

White-clawed crayfish mortality investigation

Chief Scientist's Group research report

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Dr Robert Bradburne
Chief Scientist

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Executive summary

The Environment Agency's statutory duties include protecting biodiversity. Central to this aim is the prevention, investigation, and mitigation of environmental incidents that pose risks to ecosystems, wildlife, and human health. When incidents occur, such as pollution events or sudden wildlife mortalities, the Environment Agency assesses the causes and minimises impacts to support ecological recovery.

In the summer of 2024, reports were received of significant mortalities of white-clawed crayfish (*Austropotamobius pallipes*) in the North East area of the Environment Agency. Affected white-clawed crayfish displayed unusual clinical signs of disease, including discolouration, lesions and abnormalities of the outer shell (carapace). Similar symptoms were later observed in two crayfish populations in the midland area of England

Initial investigations focused on identifying known causes of white-clawed crayfish deaths. Field surveys assessed the extent of the outbreak and investigated possible environmental factors. Investigations found no evidence of pollution, and no impacts were observed in fish or other aquatic wildlife. Laboratory testing by experts at the Centre for Environment, Fisheries and Aquaculture Science (Cefas) screened for known white-clawed crayfish diseases, including crayfish plague, white spot syndrome virus, bunya-like brown spot virus, but these were ruled out as the primary cause of the incident.

As no obvious explanation was identified, additional investigations were undertaken. White-clawed crayfish from affected populations were monitored in laboratory tanks to understand how the clinical signs of disease developed over time. Researchers from the University of Teesside and Cefas also used detailed visual assessments of body tissues (pathology), microscopic examinations and molecular (genomic) techniques to compare affected and unaffected crayfish and to search for potential pathogens, including bacteria, fungi and viruses.

The investigations found that the clinical signs of disease varied considerably between individuals. Some white-clawed crayfish had large 'burn-spot' like areas, sometimes appearing like an abscess, whereas others had lesions, marks or holes in the carapace (shell). Laboratory observations showed that many white-clawed crayfish survived and improved over time. Recovery was particularly common in younger animals, which shed their carapace or moult more frequently. Field surveys carried out during 2025 showed a substantial reduction in both the number of affected white-clawed crayfish and the severity and incidence of clinical signs of disease across surveyed populations. Incidence of shell abnormalities were even lower during 2026.

The molecular work showed a diverse microbial community (microbiome) on both healthy and affected tissues of white-clawed crayfish, including viral, bacterial, and other microorganisms. The presence of these microorganisms reflects that the carapace is open to the environment, and that organisms are able to settle and survive on the crayfish. Many of these will be harmless.

Despite extensive investigation, no single disease, pathogen, pollution event or environmental factor could be conclusively linked to the clinical signs of disease and mortalities. It is possible that multiple factors, potentially including naturally occurring pathogens and environmental stressors, may have contributed to the mortalities. For example, a new disease or a new presentation symptom of an already existing disease which may have been triggered by a change in environmental conditions such as prolonged dry weather. However, the available evidence does not allow a definitive conclusion.

Although the cause of the mortalities remains unknown, the investigation generated important new knowledge. It demonstrated the value of combining field observations, pathology and emerging genomic techniques when investigating wildlife disease events. The work also revealed that white-clawed crayfish host a diverse range of naturally occurring microorganisms, highlighting the need for a better understanding of the relationship between crayfish health and their microbiome. Such knowledge could provide a better insight into levels of infection and allow crayfish movements to be undertaken with more confidence.

White-clawed crayfish are an endangered species that host multiple potentially pathogenic or opportunistic agents that do not necessarily manifest in disease at any particular time. While the causative factor(s) of novel disease signs remain unknown, experts at Cefas recommend that white-clawed crayfish (even if not showing visible signs of disease) collected from sites where carapace abnormalities have been observed and/ or significant mortalities have occurred are not translocated between catchments unless the 'new' site is unconnected to other water bodies, and do not already harbour white-clawed crayfish. Movement of white-clawed crayfish from unaffected areas are considered on a case-by-case basis and follow guidelines that currently recommend that all donor white-clawed crayfish must be quarantined for a minimum of 4 weeks if translocated between different operational catchments and only then translocated if observed to be visually healthy.

1 Introduction

The Environment Agency plays an important role in protecting and enhancing the environment across England. Its statutory duties include regulating activities that may affect air, land, and water quality; managing flood risk; protecting biodiversity; and promoting the sustainable use of natural resources (Environment Agency, 2021a). Central to its aims are the prevention, investigation, and mitigation of environmental incidents that pose risks to ecosystems, wildlife, and human health.

When incidents occur, such as pollution events, fish kills, or unexplained wildlife mortalities, the Environment Agency responds rapidly to assess causes, minimise impacts, and support ecological recovery. This response often involves close coordination with local authorities, conservation bodies, and other stakeholders to ensure an effective and proportionate approach (DEFRA, 2022).

Amongst the wildlife incidents investigated are reports of crayfish mortalities, which can indicate disease outbreaks (e.g. *Aphanomyces astaci*, the crayfish plague), broader ecological pressures such as low flows, poor water quality, or chemical contamination (Environment Agency, 2021b; Holdich et al., 2014). In such cases, the Environment Agency undertakes detailed investigations to identify the causes and support remediation.

In July/August 2024 extensive white-clawed crayfish (*Austropotamobius pallipes*; Figure 1) mortalities were reported from the Hart Burn, a tributary of the River Wansbeck, in Northumberland and subsequently a number of other rivers in the area. The white-clawed crayfish exhibited unusual clinical signs of disease. Initial assessments were unable to determine the cause of the mortalities. This report details the subsequent investigation into the cause of the mortalities in the North East area and another similar incident in the West Midlands area.



Figure 1. White-clawed crayfish (*Austropotamobius pallipes*).

2 Background

2.1 White-clawed crayfish

White-clawed crayfish are Britain's only native species of freshwater crayfish species and the largest of its freshwater invertebrates. They have an important role in the food chain and shape the physical environment of aquatic habitats (Holdich, 2003). They were once widespread across Britain but have been lost from around 70% of their former range due to introduced non-native invasive crayfish, habitat loss and water pollution (Defra, 2001; Chadwick, 2019). Additional and increasing threats include high water temperatures, drought, flood, diseases and changing climatic conditions. White-clawed crayfish are now classified by the IUCN red list as endangered in Britain and are protected from taking and killing under the Wildlife and Countryside Act 1981 (as amended) (Wildlife and Countryside Act 1981).

The Environment Agency has taken a lead role in the conservation of white-clawed crayfish (*Austropotamobius pallipes*) since the 1990s, when they were assigned to be the lead organisation for this species Biodiversity Action Plan, as part of the UK's commitment to the global Convention on Biological Diversity (JNCC, 1994). White-clawed crayfish continue to be a flagship species for the Environment Agency. This means delivering bespoke and broad recovery actions, which include; improved water quality and habitat, translocations to create Ark sites and responding to incidents involving white-clawed crayfish, with the help of catchment partners. These actions help protect and recover this species as part of the governments legally binding targets to increase species abundance and improve the extinction risk of threatened species (Defra, 2023).

As a legally protected species incidents involving White-clawed crayfish will activate either a category 1 or 2 response under our Common Incidents Classification System (CICS) categorisation and will be investigated immediately (Environment Agency, 2025). Where mortalities are confirmed specimens are tested by Centre for Environment, Fisheries and Aquaculture Science (Cefas) Fish Health Inspectorate, for notifiable diseases.

2.2 Crayfish health

There have been a variety of pathogens (microorganism that can cause disease) described within crayfish populations across the globe ranging from viral infections to parasites (Longshaw, 2011). Of these 3 diseases are listed as notifiable to the World Organisation for Animal Health (WOAH), Crayfish plague, White Spot Syndrome Virus (WSSV), and Decapod Iridescent Virus (DIV1). Crayfish plague is perhaps the most well-known disease in crayfish species and is caused by the oomycete *Aphanomyces astaci*. This pathogen affects the cuticle and causes disease in some but not all crayfish species. White-clawed crayfish are susceptible to infection and experience mortality while other species, such as the American signal crayfish (*Pacifastacus leniusculus*) are non-susceptible carriers of the disease. The first sign of an outbreak in a population is high

level of mortality observed within a short space of time. Disease is spread by contact between crayfish individuals and downstream by spores in the water. Affected animals can unusually be seen during daylight hours, some of which may display a lack of co-ordination, falling onto their back and being unable to right themselves. In some cases, a brown colouration of the cuticle may occur, and hyphae may be visible in the affected cuticle.

White Spot Syndrome Virus is a DNA virus that affects the cuticular epithelium (layer of cells underneath the shell) of crustacean species (crabs, lobsters, shrimps) White-clawed crayfish have been shown to be susceptible to this infection (Bateman et al., 2012). This disease is also listed as notifiable in UK and European legislation. However, WSSV has not been reported in wild populations of crayfish in UK. DIV1 is also a DNA virus which has been reported in Asia: this viral infection has not been reported in white-clawed crayfish.

In addition to the WOAHA listed diseases, multiple viral infections have been reported affecting different tissues in various crayfish species. Some of these descriptions are incidental findings while others have been related to mortality events (Longshaw, 2011). Some but not all of these viral infections have been described within white-clawed crayfish. The majority of crustacean viruses have been tentatively assigned to families based upon morphological and developmental characteristics and the location within the cell, including the intranuclear bacilliform viruses (IBV). In recent years IBV infections in other crustacean species have been formally characterised as DNA viruses within the family *Nudiviridae*. It has recently been determined that *Austropotomobius pallipes* Bacilliform Virus is a member of the *Nudiviridae* family, and this virus will be renamed *Austropotomobius pallipes nudivirus* (AupNV) (Bojko, unpublished data).

A viral infection in white-clawed crayfish was reported to be associated with brown spots on the cuticle of affected animals in Eastern France (Grandjean et al., 2019). The affected population showed signs like that reported for crayfish plague infections, animals were reported to be active during daylight hours amongst multiple dead animals and the infection was shown to spread upstream of the initial outbreak. Affected animals displayed shell lesions on the abdomen and discoloured brown spots on the cuticle, the diameter of which varied from 1 to 20mm. The animals tested negative for crayfish plague and further analysis showed that the brown spots were associated with presence of a virus belonging to the *Phenuiviridae* family of negative ssRNA viruses and was named Bunya-like Brown Spot Virus (BBSV). To date this virus has not been shown to be present in UK populations of white-clawed crayfish.

Porcelain disease is an infection of muscle, including heart, in White-clawed crayfish and is caused by a microsporidian parasite *Astathelohania contejeani*, previously known as *Thelohania contejeani* (Stratton et al., 2022). Muscle tissue in affected animals looks opaque/white in appearance and can be observed through the cuticle of the abdomen. Infection develops throughout the muscles resulting in loss of muscle function leading to lethargy, the tail-flick escape reaction is impaired, and the disease can be fatal. The infection has been reported via visual observations to affect low numbers of individual white-clawed crayfish across multiple sites in UK. Prevalence in wild crayfish is thought to

be <5% (Chong, R.S., 2022), but further work is required to establish a true estimate of prevalence in UK populations of white-clawed crayfish. Details of the pathogens which have been described affecting white-clawed crayfish, the tissues affected and the associated clinical signs of disease are presented in Table 1.

Investigating mortality events and adverse health conditions in wild aquatic populations can be challenging and usually relies upon opportunistic sightings of dead or diseased animals. The first step is to gather observational evidence at the site affected, including information about environmental conditions and detailed observations from the animals themselves. This is usually followed by more detailed laboratory assessments. Detailed sampling of tissues is used for multiple diagnostic methods to identify presence of known pathogens and/or identify new and emerging diseases. Investigating dying (moribund) animals is the primary way new or emerging diseases of invertebrates are discovered.

Table 1. Known pathogens affecting white-clawed crayfish, the tissues affected and the associated clinical signs of disease.

Pathogen Name	Pathogen Type	Tissue Affected	Gross pathology	Histopathology	References
Austropotomobius pallipes bacilliform virus (ApBV)	DNA Virus	Hepatopancreas tubule cells	Associated with mortality event. No gross pathology.	Infected cells display hypertrophied nuclei with marginalised chromatin. Multiple tubules and multiple cells within a single tubule can become infected. Virions are rod-shaped with a trilamellar envelope and a lateral expansion at one end. Virions measure ~60 x 260 nm and nucleocapsids measure ~50 x 220 nm.	Edgerton et al. (2002b); Edgerton (2003)
White Spot Syndrome Virus (WSSV)	DNA Virus	Cells of mesodermal and ectodermal origin, especially the cuticular epithelium and subcuticular connective tissues	White spots can be observed on the cuticle of penaeid shrimps, spots are not commonly observed in other species such as crayfish. Affected animals are lethargic and cease feeding.	Infected cells display hypertrophied nuclei with marginalised chromatin containing eosinophilic or basophilic inclusions. Multifocal necrosis associated with pyknotic and karyorrhectic nuclei in affected areas. Virions are ovoid or ellipsoid to bacilliform in shape, and measure 80–120 nm in diameter and 250–380 nm in length. A flagella-like extension (appendage) may be observed at one end of the virion.	Bateman et al. (2012)

Pathogen Name	Pathogen Type	Tissue Affected	Gross pathology	Histopathology	References
Bunya-like Brown Spot Virus (BBSV)	RNA Virus	Haemolymph, nerve, hepatopancreas, gills and muscle tissues	Associated with mortality event. Affected animals are observed in daylight hours and show loss of coordination. Discoloured brown spots or lesions on carapace.	No histopathology details available. Virions appear spherical, with diameters generally ranging from 80 to 120 nm.	Grandjean et al. (2019)
<i>Nocardia</i> sp.	Bacteria	Single animal possesses a melanised fracture of the exoskeleton. Muscle tissues beneath were affected.	Lethargic and uncoordinated and moving around in daylight.	Gram positive, acid fast, filamentous bacteria. Several nodules formed from haemocyte infiltration contained acid-fast branching filaments	Alderman et al. (1986)
Crayfish plague <i>Aphanomyces astaci</i>	Oomycete	Soft cuticle as found on ventral abdomen and around joints	High levels of mortality in susceptible species. Affected animals are observed in daylight hours and show loss of coordination, falling onto their backs and remaining unable to right themselves. Occasionally, the infected animals can be seen trying to scratch or pinch themselves. In some cases, a brown colouration of cuticle and	<i>A. astaci</i> hyphae can be difficult to find in stained preparations and histopathology is not recommended for diagnosis. Presence of pathogen is confirmed by PCR.	WOAH (2025)

Pathogen Name	Pathogen Type	Tissue Affected	Gross pathology	Histopathology	References
			muscle may occur, or hyphae may be visible in infected cuticles in the form of fine brown (melanised) tracks in the cuticle.		
Black gill disease <i>Fusarium tabacinum</i> (Beyma) Gams.	Fungi	Gills	Delayed moulting	Gills contained numerous melanised encapsulations. containing fungal mycelia surrounded by hyaline haemocytes.	Alderman and Polglase, (1985)
Porcelain disease <i>Astathelohania contejeani</i>	Microsporidian	Muscle tissues	Muscle tissues appear white as opposed translucent and can be observed through the ventral cuticle of the abdomen. Infection results in loss of muscle function and likely death.	Microsporidian parasites can be observed within the muscle bundles. Various developmental stages can be observed, spores develop within sporophorous vesicles. Parasite replaces muscle structure as infection progresses. Spores are oval shaped and measure ~3.6 x 1.9 µm.	Edgerton et al. (2002a, Stratton et al. (2022)

2.3 White-clawed crayfish mortality in the North East area

2.3.1 Multiple reports of mortalities

The initial report of dead crayfish was made to the Animal Health Agency on the 21st July 2024 by a member of the public. On the 12th August 2024 the Environment Agency received a report of 7 dead white-clawed crayfish on Hart Burn, tributary of River Wansbeck (NZ0884486547) upstream of Hartburn Village (Figure 2). In the following 2 days the site of the reported incident was visited with both live and dead, white-clawed crayfish found. There were no visual signs of pollution and field measurements of water quality raised no concerns. Only live fish were observed and no fish in distress were seen. Seven dead white-clawed crayfish were collected, preserved and sent to Cefas for analysis (see section 2.3.2). Continued surveys found additional mortalities alongside live white-clawed crayfish including some with unusual clinical signs of disease (see section 2.3.3) in the wider Wansbeck catchment. Further investigative surveys were conducted in adjacent catchments (see section 3.1.1).

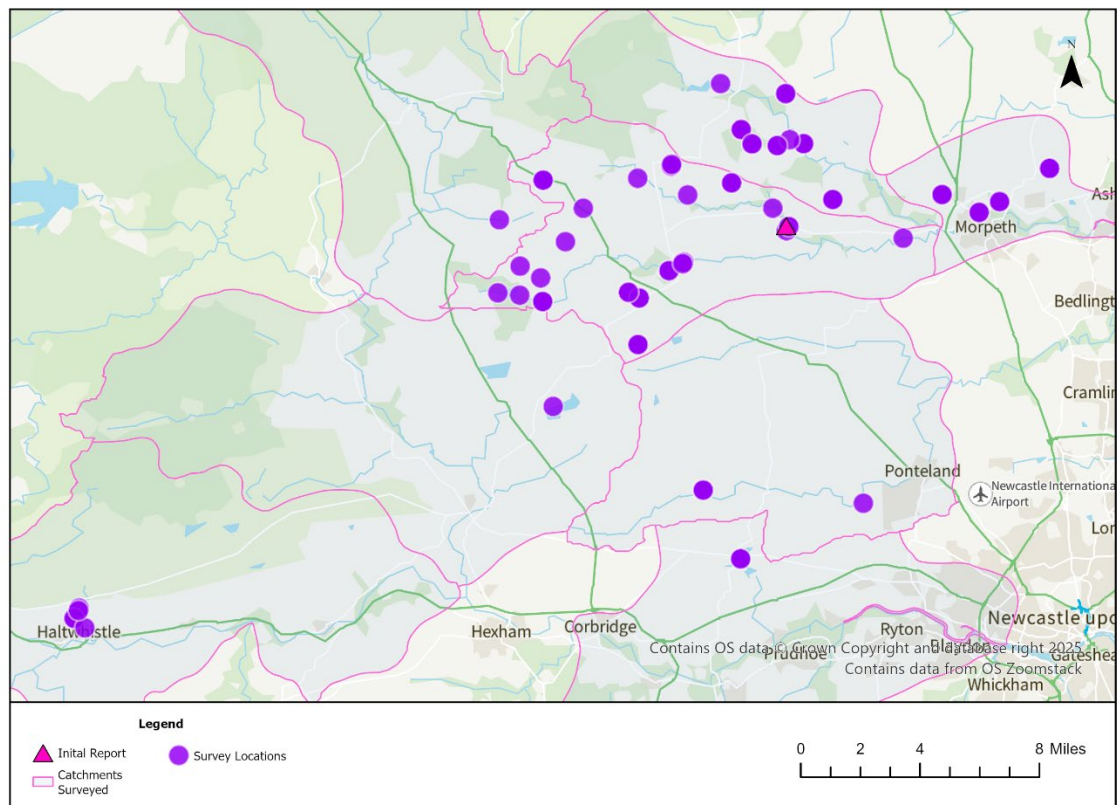


Figure 2. Survey locations across all catchments during the incident. The pink triangle indicates initial report of mortalities on the Wansbeck at Hartburn.

2.3.2 Pollution investigation

During the initial response to the mortality on 13th August 2024 a visual assessment suggested no pollution to the watercourse and a testing of the water quality with a multi-meter probe raised no concerns. There were no dead fish observed and a biological survey on 19th August 2024 indicated a healthy macroinvertebrate population in the Wansbeck. Due to the lack of other indicators and that white-clawed crayfish mortalities and clinical signs of disease occurred over a widespread area it was considered unlikely that the impacts were due to pollution. However, for a complete assessment, water quality sampling was undertaken at three locations in the Wansbeck on the 20th September 2024. Samples were collected in proximity to the initial report, upstream on the Hart Burn and downstream of the confluence of the River Font on the main River Wansbeck (Figure 3). These locations give a good indication of the water quality at the location of the initial incident as well as in adjacent watercourses. The water quality determinants measured were all within the optimal range for white-clawed crayfish (Marshall and Nightingale, 2022).

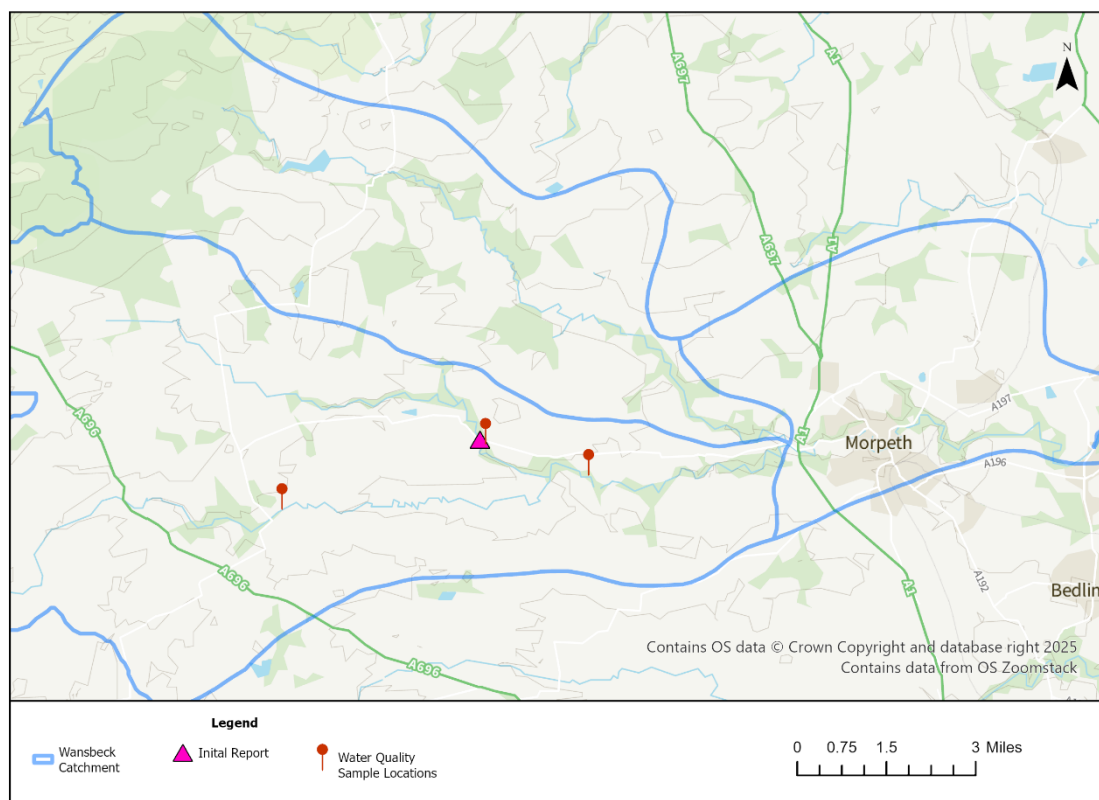


Figure 3. Location of water quality sampling undertaken on the 20th September 2024.

2.3.2 Screening for known diseases

Specimens of white-clawed crayfish were sent to Cefas for analysis of known crayfish diseases (Table 2), including white spot syndrome virus (WSSV), crayfish plague (*Aphanomyces astaci*), Austropotomobius pallipes bacilliform virus (ApBV), porcelain disease (*Astathelohania contejeani*) and Bunya-like Brown Spot Virus (BBSV). Crayfish

plague and WSSV tests were negative, ApBV and porcelain disease were shown to be present within the tissues, but infection levels were low and considered to be incidental infections.

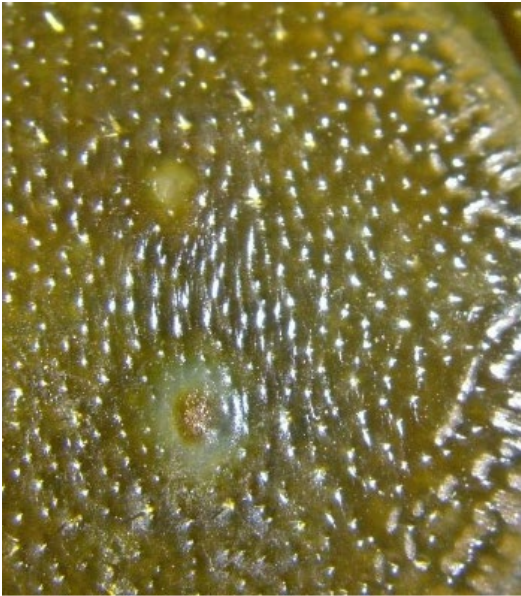
Table 2. Summary of results from analysis undertaken by Cefas

Date sampled	Date of results	Crayfish sample size	Results	
14/08/24	27/08/24	6 (dead)	Crayfish plague	Negative
			White-spot syndrome virus	Negative
			Porcelain disease	Retrospective molecular work confirmed that the sample was positive
5/09/24	27/09/24	19 (live/moribund)	Crayfish plague	Negative
			White-spot syndrome virus	Negative
			Porcelain disease	3 individuals displaying microsporidian infection by histopathology and confirmed by molecular analysis
			BBSV	No gross pathology, Molecular testing also negative.
			Bacteriology	10 samples No predominant species observed
8/10/24	1/11/24	10 (live/moribund)	Crayfish plague	Negative
			White-spot syndrome virus	Negative
			Porcelain disease	2 individuals displaying microsporidian infection
			BBSV	Negative
			Bacteriology	10 samples No predominant species observed

2.3.3 Unusual clinical signs of disease

The unusual clinical signs of disease were originally observed as patches of whiteness on the lateral area of the head and large lesions or other small orange markings on various areas of the body (Table 3; Appendix 1).

Table 3: The unusual clinical signs of disease observed on white-clawed crayfish.

Clinical signs of disease	
Small white spots with a yellow or orange centre	
	
Orange or red blotches – specifically with no obvious signs of injury such as partial consumption by a predator or crushing.	
	

Clinical signs of disease

Significant holes to the carapace which appear ulcerative in nature and not to have been caused by physical injury



Mottling to the side of the head



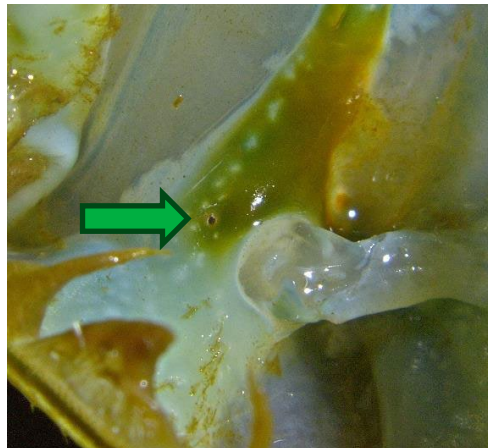
Dark patches on thorax (body)



White patching on underside of claws.



Enflamed excretory pore



Cefas Fish Health Inspectorate confirmed that the statutory disease investigation had not identified a notifiable disease. The Area crayfish experts involved with investigating the incident, considered that the carapace abnormalities were novel due to the lack of any

similar clinical signs of disease being observed in crayfish historically in the region, particularly in the last five years, during intensive surveying of crayfish

The carapace abnormalities were observed in a proportion of individuals at sites within the Wansbeck catchment and at varying degrees at sites within the wider area (see sections 3.1.1 and 4.1.1 for details).

Consultation with national and international crayfish experts and a search of published studies suggested that the brown/orange mottling clinical signs of disease observed were novel and had not been reported before in Britain. To investigate further researchers at Teesside University were commissioned to investigate the potential cause of the mortalities.

2.4 White-clawed crayfish incident in the Midlands

2.4.1 River Arrow, Herefordshire

On 10th June 2025 40-60 dead white-clawed crayfish were found during a routine biological survey by the Environment Agency at two sites, Downfield Bridge (SO 31665 57227) and Mowley (SO 34904 59444) on the River Arrow between Kington and Pembridge, Herefordshire. This section of the River Arrow was known to have historically supported a large population of white-clawed crayfish. Some of both the deceased and live white-clawed crayfish that were found showed carapace abnormalities including mottling, orange spots and burn like spots, which were considered to be comparable to the carapace abnormalities observed in the North East area mortality incident. Live fish and other invertebrates were observed in the River Arrow and there were no indications of pollution. Specimens of recently deceased white-clawed crayfish were sent to Cefas for analysis of known crayfish diseases.

On 26th June 2025 further surveying for crayfish was undertaken at four additional sites in the River Arrow between Kington and Pembridge. During 10-minute searches small numbers of white-clawed crayfish were found. All appeared to be healthy apart from a couple of individuals that had raised red spots on their carapace and a couple of single discarded claws were found which had unusual raised red blobs on them. In addition, a member of the public reported observing one dead white-clawed crayfish on the 21st June at Kington.

On 15th July 2025 baited traps were deployed at Downfield Bridge and Mowley to collect white-clawed crayfish for additional molecular testing by Cefas. Thirty animals showing minor carapace abnormalities from each site were collected from the traps or by hand on the 16th July. Macroinvertebrate kick samples at both sites resulted in expected assemblages. Water quality samples collected and analysed in the Environment Agency laboratory were within the optimal range for white-clawed crayfish (Marshall and Nightingale, 2022).

To investigate further researchers at Cefas investigated the potential cause of the carapace abnormalities by comparing individuals exhibiting clinical signs of disease from the River Arrow in the Midland with those from a population in the Candover stream, Hampshire that did not show clinical signs of disease (SU 56950 33501).

2.4.2 Whitmans Quarry, Worcestershire

On 27th July 2025 white-clawed crayfish with carapace abnormalities were observed at Whitman's Hill Quarry in the Malvern Hills, Worcestershire. This followed a hot dry summer and water levels in the quarry pool were observably low. The white-clawed crayfish population in the quarry pool was established in July 2022 as a planned and licensed ark site by translocating approximately 500 white-clawed crayfish from the River Redlake in Shropshire during a drought event. 132 adult and juvenile white-clawed crayfish were caught in baited traps, artificial refuge traps and by hand netting. 48% had unusual orange markings and legions (Table 4, Appendix 2). Field- and laboratory-analysed water quality tests raised no concerns, apart from high water temperature.

On a follow up visit on 11th October water levels in the quarry pool were very low. Twenty white-clawed crayfish were observed, including females carrying young. None of the white-clawed crayfish exhibited visual signs of disease.

Table 4: The unusual clinical signs of disease observed on white-clawed crayfish at Whitman's Hill Quarry.

Clinical signs of disease	
	

Clinical signs of disease



3 Methods

A series of approaches were used to better understand the occurrence of the carapace abnormalities and identify potential causes. Observations of the prevalence and progression of carapace abnormalities were made through field surveys and laboratory observations. Additional assessments involving the microscopic examination of crayfish tissue and genetic approaches were used to try and identify the potential causes of the abnormalities and whether it is an infection that could be spread to other populations..

3.1 Observational study

3.1.1 Field surveys

Following initial site visits in response to the reported mortalities in the North East area, 49 site surveys were undertaken, across 41 sites, during 2024 (Figure 4) to assess the extent of the occurrence of carapace abnormalities and crayfish mortalities. Surveys were mainly by manual searches but were supplemented by baited traps, artificial refuge traps (ARTs) and Pritchard traps. The methods used aligned with approaches previously used to allow population size comparisons to be made between years.

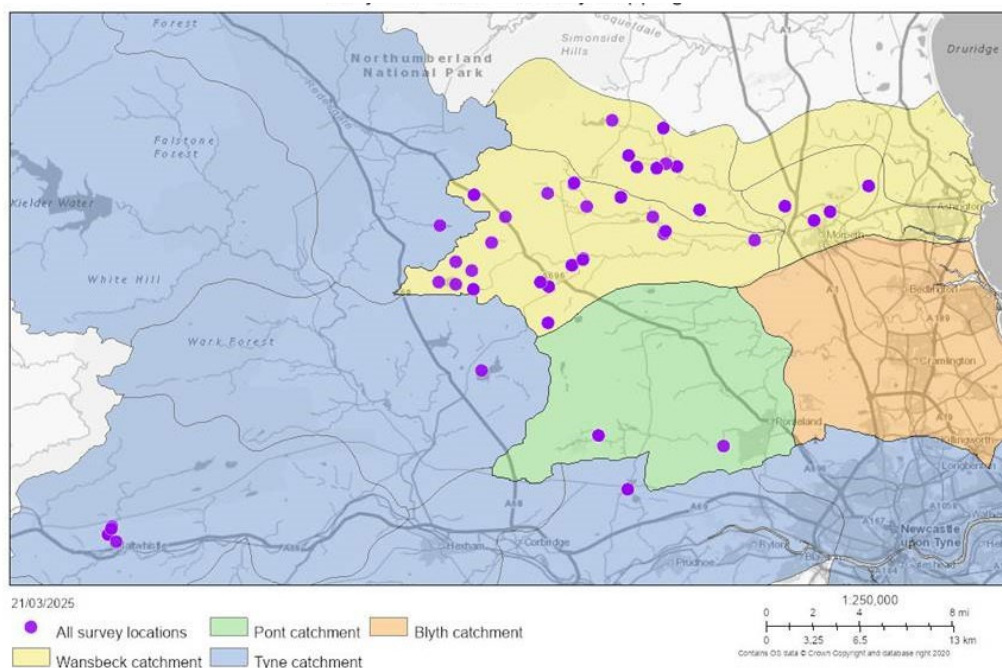


Figure 4. Location of 2024 field surveys

During the 2025 Crayfish survey season (July- October) a targeted approach to surveys was implemented (Table 5). Sites were selected according to 5 categories;

1. Areas already affected to determine likely on-going impacts.
2. Known white-clawed crayfish locations to assess wider occurrence within the area.
3. Historic Ark sites to assess population health.

4. Known signal crayfish locations to assess any occurrence of clinical signs of disease.
5. To collect samples for analysis at Teesside University.

Table 5. The location and reason for surveys in 2025.

Site	Catchment	Reason	Technique
Wansbeck (Wallington)	Wansbeck	Specimens for Teesside Uni	Baited traps
Lowick Pond complex	Northumberland	Ark site check	Baited traps
Hunting Hall	Northumberland	Ark site check	Baited traps
Brunton Burn	Northumberland	Ark site check	Baited traps
Forest Gate Burn	Coquet	Ark site check	Baited traps
Bellasis Bridge	Blyth	Signal crayfish collection	Baited traps
Langley Beck	Tees	Signal crayfish collection	Baited traps
Delf Burn	Wansbeck	Intensive survey effort	Pritchard
Wansbeck (Wallington)	Wansbeck	Intensive survey effort	Pritchard and ART
Aln	Aln	Check spatial extent	Manual search
Crook Burn	Tyne	Check spatial extent	Manual search
North Tyne	Tyne	Check spatial extent	Manual search
Hart Burn	Wansbeck	Assess mortality location	Manual search
Pont	Pont	Spot check	Manual search

3.1.2 Crayfish laboratory observations

Thirty-two crayfish from the original incident site at Hartburn, were collected on the 31st of October 2024 and held in tanks to observe the development of the carapace abnormalities. The aims were to observe the range of abnormalities, document the progression of clinical signs of disease, determine crayfish recovery and mortality rates and determine whether these varied between with crayfish size class.

The majority of the crayfish collected were male to cause minimal disruption to the breeding potential of the population as the females were with eggs and it also avoided the need to house males and females separately. Individuals across different age ranges and with and without obvious carapace abnormalities were selected (Table 6). The crayfish were housed in a 2-tank system with one subdivided so that adult, sub-adults and juveniles were housed separately but in the same water (Figure 5).



Figure 5. Laboratory observation tanks.

Table 6. Showing crayfish numbers brought to the laboratory at Tyneside House

Age	Without carapace abnormalities	With carapace abnormalities	Males	Females
Juveniles	8	3	7	4
Sub Adults	1	9	10	
Adults		11	11	
Total	9	23	28	4

Standardised documentation of crayfish anatomical features was used for the accurate recording of the advancement or variation in carapace abnormalities. Recording was undertaken weekly (adults and sub-adults) and monthly (juveniles) to minimise stress. From May 2025 observations were reduced to monthly.

The individuals brought from the river showed a variety of abnormalities (Table 3) that broadly fell into 4 categories;

1. Light markings on the claws (chelipeds), tail or hard upper section of the exoskeleton (carapace).
2. Discoloration (melanisation) of the exoskeleton.
3. Orange/red markings on the limbs on the underside of the crayfish.
4. Major deformities of the exoskeleton with or without discoloration.

It was hypothesised that the clinical signs of disease progress from lighter marking to darker lesions and deformities and eventually death (Figure 6).



Figure 6. Illustration of expected progression of the carapace abnormalities.

3.2 Looking for the cause of the disease

3.2.1 Selection of specimens for examination

North East Area/Teesside University investigation

In total, 26 white-clawed crayfish were submitted to the National Horizons Centre (Teesside University) Animal Pathology laboratory for analysis. The animals were collected from a variety of sites within the Wansbeck catchment (see Section 2.3). Upon arrival to the NHC laboratory, the animals were immediately dissected by first halving the animals and then harvesting the organs for diagnostic examinations (Table 7).

Table 7. Samples taken from each animal for the various diagnostic methods. as described in the following sections. Those indicated with a star (*) were sampled by Cefas only.

Fixative	Method	Tissues Sampled
Davidsons freshwater fixative	Histology	Hepatopancreas Gill Gut Muscle Gonad Heart Nerve Antennal gland Carapace
2.5% Glutaraldehyde in 0.1M sodium cacodylate buffer	Transmission Electron Microscopy	Hepatopancreas Gill Muscle
Ethanol	Molecular analysis (PCR and metagenomic sequencing)	Hepatopancreas Gill Gut* Muscle Carapace
RNA Later	Molecular analysis (PCR and metagenomic sequencing)	Hepatopancreas* Gill* Carapace*
Liquid nitrogen (snap frozen)	Molecular analysis (PCR and metagenomic sequencing)	Haemolymph Hepatopancreas

Midlands/Cefas investigation

The white-clawed crayfish collected from the two sites on the River Arrow in Herefordshire on the 16th July 2025 were transported back to Cefas facilities where they were housed in two separate tanks overnight, one tank per site. Males and females were separated via a central division in both tanks to limit predation and tanks were covered with nets to prevent escape (Figure 7).



Figure 7. Crayfish were held in large tanks overnight at Cefas. Tanks were divided to separate male and female animals and hides were provided to limit predation. Tanks were secured with nets to prevent escape.

Animals were sampled the following day, full examination of the carapace was undertaken, with tissues being sampled (Table 7). At site 1 14 females and 16 male crayfish were sampled, cephalothorax lengths ranging between 33 and 50 mm, total lengths between 71 and 106 mm and weights between 10 and 47 g. At site 2 19 females and 11 males were sampled, animals appearing slightly smaller with cephalothorax lengths ranging between 29 and 48 mm, total lengths between 59 and 107 mm and weights between 7 and 40 g.

Hampshire/Cefas investigation

Individuals of white-clawed crayfish exhibiting clinical signs of disease were compared with individuals from the Candover stream, Hampshire. None of the animals from the Candover stream displayed shell discolorations or lesions like those reported in the North East or Midland areas. Thirty animals were collected from the site and transported back to Cefas for processing. Fourteen females and 16 male crayfish were sampled, cephalothorax lengths ranging between 17 and 34 mm, total lengths between 44 and 74 mm and weights between 4 and 21 g. Samples were taken as detailed in Table 7.

3.2.2 Visual and microscopic examinations

These examinations involved assessing the pathology – observation of changes in the host's tissues as a result of disease and parasites etc. Gross pathology is done by general observation. This full examination of the carapace was undertaken on all animals, external deformities as detailed in section 2.3.3 were noted. The visual observations were followed by examination of tissues using histology - the study of the tissues and organs through sectioning, staining, and examining those sections under a microscope. A full histological investigation was completed in line with usual practice for disease investigations. If abnormalities are detected, organs and tissues are then further examined using appropriate methods such as molecular approaches and ultrastructural analysis. This

approach is routinely applied in the health screening of wild populations and enables the confirmation of known pathogens and description of novel pathogens observed infecting tissues and organs of animals.

3.2.3 Screening for porcelain disease *Astathelohania contejeani*

Molecular screening techniques (PCR) can be applied to look for specified pathogens. Muscle tissues were screened via qPCR for presence of porcelain disease *Astathelohania contejeani* in all animals from the Midlands and Hampshire. This work involved designing and validating different methods, a new 18S region quantitative PCR probe and an updated conventional PCR assay. Both types of PCR were then applied to the samples alongside the previously described *A. contejeani* specific conventional PCR assay (El-Matbouli and Soliman, 2006).

3.2.4 Genetic assessments

In total, 26 white-clawed crayfish were submitted to the National Horizons Centre (Teesside University), Animal Pathology laboratory for analysis. DNA and RNA extraction was conducted on tissue taken from the crayfish and were analysed to determine whether viral, bacterial, or other microorganisms, were present. These DNA and RNA samples were either pooled from multiple individuals to give an overview at the population level before processing and sequencing using an Illumina next generation platform, or were sampled separately to give individual-level detail.

Tissues selected for sequencing included either the hepatopancreas or the cuticle (including lesion, if present). Sequencing data were analysed using bioinformatic software, trimmomatic (Bolger et al., 2014) and spades (Bankevich et al., 2012). Metagenomic and metatranscriptomic sequencing was performed. Metagenomics involves untargeted sequencing and analysis of total DNA, which can be assembled into (partial) genomes. Metatranscriptomics involves untargeted sequencing and analysis of total RNA, which can be assembled into RNA components (e.g. messenger RNA which copies a gene's code from DNA) or viral genomes. A further bioinformatic step was taken using metaxa2 software (Bengtsson-Palme et al., 2018) to determine the diversity of eukaryotic species, such as fungal species or protozoans.

4 Results

4.1 Observational insights

4.1.1 North East area field surveys

The carapace abnormalities were observed in a proportion of individuals at sites within the Wansbeck catchment (Figure 8) and at varying presence at sites within the wider area (Figure 9). The average proportion of observations was 20% ranging from 3% (Wansbeck at Wallington) to 57% (Wansbeck at Raechesters).

Porcelain disease was present at 11% of sites where carapace abnormalities were observed and at 2% of sites where they were not. Injuries were also more common at sites where carapace abnormalities were observed: 26% compared to 11%. However, some variation is to be expected since the survey methods varied between sites.

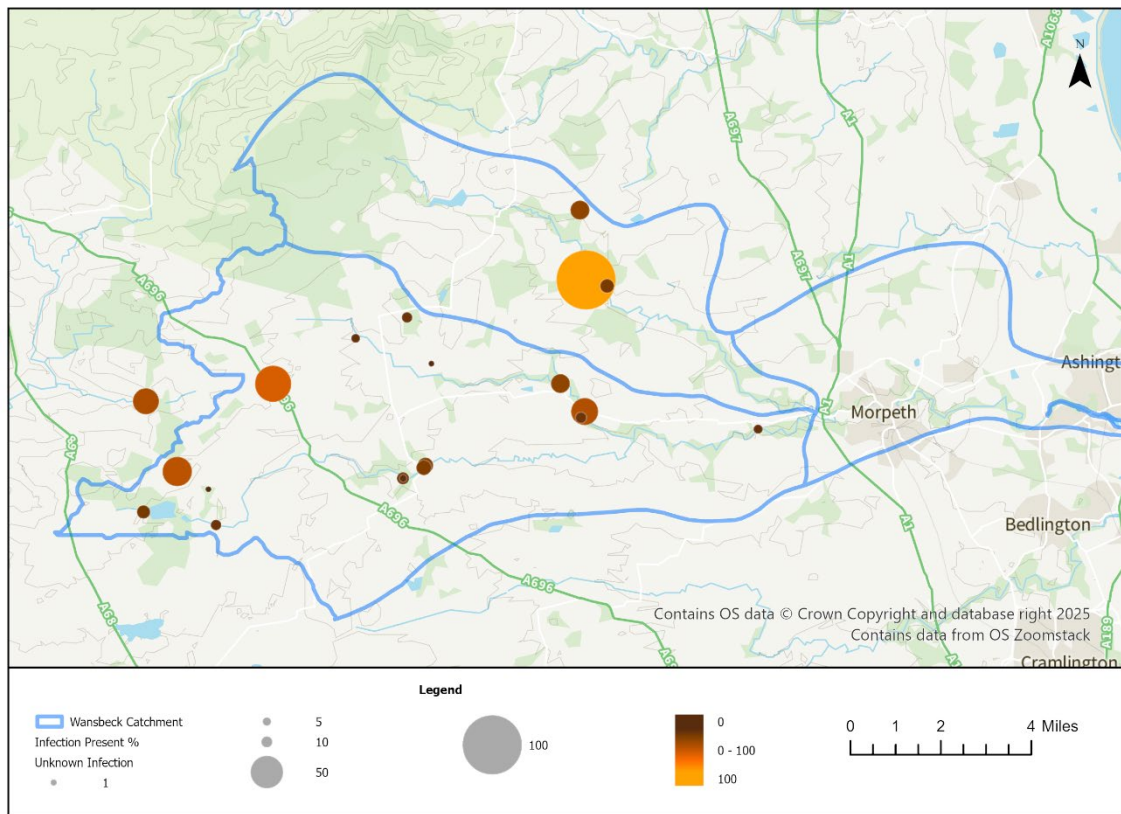


Figure 8. Percentage of crayfish with carapace abnormalities within the Wansbeck catchment

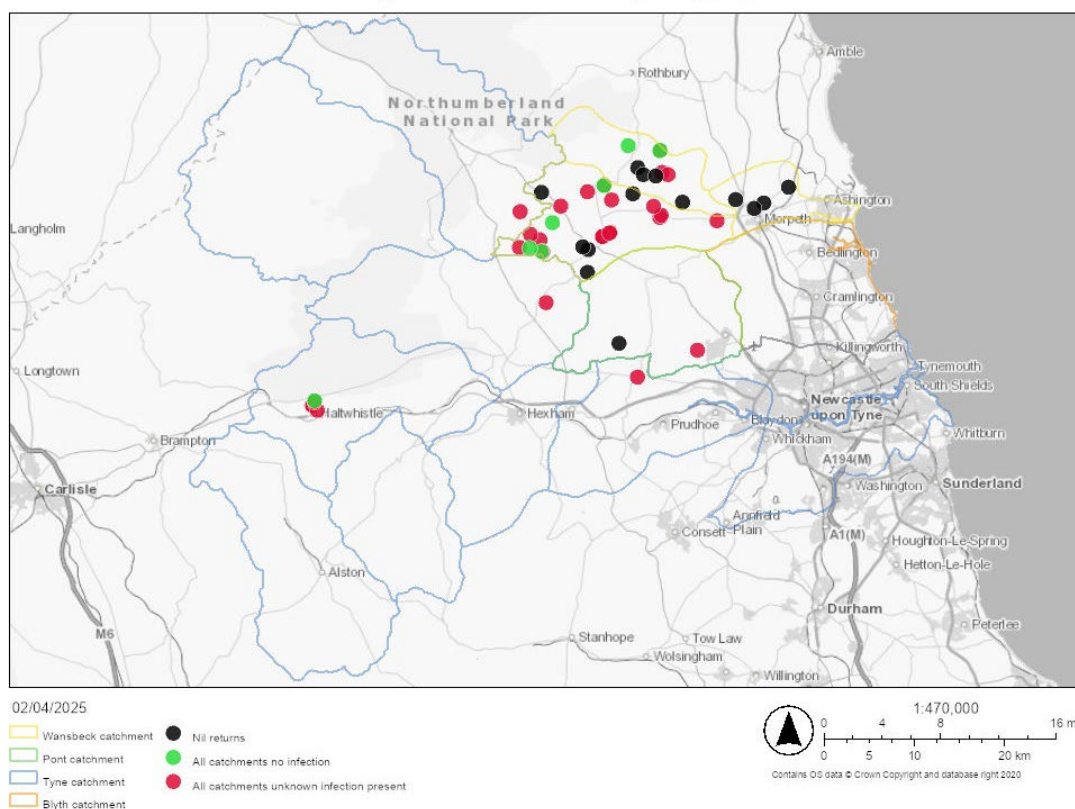


Figure 9. Occurrence of clinical signs of disease, no signs of disease and negative records from surveys across all catchments

Comparing the incidence of carapace abnormalities observation between 2024 and 2025 (Table 8) there was a large reduction in crayfish exhibiting clinical signs of disease across all catchments. Overall, there was a reduction from 26% in 2024 to 4% in 2025.

Table 8. Total crayfish surveyed by catchment in response to the incident (*no surveys were carried out on the Aln in 2024).

River Catchment	Crayfish surveyed 2004	Percentage with carapace abnormalities	Crayfish surveyed 2025	Percentage with carapace abnormalities
Wansbeck	1247	12	636	5
Tyne	140	20	103	2
Aln*	---	---	103	3
Pont	17	47	38	8

Comparing three sites surveyed in both years, with the same method, showed a similar reduction in observations of carapace abnormalities, from 16% of crayfish with clinical signs of disease in 2024 to 6% in 2025 (Table 9).

Table 9. Results from 2024 comparison sites.

Site	Crayfish surveyed 2024	Percentage with carapace abnormalities	Crayfish surveyed 2025	Percentage with carapace abnormalities	Method
Delph Burn	330	9	455	3	Pritchard traps
Hart Burn	149	20	131	9	Manual search
Wansbeck	97	18	50	6	ARTs

Three sites were surveyed repeatedly during 2025 and showed a reduction in the occurrence of crayfish with carapace abnormalities from July to September (Table 10). There was a notable reduction throughout the year at two of the sites and the main river Wansbeck stayed the same.

Table 10. White-clawed crayfish sites surveyed multiple times in the 2025 season

Site name	July numbers	Percentage with carapace abnormalities	August numbers	Percentage with carapace abnormalities	September numbers	Percentage with carapace abnormalities
Delph Burn	153	5	203	4	99	0
Wansbeck	15	7	20	5	15	7
Crook Burn	---	---	28	3.57	39	0.00

The three sites surveyed for signal crayfish recorded nearly a third of all the Signal crayfish caught were with carapace abnormalities which appeared to be similar to those observed with white-clawed crayfish (Tables 11 and 12).

Table 11. Results from the Signal crayfish surveys

Site	Crayfish numbers	Percentage with carapace abnormalities
Belasis Bridge - Blyth	317	33
Langley Beck - Tees	184	34
Langdon Beck- Tees	30	23
Total	531	33

Table 12. Carapace abnormalities observed on signal crayfish.



Carapace abnormalities



4.1.2 Crayfish laboratory observations

The progression of the carapace abnormalities and mortality were observed across the crayfish by size classes (Figure 10). One crayfish died in the first week, thought to be due to the stress of the move. Over the year four crayfish deaths were recorded – an overall mortality rate of 13%. Ten of the 23 individuals who had clinical signs of disease no longer exhibit signs suggests a recovery rate of 43%. In addition, 5 individuals (20%) who had clinical signs of disease showed an improvement. None of the 9 individuals without signs went on to develop clinical signs of disease over the year.

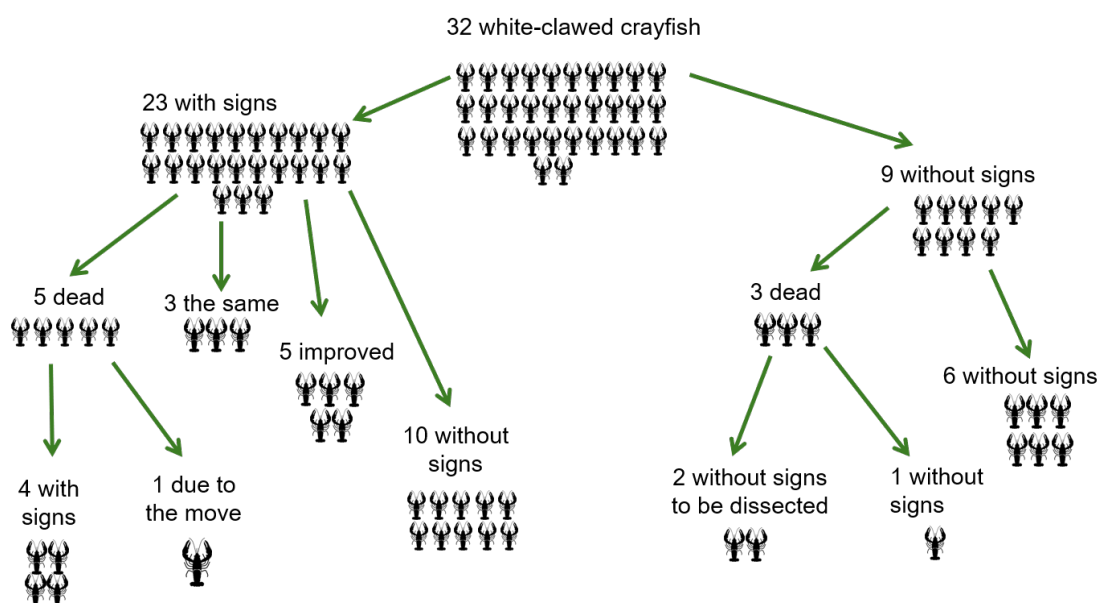


Figure 10. Showing the overall progression of clinical signs of disease seen in the crayfish within the laboratory observation study.

There was a notable difference in the mortality and recovery between the size classes. Only one juvenile died and it showed no outward signs of disease and is thought to have died from unrelated stress. Two juveniles were sent to Teesside University for comparison with those crayfish exhibiting clinical signs of disease. Removing these 3 individuals from further analysis meant that all the juvenile crayfish either recovered, improved or did not develop signs of disease (Figure 11). Notable reductions in the signs of disease were seen as the juveniles moulted which they do 4 to 5 times per year.

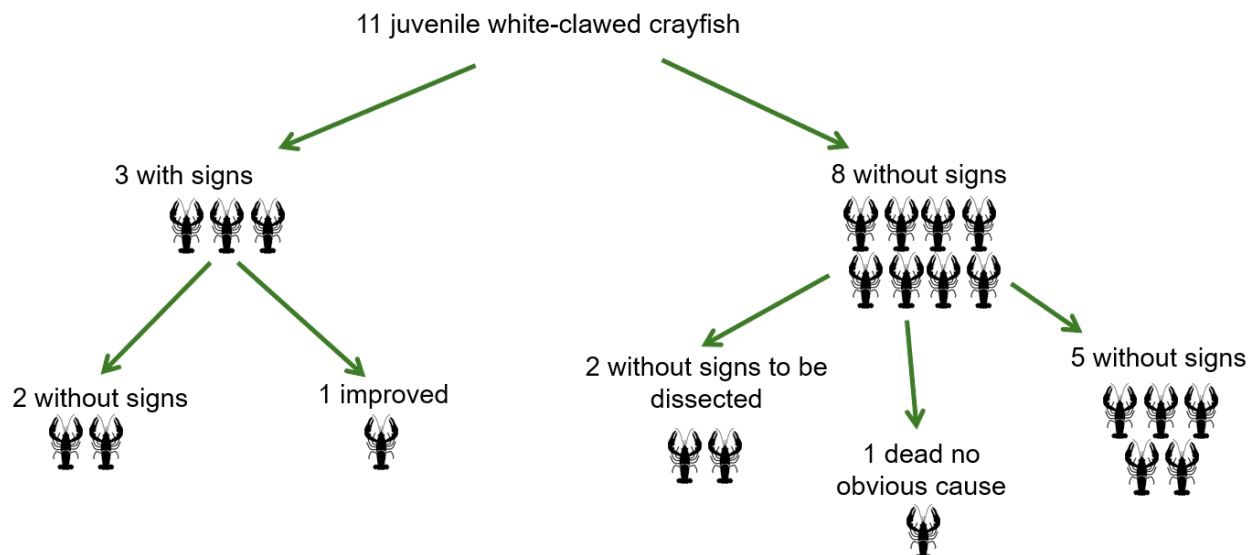


Figure 11. Showing the overall progression of clinical signs of disease seen in the juvenile crayfish within the laboratory observation study.

The 1 sub adult that had no clinical signs of disease when captured remained sign free throughout the year, 6 of the 9 individuals exhibiting signs no longer exhibited signs suggesting a recovery rate of 67% and 1 individual died (Figure 12). Individuals were noted to have incremental recovery exhibited by reduced melanisation and severity of clinical signs of disease throughout their moulting, which is 3 to 4 times per year for sub-adults.

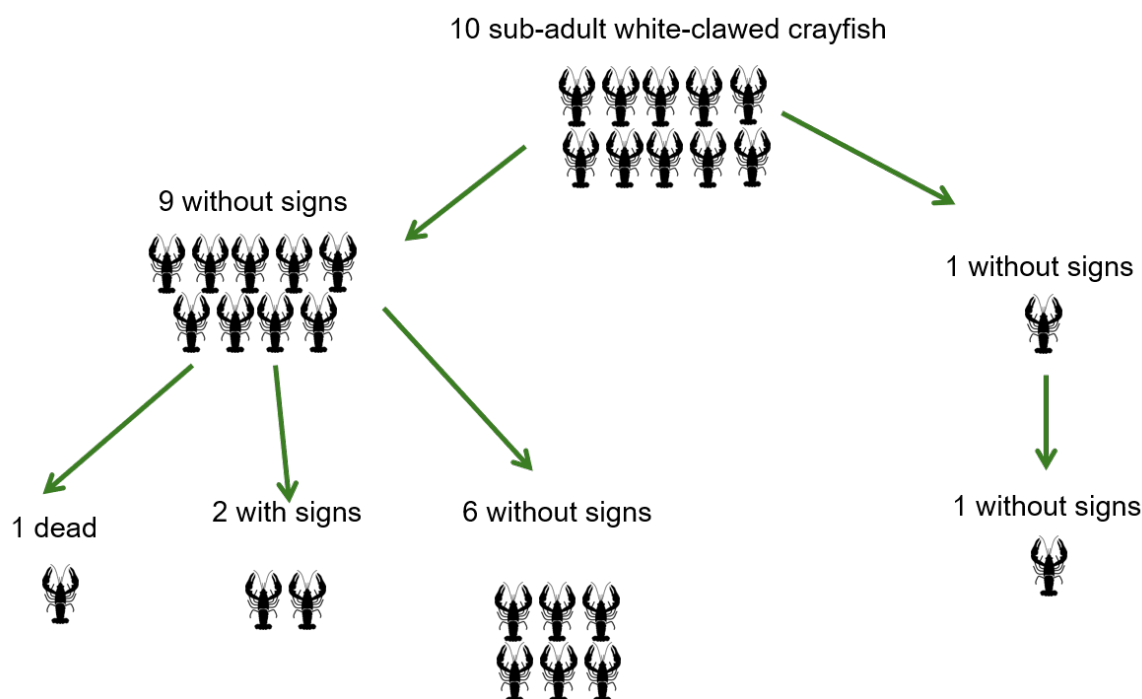


Figure 13: Showing the overall progression of clinical signs of disease seen in the sub-adult crayfish within the laboratory observation study.

All the larger adult crayfish exhibited clinical signs of disease when captured. Of these 4 died, (1 attributed to stress), suggesting a 27% mortality rate (Figure 13). Two became sign free (20%) and 4 (40%) showed some improvement.

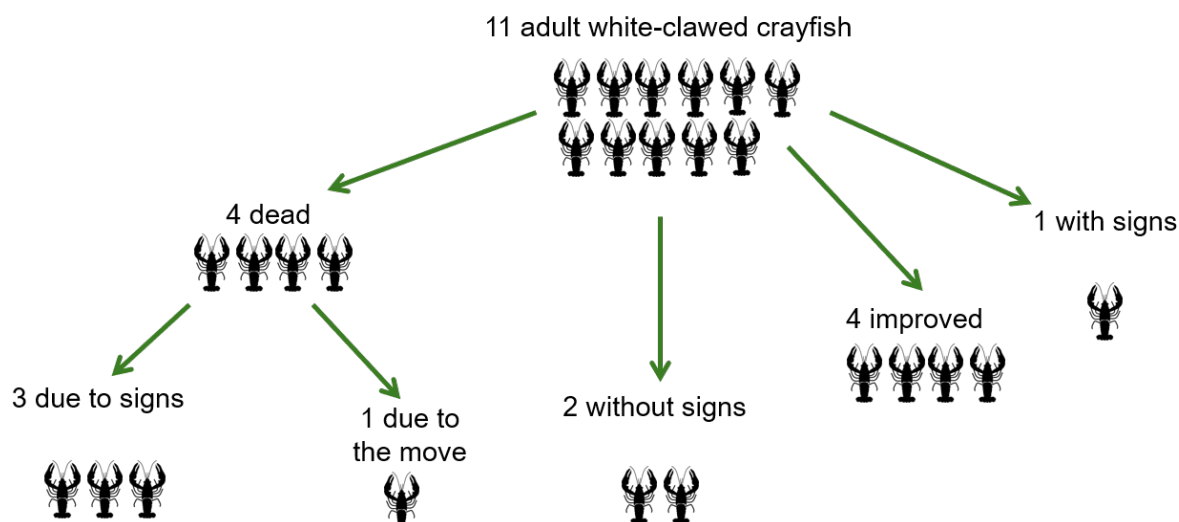


Figure 13. Showing the overall progression of clinical signs of disease seen in the adult crayfish within the laboratory observation study.

The reduction in clinical signs of disease observed through the moulting process is a reverse of the predicted progression of the carapace abnormalities (Figure 6). Observations are that when the crayfish moults, depending on the severity of the clinical signs of disease, they either reduce in size or typical dark melanisation becomes lighter and then over successive moults disappears. This pattern has been observed for the 3

categories of disease indications - light marking, melanisation and red limbs (Figure 6). Large deformities such as missing section of carapace were still evident even after moulting, though the associated melanisation reduced or disappeared over the study period.

4.2 Looking for the cause of the disease

4.2.1 Gross observations

A total of 26 white-clawed crayfish were delivered to the NHC for analysis, from sites across the North East area. These were received in 3 main batches: 1) batch 1 was delivered by Dr Josie South (University of Leeds) and included 13 individuals; 2) batch 2 was delivered at different time points from the Environment Agency, and composed of surviving animals from the original mortality event; 3) finally, batch 3001_001-003 and 016-018 included 3 “affected” and 3 “unaffected” animals from the Wansbeck site. In batch 1, one animal was noted with white muscle (porcelain disease), and one animal was noted with a significant carapace abnormality (Figure 14). In batch 2, animals surviving the original mortality event displayed small white or orange markings, with one animal displaying similar lesions on the claws. Finally, in batch 3, animals 001-003 displayed no external markings, but animals 016-018 displayed some orange markings or degraded carapace. This included animal 016, which displayed a significant abscess-like lesion noted in Figure 16.

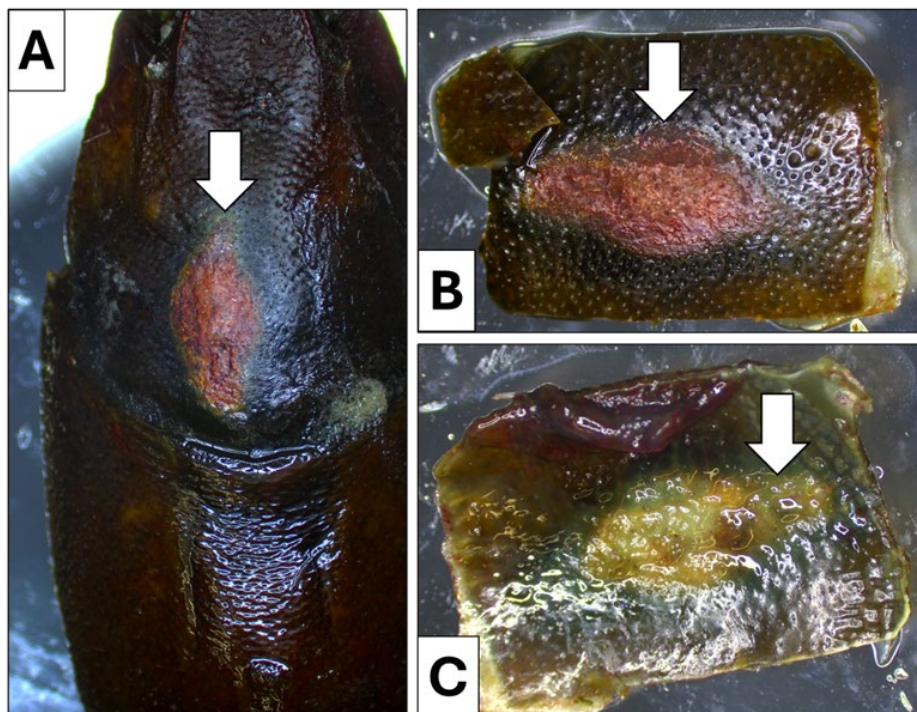


Figure 14. Observed on a white-clawed crayfish delivered to the NHC from the Wansbeck area. White arrows in the three images indicate the lesion both above (A-B) and below (C) the carapace.

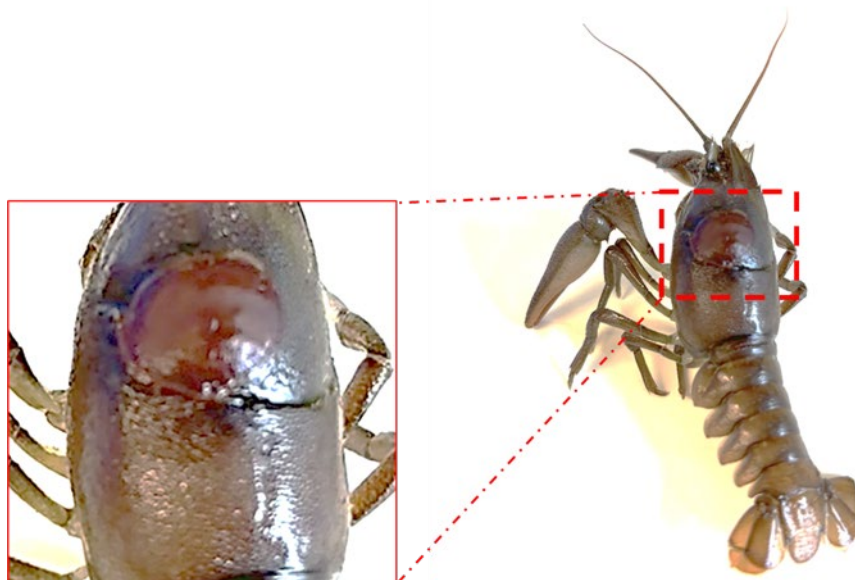


Figure 15. An image of the abscess present on animal JB25001_16.

During the external observations at site 1 from the River Arrow, Herefordshire a single animal was noted to show several red spots and discolouration on tail and claw, another animal displayed damage to the tail carapace and a third animal showed deformed carapace of the cephalothorax, orange spot on side of deformed carapace and some discolouration at the claw hinge (Figure 16). One animal was noted to display a dark mark at the bottom of the claws, but this was thought to be due to an old shell injury. At site 2 from the River Arrow 3 animals were noted to display a red colouration of the carapace on the underside and around the rostrum, pleopods and legs. A single animal showed a carapace deformity at the side of the head with granulomas noted to be present underneath the carapace in this region. A few animals were noted to show small areas of damage to the carapace. No external shell discolouration or lesions were noted in the animals sampled from Hampshire.

White muscle was observed at dissection and recorded in a single animal from site 1 and 4 animals from site 2 on the River Arrow in Herefordshire. Two animals were noted to have white muscle from the site in Hampshire.



Figure 16. Examination of an individual from the River Arrow. A) Damage to tail carapace (arrow). B) Animal was noted to show a few red spots and discolouration on tail (arrow) and claw. C) Deformed carapace of the cephalothorax (black arrow), orange spot on side of deformed carapace (white arrow) D) Damage to carapace at the top of the tail. E) Gross pathology of microsporidian infection. Affected muscle appears white in colouration. F) Uninfected muscle appears translucent (*), developing microsporidian infection can be observed in the whitened muscle at the edges of the tail (arrow).

4.2.2 Microscopic examinations

Histological analysis revealed the presence of microorganisms in the different tissues examined (Table 13; Figures 17 and 18). A viral infection thought to be *Austropotomobius pallipes nudivirus* (AupNV) was present in the hepatopancreas of animals at all sites sampled. The level of pathology varied between animals with some showing low level infection where a small number of hepatopancreas tubules were observed to contain a few enlarged nuclei within the section. While others showed more intense infections where multiple hepatopancreas tubules contained multiple infected cells with enlarged nuclei. A previous survey of white-clawed crayfish from the Hampshire site showed 40% prevalence of AupNV (Bateman, unpublished data).

The microsporidian parasite *Astathelohania contejeani* was also observed in a small number of animals, with the histological observations corresponding to the observation of white muscle which was noted at time of dissection. Microsporidian parasites could be observed developing within the muscle fibres, parasites replacing the host tissue. In severe infections the microsporidian spores (infectious units) were released from the sporophorous vesicles where they develop and could be observed within the haemolymph (blood) and dispersed throughout the tissues.

Apicomplexan protozoa thought to be gregarines were noted in the gut of multiple animals at all sites. Gregarines are single celled protists commonly observed in the guts of freshwater crayfish species and are thought to be commensal organisms (ones that live alongside a host).

Interestingly, areas of necrosis (dead or dying tissue) were noted in the cuticular epithelium of a few of the crayfish sampled from the two sites in Herefordshire. The tissues which lay just under the carapace were noted to show several areas of necrosis, identified by large numbers of deeply basophilic spherical inclusions likely to be dead and dying cells in these regions. There was no clear correlation between presence of these lesions and any discolouration noted on the cuticle in this survey. Similar pathology is noted in some RNA virus infections in shrimp tissues; further work is required to determine whether these lesions could be caused by a viral infection and whether this pathology could be related to the shell discolouration and lesions noted in some animals. In addition, areas within cells in the hepatopancreas that indicated that pathogens (microscopic organisms that can cause disease) might have been present but were not visible (cytoplasmic inclusions).

The pathogens that were detected were present in several individuals and their distribution did not correspond to those with external lesions and associated markings across all three affected sites. In particular, the crayfish that had a large lesion on the carapace from the Wansbeck region, did not have any novel or detectable parasites, and instead contained only gregarines in the gut. This means that the histological assessment could not identify a cause of the carapace abnormalities.

Table 13. Results of the microscopic examinations

Tissue	Observation	Percentage prevalence			
		North East Area (n = 26)	Site 1 Herefordshire (n = 30)	Site 2 Herefordshire (n = 30)	Hampshire (n = 30)
Muscle	Microsporidian parasite <i>Astathelohania contejeani</i>	12	3	6	6
Gut	Apicomplexan protozoans (putatively gregarines),	30	40	30	42
Hepatopancreas	A bacilliform virus <i>Austropotamobius pallipes</i> bacilliform virus.	35	63	40	39
Cuticular epithelium	Areas of necrosis in tissues underneath carapace	23	27	10	0

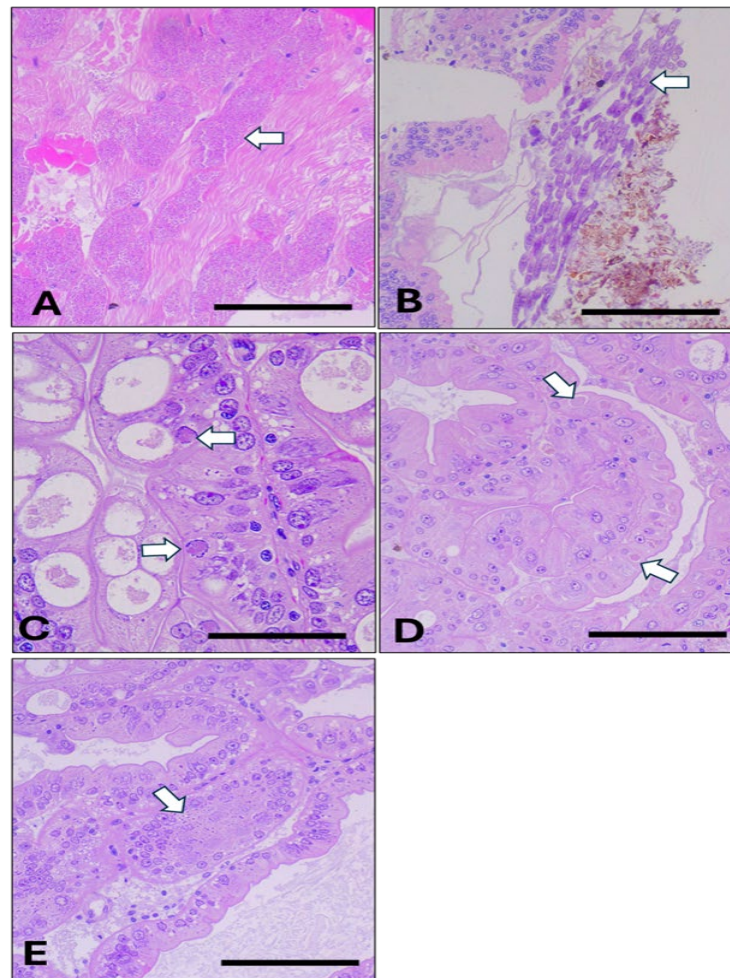


Figure 17. Histological assessment of crayfish tissues displaying parasites from the Wansbeck population. A) Microsporidian *Astathelohania contejeani* infection (white arrow) in the muscle tissue. Scale – 100µm. B) putative commensal gregarines (white arrow) in the gut lumen. Scale – 100µm. C) Intranuclear viroplasm formation and chromatin margination, indicating infection of cell nuclei. Scale – 100µm. D-E) Cytoplasmic inclusions (white arrow) in the hepatopancreatic epithelium, possibly indicating a response to a virus. Scale – 100 µm.

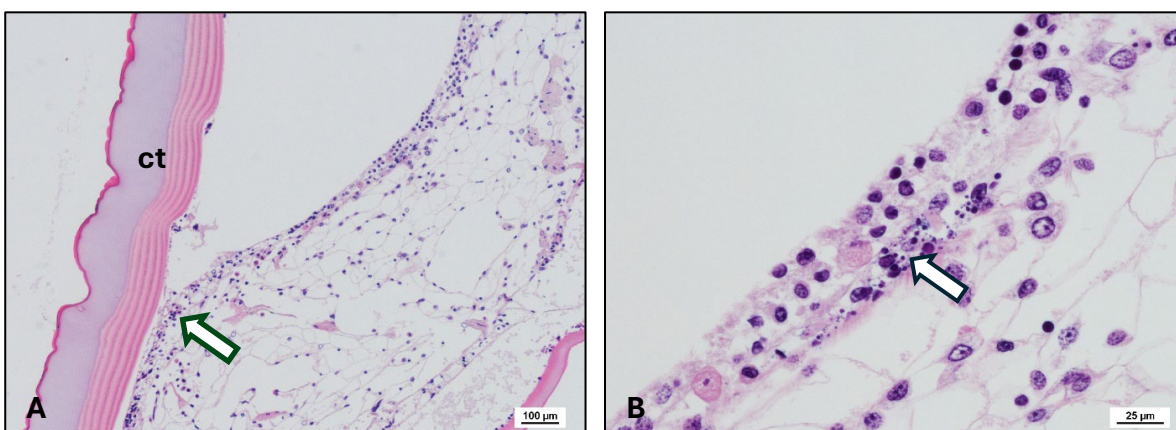


Figure 18. Histological assessment of crayfish tissues from the River Arrow. Necrotic areas observed within the cuticular epithelium (arrow). A) Cuticular epithelium can be seen lining the cuticle (ct) and contained necrotic areas (arrow). Scale bar = 100 µm. B) Higher power magnification of areas of necrosis (arrow), identified by large numbers of deeply basophilic spherical inclusions likely to be dead and dying cells in these regions. Scale bar = 25 µm.

A detailed comparison of 3 crayfish displaying the external marks considered to present the novel clinical signs of disease (JB25001_16-18) and 3 displaying no external marks, as healthy control animals (JB25001_1-3) was made.

The histology of the healthy cuticle (Figure 19) showed a double carapace layer comprising of an epithelial layer (tightly packed cells forming a protective cover of the body's surface) and spongy cuticular tissue (a tough, protective, non-living outer layer) underneath, with a small numbers of granulocytes (a type of white blood cell that respond to infections). By contrast the infected tissue, especially from the individual with the large abscess, was heavily inflamed, had melanisation (an immune response where the body produces a dark pigment), and included the aggregation of haemocytes (invertebrate blood cells) creating a “scab”, most likely due to a response to infection (Figure 19).

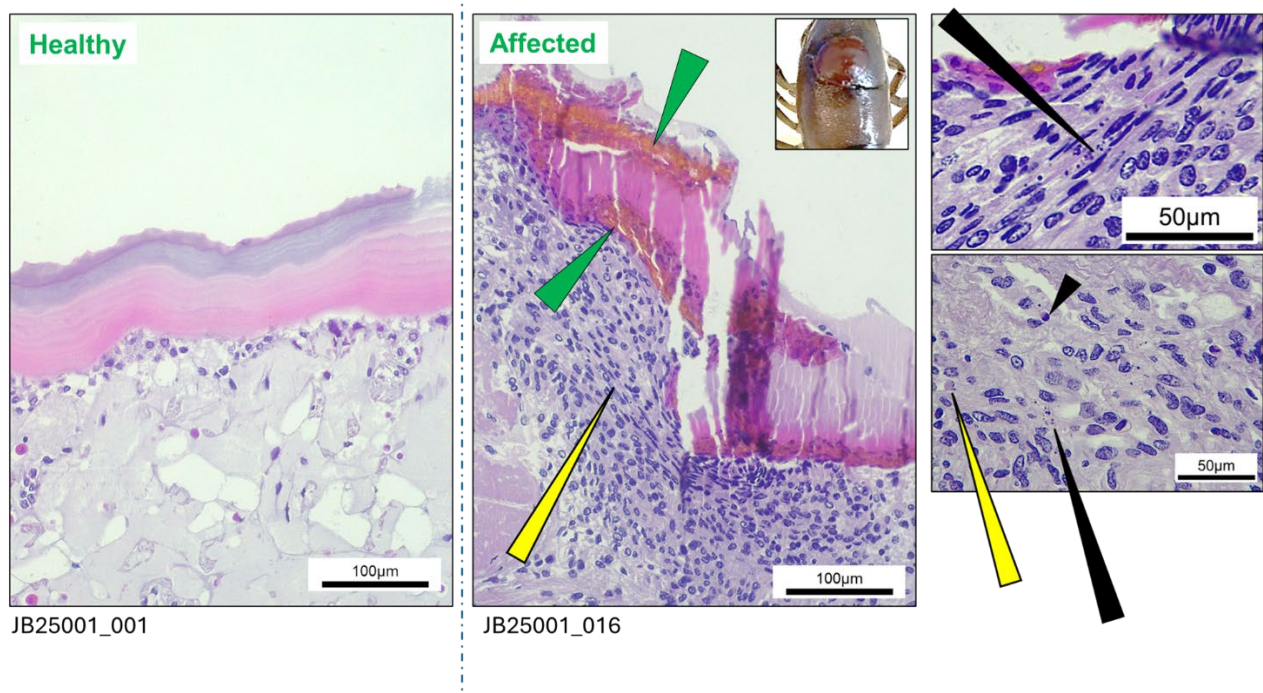


Figure 19. A histological comparison the carapace from a healthy individual (JB25001_001) and affected individual (JB25001_016). The green arrows indicate melanisation (an immune response where the body produces a dark pigment). The yellow arrows indicate the aggregation of haemocytes (blood cells) indicating an immunological response. The black arrows indicate damaged cells.

4.2.3 Screening for porcelain disease.

PCR screening of muscle tissues identified presence of *A. contejeani* in 66.7% of animals sampled at site 1 and 86.7% of animals from site 2 on the River Arrow in Herefordshire. At the site in Hampshire 64.5% of muscle tissues sampled were PCR positive. At dissection a single animal from site 1 (3.33%), 4 animals from site 2 (13.3%) from the River Arrow and 2 animals (6.67%) from Hampshire site were noted to possess white muscle, infection confirmed via histological analysis. The prevalence identified via PCR is far higher than that observed via presence of white muscle at time of dissection and the observation of microsporidian spores within cells in histology screen. PCR is a sensitive technique that

can detect low level of infection, pathogen presence detected in the tissue before the associated pathology develops. These results suggest that this pathogen has a higher prevalence in wild populations than initially thought and will require additional work to understand the significance of these findings.

4.2.4 Genetic assessments

The hepatopancreatic tissues of the first 20 crayfish animals from the North East area were combined and analysed to determine whether viral, bacterial, or other organisms, were present in the tissue. This analysis identified 8 novel RNA viruses (Table 14). However, these viruses did not directly correspond with individuals with lesions, so to determine if the lesion directly harboured a virus or other microorganism analysis was conducted on the individual (JB25001_16; Figure 14) with the prominent abscess. This analysis detected no viruses, but did find the presence of several bacterial, protozoan and fungal species (Table 15).

Table 14. Results of the metagenomic and metatranscriptomic assessments, detailing characterised and novel species of virus, bacteria, and protozoan detected from pooled samples or direct sequencing of specific samples.

Tissue	Taxa found	
Metatranscriptomics from hepatopancreatic tissues (20 individuals combined)	Viruses	Austropotamobius pallipes picornavirus 1 (AupPV1) Austropotamobius pallipes picornavirus 2 (AupPV2) Austropotamobius pallipes picornavirus 3 (AupPV3) Austropotamobius pallipes marnavirus 1 (AupMV1) Austropotamobius pallipes marnavirus 2 (AupMV2) Austropotamobius pallipes dicistrovirus 1 (AupDV1) Austropotamobius pallipes nepovirus 1 (AupNepV1) Austropotamobius pallipes secovirus 1 (AupSV1)
Metagenomics from I Legion of affected individual JB25001_016 (Figure 15).	Virus	None
	Bacteria	<i>Aestuariivirga litoralis</i> <i>Rhizobium</i> sp. XWS-28 <i>Rhodobacter</i> sp. <i>Allonocardiopsis opalescens</i> <i>Actinobacterium</i> sp. <i>Escherichia coli</i>
	Protozoa	<i>Dendrosoma</i> sp.

Tissue	Taxa found	
	Fungus	<i>Massalongia</i> sp. <i>Cladonia</i> sp. <i>Tolypocladium</i> sp.

A detailed comparison of 3 crayfish displaying the carapace abnormalities (JB25001_16-18) and 3 displaying no external marks, as healthy control animals (JB25001_1-3) was made.

There was no clear difference in viral diversity (Table 14) or the diversity of other species present (Table 15) between healthy cuticle and cuticle containing lesions. This means the result were inconclusive for determining the cause of the lesions and highlights the importance of understanding the natural variation in the array of organisms associated with the crayfish cuticle, which is rich in viral, fungal, and protozoan species. Of particular note was the presence of the fungus *Cladosporium* sp. in 5 out of 6 of the animals; this genus is considered commensal (living alongside a host). The protozoan (*Poterioochromonas andersenii*) only occurred in the individual with a significant abscess-like lesion (Figure 14). Other species of particular interest were known pathogens, or common species known to incite infection among the crayfish hosts (pink; Table 16).

Table 15. Putative viruses identified from metatranscriptomic assessment of the cuticle of 6 individuals, 3 from a healthy group and 3 of an affected group displaying the carapace lesions. Each indication represents a viral genomic sequence in the dataset that requires follow-up confirmation and validation.

Visibly healthy group	Visibly affected group
JB25001_001 • Picornavirus • Partitivirus • Sobemovirus • Wenling crustacean virus • Endornavirus • Tombusvirus • 'Shrimp virus' • Bunyavirus	JB25001_016 • 'Shrimp virus' • Two insect viruses • Bunyavirus
JB25001_002 • Picornavirus • Partitivirus • Sobemovirus • Wenling crustacean virus • Endornavirus • Tombusvirus • 'Shrimp virus' • Naravirus • Tetavirus	JB25001_017 • 'Shrimp virus' • Picobirnavirus • Tombusvirus • Bunyavirus

Visibly healthy group	Visibly affected group
• Bunyavirus	
JB25001_003 • Picornavirus • Hepevirus • Sobemovirus • Totivirus • Tombusvirus • Endornavirus	JB25001_018 • Picornavirus • Totivirus • Partitivirus • Naravirus • Tombusvirus • 'Shrimp virus' • Two insect viruses • Dicistrovirus • Bunyavirus

Table 16. The eukaryotic species detected in the sequence data for the 6 individuals, 3 from a healthy group and 3 of an affected group displaying carapace lesions. Those species that are highlighted are of particular interest as known pathogens, or common species known to incite infection among the crayfish hosts (pink), particularly numerate (yellow) or occurred only in the individual with a significant abscess-like lesion (Figure 15).

Visibly healthy group	Visibly affected group
JB25001_001 • <i>Epistylis cambari</i> (stalked protozoan) • <i>Vorticella</i> sp. (ciliated protozoan) • <i>Cladosporium</i> sp. (fungus) • <i>Paralia</i> sp. (alga) • <i>Malassezia obtusa</i> (yeast) • <i>Fulvocentrum</i> sp. (fungus)	JB25001_016 • <i>Fulvocentrum</i> sp. (fungus) • <i>Poterioochromonas andersenii</i> (protozoan) • <i>Cladosporium</i> sp. (fungus)
JB25001_002 • <i>Dactylochlamys pisciformis</i> (ciliated protozoan) • <i>Epistylis vaginula</i> (ciliated protozoan) • <i>Actinobolina radians</i> (ciliated protozoan) • <i>Didinium</i> sp. (ciliated protozoan) • <i>Coleps</i> sp. (ciliated protozoan) • <i>Paramecium</i> sp. (ciliated protozoan) • <i>Aureobasidium pullulans</i> (fungus) • <i>Dematophora necatrix</i> (fungus)	JB25001_017 • <i>Meira</i> sp (fungus) • <i>Miamiensis avidus</i> (ciliated protozoan) • <i>Naganishia</i> sp. (fungus) • <i>Epistylis cambari</i> (ciliated protozoan) • <i>Pseudofabrea</i> sp. (fungus) • <i>Cladosporium</i> sp. (fungus) • <i>Aureobasidium pullulans</i> (fungus) • <i>Dothiora cannabinae</i> (fungus) • <i>Varicosporium</i> sp. (fungus) • <i>Pseudocercospora</i> sp. (fungus) • <i>Paramecium</i> sp. (ciliated protozoan) • <i>Thecamonas</i> sp. (protozoan)
JB25001_003 • <i>Epistylis</i> sp. (ciliated protozoan) • <i>Aeolosoma hemprichi</i> (annelid) • <i>Cladosporium</i> sp. (fungus) • <i>Solicoccozyma keelungensis</i> (fungus)	JB25001_018 • <i>Cercomonas</i> sp. (ciliated protozoan) • <i>Stichotrichia</i> sp. (ciliated protozoan) (cov_60.12) • <i>Dendrosoma radians</i> (ciliated protozoan)

Visibly healthy group	Visibly affected group
• <i>Saxomyces</i> sp. (fungus) • <i>Uronema</i>-like (ciliated protozoan) • <i>Mycosphaerella punctiformis</i> (fungus) • <i>Ascochyta lentis</i> (fungus) • <i>Halteria</i> sp. (ciliated protozoan)	• <i>Leptosphaerioides guizhouensis</i> (fungus) • <i>Bacteriovorax</i> sp.(bacteria) • <i>Nuclearia</i> sp. (amoebae) • <i>Thecofilosea</i> sp. (amoebae) • <i>Epistylus cambari</i> (ciliated protozoan) • <i>Acrospermum urticae</i> (fungus) • <i>Vorticellides</i> sp. (ciliated protozoan) • <i>Rhodomonas minuta</i> (alga) • <i>Holosticha pullaster</i> (ciliated protozoan) • <i>Heliophrya erhardi</i> (protozoan) (cov_85.12) • <i>Petalomonas cantuscygni</i> (protozoan) • <i>Trebouxia aggregate</i> (alga) • <i>Cladosporium</i> sp. (fungus) • <i>Shiraia bambusicola</i> (fungus) • <i>Bryometopus atypicus</i> (ciliated protozoan) • <i>Rhynchosporium orthosporum</i> (fungus) • <i>Preussia longisporopsis</i> (fungus) • <i>Chloroidium</i> sp. (alga) • <i>Uronema</i> sp. (ciliated protozoan) • <i>Cercospora</i> sp. (fungus) • <i>Didinium nasutum</i> (ciliated protozoan) • <i>Alternaria burnsii</i> (fungus) • <i>Chlamydonellopsis calkinsi</i> (ciliated protozoan) • <i>Phaeotrichum</i> sp. (fungus) • <i>Sydowia polyspora</i> (fungus) • <i>Saprolegnia</i> sp. (Oomycete) • <i>Tetrahymena paravorax</i> (protozoan)

Visibly healthy group	Visibly affected group
	• <i>Acremonium implicatum</i> (fungus) • <i>Trebouxia aggregate</i> (alga) • <i>Arthrocladium fulminans</i> (fungus) • <i>Xanthoria parietina</i> (lichen) • <i>Allantophomopsis lycopodina</i> (fungus) • <i>Spirostomum</i> sp. (protozoa)

5 Discussion

5.1 What are the carapace abnormalities and do they change?

5.1.1 Visual observations

The carapace abnormalities can present in a wide array of different ways (Table 2, Appendix 1 and 2). Some individuals have large ‘burn-spot’ like clinical signs of disease sometimes appearing like an abscess, whereas others appear to be more progressed and lacking the abscess. The similar locations of the larger lesions (on the claws or body) suggest that there may be a progression of the clinical signs of disease (Figure 20). It was noticed that within the individuals collected for the Teesside study that moulting resulted in higher levels of mortality. Moulting can result in mortality in healthy animals, and usually larger animals are more at risk – however these burn-like spots/abscesses may increase potential mortality rate when moulting. Those that were able to moult reduced the size of their lesion, and in some cases smaller lesions were noted to almost disappear, leaving a white spot behind. As moulting is more stressful for larger individuals, it could also be a contributory factor leading to a threshold being crossed where mortality occurs.

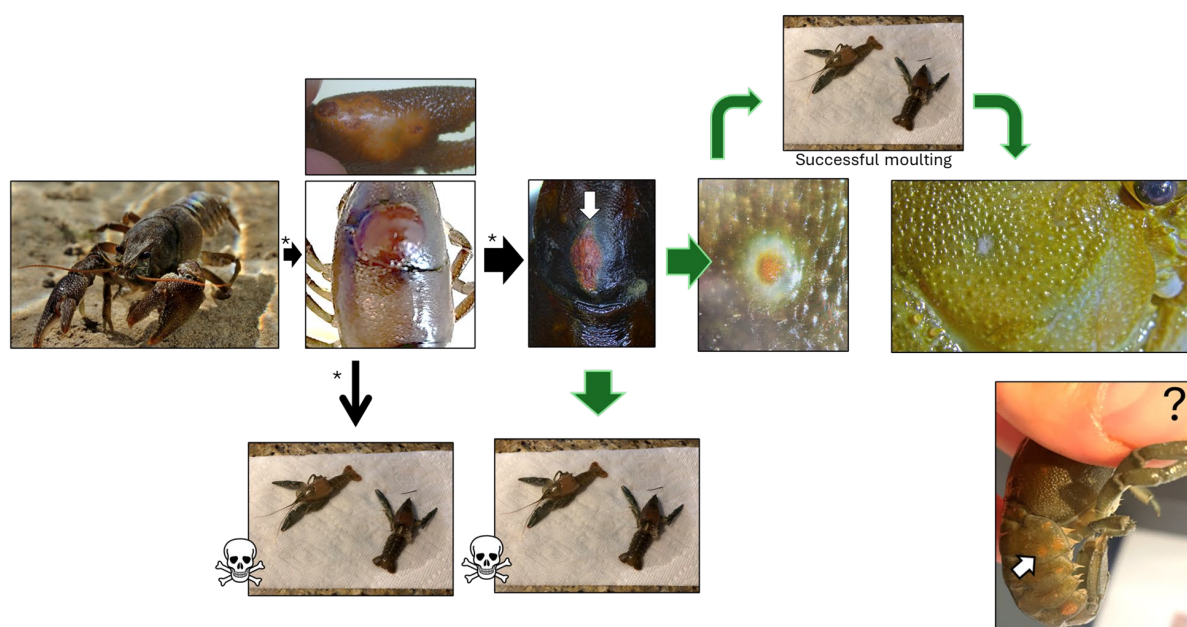


Figure 20. An overview of the carapace abnormalities. Starting from the left, a healthy individual, appears to spontaneously develop large lesions on the claws and body (cephalothorax). These may then “dry-up” and form the rust-coloured lesion noted in the centre of the image. Based on individuals retrieved from the initial mortality event, several were seen to come into captivity with large lesions, which then became small orange spots and eventually white spots after moulting. Finally, the image marked with a “?” outlines a particular stage of lesion development which has yet to be defined.

Similar conclusions were made from the observational study (section 4.1) that white-clawed crayfish may recover from the carapace abnormalities through the moulting process and that the more they moulted the better they seemed to be at reducing/recovering from the clinical signs of disease. As juveniles moult more than adult crayfish, this seemed to have enabled them to either recover, or not present with clinical signs of disease throughout the study. Larger crayfish moult less often, and this appears to have reduced their ability to recover from the clinical signs of disease. These conclusions are also supported from observations made from the field surveys. During the initial investigation into the mortality incident the majority of dead animals were larger adults. The prevalence of white-clawed crayfish exhibiting the carapace abnormalities in 2025 was much reduced from 2024 and observations during 2026 were even lower (<1%).

5.1.2 Laboratory analysis

Histology of the individual with the larger lesion (Figure 14) showed a clear immune response in the form of melanisation and increase in the presence of immune cells (granulocytes - white blood cells that respond to infections) in the affected area, relative to a control group of individuals that had no lesions. There were no obvious pathogens visible; however, viral and bacterial species are often too small to be distinguished directly at this scale.

The molecular data showed a diverse microbial community (microbiome) on the surface of the crayfish lesion, which is to be expected. The presence of these microorganisms reflects that the cuticle (shell) of crayfish are open to the environment, and that species are able to settle and survive on the white-clawed crayfish. Many of these will be harmless. As it is not possible to tell which might be harmful it makes identification of the cause of the lesions more difficult. There was also no significant difference in the microorganisms found on individuals with and without the carapace abnormalities.

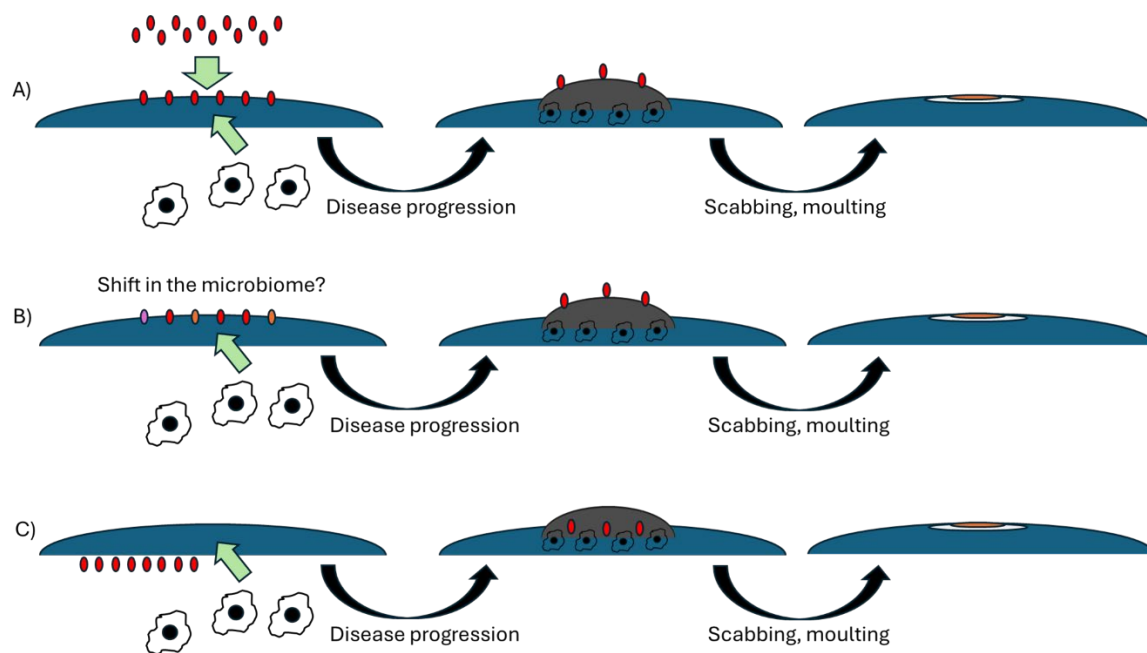


Figure 21. A graphical representation of the possible progression to lesion formation. A) a microorganism settles on the outside of the cuticle causing a lesion to develop. B) temperature or another environmental factor changing the microbiome of the white-clawed crayfish cuticle, leading to lesion formation. C) a pathogenic organism causing the lesion formation from within the animal.

5.2 What could be causing the carapace abnormalities?

As there are a range of clinical signs of disease it draws into question whether a) each of the different lesions presented are from the same cause, and b) whether different processes/causes can result in the formation of similar lesions in crayfish.

This study has identified an array of previously unknown organisms and microbial associations with the white-clawed crayfish from northern English catchment areas, including viral, bacterial, and other microorganisms. Direct examination of the large lesions (Figure 14) found immune responses - melanisation (an immune response where the body produces a dark pigment) and an increase in blood cells.

Follow-up genetic analysis identified that both healthy and affected tissues were associated with a broad range of viral, bacterial and other microorganisms, some of which could cause disease. These new data present many questions about how white-clawed crayfish interact with microbes in their aquatic environment.

5.2.1 Could the clinical signs of disease be caused by a virus?

This study identified a wide range of viruses, most of which are new to science and require further understanding and investigation. Until this is done it is not possible to say whether or not they may be associated with the carapace abnormalities.

5.2.2 Could the clinical signs of disease be caused by bacteria?

Bacteria could be associated with the carapace abnormalities since a broad diversity of bacteria were detected at the lesion site. However, it is difficult to distinguish those typical of the natural environment from those that may be correlated with the lesions. None of the affected samples analysed were dominated by known pathogenic bacteria, and there was no consistent bacterial signal in affected samples that could be defined as distinct from an environmentally-derived bacterial community.

5.2.3 Could the clinical signs of disease be caused by fungi?

A diverse range of fungal organisms were detected, but as for the bacteria, it is difficult to distinguish those typical of the natural environment from those that may be correlated with the lesions. Transmission studies and further analysis would help to determine if there are immune responses triggered by fungal spores.

5.2.4 What are other potential causes?

White-clawed crayfish like other animals may have 'allergic reactions' on the surface of their cuticle, and therefore there might be a stimulus resulting in this lesion formation. The clinical signs of disease may be a result of a cumulative impact or an imbalance of viral or bacterial loading commonly found in the species which becomes an issue due to environmental stress such as warmer temperature or low flows. Other options include potential genetic reasons for developing these spontaneous lesions.

This study found a higher-than-expected prevalence of porcelain disease (*Astathelohania contejeani*) at the two sites on the River Arrow and the site in Hampshire. Low level infections were identified via qPCR analysis in animals which did not show any external signs of disease (white muscle). Additional work is required to determine the level of infection required before the pathogen becomes detrimental to the host. Further work is also required to investigate whether porcelain disease can be associated with presence of the abnormal shell lesions.

5.3 Recommendations

5.3.1 Further observations

The field and laboratory observations so far suggest that the white-clawed crayfish can recover from the carapace abnormalities. Continued monitoring of the carapace abnormalities is required to provide a better understanding of the prevalence and survival rates of animals in the wild. Continued observation of the laboratory animals in a well-designed experimental setting would provide a more detailed resolution and to help confirm disease progression from visual clinical signs of diseases.

Recording, and potentially investigating, other instances of carapace abnormalities if they occur could provide additional valuable information of the context in which they develop. This should include exploration of the clinical signs of diseases noted in signal crayfish.

5.3.2 Additional diagnostics

It was beyond the scope of this study to examine the tissue samples using electron microscopy. Observations of putative inclusions in the cuticle could benefit in future from Transmission Electron Microscopy (TEM) application, after appropriate fixation.

Greater understanding of the bacterial microbiome would be valuable, but to generate this information there is need for a broader study using additional metabarcoding to focus on bacterial diversity in the animal and on the affected and unaffected cuticle.

Transcriptomic examination (measuring RNA to understand what genes are active) of the affected cuticular tissue will help to determine what the immune system is trying to target. Exploring which immune genes are turned on and off, could help narrow down what the crayfish immune system is attempting to combat as some genes are only turned on when combatting viral, bacterial or other infections.

5.3.3 Assessing transmissibility

Determining whether the cause of the carapace abnormalities can be transmitted to healthy populations will determine whether their cause is likely to be a biological agent.

Undertaking transmission trials from infected to healthy individuals – tank separated with a mesh – would allow the progression of the clinical signs of disease/lesions to be monitored more closely. In addition, experiments that also include signal crayfish as potential vectors would help to untangle their involvement in lesion formation, since similar lesions have been noted in that species.

5.3.4 Diagnostic development

The data collected in this study includes partial or complete genomes for viruses, offering the potential for development of diagnostics to detect the viruses in future studies, and the capacity to compare viruses within the white-clawed crayfish relative to viruses that are found only on the carapace lesions. However, this is only worth doing once a good candidate causative agent (or agents) has been identified.

5.3.5 Reviewing translocation protocols

White-clawed crayfish can be moved for conservation purposes to pre-assessed sites with agreement from Natural England (Marshall et. al 2022). Current guidelines drawn up by the England and Wales Crayfish Steering Group allow direct translocations within an operational river basin management catchment ([Environment Agency Catchment Data Explorer](#)). Movements of white-clawed crayfish between different operational catchments

are considered on a case-by-case basis informed by the guidelines that currently recommend that all donor crayfish must be quarantined for a minimum of 4 weeks and only translocated if observed to be visually healthy (Appendix 3).

This protocol is a balance between the current understanding of crayfish plague viability (WOAH. 2025), unknown infection rates of other crayfish pathogens, the ability to undertake diagnostic testing without having to kill individuals and the need to urgently create new populations of white-clawed crayfish in ark sites due to threats to existing populations. In this context determining whether the carapace abnormalities described in this study are transmissible, and if so what is the nature of the agent(s) are high priorities. Experts at Cefas recommend extreme caution around translocation of crayfish from sites where carapace abnormalities have been observed and/ or significant mortalities have occurred previously (even if none of the White-clawed crayfish show signs of disease). Advising that animals from these previously affected sites should not be translocated between catchments unless the 'new' ark site is unconnected to other water bodies, and does not already harbour crayfish.

Quarantine reduces but does not eliminate the risk of spreading diseases or infection. The need for translocations is increasing, particularly as American Signal crayfish, which outcompete white-clawed crayfish and spread crayfish plague are rapidly spreading across the country and occurrences of droughts and floods are increasing with climate change.

More ark sites with associated quarantine and captive breeding and rearing facilities may be needed to maintain resilient populations of white clawed crayfish. This would require capital, revenue and staff resources in a range of partner organisations including Zoos and aquariums. As genomic testing becomes cheaper incorporating health screening as part of the translocating populations could be beneficial.

5.3.6 Protocols for investigating future novel diseases

Incidents of new and novel diseases or the different presentation of existing diseases are anticipated to increase with environmental change and a changing climate. More incidents of unexplained mortalities and the carapace abnormalities should be expected.

This outbreak and subsequent collaborative investigations have highlighted the value of a more joined up approach to incidents where wildlife mortalities occur. Robust procedures exist for investigation of known diseases. New approaches may be needed to investigate multifactorial adverse health events.

6 Conclusions

This study investigated the potential cause of the mortalities associated with novel signs of disease (carapace abnormalities) exhibited by some white-clawed crayfish in the North East and Midlands areas of the Environment Agency. An array of previously unknown organisms and microbial associations with the white-clawed crayfish were found, presenting many questions about how crayfish interact with microbes in their watery environment, but no obvious cause of the mortalities and carapace abnormalities could be determined.

Continued and more structured monitoring of the carapace abnormalities could provide a better understanding of the prevalence and survival rates of animals in the wild. Continued observation of the laboratory animals would provide a more detailed resolution and to help confirm disease progression from visual clinical signs of disease. Additional molecular analysis may also be helpful. It could be beneficial to expand monitoring of water quality (particularly temperature, dissolved oxygen and flow levels) at important, white-clawed crayfish locations by citizen scientists to help understand potential changes in climatic conditions in any future mortality incidents or where there are novel clinical signs of disease.

Current guidance for translocations of crayfish such as to safe havens or ark sites, takes a precautionary but proactive approach to only move animals within an operational river basin management catchment. Each application is considered on a case-by-case basis. As a result of this study Cefas experts recommend extreme caution around translocation of crayfish from sites where carapace abnormalities have been observed and/ or significant mortalities have occurred (even if none of the White-clawed crayfish show signs of disease). Advising that such animals should not be translocated between catchments unless the 'new' ark site is unconnected with other water bodies, and does not already harbour crayfish.

As suitable sites for in-catchment movement are becoming limited due to the spread of signal crayfish, the need for translocations between operational catchments will increase over time. A review of current protocols could be beneficial in the light of emerging diseases, as the impacts on conservation activities are far ranging.

Stress induced by climatic conditions (temperatures and flow conditions) may have contributed to the mortality event. As the climate changes more instances of novel diseases or presentations of existing diseases or pathogens can be expected. Sensitive species such as the white-clawed crayfish may act as barometers of environmental health. It is recommended that protocols for incident response are reconsidered and reviewed to detail how any future unexplained wildlife mortalities are dealt with.

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Appendix 1 White-clawed crayfish with carapace abnormalities from the North East area

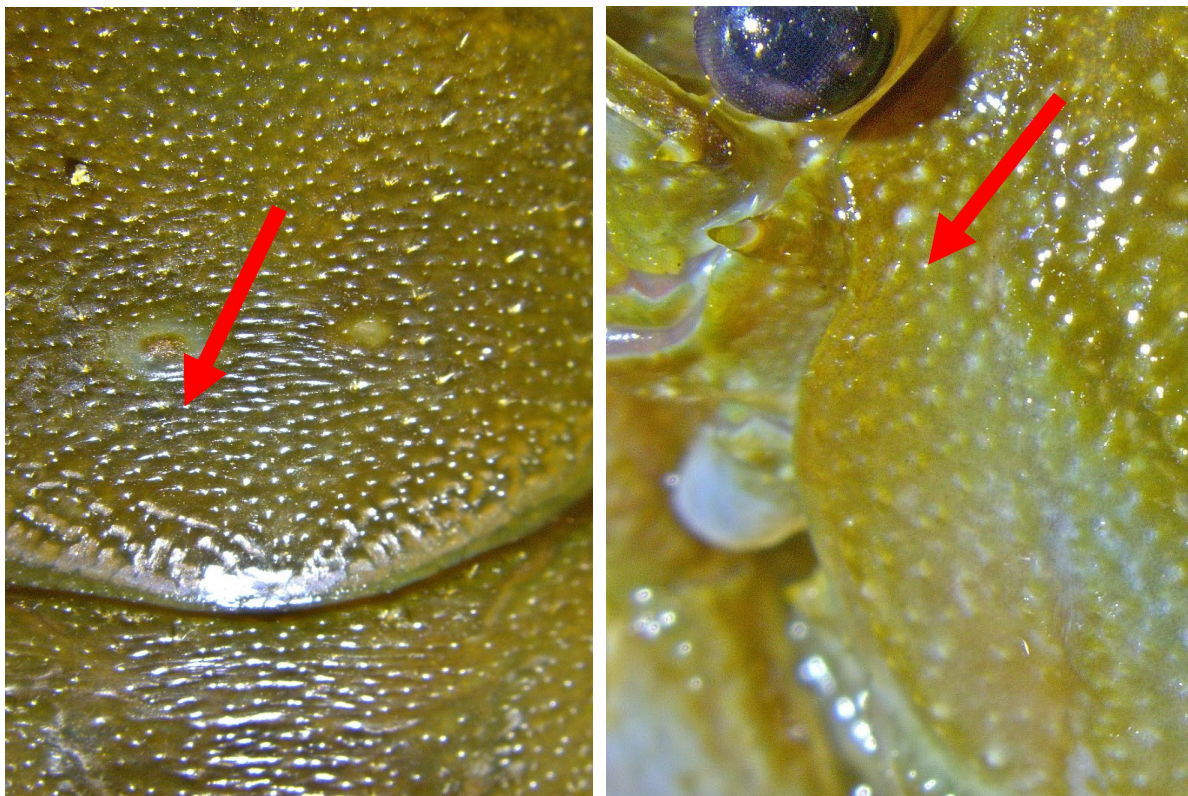


Figure A1: White-clawed crayfish specimen taken from Hart Burn on the 4th September 2024 showing discolouration and small markings on a live individual sent for analysis with Cefas. Photos taken using a microscope.



Figure A2: White-clawed crayfish specimen taken from Hart Burn on the 16th September 2024 showing discolouration and small markings on numerous live individuals.



Figure A3: White-clawed crayfish specimen taken from River Wansbeck at Wallington on the 19th September 2024. Note mottling on cheek in top left image, likely to result in holes in a carapace in top right image and inflammation of the excretory gland in bottom left.



Figure A4: Two crayfish from the Haltwhistle Burn observed with orange markings and discolouration observed in the South Tyne catchment on the 23rd October 2024.

Appendix 2 White-clawed crayfish from Whitman’s Hill Quarry

Symptom	
	
	

Symptom

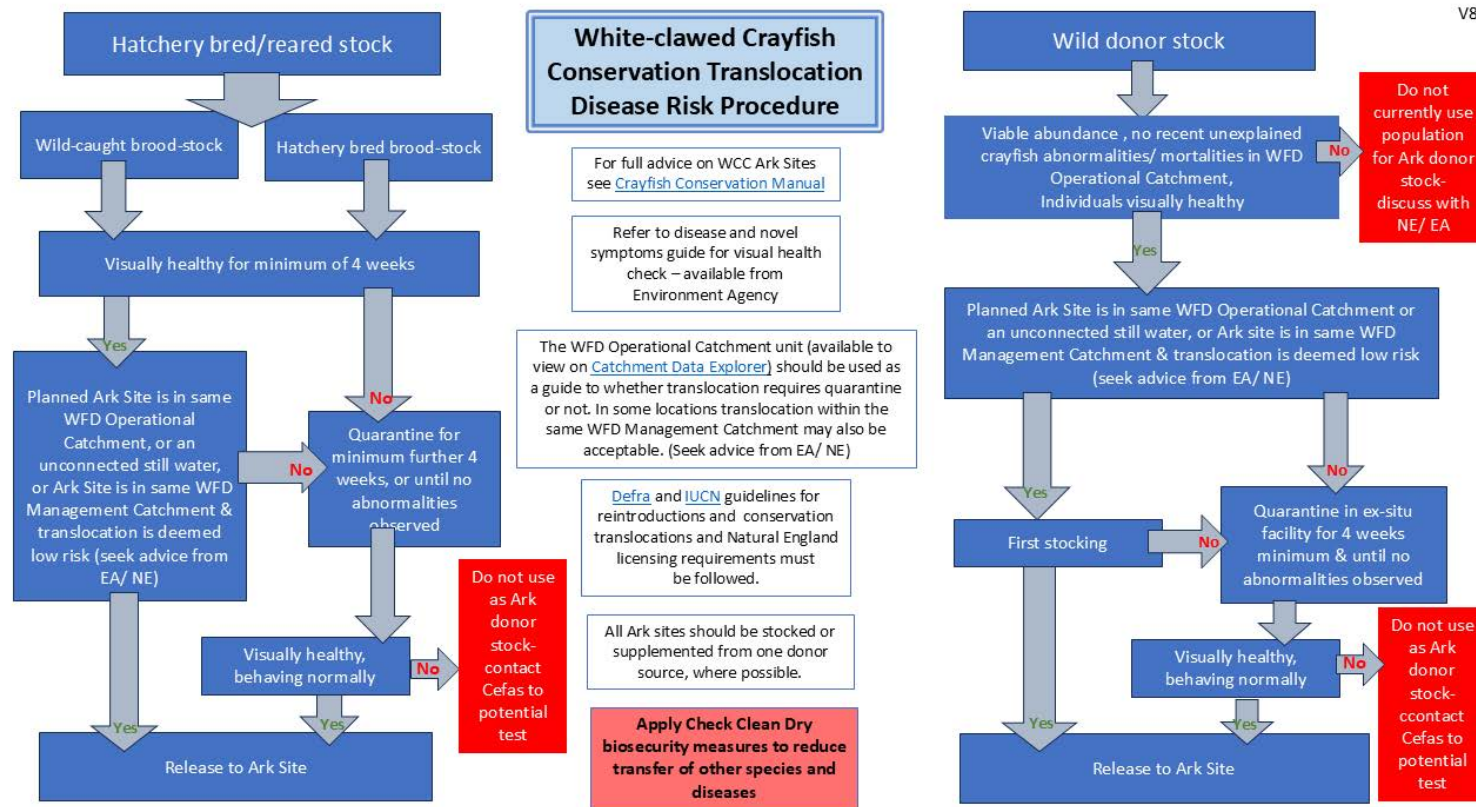


Symptom



Figure A5: White-clawed crayfish from Whitman's Hill Quarry with carapace abnormalities 27th July 2025.

Appendix 3 White-clawed crayfish Conservation translocation disease risk protocol



England and Wales Crayfish Steering Group 2025, unpublished. Links within the protocol are: Crayfish Conservation Manual (Marshall and Nightingale, 2022), [Catchment Data Explorer](#), [Defra](#) and [IUCN](#) guidelines.

Glossary

Term	Definition
Bioinformatic	The use of computational methods to explore biological data
Carapace	The external organ (or exoskeleton) surrounding an arthropod
Chelipeds	One of the pair of legs that bears the large chelae/claws
Commensal organisms	Ones that live alongside a host.
Crustacean	A large, group of mainly aquatic species, such as a crab, lobster, shrimp, or barnacle.
Cuticular epithelium/ tissues	The tough, protective, non-living outer layers produced by the underlying living epidermal cells of many organisms, notably plants and invertebrate.
Cuticular	Relating to a cuticle, which is the tough, flexible, non-mineral outer protective layer of an organism.
Cytoplasmic inclusions	A continuous sheet of tightly packed cells forming protective coverings on all body surfaces.
DNA	Deoxyribonucleic acid the genetic information for the development, functioning, growth, and reproduction of organisms.
DNA virus	DNA virus is characterised as possessing deoxyribonucleic acid-based genome and replicates within host cells by using the host cell's DNA polymerase. The genome can be single stranded DNA (ssDNA) or double stranded DNA (dsDNA).
Epithelial tissues	A continuous, protective sheets of cells that cover the body's outer surfaces.
Eukaryotic	Organisms (including animals, plants, fungi, and protists) composed of cells that contain a distinct, membrane-bound nucleus holding the genetic material (DNA).
Exoskeleton	The hard, external skeleton, made of chitin and calcium, of the crayfish. It is to shed (moulted) periodically to grow, revealing a new, soft one underneath which then hardens.
Genome	The DNA based molecule(s) that encode genes in each cell

Term	Definition
Granulocytes	A major type of white blood cell (leukocyte) characterized by enzyme-filled granules in their cytoplasm and lobed nuclei, acting as fast responders in innate immunity against infections, allergies, and inflammation by releasing these granules to destroy pathogens like bacteria and fungi.
Gut lumen	Hollow, open internal space within the intestines
Gross pathology	The overall observations of an impacted or affected tissue or organ
Haemocytes	A blood cell especially of an invertebrate animal.
Hepatopancreas	The liver/pancreas of a crustacean
Histology	The study of the microscopic structure of tissues.
Histopathology	The use of histology to define pathological parameters
Lesion	Any area of abnormal or damaged tissue, whether on the skin or inside the body.
Melanisation	An immune defence mechanism where the body produces a dark pigment called melanin to isolate foreign invaders like pathogens or to aid in wound healing.
Metagenomics	The non-biased sequencing and analysis of total DNA, which can be assembled into genomes.
Metatranscriptomics	Non-biased sequencing and analysis of total RNA, which can be assembled into RNA components (e.g. messenger RNA) or viral genomes.
Microbiome	The complement of organisms naturally present in a given system or organism
Musculature	The system or arrangement of muscles in a body.
Necrosis	Dead or dying tissue.
Oomycete	Water moulds - fungus-like eukaryotic organisms
Pathogens	Bacteria, viruses and other microscopic organisms that can cause disease.
Pathology	Visual observation of changes in the host's tissues
Protozoans	Single celled eukaryotic organisms
Putative inclusions	An abnormal structure suspected to exist within a cell

Term	Definition
RNA	Ribonucleic acid which carries instructions from DNA for controlling the synthesis of proteins. In some viruses RNA carries the genetic information rather than DNA.
RNA virus	RNA virus is characterised as possessing ribonucleic acid-based genome and replicate using virally encoded RNA-dependent RNA polymerase (RdRP). The genome can be single stranded RNA (ssRNA) or double stranded RNA (dsRNA).
Sporophorous vesicles	A membrane-bound sac formed by microsporidian parasites inside a host cell. Within it spores (infectious units) develop and mature before being released to infect new hosts.
Symbiont	A symbiont is an organism that lives on or in another organism and can either be pathogenic, parasitic, commensal, or a mutualist.
Transcriptomics	The study of all the RNA molecules that a cell or tissue produces. It looks at which genes are active by measuring the RNA made from them, helping understand what the cell is doing at a specific moment.

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