Novel technology for crayfish management

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Intellectual Property & Publication Statements

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

Chapter 4 and Chapter 7 are based on work from jointly authored publications

Chapter 4: Harwood, M., Stebbing, P.D., Dunn, A.M., Cole, Z.K., Bradbeer, S.J., Aston, B., South, J. (2025) Rapid assessment of population dynamics and monitoring methods for invasive narrow clawed crayfish Pontastacus leptodactylus in a freshwater reservoir. Knowl. Manag. Aquat. Ecosyst., 426 (2025) 22. DOI: https://doi.org/10.1051/kmae/2025017

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I was the lead author, collected specimens in field and prepared samples for analysis, curated the data, analysed the data and wrote and edited the manuscripts in both first and final draft. Collaborating authors assisted in data analysis, supervision and reviewing of the manuscript.

Signed:

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Thesis Abstract

Freshwater conservation is key for the overarching health of the globe and novel technology, such as remote underwater video (RUV), provides an opportunity to advance freshwater conservation. RUVs are an underutilised tool in freshwater environments that currently lack standardised methodologies. A systematic literature review was conducted into the use of RUV in freshwater environments and a cautious set of standards are recommended, consisting of using a standard action camera, recording at ≥30fps with a resolution of 1080p for 60 minutes. I have constructed CrayCam, a specialised RUV system for surveying crayfish. Feasibility tests of CrayCam found that it could successfully observe crayfish, with diminishing returns when crayfish are further away from the RUV system and when turbidity is higher. CrayCam was used alongside traditional surveying methods to study a population of the data deficient narrow clawed crayfish (*Pontastacus leptodactylus*). I recommend a mixed approach of methods to successfully study this species and classified the location of this population as in the 'Containment' or 'Asset Protection' stage of the Generalised Invasion Curve. A multi-method approach was also employed to assess the extent of a signal crayfish (*Pacifastacus leniusculus*) invasion. This study found that over half of the barriers within the survey area had been breached by invasive crayfish and crayfish tended to be found at locations closer to upstream barriers and had higher abundances at sites with lower ORP. Field observations of native white-clawed crayfish (*Austropotamobius pallipes*) were also made using CrayCam. These studies found white-clawed crayfish to be more active during daylight than was initially thought, with larger individuals tending to have higher aggressive intensities. Finally, it is important to understand the threats of disease invasive crayfish can bring into their new environments and a pathological study of narrow clawed crayfish has been conducted to assess their potential risk. Overall, this thesis provides new knowledge that can be practically applied across the myriad challenges of crayfish management.

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Chapter 1

Introduction

Freshwater Conservation

Approximately seventy percent of the Earth's surface is covered in water, of which only 2.5% is freshwater (Mishra, 2023). Despite making up less than 0.8% of the Earth's surface, freshwater environments support at least 100,000 species, which is approximately 6% of all described species (Dudgeon et al. 2006), including around a third of all vertebrate species (Strayer & Dudgeon, 2010). Over 50% of the world's population live within 3km of a freshwater body (Kummu et al. 2011). Freshwater biodiversity provides many services to humans including food, material goods, education, recreation and water purification (Lynch et al. 2023) and it is vital that it is conserved.

The conservation of freshwater ecosystems is vital for the health of the Earth as a whole. Policy and legislation are key to ensure the sustainable use and management of freshwater ecosystems. Freshwater conservation is an integral part of the United Nation's Sustainable Development Goals (SDGs) (https://www.un.org/sustainabledevelopment/). Of these seventeen goals, SDG 6 (Clean Water and Sanitation), SDG 12 (Responsible Consumption and Production) and SDG 15 (Life on Land) all list major routes to conserving freshwater ecosystems. Key freshwater conservation targets within these three groups are: 6.3) improve water quality by reducing pollution, 6.6) protect and restore water-related ecosystems, 12.1) implement a 10-year framework for sustainable consumption, 12.2) achieve sustainable management and efficient use of natural resources, 12.4) sound management of chemicals and waste, reducing their release into water, 15.1) ensure the conservation, restoration and sustainable use of freshwater ecosystems and their services, 15.5) take urgent action to reduce degradation of natural habitats and 15.8) introduce measure to prevent the introduction and significantly reduce the impact of invasive alien species and control or eradicate the priority species (United Nations, 2025). By achieving these targets, the future protection of freshwater ecosystems can be achieved.

Other policies and legislations are in place to raise the profile of freshwater ecosystems. The International Union for Conservation of Nature (IUCN) assesses the extinction risk of freshwater species through the IUCN Red List of Threatened Species. The IUCN also identifies the key sites that are important in the survival of these species. These classifications help governments and stakeholders to advise suitable approaches to conservation, and which species and habitats should be prioritised (IUCN, 2025). Ramsar's Convention on Wetlands is another legislation that looks to conserve wetlands through local, national and international collaboration. The term "wetlands" used by Ramsar encapsulates all waterbody types including rivers, lakes, marshes, coastal areas and human-made waterbodies. This legislation commits to the wise use of wetlands, the designation of wetlands as "Wetlands of International Importance" and the effective management of them, and international cooperation on transboundary waterbodies (RAMSAR, 2025).

Freshwater ecosystems include species which are among the most endangered in the world, with freshwater fishes having a higher proportion of species at risk of extinction than most other groups (Jenkins, 2003; Nel et al. 2009). They are vulnerable to multiple stressors and freshwater species face a rapid extinction rate (Ormerod et al. 2010). Almost half of all freshwater habitats are classified as threatened by the European Red List for terrestrial and freshwater habitats, these are either classified as critically endangered, endangered or vulnerable (Janssen et al. 2016; Grzybowski & Glińska-Lewczuk, 2019). Freshwater species declined at a faster rate than both marine and terrestrial species over fifty years between 1970 and 2020 (WWF, 2024). As opposed to their marine and terrestrial counterparts, freshwater vertebrate populations are declining twice as fast (Tickner et al. 2020). In addition to this, more aquatic invertebrate species are threatened with extinction than their terrestrial counterparts (33% of aquatic invertebrates compared to 28% of terrestrial invertebrates) (Sánchez-Bayoa & Wyckhuysb, 2019).

The key factors that are influencing the range and abundance of many freshwater species are pollution, overexploitation, climate change, habitat degradation and invasive species (Strayer & Dudgeon, 2010; Sibley et al. 2010; Tickner et al. 2020). In most cases it is not a single stressor that influences the decline of freshwater populations and ecosystems. Multiple stressors in freshwater ecosystems produce synergistic interactions that compound the damage and decline of these species and habitats (Bao et al, 2024). The conservation of these species and ecosystems is key as they play a significant role in sustaining human life and the loss of them can result in wider impacts and increased disaster risk as well as devastation caused by the loss of resources such as food supplies. Further research is required to establish the extent of loss and the factors influencing decline. By rapidly identifying areas of concern mitigation methods can be installed that reduce the potential for further decline. By understanding these problems, we can find the best possible solutions to mitigate the extent of them and provide the fragile ecosystems with the best possible chance of survival.

Invasive Non-Native Species

A non-native species is one that has been transported beyond its natural biogeographic range through human intervention. These populations become known as established non-native species if they are able to successfully establish a self-sustaining population in their new range (Soto et al. 2024). When these established non-native populations have recently spread or are spreading rapidly in their invaded range either actively or passively, they become an invasive non-native species (Soto et al. 2024). Not all non-native species are invasive as some species are not capable of surviving and reproducing in their new environments and it is theorised that ten percent of all introduced non-native species will become established, and then ten percent of those that become established will become invasive (Keller et al. 2011). The progress of an invasion is divided into four sequential stages (sensu Blackburn et al. 2011; Figure 1.1), these are: 1) Transport: how the species moves from its native range to the invaded location, 2) Introduction: when the species is first introduced to its invaded location, 3) Establishment: when the initial introduced individuals establish a self-sustaining population (Soto et al. 2024), 4) Spread: when the established non-native population expands its range either actively or passively, with or without human intervention (Blackburn et al. 2016; Soto et al. 2024). The success of an invasive non-native species is dependent on many factors. Reduced stressors, including no natural predators and reduced disease allow non-native populations to become established. There are several characteristics that make a good invasive species, these include the ability to rapidly reproduce and grow, high dispersal rates and high levels of adaptability (Chinchio et al. 2020).

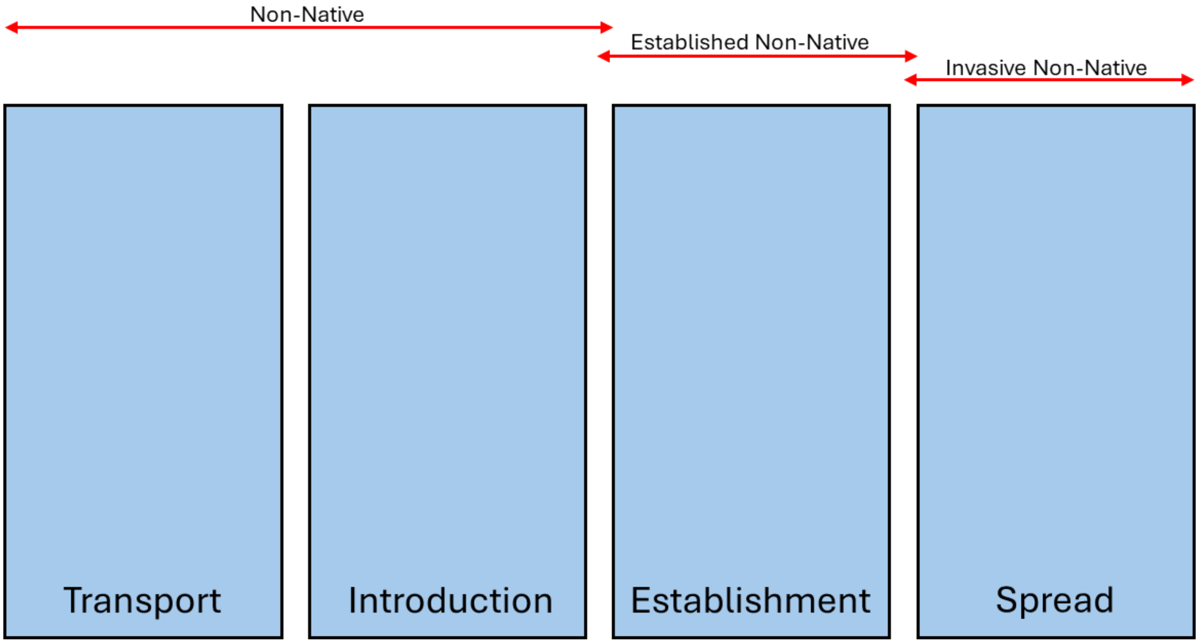


Figure 1.1. Invasion stages adapted from Blackburn et al. 2011 proposed framework for a biological invasion (blue boxes). An invasion is divided into four stages, transport, introduction, establishment and spread. The terminology used for a non-native species is dependent on the stage of an invasion (red arrows) (Soto et al. 2024)

In the past hundred years humans have broken down geographical boundaries. Major developments in technology, including commercial air travel and specialised freight shipping, have meant that trade and travel have become globalised and this has facilitated the spread of non-native species across the globe (Keller et al. 2011). Shipping and the construction of canals, including the Panama Canal that connects the Caribbean Sea with the Pacific Ocean, has facilitated the movement of non-native species to areas they could not reach unaided at a faster rate than they would naturally (Molnar et al. 2008). These fixed transport infrastructures allow biotic interchange in the same process as natural corridors (Wilson et al. 2009). There is also fear that climate change and increased anthropogenic activities threatens Arctic resilience and an Arctic passage trade route is becoming more viable due to declining summer sea ice and these activities are expected to increase the potential introduction of non-native species (Chan et al. 2019). Changes driven by climate change threaten Arctic resilience and this amplifies the potential of ecological consequences from non-native species introduction and range expansions (Kaiser & Kourantidou, 2021).

Non-native species can be transported to new locations through natural dispersal. This includes water currents, migration and hitchhiking on other species, i.e. zoochory (Ricciardi, 2012). Alternatively, the more common methods involve human activity. These can include international trade and the transportation of people and goods (Ricciardi, 2012). Non-native species are transported globally as part of international trade, with the key areas of trade being for agriculture, aquaculture, and landscape aesthetics (Xu et al. 2014). Unintentional translocations can also occur through contamination of goods, as well as species that are stowaways (Minchin et al. 2013). Trade can result in both intentional and unintentional introductions of non-native species into the wild. The introduction of newly transported non-native species into the wild occurs through either intentional or unintentional releases. Illegal activities, such as unauthorised stocking or the release of pets to the wild (Drake et al. 2015) are a leading cause of non-native species introduction. Alternatively, individuals are unintentionally released into the wild by escaping from their designated locations, either through poor containment or poor biosecurity, with individuals piggybacking onto people and objects that pass through their containment area.

Williamson & Fitter (1996) theorised that ten percent of every introduced non-native species will become established. High numbers of routes of introduction and high numbers of individual introductions across multiple locations all contribute to higher propagule pressures. These higher pressures result in a greater likelihood that a non-native species will become established (Lockwood et al. 2009; Blackburn et al. 2011). By keeping propagule pressures low, the establishment probability of potential invasive non-native species can be reduced (Stringham & Lockwood, 2021). Of the ten percent of non-native species that become established, ten percent of these are likely to become invasive (Williamson & Fitter, 1996). When these established populations spread to the wider ecosystem, they then become invasive. Habitat fragmentation and barriers, including human-made weirs and disused mills, may function as tools that can reduce the spread of invasive non-native species (Danilović et al. 2025).

Established populations need to adapt to their new range by overcoming biotic resistance in order to successfully spread (Blackburn et al. 2016). The process of the development of a non-native species becoming an invasive non-native species can be illustrated through Blackburn's stages of a biological invasion (Figure 1.1). The extent of these invasive non-native populations can also be categorised into stages on a site-by-site basis using the Generalised Invasion Curve (Figure 1.2), these stages can be used to determine potential interventions (Harris et al. 2018). The four stages of the Generalised Invasion Curve are 1.) Prevention: when the species is absent, 2.) Eradication: when populations are isolated and small. 3.) Containment: when populations are rapidly increasing, and 4.) Asset protection: when there is a widespread population (Harris et al. 2018; Harwood et al. 2025). By categorising an invasion, appropriate management responses can be introduced, and the failure to do this could result in further invasive non-native species spread and increased costs of management interventions (Cuthbert et al. 2022).

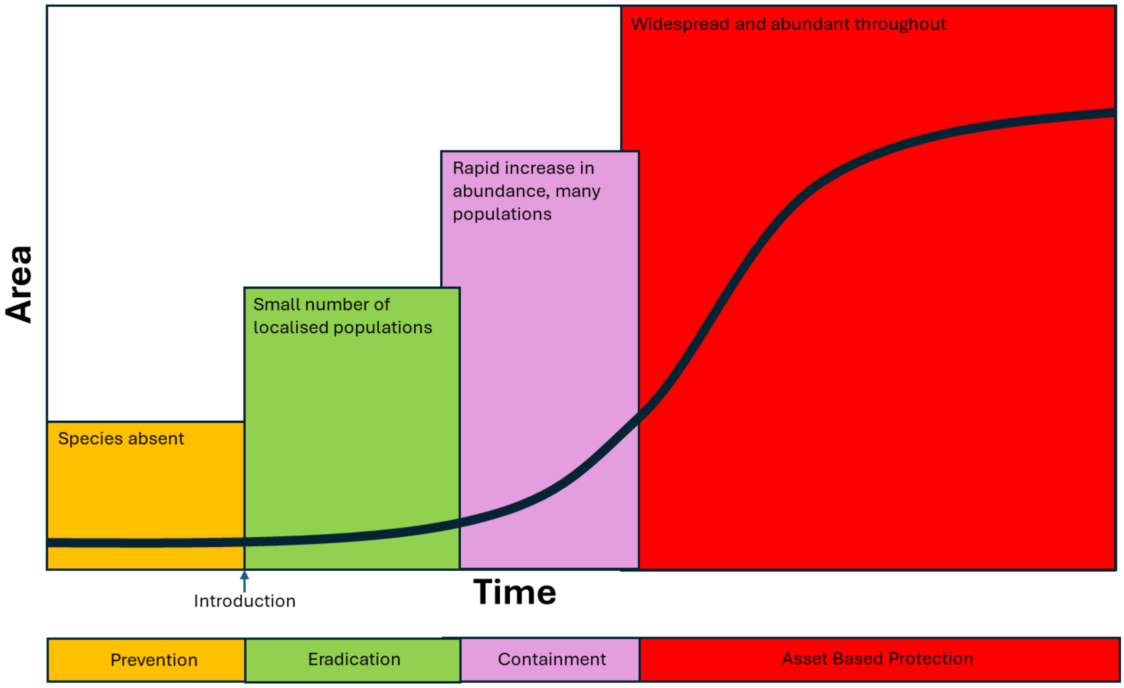


Figure 1.2. General Invasion Curve for invasive non-native species, there are four stages on the curve; 1) Prevention, when the species is absent from the asset, 2) Eradication, when populations are small and isolated, 3) Containment, when populations are rapidly increasing, and 4) Asset protection, when there is a widespread population

When an invasive non-native species is absent from a site measures can be taken to prevent its introduction. The key preventative measure is biosecurity, this is the first line of defence against invasive non-native species introduction and establishment (Dunn & Hatcher, 2015). By monitoring potential routes that invasive non-native species enter their invaded range the chance of detecting harmful species early, before they can become established, increases maximising the likelihood of successfully mitigating their impacts. However, biosecurity on a regional scale is vital to prevent the establishment of invasive non-native species (Faulkner et al. 2020). When populations are localised and small there is still potential that non-native species can be eradicated at a site level. These eradication procedures often incur high costs and the feasibility of a successful eradication is dependent on six criteria: 1) rate of removal exceeds rate of population increase, 2) immigration is prevented or reduced, 3) all reproductive individuals are at risk, 4) individuals can be detected at low densities, 5) cost-benefit analysis in favour of eradication and 6) there is a suitable socio-political environment (Bomford & O’Brien, 1995; Stebbing, 2016). When a site is in the containment stage it is important to educate users of the area about the importance of biosecurity and what measures can be taken to contain invasive non-native species to the site and prevent further spread. The “Check, Clean, Dry” initiative is one of the many biosecurity campaigns used to prevent the introduction and ensure the containment of invasive non-native species at a site

(Ovenden & Studholme, 2021). Other features, including artificial barriers can also act as a form of containment at sites that have populations of invasive non-native species (Danilović et al. 2025). During the containment stage it is important to create barriers to minimise the risk of invasive non-native populations dispersing and spreading beyond the invaded area (Robertson et al. 2020). When a site has become fully invaded by an invasive non-native species and they have spread throughout the connecting sites, asset-based protection is required. Here the key challenge is to identify the best way to allocate resources and funds to assess expected returns and uncertainty while also ensuring that assets are protected to avoid significant damage that invasive non-native species can cause (Akter et al. 2015). It is important to monitor and understand populations in order to successfully identify the steps required to mitigate their impacts.

Aquatic environments are highly vulnerable to invasive species and thousands of non-native species have been successfully translocated across the globe (Saidova et al. 2024). Humans are responsible for the transportation and introduction of many freshwater species outside of their natural habitats, with several different vectors responsible for this. Sources of introduction include angling, with species being introduced for commercial or ornamental value, as well as anglers using non-native live bait that is allowed to escape or is intentionally released into waterbodies (Lipták et al. 2023). Aquaculture and the aquarium trade are also responsible for the introduction of non-native species into environments through accidental releases and intentional "biological pollution" and unwanted pet release (Naylor et al. 2001). Ballast water from ships also acts as a vector for aquatic invasive non-native species to freshwater environments (Bailey, 2015). Large volumes of water are transported internationally with potential non-native stowaways who upon discharge are introduced to their new environment, these can access areas they could not previously have reached at a faster rate and potentially have significant negative impacts on their new ecosystems (Molnar et al. 2008).

Invasive non-native species can cause large scale impacts in their invaded range. These impacts are the consequences of the invasive non-native species and are split into six levels, 1) individuals, 2) populations, 3) species, 4) assemblages, 5) ecosystem functions/service, 6) abiotic environment (Carneiro et al. 2025). These impacts are caused both directly and indirectly (Gallardo et al. 2016). Invasive non-native species break down biogeographic realms having devastating impacts on native species, while also changing ecosystem functioning, damaging native ecosystem service (Pyšek et al. 2020). Invasive non-native species have been responsible for the local and global extinctions of native species through a range of factors (Simberloff, 2010). They also impact native species through predation, in the Atlantic Ocean Invasive non-native Indo-Pacific lionfish (*Pterois volitans & Pterois* *miles*) have reduced the abundance of small native fish species by up to 95% in some invaded sites through predation (Côté et al. 2013). Competition for resources between native species and invasive non-native species is also responsible for the displacement of native species. In Tanzania, invasive non-native Nile tilapia (*Oreochromis niloticus*) have shown strong competitive dominance over native Manyara tilapia (*Oreochromis amphimelas*) that has resulted in long term habitat displacement (Champneys et al. 2021). Invasive non-native species are also responsible for the spread of parasites and disease. The squirrelpox virus is believed to have co-invaded with the grey squirrel (*Sciurus carolinensis*) when it was introduced into Britain in the 1900s, this virus has catastrophic impacts on native red squirrel (*Sciurus vulgaris*) populations whereas invasive non-native squirrels do not show signs of infection (Atkin et al. 2010). It is important to understand what pathogens invasive non-native species may bring into their invaded environments in order to identify potential risks to native species, histological analysis is an important tool that addresses this.

Many invasive non-native species are often referred to as ecosystem engineers and can cause widescale impacts throughout an invasive ecosystem and cause massive economic burdens (Carneiro et al. 2025). Modification of habitats by invasive species can cause significant nutrient shifts and contamination that have serious impacts of wider ecosystem functions (Crooks, 2002; Pyšek et al. 2020). Red swamp crayfish (*Procambarus clarkii*) alter their invaded environment through burrowing and scavenging, resulting in increased turbidity that may contribute towards eutrophication (Geiger et al. 2005; Anastácio et al. 2011), which in turn can cause a rapid decline in aquatic macrophyte populations throughout a waterbody due to the reduced levels of light penetration (Hilt et al. 2013). Invasive non-native species also cause significant damage to operational components of many vital services. They have been found to cause damage to buildings, energy and water structures and transport infrastructure (Booy et al. 2017). Many agricultural services have been impacted by invasive non-native species. In Portuguese rice fields invasive non-native red swamp crayfish have caused economic losses due to them uprooting rice seeds and seedlings, as well as fragmenting plants and their burrows causing damage to irrigation (Anastácio et al. 2005; Anastácio et al. 2011). Water system infrastructure, including facilities that treat drinking water and wastewater, are at serious risk of damage from a range of invasive non-native species. Invasive non-native zebra mussels (*Dreissena polymorpha*) and quagga mussels (*Dreissena bugensis*) are major biofouling pests for water treatment works and irrigation systems in Europe and North America, populations of these invasive non-native mussels clog water intakes and pipes and contribution to the formation of disinfection by-products (Elliott et al. 2005; Chakraborti et al. 2016).

Damage caused by invasive non-native species to native ecosystems and human-made infrastructure is significant. Large sums of money are spent to fix the destruction caused by them and there are also many further costs associated with their control and eradication, with careful consideration required when prioritising management approaches (Booy et al. 2020). Invasive species cost national economies an estimated US$423 billion annually (Bradshaw et al. 2024) with costs likely to substantially increase over the next 15 years (Henry et al. 2023). Framework, such as the Environmental Impact Classification for Alien Taxa (EICAT), clarifies non-native taxa to the magnitude of their detrimental environmental impacts in their new ranges (Hawkins et al. 2015). By understanding these magnitudes policy can be implemented to deter illegal activities that would aid in the spread of invasive non-native species, such as The Invasive Alien Species (Enforcement and Permitting) Order 2019, which include penalties for the spread of 'species of special concern'. Other policies, including The Wildlife and Countryside Act 1981 ensure that landowners and service providers are responsible for, and can be held liable for, invasive non-native species that are present within their sites. These policies and legislations are important as invasive non-native species are an ever-growing problem globally (Ricciardi et al. 2021) and the development of strategies to mitigate future invasion threats is vital (Ricciardi et al. 2017).

Crayfish

Crayfish are a diverse group of large freshwater decapod crustaceans that were first introduced to the study of zoology by T.H. Huxley in 1880 (Huxley, 1880; Crandall & De Grave, 2017). They act as a keystone predator in a range of different habitats and ecosystems and play a key role in the transfer of energy throughout complex food webs (Momot, 1995). Crayfish naturally occur around the majority of the globe, excluding continental Africa and Antarctica (Crandall & Buhay, 2008; Ion et al. 2024; Figure 1.3). Crayfish are split into two superfamilies (Astacoidea and Parastacoidea) which consist of five families with a total of 692 unique species of crayfish (Crandall & De Grave, 2017). Crayfish consist of two main body parts, these are the cephalothorax and abdomen. The cephalothorax is protected by the carapace which is part of the exoskeleton of a crayfish. Crayfish have two large claws, referred to as chelae which tend to be larger in males than females when carapace lengths are equal (Malavé et al. 2018; Graham et al. 2023). Female crayfish can hold hundreds of eggs (Figure 1.4A), and newly hatched juveniles cling to their mother and the juvenile mass for up to two weeks after hatching (Mathews, 2011; Figure 1.4B). Crayfish grow by moulting, this involves shedding of their old exoskeleton and assuming its new length and volume by actively absorbing water. During the premoult process they can regenerate lost limbs and other damaged components (Aiken & Waddy, 1992).

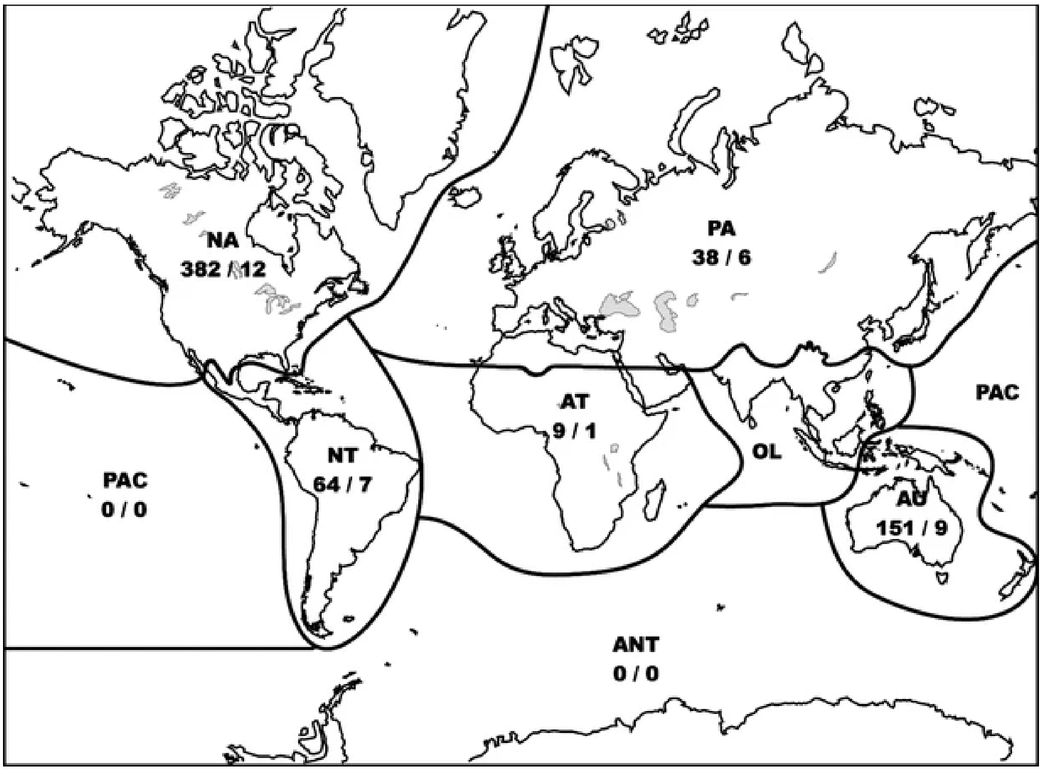


Figure 1.3. Crandall & Buhay (2008) reproduction of a map showing the geographical distribution of freshwater crayfish diversity (number of species/number of genus). PA- Palaearctic; NA – Nearctic; NT – Neotropical; AT – Afrotropical; OL – Oriental; AU – Australasian; PAC – Pacific Oceanic Islands; ANT – Antarctic

Figure 1.4. A) Female narrow clawed crayfish (*Pontastacus leptodactylus*) carrying unfertilised eggs, awaiting fertilisation B) Juvenile mass attached to a female white-clawed crayfish (*Austropotamobius pallipes*) with juveniles holding on to their mother after hatching

Crayfish are seen as ecosystem engineers and hold a crucial ecological position in systems in which they naturally occur (O'Hea Miller et al. 2024). One of the ways in which crayfish are ecosystem engineers is through their burrowing capabilities, many species of crayfish construct terrestrial burrows in which they shelter to avoid desiccation as well as overwintering (Stoeckel et al. 2011). Crayfish are seen as opportunistic omnivore feeders with plant matter and detritus making up a large portion of their diet, they have also been seen to demonstrate a predilection for animal protein (Momot, 1995; Nyström et al. 1996). This role as an ecosystem engineer makes crayfish a highly successful invasive organism that can have large scale impacts on native crayfish species, as well as the wider ecosystem as a whole.

Invasive Crayfish

Due to their ability to integrate into a food web at many levels, crayfish are a good candidate to become an invasive non-native species (Gherardi et al. 2011). As a result of a range of factors, particularly the aquarium trade and aquaculture, the global distribution of crayfish has dramatically changed (Gherardi, 2007). The first accounts of crayfish introductions beyond their natural range date back to 1746 when King John III of Sweden was reported to have imported an unspecified crayfish species into Sweden, before this they had not previously been seen in the country (Hobbs et al. 1989; O'Hea Miller et al. 2024). Globally 28 species of crayfish have successfully formed established non-native populations and in Europe almost two thirds of the crayfish species that are found are non-native (Souty-Grosset et al. 2006; Gherardi, 2010). Two of the most notorious invasive non-native crayfish species that are present within Europe are the red swamp crayfish (*Procambarus clarkii*), which is native to southeastern USA and northern Mexico (Oficialdegui et al. 2019), and the signal crayfish (*Pacifastacus leniusculus*) which is native to western North America between the Pacific Ocean and Rocky Mountains (Holdich et al. 2014).

Introductions of invasive non-native crayfish have been both intentional and unintentional. The global trade in crayfish focuses on both aquaculture and the aquarium trade, with crayfish often being a desired ornamental pet. The introduction of these non-native crayfish into the wild occurs through individuals escaping enclosures or unwanted pets and live bait being dumped into waterbodies (Holdich, 1993; Haubrock et al. 2021; Akmal et al. 2023; DiStefano et al. 2023). Freshwater crayfish are among the most problematic invasive species once established (Haubrock et al. 2025). Crayfish are good invaders due to their fast growth and high fecundity, for example, the success of the invasive non-native marbled crayfish (*Procambarus virginalis*) in Europe is significantly driven by its fast growth and frequent reproduction (Kouba et al. 2021). The generalist feeding habits of crayfish also make them good invaders, red swamp crayfish (*Procambarus clarkii*) have been shown in southern Europe to have diets that consist of plant matter and animal remains of species that dominate their invaded habitats, showing that they easily adjust to the prey items available in their new environments (Gherardi & Barbaresi, 2008). The aggressive nature of invasive non-native crayfish over native species also makes them a successful invader (Fořt et al. 2019), an example of this in Australia is smaller individuals of the invasive non-native common yabby (*Cherax destructor*) shows inherent and greater aggressiveness that the native Fitzroy Falls spiny crayfish (*Euastacus dharawalus*), and only larger individuals of the native crayfish hold a competitive advantage over invasive non-native crayfish (O'Hea Miller et al. 2023).

Invasive non-native crayfish pose several threats that are of serious concern, these threats can be split into three categories: impacts on native species, impacts on native ecosystems and, impacts on human-made infrastructure. Invasive rusty crayfish (*Orconectes rusticus*) are replacing native virile crayfish (*Orconectes virilis*) in Michigan and northern Wisconsin, USA, and abundances of native fish populations are declining as a result of the invasive rusty crayfish being a more effective predator of fish eggs than the native crayfish (Morse et al. 2013). Similar trends have also been observed in Northern Italy with invasive red swamp crayfish having a negative impact on the breeding success of native amphibians (Ficetola et al. 2011). Other than direct predation and competition, invasive non-native crayfish also can impact native crayfish populations through the spread of disease. North America species of crayfish, particularly the signal crayfish and red swamp crayfish, are vectors of the oomycete *Aphanomyces astaci*, the causative agent of crayfish plague (Bouallegui, 2021). Crayfish plague can have significant negative impacts on populations of native crayfish throughout Europe and has often led to localised extinctions of them and widespread population declines (Matthews & Reynolds, 1992; Harlioğlu, 2008; Dunn et al. 2009). Invasive non-native crayfish are ecosystem engineers, invasive non-native signal crayfish show higher rates of litter consumption and increased production of fine particulate organic matter and dissolved organic carbon, compared to their native counterpart the white-clawed crayfish (*Austropotamobius pallipes*) (Doherty-Bone et al. 2018). The burrowing of invasive non-native crayfish can also lead to wide scale damages, in the UK the intensity of bank retreat increased significantly with densities of invasive non-native signal crayfish (Sanders et al. 2021). This crayfish burrowing can also have major impacts on human-made infrastructure, as has been discussed earlier in the *Invasive Non-Native Species* section of this thesis introduction, with invasive non-native red swamp crayfish burrows causing damage to Portuguese rice field irrigation (Anastácio et al. 2005; Anastácio et al. 2011). Invasive non-native common crayfish (*Procambarus clarkii* and *Cherax quadricarinatus*) have also been associated with damage to dams and irrigation canals in South Africa (de Moor, 2002).

The first non-native crayfish to be introduced into Europe was the spiny cheek crayfish (*Orconectes limosus*) in the early 1900s in Germany and then further European countries for aquaculture purposes (Hentonnen & Huner, 1999). The decline of native noble crayfish (*Astacus astacus*) populations, that were used for aquaculture, as a result of crayfish plague led to the introduction of signal crayfish as a plague resilient species to subsidise these losses (Holdich, 2002). The first signal crayfish introduction occurred in Sweden in 1959, with further introductions occurring throughout Europe through the 1960s and 70s (Lewis, 2001). The red swamp crayfish was first introduced to Spain in 1973, from which it has rapidly spread throughout other European countries (Holdich, 2002). Many further introductions of non-native crayfish have since occurred, with the trade in ornamental crayfish rapidly growing and being responsible for the intentional and accidental release of new species into the wild (Chucholl, 2013). The marbled crayfish (*Procambarus virginalis*) is a popular exotic crayfish with a high demand and there is great fear about the possibility of it being released into new ecosystems (Faulkes, 2010). The asexual reproduction ability of marbled crayfish makes it a highly successful invasive non-native species, and they have been found to negatively alter the state of an entire ecosystem, making it a species of serious concern (Maciaszek et al. 2022).

It can be argued that crayfish plague is responsible for the introduction of invasive non-native crayfish into Europe as the loss of native stocks in aquaculture due to plague outbreaks led to the introduction of signal crayfish to subsidise them. Crayfish plague was first identified in Europe in the River Po Valley, Italy, in the 1850s, this introduction was likely accidental and the vector is unknown, but the timing coincides with early developments in using water as ballast (Jussila et al 2015). The subsidisation of noble crayfish stocks with signal crayfish also introduced a vector for crayfish plague which facilitated the spread of the disease throughout Europe and then into the UK (Holdich & Reeve, 1991; Holdich, 2003).

UK Crayfish

The white-clawed crayfish (*Austropotamobius pallipes*) is the only native species of crayfish present within the United Kingdom, although the natural occurrence of crayfish in Britain is contested (Albrecht, 1983; Holdich, 2002a). Invasive non-native species are estimated to cost the UK economy £4 billion each year (Eschen et al. 2023). Invasive non-native crayfish contribute heavily to this cost with signal crayfish costing US$15.3 million between 2000 and 2020 in the UK (Kouba et al. 2022). There are currently seven established non-native crayfish species within the UK (Stebbing et al. 2014). These are: signal crayfish (*Pacifastacus leniusculus*); narrow clawed crayfish (*Pontastacus leptodactylus*); noble crayfish (*Astacus astacus*); red swamp crayfish (*Procambarus clarkii*), virile crayfish (*Orconectes virilis*); spiny-cheek crayfish (*Orconectes limosus*) and the white-river crayfish (*Procambarus acutus*) (Stebbing et al. 2014). Historical legislation has been implemented within the United Kingdom to reduce the risk of spread of invasive non-native crayfish, the Prohibition of Keeping of Live Fish (Crayfish) Order 1996 made it an offence to keep any non-native crayfish in England and Wales without a license, excluding areas where an established population existed, defined by postal codes, and prevented the creation of new crayfish aquaculture centres unless they operate indoor (Vigneux et al. 2002). This legislation has now been superseded by the Invasive Alien Species (Enforcement and Permitting) Order 2019, which has identified non-native species of special concern and introduces penalties for the spread of them.

Study Species

One native species of crayfish, the white-clawed crayfish, and two invasive non-native crayfish, the signal crayfish and narrow clawed crayfish were used as target species to address different research questions throughout this thesis, these are outlined at the end of this introduction.

White-clawed crayfish (*Austropotamobius pallipes*)

The white-clawed crayfish are a small crayfish species (body length <12cm) with a brown/olive complexion (Figure 1.5). They are associated with stony habitats containing obvious refuge including tree roots and gaps under rocks (Holdich et al. 2006). They are believed to remain in burrows during the day, with high activity levels at night (Peay & Hirst, 2003). They are omnivorous and opportunistic with their diet consisting of easily accessible vegetation and detritus as well as invertebrates including Tricoptera and Diptera (Scalici & Gibertini, 2007).

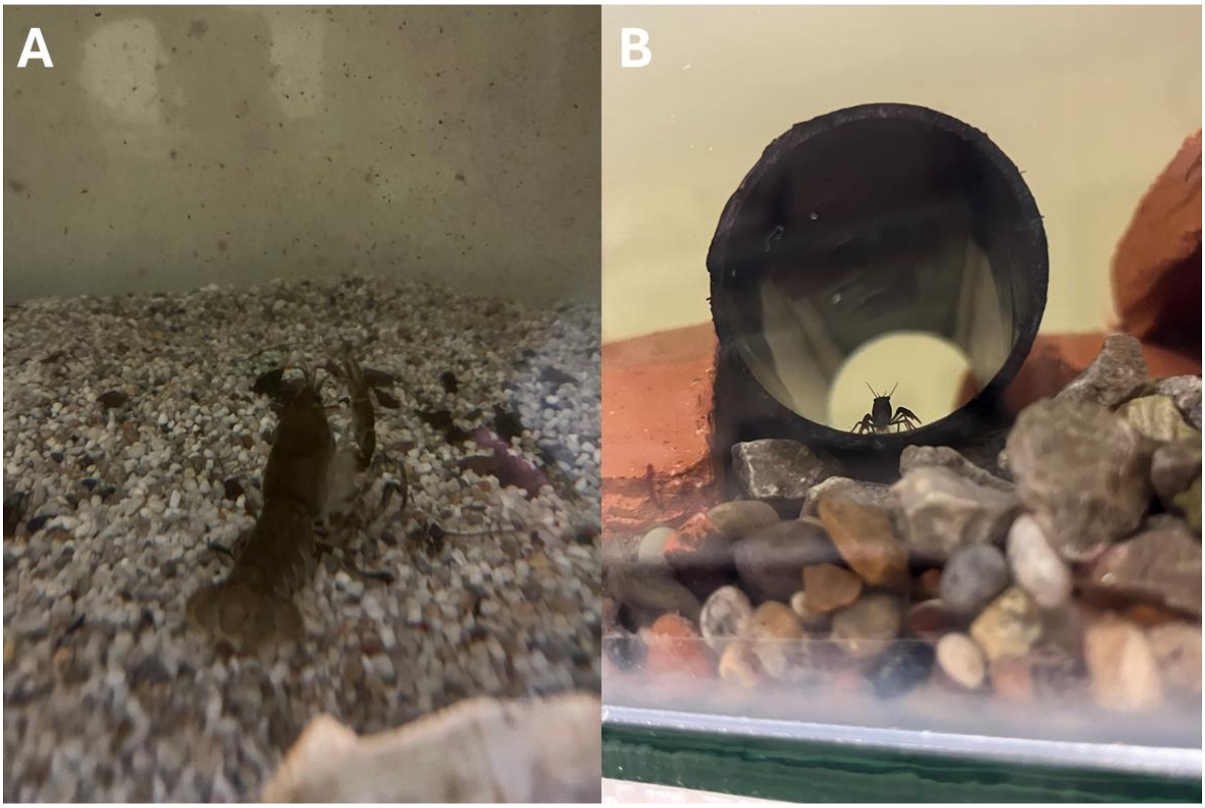


Figure 1.5. A) White-clawed crayfish (*Austropotamobius pallipes*), this individual was recovered from the wild as part of a crayfish rescue after an outbreak of crayfish plague, this is discussed in Chapter 3, B) Juvenile white-clawed crayfish, less than 1cm in length, less than a week after detaching from its mother

White-clawed crayfish are naturally found in Western Europe; however, populations have been drastically declining across the continent. The species is classified as endangered by the International Union for Conservation of Nature (IUCN) Red List and it is protected by UK legislation (Nightingale et al. 2017). There is speculation that white-clawed crayfish were not present within the UK prior to the 1500s, it is argued that the lack of genetic diversity of white-clawed crayfish in Britain, along with the strong genetic similarity with a population in France is evidence that the “native” population originated elsewhere (Grandjean et al. 1997a; Grandjean et al. 1997b; Holdich, 2002a). The time of this introduction is unknown, but the earliest written record of crayfish in Britain is the 1587 book The Chronicles of England, Scotland and Ireland by Holinshed, in which crayfish are referenced as being “plentifullie in our fresh rivers in banks” (Holinshed, 1587; Sibley et al. 2010).

Regardless of historical status, white-clawed are a keystone species in British waterways (Biasetti et al. 2021). They are the largest native freshwater invertebrate in Britain and play a key role in aquatic habitats and ecosystems (Nightingale et al. 2017). White-clawed face significant threats from a range of factors including habitat destruction, pollution and invasive species (Grandjean et al. 1997c). The slow growth rate and low fertility of white-clawed crayfish make them particularly vulnerable to habitat instability and modification and the channelisation of banks and destruction of suitable habitat through construction removes potential food sources and shelter (Biasetti et al. 2021). The introduction of invasive non-native crayfish has also introduced crayfish plague (as described in the Invasive Crayfish section of this introduction) which can cause complete localised extinctions of white-clawed crayfish (Dunn et al. 2009).

White-clawed crayfish are difficult to monitor due to their protected status and vulnerability; this has led to knowledge gaps in our understanding of them as a species and it is vital to address these in order to better conserve them as a species. There are gaps in understanding their instream behaviour and habitat preferences, I have explored this knowledge gap in Chapter 6 through instream behavioural observations. Our understanding of the distribution of white-clawed crayfish is also incomplete and there are potentially still undiscovered populations throughout the UK. In the surveys conducted as part of Chapter 5 I found an unknown population of white-clawed crayfish and have used this to inform wider protection of the area. It is important to understand where these crayfish are in order to protect them, while also considering conservation tensions, such as barrier removal for salmonids potentially aiding the spread of invasive non-native crayfish, threatening the native populations, this is one of the main aims of Chapter 5.

Signal crayfish (*Pacifastacus leniusculus*)

Signal crayfish (Figure 1.6) are a large crayfish species (body length up to 16cm) with a bluish-brown/reddish-brown complexion and the underside of their claws is a distinct red (Figure 1.6A). Signal crayfish prefer habitats with deep pools and are found in saprobel-dominated environments with a layer of sediment rich organic matter (Wutz & Geist, 2013). They are omnivorous and vegetation detritus is a consistent aspect of their diet throughout the year, during the summer and autumn they predate on small fish species, larger crayfish also show instances of cannibalism (Guan & Wiles, 1998).

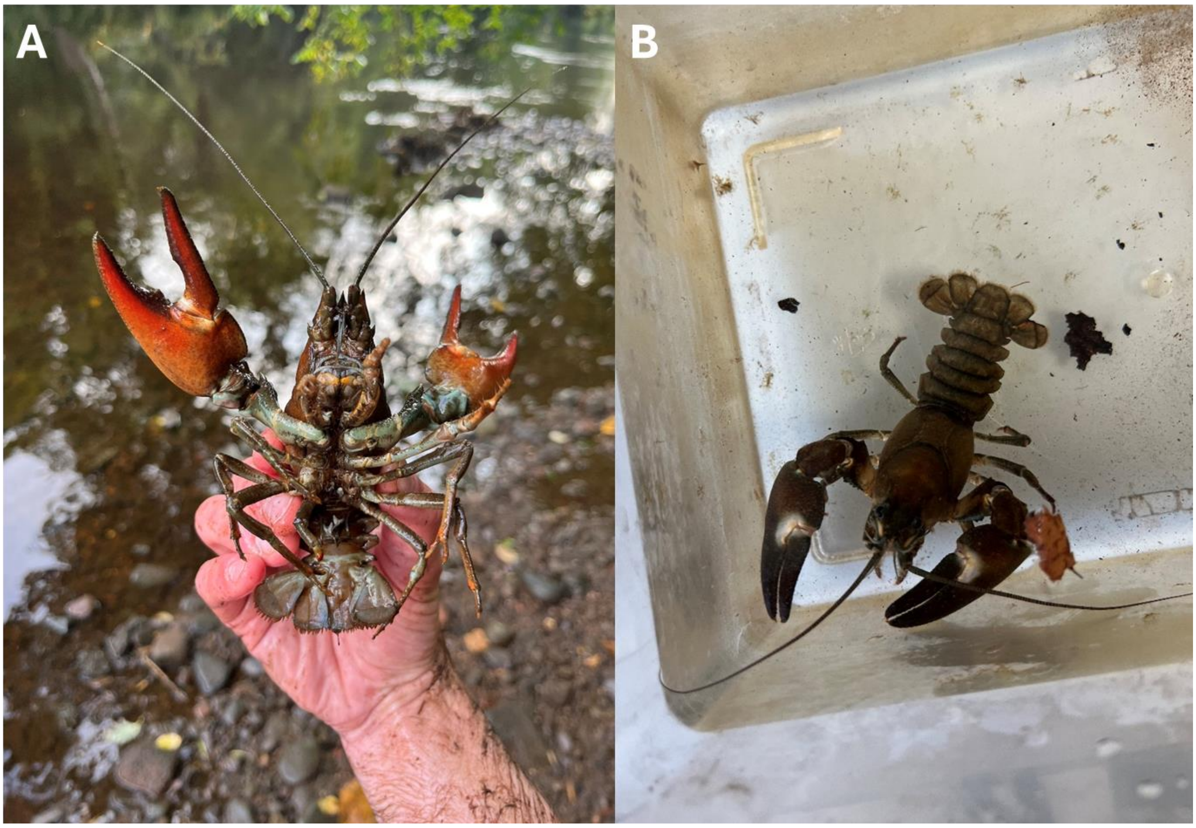


Figure 1.6. A) Signal crayfish (*Pacifastacus leniusculus*) caught in the River Calder, Yorkshire, England, signal crayfish have a distinct red underside to their claws, B) Signal crayfish commonly have white spots on the top of their claws, this is where they get their name from as these resemble the signalling signs used by train conductors

The signal crayfish is one of the most famous examples of an invasive non-native species in the UK and Europe. They are native to western Norther America and were first introduced into the UK in the 1970s to be used in aquaculture and have since formed viable established wild populations (Holdich et al. 2014). Signal crayfish can impact native populations of white-clawed crayfish through competition, with the more aggressive signal crayfish out-competing native populations for resources such as food and shelter, they are also responsible for the spread of crayfish plague, which has been discussed in the Invasive Crayfish section of this introduction (Peay et al. 2010; Dunn et al. 2009). They also have devastating effects on the wider ecosystem, with the potential to decimate benthic invertebrate and submerged vegetation populations within rivers, while also outcompeting fish species for food and shelter (Vaeßen & Hollert, 2015). Signal crayfish are also ecosystem engineers, and their burrowing causes significant bank destabilisation, as well as producing high sedimentation that causes invaded rivers and waterbodies to have high levels of turbidity (Sanders et al. 2021).

Narrow clawed crayfish (*Pontastacus leptodactylus*)

The narrow clawed crayfish is a large species of crayfish (body size can be up to 15cm and larger), it has an olive-green to honey brown complexion and distinct long thin claws (Figure 1.7). Narrow clawed crayfish can be found in both lentic and lotic environments, but they are classified as a still-water specialist (Bök et al. 2013). They utilise a range of shelters that can be found throughout their habitats, including crevices in rock and discarded waste including submerged plastic bottles (Kaya et al. 2024). Narrow clawed crayfish have a high feeding intensity and are opportunistic omnivorous feeders (Skurdal & Taugbøl, 2002; Roessler et al. 2020).

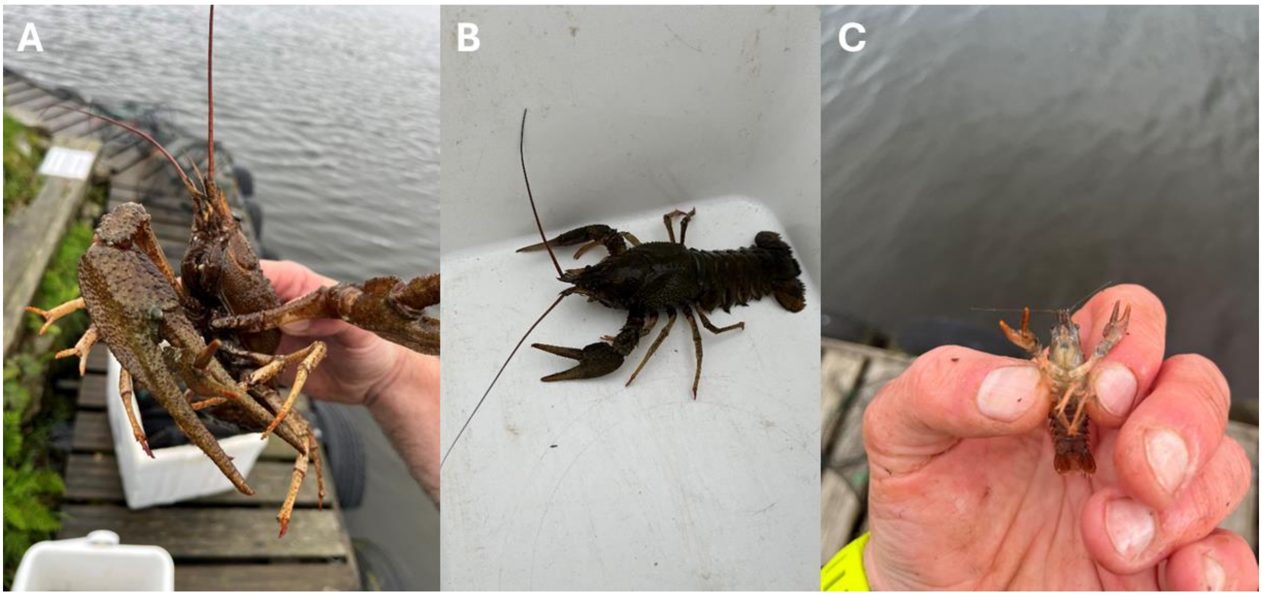


Figure 1.7. A) A large narrow clawed crayfish (*Pontastacus leptodactylus*) (body length 25cm) caught at Boshaw Whams Reservoir, Holmfirth, Yorkshire England, B) medium sized narrow clawed crayfish (body size 18cm) also caught at Boshaw Whams Reservoir, C) young of year narrow clawed crayfish (body size 2cm) this individual is likely to have been released from its mother 2 months prior to being caught

The narrow clawed crayfish is native to the Ponto-Caspian region. In its native range it is a valuable fishery resource, with around 750 tonnes harvested in Turkey in 2007, but these populations face significant threats from invasive non-native crayfish (Skurdal & Taugbøl, 2002; Mazlum et al. 2011; Ghiasvand et al. 2012). Narrow clawed crayfish were first introduced into the UK in the 1980s as part of the aquaculture industry, they have also been observed to be present within the pet trade (Peay et al. 2010). The capability and impacts of narrow clawed crayfish are understudied and they are considered data deficient in their potential as an invasive non-native species. Little is known about the disease and pathogen risks that they could introduce into their invaded range, however they are susceptible to crayfish plague, and this could provide a potential biocontrol solution (Harlioğlu & Harlioğlu, 2006). Narrow clawed crayfish are widespread outside of their native range and there is high potential that this range will expand under current conditions and monitoring them must be prioritised (Hodson et al. 2024). The narrow clawed crayfish is an understudied species and there are significant knowledge gaps regarding them as an invasive non-native species, I look to address this in Chapter 4. They are known to have a range of pathogens in their native range but it is unknown if these pathogens have co-invaded with them or if they have acquired new ones, I investigate this in Chapter 7.

Novel Technology

The products that are used in ecological studies are rapidly evolving, and thousands of new types of equipment and tools have been developed that can be used to address many different ecological and environmental questions, including instream behaviours, population dynamics and abundances (Hitt et al. 2021; O'Hea Miller et al. 2023). These technological advancements have also meant that equipment, that once would have been cripplingly expensive, are now affordable and can be obtained and used at much lower costs, making them more accessible to the scientific community. In this thesis I use three pieces of technology, these are remote underwater video, environmental DNA and histological analysis. These three tools do not encompass the entire extent of novel technology and there are several other technologies that have not been investigated further as part of this thesis.

Remote Underwater Video

Remote underwater video (RUV) is a non-intrusive, non-destructive and easily deployed tool for observing aquatic life. Similar to camera traps in terrestrial environments, RUVs record visual observations of target and non-target species in a naturalistic environment without the confounds of human presence. They provide permanent visual records of the target species that can be stored for future review. Remote underwater video is a tool that has become rapidly more accessible over the past twenty years with developments in technology and data management making them a useful tool that no longer prices out most researchers from the market. Remote underwater video research is heavily skewed towards studies being conducted in the marine environment (Mallet & Pelletier, 2014). Because of this bias there are limited studies that have been conducted in freshwater environments, and there is no set of agreed standards and methodologies that are to be adhered to when conducting freshwater remote underwater video surveys. This lack of standards reduces the comparability of different monitoring efforts and few conclusions can be made when comparing multiple studies. Factors including turbidity have been shown to limit the success of remote underwater video (Tweedie et al. 2023). On top of these limiting factors there is a major knowledge gap globally in the potential of using remote underwater video as a tool for monitoring invasive species.

Remote underwater video has not been utilised on a large-scale basis to study invasive species, and as of December 2022, only five published studies have used it as a tool to address a range of scientific questions. The most common use has been in abundance studies, these studies involved assessing the abundance of round gobies (*Neogobius melanostomus*) and rusty crayfish, while also assessing how their habitat usage varies seasonally (Andres et al. 2020). Another study involved assessing the abundance of native and invasive fish species prior to piscicide treatments for the removal of the invasive non-native fish species (Weyl et al. 2013). Following on from this abundance study, two further studies assessed the species richness of the Rondegat River following piscicide treatment for invasive non-native fish species (Weyl et al. 2016, Castañeda et al. 2020a). The remaining study focused on invasive non-native crayfish, looking at how native Fitzroy Falls spiny crayfish interacted with the invasive non-native common yabby (O'Hea Miller et al. 2023). These studies have all been conducted on known populations of invasive non-native species and remote underwater video has not previously been used to establish the presence of an invasive non-native species.

eDNA

Environmental DNA (eDNA) is defined as DNA that can be extracted from environmental samples, without initially isolating target organisms (Taberlet et al. 2012). eDNA sampling is an emerging surveying tool that holds potential to detect and quantify occurrences and diversity of rare and elusive species that may go unnoticed with direct observations (Beng & Corlett, 2020; Thivierge et al. 2025). eDNA is a non-invasive tool and it has successfully been used to identify the entire fauna composition of a series of habitats and has been identified to outperform any established surveying approaches for targeted detection of a single species (Rees et al. 2014; Hänfling et al. 2016), it requires significantly less survey effort to achieve results, compared to more intensive and extractive methods like bottom trawling (McClenaghan et al. 2020). The use of eDNA in ecology is rapidly expanding, but it still has limitations and the lack of knowledge about these limitations affects how eDNA's potential is perceived by end users (Thivierge et al. 2025). eDNA does produce conclusive results as the non-detection of species in surveys does not automatically imply the absence of them, conversely the detection of species does not automatically imply the presence of them as DNA fragments could have been transported to the area prior to the animal's death, either as bait or other sources (Roussel et al. 2014). The process of eDNA sampling in aquatic environments involves collecting water samples from the site of interest, these samples are filtered, and the nucleic acids are extracted. From here a real-time PCR assay is used to detect the presence of DNA for target species (Rees et al. 2014). The low eDNA shedding rate of arthropods with exoskeletons pose significant challenges (Lancaster et al. 2025) thus invertebrate eDNA monitoring remains technically difficult and unvalidated. I aim to compare the effectiveness of eDNA to other surveying methods in Chapter 5 to establish its ability to detect crayfish in a highly turbid river in relation to trapping, RUV and BRUV.

Histological Analysis

Histopathology is not a novel approach to research and histopathology techniques date back to 1838 when Johannes Müller, who pioneered the use of microscopes in pathology, published "On the Nature and Structure Characteristics of Cancer" (Titford, 2006). However, the application of histological analysis to invasive non-native species is a novel approach. There are many parallels between biological invasions and disease emergence (Dunn & Hatcher, 2015). Invasive non-native species may host pathogens that are not present within their new invaded range. These pathogens are then able to establish themselves within the new area and subsequently spillover onto species that are native to the invaded area (Chinchio et al. 2020). These emergent diseases can then lead to a range of negative impacts including introducing diseases that can be fatal to native species. A key example of this is crayfish plague, which I have previously discussed in the Invasive Crayfish section of this introduction. Alternatively, pathogens can be "left behind" in an invasive non-native species native range when they are translocated to their invaded environment. This is referred to as enemy release and can also lead to significant impacts on the wider ecosystem as non-native species lose what could potentially be agents of control in their native range, which means that they have better fitness and can outcompete native populations as a result (Dunn & Hatcher, 2015).

Aims of the project and thesis outline

This study aims to: 1) understand the current status of remote underwater video research in freshwater environments and the feasibility of using it as a tool in crayfish research, 2) assess the extent of crayfish invasions within two different Yorkshire waterbodies, 3) address the current knowledge gap in understanding the instream behaviours of native white-clawed crayfish and 4) understand the wider impacts that understudied invasive crayfish could introduce through disease. This thesis is divided into eight chapters. The first is this current introduction, followed by six data chapters (Chapters 2 – 7) and a final discussion in Chapter 8. When considering the extent of an invasion, each chapter can be positioned at a specific point (Figure 1.8).

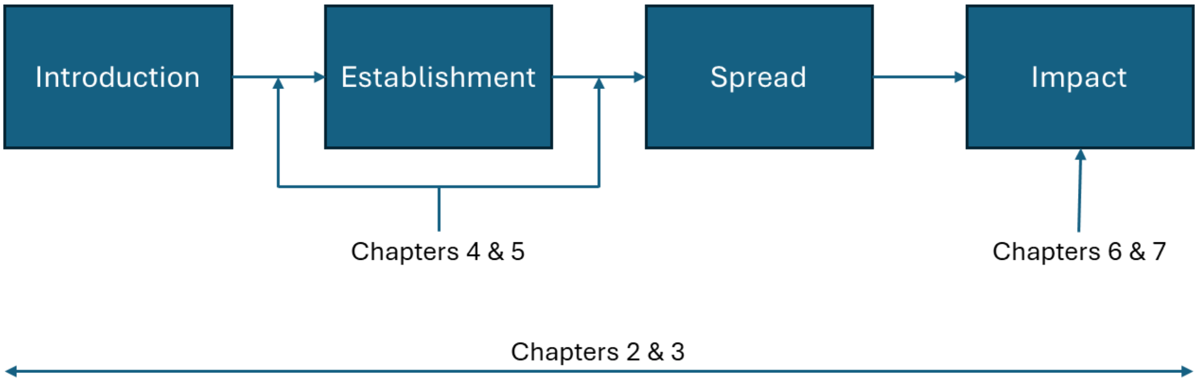


Figure 1.8. Each of the main data chapters of this PhD thesis can be positioned at a specific point during the extent of an invasion. Chapters 2 and 3 encapsulate the entire extent of an invasion. Chapters 4 & 5 fit within the Establishment stage, assessing populations between their initial introduction and their establishment, as well as between their establishment and further spread throughout the system. Chapters 6 & 7 both assess the impact of invasive species and how their actions may affect native populations and the wider ecosystem

The six data chapters investigate the following:

Chapter 2 - Application, development and opportunities of Remote Underwater Video for freshwater fisheries management

There are no agreed standards for the use of remote underwater video in freshwater environments. The current literature is heavily dominated by marine studies with all freshwater studies following different methods. In this chapter I complete a literature review to establish the studies that have been completed in freshwater environments and identify the most common methodology and equipment used to recommend a set of surveying standards. This chapter is in review in the journal Knowledge and Management of Aquatic Ecosystems:

Harwood, M., Broom, C.J., van Wyk, A., Castañeda, R.A., Wong, M.Y.L., Bernard, A.T.F., Stebbing, P.D., Dunn, A.M., South, J. (In Review) Application, development and opportunities of Remote Underwater Video for freshwater fisheries management. *Knowledge and Management of Aquatic Ecosystems*

Chapter 3 - Feasibility of Using Novel Technology to Monitor Crayfish Species

In order to address a series of questions related to crayfish, a specialist tool needs to be designed that is catered towards studying them. In this chapter I design, build and test the feasibility of CrayCam, a specially designed remote underwater video rig. It is important to understand the limitations that can impact remote underwater video deployments, such as turbidity, and here I test how detrimental it is to remote underwater video studies. I also look at how effective CrayCam is at detecting different species of crayfish, both native and invasive, in their natural environments, proposing a wider network of remote underwater video crayfish studies collaborations that can help advise future crayfish research.

Chapter 4 - Rapid assessment of abundance, distribution and spread of a data deficient Invasive Non-Native Species, the Narrow Clawed Crayfish (*Pontastacus leptodactylus*), in a freshwater reservoir in Yorkshire, United Kingdom

Narrow clawed crayfish are a data deficient species and they are critically understudied in regard to their invasion capabilities. In this chapter I look to compare remote underwater video with traditional trapping methods in terms of costs and efficiency when assessing the abundance of a crayfish population. By conducting monthly surveys of the population and a mark-recapture experiment I look to also gather information on population size and dynamics as well as any seasonal trends. This chapter was published as:

Harwood, M., Stebbing, P.D., Dunn, A.M., Cole, Z.K., Bradbeer, S.J., Aston, B., South, J. (2025) Rapid assessment of population dynamics and monitoring methods for invasive narrow clawed crayfish *Pontastacus leptodactylus* in a freshwater reservoir. *Knowledge and Management of Aquatic Ecosystems*

Chapter 5 - A multi-method approach to assessing barrier effectiveness in preventing the spread of invasive Signal Crayfish (*Pacifastacus leniusculus*)

The Calder Rivers Trust is currently facing a conservation conundrum. Barrier relief efforts are being made to support the upstream migration of salmonids. The removal of these barriers, however, opens up the opportunity for invasive signal crayfish to spread throughout the river. In this chapter I use a multi-method approach to rapidly assess the extent of the crayfish invasion within the river. I will identify the barriers that have been “breached” and the removal of which would have no detrimental effect in preventing the spread of invasion. I will also identify those that are “un-breached” and are vital in preventing the spread and act as a barricade protecting the native white-clawed crayfish populations in the upstream of the river. This chapter is in review in the journal River Research and Applications:

Harwood, M., Bray, A., Woolfenden, K., Stebbing, P.D., Dunn, A.M., South, J. (In Review) A multi-method approach to assessing barrier effectiveness in preventing the spread of invasive Signal Crayfish (*Pacifastacus leniusculus*). *River Research and Applications*

Chapter 6 - Using Remote Underwater Video to observe habitat association in a healthy population of native White-Clawed Crayfish (*Austropotamobius pallipes*)

The accepted consensus about white-clawed crayfish is that they are nocturnal and are rarely seen active during daytime. Despite this, personal observations were made that showed large numbers of white-clawed crayfish active during the day throughout the River Kent. This activity provided me with the opportunity to study the instream behaviour of white-clawed crayfish, while also assessing the intraspecies interactions that they had. In this chapter I also explore the habitat associations for this population of white-clawed crayfish to try to identify what factors influence crayfish densities.

Chapter 7 - Pathogen diversity of the non-native Narrow Clawed Crayfish (*Pontastacus leptodactylus*) in a UK water body

The transmission of disease is one of the leading reasons as to why invasive crayfish can have such a negative impact on native crayfish species. When an invasion occurs it is important to understand the pathological profile of the invader to identify potential threats that may have been brought over with them. In this chapter I look to create a pathology profile for the understudied narrow clawed crayfish population that is present within Yorkshire. I intend to identify how the disease profile differs from that in their native range, while also establishing if they carry any pathogens that would be of greater concern to native crayfish populations. This chapter has been accepted with revisions in the Journal of Invertebrate Pathology:

Harwood, M., South, J., Dunn, A.M., Stebbing, P.D., Burgess, A., Bojko, J. (2025) Pathogen diversity of the non-native narrow-clawed crayfish (*Pontastacus leptodactylus*) in a UK water body. *Journal of Invertebrate Pathology*

Chapter 2

Application, development and opportunities of Remote Underwater Video for freshwater fisheries management

Harwood, M., Broom, C.J., van Wyk, A., Castañeda, R.A., Wong, M.Y.L., Bernard, A.T.F., Stebbing, P.D., Dunn, A.M., South, J. (In Review) Application, development and opportunities of Remote Underwater Video for freshwater fisheries management. *Knowledge and Management of Aquatic Ecosystems*

Abstract

Remote Underwater Video (RUV) is a promising tool for progressing the future of freshwater fisheries monitoring and management. While uses have previously been focused on marine systems there has been a rise in application for freshwaters. Given the potential for coordinated geographical research using RUVs it is essential that standardised methodologies are describe and promoted. I therefore conducted a systematic literature review which returned 163 publications that discussed using RUVs in freshwater environments. These publications used RUVs to measure: abundance, species richness, length-frequency, spawning/mating, behaviour, migration, foraging, size, habitat use, presence and nesting. There were taxonomic and geographic biases in the results, with commercial salmonid fisheries the primary focus and 49% of published research was performed in North and Central America. While some research has investigated best practices, there are numerous gaps including: determining optimal deployment time in different systems/species compositions, determining suitable acclimation time for behavioural analysis and ascertaining the costs and benefits of using bait as an attractant and stereo-camera for photogrammetry. Until these gaps are addressed, I recommend a cautious set of standards for freshwater RUVs deployment which includes using a standard action camera, recording at ≥30fps with a resolution of 1080p for 60 minutes. This will ensure that data is broadly comparable between studies. Current bottlenecks in methodology uptake relate to data storage, processing time and cost but this may be overcome with the optimisation of computer vision and machine learning. There are broad opportunities to develop RUV application into a powerful tool for freshwater fisheries management, invasive

species detection, and ethological observations if standardised and findability, accessibility, interoperability, and reusability (FAIR) workflows are followed.

Introduction

The development of effective and feasible long-term monitoring programmes is crucial to identifying key drivers of large-scale environmental degradation and determining the efficiency of potential restoration (Lindenmayer & Likens, 2010). *In situ*, multi-dimensional observation data achieved through field monitoring can be used to link key processes and biological responses such as community composition, population dynamics, breeding ecology, foraging rates, behaviour and response to stressors at a landscape scale (Block, 2005; Caravaggi et al. 2017; Lindenmayer et al. 2022). Signals of large-scale biological change may only be detected after multiple sampling seasons and these changes may be non-monotonic, for example, boom-bust dynamics of non-native invasive species and native species population responses (Haubrock et al. 2022; Lindenmayer et al. 2022). Well-designed monitoring programmes thus need to encompass both long-term and landscape-scale processes, which means that they are extremely resource-intensive in terms of both cost and people time (Lindenmayer et al. 2022). During the current rapid rate of environmental change, finding solutions to overcome these challenges is critical for biodiversity managers to answer key ecological questions, determine long-term changes, provide robust evidence to guide management actions and unequivocally demonstrate the benefit of any intervention investment.

Freshwater ecosystems are facing a biodiversity crisis with freshwater vertebrate populations declining twice as fast as terrestrial or marine populations (Tickner et al. 2020). Monitoring programmes in aquatic environments face struggles regarding time and cost, but also require specialised equipment and training. Traditional aquatic survey methods (e.g. trawling, gill nets, electrofishing, trapping) tend to be extractive, destructive and have inherent biases which may produce an inaccurate representation of a given population (Cappo et al. 2006; Cooke & Schramm, 2007). In addition, capture methods, such as catch and release, can elicit behavioural changes which impact fitness. An example of this being the nest abandonment behaviour observed by male black bass (*Micropterus* spp.) after catch and release surveys that leads to the total loss of offspring (Hanson et al. 2007). *In situ* snorkel surveys can be completed to reduce negative animal impacts of capture, but these are biased by observer ability, water conditions and fear responses to the observer therefore, extractive methods are used in tandem to maximise reliability (Weyl et al. 2013; Ebner et al. 2015). Environmental DNA (eDNA) approaches are being increasingly utilised and presented as a solution to aquatic ecosystem sampling limitations (Beng & Corlett 2020). However, molecular analysis is costly and conclusions based on eDNA are currently restricted to detecting the presence/absence of species with available barcodes, and inferences may be spatially confounded in lotic systems due to downstream transport of genetic material (Beng & Corlett 2020). Even if analysis advances to the point where eDNA surveys can accurately estimate total abundance or biomass, it would not be able to observe the size structure of the populations, which is an important metric for fisheries.

Camera traps and remote imaging have been extensively used in terrestrial ecosystems as they increase observation likelihood of larger and rare species and remove negative impacts of capture-based methods (Feyrer et al. 2013; Caravaggi et al. 2017; Delisle et al. 2021). Aerial surveys have been used in terrestrial and marine environments to monitor large mammals, fish, and plant stands, by tracking movement and population sizes, but they are limited by weather conditions and to animals or plants that are not hidden beneath water or tree canopies (Kelaher et al. 2019; Camacho et al. 2023). Remote imaging methods remove the risk of sampling in locations that are inaccessible or unsafe (Harvey et al. 2013; Chaudoin et al. 2015). Furthermore, results can be quickly validated by reviewing video data, unlike eDNA and aerial surveys, which often need ground-truthing, and datasets are archived for future reference and analysis, thus making it ideal for long-term monitoring programmes looking to maximise data collection (Hitt et al. 2021).

In aquatic systems, above-surface cameras have been used to monitor Atlantic salmon (*Salmo salar*) farm escapes, species assemblages, migration patterns and barriers (Shortis & Otis, 2014; Morán-López & Uceda-Tolosa, 2017; Morán-López & Uceda-Tolosa, 2020). Technological advancement, such as waterproof camera housing able to withstand high pressure, has facilitated the application of Remote Underwater Video (RUV) systems. Use of RUV and Baited RUV (BRUV) has been applied broadly in marine systems and is now a common part of the marine fisheries assessment toolkit as they provide fishery-independent data which is efficient, low-cost, and comparable across locations (Mallet & Pelletier, 2014; Whitmarsh et al. 2017). However, the application of remote underwater video in freshwater lags behind that in marine environments despite the potential for innovative monitoring.

The purpose of this review is to synthesise the current literature on the application of RUVs in freshwater and recommend a standardised methodology for effective and comparable monitoring efforts. Where possible I have identified the methods and objectives of freshwater RUV studies and categorised them according to the study objectives. In doing so, I provide a roadmap for using RUVs in freshwater aquatic systems. I also provide a starting point to advance freshwater RUVs' best practices to ensure robust data collection and enhance scientific development while addressing critical knowledge gaps in conservation and fisheries science.

Methods

A comprehensive systematic literature search was conducted using the Institute of Scientific Information (ISI; Thomson Reuters) Web of Science online database and Google Scholar database. These databases were searched to find any literature that contained relevant information regarding RUVs in freshwater published up to November 2022. The search term included a range of keyword combinations (Table 2.1). The guidelines for Preferred Reporting Items for Systematic reviews and Meta-Analysis (PRISMA) (Page et al. 2021a; Page et al. 2021b) were used to report this systematic literature review. The results obtained were collected and assessed by a single reviewer.

Table 2.1. Keyword combinations used in initial literature search

Keyword Combination
“Freshwater”* AND “RUV”*
“Freshwater”* AND “BRUV”*
“Freshwater”* AND “Remote Underwater Video”*
“Freshwater”* AND “Baited Remote Underwater Video”*
“Freshwater”* AND “Underwater Camera”*
“Lake”* AND “Remote Underwater Video”*
“River”* AND “Remote Underwater Video”*
“Stream”* AND “Remote Underwater Video”*
“Reservoir”* AND “Remote Underwater Video”*

Each publication returned was examined and included if it involved using RUVs in a freshwater environment. For each publication, I recorded the following (if details were available); What was being measured/observed in the study, the year of publication, the country the study was undertaken in, the focus species of the study, the waterbody type that the study was conducted in, and the methods used. After reviewing each of the returned pieces of literature, a backward snowball of references was conducted to check for any further relevant literature, which was then incorporated into the database (Wohlin, 2014).

Each of these studies were then categorised by the focus of the study. Literature, which was relevant but did not consist of a specific measurement/observation, was also noted and explored further for information that was relevant to this review.

Results

A total of 163 unique pieces of literature were identified through database searching, backward snowballing and personal correspondence (Figure 2.1). The first published literature on freshwater applications of RUVs was in 1988, which documented swim-up and downstream movement of newly emerged Sea Trout (*Salmo trutta*) fry in the River Itchen, UK (Moore & Scott, 1988). Annual publications were sporadic and in small numbers until 2014 when there was a sharp increase in publication rate (Figure 2.2). The database search returned studies undertaken in 27 different countries, spanning all six inhabited continents.

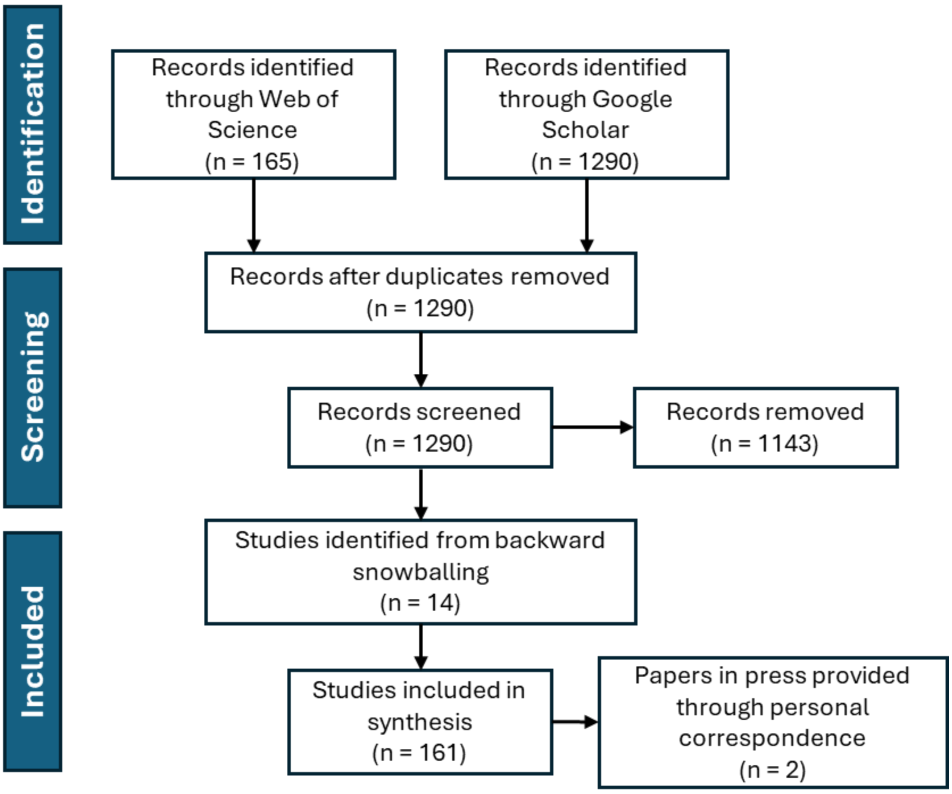


Figure 2.1. PRISMA flow diagram illustrating the different phases of the systematic literature review data identification and inclusion

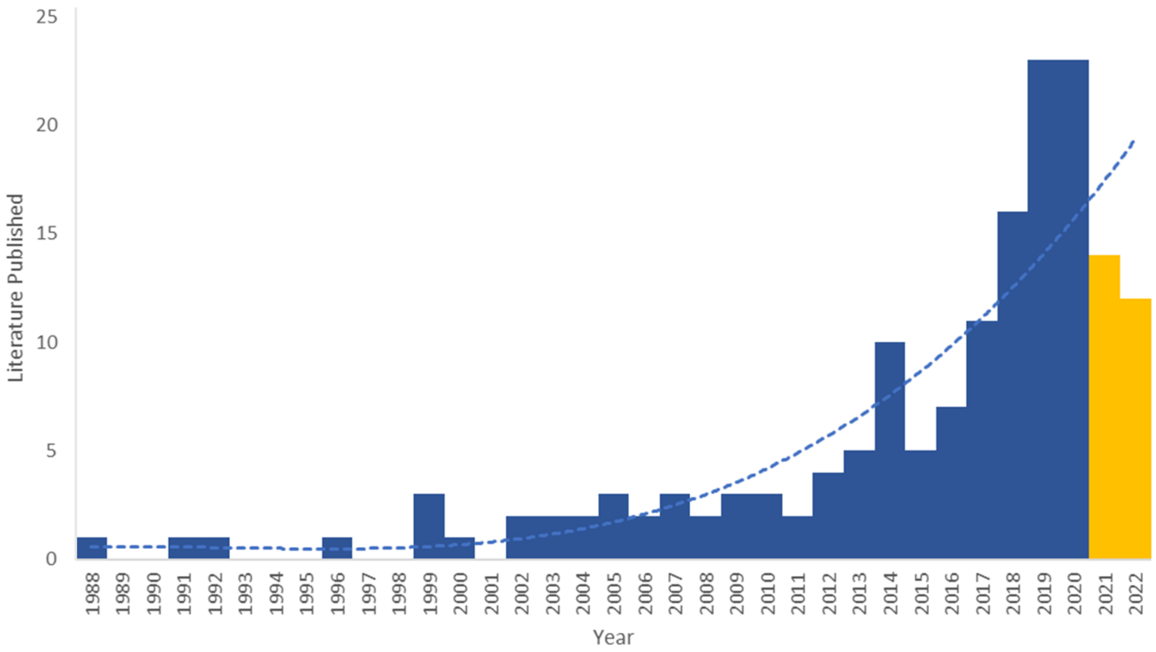


Figure 2.2. Histogram of literature related to RUVs in freshwater released each year, orange bars correspond with post COVID-19 Pandemic

Survey Design

Method specificity and reporting standards are needed for reproducible and comparable RUV research. Despite this, two thirds of the literature did not include information on at least one of these factors (Table 2.2). Prior to 2014, most studies used expensive professional cameras. Since 2014, most RUV studies in freshwater have used some form of action camera. A broad range of frame rates were returned by the literature search (Table 2.2). These ranged from a time lapse video of 1 frame every 5 seconds to capture habitat use by threatened species (Hannweg et al. 2020a), to a slow-motion video recording at 240fps trying to capture biomechanics of foraging (Moran et al. 2019). Most studies returned by the literature search did not account for any acclimation time between the initial deployment of the RUV and recording results (Table 2.2).

Table 2.2. Prevalence of technology and methods reporting in the literature

Factor	**Most common method**	**No. Using Most Common Method**	**Percentage of papers that do not specify factor**
Camera Type	Action Camera	119	20%
Frames per second	30fps	20	66%
Resolution	1080p	28	66%

Acclimation Time	0 minutes	20	61%
Deployment Time (total)	60 minutes	14	22%
Video Analysis	Human Reviewer	84	19%

Remote Underwater Videos with bait arms attached (BRUVs) within the camera's field of view to attract individuals was a common customisation (19% of surveys used some form of baited arm). Bait material and volume was varied, including: bread and marmite, cat food, freshwater fish carcasses and fish eggs.

The duration of deployments varied greatly. When the RUV is used for a rapid deployment and not left continuously recording the most common duration (9%, n = 14) was for 60 minutes and the second most common duration (9%, n = 13) was for 30 minutes. Other durations have been used in a more sporadic distribution, with camera battery life and limited budgets and tight deadlines playing a key factor in this.

A range of different video analysis methods were employed throughout the literature. The most common method (66%, n = 97) was by a human reviewer watching the footage and manually noting observations. The other method used involved the use of specialist software, e.g. EventMeasure (seagis.com.au), Everfocus (everfocus.com), Beast Software (beast.community), Argus (argussoft.org) and Tracker 5.1 (physlets.org) to review footage (15%, n = 23). These softwares range in complexity and cost.

Target Species

Fish were the observed target in 87% of the studies conducted, decapods made up 7% of the studies, and the remaining 6% consisted of a range of literature observing reptiles, amphibians, mammals, macrophytes and mussels. Research on fish was split, with 50% of studies focusing on the whole fish assemblage and the remaining 50% focused on a single target species (Figure 2.3).

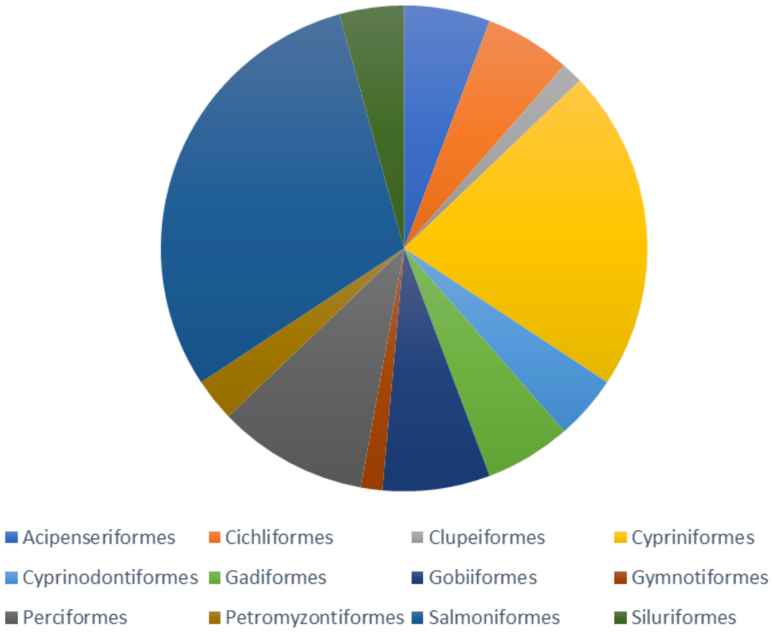


Figure 2.3. Pie chart showing the orders of target species in relevant literature

Abundance

Assessing species abundance is a critical component of conservation and wildlife management. This was the most common application of RUVs with 33% (n=53) of studies aiming to determine relative or total abundance of aquatic biota.

The most frequently used method (64%) is calculating the maximum number of individuals, of a single species, observed simultaneously in a single frame known as MaxN (Hitt et al. 2021). This value can be determined by either searching fixed time points (i.e. every 30 seconds) for a frame with the most individuals of a target species recorded (Hannweg et al. 2020b) or obtained by reviewing the entire video (Crook et al. 2021). Alternatively, SumMaxN, a cumulative sum of the MaxN for every species can be used to determine fish abundance within a given habitat or area (Work & Jennings, 2019). If duplicate counting of animals was not considered a confound, e.g. fish passing through a one-way fish pass (Johnson et al. 2007), counting the total number of fish observed (28% of records) was used to estimate species biomass by either estimating the densities and percentage-cover of target species (Karatayev et al. 2021) or using video quadrants (Andres et al. 2020).

Species Richness

Identifying species richness and diversity usually relies on being able to physically handle an animal to key it out correctly or through eDNA analysis, which is compromised in tropical localities by limited barcode libraries and cryptic species. RUVs may offer an alternative to species richness and biodiversity assessments. Overall, 22% (n=36) of studies utilised RUVs for this task with 83% either reviewing the footage in full (Glassman et al. 2022), or by selecting a random frame every minute (Robinson et al. 2019) to identify all individuals to species level and create a species list. Generally, the quality of the video allowed species to be identified to species level by experts and through consulting identification guides for diagnostic characteristics (van Wyk et al. 2017; Pedersen, 2021). In some instances, differentiation between similar species can be confounded due to visibility issues and cryptic taxonomy (Cooke & Schreer, 2002; Widmer et al. 2019). Therefore, identifying organisms to family level occurred in 11% of publications.

Spawning/Mating/Nesting

Studying reproductive behaviour of aquatic biota without causing adverse impacts on the target species is fraught with difficulties. RUVs were used in 18% (n=30) of studies where 74% deployed RUVs at known spawning sites to confirm event timings. In some cases, this was used as a solution to better understand spawning of critically endangered fish such as the Devils Hole Pupfish (*Cyprinodon diabolis*) (Chaudoin et al. 2015). Furthermore, RUVs can visually assess effects of abiotic factors such as habitat (Groves & Chandler, 1999) and the lunar cycle (Fernández et al. 2021) on salmonid spawning. RUVs can also be used in tandem with hydrophones to expand the toolkit of remote sensing in aquatic habitats; for example, a novel method of time-synchronised sound and video was also used to identify the sounds produced by spawning trout (Johnson et al. 2018). Opportunistic sampling was able to document footage of courting and mating behaviour incidentally during recording. One of these studies provided the first observation of copulation of the cryptic Andean Catfish (Mena-Valenzuela et al. 2022). Another observation was courtship of Zebrafish (*Danio rerio*) which was recorded outside of the known mating season by chance in a separate RUV study (Sundin et al. 2019). RUVs were positioned directly in front of the nests of target species to observe nesting behaviour. Behaviours ranged from nest guarding and maintenance behaviours conducted by males (Unger et al. 2020), to interactions with heterospecific species eggs placed within the nest (Yamane et al. 2016), subordinates within nests, sheltering with juveniles, and cooperative breeding (Satoh et al. 2022). One study used a less targeted approach where RUVs were deployed in the USA to opportunistically record nesting of Redbreast Sunfish (*Lepomis auratus*) (Martin & Irwin, 2010).

Behaviour

Behavioural studies comprised 14% (n=23) of the literature, where responses to external stimuli including anthropogenic sounds (Fleissner et al. 2022), chemical cues such as chemical predatory alarm cues (Friesen & Chivers, 2006), infrared lighting (O’Malley et al.

2018) and researchers shadows (Smith, 2022) formed the majority of the analysis. Beyond that, general behavioural time budget research, such as time spent swimming compared to nesting or foraging was the core focus of these papers. RUVs were used to quantify the extent of inter and intraspecific interactions, for example territorial defence behaviours in fish (Ebner et al. 2017), aggressive interactions between noble crayfish (*Astacus astacus*) individuals within the same trap (Raugstad, 2019), and competitive interactions between invasive and native crayfish species (O'Hea Miller et al. 2022a). Collective behaviour of fishes was assessed through open water RUV deployment, where a focal species was identified but data collected on the icthyofaunal community as a whole.

Migration

Migration studies comprised 10% (n=17), where 53% of this subset deployed RUVs into obstacles passed by migrating species including fish ladders (Negrea et al. 2014), fishways (Limaye, 2019), weirs (Marston, 2014) and fish passes (Hawkins et al. 2018). These RUVs were deployed primarily for commercially important species Alewives (*Alosa pseudoharengus*), Rainbow Trout and Sockeye Salmon (*Oncorhynchus nerka*). The RUVs were left to continuously record, and the footage was reviewed to count the total number of individuals that passed either upstream or downstream. In some cases, *a priori* knowledge of important migratory locations were chosen for RUV deployment whereas in others, RUVs were deployed at waterbody entrances of specialised migration swim-through chutes to monitor salmonid migration (Musslewhite, 2020). Finally, multiple camera arrays of RUVs were strategically placed along the Atlantic Salmon migration route to incorporate the spatio-temporal aspect of migrating populations (Borgstrøm et al. 2010).

Foraging

Foraging activities comprised 7% (n=11) of the overall results. These recordings provided information on trophic interactions to quantify intensity of bottom foraging (Pledger et al. 2014), feeding aggregations (Starrs et al. 2015) and species feeding on floating material including zooplankton (Marchand et al. 2002) and recently released eggs (Šmejkal et al. 2017). Baited studies comprised 27% (n=3) with the intention of observing scavenger feeding behaviour with the most common bait being a fish carcass positioned in front of the RUV (Unger & Hickman, 2019). High frame rate video was used to record predation events by Bluegill (*Lepomis macrochirus*) on live prey tethered within view of the RUV (Moran et al. 2019).

Size

Estimating length underwater of a moving object is a barrier to ascertaining critical fisheries data, thus length frequency studies made up 7% (n=11) of the total results. The most common method (64%) estimated size by comparing an individual to an object of known size that is within the frame of the video. These objects were variable and included a stake of known size (Tweedie et al. 2018), to specially mounted scale bars (Loffredo, 2018). In some instances, exact sizes were not calculated, instead individuals were either assigned a size class bin (Hopper, 2019) or estimated in relation to previously caught individuals (Skorulis et al. 2021). Specially calibrated stereo-RUVs and specialist software was used in 18% of studies to accurately estimate the size of individuals. One novel method was applied in Canada to study Shortnose Sturgeon, where parallel lasers were mounted to an RUV and used to estimate length (Usvyatsov et al. 2012). Another novel method involved counting the pixels a fish took up in footage when it passed a known location and transforming these to millimetres to estimate sizes, as done in Reunion Islands to estimate the size of Red-tailed Goby (*Sicyopterus lagocephalus*) (Boussarie et al. 2016).

Habitat Use

RUVs were used to assess habitat associations in 7% (n=11) of the results. Most studies aimed to determine differences in artificial habitat use compared to natural habitat as well as assessment of seasonal habitat changes (Pratt et al. 2005; Lintermans et al. 2013). RUVs were also used to quantify cyprinid habitat use after disturbance events from hydropeaking (Boavida et al. 2021).

Presence

A small number of studies (4%, n=7) were intended to confirm species presence in a waterbody. Most studies deployed RUVs at a fixed location within the waterbody in the hope of serendipitously detecting a species, such as the first official record of the Cleft-lipped Goby in Australia and multiple deployments were used to determine the presence of escaped farmed salmon in Norway (Ebner et al. 2017; Svenning et al 2017). Macrophyte studies were rare across the dataset, but short RUV deployments were completed in multiple locations in the UK to confirm the maximum colonisation depths of all macrophytes in the waterbody (Spears et al. 2009).

Case study applications for fisheries science and conservation

Conservation Intervention

Eradication of non-native invasive species is a high-risk, high-cost venture which relies on robust evidence-gathering and success post-intervention. A successful example of an invasive fish eradication project took place at the Rondegat River in the Cape Fold

Ecoregion, South Africa (Marr et al. 2012; Weyl et al. 2013; Weyl et al. 2014; Weyl et al. 2016), an important biodiversity hotspot characterised by high diversity and endemism (Ellender et al. 2017; Broom et al. 2023). RUVs were deployed yearly to effectively monitor the recovery of the endemic fish population in a non-destructive manner (Weyl et al. 2013; Weyl et al. 2014; Weyl et al. 2016). Using the Rondegat River's RUV yearly dataset (2011-2016), Castañeda et al. (2020) tracked the occupancy dynamics of the endemic fishes along the river, before and after the eradication of the invasive fish. They found that the strongest driver of the endemic fish's probability of occupancy in the river was the presence of an invasive fish. After the invasive fish eradication, the endemic fish were able to naturally colonise downstream sections of the river and increase in density. Two of the endemic fish populations appear to have reached population equilibrium across the river, while the third has not, suggesting it may be more sensitive to fluctuations in habitat variables (Castañeda et al. 2020). To assess the habitat associations of the three vulnerable and recovering cyprinid species, Broom et al. (2022) utilised a RUV system across 51 sites as part of a long-term monitoring project for the Rondegat River post-intervention. With repeated sampling over three seasons (2018-2019), Broom et al. (2022) were able to assess community composition and relative abundances with respect to habitat, its overlap with a protected area and species-specific abiotic predictors of relative abundance. Results confirmed a lack of re-invasion and indicated that habitat-specific interventions to reduce the impact of drought, eutrophication and sand deposition are needed along the Rondegat River to ensure the continued persistence of threatened fish. Underwater video monitoring is an effective and low-cost approach that can rapidly inform tangible conservation recommendations for vulnerable fish species in impacted or recovering river systems, especially in locations with underfunded resourcing for biodiversity management.

Ethology

O'Hea Miller et al (2022a) investigated the competitive interactions and outcomes between an invasive crayfish (*Cherax destructor*) and a critically endangered native one (*Euastacus dharawalus*), which cohabit a 7.5km stretch of creek in Wildes Meadow, located in the Southern Highlands region of NSW, Australia. Up until this point, behavioural investigations of invasive and native crayfish had largely been confined to laboratory trials, which facilitate clear and controlled observations of individuals competing over resources (Cerato et al. 2019; Lopez et al. 2019; O'Hea Miller et al. 2022b). Owing to the challenges of observing individuals in situ, particularly due to shallow and often turbid conditions, little was known about how these species interacted under natural conditions nor whether body size affected contest dynamics and outcome. By deploying 15 baited remote underwater videos along nine locations within the creek over the course of 12 months, O'Hea Miller et al (2022a) were able to extract and score 178 interspecific and intraspecific interactions from which interaction duration, maximum intensity, conclusion, outcome, and interaction initiator were quantified. All behaviours were assessed relative to an established ethogram (Bergman & Moore 2003) and relative body size of contestants was estimated from the precent difference in size as measured on the video screen (Martin & Moore, 2007). Overall, *Euastacus dharawalus* won more contests than the invasive species *Cherax destructor*; however this was largely attributed to the fact that in most cases, *E. dharawalus* was larger than *C. destructor*. Alarmingly, when considering only interactions where contestants were size-matched (i.e. within 10% body size), *C. destructor* was more likely to win interactions than *E. dharawalus*. Additionally, *C. destructor* were more willing to initiate contests than *E. dharawalus*, even if *C. destructor* was the smaller contestant, and they were more willing to continue fighting than *E. dharawalus* in intraspecific contests, demonstrating a greater inherent aggressiveness of the invasive species. This study highlights the capacity of BRUVs to quantify complex behavioural interactions in challenging freshwater systems, but also a key consideration of using BRUVs for behavioural studies – namely the potential for limited and unbalanced sample sizes. Firstly, some BRUV deployments had to be discarded from the analyses due to high turbidity and time of year (i.e. winter crayfish inactivity). Secondly, O'Hea Miller et al. (2022a) reported only one interaction where *E. dharawalus* was smaller than *C. destructor* (compared to 29 interactions where *C. destructor* was smaller than *E. dharawalus*). Future considerations must, therefore, involve increasing the number of BRUV deployments over time and the use of stereo-BRUVs to enable scoring of relative body sizes of all individuals in the frame. Both these considerations will help boost replication of behavioural observations and hence the efficacy of BRUVs for understanding behaviour in freshwater systems. Incorporating ethological studies and interspecific interactions into fisheries management plans has been a persistent and key challenge which may be tackled by appropriate use and deployment of BRUV systems.

Fisheries Monitoring

Chambo (*Oreochromis* spp.) is a key fishery in Lake Malawi and is a key target for management and conservation efforts. However, traditional monitoring methods for Chambo, such as gillnetting and trawling, are destructive and can negatively impact fish populations (Tweddle & Magasa, 1989; Banda et al. 2005; Weyl, 2005; Weyl et al. 2010).

Van Wyk (2019) evaluated the potential for using stereo-BRUVs to monitor Chambo populations across different management zones of Lake Malawi (Mozambique and Malawi) by assessing the effectiveness of stereo-BRUVs for monitoring Chambo populations and to determine the optimal sampling design for annual monitoring. Both Chambo abundance and size differed significantly between Malawi and Mozambique - which may be attributed to differences in fisheries pressure. Malawi experiences greater levels of fishing pressure compared to Mozambique, resulting in a decline in Chambo populations and a decrease in the size of sexually mature individuals. In contrast, Mozambique has relatively low fishing pressure due to low population densities, weak market forces, and a history of civil war. This has resulted in higher Chambo abundance and larger sexually mature individuals in Mozambique compared to Malawi (Halafo et al. 2004; van Wyk, 2019; Weyl, 2005; Weyl et al. 2010). In this system, an acceptable stereo-BRUVs deployment time was 15 minutes and required a maximum of 120 annual video samples to detect a 10% change in Chambo abundance over a hypothetical 10-year monitoring scenario. This suggests that stereo-BRUVs can be used as a cost-effective long-term monitoring tool for economically and ecologically important fisheries, provide evidence-based recommendations for the establishment of closed sanctuary areas, and monitor intervention outcomes.

This case study highlights the importance of effective, standardisable monitoring methods for fisheries management and conservation, and the potential of stereo-BRUVs technology to provide robust data for monitoring and managing complex inland fisheries.

Discussion

Despite RUV research being completed across a broad range of subject matter and spanning continents, there is a distinct lack of cohesion in method standardisation between research groups. This is a stark contrast to the marine environment, where proven methodological standards are in place and coordination levels at a global scale are high. The lack of standardisation within the freshwater environment limits the value and use of the datasets, thus hampering RUV work being used to its full capacity in freshwater fisheries. Research so far has, therefore, been fairly ad-hoc, with a sharp increase in the literature published annually from 2014, which likely reflects a technological trend in better video quality, combined with a decrease in the cost of cameras, which has made action cameras more available. The drop in frequency of literature using freshwater RUVs between 2021 and 2022 is most likely a result of COVID-19 lag in publishing, with limited studies occurring during the pandemic lockdowns.

Use of RUVs

Freshwater research utilising RUVs has the capacity to be a one size fits all method for ecological assessments and fisheries science, if deployed correctly and in a standardised manner. Development of a freshwater RUV consortium following a standardised methodology could result in globally coordinated research which spans broad spatio-temporal ranges with the capacity to answer pressing questions in fisheries science. Similar consortiums have been created, for example: acoustic tracking in marine systems and BRUV census of global shark populations, which have resulted in unexpected natural history observations (Phillips et al. 2019; MacNeil et al. 2020; Lennox et al. 2023). As RUVs represent a long-term low-cost data acquisition method they are an excellent tool for post conservation intervention monitoring, as once the initial costs of purchasing the equipment are covered, future costs are limited to staff cost, travel cost and data storage and processing. They can also be used to complete rapid baseline assessments of freshwater environments, perform freshwater fisheries stock assessments or monitor the escapements of farmed fish (Weyl et al. 2013; Svenning et al. 2017). This is especially true for stereo-B/RUVs which provide accurate length estimates for length frequency and biomass estimations which can be used to support fisheries independent assessments.

Many lessons can be learned from the success of using RUV in marine environments. In contrast to more traditional visual censuses, researchers are now able to sample in locations without constraints of time and depth, allowing new areas to be surveyed that previously would have been impossible (Nalmpanti et al. 2023). These lessons can be taken into freshwater environments, allowing surveys to be conducted in hard-to-reach locations that potentially pose risks to surveyors including the beds of deep lakes, as well as locations with strong flow or potential predator threats. Terrestrial camera traps can also provide lessons in long term deployments. Motion triggered recordings are a solid solution to extending total deployment times and saving both battery life and space on memory cards as they remove the need to continuously record to make an observation, while also only providing footage that contains an individual (Ortmann & Johnson, 2021). These lessons could potentially be used in freshwater environments, however there is risk that the continuous flow of water may trigger the motion capture sensors, as well as detritus, such as leaves, that may float past the sensor and trigger wasted recordings. Ecological soundscapes are beyond the scope of this review, however when paired with video recording, they can potentially be used to address new ecological questions, such as the sound produced by spawning trout (Johnson et al. 2018).

Limitations of RUVs

With most modern action cameras maximum battery life is around 80 minutes, however, this can be lower in colder waters, meaning that RUVs with action cameras cannot be deployed for extended periods of time, which can result in key events being missed. Other issues include malfunctioning memory cards, water damage, unfocused images and obstacles within the field of view, all of which can result in wasted effort (Struthers, 2015). Although depending on the specific set up of the rig, i.e. commercial or home-made, there may be low-tech ways to mitigate these, such as the AquaticVid set-up, a low-cost extended battery life camera system (Fetterplace et al. 2023). There are also environmental limitations into RUV surveys, such as light levels and turbidity limiting data capture. Turbid water greatly reduces an RUVs field of view which reduces probability of event observation. Similarly suitable lighting and hours of daylight restricts timing of many surveys and introduces bias against nocturnal species - which may also avoid artificial lighting (Struthers et al. 2015). This highlights a priority question to advance methodology by assessing the effectiveness of white, red and blue lights for RUV surveys.

Camera Setup and Analysis

A set of standards for using stereo-BRUVs in marine environments has been proposed (Langlois et al. 2020) and these have been used as a framework to guide the standards described here, to ensure reliable deployments of all forms of RUVs in freshwater. To be able to create a standardised method for RUVs in fisheries research there are several systematic methodological developments that need to be considered (Table 2.3).

Table 2.3. Recommended standards and reasoning

Factor	**Recommendation**	**Why?**
Camera Type	Action Camera	Most common in reviewed literature
Frames per second	30fps lower limit	To reduce blur produced by fast moving individuals
Resolution	1080p lower limit	Suitable resolution to identify species while saving on storage space
FOV	109° and 120°	Creates a suitable visible area without distorting the image
Acclimation Time	1 minute	Majority of literature does not have an acclimation time incorporated, but to avoid issues with disturbed sediment obscuring field of view, an acclimation time is recommended
Deployment Time (total)	60 minutes	Most common in reviewed literature and standard in marine systems. Over collection of data is preferred over under sampling until sampling efficiency is assessed systematically in freshwater
Video Analysis	Human Reviewer	Most accessible option in terms of cost

Any action camera which can record at the recommended settings can be used for future standardised surveys. Framerate, resolution and field of view (FOV) are all important considerations as they influence a video analyst's ability to accurately identify, count and measure fish, as well as the size of the visible area (in the case of FOV). At a minimum, high definition (1920x1080p) resolution with 30 frames per second, with a field of view between 109° and 120°, appear to be an adequate standard (Langlois et al. 2020). While 4K resolutions might be tempting, researchers will run into challenges with cameras overheating and space for data storage. For measuring fish length with stereo-camera any settings that automatically adjust the pixel size (e.g. image stabilisation), frame rate (e.g. auto low light) or distort the image (e.g. fish-eye or ultra-wide FOV) should be disabled (Langlois et al. 2020).

Acclimation time is likely to vary between species and communities depending on their exposure to disturbance and life history traits. Most studies reviewed here did not include an acclimation time as most species entered the RUV's field of view a short period of time after deployment. This is a methodology priority question - to determine a suitable baseline acclimation time for different species and purposes to ascertain whether a standard can be achieved. Similarly, a standard operating procedure for total deployment time has not yet been determined, thus I recommend the most common deployment duration time, i.e. 60 min. However, van Wyk (2019) found that 15 minutes was sufficient for fisheries monitoring in Lake Malawi, whereas 60 and 30 minutes is a recommended deployment time in marine systems to reduce diminishing returns (Langlois et al. 2020). Sampling efficiency analyses such as time - species accumulation curves in freshwaters are required urgently.

Video analysis by Artificial Intelligence is in development, this AI would be able to identify frames of the footage that hold a target species that can then be analysed by a human reviewer. Potentially, in the future there is even the scope that it can fully review footage identifying all species and individuals that occur throughout a video sample, but prior to optimisation and validation of these models human review should be prioritised. Ideally, reviewers will have undergone species identification and software training prior to analysis. Random review should be implemented for quality assurance, and if a complex community is present then two independent reviewers should be used to ensure accuracy. While human review is currently the only viable option, researchers should invest effort into developing training datasets to enable AI applications when the technology is mature.

Compared to RUVs, BRUVs achieve higher MaxN and species richness estimates. Therefore, BRUVs should be used for abundance, species richness and presence absence studies. Due to bait-attraction altering natural behaviour I do not advise their use for ethological observations or habitat use, nesting and migration. On the other hand, BRUVs can be used effectively for some ethology experimental purposes such as scoring competitive behaviours and aggression (O'Hea Miller et al. 2022a).

Experimental bait efficiency assessments need to be completed to recommend a data-driven standard, where bait type, volume, local hydrology and survey purpose must be considered. Using a bait local to the area is recommended for practical purposes, small oily food fish species work well as the scent plume travels well in the water, and they are usually inexpensive and readily available. The use of local fish reduces the risk of the introduction of disease or invasive species while conducting surveys. Nonetheless, non-natural bait has also been used, e.g. marmite™ and bread (Bajaba et al. 2020), variations of this, or indeed canned oily fish may be preferable if surveying in remote locations. The use of marine species as bait in freshwater has been promoted in the UK during crayfish surveys to reduce chances of disease introduction through freshwater bait (J South pers. comm). Furthermore, depending on survey location, safety concerns should also be considered when using BRUVs as bait has been known to attract large predators, like crocodiles, that pose a risk to researchers and BRUVs should be avoided when these risks are present (King et al. 2018). Effort should be applied to follow FAIR (Findable, Accessible, Interoperable and Reusable) data workflows to the large amount of data produced in RUV surveys (de Visser et al. 2023).

As standard, all data should be suitably annotated with meta-data for location, date and surveyor, and saved in raw video format. Good practices regarding data management and storage are crucial and data ought to be stored along with off-site back-ups in both physical and cloud repositories. However, this may incur unforeseen costs for practitioners and researchers. I strongly recommend the creation of a global freshwater RUV repository, following open data principles, similar to those suggested for marine systems (Langlois et al. 2020). Standard approaches to analysis will enhance the usability and interoperability of datasets and analysis codes. All species should be identified to the lowest taxonomic level possible. MaxN analysis methods should be used as standard in abundance studies, as it is less likely to overestimate true abundance (van Wyk, 2019). Efficiency and robustness of MaxN calculation approaches, such as snapshots vs total video, need to be assessed to recommend best practice. Specialist software, such as EventMeasure, can be used to annotate video which can ensure that fish are not counted multiple times or missed when reviewing footage to reduce the risk of miscounts when calculating MaxN values. This software can also be used with stereo-RUVs to obtain accurate fish lengths after careful calibration (Langlois et al. 2020). When possible, specialist software should be used to ensure that results are consistent; however, the high costs of licences for this software often makes it implausible. Regardless of the use of specialist software, the suggested standard methods should be used when reviewing footage, with footage saved for future review if requested. Behavioural studies should follow published ethograms when possible, however, the reviewing process can be lengthy and subject to observer bias. Using free software such as BORIS (Friard and Gamba, 2016) the recommended standard method of analysis would be to review footage back at an increased speed, until a desired event is observed, and then reviewing the footage at normal speed to score results, as in O'Hea Miller et al (2022a). Automated behavioural analysis software is available, but the cost is often prohibitive for environmental managers and negates the initial RUV cost saving.

Future Steps and Potential Developments

The prospect of low cost, high data acquisition methods for fisheries monitoring and management means that technology is constantly developing for both research and commercial applications. Novel methods including a Raspberry-Pi platform can allow automated data acquisition through scheduling and automatically uploading results to an online database (Almero et al. 2021), or through a video streaming link that listens for requests to connect (Dadios et al. 2022), removing the need for researchers to replace batteries and storage cards. This technology is in the initial trial stages and currently the video is limited to 6fps, which does not adhere to the standards suggested.

Time spent reviewing footage is a major bottleneck in RUV methodology, this may be overcome with optimisation of artificial intelligence applications and machine learning to automate the process. Through deep-learning, AI can be trained to categorise behaviours and identify fish species with the most recent versions being able to detect fish and categorise species to almost a human-like accuracy (Abangan et al. 2023). Approaches such as Convolutional Neural Networks or the You Only Look Once (YOLO) algorithm can be optimised to identify species passing through fish passes or in RUV footage to automatically identify species (Soom et al. 2022; Ovalle et al. 2022). For instances when species identification is not plausible AI, could be used instead to flag instances when individuals are present on the screen so that a human observer can manually review a smaller subsection of the video file with confirmed presence, rather than reviewing footage without species present. Furthermore, this could be developed further to enable the monitoring and tracking of individual fish over time, calculate rapid biomass estimated by deriving length-weight relationships, and expedite the accurate detection of invasive fish through catchments, thus potentially revolutionising the way we monitor and manage aquatic ecosystems. Environmental factors including turbidity and lighting limit the effectiveness of RUVs, specialist lighting rigs can be developed to address lighting issues; for example, a clear liquid optical chamber to improve underwater visibility (Jones et al. 2019).

Remote Operated Vehicles are a move away from the static camera approach and should be considered a separate methodology entirely, with method development focused on in their own right. These have almost exclusively been used in marine environments but are increasingly being exploited in commercial applications in freshwater. For example, ROVs have been deployed effectively in reservoirs to assess the presence and distribution of target species, such as Signal Crayfish (*Pacifastacus leniusculus*) (P Stebbing pers. comm). Information gathered from such surveys has facilitated determining the risk of spread of invasive non-native species from reservoir assets and their distribution within the asset providing valuable information for the development of biosecurity and management plans. Additionally, ROVs are able to detect signs of crayfish, such as burrows, burrow bound animals, and parts of animals, such as claws and carapaces, which trapping or static video may miss. This provides much more detailed information on the size and distribution of the population, in addition to key information on meta-population distribution, which is vital in the development of management plans. The deployment of traditional monitoring methods, such as trapping for crayfish, are not suitable for assessing key locations in reservoirs which are often hard and dangerous to access. Draw off towers and scour values present key points of risk for the potential dispersal of invasive non-native species from impounded reservoirs but are difficult to monitor due to health and safety risks presented by the infrastructure and its operation, in addition to the depth of water in which they are often situated. As ROVs can be deployed at a distance and at depth, with umbilical cords of 100m being common, these issues are overcome. The large size and weight of currently available ROVs does not make them an ideal tool for monitoring smaller freshwater environments. While ROVs are becoming smaller and more accessible, they are preferable in still water or without their umbilical cords to avoid becoming entangled on submerged objects. The biggest issue currently faced by ROVs is that they cannot handle strong water movement, which prevents standardised sampling protocols being followed. Both RUV and ROV, as well as the field of research in freshwater ecosystems, would benefit from method comparison studies to better understand the pros and cons and how these technologies could be used together to provide more holistic ecosystem assessments.

Conclusion

RUVs offer a non-destructive and effective method for monitoring freshwater fisheries species in non-turbid waters. They can provide us with very useful information to address a range of scientific questions. All future RUV surveys should consist of an action camera, set to record at 30fps, 1080p being deployed for 60 minutes. By having a consistent methodology all future surveys can be accurately compared. These standards that I have recommended will ensure that RUV becomes a vital tool in the future of freshwater surveys.

Rapid technological advances have the potential to vastly transform fisheries research to become streamlined, automated, and standardised which will improve both the quality and granularity of data that environmental managers have access to. This can greatly advance the robustness of management plans and capacity for evidence-based interventions. With the declining state of global freshwater fisheries and lack of management incentive I promote the creation of an international freshwater RUV consortium to increase standardisation, collaboration and method development to improve data availability and implement baseline monitoring programmes (Barbarossa et al. 2021; Ainsworth et al. 2023).

Chapter 3

Feasibility of using novel technology to monitor crayfish species

Abstract

The continuous upgrades of camera equipment have meant that it is becoming a readily affordable tool that can be utilised by researchers to address freshwater ecosystem questions. Few studies have employed remote underwater video (RUV) as a tool to survey crayfish. As part of this research the aim was to address the limitations of this tool for surveying this organism and identify its effectiveness. By developing the current literature CrayCam has been designed, a specialised RUV tool for surveying crayfish. Turbidity tests found that both distance and turbidity have a significant impact on the effectiveness of RUVs, with higher turbidity waterbodies, and subjects being further away from the RUV system, being less effective at observing the target organism. Time to first observation for narrow clawed crayfish (*Pontastacus leptodactylus*) in a freshwater reservoir with limited turbidity occurred mainly within the first25 minutes when using a RUV and 17 minutes when using a baited RUV (BRUV). Not enough observations occurred to clear trends graphs when using the RUV to observe signal crayfish (*Pacifastacus leniusculus*) in a highly turbid river, however three observations were made using the RUV and a further three using the BRUV. Time to first observation for white-clawed crayfish (*Austropotamobius pallipes*) in a clear river occurred mainly within the first 12 minutes for RUVs and 15 for BRUVs, making several observations of this species of crayfish. This research has shown that RUV is a tool that can be used to successfully monitor crayfish populations and the rapid deployment of CrayCam successfully identified a surviving population of endangered crayfish in time to conduct a multi-agency rescue event.

Introduction

Technological advances in the last twenty years have allowed the use of underwater video to become a more accessible tool in aquatic research. Progress in the size of batteries and storage, as well as improved video qualities of equipment that costs less than £1000 have made it a useful piece of tool for water-based surveys (Mallet & Pelletier, 2014). As a tool Remote Underwater Video (RUV) has been predominantly used in marine environments, with publications mentioning its use as early as 1952 (Barnes, 1952; Mallet & Pelletier, 2014). Despite the widespread use in marine environments, remote underwater video has been under utilised in freshwater environments, as of 2022 only 167 publications used remote underwater videos in freshwater environments (Chapter 2). The earliest publication using remote underwater video was from 1988, however in recent years trends have been rapidly increasing, with an annual increase in the number of RUV studies in freshwater publications per year (Moore & Scott, 1988; Chapter 2).

Freshwater ecosystems face a major biodiversity crisis, with populations declining at a rapid rate (Tickner et al. 2020). There are several factors that influence biodiversity decline in freshwater ecosystems, including pollution, climate change and invasive species (Collier et al. 2016). Invertebrates play a key role in freshwater environment biodiversity and provide ecosystem services across them. In the UK species such as the native white-clawed crayfish (*Austropotamobius pallipes*) are a keystone species in aquatic habitats (Matthews et al. 1993). Despite their importance they are classified as Endangered by the International Union for Conservation of nature (IUCN) (Nightingale et al. 2017; IUCN, 2025). Many crayfish species have been introduced outside of their native range, putting native crayfish populations at risk of extinction (Dunn et al. 2009). I looked to create a specialised tool that could be rapidly deployed throughout waterbodies in order to successfully answer questions about both native and invasive UK populations of crayfish, which could then be further deployed to answer global questions.

Globally there have been few studies that have implemented remote underwater video as a tool to monitor crayfish populations, both native and invasive. Previous studies have concluded that baited remote underwater video are more effective than traditional crayfish traps when surveying abundance of crayfish (Fulton et al. 2012). As of 2022 there have only been eight studies that have used remote underwater video to address a range of questions about crayfish populations. Of these studies two looked at assessing the abundance of invasive crayfish in the USA (Robinson, 2014; Loffredo 2018). Two assessed the abundance of native crayfish in Australia (Fulton et al. 2012; Skorulis et al. 2021), and one assessed the presence/absence of native crayfish at an ark site within the UK (Rosewarne, 2013). Two studies looked at the behaviour of native crayfish in both Australia (Starrs et al. 2015) and Norway (Raugstad, 2019). The final study looked at the interspecies behaviours between a native and invasive crayfish (O'Hea Miller et al. 2022).

There are many limitations to the success of remote underwater video, with factors like high turbidity limiting successful identifications (Tweedie et al. 2023). Different crayfish often require different habitat types, native white-clawed crayfish are often found in areas with large amounts of stone refuge (Holdich & Rogers, 2000). Invasive signal crayfish

(*Pacifastacus leniusculus*) influence suspended sediment yields (Harvey et al. 2014) and are often found in more turbid waters. Finally invasive narrow clawed crayfish (*Pontastacus leptodactylus*) are an understudied species in terms of invasion (Harwood et al. 2025) and little is known about their activity. This chapter aims to identify the extents of these limitations and identify how the effectiveness of remote underwater video varies between different crayfish species and habitat types.

Methods

RUV design

A systematic literature review was conducted into the different uses of RUVs in freshwater environments (Chapter 2). All described designs were reviewed to identify the best structures required for stability and ensuring the camera settled in an upright position. Upon identification of requirements an initial design was drawn and a first setup was constructed by qualified fabricators and finalised using parts acquired from a hardware store. The final designed product was termed “CrayCam”.

Turbidity tests

To test how far a camera could observe in different turbidity levels a plastic tub (63cm x 38cm x 20cm) with a maximum capacity of 50 litres was filled with 30 litres of water. A GoPro Hero 10 camera was placed in a fixed position at one side of the tub, the tub was split into eight seven-centimetre line segments to establish maximum distance visible at different turbidity levels, with the furthest point being 56 centimetres from the camera. Following the recommended camera settings from Chapter 2, the camera was set to record at 1080p resolution at 30 frames per second. A model crayfish was positioned at the furthest point from the camera and left for five seconds. After five seconds the model was moved towards the camera, remaining in place for five seconds at each point. Recording was stopped once the model was in the location closest to the camera.

Fifteen grams of fine soil was then placed into the water and thoroughly stirred to ensure complete diffusion throughout the water. Recording was repeated as described previously using the new “turbid” water. This process was further repeated using 15-gram increments of fine soil until a total of 120 grams of fine soil was in the water. Upon completion all footage was manually reviewed, noting the position at which the crayfish model became visible for each turbidity level. A binomial GLM was constructed to establish if either turbidity or distance, or a combination of both, could predict the success of observing a crayfish.

Time to first observation histograms

Remote underwater video (RUV) and baited remote underwater video (BRUV) surveys were conducted as part of three separate surveys (Chapter 4, 5, 6). The first surveys were conducted at Boshaw Whams Reservoir (53°32′N; 001°46′W) a freshwater reservoir located near to Holmfirth in Kirklees, West Yorkshire, United Kingdom. This reservoir has limited turbidity and is home to the only known population of invasive narrow clawed crayfish (*Pontastacus leptodactylus*) in Yorkshire (Chapter 4, Harwood et al. 2025). The second surveys were conducted on the River Calder (53°44′N; 002°00′W), a tributary of the River Aire, running through Yorkshire, United Kingdom (Chapter 5). The river has high levels of turbidity and invasive signal crayfish (*Pacifastacus leniusculus*) were first reported in the lower regions of the river in 2023 (Calder Rivers Trust, pers. comm.). The final surveys were undertaken on the River Kent (54°22′N; 002°47′W) a river that runs through the Lake District National Park, Cumbria, United Kingdom (Chapter 6). This is a clear, shallow river that runs through rural countryside, it is home to a healthy population of native white-clawed crayfish (*Austropotamobius pallipes*).

RUVs were deployed at each site for an hour, upon completion of the RUV survey the BRUV was baited with wet cat food and then deployed for an hour at the same location. Each deployment used a GoPro HERO 10 Black (GoPro, Inc., USA) camera recording at 30 frames per second and a resolution of 1080p, following standards recommended in Chapter 2. All footage was manually reviewed by the same surveyor and the until first observation was noted for each video. Video footage that did not include any records of crayfish was discarded for time to first observation analysis. Each survey site and method were separated into a unique dataset and histogram was plotted. These results can then be used to establish maximum deployment durations to ensure observations for each species, method and habitat type.

Results

RUV design

An initial design described in van Wyk (2019) was used as the foundation of the design for CrayCam. Rather than use acrylic piping like van Wyk (2019) my setup was constructed using stainless steel to eliminate the need for a weighted anchor system. As crayfish are the target species of CrayCam the orientation of the design was modified to ensure that the camera faced towards substrate and benthic zone rather than midwater (Figure 3.1).

Figure 3.1. Final construction of the CrayCam system with. A) Removable PVC bait arm, B) GoPro Hero10 with action mount, C) removable and posable legs, D) Base plate, E) rope for attachment to bank

Turbidity tests

When the model was in the segments furthest from the camera, 56cm, it could be seen with a maximum siltation of 15g. When the model was in the segment closest to the camera, 7cm, it could be viewed at a different maximum siltation of 105g (Figure 3.2). Distance (χ^2 = 104.58, df = 1, p < 0.01; Table 3.1) and turbidity (χ^2 = 198.09, df = 1, p < 0.01; Table 3.1) both had a high significance in whether the crayfish model could be seen. With lower levels of turbidity increasing the likelihood of a successful observation, and the model is more likely to be successfully seen at locations closer to the camera.

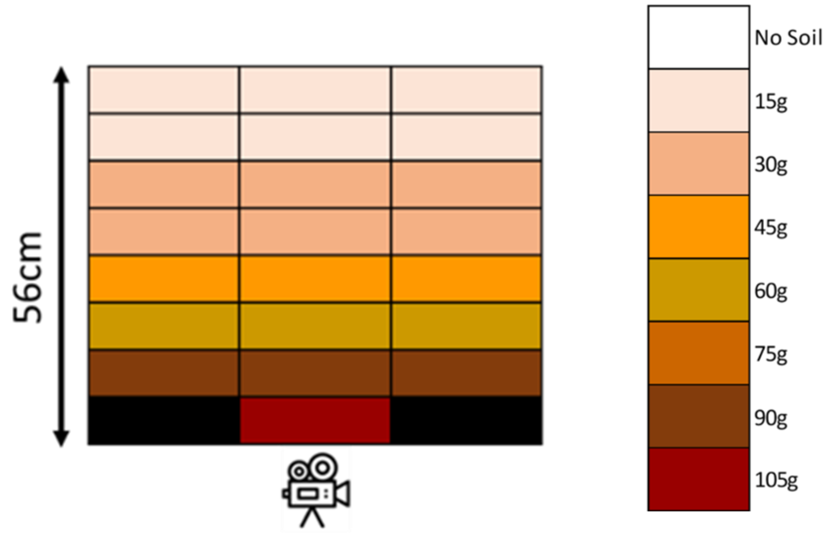


Figure 3.2. Maximum turbidity levels that the crayfish model could be observed at, the black rectangles represent areas of tank outside of the camera's field of view

Table 3.1. Type II ANOVA for a binomial GLMs assessing effect of distance and turbidity on likelihood to see an object

	χ^2	**df**	**p**
Distance	104.58	1	< 0.01
Turbidity	198.09	1	< 0.01

Time to first observation histograms

A total of eight RUV deployments at Boshaw Whams Reservoir observed invasive narrow clawed crayfish. The earliest observation occurred within the first minute, and the latest observation occurred in the fiftieth minute. The histogram shows most observations to occur before the 25-minute mark, with sporadic times after this (Figure 3.3A). A total of seven BRUV deployments at this site observed crayfish, with the earliest observation occurring in the first minute, and the latest observation occurring in the twenty-fourth minute. The histogram is heavily weighted towards earlier times for this site and species when using the BRUV, with all observations occurring within 25 minutes (Figure 3.3B).

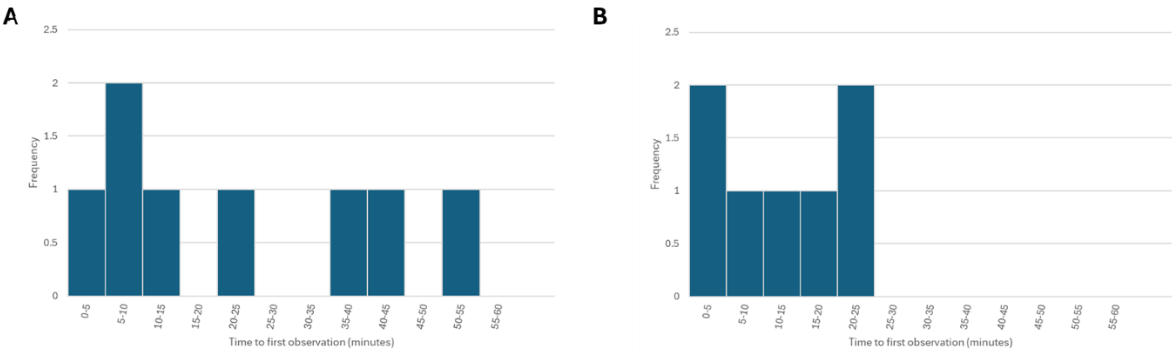


Figure 3.3. Time to first observation histograms for narrow clawed crayfish at Boshaw Whams reservoir using A) RUV B) BRUV

Surveys on the River Calder, observing invasive signal crayfish obtained fewer crayfish observations than other sites and species. Three RUV deployments observed crayfish with the earliest observation in the eighth minute and the latest in the fifty-seventh minute. There were also three crayfish observations made by the BRUV, the earliest of these in the sixth minute and the latest in the fifty-third minute. Neither method made enough observations to identify a trend in the results (Figure 3.4).

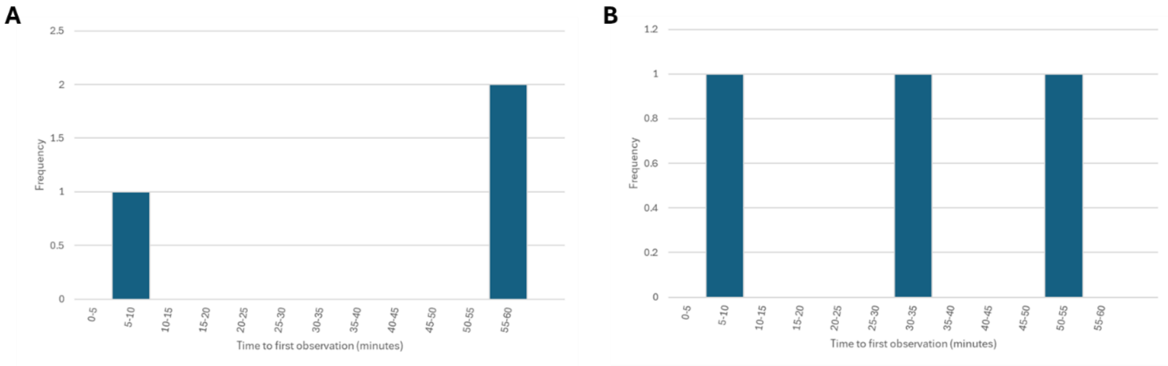


Figure 3.4. Time to first observation histograms for signal crayfish in the River Calder using A) RUV B) BRUV

Native white-clawed crayfish surveys on the River Kent made twelve observations using a RUV and fifteen using a BRUV. The earliest observations for the RUV occurred in the first minute and the latest occurred in the fifty-seventh minute. For RUVs almost all observations happened within the first 40 minutes with a single record after this (Figure 3.5A). The earliest observations for the BRUV also occurred within the first minute and the latest in the forty-third minute, most observations occurred within the first 20 minutes with sporadic occurrences later, all observations were made before the 45 minute mark (Figure 3.5B).

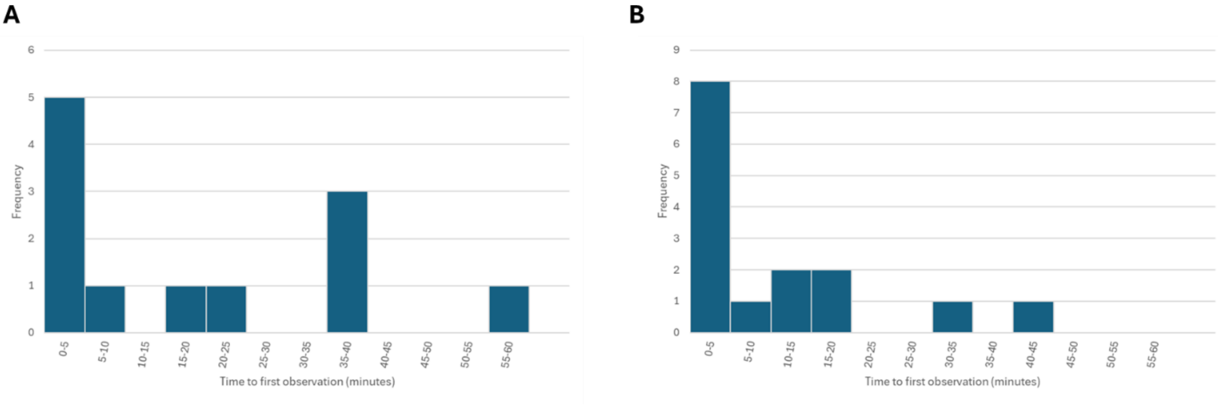


Figure 3.5. Time to first observation histograms for native white-clawed crayfish in the River Kent using A) RUV B) BRUV

Case study: Rapid deployment of CrayCam

Monthly water quality assessments completed by the Environment Agency in November 2022 at Meanwood Beck, Leeds, West Yorkshire, found traces of crayfish plague. Meanwood Beck is home to a well-documented population of white-clawed crayfish (Peay, 2004) and it was feared that this outbreak of crayfish plague would result in the population becoming extinct. Traditional trapping methods could not be used at Meanwood Beck due to a resident population of water voles (*Arvicola amphibius*), and it was therefore difficult to confirm the extent of the spread of crayfish plague, and if the white-clawed crayfish were still present within the beck while also maintaining good biosecurity. This presented the first opportunity to field test CrayCam to see if it could feasibly assess an area to determine the presence of crayfish. During August 2023 eight CrayCam deployments were made over two days. Each day the RUV was deployed at two separate locations along the beck for 60 minutes, upon completion of these deployments two BRUVs was then deployed at the same locations for a further 60 mintues. This initial trial successfully confirmed the presence of white-clawed crayfish along Meanwood Beck, with a single record being made of white-clawed crayfish on one of the BRUV deployments (Figure 3.6) and led to a multi-agency crayfish rescue event in which the beck was dewatered. During this dewatering several adult white-clawed crayfish were retrieved, including females carrying young. These individuals were transported to the University of Leeds for quarantine, during which the females released their young and these juveniles were reared so that they could be reintroduced to the wild. Post quarantine the crayfish were released into two new ark sites, one a Yorkshire Water owned reservoir, and the other a pond next to the University of Leeds playing fields. It is hoped that these ark sites will allow this population to survive and once crayfish plague has burnt out within Meanwood Beck the ark populations can be used to restock the beck.

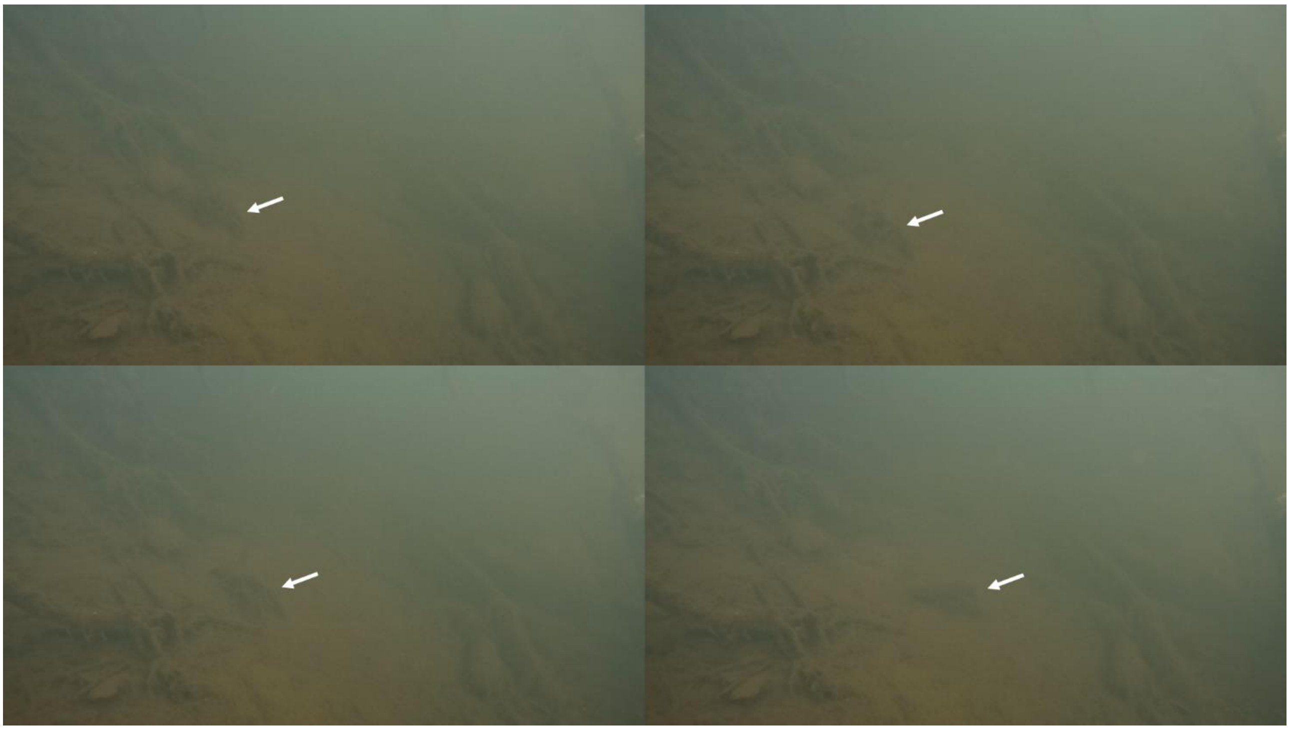


Figure 3.6. Successful observation of a white-clawed crayfish (*Austropotamobius pallipes*) in Meanwood Beck, Leeds, West Yorkshire, obtained using Baited Remote Underwater Video

Discussion

This study aimed to assess the feasibility of using remote underwater video to monitor crayfish populations. In this study I have developed CrayCam, a successful and effective tool for monitoring crayfish, this has been successfully tested in Meanwood Beck, Leeds, West Yorkshire, and proven to be a useful tool to identify crayfish when alternative surveying methods are not possible. Here I have shown that even in highly turbid waterbodies the RUV can successfully identify crayfish presence. The effectiveness efficiency in which the RUV can detect different crayfish species in different habitat types is variable and is dependent on both the target species of crayfish and the environments that are being surveyed.

RUV design

The orientation of a camera is a vital aspect that should be considered when designing a remote underwater video rig (Whitmarsh et al. 2017). Crayfish are a bottom dwelling species and only occupy the bed of the waterbodies that they are present in. Since crayfish are the only target species for CrayCam an angled downward facing orientation was chosen to ensure that a larger waterbody bottom field of view was achieved, removing "wasted" areas such as the midstream. The downward facing orientation was achieved using a tripod design with two shorter legs at the front and one taller one at the back, this tripod also allows for stability in fast flowing waterbodies, while also allowing the rig to remain upright as it descends to the bottom of deeper waterbodies. I have also created two designs for the remote underwater video, the first is the standard tripod design, the second is a baited remote underwater video rig, which includes a removable bait arm. Bait acts as a stimulus for attracting individuals towards a camera's field of view (Jones et al. 2020). I opted for these two designs in order to answer different research questions. Baited remote underwater video provides a vital tool in rapidly detecting if populations are present, white also accessing abundances of crayfish populations. The presence of bait is not always ideal as it can influence crayfish behaviours (O'Hea Miller et al. 2022, Chapter 6). Studies looking to observe crayfish in their natural habitat could witness unnatural behaviour as a response to these baits. On top of bait bias, there is also risk that bait could attract dangerous predators to the survey area, like crocodiles, putting surveyors at risk (Ebner et al. 2015). Although this is not a risk that is present within the UK waterbodies, it is an important consideration to make when wanting to globalise the use of CrayCam.

Turbidity tests

There is a direct correlation between turbidity and the distance at which the remote underwater video can successfully detect crayfish, with further distances becoming less accessible as turbidity increases. Despite this the model crayfish could still be seen in the closest square to the camera in the highest turbidity ratio, this level of turbidity is likely to represent that of some of the highest turbidity watercourses that may host crayfish throughout the United Kingdom (Harvey et al. 2014). Given that crayfish observations could still be made at the highest turbidity, this shows that CrayCam is a potential tool for determining presence/absence of crayfish at sites throughout even those with high levels of turbidity. In these high turbidity levels remote underwater video is only able to obtain silhouettes of crayfish and more distinct features, such as key species identifiers, cannot be observed. The other issue that turbidity could present is when using baited remote underwater video, here the bait arm may be positioned far enough away from the camera that it is no longer visible, this can then lead to individuals being missed that visit the bait without passing the front of the camera. Historically visual methods, such as the Secchi disk, have been used to assess turbidity. However, advancements in technology have meant that more reliable and quantitative methods have been developed to measure turbidity. Although they produce more reliable and accurate results, specialised probes that can be used to measure turbidity levels are prohibitively expensive and were not a part of the toolkit for CrayCam. Surveys using RUVs look to focus on a horizontal plane and tools like a Secchi disk, which can be used to measure the transparency of turbid water, only work on a vertical plane. By developing a "Secchi stick" a horizontal Secchi disk alternative has been created. Previous studies have found that Secchi disk results are inversely proportional to turbidity probe measurements in Nephelometric Turbidity Units (NTU) (Golubkov & Golubkov, 2024). This finding maps closely to the results shown in Figure 3.2. In my turbidity experiment trials with lower levels of soil are easier to distinguish between, as shown by the double grid square spacing for 15g and 30g in Figure 3.2. This relates to past literature that has found that when turbidity levels are below 20 NTU Secchi disks can easily distinguish differences in turbidity (Golubkov & Golubkov, 2024). This can then relate to in the field studies where the Secchi stick can be used to accurately measure variations in water transparency for low/moderately turbid waterbodies. When turbidity measurements exceed 40 NTU the Secchi stick can no longer be used to establish differences in water transparency, as has been shown by the more compact grid square spacing for higher soil volumes in Figure 3.2 (Golubkov & Golubkov, 2024). This has shown that the camera can confidently record the presence of crayfish when the Secchi stick shows low levels of turbidity but is less likely to observe crayfish when fewer markers are visible on the stick.

Time to first observation histograms

During my in-field trials both remote underwater video (RUV) and baited remote underwater video (BRUV) successfully observed all three target species of crayfish. Both methods also successfully made at least one observation of all three target species of crayfish within the first ten minutes of deployments, showing that the crayfish are both present and active, but also show a naturally inquisitive nature (Parkyn et al. 2011), which has been seen here when a foreign object is placed within their territory. Of the three species of crayfish tested in my study narrow clawed crayfish achieved observations the quickest for both RUV and BRUV deployments. These results are due to the high feeding intensity of narrow clawed crayfish with them active during both day and night (Skurdal & Taugbøl, 2002), the water clarity at Boshaw Whams reservoir, where the crayfish have been surveyed, is clear with limited turbidity and suspended sediments as a result of it being a UK water company owned freshwater reservoir (Harwood et al. 2025). Surveys for white-clawed crayfish also achieved several successful observations with twelve RUV surveys observing crayfish and fifteen BRUV surveys observing crayfish. These daytime observations of white-clawed crayfish go against the current understanding that white-clawed crayfish are active at night, remaining in burrows during daylight hours (Peay & Hirst, 2003). Despite these findings white-clawed crayfish have been observed to show high levels of activity and these observations have led to further studies (Chapter 6) to gain an understanding of white-clawed crayfish instream behaviour. The clear nature of the River Kent, where these observations have been made, provides suitable conditions for successful observations, with limited suspended sedimentation providing visibility of more than 2 meters. Less observations have been made when looking at signal crayfish and the species accumulation curves that have been produced do not appear to show any trends. Signal crayfish are ecosystem engineers and their burrowing yields high sedimentation (Harvey et al. 2014). The survey area for this species was a highly turbid river, with most observations being made when a crayfish silhouette was observed close to the camera and species confirmation was only made by trapping surveys conducted simultaneously. The turbid river and the fact that signal crayfish are highly nocturnal (Johnson et al. 2014) provides several factors making them a difficult species to study using remote underwater video, but not impossible.

Future Steps

By establishing a network of global contributors that can share time to first observations for all crayfish remote underwater video surveys, I propose a large-scale database of remote underwater video studies looking at all species of crayfish in a range of environments. By producing species accumulation curves for different species and habitat types an understanding of crayfish activity can be obtained, while also identifying the minimum effort that needs to be employed to confidently survey an area and establish if a specific species of crayfish is present. The manual annotation of wildlife is a time-consuming process (Njathi et al. 2023) and reviewing footage to obtain time to first observation data can be tedious. Developments are being made, and algorithms are being trained to classify images (Gitau et al. 2024), if these can be developed to identify the first record of crayfish, then the process could be automated to ensure standard protocol and reduce the effort required to conduct remote underwater video surveys of crayfish.

A closely linked novel application that I do not investigated throughout this feasibility study, or my wider thesis, is the use of remote operated vehicles (ROV) to monitor invasive non-native crayfish populations (P Stebbing pers. comm). These currently play a role in invasive non-native species management and could potentially play a wider role in the future. Despite its wide application in commercial sectors there is a large disconnect between the published literature and the wider applied field of work for ROV surveys. The objectives of these surveys are to: 1) identify where in the water column the invasive non-native populations are, 2) assess the level of risk of the of the non-native populations spreading out of the target waterbody and 3) identify the level of impact that the invasive non-native species have on the wider environment. The risk of transport and spread are examined using ROVs by identifying pathways for raw water transport and assessing the risk of a target species being able to pass them. In a reservoir in the Southwest of England ROVs have been used to assess the heights of submerged pipes and reservoir spillways to establish if they were at levels that would allow signal crayfish to walk over them, and the level of risk of it being a pathway for further spread (P Stebbing pers. comm). By identifying these risks and confirming areas of concern suitable mitigation measures can then be advised. Similar processes are followed on a species-by-species level depending on the motor patterns of the target species (P Stebbing pers. comm). In Scotland ROVs have been deployed into a reservoir to assess the distribution of an invasive non-native crayfish. Here the distribution of a population has been mapped and suitable habitat throughout the reservoir has been identified to estimate potential spread throughout the wider waterbody (P Stebbing pers. comm). Gradual spread of invasive non-native crayfish can be predicted if the entire reservoir displays a continuous distribution of suitable habitat, alternatively fragmented patches of suitable habitat will reduce the rate of spread as crayfish are less likely to move across unsuitable areas to get to them (P Stebbing pers. comm).

Conclusion

This feasibility study has shown that remote underwater video is a tool that can be used to survey several different crayfish species in a range of aquatic habitats. Despite limitations through turbidity, it still offers a cost and time efficient alternative to more traditional methods such as trapping and hand searching, when wanting to conduct rapid assessments of invasion spread, or assess the instream behaviours of native species. Remote underwater video offers a non-invasive alternative that can be used to observe endangered species, like the white-clawed crayfish, in their native environments (Broom et al. 2023). Trapping can determine aspects such as length frequency and sex ratios which standard RUV cannot deliver, however implementation of a stereo-RUV system would facilitate length frequency assessments. Overall technological advances have meant that remote underwater video has become a more accessible and affordable piece of survey equipment.

Chapter 4

Rapid assessment of population dynamics and monitoring methods for invasive narrow clawed crayfish (*Pontastacus leptodactylus*), in a freshwater reservoir in Yorkshire, United Kingdom

Harwood, M., Stebbing, P.D., Dunn, A.M., Cole, Z.K., Bradbeer, S.J., Aston, B., South, J. (2025) Rapid assessment of population dynamics and monitoring methods for invasive narrow clawed crayfish *Pontastacus leptodactylus* in a freshwater reservoir. Knowl. Manag. Aquat. Ecosyst., 426 (2025) 22. DOI: https://doi.org/10.1051/kmae/2025017

Abstract

Narrow clawed crayfish (*Pontastacus leptodactylus*) are a data deficient invasive non-native species in the UK. Boshaw Whams (West Yorkshire, UK) contains the only known population of narrow clawed crayfish in Yorkshire. The risk of further spread of these crayfish is high and it is important to establish the extent of the current invasion on the Generalised Invasion Curve to identify potential management options. I used a combination of methods over a 15-month period including trapping, Remote Underwater Video (RUV) and Baited RUV (BRUV) to establish the most efficient method for narrow clawed crayfish monitoring and determine annual population dynamics. There was no significant difference between the three methods in terms of detection efficiency thus I recommend a mixed approach in the future dependent on practitioner capacity. Significantly more males were observed through trapping than females and berried females were detected between February and April. A mark-recapture survey estimated the population to have a minimum size of 10,045±5602 (95% CI) individuals in a waterbody spanning 50,000 m2. Boshaw Whams Reservoir should be considered as in the 'Containment' or 'Asset Protection' stage of the Generalised Invasion Curve, and action urgently required to prevent further spread.

Introduction

The ongoing spread of invasive non-native species (sensu Soto et al. 2024) is a major driver of biodiversity loss and economic strain globally (Roy et al. 2023). Invasive non-native species have negative impacts on their new environments, and it is estimated that they cost the British economy £4 billion each year (Eschen et al. 2023). Of these, invasive crayfish can cause significant damage to irrigation structures and banks of rivers and lakes through burrowing activity and may alter community composition through predation, competition and ecosystem engineering (Holdich, 1999; Bubb et al. 2004; O'Hea Miller et al. 2024). In the United Kingdom (UK), the signal crayfish (*Pacifastacus leniusculus*) alone, was attributed to have caused US$15.3 million between 2000-2020 (Kouba et al. 2022). There is less information on other invasive crayfish, and their environmental and economic impacts, including the focal species of this study, the narrow clawed crayfish (*Pontastacus leptodactylus*). The narrow clawed crayfish is a data deficient invasive non-native species considered native to the Ponto-Caspian region (Skurdal & Taugbøl, 2002). They are a large freshwater crayfish species found in both lentic and lotic environments but considered to be still-water specialists (Bök et al. 2013). Invasive populations are widespread throughout Europe and are predicted to spread through central-western Europe under future climate scenarios (Hodson et al. 2024).

There are 16 main water and sewage companies supplying the UK with water resource management, including 273 major reservoirs which account for 90% of total water storage capacity (Williams et al. 2010; Durant & Counsell, 2018). These reservoirs can indirectly act as sources, sinks and stepping stones for the spread of invasive non-native species (Havel et al. 2005). Reservoirs can be easily invaded due to regulated flow, low biotic diversity, and high anthropogenic influence (Moyle and Light, 1996; Havel et al. 2005; Clavero et al. 2013). Some reservoir sites also have high numbers of anthropogenic pathways, such as recreational activities including boating, kayaking and angling. Where they are required, compensation flows from reservoirs allows the release of water from a reservoir to the connected waterbodies downstream. In addition to compensation flows, the Reservoir Safety Act 1975 requirements include operating scour valves to maintain the safety at reservoirs. These are key biosecurity issues as a large volume of water is released into connecting waterways. The Wildlife and Countryside Act 1981 outlines responsibilities and liabilities for water companies around managing invasive non-native species at their sites. The Invasive Alien Species (Enforcement and Permitting) Order 2019 also introduced penalties for the spread of 'species of special concern'.

The General Invasion Curve illustrates the process of invasion in a given area to categorise threat and determine possible interventions (Harris et al. 2018). The key aim of the graph is to establish the threat of an invasive population on a national scale, here I apply it on a more local scale, looking at a single site. When a waterbody is invaded, management actions are often working on a time lag between invasion arrival, detection, and impact. Assessing the stage of invasion must be prioritised and determined rapidly to inform appropriate decisions following the General Invasion Curve (Figure 4.1; Harris et al. 2018) which guides mitigation measures along stages related to the progression of the invasion: 1) prevention, 2) eradication, 3) containment and 4) asset protection (Harris et al. 2018). Further frameworks provide summarises of management options relative to the stage of invasion, for example Robertson et al. (2020). Failure to enact appropriate management may result in further invasive non-native species spread and increased cost of management interventions (Cuthbert et al. 2022). Appropriate management is relative to the stage of invasion of the population, and can include different key forms of management, including pathway management and population suppression (Robertson et al. 2020). It is important to note that these frameworks and curve can be applied at different spatial scales, thus whilst assessing one site within this study, when considering management options, it is imperative to understand the overall spread and impact of the species, especially at a catchment/regional scale, with connected waterbodies potentially facilitating further spread in the absence of anthropogenic pathways.

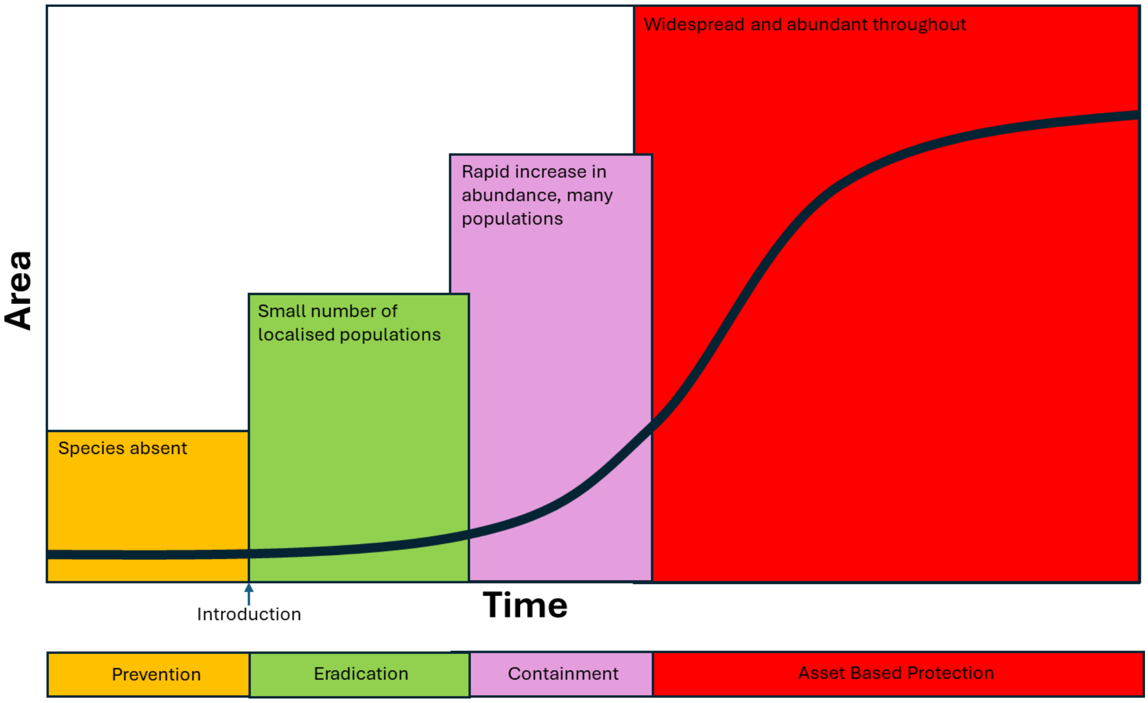


Figure 4.1. General Invasion Curve for invasive non-native species, there are four stages on the curve; 1) Prevention, when the species is absent from the asset, 2) Eradication, when

populations are small and isolated, 3) Containment, when populations are rapidly increasing, and 4) Asset protection, when there is a widespread population

Boshaw Whams, a Yorkshire Water owned reservoir in Holmfirth, West Yorkshire, United Kingdom, contains the only known population of invasive narrow clawed crayfish in Yorkshire. This population was illegally introduced around 2014, and local anglers started to report them as a nuisance in 2019 (pers. comm Huddersfield Angling Club). Although the impact that other invasive non-native crayfish have on narrow clawed crayfish within their native range has been studied (Lele & Pârvulescu, 2017; Hudina et al. 2016), *Pontastacus leptodactylus* is a data deficient species regarding its invasion dynamics and ecological impacts. Thus, given the data deficiency of the species' invasion ecology and the likelihood of further spread (Hodson et al. 2024) my overarching aim was to rapidly assess the stage of the invasion on the Generalised Invasion Curve to advise management at this study site. In doing so, I tested two bait types and compared traditional (trapping) monitoring methods with Remote Underwater Video (RUV) and baited RUV (BRUV) for efficiency in detecting presence/absence and abundance dynamics of narrow clawed crayfish throughout the year at Boshaw Whams reservoir. I then used mark-recapture to estimate population size and characterise reproductive ecology over a year. Finally, I provide comments on the economic resources needed to assess a crayfish invasion in a small reservoir and how to optimise invasion related costs.

Methods

Study Site

Boshaw Whams (53°32′N; 001°46′W) (Figure 4.2) is a freshwater reservoir, and is used for angling and boating recreational activity, located near to Holmfirth in Kirklees, West Yorkshire, United Kingdom. The reservoir is situated at an altitude of 300m and has a circumference of ~ 900m and a maximum depth of ca. 6m. The reservoir is stocked with triploid rainbow trout (*Oncorhynchus mykiss*) and brown trout (*Salmo trutta)* monthly from March to July with a final stocking in September, on an annual basis, with approximately 200 fish stocked per event (Huddersfield Angling Club, pers. comm.). There is a wooden jetty along one side of the reservoir with the remaining sides consisting of a grass bank with a gradual incline above the water and cobbles below. A population of native white-clawed crayfish has been reported at Armitage Bridge (Figure 4.2) WoC ID: WK9180 (for *A. pallipes*, observation date: 2022) (Ion et al. 2024) approximately 10km downstream from Boshaw Whams reservoir.

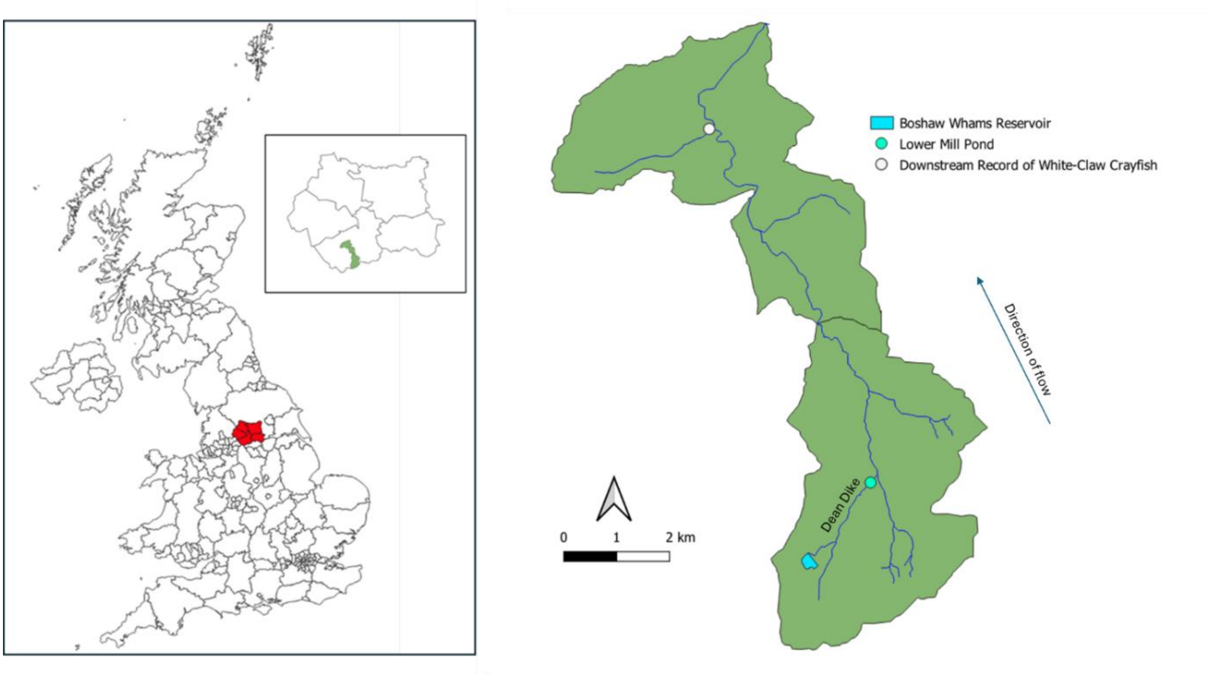


Figure 4.2. Map showing Boshaw Whams Reservoir location and downstream waterbodies as well as closest population of native white-clawed crayfish (*Austropotamobius pallipes*) species

Twice a year a scour valve test is conducted to ensure that the reservoir is managed in compliance with the Reservoir Safety Act (1975). During a scour valve test, water is released from the reservoir into Upper House Dike, which is 250m long and then flows directly into Dean Dike. Dean Dike is a small stream that runs through woodland, Strahler order 2 from Boshaw Whams reservoir. Dean Dike is 1.5m wide at the widest point and flows for 1km before entering Lower Mill Pond. The banks consist of woody vegetation and are bordered by farmlands.

Bait Comparison

Method comparison between bait types and equipment types were completed to determine best operating procedures for detecting crayfish and method efficiency. This data was also used to describe the population dynamics in relative abundance over the course of a year. To compare bait types, crayfish trapping was conducted between October 2022 and February 2024 using collapsible fladen crayfish traps (570mm x 290mm, 25mm mesh size). Bait comparison tests were conducted within a subsection of these surveys between October 2022 and June 2023, when both dry meat flavoured dog food and wet poultry flavoured cat food were used in the traps. Up to 49 traps were deployed per survey, all traps were deployed for 18 hours overnight. Half of the traps were baited with dry food and deployed on one side of the jetty and the other half were baited with wet food and deployed on the other side of the jetty. The side of the jetty the bait type was deployed on was alternated monthly. The jetty split the two bait types by approximately ten meters, and the olfactory dispersal of bait plumes was not enough to cause overlaps. The catch per unit effort (CPUE) i.e. – total catch per trap per night was determined for both bait types. All crayfish caught were removed from the waterbody and either euthanised with an overdose of MS-222 or brought to the facilities at the University of Leeds. A paired t-test was used on square root transformed CPUE to assess differences between in CPUE wet and dry bait types. Olfaction plays a key role in crayfish foraging (Willman et al. 1994) thus I predicated that wet bait would have a higher CPUE due to the scent diffusing more rapidly.

Gear Comparison

I test the potential of Remote Underwater Video (RUV) and Baited Remote Underwater Video (BRUV) surveys as an alternative to trapping (Figure 4.3).

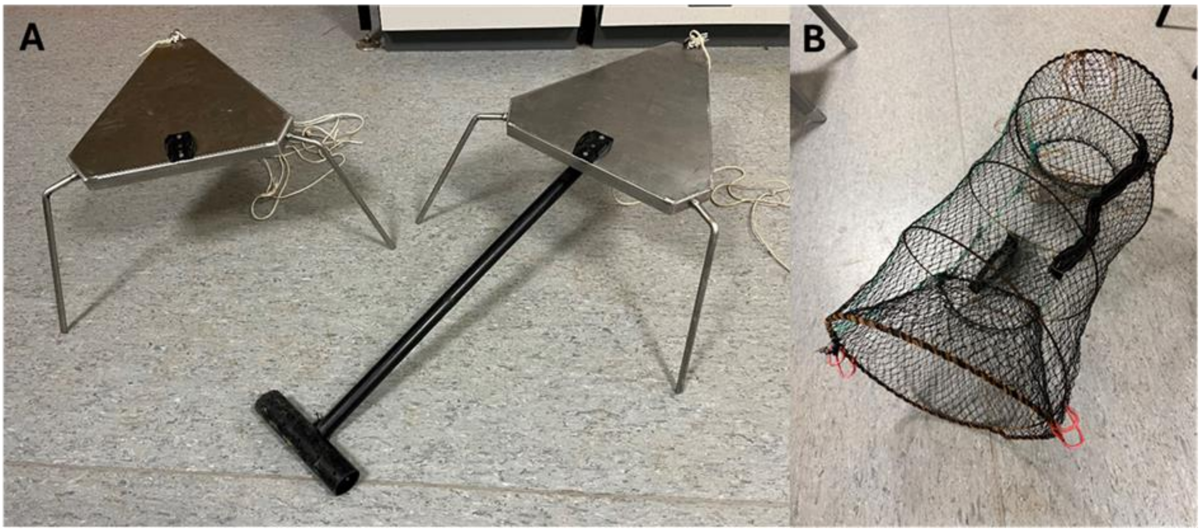


Figure 4.3. A) Photograph of the Remote Underwater Video (left) and Baited Remote Underwater Video (right) rigs used in the surveys and B) a standard crayfish trap used throughout the surveys (570mm x 290mm, 25mm mesh size)

B/RUV surveys were conducted once a month between March 2023 and February 2024. The BRUV was baited using wet poultry flavoured cat food. Both cameras were deployed at separate locations within the reservoir, not simultaneously, for one hour, using a GoPro HERO10 Black (GoPro, Inc., USA) recording at 30 frames per second with 1080p resolution. Cameras were deployed between 12:00 and 14:00 before traps as narrow clawed are active during both day and night (Skurdal & Taugbøl, 2002) and this allowed for suitable light levels for video analysis. They were also deployed at different times to avoid interference between gear types. All footage was manually reviewed by the same observer noting the maximum number of individuals present in frame throughout the deployment (MaxN).

Monthly trapping was conducted as describe in section 2.2. The CPUE for trapping was calculated by taking the total number of crayfish caught in traps and dividing it by the total number of traps (number of crayfish per trap). To account for the different time of deployment taken for each gear I also compared the detection probability of each gear to accurately compare gear. CPUE was calculated and compared between each of the gears to assess the effectiveness of B/RUVs at detecting crayfish to traditional (trapping) methods. Detection probability of each gear was calculated (Equation 1). Due to high number of zero observations delta-X corrections were applied to normalise the dataset (Madzivanzira et al. 2021; Nawa et al. 2024), I therefore used ANOVA to compare CPUE and detection probability between gear. A chi-squared test was used to establish if there was a relationship between the number of crayfish caught in traps and the detection probability of either the RUV or BRUV.

$$Detection\ Probability = \frac{Deployments\ observing\ crayfish}{Total\ Deployments} \qquad \text{eqn 1}$$

Mark-Recapture Survey

A mark-recapture experiment was conducted in March 2023 following the methods of Guan (1997) and using trapping protocols described previously to obtain an estimate of the total population size of the crayfish present within the reservoir. An important assumption for mark-recapture surveys is that there is no immigration or emigration (Rabeni et al. 1997). Boshaw Whams is an isolated reservoir with little external influence and no other populations of narrow clawed crayfish locally. Forty-nine traps were deployed daily over a three-day period. Each trap was checked at 24-hour intervals and all crayfish present within the traps were removed, and morphometric measurements taken for all crayfish, measuring the carapace length (mm), carapace width (mm), total length (mm), claw length (mm) and mass (g). After measuring, a triangular segment was cut from the tail, changing the segment of tail that had a segment removed each day so that the date of capture could be identified on any recaptures. Each marked crayfish was returned to the waterbody in a similar location as to where it was captured. Tail marks on crayfish are distinct and have durability for between two and three moults (Nowicki et al. 2008).

All crayfish captured during the monthly trapping events (total 10 months) conducted after the mark-recapture survey were also checked for marked tails. Chapman's corrected mark-recapture formula (Equation 2) with a normal approximation for 95% confidence interval was then used to estimate the population size to help mitigate bias under low recapture numbers.

$$N = \frac{((M+1)*(C+1))}{(R+1)} - 1 \qquad \text{eqn 2}$$

Where N is the estimated population size, M is the number of marked individuals, C is the total captured after marking and R is the number recaptured.

Population Dynamics

Monthly trapping was used to gain an understanding of the population dynamics at the reservoir. Traps were deployed as described in Section 2.2. After June 2023 all traps were baited solely with wet cat food. All traps were deployed in the afternoon and left overnight for 18 hours. Traps were retrieved, noting the number of individual crayfish in each trap, the sex of each crayfish and if females were berried. Morphometric measurements were taken for all crayfish as described in Section 2.4. The air temperature was recorded during each survey to compare how season and temperature affected the population dynamics and CPUE.

A chi-squared test was conducted to assess the male/female sex ratio in the trapped individuals. Two linear regressions F-tests with were conducted to establish whether number of crayfish removed over time and air temperature affected the trapping CPUE, all model assumptions were checked via QQ plots.

Economic Costs

In order to compare the economic costs of the different survey methods, the economic costs were calculated using the initial equipment and startup costs in combination with the annual cost to conduct monthly surveys. Economic costs of assessing the population dynamics of narrow clawed crayfish at one reservoir site were calculated first by identifying the equipment needed for surveys using; 1) traps, 2) RUV, 3) BRUV and the initial startup costs. The monthly cost of each survey was then calculated, accounting for travel to and from the site, car hire, fuel costs (one journey required for camera surveys but two for trapping surveys), bait, and surveyor time (at a minimum rate of £12/ hour). The values presented here were typical for the survey undertaken by the University of Leeds and fuel and distances are calculated appropriately, distance from the University of Leeds to Boshaw Whams is 48km. The initial costs and monthly costs were then scaled up to establish the total costs for 12 months' worth of surveys for each of the three different gears. The costs for each method are assessed and presented as either initial one-off costs or continuous monthly costs.

Results

Bait Comparison

For baited traps, there was no significant difference between the catch per unit effort (CPUE) obtained by the two bait types, dry dog food and wet cat food (t = 1.23, df = 8, p = 0.25).

Gear Comparison

There was no significant difference between the CPUE of any of the three gears (trapping, RUV, BRUV) (ANOVA, F = 0.979, p = 0.388; Figure 4.4, Table 4.1, Table 4.2). There was also no significant difference between the detection probability of any of the three gears (trapping, RUV, BRUV) (ANOVA, F = 0.066, p = 0.936; Figure 4.5, Table 4.1, Table 4.2).

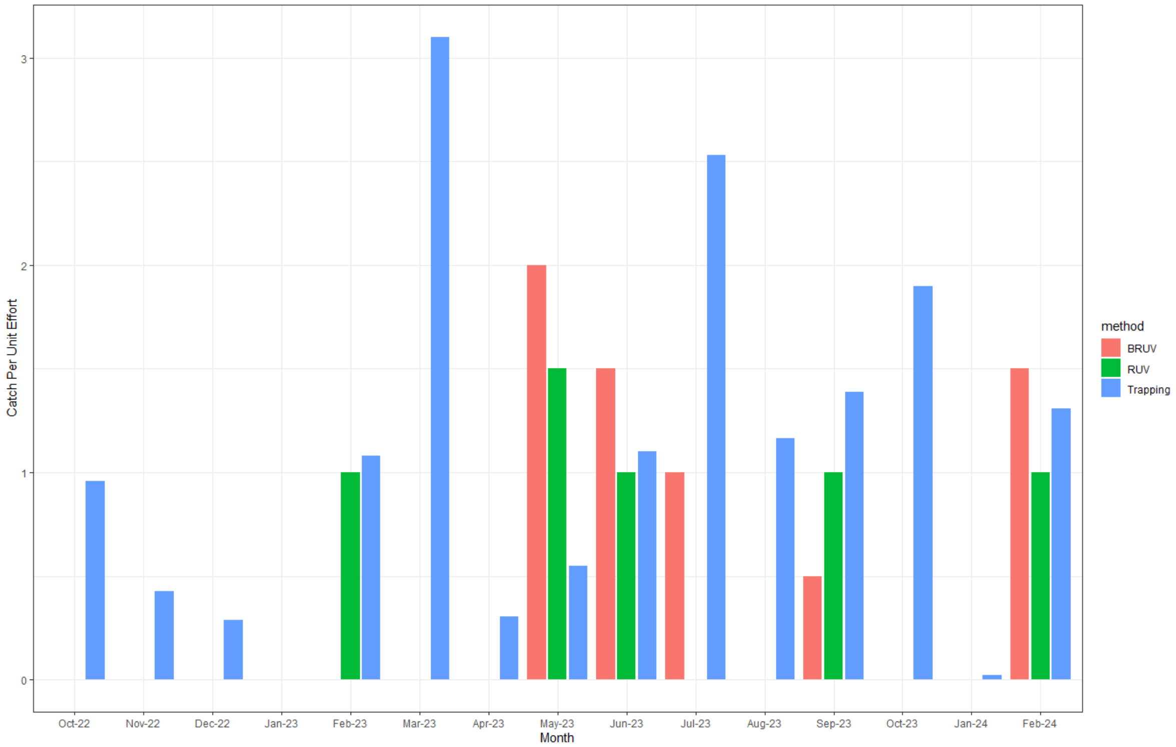


Figure 4.4. Monthly Catch Per Unit Effort (CPUE) of narrow clawed crayfish (*Pontastacus leptodactylus*) for Baited Remote Underwater Video (BRUV), Remote Underwater Video (RUV) and trapping surveys. CPUE for BRUV and RUV is the sum of MaxN (maximum number of individuals in frame at a single moment in deployment) divided by the number of deployments for each survey. CPUE for trapping is the total number of crayfish caught divided by the number of traps deployed for each survey. Note only trapping was conducted during the first four months of the survey (Oct-22, Nov-22, Dec-22 and Jan-23)

Table 4.1. The overall Catch Per Unit Effort (CPUE) and Detection Probability of each crayfish surveying methods, values have been calculated using all deployments of each method type

Gear	**Dates Deployed**	**No. Surveys**	**Total Deployed**	**Detection Probability**	**CPUE**
Trapping	October 2022 – February 2024	15	682	0.46 ± 0.25	1.11 ± 0.87
RUV	March 2023 – February 2024	9	15	0.53 ± 0.39	0.67 ± 0.75
BRUV	April 2023 – February 2024	8	14	0.43 ± 0.47	0.86 ± 0.57

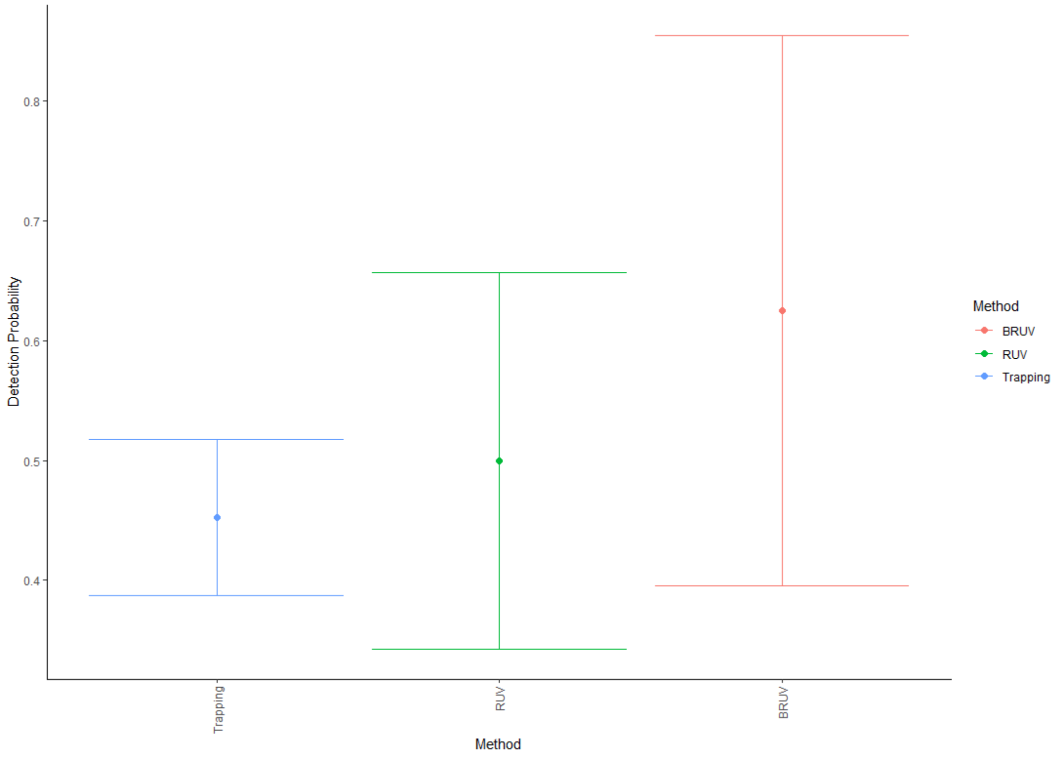


Figure 4.5. Mean monthly detection probability for Baited Remote Underwater Video, Remote Underwater Video and trapping of narrow clawed crayfish (*Pontastacus leptodactylus*). Error bars are the standard error of detection probability for each method

Table 4.2. Monthly Catch Per Unit Effort and Detection Probability and water quality parameters for each surveying method. Trapping CPUE is the total number of crayfish caught in traps, divided by the total number of traps deployed. Both RUV and BRUV CPUE is the sum of MaxN for all B/RUV deployments made during a survey (maximum number of individuals on screen throughout a deployment) divided by the total number of camera deployments for each gear

Month / Year	**Trapping Detection Probability**	**Trapping CPUE**	**BRUV Detection Probability**	**BRUV CPUE**	**RUV Detection Probability**	**RUV CPUE**	**No. Traps**	**Survey Date Average Air Temp (°C)**
Oct-22	0.50	0.96					22	16.0
Nov-22	0.31	0.43					35	6.1
Dec-22	0.20	0.29					35	6.1
Jan-23	0.00	0.00					49	7.1
Feb-23	0.61	1.08			1.00	1.00	49	4.0
Mar-23	0.80	3.10					49	8.6
Apr-23	0.27	0.31	0.00	0.00	0.00	0.00	49	14.0
May-23	0.41	0.55	0.50	2.00	1.00	1.50	49	19.0
Jun-23	0.47	1.10	0.50	1.50	1.00	1.00	49	17.0
Jul-23	0.86	2.53	1.00	1.00	0.00	0.00	49	17.0
Aug-23	0.39	1.16	0.00	0.00	0.00	0.00	49	16.0
Sep-23	0.65	1.39	0.50	0.50	0.50	1.00	49	13.0
Oct-23	0.69	1.90					49	12.0
Jan-24	0.02	0.02	0.00	0.00	0.00	0.00	49	0.7
Feb-24	0.61	1.31	1.00	1.50	1.00	1.00	49	10.0

There was no relationship between the number of crayfish caught in traps and the detection probability of either camera method ($\chi^2 = 1.550$, df = 1, p = 0.2131). The cumulative removal of crayfish from the waterbody after each trapping survey did not bias the results.

Mark-Recapture Experiment

A total of 286 crayfish were marked over a three-day trapping period (Table 4.3). Over the six-month period following the initial marking survey 384 crayfish were trapped, ten of these were recaptured marked individuals (3.5% of those marked). The current population size of narrow clawed crayfish at Boshaw Whams reservoir is estimated at 10,045 ± 5602 (95% CI) individuals in a 50,000m^2 reservoir, equating to approximately 0.20 crayfish per meter squared.

Table 4.3. Mark-recapture experiment summary of narrow clawed crayfish (*Pontastacus leptodactylus*) at Boshaw Whams reservoir. Three-day trapping and marking period highlighted in green

Date	Total Caught	Males	Females	Total Recaptured
31/03/2023	152	78	74	n/a
04/04/2023	78	43	34	0
05/04/2023	56	37	19	0
06/04/2023	39	29	10	1
29/04/2023	15	10	5	0
26/05/2023	27	23	4	0
30/06/2023	54	26	28	2
01/08/2023	124	88	36	3
01/09/2023	57	28	29	1
13/10/2023	68	43	25	3

The distinctiveness of marks on crayfish varied over time. Markings on the tails of individuals recaptured closer to the initial experiments were clear, but after a six-month period the markings on the tails of some individuals became fainter and less distinct (Sup Mat. 1) after potential healing and moulting. All captured individuals were thoroughly checked to identify any potential markings, and I am confident that no marked individuals recaptured were missed.

Population Dynamics

I deployed a total of 682 traps at Boshaw Whams reservoir during this survey. In which, 756 crayfish were captured, of these 490 were males, 266 females and 30 of which were berried. Carapace length ranged from male 22.5 - 99.0mm and females: 23.8 - 79.1mm (Figure 4.6). Traps caught significantly more males that females (χ^2= 66.370, df = 1, $p < 0.05$), and these trends are visible when comparing the monthly CPUE (Figure 4.6).

Females carrying young were recorded between February and April (Table 4.4). The range of carapace lengths for berried females was 44.8 - 79.1mm (Figure 4.6).

There was no relationship between cumulative number crayfish removed and trapping CPUE (F(1,13) = 0.2356, p = 0.6355) and air temperature did not have a relationship with CPUE (F(1,13) = 1.273, p = 0.2796).

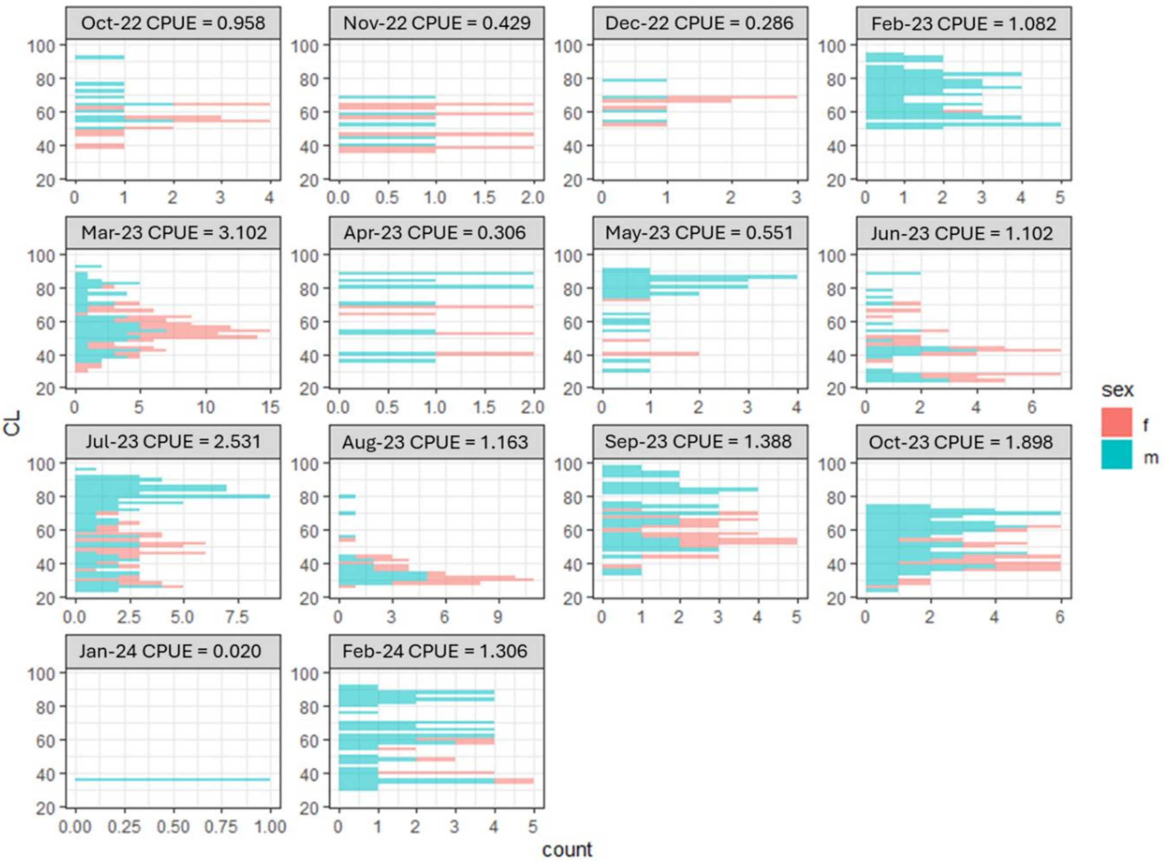


Figure 4.6. Histograms comparing the monthly sex ratios and size distributions with catch per unit effort

Table 4.4. Monthly trapping and crayfish morphometrics on the narrow clawed crayfish (*Pontastacus leptodactylus*) at Boshaw Whams Reservoir

Month / Year	**Total Caught**	**Male**	**Female**	**Berried Female**	**Mean Carapace Length ± SD**	**Mean Carapace Width ± SD**	**Mean Total Length ± SD**	**Mean Claw Length ± SD**	**Mean Weight ± SD**
Oct-22	23	10	13	0	59.3 ± 11.6	31.6 ± 7.0	115.5 ± 21.3	43.0 ± 17.7	60.2 ± 31.2
Nov-22	15	5	10	0	51.6 ± 10.6	27.0 ± 6.0	101.4 ± 21.3	32.0 ± 9.2	40.3 ± 22.6
Dec-22	10	4	6	0	64.1 ± 7.8	34.0 ± 4.7	126.4 ± 14.7	46.2 ± 13.2	67.6 ± 24.4
Jan-23	0	0	0	0					

Feb-23	53	52	1	1	69.1 ± 12.6	37.3 ± 7.7	131.8 ± 23.3	58.7 ± 20.5	89.5 ± 47.0
Mar-23	152	78	74	26	57.0 ± 13.2	30.3 ± 7.9	110.5 ± 24.0	41.5 ± 17.6	55.7 ± 35.9
Apr-23	15	10	5	2	65.6 ± 17.0	35.5 ± 9.7	128.1 ± 32.0	53.0 ± 22.9	84.1 ± 56.4
May-23	27	23	4	0	70.5 ± 17.7	38.5 ± 10.3	136.6 ± 32.9	63.1 ± 24.9	106.5 ± 59.0
Jun-23	54	26	28	0	44.2 ± 16.6	22.6 ± 9.5	87.9 ± 33.0	28.7 ± 17.1	29.2 ± 36.4
Jul-23	124	89	35	0	60.5 ± 20.6	31.9 ± 11.9	117.3 ± 39.2	47.6 ± 26.5	69.4 ± 58.2
Aug-23	57	28	29	0	36.7 ± 10.30	18.6 ± 6.3	71.3 ± 19.3	20.5 ± 7.5	13.3 ± 17.7
Sep-23	68	42	26	0	66.2 ± 16.0	35.3 ± 9.4	129.5 ± 28.5	55.5 ± 27.9	79.6 ± 57.5
Oct-23	93	68	25	0	51.5 ± 13.2	27.2 ± 7.67	100.5 ± 24.3	36.3 ± 16.5	48.6 ± 32.5
Jan-24	1	1	0	0	36.6	17.5	69.5	17.9	9.0
Feb-24	64	54	10	1	59.7 ± 18.6	31.6 ± 11.0	114.7 ± 33.7	48.4 ± 26.0	64.9 ± 54.1

Economic Costs

Trapping had the lowest initial set-up cost, but the highest survey costs and the highest 12-month survey cost (Table 4.5) compared to RUV and BRUV.

Table 4.5. Total cost for each method for monthly surveys over 12 months of surveying, where method is deployed once a month

Monitoring Method	Total Initial Cost	Monthly Cost	12 months of survey cost
Trapping	£494.41	£293.02	£4010.65
RUV	£611.00	£146.36	£2367.32
BRUV	£631.04	£146.96	£2394.56

Discussion

This study aimed to rapidly assess the population ecology of narrow clawed crayfish (*Pontastacus leptodactylus*) at Boshaw Whams reservoir. Here I provide the first year-round monitoring data of population dynamics of a narrow clawed crayfish invasion in the UK and report a large and established population at Boshaw Whams reservoir. All tested monitoring methods (traps and B/RUVs) had comparable efficacy in detecting narrow clawed crayfish in this reservoir. Narrow clawed crayfish are highly abundant in this reservoir which may have masked the differences in surveying methods. I recommend a multi-method approach to monitoring as well and suggest that biodiversity managers assess their capacity for monitoring (cost, people time, accessibility) and specific information needed to inform the best approach to designing and monitoring campaigns for narrow clawed crayfish.

Bait Comparison

I predicted that due to wet food solubility that it would have a higher efficacy in attracting crayfish (Willman et al. 1994) but in this instance there was no difference in CPUE between bait type. There is a high degree of variation in bait efficiency with some reporting no difference (Somers and Stechey 1986), whilst other studies have concluded both that wet bait achieved higher CPUE (Beecher and Romaire, 2010) or that dry bait achieved a higher CPUE (Rach and Bills, 1987). I opted to use wet cat food for the remainder of the surveys due to the ease of use in distributing into mesh pouched to place in the traps. However, it is likely that the amino acid content of the bait, scent plume diffusion and physical properties of the water body all mediate efficacy (Westerberg & Westerberg, 2011).

Gear Comparison

All trialled methods had comparable detection probability therefore in relatively clear, still-waters I would recommend any of the methods for detecting presence/absence of invasive crayfish populations. To assess relative abundance, I recommend a multi-method approach as traditional trapping as the MaxN value from the B/RUV setups provides a conservative estimate of abundance whereas trapping can provide more nuanced information regarding population demographics. My results are similar to other attempts to compare novel monitoring methods to traditional approaches in freshwaters in that there is not a one size fits all monitoring method, but rather, specific methods may be better suited dependending on the question. For example, Castañeda et al. (2020) found that underwater cameras were more time consuming than snorkel surveys but that cameras were better for detecting rare freshwater fish species. Thus, choice of monitoring methods depends on what information the surveyor is aiming to achieve.

Trapping can be a time-consuming method which may or may not require entering waterbodies. Furthermore, it requires a minimum of two days of staff time to conduct a single survey, which can limit the total number of surveys that can be conducted in a fixed timeframe. Cameras therefore provide an alternative survey method which, although initially costly, can reduce the amount of labour needed to complete the surveys and allow video data to be assessed at any time in the future, thus removing immediate time constraints – especially in remote locations (Ebner et al. 2014; Castañeda et al. 2020b; Broom et al. 2023). Video data still requires human time to assess but developments in artificial intelligence image recognition may remove this barrier (Siri et al. 2024).

Lighting and water turbidity can limit the effectiveness of RUV as this hampers the capacity to obtain usable video (Ebner et al. 2014; King et al. 2018), this was not an issue for my surveys at Boshaw Whams as the nature of the reservoir meant that underwater visibility averaged >1m for all surveys. Crayfish species are largely nocturnal thus the need for overnight sampling has been a barrier to developing rapid surveying methods, besides eDNA approaches, due to light level limitations (Fanjul-Moles & Prieto-Sagredo, 2003; Mallet & Pelletier, 2014). Narrow clawed crayfish show high feeding intensity during both day and night (Skurdal & Taugbøl, 2002) and were regularly observed on the cameras. Studies must account for these limitations when designing and deploying RUVs for crayfish monitoring to avoid wasted effort and costs.

Mark-Recapture Experiment

The mark-recapture experiment indicated that there were over ten-thousand narrow clawed crayfish individuals in the reservoir (one per 5 metres squared (0.20 crayfish/m^2)). Considering only 10 individuals were recaptured, representing 3.5% of all trapped individuals after the mark-recapture event, this data is likely unreliable but offers an indication of the extremely high density of crayfish. This is reinforced by the large confidence intervals post Chapman correction implementation. There is limited information about the densities of narrow clawed crayfish found in waterbodies in their native range. However, in comparison to other invasive crayfish populations in lotic waterbodies (signal crayfish; *Pacifastacus leniusculus*) in the UK, they are much lower (3-20/m^2 (Guan & Wiles, 1997), 20/m^2 (Bubb et al. 2004), 21-110/m^2 (Chadwick et al. 2021).

Trapping and marking can have a short-term negative effect on crayfish recapture chances (Nowicki et al. 2008), with marked individuals potentially having a negative association with traps and avoiding them. Individuals found over six months after the marking event only had faint markings (Suppl. Fig. 1). This is supported by other studies which found that markings from mark-recapture events tend to last two to three moult cycles (Nowicki et al. 2008).

Population Dynamics

The narrow clawed crayfish population in Boshaw Whams is established, detectable throughout the year and reproducing at small sizes. The number of male narrow clawed crayfish caught was 1.84 times higher than females. This sex bias may be a result of the aggressive behaviour of larger males resulting in females and smaller males avoiding the traps (Hilber et al. 2020; Hein et al. 2007; Momot & Gowing, 1977; Holdich, 2002b). The mean size of individuals remained fairly consistent throughout the survey, with slight declines during June and August 2023 (Table 4.4). During these months size dynamics shifted towards higher volumes of small individuals (Figure 4.6), likely as the previous year's young of year became big enough to actively search for food with reduced risk of cannibalisation and easy predation from smaller fish. The mean total length of crayfish found each month was consistently around 12cm, which is the maximum size of native white-clawed crayfish (Chapter 1). This shows that this population of invasive narrow clawed crayfish are consistently larger than native species. The size difference between the two species would likely to result in the larger narrow clawed crayfish winning any competitions with smaller white-clawed crayfish, if their populations were to overlap. It is hoped that the morphometric data collected alongside this study can be used in future work and has been submitted to the CRAFT network that is compiling individual data on a site-by-site basis for as many species of crayfish as possible. Except for January 2023, crayfish were caught each month throughout the year with an increase in CPUE between March and May (2023), which was also when the first observations of berried females occurred. In their native range, berried females can be found between December and May (Cìlbìz, 2020) and reproduction generally occurs in cold water between 7-12 °C (Farhadi and Harlioglu, 2018). In my study most berried females were caught during these months and a small number of berried individuals were observed in subsequent months. In their native range females reach sexual maturity at 64.3 mm total length but spawning only occurred for crayfish with a total length ≥ 82 mm (Berber & Mazlum, 2009). This is smaller than the total length of the smallest berried individual found in the present study (carapace length: 44.8 mm, total length of 90.8 mm), suggesting that reproduction happens at smaller sizes in the invasive range.

My monitoring campaign did not appear to have had an impact on the population size at Boshaw Whams reservoir. Despite removing over 700 crayfish there was no negative trend in the monthly CPUE which indicates that removal of crayfish did not bias my results. This is due to the relatively low monthly trapping effort (Peay, 2001; Green et al. 2018).

Economic Costs

The largest cost that goes into crayfish sampling is staff-time (Peay, 2004). I recommend that surveyors first determine their data needs such as whether rapid detection is the aim or whether population level information is needed at each stage of assessment. Beyond this, accessibility issues, economic and time constraints can also influence choice of method. In this scenario, i.e. a high-density invasion in clear water, all three methods were able to detect crayfish, however, more granular information on breeding period, length frequency, sex ratio were only available with trapping. Thus, a staggered approach to monitoring where cameras are first deployed and this data used to inform trapping may be the most effective.

Conclusion

Boshaw Whams reservoir should be considered in the ‘Containment’ or ‘Asset Protection’ stage of the General Invasive Curve, which means there are large populations within the waterbody, but I cannot confirm that they have colonised the entire area of it. The population of narrow clawed crayfish at Boshaw Whams reservoir is estimated to be 10,045, and breeding activity was seen between February and April. Potentially this could mean that the opportunity to eradicate this population has passed and eradication is no longer viable, however the feasibility of eradication would need to be assessed on a site and catchment specific scale, beyond the scope of this study. Narrow clawed crayfish ranges are expected to expand and shift with predicted climate change therefore mitigation of further spread is required urgently (Hodson et al. 2024).

Chapter 5

A multi-method approach to assessing barrier effectiveness in preventing the spread of invasive signal crayfish (*Pacifastacus leniusculus*)

Published Reference

Harwood, M., Bray, A.W., Woolfenden, K.A., Stebbing, P.D., Dunn, A.M., South, J. (in review) A multi-method approach to assessing barrier effectiveness in preventing the spread of invasive Signal Crayfish (*Pacifastacus leniusculus*). *River Research and Applications*

Abstract

The United Kingdom government is seeking to restore salmon stocks throughout the country. One of the objectives of this initiative is to restore the free-flowing state of UK rivers by removing artificial structures, referred to as barriers. These barriers have been seen to act as a mitigation measure for the spread of invasive species and it is feared that removal of them could aid in the spread of invasive signal crayfish (*Pacifastacus leniusculus*). I used a multi-method approach to test for crayfish presence and found there was no difference between the detection probability of trapping, remote underwater video (RUV) and baited RUV (BRUV), however I found that trapping had a significantly higher detection probability than eDNA. Distance from barriers played a key role in determining crayfish presence, with populations of crayfish more likely to be found directly below upstream barriers. Temperature also influences the presence of crayfish with higher temperatures more likely to detect them. ORP influenced crayfish abundance, with higher densities of crayfish at locations with lower ORP. Over half (50.9%) of the barriers that were within my study were found have invasive crayfish both above and below them (breached), and a further 17% had crayfish directly below them and were at risk of becoming breached. Both tributaries that had reported presence of native white-clawed crayfish (*Austropotamobius pallipes*) had several barriers between them and the nearest record of signal crayfish, it is recommended that these barriers remain in place and are potentially reinforced, while those that have been identified as breached be the focus of barrier alleviation.

Introduction

There are many stressors that are negatively and simultaneously impacting global river systems; including pollution, climate change, invasive species and habitat fragmentation (Reid et al. 2019). The demands of rapid urbanisation have resulted in rivers being harnessed for resources including electricity, water supply and flood control (Jumani et al. 2020). To do so requires construction of artificial structures within the watercourse, however, some of these structures (termed *barriers* in this paper) fragment connectivity, with only 37% of rivers over 1000 km long remaining free flowing (Stoffers et al. 2024). The European Union's "Nature Restoration Law" looks to restore 25,000 kilometres of free-flowing rivers by 2030 (Darre et al. 2025). In association with this the UK government has set a goal of restoring salmonid stocks throughout England and one of the main points in the Environment Agency's five-point approach is removing barriers to migration (Angling Trust, 2016). Habitat loss through fragmentation is a key factor in biodiversity decline and often poses other threats, including altering the habitat suitability and isolating populations, leading to less genetic diversity (Kuipers et al. 2021).

The loss of free-flowing rivers as a result of barriers has been a major driver in the global decline in migratory fish populations which has seen an 80% decline in the past 50 years (Deinet et al. 2024). Salmonids are migratory and rely on habitat connectivity to travel long distances for spawning, thus unhindered migratory passage is necessary to maintain healthy populations of ecologically and economically important migratory fish (Lehnert et al. 2019; Cooper et al. 2021). Migratory fish species including Atlantic salmon (*Salmo salar*), brown trout (*Salmo trutta*), northern pike (*Esox lucius*) and alewife (*Alosa pseudoharengus*) have been estimated to have a maximum jumping heights of less than two meters (Meixler et al. 2009). When their historical migratory routes are fragmented by barriers larger than these sizes it can have devastating impacts on the species, therefore barrier removal is a critical intervention to improve river biodiversity (Clark et al. 2020; Dolan et al. 2025).

Despite the negative aspects of artificial barriers, they may also function to reduce the spread of harmful invasive aquatic non-native species (Danilović et al. 2025). Riverine barriers pose a clear obstacle to not only fish species but also crayfish. Invasive non-native crayfish contributed to US£15.3 million worth of costs to the United Kingdom between 2000 and 2020 (Kouba et al. 2022). Signal crayfish (*Pacifastacus leniusculus*) are expert invaders due to their ability to integrate into a food web at many levels (Gherardi et al. 2011) and there is fear that barrier removal will aid their spread by reducing fragmentation and providing crayfish with an unobstructed route to invade further upstream. Signal crayfish are habitat engineers that can have significant negative impact on the ecosystem through riverbank burrowing which causes bank degradation, erosion, and increased turbidity (Gallardo et al. 2016). In the UK and Europe, signal crayfish also pose a risk to endangered native white-clawed crayfish (*Austropotamobius pallipes*) populations through competition and disease transmission (Vaeßen & Hollert, 2015). While there is limited information regarding crayfish invasions and barriers, it is considered that physical characteristics such as smooth, vertical walls with an overhanging lip are the most effective barriers at reducing invasive crayfish movements (Krieg & Zenker, 2020). Thus, these barriers can reduce the pressure brought by invasion establishment, potentially allowing for coexistence of invasive and native species in fragmented rivers (Morissette et al. 2023). Therein, the removal of barriers introduces a potential conflict of conserving one species at the expense of another.

If a compromise is to be made, then it is important to establish areas that have been invaded, and those that are at risk of invasion. When an invasion occurs, assessing the stage and extent of it must be prioritised to ensure that appropriate decisions are made to mitigate impacts (Harris et al. 2018), it is also important to understand how crayfish respond to the barrier landscape, and where they are in the network. Establishing the magnitude of population spread, and potential spread range, are vital and provide critical influence on the most effective management strategy (Larson et al. 2022). Studies into instream crayfish distribution are novel and, through understanding the extent of an invasion, appropriate monitoring and management plans can be implemented. Using the River Calder, West Yorkshire, UK as a case study for a barrier removal plan, I have used a multi-gear approach to survey crayfish and used my data to predict barriers that have been breached by invasive signal crayfish, and therefore advise future management and frameworks, while also identifying key barriers that could potentially act as a preventative measure in the spread of invasive species. My overarching aims were to: 1) characterise environmental drivers of crayfish distribution patterns within a fragmented river system, 2) compare the efficiency of four different surveying techniques (Remote Underwater Video, Baited Remote Underwater Video, Trapping, eDNA) to detect crayfish. This approach will allow me to assess the River Calder for barrier removal options in the future, and guide other removal efforts in invasive crayfish appraisal and environmental impact assessment and prioritisation.

Methods

Study site

The River Calder (53°44′N; 002°00′W) (Figure 5.1) is a 72-km-long, Strahler order 6 tributary of the River Aire, with the point of confluence in Castleford. The source of the river is in Heald Moor. Historically the river has been heavily polluted through industrial developments, including textile and chemical works (Hudson et al. 1988). The river is larger than twenty metres at its widest point and progresses through deciduous woodland. The main stem of the River Calder is fragmented by 39 weirs (14 of which have either been eased or have collapsed), with over 500 weirs across the whole river catchment's tributaries (Calder Rivers Trust, pers. comm.). Many of these weirs are remnants of textile millworks. Alongside and within navigable reaches of the River Calder run the Rochdale Canal, Calder & Hebble Navigation, and Aire & Calder Navigation canals, which connect to the Huddersfield Broad & Narrow, and the River Aire catchment. One of the tributaries of the River Calder with a documented population of white-clawed crayfish is Luddenden Brook, a typical steep bedrock and boulder dominated Pennine stream which hosts at least 14 weirs, and flows through pasture and dense woodland.

Invasive signal crayfish were first reported in the lower regions of the River Calder (Calder Rivers Trust, pers. comm.) and it has been assumed that they have been invading from lowland to upstream. My survey sites were chosen based on previous knowledge of the area, choosing sites along the main river, as well as sites along three tributaries of the river, Cragg Brook, Hebble Brook and Luddenden Brook. Site 1 at Hebble Brook was chosen as this was the first site with a positive record of invasive signal crayfish on the River Calder (Calder Rivers Trust, pers. comm.). A tributary with a known population of white-clawed crayfish present is Luddenden Brook, a third order tributary of the River Calder. Barriers, including waterfalls and dams, have previously been seen to limit crayfish movement (Stebbing et al. 2014). The in-channel barriers along the River Calder and Luddenden Brook may be inhibiting the spread of invasive crayfish and protecting native populations. The international push for removal of artificial barriers and restoration of free-flowing rivers should consider negative ecological impacts, such as the potential spread of invasive crayfish.

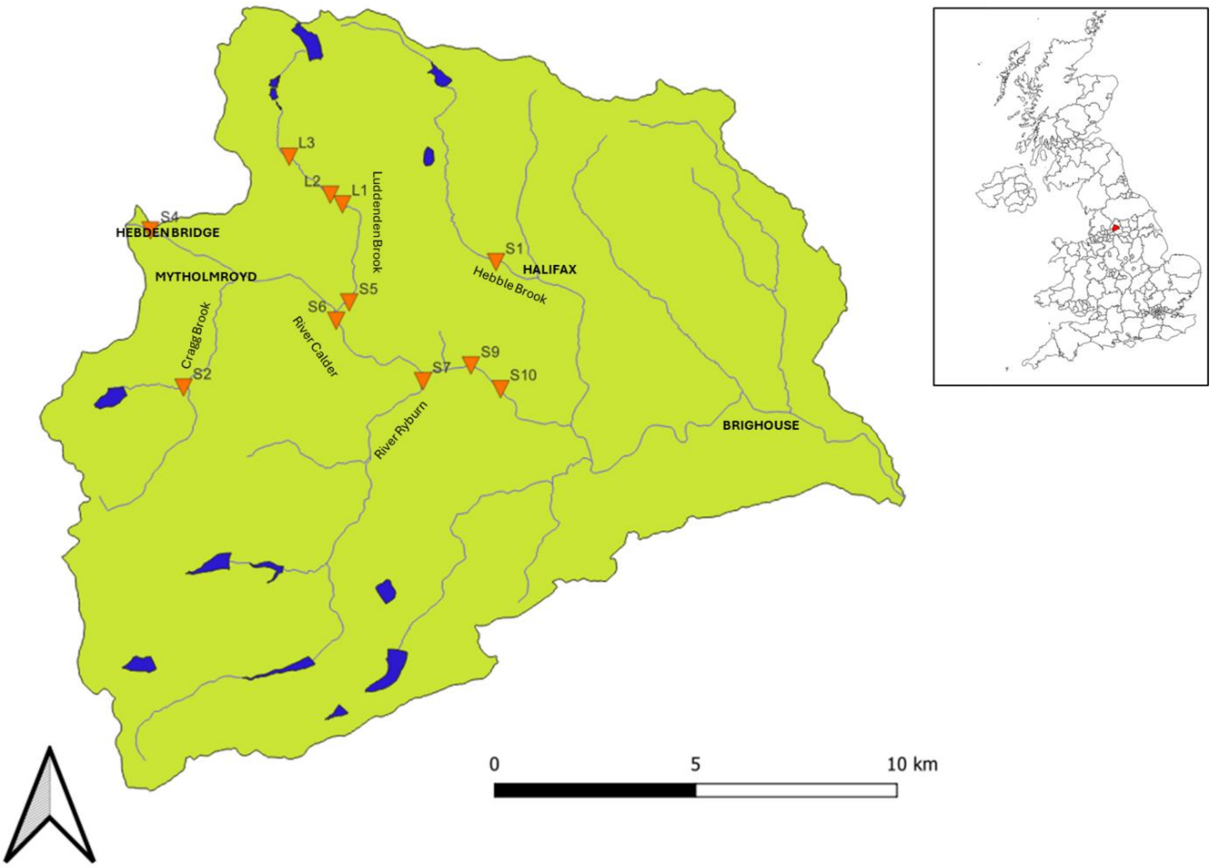


Figure 5.1. A map showing survey locations along the River Calder, main towns and tributary names

Crayfish distribution and abundance

All survey methods were deployed in each of the locations at each site. First, eDNA samples were collected to avoid contamination from traps and bait, Remote Underwater Video (RUV) deployments were then deployed for one hour, followed by Baited Remote Underwater Video (BRUV) deployments for one hour. Finally, traps were deployed at sites and left overnight. Surveys were completed in two seasons: autumn-winter 2023 and summer 2024 (Table 5.1).

Table 5.1. Overview of sampling effort for each method across sites, for trapping this is the total number of traps deployed per survey, B/RUV is the total number of 60-minute camera deployments and eDNA is the number of water samples taken and filtered at each site

Site	**Trapping**	**RUV**	**BRUV**	**eDNA**
Autumn-Winter 2023				
Site 1	15	2	2	4
Site 2	5	2	2	1

Site 4	15	2	2	4
Site 5	15	2	2	4
Site 6	9	2	2	
Site 7	9	1	1	
Site 9	9	2	2	4
Site 10	15	1	1	
Lud 1			2	2
Lud 2			2	2
Lud 3			2	2
Summer 2024				
Site 1	12	1	1	3
Site 2	9	1	1	3
Site 4	12	1	1	3
Site 5	12	1	1	3
Site 6	12	1	1	3
Site 9	12	1	1	3
Lud 1			1	
Lud 2			1	3

Trapping

Collapsible cylindrical mesh crayfish traps (570mm x 290mm, 25mm mesh size), permit reference EP/EW065-X-049/26415/01 were used at each site. The number of traps deployed at each site varied, depending on site size and accessibility (Table 5.1). Traps were baited with wet cat food and left overnight for 18 hours. All traps were collected the following morning. Any crayfish found in traps were identified to species level, taking measurements of their carapace length (mm) and sex.

Camera surveys

For each survey the RUV was deployed for one hour before being retrieved. Once the RUV was retrieved the BRUV was baited using wet cat food and then deployed for an hour in the same location as the RUV had been. Each GoPro HERO10 Black (GoPro, Inc., USA) camera was set to record at 30 frames per second with 1080p resolution, following standards recommended by Harwood et al. (in review, Chapter 2). Footage was manually reviewed by the same observer noting the total number of times crayfish entered the camera's field of view, the time taken for first crayfish observation to be made and the MaxN, which is the maximum number of crayfish on screen at any time during the deployment.

eDNA surveys

eDNA samples were taken at eight sites in the Autumn-Winter survey and seven sites in the summer survey (Table 5.1). For each eDNA sample 100ml water samples were collected from twenty evenly distributed points throughout the site, all twenty samples were placed into a whirl-pak and mixed thoroughly. 1.2 litres of the mixed sample was then filtered using standard eDNA kits provided by FERA Science Ltd (fera.co.uk/). A field blank sample was also collected at each site. All samples were sent to FERA for analysis where the nucleic acids were extracted from the buffer using CTAB/chloroform method and a real-time PCR assay was used to detect the presence of DNA for specific target species, white-clawed crayfish and signal crayfish.

Gear comparison

Gear effectiveness and comparisons were conducted by comparing Catch Per Unit Effort (CPUE) for each survey technique. For trapping surveys CPUE was calculated as the total number of crayfish caught divided by the total number of traps deployed for each survey (number of crayfish per trap) (Equation 3). For both BRUV and RUV surveys the CPUE was calculated as the MaxN of crayfish for each survey. For eDNA the values were taken as either present or absent. The detection probability of each of these methods was also calculated, to account for the different lengths of deployment, by dividing the number of instances the gear observed crayfish by the total number of gear deployments for each survey (Equation 4).

$$CPUE = \frac{Total\ crayfish\ caught}{Total\ number\ of\ deployed\ traps} \quad \text{eqn 3}$$

$$Detection\ Probability = \frac{Deployments\ observing\ crayfish}{Total\ Deployments} \quad \text{eqn 4}$$

ANOVA was used to compare the detection probability (PC) for each of the methods. Linear regressions were also conducted between the trapping CPUE and both the BRUV and RUV CPUE to identify whether B/RUV deployments can be equivalent to trapping.

Determinants of crayfish abundance

To establish if habitat and water quality variables influence the presence/absence of crayfish and the abundance of populations throughout the River Calder temperature (˚C), Total Dissolved Solids (TDS), Oxidation-Reduction Potential (ORP) and Practical Salinity Unit (PSU) readings were taken using a Hanna HI9819X Multiparameter meter at all sites during the deployment of the equipment. If specific environmental factors and water quality scenarios are more likely to indicate the presence of invasive crayfish, then the results from this section can be used to identify priority sites that can be surveyed first when trying to establish the extent of an invasion. It is hypothesised that crayfish are less active during winter months, temperature was measured to identify if crayfish are therefore harder to observe in colder temperatures. Invasive non-native signal crayfish are ecosystem engineers that produce high levels of sedimentation (Johnson et al. 2011). I hypothesise that sites with denser populations of signal crayfish will have higher values for TDS and lower values for ORP as a result of sedimentation reducing light levels in the waterbody and causing reduced vegetation leading to lower oxygen levels at the site. Salinisation of freshwater ecosystems is a growing problem that can have a negative impact on the health of freshwater species (Luo et al. 2024). It is hypothesised that sites with higher salinity levels will be less likely to contain vulnerable native white-clawed crayfish, and the hardier invasive non-native signal crayfish may have a higher likelihood of being present. Environmental factors extracted for each site to establish what conditions influenced crayfish abundances and presence/absence. Void Filled Digital Elevation Model for the UK was taken from the HydroSHEDS online database (Lehner et al. 2008). I then used the Sample Raster Values feature in QGIS to extract elevation data for each site from this data. Slope was calculated by taking the elevation values 100 metres upstream and 100 metres downstream and dividing the difference by 200. For each survey point the distance to both the nearest upstream and downstream barrier were calculated by using the in-built measure function in QGIS.

A scaled PCA analysis was conducted to establish if the sites were unique in environmental factors. Pearson's correlation was used to establish correlations between variables. Variables with significant correlations ($p < 0.05$) were removed from the PCA. Pearson's correlation showed a significant correlation between elevation and slope (Figure 5.2) and that PSU and TDS both were significantly correlated between multiple variables. The decision was made to drop PSU, TDS and elevation as slope and distance to barriers were key variables to retain to establish what could potentially influence invasion capabilities.

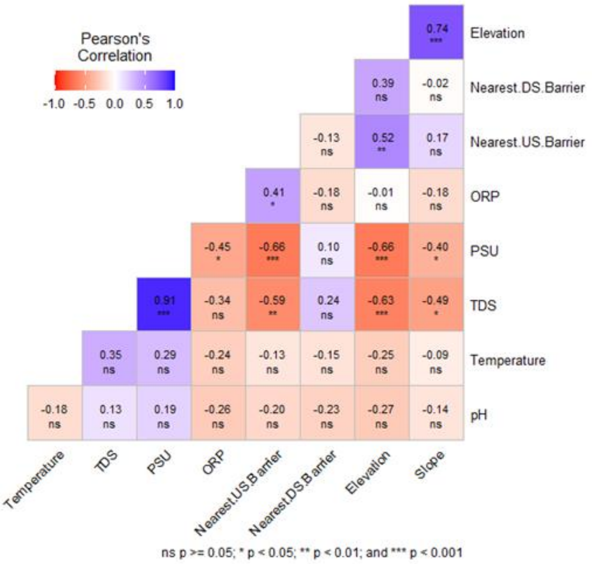


Figure 5.2. Pearson’s Correlation results between unique factors at each site

Initially, all data was scaled and centred and an analysis of variance model was run on it. Then Q-Q plots of the theoretical quantiles were visually inspected for residual deviance. After a visual inspection the decision was then made to split variables into environmental factors (temperature, pH, ORP, slope) and barriers (distance to nearest downstream barrier, distance to nearest upstream barrier). New Q-Q plots were visually inspected for residual deviance, barrier variables for trapping were heavily skewed due to almost all sites detecting crayfish presence leading to almost all data being singular values (i.e. all 1). For this case I dropped the barrier analysis for the trap data.

Binomial GLMs were used to determine if environmental factors or barriers significantly predicted crayfish presence. A type 2 ANOVA was then used to simplify these binomial GLMs to identify if there were any factors that had a significantly predicted crayfish detection reporting χ^2 for effect size. Quasi-Poisson GLMs were used to determine what factors influenced signal crayfish abundances (i.e. CPUE) throughout sites.

Barrier assessment

A map of all the barriers throughout the Calder catchment was provided by the Calder Rivers Trust. These locations were then mapped onto the river in QGIS alongside survey points and locations that had any crayfish, either signal crayfish (*P. leniusculus*) or white-clawed crayfish (*A. pallipes*) collected from both this survey and historical citizen science records provided by the Calder Rivers Trust’s River Health Partnership data dashboard (https://lookerstudio.google.com/reporting/1RXBvLxMavfiLq06PdMqjX5QKkhP1G7QW/page/qwSp?s=nD9rQathFGM). Only citizen science crayfish records that had expert confirmation and photographic evidence were used in this study. Although my surveys did not detect any crayfish species in Luddenden Brook, I have used records of white-clawed crayfish presence from the Environment Agency in 2022 to assume their presence there. Each barrier was then classified to determine its risk of breaching. A barrier was considered breached if there was a signal crayfish record upstream of it, at risk if signal crayfish are directly below it, or un-breached if there are no records of signal crayfish either above or below it.

The percentage of barriers breached was calculated by dividing the number of breached barriers by the total number of barriers within the survey area.

Results

A total of twenty-eight site surveys were conducted over three separate survey periods, Autumn 2023, Winter 2023/24 and Summer 2024. Environmental parameters at each sampling instance were varied, with winter and autumn surveys being similar and summer surveys distributed within these (Figure 5.3). The first principal component explained 40.4% (eigen value = 3.6397) of the variation in survey sites and was most strongly related to PSU. The second principal component explained 18.2% (eigen value = 1.6402) of the variation in survey sites and was most strongly related to the distance to the nearest downstream barrier (Figure 5.3).

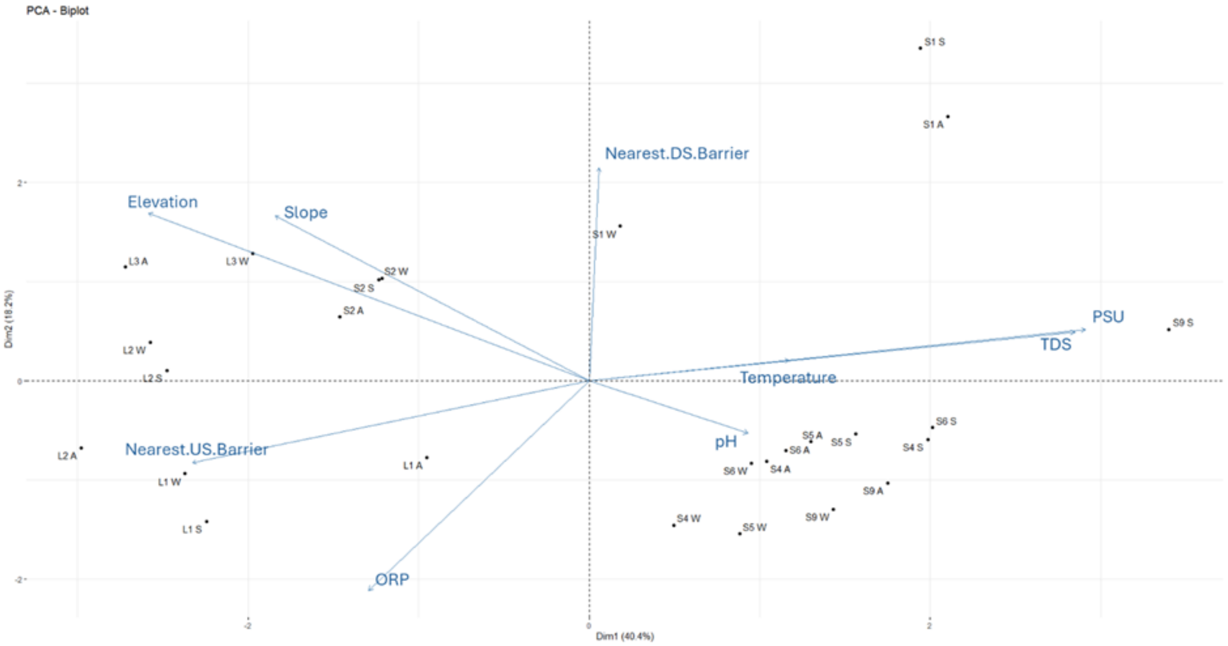
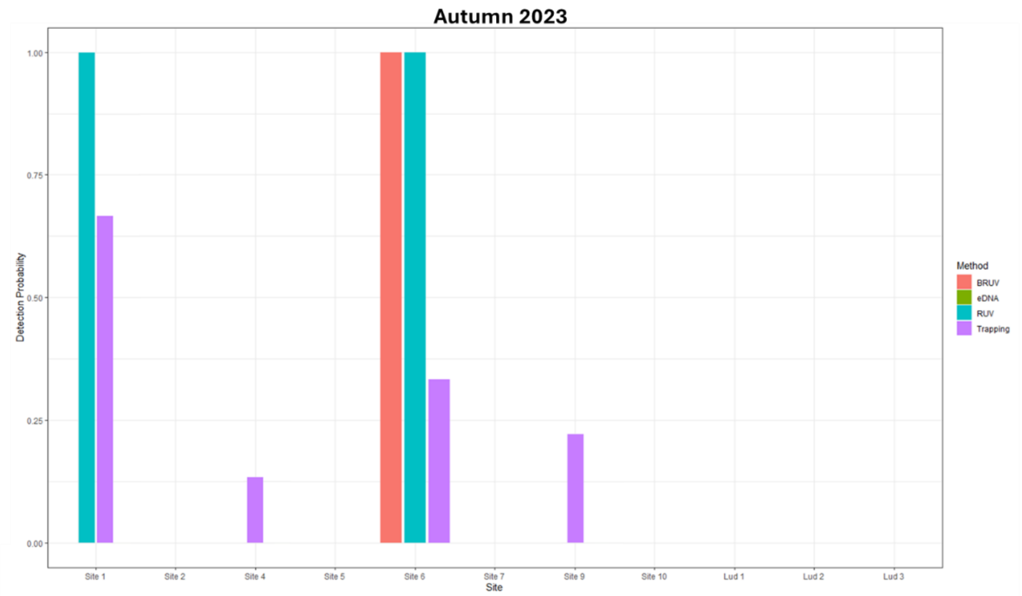


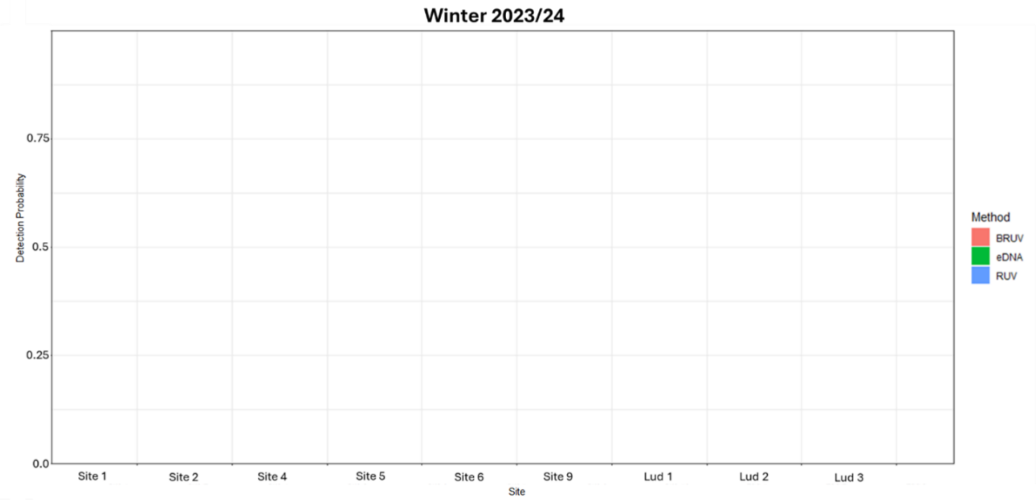
Figure 5.3. Biplot of Principal Component Analysis for factors present at each site. Each point represents a unique site and season. Survey points are represented by black points, the first letter of each label represents the site location, L are the sites on Luddenden Brook and S are the sites on the main River Calder. The last letter of the name represents the survey

season, W for winter, A for autumn and S for summer. ORP is the Oxidation Reduction Potential, TDS Total Dissolved Solids and PSU Photosynthetic Unit

Gear comparison

64% of trapping surveys, 18% of RUV surveys, 11% of BRUV surveys and zero eDNA surveys detected the presence of crayfish. There was no difference between the detection probability of trapping, RUV and BRUV ($\chi^2 = 0.001$, df = 1, p = 0.976; Figure 5.4). However, trapping had a significantly higher detection probability than eDNA ($\chi^2 = 8.15$, df = 1, $p < 0.01$; Figure 5.4).


Autumn 2023
Detection Probability
1.00
0.75
0.50
0.25
0.00
Site 1
Site 2
Site 4
Site 5
Site 6
Site 7
Site 9
Site 10
Lud 1
Lud 2
Lud 3
Site
Method
BRUV
eDNA
RUV
Trapping


Winter 2023/24
Detection Probability
0.75
0.5
0.25
0.0
Site 1
Site 2
Site 4
Site 5
Site 6
Site 9
Lud 1
Lud 2
Lud 3
Site
Method
BRUV
eDNA
RUV

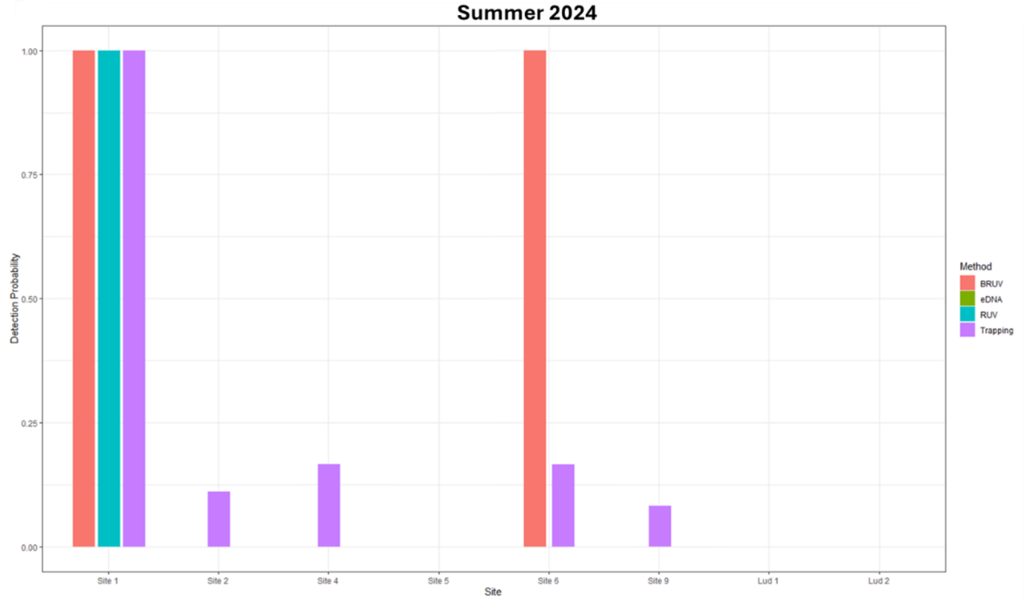

Summer 2024
Detection Probability
1.00
0.75
0.50
0.25
0.00
Site 1
Site 2
Site 4
Site 5
Site 6
Site 9
Lud 1
Lud 2
Site
Method
BRUV
eDNA
RUV
Trapping

Figure 5.4. Detection Probability at each site for each unique method. BRUV, eDNA, RUV and Trapping used at each site across three different survey seasons. A) Autumn 2023, B) Winter 2023/24, C) Summer 2024

Determinants of crayfish abundance

Determinants of crayfish abundance through trapping

ORP significantly predicted the CPUE of trapping (Table 5.2), with lower ORP values having a higher CPUE (Table 5.2; Figure 5.5). Distance to the nearest downstream barrier also significantly predicted trapping CPUE, with sites further away from a downstream barrier having higher CPUE (Table 5.2; Figure 5.5).

Table 5.2. Type 2 ANOVA for quassipoisson GLM assessing the effect of environmental factors and barriers on determining crayfish abundance using trapping

Variable	χ^2	**df**	**p**
Temperature	0.85	1	0.36
pH	0.12	1	0.73
ORP	6.55	1	0.01
Slope	0.04	1	0.85
Nearest DS Barrier	64.11	1	< 0.05
Nearest US Barrier	0.65	1	0.42

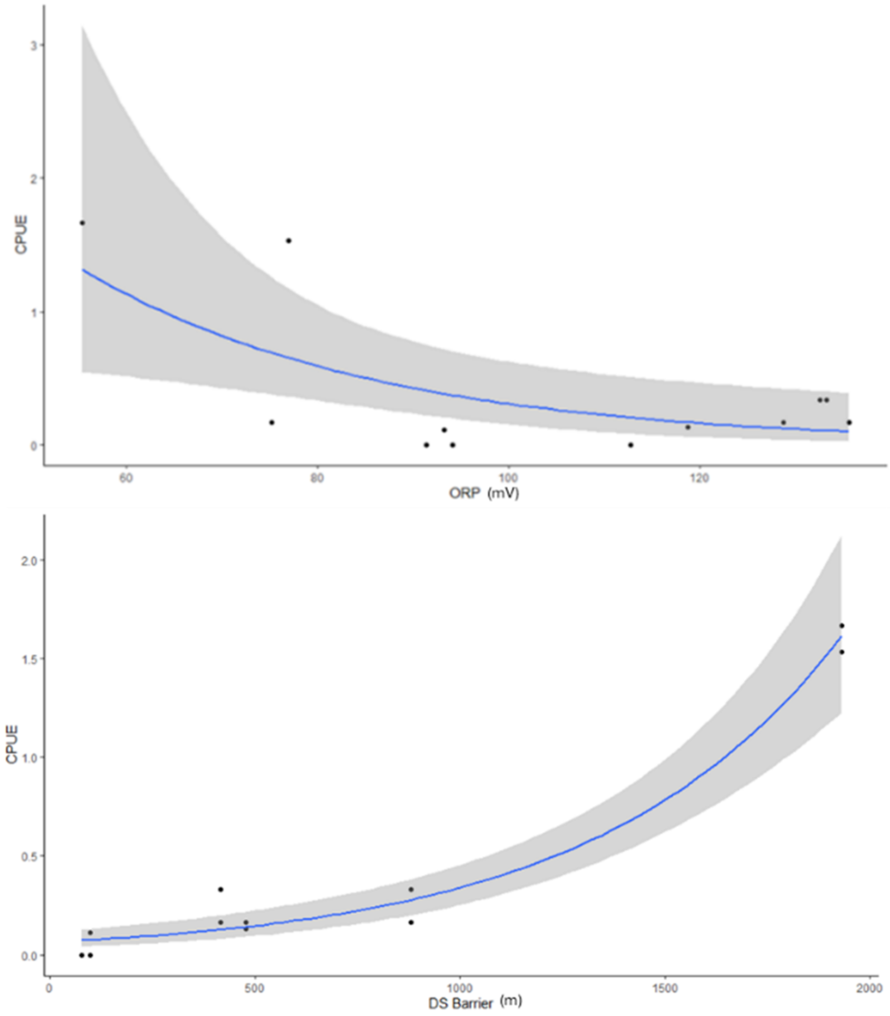


Figure 5.5. Generalised Linear Model depicting how A) ORP and B) the distance from a downstream barrier influences the Catch Per Unit Effort (CPUE) of trapping surveys

Determinants of crayfish abundance through BRUV

The distance to the nearest upstream barrier had a significant impact on the BRUV detecting presence/absence (χ^2= 6.4502, df = 1, $p < 0.05$; Figure 5.6; Table 5.3), with the BRUV being more likely to detect presence/absence closer to an upstream barrier.

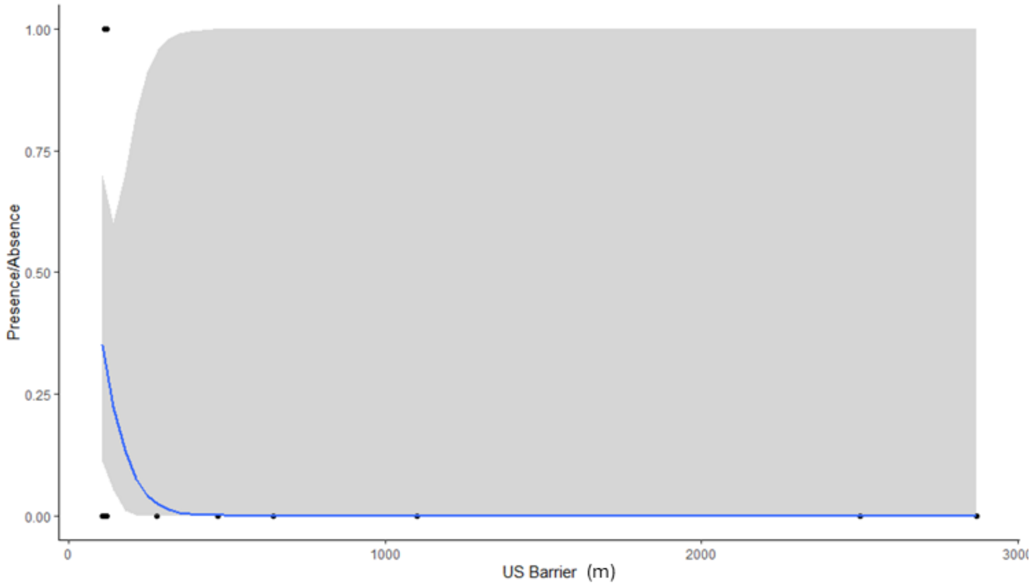


Figure 5.6. Binomial Generalised Linear Model depicting how the distance from an upstream barrier influences the chance of a BRUV detecting the presence/absence of crayfish

ORP had a significant influence in determining BRUV CPUE with lower ORP values achieving higher CPUE (Table 5.3; Figure 7.7). Distance to the nearest downstream barrier also had a significant impact on BRUV CPUE, with higher CPUE for values further away from barriers (Table 5.3; Figure 5.7).

Table 5.3. Type 2 ANOVA for quassipoisson GLM assessing the effect of environmental factors (Model 1) and barriers (Model 2) on determining crayfish abundance using BRUV

Variable	χ^2	**df**	**p**
Model 1			
Temperature	1.16	1	0.28
pH	3.07	1	0.08
ORP	8.82	1	< 0.05
Slope	0.59	1	0.44
Model 2			
Nearest DS Barrier	4.51	1	< 0.05
Nearest US Barrier	12.39	1	< 0.05

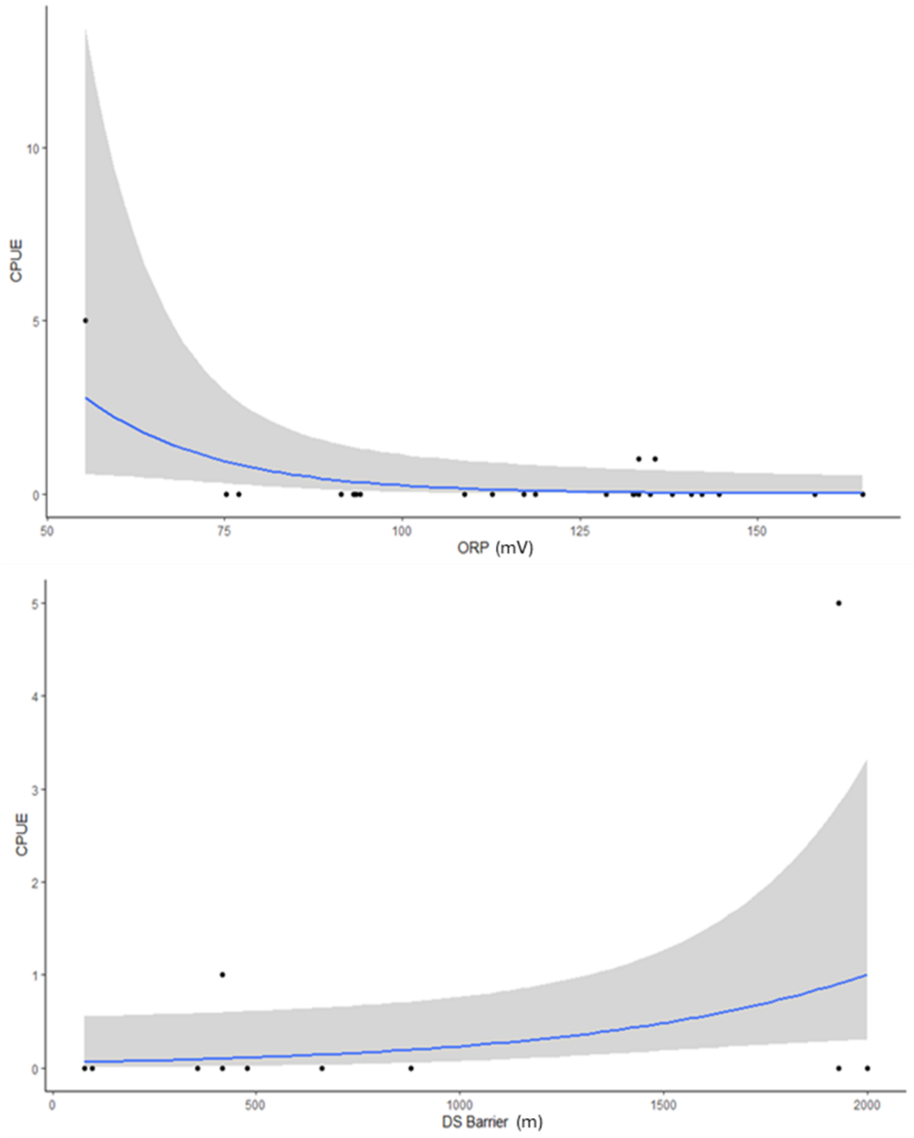


Figure 5.7. Generalised Linear Model depicting how A) ORP and B) the distance from a downstream barrier influences the Catch Per Unit Effort of the BRUV

Determinants of crayfish abundance combined methods

Temperature had a significant role in determining the presence/absence of crayfish (χ^2= 12.0590, df = 1, $p < 0.05$; Figure 5.8) with colder temperatures having a lower probability of observing a crayfish. Nearest upstream barrier also had a significant role in determining crayfish presence/absence (χ^2= 8.5239, df = 1, $p < 0.05$; Figure 5.8) with crayfish more likely to be present nearer to an upstream barrier.

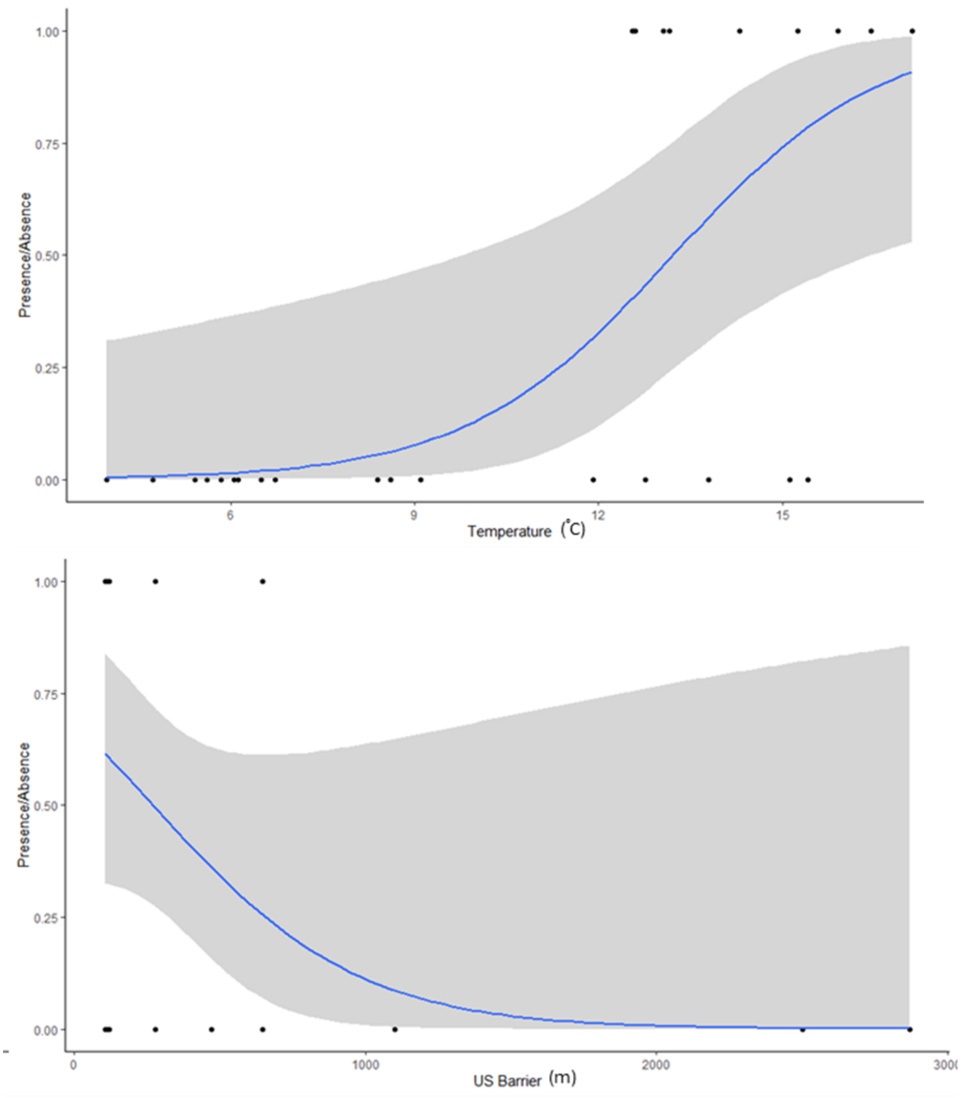


Figure 5.8. Binomial Generalised Linear Model depicting how A) temperature and B) the distance from an upstream barrier influences the chance of detecting the presence/absence of crayfish

Three variables had a significant impact on the CPUE of all methods combined. ORP (χ^2= 11.3450, df = 1, $p < 0.05$; Figure 5.9) where CPUE was higher at lower ORP values. Distance to nearest downstream barrier (χ^2= 10.561, df = 1, $p < 0.05$; Figure 5.9), where CPUE was higher the further away from a downstream barrier the survey was conducted. The opposite to this was true for distance to the nearest upstream barrier (χ^2= 15.985, df = 1, $p < 0.05$; Figure 5.9) with sites closer to an upstream barrier having higher CPUE.

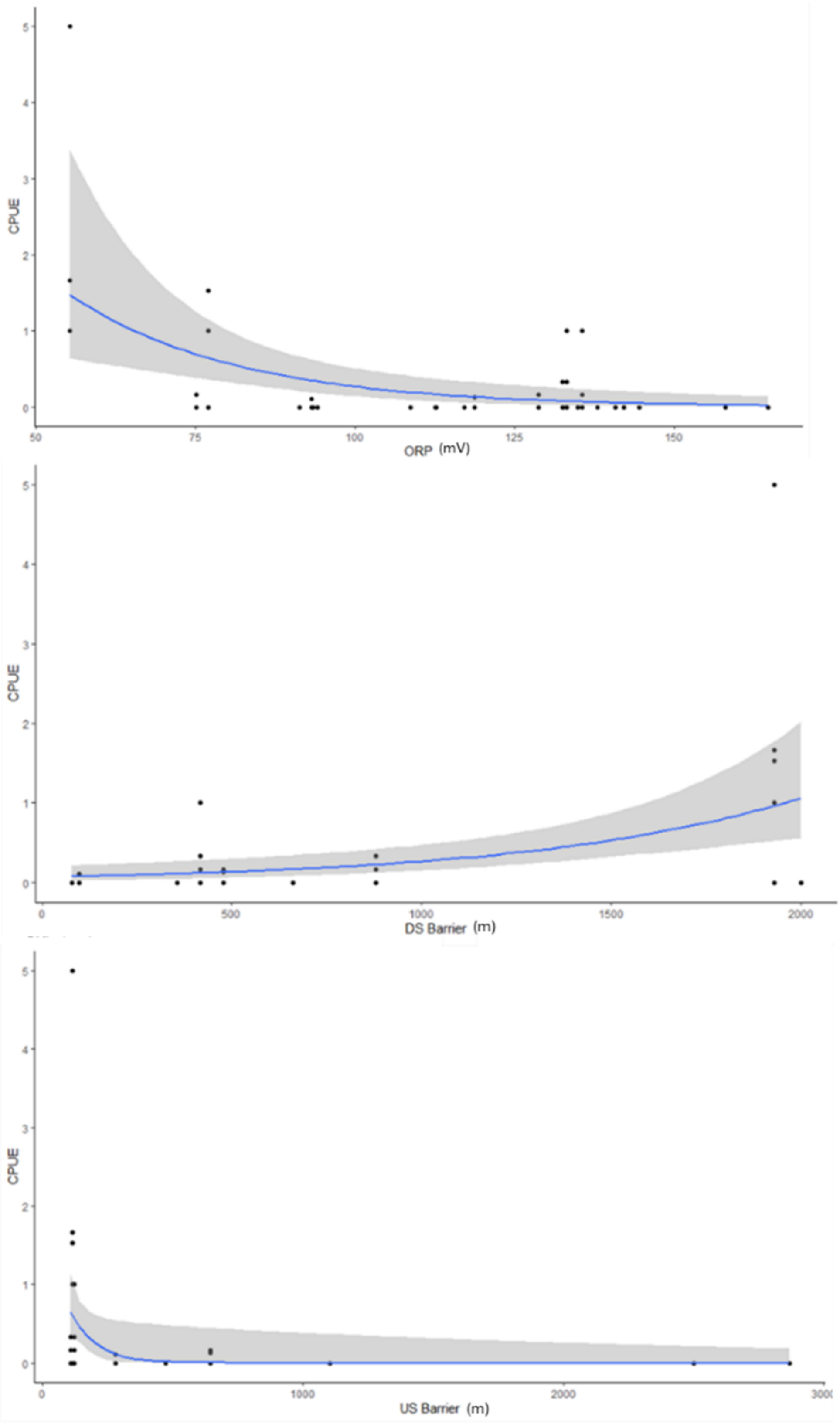


Figure 5.9. Generalised Linear Model depicting how A) ORP, B) the distance from a downstream barrier and C) the distance from an upstream barrier influences the Catch Per Unit Effort of all surveying methods

Crayfish distribution

Throughout the Calder River catchment a total of nine locations were identified as having a species of crayfish present (Figure 5.10). Seven of these locations had positively identified signal crayfish records. Of these my surveys detected four sites, and three sites were confirmed through citizen science and the Calder Rivers Trust's River Health Partnership data dashboard. The remaining two sites had records of white-clawed crayfish, one of which was the historical record from extensive surveys reported by the Calder Rivers Trust, Yorkshire Wildlife Trust, and Calderdale local government in 2022. The other white-clawed site was an unknown population discovered by the authors during this study WoC ID: WK9185 (for *A. pallipes*, observation date: 2024) (Ion et al. 2024).

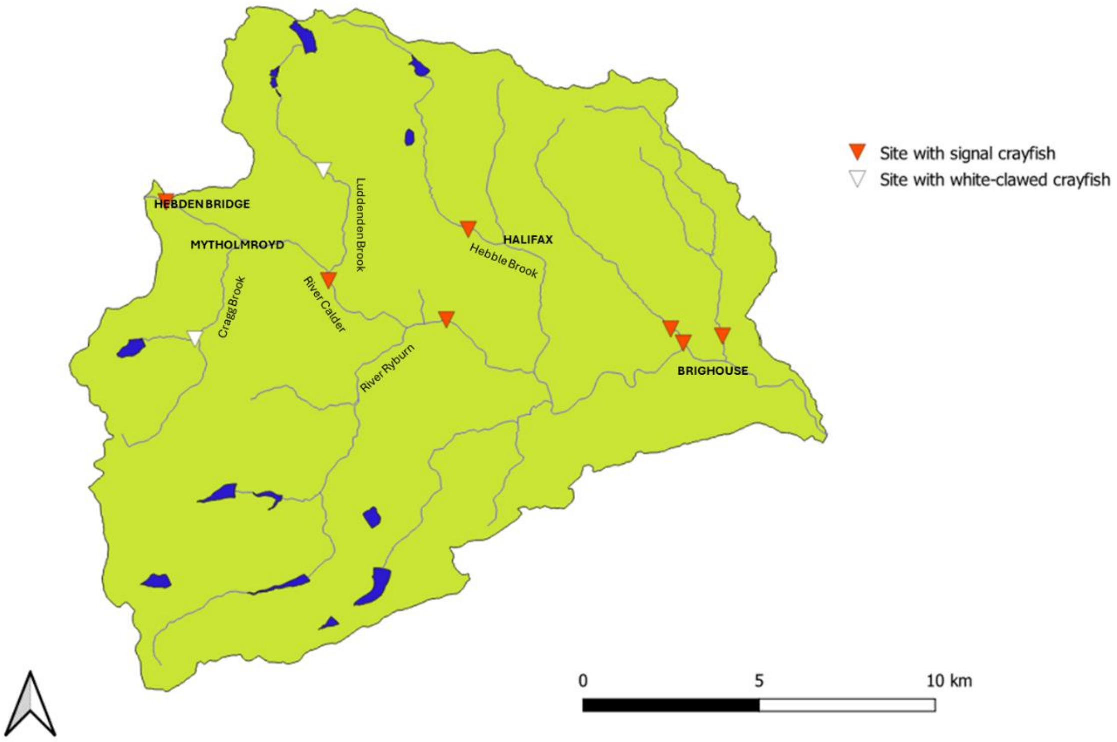


Figure 5.10. Locations of invasive signal crayfish and native white-clawed crayfish throughout the Middle Calder Water Framework Directive Operational catchment. Five records of crayfish come from surveys conducted during this research, four are historical records since Summer 2022

Barrier assessment

There are 122 barriers throughout the Calder catchment (Figure 5.11). Of these 53 were encapsulated by my survey sites and records from citizen science surveys. Of these barriers, a total of 27 were categorised as breached (50.9%) and 9 as at risk (17.0%). All barriers within the main river were breached. The two tributaries that have white-clawed crayfish present in the upper reaches have a series of barriers that do not appear to have been breached and are also not yet classified as at risk (Figure 5.12).

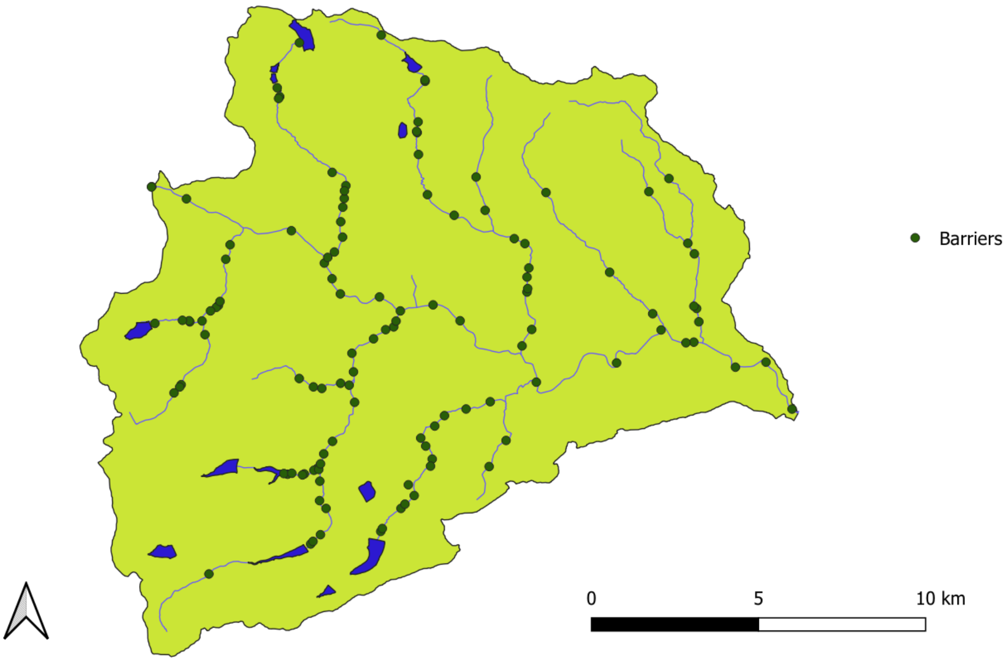


Figure 5.11. Locations of barriers throughout the Middle Calder Water Framework Directive Operational catchment. Barriers are classified as a feature fragmenting the waterway, these are either disused mills or weirs

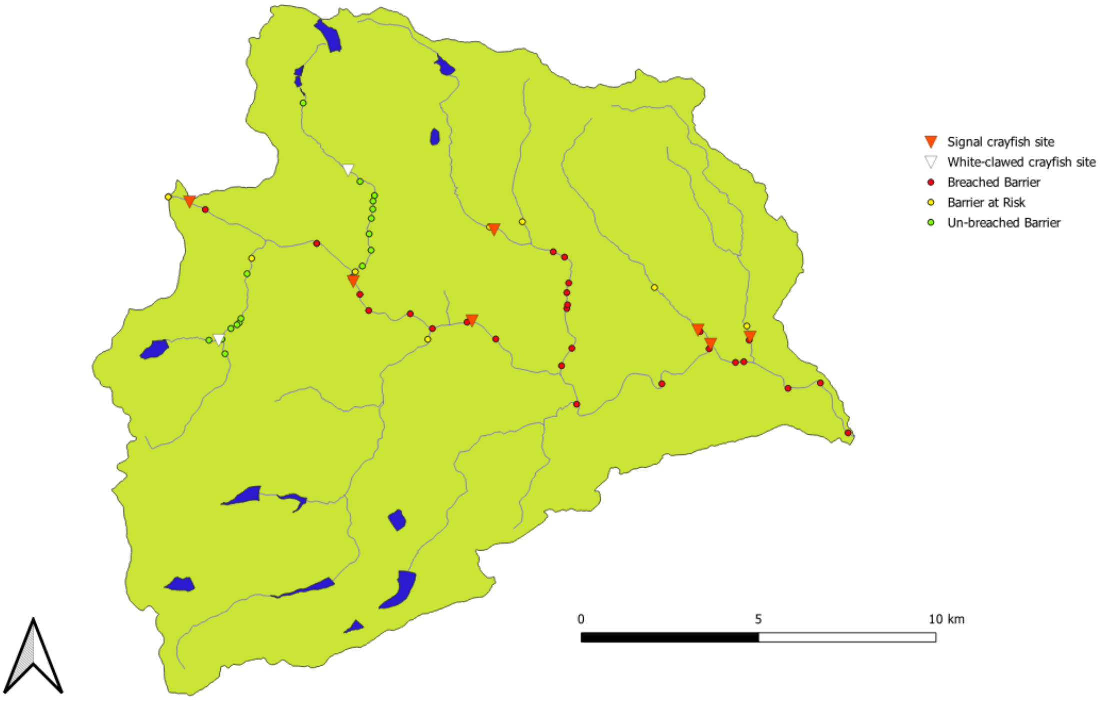


Figure 5.12. Locations of barriers assessed throughout the Middle Calder Water Framework Directive Operational catchment. Barriers are classified as breached if there are records of invasive crayfish both upstream and downstream of it. A barrier is at risk if there are records of invasive crayfish directly downstream of it, but none upstream. It is classified as un-breached if there are no invasive crayfish upstream of it and there is at least one barrier between it and the nearest downstream record

Discussion

Removal of instream barriers can be a powerful and feasible intervention to increase biodiversity and reverse the trend of declining freshwater fishes (Bellmore et al. 2017). Removal of one threat, i.e. barriers, may promote the spread of another, i.e. invasive species. Here, I pioneer an assessment method to appraise river systems for invasive species spread, to inform barrier removal/easement strategies. Multi-method assessment of crayfish distribution and abundance showed that crayfish appear to aggregate downstream of the nearest upstream barrier, thus posing a persistent threat to uninvaded upstream reaches. Targeting areas below barriers with refugia from turbulent flow should be a priority for any pre-barrier removal management. Besides eDNA, surveying methods compared could ostensibly be deployed interchangeably due to similarity in detection efficiency during environmental impact assessments. In this case, the distribution of invasive crayfish throughout the sampled reaches of the River Calder indicates that barrier removal in the mainstem would not drive any negative impacts by way of invasive crayfish range expansion, and enhanced flow regimes may confer secondary benefits.

Gear comparison

There was no significant difference between the detection probability or CPUE for trapping or either camera method. These three methods all showed similar results when determining the presence/absence of crayfish at a site, as well as estimating population sizes. This is similar to results from a still-water reservoir which found similar efficacies in these methods (Harwood et al. 2025). A major limitation with using remote underwater video is turbidity, with high levels of turbidity limiting the successful identification and measurements of target species (Tweedie et al. 2023). The high turbidity found in some of the sites along the River Calder did not impact the effectiveness of the B/RUV deployments detecting the presence of crayfish, with individuals observed in footage with a visibility less than 15cm. However, turbidity did limit the effectiveness of identifying crayfish to species level, and trapping surveys conducted at the same site were needed to confirm species type. Trapping is the most time consuming of these three methods, but it was also the only method to detect crayfish at sites that other methods did not. Furthermore, trapping provides data on population structure which would only be possible for B/RUV if a stereo camera setup was used (Langlois et al. 2020).

Environmental DNA (eDNA) has been seen as a powerful tool that has been used extensively to detect the presence of invasive crayfish throughout a range of waterbodies (Dougherty et al. 2016; Cai et al. 2017; Larson et al. 2017; Harper et al. 2018; Ikeda et al. 2019; Porco et al. 2022). eDNA in my study did not report any evidence of crayfish presence. Invertebrates are a difficult organism to detect using eDNA due to their lack of mucous producing structures (Harper et al. 2018). Crayfish exoskeletons limit the release of extracellular DNA (Harper et al. 2018) and during times when crayfish do not moult as frequently, such as winter and other periods of cold temperature, it is less likely that there will be a significant amount of DNA in the water to allow for a successful detection. The River Calder is a highly turbid watercourse and eDNA sampling required a lot of physical effort, with filters clogging up very quickly, although this would have been negated with the use of a peristaltic pump. Turbid water samples have been found to contain high concentrations of suspended organic and inorganic material, while also exhibiting high levels of PCR inhibition, in comparison to clear water samples (Kumar et al. 2022). In this case, eDNA proved to be the least effective and cost-effective assessment method.

Presence/absence and relative abundance data of non-native species are the pre-requisites for environmental impact assessments of barrier removals. Therefore, rapid B/RUV deployments could be made throughout a catchment to identify sites where invasive crayfish are present to rapidly identify distribution patterns and sites of concern. By identifying crayfish risk sites, schedules can be created in order to conduct more time-consuming trapping events to mechanically reduce high abundance populations in necessary prior to barrier removal.

Determinants of crayfish abundance

Crayfish abundance was related to barrier positioning, temperature and ORP. In the River Calder, crayfish tended to be present closer to their upstream barriers and have higher densities further away from downstream barriers. Signal crayfish shelter from strong flow in either burrows or below rocks on the riverbed (Johnson et al. 2010). Areas directly above barriers tend to have fast flows as the water cascades over them, these conditions are not favourable to signal crayfish and after breaching a barrier they will opt to move away from this high flow, either further upstream or back over the barrier (Johnson et al. 2010). Opposed to this, directly below barriers, there are pools and riffles (Salant et al. 2012) that provide shelter with low flows that crayfish could potentially move to and accumulate in (Maude & Williams, 1983; Foster & Keller, 2011; Rosewarne et al. 2013). Crayfish abundance was higher in sites with lower ORP, ORP relates to the cleanliness of water and the amount of oxygen in it. This likely reflects the burrowing and foraging behaviour of signal crayfish which increases sedimentation (Johnson et al. 2011). This sedimentation can lead to a reduction in macrophyte growth (Roessinl et al. 2017), through reduced light levels (Madsen et al. 2001) and the altering of the structure of the riverbed (Jones et al. 2012). Less macrophytes also results in less oxygen in a waterbody, which in turn results in lower ORP values. Crayfish abundance was linked to seasonality and temperature whereby they were not detected in any B/RUV or eDNA sampling in the winter. This is related to metabolic processes reducing foraging and movement in the colder seasons (Bubb et al. 2002; Bubb et al. 2004; Johnson et al. 2014).

Crayfish distribution

Along with being present on Hebble Brook, my study found that signal crayfish are present throughout the entirety of the main River Calder with records from Hebden Bridge all the way downstream to Brighouse. This site along Hebble Brook, Halifax, achieved the highest catch per unit effort across all three method types. Despite pressure from invasive species, the United Kingdom contains some of the best white-clawed crayfish stocks in Europe (Holdich & Rogers 1997). Populations of white-clawed crayfish continue to be in a state of decline, while invasive signal populations are expanding (Chadwick 2019). This project discovered a previously unknown population of white-clawed crayfish along Cragg Brook. With correct protection this site could potentially provide a new stronghold to preserve the population. This also provides hope that there are more unknown populations like this throughout the UK.

Native white-clawed crayfish were detected at Luddenden Brook in 2022, however, my surveys did not obtain any records of crayfish at these sites. Although this result is of concern, a more thorough survey would be needed to assess the site, involving trapping, hand searching and torchlight surveying. These methods are both intensive and potentially cause unwarranted stress to the endangered white-clawed crayfish (Peay 2000). The three records of signal crayfish closest to Brighouse were obtained through citizen science contributions. Only citizen science records with photographic and reliable confirmation have been considered throughout this study. Citizen science offers a low-cost solution to addressing river monitoring challenges, including invasive species (Gurnell et al. 2019).

Barrier removal

By identifying all barriers that have been breached, informed decisions can be reached as to what barrier easement can occur to aid salmonid migration, while also protecting native white-clawed crayfish populations. The barriers that have been identified as breached will be seen as priority targets for future barrier relief projects. Just over half (27/53) of the barriers encapsulated within our survey area were breached. All barriers within the main River Calder between Hebden Bridge and Brighouse had a record of signal crayfish both above and below them.

Smooth, vertical walls with overhanging lips above the water surface are the most effective type of barrier to crayfish migration (Krieg & Zenker 2020). The barriers that are present along Luddenden Brook and Cragg Brook follow this design and conservation efforts should be advised to ensure that these barriers remain like this. Any future breaches or degradation could provide opportunity for signal crayfish to pass and put white-clawed crayfish populations at risk. When barriers are too steep for a crayfish to cross in the water, they can easily crawl out of the water to circumnavigate them before re-entering the river (Kerby et al. 2005). Therefore, it is also important when conserving these barriers that the surrounding terrain is also conserved. Steep terrain surrounding barriers that takes crayfish extended time to navigate, putting them at risk of desiccation, also helps to ensure that barriers are effective at preventing crayfish spread (Thomas et al. 2019). This steep terrain does not completely eradicate the movement of crayfish over these barriers as many factors, including humans and birds can pick up individuals and move them above (Anastácio et al. 2014).

With our findings I can advise that complete barrier relief can happen within the main River Calder. Local restoration efforts can focus on these without fear of accidentally further spreading signal crayfish populations. However, the barriers that are present along the two tributaries, Luddenden Brook and Cragg Brook, should be left untouched as they appear to be successfully preventing signal crayfish from spreading up the tributaries. Management focus should instead be towards alleviating barriers for salmonid migration, while also reinforcing barriers that act as a buffer between native and invasive crayfish to try and reduce the risk of further invasion. Caution must be advised however while considering the potential spread of crayfish plague. Biosecurity is the first line of defence against invasive non-native species (Dunn & Hatcher, 2015) and it is vital that signage is clear to prevent accidental translocation of both invasive crayfish and their pathogens. There is clear biosecurity signage around Luddenden Brook but further information could be added and more signage could be included along the main River Calder to warn about the potential risks.

Conclusion

Invasive non-native crayfish are among the most invasive aquatic species in the world (Galib et al. 2022), and they can have a devastating impact on their invaded ecosystems. Artificial barriers can act as a buffer to their migrations, slowing their spread, but these are only a temporary barrier, and they can become breached. While reducing the spread of invasive non-native species these barriers also prevent the upstream migration of salmonids. Trade-offs between barrier easement for salmonid migration and keeping barriers to act as a buffer for upstream invasive crayfish migration need to be made in ensuring the best possible outcomes when planning to remove these barriers. By identifying the key environmental factors that influence the presence and abundance of crayfish throughout this survey, areas at risk can be modelled to predict where populations may be on a watercourse to influence rapid surveys. A combination of trapping and both RUV and BRUV surveys conducted during summer, when signal crayfish are more active, are likely to achieve the best results when assessing the extent of an invasion. Upon establishing the extent of these invasions, decisions can be made of barrier alleviation.

Chapter 6

Remote underwater video as a tool for assessing behavioural interactions and habitat associations in white-clawed crayfish (*Austropotamobius pallipes*)

Abstract

It is widely believed that white-clawed crayfish (*Austropotamobius pallipes*) are a nocturnal species. However, in this chapter firsthand infield observations have been made showing them to be highly active during daylight. The River Kent is home to a healthy population of white-clawed crayfish and remote underwater video (RUV) and baited RUV (BRUV) deployments successfully observed white-clawed crayfish activity during daylight. BRUVs achieved higher total counts, maximum number of individuals on screen at once and higher cumulative times than RUVs, but there was no difference in the time it took for either method to first observe a crayfish. No variables were found to have an impact on determining crayfish abundances and it is believed that the healthy nature of the River Kent catchment makes it a suitable site for white-clawed crayfish throughout. Individuals will assess the value of rewards against the potential risks of an interaction before initiating aggressive behaviour. Camera observations revealed that significantly more aggressive behaviours occurred when bait was present as opposed to when it was not. Most intraspecific aggressive behaviours involved evenly matched individuals in terms of size, with larger individuals winning in significantly more interactions when sizes were different. Meral spread was the most commonly observed behaviour throughout the study, which is also the least intense of the behaviours observed. Each behaviour type had a varying duration, with grappling interactions tending to occur for longer periods. It is hoped that the observations made throughout this study can be used to guide future white-clawed crayfish management and conservation.

Introduction

Freshwater biodiversity is declining globally because of habitat destruction, biological invasions and climate change (Tickner et al. 2020). Many conservation initiatives are species-focused, which requires a grounding in baseline ecological requirements and intraspecific behaviour to guide welfare in captive breeding and population management-based interventions (Hare et al. 2011; Tobias & Pigot, 2019). Behavioural ecology plays a key role in conservation and can be used to quantify population and community level impacts caused by human threats to biodiversity with shifts in behaviour, such as feeding or habitat associations, often a result of having to adapt to negative changes (Bro-Jørgensen et al. 2019). The habitat requirements of species of conservation concern are often influenced by breeding, diet choice, home range and social systems. Thus, understanding what is needed to sustain these behaviours is necessary to inform conservation policies and restoration projects (Sutherland, 1998). Several factors can cause behavioural shifts, for example invasive species can influence negative evolutionary and behavioural shifts in response to competition or predation, this can be as a result of habitat selection changes due to habitat structural changes made by invasive species or shifts in diet (Caro & Sherman, 2011). In these cases, there may be increased frequency and magnitude of aggressive interactions and individuals having to interact more for reduced resources (Greggor et al. 2016). Variations in feeding behaviours can also be an indication of shifts in the abundance of nutrient rich resources (Kotler et al. 2016; Malone & Polivka, 2022). Understanding habitat preferences and behavioural interactions can thereby be used to guide management actions such as population management to reduce competition, food supplementation or conservation of specific habitat types that provide more nutritious food sources.

Game theory states that the value of rewards for behavioural reactions and decisions can be assessed from a range of factors and the probability of a future reward (Schultz, 2004). Game theory is important as the decisions individuals make in response to behaviours of other individuals will have a significant impact on fitness consequences (Goss-Custard & Sutherland, 1997). This theory relates directly to animal behaviour when assessing likelihood of winning a competitive interaction. For example, prior to an interaction, individuals must assess if their chance of victory is high and if their resource holding potential (RHP), also known as resource defence, has a selective advantage to that of their opponent. If their opponent's RHP exceeds their own by a sufficient amount then it can avoid the competition completely and eliminate a risk of damage (Parker, 1974). It is hypothesised that larger individuals, with larger "weapons" are more likely to win interactions, while fights are more likely to be conducted by evenly sized individuals, with individuals only initiating contact that it believes it can win (Sneddon et al. 1997). By making field observations of species an understanding of the baseline activities and competition can be constructed.

Crayfish are keystone predators and play an important role in the transfer of energy throughout complex food webs (Momot, 1995). Understanding crayfish behavioural patterns can provide key knowledge on the habitat and breeding requirements of native crayfish, as well as identifying the success of invasive non-native crayfish species (Kubec et al. 2019). Several studies have been conducted assessing behavioural interactions between crayfish, with invasive non-native crayfish species of particular interest. Laboratory trials have shown that between two invasive non-native crayfish species, signal crayfish (*Pacifastacus leniusulus*) and spiny-cheek crayfish (*Orconectes limosus*), signal crayfish were more dominant and could showed the potential to establish dominant populations (Hudina et al. 2011). Field studies have observed that invasive non-native common yabby (*Cherax destructor*) was more dominant that the native Fitzroy Falls spiny crayfish (*Euastacus dharawalus*) when within a 10% size difference, while the invasive non-native crayfish also had significantly longer intraspecific interactions than the native crayfish (O'Hea Miller et al. 2023). Limited studies exist that investigate the intraspecific interactions of crayfish, and these studies can offer important information regarding native crayfish behaviour, that can help to influence conservation. Chelae scarring on white-clawed crayfish (*Austropotamobius pallipes*) is often the result of intraspecific agonistic encounters and chelae play a key role in social contests and wider crayfish interactions that can provide key information on population dynamics (Gherardi et al. 2000).

The white-clawed crayfish is the United Kingdom's only native species of crayfish (Holdich & Rogers. 1997). They are protected under national and European legislation and are classified as endangered by the International Union for Conservation of Nature (IUCN) (Rosewarne et al. 2017; IUCN, 2025). Populations of native crayfish are declining across Europe with human induced causes being a key reason for this decline (Biasetti et al. 2021). There are many stressors that negatively impact populations of white-clawed crayfish including habitat loss, pollution, climate change, poaching and invasive species, through either disease such as crayfish plague (*Aphanomyces astaci*) or competition for food and habitat (Gherardi & Barbaresi, 2008; Sibley et al. 2010; Vaeßen & Hollert, 2015). Understanding the habitat requirements of white-clawed crayfish is vital to their conservation (Smith et al. 1996) and having knowledge of the associations and drivers of species abundance is critical for species protection (Broom et al. 2023). Previous field observations have found the frequency of white-clawed crayfish activity to be high at night, with limited observations made while the sun is up, similar trends were also observed in a laboratory setting, with significantly higher activity levels in simulated night-time when lights were off (Barbaresi & Gherardi, 2001). Boulder and pebble coverage plays an important role in white-clawed crayfish habitat usage, with the presence of shelter having previously been identified as the only habitat parameter correlated with crayfish distribution (Broquet et al. 2002; Rosewarne et al. 2017). White-clawed crayfish tend to remain in burrows during the day and are most active at night (Peay & Hirst, 2003). This nocturnal nature makes them a difficult to observe species. Various monitoring approaches are recommended: trapping, artificial refuge surveys, manual searching, Pritchard trapping and, torchlight surveys (Peay, 2004; Pritchard et al. 2021). These approaches often require large amounts of effort by surveyors due to extended nighttime searching. Manual searching and night viewing also require specialised training and Environment Agency licensing to ensure the risk to crayfish are mitigated and effective biosecurity protocols are followed. Trapping also has limitations as it is often size biased and can be stressful for animals (Holdich et al. 2006). Habitat fragmentation can also influence the distribution of crayfish, with artificial barriers often reducing the ability of them to move upstream. Previous observations made by Harwood et al. (Thesis Chapter 5) have found that distance to upstream barriers had a significant influence in crayfish abundances, with higher abundances of crayfish found closer to an upstream barrier.

Throughout England and Wales there are fewer than twelve catchments that are thought to be populated by native white-clawed crayfish and free from invasive signal crayfish (Ellis & England, 2009). Remote Underwater Video (RUV) and Baited Remote Underwater Video (BRUV) offers a potential non-extractive method for assessing native crayfish abundances and behaviours. Trapping and manual searching are not able to assess natural instream behaviours of white-clawed crayfish, thus there is a major gap in the current literature causing a barrier to conservation planning (Rosewarne et al. 2017). Here I sought to address whether underwater cameras could be used to successfully document white-clawed crayfish during daylight hours and assess habitat associations with respect to environmental parameters, barriers and stressors (e.g. sewage outflow). Furthermore, using baited and unbaited cameras can act as an in-field experiment to determine differences in intraspecific behavioural interactions in competitive scenarios to address game theory and behavioural decision making in white-clawed crayfish. I hypothesize that remote underwater video and baited remote underwater video will be able to successfully observe crayfish and gain estimates for population abundances throughout each site, as we have previously seen in Chapter 4 and Chapter 5. However, I do not expect there to be a significant difference in the abundances estimated between the RUV and BRUV as this has not been observed in either of these previous studies. Finally, I expect the presence of bait to increase the intensity of intraspecific interactions, with larger individuals showing higher levels of dominance as has been witnessed in other crayfish behaviour studies, and more evenly matched individuals having longer interactions (O'Hea-Miller et al. 2023).

Methods

Study Site

The River Kent (54°22′N; 002°47′W) (Figure 6.1) is a 32-km-long river in Cumbria, United Kingdom, that empties into Morecambe Bay. The source of the river is in Kentmere within the Lake District National Park. The river runs through the Lake District National Park, progressing through grassland, pasture fields and rural settlements before reaching more urban areas near to the river mouth. Prolonged rainfall in 2009 around the Lake District resulted in wide scale flooding and severe human impact, with many rivers within the flooded area. During this time the River Kent reached levels 87% from its highest recorded maxima, while other rivers reached levels of up to 200% (Miller et al. 2013). The surrounding geology consists of Silurian Slates and Kirby Moor Flags as well as gravel and boulder clay (Ridings & Eloy, 2008). The river has Strahler order 1 and is shallow and no more than five metres wide in the upper regions, where this study was undertaken, before becoming deeper and wider prior to emptying into Morecambe Bay. The river has populations of native white-clawed crayfish (*Austropotamobius pallipes*), freshwater pearl mussels (*Margaritifera margaritifera*), bullhead (*Cottus gobio*) and water crowfoot (*Ranunculus fluitantis*) throughout the catchment and it has been declared a Special Area of Conservation (SAC) to protect these populations.

Five sites were chosen along the River Kent to conduct these surveys. Sites were chosen to include both pools and riffles to be able to assess how these habitat features influenced behaviours and abundances and at locations where the water was accessible. The effect of two other environmental factors were also considered, a waterfall between sites 2 and 3, as well as a sewage treatment works upstream of site 1. These features were considered in analysis to identify if they had any influence on results for sites nearest to them.

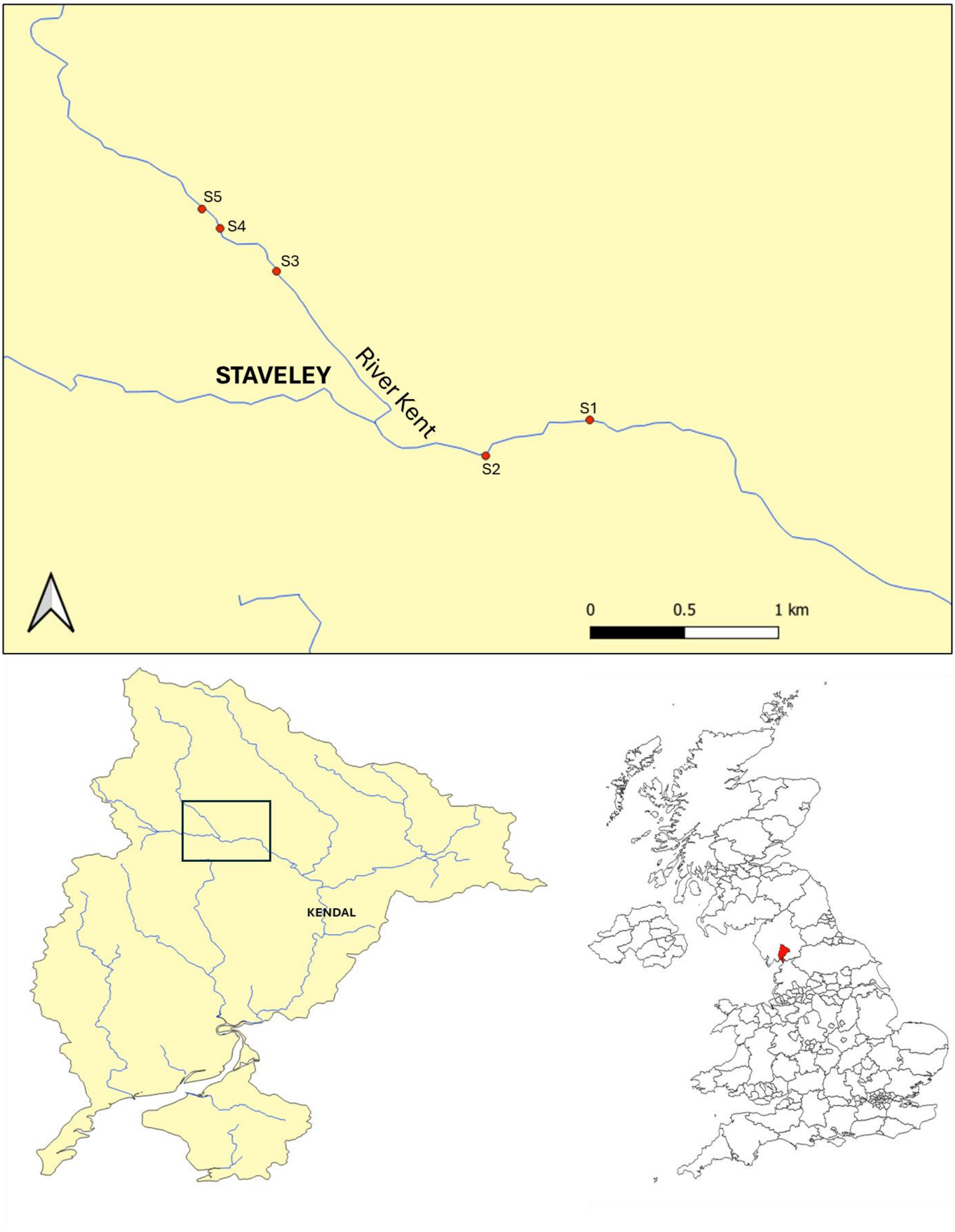


Figure 6.1. Locations of all five survey sites on the River Kent

Remote Underwater Video Surveys

Remote Underwater Video (RUV) were deployed at five sites in the upper regions of the river Kent on three occasions, September 2023, May 2024, and September 2024. Initially the third survey was scheduled for August 2024, but flash flooding resulted in unsafe surveying conditions. The RUV was deployed for one hour at each of the sites between 10:00 and 15:00, recording at 30 frames per second with 1080p resolution. These settings are following standards recommended by Harwood et al. (Chapter 2, in review).

Baited Remote Underwater Video (BRUV) were also deployed at the same five sites. Each BRUV deployment was made 24 hours after the RUV deployment at the same site. Each BRUV was baited using wet cat food and deployed for one hour, with the same settings as described previously (Chapter 4, 5).

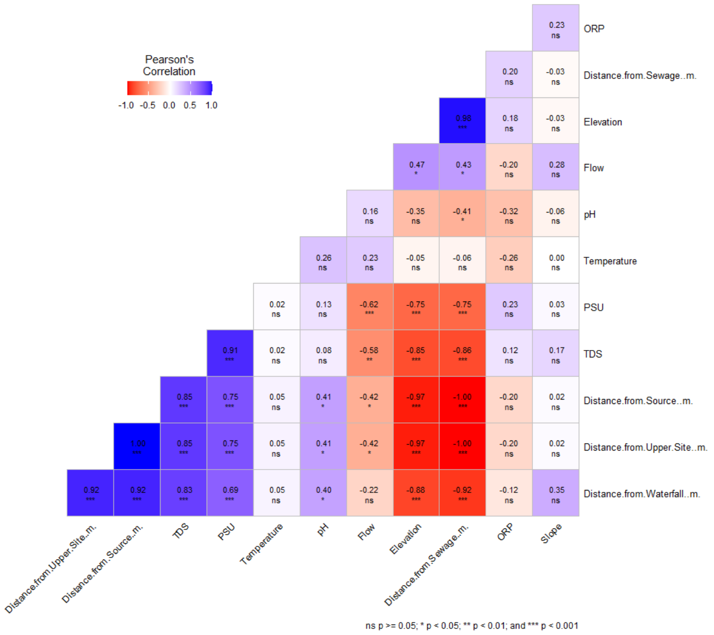
All footage from the RUV and BRUV deployments were manually reviewed by the same observer. Initial population observations were made, first by noting the time taken for the first observation of a crayfish to occur for each deployment. The total number of instances crayfish entered the camera's field of view was also recorded as a cumulative tally. MaxN, which is the maximum number of individuals on screen at any single time, was also noted for each deployment to establish the area with the densest population of crayfish. Wilcox tests were used to establish if the use of bait impacted observations including MaxN, total time on screen, time to first observation and the total count of crayfish entering the screen.

Habitat Drivers

To establish if habitat and water quality variables affect abundance of white-clawed crayfish I measured water temperature (°C), pH, ORP, TDS, PSU, PSI and depth (cm) using a Hanna HI9819X Microparameter meter, and flow was measured with a Geopacks Flowmeter. Due to an error with the probe, values could not be obtained for PSI at some sites. Values for presence/absence were also noted, however all sites had crayfish present, so presence/absence analysis was irrelevant. At each site stream features were observed, noting whether it was deployed in a pool or riffle habitat. Site location was also noted in relation to a sewage treatment works (54°22′N, 002°47′W), distance from the waterfall barrier (54°38′N, 002°80′W) and distance from source (54°46′N, 002°86′W). Values for site elevation were taken from the HyroSHEDS online database (Lehner et al. 2008), and slope was the change in elevation over a distance of 200m (Equation 1).

$$slope = \frac{change\ in\ elevation}{distance} \text{ eqn 5}$$

All variables were scaled prior to analysis. A PCA was completed to identify the uniqueness of each site and differences between surveying sessions regarding environmental and water quality factors. Pearson's correlation was also used to establish variables that were correlated and therefore had a strong relationship. Variables with strong relationships were identified and only one of those variables was included in further analysis. All distance measurements and elevation were significantly correlated, and distance from source was chosen as a key variable (Figure 6.2). Distance from source was chosen over the distance from sewage as they are the reverse of each other, with the sewage treatment works being at the most downstream site and distance from source shows how populations vary throughout the watercourse. Quasi-Poisson GLMs were used to determine what variables influenced white-clawed crayfish abundances. The variables were scaled and those included in the GLM were temperature, pH, ORP, slope and distance from source using MaxN as the response variable.



	Distance.from.Upper.Site..m.	Distance.from.Source..m.	TDS	PSU	Temperature	pH	Flow	Elevation	Distance.from.Sewage..m.	ORP	Slope
ORP											0.23 ns
Distance.from.Sewage..m.										0.20 ns	-0.03 ns
Elevation									0.98 ***	0.18 ns	-0.03 ns
Flow								0.47 *	0.43 *	-0.20 ns	0.28 ns
pH							0.16 ns	-0.35 ns	-0.41 *	-0.32 ns	-0.06 ns
Temperature						0.26 ns	0.23 ns	-0.05 ns	-0.06 ns	-0.26 ns	0.00 ns
PSU					0.02 ns	0.13 ns	-0.62 ***	-0.75 ***	-0.75 ***	0.23 ns	0.03 ns
TDS				0.91 ***	0.02 ns	0.08 ns	-0.58 **	-0.85 ***	-0.86 ***	0.12 ns	0.17 ns
Distance.from.Source..m.			0.85 ***	0.75 ***	0.05 ns	0.41 *	-0.42 *	-0.97 ***	-1.00 ***	-0.20 ns	0.02 ns
Distance.from.Upper.Site..m.		1.00 ***	0.85 ***	0.75 ***	0.05 ns	0.41 *	-0.42 *	-0.97 ***	-1.00 ***	-0.20 ns	0.02 ns
Distance.from.Waterfall..m.	0.92 ***	0.92 ***	0.83 ***	0.69 ***	0.05 ns	0.40 *	-0.22 ns	-0.88 ***	-0.92 ***	-0.12 ns	0.35 ns

ns p >= 0.05; * p < 0.05; ** p < 0.01; and *** p < 0.001

Figure 6.2. Pearson’s Correlation results between unique factors at each site

All models were assessed for assumptions through Q-Q plots (Figure 6.3).

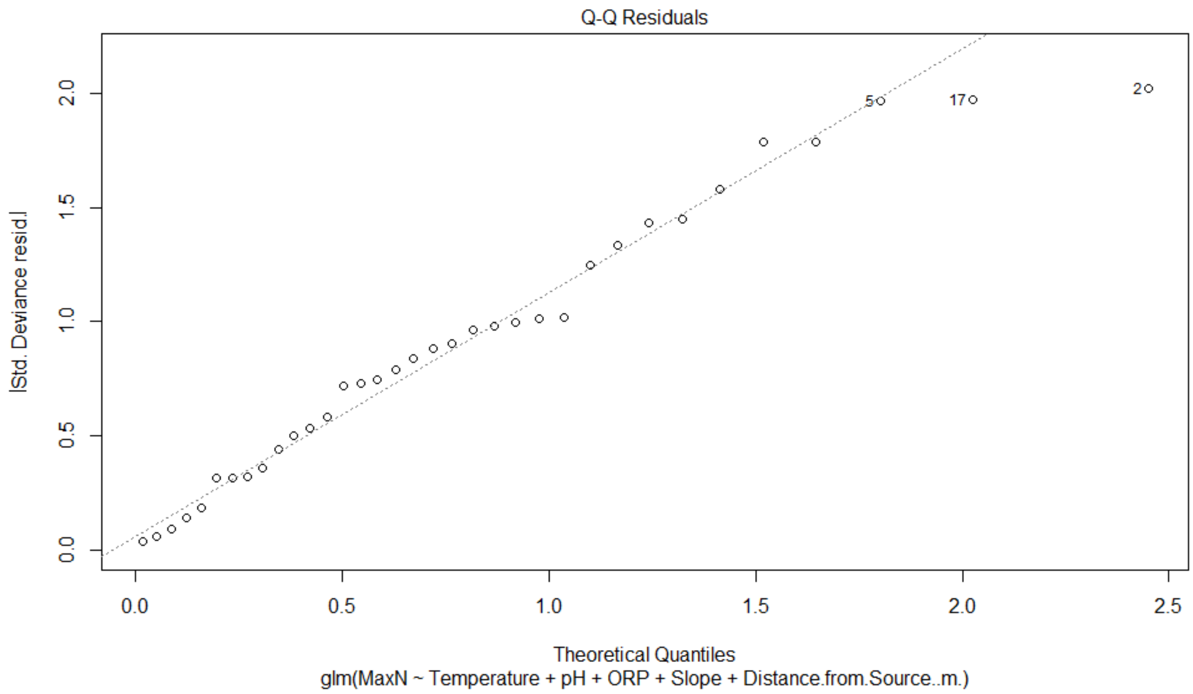


Figure 6.3. Q-Q plot of residual deviance for values to test the assumptions and quality of the model

Behaviour Analysis

All footage was reviewed to identify interaction types demonstrated by the white-clawed crayfish. Each behaviour type was classified into categories: "intraspecific aggressive behaviour" and "inter-species aggressive behaviour". Each behaviour was described and then assigned an intensity score using an ethogram and the duration of each behaviour and intraspecific interaction was recorded (Table 6.1). Intensity scores were assigned based on the level of aggression, with more aggressive behaviour obtaining higher score and negative values were assigned for retreating behaviours, a cumulative score for each interaction was calculated and the total score was used in analysis. Each interaction was categorised to establish if it was between two evenly sized individuals, or if there was a difference in size each was categorised as small or large. The loser of the interaction was determined by the first individual to disengage and back away. A χ^2 test was used to establish if the larger or smaller individuals were more likely to win the contest, and another χ^2 test was used to establish if larger or smaller individuals were more likely to initiate a contest.

A Wilcox test was used to identify if there was a significant difference between the intensity of crayfish interactions when bait was present versus when it was not. For each interaction a total score was calculated for both the winner and loser by summing the intensity score of each behaviour observed during the interaction.

To assess whether white-clawed crayfish use size to assess competitive interactions I used generalised linear models (GLM). To determine whether result is influenced by crayfish size and total interaction intensity, a binomial GLM was constructed using result (i.e. winner/loser: 1/0) as the response variable and size and total intensity score as the predictor variables. The model was fitted with full interaction effects and simplified if non-significant. To assess factors which determine the duration of an intraspecific agnostic bout, I used a GLM with quasipoisson error distribution to account for over dispersion. Whereby duration was the response variable and crayfish sizer and behaviour type as the predictor variables. Post hoc assessments were completed for each model on the categorical factors using the R package "emmeans" (Lenth, 2025).

Table 6.1. Ethogram of behaviour types observed throughout this study and the intensity score assigned to each behaviour type

Behaviour	***Description***	***Intensity Score***
Tail flip	Rapidly retreat through the water column through contraction of the abdomen (Lopez et al. 2019)	-2
Back away	Backwards retreat from interaction by walking	-1
Approach with threat display (Meral spread)	Individuals spread major chelae. The individual appears as a Y shape from above. (Bergman & Moore, 2003)	2
Boxing, pushing, touching	Individual pushes and touches opponent with closed chelae.	3
Grappling opponent	Individual grabbing and pulling at opponent appendages (Mohammed et al. 2023).	4

Results

RUV Surveys

Of the nineteen RUV deployments made throughout this survey, 63% (n = 12) obtained at least one record of crayfish, with a maximum of 5 individuals on screen at a single time (Table 6.2). Of the sixteen BRUV deployments made, 94% (n = 15) obtained at least a single record of crayfish, with a maximum of fifteen individuals on screen at a single instance (Table 6.3).

Baited RUVs obtained significantly higher values for MaxN (Wilcox, W = 177.0, $p < 0.05$) than un-baited ones. Baited RUVs achieved a significantly higher total count of instances when crayfish entered the field of view (Wilcox, W = 179.5, $p < 0.05$) than un-baited. The total time in which crayfish spent within the field of view of the camera rig was also significantly higher when a baited RUV was deployed (Wilcox, W = 184.0, $p < 0.05$).

There was no significant difference between baited and un-baited RUV deployments when regarding the time it took for a crayfish to first appear within the field of view of the camera rig (Wilcox, W = 60.0, $p = 0.875$).

Table 6.2. White-clawed crayfish (*Austropotamobius pallipes*) observations collected using Remote Underwater Video. MaxN is taken as the maximum number of crayfish present in frame throughout the deployment. Total count is the total number of instances that a crayfish enters the frame throughout the deployment. Rows highlighted in yellow are dates when the complete survey could not be conducted fully due to flash flooding

Date	Site	Start Time	First Observation	MaxN	Total Count	Cumulative Time
08/09/2023	Site 1	13:54		0	0	00:00
08/09/2023	Site 2	12:33	03:59	2	4	01:52
08/09/2023	Site 3	17:14	39:41	2	6	05:31
08/09/2023	Site 5	15:50	00:00	5	28	30:24
29/05/2024	Site 1	10:00		0	0	00:00
29/05/2024	Site 2	11:13	09:55	2	14	17:00
29/05/2024	Site 3	12:37		0	0	00:00
29/05/2024	Site 4	15:03	00:01	2	12	23:11
29/05/2024	Site 5	14:00	20:29	1	1	00:03
21/08/2024	Site 1	09:34		0	0	00:00
21/08/2024	Site 2	10:45	36:02	2	4	13:13
21/08/2024	Site 3	12:02	18:37	3	14	31:58
21/08/2024	Site 4	13:19	57:08	1	1	02:52
21/08/2024	Site 5	14:24	00:00	3	18	35:24
11/09/2024	Site 1	09:18		0	0	00:00
11/09/2024	Site 2	10:28		0	0	00:00
11/09/2024	Site 3	11:42		0	0	00:00
11/09/2024	Site 4	12:54	38:28	1	1	00:46
11/09/2024	Site 5	13:59	00:00	3	19	45:16

Table 6.3. White-clawed crayfish (*Austropotamobius pallipes*) observations collected using Baited Remote Underwater Video. MaxN is taken as the maximum number of crayfish present in frame throughout the deployment. Total count is the total number of instances that a crayfish enters the frame throughout the deployment

Date	Site	Start Time	First Observation	MaxN	Total Count	Cumulative Time
08/09/2023	Site 4	18:36	01:06	15	66	58:54
09/09/2023	Site 1	10:50	13:02	5	20	40:14
09/09/2023	Site 2	12:08	06:09	4	28	51:44
09/09/2023	Site 3	14:22	01:44	8	52	56:01
09/09/2023	Site 4	17:07	00:35	3	9	22:58
09/09/2023	Site 5	15:48	00:53	7	41	59:07
30/05/2024	Site 1	10:00		0	0	00:00
30/05/2024	Site 2	11:15	02:18	5	38	53:41
30/05/2024	Site 3	12:41	01:34	3	17	25:05
30/05/2024	Site 4	15:07	15:40	1	3	06:40
30/05/2024	Site 5	14:03	34:49	1	2	01:49
10/09/2024	Site 1	09:26	14:57	1	5	09:57
10/09/2024	Site 2	10:41	16:33	7	48	43:27
10/09/2024	Site 3	12:06	02:21	2	7	22:24
10/09/2024	Site 4	13:26	43:46	2	5	07:50
10/09/2024	Site 5	14:34	04:26	5	46	47:02

Habitat Drivers

More than half of the variation between survey was explained by the first principal component (55.5%). This was strongly related to distances and elevation. The second principal component explained 16.1% of the variation with this being characterised by temperature and ORP (Figure 6.4).

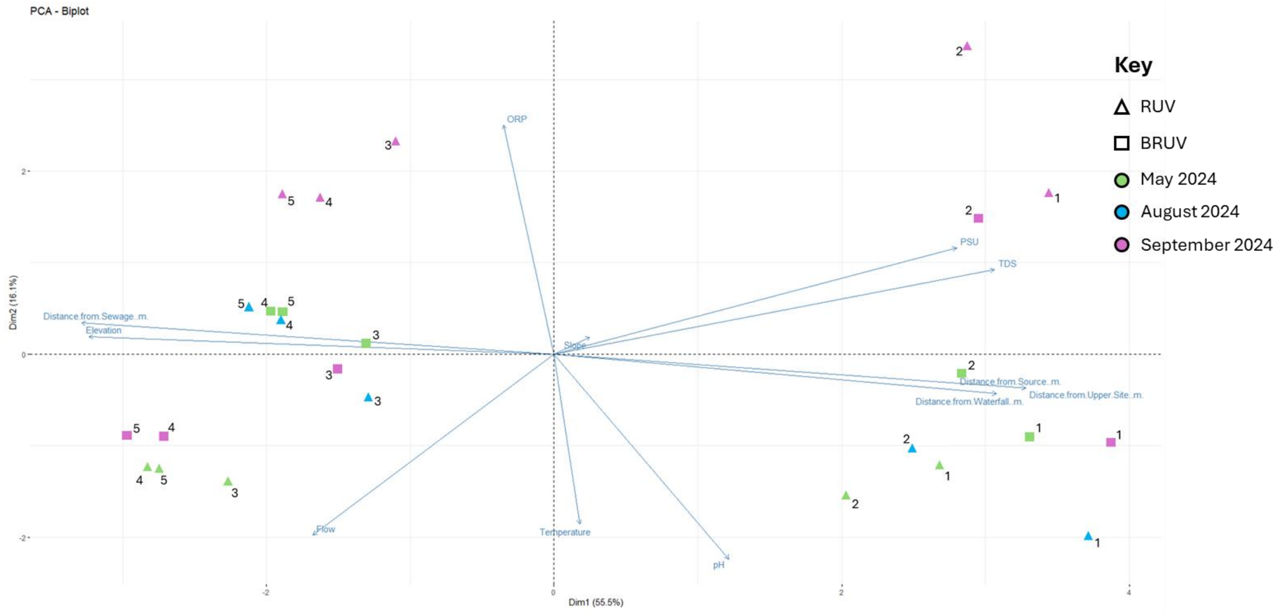


Figure 6.4. Principal Component Analysis biplot of factors present for each survey, surveys are illustrated by method and surveying month. Values next to each point signifies the survey site

No variables had a significant impact on determining the MaxN of white-clawed crayfish (*Austropotamobius pallipes*) on any camera survey (Table 6.4).

Table 6.4. Type 2 ANOVA results for Quasi-Poisson GLMs to identify if variables had a significant impact on the MaxN of surveys

Variable	**χ^2**	**p**
Temperature	2.644	0.104
pH	1.829	0.176
ORP	0.930	0.335
Slope	0.637	0.425
Distance from Source	0.482	0.488

Behaviour Observations

Throughout all surveys, a total of 165 instances of aggressive crayfish behaviours were observed. Of these, two were classified as inter-species aggressions and 163 were intraspecific aggressions. Significantly more aggressive behaviours occurred when bait was present (Wilcox, $W = 221.5$, $p < 0.01$) with 98% of the aggressive behaviours being recorded during BRUV deployments.

Both inter-species observations occurred between white-clawed crayfish and small species of fish (*Phoxonius phoxonius* & *Cottus gobio*). The first involved a crayfish sitting on the bait arm of the BRUV and when a *Phoxonius phoxonius* attempted to approach the bait the crayfish displayed meral spread towards the fish before the fish swam away (Figure 6.5A). The second instance of inter-species interactions involved a *Cottus gobio* approaching a crayfish from behind and biting it on the carapace, this crayfish then escaped the mouth of the fish and showed meral spread towards the fish while backing away (Figure 6.5B).

Figure 6.5. Inter-species interactions observed during deployments. A) Crayfish aggressively moves chelae in an attempt to grab a *Phoxonius phoxonius* that approaches the bait. B) *Cottus gobio* attacks a crayfish from behind before crayfish escapes and waves chelae aggressively at it

Most intraspecific aggressive behaviours observed involved individuals that were evenly matched in size (n = 111, 68%). During instances in which there were differences in size the smaller crayfish either retreated, backed away or deferred in most interactions, resulting in the larger individual being classified as the "winner" in significantly more interactions with mismatched sizes ($\chi^2 = 62$, df = 1, $p < 0.01$). In interactions with a difference in size the initiator was usually larger (n = 43, 83%; $\chi^2 = 12.72$, df = 1, $p < 0.01$).

The most common aggressive behaviour observed throughout all surveys was classified as a "meral spread" (score 2; Table 6.1) (n = 193, 50%). Examples of this behaviour are shown in Figure 6.6C. The second most observed behaviour was "grabbing opponent" (score 4; Table 6.1) (n = 136, 35%). Examples of this behaviour are shown in Figure 6.6A and 6.6B. The final type of aggressive behaviour observed was a "push" (score 3; Table 6.1) (n = 57, 15%).

An example of this is shown in Figure 6.6D. Instances of "Meral spread" escalated into either "grabbing opponent" or "pushing" in just under half of the occurrences (n = 88, 46%). Of the two types of submission behaviour "backing away" (score -1, Table 6.1) was the most common (n = 106, 63%), and "tail flip" (score -2, Table 6.1) was less common (n = 61, 37%).

Figure 6.6. Intraspecific interactions observed throughout surveys. A) Two evenly sized crayfish grapple on the bait arm, they are both holding the claw of their opponent. B) Two crayfish grapple near to the bait while a third crayfish watches from the side. C) Crayfish sat on BRUV bait arm raises chelae to intimidate approaching crayfish and stop it from approaching further. D) Larger crayfish approaches a smaller individual and pushes it aside using a closed chela

There was no interaction effect between total score and size, but there were significant main effects of total score and size (Table 6.5). Larger individuals are more likely to be the victor achieving higher intensity total scores than smaller individuals as well as obtaining higher intensity scores than interactions that involve evenly sized individuals (Table 6.6; Figure 6.7).

Table 6.5. Type 3 ANOVA table from a binomial GLM reporting χ^2 effect size and total score on outcome of an interaction

	χ^2	df	p
Total Score	84.14	1	< 0.01
Size	50.71	2	< 0.01
Total Score:Size	4.40	2	0.11

Table 6.6. A post hoc assessment on the categorical factors using emmeans for the relationship between size and intensity scores

Contrast	**z-ratio**	**p**
Even – Large	-4.38	< 0 .01
Even – Small	1.85	0.16
Large - Small	4.74	< 0.01

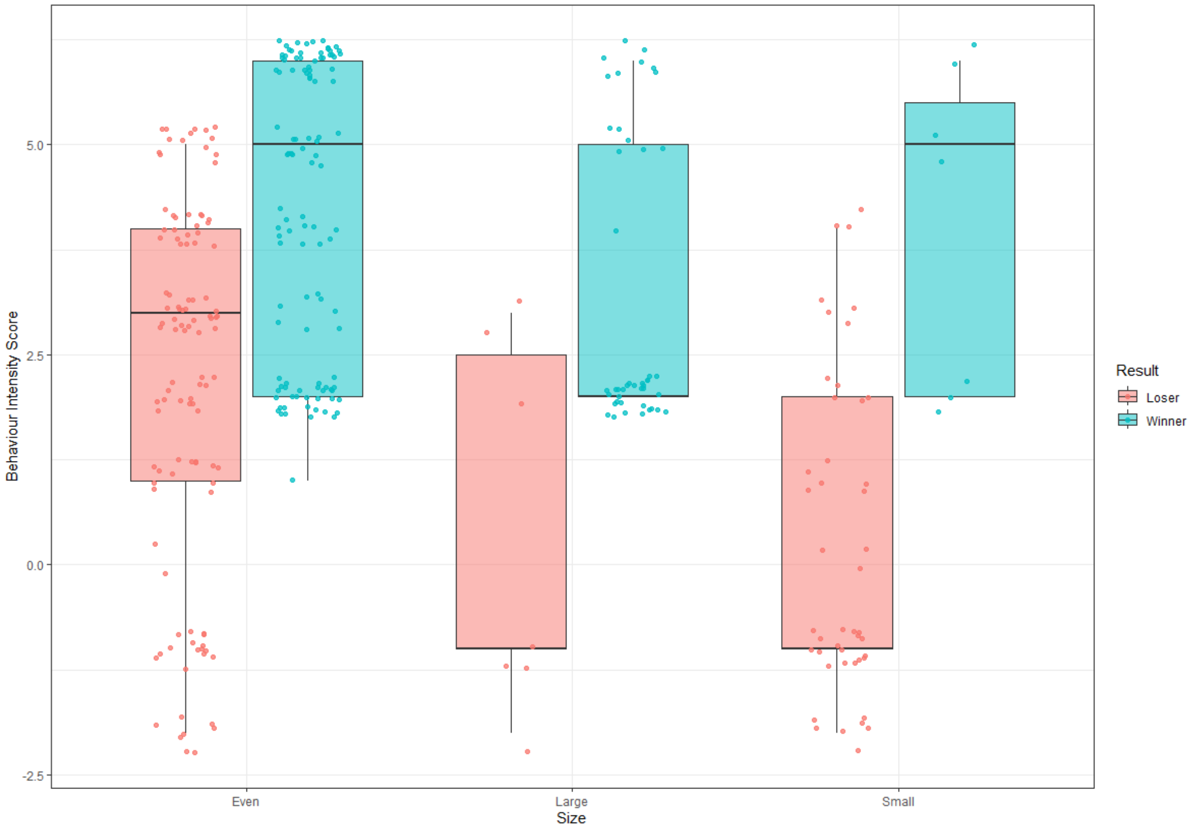


Figure 6.7. Boxplot showing the variation in behaviour intensity scores for each size range and the individuals that were determined either the winner or the loser. The plot shows the median and interquartile range values for each size and result

The least intense behaviour, Meral spread, was observed to last between 1 and 34 seconds with a mean duration of 6.25 seconds (Figure 6.8). Pushing lasted between 1 and 31 seconds with a mean duration of 7.28 seconds (Figure 6.8). Grappling, the most intensive and energy exerting behaviour, was observed to last between 1 and 80 seconds with a mean duration of 9.35 seconds (Figure 6.8).

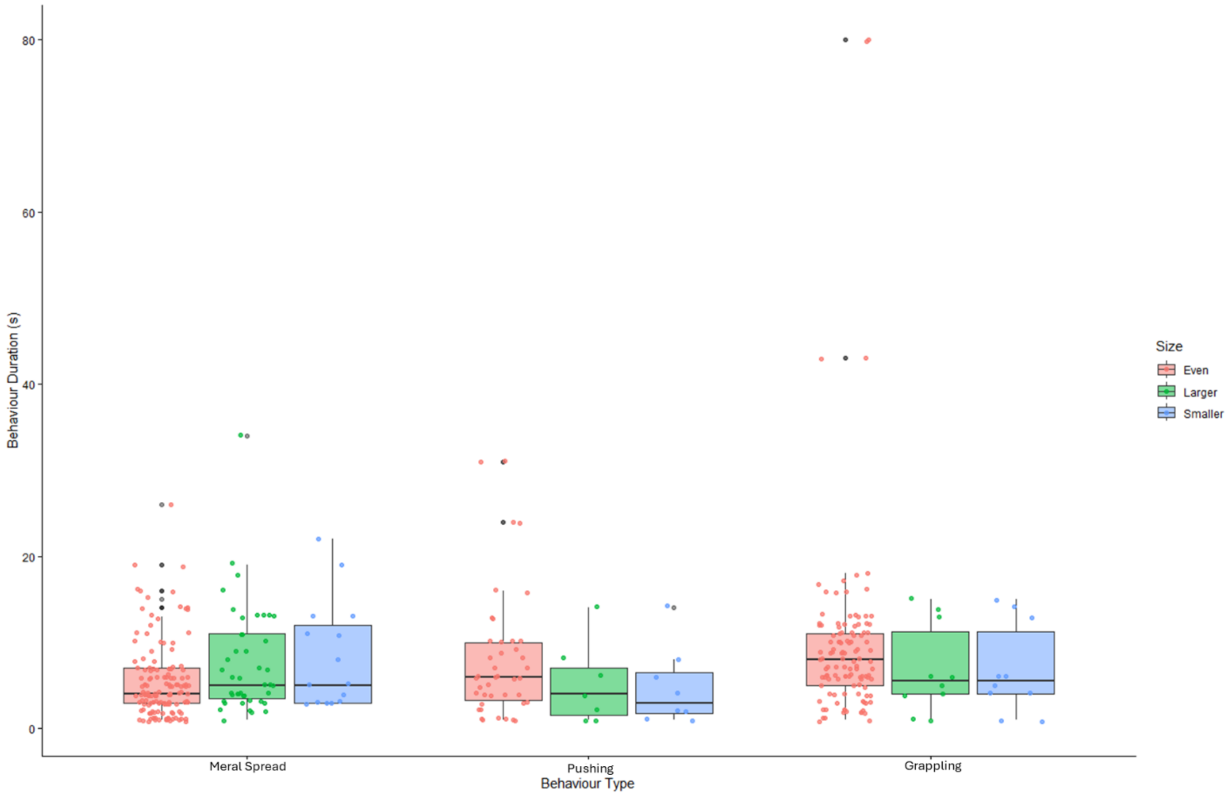


Figure 6.8. Boxplot showing the variation in duration for each aggressive type of behaviour. Durations are split by the size of the individual. The plot shows the median and interquartile range values for each behaviour type and size category. Only the duration of aggressive behaviours has been analysed as submissive behaviours are the act of disengagement from an interaction and these occur instantly and are not a timed event

There was a significant interaction between behaviour type and size on duration of an intraspecific interaction (Table 6.7; Figure 6.8). Size alone had no main effect but behavioural type did. Therefore, I discuss only the interaction effect. Grappling interactions had longer durations than pushing for all size categories, when size disparity occurred meral spread tended to have a longer duration than either grappling or pushing, with pushing interactions having the shortest durations of the three behaviours (Table 6.8; Figure 6.9). When interactions occurred between individuals of even size grappling interactions grappling interactions had a longer duration than either pushing or meral spread, with meral spread interactions being the shortest duration of the three behaviour types (Table 6.8; Figure 6.9).

Table 6.7. Type 3 ANOVA table from a quasipoisson GLM reporting χ^2 effect on size and behaviour type on the duration of an interaction

	χ^2	**df**	**p**
Behaviour	14.27	2	< 0.01
Size Variation	0.03	1	0.86
Behaviour:Size Variation	9.56	2	< 0.01

Table 6.8. A post hoc assessment on the categorical factors using emmeans for the relationship between behaviour type and duration

Contrast	**Size Categories**	**z-ratio**	**p**
Meral Spread – Pushing	mismatch – mismatch	1.42	0.72
Meral Spread – Grappling	mismatch – mismatch	0.52	1.00
Meral Spread – Meral Spread	mismatch – even	2.24	0.22
Meral Spread – Pushing	mismatch – even	-0.16	1.00
Meral Spread – Grappling	mismatch – even	-1.47	0.68
Pushing – Grappling	mismatch – mismatch	-0.88	0.95
Pushing – Meral Spread	mismatch – even	-0.37	1.00
Pushing – Pushing	mismatch – even	-1.47	0.68
Pushing – Grappling	mismatch – even	-2.16	0.26
Grappling – Meral Spread	mismatch – even	0.89	0.95
Grappling – Pushing	mismatch – even	-0.61	0.99
Grappling – Grappling	mismatch – even	-1.46	0.69
Meral Spread – Pushing	even – even	-2.21	0.23
Meral Spread – Grappling	even – even	-4.56	< 0.01
Pushing – Grappling	even – even	-1.14	0.87

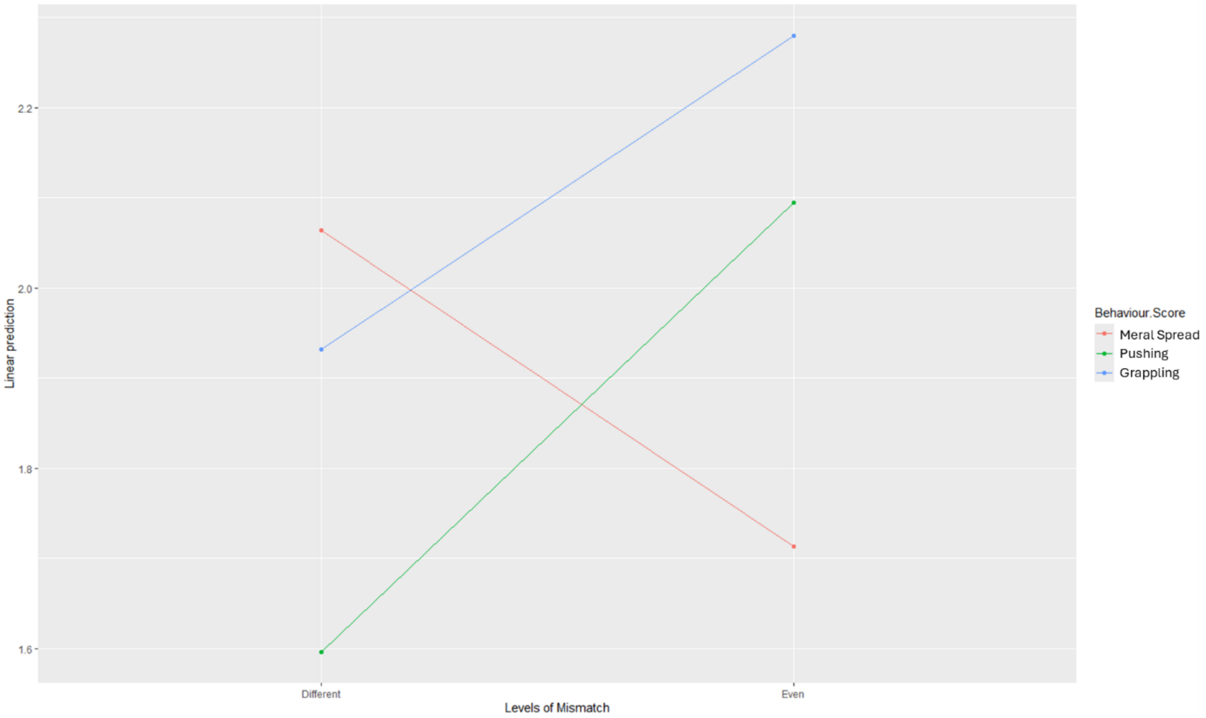


Figure 6.9. Plot showing the nature of interaction between behaviour type and size on duration of an intraspecific interaction

Discussion

Baseline understanding of endangered species natural distribution and behaviour is critical for designing more effective conservation measures, such as resource reintroduction events. The surveyed stretch of the River Kent showed no habitat variable which predicted white-clawed crayfish abundance and demonstrated a healthy and abundant population within these reaches. My results conform to expected game theory whereby larger individuals hold competitive advantage over smaller individuals for resource holding potential. White-clawed crayfish are vulnerable to predation by birds and fish and are believed to remain in refuges during daylight hours to avoid them (Peay & Hirst, 2003). Despite this understanding I obtained several records of white-clawed crayfish being active during the day. It was initially feared this observed abnormal behaviour of daytime activity was a symptom of crayfish plague (Oidtmann et al. 2002) and the population was at risk of extinction. However, the population was surveyed in September 2023 and no evidence was recorded of any other crayfish plague behavioural abnormalities including “walking on stilts” or slow tail escape response (Collas et al. 2016). No mass mortality events or further abnormal behaviours were observed and it was concluded that the population did not have crayfish plague. Similar daylight activity had also been noted by other institutes throughout the UK (J. Nightingale pers. comm), suggesting that this activity is more common than was initially believed. These results can be used to guide future guidance for animal welfare in captive breeding initiatives and reintroduction to ark sites.

RUV Surveys

By using remote underwater video and baited remote underwater video I can introduce a rapid, easy to sterilise tool that can be deployed for short time periods, improving biosecurity and reducing the risk of the spread of disease, including crayfish plague. Baited remote underwater video surveys are more likely to observe species, than those deployed without bait (Donaldson et al. 2020; Harwood et al. 2025, Chapter 2, in review). Despite this, the present study did not observe any difference in the likelihood of a white-clawed crayfish being observed during either an RUV or BRUV deployment as they were present in almost all deployments making presence/absence irrelevant and suggested a very high abundance of crayfish within the River Kent. In this study BRUVs achieved a significantly higher MaxN of white-clawed crayfish than the RUVs did. There are no other comparisons of white-clawed crayfish abundances when using these two methods, however similar trends have been observed in Australia when studying native crayfish (*Euastacus* spp.), with BRUVs detecting significantly more crayfish than RUVs (Skorulis et al. 2021). Other studies comparing BRUVs and RUVs in the UK, that have been conducted on two different invasive crayfish species, the narrow clawed crayfish (*Pontastacus leptodactylus*) and signal crayfish (*Pacifastacus leniusculus*), did not find a significant difference in the densities of crayfish observed by either the RUV or BRUV (Harwood et al. 2025; Harwood et al. in prep). The River Kent catchment in Cumbria is one of the few last UK catchments with healthy white-clawed crayfish populations throughout the main river and tributaries with only a single historical record of an individual signal crayfish from 2012 within the catchment, but it is unknown if this record has been eradicated (Hutton et al. 2023). Total counts and cumulative time on screen values were also significantly higher during BRUV surveys, as opposed to RUV surveys. This trend is expected as total counts and cumulative time on screen are highly correlated to the MaxN and crayfish densities. The high cumulative time on screen is also related to the fact that scavenging behaviour is common among crayfish species, this is likely also related to high population densities increasing intraspecific competition (Imhoff et al. 2012). This is supported by observations made during BRUV deployments for this study saw crayfish investing high levels of energy and spending large amounts of time on the sealed bait arm attempting to access the wet cat food that was present within it, with individuals only giving up after extended periods of time, with interactions lasting as long as 80 seconds.

Despite the presence of bait influencing abundances and duration the total time crayfish were present on screen, there was no significant difference in the time it took for an individual to first be observed. Studies of northern koura crayfish (*Paranephrops planifrons*) concluded that crayfish are naturally inquisitive (Parkyn et al. 2011). This result could also provide an explanation as to why there is no difference in the time to first observation between BRUVs and RUV surveys, with individuals displaying limited neophobia. This may be a result of high crayfish densities within the River Kent reducing shy phenotypes (Su et al. 2024). Furthermore, limited neophobia in white-clawed crayfish could be a reason for population declines, making them more susceptible to predation, or at greater risk of poaching.

Habitat Drivers

This study supports the findings of Hutton et al. (2023) and shows that the white-clawed crayfish population remains strong in the section of the catchment that was studied. High abundances of crayfish, achieving MaxN values higher than previous studies conducted on protected population of white-clawed crayfish in Europe (Grandjean et al. 2000), were found throughout all the study sites and no habitat or environmental variables had a significant influence on the maximum number of crayfish observed in a single frame (MaxN). Unpolluted waterbodies with suitable water chemistry are key factors in white-clawed crayfish survival and population sizes (Smith et al. 1996). Despite distance to sewage treatment works not having a significant impact on determining crayfish abundances, site 1, the only site downstream of the sewage treatment works outflow, obtained far fewer crayfish observations than any other site. Zero observations of crayfish were made by the RUV at site 1 (i.e. closest downstream from the sewage treatment) and the single BRUV deployment that made no crayfish observations was also conducted at site 1. It is possible that the sewage work may be having a negative impact on white-clawed crayfish abundances and solutions should be considered as to how to minimise these, however this survey obtained no conclusive results and using distance from sewage as a variable in analysis instead of distance from source made no difference to the results obtained.

Behavioural Observations

Individuals will compete for limiting resources including food and shelter, the outcome of these is determined by an individual’s fighting ability (Pavey & Fielder, 1996). Engaging in competition poses the risk of injury to an individual, while also exerting high amounts of effort (Lopez et al. 2019). Intraspecific aggression is caused by competition over mates or limited resources, these surveys were conducted outside of the standard breeding season for white-clawed crayfish, therefore it is more likely the competition was for resources (O’Hea Miller et al. 2023). Almost all interactions observed throughout my study occurred during deployments in which bait was present, showing that they were competing for food over other resources. Bait played a key role in initiating the behaviours seen, with individuals either attempting to defend the resource and prevent newcomers getting to it or approaching individuals already on the bait and attempting to take it over. Crayfish have been seen to initiate intraspecific interactions when strangers invade their territory (Bruski & Dunham, 1987) and the limited behaviours observed without bait were in relation to other resource protection, specifically shelter and territory.

The most common aggressive behaviours occurred between individuals of similar size. Individuals of similar size are likely to see each other as a threat due to having comparable abilities to maintain possession of a resource (Gruber et al. 2016). In these situations, initiating an interaction with an individual of similar size is within the best interest of the crayfish as it gives them a higher likelihood of winning and either successfully defending their position on the bait, or successfully overtaking it (Pavey & Fielder, 1996; O'Hea Miller et al. 2023). Body size also plays a factor in a crayfish individual's ability to obtain resources (Rabeni, 1985). In instances in which there was a size variation between crayfish the larger individual "won" the interaction on most occasions. These interactions would require less energy from them than they are likely to expend (Ferderer et al. 2022).

Contact and non-contact interactions were observed. Crayfish can only tolerate certain levels of damage that occurs as a result of fighting and are selected to minimise confrontations that risk damage (Kubec et al. 2019). The most common behaviour observed was Meral spread, the mean duration of which was lower than the other two forms of aggressive behaviour and in just under half of the Meral spread observations the interaction escalated to a more intense fight. In instances when escalation did not occur one of the individuals opted to retreat before any contact was initiated, likely due to assessing the physical costs of the interaction and the resulting reward not being worth the effort (Kubec et al. 2019). The duration of Meral spread behaviour between evenly sized individuals was slightly shorter than instances with size variation and more aggressive interactions had longer durations in evenly matched individuals as these individuals assessed that they were more likely to win an evenly matched interaction and therefore are willing to commit more energy to them. The high scoring grappling posed the highest risk of physical damage, including the loss of a body part. Although I did not witness any loss of body parts throughout my surveys, individuals were observed in the footage missing chelae and parts of their rostrum, likely because of intraspecific competition.

My surveys made several observations of high densities of white-clawed crayfish during daytime suggesting that the crayfish are highly active during daylight hours. There are several possibilities for this outcome, firstly, our understanding of white-clawed crayfish being predominantly nocturnal could be incorrect, and they are more active during daylight hours than originally believed. Alternatively, it could potentially be due to the high density of crayfish at this site, meaning refuge is at a premium. The rocky cobble nature of the substrate in the River Kent could potentially have resulted in fewer shelter opportunities for the white-clawed crayfish. The availability of shelter has previously been seen to influence white-clawed crayfish activity around artificial sunrise events in a laboratory, with peaks being observed in simulations with an absence of refuge, and not in those with refuge (Barbaresi & Gherardi, 2001). Another option could be because of reduced predator stressors within this study site. Only one instance of a predator stressor was observed during the entire survey. This instance of a *Cottus gobio* attacking a smaller crayfish was unsuccessful but shows that this population of crayfish is not completely devoid of predator stressors. The upper sections of my survey sites are shallow and there is clear separation, through a waterfall, from the lower regions. This fragmentation could mean there are fewer larger salmonid, eels, perch and pike, all of which are known to be predators of white-clawed crayfish (Holdich & Lowery, 1988). Other known predators, including otter and heron (Reynolds, 1998) are less likely to have been observed by the camera systems due to their terrestrial nature, but otters are known to be present within the area as several otter scats were observed during the surveys. Interspecies interactions were far fewer than initially predicted, with only one other interspecies interaction being observed. This instance showed a crayfish aggressively warning *Phoxonius phoxonius* approaching the bait, demonstrating resource defence one of the main reasons for interspecies interactions (O'Hea Miller et al. 2023).

Conclusion

Underwater videos provide a non-invasive low-cost method that can be used to observe vulnerable species in their natural environments (Broom et al. 2022). By observing native white-clawed crayfish in their natural environment I have been able to show that they have high levels of activity during daylight hours, despite what current and historical literature suggests. I made several observations of crayfish active during daylight hours, with high volumes of intraspecific aggressive interactions occurring. Before the crayfish commit to intraspecific interactions they assess the likelihood of winning the interaction and will only compete if they believe they can win, following game theory, assessing if the reward is worth the risk. In a waterbody like the River Kent, where conditions are suitable and external stressors are limited, the population of white-clawed crayfish has been allowed to thrive and the River Kent catchment is one of the last strongholds of white-clawed crayfish within the United Kingdom. Appropriate signage and information must be distributed throughout the catchment to ensure biosecurity protocols are followed by the large volume of tourists that visit the area each year to reduce the risk of population loss through unintentional spread of crayfish plague or invasive crayfish species. If biosecurity can be reinforced throughout the catchment, then I would also recommend the River Kent catchment as a potential white-clawed crayfish haven, with opportunities for river supplementation and crayfish ark sites throughout.

Chapter 7

Pathogen diversity of the non-native narrow clawed crayfish (*Pontastacus leptodactylus*) in a UK water body

Harwood, M., South, J., Dunn, A.M., Stebbing, P.D., Burgess, A., Bojko, J. (2025) Pathogen diversity of the non-native narrow-clawed crayfish (*Pontastacus leptodactylus*) in a UK water body, Journal of Invertebrate Pathology, 2025, 108458, ISSN 0022-2011, https://doi.org/10.1016/j.jip.2025.108458.

Abstract

Biological invasions are intrinsically linked to introducing associated symbiotic organisms, some of which can be parasitic or pathogenic. The pathogenic risk of an 'invasive parasite' (aka. exotic pathogen) stems from its potential to infect native hosts and induce behavioural change or mortality, with the pathogen potentially presenting a greater risk than the host. Conversely, parasites translocated by invasive hosts may also reduce the impact of their host, indirectly curbing the hosts impact on the invaded ecosystem. In this study, I develop a pathogen profile for the narrow clawed crayfish, *Pontastacus leptodactylus*. This is a non-native species in the United Kingdom, and poses a possible risk as a sink for invasive parasites. I use histopathology, metagenomics and metratranscriptomics to outline the symbiotic diversity harboured by a *P. leptodactylus* population from West Yorkshire, England. I discovered several protozoan and bacterial species that appear to be putatively commensal with this invader, as well as several RNA viruses (*Hepelivirales*; *Picornavirales*; *Nodaviridae*, and others) that may be more pathogenic in nature. Microsporidia and *Nudiviridae* were absent in my population sample set, as were all metazoan obligate parasites, such as trematodes and acanthocephalans. Using the novel genomic and pathological data available, I have explored the evolutionary history of each symbiotic species and provided an initial assessment on the putative risk to native species.

Introduction

Invasive Non-Native Species (INNS) pose a significant risk to native wildlife, cultured species, and human health, due to their capacity to carry and transmit exotic symbionts, such as parasites and pathogens (also termed, "invasive parasites"; Dunn et al. 2009a; Dunn and

Hatcher, 2015; Dunn et al. 2023). These invasive parasites have been shown to affect invasion systems in diverse ways: they can hinder an invader by eliciting population control through behavioural modification, or a reduction in survival (Bojko et al. 2019); or they may go on to infect native or economically important species (Wood et al. 2023), causing population declines (Svoboda et al. 2017) or other wildlife health impacts (Hatcher et al 2019). Most notably from freshwater environments, *Aphanomyces astaci* (causative agent of crayfish plague) can result in mass white-clawed crayfish (*Austropotamobius pallipes*) mortalities, and originates from the invasive asymptomatic host, the signal crayfish (*Pacifastacus leniusculus*; Svoboda et al. 2017).

Outside of the well-studied crayfish plague pathogen, crayfish invasions are also commonly associated with co-invasive symbionts within the groups: Microsporidia (Bojko et al. 2020; Stratton et al. 2021; Stratton et al. 2022a-b; Stratton et al. 2023a-b; Stratton et al. 2024a); *Nudiviridae* (Stratton et al. 2024b; Petersen et al. 2024); *Psorospermium* sp. (Anaya, 2021; Longshaw et al. 2012); Branchiobdella (Rosawarne et al. 2012); and trematodes (Reisinger et al. 2015). For example, the *P. leniusculus* invasion of the UK has been linked closely with the spread of *A. astaci*, but also the presence of a 'bacilliform virus', *Psorospermium* sp., branchiobdellids, and the likelihood of acquiring native microsporidian species such as *Astathelohania contejeani* (Dunn et al. 2009b; Anderson et al. 2021). Studies such as Anderson et al. (2021) provide geographical detail on symbiont dispersal through invasive/native networks that help to define possible emerging disease risk in wildlife.

Of recent concern to the UK is the narrow clawed crayfish (*Pontastacus leptodactylus*) and the pathogens that it may harbour. Narrow clawed crayfish have been broadly introduced across Europe for aquaculture. They are considered data deficient in terms of ecological impact. However, species range is predicted to shift following climate niche changes expected across Europe making them a cause for concern in the future (Hodson et al. 2024). Although *P. leptodactylus* are distrubted across England (Peay et al. 2010), we do not know what pathogens may have been co-introduced, or whether they have acquired pathogens in the new range.

Bojko et al. (2021) identified 23 symbionts associated with *P. leptodactylus* from their native and invasive ranges from literature published up to 2017. These included: Fungi (*Saprolegnia parasitica* and *Acremonium* sp.); bacteria (*Listeria monocytogenes* and *Aeromonas hydrophila*); Microsporidia (*A. contejeani*); Protozoa (*Psorospermium haeckeli*, *Branchiobdella* spp., *Chilodonella* spp., *Cothurnia sieboldii*, *Epistylis* spp., *Histricosoma chappuisi, Opercularia articulata, Podophrya fixa, Pyxicola annulata, Tetrahymena pyriformis, Vorticella similis, Zoothamnium intermedium,* and *A. astaci*); Trematoda

(*Astacotrema tuberculatum*); and *Nimaviridae* (WSSV). Post 2017, four new symbionts have been identified to date. These include: the Fungi *Fusarium solani* (Salighehzadeh et al. 2019), *Fusarium avenaceum* (Taştan and Akhan, 2021); the bacterium *Shewanella putrefaciens* (Kuzucu and Özcan, 2025), and a nudivirus detected through transcriptomic data: 'Astacus leptodactylus nudivirus', which should be putatively termed: 'Pontastacus leptodactylus nudivirus' (AlNV to PlNV; Petersen et al. 2024) due to the recent taxonomic change.

A combination of technologies can be used to develop pathological surveys of invasive populations, increasing our understanding of pathogen risk by building a baseline pathological view of invasive populations (Foster et al. 2021; Bojko et al. 2023). Such technologies can include classical pathological techniques, such as histology and electron microscopy, and also encompassing more recent techniques, such as metagenomics and metatranscriptomics, which can provide detailed sequence data from an array of pathogens in an unbiased manner.

The aim of this study is to use histology, metagenomics and metatranscriptomics, to screen narrow clawed crayfish for symbionts, to better understand the potential for control, and to determine whether they house pathogens that may pose a risk to native species.

Methods

Specimen collection and husbandry

Narrow clawed crayfish (n=20), *P. leptodactylus*, were collected from Boshaw Whams Reservoir, Holmfirth, West Yorkshire, UK (Lat 53°32′52″N, Long 001°46′23″W) between October 2022 and December 2022. *P. leptodactylus* were first detected around 2014 and local anglers started to report them as a nuisance in 2019 (pers. comm Huddersfield Angling Club). Crayfish were collected under a Natural England trapping license using collapsible fladen crayfish traps (570mm x 290mm, 25mm mesh size) deployed overnight at the reservoir and retrieved after 18 hours. All crayfish were transported to the University of Leeds, West Yorkshire and housed in sex segregated holding tanks. The animals were anaesthetised before dissection by being placed in a -20°C freezer for 10 minutes, following the methods described by Bojko et al. (2022).

Histological preparation

Twenty *P. leptodactylus* were prepared for histological analysis, where the muscle, nerve, gill, gonad, heart, gut, hepatopancreas, and antennal gland were biopsied and placed into a single labelled cassette, per crayfish. The tissues were fixed in Davidson's freshwater fixative, and then moved into 70% ethanol after 24h. Tissue processing included 90% and

100% ethanol infiltration, prior to 2 baths of xylene-substitute, and finally paraffin wax. The tissues were solidified into a block of paraffin wax including the labelled cassette. Each block was sectioned at 3µm, and the resulting sections were adhered to glass slides. The slides were stained using a haemotoxylin and alcoholic eosin protocol (see Bojko et al. 2022). The slides were read on a Leica compound microscope and images were taken using a Leica integrated camera.

Next generation sequencing and bioinformatics

The same 20 crayfish that were prepared for histology, also had corresponding muscle, gill and hepatopancreas preserved in 2 ml of 99% ethanol. These tissues underwent both DNA and RNA extraction using Wizard extraction kits (Promega). The hepatopancreas of samples C6, C13, and C16 were submitted as individual RNA and DNA extracts for metagenomic and metatranscriptomic analysis. The remaining DNA and RNA extracts from all tissues were pooled into two corresponding batches for sequencing: crayfish samples C1-C10 (excluding C6) were pooled, and crayfish samples C11-C20 (excluding C13 and C16) were pooled, separately for RNA and DNA. This resulted in 5 DNA samples for metagenomics, and 5 RNA samples for metatranscriptomics. The samples were submitted to Novogene, where they underwent library preparation and were sequenced on an Illumina NovaSeq. Each sample provided 10Gb of paired data, which were delivered to the laboratory for bioinformatic processing.

The files were initially trimmed using Trimmomatic (Bolger et al. 2014; parameters: LEADING:3 TRAILING:3 SLIDINGWINDOW:4:15 MINLEN:36) and then assembled using SPAdes v4.0.0 (Bankevich et al. 2012). The contiguous sequence files from each sample, including samples 3, 13, 16, and the two pooled samples, for both DNA and RNA data, were then used to screen for the presence of symbionts. For the DNA samples, metaxa2 (Bengtsson-Palme et al. 2015) was used to mine out the presence of bacterial and eukaryotic species, by searching for 16S/18S sequences. The DNA datasets were also screened for DNA viruses, using a bespoke DNA virus database, which was built from Refseq DNA viruses on NCBI (August 2024). The RNA datasets were screened for RNA viruses using a bespoke RNA virus database, built from Refseq RNA viruses on NCBI (August 2024). Sequences that indicated possible viral genomes were collected from the contiguous files and completed where necessary. Each viral contig was mapped using the trimmed forward and reverse data in CLC genomics v.12, and then annotated using GeneMarkS (Besemer et al. 2001).

Phylogenetics and sequence analysis

Viral genomes and their annotations were used to explore their evolutionary origin using blastn, blastp, and blastx. The available protein sequences were used to develop maximum-likelihood phylogenetic trees, all of which underwent 1000 bootstraps and took place using IQ-TREE (Nguyen et al. 2015), after MAFFT alignment. Each viral group was separately explored and the specific details pertaining to each tree are provided in the caption of the relevant figure, including the evolutionary model used, which was predicted in IQ-TREE using Bayesian Information Criterion. Determination of protein function was explored using InterProScan (Jones et al. 2014), and HHpred (Zimmermann et al. 2018), where the following parameters were used to reduce unlikely function assignment: >75% probability; e-value >0.1e^{-5}; p-value <0.05 (database: PDB_mmCIF70_3_Jan).

Data are available from project code PRJNA1246988 and related biosamples, stored on NCBI.

Results

Protozoan associations

Histological preparation of *P. leptodactylus* tissues resulted in the detection of two symbiont groups: gregarines (Figure 7.1A) and ciliated protozoans (Figure 7.1B). Gregarines were detected in 15/20 and ciliates in 8/20 individuals The gregarines presented within the gut tissue as elongate single-celled masses, lined against the gut epithelium and bolus (Figure 7.1A), but no molecular detection of this species was made in the metagenomic or metatranscriptomic data from HP, gill and muscle tissues. There wasn’t evidence of pathology due to the presence of the gregarines alongside the gut epithelial tissues. The ciliated protozoans detected histologically in the gill (Figure 7.1B) with no relation to any athological effect, and were also detected within my metagenomic data, as *Epistylis cambari* (Ciliophora; OQ924989; 61% cov.; 98.43% sim.; e-value: 0.0; Table 7.1). This species was not detected in the HP metagenomic assessments, but was detected in my pooled sample approach which included gill, muscle, and HP together (Table 7.1).

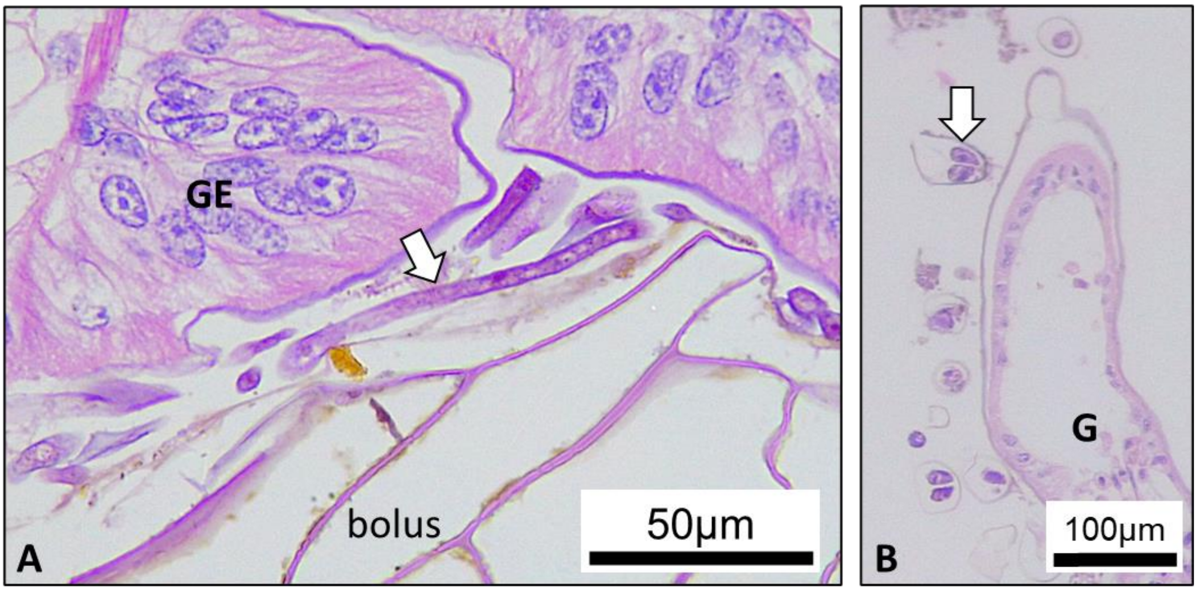


Figure 7.1. Protozoa detected in histological section, from *Pontastacus leptodactylus*. A) Gregarine parasites (white arrow) lined against the gut epithelium (GE) and bolus. B) Ciliated protozoa (likely Ciliophora; white arrow), stemming from a single gill lamella (G)

Table 7.1. Protozoan 18S data derived from the metaxa2 analysis of available NGS datasets collected within this study. The BLAST analysis results are included in this table, highlighting the relevant taxonomy and closest identified organism

Isolate	**Organ**	**Animal**	**Length (bp)**	**Contig Coverage**	**NCBI Accession**	**Similarity (%)**	**Coverage (%)**	**E Value**	**Associated Species**
32372	Gill/Muscle/HP	C1-10 pool	628	1.324864	AY753614	99.45	56	0.0	*Neobodo designis*
82	Gill/Muscle/HP	C1-10 pool	2597	16.96123	OQ924989	98.43	61	0.0	*Epistylis cambari*
154056	Gill/Muscle/HP	C1-10 pool	354	1.054152	AF484686	100.00	100	0.0	*Nuclearia moebiusi*
129	Gill/Muscle/HP	C11-20 pool	2597	5.115136	OQ924989	98.43	61	0.0	*Epistylis cambari*

Metagenomic analysis of DNA extracts from hepatopancreas preps from individuals 6, 13, and 16 did not reveal any detectable protozoan diversity, suggesting a lack of protozoa in this tissue type from these three individuals. However, pooled samples that included both gill and muscle in two batches, 1-10 and 11-20, both revealed further diversity. The pooled sample 11-20 only picked up the *E. cambari* noted above. Pooled sample 1-10 picked up greater diversity, including *Neobodo designis* (Excavata; AY753614; 56% cov.; 99.45%; e-value: 0.0) and *Nuclearia moebiusi* (Choanozoa; AF484686; 100% cov.; 100% sim.; e-value: 0.0). The presence of these final two species were not detected histologically.

Putative bacterial associations

In the histological sections, a series of undetermined pathologies within the hepatopancreas were detected in single indidivuals (Figure 7.2). Healthy tissue from one individual (Figure 7.2A) was compared to four other animals, which presented abnormal pathologies with no specific designation. Putative assignment may involve bacterial or viral origin; however, this is discussed later. Cytoplasmic inclusions were present in specimen 6 (Figure 7.2B); however, no bacterial symbiont was detected within the metagenomic data. Agents of viral origin are explored in section 3.3. Specimen 16 displayed hypertrophic basophilic nuclei (Figure 7.2C), and a deep eosinophilic staining hepatopancreatic cytoplasm. Again, molecular analysis via metagenomics did not detect bacterial symbionts in this HP DNA preparation. In specimen 13, the hepatopancreas displayed large clear cytoplasmic inclusions alongside smaller eosinophilic inclusions (Figure 7.2D), where typical basophilic staining nuclei of an appropriate size are also seen. Metagenomic data for this sample did pick up one bacterial species, which was *Staphylococcus epidermidis* (Staphylococcaceae; CP052985; 100% cov.; 100% sim.; e-value: $6e^{-117}$). Specimen 20 presented small, long green particles within the HP cytoplasm, which were considered unknown in origin. This specimen was a part of the pooled 11-20 batch, which was the source of significant bacterial diversity (Table 7.2). In addition, pooled batch 1-10 also presented significant bacterial diversity (Table 7.2).

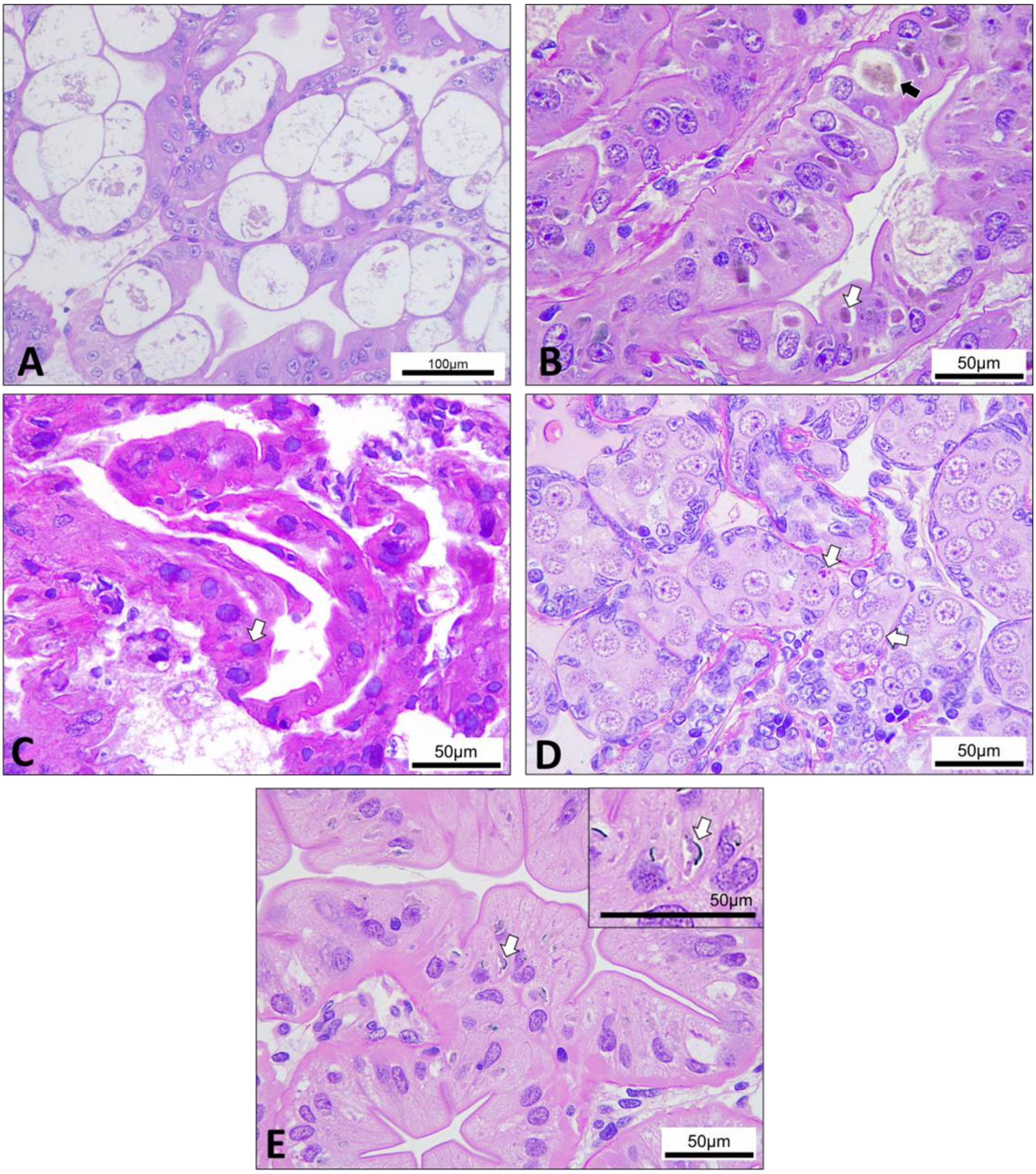


Figure 7.2. Various unclassified pathologies located in the hepatopancreas of *Pontastacus leptodactylus* under haemotoxylin and eosin staining, where each was only observed once in my 20-crayfish sample. A) A view of a healthy 'normal' hepatopancreas for comparison. B) Basophilic cytoplasmic inclusions within the hepatopancreatocytes (white arrow), which may develop further to result in cell degradation (black arrow). C) Heavily degraded hepatopancreas with hypertrophic basophilic nuclei in the hepatopancreas (white arrow). D) Large cytoplasmic inclusions in the hepatopancreas appear spherical and clear, or as small eosinophilic inclusions (white arrows). E) Green-staining elongate cytoplasmic inclusions within the hepatopancreas

Table 7.2. A blast results table of the bacterial 16S sequences identified by Metaxa2, from the various NGS datasets collected during this study

Isolate	**Organ**	**Animal**	**Length (bp)**	**Contig Coverage**	**NCBI Accession**	**Similarity (%)**	**Coverage (%)**	**E Value**	**Associated Species**
646264	Hepatopancreas	C13	234	0.929936	CP052985	100.00	100	6e-117	*Stphyloccocus epidermidis*
10150	Gill/HP/Muscle	Pooled	823	1.43572	JQ692099	91.75	96	0.0	*Flavobacterium terrigena*
					FJ718901	94.33	96	0.0	Uncultured bacterium clone
102633	Gill/HP/Muscle	Pooled	160	2.01548	MH838009	91.25	100	2e-52	*Runella aurantiaca*
					LR636054	100.00	100	5e-76	Uncultured bacterium
181617	Gill/HP/Muscle	Pooled	260	1.403846	MW142058	100.00	100	2e-131	*Xanthomonas maliensis*
190139	Gill/HP/Muscle	Pooled	332	2.078431	KF228157	96.32	98	7e-148	*Nitrosomonas oligotropha*
					EU224342	98.12	96	1e-154	Uncultured bacterium clone 4D
228968	Gill/HP/Muscle	Pooled	314	1.236287	OK342099	93.65	100	6e-128	*Sediminibacterium* sp.
					KX652468	94.90	100	1e-134	Uncultured bacterium clone OTU 61
28251	Gill/HP/Muscle	Pooled	677	1.041667	CP042433	96.41	76	3e-69	*Flavisolibacter ginsenosidimutans*
2983	Gill/HP/Muscle	Pooled	1457	1.906166	KX505858	96.37	100	0.0	*Rhodoferax lacus*
31410	Gill/HP/Muscle	Pooled	499	2.342246	PQ782258	100.00	100	0.0	*Pseudomonas fluorescens*
317378	Gill/HP/Muscle	Pooled	284	1.057971	OR136292	99.65	100	6e-143	*Rhizobacter profundi*
344660	Gill/HP/Muscle	Pooled	277	1.825000	AB355702	100.00	100	9e-141	*Thermomonas brevis*
370875	Gill/HP/Muscle	Pooled	272	1.497436	NR074303	99.13	84	9e-111	*Leadbetterella byssophila*
386952	Gill/HP/Muscle	Pooled	269	1.161458	MT910335	98.14	100	5e-128	*Microvirga* sp.
387633	Gill/HP/Muscle	Pooled	269	1.119792	PP341839	100.00	100	2e-136	*Tabrizicola* sp.
429055	Gill/HP/Muscle	Pooled	259	0.886486	MW486538	98.07	100	2e-122	*Chryseobacterium* sp.
454119	Gill/HP/Muscle	Pooled	258	0.839779	CP034328	91.63	82	4e-75	*Tabrizicola piscis*
					CP136571	98.21	43	1e-45	*Fuscovulum* sp.

494379	Gill/HP/Muscle	Pooled	252	0.834286	CP016592	97.24	100	9e-116	*Ketogulonicigenium*
578718	Gill/HP/Muscle	Pooled	241	0.890244	AB920567	100.00	100	8e-121	*Arthrobacter alpinus*
620735	Gill/HP/Muscle	Pooled	236	1.377358	NR064420	99.15	100	1e-114	Haliscomenobacter hydrossis
64403	Gill/HP/Muscle	Pooled	467	1.241026	CP030850	96.35	77	3e-162	*Runella rosea*
659587	Gill/HP/Muscle	Pooled	232	1.412903	KX981406	86.70	100	4e-64	*Niastella* sp.
					LR637787	97.84	100	2e-107	Uncultured bacterium partial
683464	Gill/HP/Muscle	Pooled	230	0.954248	EF540472	98.91	80	3e-85	*Flavobacterium* sp.
					KC255331	99.51	88	5e-98	Uncultured bacterium clone GMM_40
685155	Gill/HP/Muscle	Pooled	135	0.856209	MN620434	100.00	100	3e-62	*Thermomonas* sp.
86231	Gill/HP/Muscle	Pooled	317	0.959538	CP060007	97.48	100	1e-149	*Lacibacter sediminis*
9363	Gill/HP/Muscle	Pooled	852	2.231717	CP015225	93.15	100	0.0	*Pseudomonas fluorescens*
110442	Gill/HP/Muscle	Pooled	351	0.532847	OQ359397	94.17	87	3e-127	*Simplicispira piscis*
15946	Gill/HP/Muscle	Pooled	461	0.854975	JN679215	93.51	83	9e-158	*Terrimonas* sp.
27873	Gill/HP/Muscle	Pooled	533	0.791667	NR148792	94.65	98	0.0	*Lampropedia cohaerens*
289926	Gill/HP/Muscle	Pooled	267	0.768421	CP042582	93.55	52	8e-42	*Hypericibacter adhaerens*
					HQ114192	100.00	49	1e-59	Uncultured bacterium clone
440529	Gill/HP/Muscle	Pooled	151	0.790123	CP002104	100.00	100	5e-71	*Gardnerella vaginalis*
515085	Gill/HP/Muscle	Pooled	229	0.789474	PP345989	93.39	99	5e-88	*Flavobacterium granuli*
68997	Gill/HP/Muscle	Pooled	400	0.804954	AM492750	97.20	63	7e-114	*Flavobacterium hercynium*
					CP038810	87.68	100	1e-125	*Flavobacterium sangjuense*

Viral associations

A blastx analysis of the metagenomic datasets revealed no presence of complete viral genomes, outside of small fragments with low levels of similarity and e-value support. However, metatranscriptomic analysis of the available RNA sequence data revealed a range of complete and partial RNA viruses within the series of specimens. The complete genomes of eight RNA viruses were identified from metatranscriptomic data from *P. leptodactylus*, alongside a further four partial RNA virus genomes (Suppl. Fig. 2). Complete genomes included five hepe-like viruses, two nodaviruses, and a tombusvirus. Partial genomes included a dicistrovirus, a tombusvirus, and two toti-like viruses. Each virus showed some protein similarity to viruses stored in NCBI (Table 7.3).

Table 7.3. RNA virus proteins are included in this table, alongside their blastp comparison result. The table indicates the most closely related known virus, identified prior to this study

Virus Name	**ORF**	**Animal**	**Length (aa)**	**NCBI Accession**	**Similarity (%)**	**Coverage (%)**	**E Value**	**Associated Taxon**
Pontastacus_leptodactylus_Dicistrovirus_C16-455	1	C16	826	XII42478	69.24	100	0.0	clirnapec virus 239
Pontastacus_leptodactylus_Hepevirus_C13-48	1	C13	1897	CAJ2444841	53.64	31	2e-180	*Astacus astacus hepevirus*
Pontastacus_leptodactylus_Hepevirus_C13-48	2	C13	471	CAJ2358127	74.57	99	0.0	*Astacus astacus hepevirus*
Pontastacus_leptodactylus_Hepevirus_C13-48	3	C13	254	CAJ2444836	54.00	98	6e-89	*Astacus astacus hepevirus*
Pontastacus_leptodactylus_Hepevirus_C13-48	4	C13	95	No significant similarity found				
Pontastacus_leptodactylus_Hepevirus_C13-48	5	C13	107	No significant similarity found				
Pontastacus_leptodactylus_Hepevirus_C13-48	6	C13	415	CAJ2444837	72.02	99	0.0	*Astacus astacus hepevirus*
Pontastacus_leptodactylus_Hepevirus_C13-48	7	C13	155	CAJ2444838	56.21	99	1e-51	*Astacus astacus hepevirus*
Pontastacus_leptodactylus_Hepevirus_C13-319	1	C13	1855	WAY16406	50.30	9	3e-40	*Hepelivirales* sp.
Pontastacus_leptodactylus_Hepevirus_C13-319	2	C13	540	WAY16407	43.37	83	1e-106	*Hepelivirales* sp.
Pontastacus_leptodactylus_Hepevirus_C13-376	1	C13	1894	CAJ2444841	64.79	91	0.0	*Astacus astacus hepevirus*
Pontastacus_leptodactylus_Hepevirus_C13-376	2	C13	550	CAJ2358127	81.93	100	0.0	*Astacus astacus hepevirus*

Pontastacus_leptodactylus_Hepevirus_C13-376	3	C13	202	CAJ2358133	71.29	100	3e-94	*Astacus astacus hepevirus*
Pontastacus_leptodactylus_Hepevirus_C13-376	4	C13	418	CAJ2358134	65.16	100	0.0	*Astacus astacus hepevirus*
Pontastacus_leptodactylus_Hepevirus_C13-376	5	C13	123	No significant similarity found				
Pontastacus_leptodactylus_Hepevirus_C13-376	6	C13	169	CAJ2358130	53.21	92	8e-52	*Astacus astacus hepevirus*
Pontastacus_leptodactylus_Hepevirus_C16-8	1	C16	1897	CAJ2444841	53.64	31	2e-180	*Astacus astacus hepevirus*
Pontastacus_leptodactylus_Hepevirus_C16-8	2	C16	471	CAJ2358127	74.57	99	0.0	*Astacus astacus hepevirus*
Pontastacus_leptodactylus_Hepevirus_C16-8	3	C16	254	CAJ2444836	54.00	98	6e-89	*Astacus astacus hepevirus*
Pontastacus_leptodactylus_Hepevirus_C16-8	4	C16	95	No significant similarity found				
Pontastacus_leptodactylus_Hepevirus_C16-8	5	C16	107	No significant similarity found				
Pontastacus_leptodactylus_Hepevirus_C16-8	6	C16	415	CAJ2444837	72.02	99	0.0	*Astacus astacus hepevirus*
Pontastacus_leptodactylus_Hepevirus_C16-8	7	C16	155	CAJ2444838	56.21	99	1e-51	*Astacus astacus hepevirus*
Pontastacus_leptodactylus_Hepevirus_C16-9	1	C16	1894	CAJ2444841	64.79	91	0.0	*Astacus astacus hepevirus*
Pontastacus_leptodactylus_Hepevirus_C16-9	2	C16	550	CAJ2358127	81.57	100	0.0	*Astacus astacus hepevirus*
Pontastacus_leptodactylus_Hepevirus_C16-9	3	C16	202	CAJ2358133	71.29	100	3e-94	*Astacus astacus hepevirus*
Pontastacus_leptodactylus_Hepevirus_C16-9	4	C16	418	CAJ2358134	65.16	100	0.0	*Astacus astacus hepevirus*
Pontastacus_leptodactylus_Hepevirus_C16-9	5	C16	123	No significant similarity found				

Pontastacus_leptodactylus_Hepevirus_C16-9	6	C16	169	CAJ2358130	53.21	92	8e-52	*Astacus astacus hepevirus*
Pontastacus_leptodactylus_Nodamuravirus_C13-3553	1	C13	967	XKB76444	80.60	99	0.0	*Nodamuvirus*
Pontastacus_leptodactylus_Nodamuravirus_C13-3555	1	C13	1031	XKB76444	80.74	100	0.0	*Nodamuvirus*
Pontastacus_leptodactylus_Tombusvirus_C13-873	1	C13	260	YP_009336878	30.77	45	3e-07	Hubei tombus-like virus
Pontastacus_leptodactylus_Tombusvirus_C13-873	2	C13	409	UBJ25992	28.40	37	5e-05	Sichuan mosquito tombus-like virus
Pontastacus_leptodactylus_Tombusvirus_C13-873	3	C13	502	UBJ25993	58.08	67	5e-142	Sichuan mosquito tombus-like virus
Pontastacus_leptodactylus_Tombusvirus_C13-873	4	C13	627	XKB76289	41.41	16	7e-16	*Tombusviridae*
Pontastacus_leptodactylus_Tombusvirus_C13-5563	1	C13	296	WRQ65157	27.78	49	8e-07	*Tombusviridae*
Pontastacus_leptodactylus_Tombusvirus_C13-5563	2	C13	465	WRQ65158	42.70	78	9e-80	*Tombusviridae*
Pontastacus_leptodactylus_Totivirus_C16-10330	1	C16	268	UHS72454	51.06	18	3e-07	*Totiviridae*
Pontastacus_leptodactylus_Totivirus_C16-11810	1	C16	248	UHS72490	53.56	94	2e-66	*Totiviridae*

The partial dicistrovirus genome (‘Pontastacus leptodactylus Dicistrovirus C16-455’; PlDC16-455) (PV454206) isolated from crayfish C16 was 3547 bp in length (GC% = 51%), and encoded a partial single polyprotein (826 aa). This protein showed greatest similarity to a polyprotein from ‘clirnapec virus 239’ (XII42478; sim. 69.24%; cov. 100%; e-value: 0.0). InterProScan analysis of the partial polyprotein revealed the following categorised domains: 1-216 region, *Picornavirales* 3C/3C-like protease domain profile (IPR044067); 352-736 region, ‘*Dicistroviridae*_RdRp’ (IPR001205). The uncategorised region (217-351) was assessed using HHpred to determine possible function. This analysis determined that this region likely encodes an undetermined transferase (HHpred; probability: 99.84; e-value: $3.1e^{-20}$). The uncategorised region (737 - 826) was also assessed using HHpred to determine possible function, determining that it may have a hydrolase function, but with low probability (HHpred; probability: 17.61; e-value: 84). Phylogenetic comparison and sequence demarcation analysis of the RdRP region of the novel dicistrovirus, determined that it grouped with viral isolates collected from freshwater bivalves (*Ortmanniana pectorosa*) originating from Virginia (USA), and that it forms a sister branch to the *Cripavirus* genus within the *Dicistroviridae* (Figure 7.3).

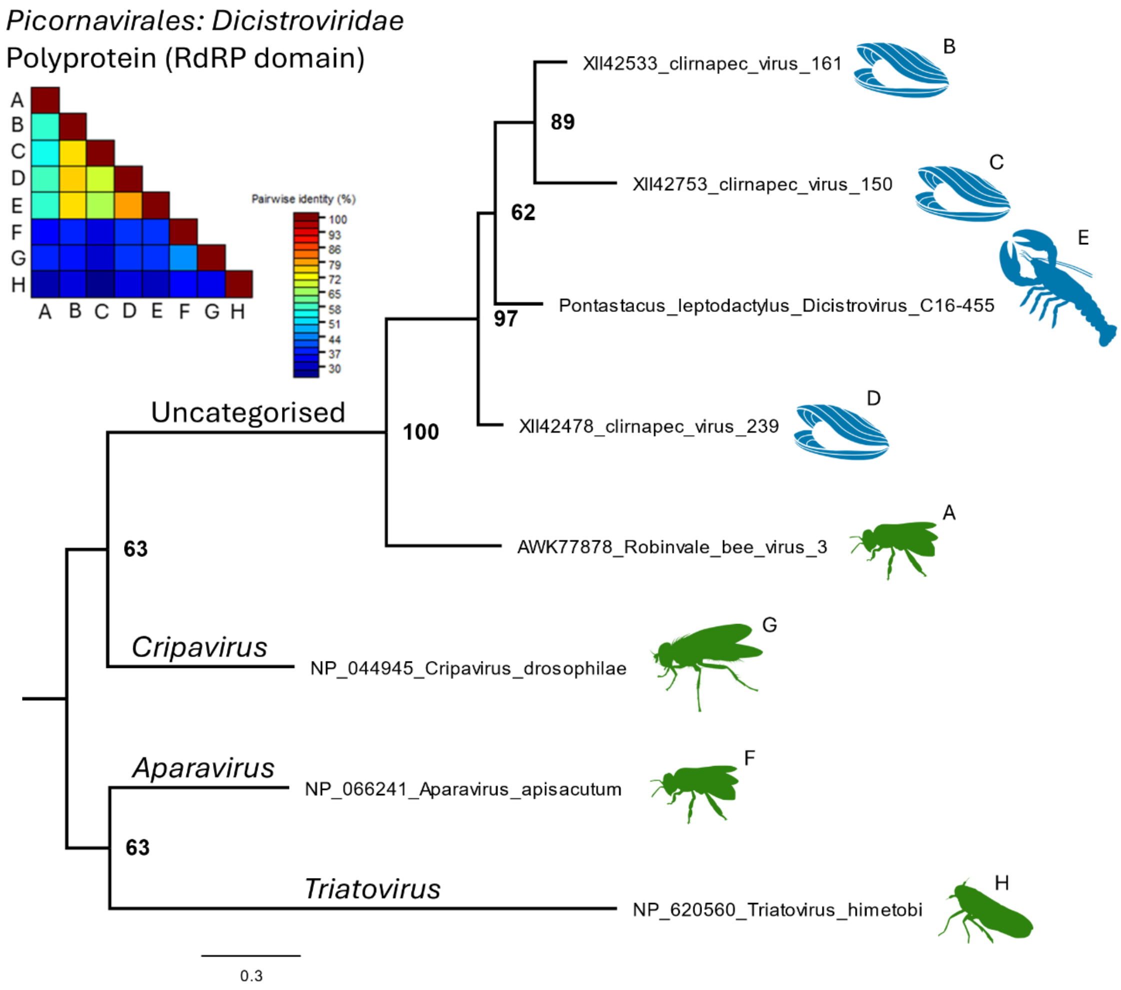


Figure 7.3. A maximum-likelihood phylogenetic tree representing the phylogenetic position of a partial dicistrovirus genome from *Pontastacus leptodactylus*, based on the RNA-dependent RNA polymerase (RdRP) region of the polyprotein. The tree is midpoint rooted. The tree includes 8 viral isolates, including representatives from the *Triatovirus*, *Aparavirus*, and *Cripavirus*. The tree is based on the best-fit model: LG+G4, chosen according to Bayesian information criterion. The original alignment included 340 columns, 316 distinct patterns, 152 parsimony-informative sites, 130 singleton sites, and 58 constant sites. The tree was developed using IQ-TREE and annotated using FigTree. In addition, a sequence demarcation plot is presented, highlighting the approximate percent similarity between the RdRP regions of the viruses included in the analysis. Small animal icons are present to

indicate the host and they are coloured in either green (terrestrial) or blue (aquatic) to represent their environmental origin

Five hepe-like viruses were identified, one more closely associated with the *Hepelivirales* than the others (Figure 7.4), which was termed: '*Pontastacus leptodactylus* Hepevirus C13-319' (PlHC13-319) (PV454208). This isolate was 7480 bp in length (GC% = 47%) and encoded two proteins, a polyprotein and a capsid protein (Suppl. Fig. 2). Using InterProScan, the viral polyprotein was identified to have the following functional predictions: region 65-296, methyltransferase (IPR002588); region 1129-1301, viral helicase (IPR027351); region 1493-1799, RdRP (IPR001788). This left two major regions without function clarification: region 297-1128, and region 1302-1492. HHpred prediction of the uncategorised 297-1128 region revealed possible tRNA methyltransferase (HHPred; probability: 99.97; e-value: $3e^{-29}$), and capping enzyme (HHpred; probability: 93.6; e-value: $5e^{-1}$) functions. HHpred revealed no confident output for region 1302-1492. The phylogenetic analysis of this virus among the *Hepevirales*, determined that it clusters within an uncategorised section of the phylogeny, but most closely with the *Alphatetraviridae* (Figure 7.4). The phylogenetic lineage it sits within houses aquatic mollusc and crustacean-infecting viruses, and environmental samples, including other viruses derived from crayfish.

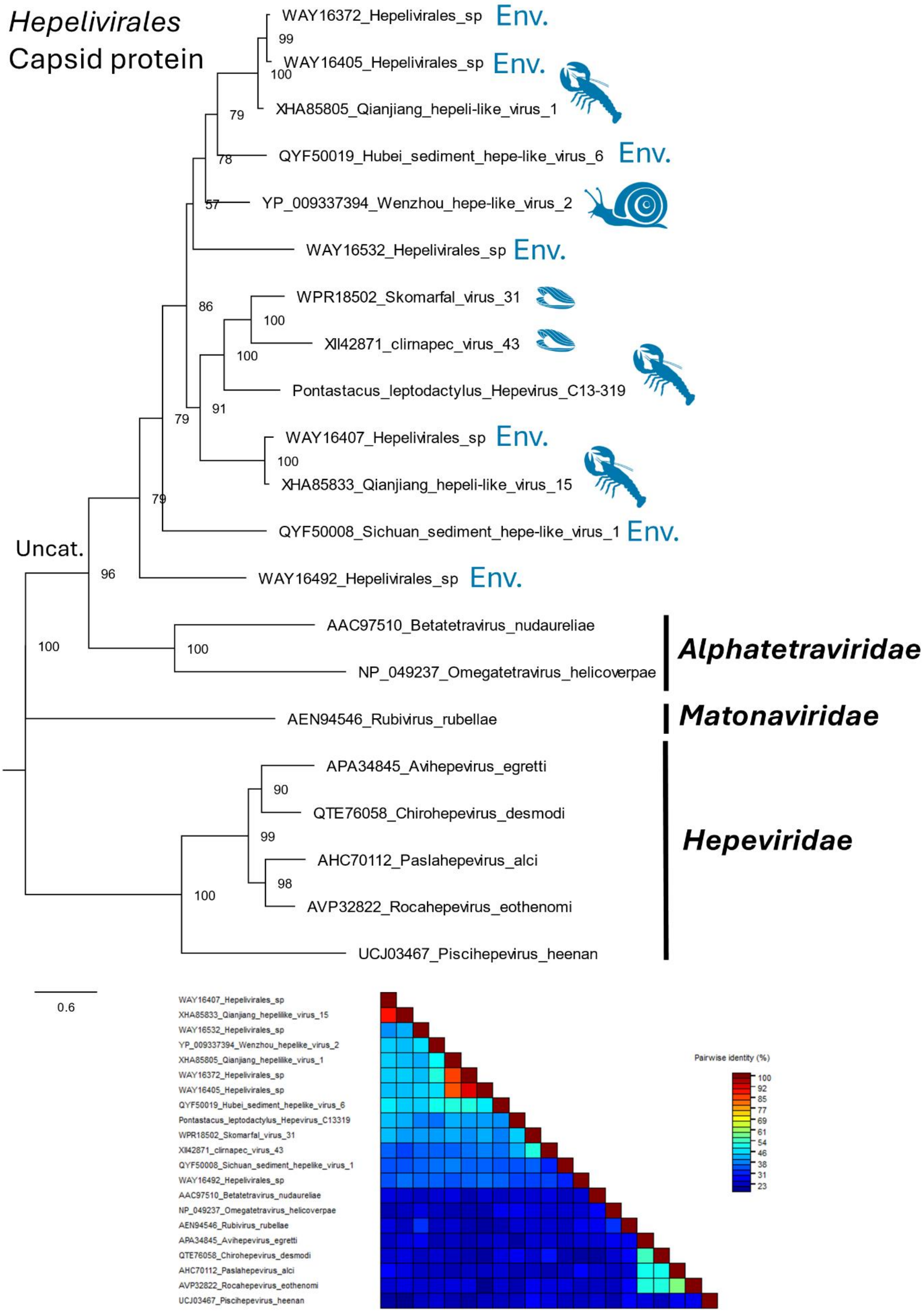


Figure 7.4. A maximum-likelihood phylogenetic tree representing the phylogenetic position of a new hepe-like virus from *Pontastacus leptodactylus* within the *Hepelivirales*, based on

the capsid protein. The tree is midpoint rooted. The tree includes representatives from the *Alphatetraviridae*, *Matonaviridae*, and *Hepeviridae*. The tree is based on the best-fit model: LG+F+I+G4, chosen according to Bayesian information criterion. The original alignment has 21 sequences with 1329 columns, 1029 distinct patterns, 624 parsimony-informative sites, 291 singleton sites, and 413 constant sites. The tree was developed using IQ-TREE and annotated using FigTree. In addition, a sequence demarcation plot is presented, highlighting the approximate percent similarity between the capsid proteins of the viruses included in the analysis. Small animal icons are present to indicate the host and they are coloured in blue to represent their aquatic origin. Inclusion of the 'Env.' Term after some isolates indicates an environmental sample

Four other "hepe-like" viruses were more closely associated with several "hepe-like" viruses from aquatic arthropods, such as crayfish, crab, and octopus (Figure 7.5; Table 7.3; Suppl. Fig. 2). The four similar viruses ranged in length between 11,018 - 11,236 bp (GC% range of 48%-50%), and were derived from two crayfish (C13 and C16) in my study. Viruses 'Pontastacus leptodactylus Hepevirus C16-9' (PlHC16-9) (PV454211) and 'Pontastacus leptodactylus Hepevirus C13-376' (PlHC13-376) (PV454209) were 99.9% similar at the nucleotide level and encoded six open reading frames (ORFs); whereas viruses 'Pontastacus leptodactylus Hepevirus C16-8' (PlHC16-8) (PV454210) and 'Pontastacus leptodactylus Hepevirus C13-48' (PlHC13-48) (PV454207) were 99.63% similar at the nucleotide level and encoded seven ORFs; indicating two groups of similar viruses from two individuals (Figure 7.5). For PlHC16-9 and PlHC13-376, the Polyprotein region 175-360 encoded a methyltransferase (IPR002588); and region 1541-1774 encoded a helicase (IPR027351). ORF2 encoded the viral RdRP and ORF3 encoded a transmembrane protein. ORF4-6 had an undetermined function. For PlHC16-8 and PlHC13-48, the Polyprotein region 177-362 encoded a methyltransferase (IPR002588); and region 1541-1774 encoded a helicase (IPR027351). ORF2 encoded the viral RdRP. ORF3-5 all encoded transmembrane proteins. ORF6 and ORF7 had an undetermined function. HHpred analysis did not identify confident predictions for function for the uncharacterised polyprotein regions or ORFs. The phylogenetic tree including these four novel viruses grouped them with other crayfish-infecting viruses, in a lineage separate from those that infect crab and octopus (Figure 7.5).

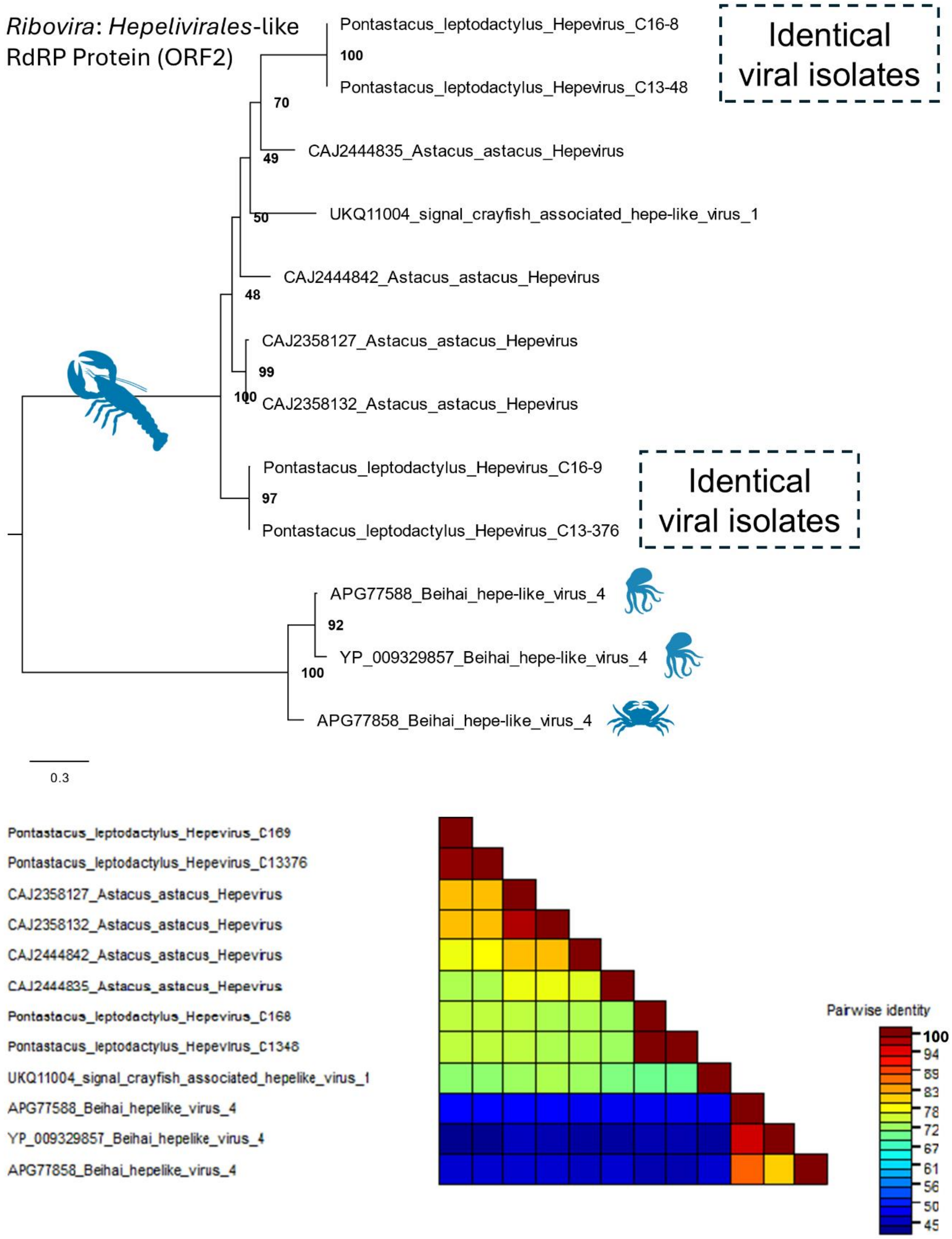


Figure 7.5. A maximum-likelihood phylogenetic tree representing the phylogenetic position of several *Riboviria* (*Hepevirales*-like) viruses from *Pontastacus leptodactylus*, based on the RNA-dependent RNA polymerase (RdRP) protein (open reading frame 2). The tree is midpoint rooted. The tree includes 12 viral isolates, including representatives from NCBI, but with no specific taxonomic detail provided to date by the ICTV. The tree is based on the best-fit model: LG+G4, chosen according to Bayesian Information Criterion. The original

alignment includes 693 columns, 435 distinct patterns, 308 parsimony-informative sites, 70 singleton sites, 315 constant sites. The tree was developed using IQ-TREE and annotated using FigTree. In addition, a sequence demarcation plot is presented, highlighting the approximate percent similarity between the RdRP proteins of the viruses included in the analysis. Small animal icons are present to indicate the host and they are coloured in blue to represent their aquatic origin

Two distinct nodaviruses were sequenced from the same crayfish specimen (C13). These viruses were termed '*Pontastacus leptodactylus* alphanodavirus C13-3553' (PlAC13-3553) (PV454212) and '*Pontastacus leptodactylus* alphanodavirus C13-3555' (PlAC13-3555) (PV454213). Both encoded a single polyprotein and consisted of 3113 bp (GC% = 40%) and 3210 bp (GC% = 41%), respectively. The two were 87.32% similar at the nucleotide level. The polyprotein encoded by the two viruses exhibited the following functional regions (based on PlNC13-3555): 100-241 encoded a methyltransferase (IPR043647); and region 509-749 encoded an RdRP. HHpred prediction of the uncharacterised regions suggested that region 242-508 of the polyprotein is an extension of the RdRP prediction, including a capping enzyme (HHpred; probability: 100; e-value: $1.1e^{-63}$). The remaining region (750-1020) is also an extension of the InterProScan-predicted RdRP site, but specific to transferase function (HHpred; probability: 99.17; e-value: $5.3e^{-11}$). Phylogenetic analysis using the entire polyprotein determined that the two nodaviruses branch closely together, on a sister branch to the Leuven nodavirus (QZZ63349) and an environmental sample (XKB76444; Figure 7.6). *Alphanodavirus flockense* branches at the base of the cluster containing the two *P. leptodactylus*-infecting nodaviruses (support: 67%). This cluster also contains a nodavirus sequenced from a freshwater bivalve (Chemarfal virus 256; WPR18356; Figure 7.6).

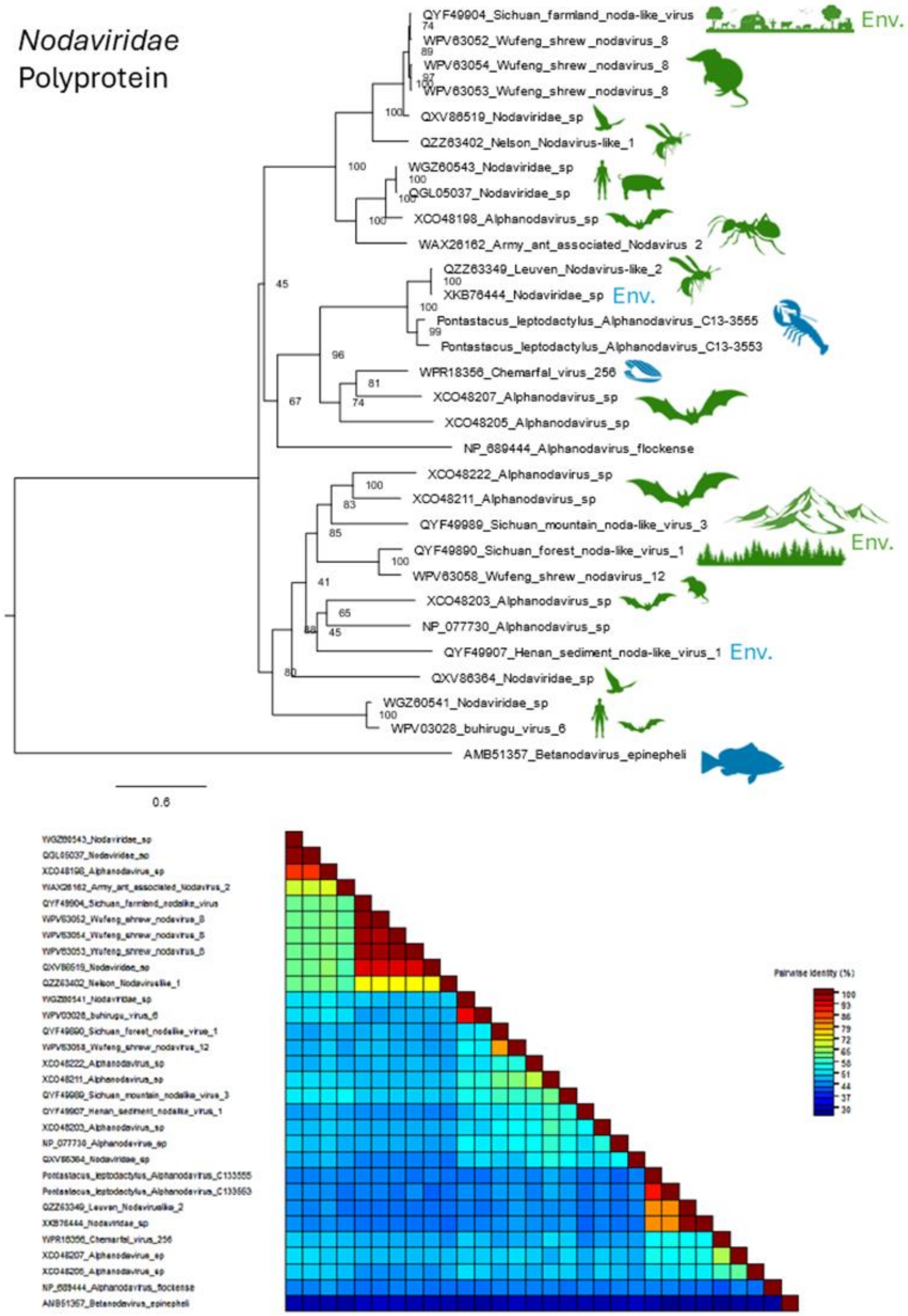

Nodaviridae
Polyprotein
QYF49904_Sichuan_farmland_noda-like_virus
Env.
74
WPV63052_Wufeng_shrew_nodavirus_8
89
WPV63054_Wufeng_shrew_nodavirus_8
97
100
WPV63053_Wufeng_shrew_nodavirus_8
100
QXV86519_Nodaviridae_sp
QZZ63402_Nelson_Nodavirus-like_1
100
WGZ60543_Nodaviridae_sp
100
100
QGL05037_Nodaviridae_sp
100
XCO48198_Alphanodavirus_sp
WAX26162_Army_ant_associated_Nodavirus_2
45
QZZ63349_Leuven_Nodavirus-like_2
100
XKB76444_Nodaviridae_sp
Env.
100
Pontastacus_leptodactylus_Alphanodavirus_C13-3555
99
Pontastacus_leptodactylus_Alphanodavirus_C13-3553
96
WPR18356_Chemarfal_virus_256
81
XCO48207_Alphanodavirus_sp
67
74
XCO48205_Alphanodavirus_sp
NP_689444_Alphanodavirus_flockense
XCO48222_Alphanodavirus_sp
100
83
XCO48211_Alphanodavirus_sp
QYF49989_Sichuan_mountain_noda-like_virus_3
85
QYF49890_Sichuan_forest_noda-like_virus_1
Env.
100
WPV63058_Wufeng_shrew_nodavirus_12
41
XCO48203_Alphanodavirus_sp
65
NP_077730_Alphanodavirus_sp
88
45
QYF49907_Henan_sediment_noda-like_virus_1
Env.
80
QXV86364_Nodaviridae_sp
WGZ60541_Nodaviridae_sp
100
WPV03028_buhirugu_virus_6
AMB51357_Betanodavirus_epinepheli
0.6
WGZ60543_Nodaviridae_sp
QGL05037_Nodaviridae_sp
XCO48198_Alphanodavirus_sp
WAX26162_Army_ant_associated_Nodavirus_2
QYF49904_Sichuan_farmland_nodalike_virus
WPV63052_Wufeng_shrew_nodavirus_8
WPV63054_Wufeng_shrew_nodavirus_8
WPV63053_Wufeng_shrew_nodavirus_8
QXV86519_Nodaviridae_sp
QZZ63402_Nelson_Nodaviruslike_1
WGZ60541_Nodaviridae_sp
WPV03028_buhirugu_virus_6
QYF49890_Sichuan_forest_nodalike_virus_1
WPV63058_Wufeng_shrew_nodavirus_12
XCO48222_Alphanodavirus_sp
XCO48211_Alphanodavirus_sp
QYF49989_Sichuan_mountain_nodalike_virus_3
QYF49907_Henan_sediment_nodalike_virus_1
XCO48203_Alphanodavirus_sp
NP_077730_Alphanodavirus_sp
QXV86364_Nodaviridae_sp
Pontastacus_leptodactylus_Alphanodavirus_C133555
Pontastacus_leptodactylus_Alphanodavirus_C133553
QZZ63349_Leuven_Nodaviruslike_2
XKB76444_Nodaviridae_sp
WPR18356_Chemarfal_virus_256
XCO48207_Alphanodavirus_sp
XCO48205_Alphanodavirus_sp
NP_689444_Alphanodavirus_flockense
AMB51357_Betanodavirus_epinepheli
Pairwise identity (%)
100
93
86
79
72
65
58
51
44
37
30

Figure 7.6. A maximum-likelihood phylogenetic tree representing the phylogenetic position of two new nodaviruses from *Pontastacus leptodactylus*, based on a whole polyprotein alignment. The tree is midpoint rooted. The tree includes representatives from the *Betanodavirus* and *Alphanodavirus* genera. The tree is based on the best-fit model: LG+I+G4, chosen according to Bayesian information criterion. The original alignment has 30 sequences with 1250 columns, 1138 distinct patterns, 824 parsimony-informative sites, 233 singleton sites, and 193 constant sites. The tree was developed using IQ-TREE and annotated using FigTree. In addition, a sequence demarcation plot is presented, highlighting the approximate percent similarity between the polyproteins of the viruses included in the analysis. Small animal icons or environmental icons are present to indicate the host or origin of the sample, and they are coloured in either green (terrestrial) or blue (aquatic) to represent their environmental origin. Inclusion of the 'Env.' Term after some isolates indicates an environmental sample – green refers to terrestrial, blue refers to aquatic

Two tombusvirus sequences were identified from animal C13. A partial genome encoding two ORFs is termed 'Pontastacus leptodactylus tombusvirus C13-5563' (PlTC13-5563) (PV454215), which was 2518 bp in length (GC% = 39%). A complete viral genome containing four ORFs is termed 'Pontastacus leptodactylus tombusvirus C13-873' (PlTC13-873) (PV454214), which was 5600 bp in length (GC% = 41%). The proteins encoded by the two viruses show varied levels of similarity to other tombusviruses (BLAST TABLE). The two ORFs encoded by PlTC13-5563 consist of a transmembrane protein (ORF1) and an RdRP (ORF2) according to InterProScan. The complete tombusvirus genome (PlTC13-873) encoded four ORFs (Suppl. Fig. 2), which appear to function in the following ways based on InterProScan and HHpred predictions: ORF1, undetermined; ORF2, undetermined; ORF3 encodes an RdRP; and ORF4 encodes a peptidase A21 (IPR005313). The tombusvirus phylogeny determined that the complete and partial viruses sequenced in this study group separately across the *Tombusviridae* (Figure 7.7). PlTC13-5563 groups with other crustacean and mollusc-infecting tombusviruses from aquatic environments, most closely associated with the *Regressovirinae* and *Calvusvirinae*. PlTC13-873 groups in a different part of the tree, alongwith related viruses from mosquito, bird, and molluscan origin (Figure 7.7).

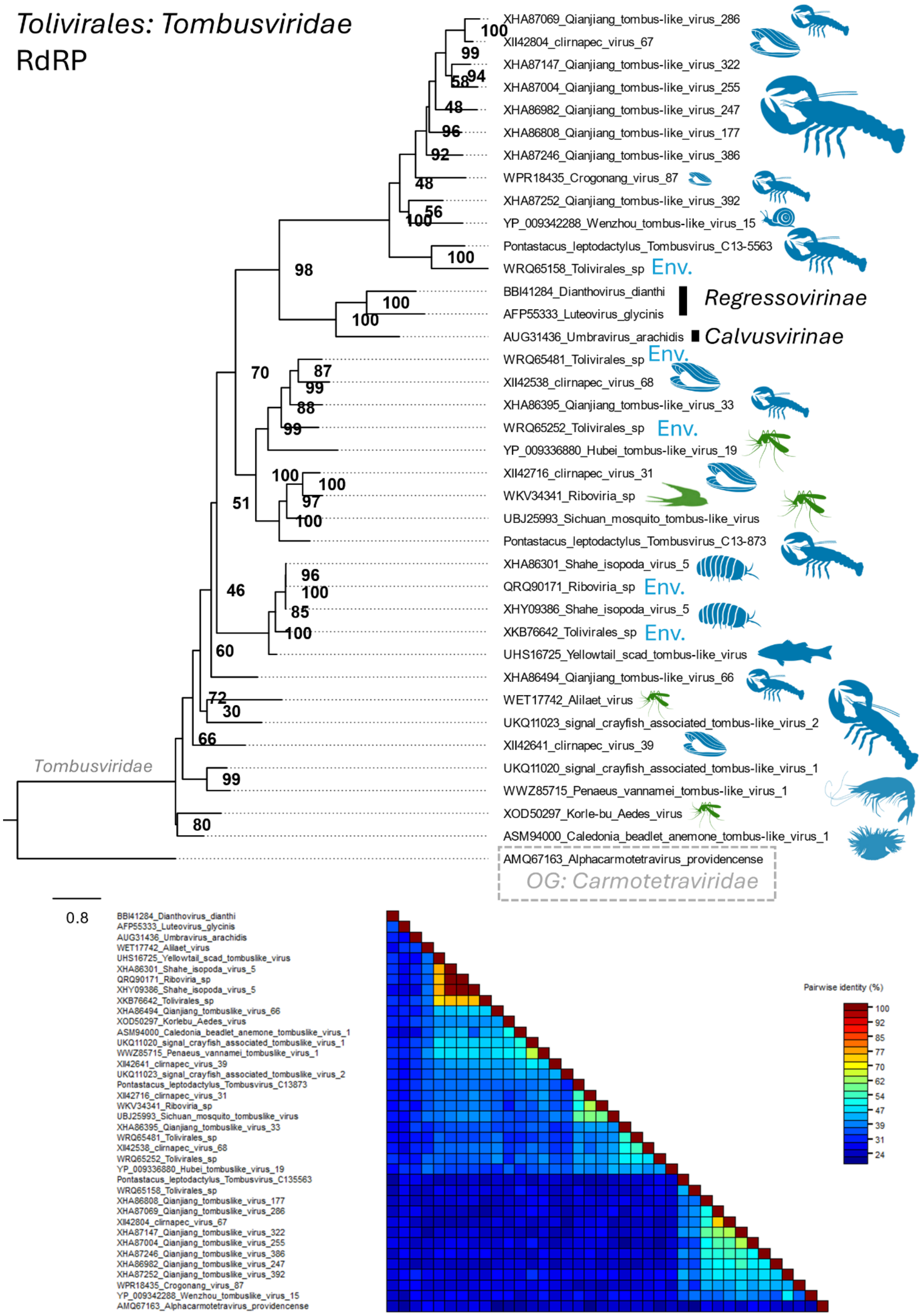


Figure 7.7. A maximum-likelihood phylogenetic tree representing the phylogenetic position of two new tombusviruses from *Pontastacus leptodactylus*, based on RNA-dependent RNA

polymerase protein alignment. The tree includes representatives from the *Regressovirinae* and *Calvusvirinae*, and includes a member of the *Carmotetraviridae* as an out-group. The tree is based on the best-fit model: LG+I+G4, chosen according to Bayesian information criterion. The original alignment has 38 sequences with 1399 columns, 1261 distinct patterns, 625 parsimony-informative sites, 506 singleton sites, and 268 constant sites. The tree was developed using IQ-TREE and annotated using FigTree. In addition, a sequence demarcation plot is presented, highlighting the approximate percent similarity between the polyproteins of the viruses included in the analysis. Small animal icons or environmental icons are present to indicate the host or origin of the sample, and they are coloured in either green (terrestrial) or blue (aquatic) to represent their environmental origin. Inclusion of the 'Env.' Term after some isolates indicates an environmental sample – green refers to terrestrial, blue refers to aquatic

Finally, two genomic fragments with greatest similarity to the *Totiviridae* (Table 7.3) (PV454216) (PV454217) were isolated from sample C16. These two fragments are considered partial sequences of a viral polyprotein and consisted of 805 bp and 746 bp.

Discussion

This study explores symbiotic organisms in the only known population of non-native narrow clawed crayfish, *P. leptodactylus* in West Yorkshire, UK. Invasive crayfish pose a significant threat to native crayfish through both competition and disease (Everard et al. 2009) and this recently established population could pose a risk to native white-clawed crayfish populations, with the nearest known population of the native species only ten km downstream (WoC ID: WK9180; for *A. pallipes*, observation date: 2022; Ion et al. 2024). Information on the disease profile of this population will assist in further elucidating the level of risk presented.

Symbionts in an early invading population of narrow clawed crayfish

Previous records associate a range of symbionts with *P. leptodactylus* (reviewed in Bojko et al. 2021). My study found a lack of nudiviruses, Microsporidia, Fungi, some protozoans (including *Psorospermium*), and Metazoa, such as trematodes, from the UK population, which are present in *P. leptodactylus* in its native range (Bojko et al. 2021). The absence of these pathogens may reflect enemy release (Williamson, 1996; Keane & Crawley, 2002; Colautti et al. 2004; Miura and Torchin, 2023), where the founding population 'escaped' pathogens as a results of stochastic and selective pressures during invasion. However, I did detect several protozoans, bacterial species, and RNA viruses.

The protozoan, *E. cambari*, infests the gill filaments of crayfish (Abd El-Moaty et al. 2016), with no known negative effects; however, high burden of *Epistylis* sp. has been associated with lower concentrations of dissolved oxygen within a waterbody, potentially indicating increased organic matter decomposition (Quaglio et al. 2004). Ciliated protozoa (*Epistylis* sp., etc.) were commonly noted in the gill histology, and are likely commensal associations. The Protozoa, *Neobodo designis and Nuclearia moebiusi* may also be considered commensal associates and are commonly found in aquatic biomes (Chavez-Dozal et al. 2013; Gabaldon et al. 2022). My detection of these two species increases their known habitation to crayfish, as symbionts.

Of the bacteria that I detected, two show genetic similarity to opportunistic human pathogens: *Gardnerella vaginalis* and *Staphylococcus epidermidis*. *Gardnerella vaginalis* has previously been seen in humans and is linked to sexually transmitted infections and public health complications (Schwebke et al. 2014). *Staphylococcus epidermidis* has also previously been seen in humans and colonises skin (Otto, 2012). It is likely that both of these bacteria have entered the waterbody, and therefore the crayfish, through either human waste entering the reservoir, or human bathing. The other bacteria observed in this study likely form a part of the more natural crayfish microbiome, within UK waters. *Xanthomonas maliensis* (Triplett et al. 2015)*, Pseudomonas fluorescens* (Rainey, 1999;) and *Leadbetterella byssophila* (Weon et al. 2015) have all previously been found in vegetation and agricultural samples with no notable effects on their host or environment. *Thermomonas brevis* (Mergaert et al. 2003) and *Haliscomenobacter hydrossis* (Daligault et al. 2011) have both been found in water samples with no notable effects, and *Arthobacter alpinus* has previously been found in soil samples, with no notable effects (Zhang et al. 2010). I believe that these species are likely to be commensal or mutualists with regard to their crayfish host.

We did not identify any DNA viruses from metagenomic data collected from my samples, despite previous detection of viruses in this species (Petersen et al. 2024). However, I did sequence and identify several RNA viruses from the population. This included a dicistrovirus, several hepe-like viruses (~ *Hepelivirales*), nodaviruses, tombusvirus, and a totivirus. For the majority of these viruses, this is their first detection and knowledge of their pathological effect is limited.

The Pontastacus_leptodactylus_Dicistrovirus_C16-455 and partial totivirus sequences were detected only in specimen 16, which had an hepatopancreatic pathology visible in Figure 7.2C. No other hepatopancreas from specimens in my sample set presented this way, and follow-up of this pathology will be valuable to gain further detail on whether one of these virus groups drove such hepatopancreatic presentation in the histological section.

Dicistroviruses have previously been shown to cause mortality in arthropods (Sun et al. 2021) and such a discovery may have relevance to crayfish population control.

Pontastacus_leptodactylus_Hepevirus_C13-319 had a unique genome organisation among the hepe-like viruses I detected, with two open reading frames, and was only detected in specimen 13. Two nodavirus-like genomes were specifically detected in specimen 13: Pontastacus_leptodactylus_Nodamuravirus_C13-3553 and Pontastacus_leptodactylus_Nodamuravirus_C13-3555, as was a tombusvirus (Pontastacus_leptodactylus_Tombusvirus_C13-873). It is more difficult in this situation to determine if these viruses were involved in the pathology in the hepatopancreas of specimen 13 (Figure 7.2D). In-situ hybridisation methods may aid to untangle this detail in future studies.

The detail provided above gives an overview of the symbiotic community housed within invasive *P. leptodactylus* at a freshwater site in West Yorkshire. They house a broad array of protozoans (gregarines; Table 7.1), bacteria (Table 7.2), and RNA viruses (Table 7.3), but lack microsporidians, DNA viruses, and common metazoan groups, like trematodes and acanthocephalans.

Virological novelties

Each of the viral genomes that I have uncovered will require formal ratification by the International Committee on Taxonomy of Viruses (ICTV). I estimate that the complete viral genomes I provide herein will increase known diversity within the *Riboviria*, with specific detail relevant to the *Nodaviridae*, *Dicistroviridae*, *Hepelivirales*, and *Tombusviridae*, alongside of partial genomes for viruses that require follow-up study (i.e. *Totiviridae*).

In each of my phylogenetic trees (Figure 7.3-7.7), I have provided relative ICTV accepted species as anchor points to determine the likely taxonomic relevance of each new discovery. In all but two cases, the viruses I have identified sit outside of current taxonomic boundaries and, for the majority, have only been comparable using predicted protein sequence data. The nodavirus-like genomes that I sequenced from specimen 13 are the only viruses with comparable nucleotide similarity to other sequenced viruses, which show ~79% nucleotide similarity with a nodavirus that had been identified in wasps (Leuven nodavirus; MZ443597). This virus has been seen in the genus *Vespula*, a group of predatory wasps that are also invasive (Remnant et al. 2021) and I highlight that this group is now also associated with aquatic invertebrates alongside terrestrial species. The crayfish nodaviruses appear to group into a lineage of the *Alphanodavirus* genus (Figure 7.6), branching with *Alphanodavirus flockense* (aka. ‘Flock House virus’), a virus from insects with control applications (Jiang et al. 2023).

In other cases, my viruses group with others emerging from large studies into viral diversity; however, one particularly unique observation is that of the hepe-like viruses, which each encode 6-7 open reading frames (Figure 7.5). I provide genomes for four hepe-like viruses, which group with similar viruses only found in crayfish to date (Bačnik et al. 2021 and direct NCBI submissions). The crayfish hosts identified to date are *P. leniusculus* and *A. astacus*. Outside of this group are only three comparable viruses, two from octopi and one from a crab. My findings here support a unique group of viruses within the crayfish virome that requires further exploration and possibly the erection of a novel taxonomic group as sister to the *Hepelivirales*.

Conclusion and future directions

Pontastacus leptodactylus in the UK are associated with a diverse range of symbionts. This invader appears to have rapidly acquired these symbioses from the UK freshwater environment during a short time period (introduced to Boshaw Whams in 2014), but several of these symbionts may have co-invaded along with the founder population. My approach to screening this invasive population presents a pathway for considering a rapid method to determine symbiont introduction risk via an invasive species, saving cost by pooling tissue nucleotide extracts for individual next generation sequencing runs, with supporting histopathology (following Bojko et al. 2023). I conclude that, despite the population harbouring a diverse array of RNA virus, bacterial, and protozoan symbionts, the population appears to have escaped mortality-inducing groups, such as Microsporidia (Stratton et al. 2024) and *Bunyavirales* (Grandjean et al. 2019).

Chapter 8

Discussion

Synthesis

This thesis focused on how we can use novel technology to monitor and manage crayfish. The thesis can be divided into four overarching themes: status and feasibility of using remote underwater video (Chapter 2 – 3); assessing the extent of crayfish invasions and providing guidance on appropriate actions (Chapter 4 – 5); identifying the instream behaviours of native crayfish (Chapter 6), and understanding the wider impacts understudied invasive crayfish could bring with them (Chapter 7). Extensive field surveys were undertaken throughout this thesis with almost 100 days of surveys completed. Despite the increase in the use of remote underwater video in freshwater environments, there is no set of standards for these surveys. A systematic literature review was completed to understand what research has previously been conducted using remote underwater video in freshwater environments and to produce a set of standards that could be followed for all future remote underwater video studies (Chapter 2). This review found that as of November 2022 a total of 163 unique pieces of literature had used remote underwater in freshwater environments. This review concluded that all future remote underwater video studies in freshwater environments should follow the standards of recording in 1080p and 30 frames per second. Deployments should last for 60 minutes without the need for any acclimatisation times, and all footage should be manually reviewed by a single human reviewer (Harwood et al. In Review; Chapter 2).

After identifying a set of standards that could be used in remote underwater video the next step was to develop a remote underwater video tool that could be used to monitor crayfish. CrayCam was designed and tested (Chapter 3) to establish if remote underwater video would be a feasible tool for monitoring both native and invasive crayfish within the United Kingdom. Laboratory testing found that remote underwater video could successfully detect crayfish, even in highly turbid simulations, with increases in turbidity and distance from the camera reducing the effectiveness (Chapter 3). Surveys conducted for future chapters (Chapter 4, 5, 6) all successfully detected crayfish with each of these chapters focussing on a different species of crayfish. Chapter 4 had a target species of the invasive narrow clawed crayfish (*Pontastacus leptodactylus*), Chapter 5 the invasive signal crayfish (*Pacifastacus leniusculus*) and Chapter 6 the native white-clawed crayfish (*Austropotamobius pallipes*).

These observations found that each species had a unique species accumulation which differed between baited and non-baited remote underwater video deployments (Chapter 6).

Using a combination of crayfish surveying methods is the most effective approach to monitor invasive crayfish populations (Harwood et al. 2025; Chapter 4). It is important to understand the extent of an invasion in order to advise appropriate management. Chapter 4 has identified that Boshaw Whams, a Yorkshire Water owned reservoir, can be classified as being in the “Containment” or “Asset Protection” state. The population of invasive narrow clawed crayfish has been estimated at 10,045 ± 5602 (95% CI) individuals with breeding activity observed between February and April (Harwood et al. 2025; Chapter 4). This suggests that the potential for eradication at this site is highly unlikely and biosecurity measures must be implemented to ensure that the population remains contained and that mitigation for potential further spread is required. This multi-method approach was also used across a large portion of the River Calder catchment to establish the extent of a signal crayfish invasion (Harwood et al. In Review; Chapter 5). The multi-method approach found no difference in the effectiveness of trapping and RUV and BRUV surveys when detecting the presence of signal crayfish, however it did find that these methods were significantly more effective than eDNA. Temperature, Oxidation Reduction Potential (ORP) and distance from upstream barriers all influenced the abundance of crayfish found at each site, with more signal crayfish present in warmer temperatures, lower ORP values, and closer to upstream barriers (Harwood et al. In Review; Chapter 5). These variables can be considered when conducting future surveys, identifying the sites that are more likely to have suitable conditions for their presence. Signal crayfish were found to be present throughout the extent of the main River Calder and 51% of all barriers found within the survey area had been breached by invasive crayfish (Harwood et al. In Review; Chapter 5). The removal of these barriers to aid in the migration of salmonids would have limited influence on the continued spread of invasive signal crayfish throughout the Calder as they have already been breached. It is, however, important that barriers along two of the tributaries, Luddenden Brook and Cragg Brook, remain as signal crayfish have not been found above these. Those along Luddenden Brook are protecting a historical site for native white-clawed crayfish, while those along Cragg Brook are protecting a newly identified population of white-clawed crayfish that was discovered while conducting these surveys.

My thesis has shown that a mixed method approach to crayfish monitoring is effective and informative and can offer a rapid and cost-effective solution to establish the extent of crayfish invasions. RUVs and BRUVs can be used to initially identify sites with crayfish presence and then trapping can be used to answer more specific questions about the populations that have been identified.

A suggested approach for using mixed methods when surveying would be to identify potential crayfish locations throughout the survey area, such as sites with low ORP or close to an upstream barrier, as Chapter 5 has shown these are closely related to crayfish presence and abundances. Upon the identification of these sites rapid RUV and BRUV deployments can be made at multiple sites during a single day, with rigs being rinsed with Virkon between sites to avoid potential biosecurity risks. Footage can then be reviewed to confirm the presence of crayfish at any of these sites and more time-consuming trapping can then be conducted at sites with confirmed crayfish presence to establish species, population dynamics and densities. Unless environmental DNA methods are improved and environmental conditions are ideal eDNA surveys should not be considered when trying to rapidly establish the extent of invasive crayfish populations as the high costs and long turn-around time are prohibitive.

Comprehensive field observations were made to identify the in-stream behaviours of native white-clawed crayfish and the factors that influence their abundance (Harwood et al. In Prep; Chapter 6). This chapter offers the first evidence that white-clawed crayfish are highly active during daylight, going against the current understanding that they are nocturnal, with high levels of activity during twilight. Chapter 6 found that no single habitat variable influenced white-clawed crayfish abundance. The entire area of River Kent catchment that was studied offered suitable habitat for healthy white-clawed crayfish. The use of an ethogram provided insight into the intra-species behaviours that white-clawed crayfish demonstrate through a healthy environment. This found that aggressive interactions were more likely to occur when individuals are evenly matched in size, and when there was a difference in size, the larger individual was more likely to win the contest (Harwood et al. In Prep; Chapter 6). These results can help influence conservation efforts, especially in crayfish Ark sites, ensuring that conditions are set to reduce the likelihood of competition and the potential damage and risks that are associated with it.

One of the major risks of invasive species is disease and it is important to understand the pathological profile of an invasive species in order to identify the impacts that it may have on native populations, and the ecosystem as a whole. Several protozoan and bacterial species were found to be common throughout the invasive population of narrow clawed crayfish present at Boshaw Whams, the site that has been extensively surveyed as part of Chapter 4. The pathogens that have been identified within this population have either co-invaded with them or have been acquired during a short-time period since invasion (Harwood et al. 2025b; Chapter 7). Despite the presence of these symbionts, it would appear that this population has avoided mortality-inducing groups. Despite the lack of symbionts that could have devastating impacts on native populations, there is still risk as several symbionts, including

Microsporidia and *Nudiviridae* are not present within this population and therefore they face reduced natural control methods, which could lead them to be a successful invasive species (Harwood et al. 2025b; Chapter 7). This shows that it is vital that biosecurity measures are in place and the mitigation approach recommended in Chapter 4 is important.

Conclusion

This thesis contributes to the understanding of crayfish throughout the United Kingdom and has addressed the knowledge gap in how we should conduct freshwater remote underwater video research providing a set of standards and guidance for all future freshwater RUV surveys. The creation of CrayCam has produced a tool that has successfully monitored both native and invasive species of crayfish throughout the UK and is a tool that will continue to be used in future research that will be conducted within the South Aquatic Interaction Lab at the University of Leeds. The results found in Chapter 4 and Chapter 5 have proven that remote underwater video is a useful tool that can be used in combination with traditional crayfish surveying methods.

My findings will allow environmental consultants to confidently use remote underwater videos in their future surveys to address a range of questions. Also introducing remote underwater video as a part of the wider crayfish surveying toolkit utilised by practitioners such as the Environment Agency, local rivers trusts and regional water suppliers. Chapter 4 established that Boshaw Whams is in “containment” and “asset protection” status and has led to Yorkshire Water commissioning an eradication feasibility assessment on Boshaw Whams reservoir, guided by the work produced in Chapter 4. Observations made have found that invasive narrow clawed crayfish are now present within Dean Dike, the waterbody directly connected to the reservoir. Future work is needed to monitor and assess this population, and my initial findings have been used in MSc student projects monitoring this potential invasion front. Future PhD projects, and wider Environment Agency and River Holme Connections Trust monitoring, have also been initiated to continue to monitor this population and establish the impacts that this frontline population may have on the wider ecosystem.

CrayCam has also benefitted the conservation and species recovery of native white-clawed crayfish (*Austropotamobius pallipes*) through the Natural England Species Recovery Programme, which provided a grant that contributed significantly to the funding for this project. Significant contributions have been made into the conservation of species through the initial testing of CrayCam resulting in a large-scale crayfish rescue and recovery project. This rescue project led to the successful creation of two crayfish ark sites and received wide media attention (Figure 8.1) and was featured on BBC Look North (Figure 8.2) and ITV

Calendar (https://www.itv.com/news/calendar/2023-04-28/rescue-mission-to-save-crayfish-from-deadly-plague). The story was also covered by the University press and the government's official website (Figure 8.3). The first concrete proof that white-clawed crayfish have high levels of daytime activity has also been proven which is contradictory to what the current literature believes. This discovery can help influence future conservation approaches and monitoring. It is hoped that this new understanding can be used to provide evidence that can support the need to update how we monitor and survey white-clawed crayfish and influence future policy changes. By introducing remote underwater video as a tool for surveying white-clawed crayfish, legislation and policy can be amended to ensure that the future surveying approaches have lower risks of causing stress on white-clawed crayfish. As well as this policy can be put into place that ensure areas with known white-clawed crayfish populations have limited foot traffic at all times of the day.

Figure 8.1. Film crews record me and Daniel Chadwick from the Environment Agency releasing the white-clawed crayfish (*Austropotamobius pallipes*) into the Yorkshire Water owned reservoir ark site

Figure 8.2. Screenshot from the BBC Look North bulletin that was aired in Summer 2023 describing the crayfish rescue and subsequent release into the ark site at the University of Leeds

FACULTY OF BIOLOGICAL SCIENCES NEWS /// FRIDAY 5 MAY 2023

Researchers join race to save endangered species from deadly disease

SHARE

FBS researchers joined an operation to rescue populations of endangered white-clawed crayfish in Leeds last week.

Rescued crayfish find new home at Bodington Pond

Environment news

28 June 2023

A rescued population of endangered white clawed crayfish that were in danger of being wiped out have been moved to the University's Bodington Pond, where they can thrive and breed.

Press release

Rescued endangered native crayfish moved to safe haven

A rescued population of endangered white clawed crayfish that were in danger of being wiped out have been moved to a safe haven where they can thrive and breed.

From: Environment Agency

Published 25 August 2023

A native white clawed crayfish rescued as part of the River Aire catchment operation

Figure 8.3. Screenshots of news articles featuring coverage of the crayfish rescue and ark site introduction from 1) The University of Leeds Biological Science website (https://biologicalsciences.leeds.ac.uk/biological-sciences/news/article/372/researchers-join-race-to-save-endangered-species-from-deadly-disease), 2) The University of Leeds website (https://www.leeds.ac.uk/news-environment/news/article/5334/rescued-crayfish-find-new-home-at-bodington-pond), 3) The Government website (https://www.gov.uk/government/news/rescued-endangered-native-crayfish-moved-to-safe-haven)

The implications that work from this PhD thesis will have on stakeholders relates heavily to how stakeholders will plan future surveys and assess the best approaches to be taken to answer their questions. Remote underwater video should now be considered as a potential tool by stakeholders when they are planning future crayfish related surveys as I have shown that it can successfully answer a series of questions relating to crayfish and has proven to be a valid alternative to traditional trapping methods when conditions have prevented the use of traps. Stakeholders such as environmental consultants, local wildlife trusts and the Environment Agency should look to update their crayfish surveying methodologies to include assessing if RUVs should be used first to address questions, before opting for more time extensive methods such as trapping. The findings from Chapter 6 also suggest that the current understanding of the activity of native white-clawed crayfish is wrong and stakeholders should look to alter their surveying methods to account for this.

This work has also shown that the use of environmental DNA should be thoroughly considered by stakeholders before committing to the high costs of them. My work has shown that it does not provide as conclusive and reliable results as RUVs and more traditional methods (as shown in Chapter 5). My results had a high volume of false negatives, with results stating that crayfish were absent from sites, despite trapping and RUV both confirming the presence of them there. Unless eDNA suppliers can improve the detectability of eDNA kits, and habitat variables such as reduced turbidity and freshly moulting individuals are present, then stakeholders should look to avoid using it.

There is also the potential for a major future project which has been initiated by the work completed in Chapter 3. I have been fortunate enough to become part of a major network of crayfish researchers around the world and by assessing the time to first observation for a range of different crayfish species, both native and invasive, in a range of different habitat types, there is potential to create a major database showing the likelihood of detecting a target crayfish in a specific environment using remote underwater video.

The next steps for this project will be to share the specifications for CrayCam with this international network of crayfish researchers. Using the standards and methodology that were identified in Chapter 2, collaborators will then make RUV and BRUV deployments using CrayCam at sites that they have previously surveyed and have confirmed presence of either native or invasive non-native crayfish. Footage from these deployments should be reviewed, taking note of the time to first observation, MaxN, cumulative time on screen and total number of crayfish, as described in Harwood et al. 2025, Chapter 4, Chapter 5 and Chapter 6. Details should also be taken for the target species of crayfish, habitat type, water turbidity and if the waterbody is lentic or lotic. If environmental factors allow it, trapping should also be conducted at these sites to gain a value for trapping Catch Per Unit Effort as well as population dynamics and mean crayfish carapace lengths.

The results from these surveys will be collated into a large global database and will be analysed to identify trends in how CrayCam works with regard to population densities, species and habitat type. The goal will be to establish if species observations are locally specific, have national trends or international trends. Results can be used on a case-by-case basis to guide the best approach to answer specific questions related to different crayfish species with varying densities in different locations. This is a project that I intend to continue with after the completion of this PhD, starting with collaborations with Bristol Zoo and local wildlife trusts, looking to expand on the results found for native white-clawed crayfish in Chapter 6.

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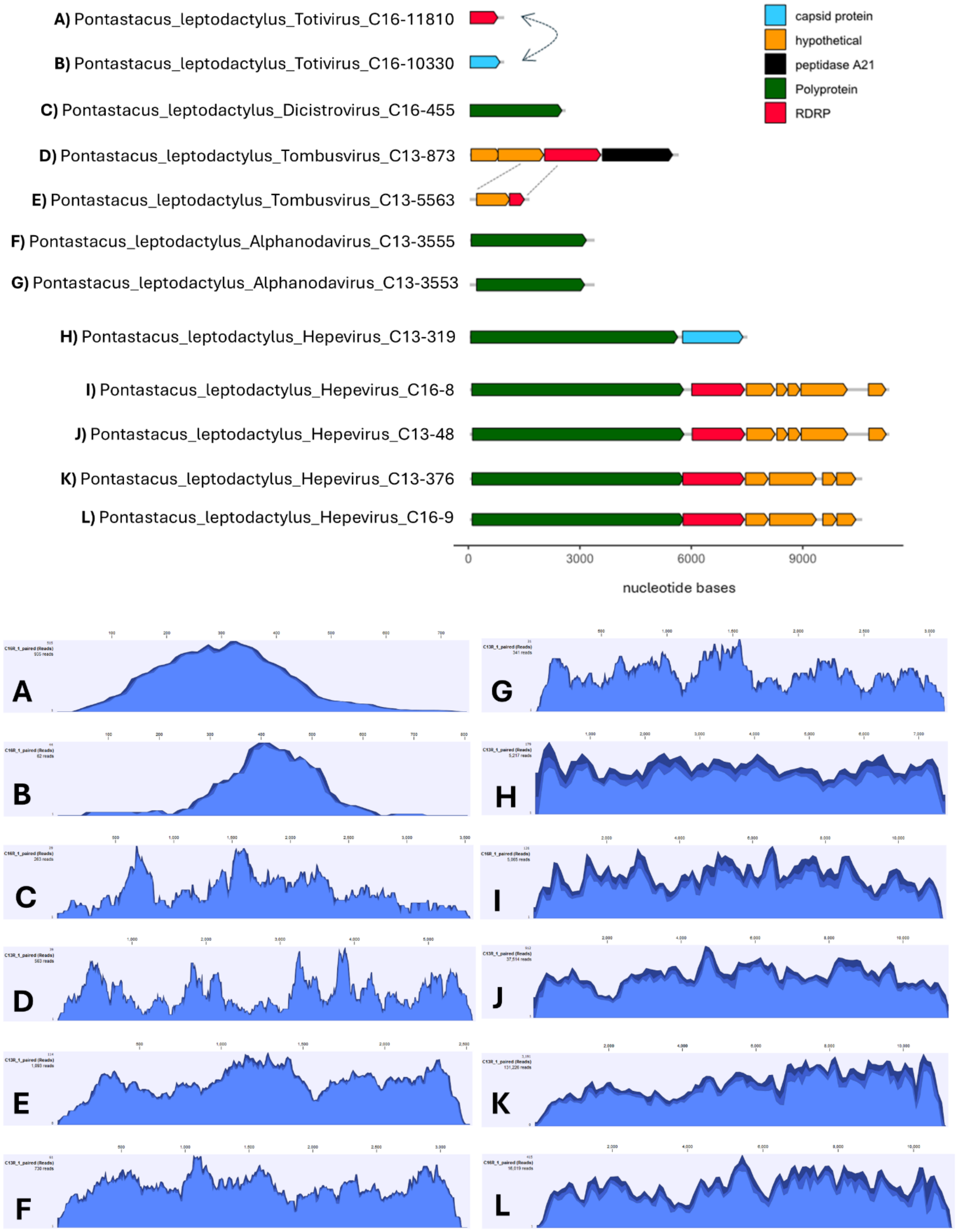
Supplementary Material

Supplementary Figure 1. Marks remaining on individuals captured on 13/10/2023, six months after the initial marking experiment.



Supplementary Figure 2. An overview of the gene organisation for the RNA viruses that were detected in the *Pontastacus leptodactylus* samples collected from the UK. The figure outlines 12 partial or complete viral genomes and the genes that they encode. The key identifies common protein functions, or highlights those with no determined function (hypothetical). The coverage and mapping results for each viral contig are included in sections A-L.