To date, there is no technique available to successfully eradicate signal crayfish or the associated crayfish plague, *A. astacai,* once they have become established (Stebbing, Longshaw and Scott 2014). However, as an invasive species with potentially severe ecological, economic and social impacts, a variety of methods have been proposed and trialled to at least manage and control populations of invasive crayfish in the wild. Control methods fall into six distinct categories; 1) mechanical control (trapping), 2) physical control (draining, electrocution, habitat modification, barriers) 3) biological control (pathogens and predation) 4) biocidal control (pesticides) 5) autocidal control (male sterilisation) and 6) legislative control (Stebbing *et al.* 2012; Stebbing, Longshaw and Scott 2014). Each method presents its own limitations. For example, many of these methods are not species-specific and can have severe implications for the entire ecosystem, particularly habitat modification, the use of pesticides and introduced predators.

For decades, trapping and removing crayfish using baited funnel traps has been the most commonly employed ‘management’ technique (Manfrin *et al.*

2019). However, this requires a long-term, sustained high effort of trapping and has not yet proven effective in open lotic systems (Stebbing, Longshaw and Scott 2014; Manfrin *et al.* 2019). Furthermore, the aforementioned size-selectivity presents a fundamental flaw in trapping for control, as only a fraction of the population can be removed. This was highlighted in the development of the TDD method, where Chadwick *et al.* (2021) found only 2.3% of the surveyed population to be large enough to be caught in standard traps. Although not all invasive crayfish populations necessarily are expected to adopt the same juvenile-dominated population structure as observed in the study system shared by Chadwick *et al.* (2021) and this thesis, the results further emphasise the importance of quantitative survey techniques to inform management, and to evaluate the effectiveness of control strategies.

The collection of accurate, quantitative survey data is in my opinion the key underlying process through which effective management can be attained. A solid understanding of invasive population density, structure and distribution enables managers to predict the ecological impact and invasiveness of introduced species, forecast the spread and improve detection and control, which are all crucial components necessary to mitigate the impact of biological invasions (Manfrin *et al.* 2019).

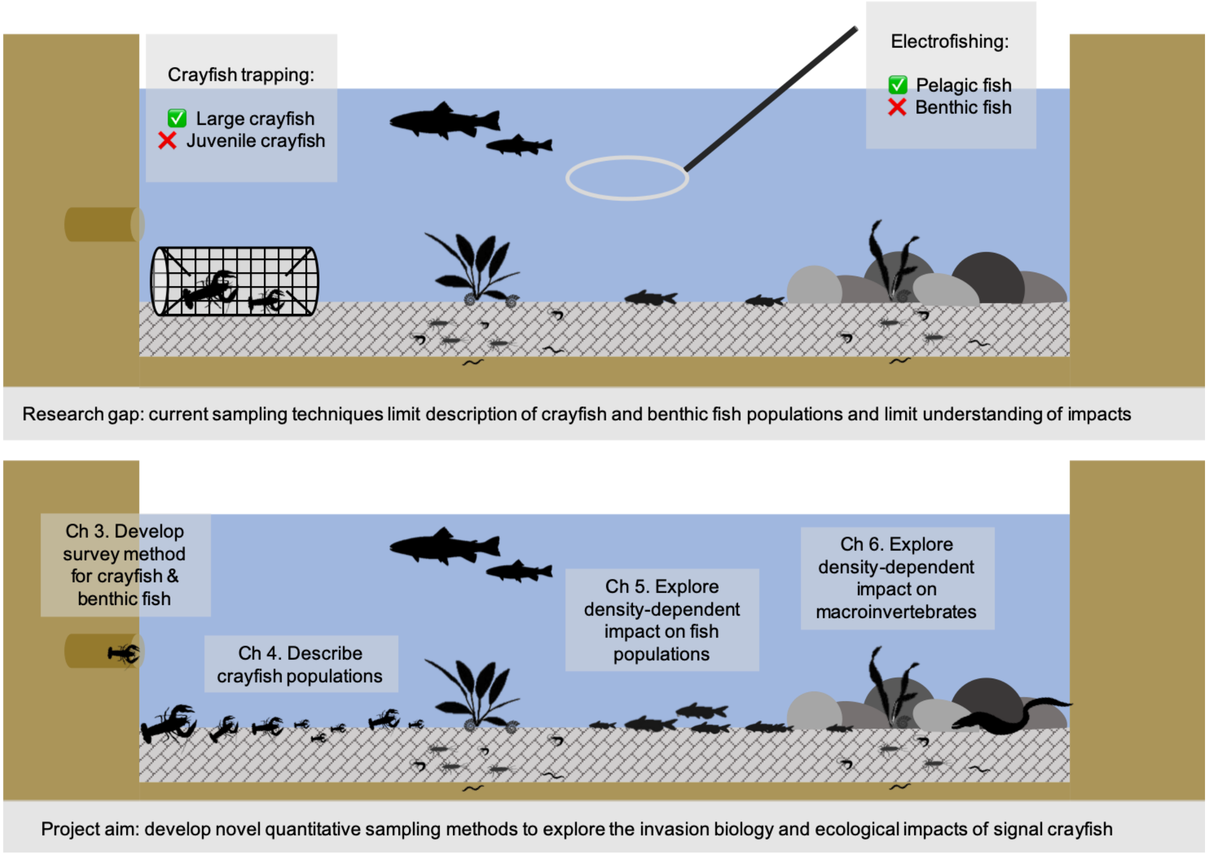
1.6 Research aims

As outlined above, the ability to accurately describe crayfish population demographics is crucial to understand their invasion biology and quantify subsequent ecological impacts. Methodological constraints have been a major limitation in this context, effectively preventing the quantitative assessment of crayfish populations *in situ* (Figure 1.6). Similar limitations prevent the quantitative assessment of native biota at invaded ecosystems, particularly the sampling of benthic fish through electrofishing (Figure 1.6). The development of the TDD technique presented a significant advancement in this context, providing means to collect robust, quantitative data on crayfish populations. It also highlighted the importance of such data, with major implications for management and the effectiveness of trapping for control (Chadwick, Pritchard, *et al.* 2021). However, logistical and practical requirements of the TDD method limit its widescale application and accessibility to the wide range of scientists, practitioners and river managers who would benefit from such survey data. **As a result, the first overarching aim of this thesis was to develop and test a novel, accessible sampling technique that is able to describe signal crayfish populations *in situ* and explore crayfish density as a driver of ecological impact (Figure 1.6). The second overarching aim was then to evaluate the links between crayfish population densities in the study system and observed impacts on macroinvertebrate and fish populations**.

The first objective of the study was to develop and test a novel sampling technique, which forms the basis of Chapter 3. In the formulation of designs, it was important to prioritise ease-of-use and cost-effectiveness, without compromising data quality. A pilot study testing several designs was carried out and one design was taken forward for further testing. The TDD method was used to gather ‘true’ population estimates, and to subsequently evaluate the efficacy of the selected novel method. This method proved successful at sampling invasive crayfish, native crayfish and benthic fish.

The second objective of the study was to apply the novel method to an in-depth case study, whereby quantitative data on crayfish would be collected and used to explore the invasion biology and density-dependent ecological impacts of signal crayfish. The research was carried out in a system of connected headwater streams in North Yorkshire, UK (full details provided in Chapter 2). Signal crayfish were illegally introduced to the system in approximately 1995, and they have since spread downstream, creating a distinct invasion gradient from well-established populations to the invasion front. Crayfish populations were described and compared along the invasion gradient over a three-year period (Chapter 4). A thorough understanding of the crayfish populations enabled the evaluation of crayfish density as a driver of ecological impact within the headwater system. The differences in fish assemblages along the invasion gradient were therefore explored in Chapter 5, followed by impacts on macroinvertebrate communities in Chapter 6.

The final synthesis in Chapter 7 contextualises and discusses how the overall findings of my research can contribute to the field, and I evaluate the implications for the management and conservation of freshwater ecosystems.



Research gap: current sampling techniques limit description of crayfish and benthic fish populations and limit understanding of impacts

Project aim: develop novel quantitative sampling methods to explore the invasion biology and ecological impacts of signal crayfish

Figure 1.6 Main research gaps (top) that the thesis addresses, with descriptions of how each chapter contributes to the overarching aim of the thesis (bottom).

Chapter 2. General Methodology

2.1 Study system

The study was conducted at the connected upland headwater streams Bookill Gill Beck (BGB) and Long Preston Beck (LPB) in the Ribble catchment of North Yorkshire, England (Figure 2.1). BGB is a small (0.7 – 1.9 m wide), fast-flowing stream and runs approximately 5.1 km from its source to a confluence with Scaleber Beck to form LPB. LPB is a larger watercourse (4 – 5 m wide) that runs for approximately 3.8 km before meeting the main River Ribble. BGB and LPB are situated in a sub-catchment of unimproved or semi-improved grazed pasture (Figure 2.2). There is a small conifer plantation near the upper reaches of BGB and small coppices of broadleaf trees along the beck in places, but most of BGB and LPB are open and unshaded, running through grassland pasture. The sites are relatively isolated, with access limited to foot or 4x4 vehicles through private farm yards and tracks. Landowner permission was sought and granted prior to all fieldwork.

Figure 2.1 Confluence of Scaleber Beck (left tributary) and Bookill Gill Beck (right tributary) to form Long Preston Beck (foreground).

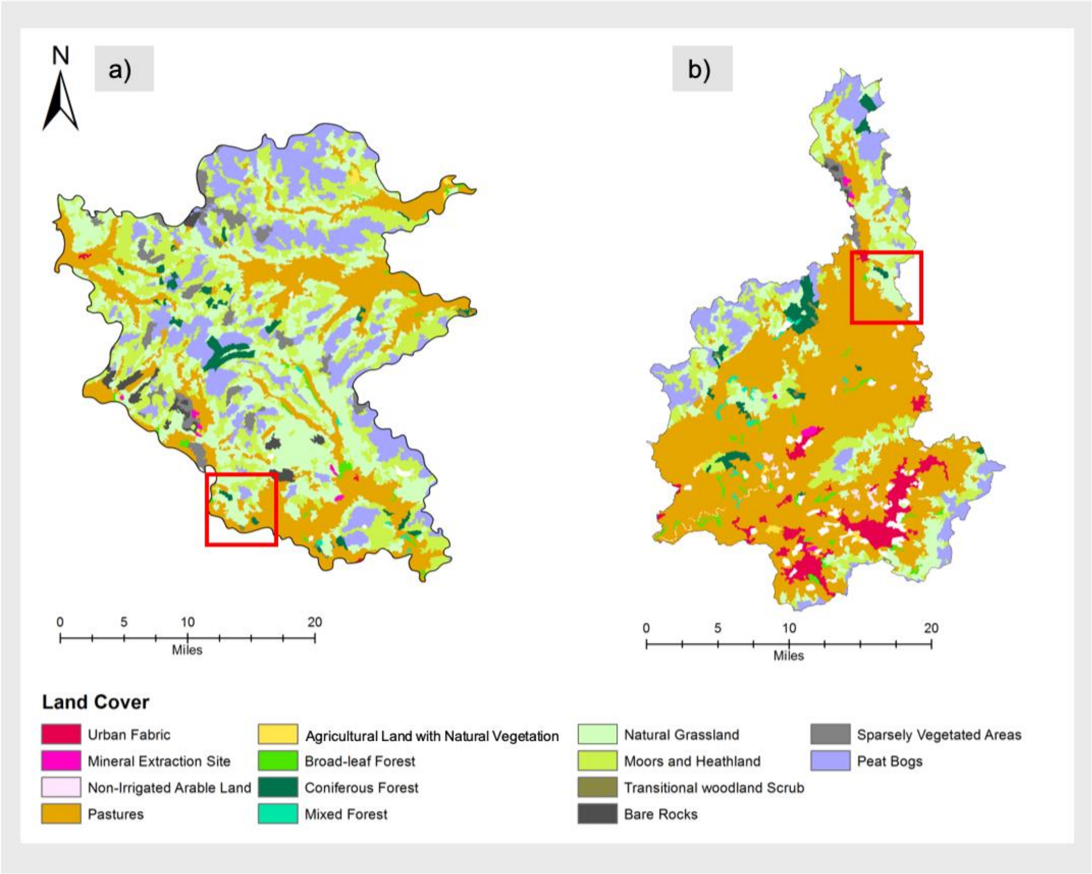


Figure 2.2 Land cover within a) the Yorkshire Dales National Park boundary and b) the Ribble catchment boundary, with broad study area highlighted in a red box. Figure adapted from Pritchard (2016) using Corine data, Environment Agency.

Historically, strong populations of white-clawed crayfish and diverse macroinvertebrate and fish communities were present along BGB (Peay *et al.* 2009). An illegal introduction of signal crayfish occurred in circa 1995, when a small number of individuals were released approximately 2.3 km downstream from the source of the stream, near a conifer plantation (reported in Peay *et al.* 2009). Since the initial introduction signal crayfish have spread both up and downstream and have become well established along the entire length of BGB (Pritchard 2016). At present, this is the only known population of signal crayfish in the Ribble catchment.

This study set out to establish sites along the invasion gradient from well-established to the invasion front. Four sites were selected, with one site on

BGB and three sites along a 1.5 km stretch of LPB. The sites were named based on location 1) Double Gate Bridge (DGB), 2) Confluence, 3) Footbridge and 4) Farm (Figure 2.3). DGB is the closest site to the signal crayfish introduction point, and populations have been well-established for approximately 20 years. Signal crayfish were confirmed present at Confluence in 2009 (Peay *et al.* 2009), near to Footbridge in 2015 (Taylor 2016) and extensive manual searches located what was believed to be the invasion front at Farm in 2018 (Table 2.1).

Table 2.1 Distance (km) from signal crayfish introduction point to study sites and date the signal crayfish population was confirmed as present on the site.

River	Site	Distance from signal crayfish introduction point (km)	Signal crayfish confirmation date
Bookill Gill Beck	Double Gate Bridge	1.10	2002 (Peay *et al.* 2009)
Long Preston Beck	Confluence	2.95	2009 (Peay *et al.* 2009)
Long Preston Beck	Footbridge	3.52	2015 (Taylor 2016)
Long Preston Beck	Farm	4.43	2018 (pers obs)

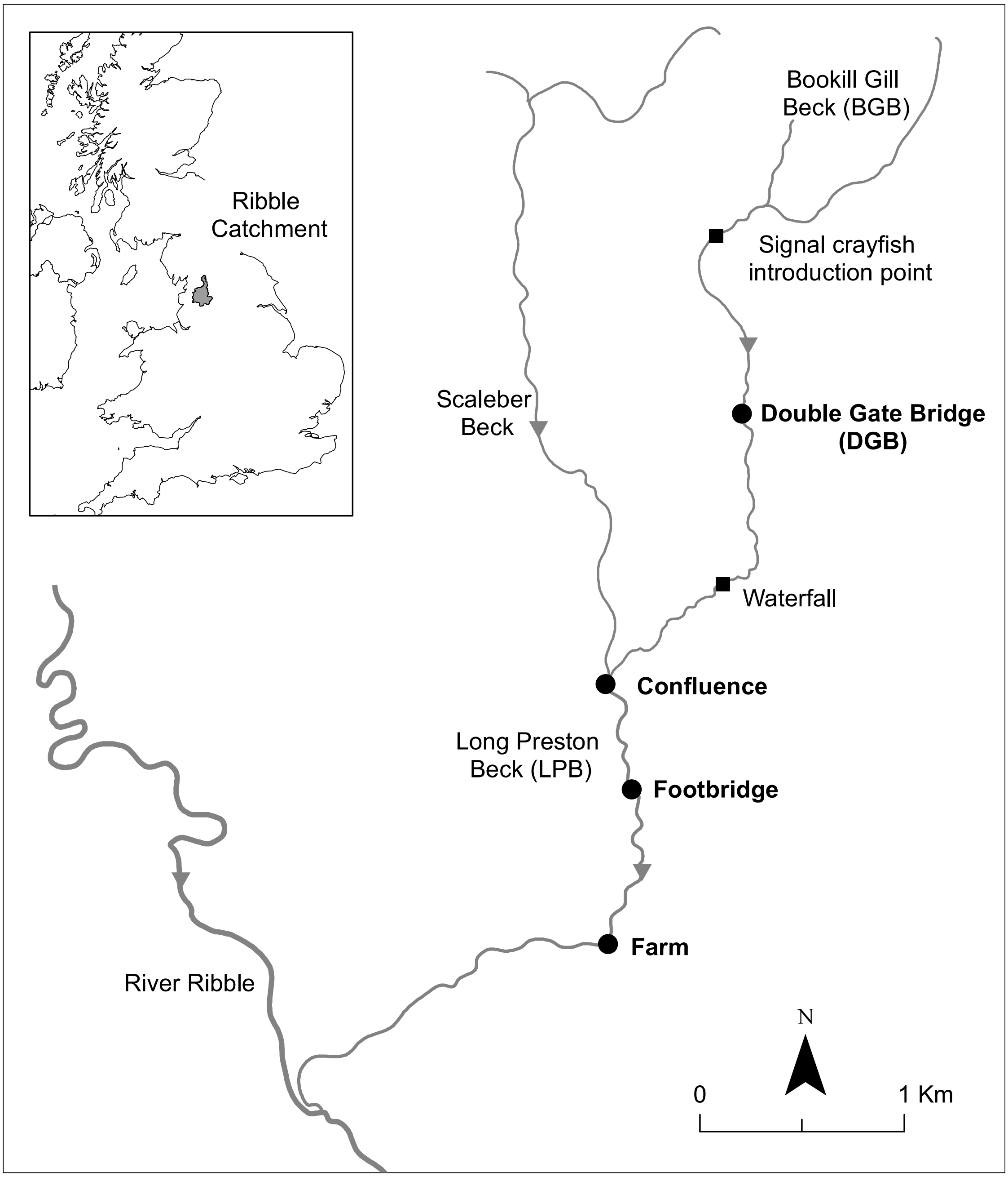


Figure 2.3 Site map of the study area, including the four study sites, Double Gate Bridge (DGB) on Bookill Gill Beck (BGB) and Confluence, Footbridge and Farm along Long Preston Beck (LPB).

2.2 Site conditions

Detailed habitat assessments were conducted at all sites in 2018 to identify any differences between sites that may need to be taken into consideration when interpreting results of the study. Water chemistry readings including pH, dissolved oxygen (mg/L), conductivity (µS/cm) and temperature (°C) were recorded in triplicate at the study sites using a HACH HQ30d flexi probe. An in-field alkalinity titration kit was also used to measure alkalinity. The water chemistry remained relatively consistent between sites (Table 2.2). It is important to note that throughout summer 2018, droughts were experienced in Northern England and as such, water chemistry results reported here may not represent normal conditions expected at these sites. For example, temperature was especially high reaching ~21°C at all sites.

Physical habitat surveys were undertaken at the sites, recording flow (m/s), depth (cm), wetted width (m) and in-channel substrate type (% cover). Wetted width was recorded at 2 m increments along the length of the site using a 50 m measuring tape. The wetted width and site length were used to calculate the site area (m^2). Flow and depth were recorded at the left margin, centre and right margin every 3 m along the site (n = 18). Flow velocity was measured using a Valeport electromagnetic flow meter (Model 801), producing an average of 30 readings taken once per second. DGB is situated on BGB which is a smaller stream than LPB and therefore wetted width was lower than at the other sites. Flow and water depth were very low due to drought conditions, especially at DGB (Figure 2.4), but conditions were consistent at the LPB sites (Figures 2.5 – 2.7).

In-channel substrate type was recorded using a quadrat viewfinder and estimating the percentage cover of cobble (64 – 256 mm intermediate axial length), gravel (4 – 64 mm intermediate axial length), silt/sand and macrophytes at the left margin, centre and right margin every 2 m along the length of the site (n = 18). Cobble was the dominant substrate type, comprising over two thirds of the in-channel substrate at all sites (67.6 –

87.5%). Macrophytes were scarce or absent at most sites, except for Farm where there was approximately an 9% cover in-channel at the beginning of my study (Table 2.2).

Table 2.2 Site characteristics, including physical habitat features, water chemistry and percentage substrate cover. Standard deviation is denoted in parentheses.

Survey type	Site descriptor	Double Gate Bridge	Confluence	Footbridge	Farm
Physical habitat features	Area (m^2)	15.0	45.5	45.5	50.0
	Wetted width (m)	1.5 (± 0.35)	2.84 (± 0.94)	2.84 (± 0.00)	4.03 (± 0.85)
	Depth (cm)	7.64 (± 4.86)	11.90 (± 4.25)	12.83 (± 4.60)	10.95 (± 3.22)
	Flow (m/s)	0.05 (± 0.02)	0.15 (± 0.17)	0.09 (± 0.08)	0.17 (± 0.13)
Water chemistry	pH	8.15 (± 0.03)	8.30 (± 0.01)	8.15 (± 0.01)	8.05 (± 0.12)
	Conductivity (µS/cm)	292.67 (± 0.58)	319.33 (± 0.58)	312.00 (± 0.00)	271.00 (± 0.00)
	Dissolved oxygen (mg/L)	9.64 (± 0.03)	10.01 (± 0.01)	9.82 (± 0.10)	7.31 (± 0.03)
	Temperature (°C)	22.10 (± 0.10)	21.30 (± 0.00)	21.90 (± 0.20)	21.50 (± 0.10)
	Alkalinity	132	132	131	142
In-channel substrate	Cobble (% cover)	80.3 (±19.24)	71.5 (± 27.51)	87.5 (± 14.06)	67.6 (± 23.87)
	Gravel (% cover)	12.12 (±14.74)	21.2 (± 22.05)	11.7 (± 13.72)	21.9 (± 20.15)
	Silt/sand (% cover)	7.58 (± 9.36)	7.3 (± 13.35)	0.8 (± 1.92)	6.9 (± 12.50)
	Macrophytes (% cover)	0.0 (± 0.0)	0.3 (± 1.74)	0.0 (± 0.00)	9.3 (± 12.87)

Figure 2.4 Photographs of Double Gate Bridge (DGB) in: a) low flow conditions, b) normal conditions and c) moderate to high flow conditions.

Figure 2.5 Photographs of Confluence in: a) normal conditions downstream, b) low flow conditions upstream, c) normal conditions upstream and d) moderate to high flow conditions.

Figure 2.6 Photographs of Footbridge: a) prior to field surveys, b) normal conditions and c) moderate flow conditions.

Farm, Long Preston Beck

Figure 2.7 Photographs of Farm in: a) low flow conditions, b) moderate flow conditions, c) prior to field surveys and d) normal conditions.

2.3 Research design

This research project comprises three major field campaigns, in the summers of 2018, 2019 and 2020, respectively (Table 2.3). The 2018 field season focussed on gathering data to investigate the crayfish populations, native biota and environmental conditions along the signal crayfish invasion gradient. The field season included extensive surveys, including characterising site conditions such as water quality and in-channel substrate type. Macroinvertebrate communities were sampled through Surber sampling, and thorough surveys of crayfish were undertaken using the Triple Drawdown Technique (TDD). Fish communities were surveyed through electrofishing and TDDs. Pilot studies were also undertaken to test a range of novel sampling technique designs for crayfish and benthic fish. One design in particular, subsequently termed the ‘Pritchard Trap’ (PT), showed great potential, and the 2019 field season focussed on more thoroughly testing the PT method, and on using it to monitor crayfish and benthic fish over the summer. The 2020 field season saw the collection of data for temporal comparisons of crayfish and native biota along the invasion gradient, including crayfish and benthic fish surveys using the PTs, and collection of macroinvertebrate samples.

Table 2.3 Summary table of fieldwork undertaken over the three field campaigns in 2018, 2019 and 2020, including survey type, method, site and which thesis chapter the fieldwork contributed to.

Year	Survey type	Method(s)	Site(s)	Chapter contribution
2018	Crayfish pilot study	Three novel crayfish trap designs	DGB	Ch 2. Methods
	Water chemistry	Hach probe and in field alkalinity titration kit	All	Ch 2. Methods
	Physical habitat features	Measuring tape and flow meter	All	Ch 2. Methods
	In-channel substrate	Percentage cover using quadrat viewfinder	All	Ch 2. Methods
	Crayfish	Triple Drawdowns (TDDs) Pritchard Traps (PTs)	All All	Ch 3. Novel methods & Ch 4. Crayfish populations
	Fish	Electrofishing using Smith Root backpack system Triple Drawdowns (TDDs) Pritchard Traps (PTs)	LPB sites All All	Ch 3. Novel methods & Ch 5. Impacts on fish
	Macroinvertebrates	Surber samples	LPB sites	Ch 6. Impacts on macroinvertebrates

2019	Crayfish	Pritchard Traps (PTs) for deployment time experiment	All	Ch 3. Novel methods
		Pritchard Traps (PTs) for crayfish monitoring throughout season	All	Ch 3. Novel methods & Ch 4. Crayfish populations
	Fish	Pritchard Traps (PTs) for deployment time experiment	LPB sites	Ch 3. Novel methods
		Pritchard Traps (PTs) for benthic fish monitoring throughout season	LPB sites	Ch 3. Novel methods & Ch 5. Impacts on fish
	Substrate	Collection of substrate from Pritchard Traps for laboratory analysis	All	Ch 4. Crayfish populations
2020	Crayfish	Pritchard Traps (PTs)	All	Ch 4. Crayfish populations
	Fish	Pritchard Traps (PTs)	LPB sites	Ch 5. Impacts on fish
	Macroinvertebrates	Surber samples	All	Ch 6. Impacts on macroinvertebrates

2.4 Surveys of biota

2.4.1 Macroinvertebrate sampling

Freshwater macroinvertebrates are good indicators of ecosystem health, with many taxa exhibiting sensitivity to specific environmental conditions. Invasive crayfish are also shown to negatively affect invertebrate communities (Crawford, Yeomans and Adams 2006). Therefore, macroinvertebrate analysis was an important component of assessing the impact of invasive crayfish on native biota. Surber sampling (Figure 2.8) was selected as the preferred method of invertebrate sampling as it is a quantitative method and all sites under typical conditions satisfied the technical requirements of river depth < 30 cm, rocky substrate and moderate flow. Ten repeat samples were collected for thorough assessment of macroinvertebrate assemblages. Samples were picked and stored in Industrial Methylated Spirits (IMS). Where possible, specimens were identified to species level under a dissecting microscope using appropriate keys and literature (e.g. Dobson and Crowden 2012). The total body length (mm) was also recorded for all invertebrates so that biomass (Ash Free Dry Weight, mg) could be calculated using published length-weight regressions (e.g. Benke *et al.* 1999). Full details of macroinvertebrate sampling and identification can be found in Chapter 6.

Figure 2.8 Surber sampler used in field surveys.

2.4.2 Fish surveys

Fish strongly influence the structure and functioning of freshwater ecosystems (Reynolds 2011). At the same time, habitat modifications, pollution, overfishing, alien species invasions and climate change render freshwater fish one of the most threatened groups of vertebrates (Reid et al., 2013). Given the reported impacts of invasive crayfish on fish populations (Peay *et al.* 2009; Reynolds 2011), it is crucial to understand how invasive crayfish are affecting native fish communities on both a temporal and spatial scales. Electrofishing is a widely accepted method for sampling fish, and it has become standard practice for fishery studies and management (Beaumont 2016). Electrofishing involves using a back-pack or bankside generator to create an electrical current in the watercourse. The operator works with the anode in front of them and the cathode submerged behind them. The electrical current stuns fish, allowing (an) additional team member(s) to catch the fish using nets. A typical electrofishing team will comprise of 2 - 3 team members, depending on the size of the river or beck investigated. Team members will stand abreast from one another and work progressively upstream (Figure 2.9). To minimise fish mortality and achieve optimal catch efficiency, it is important to adjust the voltage settings depending on the water conductivity (Beaumont 2016). As a first step in electrofishing surveys, a site is selected and stop nets are placed at both the upstream and downstream limit of the site to enclose the local fish community. The site is then electrofished using three consecutive sweeps. From the three-sweep depletion, population density and abundance can be estimated through specialised formulae, providing more detailed information than single sweep runs or alternative methods such as seine netting (Carrier *et al* 2009). Electrofishing was used in 2018 at multiple sites during the field campaign. Consent to conduct electrofishing surveys was authorised by the Environment Agency (FR2 licence). Further details of electrofishing methodology are outlined in Chapters 3 and 5.

Figure 2.9 Electrofishing team ‘in action’ (photograph taken in 2016 at White Beck, Wharfe catchment).

2.4.3 Triple Drawdown (TDD)

The TDD method involves isolating a small section of a watercourse with nets and dams, then using pumps to gradually dewater the site (Figure 2.10, Chadwick *et al.* 2021). As the water drains, all suitable crayfish refugia are removed, such as cobbles, pebbles and woody debris, and all exposed crayfish are collected by hand or net. The isolated study site is then rewetted to entice any hidden crayfish to remobilise into the channel. The process of dewatering and rewetting is repeated until no more crayfish are encountered. Upon completion of the final ‘sweep’ of dewatering and crayfish collection, all refugia materials are returned to the channel, any barriers are removed and the site is rewetted. The reduction in crayfish numbers over each sweep allows depletion analyses to be conducted and total crayfish estimates to be made. The total number of crayfish divided by the site area allows a quantitative crayfish density (individuals m^{-2}) to be calculated (Chadwick, Pritchard, *et al.* 2021). TDDs were conducted at all study sites (Figures 2.11 - Figures 2.14). Further details of TDD methodology are described in Chapter 3.

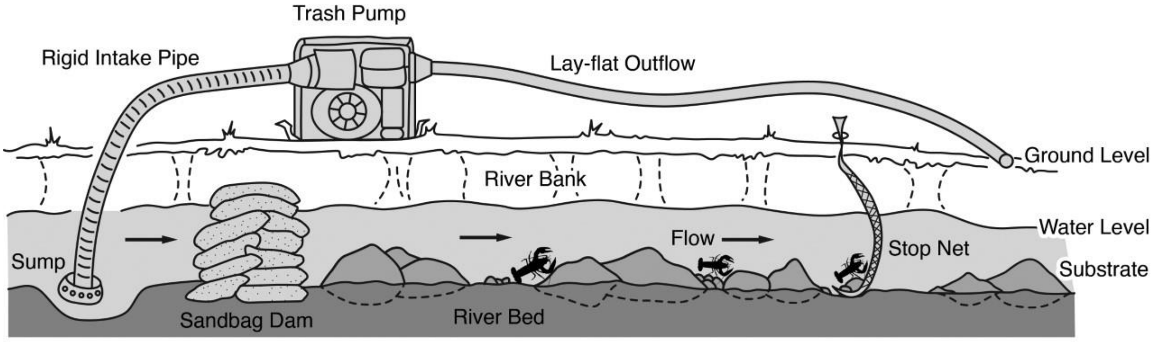


Figure 2.10 Schematic diagram of the Triple Drawdown (TDD) site and equipment setup (source: Chadwick *et al.* 2021).

Figure 2.11 Triple Drawdown (TDD) at Double Gate Bridge (DGB), Bookill Gill Beck (BGB), a) before and b) during dewatering.

Triple drawdown at Confluence, Long Preston Beck (LPB)

Figure 2.12 Triple Drawdown (TDD) at Confluence, Long Preston Beck (LPB), a) before dewatering and b) during dewatering.

Triple drawdown at Footbridge, Long Preston Beck (LPB)

Figure 2.13 Triple Drawdown (TDD) at Footbridge, Long Preston Beck (LPB), a) before dewatering and b) during dewatering.

Triple drawdown at Farm, Long Preston Beck (LPB)

Figure 2.14 Triple Drawdown (TDD) at Farm, Long Preston Beck (LPB), a) during dewatering and b) after the TDD.

2.4.4 Biosecurity

Biosecurity is paramount for all field work and as such a strict biosecurity protocol was followed throughout the project. The ‘Check, Clean, Dry’ campaign (NNSS 2018) was adhered to and all equipment was disinfected with Virkon™ Aquatic S between uses. Wherever possible, equipment was left outside for exposure to UV radiation to dry fully.

Chapter 3. Novel methods

The content of this chapter also contributes to two manuscripts published in *Ecological Solutions and Evidence*:

Pritchard, E. G., Chadwick, D. D. A., Patmore, I. R., Chadwick, M. A., Bradley, P., Sayer, C. D., & Axmacher, J. C. (2021). The ‘Pritchard Trap’: A novel quantitative survey method for crayfish. *Ecol Solut Evidence*, 2: e12070, https://doi.org/10.1002/ 2688-8319.12070 (manuscript summary available in Appendix 2).

Pritchard *et al.* (2021) Supporting Information (Appendix 3).

Pritchard, E. G., Chadwick, D. D. A, Chadwick, M.A, Bradley, P., Sayer, C. D, Axmacher, J.C. (2021b) Assessing methods to improve benthic fish sampling in a stony headwater stream. *Ecol Solut Evidence, 2, e12111. https://doi.org/10.1002/2688-8319.12111* (manuscript summary available in Appendix 4).

3.1 Summary

1. As crayfish invasions continue to threaten native freshwater biota, a detailed understanding of crayfish distribution and population structure becomes imperative. Nonetheless, most current survey methods provide inadequate demographic data. The quantitative ‘Triple Drawdown’ (TDD) dewatering method has highlighted the importance of such data, yet practical constraints prevent the large-scale application of TDDs. Methodological constraints present a similar limitation on the quantitative assessment of benthic fish.
2. Here, we introduce the ‘Pritchard Trap’ (PT), a novel passive sampling device that reliably allows the generation of quantitative crayfish population data while requiring substantially lower sampling effort than TDDs. The PT was trialled over several time intervals to determine the minimum required trap deployment time. TDDs at the same sites allowed for a robust evaluation of PT sampling accuracy in representing crayfish densities and population structure. PTs and TDDs were also tested in their ability to survey benthic fish in comparison to the conventional method of electrofishing.
3. PTs successfully sampled both invasive and native crayfish (8 – 42 mm carapace length). A minimum passive deployment time of four days was required. At low crayfish densities (0.5 ind. m^{-2}), increased trapping effort was necessary to achieve accurate population density and size class distribution estimates. The PT required substantially less sampling effort (working hours) and resources than the TDD, whilst also posing less risk to non-target species.
4. Both the PT and TDD methods successfully sampled benthic fish and provided similar high-density population density estimates for European bullhead, which were at least 2.5 - 5 times higher than predicted from electrofishing-derived population estimates.
5. The PT, for the first time, affords logistically simple, truly quantitative investigations of crayfish and benthic fish population demographics for headwater systems. The method could be integrated into research, conservation and management, for example to monitor populations

and explore density-dependent ecological impacts of invasive crayfish on native ecosystems, as also explored in Chapters 4, 5 and 6 of my thesis.

3.2 Introduction

3.2.1 Crayfish surveys

Crayfish surveys have employed a variety of methods and approaches (see Parkyn 2015), including baited traps (e.g. De Palma-Dow *et al.* 2020), passive traps (e.g. artificial refuge traps (ARTs) - Green *et al.* 2018), manual handsearches (Bradley *et al.* 2015; Hilber *et al.* 2020), electrofishing (e.g. Alonso 2001) and environmental DNA (eDNA; Chucholl *et al.* 2021). However, these methods each have inherent limitations and biases, such as low spatial resolution (eDNA - Harper *et al.* 2018), or selecting for specific crayfish life stages, sexes or species (Rabeni *et al.* 1997; Price and Welch 2009). Baited trapping is the most widely used crayfish survey method (Parkyn 2015). Their low cost, ease of use and suitability across a wide range of habitats make baited traps generally a convenient tool for basic survey and management. However, standard trap samples are biased towards large (≥ 35 mm carapace length CL), active males (Gherardi *et al.* 2011; García-De-Lomas *et al.* 2020; Chadwick *et al.* 2021), generating semi-quantitative catch-per-unit-effort abundance estimates limited to large size classes.

A range of modified equipment and new methods have been suggested to survey small crayfish size classes in various aquatic systems. These include finer mesh sizes for baited traps (Stebbing *et al.* 2016), and trials of quadrat samplers (Distefano *et al.* 2003) and enclosure traps (Byrne *et al.* 1999; Fjälling 2011). For example, Stebbing *et al.* (2016) observed smaller signal crayfish to sometimes be retained when using a decreased mesh size in baited funnel traps. Nonetheless, larger crayfish present in the traps deterred juvenile and female crayfish, and a strong sample bias remained. A modified quadrat design somewhat resembling a large Surber sampler has been tested (Rabeni *et al.* 1997; Distefano *et al.* 2003; Larson *et al.* 2008). Here, the survey area is enclosed with a mesh-sided frame, and the substrate is methodically disturbed to collect the crayfish within the frame. This method revealed spatial and temporal differences in crayfish population densities in

large (20 - 25 m width) streams (Distefano *et al.* 2003), but was prone to both under- and over-estimations of the overall population size (Larson *et al.* 2008), as well as bias towards small size classes (Rabeni *et al.* 1997).
An enclosure trap was designed by Fjälling (2011) and further tested by Engdahl *et al.* (2013) with the explicit aim of sampling juvenile signal crayfish in Swedish lentic systems. Small circular traps (0.09 m^2) were filled with suitable juvenile refugia (small gravels, then naturally occurring bed materials). Traps were left *in situ* at depths of 1 – 3 m along the littoral margin of a lake for several weeks to allow for passive colonisation. This method proved highly effective at sampling small crayfish, with the juvenile size class (< 37.5 mm total length) comprising 97.8 – 98.6 % of the total catch. The reported juvenile densities were strongly influenced by the substrate type, and very few adult crayfish were captured – likely in response to the substrate composition (Engdahl *et al.* 2013). Therefore, whilst effectively capturing juvenile crayfish, this enclosure trap remains unsuitable for generating whole population density or structure estimates.

Chadwick *et al* (2021) assessed *in-situ* crayfish demographics using a triple drawdown (TDD) approach in headwater streams in Northern England. The TDD involves isolating small sections of stream and sequentially dewatering them to form depletion ‘sweeps’. Crayfish refugia, including cobbles, boulders and woody debris, are removed from the channel and exposed crayfish are captured by hand or net. The TDD enabled robust estimates of the total crayfish population and its structure, sampling on average 92% of the estimated crayfish population. The TDD revealed extremely high crayfish densities (21 – 110 individuals m^{-2}), with juveniles and sub-adults comprising the majority of the population (90% of individuals < 25 mm CL). The TDD is an extremely resource-intensive sampling approach, making it widely impractical for use in frequent or remote surveys.

3.2.2 Benthic fish surveys

Traditional survey methods present similar limitations for the assessment of benthic fish communities.
Fish populations can be surveyed using again a variety of methods, including netting (e.g. seine netting, Neilson and Johnson 1983; Pierce et al., 1990), trapping (e.g. minnow traps, Bloom 1976; Bryant 2000) acoustic telemetry (Crossin *et al.* 2017) and electrofishing (Reynolds 1996; Beaumont 2016). Electrofishing, widely used in stream biological monitoring, involves applying an electric field in the water to temporarily incapacitate fish, allowing them to be caught (see Ch. 2; Beaumont 2016). Many physical factors affect the efficiency of electrofishing, including water clarity, depth and conductivity, substrate type, and fish species. Benthic fish are notoriously difficult to capture by electrofishing, owing to their relatively small body size, behaviour and preference for staying close to the riverbed. Some benthic fish additionally show a poor electrotactic response (Cowx 1983; Beaumont 2016), with some taxa also lacking a swim bladder (e.g. species in the Cottidae), reducing their buoyancy and thus the effectiveness of the anodes' pull. Further limitations to electrofishing relate to benthic species being associated with structures like cobbles and boulders that partially shield them from electric fields, rendering incapacitated animals inaccessible. Whilst electrofishing and other contemporary methods have proven suitable and effective in sampling many fish species in various freshwater systems, a strong need persists for new methodologies that generate reliable quantitative data on benthic fish populations.

In some instances, benthic invertebrate sampling techniques have been adapted to sample benthic fish - for example Hess samplers and Surber samplers being employed to survey European bullhead *Cottus gobio* in English chalk streams (Harrison et al. 2005; Woodward et al. 2008). These benthic invertebrate survey methods proved successful at quantitatively sampling bullhead, chiefly due to their sedentary nature. Recent methodological advances in surveying freshwater crayfish also show potential promise for benthic fish survey. The habitat requirements of benthic fish and crayfish often strongly overlap (Bubb et al. 2009; Ruokonen et al., 2014), and methods that successfully survey crayfish within benthic habitats could hence reasonably be expected to also catch benthic fish.

3.2.3 Rationale

The biases associated with conventional crayfish sampling methods and the technical limitations of the TDD highlight a crucial need for an intermediate method that combines the simplicity and cost-efficiency of conventional trapping techniques with the data quality of the TDD. There is a similar need for new methods to quantitatively survey benthic fish populations, as biases with conventional techniques currently hinder the efficient recording of their population densities and structure, too. In response to these needs, I designed and tested a novel method for the sampling and subsequent characterisation of crayfish and benthic populations in rocky streams.

3.2.4 Research aims and hypotheses

The primary aim of this work was to design an effective, quantitative crayfish survey method. Initially, three designs were tested in a pilot study, where one design, subsequently named the ‘Pritchard Trap’ (PT), showed particular promise and was taken further. All three methods are summarised in 3.3.1. Therefore, the aims of the research covered in this chapter were to assess the performance and practicalities of the method and determine whether PTs could provide robust quantitative data on crayfish and benthic fish populations.

Several hypotheses were formulated to address this;
HI. PTs successfully sample crayfish across a range of size classes.
HII. Crayfish numbers in PTs reach a stable equilibrium once a ‘minimum deployment time’ is exceeded.
HIII. The population demographics of crayfish surveyed in PTs is similar to the ‘true’ population demographics generated using the TDD technique at the same study site.
HIV. PTs successfully sample benthic fish across a range of size class.

HV. PTs and TDDs generate quantitative population data on benthic fish, while electrofishing underestimates population densities.

3.3 Methods

3.3.1 Pilot study to test different trap designs (2018)

Three novel trap designs were tested in a pilot study in May 2018; a quadrat trap, later named the ‘Pritchard Trap’ (PT), a basket trap and a modified trappy trap. The designs were tested at DGB on BGB (Ch. 2 Figure 2.3), as previous work had provided a detailed insight into the population density and structure of invasive crayfish at this site (Triple Drawdowns in 2017; Chadwick *et al.* 2021). The pilot study was carried out in May, and it was expected that population densities would be lower than the 2017 TDD-derived density estimates, which took place later in the summer when berried females had mobilised and released young-of-year. The site provided a great opportunity to test the different designs, check whether they sampled a range of crayfish size classes and identify any logistical constraints. A method statement and licence (CR1) was authorised for all trap designs by the Environment Agency.

3.3.1.1 Pritchard Trap design

The Pritchard Trap (PT) consists of a 5-panel mesh bag and rigid plastic quadrat frame. The quadrat comprises four detachable plastic pipes with corner piece sections (50 cm in length), attached to the mesh bag through webbing loops (Figure 3.1). The base of the mesh bag measures 50 cm x 50 cm, creating a base trap area of 0.25 m^2. Mesh bag panels measure 30 cm in height and are reinforced with strong webbing – this height could be adjusted in response to water depth requirements. While the mesh is strong and rigid, it can be compressed flat during deployment. The mesh bag is green in colour (Figure 3.2) with a 1.9 mm x 1.9 mm mesh size to allow passage of water and small invertebrates, whilst retaining all crayfish. This quadrat can be dismantled and the mesh bag folded down for convenient travel and storage. Details of materials and the manufacturing process are provided in Appendix 3.

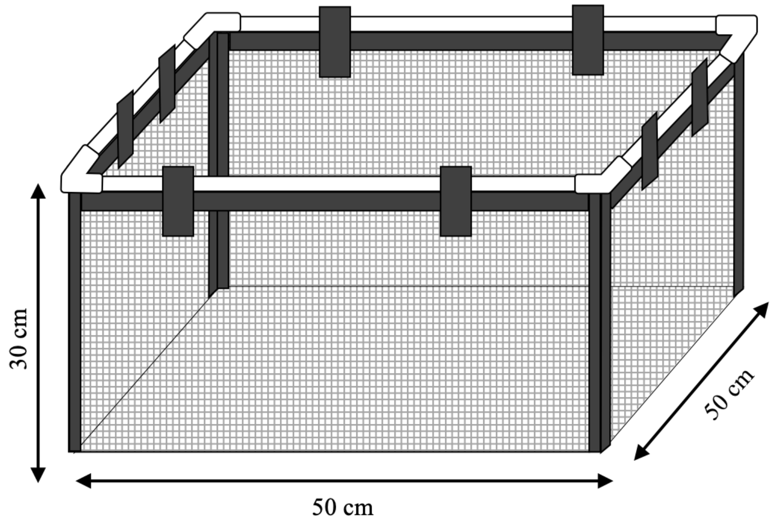


Figure 3.1 Technical drawing of the Pritchard Trap (PT) illustrating the square mesh bag, webbing hems and loops and plastic quadrat.

To set a trap, substrate (boulders, cobbles, gravel, woody debris) is first removed from the 0.25 m^2 trap footprint and collected into a bucket to expose the riverbed (typically 10 – 20 cm substrate depth, Figure 3.2b). The PT is then pressed flat into the created depression so that the mesh panels are fully collapsed, being folded over themselves underneath the plastic quadrat frame, to maintain a consistent base area. Quadrat corners are weighted down with large cobbles, and the collected substrate is placed on top of the flattened PT to reform the original channel profile (Figure 3.2c). Throughout the study, placement of PTs within the channel was never observed to encourage or impede the mobilisation of bed materials. Crayfish were able to freely enter and exit the trap area, accessing the substrate for refuge and foraging. Upon retrieval, the quadrat is then sharply pulled upwards, so that the mesh panels are fully extended, thus entrapping any crayfish residing within the PT (Figure 3.2d). The entire contents of the PT including substrate and crayfish are emptied into buckets to be processed. Substrate and PTs can be redeployed to the same position between sampling events, with substrate returned following the sampling completion. Both the initial deployment and subsequent retrievals and re-deployments of a PT typically took one operative 15 minutes.

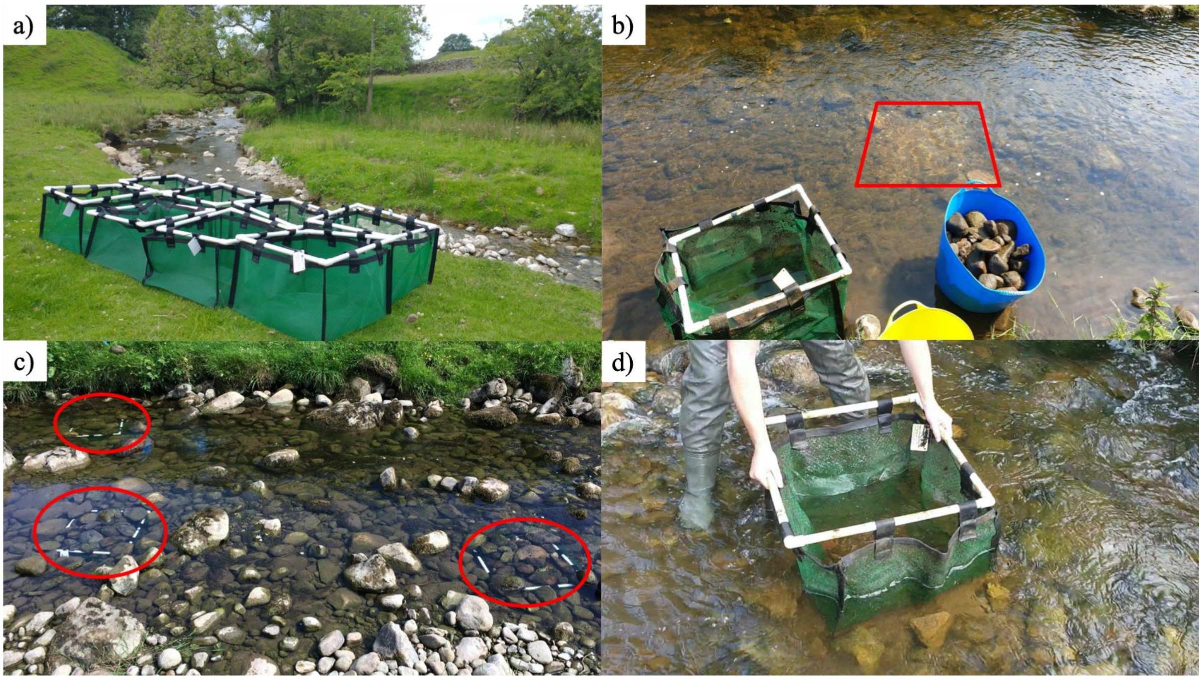


Figure 3.2 Photographs of Pritchard Traps (PTs) in the field; a) PTs assembled ready to set, b) substrate collected in a bucket from the footprint of the PT to be set (0.25 m^2 sample location outlined), c) PTs set in the river (red circles) and d) retrieval of PT from a river.

Four PTs were deployed at Double Gate Bridge (DGB), Bookill Gill Beck (BGB) for 24 hours (n = 2) and 48 hours (n =2) in the pilot study. The PTs proved successful at sampling a range of size classes including juveniles, with a 100% detection rate capturing crayfish in every PT deployed (Table 3.1). The materials were robust and functioned well in the field and the base sampling area seemed suitable to encompass naturally occurring substrate such as large cobbles and small boulders. The PT showed potential as a quantitative survey technique and was selected for further testing which is described later in this Chapter.

Table 3.1 Summary statistics from the Pritchard Trap (PT) design pilot study, including size range of carapace lengths, density estimates and detection rates.

	24-hour deployment	48-hour deployment
Minimum carapace length (mm)	8	8
Maximum carapace length (mm)	33	30
Lower density estimate (m^{-2})	12	8
Upper density estimate (m^{-2})	24	16
Average density estimate (m^{-2})	18	12
Detection rate (% of PTs with ≥1 crayfish)	100%	100%

3.3.1.2 Basket trap

The second trap design tested was a fine mesh basket that was filled with substrate from the site and set within the river bed, in a similar manner to the PT method described above (Figure 3.3). Nonetheless, each basket measured only 41 cm in diameter, resulting in a base sample area of 0.13 m^2. The basket was again collapsible and could be set completely flat to allow crayfish to enter and exit the trap and use the substrate as normal. In the pilot study, the basket traps were set at DGB and deployed for 24 hours (n = 6) and 48 hours (n = 3). Upon retrieval, the handle was pulled sharply upwards so that the mesh sides extended and the basket sides enclosed any crayfish utilising the substrate within.

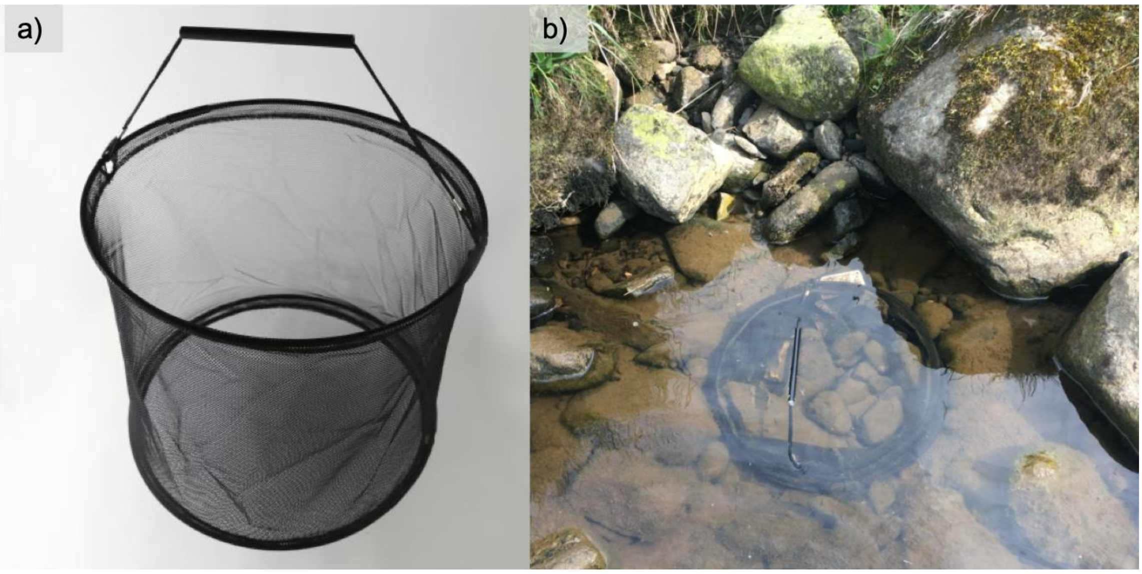


Figure 3.3 Basket trap design; a) basket trap fully expanded, b) basket trap deployed flat and filled with small cobbles and pebbles.

The results of the pilot study showed that the concept of the method was good, and the basket traps successfully sampled juvenile crayfish (8 mm CL, Table 3.2). However, the small base sampling area proved problematic for several reasons. Firstly, large cobbles and boulders did not fit within the sampling area, which may limit the chance of catching larger crayfish that may utilise larger substrate. Furthermore, the calculation of density estimates (number of crayfish per m^2) caused large variations in estimates due to the multiplication factor (number of crayfish in one basket multiplied by 7.69 to gain density m^{-2}). To compensate for this, large numbers of baskets would need to be deployed to gather more reliable estimates, as with a small sample size, the difference in one crayfish per trap would make a large difference in density estimates. A final consideration was the durability of the mesh, which felt brittle and showed signs of wear after a short deployment time. It was concluded that the principle of the method had potential, but this design was not suitable and was therefore not subject to further testing.

Table 3.2 Summary statistics from the basket trap design pilot study, including size range of carapace length, density estimates and detection rate.

	24-hour deployment	48-hour deployment
Minimum carapace length (mm)	8	8
Maximum carapace length (mm)	26	17
Lower density estimate (m^{-2})	0	0
Upper density estimate (m^{-2})	15.4	15.4
Average density estimate (m^{-2})	5.1	5.1
Detection rate (% of baskets with ≥1 crayfish)	33.3	66.7

3.3.1.3 Modified trappy trap

The final design involved modifications of a standard Swedish style Trappy trap (Fjälling 1995). Additional mesh, with a finer mesh size (5 mm), was added to the inside of the traps to prevent smaller crayfish escaping through the trap walls. The aperture size on one end of the trap was reduced from 5 cm to 2 cm in an effort to only permit small crayfish individuals to enter the trap on this side. The other end of the trap was left unaltered to allow large individuals to enter (Figure 3.4). A plastic divider was inserted into the middle of the trap to separate the trap into two compartments. The aim was to have two separate sides to the trap, the ‘small side’ with a modified entrance aimed at catching smaller size classes and the ‘standard’, unmodified side aimed at catching larger size classes. The barrier was installed to prevent cannibalism of smaller crayfish and to check if the small entrance to the trap functioned as expected in limiting access only to smaller specimens.

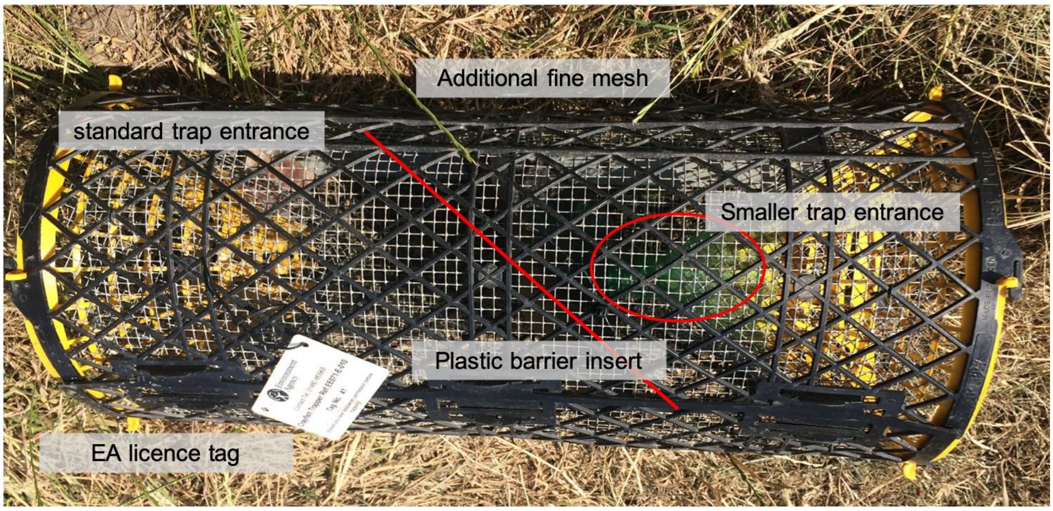


Figure 3.4 Modified Trappy trap, with key features labelled and modifications highlighted with red markers.

Ten modified trappy traps were prepared and tested in the pilot study. Traps were deployed at DGB, BGB. They were baited with oily fish (mackerel), submerged in the channel and anchored to the bank with rope and pegs. The traps were set overnight and left for approximately 12 hours before retrieval. Crayfish were collected and processed for sex, carapace length (mm) and mass (g).

The pilot study results showed that the ‘small side’ of the trap resulted in a much lower Catch-Per-Unit-Effort (CPUE, Table 3.3) and there were several occasions where no crayfish were caught on that side of the trap. Furthermore, the average size of crayfish did not differ between the two sides, indicating that large crayfish were still accessing both sides (Figure 3.5). Yet the maximum size captured was much higher for the ‘standard side’, which suggests that the barrier was preventing mixing of crayfish between compartments in the trap and the ‘small’ entrance was excluding very large adults (e.g. > 40 mm CL). The minimum carapace length of any crayfish caught in the modified traps was 20 mm CL, confirming that the design was ineffective at sampling juvenile size classes and therefore did not satisfy the desired requirements of a novel crayfish sampling method in this study. As a result, the decision was made not to test this method any further.

Table 3.3 Summary statistics from the modified trappy design pilot study (n = 10), including size range of carapace length, catch-per-unit-effort, and number of no catch incidences.

	Small side	Standard size
Minimum carapace length (mm)	21	20
Maximum carapace length (mm)	39	53
Mean carapace length (mm)	32.1	32.7
Standard deviation	4.29	6.40
Median carapace length (mm)	32.5	32
CPUE	1.6	5.8
Number of ‘no catch’ incidences	5	2

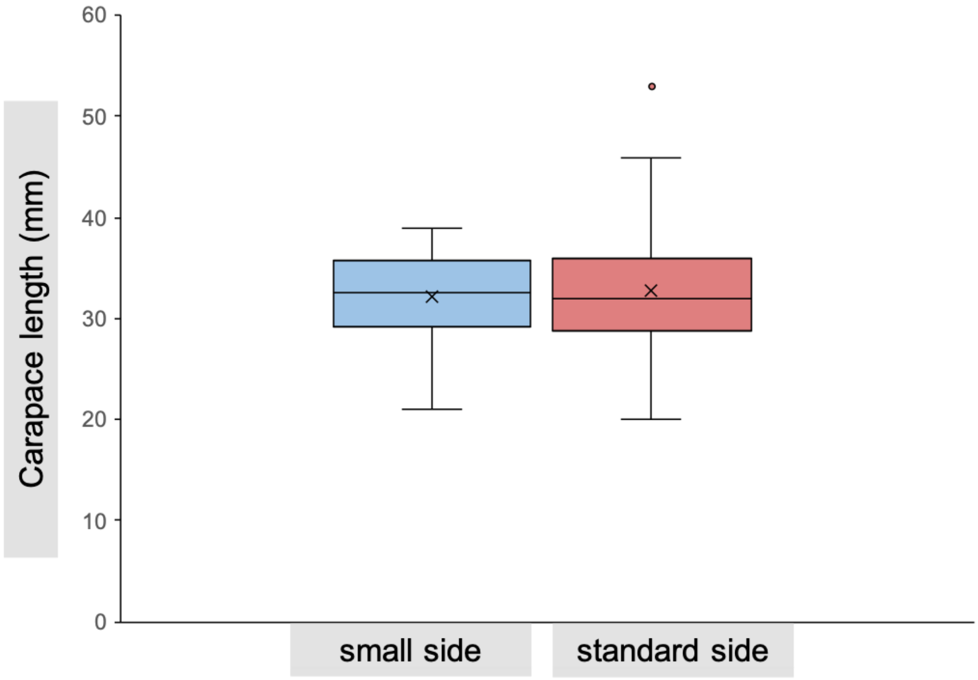


Figure 3.5 Boxplot of signal crayfish carapace length (mm) captured in the ‘small’ and ‘standard’ sides of the modified trappy trap.

3.3.2 Pritchard Trap-based crayfish assessments

3.3.2.1 Experimental design

Following pilot testing, the Pritchard Trap method was selected for more vigorous testing across the study system. Fieldwork was undertaken at DGB, Confluence, Footbridge and Farm (Ch. 2 Figure 2.3), during the summers (June – September) of 2018 and 2019. The use of the PTs was authorised by the Environment Agency (CR1 licence). Two experiments were undertaken to evaluate PT performance for surveying crayfish. A deployment time experiment established the minimum trap deployment time for crayfish densities in the PTs to stabilise. The second experiment enabled comparisons of crayfish population density and structure reflected by PT and TDD samples (Table 3.4). PTs were used at a higher sampling effort (7.5 m^2) for population structure analyses (2019, all sites) and a low sampling effort (0.75 – 1 m^2) for all other analyses (Table 3.4). The sampling effort was driven by the number of PTs that could be built prior to the sampling season and the number of re-deployments that could take place over the summer.

Table 3.4 Summary table of field studies, including deployment time analyses, density estimates and population structure comparisons at sites along Bookill Gill Beck (BGB) and Long Preston Beck (LPB).

Experiment	Method	Year	Months	Site(s)	Sample size (m^2)
Deployment Time	PT	2019	June-September	All	n = 3 (0.75 m^2) per site
PT vs TDD comparison	PT (density)	2018	July-August	All LPB	n = 4 (1 m^2) per site
	PT (density)	2019	August	Double Gate Bridge (DGB), BGB	n = 3 (0.75 m^2)
	PT (structure)	2019	June-September	All	n = 30* (7.5 m^2) per site
	TDD	2018	August	Double Gate Bridge, BGB	n = 1 (15 m^2)
	TDD	2018	July	Confluence, LPB	n = 1 (45.5 m^2)
	TDD	2018	July	Footbridge, LPB	n = 1 (45.5 m^2)
	TDD	2018	July	Farm, LPB	n = 1 (50 m^2)

* comprised of 3 individual traps lifted 10 times each over the 2019 field season.

3.3.2.2 Processing of crayfish

All crayfish were identified to species level in the field. White-clawed crayfish were measured on the bankside and immediately released back at the site in a safe, undisturbed area. All PT-sampled signal crayfish were processed and then released back at the site to prevent skewing catch results of the next repeat (method statement approved by Environment Agency). Signal crayfish captured in TDDs were stored on ice and humanely destroyed by freezing, before processing in the laboratory. Carapace length (CL mm), mass (g) and cheliped damage (absent or regenerating) were recorded for all crayfish. Crayfish over 12 mm CL were processed for sex (male/female), while crayfish ≤ 12 mm CL were too small to be reliably sexed and were classified as juveniles in this study.

The PT crayfish samples from the mixed population at the Farm site were split into two separate datasets, one for each species present (‘Farm WCC’ for white-clawed crayfish and ‘Farm SC’ for signal crayfish).

3.3.2.3 Deployment time experiment

PTs were set at all four sites (n = 3 per site) to estimate the minimum deployment time. All traps were set by the same two personnel for consistency, across a range of habitat types including pools, riffles, central channel, margins, shaded and unshaded areas. Five different time interval treatments were used; 1, 2, 4, 7 and 10 days over which time PTs were left submerged without disruption. On experiment completion, all traps were retrieved and disinfected with Virkon™ Aquatic S.

The deployment time experiment was conducted over the summer period (2019), when young-of-year (YOY) hatch, potentially leading to considerable variations in crayfish numbers at each site through hatching events and subsequent mortality. The PT catch data (density per time interval) was therefore presented as two size groups (≥ 13 mm CL and ≤ 12 mm CL), with the population structure of larger individuals expected to remain comparatively stable during the summer months. Detection rates were calculated as the percentage of PTs that caught at least one crayfish individual at each site. Two density estimates were provided for each site (1. sub-adults and adults ≥ 13 mm CL and 2. all sizes) at 95% of the respective TDD catch (see below for approach) to give a broad indication of expected density.

3.3.2.4 Pritchard Trap and Triple Drawdown comparison

TDDs were undertaken in July - August 2018 (Table 3.4). The TDD at DGB was conducted following the method described in Chadwick *et al* (2021) with one pump (Honda Trash pump 3 inch), three consecutive sweeps and four operatives. However, due to the larger area of the sites along LPB (45.5 – 50 m^2), some adjustments to the TDD method were required. Firstly, two pumps (Honda Trash pumps 2 inch and 3 inch) were used simultaneously at each site to overcome the greater inflow of water. Secondly, a total of four dewatering ‘sweeps’ were conducted to ensure a satisfactory depletion in crayfish numbers, with a total of 6 - 10 operatives required for each TDD. In this study, a TDD was also carried out at a white-clawed crayfish site (Farm) under licence (licence number 2016-21910-CLS-CLS), with all work overseen by the licence holder.

Triple drawdown depletion calculations and total crayfish density estimates were made using the Carle Strub maximum weighted likelihood estimation method (Eqn 1; Carle and Strub 1978) in the FSA package (Ogle 2018) in R (version 3.5.1). The Carle Strub method uses the values from subsequent removal events e.g. TDD sweeps, and associated depletion curve to estimate the total population size. The efficiency of the TDD method was calculated using the total number of crayfish caught in a TDD as a fraction of the Carle Strub-derived total estimated population.

$$\frac{N_0 + 1}{N_0 - T + 1} \prod_{i=1}^{k} \frac{kN_0 - X - T + \beta + k - i}{kN_0 - X + \alpha + \beta + k - i} \leq 1 \qquad (1)$$

Equation 1. Carle Strub maximum weighted likelihood estimate method.

Density

To determine density estimates, PTs (n = 4) were deployed for four days at each site before the TDDs in 2018. Pritchard Traps were retrieved and processed immediately before the start of the TDD. Effective trap deployment at DGB in 2018 was disrupted by low water levels due to a drought event (NHMP, 2018), and as such, PT data from 2019 (0.75 m^2) at the same location was used for density comparisons. Whilst TDD and PT data were therefore collected in subsequent years, typical seasonal conditions were observed in 2019, and 2016 - 17 TDD samples indicate consistently high crayfish population densities (86 – 110 m^{-2}) at this site (Chadwick et al., 2021). The DGB 2019 traps were retrieved at a similar date to the 2018 TDD (05/08/2019 and 06/08/2018 respectively), to standardise for seasonal fluctuations in population density related to juvenile release and mortality. Pritchard Traps in LPB remained fully submerged during the sampling interval, and hence the original 2018 data were used in comparisons.

Structure

Repeat PT sampling was undertaken between June - September 2019 to assess the ability of PTs to determine crayfish population structure. Pritchard Traps (n = 3) were set at all sites and retrieved a total of 10 times throughout the summer to increase sampling effort (n = 30 lifts, total sampling area 7.5 m^2). Traps were set for a minimum soak time of four days. SPSS was used to derive statistical descriptors such as minimum, maximum, mean and median carapace length and undertake post hoc analyses on demographic data including sex ratios (Chi-squared). Graphical representation of the population structures was achieved through ggplot2 package (Wickham 2016) in R (version 3.5.1).

3.3.3 Pritchard Trap-based fish sampling

3.3.3.1 Experimental Design

The PT and TDD techniques were also tested as alternative methods to electrofishing to survey benthic fish populations. Fish were not present at DGB (see Ch. 5 for more detail). Therefore, all fieldwork was undertaken at Confluence, Footbridge and Farm (Ch. 2 Figure 2.3), during the summers (June – September) of 2018 and 2019 (Table 3.5). The fish study again comprises two main components. Firstly, the performance of each survey method (electrofishing, TDDs and PTs) was assessed individually. Sweep depletions were evaluated for electrofishing and TDDs to generate total fish population estimates and to assess method efficiencies. PTs were again tested over a range of deployment intervals (see below) at all sites to establish the minimum deployment time required to reach stable fish density estimates. Secondly, the three survey methods were compared to evaluate their ability to generate robust density and population demographic data for benthic fish. Resident fish at LPB include the pelagic species brown trout *Salmo trutta*, Atlantic salmon *Salmo salar* and minnow *Phoxinus phoxinus*, and benthic species comprise European bullhead *Cottus gobio* (hereafter bullhead), stone loach *Barbatula barbatula* and European eel *Anguilla anguilla*.

3.3.3.2 Processing of fish

Surveyed fish were collected by net (TDDs and electrofishing) or by hand (PTs) and placed in large buckets filled with frequently replaced cool, well-oxygenated water, that were kept in the shade. All fish were identified to species level, and total length (TL, mm) was measured and recorded on site. For electrofishing and TDDs, all young-of-year (YoY) bullhead were recorded as 20 mm TL based on measurements of a sub-sample of YoY at site. Once processed, fish were released immediately downstream of the site. A method statement and FR2 fishing licence was approved by the Environment Agency. The “Check, Clean, Dry” (NNSS, 2018) procedure was strictly followed, and all equipment was disinfected with either Virkon S Aquatic™ or FAM® 30 (iodophor based) between each use.

Table 3.5 Summary of field studies including sites, survey methods, survey area and year.

Sampling Method	Year	Months	Site(s)	Sample size (m^2)
Electrofishing	2018	July-August	Confluence Footbridge Farm	1 sweep (~45.5 m^2) 3 sweeps (~45.5 m^2) 4 sweeps (~50 m^2)
TDDs	2018	July - August	All	45.5 – 50 m^2 4 sweeps
PT (deployment time experiment)	2019	June – September	All	n = 3 (0.75 m^2)
PT (low sampling effort to assess population densities)	2018	July - August	Footbridge and Farm	n = 4 (1 m^2)
PT (repeat sampling to assess population structure)	2019	June – September	All	n = 30 (7.5 m^2)

3.3.3.3. Performance of electrofishing, TDDs and PTs

Electrofishing was undertaken at each site (summer 2018, Table 3.5) by three trained (Institute of Fisheries Management) and experienced operatives using a Smith-Root 400w LR-20B Electrofishing backpack system. Stop nets (2 mm mesh size) were installed at the upstream and downstream limits of the site to prevent immigration or emigration of fish during electrofishing sampling and between electrofishing and TDD, with the electrofishing carried out in preparation of the subsequent TDDs (see below). A single electrofishing sweep was undertaken at Confluence, while three consecutive sweeps were undertaken at Footbridge and four consecutive sweeps were undertaken at Farm. The multiple sweeps at Footbridge and Farm allowed depletion analyses to be carried out using the Carle Strub maximum weighted likelihood method (Carle and Strub 1978) in the FSA package (Ogle 2018) in R (version 3.5.1). Total population estimates were generated, which allowed method efficiency to be calculated as the total number of fish caught as a percentage of the total estimated population. Density estimates were then calculated as the number of fish caught over the site area – and the expected density using the estimated total population over the site area.

TDDs were undertaken at each site immediately after the electrofishing surveys on each isolated stretch of LPB (summer 2018, Table 3.5). TDDs were carried out as described above for crayfish. Multiple sweeps at each site allowed depletion analyses to be calculated using the same method as described above. This allowed for the generation of total population estimates, method efficiency and, in combination of site area measurements, fish density estimates for the TDD. Specifications and general operation of PTs followed the approach described above for crayfish. A deployment time experiment was undertaken (summer 2019, Table 3.1) to establish the minimum deployment time required for fish numbers recorded in the PTs to stabilise. PTs were deployed at each site (n =3 per site) for five time intervals; 1, 2, 4, 7 and 10 days. Upon retrieval, the traps were carefully emptied, with substrate from the traps separated into one bucket, and fish specimens into another. Fish were processed as described above and then released back into the site. PTs were reset in the same position using the same substrate for each time interval. Benthic fish species were grouped together to generate total fish numbers captured during each trapping event. Detection rates were calculated as the percentage of PTs with at least one individual benthic fish captured.

3.3.3.4 Comparison of methods (electrofishing, TDD and PT)

The fish data generated through each method were compared to determine differences in estimated community species structure. Density estimates and population size structure of bullhead as the dominant benthic fish species in the system were also explored across all three methods. Additional PT sampling was undertaken in 2018 with a low sampling effort (n = 4) for density estimates at Footbridge and Farm prior to secondary sampling by electrofishing and TDD. Repeat PT sampling was also undertaken in summer 2019 (Table 3.5) to increase sample size (n = 30, 7.5 m^2) in order to enable robust comparisons of population demographics. In all these sampling events, PTs were deployed for a minimum of four days. An estimate of true fish density for the three sites was generated through summing all fish physically removed via electrofishing prior to the TDD and the total TDD-derived population estimate (Carle Strub) for each site. The density estimates from individual methods was then compared to the ‘true fish density’ to assess effectiveness. Furthermore, the population size structure generated from each method was compared. Comparative analyses of the methods were carried out in SPSS (version 27) for statistical descriptors on demographic data. The ggplot2 package (Wickham 2016) in R (version 3.5.1) was used for the graphical representation of the population structures.

3.4 Results

3.4.1 Crayfish

3.4.1.1 Deployment time experiment

Pritchard Traps (PTs) successfully sampled signal crayfish at all study sites and white-clawed crayfish at Farm. The PTs detected both species at the minimum tested deployment time of one day (Figure 3.6). Crayfish were consistently detected by PTs (n = 3) across all sampling times and sites except for signal crayfish at Farm two-day deployment time (Figure 3.6). At the signal crayfish-only sites, PTs consistently (44/45 PTs) detected crayfish presence. At the mixed-population site (Farm), individual PT detection was more variable, but the detection rate for each deployment time treatment remained high.

At the high-density DGB site, only a two-day deployment time was required to generate density values that were comparable with subsequent samples representing longer deployment times (Figure 3.6a). At lower density sites, crayfish numbers in PTs stabilised after 2 - 4 days for signal crayfish, again providing densities broadly within expected ranges (Figure 3.6b-d). The PTs successfully detected white-clawed crayfish at Farm, where numbers also stabilised after 2 - 4 days (Figure 3.6e).

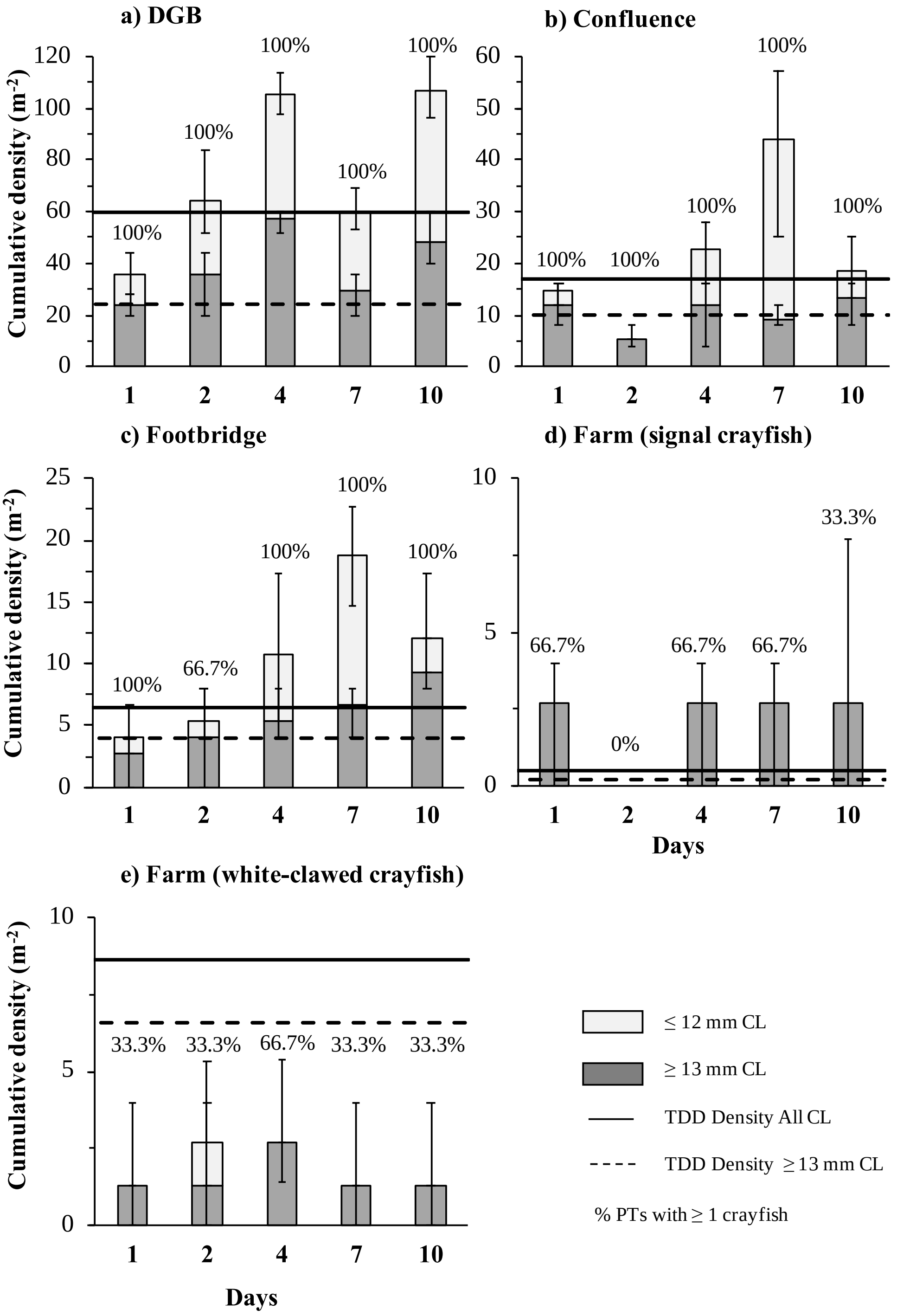


Figure 3.6 Cumulative density of crayfish (≥ 13 mm CL, dark grey and ≤ 12 mm CL, pale grey) derived from various deployment time intervals (days) of PTs (n = 3) across all sites (June - September 2019). Error bars show the deviation of minimum and maximum from average catch densities (m^{-2}). Crayfish detection rates (% PTs containing ≥ 1 crayfish) are presented above each bar. Past density estimates (95% of 2018 TDDs) are provided for reference (≥13 mm CL, dashed line and all crayfish, solid line).

3.4.1.2 Pritchard Trap and Triple Drawdown comparison

Crayfish population density

Based on Carle Strub depletions, the TDDs consistently caught >90% of the total estimated population (DGB 99.0%, Confluence 90.5%, Footbridge 96.6%, Farm SC 96.3%, Farm WCC 98.9%), and thus allowed accurate total population estimates for each site (Table 3.6). Similar to past work on the study system (Chadwick, Pritchard, *et al.* 2021), the TDDs confirmed a wide range of signal crayfish densities along the invasion gradient, including 63 m^{-2} for the well-established, high-density population (DGB), medium densities at Confluence and Footbridge (19.9 and 7.1 m^{-2} respectively), and a very low density of 0.5 m^{-2} at the invasion front (Farm). The TDD also revealed a strong population of native white-clawed crayfish at the invasion front (9 m^{-2} at Farm). At a lower sampling effort (n = 4, 1 m^2 at LPB, n = 3, 0.75 m^2 at BGB) the PTs produced density estimates congruent with TDD estimates derived over a much larger area (15 - 50 m^2 survey area). In addition, the PTs estimated the same changes in density along the invasion gradient as derived from TDDs (Table 3.6). The total estimated crayfish density calculated from TDD depletion curves was within the PT lower and upper density estimates for DGB, Confluence and Footbridge (Table 3.6). However, at Farm, PTs failed to detect the low-density signal crayfish population (< 1 crayfish m^{-2}) and slightly underestimated the density of white-clawed crayfish.

Table 3.6 Crayfish population density values (m^{-2}) generated from Pritchard Traps (2018/2019) and Triple Drawdowns at all sites (2018). Average, lower and upper densities are provided from PT catches. The ‘raw’ densities of crayfish caught during TDDs are provided, as well as the total, lower and upper estimates based on Carle Strub depletion analyses.

Site	Pritchard Trap (PT) Density estimate (m^{-2})			Triple Drawdown (TDD) Density estimate (m^{-2})			
	Average	Lower	Upper	Raw	Total	Lower	Upper
Double Gate Bridge (DGB) (n = 3, 2019)	54.7	32	72	62.7	63.3	62.8	63.7
Confluence (n = 4, 2018)	23	12	32	18.0	19.9	19.1	20.6
Footbridge (n = 4, 2018)	6	4	8	6.9	7.1	6.9	7.3
Farm SC (n = 4, 2018)	0	0	0	0.5	0.5	0.5	0.6
Farm WCC (n = 4, 2018)	6	4	8	9.0	9.1	9.0	9.2

Crayfish population structure

Repeat PT sampling (7.5 m^2, 2019) provided larger sample sizes (820 crayfish sampled in total) from which population demographic data could be explored (Table 3.7). Male to female sex ratios generated from PT surveys were consistent with those from the TDDs (χ^2, $p > 0.05$) at all sites, apart from Footbridge, where PTs showed a female-biased sex ratio ($\chi^2 = 5.439$, df = 1, $p = 0.02$). The incidence of cheliped damage reported through PT sampling was lower than for the TDDs for signal crayfish, but was slightly higher for white-clawed crayfish (Table 3.7). The PTs sampled crayfish from a wide size range (8 – 42 mm CL). The median CL obtained through PTs was similar to that produced by the TDDs for both species, except for signal crayfish present at an extremely low density at Farm (Table 3.7). Crayfish size class distribution derived from PT sampling was analogous to that from the TDDs at DGB, Confluence and Footbridge (Figure 3.7), showing the majority of the population to be juvenile or sub-adult (≤ 25 mm CL) with very few large adults (≥ 35 mm CL). At Farm, however, the number of white-clawed crayfish and signal crayfish sampled was too low to permit a robust evaluation of the data. The repeated PT sampling also provided density estimates congruent with the TDD values for signal crayfish, despite sampling occurring throughout summer, and thus population density estimates being vulnerable to fluctuations due to recruitment and predation. The PTs were able to detect and accurately report the low-density population (0.5 m^{-2}) of signal crayfish at Farm. The PTs recorded a lower density of white-clawed crayfish at Farm in comparison to density estimates from the TDD in the previous year.

Table 3.7 Population demographics from Pritchard Trap repeat sampling (7.5 m^2 in 2019, June - September) and Triple drawdowns (2018) at each site, including density, range of carapace lengths (CL), sex ratios and percentage cheliped damage. PT estimates are compared to TDD baselines.

Parameter	Double Gate Bridge (DGB)		Confluence		Footbridge		Farm WCC		Farm SC	
	PTs	TDD	PTs	TDD	PTs	TDD	PTs	TDD	PTs	TDD
Density (m^{-2})	75.2	62.7	21.2	18.0	10.3	6.9	2.1	9.0	0.5	0.5
Minimum CL (mm)	8	8	8	8	8	8	8	8	23	8
Maximum CL (mm)	42	44	37	50	42	51	32	39	29	52
Median CL (mm)	8	12	15	17	19	19	18.5	15	24	12
Mean CL (mm) Standard deviation (2 d.p)	13.1 7.44	13.6 6.72	15.3 7.17	16.7 9.06	17.8 8.39	18.3 10.15	19.6 8.57	16.9 6.92	25.0 2.83	20.7 13.58
M:F ratio	45.7:54.3	45.3:54.7	37.9:62.1	42.6:57.4	28.9:71.1	48.1:51.9	50:50	46.5:53.5	50:50	50:50
Cheliped damage of >12 mm CL (%)	29.1	30.4	26.4	30.8	20.0	34.8	16.6	12.4	0.0	40.0

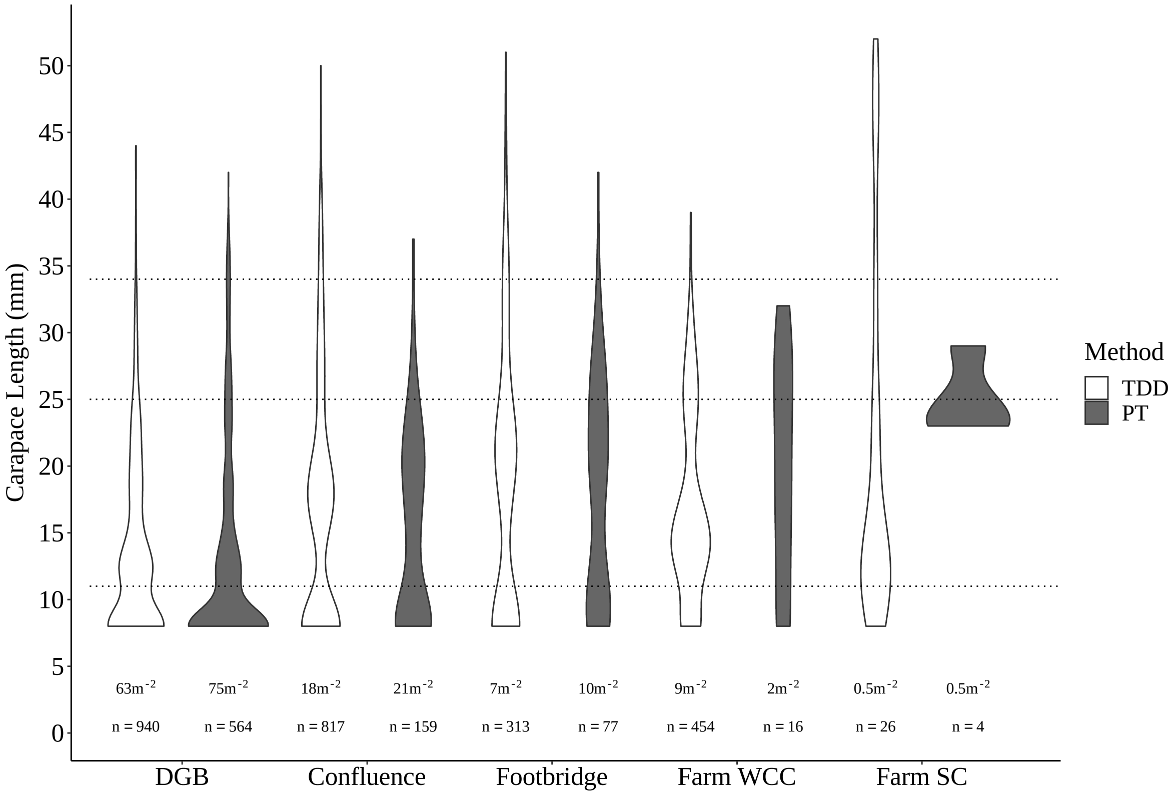


Figure 3.7 Bean plot (i.e. probability density of the catch data) of crayfish size class distribution (mm CL) captured through Triple Drawdowns (2018) and Pritchard Traps (2019) across all study sites. The density of crayfish (m^{-2}) and the number captured (n) are also denoted.

3.4.2 Benthic fish

3.4.2.1 Performance of methods

Electrofishing

At Confluence, 51 benthic fish (Appendix 5) were caught in a single electrofishing sweep (Figure 3.8a). At Footbridge, a total of 241 benthic fish (Appendix 5) were caught over three sweeps (138, 55, and 48, respectively, Figure 3.8b). Capture efficiency was estimated at 84%, resulting in a total population estimate of 287 (Standard Error (SE) 16.19), with lower and upper 95% confidence intervals of 255.3 (SE 0.36) and 318.7 (SE 0.55) specimens. At Farm, a total of 259 benthic fish (Appendix 5) were caught over four sweeps (70, 72, 75, and 42, respectively, Figure 3.8c). Capture efficiency was estimated at 45.1%, resulting in a total population estimate of 574 (SE 160.07) specimens, with lower and upper intervals of 260.3 (SE 0.04) and 887.7 (SE 0.23). All fish were released outside the isolated river stretches following recording of species and size.

Triple Drawdowns

Following the completion of electrofishing at the isolated river sections, the four subsequent, consecutive TDD sweeps at Confluence caught an additional 352 benthic fish individuals (152, 100, 54 and 46, respectively, Figure 3.8a). Capture efficiency was estimated at 83.2%, resulting in a total population estimate of 423 (SE 20.12) specimens with lower and upper intervals of 383.6 (SE 0.29) and 462.4 (SE 0.42). At Footbridge, an additional 1253 benthic fish were caught over four TDD sweeps (837, 302, 54 and 60, respectively, Figure 3.8b, Appendix 5) following the electrofishing. Capture efficiency was estimated at 98.6%, resulting in a total population estimate of 1271 (SE 5.10) individuals, and with lower and upper intervals of 1261 (SE 0.63) and 128 (SE 0.68), respectively. At Farm, 1332 benthic fish were caught over four TDD sweeps (637, 309, 256 and 130 respectively, Figure 3.8c). Capture efficiency was estimated at 86.9%, resulting in a total population estimate of 1532 (SE 29.36), with lower and upper intervals of 1475 (SE 0.37) and 1590 (SE 0.43), respectively.

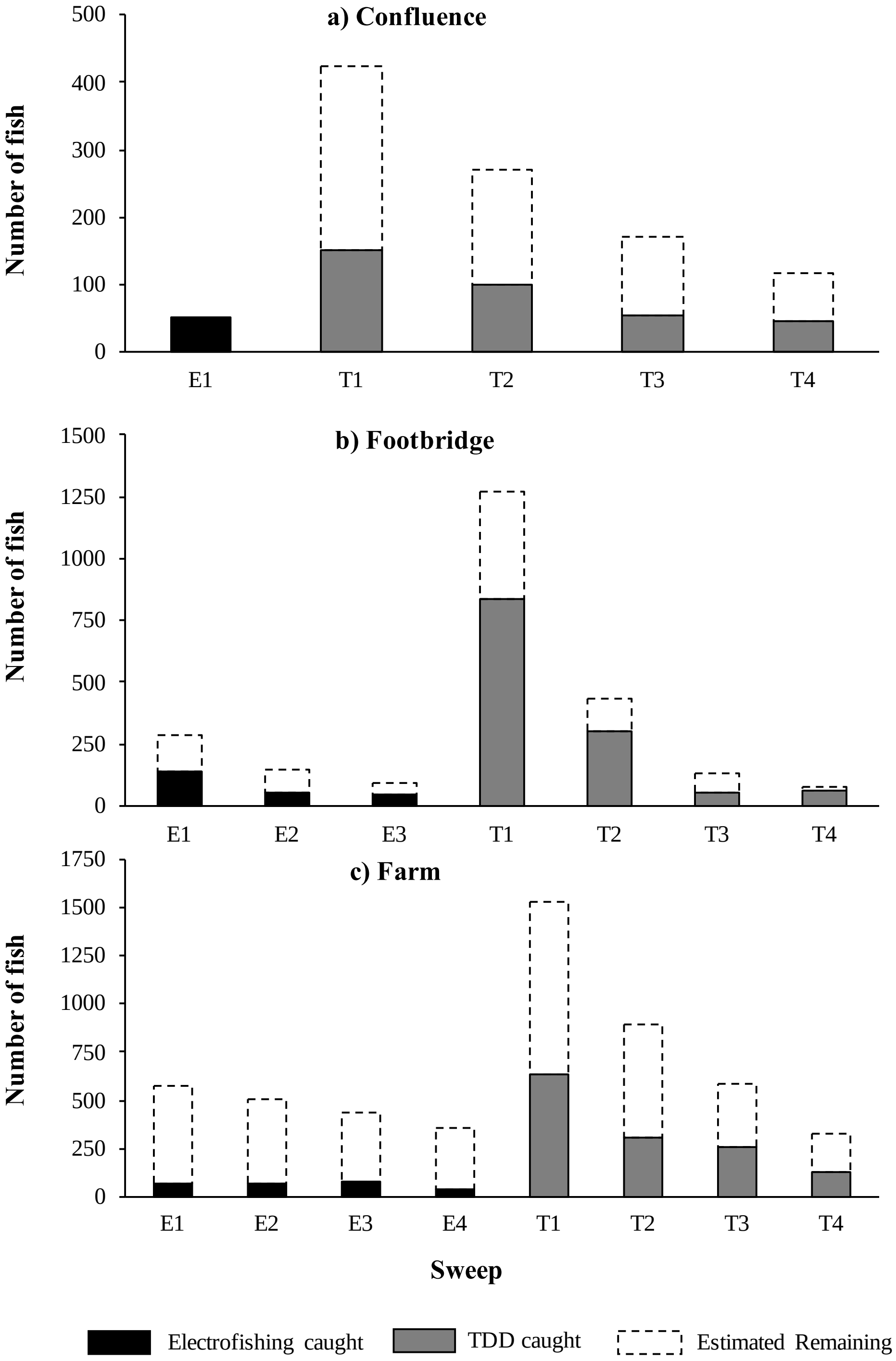


Figure 3.8 Number of benthic fish caught in each electrofishing sweep (E, black bar) and by the subsequent TDD sweeps (T, grey bar) in the same, isolated river sections. Carle Strub depletion-based total population estimates, calculated separately for each method (dashed lines), indicate how many fish were available to be caught in each sweep according to the depletion curves for the respective method.

Pritchard Trap deployment time

Pritchard Traps (PTs) successfully sampled benthic fish at all sites (Confluence, Footbridge and Farm). Benthic fish were reliably detected after the minimum deployment time of one day at all the sites (Figure 3.9). At Footbridge and Farm, PTs consistently (29/30) detected fish presence across all time intervals. At Confluence, individual detection was more variable (6/15), but fish were detected at each time interval except 7 days, where no fish were captured.

Fish numbers generally stabilised after two days, but with high fluctuations at Confluence where the overall lowest density of fish was recorded. Furthermore, while a high density was recorded at Footbridge after only one day, subsequent data showed an increase in observed numbers with time, and peak density estimates for every site were only reached at the maximum exposure time interval of 10 days.

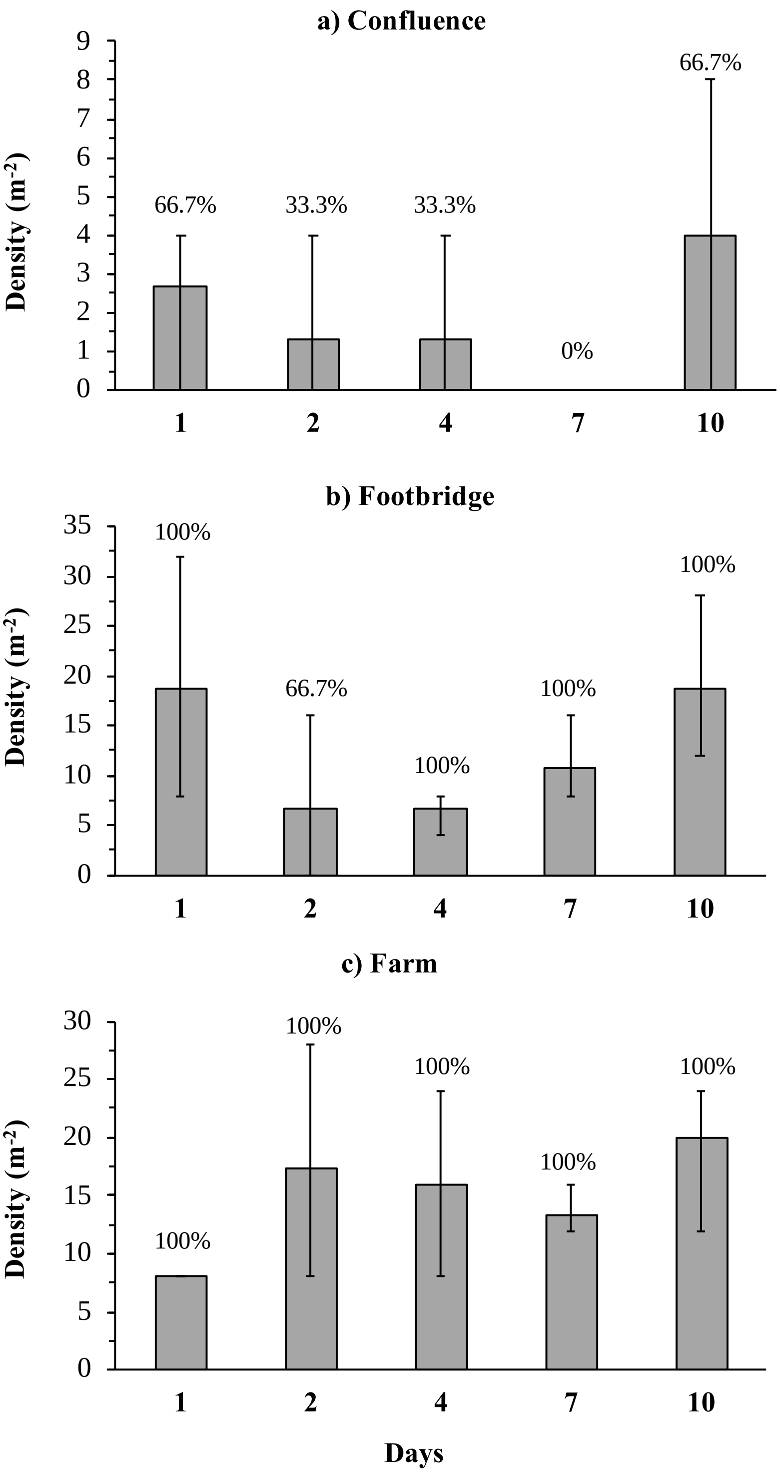


Figure 3.9 Density of benthic fish (m^{-2}) generated from Pritchard Traps (2019, n = 3, 0.75 m^2) after deployment times of 1, 2, 4, 7 and 10 days. Error bars show deviation from average of minimum and maximum catch densities. Benthic fish detection rates (as % of PTs containing any benthic fish specimens) are presented above each bar.

3.4.2.2 Comparison of electrofishing, TDD and PT-based fish surveys

Population structure and density

Three benthic fish species were sampled at the sites: bullhead, stone loach and European eel (hereafter eel). All sampling methods consistently found bullhead to be the most abundant species at all study sites. Of the electrofishing-derived population, bullhead comprised 82.4 – 94.2% of the population and stone loach 5.8 – 17.6%. Electrofishing caught one eel at Farm (0.4% of the population). Of the individuals captured during the TDDs, 81.8 – 97.5% were bullhead, 2.2 – 16.5% stone loach and 0.3 – 1.7% eel. Eel were sampled at all sites through the TDDs (4 – 6 individuals/site). In the PT-derived benthic fish population based on repeat sampling in 2019, 76.3 – 86.1% of individuals represented bullhead, with the remaining 13.9 – 23.7% representing stone loach. No eels were captured by the PTs.

All methods detected a much lower density of fish at Confluence relative to Footbridge and Farm (Table 3.8). Overall, electrofishing generated density estimates that represented only ~ 20% of the estimates generated by both TDDs and PTs at each respective site in the same year (Table 3.8). The TDD and PTs at Confluence produced very similar density estimates, as did the TDD and 2018 PT surveys at Footbridge and Farm. The 2019 intensive repeat PT sampling generated lower benthic fish densities than PT sampling in the previous year – although still considerably higher than the 2018 electrofishing derived density estimates. When compared to total density estimates, electrofishing caught 10.6 – 15.9% of all available fish at the sites, while TDDs caught 74.1 – 82.7% and 2018 PTs generated density estimates of between 69.8 – 84.3% of all available fish.

Table 3.8 Benthic fish densities recorded from electrofishing, Triple Drawdowns (TDDs) and Pritchard Traps (PTs, n = 4 in 2018 and n = 30 in 2019). Estimated totals result from adding the specimens caught by electrofishing to the estimates resulting from the TDDs on the same, isolated stretch of river.

Site	Method	Bullhead density (m^{-2})	Stone loach density (m^{-2})	Eel Density (m^{-2})	Total benthic fish density (m^{-2})
Confluence	Electrofish	0.9	0.2	0.0	1.1
	TDD	6.3	1.3	0.1	7.7
	Total estimate	8.4	1.9	0.1	10.4
	PT (2019)	6.0	1.9	0.0	7.9
Footbridge	Electrofish	5.0	0.3	0.0	5.3
	TDD	26.7	0.7	0.1	27.5
	Total estimate	32.1	1.0	0.1	33.2
	PT (n = 4, 2018)	26.0	2.0	0.0	28.0
	PT (n =30, 2019)	9.1	1.5	0.0	10.5
Farm	Electrofish	4.6	0.5	0.02	5.2
	TDD	26.0	0.6	0.1	26.6
	Total estimate	34.6	1.1	0.1	35.8
	PT (n =4, 2018)	25.0	0.0	0.0	25.0
	PT (n =30, 2019)	14.3	2.4	0.0	16.7

All methods captured bullhead across a wide range of size classes (20 – 89 mm total length). Bullhead size class distributions derived from electrofishing and TDDs were widely analogous, showing juvenile (20 - 25 mm TL) dominated populations, with further, distinct cohorts between 30 - 40 mm TL and 60 - 70 mm TL (Figure 3.10). The repeated PT sampling (2019), also detected these distinct cohorts, despite being deployed June to September and therefore sampling throughout the main growth season for the species. However, proportionally, PTs did not catch as many juvenile fish as electrofishing and TDDs, instead catching more of the larger two cohorts.

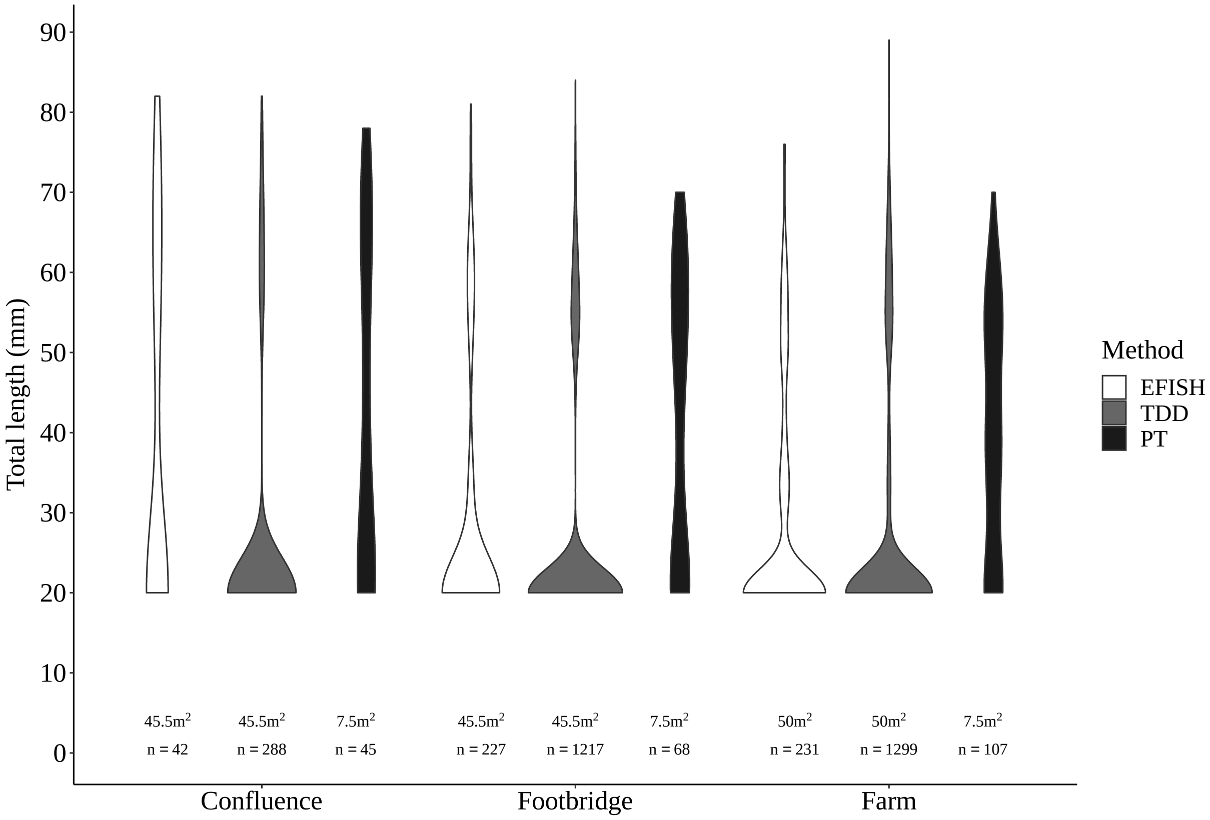


Figure 3.10 Bean plot (i.e. probability density of the catch data) of bullhead size class distribution (mm TL) captured through electrofishing (2018), Triple Drawdowns (2018) and repeated Pritchard Traps (2019) across study sites. The area sampled using each method (m^2) and the number of bullhead captured (n) are also denoted.

3.5 Discussion

3.5.1 Performance of the Pritchard traps

3.5.1.1 Crayfish

The Pritchard Trap (PT) presents a novel and accurate method for surveying freshwater crayfish in rocky headwater streams in our study area, as was demonstrated by its ability to produce quantitative survey data. PTs detected the presence of crayfish after the minimum tested deployment time of one day using a relatively small (1 m^2) sampling area (4 PTs) across a range of densities. In medium to high density populations, a small sampling area ($\leq$ 1 m^2) sufficed to accurately report population densities. However, an increased sampling intensity was required to accurately report the density of signal crayfish at Farm (0.5 individuals m^{-2}). I therefore recommend for practitioners to plan with a deployment time of four days where detailed demographic data are required (i.e. to evaluate links between crayfish demographics and environmental and ecological impacts; Bubb *et al.* 2009). Demographic estimates can be further improved with increased sampling effort, for example using more traps and repeated lifting of traps over successive 4-day periods.

Crayfish show a strong association with in-channel substrate, and habitat features that provide shelter like boulder substrates positively correlate with crayfish presence (Rosewarne *et al.* 2017). The success of PTs in sampling crayfish populations in the surveyed headwater streams is therefore likely also related to abundant in-channel rocky substrate. Under these conditions, retrieving PTs during daytime hours when crayfish are typically less active and hide under boulders and cobbles as refugia from predation (Barbaresi and Gherardi 2001) was highly successful. However, there remains a need to evaluate the effectiveness of PTs in systems that widely lack suitable in-channel crayfish refugia, and where crayfish consequently tend to live in burrows in the banks. Here, night-time retrieval of PTs when crayfish are expected to emerge to forage may prove effective (Hilber *et al.* 2020), but needs to be tested.

One big advantage of the PTs, particularly in relation to the TDD approach, is the possibility of estimating microhabitat-use within sites. In heterogeneous environments, the clearly defined sampling area of the PTs for the first time enables the direct investigation of associations between crayfish density and microhabitat usage (see Ch. 4 for crayfish association with substrate). Microhabitat associations can be explored by setting PTs at distinct specific habitats, or by varying the substrate used in the traps. To increase capture rates, PT deployment could also specifically target areas with refugia considered suitable for crayfish like rocky substrate, woody debris and shaded areas (Rosewarne *et al.* 2017). In these latter instances, density can only be considered in the context of habitat suitability and should not be extrapolated across the entire site.

The PTs consistently showed a lower density of white-clawed crayfish at Farm than TDD estimates from the previous year. This could represent a notable temporal decline in white-clawed crayfish or behavioural responses (ousted from refuges and the seeking of alternative refuges – see Bubb *et al.* 2006), both potentially linked to active signal crayfish invasion. Invasive signal crayfish are known to dominate over native crayfish species in interspecific competition for shelter, eventually contributing to the displacement of native species (Holdich *et al.* 2009). Further sampling of white-clawed crayfish and mixed-species populations will be required to specifically investigate crayfish habitat use and its implications for PT sampling efficiency of co-occurring species.

3.5.1.2 Benthic fish

Evaluation of the TDD and PT techniques clearly shows their ability to generate valuable information not only on crayfish, but also, and simultaneously, on benthic fish population density and structure in rocky headwaters. Whilst the assumed capture efficiency generated through electrofishing data ranged from 45 – 84%, and the assumptions of the depletion analyses were satisfied, these two novel, alternative techniques also confirmed that the total number of benthic fish actually available to be caught was severely underestimated by the electrofishing depletion curves. Indeed, subsequent TDDs revealed total population estimates to predict 3.2 – 5.3 times more fish than were estimated to be present based on the electrofishing surveys – with the fish actually caught in the TDD repeats already significantly outnumbering the total predicted population based on the electrofishing depletions. This shows that the reduction in fish captured during electrofishing sweeps was not chiefly driven by the reduction in fish present in the site via removal, but that the fish present became progressively less easily detectable and catchable. Fish catchability could be affected by a number of factors, for example behavioural responses to repeated electric shock, or the physical disturbance of prior sweeps causing fish to seek shelter. The TDD, however, systematically removes available refugia from the channel, leaving no place for fish to hide, resulting in a much higher catchability. While the ability to produce total population estimates through sweep depletion analyses is a valuable tool in fish stock assessments for both monitoring and managing wild and stocked fisheries (Cowx 1983; Vehanen *et al.* 2013), my results highlight methodological constraints potentially limiting the reliability of such assessments.

The PTs produced density values generally congruent with the TDDs and sampled a wide range of size classes even after a minimum deployment time of just one day. However, the observed population structure of bullhead in PTs showed an even size class distribution – which differed strongly from both other methods that indicated a strong juvenile dominance. These pronounced observed differences can be related to a number of potential causes. When compared with the ‘single point in time’ samples generated by electrofishing and TDD surveys, these differences could for example represent real changes in the population structure over the PT sampling season that included the summer months when bullhead growth rates are highest. It has been noted that, in productive systems, bullhead can attain lengths of 50 mm within their first year (Mills and Mann 1983). As such, we can therefore expect much smoother population structures due to growth effects in the PT samples. Furthermore, antagonistic interactions and competition between bullhead and invasive signal crayfish could influence PT samples. Signal crayfish have been shown to be dominant over bullhead and exclude bullhead from refugia (Bubb *et al.* 2009). Although Bubb *et al,* (2009) found no evidence of changes in the response of bullhead to different sized crayfish, it is possible that juvenile bullhead are more sensitive to crayfish presence, especially given crayfish can predate on small bullhead (Guan and Wiles 1997; Guan and Wiles 1998). Predation of smaller size classes of bullhead over the summer season could also cause a smoothing effect on the population structure.

Alternatively, differences in size structure could be due to bullhead behaviour. The PTs function by passively sampling specimens that are utilising the specific substrate within the trap area. Therefore, PT catches are likely to be strongly associated with the immediate habitat where they are set. Juvenile bullhead may have strong habitat preferences, for example preferring areas with low water depth or areas near the edge of the river, that were potentially underrepresented by the PT locations. Moreover, juvenile fish may be more sensitive to disturbance and take longer to re-colonise the recently disturbed habitats within PTs. The fact that the number of fish sampled in PTs peaked at all sites at the maximum deployment time (10 days) supports this theory, but further research using PTs within various microhabitats and over longer deployment times is required for verification. Overall, both TDDs and PTs in my view present promising new tools to survey and monitor benthic fish communities, generating much more representative data than traditional electrofishing surveys. The TDD may be better suited to reach scale assessments and broad community structure, whereas the PT can function at a microhabitat level, with both approaches providing strong insights into local population densities.

3.5.2 Practicalities of the PT method

Further to the performance, consideration of practical requirements, resources and risks associated with survey methods strongly influence their suitability. Overall, the PTs performed very well and the materials proved robust and hard-wearing through repeat sampling over several months. The traps were also easy to clean and quick-drying, aiding thorough biosecurity procedures. The small size and lightweight collapsible design of the trap (~700 g weight, 0.25 m^2 sampling area per trap) provided a good balance between sampling effort, data quality and suitability for remote fieldwork. The PT design trialled here is adaptable, with shape and size open for modifications to tailor the traps to specific site conditions e.g. using a rectangular shape for narrow streams or shorter/taller panels for different water depths. At approximately £15 per trap to self-manufacture, PTs are accessible and competitively placed within the current trap market (NHBS 2020) - and remain significantly cheaper than methods that require specialist equipment such as electrofishing (Evans *et al.* 2017) and TDDs (Chadwick *et al.* 2021).

Deployment of PTs requires the surveyor to enter the watercourse and manually lift in-channel substrate. In this regard, setting PTs requires additional time and labour in comparison to baited funnel traps (e.g. Fjälling 1995), but in my experience, this represents a comparable effort to other methods also suited to shallow rocky systems, such as handsearches or quadrat sampling (e.g. Distefano *et al.* 2003; Bradley *et al.* 2015). The applicability of PTs in other aquatic systems, such as in larger waterbodies with deeper water or less available refugia, remains to be tested, with modifications to trap design and deployment (e.g. scuba as in Engdahl *et al.* 2013) potentially required. Although PTs should be deployed for a minimum time (four days), this is still relatively short compared to other passive techniques like ARTs and enclosure traps that commonly require deployment for entire months or seasons (e.g. Engdahl *et al.* 2013; Green *et al.* 2018).
A key aspect of the PT design is that they are passive, designed to survey crayfish in their natural environment, and only entrapping crayfish upon retrieval. This avoids issues of unknown bait attractancy (Rach and Bills 1987) and bycatch, which are recognised survey concerns, especially for baited funnel trapping (De Palma-Dow *et al.* 2020). Mitigating risks to non-target organisms is a key consideration for more intrusive methods such as TDDs (Chadwick, Pritchard, *et al.* 2021) and electrofishing (e.g. burns, fractures and crayfish losing chelipeds; Alonso 2001). In this context, the PT poses minimal risk of harm to non-target organisms when operated following strict biosecurity protocols. During testing, several non-target species,

including macroinvertebrates and fish species, were recorded entirely unharmed in the PTs (Appendix 3 – Pritchard *et al.* 2021 Supporting Information).

3.5.3 Implications for conservation

The biases associated with conventional crayfish sampling techniques have hindered quantitative assessments and thus meaningful comparisons of crayfish populations. With 32% of the world’s crayfish species vulnerable to extinction and a further 21% considered data deficient (Richman *et al.* 2015), and with many other crayfish species being invasive and threatening native ecosystems (Twardochleb *et al.* 2013), the ability to accurately describe the structure of crayfish populations, including their recruitment and overall size class distributions, is becoming paramount. The PT presents a promising tool to determine crayfish demographics that is applicable in a range of scenarios in research, management and conservation.

PTs can be used for long-term monitoring campaigns, mark-recapture experiments and substrate / microhabitat preference studies to advance our understanding of crayfish behaviour and invasion ecology, ultimately benefitting any control programmes. Equally, such information on threatened crayfish species could be beneficial to enhance conservation efforts and their effectiveness. Given that PTs are passive and have limited impact on sensitive species or non-target organisms, they can be used repeatedly within protected areas with minimal wider environmental risk. Therefore, PTs are well suited for long-term monitoring programmes of native crayfish, and for evaluating translocations and reintroductions (Seddon *et al.* 2007; Rosewarne *et al.* 2017).

PTs can also be applied to fish conservation and monitoring. Bullhead are the only freshwater cottid found in the UK (Tomlinson and Perrow 2003). They are a protected species listed on Annex II of the European Commission Habitats Directive (Boon *et al.* 2005; Knaepkens *et al.,* 2005). The ability to monitor their populations is crucial to understand population trends and assess conservation status in the face of various stressors, including invasive species like the signal crayfish (see Ch. 5; Guan and Wiles 1997). Methodological constraints and poor catchability have limited effective population assessments of benthic fish, and the importance of such species within ecosystems has likely been underestimated (Harrison *et al.,* 2005). Benthic fish such as bullhead may be considered keystone species in some systems where they attain high abundances and have an intermediate trophic position (Harrison *et al.* 2005; Woodward *et al.* 2008). Bullhead have strong associations with substrate type, often preferring coarse gravel and cobble (Welton *et al.* 1983) which can vary between the seasons (Harrison *et al.* 2005). As a result, the ability to accurately record their densities within small patches of microhabitat is paramount to better understand their behaviour and ecology. While the shock of electrofishing can cause fish to rapidly dart between habitats (Harrison *et al.* 2005), the PTs now offer a passive method to explore specific habitat preferences, recruitment patterns and response to stressors.

3.5.4 Implications for management

A thorough understanding of the impacts of invasive crayfish on biodiversity and ecosystem functioning is vital to inform management decisions (Lodge *et al.* 2012; Jackson *et al.* 2014; Galib, Findlay and Lucas 2021). In this respect, the population size of the invader is likely a key determinant of the extent of impact and associated management costs (Yokomizo *et al.* 2009). For example, the hyper-dense signal crayfish populations established at BGB corresponded with severe declines in native biota (Peay *et al.* 2009; Chadwick *et al.* 2021). However, the degree to which this scenario plays out elsewhere, and hence the true extent of the ‘signal crayfish problem’, is little known in the UK. Furthermore, as the evidence of ecosystem impacts from multiple invasive crayfish species worldwide continues to grow (e.g. Lodge *et al.* 2012; Haubrock *et al.* 2021), there is an ever-growing demand for accurate data on crayfish distribution and population structure to drive effective management (Madzivanzira *et al.* 2020).

The PT method presents a new means of exploring crayfish density-dependent impacts in the field and hence of evaluating crayfish population dynamics and community-scale impacts, when coupled with other environmental surveys. This approach could be employed for spatial comparisons along invasion gradients (e.g. Ch. 4; Hudina *et al.* 2012) and used to investigate temporal impacts of invasion (see Ch. 4). Whilst such combined studies are scarce (see Mathers *et al.* 2016; Galib, Findlay and Lucas 2021), they are vital in understanding the processes by which invasive crayfish become established, dominant and impactful.

Whole population assessments are necessary to directly inform the effectiveness of invasive species control measures, with meaningful evaluations requiring before-and-after density and population structure estimates. The PT approach provides a robust foundation for such surveys, as well as for assessing and optimising invasive crayfish control techniques. Knowledge of crayfish behaviour, activity levels and seasonal trends will help identify times when management efforts can have greatest impacts (Rogowski *et al.* 2013). For example, targeting berried females could substantially reduce juvenile recruitment, which has also been suggested as a use and benefit of ARTs compared to other techniques (Green *et al.* 2018). Similarly, the ability of PTs to capture a wide range of size classes across different densities may facilitate their role in the physical management of crayfish populations.

Chapter 4. Crayfish population dynamics through space and time

4.1 Summary

1. Novel survey methods were employed to explore population demographics of signal crayfish along an invasion gradient from well-established (20+ years) to invasion front. Surveys were repeated in the following years to monitor changes over a short temporal scale (2018 – 2020).
2. Population density varied greatly along the invasion gradient (<5 km), with consistently high density signal crayfish populations (63 – 85 m^{-2}) at the well-established DGB site, decreasing to 0.5 m^{-2} at the invasion front. Signal crayfish densities increased at all sites over the three-year period, except for Confluence which remained consistent. White-clawed crayfish were recorded at Footbridge and Farm in 2018, but disappeared from Footbridge in subsequent years and severely declined at Farm.
3. Signal crayfish populations were juvenile dominated at DGB and comprised large numbers of juveniles and sub-adults at the Confluence and Footbridge. The majority of the crayfish biomass was made up of sub-adult and adult size classes. Populations structures were relatively stable over the years. At Farm, such low densities made it difficult to examine population dynamics, however it is clear that the population is surviving and recruiting.
4. The substrate used in PTs comprised of the material present on site, within the footprint of the trap. Substrate composition within all PTs was largely cobble based, with some pebble and granule material and the occasional boulder. No obvious association between substrate composition and crayfish size structure was found.
5. This study allows one of the first detailed investigations of signal crayfish invasion biology in a rocky headwater. Population density can vary massively even along a very small spatial scale (<5 kms). Population structure varied by abundance and biomass, emphasising the importance of accurately recording and considering both when assessing and predicting ecological impact and forming targeted management plans.

4.2 Introduction

4.2.1 Population density

A long-standing theory in invasion science proposes that the total impact of an invader is determined by three fundamental elements, range, abundance and the specific per-capita or per-biomass effect of the invader on the ecosystem (Parker *et al.* 1999). The importance of theoretical and experimental investigations of the relationship between an invasive species per-capita effect and its abundance in order to predict spatiotemporal variation in impact is still recognised as a major priority area of invasion science (Ricciardi *et al.* 2021).

However, as keystone species that also display an ontogenetic shift in feeding behaviour, crayfish present a particularly complex taxon to investigate per-capita effects. This is further compounded by methodological survey limitations preventing quantitative population assessments. The lack of quantitative data on crayfish populations in the wild mean we have a limited understanding of the abundance/density a species can attain in its native or invaded range. It is assumed that invasive crayfish can attain higher densities than analogous native species due to higher fecundity, rapid growth and generalist diets (Holdich *et al.* 2014). Any reports of crayfish densities are scarce, varied and often limited to older literature. Published density estimates for UK-native white-clawed crayfish range from 0.4 to 26 white-clawed crayfish m^{-2} in France (Daguerre de Hureaux and Roqueplo 1981; Reynolds and Demers 2006), while a density of 1.67 m^{-2} was reported from Spain (Rallo and Garcia-Arberas 2000), a density of 2.6 m^{-2} from England (Hogger and Lowery 1982) and densities of 3.3 - 5.1 m^{-2} in Ireland (Demers, Reynolds and Cioni 2003). Density estimates for signal crayfish were until recently comparable in their estimates, with 0.9 – 1.1 signal crayfish m^{-2} reported from its introduced range in the US (Abrahamsson and Goldman 1970; Flint 1975), 4.3 – 7.3 m^{-2} from France (Laurent and Vey 1986), 0.8 – 20 m^{-2} from England (Guan *et al.* 1996; Bubb, Thom and Lucas 2004), and 0

– 16 m^{-2} in their native range (Wooster, Snyder and Madsen 2012). However, investigations directly forming the foundation of this PhD thesis, published in Chadwick *et al,* (2021)*,* revealed signal crayfish densities ranging between 20 to 110 crayfish m^{-2} along a short section (~5 km) of Bookill Gill Beck, North Yorkshire, using the novel TDD method (see Ch. 2). This has highlighted that more evidence and data is urgently needed to fully understand and compare the range of densities that both native and invasive species can attain. Further, this data enables robust study of the environmental factors that may regulate these population densities, for example in relation to available refugia, food or predator’s presence.

4.2.2 Population structure

Population demographics such as size structure are crucial factors to consider when assessing ecological impacts of an invader. Both signal crayfish and white-clawed crayfish display ontogenetic shifts in feeding preferences, with a diet dominated by macroinvertebrates observed in juveniles and plant-based materials in adults (in Reynolds 1979 and Lewis 2002, respectively). Therefore, the relative proportions of each life stage within a population will strongly determine the level and type of ecological impact exhibited. Differences in population structure between native and invasive species may further explain why invaders cause different effects on the ecosystem compared to native species.

Population size structure is also important when combining per-capita/per-biomass impact with total population abundance to understand total impact. One facet of invasion science research has been to investigate functional responses (per-capita impact, e.g. feeding rate) of invasive species and combine these with abundance estimates to calculate ‘Impact Potential’ (Dick *et al.* 2017). This work has been developed further to include the metric ‘Relative Impact Potential’ (RIP) to compare impact potential of invasive species with analogous native species (Dickey *et al.* 2020). Functional response experiments have been undertaken in the laboratory to compare signal crayfish and white-clawed crayfish (Taylor and Dunn 2018). However, there is little current work that explores crayfish functional responses across various size classes to account for the aforementioned ontogenetic shift in feeding behaviour, though this has been identified as an important factor in other taxa (Hohberg and Traunspurger 2005; Ranjan, Bagchi and Kellogg 2016). There is therefore an urgent need for *in situ* data on crayfish population density and structure to contextualise this information.

Population density and size structure are likely to vary across different types of aquatic system. The size classes of crayfish captured in passive trapping methods (similar to the PT in concept) have been shown to be strongly influenced by substrate type (Engdahl *et al.* 2013). Crayfish show a strong association with substrate type, and habitat features that provide shelter, such as boulders, woody debris and exposed roots are positively correlated with crayfish presence (Rosewarne, Mortimer and Dunn 2017). Furthermore, crayfish individuals differing by sex and size classes have been shown to utilise habitat differently (Demers, Reynolds and Cioni 2003; Rosewarne, Mortimer and Dunn 2017). Presence of substrates that provide suitable crayfish refugia will be a strong limiting factor on total population density - and this density will therefore differ vastly between different habitats and freshwater systems (Lodge and Hill 1994; Holdich 2003).

4.2.3 Invasion front and dispersal

Anthropogenic introductions and subsequent ‘natural’ dispersal of signal crayfish have resulted in a fast rate of overall and localised range expansion, with populations now widespread across Europe (Kouba, Petrusek and Kozák 2014). Once introduced into a watercourse, crayfish will spread in both upstream and downstream directions. However, the mechanisms by which they spread are not well understood. The rate of dispersal is highly variable, though dispersal downstream generally seems to be faster than upstream colonisation (Bubb, Thom and Lucas 2004; Peay *et al.* 2009; Hudina *et al.* 2009). Estimates of rate of expansion range from 0.1 - 0.5 km per annum (Peay *et al.* 2009) and 0.4 – 1.8 km per annum (Bubb, Thom and Lucas 2004) observed for English upland rivers, to much faster expansion rates of 18 – 24.4 km per annum in the Mura River, Croatia (Hudina *et al.* 2009). There has been much debate on the way in which signal crayfish expand their range, whether particular size classes or sexes drive the invasion front or whether passive dispersal during high flows facilitates spread. Downstream drift contributes to the dispersal of many macroinvertebrate species (Bilton, Freeland and Okamura 2001). However, Bubb et al. (2004) suggest that even at periods of high discharge, adult crayfish are able to remain protected by persisting in burrows and refugia and as such, passive dispersal of adult crayfish is not believed to form a major component in their dispersal. Yet, very little is understood on how juvenile and sub-adult size classes of crayfish contribute to the dispersal of the species, particularly through downstream drift.

Knowledge of the invasion biology and mechanisms of natural range expansion is crucial for understanding crayfish behaviour, spread, impact and, ultimately, for the formulation of effective management interventions. However, there is relatively little information on signal crayfish population characteristics and structure along its invasion pathway (Hudina *et al.* 2012). Traditional sampling methods have presented a major limitation in this respect, as they cannot provide quantitative data across the full range of size classes, and low-density populations at the leading edge of invasion may fall below the minimum detection rate of many methods. A knowledge of the introduction event is also extremely valuable, to know the location, size and approximate date of introduction in order to monitor time to establishment, dispersal rates and population changes during expansion and establishment. The population demographics of signal crayfish are likely to differ along their invasion gradient, due to selection of specific dispersal phenotypes from a source population (Pintor, Sih and Kerby 2009; Cote *et al.* 2010) and the trade-offs that individuals will experience during population establishment and growth in new habitats and conditions as it spreads (Burton, Phillips and Travis 2010). In addition to differences along a spatial gradient, population demographics will change over time as a species becomes established and population size and resource availability change (Bøhn *et al.* 2004).

Established populations may be expected to attain higher densities than those nearer the invasion front, as found by Hudina et al (2012) in the Mura River, a tributary of the large European Drava River. However, the ‘boom-bust’ concept used widely in invasion biology may contradict this, as invaders are generally observed to go through an initial outbreak (boom) phase where the population becomes very large very quickly, that is then followed by a sharp decline (bust) to a much lower population size that can then remain relatively stable (Figure 4.1; Strayer *et al.* 2017). In a solitary boom-bust scenario, the population density of the invader may be fairly stable along the invasion gradient, except for the spike representing the ‘boom’ phase lagging somewhat behind the actual invasion front. Alternatively, in a recurring boom-bust dynamic, the population size of the invader will undergo repeated episodes of boom-bust, which may be cyclic or irregular. According to the specific case, population size may vary drastically along the invasion front. The variation in population size along an invasion pathway in turn will have direct consequences for the native ecosystem. Solitary boom-bust scenarios imply the impact of the invader during the initial boom is temporary and potentially reversible, whereas recurring boom-bust cycles may result in more prominent impact.

The identification of population dynamics along a spatio-temporal invasion gradient (distance and time since introduction) are therefore of significance for the understanding of the underlying drivers of dispersal, the extent and permanence of ecological impacts, and for designing targeted management approaches.

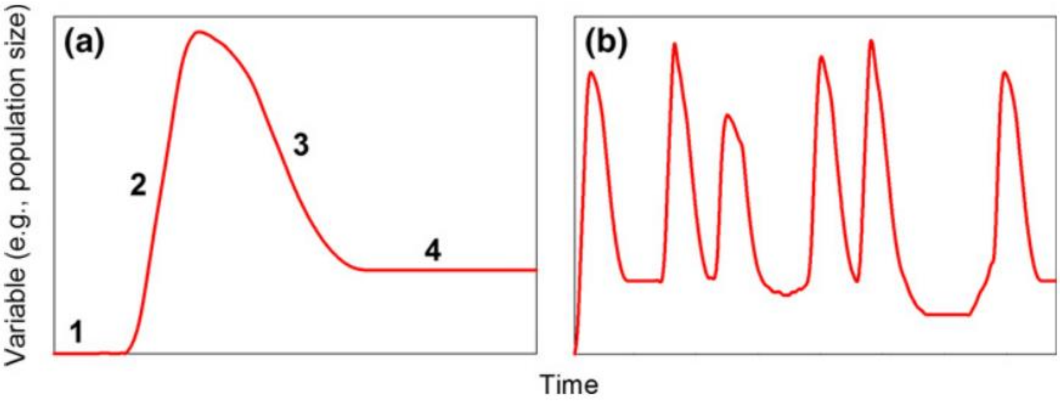


Figure 4.1 Essential characteristics of boom-bust dynamics; a) solitary and b) recurring. The four phases of the solitary boom-bust are 1) pre-invasion and lag phase (pre-boom), 2) boom, 3) bust, 4) post-bust. These phases can be repeated in the recurring scenario. Adapted from Strayer *et al* (2017).

4.2.4 Rationale

As outlined above (Ch. 3), biases associated with contemporary sampling techniques have limited the quantitative assessments of invasive and native crayfish populations. To date, density and structure of signal crayfish populations along an invasion gradient have not been described, nor has their population density and structure been compared to that of the “natural state” of established white-clawed crayfish populations. The development of Triple Drawdowns (Ch. 2) and Pritchard Traps (Ch. 3) now enable us to describe crayfish populations in rocky headwaters *in situ* and to explore changes in population demographics along invasion gradients from well-established populations to the invasion front, as well as inter-annual fluctuations in these populations. This will provide first time insight into the invasion biology of signal crayfish in the UK.

4.2.5 Research aims and hypotheses

The aim of this chapter was to investigate how signal crayfish populations differed at sites along an invasion gradient and over time. Several hypotheses were formulated to address this;

HI. The density and population demographics of signal crayfish will predictably change along the invasion gradient, with a high-density ‘boom’ lagging behind a low-density invasion front. The densities at the well-established sites may also fluctuate with recurring boom-bust cycles since high densities have been recently observed at DGB on Bookill Gill Beck (Chadwick, Pritchard, *et al.* 2021).

HII. When crayfish plague is lacking, as in the study populations, the density of white-clawed crayfish will be negatively associated with signal crayfish density, with the latter out-competing the former, and densities of white-clawed crayfish accordingly decreasing over time.

HIII. The population demographics, including size class distribution, sex ratios and incidence of cheliped damage will vary along the invasion gradient, with a dominant sex and size class found at the invasion front. The demographics at more recently invaded sites will fluctuate substantially over time, as the population becomes established. In contrast, sex ratio and size structure will be more stable at established sites and incidence of cheliped damage will be higher at high density sites where intra-specific competition is highest.

HIV. Crayfish population size structure will be strongly related to site-specific substrate composition. Larger crayfish will be found in PTs that contain larger substrate (coarse cobbles and boulders), whereas juvenile crayfish will be associated with finer substrate such as granules and pebbles.

4.3 Methods

4.3.1 Experimental design

This study involved surveying and monitoring crayfish populations along Bookill Gill Beck (BGB) and Long Preston Beck (LPB) over consecutive years (2018/19/20), with the aim of identifying differences in crayfish demographics along the invasion gradient and over time. Triple drawdowns (TDDs) in 2018 (Table 4.1) allowed for thorough baseline data to be collected and provided the first reliable density estimates at the study sites. Pritchard Traps (PTs) were used at a low sampling effort (n =4) at the LPB sites in 2018 as part of the proof-of-concept work (see Ch. 3). Unfortunately, the PTs at DGB in 2018 had to be discounted from the study due to drought conditions drying up trap locations. PTs were used at a higher sampling effort in 2019 (n = 30, with sets of 3 PTs deployed and lifted on 10 occasions) and in 2020 (n = 21-24, with sets of 7 – 10 PTs being deployed and lifted on three separate occasions over the summer months). In 2020, sets of 8 PTs were deployed at each site. However, interference or removal of PTs resulted in varying n values (23 at Confluence, 21 at Footbridge and 22 at Farm).

Table 4.1 Summary table of field studies, including crayfish population demographic comparisons at sites along Bookill Beck (BGB) and Long Preston Beck (LPB) in 2018, 2019 and 2020. Crayfish associations with substrate were also explored in 2019.

Experiment	Method	Year	Months	Site(s)	Sample size (m^2)
Crayfish demographics 2018	TDD	2018	August	Double Gate Bridge, BGB	n = 1 (15 m^2)
	TDD	2018	July	Confluence, LPB	n = 1 (45.5 m^2)
	TDD	2018	July	Footbridge, LPB	n = 1 (45.5 m^2)
	TDD	2018	July	Farm, LPB	n = 1 (50 m^2)
	PT	2018	July - August	All LPB	n = 4 (1 m^2)
Crayfish demographics 2019	PT	2019	June - September	All	n = 30 (7.5 m^2)
Crayfish demographics 2020	PT	2020	July – September	Double Gate Bridge	n = 24 (6 m^2)
				Confluence, LPB	n = 23 (5.75 m^2)
				Footbridge, LPB	n = 21 (5.25 m^2)
				Farm, LPB	n = 22 (5.5 m^2)
Crayfish – substrate association	PT & substrate analysis	2019	June – September	All	n = 1 - 3 (0.25 – 0.75 m^2)

4.3.2 Population density and structure

TDDs were carried out as described in Chapter 3 (Novel Methods), with multiple sweeps allowing for Carle Strub depletion analyses and total populations estimates. PTs were used as described in Chapter 3 (Novel methods), using only natural substrate from the site and deployed for a minimum of four days. All crayfish surveyed were first identified to species level. White-clawed crayfish were processed on site and released back to the site immediately afterwards. Signal crayfish sampled during TDDs were stored on ice and processed in the laboratory. Signal crayfish sampled in PTs in 2019 and 2020 were processed on site and returned to the site. All work was authorised by the Environment Agency (CR1 licence and method statement approved). All equipment was disinfected following use.
For all crayfish surveyed, species, carapace length (CL mm), sex (male, female or juvenile for individuals ≤ 12 mm CL), weight and cheliped damage (absent/regenerating) were recorded. These parameters allowed crayfish population density, size structure, sex ratios, biomass and incidence of cheliped damage to be explored along the invasion gradient and over time.

Depletion analyses for the multiple pass TDDs were undertaken following the Carle Strub method in the Fisheries Stock Assessment (FSA) package in R. Density values from the TDDs were calculated as the number of crayfish divided by the site area. Density values were calculated from PT survey data as the number of crayfish divided by the trapped survey area (0.25 m^2 per PT), with lower and upper estimates as the minimum and maximum number of crayfish captured in one PT. Population size structure was explored through bean plots, reflecting probability density of the catch data of size class distribution, using ggplot2 (Wickham 2016) in R.

Biomass was calculated for crayfish surveys at each site. Length weight regressions were generated for signal crayfish ($R^2 = 0.98$, n = 3572, Appendix 6) and white-clawed crayfish ($R^2 = 0.91$, n = 477, Appendix 6) using raw length and weight data from TDD and PT sampling combined. Due to weighing scale malfunctions in the field, some weight values were missing from crayfish datasets across the three field seasons (signal crayfish n = 52 and white-clawed crayfish n = 11). Therefore, length-weight regressions were calculated for existing datasets and the line of best fit was used to interpolate weight estimates based on their respective carapace lengths. Total crayfish biomass (g/m^2) was calculated using the weight of crayfish divided by sampling area. Male to female sex ratios were calculated using all animals over 12 mm CL. Incidence of cheliped damage was calculated as the number of crayfish displaying damage (at least one cheliped absent or regenerating) divided by the total number of crayfish sampled. It is important to note cheliped damage was only recorded for subadult and adult crayfish (excluding < 12 mm CL).

4.3.3 Substrate association

The substrate contained in the PTs during the 2019 sampling period was collected upon survey completion (n = 3 at each of DGB, Confluence and Farm, n = 1 at Footbridge due to trap interference). Substrate was emptied from PTs directly into heavy-duty rubble sacks, labelled and transported back to the laboratory at PBA Applied Ecology for analysis. One rubble sack was placed in a large bucket and contents were emptied onto white trays for processing (photos in Appendix 7). The Udden Wentworth Scale was used to define substrate categories as; granules, pebbles, cobbles and boulders with sizes of fine, medium, coarse and very coarse (Udden Wentworth Scale Appendix 7). A 30 cm rigid ruler was used to record the a (longest) and b (intermediate) axis to the nearest mm for all stones with a b axis ≥ 32 mm (ie very coarse pebbles, cobbles or boulders). The volume of the stone was recorded using the water displacement technique (ie volume of stone = water level with submerged stone – water level without stone). The level of precision for stone volume measurements was dependent on the size of the stone and required container size. Large stones were measured to the nearest 250 ml (container capacity 13 litres), medium stones were measured to the nearest 50 ml (container capacity 2 litres) and small stones were measured to the nearest 25 ml (container capacity 0.57 litres). For stones under 25 ml volume, a number of stones were measured together and the total volume was divided by the number of stones provided to generate an average volume per stone.

All ‘coarse pebbles’ (16 – 32 mm b axis) were placed into a white tray (3 litre volume) and a 15 cm ruler was used to confirm a 16 – 32 mm b axis length. All pebbles were counted and the total volume was measured which also provided an average volume per pebble estimate. All granules/pebbles with a b axis < 16 mm were sorted using a stack of two Eisco sorting sieves (4000 micron / 4 mm mesh size and 2000 micron / 2 mm mesh size). Substrate collected in the 4 mm sieve was then sorted into two categories, medium pebbles (8 -16 mm b axis) and fine pebbles (4 – 8 mm b axis) using a 15 cm rigid ruler. The medium pebbles were all counted and a total volume measurement was recorded to provide an average volume per pebble. A subsample of the fine pebbles was counted (either 1/8th or 1/16th of a white tray) and the coverage of a white tray was recorded to the nearest 1/16th of a tray. The total volume of fine pebbles was recorded. Substrate retained in the 2 mm sieve was defined as granules (2 – 4 mm b axis) and a total volume of all granules was recorded along with the white tray coverage. Substrate (silt/sand) with a b axis less than 2 mm was not recorded in this study as this would be too fine to be retained in PTs so the amount collected will not be representative of true field conditions. Once cobbles/pebbles/granules were processed they were placed into a separate bucket, then decanted back into rubble sacks for storage.

The substrate composition was explored by calculating the total volume (cm^3) of each grain size in the Udden Wentworth Scale within each PT ($n = 3$ at all sites, except Footbridge $n = 1$). These were grouped into a coarser scale (Granules/Pebbles/Cobbles/Boulders) for more general comparisons of relative proportions (% substrate) within PTs. Size class distributions of crayfish captured within each PT were generated through bean plots generated in ggplot2 in R.

4.4. Results

4.4.1 Population density

There was a large difference in crayfish density at sites along the invasion ‘timeline’ reflected by the river sampling sites from the release point to the current invasion front (Table 4.2). The density was highest at DGB (63 – 85 m^{-2}), closest to where signal crayfish were released and have hence first become established over 20 years ago. Crayfish density sharply decreased at sites downstream along LPB, to the invasion front at Farm, where only 0.5 signal crayfish m^{-2} were found in 2018. Signal crayfish density generally increased at each site over consecutive years (Table 4.2), with the exception of Confluence in 2020 which declined slightly, but remained consistent with previous years.

White-clawed crayfish were only found at two sites, Footbridge and Farm. Very few individuals were sampled at Footbridge during the TDD in 2018 (0.15 m^{-2}), and no white-clawed crayfish were found at Footbridge in the following two years. A large population of white-clawed crayfish were found at Farm in 2018 (9.1 m^{-2}). However, the density of white-clawed crayfish declined sharply in subsequent years.

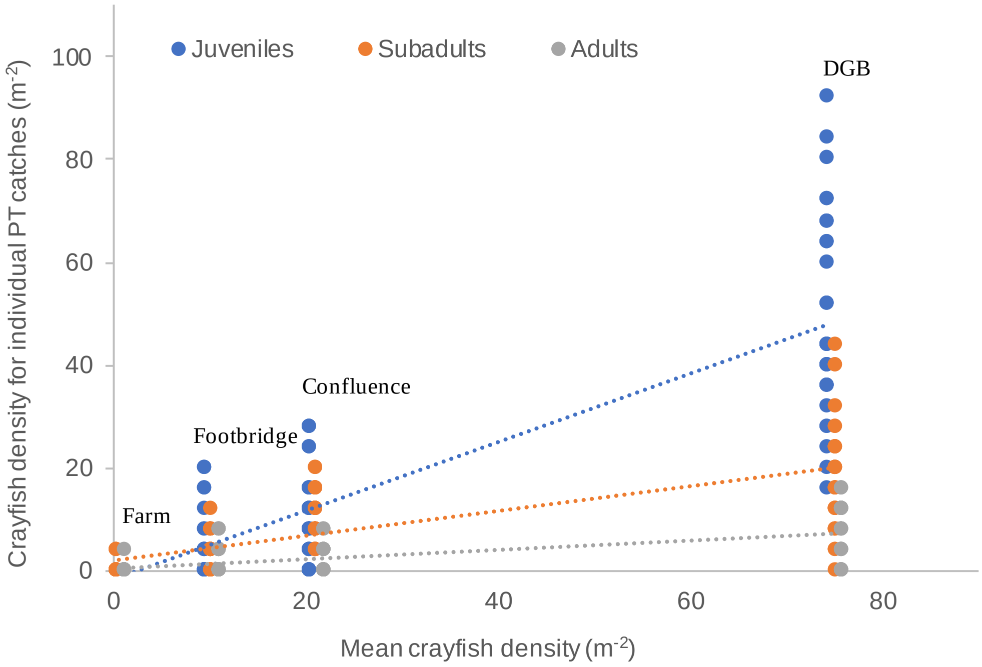
Repeat PT sampling in 2019 and 2020 allowed thorough investigations of crayfish density patterns and their variance between PT repeats. The density of crayfish did show distinct variations between PT repeats in 2019 (30 PT lifts; Figure 4.2a) and in 2020 (24 PT lifts; Figure 4.2b) across the sites. The variance of crayfish density for all size categories (juveniles, sub-adults and adults) increased as average crayfish density increased. Comparatively, juvenile density showed the highest variance at each site, whereas adult densities were more stable.

Lifting of three PTs for 10 times across the summer months in 2019 revealed distinct seasonal changes in crayfish densities with particularly large changes in the number of juveniles (Figure 4.3). Numbers of sub-adults generally increased over the summer, likely recruiting numbers from the juvenile size class as these grow rapidly during the first year (Holdich *et al.* 2014). Whilst adult densities did vary between PT lifts over the summer, they only comprised a small fraction of the total population.

Table 4.2. Density (m^{-2}) of signal crayfish and white-clawed crayfish along the invasion gradient in 2018, 2019 and 2020, with standard deviation denoted below.

Site	Crayfish Species	Crayfish density (m^{-2})		
		2018 (TDD)	2019 (PT)	2020 (PT)
Double Gate Bridge	Signal crayfish	63.3 ± 0.45	75.2 ± 20.02	84.8 ± 15.78
Confluence	Signal crayfish	19.9 ± 0.75	21.2 ± 11.70	19.7 ± 14.61
Footbridge	Signal crayfish	7.1 ± 0.20	10.3 ± 6.62	15.8 ± 9.65
Farm	Signal crayfish	0.5 ± 0.06	0.5 ± 1.38	3.1 ± 3.00
Footbridge	White-clawed crayfish	0.15 ± 0.00	0.0 -	0.0 -
Farm	White-clawed crayfish	9.1 ± 0.10	2.1 ± 3.44	2.2 ± 3.20

a) Signal crayfish, 2019



b) Signal crayfish, 2020

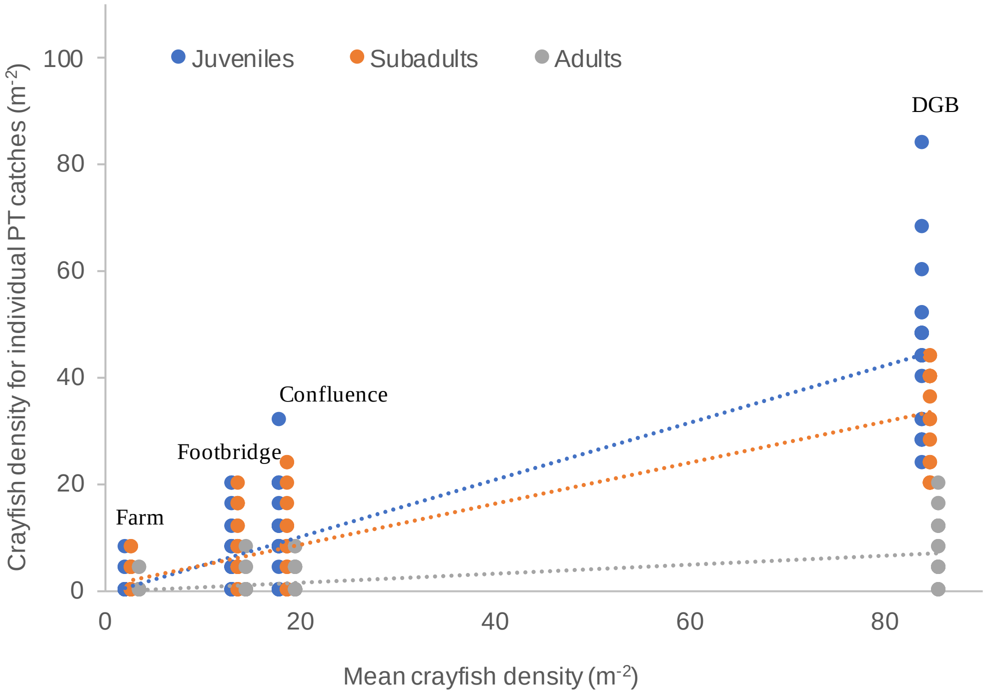


Figure 4.2 Densities of signal crayfish split by size classes; juveniles (≤ 12 mm CL), sub-adults (13 – 25 mm CL) and adults (≥ 26 mm CL), from all samples in a) 2019 and b) 2020. Clusters of data points represent study sites with Farm, Footbridge, Confluence and DGB with increasing mean densities and variance of crayfish densities between PT repeats shown on the y axis.

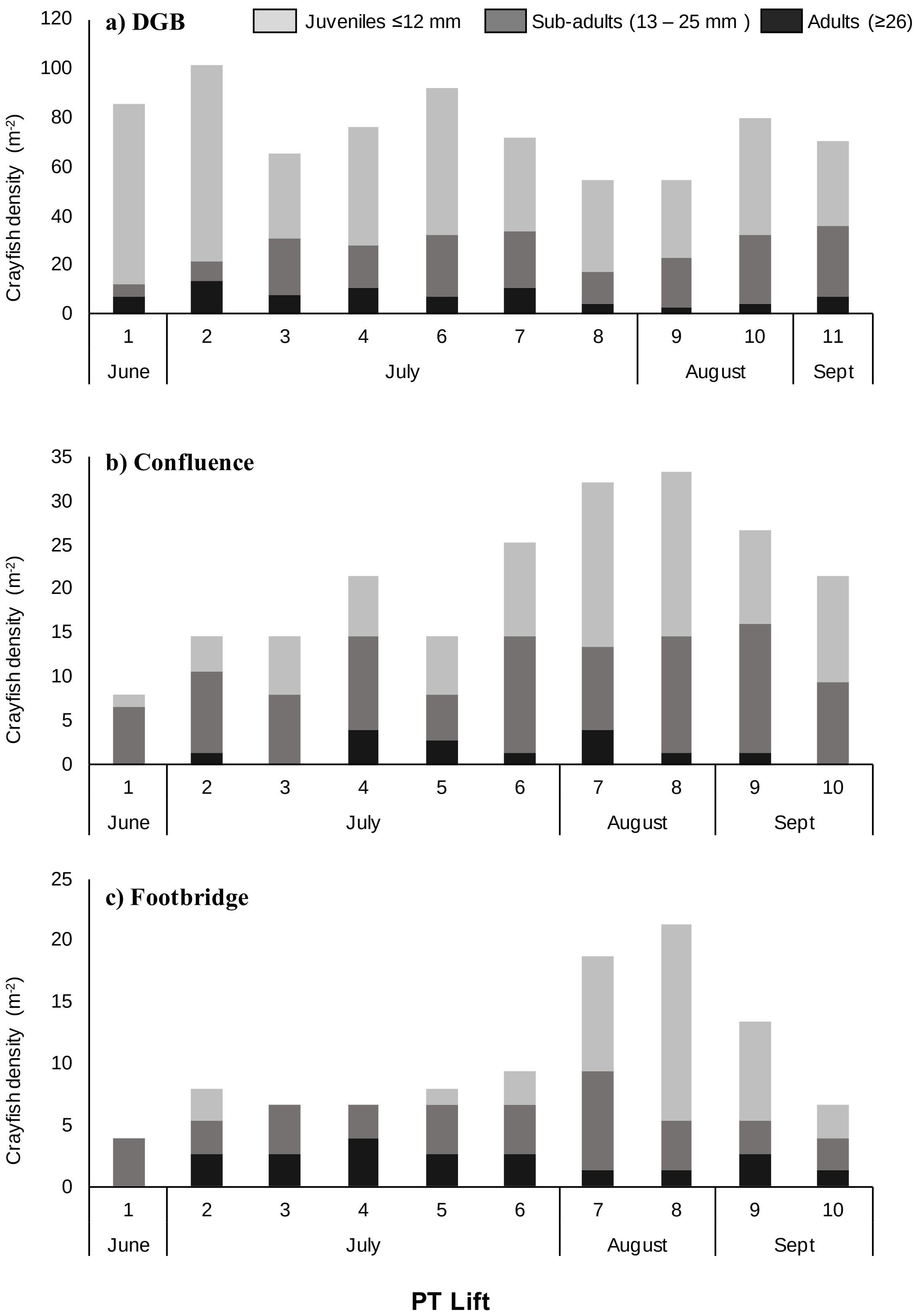


Figure 4.3 Crayfish densities by size class category; juveniles (≤ 12 mm CL), sub-adults (13 – 25 mm CL) and adults (≥ 26 mm CL) from PT lifts (n = 10) over summer months in 2019 at a) DGB, b) Confluence and c) Footbridge.

4.4.2 Population structure

Population demographics for crayfish at all sites were recorded in 2018, 2019 and 2020 (Table 4.3). Young-of-year crayfish (8 mm CL) were caught in PTs from all sites, across all years, except for signal crayfish at Farm in 2019. The largest signal crayfish captured in the system during sampling over the three years ranged between 27 and 52 mm carapace length, whereas the largest white-clawed crayfish sampled ranged between 32 and 39 mm. Median size class ranged between 8 and 24 for signal crayfish and 8 and 18.5 for white-clawed crayfish. The incidence of cheliped damage for crayfish > 12 mm CL ranged between 0 – 35% in signal crayfish and 12 – 20% in white-clawed crayfish. The ratio of male to females was either fairly evenly split, or female biased, with the exceptions of signal crayfish at Confluence in 2020 and white-clawed crayfish at Farm in 2020 that showed a male-dominant ratio. Crayfish biomass (g/m^2) followed a similar trend to the crayfish density, with signal crayfish biomass decreasing along the invasion gradient (highest at DGB and lowest at Farm) and increasing at each site in subsequent years (except Confluence which stayed consistent between 2019 and 2020).

Table 4.3 Summary data for crayfish populations in 2018, 2019 and 2020 at all sites, including size range of carapace lengths (CL), biomass, sex ratios and percentage of cheliped damage in the population.

Site	Year	Minimum CL (mm)	Maximum CL (mm)	Mean CL (mm)	Median CL (mm)	Biomass (g/m^2)	M:F ratio	Cheliped damage of >12 mm CL (%)
DGB	2018	8	44	14	12	89.1	45.3 : 54.7	30.4
	2019	8	42	13	8	108.2	45.7 : 54.3	29.1
	2020	8	42	14	12	119.3	44.9 : 55.1	17.3
Confluence	2018	8	50	17	17	48.9	42.6 : 57.4	30.8
	2019	8	37	15	15	36.9	37.9 : 62.1	26.4
	2020	8	37	16	13	36.6	71.8 : 28.2	29.3
Footbridge	2018	8	51	18	19	24.4	48.1 : 51.9	34.8
	2019	8	42	18	19	28.0	28.9 : 71.1	20.0
	2020	8	34	16	17	28.6	43.8 : 56.3	27.1
Farm SC	2018	8	52	21	12	3.6	50 : 50	40.0
	2019	23	29	25	24	2.3	50 : 50	0.0
	2020	8	27	20	22	8.7	30.8 : 69.2	23.1
Farm WCC	2018	8	39	17	15	24.5	46.5 : 53.5	12.4

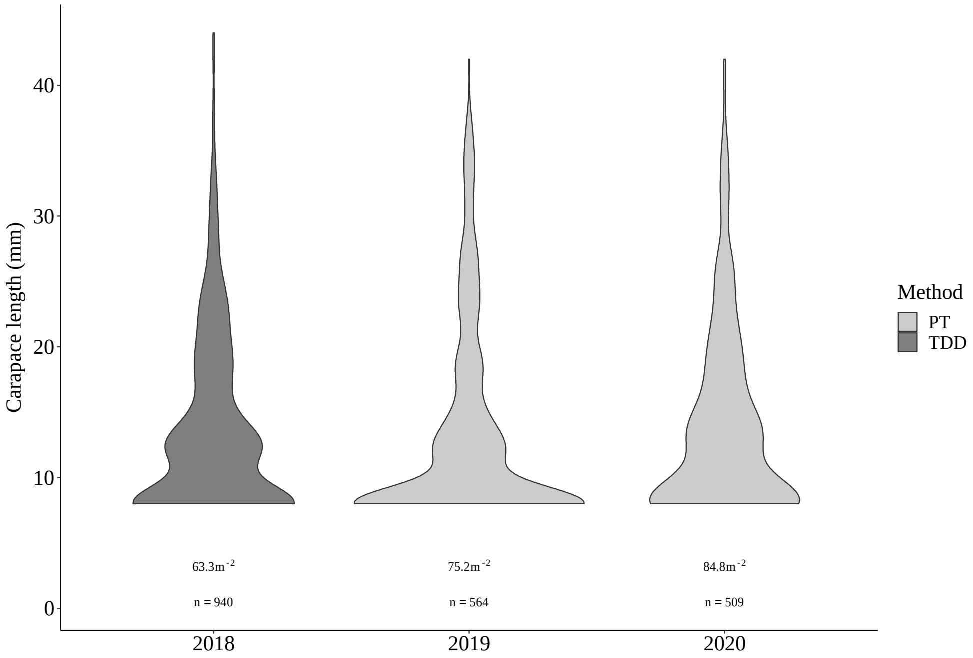
	2019	8	32	20	18.5	7.1	50 : 50	16.6
	2020	8	35	15	8	5.7	60 : 40	20.0

The crayfish population size structures did vary between site. At DGB the population size structure by abundance was relatively stable across the three-year period, with a distinct juvenile-dominated size class distribution (52 – 65% ≤ 12 mm CL) and very few large individuals (1 – 2% ≥ 35 mm CL; Figure 4.4a). The biomass of crayfish of different sizes resulted in a very different distribution, with the bulk of the weight being provided by individuals falling between 15 and 35 mm CL (Figure 4.4b). In 2018, the bulk of the biomass was made up of juveniles (17% ≤12 mm CL) and sub-adults (45% 13 – 25 mm CL), whereas in 2019, the bulk was made up of sub-adults (32% 13 – 25 mm CL) and small adults (38% 26 – 34 mm CL) and again in 2020, with the bulk comprised of sub-adults (45% 13 – 25 mm CL) and small adults (32% 26 – 34 mm CL).

At Confluence, the population structure was relatively stable over the three years, with fewer large individuals (1 – 2% ≥ 35 mm CL) captured in 2019 and 2020, compared with 2018 (6%; Figure 4.5a). Juveniles (41 – 49 %, ≤ 12 mm CL) and sub-adults (41 – 47%, 13 – 25 mm CL) were the dominant size classes by abundance at Confluence. The distribution of size classes by biomass did reflect the large number of sub-adults (28 – 62% 13 – 25 mm CL), but not the juveniles (2 – 5% ≤12 mm CL; Figure 4.5b). In 2018, the biomass was more evenly split amongst sub-adults and adults (28% 13 – 25 mm CL, 34% 26- 34 mm CL and 36% ≥35 mm CL). Whereas in 2019 and 2020 the majority of the biomass is comprised of sub-adults (62 – 51%, 13 – 25 mm CL) and small adults (23 – 32% 26 - 34 mm CL).

At Footbridge, the population structure by abundance was relatively similar between years. However, progressively fewer large animals (≥ 35 mm CL) were caught over the three years (9%, 1%, 0% in 2018, 2019 and 2020 respectively. There were two dominant size cohorts, juveniles (40 – 42%, ≤ 12 mm CL) and sub-adults (38 – 52%, 13 – 25 mm CL), when looking at abundances (Figure 4.6a). The biomass distribution across size classes did not reflect the large numbers of juveniles (1 – 4 % ≤12 mm CL). In 2018, there were similar amounts of sub-adults (28% 13 – 25 mm CL) and small adults (23% 26 – 34 mm CL) and a large biomass of large adults (48% ≥35 mm CL). In 2019, there was a greater biomass of small adults (48% 26 – 34 mm CL), whereas in 2020 the majority of the biomass was within the sub-adult category (69% 13 – 25 mm CL; Figure 4.6b).

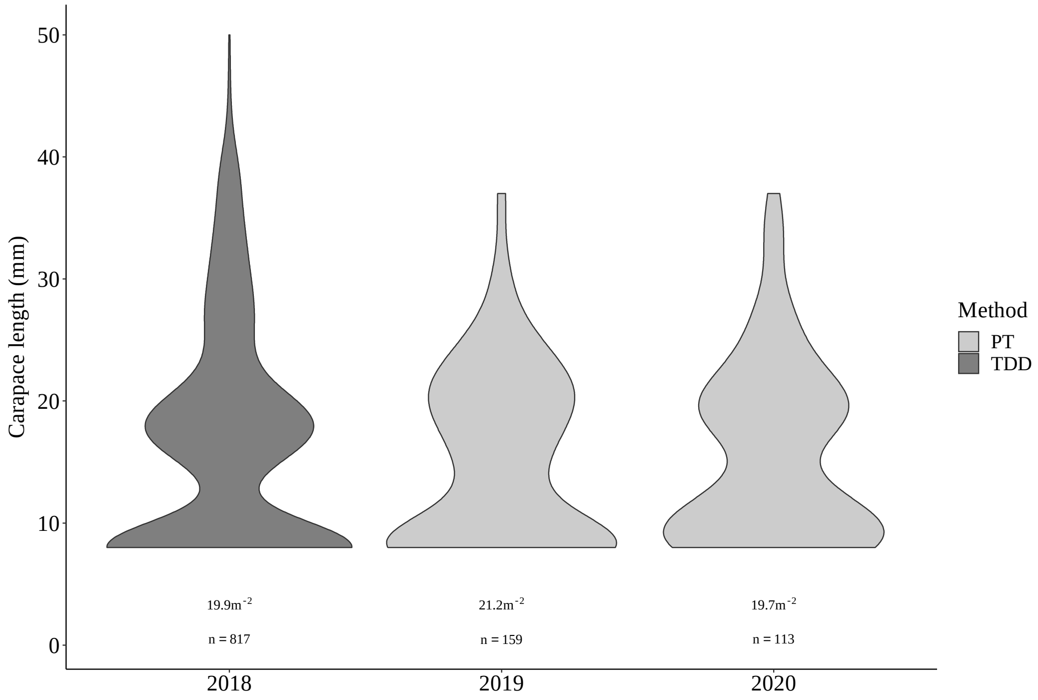
At the site with the lowest density of signal crayfish, Farm, the size class distribution of these invasives changed substantially over the three-year period (Figure 4.7a). In 2018, large adults (19%, ≥35 mm) and juveniles (62% ≤12 mm CL and 15% 13 – 25 mm CL) were sampled, while in 2019, no large adults or juveniles were recorded in the PTs. Instead, all crayfish represented the 20- and 25-mm CL size class. In 2020, whilst there were again no large adults present in the traps, some juveniles were recorded again (24% ≤ 12 mm CL). The size class distribution by biomass showed a large proportion of the biomass in 2018 was made up of large individuals (82% ≥35 mm CL), while biomass distribution in 2019 and 2020 showed distinct maxima in the sub-adult size class (61 – 74% 13 - 25 mm CL; Figure 4.7b). It is important to note that the very low density of crayfish at the site does limit the evaluation of the data. The white-clawed crayfish population at Farm contained a large number of juveniles (26% ≤ 12 mm CL) and sub-adults (58% 13 - 25 mm CL) in 2018, but a more even distribution in 2019 and 2020 (Figure 4.8a). Small adult crayfish made up the vast majority of the white-clawed crayfish biomass (40 – 67% 26 – 34 mm CL), with a decrease in juvenile biomass observed over time (6% ≤12 mm CL in 2018 and 2% ≤12 mm CL in 2019 and 2020; Figure 4.8b). Again, the low density of the populations limits the strength of the data evaluation.



a) DGB – size structure by frequency

b) DGB - size structure by biomass

Figure 4.4 Bean plots (probability density of the catch data) of signal crayfish size class distribution (mm CL) captured through TDD in 2018 and PTs in 2019 and 2020 at Double Gate Bridge (DGB) - a) size class distribution by frequency with crayfish density (m^{-2}) and number of crayfish (*n*) also denoted, and b) size class distribution by biomass with total crayfish biomass (g/m^{2}) denoted.


a) Confluence – size structure by frequency

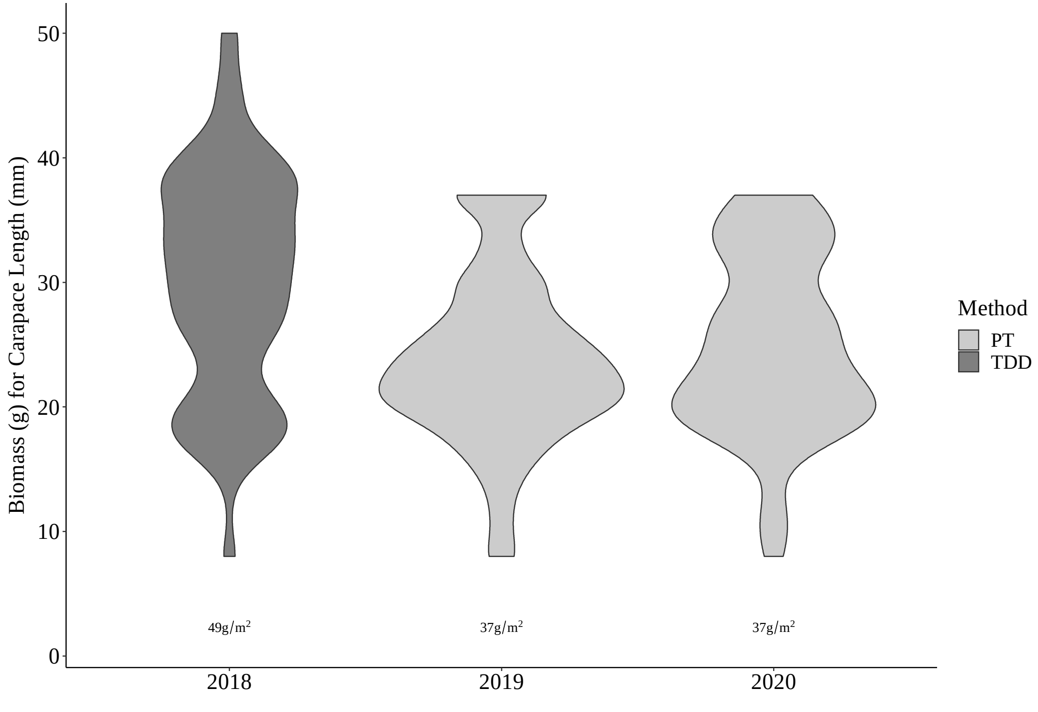
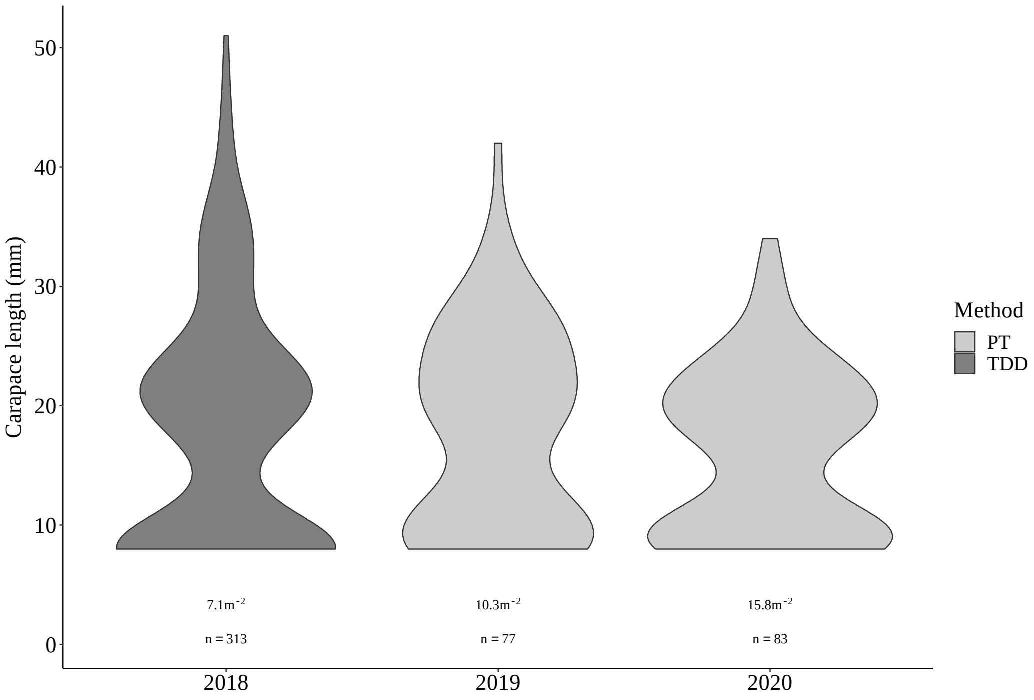

b) Confluence - size structure by biomass

Figure 4.5 Bean plots (probability density of the catch data) of signal crayfish size class distribution (mm CL) captured through TDD in 2018 and PTs in 2019 and 2020 at Confluence - a) size class distribution by frequency with crayfish density (m^{-2}) and number of crayfish (*n*) also denoted, and b) size class distribution by biomass with total crayfish biomass (g/m^2) denoted.

a) Footbridge – size structure by frequency



b) Footbridge - size structure by biomass

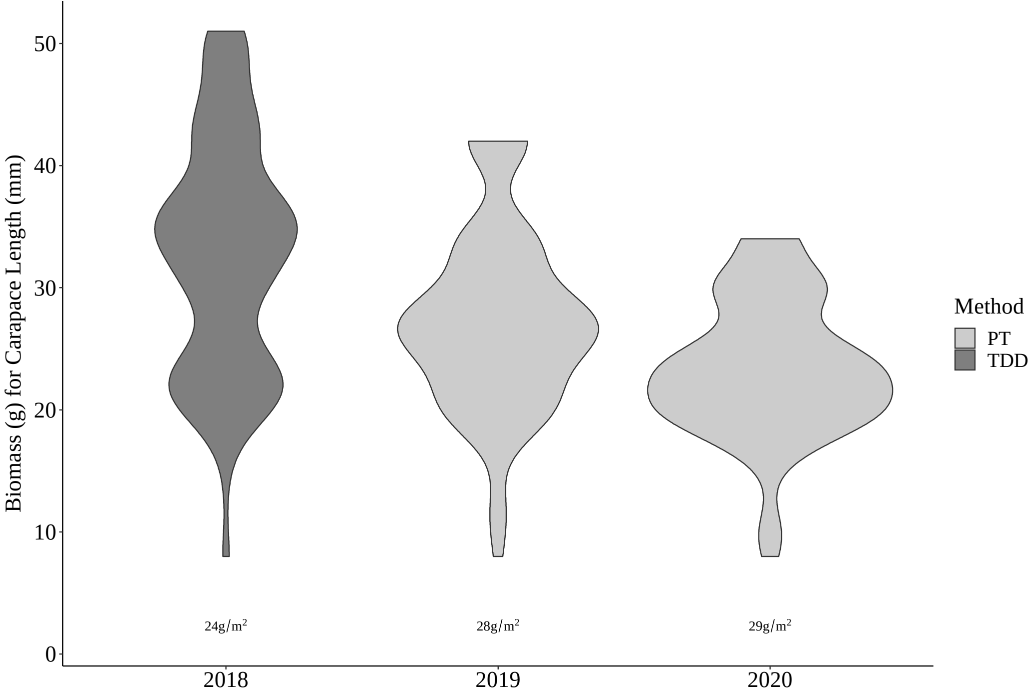
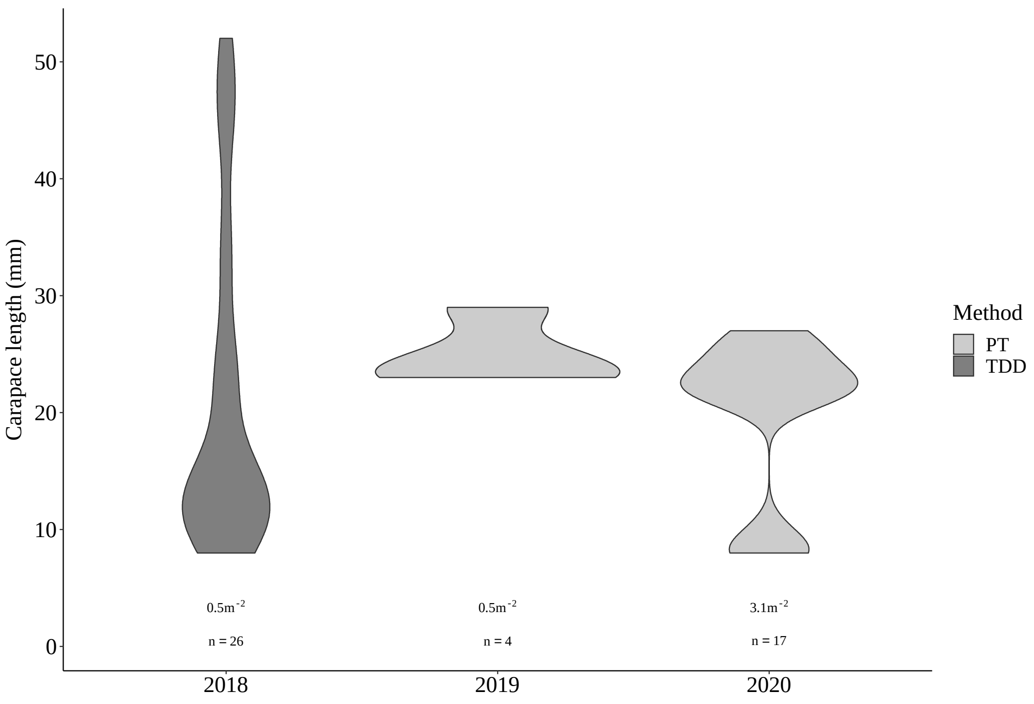


Figure 4.6 Bean plots (probability density of the catch data) of signal crayfish size class distribution (mm CL) captured through TDD in 2018 and PTs in 2019 and 2020 at Footbridge - a) size class distribution by frequency with crayfish density (m^{-2}) and number of crayfish (*n*) also denoted, and b) size class distribution by biomass with total crayfish biomass (g/m^2) denoted.

a) Farm (SC) – size structure by frequency



b) Farm (SC) - size structure by biomass

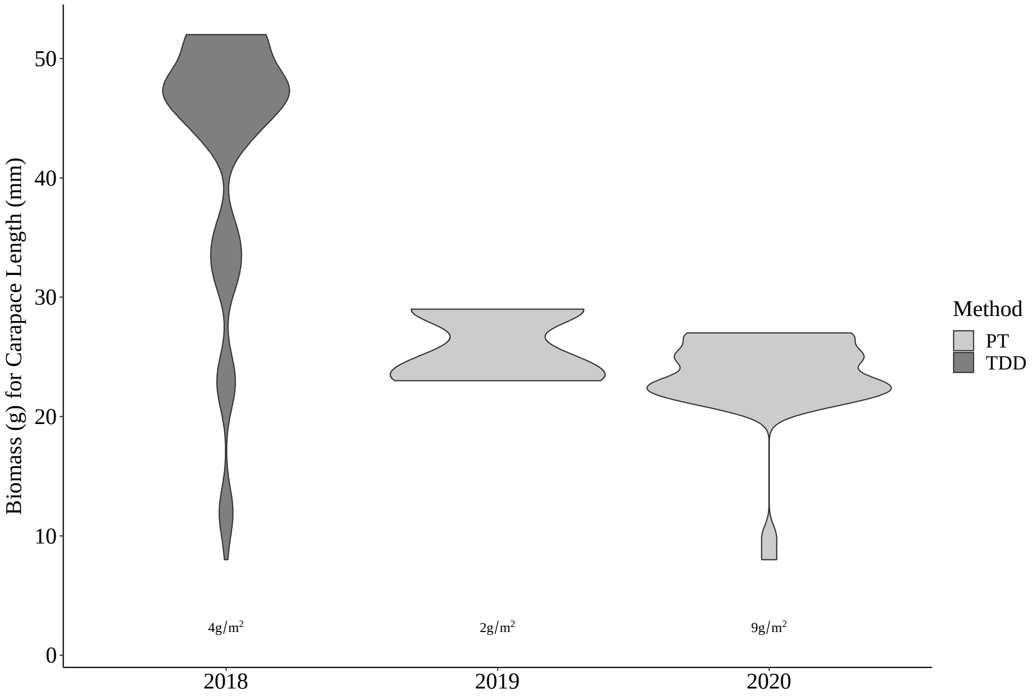
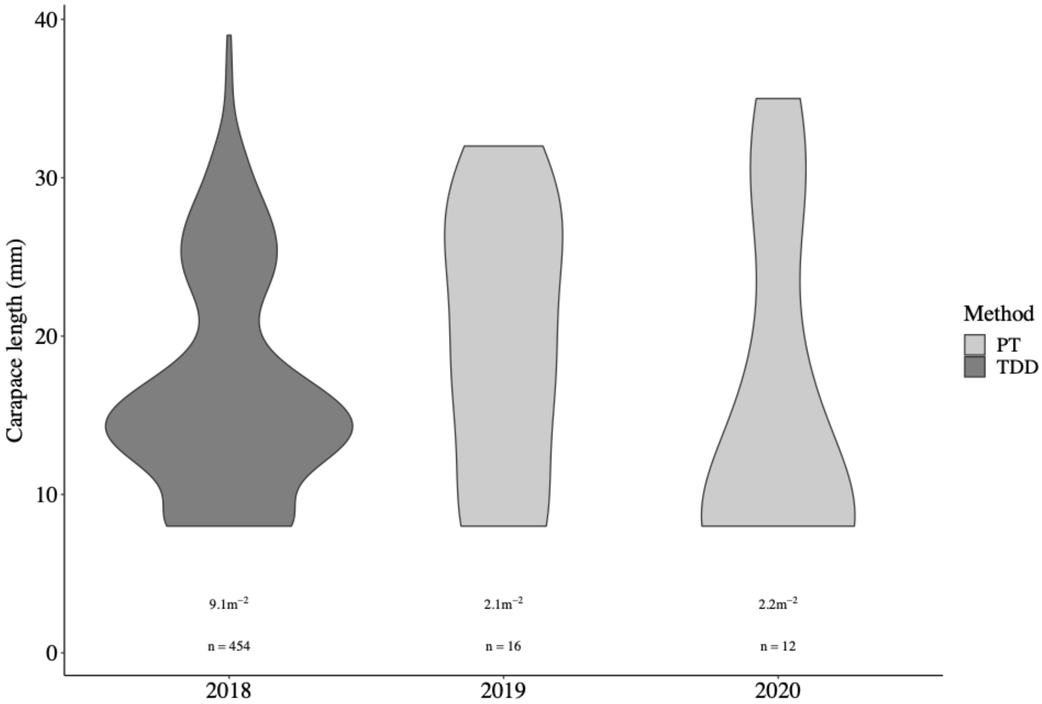


Figure 4.7 Bean plots (probability density of the catch data) of signal crayfish size class distribution (mm CL) captured through TDD in 2018 and PTs in 2019 and 2020 at Farm - a) size class distribution by frequency with crayfish density (m^{-2}) and number of crayfish (*n*) also denoted, and b) size class distribution by biomass with total crayfish biomass (g/m^2) denoted.

a) Farm (WCC) – size structure by frequency



b) Farm (WCC) - size structure by biomass

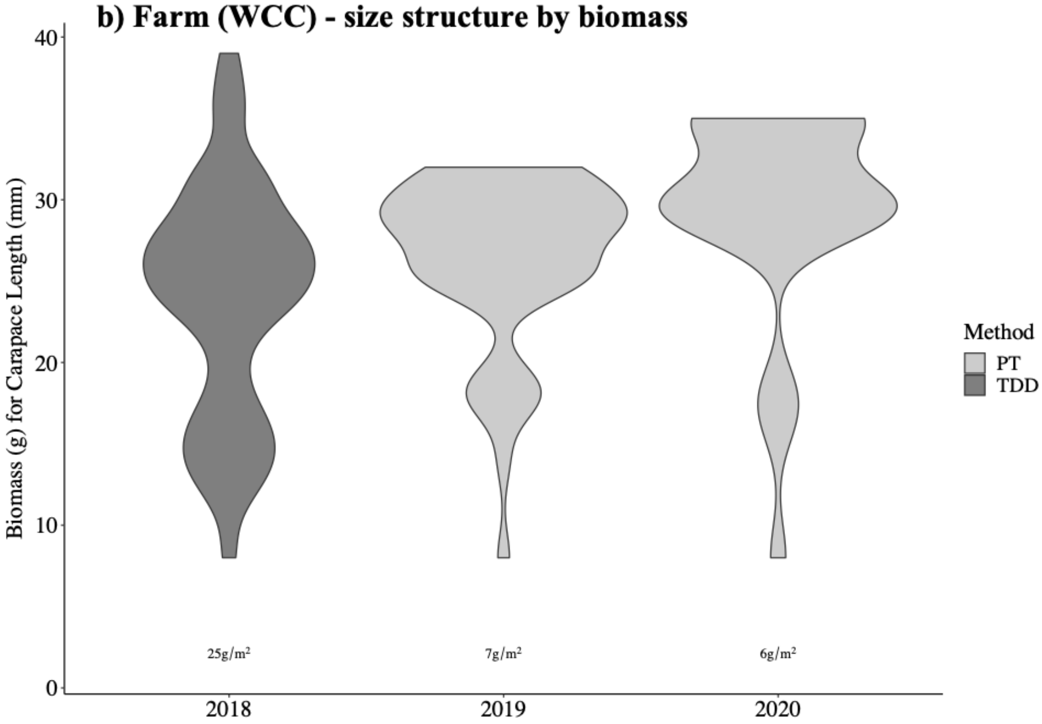


Figure 4.8 Bean plots (probability density of the catch data) of white-clawed crayfish size class distribution (mm CL) captured through TDD in 2018 and PTs in 2019 and 2020 at Farm - a) size class distribution by frequency with crayfish density (m^{-2}) and number of crayfish (*n*) also denoted, and b) size class distribution by biomass with total crayfish biomass (g/m^2) denoted.

4.4.3 Substrate association (2019)

At all PTs across the four study sites, fine and coarse cobble were the dominant substrate types. This was also in line with a set of in-channel substrate cover surveys undertaken in 2018 (Ch 2; Table 2.2), which showed cobble to be the main substrate type at all sites. Nevertheless, microhabitats varied somewhat between individual PT locations, with some placed at river margins, others at the central channel, and locations also varied between riffles and pools. Fine boulders were present at the sites, but only occurred within PTs on two occasions (DGB PT3 and Confluence PT2). In these PTs, the boulders comprised a large proportion of substrate volume within the trap (29 – 35% of total volume).

At DGB, cobble was the dominant substrate type across the three PTs (55 – 84% of total substrate volume; Figure 4.9c), with 17 – 40% of the volume taken up by fine and 30 – 50% by coarse cobble. PT3 contained one fine boulder, which equated 29% of the total volume in that trap, while boulders were absent from the two other PTs. PT2 contained more granules (13%) and fine pebbles (11%) than PT1 (5 and 3% respectively) and PT3 (6 and 4% respectively). Total substrate volume was 17,025 cm^3, 19,950 cm^3 and 20,588 cm^3 at PTs 1, 2 and 3 respectively (Figure 4.9c).

The average density of crayfish was consistent across PTs (73 – 77 m^{-2}). The size class distribution was also similar between PTs (Figure 4.9b), although it is worth noting that PT2 with the highest proportion of fine substrate size classes contained more juveniles (52 juveniles m^{-2} in PT2 compared to 47 juveniles m^{-2} in PT1 and PT3) and a slightly higher population density than the other two traps, and the largest individual (42 mm CL) was captured in PT3, where the boulder was present. However, this individual was only caught on one occasion, so it is unclear whether this was related to substrate and not a random sampling effect.

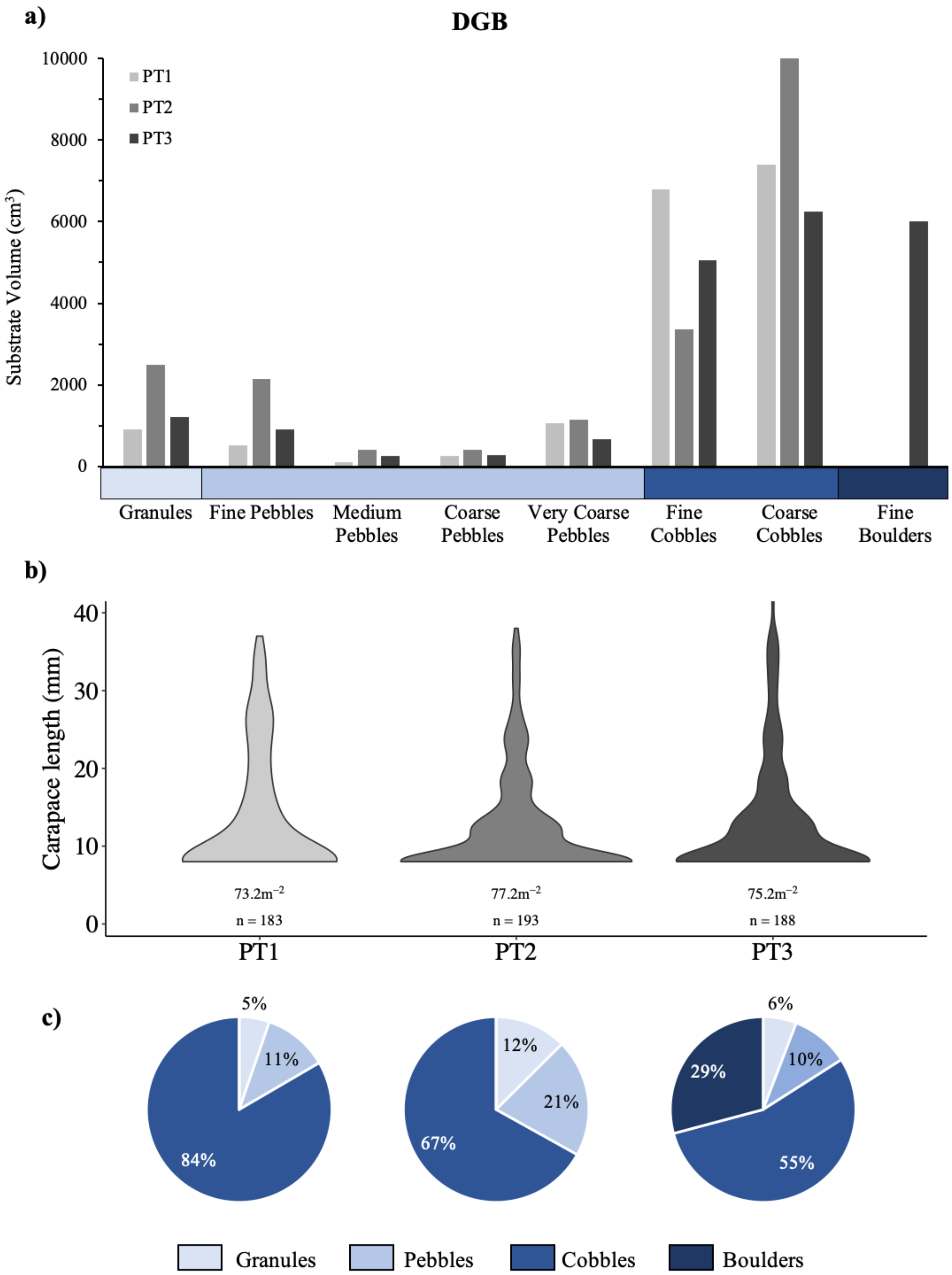


Figure 4.9 Substrate grain size and crayfish size class distribution in PTs at DGB; a) volume (cm^3) of substrate within each Udden Wentworth grain size category; b) bean plot (probability density of the catch data) of signal crayfish size class distribution (mm CL) captured in three PTs in 2019. The average density (m^{-2}) and number of crayfish (*n*) are also denoted; c) pie charts with relative proportion (%) of main substrate types.

At Confluence, cobble was again the dominant substrate size (55 – 75 % of total substrate volume; Figure 4.10c), with 37 – 45 % of the volume representing fine cobbles and 18 – 31% coarse pebbles. One fine boulder was present in PT2, which comprised 36% of the total substrate volume, while boulders were absent from PT1 and PT3. There was a higher proportion of granules (7%) and fine pebbles (11%) at PT1, compared to PT2 (0.1 and 0.7% respectively) and PT3 (2 and 6% respectively). Although the total volume of fine cobbles was higher in PT1 compared to PT2 and PT3, the relative proportion was similar across traps (37 – 45%). Total substrate volume was 22,000 cm^3, 16,900 cm^3 and 14,350 cm^3 at PTs 1, 2 and 3 respectively (Figure 4.10a).

The density of crayfish sampled in each PT was more variable, with 27.2 m^{-2}, 22.8m^{-2} and 13.6m^{-2} recorded at PTs 1, 2 and 3 respectively. The size class distribution was generally similar between the PTs, with juveniles being the dominant size class (Figure 4.10b). However, the maximum carapace length captured in PT2 was 26 mm compared to 37 mm in both PTs 1 and 2, despite PT2 being the only PT to contain a boulder. Also, PT1 with the highest volume of fine granules (7%) again contained a higher density population than the other two PTs, with a very large representation of the small size classes when compared to the other two traps.

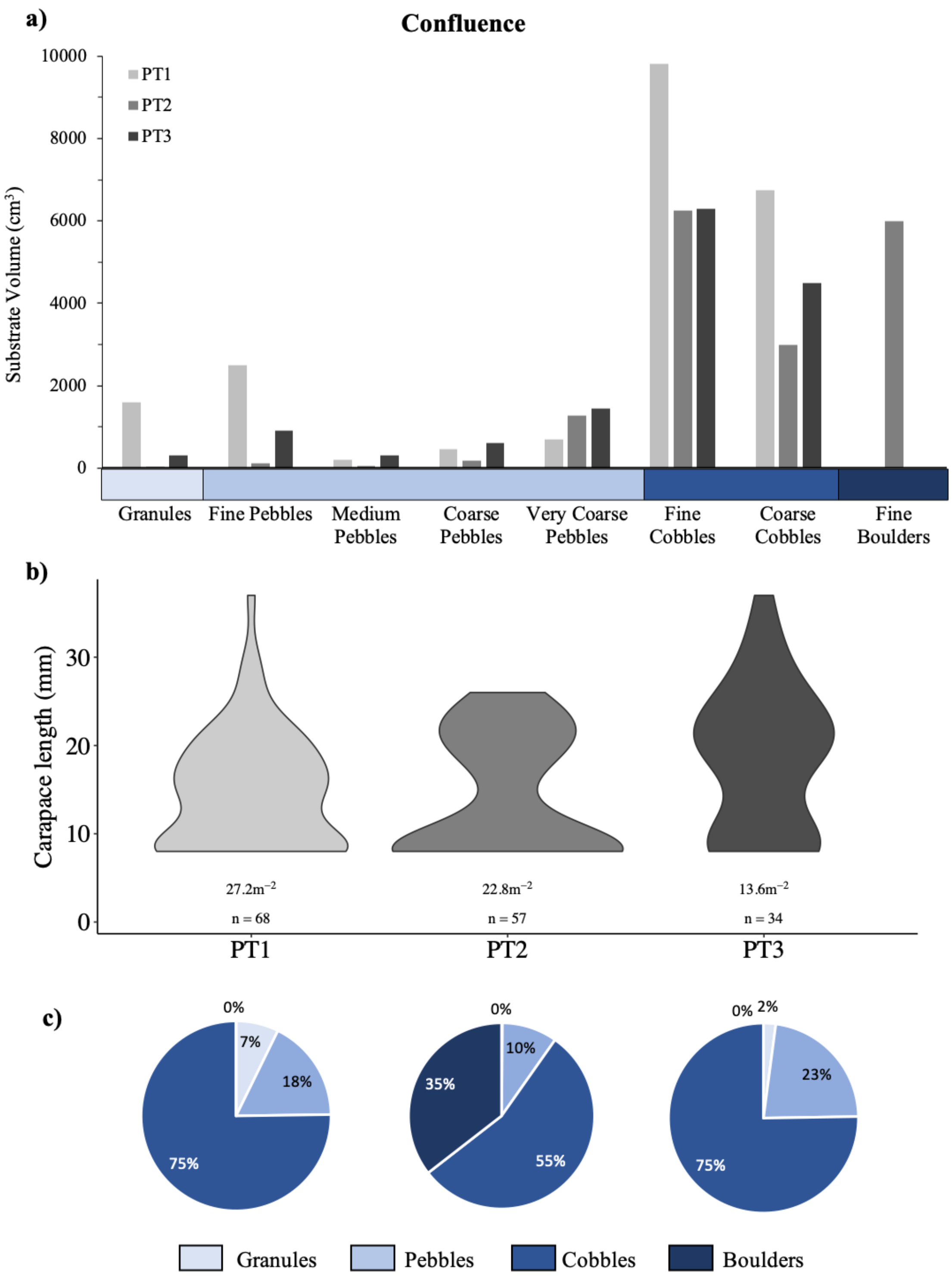


Figure 4.10 Substrate grain size and crayfish size class distribution in PTs at Confluence; a) volume (cm^3) of substrate within each Udden Wentworth grain size category; b) bean plot (probability density of the catch data) of signal crayfish size class distribution (mm CL) captured in three PTs in 2019. The average density (m^{-2}) and number of crayfish (*n*) are also denoted; c) pie charts with relative proportion (%) of main substrate types.

At Footbridge, substrate was only processed for one PT due to the disappearance of the other two PTs at this site. Within this PT, cobbles comprised 70% of the total substrate volume, with 32% fine cobbles and 38% coarse cobbles. No boulders were present (Figure 4.11c). Total substrate volume at PT2 was 17,650 cm^3 (Figure 4.11a). In PT2, the average density of crayfish was 9.2 m^{-2} with a maximum carapace length of 34 mm (Figure 4.11b).

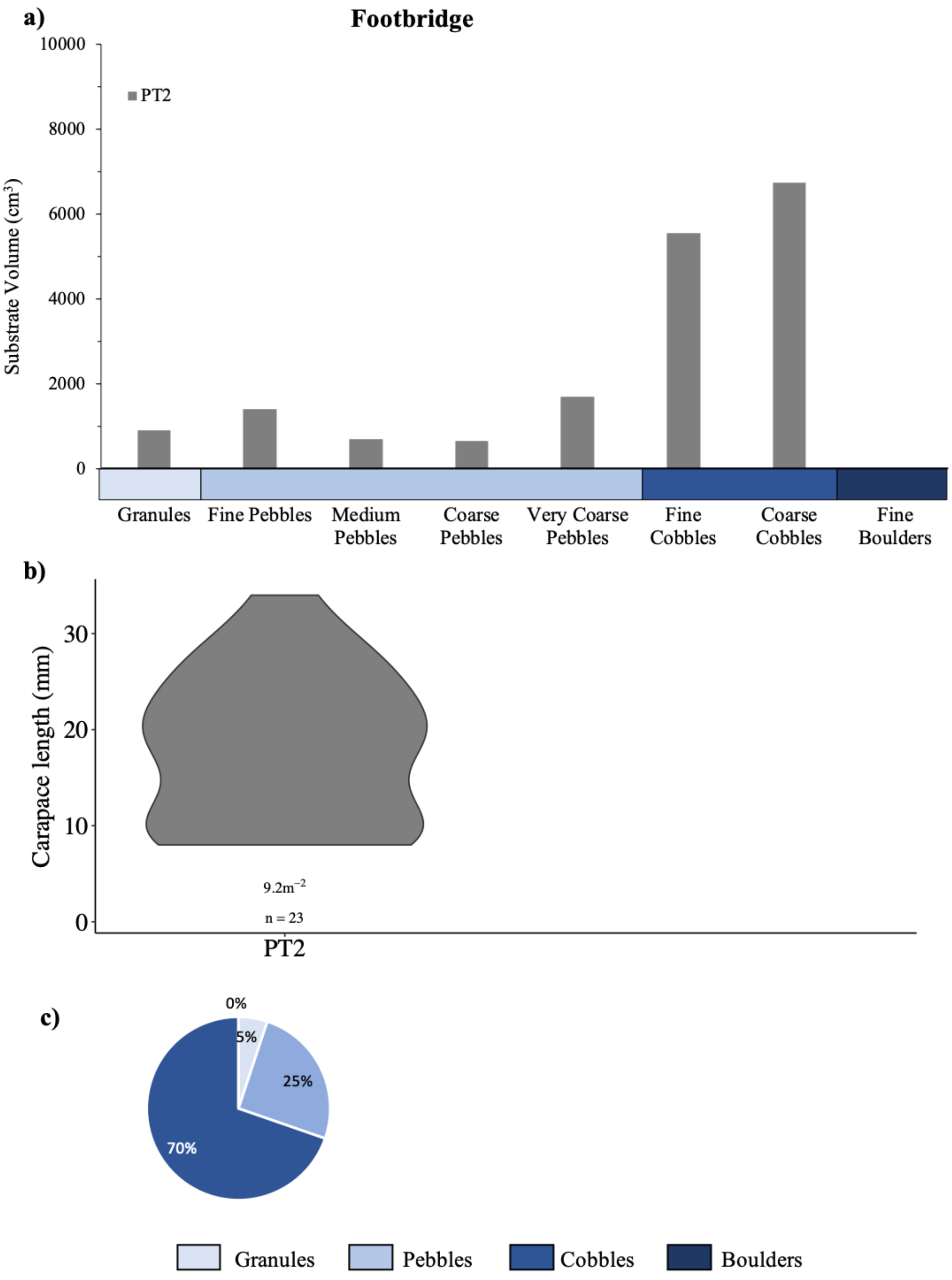
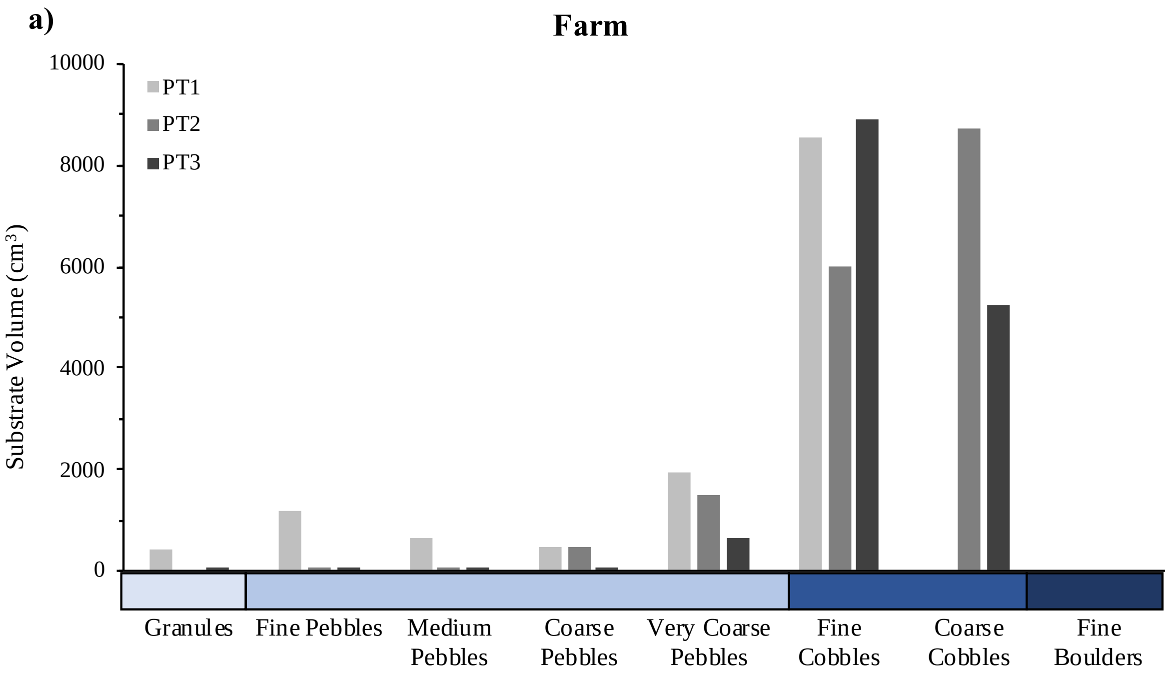


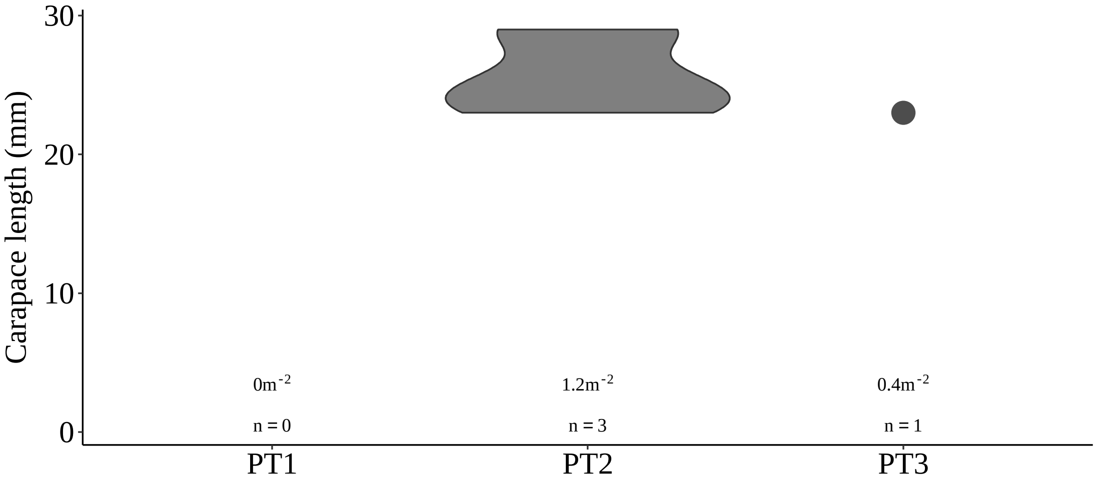
Figure 4.11 Substrate grain size and crayfish size class distribution in PTs at Footbridge; a) volume (cm^3) of substrate within each Udden Wentworth grain size category; b) bean plot (probability density of the catch data) of signal crayfish size class distribution (mm CL) captured in three PTs in 2019. The average density (m^{-2}) and number of crayfish (*n*) are also denoted; c) pie charts with relative proportion (%) of main substrate types.

At Farm, cobble comprised the majority of the total substrate volume (65 – 94 % of total substrate volume; Fig 12d). Fine cobbles accounted for 36 – 65 % of the substrate volume. PT2 and PT3 contained 35 – 52% coarse cobble, but unusually, no coarse cobbles were present in PT1. Total substrate volume was 13,225 cm^3, 16,760 cm^3 and 15,025 cm^3 at PTs 1, 2 and 3, respectively (Figure 4.12a).

Only four signal crayfish individuals were sampled during the 30 PT lifts in 2019. No signal crayfish were caught in PT1 (0 m^{-2}), whilst three of the crayfish were sampled in PT2 (1.4 m^{-2}) and the remaining one in PT3 (0.4 m^{-2}). All crayfish were of a similar size class (23 – 29 mm CL; Figure 4.12b). An additional 16 white-clawed crayfish were sampled over the 2019 period. Overall, due to the low numbers of individuals caught for both species, caution must be taken when drawing conclusions from this data. Density of white-clawed crayfish was consistently low and ranged from between 2.4 m^{-2} (PT1 and PT2) to 1.6 m^{-2} (PT3). However, the size class distribution was quite varied between PTs. PT1 only sampled adults (25 – 32 mm CL; Figure 4.12c), despite having no coarse cobbles or boulders present. PT2 sampled across the main size classes (14 – 29 mm CL; Figure 4.12c), whereas PT3 tended to only catch juveniles and sub-adults (8 – 17 mm CL; Figure 4.12c).



b) Signal crayfish



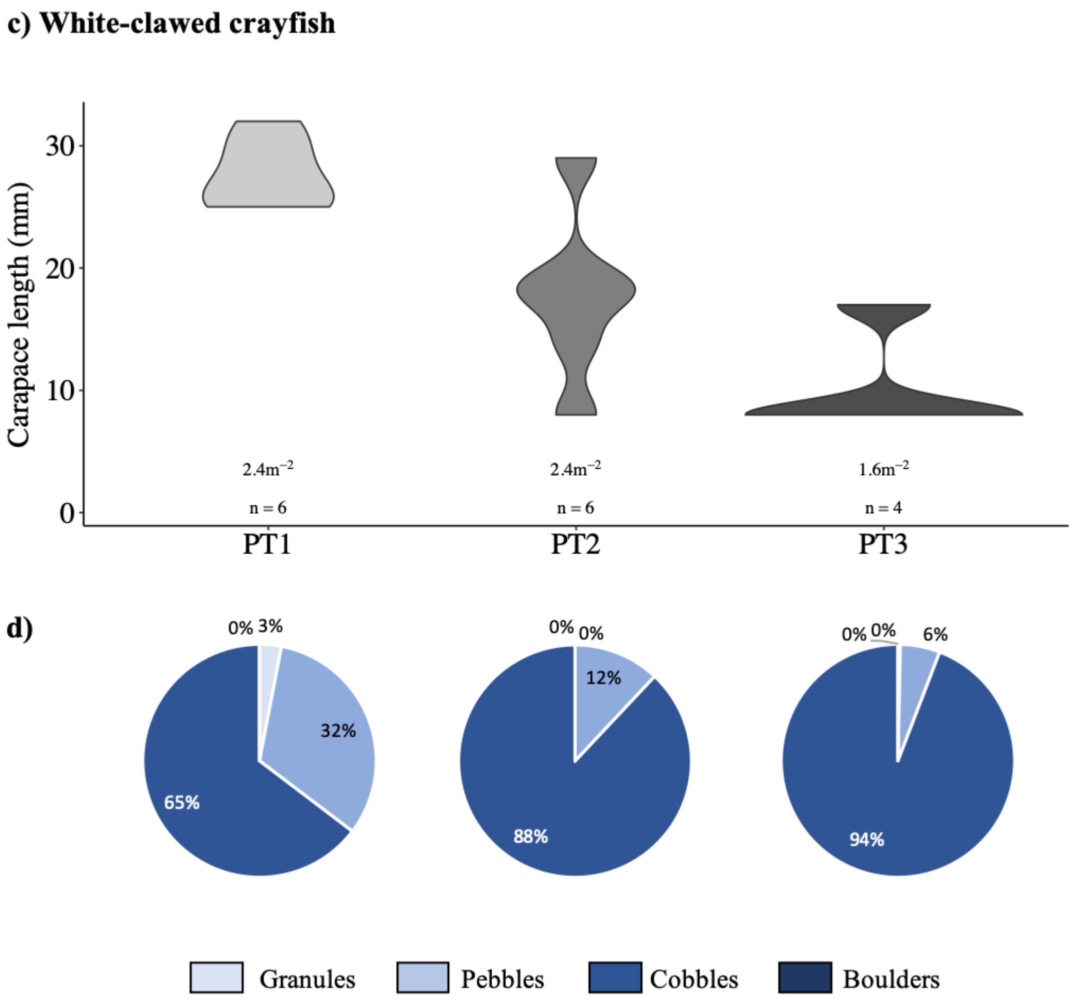


Figure 4.12 Substrate grain size and crayfish size class distribution in PTs at Confluence; a) volume (cm^3) of substrate within each Udden Wentworth grain size category; b) bean plot (probability density of the catch data) of signal crayfish size class distribution (mm CL). The average density (m^{-2}) and number of crayfish (*n*) are also denoted; c) bean plot (probability density of the catch data) of white-clawed crayfish size class distribution (mm CL). d) pie charts with relative proportion (%) of main substrate types.

4.5 Discussion

4.5.1 Population density

In contradiction to the hypothesised boom-bust population dynamics (Strayer *et al.* 2017), the population density at all invaded sites remained stable or increased each year (2018 – 2020). The population at DGB has been established for over two decades, attaining consistently high densities over the past five years with no sign of population crashes (110 - 86 m^{-2} in 2016 and 2017; Chadwick *et al.* 2021). The population density gradually decreases towards the invasion front suggesting that as the population spreads and becomes established, the population density steadily increases over time, reaching incredibly high densities at the well-established sites. We can therefore reject the proposition that isolated or recurring boom-bust population dynamics, as suggested by invasion biology theory (Strayer *et al.* 2017) are occurring within the study system. This has significant implications for understanding the potential impact and management of signal crayfish. The boom-bust dynamic has been argued as a reason not to manage biological invasions, under the assumption that the ‘boom’ is a transient harmful phase that will shift to a persistent harmless phase following the population ‘bust’ (Strayer *et al.* 2017). This concept assumes that the ecological impacts of the boom are somewhat reversible and the best course of action may be to implement no management and wait for the population to diminish on its own (Thompson 2014; Pearce 2015). In our study system however, signal crayfish populations spread, establish and grow to extremely high population densities with no sign of abating, and thus the corresponding ecological impact will be persistent, and likely worsening with time, especially if density-dependent.

Whilst overall population densities remained stable or gradually increased over time, signal crayfish density did fluctuate within sites over the summer period of 2019, with juvenile size classes showing the largest variance over the season. The spike in juvenile density was exceptionally pronounced at the high-density site (DGB) which is likely directly related to the high number of sexually mature females (usually 2 -3 years of age) which typically carry between 200 and 400 eggs in one brood (Holdich *et al.* 2014). The subsequent decline in juvenile numbers over the summer will partly be due to fast growth, with young-of-year typically undergoing up to 11 moults in their first year (Lewis 2002a) and consequently moving up into the sub-adult (13 – 25 mm CL) size class. However, mortality is also going to play a large part in juvenile decline over the summer, with estimates of survivorship to two years varying between 10 to 52% depending on environmental conditions (Holdich *et al.* 2014). The differences in available resources and competition could explain the differences in juvenile seasonal trends between DGB, Confluence and Footbridge, with juveniles peaking earlier in the season at DGB. At DGB, the high density of signal crayfish will result in limited resources and a high level of intra-specific competition and the incidence of cannibalism is expected to increase with population density, with larger crayfish preferentially cannibalising juvenile size classes (Houghton, Wood and Lambin 2017). At the other sites, whilst intra-specific competition and cannibalism may be relatively lower, the resident fish communities (see Ch. 3 and Ch. 5) may lead to different juvenile behaviour, for example utilising different habitats or reduced range of movement. Competition for resources (Bubb *et al.* 2009) and predation (Reynolds 2011) may also lead to variable mortality rates.

4.5.2 Invasion front and interaction with native crayfish

The decline and ultimate displacement of white-clawed crayfish at sites along the study system support the hypothesis that when crayfish plague is lacking, the density of white-clawed crayfish will be negatively associated with signal crayfish density. In my study system, the replacement of white-clawed crayfish with signal crayfish takes approximately 4 – 6 years, in keeping with estimates from the literature (5 years in Holdich and Domaniewski 1995; 4 - 7 years in Peay and Rogers 1999). In 2008, white-clawed crayfish were recorded present and abundant at the Confluence and downstream in Long Preston Beck (annual surveys 2002 - 2008 inclusive, in Peay *et al.* 2009).

Signal crayfish were first recorded at Confluence in 2009 but remained at low-density until 2012 (Taylor 2016). White-clawed crayfish were found to be absent at Confluence in 2015, whereas signal crayfish were abundant, therefore displacing their native counterparts within six years of arrival at the site (Taylor 2016). Although lack of intermediate surveys (between 2012 and 2015) means that this extinction could have occurred sooner and remained undetected. In 2015, signal crayfish were recorded through trapping and manual handsearches at very low densities immediately upstream of Footbridge, with no signal crayfish recorded downstream of Footbridge (Taylor 2016). In 2018, only a few remnant white-clawed crayfish were found at Footbridge during the TDD, with none found in following years, showing a complete displacement within four years. At Farm, signal crayfish were found at low densities in 2018, where a population of white-clawed crayfish remained. However, in the following two years signal crayfish density has increased gradually, and white-clawed crayfish density has declined sharply. Based on the timeframe observed upstream, white-clawed crayfish are likely to become locally extinct at Farm by 2022 - 2024. The patterns observed along this study system suggest that as signal crayfish become established, they may remain at relatively low densities for a number of years before rapidly increasing and completely displacing native crayfish.

If the limit of signal crayfish downstream dispersal in 2018 was indeed Farm, then it has taken nearly 10 years for them to spread 1.5 km downstream since the population was confirmed at the Confluence in 2009 (Peay *et al.* 2009; Taylor 2016). It is important to note that the populations at Confluence and Farm were recorded in 2009 and 2018, respectively, and may have been present for longer, yet remained undetected. This rate of downstream dispersal (0.15 km year^{-1}) is in keeping with previous estimates within the study system, with a rate of expansion of 0.1 km year^{-1} in Bookill Gill Beck between 1995 and 2002, which then increased to 0.46 km year^{-1} between 2002 and 2008 (Peay *et al.* 2009). A slightly higher rate of downstream dispersal was reported for signal crayfish in the River Wharfe, North Yorkshire, at 1.5 km year^{-1} between 1987 and 2002 (Bubb, Thom and Lucas

2004), despite the environmental conditions being very similar to those in this study (rocky substrate, upland rivers). This highlights the variability in signal crayfish expansion rates within a system and between similar systems, and based on these estimates signal crayfish could reach the main River Ribble (2.3 km downstream from Farm) in anywhere between 1.5 and 15 years, showing that we cannot rely on expected or anticipated dispersal rates to provide a timeframe for conservation action and management. Nevertheless, the dispersal rates for these English upland rivers are still significantly lower than estimates elsewhere, with downstream dispersal rates of 18 to 24.4 km year^{-1} in the Mura River, Croatia (Hudina *et al.* 2009). The slow dispersal rates may be due to abundant refugia and food in the system, which may present a limiting factor on crayfish populations and cause greater pressure to disperse when resources are low. The factors driving expansion and dispersal in natural, rocky, headwater streams may be limited, thus presenting a slightly larger window for management interventions to be taken. For example, a bespoke crayfish barrier has been installed on the River Clyde in Scotland, to prevent the migration of signal crayfish to a neighbouring catchment, the River Annan, and protect important headwater habitats and salmonid populations (Rahel 2013), though notably this is only appropriate to prevent upstream colonisation (Krieg and Zenker 2020).

4.5.3 Population demographics

The hypothesis that signal crayfish population demographics would vary along the invasion gradient was supported by the results of this study, showing a distinct phenotype at the invasion front. The low number of individuals that were found at the site in coexistence with an initially strong population of white-clawed crayfish in 2018, was chiefly composed of large adults and young-of-year, with the juvenile/subadult (9 – 18 mm CL) size class missing completely. This suggests that larger individuals have dispersed downstream and become established in a new territory. Large crayfish may be able to establish themselves faster than smaller individuals, as they can outcompete native crayfish and small fish to obtain refuge and resources (Bubb *et al.* 2009), and they are significantly less prone to being predated by large fish than smaller size classes, with small crayfish individuals hence more vulnerable to both predation and starvation. The equal sex ratio (50:50) would indicate that the young-of-year were a result of *in situ* reproduction at the invasion front, as opposed to the theory that berried females actively disperse to new territory with greater resources to overwinter and release young (Almeida *et al.* 2013). Our results here also contradict the idea that males are more important for dispersal, with a male dominated invasion front reported in other studies (Hudina *et al.* 2012; Hudina, Zganec and Hock 2015).

The instances of cheliped damage were variable but generally high across all densities of signal crayfish populations in this study, especially in comparison to the low level of damage recorded in the white-clawed crayfish population at the Farm in 2018. This supports the understanding that signal crayfish are far more aggressive than the native white-clawed crayfish (Holdich *et al.* 2009). The high level of cheliped damage could be a result of high intra-specific competition, particularly at the high density sites. Whilst crayfish will interact with native fish through competition and predation (Reynolds 2011), and antagonistic interactions could result in cheliped damage, the predators are unable to coexist in a balance with signal crayfish as evidenced by the significant increase in population size to incredibly high densities.

The size structure of signal crayfish at Farm changed considerably between 2018 and 2020, with a lack of large adults in 2019 and 2020 and the juvenile size class absent from 2019 samples, although very small sample sizes limit clear evaluation. Large adults could potentially move into burrows as preferred habitat, although the removal and destruction of individuals captured during the TDD in 2018 could also have potentially affected population dynamics. Whilst populations recolonised at the other study sites very quickly following TDDs and crayfish removal (pers. obs), the low-density population at Farm may have required more time to recolonise and form the same population structure. Further work is required to investigate the legacy effects of crayfish removals during TDDs. Nevertheless, a similar ‘invasion front’ density was recorded in 2019 (0.5 m^{-2}) which then accelerated to over 3 m^{-2} in 2020, showing establishment and population growth. The presence of sub-adults and small adults in 2019 shows the original invasion front demographic is no longer dominant, and represents a more typical population and by 2020, the population was expanding and recruiting with young-of-year recorded in the samples. The population of white-clawed crayfish had declined severely over the three-year period, and although it may take 4-6 years to become locally extinct, the population can decrease very quickly, with only smaller numbers persisting as signal crayfish become dominant. The invasion of signal crayfish could potentially cause white-clawed crayfish to disperse downstream to areas where they are not yet present, resulting in a two-fold decline due to out-competition and emigration. However, signal crayfish have been observed to disperse twice as far as white-clawed crayfish (Bubb, Thom and Lucas 2006) and can outcompete them for shelters (Holdich and Domaniewski 1995), showing that they will eventually become dominant over white-clawed crayfish throughout the system.

In contrast to the variable signal crayfish population at the invasion front, it was hypothesised that population structure would be stable at well-established sites. The established signal crayfish populations, even at low-densities (e.g. Footbridge) developed a “typical” population structure, that remained relatively stable even as numbers increased over time. All signal crayfish populations were dominated by young-of-year, whereas only 15% of the healthy white-clawed crayfish population (2018) were young-of-year. This could be due to the high fecundity of signal crayfish relative to white-clawed crayfish (Holdich *et al.* 2014), or due to signal crayfish preferentially predating on juvenile white-clawed crayfish. The juvenile dominated signal crayfish population structure could have strong implications for understanding the ecological impact of the species. Signal crayfish undergo an ontogenetic shift in feeding behaviour with the juveniles primarily predating on invertebrates and adult diet comprising of plant material.

Therefore, a high impact on macroinvertebrate communities within our study system may be anticipated (discussed further in Ch. 6).

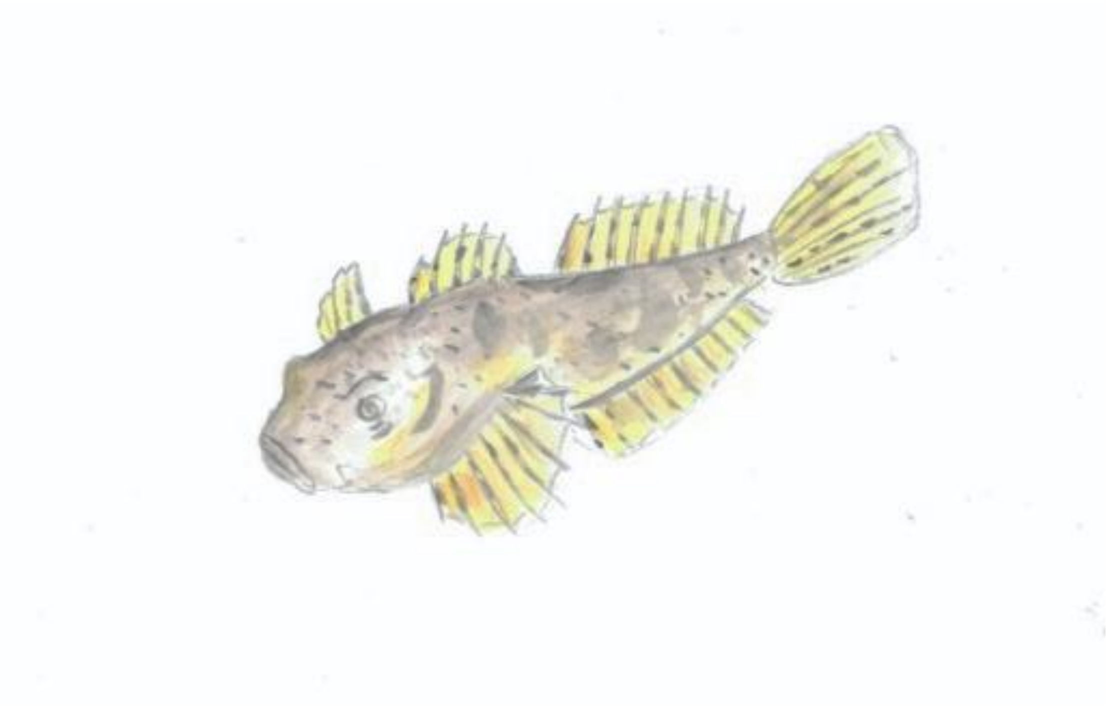
However, this assumes that the per-capita effects are driving the ecological impact, whereas it could be per-biomass (Parker *et al.* 1999). In this study system, the total biomass of crayfish (g/m^2) was chiefly comprised of sub-adult and adult individuals, and the vast number of juveniles were not reflected in the biomass size structure given their individual light weight (~ 0.1 – 0.3 g per juvenile). Previous work has highlighted the importance of population size structure when understanding and predicting impacts of invasions, yet the relative contributions of biomass and abundance across size classes are poorly understood (Fritschie and Olden 2016). This highlights the importance and urgency of further research that will investigate and compare the per-capita vs per-biomass impact of signal crayfish. Functional response experiments that generate feeding rates for invasive species should be undertaken for signal crayfish at various size classes, to better understand the ecological impacts across the populations and how ontogenetic shifts in feeding behaviour may influence this. This kind of research could further inform whether it is more effective to remove more numbers or biomass of crayfish and which size classes may be most damaging and therefore priority for targeted management.

4.5.4 Substrate association

In contradiction to the hypothesised relationship between crayfish size class distribution and substrate composition within PTs, no clear association was observed. I expect this is due to the relatively homogenous spread of substrate across the sites, with cobble being the dominant substrate type in all PTs. Furthermore, whilst substrate type is important for habitat preference for crayfish, previous work has highlighted that rocky substrate such as gravels, pebbles, cobbles and boulders are often the preferred substrate (Engdahl *et al.* 2013; Rosewarne, Mortimer and Dunn 2017), and will contain crayfish of all sizes (Demers, Reynolds and Cioni 2003). Whereas, soft substratum, leaf litter and vegetation may provide shelter for juvenile crayfish and less preferable for larger crayfish (Kershner and Lodge 1995; Demers, Reynolds and Cioni 2003). Therefore, the lack of obvious relationship between crayfish size class distribution and substrate composition could also be due to a lack of habitat types such as vegetation and all habitat is suitable and high quality, leading to little preference between grain sizes and providing a high carrying capacity.

The consistently high density of signal crayfish between PTs at DGB is also likely related to the extremely high overall density of crayfish at this site, causing colonisation pressure to be very high and resulting in all available habitat being utilised. Confluence, however, had a wider variance in density between PTs, which could reflect the lower density relative to DGB, resulting in reduced colonisation pressure. On two occasions, PTs with a higher volume of granule and fine pebbles (DGB PT2 and Confluence PT1) also contained a large number of juvenile crayfish, potentially demonstrating a weak relationship. Finer material may provide more efficient opportunities to hide from large crayfish and reduce intra-specific competition with larger con-specifics. Larger crayfish may then utilise larger substrate including cobbles and boulders which have been suggested to provide the most desirable dimensions (Watson and Rogers 2003). Previous work has found that juvenile crayfish preferentially selected cobbles (Brusconi *et al.* 2008), whereas adults have a stronger association with boulders than cobble (Naura *et al.* 1998; Brusconi *et al.* 2008). Substrate that provides suitable refugia is a key limitation on crayfish abundance (Lodge and Hill 1994; Holdich 2003) and therefore the associations between juvenile crayfish and cobble could potentially explain why this size class are so successful in this system. Abundant optimal refugia allows juveniles to forage and evade predators, whereas in other systems where such refugia is limited, juvenile mortality may be higher and overall population size structure may be different.

Chapter 5. Signal crayfish impacts on fish



5.1 Summary

1. Headwater streams are important ecosystems, providing crucial spawning grounds for economically valuable fish species, such as brown trout (*Salmo trutta*) and Atlantic salmon (*Salmo salar*). They also offer important habitats for protected fish species such as the European bullhead, (*Cottus gobio*) in the UK and Europe.
2. Previous work has demonstrated that invasion by signal crayfish threatens the integrity of these habitats and their associated fish species, potentially explaining declines of both bullhead and salmonids in English upland streams. However, the lack of quantitative survey methods for both crayfish and benthic fish have made it difficult to quantify invasion impacts.
3. Here, I employ the new techniques – PTs and TDD (see Ch. 2 & 3) to explore density-dependent impacts of invasive signal crayfish on fish populations in a small headwater stream.
4. Despite seasonal changes in fish populations, fish communities did differ along the invasion gradient, with densities, especially of bullhead, declining as the signal crayfish population increased.
5. This study highlights the potential severe impact of signal crayfish on native fish communities and shows that fish populations may be lost when headwater river sections are heavily invaded. Further work should prioritise more detailed investigations into density-dependent impacts and the identification of environmental conditions that support high-density populations of crayfish invaders, in order to inform fish management and conservation efforts.

5.2 Introduction

5.2.1 Interactions between crayfish and fish

Ecosystem-wide effects related to signal crayfish invasions include the transmission of crayfish plague (Holdich, Reynolds, *et al.* 2009), displacement of native crayfish (Sibley, Holdich and Richman 2011; Richman *et al.* 2015), reduction of macrophytes (Nyström and Strand 1996) and macroinvertebrates (Crawford, Yeomans and Adams 2006; Mathers *et al.* 2016). Signal crayfish can also impact native fish communities, although relationships between fish and crayfish are highly complex. For example, crayfish and fish are known to interact directly through reciprocal predation and competition, and also indirectly by crayfish altering habitat structure and food resources that are available to fish (Reynolds 2011). Competition between crayfish and fish also regularly occur for both, shelter and resources (Reynolds 2011). Native crayfish and fish can co-exist in an ecosystem and interactions are generally balanced, fluctuating with various stressors (Reynolds 2011). In contrast, the replacement of native crayfish with invasive crayfish, or the introduction of crayfish to a previously crayfish-free environment, can affect ecosystem processes (Kouba, Petrusek and Kozák 2014).

Signal crayfish have been shown to predate on native fish species, including on their eggs and juvenile and adult life stages. Laboratory experiments have shown direct predation of buried salmonid eggs, with a 25% reduction of egg survival in the presence of signal crayfish compared with a control (Edmonds, Riley and Maxwell 2011). However, in other studies, signal crayfish appeared unable to identify salmonid eggs located in artificial redds (Gladman *et al.* 2012). Furthermore, gut content analysis has revealed significant signal crayfish predation on adult European bullhead and stone loach (*Barbatula barbatula;* Guan and Wiles 1997; Guan and Wiles 1998) and it can be expected many other species are consumed by signal crayfish. However, fish will also predate on crayfish (Reynolds 2011), and in a study of signal crayfish predation by chub (*Squalius cephalus*) effects of signal crayfish were shown to be highly life stage-specific (Wood *et al.* 2017). Both species were found to be omnivorous, occupying similar trophic niches, with crayfish representing important food items for chub. While growth rates of young chub were typically lower at signal crayfish sites, growth rates of older chub were generally higher. In contrast, generally reduced growth rates were observed in a native sculpin species (*Cottus beldingi*) in California, North America (Light 2005). This was attributed to competition with signal crayfish for refuge, leading to increased energy expenditure and fleeing of fish to high velocity microhabitats. Signal crayfish were also observed to displace Atlantic salmon from refugia in a controlled mesocosm experiment, with refugia usage rates found to be crayfish density-dependent (Griffiths, Collen and Armstrong 2004). Similarly, signal crayfish displaced European bullhead from shelter in a laboratory trial (Bubb *et al.* 2009).

The local composition of fish populations may furthermore alter the effect of crayfish and cause interactions to vary, with small fish species, for example, displaced from shelter by the crayfish, which in turn increases their vulnerability to predation by piscivorous species (Rahel and Stein 1988; Light 2005).

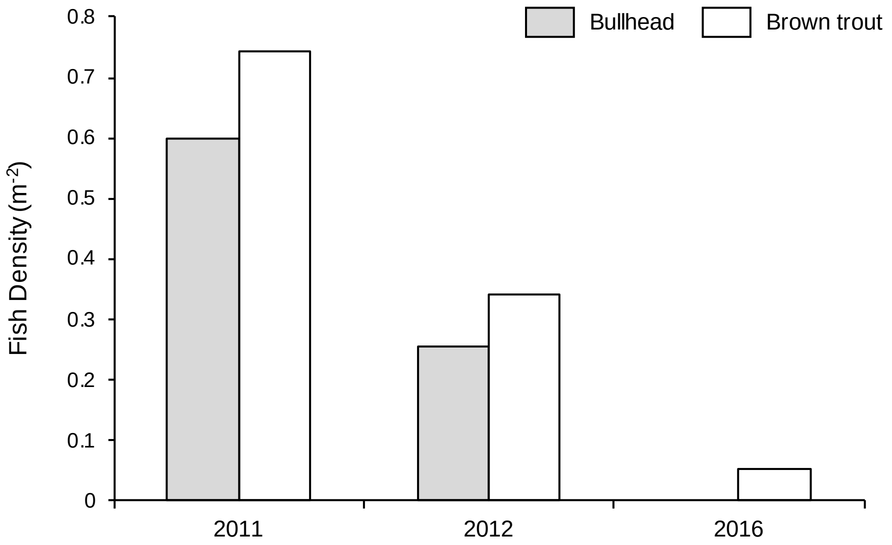
The observed negative impact of signal crayfish on salmonid fish in upland streams (Peay *et al.* 2009; Galib, Findlay and Lucas 2021) can again be related to both direct interactions such as predation or competition for resources, as well as indirect effects. For example, signal crayfish act as geomorphic agents, and their burrowing behaviour can increase sediment load in watercourses (Harvey *et al.* 2011; Sanders, Rice and Wood 2021). Increased bioturbation and suspended sediment can degrade important in-stream habitats such as spawning gravels for fish such as brown trout and Atlantic salmon (Soulsby *et al.* 2001). As a result, the impact that signal crayfish exert on salmonid fish is likely accentuated in headwater streams that provide important spawning and nursery grounds.

Crayfish density may be a key determinant in the level of impact on fish. However, previous studies have lacked the ability to generate quantitative in-situ data on crayfish populations, which could explain the great variation in reported results. Furthermore, density-related impacts take time to fully develop (Simberloff *et al.* 2013) as populations grow and expand following introduction. Yet, very few studies monitor long-term invasion impacts. Responses to invasion are often investigated through spatial comparisons, furthermore focusing on comparisons between invaded and uninvaded sites (e.g. Crawford, Yeomans and Adams 2006; Ercoli *et al.* 2015), but do not provide information on temporal impact patterns. Studies focussing on such temporal variations in impact are therefore crucial (Ricciardi *et al.* 2021; Galib, Findlay and Lucas 2021). Galib *et al* (2021) highlighted the importance of such work in a long-term study of signal crayfish impacts on fish populations in upland streams. They recorded significant impacts on benthic fish and young-of-year salmonids over a seven-year timescale, with a complete disappearance of bullhead in some instances. They concluded that crayfish abundance was a key factor influencing fish communities, with small benthic fish particularly vulnerable; thus highlighting the need for further quantitative investigation of density-dependent impact over long-term timescales.

5.2.2 History of impact of study system

Previous work within the Bookill Gill Beck study system (see Ch.2, Figure 2.3) has shown a negative relationship between signal crayfish and salmonids, with both salmonid abundance and recruitment severely impacted by the replacement of white-clawed crayfish with signal crayfish (Peay *et al.* 2009). A study conducted in 2009 showed that signal crayfish had already impacted fish communities in the decade following introduction (Peay *et al.* 2009). Data from the Ribble Rivers Trust and other surveys (Pritchard 2016) reported further declines in fish communities along Bookill Gill Beck at crayfish-invaded sites approaching the confluence with Long Preston Beck (Figure 5.1; Pritchard 2016). These findings suggest a strong and negative relationship between invading signal crayfish and salmonids, revealing a severe reduction in bullhead abundance and complete displacement of this species at one upstream site (Figure 5.1). This highlights how small benthic species may be particularly vulnerable to the presence of signal crayfish in upland streams, as also demonstrated by Galib *et al* (2021). Nonetheless, these relationships have been under-explored due to methodological constraints. There is therefore a crucial need for more research to understand the temporal impact patterns of signal crayfish invasions in upland streams (Galib, Findlay and Lucas 2021).

a) BGB – 350 m u/s of Confluence



b) BGB – 50 m u/s of Confluence

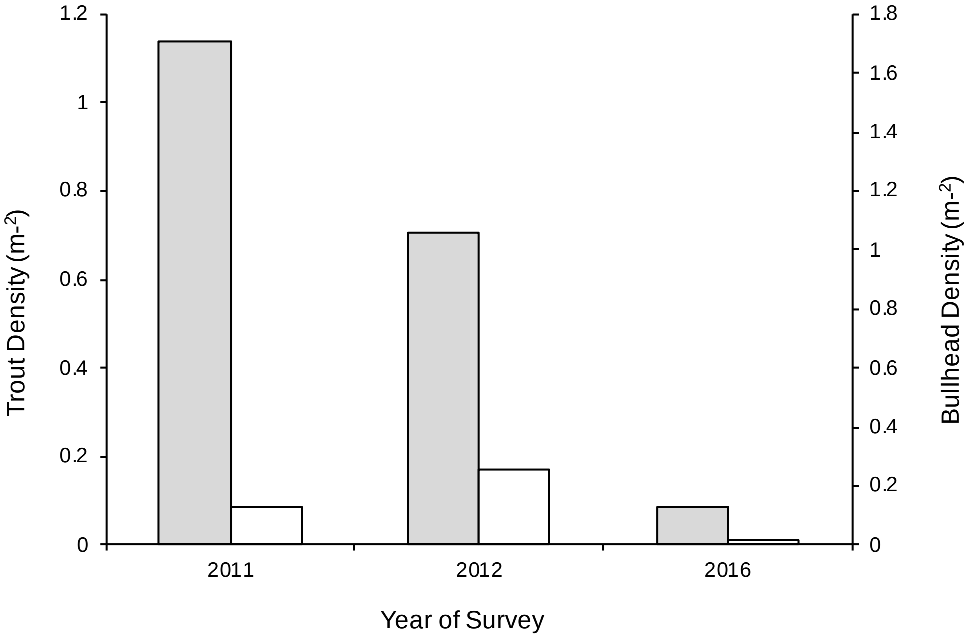


Figure 5.1 Electrofishing-derived density estimates (m^{-2}) of bullhead (grey) and brown trout (white) in 2011, 2012 and 2016 at Bookill Gill Beck (BGB); a) ~350m upstream of the confluence and b) ~50 m upstream of the confluence. Figure adapted from the author's MSc thesis (Pritchard 2016), including 2011 and 2012 data from the Ribble Rivers Trust and 2016 data originating from the author's surveys.

5.2.3 Rationale

Signal crayfish presence has been shown to negatively impact fish communities, but methodological constraints have limited the understanding of crayfish density as a driver and determinant of impact. There is a history of fish decline within the Bookill Gill Beck / Long Preston Beck study system following the introduction of signal crayfish in 1995. The survey methods developed in this project (Ch. 3) allow us, for the first time, to quantitatively assess the crayfish and benthic fish populations and provides a novel opportunity to explore the temporal ecological impact of signal crayfish along an invasion gradient.

5.2.4 Research aims and hypotheses

The first aim of this chapter was to describe the fish communities (pelagic and benthic) along the known signal crayfish invasion gradient (2018). The second aim was to investigate how invasion by signal crayfish has impacted benthic fish communities over the period 2018 - 2020.

In particular, three hypotheses were tested in this chapter:
HI. Presence of signal crayfish negatively impacts fish populations
HII. Signal crayfish density is a driver of impact on fish. Therefore, fish community structure differs along the signal crayfish invasion gradient, with a lower density of benthic fish at high density signal crayfish sites where populations are well-established.
HIII. The density of benthic fish declines at each study site over time, as the density of signal crayfish increases.

5.3 Methods

5.3.1 Study design

The study was carried out at the four sites along Bookill Gill Beck (BGB; one site – Double Gate Bridge (DGB), Ch.2 Figure 2.3) and Long Preston Beck (LPB; three sites – Confluence, Footbridge and Farm, Ch.2 Figure 2.3) over the summers (June – September) of 2018, 2019 and 2020. There is a well-defined signal crayfish invasion gradient along these sites (Table 5.1) as described in Chapter 4.

Table 5.1 Summary of signal crayfish and white-clawed crayfish density (m^{-2}) along the invasion gradient in 2018, 2019 and 2020.

Site	Crayfish Species	Crayfish density (m^{-2}) by year		
		2018	2019	2020
Double Gate Bridge	Signal crayfish	63.3	75.2	84.8
Confluence	Signal crayfish	19.9	21.2	19.7
Footbridge	Signal crayfish	7.1	10.3	15.8
Farm	Signal crayfish	0.5	0.5	3.1
Footbridge	White-clawed crayfish	0.15	0.0	0.0
Farm	White-clawed crayfish	9.1	2.1	2.2

Extensive fish surveys, including electrofishing (2018), Triple drawdowns (TDDs; 2018) and Pritchard Trap (PT) sampling (2018 and 2019) were undertaken to test the ability of PTs and TDDs to survey benthic fish and compare the effectiveness of each method (Ch. 3). These surveys generated detailed data on the community structure and population densities of benthic and pelagic fish. They also showed the ability of PTs to produce quantitative data on benthic fish with relatively low sampling effort concurrently to crayfish surveys. Given that European bullhead were by far the most abundant species in Long Preston Beck, the 2019 and 2020 surveys focussed on benthic species through repeat PT sampling. Therefore, these surveys allow for investigations of temporal changes in benthic fish populations along the signal crayfish invasion gradient.

Electrofishing and TDD surveys were undertaken as described in Chapter 3. Electrofishing operatives were Institute of Fisheries Management (IFM)-trained and used a Smith-Root 400w LR-20B Electrofishing backpack system. Stop nets (2 mm mesh size) were installed at the upstream and downstream limits of the sites to prevent the immigration or emigration of fish. A single electrofishing sweep was carried out at Confluence, while three sweeps were undertaken at Footbridge and four sweeps were undertaken at Farm (summer 2018,Table 5.2). No electrofishing surveys were undertaken at DGB. Triple Drawdowns were undertaken at all sites in 2018 (Table 5.2), comprising three consecutive sweeps at DGB and four consecutive sweeps at Confluence, Footbridge and Farm. Following electrofishing and TDDs, all fish were processed and released immediately downstream of the site. The PTs used in this study during 2019 and 2020 followed the same specifications as described in Chapter 3, providing a sampling area of 0.25 m^2 per PT. PTs were set at various locations across the sites, including riffles, pools and central channel and channel margin areas. PTs were filled using the substrate naturally occurring within the base footprint of the trap. Cobble was the dominant substrate type in all PTs.

In 2019, PTs were deployed (n = 3) and lifted on 10 occasions through the summer (once in June, five times in July, twice in August and twice more in September, n = 30, 7.5 m^2, Table 5.1). In 2020, PTs were deployed (n = 8) and lifted on three occasions through the summer (twice in August, once in September; Table 5.2). However, some PTs were interfered with, or removed from the site leaving a sampling area of 6 m^2 at DGB (n = 24), 5.75 m^2 at Confluence (n = 23), 5.25 m^2 at Footbridge (n = 21) and 5.5 m^2 at Farm (n = 22). All fish sampled in PTs were processed and then released back into the site.

Fish were captured by net or by hand and stored in fresh, well-oxygenated water in shaded buckets. All fish were identified to species level and total length (TL, mm) was recorded before all fish were released again. A method statement and FR2 fishing licence was approved by the Environment Agency for all fish surveys over the three-year period. The “Check, Clean, Dry” (NNSS, 2018) procedure was strictly followed, and all equipment was disinfected with either Virkon S Aquatic™ or FAM® 30 (iodophor based) between each use.

Table 5.2 Summary of field studies including comparisons of fish communities at sites along Bookill Gill Beck (BGB) and Long Preston Beck (LPB) in 2018, 2019 and 2020.

Sampling Method	Year	Months	Site(s)	Sample size (m^2)
Electrofishing	2018	July-August	Confluence Footbridge Farm	1 sweep (~45.5 m^2) 3 sweeps (~45.5 m^2) 4 sweeps (~50 m^2)
TDDs	2018	July - August	DGB Confluence Footbridge Farm	3 sweeps (15 ^{m2}) 4 sweeps (45.5 m^2) 4 sweeps (45.5 m^2) 4 sweeps (50 m^2)
PT (low sampling effort to assess population densities)	2018	July - August	Footbridge and Farm	n = 4 (1 m^2)
PT (repeat sampling)	2019	June - September	All	n = 30 (7.5 m^2)
PT (repeat sampling)	2020	July – September	DGB Confluence Footbridge Farm	n = 24 (6 m^2) n = 23 (5.75 m^2) n = 21 (5.25 m^2) n = 22 (5.5 m^2)

5.3.2 Data Analysis

5.3.2.1 Fish community structure (2018)

Fish data from electrofishing and TDDs in 2018 were combined for the best estimates of both benthic and pelagic fish species abundances. Brown trout and Atlantic salmon were grouped together as ‘salmonids’ for analysis. Locally derived length-weight regressions were used to calculate fish biomass (g/m^2) estimates (Chadwick, unpublished; Appendix 8). Raw capture data were used for community structure analysis, including relative abundance and biomass. Chi-squared tests were used to compare proportions of categorical data across the sites, including the number of fish of each species recorded and fish biomass. Post hoc tests were used to identify which results were significant using adjusted residual values and adjusted Bonferroni p values to allow for the number of tests run (e.g. 5 fish species categories x 3 site categories = 15 pairwise tests, 0.05/15 results in an adjusted p = 0.003). This analysis was undertaken in IBM SPSS (version 25).

5.3.2.2 Changes in benthic fish populations (2018 - 2020)

The multiple sweep TDDs (2018) allowed depletion analyses to be undertaken using the Carle Strub method (Carle and Strub 1978) in the FSA package (Ogle 2018) in R (version 3.5.1). Total fish density estimates for each benthic fish species surveyed in 2018 were calculated by summing all fish physically removed via electrofishing prior to the TDD and the total TDD-derived population estimate for each site. PTs were used to estimate benthic fish densities in 2019 and 2020 and densities across the years were compared. The size class distribution of bullhead sampled in 2018, 2019 and 2020 was also explored through bean plots in ggplot2 in R. The size class distribution of bullhead through the summer months of 2019 were also explored.

5.4 Results

5.4.1 Community structure (2018)

Six fish species were recorded in Long Preston Beck (LBP; Ch.2 Figure 2.3): brown trout, Atlantic salmon, minnow (*Phoxinus phoxinus*), stone loach (*Barbatula barbatula*), bullhead and European eel (*Anguilla anguilla*). Minnow were only recorded at Farm in 2018, whilst all other species were present at all sites on LPB. No fish were recorded at site Double Gate Bridge (DGB) on Bookill Gill Beck in 2018. In 2018, a total of 3,588 fish were sampled across the three other sites, with 443 individuals sampled at Confluence, 1,515 at Footbridge and 1,630 at Farm. Bullhead was by far the most abundant species at all sites, comprising 74.5% of the total fish specimens at Confluence (Figure 5.2a), 95.3% at Footbridge (Figure 5.2b) and 93.9% at Farm (Figure 5.2c). Of the remaining fish community, stone loach was the most abundant species, followed by salmonids, minnow (at Farm) and eel (Figure 5.2d-f).

The proportion of the fish community (counts) made up by each species differed significantly between sites in 2018 (χ^2 <0.001). At Confluence, samples contained significantly fewer bullhead, more stone loach and more salmonid specimens. At Footbridge, there were more bullhead, fewer stone loach, fewer salmonids and fewer minnow specimens recorded. At Farm, there were more bullhead, fewer stone loach, fewer salmonids and more minnow recorded when compared to surveys from the other sites.

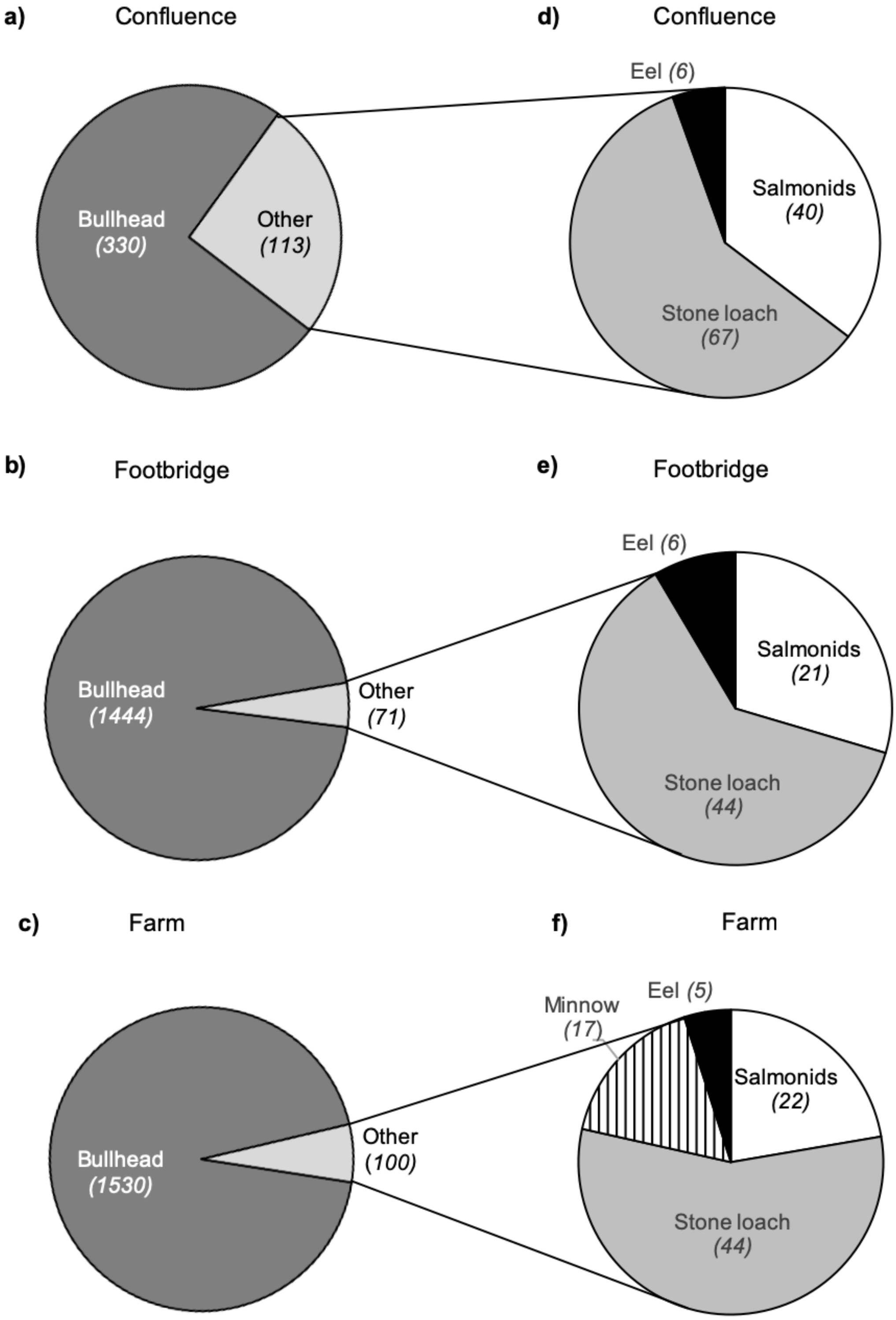


Figure 5.2 Proportion of fish abundance by species at each site in 2018, with n values denoted; Bullhead and other species (left) at a) Confluence, b) Footbridge and c) Farm; and proportion of “other” fish species (right) at d) Confluence, e) Footbridge and f) Farm.

The proportion of total fish biomass (wet weight) that each fish species contributed, was significantly different between sites (χ^2 <0.001,Table 5.3). At Confluence there was significantly less bullhead, less stone loach, more salmonid and more eel biomass present in the sampled communities. At Footbridge, there was more bullhead, less stone loach and less salmonid biomass. At Farm, samples contained a higher biomass of bullhead, stone loach and minnow, but less salmonid and eel biomass.

Table 5.3 Biomass (wet weight, g/m^2) of fish by species at each site along Long Preston Beck in 2018.

Site	Salmonids (g/m^2)	Bullhead (g/m^2)	Stone loach (g/m^2)	Minnow (g/m^2)	Eel (g/m^2)
Confluence	7.8	5.1	0.4	0	18.5
Footbridge	1.3	16.8	0.6	0	18.1
Farm	1.1	17.2	1.9	0.1	12.7

The fish density estimates, based on electrofishing catch combined with TDD-derived total estimates varied between sites in 2018 (Table 5.4). The density of salmonids was <1 m^{-2} at all sites along LPB, with the highest density at Confluence. Minnows were only present at Farm, where they also only reached low densities (0.3 m^{-2}). Benthic fish were more abundant across the sites, primarily due to a high abundance of bullhead. Eel density was consistent between the sites (0.1 m^{-2}). Stone loach density was consistent between Footbridge and Farm (~1 m^{-2}), but higher at Confluence (1.9 m^{-2}). Bullhead was the most abundant species, attaining much higher densities than the other fish species (8.4 – 34.6 m^{-2}). Despite this, there were over four times as many bullhead at Footbridge and Farm than at Confluence.

Table 5.4 Total density estimates (m^{-2}) of each fish species at sites in 2018. Estimates based on electrofishing catch combined with Triple drawdown-derived total estimates.

Site	Pelagic		Benthic			Total
	Salmonids (m^{-2})	Minnow (m^{-2})	Bullhead (m^{-2})	Stone loach (m^{-2})	Eel (m^{-2})	(m^{-2})
DGB*	0.0	0.0	0.0	0.0	0.0	0.0
Confluence	0.9	0.0	8.4	1.9	0.1	11.3
Footbridge	0.5	0.0	32.1	1.0	0.1	33.7
Farm	0.4	0.3	34.6	1.1	0.1	36.5

* There were no fish in any of the samples extracted from the DGB site.

5.4.2 Benthic fish populations (2018 – 2020)

No fish were recorded at DGB on Bookill Gill Beck in any of the 2018, 2019 and 2020 surveys. The density of benthic fish recorded in the surveys varied greatly over the three-year period at all sites along LPB (electrofishing and TDD-derived total estimates in 2018 and PTs in 2019 and 2020; Table 5.5). There was a large decline in fish abundance in 2019, followed by an increase in 2020. There was also a large variance in fish density estimates between the PT-derived fish samples in 2019 and 2020 at all sites. No eel were recorded in PTs at any time. At Confluence, the density of benthic fish dropped from 11.3 m^{-2} in 2018, to 7.9 m^{-2} in 2019, with lower and upper density estimates of 0 m^{-2} and 20 m^{-2} respectively. The density then increased to an average of 17.4 m^{-2} in 2020, with estimates ranging from 0 to 56 m^{-2}. At Footbridge, a high density of 33.7 benthic fish m^{-2} was recorded in 2018, but this decreased to 10.5 m^{-2} in 2019, with upper and lower estimates of 0 and 24 m^{-2}. In 2020, the density of benthic fish increased to 17.1 m^{-2} with lower and upper estimates of 4 and 28 m^{-2}. At Farm, a high density of 36.5 benthic fish m^{-2} was recorded in 2018, but this decreased by over 50% to an average density of 16.7 m^{-2} in 2019, with PT estimates ranging between 4 m^{-2} and 48 m^{-2}. In 2020, the benthic fish population had returned to a similarly high density to 2018 of 35.3 m^{-2}, with density estimates ranging from 20 to 72 m^{-2}.

Table 5.5 Density of benthic fish (m^{-2}) at each site along Long Preston Beck in 2018, 2019 and 2020 with standard deviation denoted below.

Site	Method	Year	Bullhead density (m^{-2})	Stone loach density (m^{-2})	Eel density (m^{-2})	Total benthic fish density (m^{-2})
Confluence	Electrofishing and TDD-derived total	2018	8.4 ± 0.69	1.9 ± 0.62	0.1 ± 0.02	11.3 ± 1.33
	PTs	2019	6.0 ± 5.43	1.9 ± 3.27	0.0 -	7.9 ± 6.84
	PTs	2020	13.9 ± 9.17	3.5 ± 5.70	0.0 -	17.4 ± 13.62
Footbridge	Electrofishing and TDD-derived total	2018	32.1 ± 0.22	1.0 ± 0.02	0.1 ± 0.00	33.7 ± 0.24
	PTs	2019	9.1 ± 6.47	1.5 ± 2.67	0.0 -	10.5 ± 6.60
	PTs	2020	14.1 ± 5.46	3.0 ± 3.56	0.0 -	17.1 ± 6.09
Farm	Electrofishing and TDD-derived total	2018	34.6 ± 1.18	1.1 ± 0.04	0.1 ± 0.02	36.5 ± 1.24
	PTs	2019	14.3 ± 10.59	2.4 ± 2.49	0.0 -	16.7 ± 10.51
	PTs	2020	34.4 ± 11.02	0.9 ± 2.11	0.0 -	35.3 ± 10.74

The size structure of bullhead populations also changed over the three-year period. In 2018, the population was largely juvenile-dominated (20 – 30 mm) at all sites (Figure 5.3 - Figure 5.5), with relatively fewer individuals in the larger size classes. In 2019, the population structure at all sites exhibited a more even distribution in terms of size class structure. In 2020, the bulk of the population were between 30 and 45 mm in length at all sites. At both Confluence (Figure 5.3) and Footbridge (Figure 5.4), there were fewer small (<30 mm) and fewer large (>60 mm) bullhead in 2020 compared to previous years and to the population structure observed at Farm in 2020 (Figure 5.5).

In 2019, bullhead density reached a minimum at all sites. The proportion of bullhead of different size classes furthermore varied throughout the 2019 summer season (Figure 5.6). At Confluence, PT samples in July were mainly comprised of small (≤ 30 mm) bullhead individuals, but contained a greater proportion of medium and large specimens (31 – 60 mm and ≥61 mm) in August and September samples. At Footbridge, the number of small bullhead was highest in July, whereas at Farm, the highest number of small bullhead were sampled in the first August PT lift. At Footbridge and Farm, animals ≥ 61 mm were sampled throughout the season, with small (≤ 30 mm) and medium-sized (31 – 60 mm) animals making up most of the catch.

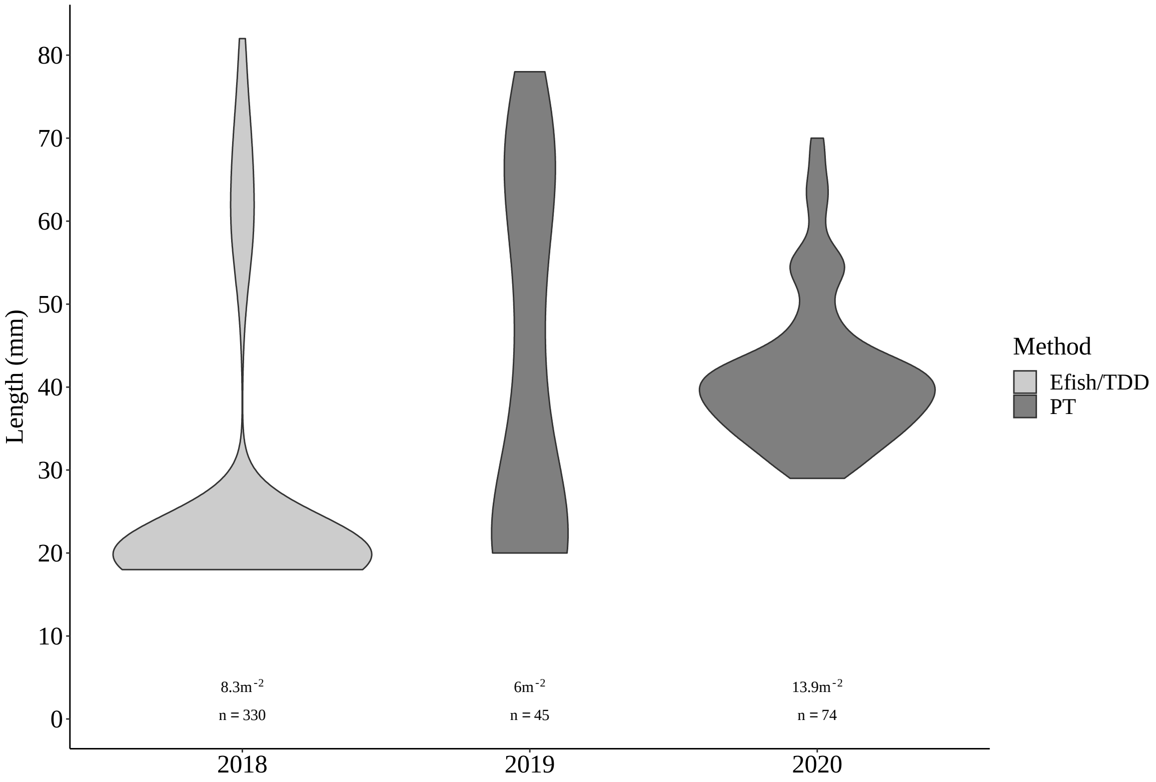


Figure 5.3 Bean plot (i.e. probability density of the catch data) of bullhead size class distribution (mm) captured through electrofishing and TDDs in 2018, and repeated PTs in 2019 and 2020 at Confluence. The density (m^{-2}) and the number of bullhead captured (n) are also denoted.

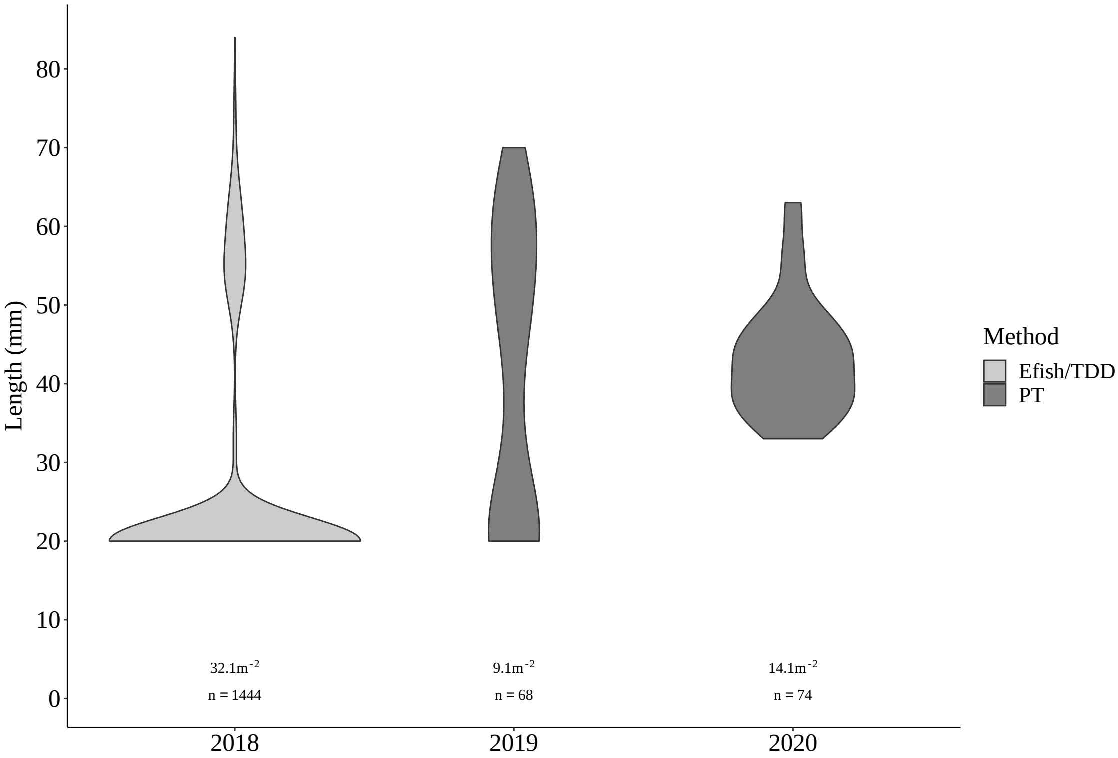


Figure 5.4 Bean plot (i.e. probability density of the catch data) of bullhead size class distribution (mm) captured through electrofishing and TDDs in 2018, and repeated PTs in 2019 and 2020 at Footbridge. The density (m^{-2}) and the number of bullhead captured (n) are also denoted.

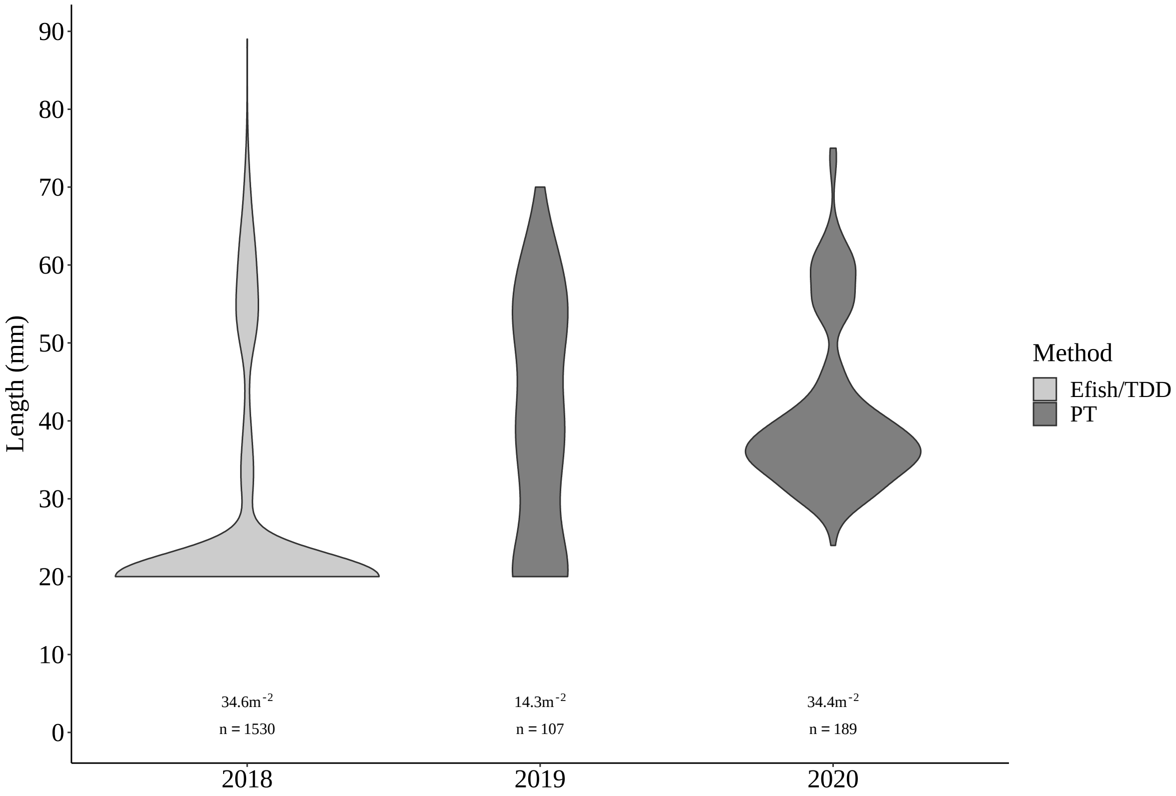


Figure 5.5 Bean plot (i.e. probability density of the catch data) of bullhead size class distribution (mm) captured through electrofishing and TDDs in 2018, and repeated PTs in 2019 and 2020 at Farm. The density (m^{-2}) and the number of bullhead captured (n) are also denoted.

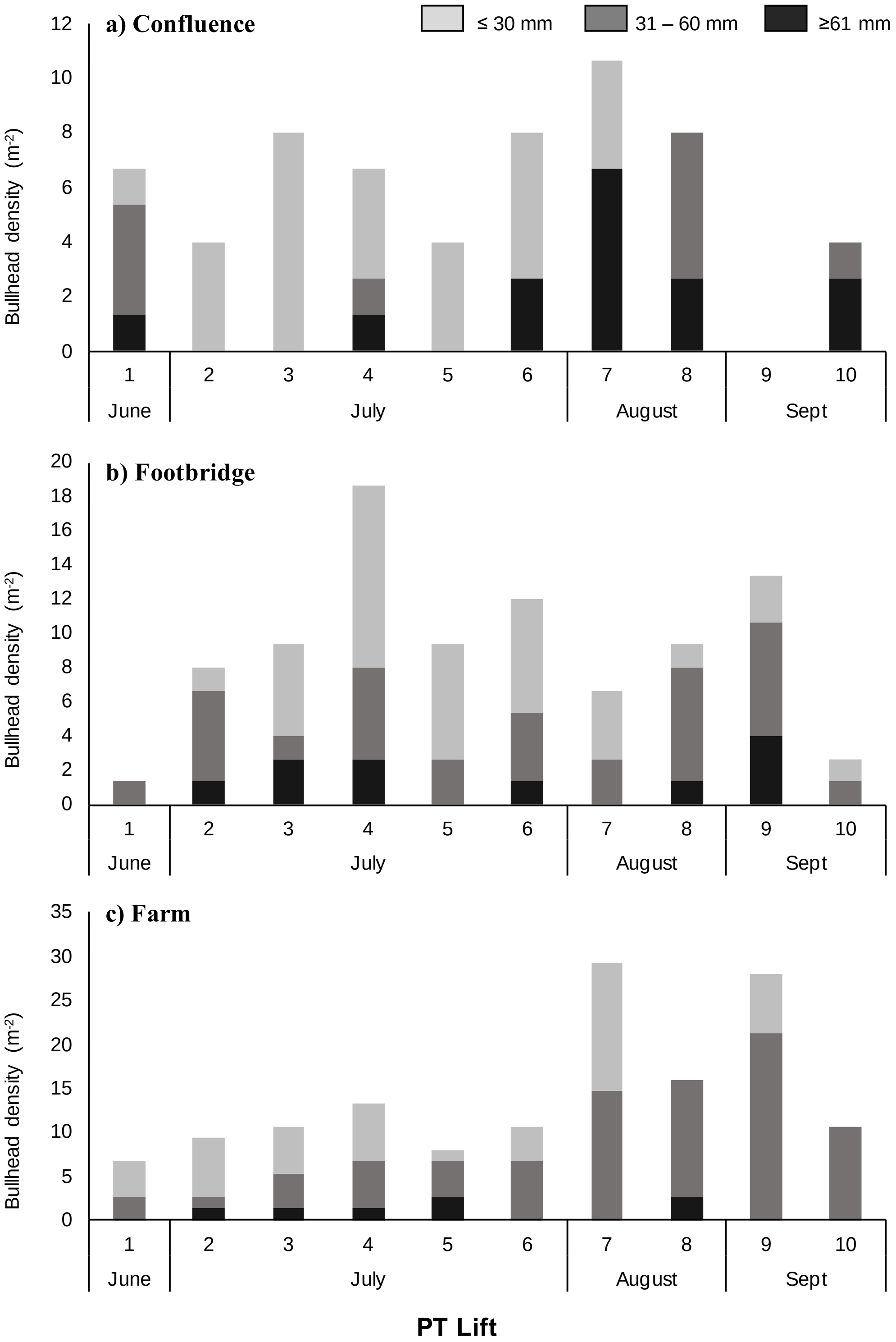


Figure 5.6 Bullhead densities by size class category; ≤ 30 mm, 31 – 60 mm and ≥ 61 mm, from PT lifts (n = 10) over summer months in 2019 at a) Confluence, b) Footbridge and c) Farm.

5.5 Discussion

5.5.1 Signal crayfish impacts on fish communities

In this study, the quantitative data on fish communities along the studied invasion gradient, as sampled in 2018, clearly supports the hypothesis that signal crayfish presence negatively impacts fish populations, in particular benthic species. The negative correlation between signal crayfish and fish observed along Bookill Gill Beck (Peay *et al.* 2009) has got progressively worse, leading to the complete displacement of all fish species along the headwater stream. The lack of fish present along BGB in all surveys since 2016 indicate that fish communities are unable to recover once signal crayfish have become hyper-dominant. Due to extreme drought conditions experienced during 2018 (National Hydrological Monitoring Programme 2018), differences in pelagic species, and especially in salmonids, should not only be attributed to differences in crayfish abundance between sites, however. Salmonids are mobile species and consequently retreat to deep, cool pools during high temperatures and low flows. A large, deep, shaded pool was situated within the Confluence site, but such habitats were absent from the Footbridge and Farm sites. This pool likely provided a key locally-important refuge for salmonids during the drought and attracted fish from a wider area and this likely explains the high number of young salmonids observed at Confluence during this year.

Benthic species such as bullhead and stone loach are less mobile and therefore the distribution was likely less affected by the drought. Thus, the abundance and distribution of fish recorded in this study likely closely resembles the local population density and structure. The hypothesis that signal crayfish density is a driver of impact on benthic fish, resulting in a lower density of benthic fish at more well-established signal crayfish sites, was supported by the results of this study. Bullhead appear to be most affected by crayfish invasion in the studied system, with, for example, only a third of the density present at the Confluence site, compared to Footbridge and Farm, which also has the highest crayfish density when compared to the other sites on Long Preston Beck (Ch. 4). The high density of benthic fish at Farm, despite a healthy population of white-clawed crayfish in 2018 (9 m^{-2}) and a mixed white-clawed crayfish and signal crayfish population at low density in 2019 and 2020 supports the theory proposed by Bubb *el al* (2009) that the high densities attained by signal crayfish provide a greater threat to bullhead than the relative impacts of signal crayfish or white-clawed crayfish at low densities.

Bullhead frequently interact with crayfish, through competition for shelter and food, and by direct crayfish predation (Bubb *et al.* 2009). These various interactions are likely impacted differently according to species' life stages, with juvenile bullhead more vulnerable to predation by large crayfish. Therefore, it is important to consider the population demographics of both the native (bullhead) and invasive (signal crayfish) components of the interaction. Population size structure and sex ratio can influence the pressures of signal crayfish invasion (Light 2003; Wutz and Geist 2013), with a higher proportion of large crayfish expected to increase the amount of fish predation (Guan and Wiles 1997). Large crayfish also tend to burrow more, therefore altering both stream communities and habitat conditions (Guan 1994). However, the signal crayfish populations sampled in our study system are dominated by juvenile size classes, with relatively few large individuals (Ch. 4). This could suggest that predation on juvenile bullhead by large crayfish does not represent the main antagonistic interaction between the species. The impact of signal crayfish on bullhead could instead be driven by indirect interactions such as competition for food and shelter. Signal crayfish have indeed already been shown to competitively displace bullhead from shelter, rendering them more vulnerable to predation, not only by large crayfish, but also by piscivorous fish (Bubb *et al.* 2009). Although cobble refugia are abundant in the study system, signal crayfish are intensively utilising this habitat across all study sites (see Ch. 4), especially when present at high densities (e.g. Confluence). This undoubtedly results in strong competition for shelter between crayfish and fish, particularly at higher-density crayfish sites. Furthermore, signal crayfish are thought to exhibit an ontogenetic shift in feeding behaviour, with juveniles preferentially selecting invertebrate-based diets (Guan and Wiles 1998). A large number of juvenile crayfish will therefore likely have a significant impact on local invertebrate populations, causing a change in their community composition and a reduction in abundance (see Ch. 6), potentially providing less food sources for bullhead.

The population structure of bullhead also varied greatly between the years, with juvenile dominated populations at all sites, demonstrating successful recruitment. In 2019, there were relatively few juveniles, indicating not only a reduced population density following the drought, but also a lack of recruitment. The lack of recruitment continued into 2020 at Confluence and Footbridge, whereas Farm showed some evidence of small individuals within the population again. Bullhead life history traits including age, growth rates and reproduction, vary greatly in different habitat types. In upland streams, young-of-year bullhead will grow rapidly, often reaching 50 mm in their first year, yet may only live to three or four years old, compared to four to ten years old in soft, lowland waters (Mills and Mann 1983). With fewer breeding cohorts present in the population (1 – 4 years old, compared to 1 -10 years), it may become more difficult for bullhead populations to recover from disturbances that reduce recruitment in a given year e.g. the 2018 drought. Furthermore, if signal crayfish are predating on juvenile bullhead (Guan and Wiles 1998), reduced numbers reaching sexual maturity will not only reduce the current population density, but will also reduce recruitment in the future. Therefore, life history traits may deem bullhead populations in headwater streams more vulnerable to impact from signal crayfish, especially in combination with extreme flow regimes.

Given that benthic fish and crayfish occupy such similar niches, there may be a combined carrying capacity for such taxa in river ecosystems. In support of this assumption, combined densities of signal crayfish and benthic fish were observed at around 30 to 40 individuals m^{-2} in ‘normal’ years, and a lower combined density of 20 to 30 individuals m^{-2} during the drought at sites along

Long Preston Beck (Table 5.6). Although, in contrast to this, signal crayfish densities at DGB on Bookill Gill Beck remained high and stable throughout the drought period (63 – 85 m^{-2}; Ch. 4). At Confluence, signal crayfish were more abundant than fish during the three years, whereas at Farm, fish were far more abundant than crayfish (Table 5.5). At Footbridge, however, a shift from fish to crayfish dominance was observed over the three-year period (Table 5.5). This theory of a combined carrying capacity could also translate to a combined capacity of biomass, or metabolic rate, which may explain the exceptionally high densities at DGB, if biomass is comparable to sites on the Long Preston Beck. Once signal crayfish become dominant, they may continue to outcompete and displace fish entirely, as seen upstream along Bookill Gill Beck (Pritchard 2016) and also reported in other upland streams (Galib, Findlay and Lucas 2021).

Table 5.6 Summary of signal crayfish, white-clawed crayfish and benthic fish densities at sites along BGB and LPB in 2018, 2019 and 2020. Colour codes represent invasive (red) and native (blue) species and the dominant taxa of the combined total.

Site	Year	Signal crayfish density (m^{-2})	White-clawed crayfish density (m^{-2})	Benthic fish density (m^{-2})	Total combined density (m^{-2})
DGB, BGB	2018	63	0	0	63
	2019	75	0	0	75
	2020	85	0	0	85
Confluence	2018	20	0	11	31
	2019	21	0	8	29
	2020	20	0	17	37
Footbridge	2018	7	0.1	34	41
	2019	10	0	10	20
	2020	16	0	17	33
Farm	2018	0.5	9	36	45
	2019	0.5	2	17	19
	2020	3	2	35	40

5.5.2 Influence of 2018 drought

The hypothesis that benthic fish density would decline progressively in subsequent years (2018 to 2020) was not supported by the results of my study. Instead, a decline in 2019 was observed, followed by a recovery in population numbers by 2020. This decline and recovery is likely due to the severe local drought conditions experienced in summer of 2018. River flow levels in Northern England, including the Ribble catchment, were classified as ‘notably low flow’, or ‘exceptionally low flow’ in 2018 based on hydrological data for 1981 to 2010 (National Hydrological Monitoring Programme 2018). Hydrological variability plays an important role in structuring aquatic ecosystems (Poff 2018). Whilst native biota have adapted to natural variations in flow and have the resilience to survive extreme events, such as droughts (Bogan, Boersma and Lytle 2015), biological invasions, as for signal crayfish in small streams, may act as additional stress factors, that could exacerbate the impact of low flow impacts (Mathers, White, Fornaroli, *et al.* 2020). Interestingly, whilst fish densities returned to a high density at Farm in 2020 (~35 m^{-2}) that were comparable to 2018 values, the population at Footbridge only returned to roughly half the 2018 density, reaching values more similar to Confluence in 2020. During this time period, the density of signal crayfish at Footbridge more than doubled, from approximately 7 to 16 crayfish m^{-2} (see Ch. 4 for more detail). Thus, whilst drought conditions likely caused a temporary reduction in fish numbers, signal crayfish were seemingly unaffected by these conditions, with densities seen to increase between 2018 and 2019 (Ch. 4). Indeed, invasive crayfish, including signal crayfish, have been shown to be highly tolerant to low-flows, stream drying and drought events (Larson *et al.* 2009; Kouba *et al.* 2016). In this study signal crayfish clearly showed this expected resilience to low flow conditions, but also the decrease in fish during the low flow interval may have positively affected the crayfish, resulting in less competition and predation of juvenile crayfish. The remaining fish will have been concentrated in pools and therefore predation on juvenile fish by crayfish may have also increased. Drought may therefore have enhanced signal crayfish population growth at Footbridge to a degree that, by the time fish populations could recover, signal crayfish had become dominant, limiting the recovery of fish numbers to lower densities (17 m^{-2}) than previously attained (34 m^{-2}). In fact, in 2020, the density of signal crayfish was greater than, or approximately equal to, the density of benthic fish at both Confluence and Footbridge. This theory is partially supported by Mathers *et al* (2020), who found that low-flow events potentially exacerbated the effects of signal crayfish on macroinvertebrate communities and facilitated the spread and establishment of signal crayfish. It is possible that extreme weather events that are becomingly increasingly severe and frequent (Fischer, Sippel and Knutti 2021) could potentially facilitate the establishment and ecological impact of invasive species such as crayfish by temporarily decreasing predation pressure from piscivorous fish and competition with benthic fish.

5.5.3 Implications for conservation and management

This study shows that invasive signal crayfish can severely impact fish communities in upland headwater streams with effects potentially exacerbated by drought, which may accelerate the impact of signal crayfish on fish. Headwater streams are important habitats for fish communities, providing vital spawning grounds for salmonid fish. Signal crayfish invasions clearly pose a significant threat to headwater systems and associated fish communities, with substantial consequences for ecosystem functioning and recreational angling (Peay *et al.* 2009).

The impact that signal crayfish invasions can cause on bullhead populations is a serious concern. Bullhead are a protected species and of high conservation importance. They are listed as a Special Area of Conservation (SAC) Annex II species, listed on Annex II of the European Commission Habitats Directive and listed on the International Union for Conservation of Nature (IUCN) Red List of Threatened Species (Habitats Directive 1992; Freyhof 2011). Galib *et al.* (2021) also recorded severe declines and even complete disappearance of bullhead at sites invaded with signal crayfish in the River Tees catchment, Northern England. This highlights that this phenomenon is not limited to the Bookill Gill Beck / Long Preston Beck system and is occurring elsewhere.

Further work is urgently needed to assess the scale of impact that signal crayfish have on native fish communities, especially over long timescales. There are currently no effective means to eradicate or manage signal crayfish populations in the wild, and no clear natural declines due to boom-bust population dynamics or in response to drought (Ch. 4) are apparent in this species. Further research is required to better understand the density-dependent ecological impacts that signal crayfish have on native ecosystems. Isotopes and molecular tools could be used to investigate predation as a driver of antagonism between species. Enclosure trials could be established to observe the interactions between crayfish of different sizes and densities with fish species to better understand the mechanisms driving the impact. Monitoring at the study system should be continued and the approach adopted here of using quantitative data to assess both crayfish and benthic fish population demographics should be applied to monitoring on a wider geographical scale.

Chapter 6. Signal crayfish impacts on macroinvertebrate communities

6.1 Summary

1. Signal crayfish are known to negatively impact macroinvertebrate communities both directly through predation and competition, and indirectly through trophic cascades and habitat degradation. Previous work has focussed on comparisons between sites where crayfish are present and absent, or on temporal comparisons of sites pre- and post-invasion. The development of robust, quantitative crayfish survey methods now allows for a comprehensive exploration of crayfish density as a driver to impacts on macroinvertebrate communities.
2. Macroinvertebrate communities differed strongly along the invasion gradient of Bookill Gill Beck and Long Preston Beck, with a severely depleted community at the high-density signal crayfish site, with decreased species diversity, richness, abundance and biomass. The depleted community was dominated by mainly small-bodied, fast-moving taxa in the families *Heptageniidae* and *Baetidae,* suggesting a higher tolerance to signal crayfish even at high densities, and potentially beneficial effects from the absence of other predatory invertebrates.
3. The differences in macroinvertebrate communities at sites along LPB were more subtle than BGB, but there was a temporal shift to a more homogenous community composition at all sites by 2020. Species richness declined between 2018 and 2020, but the increase in numbers of certain taxa such as Ephemeroptera resulted in similar overall abundance and biomass between the two time periods. However, the differences between LPB sites were not as great as expected given the wide variation in signal crayfish density recorded for these sites.
4. The relatively short time since signal crayfish invasion of LPB may play an important role in explaining these observations, with impacts taking longer to become apparent as the population of signal crayfish establishes and grows. Furthermore, individual responses to crayfish invasion may affect populations, but may not translate to community-level impacts until the population-level effects become more extreme.

Environmental conditions may play a significant role in determining the level of impact observed, with the 2018 drought potentially exacerbating the impact at low-density signal crayfish sites.

5. The 2018 drought did not appear to affect water quality, and biomonitoring scores indicated good water quality and environmental conditions across the study sites. However, the decline in low-scoring taxa as a result of the signal crayfish invasion may have affected some scores as the methods do not take such changes into account. This questions the reliability and effectiveness of biomonitoring indices in heavily invaded sites and has implications for the interpretation of such scores when determining environmental stressors and disturbances. The incorporation of invasive species into existing biomonitoring indices, or the development of invasive species-specific metrics, is therefore recommended as a priority for the monitoring and management of freshwater systems.
6. Overall, I found that signal crayfish invasion can negatively impact macroinvertebrate communities, with particularly severe impact at high-density signal crayfish sites that appear long-term and irreversible. However, levels of impacts can be context-specific with factors like time since invasion and abnormal water levels affecting the overall condition of macroinvertebrate communities.

6.2 Introduction

6.2.1 Impact of invasive crayfish on macroinvertebrates

Signal crayfish have a broad polytrophic diet, as they consume detritus, macrophytes, macroinvertebrates, fish and other crayfish (Momot, Gowing and Jones 1978; Guan and Wiles 1998; Nyström and Strand 1996; Dorn 2013; Houghton, Wood and Lambin 2017). Crayfish also exhibit ontogenetic shifts in feeding behaviour, with juveniles typically consuming more invertebrates and adults consuming more plant-based material. Signal crayfish are also ecosystem engineers, and their burrowing and foraging behaviour can modify habitats (Harvey *et al.* 2011; Sanders, Rice and Wood 2021) and trigger trophic cascades (Creed and Reed 2004), for example by altering detrital processing rates and reducing macrophyte cover (Nyström and Strand 1996; Creed and Reed 2004; Dorn and Wojdak 2004). Overall, signal crayfish therefore have the potential to impact benthic macroinvertebrate communities both directly through predation and activity (Mathers, Rice and Wood 2018; Beatty *et al.* 2020), and indirectly through alterations of habitats and trophic resources (Creed and Reed 2004). Indeed, there is a large body of evidence to show that the invasion and establishment of signal crayfish results in negative impacts on macroinvertebrate communities.

Invasive crayfish have been documented to reduce overall macroinvertebrate species richness and abundance (Crawford, Yeomans and Adams 2006; Ercoli *et al.* 2015). Yet, taxon-specific impacts are also commonly reported, typically with a reduction of large-bodied, slow moving taxa such as Gastropods (Nyström *et al.* 2001; Dorn 2013) and Trichoptera (Ercoli *et al.* 2015). Crawford *et al.* (2006) found a severe negative impact on macroinvertebrates in a study that compared communities at invaded and uninvaded sites in the headwaters of the River Clyde, Scotland. They found that invertebrate density was 40% lower at signal crayfish sites compared to uninvaded sites, and again with taxon-specific variations in observed impacts. The species richness of Plecoptera, Chironomidae, Diptera and

Hirudinea was lower at invaded sites, with reduced densities of Plecoptera, Hirudinea, Tricladida and Hydracarina, all indicative of these selective taxon-specific effects (Crawford, Yeomans and Adams 2006). Galib *et al.* (2021) also found an overall reduction in taxonomic richness and shift in community structure following the invasion of signal crayfish in upland tributaries of the River Tees in Northern England. Changes were strongly attributed to declines in chiefly sedentary taxa such as case-bearing Trichoptera and molluscs.

A long-term study conducted by Mathers *et al*. (2016) exploring the temporal and spatial extent of signal crayfish impacts in English lowland rivers again found significant changes in macroinvertebrate community composition following signal crayfish invasion. Results were consistent through seasons, although with strongest alterations recorded during autumn months. This suggests that sampling period could be an important factor to consider when quantifying crayfish invasion impacts. Again, a selective effect on certain taxa was found, with community shifts due to declines in Hirudinea, Gastropoda, Ephemeroptera (Caenis spp.) and Trichoptera. The changes in community composition appeared persistent, with no sign of recovery during the study timeframe, indicating that signal crayfish can permanently impact invaded ecosystems (Mathers *et al*. 2016). Subsequent work by Mathers *et al.* (2020a, b) indicated that the functional composition of macroinvertebrate communities is also effected by signal crayfish, and that additional stressors such as low flow may facilitate the expansion of signal crayfish, exacerbate invasion impacts on the native biota and limit recovery.

In summary, signal crayfish can cause a decrease in macroinvertebrate species richness, diversity and abundance, causing an overall change in community composition with no sign of recovery over time (Guan and Wiles 1998; Crawford, Yeomans and Adams 2006; Mathers *et al.* 2016). However, given previous methodological constraints, these studies do not incorporate the local density of invasive crayfish populations and its role in determining the level of impact observed. Crayfish density, but also their population structure - especially given the ontogenetic shift in crayfish feeding preferences - are nonetheless likely key determining factors when quantifying and understanding the ecological impact of their invasion. Similarly, work exploring variations of the invasion impact along the invasion gradient is limited, too. However, this work is extremely important to better understand the processes by which crayfish become dominant, and which species become vulnerable to impact at different stages of the spatio-temporal invasion trajectory.

6.2.2 Biomonitoring tools

Macroinvertebrate communities are important indicators of ecological and hydrological quality (Cairns and Pratt 1993; Hawkins *et al.* 2000). The life history and functional traits of particular taxa mean they are more tolerant of, or sensitive to specific conditions. Therefore, the presence, and in some cases also the abundance, of particular taxa can provide valuable insight into the broader, long term environmental conditions within a watercourse which can directly inform management and conservation (Jandry *et al.* 2014). As a result, a wide range of biomonitoring tools and indices have been developed that rely on assessments of macroinvertebrate communities in order to identify and quantify a range of stressors and disturbances to aquatic habitats (Bonada *et al.* 2006). The Biological Monitoring Working Party (BMWP) index, established in the UK, is a typical example that is widely used (Chesters 1980; Arslan *et al.* 2016). The BMWP scores macroinvertebrate families based on their sensitivity to pollution, producing an overall score (sum of the values for all families present) from which water quality is inferred (e.g. > 100 indicates clean , < 10 indicates heavily polluted) (Chesters 1980). Other derivatives of the BMWP include the number of BMWP scoring families present, and the average sensitivity of the families present as the Average Score Per Taxon (ASPT). Further biomonitoring indices have been developed to identify specific stressors, including The Lotic Invertebrate index for Flow Evaluation (LIFE) and the Proportion of Sediment-sensitive Invertebrates (PSI) index. The LIFE index quantifies hydrological regime pressures such as low flows (Extence, Balbi and Chadd 1999) and droughts, while the PSI index quantifies the level of fine sediment transported by the river system (Extence *et al.* 2013).

Biomonitoring indices are commonly employed by statutory and conservation organisations such as the Environment Agency and Rivers Trusts alike, to monitor the status of lotic systems across the UK. However, the changes in macroinvertebrate communities triggered by invasions of non-native species like signal crayfish, as outlined above, could potentially compromise the effectiveness of biomonitoring tools and the use of macroinvertebrates as bioindicators (Macneil *et al.* 2013; Mathers *et al.* 2016; Guareschi *et al.* 2021). My research presents an important opportunity to test the impact that invasive species can have on the reliability of biomonitoring tools – not only in terms of presence of invasive species per se, but also in view of potential population thresholds. My study sites are situated in relatively close proximity of approximately 3 km along a connected river system. Given that the land use does also not change significantly along this river system, water quality and environmental conditions can be assumed to remain stable, with the substantial signal crayfish invasion gradient from well-established high-density populations down to the invasion front likely representing the strongest driver of changes in macroinvertebrate communities. Therefore, differences in biomonitoring index scores that might result from these community changes will likely be due to this signal crayfish invasion gradient, rather than to changes in water quality.

6.2.3 Rationale

There is an array of convincing evidence that signal crayfish invasions alter the invertebrate community structure in river systems. However, previous work has chiefly used either spatially (crayfish presence / absence) or temporal (pre- and post-invasion) binary comparisons (Crawford, Yeomans and Adams 2006; Mathers *et al.* 2016). The development of quantitative sampling methods (Ch. 3) now provides the exciting opportunity to directly explore crayfish density as a driver of impact on invertebrate communities.

6.2.4 Research aims and hypotheses

The aim of this chapter was to investigate the impact of signal crayfish on macroinvertebrate communities along an invasion gradient, with an additional temporal dimension.

In particular, the following hypotheses were tested in this chapter:

HI. Signal crayfish negatively impact invertebrate communities, resulting in a reduction in macroinvertebrate species richness, species diversity, abundance and biomass with an increasing density of signal crayfish, with the strongest impact observed at the highest-density signal crayfish site.

HII. Signal crayfish will cause a change in the relative proportions of functional feeding groups in macroinvertebrate communities along the invasion gradient. Signal crayfish are ecosystem engineers and their activity can influence the functioning of an ecosystem.

HIII. The temporal changes in macroinvertebrate community structure vary along the invasion gradient. Invertebrate community composition will remain stable at well-established signal crayfish sites. Whereas, community composition will change over the three year time period at sites where signal crayfish are less well-established, particularly the invasion front.

HIV. Biomonitoring indices such as the BMWP and the associated ASPT scores reflect the degradation of the invasive species on the system, and the impact signal becoming stronger with increasing crayfish population densities.

6.3 Methods

6.3.1 Experimental design

Invertebrate samples were collected using a Surber sampler (dimensions 25 cm x 25 cm x 25 cm, 0.0625 m^2 sampling area, 500 µm mesh size), in the summer period of 2018 and 2020. In 2018, Surber samples (n = 10) were collected at Confluence, Footbridge and Farm (Ch. 2 – Figure 2.3). Surber samples were not collected at Double Gate Bridge (DGB) in 2018 due to inadequate sampling conditions (no flow) due to the drought. To substitute for the lack of samples collected from DGB in 2018, Surber samples (n = 10, same Surber sampler specifications) from the DGB site collected in 2016 (Chadwick 2019) were included in the analysis. In 2020, Surber samples (n = 10) were collected at DGB, Confluence, Footbridge and Farm. All Surber samples were collected during the summer period of July - August. The datasets from the two years (2016/2018 and 2020) allow the investigation into differences in invertebrate communities along an invasion gradient on a spatial scale (differences between sites) and a glimpse into temporal changes within sites as the signal crayfish population grows and its density increases (Table 6.1) as described in Chapter 4.

Table 6.1 Summary of signal crayfish and white-clawed crayfish density (m^{-2}) along the invasion gradient in 2018, 2019 and 2020.

Site	Crayfish Species	Crayfish density (m^{-2}) by year		
		2018	2019	2020
Double Gate Bridge	Signal crayfish	63.3	75.2	84.8
Confluence	Signal crayfish	19.9	21.2	19.7
Footbridge	Signal crayfish	7.1	10.3	15.8
Farm	Signal crayfish	0.5	0.5	3.1
Footbridge	White-clawed crayfish	0.15	0.0	0.0
Farm	White-clawed crayfish	9.1	2.1	2.2

6.3.2 Invertebrate sampling and identification

Surber sample locations were randomly selected using a random number generator to create coordinates within the site (channel width 1 – 4 and length along site 1 – 50) to avoid sampling bias. The metal frame of the Surber sampler was pressed into the channel substratum, cobbles were washed into the mesh bag and the substrate was disturbed for 90 seconds. The sample was then transferred into a sample pot (500 ml) and labelled with date, site and sample number. Any fish or crayfish encountered in the samples were removed on site to prevent any predation and thus deterioration of samples prior to sorting.

In 2018, samples were stored in the fridge and live-picked within 24 hours of collection, with specimens stored in Industrial Methylated Spirits (IMS) at a 2:1 ‘preservative to sample’-ratio. Samples were identified using a Leica MZ 95 microscope plugged into a CLS 150 xe light source in the King’s College London (KCL) laboratory in Autumn 2018. The 2016 DGB Surber samples (n = 10) were collected in the same manner, with randomly selected sample locations at DGB, 90 second sampling and samples live picked at the Field Studies Council (FSC) Malham Tarn Field centre laboratory by Chadwick and Pritchard. Samples were stored in IMS and identified by Chadwick in the KCL laboratory using the same Leica microscope (Chadwick 2019).

In 2020, samples were mixed with IMS on site, as Covid-19 restrictions did not permit immediate laboratory access. Samples were then picked over the following month and stored in IMS at a 2:1 ratio. Samples were identified using an Olympus SZ61 microscope with additional light source in the PBA Applied Ecology Ltd laboratory in Spring 2021. Relevant keys and literature were used to identify individuals to species level wherever possible (e.g. Dobson and Crowden 2012). However, species level-identification was not always possible due to the level of expertise required and damage encountered to some specimens that resulted in key identifying features missing, and thus the lowest possible level of identification was used for each specimen. In this context, most Diptera (true flies) were identified to family level, only. *Chironomidae* were identified to tribe level (e.g. *Tanypodinae*), *Pediciidae* were identified to genus (e.g. *Dicranota, Pedicia*), as were *Antocha* of the family *Limoniidae*. Identification was quality-assured by a set of experienced colleagues (Dr D. Chadwick, Dr D. Mills, Dr M. Chadwick) and with checks against the National Biodiversity Network (NBN) Atlas records, which provide up to date species distribution data in the UK (https://nbnatlas.org/). The total body length (to the nearest 0.5 mm) was recorded for each specimen to allow for biomass estimates to be calculated using published length-weight regression equations. Following identification, voucher specimens were stored in individual scintillation vials in IMS, and the rest of the sample was stored in a labelled vial in IMS.

6.3.3 Data analysis

Data sorting

In an excel database, density values were calculated as the number of individuals per m^2 (Surber sampling area multiplied by 16 for m^2 estimates) for each Surber sample, site and year. A full species list / taxon list was generated in the process (Appendix 9). Biomass (Ash Free Dry Weight, AFDW, mg) was calculated for each specimen using the total body length and published length-weight regression equations (Smock 1980; Benke *et al.* 1999; Baumgärtner and Rothhaupt 2003; Stagliano and Whiles 2008; Edwards, Jackson and Somers 2009; Greiner, Costello and Tiegs 2010). The biomass of each taxon per Surber was multiplied by 16 to give a biomass estimate of mg/m^2.

Species/taxa richness and diversity

Total species richness for taxa identified to species level was estimated using the Chao2 estimator in EstimateS (Colwell and Coddington 1994; Colwell and Elsensohn 2014). Chao2 was selected as a non-parametric estimator based on incidence data. Simpsons diversity (Equation 1) and Shannon’s diversity (Equation 2) were used as complementary indices of evenness and richness. Both Shannon and Simpsons diversity were calculated in EstimateS.

$$l = \frac{\sum_{l=1}^{s} ni(ni-1)}{N(N-1)} \qquad (1)$$

$$D_s = 1 - l$$

Equation 1. Simpons diversity (l) as measure of evenness, where s is the number of classes observed, n_i is the number observed from the ith class and N is the total number of individuals observed in the sample. D_s is the probability that two randomly sampled individuals are from two different classes.

$$H' = \sum_{i=1}^{s} \frac{n_i}{N} \ln\left(\frac{n_i}{N}\right) \qquad (2)$$

Equation 2. Shannon's index of diversity combining evenness and richness (H'), where s is the number of classes observed, n_i is the number observed from the ith class and N is the total number of individuals observed in the sample.

It is important to note that only species-level data was used for analysis in EstimateS, i.e. all taxa where species-level identification was impossible were excluded from this analysis. The sample order was randomised and 1000 randomised runs were run when computating species richness estimates and diversity indices.

Invertebrate density and biomass

Overall macroinvertebrate density (individuals m^{-2}) and biomass (AFDW, mg/m^2) were calculated for each Surber sample (n = 10) at each site, in each sampling year. The significance of differences in overall macroinvertebrate density and biomass were examined using Kruskal-Wallis Tests, with additional pairwise comparisons to identify specific differences between sites and years. Significance values were adjusted using the Bonferroni correction factor. Analyses were undertaken in IBM SPSS version 27.

Functional Feeding Groups

Macroinvertebrate taxa were sorted into five main ‘Functional Feeding Groups’, including ‘Collector’, ‘Shredder’, ‘Scraper’, ‘Predator’ and ‘Parasite’, following Cummins (1973) and Tatchet *et al.* (2000). The relative proportions of each functional feeding group were calculated by the abundance of each group, and also the biomass.

Community composition

Differences in macroinvertebrate community composition by site and by year were explored using Non-metric Multidimensional Scaling (NMDS) based on Bray-Curtis similarity coefficients. NMDS-derived stress values <0.2 indicate non-random distribution, with the ordination plot in these cases providing a good representation of the overall differences in communities between samples. A one-way ANOSIM (analysis of similarities) was undertaken to examine whether ‘Site’ or ‘Year” were drivers of differences in the communities. ANOSIM-derived P and R values were examined to determine significance ($p < 0.05$) and level of separation between groups (R; $R<0.25$ indicates barely distinguishable groups, $R = 0.25 - 0.75$ indicates separate groups with overlapping values, and $R > 0.75$ indicates a strong separation to exist between individual groups, Mathers *et al.* 2016). SIMPER (similarity percentage) analyses were then undertaken to identify specific taxa contributing to the differentiation of communities between sites and years. NMDS analyses were undertaken using the density (m^{-2}) and biomass (AFDW, mg/m^2) of all recorded macroinvertebrate taxa in the individual Surber samples to explore macroinvertebrate community composition. Statistical analysis to explore the community composition, including NMDS, ANOSIM and SIMPER, were performed in R using the vegan package (Oksanen 2018), and graphical representations were generated using ggplot2 (Wickham 2016).

Biomonitoring indices

The biomonitoring scores are designed to detect variations in water quality and environmental stressors such as pollution and sedimentation. Water quality is consistently high across all study sites (Ch 2), so biomonitoring indices and scores were determined in order to explore whether signal crayfish were affecting the efficiency or reliability of biomonitoring indices. Data was organised as counts per family at each site in each sampling year, using “NA” if there were no records for a specific family at the site. The data was analysed using the biotic package in R (Briers 2016) to calculate the Biological Monitoring Working Party (BMWP), Average Score Per Taxon (ASPT), number of scoring taxa, Proportion of Sediment-sensitive Invertebrates (PSI) and Lotic-invertebrate Index for Flow Evaluation (LIFE) scores. The BMWP scoring system produces a BMWP score (0 – 100+) that falls within a category (very poor, poor, moderate, good and very good) that can be used to infer the level of pollution or impact. The ASPT score can vary from 0 (grossly polluted) to 6+ (excellent quality). LIFE scores are calculated for a sample from the sum of the individual species/family flow scores divided by the number of scoring species/families. LIFE scores <6 generally indicate slow or still water conditions and scores >7.5 indicate very fast flows. PSI scores range from 0 to 100, with categories (0-20, 21-40, 41-60, 61-80 and 81-100) indicative of river bed condition. The lower the score, the more heavily sedimented.

6.4 Results

A total of 7,548 macroinvertebrate specimens were included in the analysis of this study. These include 192 records from the 2016 DGB data (Chadwick 2019) and 7,356 collected, identified and measured in the framework of this project (DGB in 2020 and Confluence, Footbridge and Farm in 2018 and 2020). A total of 15 different Orders/Classes were present across the system (Table 6.1), with Ephemeroptera being the most abundant Order, accounting for 32.1% of all specimens, followed by Diptera (25.0%) and Plecoptera (14.4%). The relative proportion of different Orders varied strongly between sites and years (Table 6.2). For example, Ephemeroptera were dominant at DGB in both 2016 and 2020 (59.6 – 74.0%), and they increased in dominance at the other sites between 2018 (11.0 – 34.3%) and 2020 (22.0 –

47.9%). Gastropods were generally scarce at all sites (0 – 0.1%) except Farm, where they comprised 16.7% of individuals in the 2018 sample. However, this value declined sharply to only 1.5% in 2020. Furthermore, Odonata and Megaloptera were only sampled at Farm in 2018.

Table 6.2 Summary of macroinvertebrate Orders present at each site in each year. The number of animals from the Surber samples (n = 10) at each site is presented, and the percentage of the sample total (Site per year) that each Order represents is also presented in parentheses (%).

Class/Order	DGB		Confluence		Footbridge		Farm	
	2016	2020	2018	2020	2018	2020	2018	2020
Amphipoda	1 (0.5 %)	0 (0 %)	23 (2.6 %)	42 (3.2 %)	3 (0.4 %)	21 (1.4 %)	23 (2.5 %)	18 (1.6 %)
Coleoptera	0 (0 %)	2 (0.2%)	143 (16.5%)	140 (10.7 %)	50 (7.0 %)	116 (7.7 %)	155 (16.6 %)	297 (25.6 %)
Diptera	2 (1%)	94 (10.8 %)	236 (27.2 %)	473 (36.3 %)	130 (18.2 %)	422 (27.9 %)	199 (21.3 %)	333 (28.7 %)
Ephemeroptera	142 (74.0 %)	517 (59.6 %)	98 (11.3 %)	341 (26.2 %)	245 (34.3 %)	724 (47.9 %)	103 (11.0 %)	255 (22.0 %)

Gastropoda	0 (0 %)	0 (0 %)	1 (0.1 %)	0 (0 %)	1 (0.1 %)	1 (0.1 %)	156 (16.7 %)	17 (1.5 %)
Hemiptera	0 (0 %)	0 (0 %)	34 (3.9 %)	0 (0 %)	72 (10.1 %)	0 (0 %)	1 (0.1 %)	0 (0 %)
Hirudina	0 (0 %)	4 (0.5 %)	1 (0.1 %)	0 (0 %)	0 (0 %)	0 (0 %)	3 (0.3 %)	15 (1.3 %)
Megaloptera	0 (0 %)	0 (0 %)	0 (0 %)	0 (0 %)	0 (0 %)	0 (0 %)	3 (0.3 %)	0 (0.0 %)
Odonata	0 (0 %)	0 (0 %)	0 (0 %)	0 (0 %)	0 (0 %)	0 (0 %)	1 (0.1 %)	0 (0 %)
Oligochaeta	0 (0 %)	20 (2.3 %)	29 (3.3 %)	14 (1.1 %)	37 (5.2 %)	27 (1.8 %)	89 (9.5%)	48 (4.1 %)
Plecoptera	46 (24.0 %)	225 (26.0 %)	126 (14.5 %)	197 (15.1 %)	134 (18.8 %)	137 (9.1 %)	87 (9.3 %)	133 (11.5 %)

Sphaeriida	0 (0 %)	0 (0 %)	1 (0.1 %)	1 (0.1 %)	1 (0.1 %)	0 (0 %)	5 (0.5 %)	0 (0 %)
Trichoptera	1 (0.5 %)	5 (0.6 %)	101 (11.6 %)	96 (7.4 %)	36 (5.0 %)	62 (4.1 %)	69 (7.4 %)	43 (3.7 %)
Tricladida	0 (0 %)	0 (0 %)	0 (0 %)	0 (0 %)	0 (0 %)	0 (0 %)	18 (1.9 %)	0 (0 %)
Trombidiformes	0 (0 %)	0 (0 %)	75 (8.6 %)	0 (0 %)	5 (0.7 %)	0 (0 %)	22 (2.4 %)	0 (0 %)
Total	192	867	868	1304	714	1510	934	1159

6.4.1 Species richness and diversity

The estimated species richness (Chao2) varied between site and between years (Figure 6.1). DGB had the lowest species richness, which remained consistent between 2016 and 2020. The species richness at Confluence was high in 2018 but this decreased significantly in 2020. The species richness at Footbridge was consistent between years and was similar to the estimated number of species at Confluence in 2020. The species richness at Farm in 2018 was the highest recorded at any site (n = 34; Table 6.3). However, this had decreased strongly by 2020 (n = 26).

Shannon diversity values remained relatively consistent between 2016 and 2020 at DGB, whereas they decreased strongly between 2018 and 2020 at Confluence, Footbridge and Farm (Table 6.2). This pattern was also observed for the Simpsons Diversity index (Table 6.3).

Table 6.3 Summary statistics for species richness and diversity, based on macroinvertebrate data to species level identification only. Estimated species richness has been rounded to the nearest whole value.

Site	Year	Estimated species richness (Chao2)	Shannon Diversity (exponential mean)	Simpsons Diversity
DGB	2016	13	5.01	4.16
	2020	11	4.70	3.81
Confluence	2018	33	14.00	9.90
	2020	22	6.85	4.72
Footbridge	2018	22	10.14	6.64
	2020	23	3.90	2.32
Farm	2018	34	16.44	9.32
	2020	26	8.67	5.11

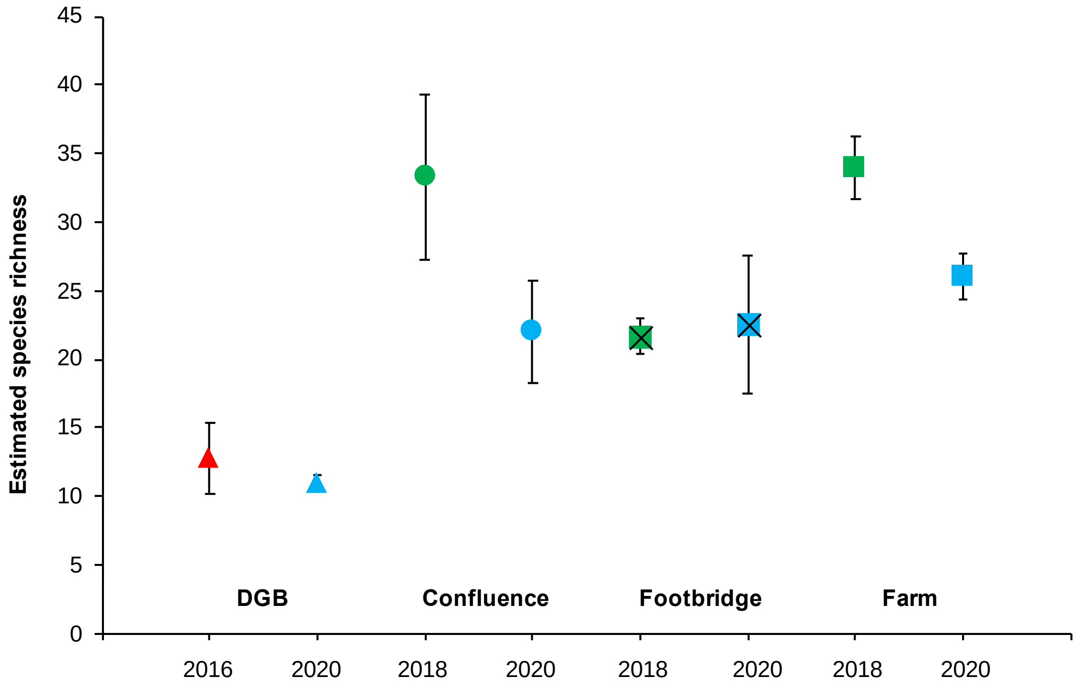


Figure 6.1 Estimated macroinvertebrate species richness (Chao2 for each site) at four sites over two years; DGB (triangles) 2016 (red) and 2020 (blue) and Confluence (circles), Footbridge (crosses) and Farm (squares) in 2018 (green) and 2020 (blue). Error bars denote standard deviation.

6.4.2 Invertebrate density and biomass

The density of macroinvertebrates was significantly different between sites and years (Kruskal-Wallis density across site, $p < 0.05$; Figure 6.2). This was due to a very low macroinvertebrate density at DGB in 2016, which was significantly lower than the density at DGB in 2020 ($p = 0.016$) and all other 2020 samples (Confluence $p<0.001$, Footbridge $p < 0.001$ and Farm $p=0.001$). The DGB 2016 density was also significantly lower than the density of macroinvertebrates at Farm in 2018 ($p=0.013$). The macroinvertebrate density was not significantly different between other sites, or within sites, over the two-year sampling period, although there was a consistent trend towards an increase in macroinvertebrate densities between the earlier and later sampling events at all sites.

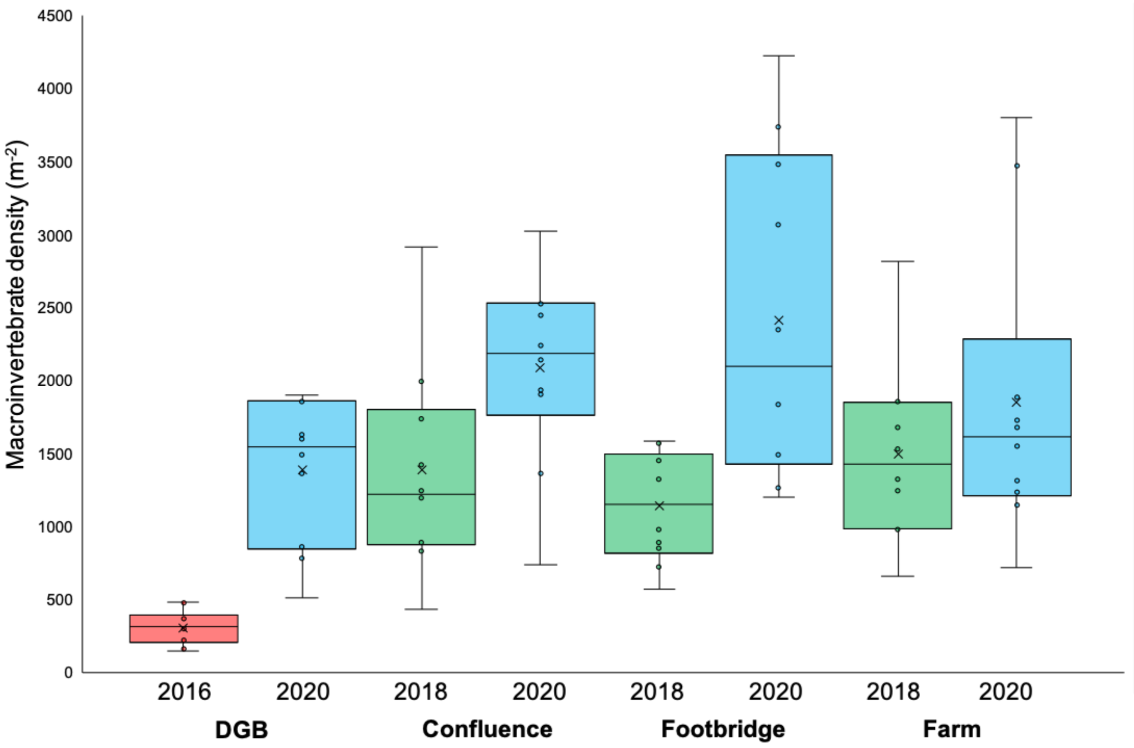


Figure 6.2 Boxplot of macroinvertebrate density (m^{-2}) at each site in each sampling year (2016, red; 2018, green; 2020, blue).

The biomass of macroinvertebrates was significantly different between sites and years (Kruskal-Wallis density across site, $p < 0.05$; Figure 6.3). Again, the low biomass at DGB in 2016 drove most of the differences, with significantly lower biomass than Farm in 2018 ($p = 0.002$), Confluence in 2020 ($p = 0.002$), Footbridge in 2020 ($p = 0.001$) and Farm in 2020 ($p < 0.001$). The biomass at Farm in 2020 was significantly higher than Confluence in 2018 ($p = 0.030$) and Footbridge in 2018 ($p = 0.019$).

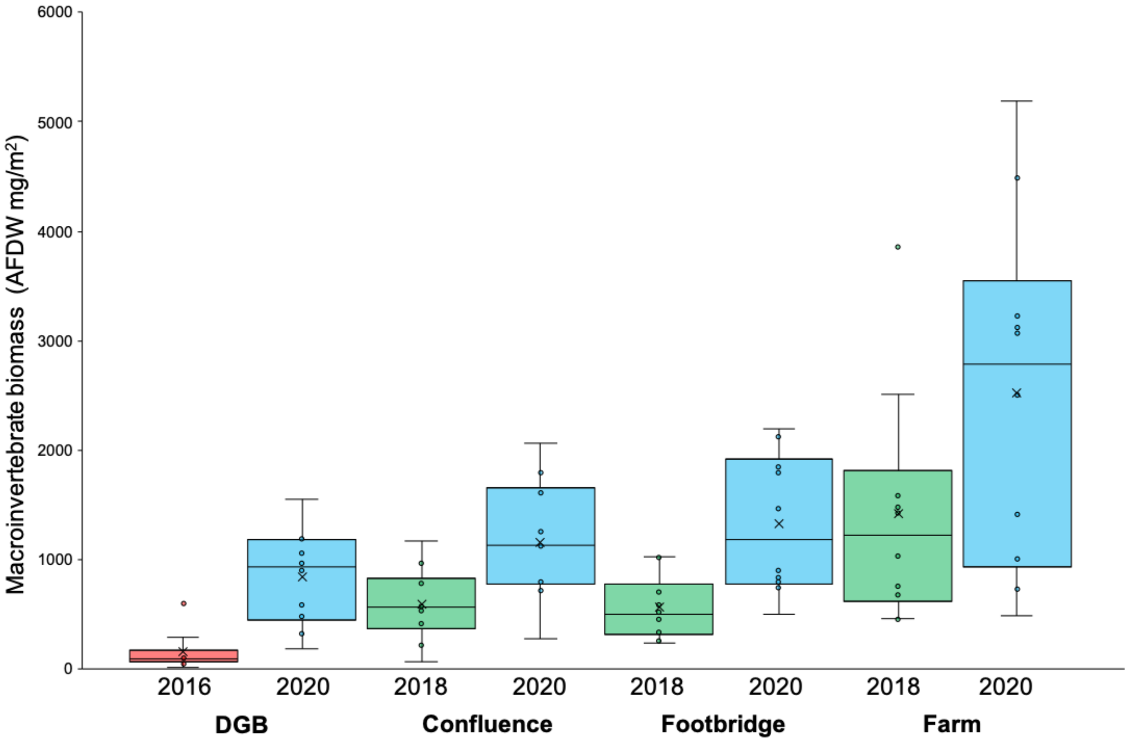


Figure 6.3 Boxplot of macroinvertebrate biomass (AFDW mg/m^2) at each site in each sampling year (2016, red; 2018, green; 2020, blue).

6.4.3 Functional feeding groups

The relative proportions of macroinvertebrate functional feeding groups by abundance (counts per site) varied between sites and years (Figure 6.4). At DGB in 2016, Scrapers were by far the most dominant group (72.4%), followed by Shredders (24.5%), with few Collectors (1.6%) and Predators (1.6%). The composition of functional feeding groups was more evenly distributed at the three LPB sites in 2018, with Scrapers comprising 14.8 – 37.7%, Shredders comprising 16.9 – 20.4%, Collectors comprising 25.8 – 46.4%, Predators comprising 10.5 – 19.2%, and with only few Parasites (0.7 – 8.6%) being present in the communities. However, in 2020, the composition of functional feeding groups was far more homogenous across all four sites - and much more similar to that of DGB in 2016; Scrapers were the dominant group across all sites (56.8 – 68.8%), followed by Shredders (13.9 – 30.7%) and Collectors (8.3 – 14.7%), with Predators generally being rare (3.3 – 7.6%), and no Parasites being present in any of the Surber samples taken at any of the sites.

The relative proportions of macroinvertebrate functional feeding groups by biomass (mg/m^2) also varied between sites and over years (Figure 6.5). The structure at DGB in 2016 mirrored the composition by abundance, with Scrapers being the most dominant group (65.8%), followed by Shredders (25.5%) with few Collectors (4.6%) and Predators (4.1%), and no Parasites being present in the samples. The composition of functional feeding groups at the three LPB sites in 2018 was again more varied. Whilst Scrapers were abundant in the communities, accounting for 31.3 – 70.0% of the biomass, a moderate proportion of the macroinvertebrate biomass was comprised of Predators (13.4 – 44.1%) and Collectors (7.1 – 15.8%), with some Shredders (5.5 – 11.5%) and few Parasites (3.3 – 5.4%). In 2020, the composition had changed at all sites (Figure 6.5), but did not reflect the same pattern seen in functional feeding groups by abundance, nor did it lead to a homogenous structure across sites (Figure 6.4). At DGB, roughly half of the biomass was comprised of Collectors (49.7%) in 2020, whereas this group was less dominant at the other sites (2.5 – 8.2%). Scrapers were the dominant group at Confluence (90.0%) and Footbridge (52.1%), but only made up 14.9% at Farm, where Predators (38.1%) and Shredders (38.8%) were the dominant groups.

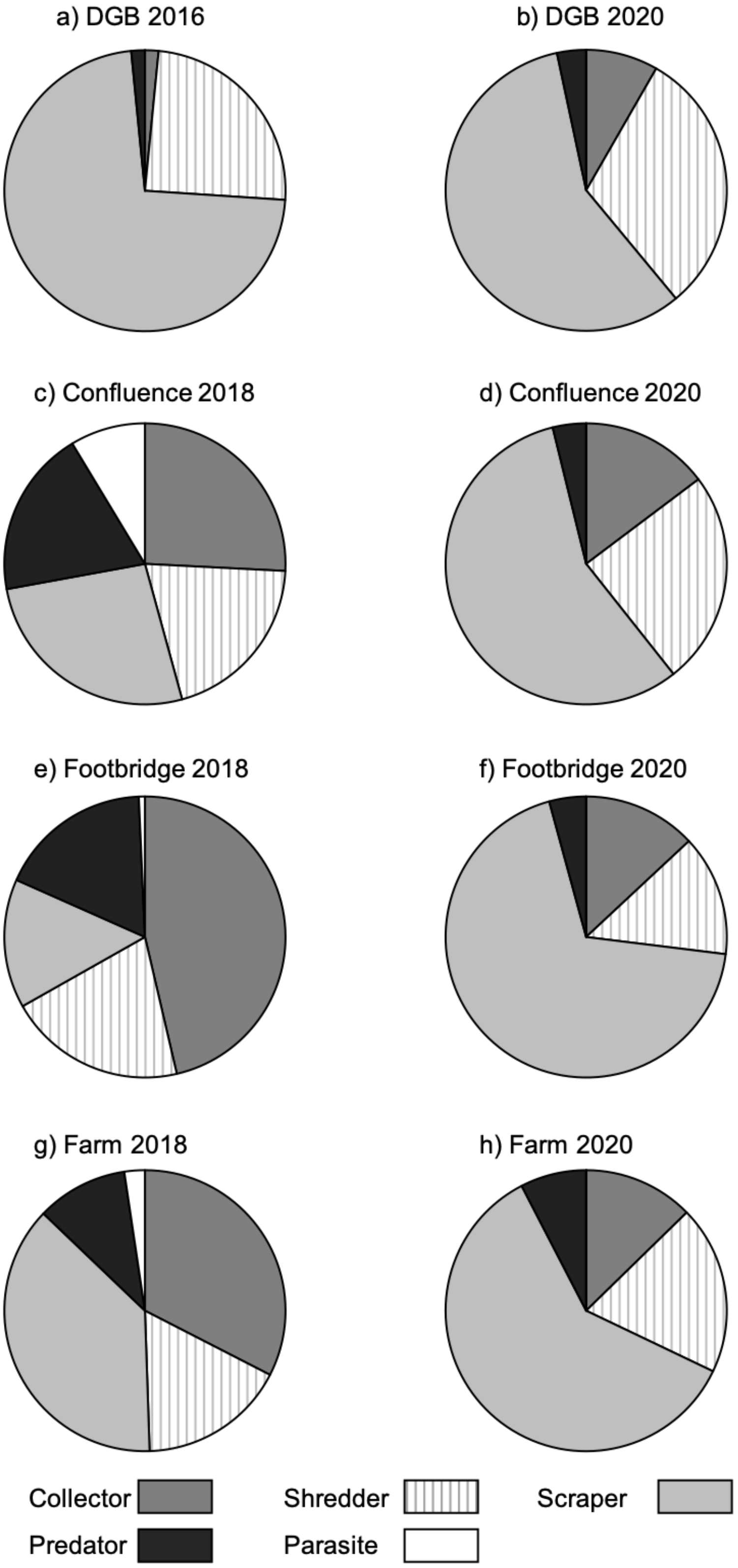


Figure 6.4 Relative proportions of macroinvertebrate abundance by functional feeding group including; Collectors, Shredders, Scrapers, Predators and Parasites, at each site (DGB, Confluence, Footbridge and Farm) in both sampling years (2016/2018 and 2020).

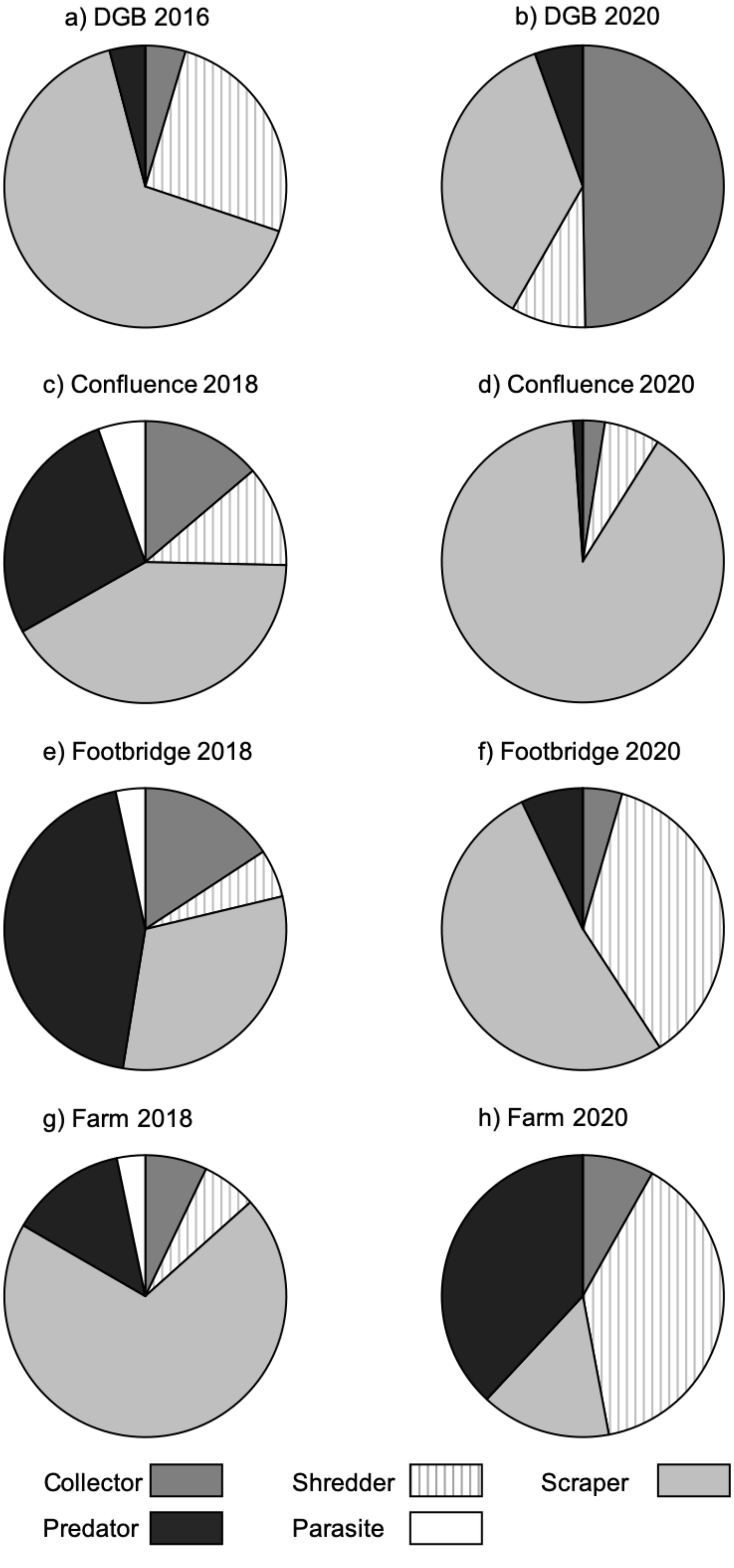


Figure 6.5 Relative proportions of macroinvertebrate biomass by functional feeding group including; Collectors, Shredders, Scrapers, Predators and Parasites, at each site (DGB, Confluence, Footbridge and Farm) in both sampling years (2016/2018 and 2020).

6.4.4 Community composition

The NMDS analyses based on the density of macroinvertebrates (m^{-2}) between sites and for the different sampling years revealed non-random distributions, with a stress value of 0.16 for the 2-dimensional scaling of the dissimilarity data. NMDS ordination diagrams accordingly showed a distinct clustering of macroinvertebrate communities at DGB in 2016 and 2020, although the 2016 samples were more highly separated from the other sites (Figure 6.6). The 2018 LPB samples were generally clustered together, with Confluence showing the largest spread amongst samples, and with a tighter clustering of the Footbridge samples. The 2020 samples from all sites clustered in an intermediate position in the ordination plot, with DGB 2020 sitting closest to the DGB 2016 cluster, and with samples from LPB (Confluence, Footbridge and Farm) closer to the LPB 2018 cluster. The ordination therefore indicates that the macroinvertebrate community at DGB is distinctly different to the sites along LPB in both years, but that the community composition has changed between 2016 and 2020 at DGB and between 2018 and 2020 at all other sites, resulting in an overall homogenization of the communities over time. The analysis of similarity (ANOSIM) confirmed that the degree of separation between the different groups was significant. Accordingly, site was identified as a significant driver of differentiation (p = 0.001) between the separate groups, with some overlapping values (R = 0.34). Year was also a significant driver (p = 0.001) of strong separation between groups (R = 0.75).

The similarity percentage (SIMPER) analyses by site showed strong dissimilarities across all sites, ranging from 70.1% to 83.2% similarity (Table 6.4).

Table 6.4 Percentage dissimilarity (%) between macroinvertebrate communities based on invertebrate density at sites based on SIMPER analysis.

Site	DGB	Confluence	Footbridge	Farm
DGB	-	78.8	76.7	83.2
Confluence	78.8	-	69.7	70.1
Footbridge	76.7	69.7	-	70.4
Farm	83.2	70.1	70.4	-

The three taxa that contributed the most to differentiation in communities between sites were consistent in all pairwise comparisons, with *Baetis rhodani* (Ephemeroptera) contributing 7.6 – 16.3% of dissimilarity, *Orthocladiinae* (Chironimidiae, Diptera) contributing 5.5 – 8.1% of dissimilarity and *Leuctra hippopus* (Plecoptera) contributing 4.4 – 8.0% of dissimilarity.

The SIMPER analyses by year showed 2016 and 2018 to be 90.0% dissimilar, 2016 and 2020 to be 81.8% dissimilar and 2018 and 2020 to be 78.5% dissimilar. The three taxa that contributed most to the dissimilarity between communities in 2016 and 2018 were *Chironomini* (9.2%), *L. hippopus* (6.5%) and *Habrophlebia fusca* (5.8%). The three taxa that contributed most to the dissimilarity between communities between 2016 and 2020 and also 2018 and 2020 were again *B. rhodani* (14.3 – 18.4%), *Orthocladiinae* (7.7 – 11.5%) and *L. hippopus* (6.5 – 9.1%) that also strongly determined between-site dissimilarity.

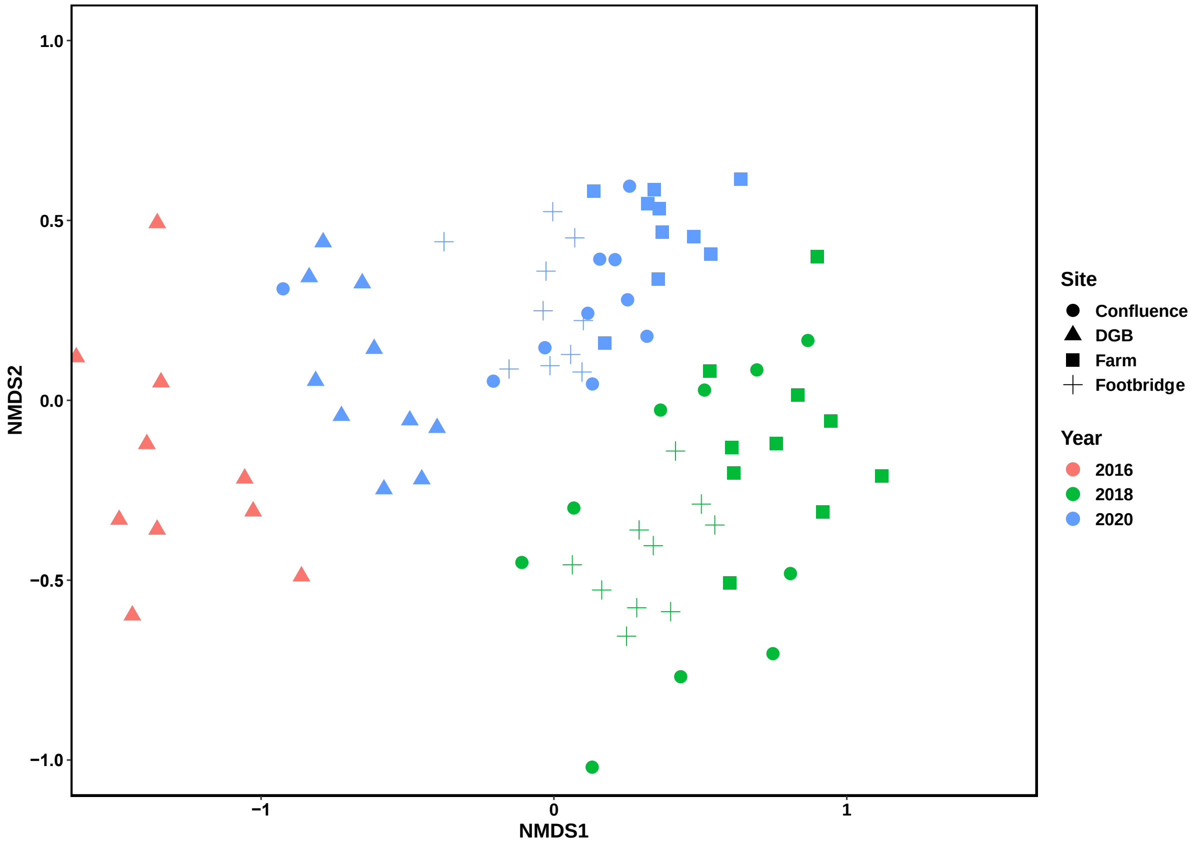


Figure 6.6 NMDS ordination of macroinvertebrate community data based on invertebrate density (m^{-2}) for each surber sample (n = 10) at four sites over two years; DGB (triangles) 2016 (red) and 2020 (blue) and Confluence (circles), Footbridge (crosses) and Farm (squares) in 2018 (green) and 2020 (blue).

The NMDS analyses based on the respective biomass of macroinvertebrates (mg/m^2) between site and year again revealed a non-random distribution with a stress value of 0.16. The invertebrate biomass NMDS ordination diagram showed a very similar distribution to the invertebrate density-based ordination. There were three distinct clusters that represented the three different sampling years (Figure 6.7). DGB 2016 was the most distinctly separated cluster, with the 2018 Confluence, Footbridge and Farm samples again clustered on the opposite side of the ordination, and the 2020 samples clustered in the middle. The ordination indicates that the macroinvertebrate community at DGB is distinctly different to the sites along LPB in both years, that the community composition has changed between 2016 and 2020 at DGB and between 2018 and 2020 at all other sites, and that there is again a strong homogenization in the communities with time. The analysis of similarity (ANOSIM) confirmed that the degree of separation between the groups was significant. Site was a significant driver of differentiation (p = 0.001) of separate groups, with some overlapping values (R = 0.33). Year was also a significant driver (p = 0.001) of differentiation of separate groups with overlapping values (R = 0.64).

The SIMPER analyses by year showed 2016 and 2018 to be 93.3% dissimilar, 2016 and 2020 to be 86.9% dissimilar, and 2018 and 2020 to be 85.1% dissimilar. The three taxa that contributed most to the dissimilarity between communities in 2016 and 2018 were *Ephemera danica* (9.7%), *Oligochaeta* (8.1%) and *Ecdyonurus dispar* (7.6%). The three taxa that contributed most to the dissimilarity between communities between 2016 and 2020, and also between 2018 and 2020, were *Oligochaeta* (13.8 - 16.1%), *E. dispar* (8.2 - 13.1%) and *B. rhodani* (8.3 - 10.7%). The similarity percentage (SIMPER) analyses by site showed a high level of dissimilarity (77.1 – 90.6%, Table 6.5). The taxa most highly contributing to the dissimilarity were fairly constant between the pairwise comparisons (Table 6.5).

Table 6.5 Pairwise site comparisons with overall percentage dissimilarity and the three taxa most highly contributing to dissimilarity based on SIMPER analyses. Percentage dissimilarity denoted in parentheses.

Site comparison	Percentage dissimilarity	Highest contributing taxa	Second highest contributing taxa	Third highest contributing taxa
DGB x Confluence	84.4	*Ecdyonurus dispar* (11.2)	*Baetis rhodani* (8.8)	*Hydropsyche instablilis* (7.7)
DGB x Footbridge	80.9	*E. dispar* (12.2)	*Oligochaeta* (10.9)	B. rhodani (10.7)
DGB x Farm	90.6	*Oligochaeta* (22.6)	*Radix balthica* (9.2)	*E. dispar* (8.5)
Confluence x Footbridge	77.1	*Oligochaeta* (8.5)	*H. instablilis* (7.6)	*B. rhodani* (7.0)
Confluence x Farm	83.0	*Oligochaeta* (19.9)	*R. balthica* (7.4)	*H. instabilis* (5.0)
Footbridge x Farm	79.6	*Oligochaeta* (20.3)	*R. balthica* (7.2)	*E. dispar* (5.4)

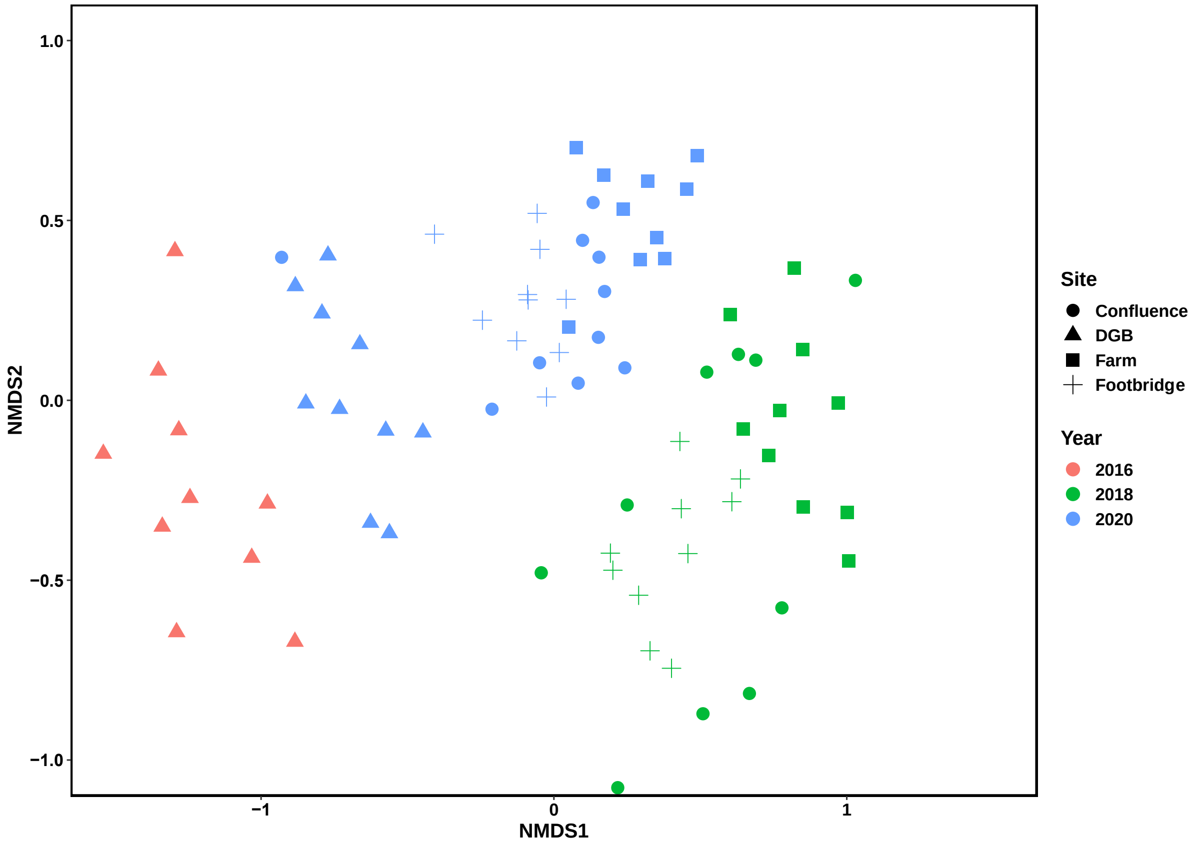


Figure 6.7 NMDS ordination of macroinvertebrate community data based on invertebrate biomass (mg/m^2) for each surber sample (n = 10) at four sites over two years; DGB (triangles) 2016 (red) and 2020 (blue) and Confluence (circles), Footbridge (crosses) and Farm (squares) in 2018 (green) and 2020 (blue).

6.4.5 *Biomonitoring indices*

The monitoring indices do show differences between sites and over the two sampling years (Table 6.6). DGB generated the lowest BMWP score, falling within the ‘Moderate’ category (41 -70) in 2016 and the ‘Good’ category (71 – 100) in 2020, indicating slight-to-moderate impact. Confluence, Footbridge and Farm all scored >100, falling into the ‘Very good’ category, indicating unimpacted or unpolluted conditions according to the BMWP scale. The Average Score Per Taxon (ASPT) was ≥ 6.00 at all sites in both years, indicating excellent water quality. The Lotic-invertebrate Index for Flow Evaluation (LIFE) score were high at all sites, indicating fast flows (>7) at Farm in 2018 and very fast flows (> 7.5) in all other instances (Extence, Balbi and Chadd 1999). The PSI score indicates the level of sedimentation at the site (Extence *et al.* 2013). The macroinvertebrate samples from DGB in 2016 indicate the river bed is ‘minimally sedimented or unsedimented’ (PSI 81 - 100), which changes to ‘slightly sedimented’ (61 – 80) in 2020. Confluence and Footbridge both score ‘slightly sedimented’ in 2018 and 2020. Farm scored as ‘moderately sedimented’ (PSI 41 – 60) in 2018, which changed to ‘slightly sedimented’ in 2020.

Table 6.6 Macroinvertebrate biomonitoring index scores for BMWP, Average Score Per Taxon, PSI and LIFE indices at each site in each sampling year.

Site	Year	BMWP	Number of scoring taxa	ASPT	PSI	LIFE
DGB	2016	59	8	7.38	87.50	8.57
	2020	85	13	6.54	75.76	8.45
Confluence	2018	131	20	6.55	75.00	8.00
	2020	121	19	6.37	79.07	8.06
Footbridge	2018	114	19	6.00	63.41	7.71
	2020	114	18	6.33	73.81	8.12
Farm	2018	171	27	6.33	52.63	7.32
	2020	134	21	6.38	74.42	7.84

6.5 Discussion

6.5.1 Macroinvertebrate communities along the invasion gradient

Previous work addressing the impact of signal crayfish on macroinvertebrate communities has focussed on presence/absence or pre-/post-invasion comparisons, producing a large body of evidence demonstrating that signal crayfish impact macroinvertebrate communities in invaded aquatic systems (Crawford, Yeomans and Adams 2006; Mathers *et al.* 2016; Galib, Findlay and Lucas 2021). Here, I describe, for the first time, how the macroinvertebrate community composition changes along a defined density gradient of signal crayfish “in situ”. Based on the literature, a negative impact as a result of signal crayfish presence was expected, and I hypothesised that the level of impact would correlate directly with crayfish population density. However, the results of this study do not support this hypothesis unequivocally. Whilst the results indisputably show DGB, the site with the highest crayfish density, to also be the most severely impacted, reflected in a significantly lower macroinvertebrate richness, diversity, abundance and biomass, and with a distinct community composition when compared to the other sites especially in 2016, the differences between the sites located along LPB were much subtler. There were no significant differences observed in abundance between the LPB sites, and with a trend of increasing macroinvertebrate biomass between 2018 and 2020 despite continued presence, and increasing density, of signal crayfish at these three sites.

The status of the macroinvertebrate community at DGB is almost certainly strongly governed by the hyper-dense population of signal crayfish present at the site, as the water quality is very good (see Ch. 2 and Chadwick 2019), and there have been no known pollution incidents or land-use changes during the duration of my research. If a pollution incident had occurred previously, recolonisation from more diverse communities downstream would have been expected, resulting in a strong diversity increase over time – which again was missing from the data. The community at DGB was comprised predominantly of small-bodied, fast moving taxa including

Plecoptera/stoneflies (e.g. *Leuctridae*) and Ephemeroptera/mayflies (e.g. *Baetidiae* and *Heptageniidae*). In 2020, high numbers of these taxa were present at the site, suggesting that they are tolerant to the presence of signal crayfish. These taxa can be assumed to be able to evade predation by signal crayfish due to their small body size, high mobility and potentially also their micro-habitat preferences. Peay *et al* (2009) suggest that some species may even benefit from the presence of signal crayfish due to the exclusion of other predatory invertebrates in crayfish populations of high density through competitive exclusion and predation pressure on these predators exerted from the crayfish population. At DGB, some species of Ephemeroptera may indeed gain such an advantage under high signal crayfish densities, as I observe a much higher proportion (60 – 74%) of the overall macroinvertebrate community to be comprised of members from this Order at DGB when compared to the low-density site (11 – 22% at Farm).

The depleted and impacted macroinvertebrate community at DGB, coupled with the local extinction of fish at the site (Ch. 5 & 6), provokes a crucial question: how can the crayfish population maintain such high densities with such limited food resources being present? In 2016, the signal crayfish density was recorded at 110 m^{-2} (Chadwick, Pritchard, *et al.* 2021), and still amounted to 85 m^{-2} in 2020 (Ch. 4). The consistently high crayfish density rules out any boom-bust dynamics (Ch. 4), indicating that the crayfish impact on macroinvertebrates will likely be permanent and irreversible for as long as the crayfish remain. This supports the findings of Mathers *et al* (2016), who found permanent changes to macroinvertebrate communities with no sign of recovery in crayfish-invaded lowland systems, and who also show similar trends occurring in upland headwater systems. In the persistent super-high density population observed at DGB, it is likely that cannibalism becomes an important trophic mechanism, and it has already been suggested that the incidence of cannibalism increases with increasing crayfish density (Houghton, Wood and Lambin 2017; Chadwick 2019). Furthermore, algae and detritus are known to form an important food resources for signal crayfish (Bondar and Richardson 2009). Algae proliferate under drought conditions where waters become warmer and nutrients are concentrated into the remaining pools. This may also be linked to the increased dominance of Scrapers reported in the 2020 Surber samples across all sites in this study, and the prevalence and role of algae as a basal resource during drought events within the system requires further scrutiny in the future.

I furthermore hypothesised that the invasion impact on the macroinvertebrate community in terms of biomass and abundances would correlate negatively with the density of signal crayfish. However, the overall macroinvertebrate abundance and biomass remained relatively constant between all the sites on Long Preston Beck in both, 2018 and 2020, with a slight increase in mean values observed at all three sites. Yet, in line with my hypothesis, species richness of macroinvertebrates did decline at Confluence and Farm over the two years, reaching a level more consistent with Footbridge from 2018 to 2020. This indicates that, although certain taxa are being lost in the early stages of crayfish invasion, a lot of taxa are relatively tolerant to low-to-intermediate crayfish densities, and their numbers and biomass increases, resulting in a relatively stable overall abundance and biomass across sites and over time. The NMDS results further corroborate a shift in community composition, leading to more homogenous communities at the LPB sites in 2020. Yet the NMDS did not indicate a clear initial gradient in communities that reflects the differences in crayfish density in 2018 or 2020. A community shift was also reflected in the relative proportions of functional feeding groups, which varied along the invasion gradient as hypothesised, but became more similar by 2020.

The SIMPER analysis identified a small set of particular taxa that are strongly driving the dissimilarity of communities, many of which were Ephemeroptera (*E. dispar, B. rhodani, H. fusca*). The community is shifting to a state where Ephemeroptera are increasingly abundant. A very striking observation is the lack of Gastopoda (snails, e.g. *R. balthica*) at all sites apart from Farm, and the severe decline of members of this taxon at the Farm site with time, where they comprised 16.7% of the entire macroinvertebrate community in 2018, but only 1.5% in 2020. These patterns suggest that snails are amongst the taxa most vulnerable to signal crayfish invasions, with numbers declining shortly after signal crayfish establishment. This pattern is particularly noteworthy, given that this site initially had a healthy population of the native white-clawed crayfish present, indicative of a highly differential impact of native and invasive crayfish species on native river biota.

Overall, the results of this study show that signal crayfish affect macroinvertebrate communities, with severe impacts observed especially at hyper-dense signal crayfish sites. However, the level of impact did not clearly correlate directly to signal crayfish density, with very similar communities observed along a wide gradient of 0.5 to 20 crayfish m^{-2}. The low impact observed at Confluence may be particularly unexpected, given the relatively high crayfish densities at this site, while the impacts observed at Farm were larger than expected, given the very low density of signal crayfish on this invasion front – and their co-existence with native crayfish. Time since invasion and invader density are often closely linked, but the full impact of invaders may take time to become fully apparent, especially when this impact is density driven (Simberloff *et al.* 2013). Yet the impact at the LPB site is similar, while signal crayfish have been established at Confluence for 10+ years and only arrived at Farm in the last 0 - 3 years. Therefore, other factors appear at play that differentiates these sites from DGB where the population has been established for 20+ years, and where observed impacts are clearly very severe. Further work could look to explore the role of the hyporheic zone in supporting crayfish and macroinvertebrate communities. Evaluation of the hyporheic zone and hyporheos was beyond the scope of this study, however, the difference in channel gradient and bedrock between Bookill Gill Beck and Long Preston Beck could have a role in the difference between BGB and LPB crayfish and macroinvertebrate communities.

Nonetheless, species richness has declined at all sites along LPB both along the invasion gradient and over time, while the impact on community composition and overall abundance and biomass appears to occur at much longer timeframes (> 10 years). Supporting this observation, Galib *et al.* (2021) found increasing effects of invasive crayfish on macroinvertebrate communities with time, however, no effect was seen in the first 7 years of invasion, while changes became evident at streams invaded by crayfish for ~20 years. Mathers *et al.* (2016) found that major changes became evident after about 5 - 10 years following signal crayfish invasion.

Types of antagonistic interaction between native communities, alien invader and the abiotic environment may influence the time it takes for impacts to become evident, and I propose that the relative impact of signal crayfish invasion occurs in three distinct stages (Figure 6.8), with taxon-specific impacts occurring throughout the establishment, but are the main initial impact as crayfish move into and establish an initial population within the habitat, escalating to community changes as the crayfish population grows, and eventually impacting on ecosystem functioning once crayfish become dominant, or even hyper-dominant, in the system (Figure 6.8). This relates to impacts caused by direct interactions such as predation occurring relatively quickly post invasion, initially targeting “easy prey”, i.e. large, slow-moving taxa such as Gastropods (Dorn 2013), while creating an overall relatively small ecosystem impact. When crayfish have become established and the population grows, indirect effects such as trophic cascades (Bondar 2005, Jackson 2014), habitat degradation through increased sedimentation, particularly in environments where crayfish burrow into the banks (Harvey *et al.* 2011; Sanders, Rice and Wood 2021) and loss of macrophytes (Nyström and Strand 1996) will start to occur. The combination of direct and indirect effects at this later invasion stage may induce community level shifts. As the effects mount and crayfish become dominant in the system, as seen at DGB, crayfish may then strongly alter ecosystem functioning by dominating benthic biomass and altering the rates at which resources are made available to other species (Creed and Reed 2004).This is the final stage of invasion, which become the persistent state for as long as crayfish remain.

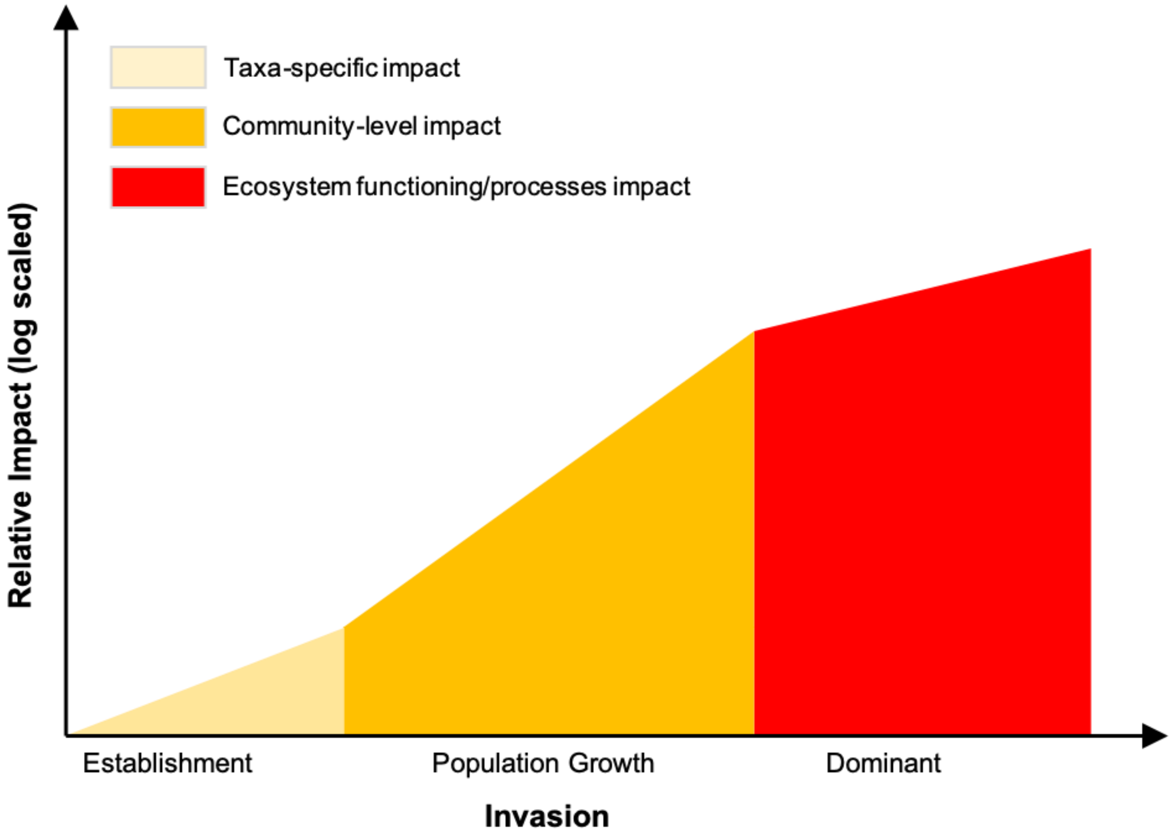


Figure 6.8 Theoretical diagram demonstrating changes in invasion impacts (taxa-specific, community-level and ecosystem scale) through the different stages of invasion (establishment, population growth and dominance.

Environmental conditions may further influence the speed and severity of signal crayfish invasion and consequent impacts. In my study, Long Preston Beck is a larger watercourse than Bookill Gill Beck, with a channel wetted width of approximately 4 - 5 m at the former compared to 1 – 2 m at the latter. BGB and Scaleber Beck feed into LPB, resulting in a larger discharge of water than that of BGB alone. During summer low flows, especially during the 2018 drought, BGB was reduced to a series of isolated pools with no continuous flow. In comparison, a low base flow was maintained at LPB, albeit at very low levels (pers. obs). In Chapter 5, I suggest that low flow or drought conditions impact native biota, but allow invasive signal crayfish to continue recruiting, with the large resulting crayfish populations then limiting the recovery of, and recolonisation by native biota, ultimately tipping the balance in the favour of the invader. The impacts of low flow or drought may be more pronounced at BGB and similar small streams compared to larger watercourses with a higher discharge like LPB. As a result of the aforementioned developments, the drought-mediated benefits to signal crayfish are also higher in small rivers since low flows will be more severe. Mathers *et al.* (2020) similarly argued that low flow events facilitated the establishment of invasive crayfish, which may in turn have exacerbated the impact of signal crayfish on macroinvertebrate communities in invaded lowland streams. This process may be accentuated in upland headwaters, as the macroinvertebrate community will likely comprise predominantly of flow-tolerant species which will already be heavily impacted from stress during low flow or drought events. Indeed, the 2018 drought may have accelerated the impact to macroinvertebrate communities at sites along LPB, with the low flows potentially severely altering the macroinvertebrate community and the presence of signal crayfish subsequently impeding the recovery of these communities. This could explain the observed, general shift to more homogenous macroinvertebrate communities in 2020 at sites with strongly differing crayfish densities. It is also possible that the severely degraded macroinvertebrate community at DGB has been created similarly by a series of low flow events in consecutive summers in the past, allowing signal crayfish to become dominant and in turn preventing the recovery of the native communities. Further research into the combined effects of multiple stressors such as invasive species and extreme weather events should be a priority of future research, to better understand the processes, impacts and risks to native ecosystems.

6.5.2 Biomonitoring tools

A loss of specific taxa such as Gastropods and Oligochaetes at signal crayfish-invaded sites could furthermore explain the observed differences in some of the water quality monitoring index scores. I hypothesised that biomonitoring indices reflect the impacted state of the system related to the presence of signal crayfish. Whilst DGB had the lowest BMWP scores, it also attained the highest ASPT scores. The BMWP at DGB indicated moderate-to-slight impact compared to the ‘very good’, unimpacted status at the LPB sites, while the ASPT suggested excellent quality at all sites. It could be argued that, given the water quality in the system is likely very homogenous and high (Ch. 2), the BMWP and ASPT are producing fair and accurate results. Indeed, in a study investigating implications of signal crayfish on biomonitoring tools, Mathers *et al* (2016b) also reported that the effectiveness of the BMWP and ASPT water quality indices was unaffected by the presence of signal crayfish. However, the lack of ‘low-scoring’ taxa at DGB, which in my view can at least partly be attributed to signal crayfish, has likely artificially inflated the ASPT. While this might not be problematic as it stands, there remains the question if subsequent pollution events would be adequately reflected in the invertebrate community, or if the strong governing impact of signal crayfish on the macroinvertebrate community composition might consequently produce a biased or misleading result. It could also be argued that despite good water quality, the river system is clearly heavily impacted, and the biomonitoring scores fail to detect this impact, which in itself is a problem when considering standard statutory monitoring procedures going forward.

Signal crayfish are known to burrow and mobilise sediment during foraging activity (Harvey *et al.* 2011; Sanders, Rice and Wood 2021). Therefore, higher rates of sedimentation would be expected at a high-density signal crayfish site. In contrast to this, DGB had the highest PSI score, indicating minimally sedimented conditions compared to slightly sedimented conditions at the other sites. However, the score is unlikely to reflect actual differences in sedimentation levels, and is instead biased by the shift in macroinvertebrate community composition and general communities’ indicative of the headwater system which would typically have low sedimentation. Similarly, the LIFE scores indicated very high flows, hence not reflecting the severe 2018 drought conditions. This could again be due to the homogenising impacts of crayfish populations on the community composition in my headwater systems - which will likely generally have more high flow tolerant species than lowland systems. However, this general pattern could well be inflated by the community shifts towards a higher proportion of flow-tolerant species such as Ephemeroptera species, that I strongly believe is at least partly linked to the signal crayfish presence. The inflation of the scores may have compensated for the effect of the 2018 drought and potentially higher than expected sediment levels due to signal crayfish activity. Mathers *et al* (2016b) similarly argued that the presence of signal crayfish caused inflation of scores weighted by abundance, such as LIFE and PSI, and suggested this may be due to the local extinction of taxa known to be preferentially predated by crayfish like Gastropda, Bivalvia and Hirudinea, coupled with a general crayfish-mediated shift in community compositions.

Mathers *et al.* (2016b) accordingly suggest to use a multi-metric approach to aid the accurate identification of stressors on invaded lotic systems, especially in the context of wider environmental and ecological conditions. The results of this study strongly support this suggestion, and also the recommendation that biomonitoring indices should be modified to consider the potential effect of invasive species (Mathers *et al.* 2016b). Biomonitoring indices are commonly employed for routine statutory monitoring by the Environmental Agency, as well as by conservation organisations such as The Riverfly Partnership. If the presence of invasive species can impact the effectiveness of biomonitoring indices - and potentially even mask other environmental stressors, then the results and interpretations are not representative or reliable. This will have significant implications for conservation and management, as it may lead to issues within systems going unnoticed with more severe ecological consequences in the future. To address this, new biomonitoring indices should be generated that incorporate the potential effects of aquatic invaders and identify risks that these species could pose to a system, but also to key taxa used in water quality monitoring.

Chapter 7. Final synthesis

7.1 Setting the scene

In my thesis, I sought to address a major research gap whereby sampling biases had limited the understanding of signal crayfish invasion biology and associated ecological impacts. This has been further confounded by similar biases in sampling some of the native biota, namely benthic fish species, which may be subject to impact. If we cannot quantify the size or structure of an invasive population or impacted native species, how can we understand or quantify the impact it is having? Furthermore, if impacts vary according to invasive crayfish' population size or structure, how can we determine the cause?

The development of the Triple Drawdown (TDD) formed a strong first basis for exploring the aforementioned questions, and involvement in that work, which partly pre-dated the core fieldwork for my thesis, was a source of much inspiration. However, it became evident that whilst the TDD produced robust, quantitative data on invasive crayfish, as well as the native species assemblages, the logistics and practicalities of this approach are challenging. The TDD requires expensive, large and heavy equipment, a large team of trained operatives and an accessible site with flows low enough to overcome with pumps. As a result, the method may not be accessible to a wide range of scientists, practitioners and river managers who would benefit from quantitative crayfish surveys.

I therefore decided, as a main first step in my work, to design and test a cost-effective, user-friendly, passive crayfish trap that permits quantitative *in situ* surveys of crayfish populations (Ch 3). The resulting "Pritchard Trap" (PT; Pritchard *et al.* 2021) proved successful also at sampling benthic fish populations, which conventional methods such as electrofishing have struggled to in the past (Ch 3). This method was then applied to an in-depth case study along a signal crayfish invasion gradient in a headwater system in North Yorkshire, England. The study system, Bookill Gill Beck leading into Long Preston Beck, presented a unique opportunity for research, since previous work has provided a detailed history of the introduction and spread of signal crayfish through this stream system. A combination of TDDs (2018) and PTs (2018 – 2020) were therefore employed in this system over three field seasons to explore signal crayfish invasion biology (Ch 4), population dynamics and density-dependent impacts on native fish (Ch 5) and macroinvertebrate communities (Ch 6).

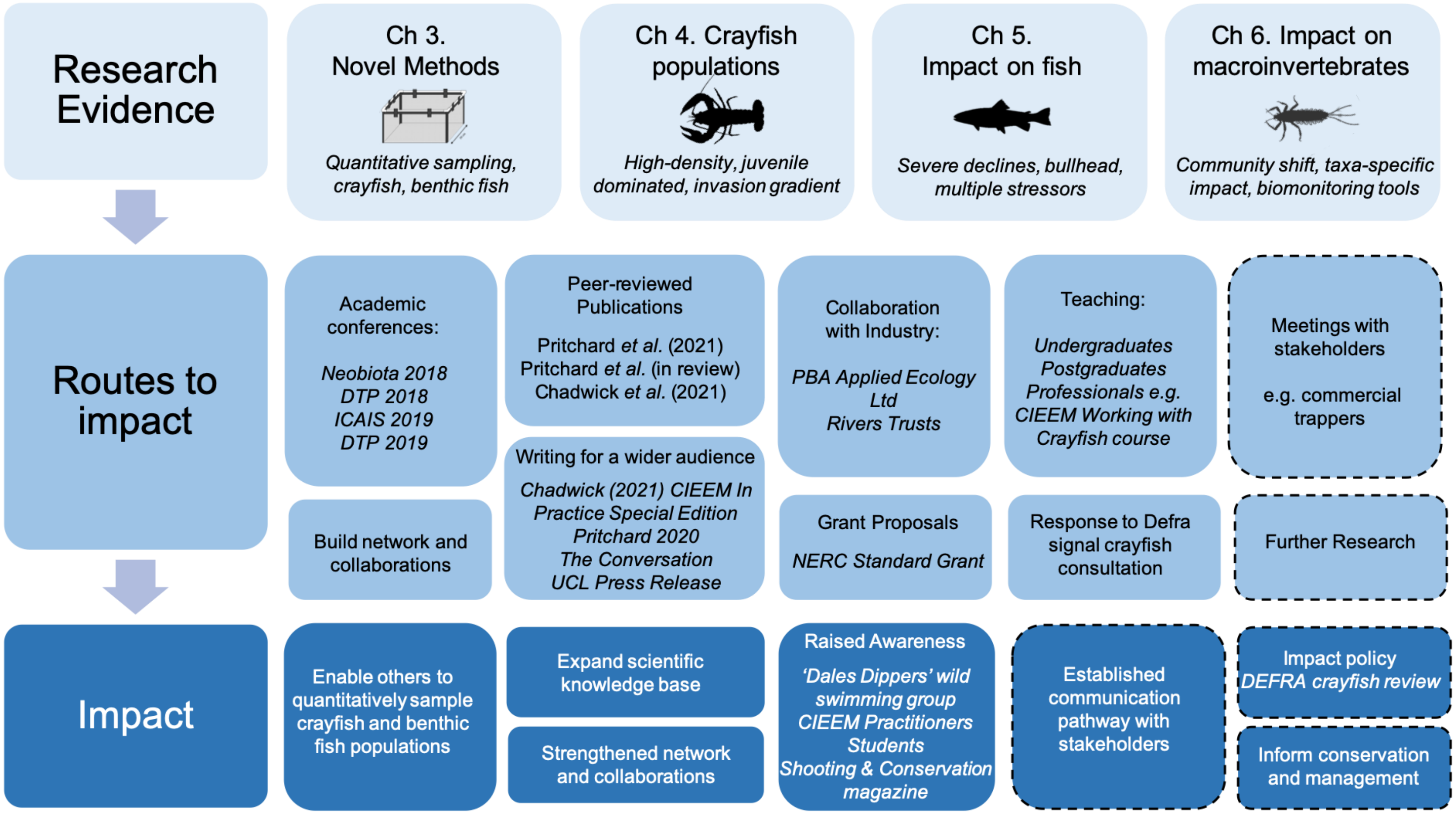
7.2 Routes to impact

This project has a strong applied element, aiming to inform freshwater conservation and management. The development of a novel survey technique will enable other scientists and practitioners to gather quantitative population data and address their own questions. Effective dissemination of findings is vital to achieve the desired outcome and have an impact. Throughout the PhD, I have made conscious efforts to communicate my research and disseminate findings to a range of audiences. The various 'Routes to Impact' have been targeted at scientists, practitioners, students and the general public (Figure 7.1). I have sought to build my professional network and forge collaborations with academics, industry and conservation organisations, attending and presenting at several academic conferences.

I have led and co-authored a number of publications including peer-reviewed publications (Chadwick, Pritchard, *et al.* 2021; Pritchard *et al.* 2021; Pritchard *et al.* 2021b), a feature article in an 'Invasive Species and Biosecurity' Special Edition of *In Practice*, the quarterly membership publication of The Chartered Institute of Ecology and Environmental Management (CIEEM: Appendix 10; Chadwick, Eagle, Pritchard *et al.* 2021) and a blog article for *The Conversation* (Appendix 11). I have co-ordinated a press release with UCL Communications & Marketing which led to news articles and public engagement with the research. As a result, communication pathways with stakeholders including commercial crayfish trappers have been established. We aim to organise a 'Town Hall' style meeting to actively engage with stakeholders and share knowledge and perspectives between parties.

I have taught undergraduate and postgraduate students delivering lectures, seminars and fieldwork training. Through my collaboration with PBA Applied Ecology, I have also helped deliver the CIEEM ‘Working with crayfish’ course. I have endeavoured to inform policy decisions by preparing an evidence-based response to Defra’s signal crayfish consultation. Finally, in collaboration with my supervisory team I have contributed to the preparation of a grant proposal with the aim of securing funding to continue this research.

This PhD addresses important research questions and provides a detailed case-study, and in doing so builds a strong foundation to continue the investigation of signal crayfish invasion biology and ecological impact. Implications for the field and recommendations for future work are discussed below.



Research Evidence

Ch 3. Novel Methods

Quantitative sampling, crayfish, benthic fish

Ch 4. Crayfish populations

High-density, juvenile dominated, invasion gradient

Ch 5. Impact on fish

Severe declines, bullhead, multiple stressors

Ch 6. Impact on macroinvertebrates

Community shift, taxa-specific impact, biomonitoring tools

Routes to impact

Academic conferences:

Neobiota 2018
DTP 2018
ICAIS 2019
DTP 2019

Build network and collaborations

Peer-reviewed Publications

Pritchard *et al.* (2021)
Pritchard *et al.* (in review)
Chadwick *et al.* (2021)

Writing for a wider audience

Chadwick (2021) CIEEM In Practice Special Edition
Pritchard 2020
The Conversation
UCL Press Release

Collaboration with Industry:

PBA Applied Ecology Ltd
Rivers Trusts

Grant Proposals

NERC Standard Grant

Teaching:

Undergraduates
Postgraduates
Professionals e.g. CIEEM Working with Crayfish course

Response to Defra signal crayfish consultation

Meetings with stakeholders

e.g. commercial trappers

Further Research

Impact

Enable others to quantitatively sample crayfish and benthic fish populations

Expand scientific knowledge base

Strengthened network and collaborations

Raised Awareness

'Dales Dippers' wild swimming group
CIEEM Practitioners
Students
Shooting & Conservation magazine

Established communication pathway with stakeholders

Impact policy
DEFRA crayfish review

Inform conservation and management

Figure 7.1 Summary of research conducted, routes to impact and impact. Shapes with no outline denote an action has already been achieved, and shapes with a dashed outline indicate actions in progress and intended outcomes.

7.3 Ecological impact of signal crayfish invasions

The signal crayfish invasion along Bookill Gill Beck and Long Preston Beck has caused significant ecological harm. However, the extent of ecological impact did not correlate directly with signal crayfish density, as was expected. It was hypothesised that the species richness, abundance and biomass of macroinvertebrate communities and fish populations would decline proportionately and inversely to the increase in signal crayfish density. Instead, it appears some impacts occurred quickly following signal crayfish colonisation and establishment, followed by a more gradual shift in community structure as the crayfish population grew further. Once signal crayfish become dominant, extreme ecosystem-wide impacts were observed.

In my study system, macrophytes were particularly sensitive to the early stages of signal crayfish establishment. Scarcely any macrophytes were recorded at DGB, Confluence or Footbridge, while they were prevalent in Scaleber Beck – the uninvaded tributary of Long Preston Beck (see Ch. 2 - Figure 2.3). Given the similarities between Scaleber Beck and Bookill Gill Beck, this indicates that, if signal crayfish were not present, Bookill Gill Beck and Long Preston Beck would also support healthy macrophyte stands. In-channel river substrate surveys revealed a decline from 9.3% macrophyte cover at Farm in 2018 (Figure 7.2), to only 0.4% cover in 2020. Therefore, it appears that within this system, macrophytes were severely impacted by signal crayfish during the establishment phase of invasion (Figure 7.3a). This reduction in macrophyte cover will have consequent direct effects for native biota that utilised the plant material for food or shelter (Burks, Jeppesen and Lodge 2001). It could also disrupt ecosystem processes through the alteration to flow heterogeneity, nutrient availability in the water, and sedimentation rates (Carpenter and Lodge 1986). The reduction and even complete loss of macrophytes, coupled with the increased sediment load and bank destabilising from signal crayfish burrowing (Sanders, Rice and Wood 2021) presents a significant threat to ecosystem functioning and potential flood risk.

Figure 7.2 Difference in macrophyte abundance between sites; a) lack of macrophytes at Footbridge and b) and evidence of large macrophyte beds at Farm site during the drought in 2018.

Changes in macroinvertebrate communities were observed along the invasion gradient and over time (Ch. 6). Species richness decreased relatively early on in the invasion process, suggesting that some taxa are more sensitive to signal crayfish presence. The taxa-specific impacts were consistent with observations in the literature and add to the growing evidence that signal crayfish negatively impact especially large-bodied, slow-moving invertebrates (Crawford, Yeomans and Adams 2006; Ercoli *et al.* 2015; Mathers *et al.* 2016). In this study a sharp decline in Gastropoda abundance was recorded during the 'Establishment' phase at the invasion front (Figure 7.3). Predation may have been a direct driver of this loss (Dorn 2013), with the simultaneous reduction in macrophyte cover potentially further accelerating the impact. Gastropoda were extremely scarce or completely absent at all other sites suggesting that molluscs are particularly vulnerable to signal crayfish invasion and can become locally extinct at relatively low signal crayfish densities (Figure 7.3c). Oligochaeta also declined substantially at the invasion front, but were present in lower numbers at the other sites, suggesting that abundance of this taxon is heavily impacted during signal crayfish establishment, but that some species can persist at low abundances (Figure 7.3c). A more general shift in community composition was observed during the signal crayfish 'Population Growth' phase. However, it is difficult to fully disentangle the effect that the 2018 drought may have had on observed patterns. It is possible that the 2018 drought negatively impacted macroinvertebrate communities, while the simultaneous expansion and growth of signal crayfish populations prevented, or at least altered any subsequent recovery, hence, as reported elsewhere (Mathers, White, Fornaroli, *et al.* 2020), accentuating the impact. It is possible that this effect is more pronounced in upland systems, with a high number of high flow-adapted species that may be more sensitive to low flow conditions.

The most depleted macroinvertebrate communities were recorded at DGB on Bookill Gill Beck, where signal crayfish are dominant and present hyper-dense populations, and low flow events are more frequent in summer months. Non-crayfish macroinvertebrate abundance and biomass were considerably lower than at sites along Long Preston Beck, while interestingly, some small-bodied, fast-moving macroinvertebrate taxa were identified to be more tolerant of signal crayfish. For example, some Ephemeroptera species (*Baetis rhodani* and *Ecdyonurus dispar*) were present at much higher relative proportions at the high population density-signal crayfish site than at the other sites (Fig 3c). Indeed, such species may benefit from the signal crayfish dominance as they can evade predation from crayfish themselves, but crayfish predation has reduced numbers of their invertebrate predators or competitors, as suggested by Peay *et al.* (2009).

The shift in macroinvertebrate community composition at the invasion front (Farm) provides evidence that, despite their strong physiological similarities, signal crayfish are not a like-for-like replacement of native white-clawed crayfish. A healthy population of white-clawed crayfish had been present at the study system for a substantial amount of time co-existing with an abundant and diverse macroinvertebrate community. However, the colonisation and establishment of signal crayfish caused the aforementioned, taxa-specific impacts and an overall shift in community composition. This is not to say that white-clawed crayfish do not affect the macroinvertebrate community, and indeed the communities of white-clawed crayfish sites compared to no crayfish sites may present differences (Chadwick 2019). However, my results show that the effects of signal crayfish on native species assemblages are different to effects linked to white-clawed crayfish, and the invasive's presence can negatively impact a native crayfish-adapted community. The relative impacts of native crayfish and invasive crayfish are understudied, and here I provide compelling evidence that they have highly distinct effects.

The prolonged co-occurrence of signal crayfish and white-clawed crayfish in this study system indicates that signal crayfish population are not currently carriers of crayfish plague, *A. astaci* in the field sites. As a result, the impact of signal crayfish on white-clawed crayfish stems solely from competition. Signal crayfish have displaced white-clawed crayfish from the entirety of

Bookill Gill Beck, and are slowly outcompeting their local populations as they invade down the system. During the study period (2018 – 2020), white-clawed crayfish were lost at Footbridge and declined substantially at Farm. It was identified that in this system, it has taken between 4 and 6 years for signal crayfish to displace white-clawed crayfish following their arrival. Yet, at Farm, the population density of white-clawed crayfish declined from 9 m^{-2} in 2018 to 2 m^{-2} in 2019 and 2020. This suggests that white-clawed crayfish are impacted substantially during the establishment phase, but that some individuals across all size classes can persist at relatively low densities for a number of years before becoming locally extinct (Fig 3b).

Fish communities were also impacted by the signal crayfish invasion, although the effects only became clear late in the invasion process once signal crayfish attained high population densities (Ch. 5). Benthic fish, particularly European bullhead, were most abundant in the system prior to invasion and were the most impacted (Ch. 5). Juvenile bullhead were affected first (Figure 7.3d), likely due to a combination of direct predation by signal crayfish and competition for shelter, leading to increased energy expenditure and a high risk of predation by piscivorous fish. Adult bullhead can predate on juvenile crayfish, but were also negatively impacted by signal crayfish invasions (Figure 7.3d). Impacts on fish can be highly complex and linked to specific local life history traits. For example, bullhead in hard water systems may only live to 3 – 4 years, compared to up to ten years in soft water systems. Therefore, there are fewer years available for sexually mature bullhead in hard water systems like the one found in the study area to reproduce and recruit into the population once they reach sexual maturity and before they die. If signal crayfish are reducing juvenile numbers, fewer fish are reaching sexual maturity in the first place, thus reducing the capacity to recruit. So as adult bullhead die off (naturally or signal crayfish-related), fewer and fewer juveniles replenish the pool of adults and the overall population size decreases. Bullhead are a Special Area of Conservation (SAC) Annex II species, listed on Annex II of the European Commission Habitats Directive and listed on the International Union for Conservation of

Nature (IUCN) Red List of Threatened Species (Habitats Directive 1992; Freyhof 2011). As such, the impact of signal crayfish on bullhead is of serious concern for freshwater conservation.

Salmonid populations only appeared to be impacted once signal crayfish were approaching dominance in the system (Figure 7.3d). A negative correlation between salmonid abundance and signal crayfish density has been reported previously at the study system (Peay *et al.* 2009), and they were completed displaced from most of Bookill Gill Beck by 2016 (Pritchard 2016). The impact of signal crayfish on salmonids is expected to be via multiple pathways, triggering both positive and negative responses: negative pressure through predation of eggs, alevin, fry or parr, in turn reducing recruitment, and/or indirectly through degradation of spawning gravel habitat, while crayfish in turn can also be expected to provide a food resource particularly for large salmonids. Nevertheless, it is evident that, overall, signal crayfish are negatively impacting native fish populations, including economically important species such as brown trout and Atlantic salmon, which will have serious implications for headwater systems and recreational fishing.

In summary, the signal crayfish invasion process correlated with distinct impacts at each stage of invasion. Taxa-specific impacts were observed during the ‘Establishment’ phase, with the loss of macrophytes and decline in white-clawed crayfish and some macroinvertebrate groups. During the ‘Population Growth’ phase, a more general community shift was observed in the macroinvertebrate community, likely partly moderated by the complete lack of macrophytes and associated habitats and the total displacement of white-clawed crayfish, with a strong decline in juvenile bullhead. As signal crayfish become ‘Dominant’ in the system, extreme impacts were seen, with an overall depletion in macroinvertebrate abundance and biomass, a decline in fish populations, leading to eventual localised extinctions at the highest signal crayfish population densities.

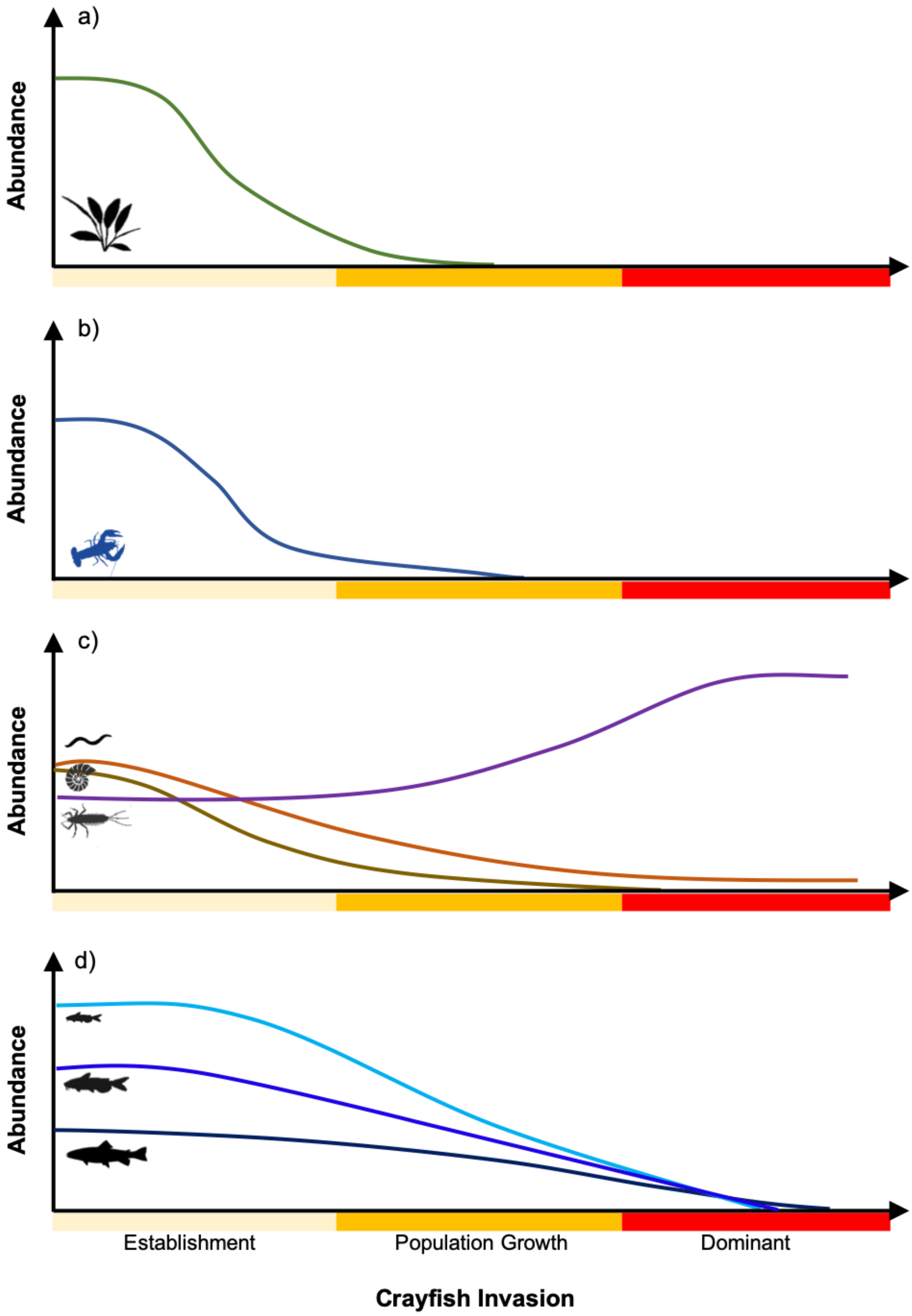


Figure 7.3 Conceptual diagram of the crayfish invasion pathway: Establishment, Population Growth and Dominant, and the associated impact on native biota including a) macrophytes, b) white-clawed crayfish, c) macroinvertebrates such as oligochaetes (medium brown), gastropods (dark brown) and some Ephemeroptera species (purple), and d) fish such as juvenile bullhead (pale blue), adult bullhead (royal blue) and salmonids (dark blue).

7.4 Signal crayfish invasion process

Along the invasion gradient, three main phases of the invasion process were identified; population establishment as signal crayfish dispersed downstream, followed by population growth, and finally signal crayfish 'dominance'. Recorded dispersal rates were consistent with previous estimates for the area and similar systems, yet considerably slower than reported in other countries (Ch 4; Hudina *et al.* 2009). Large adult male and female crayfish were driving the expansion, with subsequent recruitment in the newly invaded river sections. As signal crayfish became established, a 'typical' juvenile-dominated population structure developed, and population density increased (Ch. 4). In the study system, the substrate was dominated by cobble and boulders which provided plentiful crayfish refugia, and food resources were abundant in the lower reaches. Signal crayfish density increased slowly during the 'Establishment' phase, and then gradually increased over the following two years (Figure 7.4a). The hyper-dense crayfish stage was only observed at the well-established DGB site where signal crayfish have been present for >20 years. I hypothesise as a result of the findings in this thesis that food and shelter are not the only limitations on signal crayfish density and population growth, but that other biotic and abiotic factors also play important roles, especially competition with native species and periods of environmental stress, such as drought.

The presence of white-clawed crayfish at the invasion front (Farm, 2018; Ch. 4) will have resulted in substantial inter-specific competition between invasive and native crayfish. Individually, and in the absence of crayfish plague, signal crayfish clearly out-compete white-clawed crayfish, due to higher maximum feeding rates (Taylor and Dunn 2018) and the ability to oust white-clawed crayfish from refugia (Bubb, Thom and Lucas 2006). However, on a population scale, the relative densities (9.1 white-clawed crayfish m^{-2} and 0.5 signal crayfish m^{-2}) may slow initial establishment. Yet, once the invader has established and is successfully recruiting, white-clawed crayfish quickly decline and signal crayfish populations then expand relatively uninhibited (Figure 7.4b). Furthermore, crayfish and bullhead occupy overlapping niches with similar habitat requirements, and the presence of high-density bullhead populations may also slow the initial recruitment of invasive crayfish due to high competition.

Environmental conditions also determine the invasion process, and in the study system, a major drought in 2018 clearly accelerated the invasion process (Figure 7.4c). Like many species of invasive crayfish, signal crayfish appear tolerant to low flow regimes (Larson *et al.* 2009) and were unaffected at a population level by the drought (Ch. 4,Table 4.2). However, native biota may be less resilient to such conditions, and large fish that would usually prey on crayfish retreated to deeper waters, thus reducing predation pressure on the local signal crayfish population. For example, the signal crayfish population density at the Footbridge site more than doubled in the years following drought to levels seen at sites that have been invaded already for 10+ years. I believe this to be not only due to the emigration of fish during the drought, but also due to the recent displacement of white-clawed crayfish. Few remnant white-clawed crayfish were recorded at Footbridge in 2018 (0.15 m^{-2}), and none were recorded thereafter. The recent lack of inter-specific competition between crayfish species, coupled with reduced predation pressure from fish, may have resulted in a two-fold easing of constraints on signal crayfish population growth, allowing their populations to quickly expand. The rapid increase in crayfish population density may in turn limit the full recovery of fish populations once normal flow regimes resume.

7.5 Signal crayfish and multiple stressors

The ability of low flow events to facilitate the establishment and expansion of signal crayfish, and to amplify their ecological impacts, presents a major threat to freshwater systems, especially in the face of climate change and other anthropogenic stressors. The chances of seeing a summer as hot as 2018 in the UK has already increased to 12 – 25% due to climate change, yet, under climate projections of future warming, this could be closer to 50%

by mid-century (Met Office 2021). In fact, recent climate projections predict that summer temperatures could rise by 0.9 - 5.4 ºC, and summer rainfall could decrease by up to 47%, further increasing the frequency and severity of drought events (Met Office 2021). To further accentuate this, it is estimated that one in five waterbodies in the UK are also over-abstracted, resulting in physical changes to the waterbody and threatening biodiversity (Defra 2019). Yet the demand of water increases still, as the human population in England is forecast to have grown by 10 million by 2050. Climate change, abstraction and demand will place increasing stress on our freshwaters, potentially exacerbating other pressures such as from biological invasions. It therefore becomes increasingly important to regulate flow regimes and carefully manage abstraction. It is also important to identify even longer term ‘ark’ sites for native crayfish populations, as their range rapidly diminishes with the drop in climate-suitable areas and increasing overlap with invasive crayfish distributions (Capinha *et al.* 2013). Ecological niche modelling (ECM) could play an important role in identifying suitable areas for native crayfish conservation action, whilst also factoring in the suitability of the environment for competitors such as invasive crayfish and the additional effects of climate change (Préau *et al.* 2020).

Here, I highlight how an additional ‘stressor’ event (drought) has accentuated the impact of signal crayfish. Effects of other stressors such as water quality issues or habitat degradation could potentially also facilitate signal crayfish expansion, but more research is needed in these areas. Net effects of multiple stressors are typically categorised as ‘synergistic’ when the effect is greater than the sum of their single effects, and ‘additive’ when the effect equals the sum of their effects (Jackson *et al.* 2016). Net effects can also be ‘antagonistic’ when the effect is less than the potential additive effect, or ‘reversed’ when the net effect acts in a direction opposite to that which is predicted based on individual stressor effects. In a meta-analysis of net effects of multiple stressors in freshwaters, antagonistic interactions were most common (41%), followed by synergistic (28%), additive (16%) and reversed (15%) (Jackson *et al.* 2016). However, additive interactions were common with paired biological invasions (~30%), indicating that invasions present a significant threat to recipient ecosystems (Mainka and Howard 2010). The number of aquatic invasive species in freshwater ecosystems is predicted to increase, as new invaders are moved outside of their native ranges through various anthropogenic pathways (Hulme 2009; Turbelin, Malamud and Francis 2017) and established invaders expand towards the boundaries of their potential invasion ranges (Strayer 2010). Stressed systems are considered more susceptible to biological invasions and their impacts, because disturbance is often thought to favour invasions of non-native species (Strayer 2010). An ‘invasional meltdown’ has been hypothesised whereby the presence of an invasive species may facilitate the establishment of additional invasive species, resulting in greater and accelerated ecological impacts (Simberloff and Von Holle 1999). A recent study on signal crayfish predation of native and non-native amphipods has shown native amphipods to have a lower survival rate than non-native species (Beatty *et al.* 2020). This emphasises the importance of further work exploring how signal crayfish interact with other invasive species (Figure 7.4d), and indeed the cumulative effect of signal crayfish invasions and other disturbances (e.g. extreme weather events) which will become increasingly common in the future.

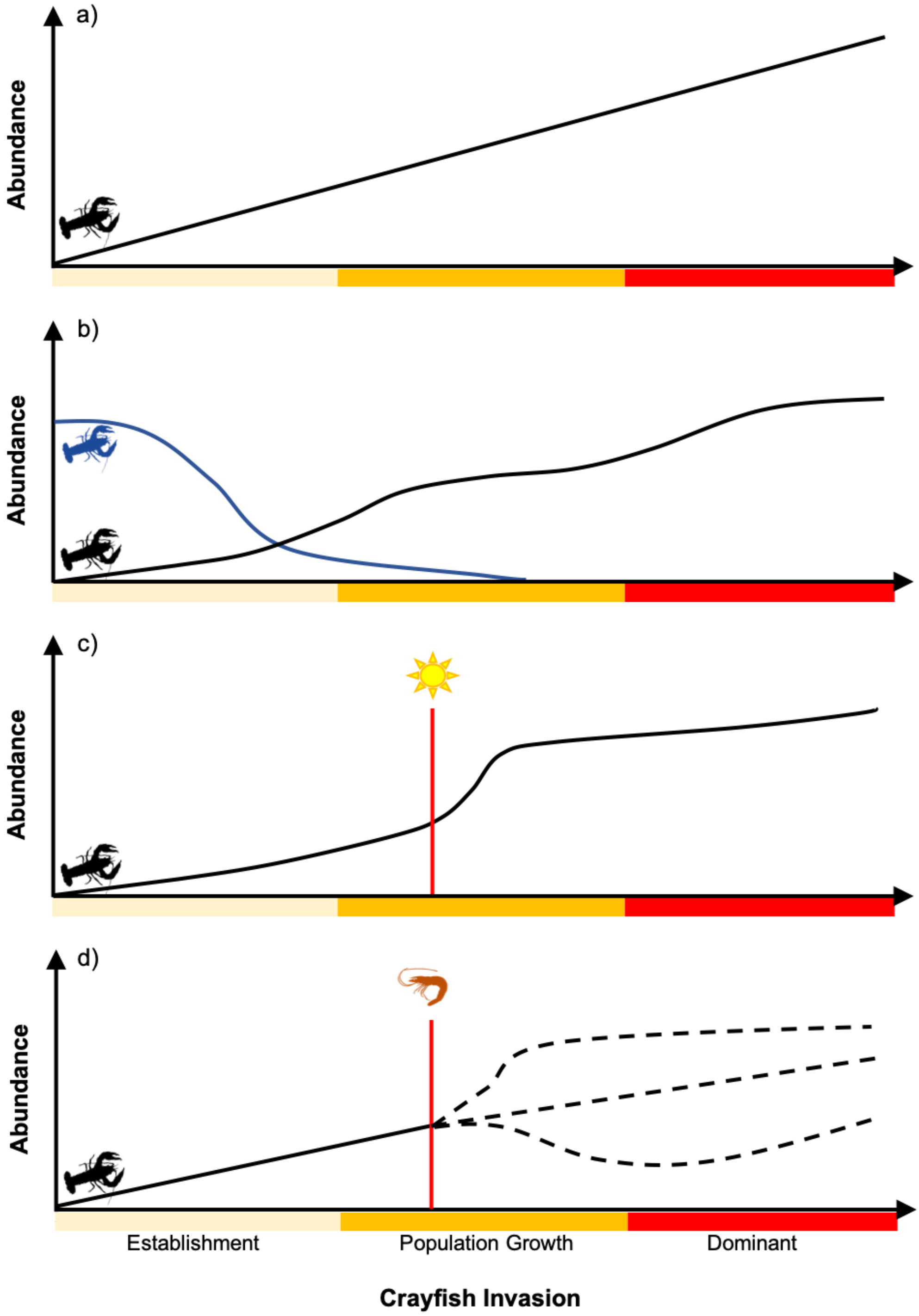


Figure 7.4 Conceptual diagram of crayfish density throughout the main stages of invasion; Establishment, Population Growth and Dominant. Factors affecting the invasion process are hypothesised: a) no factors affecting invasion, b) presence of native crayfish causing inter-specific competition during establishment, c) multiple stressor events that positively impact invasive crayfish e.g. drought, d) multiple stressor events within unknown effects e.g. multiple biological invasions.

7.6 Application of PTs and Future directions

7.6.1 Testing PTs in other aquatic systems

The PT presents a promising approach to survey crayfish, combining ease and cost-effectiveness of some traditional trapping techniques with the generation of quantitative data on crayfish population structure and density. The passive nature of the PT method reduces impact on by-catch and eliminates bias regarding bait attractancy – two factors regarded as major limitations of conventional crayfish survey methods. The PT performed very well in the studied rocky headwater streams, and future work should evaluate the efficacy of this novel trapping technique in other aquatic systems. Modifications could be made to the PT specifications and deployment method to adapt it to better suit environmental conditions at different sites. The base sampling area could be made larger or smaller and the side panels could be made taller for deeper water. Scuba or snorkelling could be used to deploy PTs in deep water and in systems dominated by fine sediments where crayfish rest in their burrows in the river bank during daytime. PTs could be buried in the fine sediment of the river and then be retrieved at night time when crayfish have left burrows to forage amongst the benthos. TDDs and PTs also offer a way to ground-truth any new methods that may be better suited to different aquatic systems. Use of the PT method allows, for the first time, large-scale accessibility to density and demographic data for astacology, conservation and management of freshwater crayfish.

7.6.2 Widescale distribution monitoring

Standard trapping as a crayfish survey technique and management option is so deeply engrained in the field, it presents a huge challenge to effect change. Indeed, the uptake of any new method provides a major obstacle, but, if successful, widespread PT use could have a huge impact and holds so much potential for advancing our understanding of crayfish distribution, invasion biology, density-dependent impacts, relationships with environmental conditions and efficiency of management techniques.

There is currently no standardised approach to crayfish monitoring or reporting in the UK. Surveys are ad hoc and records often rely on licence catch-returns. However, the Environment Agency (EA) routinely monitors macroinvertebrate and fish communities in UK main rivers up to every two years. If it were possible to incorporate quantitative crayfish surveys (and the parallel surveys of benthic fish provided by PTs) into this biannual survey process, we would soon have a much clearer understanding of crayfish distribution in the UK. It would highlight where populations of white-clawed crayfish were persisting and monitor their recruitment. It would help us understand the extent of signal crayfish spread, and indeed of other invasive crayfish species present in the UK. It may also highlight areas that do not currently have crayfish present, but may be suitable Ark sites for white-clawed crayfish conservation, although caution should always be exercised whenever concluding absence from surveys. The crayfish survey data could be used to explore density-dependent impacts using the accompanying database of macroinvertebrate and fish surveys.

7.6.3 Signal crayfish association with environmental conditions

One key result that became evident during this study was the severely impacted state that the extreme density of crayfish caused on Bookill Gill Beck (Ch. 5 and 6). Whilst impacts were clearly visible along Long Preston Beck, too, the extent was not as extreme as seen on Bookill Gill Beck. This raises the question of the exact cause for the differential impacts observed? Time since invasion could play a significant role in the variable densities and impacts observed. Signal crayfish have been present at Confluence for ~10 years, yet densities (20 crayfish m^{-2}) were considerably lower than the 65 – 110 crayfish m^{-2} recorded at DGB over the past five years (see also Chadwick *et al.* 2021). Perhaps in another ten years, the signal crayfish population at Confluence will more closely resemble that of DGB. However, the fact that the density at Confluence has remained widely stable at ~20 m^{-2} for the last three years may counter this argument and suggest that other factors are at play. I hypothesise instead that, as well as time, environmental conditions play an important factor in determining signal crayfish impacts even within the same catchment system. I believe that the specific site characteristics and environmental conditions at Bookill Gill Beck have greatly facilitated the invasion of signal crayfish and the subsequent establishment of hyper-dense populations. Whereas the conditions at Long Preston Beck are clearly capable of supporting high signal crayfish population densities of at least 20 m^{-2}, they appear less suitable in facilitating crayfish dominance at this time. Water quality and substrate type are believed to be consistent between the two becks. This leaves the size of the beck and the river flow as main differentiating parameters. I therefore propose that flow is a major factor influencing the success of signal crayfish within this system. Particularly low flow conditions, as observed during the dry summer of 2018, will greatly impact native aquatic biota, whereas the population of signal crayfish that are much more tolerant to dry conditions will grow and ultimately widely prevent the recovery of invertebrate and fish populations to pre-drought levels. Continued long-term monitoring at the study sites used in this project could help address these questions and determine causes of variable densities and differential impacts.

Moreover, the application of PTs on a wide spatial scale, coupled with environmental surveys, could be used to explore in detail which systems support high density crayfish populations and if any site characteristics provide more resilience to invasion. The PTs would be deployed to gather quantitative assessments on crayfish demographics including density and size structure, as well as information on benthic fish. Extensive site surveys should characterise bank height, aspect and material, in-channel substrate, river width, depth and flow. Water quality surveys should record water temperature, conductivity, dissolved oxygen, alkalinity and pH. Surveys of native biota should describe macrophyte, macroinvertebrate and fish communities. This information could be fed into detailed ordinations and models to identify site characteristics that correlate with low, medium or high-density crayfish populations. Outputs from such a study would have strong implications for conservation and management. Given high-density crayfish populations correlate with severe ecological impact, the understanding of what site characteristics specifically facilitate or support high density populations would allow particularly ‘at risk’ areas to be identified and mapped. Conservation efforts could then be prioritised to either protect crayfish encroachment on these sites, or, if certain conditions appear to limit the population growth and prevent signal crayfish from becoming dominant, such conditions could be created to increase ecosystem resilience. For example, if habitat heterogeneity was identified as a limiting factor on signal crayfish dominance, this could inform river restoration or river management projects to increase habitat diversity in an effort to increase resilience. Given that there is currently no effective means to eradicate signal crayfish once they are established, understanding ways in which we can live with the existing signal crayfish populations and mitigate their impacts is extremely important.

7.6.4 Informing management

The PT presents a useful tool to directly inform management of invasive crayfish populations. Knowledge of crayfish behaviour, activity levels and seasonal trends will help identify times when management efforts can have greatest impacts (Rogowski, Sitko and Bonar 2013). PTs could then be used to assess the effectiveness of signal crayfish control strategies (e.g. trapping and male sterilisation) on different parts of the crayfish population by providing ‘before’ and ‘after’ assessments of population density and structure. Not only would this determine any reduction in crayfish abundance following control, but PT data would show any differential impact across the different size classes. This could further inform which control strategies would complement each other to optimise results. For example, trapping to remove large animals coupled with management of predatory fisheries targeting juvenile crayfish has proved successful in reducing invasive rusty crayfish abundance in a lake system in the US (Hein, Vander Zanden and Magnuson 2007), and similar approaches in invaded UK systems could be closely monitored in their effectiveness using PTs.

Trapping has long been a favoured method to manage invasive crayfish populations. It is now evident that, especially in juvenile dominated populations, trapping alone will never be successful in eradicating signal crayfish given the size selectivity (Chadwick *et al.* 2021). Overall, further work is urgently required to assess its efficacy in managing populations to a level that mitigates their impacts. Invasion theory, specifically the Parker-Lonsdale equation, suggests that the total impact of an invader includes three fundamental dimensions: range, abundance and specific per-capita or per-biomass effects (Parker *et al.* 1999). The per-capita or per-biomass effects are incredibly important in this equation especially when applied to the population context and where shifts in preferred food resources are size- or age-specific. In my study system, the signal crayfish populations were dominated by juvenile size classes by abundance (Ch. 4). However, the population structure by biomass did not replicate the dominance of juveniles given their small individual weight (Figure 7.5). Instead, the majority of the crayfish biomass was comprised of crayfish > 20 mm and < 40 mm CL (Figure 7.5). If per-capita effects are more important, then trapping should be abandoned as a management technique and efforts should focus on eliminating juvenile size classes. Conversely, if per-biomass effects are greater, perhaps trapping yet holds a place in crayfish management, and although it will not eradicate a population, it may remove enough crayfish biomass from the larger size classes to significantly reduce ecological impacts observed. Laboratory feeding trials and functional response experiments may provide key insights into the differences between per-capita and per-biomass effects given the large disparity between population size structures by abundance and biomass.

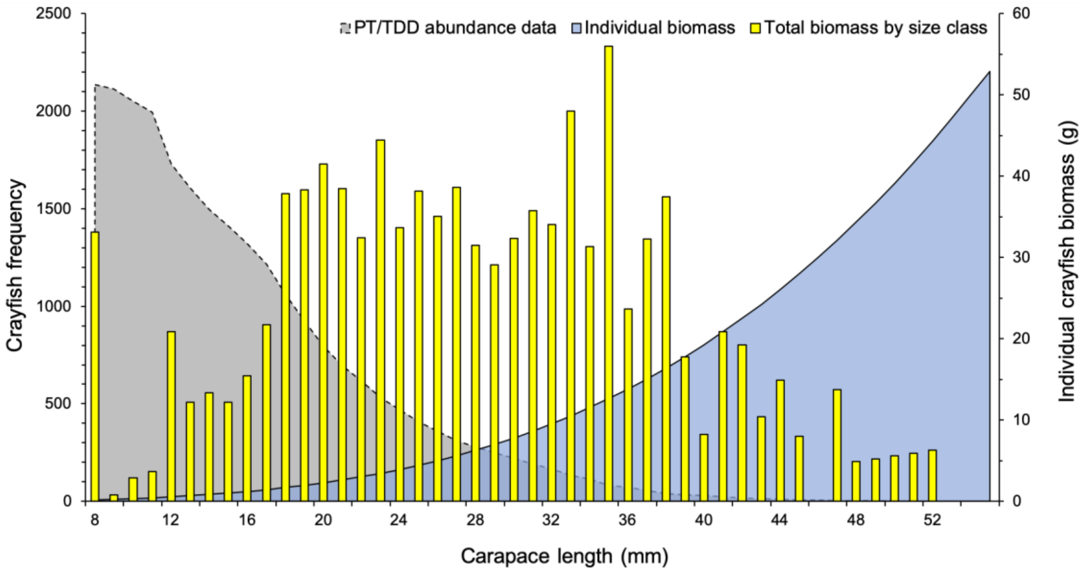


Figure 7.5 Size class distribution and relationship between size and biomass of signal crayfish from study sites. Grey area depicts crayfish size class by abundance. Blue area demonstrates the signal crayfish length-weight relationship. Yellow bars demonstrate the total biomass of size classes.

7.6.5 Testing invasion biology theories

The aforementioned Parker-Lonsdale equation is a long-standing theory in invasion science, in which the abundance or density of an invasive species is considered an important element in determining ecological impact (Parker *et al.* 1999). Future research should seek to explore, in more detail, density-dependent impacts, and whether similar patterns of progressive impacts are observed outside the study system. PTs could be employed across a wide geographic region in this context. Additional laboratory and mesocosm experiments could be used to explore the mechanisms driving the impact. For example, functional response experiments could explore the differences between crayfish size classes, and this, coupled with feeding trials with crayfish stocked at varying population structures and densities, could be used to explore predation as a driver of impact. Furthermore, molecular and isotopic tools could be used to build food webs and better understand the trophic interactions within invaded systems.

Such information could help generate a signal crayfish ‘abundance-impact curve’, whereby a measure of abundance (e.g. density) of an invader is plotted against some measure of its total impact (Sofaer, Jarnevich and Pearse 2018; Strayer 2020). This approach builds on the Parker-Lonsdale equation by accommodating non-linear relationships between abundance and impact as for example, the per-capita effect may vary with invader abundance (Strayer 2020). The ‘abundance-impact curves’ can be used to inform management by estimating the level of benefit that may be seen by reducing the population abundance by a specific amount and consequently informing the cost-benefit analysis of the management strategy (Yokomizo *et al.* 2009; Sofaer, Jarnevich and Pearse 2018). A recent development in this invasion theory, is that there is no single abundance-impact curve for a given invasive species, but instead the shape of the curve is jointly determined by the invaded ecosystem, resulting in various curves depending on the invader and invaded system (Strayer 2020). This updated theory also allows for the incorporation of many factors that have been discussed in this thesis as factors affecting the success of signal crayfish and will allow for a wider understanding of invasion biology. For example, the diversity of the recipient ecosystem has long been thought to positively correlate with resilience to invasion (Elton 1958), and the impact of multiple stressors (Jackson *et al.* 2016) such as climate-change related extreme weather, or multiple biological invasions resulting in invasional meltdown (Simberloff and Von Holle 1999). The ability to generate quantitative, demographic data on crayfish populations through the work presented in this thesis, now provides a platform to test such invasion science theories and better understand crayfish invasion biology, ecological impacts and potential management.

7.8 Key Take Home Messages

Overall, the employment of novel methods including the Triple Drawdown (TDD) and Pritchard Trap (PT) has generated unique insights into signal crayfish invasion biology and ecological impacts in rocky headwaters. From the results of my investigations, it can be concluded that;

1. The PT presents a suitable sampling device for invasive crayfish, native crayfish and benthic fish.
2. Signal crayfish can form hyper-dense, juvenile dominated populations that persist over several years with no sign of boom-bust population dynamics.
3. Signal crayfish can have severe impacts on macroinvertebrate communities and fish populations, especially benthic species such as European bullhead.
4. External disturbance events like droughts can accelerate the impact that signal crayfish have on native biota.
5. The overall approach used in this thesis and detailed case study in North Yorkshire, provides a template for future investigation of invasive species and their impacts across a wider geographical scale.
6. The evaluation of quantitative population demographic data of an invasive species, coupled with environmental surveys of native ecosystems, provides a foundation to test invasion science theories and advance our understanding of biological invasions.

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Appendices

Appendix 1. Chadwick, Pritchard *et al.* (2021) Paper

Open access publication available at:

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Journal of Applied Ecology

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A novel ‘triple drawdown’ method highlights deficiencies in invasive alien crayfish survey and control techniques

Daniel D. A. Chadwick, Eleri G. Pritchard, Paul Bradley, Carl D. Sayer,
Michael A. Chadwick, Lawrence J. B. Eagle, Jan C. Axmacher
First published: 12 October 2020

Abstract

1. Freshwater crayfish can be successful invaders that threaten native biota and aquatic ecosystems in numerous countries worldwide. Nonetheless, the inability of conventional crayfish survey techniques like trapping and handsearching to yield quantitative population data has limited the understanding of crayfish invasion biology and associated ecological impacts.
2. Here, we employed a novel ‘triple drawdown’ (TDD) method to sample invasive populations of signal crayfish *Pacifastacus leniusculus* in a headwater stream in Northern England. The method was compared with conventional techniques of trapping and handsearching.
3. The TDD method proved to be an effective technique with high capture efficiency, reporting signal crayfish densities from 20.5 to 110.4 animals/m^2 at our study sites. These numbers exceed any previous estimates for similar streams.

4. The TDD showed the vast majority of individuals across all sites were juvenile or sub-adult (<26 mm CL), with only 2.3% of the population large enough (≥35 mm CL) to be caught in standard traps.
5. *Synthesis and applications*. The triple drawdown (TDD) method demonstrates strong inefficiencies and biases in conventional crayfish survey and management techniques. Trapping is not recommended for representative sampling or control of juvenile dominated populations. TDDs, which can be adapted and modified to operate in multiple habitat types and freshwater systems, generate robust quantitative data on invasive crayfish population demographics in situ. This can advance our understanding of the biology of an important invader of freshwater systems around the world. Obtaining this data prior and post-intervention is fundamental to evaluate invasive crayfish management, and we recommend the TDD method to assess the effectiveness of future control measures.

Appendix 2. Pritchard *et al.* (2021) Paper

Open access publication available at:
https://doi.org/10.1002/2688-8319.12070

Ecological Solutions and Evidence
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The 'Pritchard Trap': A novel quantitative survey method for crayfish

Eleri G. Pritchard, Daniel D. A. Chadwick, Ian R. Patmore, Michael A. Chadwick, Paul Bradley, Carl D. Sayer, Jan C. Axmacher
First published: 07 June 2021

Handling Editor: Michelle Jackson

Abstract

1. As crayfish invasions continue to threaten native freshwater biota, a detailed understanding of crayfish distribution and population structure becomes imperative. Nonetheless, most current survey methods provide inadequate demographic data. The quantitative 'Triple Drawdown' (TDD) dewatering method has highlighted the importance of such data, yet practical constraints prevent its large-scale application.
2. Here, we introduce the 'Pritchard Trap', a novel passive sampling method that reliably generates quantitative crayfish population data while requiring substantially lower sampling effort than TDDs. This quadrat-style sampler was extensively tested in headwater streams of North Yorkshire, England, along an invasion gradient for signal crayfish (*Pacifastacus leniusculus*) from well-established sites to

mixed populations of signal crayfish and native white-clawed crayfish (*Austropotamobius pallipes*).

3. The Pritchard Trap was trialled over several time intervals to determine the minimum required trap deployment time. TDDs at the same sites allowed for a robust evaluation of Pritchard Trap sampling accuracy in representing crayfish densities and population structure.
4. The Pritchard Trap successfully sampled both invasive and native crayfish (8–42 mm carapace length). A minimum passive deployment time of 4 days was required. At low crayfish densities (0.5 individuals m^{-2}), increased trapping effort was necessary to achieve accurate population density and size class distribution estimates. The Pritchard Trap required substantially less sampling effort (working hours) and resources than the TDD, whilst also posing less risk to non-target species.
5. The Pritchard Trap, for the first time, affords logistically simple, truly quantitative investigations of crayfish population demographics for headwater systems. It could be integrated into crayfish research and management, for example to explore density-dependent ecological impacts of invasive crayfish and their management responses or to monitor populations and recruitment in native crayfish conservation initiatives.

Appendix 3. Pritchard *et al.* (2021) Paper Supporting Information

Published and open access via the BES Journals website, in association with Pritchard *et al.* (2021).
Filename: eso312070-sup-0001-SuppMat.docx

The ‘Pritchard Trap’: a novel quantitative survey method for crayfish

Ecological Solutions and Evidence
Eleri G. Pritchard, Daniel D. A. Chadwick, Ian R. Patmore, Michael A. Chadwick, Paul Bradley, Carl D. Sayer & Jan C. Axmacher

Supporting Information
Includes;

1. Specifications of Pritchard Trap (PT) Design
2. Manual to manufacture Pritchard Trap (PT)
3. Carle Strub Equation
4. List of non-target organisms captured during Pritchard Trap (PT) Sampling

Appendix 4. Pritchard *et al.* (2021b) Fish Paper

Open access publication available at:
https://doi.org/10.1002/2688-8319.12111

Ecological Solutions and Evidence
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Assessing methods to improve benthic fish sampling in a stony headwater stream

Eleri G. Pritchard, Daniel D. A. Chadwick, Michael A. Chadwick, Paul Bradley, Carl D. Sayer & Jan C. Axmacher

First published: 19 November 2021

Handling Editor: John Murray-Bligh

Abstract

1. Electrofishing is a well-established and widely used method for surveying fish populations. Nonetheless, its effectiveness is impacted by numerous factors, including water chemistry, habitat type and fish species. Both physiological and behavioural responses make bottom-dwelling “benthic” fish which lack swim bladders (e.g. European bullhead *Cottus gobio*) particularly difficult to survey by electrofishing.
2. We compare the performance and practicalities of electrofishing for benthic fish at a rocky northern English headwater stream with two sampling methods originally designed for crayfish surveys; the Triple Drawdown method which involves repeated dewatering of a site, and the Pritchard Trap method which involves sunken traps filled with natural substrate that samples a small, fixed (0.25 m^2) area of river bed.

3. Both the Pritchard Trapping and Triple Drawdown methods provided similar high-density population density estimates for bullhead which were at least 2.5-5 times higher than predicted from electrofishing derived sweep depletion curves.
4. Electrofishing and the Triple Drawdown method are both resource-intensive, requiring expensive equipment and a team of trained operatives. These approaches also pose a risk to fish and non-target organisms. In contrast, Pritchard Traps provide a cost-effective passive, low risk survey method requiring minimal training and only one operative. Pritchard traps therefore show particular promise for benthic fish surveying and monitoring.

Keywords

Bullhead ∙ Electrofishing ∙ Density estimates · Population demographics · Pritchard Trap ·Sampling bias · Triple drawdown

Appendix 5. Pritchard *et al.* (2021b) Fish Paper Supporting Information

Assessing methods to improve benthic fish sampling in a stony headwater stream

Eleri G. Pritchard, Daniel D. A. Chadwick[1], Michael A. Chadwick, Paul Bradley, Carl D. Sayer & Jan C. Axmacher

Supporting Information
Including

1. Fish species richness and abundance
    a) Electrofishing
    b) Triple Drawdown
    c) Pritchard Traps

1. Fish species richness and abundance

a) Electrofishing

Site	Survey area (m^2)	Bullhead	Stone loach	Eel	Total
Confluence	45.5	42	9	0	51
Footbridge	45.5	227	14	0	241
Farm	50	231	27	1	259

b) Triple Drawdown

Site	Survey area (m^2)	Bullhead	Stone loach	Eel	Total
Confluence	45.5	288	58	6	352
Footbridge	45.5	1217	30	6	1253
Farm	50	1299	29	4	1332

c) Pritchard Traps

i) 2018

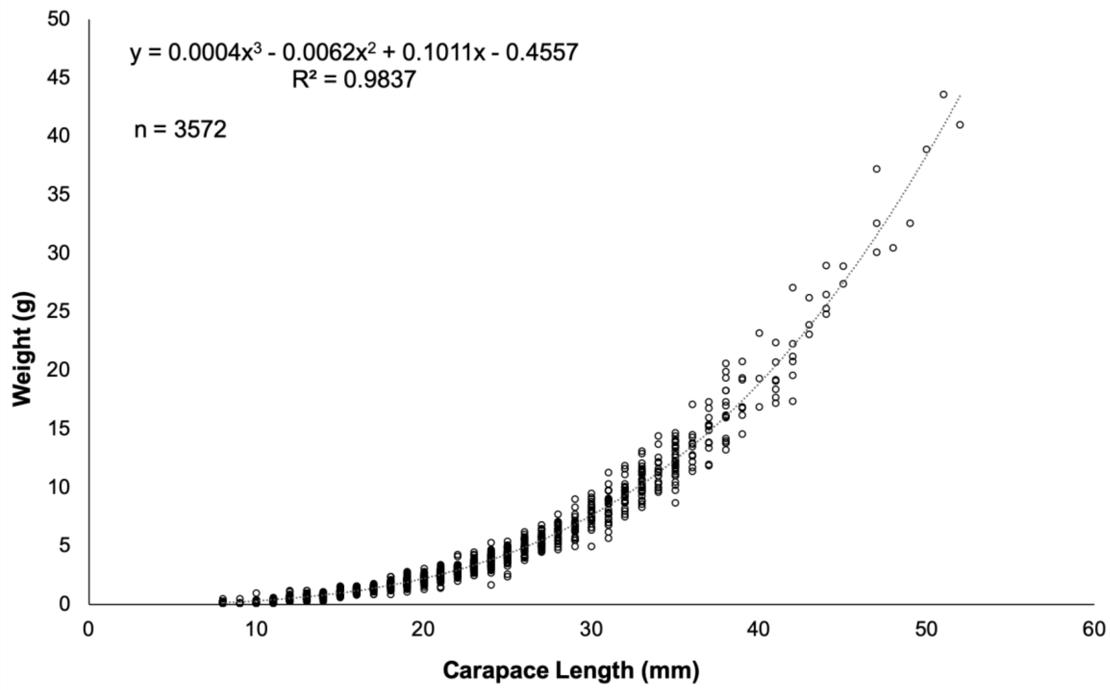
Site	Survey area (m^2)	Bullhead	Stone loach	Eel	Total
Footbridge	1	26	2	0	28
Farm	1	25	0	0	25

ii) 2019

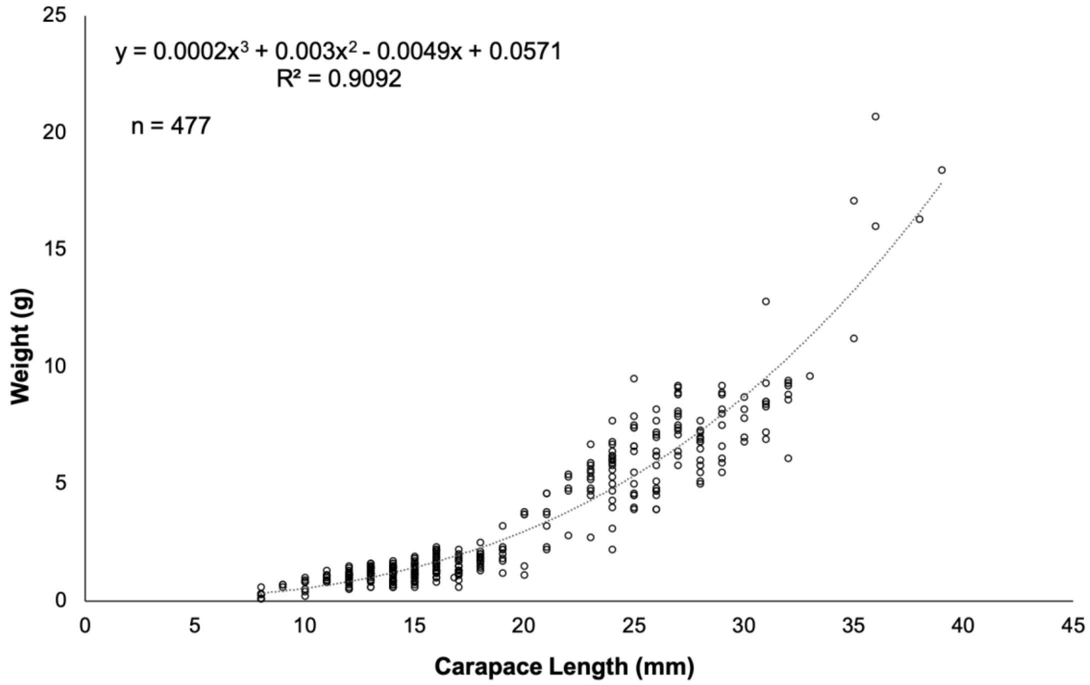
Site	Survey area (m^2)	Bullhead	Stone loach	Eel	Total
Confluence	7.5	45	14	0	59
Footbridge	7.5	68	11	0	79
Farm	7.5	107	18	0	125

Appendix 6. Crayfish length-weight regressions

Crayfish length-weight regressions used in Chapter 4.



Length-weight regression for signal crayfish (n = 3572), R^2 = 0.98, with equation of the line.

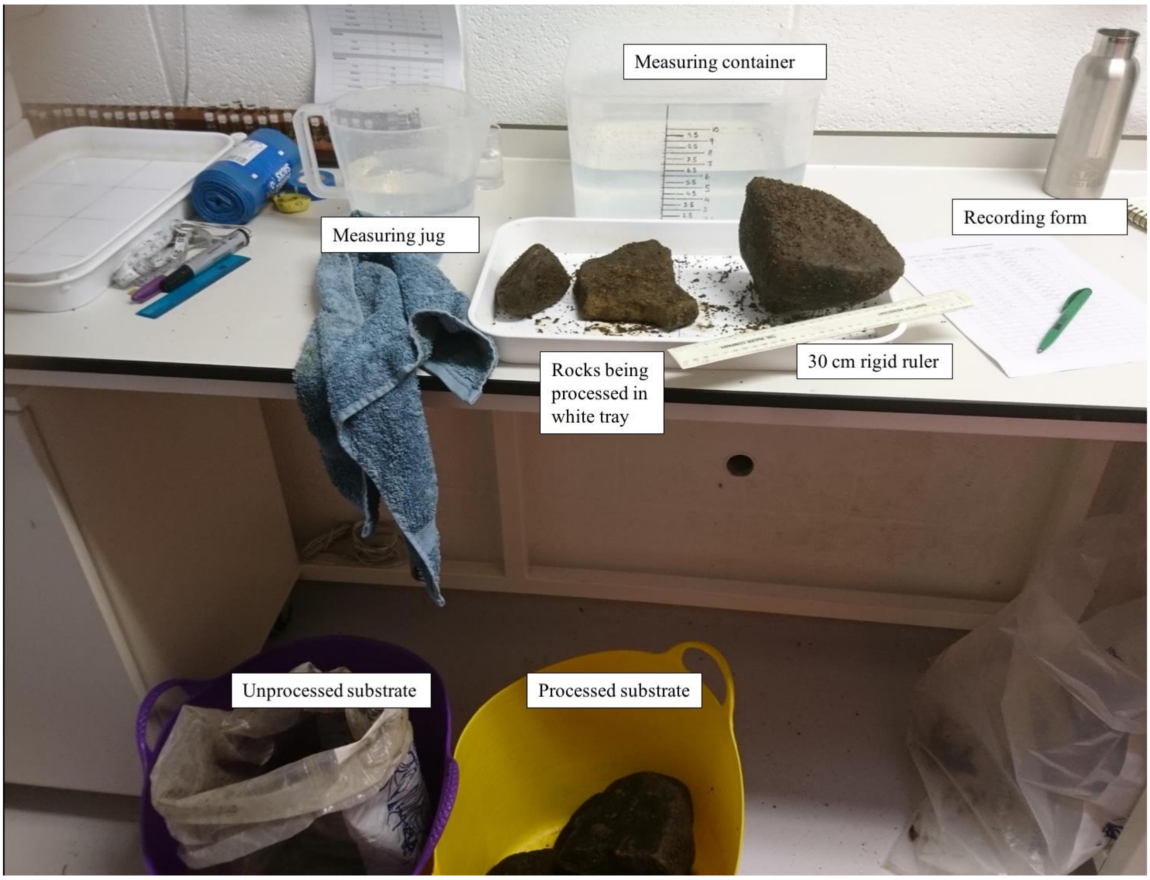


Length-weight regression for white-clawed crayfish (n = 477), R^2 = 0.91, with equation of the line.

Appendix 7. Substrate Association Methods

7.1 Photographs

Photograph of typical substrate from one trap.



Photograph of laboratory set up including; buckets of unprocessed and processed substrate from PTs, volume measuring containers, stones being processed in white trays and recording forms.

Photograph of substrate in white trays, including; coarse pebbles (13 – 32 mm), medium pebbles (8 – 16 mm), fine pebbles (4 – 8 mm) and granules (2-4 mm).

7.2 Udden Wentworth Scale

Udden Wentworth Grain Size Scale, based on particle intermediate axial length (d_1)

Substrate Category	Minimum Size (mm)	Maximum Size (mm)
Granules	2	4
Pebbles		
Fine	4	8
Medium	8	16
Coarse	16	32
Very Coarse	32	64
Cobbles		
Fine	64	128
Coarse	128	256
Boulders		
Fine	256	512
Medium	512	1024
Coarse	1024	2048
Very Coarse	2048	4096

Appendix 8. Fish length-weight regressions

Length Weight Regressions used for fish biomass calculations in Chapter 5:

Brown trout: n = 342, $R^2 = 0.971176$, Equation of the line $y = 0.000009x^{3.041482}$

Atlantic salmon: n = 67, $R^2 = 0.960868$, Equation of the line $y = 0.000011x^{3.000292}$

European bullhead: n = 1479, $R^2 = 0.944825$, Equation of the line $y = 0.000010x^{3.073898}$

Stone loach: n = 20, $R^2 = 0.870221$, Equation of the line $y = 0.000016x^{2.862200}$

Minnow: n = 25, $R^2 = 0.924286$, Equation of the line $y = 0.000002x^{3.347349}$

European Eel: n = 10, $R^2 = 0.912778$, Equation of the line $y = 0.000045x^{2.455744}$

Formulae calculated from database jointly owned by Pritchard, Chadwick and Eagle from consecutive field seasons (2015 – 2018).

Appendix 9. Macroinvertebrate Species Lists

9.1 – Macroinvertebrate Species/Taxa List

Order/Class	Family	Taxa (lowest level of identification)
Amphipoda	Gammaridae	*Gammarus pulex*
Coleoptera	Chrysomelidae	*Chrysomelidae (adult)*
		Chrysomelidae (larvae)
	Dytiscidae	*Dytiscidae (adult)*
	Elmidae	*Elmis aenea (adult)*
		Elmis aenea (larvae)
		Limnius volckmari (adult)
		Limnius volckmari (larvae)
		Oulimnius (adult)
		Oulimnius (larvae)
	Gyrinidae	*Gyrinidae (larvae)*
		Gyrinus (adult)
		Gyrinus (larvae)
	Scirtidae	*Scirtidae (adult)*
		Scirtidae (larvae)
Diptera	Ceratopogonidae	*Ceratopogonidae*
	Chironomidae	*Chironomini*
		Diamesinae
		Orthocladiinae
		Podonominae
		Prodiamesinae
		Tanypodinae
		Tanytarsini
	Dixidae	*Dixella*
	Empididae	*Empididae*
	Limoniidae	*Antocha*
		Limoniidae
	Muscidae	*Limnophora*

	Pediciidae	*Dicranota*
		Pedicia
	Psychodidae	*Psychodidae*
	Simuliidae	*Simuliidae*
	Tipulidae	*Tipulidae*
Ephemeroptera	Baetidae	*Baetis rhodani*
		Centroptilum luteolum
		Procloeon pennulatum
	Caenidae	*Caenis rivulorum*
	Ephemerellidae	*Serratella ignita*
	Ephemeridae	*Ephemera danica*
	Heptageniidae	*Ecdyonurus dispar*
		Rhithrogena semicolorata
	Leptophlebiidae	*Habrophlebia fusca*
		Paraleptophlebia submarginata
Gastropoda	Lymnaeidae	*Radix balthica*
	Planorbidae	*Ancylus fluviatilis*
	Tateidae	*Potamopyrgus antipodarum*
Hemiptera	Corixidae	*Corixidae*
Hirudinia	Glossiohoniidae	*Glossiphoniidae*
		Helobdella stagnalis
Megaloptera	Sialidae	*Sialis fuliginosa*
Odonata	Calopterygidae	*Calopterygidae*
Oligochaeta	Oligochaeta	*Oligochaeta*
Plecoptera	Leuctridae	*Leuctra geniculata*
		Leuctra hippopus
		Leuctra inermis
	Nemouridae	*Nemouridae*
		Protonemura meyeri
	Perlidae	*Chloroperla tripunctata*
		Perla bipunctata
	Perlodidae	*Isoperla grammatica*

Sphaeriida	Sphaeriidae	*Sphaeriidae*
Trichoptera	Brachycentridae	*Brachycentrus subnubilis*
	Goeridae	*Silo pallipes*
	Hydropsychidae	*Hydropsyche instabilis*
		Hydropsyche siltalai
	Hydroptilidae	*Hydroptila*
		Hydroptilidae
	Leptoceridae	*Mystacides azurea*
	Limnephilidae	*Drusus annulatus*
		Glyphotaelius pellucidus
		Halesus radiatus
		Potamophylax latipennis
	Odontoceridae	*Odontocerum albicorne*
	Polycentropidade	*Polycentropus flavomaculatus*
	Rhycophilidae	*Rhyacophila dorsalis*
	Sericostomidae	*Sericostoma personatum*
Tricladida	Planariidae	*Polycelis*
Trombidiformes	Hydrachnidae	*Hydrachnidae*

9.2 – Counts of species/taxa at each site, in each sampling year

Species / Taxa list and individual counts from Surber samples (n = 10) at all sites in each sampling year.

Species /Taxa	DGB		Confluence		Footbridge		Farm	
	2016	2020	2018	2020	2018	2020	2018	2020
Anclylus fluviatilis	0	0	0	0	1	0	5	4
Antocha	0	0	4	10	3	3	6	2
Baetis rhodani	51	265	23	260	26	609	18	215
Brachycentrus subnubilis	0	0	0	0	0	0	0	1
Caenis rivulorum	1	0	1	0	9	0	10	0
Calopterygidae	0	0	0	0	0	0	1	0
Centroptilum luteolum	6	0	3	0	8	0	1	0
Ceratopogonidae	0	0	0	0	1	0	0	0
Chironomini	0	3	99	28	63	4	107	12
Chloroperla tripunctata	1	0	0	0	0	0	0	0
Chrysomelidae (adult)	0	0	0	0	0	0	0	1
Chrysomelidae (larvae)	0	0	0	0	0	1	0	0
Corixidae	0	0	34	0	72	0	1	0
Diamesinae	0	0	0	4	0	0	0	0

Dicranota	0	36	13	42	3	26	2	6
Dixella	0	0	2	0	0	0	0	0
Drusus annulatus	0	0	2	0	0	0	3	0
Dytiscidae (adult)	0	0	0	0	0	1	0	0
Ecdyonurus dispar	20	172	21	48	17	84	14	26
Elmis aenea (adult)	0	0	4	2	2	1	8	11
Elmis enea (larvae)	0	0	11	11	8	21	18	26
Empididae	0	0	0	10	0	11	0	35
Ephemera danica	0	3	4	2	131	2	23	2
Gammarus pulex	1	0	23	42	3	21	23	18
Glossiphoniidae	0	0	0	0	0	0	0	1
Glyphotaelius pellucidus	0	0	0	0	1	0	0	0
Gyrinidae (larvae)	0	0	0	0	0	0	0	10
Gyrinus (adult)	0	0	2	0	0	0	0	0
Gyrinus (larvae)	0	0	4	0	10	0	15	0
Habrophlebia fusca	60	36	8	0	0	0	0	0
Halesus radiatus	0	0	1	1	0	0	3	1
Helobdella stagnalis	0	4	1	0	0	0	3	14
Hydrachnidea	0	0	75	0	5	0	22	0

Hydropsyche instabilis	0	1	1	73	11	27	5	17
Hydropsyche siltalai	0	3	35	0	11	0	21	0
Hydroptila	0	1	0	9	0	22	0	8
Hydroptilidae	0	0	8	0	3	0	1	0
Isoperla grammatica	0	12	0	0	0	0	0	0
Leuctra geniculata	0	0	9	6	28	5	8	15
Leuctra hippopus	45	197	96	186	80	131	60	110
Leuctra inermis	0	0	21	2	26	0	12	2
Limnius volckmari (adult)	0	0	21	2	2	0	9	4
Limnius volckmari (larvae)	0	0	38	61	10	17	29	81
Limnophora	0	0	0	1	0	0	2	4
Limoniidae	0	3	0	10	1	5	1	1
Mystacides azurea	0	0	0	0	0	0	2	0
Nemouridae	0	16	0	3	0	1	2	6
Odontocerum albicorne	0	0	4	5	0	2	8	2
Oligochaeta	0	20	29	14	37	27	89	48
Orthocladiinae	0	17	4	276	7	228	13	157
Oulimnius (adult)	0	1	35	3	3	2	54	37

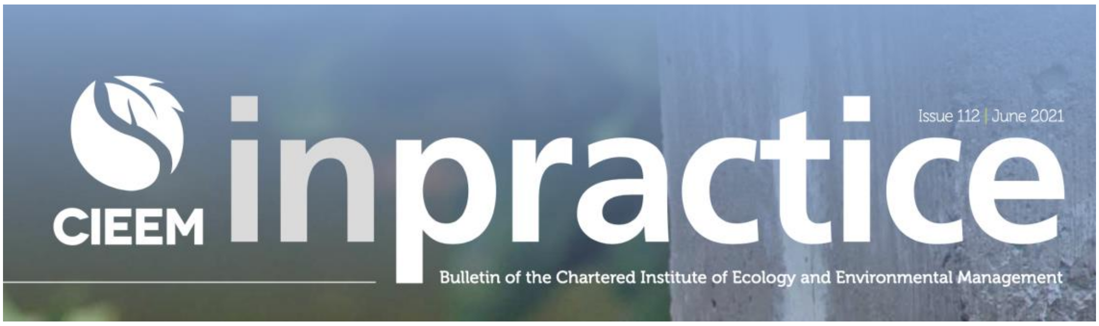
Oulimnius (larvae)	0	1	28	60	15	72	22	123
Paraleptophlebia submarginata	2	33	11	14	47	25	18	4
Pedicia	0	4	1	0	0	0	0	1
Perla bipunctata	0	0	0	0	0	0	4	0
Polycelis	0	0	0	0	0	0	18	0
Podonominae	0	0	0	0	0	0	4	0
Polycentropus flavomaculatus	0	0	45	7	9	7	16	6
Potamophylax latipennis	0	0	0	0	0	0	2	0
Potamopyrgus antipodarum	0	0	1	0	0	0	14	6
Procloeon pennulatum	0	8	0	13	0	3	0	3
Prodiamesinae	0	0	1	1	0	0	2	0
Protonemura meyeri	0	0	0	0	0	0	1	0
Psychodidae	0	2	0	1	0	2	1	9
Radix balthica	0	0	0	0	0	1	137	7
Rhithrogena semicolorata	2	0	0	0	0	0	0	0
Rhycophila dorsalis	1	0	1	1	0	3	1	5

Scirtidae (adult)	0	0	0	1	0	0	0	0
Scirtidae (larvae)	0	0	0	0	0	1	0	4
Sericostoma personatum	0	0	1	0	1	1	7	3
Serratella ignita	0	0	27	4	7	1	19	5
Sialis fuliginosa	0	0	0	0	0	0	3	0
Silo pallipes	0	0	3	0	0	0	0	0
Simuliidae	0	5	1	19	13	82	2	55
Sphaeriidae	0	0	1	1	1	0	5	0
Tanypodinae	2	20	69	33	31	31	35	43
Tanytarsini	0	4	42	37	8	30	24	8
Tipulidae	0	0	0	1	0	0	0	0
Total	192	867	868	1304	714	1510	934	1159

Appendix 10. CIEEM Feature article

Feature article available upon request.

Feature article published in *In Practice: Biosecurity and invasive species* Special Edition, bulletin of the Chartered Institute of Ecology and Environmental Management.



Invasive Signal Crayfish in the UK: Survey Methods to Inform Evidence-based Management.

Daniel D.A. Chadwick, Lawrence J. Eagle, Eleri G. Pritchard, Carl D. Sayer, Michael, A. Chadwick, Jan C. Axmacher, Paul Bradley
Published June 2021.

Summary

With invasive crayfish becoming increasingly widespread, evidence- based management is crucial to protect freshwater ecosystems. Knowledge of the structure and function of invasive crayfish populations allows for an effective evaluation of management efforts. Recent methodological developments have enabled the first truly quantitative studies of UK invasive crayfish populations in the field. This was achieved by the triple drawdown (TDD) survey approach. In this article, we explore current survey approaches and their limitations, and we introduce the TDD method with its implications for crayfish survey, policy development and management.

Appendix 11. The Conversation article

Pritchard (2020) open access and available at;
https://theconversation.com/invasive-species-why-britain-cant-eat-its-way-out-of-its-crayfish-problem-147961

THE CONVERSATION

Invasive species: why Britain can’t eat its way out of its crayfish problem

October 13, 2020 4.25pm BST

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Disclosure statement

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