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REDCLAW CRAYFISH (*Cherax quadricarinatus*): DISTRIBUTION, CRAYFISH PLAGUE, AND DIAGNOSTIC APPROACHES - A REVIEW

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ABSTRACT: *The crayfish plague, caused by Aphanomyces astaci, represents a serious threat to redclaw crayfish populations in Malaysia. This review examines the distribution of redclaw crayfish, the incidence of crayfish plague, and insights into its diagnosis. Previous research has established that A. astaci is the primary agent behind crayfish plague in both Asia and Europe. Gaining a comprehensive understanding of these biological threats, including A. astaci and other infections, is vital for protecting the redclaw crayfish industry. Implementing effective diagnosis and management strategies is crucial for preserving crayfish populations and ensuring the industry's sustainability. It is important to recognize that fungal plagues, like those caused by A. astaci, often show few symptoms until significant mortality occurs. Additional research is necessary to grasp the complex immune system of crayfish and to investigate potential therapeutic measures for managing inflammation. Collaboration and data sharing among researchers studying crayfish across different regions would significantly enhance progress in this field.*

KEYWORDS: *Aphanomyces astaci*, crayfish plague, crustacean disease, redclaw crayfish

INTRODUCTION

Inland fish farming has experienced significant growth, outpacing marine aquaculture, particularly in the South Asian region. In 2018, inland aquaculture yielded 51.3 million tonnes of aquatic animals, accounting for 62.5% of the global production of farmed food fish. The share of finfish in this production dropped from 97.2% in 2000 to 91.5% (47 million tonnes) in 2018, indicating the more

rapid expansion of other species, especially crustaceans like shrimps, crayfish, and crabs in Asia. Therefore, understanding the biological characteristics of *C. quadricarinatus* is essential for producing healthy redclaw crayfish (Tarun, 2021).

Over the past two decades, the dominance of finfish in inland aquaculture has diminished, giving way to other species, notably freshwater crustaceans such as redclaw crayfish (Naquiddin *et al*., 2016). *Cherax quadricarinatus*, the redclaw crayfish, is classified as a freshwater crustacean within the genus Parastacidae (Holdich, 2002; Haubrock, 2021). This species gained commercial importance following its successful cultivation in Australia in 1985, leading to its commercialization and farming worldwide, particularly in Asia, including Malaysia (Johan *et al*., 2012; Naquiddin *et al*., 2016). Overall, this species is known for its robustness, resilience to various diseases (both parasitic and non-parasitic), and its ease of cultivation. Additionally, the rapid growth rate of *C. quadricarinatus* allows for more frequent harvesting compared to other species, making it a key driver of the industry.

The growth and development of *C. quadricarinatus* are directly influenced by its lack of a larval stage. This species can adapt to various climates and reproduce in alkaline waters with a pH range of 7.0 to 8.5. Naguib *et al*. (2021) discovered that *C. quadricarinatus* can thrive in a variety of temperatures and low dissolved oxygen levels. The species exhibits a range of phenotypic variations, including size and reproduction rates, in diverse biotic and abiotic conditions, from tropical to temperate regions. Mature males are characterized by a decalcified red spot on their chelae, while both sexes feature reddish highlights on their bluish-green outer bodies (Belle & Yeo, 2010). Female redclaw crayfish have three to five distinct horizontal cervical spines along their cervical groove.

Economically, redclaw crayfish play a significant role in aquaculture and are also valued as ornamental species (Füreder, 2013). Production in Peninsular Malaysia is estimated to be around 12 tons annually (Johan et al., 2012). The price of redclaw varies based on size and quality, potentially reaching up to MYR 120 per kilogram (approximately USD 30 per kilogram), making it a lucrative industry (FAO, 2020). The sector has grown consistently, partly due to the involvement of private entrepreneurs as hotel and restaurant chains have expanded in Peninsular Malaysia. The species cultivated by local farms is imported from Australia and Indonesia, benefiting from the redclaw crayfish's ability to adapt to tropical conditions, which are conducive for reproduction.

The cultivation, harvesting, and commercial distribution of redclaw crayfish have become an increasingly important economic component of the aquaculture industry. However, the fungus *A. astaci* causes the crayfish plague, which results in high mortality rates not only among various crayfish species in Europe but also in several regions of Southeast Asia, including Malaysia.

Therefore, it is essential to understand the biological threats, such as the oomycete *A. astaci* and other infections, that could jeopardize the industry.

Redclaw crayfish (Cherax quadricarinatus) belong to the arthropod families Astacidae, Cambaridae, and Parastacidae, and are freshwater crustaceans that resemble smaller versions of marine lobsters (Holdich, 2002; Haubrock, 2021). There is a notable difference in size and growth rate between male and female *C. quadricarinatus,* with males generally being larger and growing more rapidly than females. The nutritional intake of these crustaceans often influences their sexual characteristics (Sun *et al*., 2023). As the species is harvested and consumed by humans, an entire industry has developed around them, emphasizing the need for a deeper understanding of crayfish, which encompass approximately six hundred known species (Aoki *et al*., 2018).

Like other crustaceans, crayfish possess a hard exoskeleton, or "shell," which they must molt periodically to grow. This exoskeleton serves to protect the crayfish from predators and provides structural support. Despite their armoured exterior, crayfish maintain agility and speed thanks to their flexible, jointed segments (Holdich, 2002; Louis & Robert, 2020; Haubrock, 2021). Figure 1 displays the anatomical structure of the crayfish, which consists of two main parts: the cephalothorax and the abdomen.

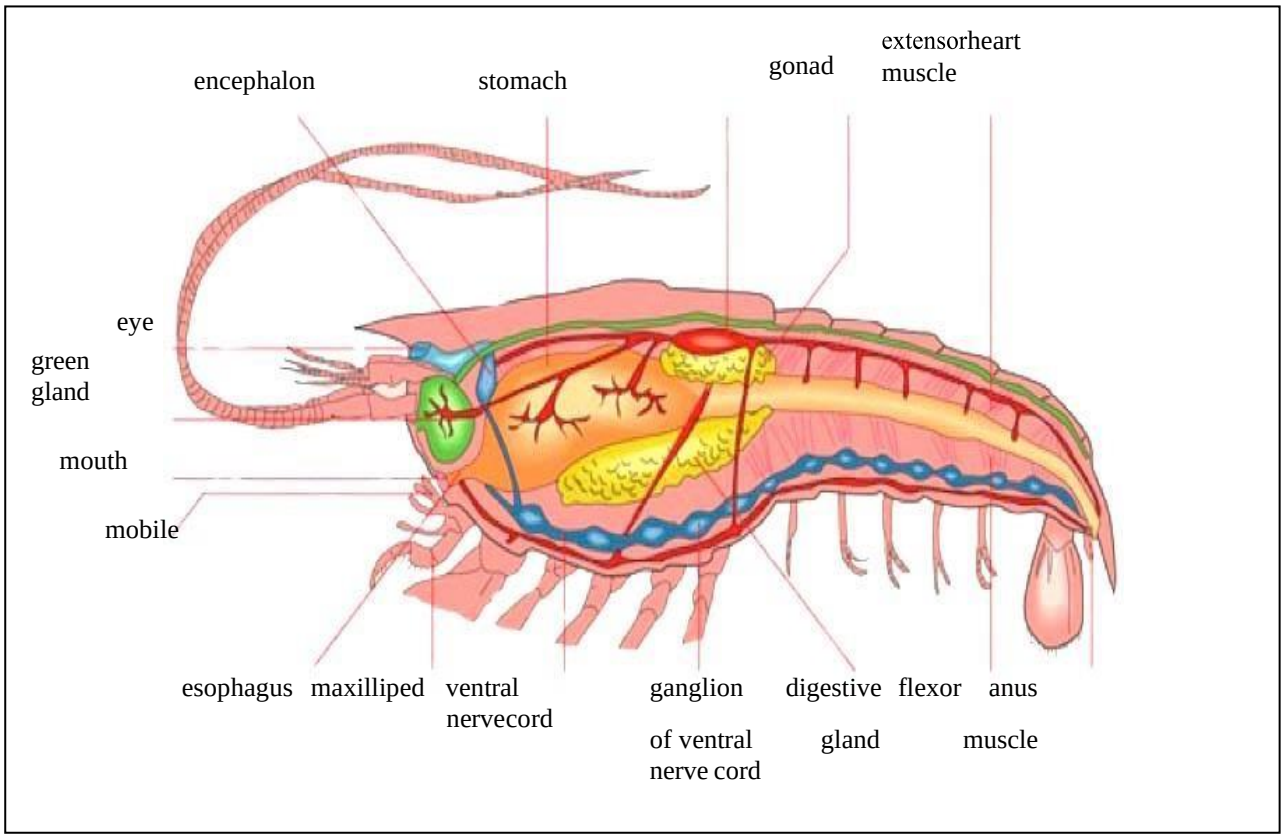

Figure 1: The internal anatomy of a crayfish. Source: www.enchantedlearning.com

The encephalon regulates the mental functions of the crayfish, while the stomach, situated in the upper region, is part of the crustacean's digestive tract, positioned between the esophagus and the intestine. The crayfish's heart is in the midsection, pumping blood to the extensor muscles and other organs, extending all the way to the tail. The gonad, which serves as the sex gland, is connected to the ventral nerve cord and the flexor muscles, which facilitate the movement of the crayfish's tail (Pliego *et al*., 1998). The cephalothorax, which includes the head and thorax, consists of 13 segments, while the abdomen is made up of six distinct segments and is located posterior to the cephalothorax. Each segment of the cephalothorax and abdomen has two appendages. In the head region, there are five pairs of appendages known as cephalic appendages. The shorter antennae, or antennules, house organs responsible for balance, touch, and taste, while the longer antennae contain organs for touch, taste, and smell (Johan *et al*., 2012; Louis & Robert, 2020; Haubrock, 2021).

Crayfish possess mandibles, or jaws, that are used for capturing and processing food. Additionally, the thoracic region has maxillipeds that help secure food during feeding, and large claws, known as chelipeds, which serve for hunting and defence. The decapod crustacean also has four pairs of walking legs and five pairs of swimmerets, or pleopods. Each leg features an attached gill to facilitate water circulation as the legs move. The gender of the crayfish can be determined by examining the first pair of swimmerets: males have larger and more robust first swimmerets for depositing sperm into the female's oviducts, whereas females have softer swimmerets to carry fertilized eggs and newly hatched young. Crayfish also utilize their tail fans, modified from a pair of uropod, to propel water forward, allowing them to move backward (Aoki *et al*., 2018)

C. quadricarinatus Habitat Conditions

Most crayfish species thrive in flowing freshwater environments, such as streams and rivers with manageable currents, rather than stagnant waters (Haubrock, 2021). This preference is due to their intolerance for high levels of ammonia and heavy metals commonly found in still water bodies (Barnett *et al*., 2017). However, certain varieties of crayfish, such as Procambarus clarkii, exhibit resilience to pollution and can survive in contaminated waters (Dorret *et al*., 2006). Additionally, crayfish favour dark, cool habitats and are frequently found hiding under rocks and vegetation. They struggle to survive in extremely cold temperatures or in aquatic environments with low oxygen levels (Belle & Yeo, 2010; Füreder, 2013). As a result, crayfish farming for industrial purposes necessitates specific temperature, pH, and salinity levels, in addition to adequate oxygen supply.

The optimal temperature range for raising healthy crayfish is between 18 °C and 25 °C, while pH levels should be maintained between 7.5 and 8.5. Lower pH levels and insufficient calcium can lead to poor molting of the crayfish shell. Adequate oxygen in the water is another crucial factor for

survival, as crayfish use gills to extract oxygen from the water (Hossain et al., 2018). If oxygen levels are insufficient, crayfish may resort to obtaining oxygen from the air above the water's surface (Holdich, 2002). Although they typically inhabit freshwater and have adapted to a stenohaline lifestyle, crayfish have demonstrated the ability to tolerate slightly elevated salinity levels (Suryanto *et al*., 2023). Hossain et al. (2018) recorded the presence of *Astacus leptodactylus* and *Astacus pachypus* in waters with salinity levels up to 14 parts per thousand (ppt) in the Caspian Sea (Yavuzcan *et al*., 2004). However, the capacity of crayfish to withstand higher salinity is directly related to their size.

Crayfish are known to be nocturnal, primarily foraging for food during the night. Most freshwater crayfish inhabit burrows, lakes, and rivers, typically seeking refuge under rocks or logs during the day and becoming more active at night (Louis & Robert, 2020; Suryanto *et al*., 2023). They are omnivorous and detritivorous, meaning they do not adhere to a specific diet but will consume a variety of plant and animal organisms, including fish, shrimp, worms, elodea, plankton, insects, and snails. Additionally, they may exhibit cannibalistic behaviour if there is an insufficient supply of food or shelter in their environment (Belle & Yeo, 2010; Neculae *et al*., 2024).

Redclaw Crayfish Distribution in Malaysia

In Malaysia, the term "freshwater lobster" is commonly used for redclaw crayfish due to their habitat and resemblance to lobsters. While it's unclear when the redclaw species was first introduced to the country, commercial-scale cultivation has been reported since 2003 in the southern region of Peninsular Malaysia (Alimon et al., 2003; Naqiuddin et al., 2016; Norshida et al., 2021). During the late 2000s, when the species was first being introduced, redclaw aquaculture thrived in southern Peninsular Malaysia, largely due to the regular importation of broodstocks from Indonesia (Naqiuddin et al., 2016). According to the FAO in 2020, Malaysia has emerged as the leading producer of redclaw, with production increasing from 2013 to 2017, driven by prices that can reach as much as MYR 120 per kilogram (Norshida et al., 2021).

Unintentional escapes or deliberate releases from aquaculture facilities and aquariums have allowed redclaw to establish themselves in natural habitats. Currently, redclaw is the only non-native crayfish species to have formed natural populations in Malaysia, a successful invasion likely due to environmental conditions that mirror their native habitat (Norshida et al., 2021). Additionally, their ability to be easily transported by humans in large quantities (Yuliana et al., 2021), alongside the country's geographical features and frequent flooding, facilitates their expansion into new areas (Mohd Dali et al., 2023). Since their introduction, wild redclaw populations have been reported not

only in Johor, in the southern part of the country, but also in Selangor along the West Coast of Peninsular Malaysia and in Sarawak on Borneo Island (Johan et al., 2012). Historical data suggest that the species was initially introduced to Malaysia in the 1980s in Johor, from where it migrated northward along the western coast of the Peninsula (Naqiuddin et al., 2016). Although no wild populations have yet been observed in states outside Johor, Melaka, and Sarawak, the spread of this species may increase with the growing number of redclaw aquaculture facilities in the country (Naqiuddin et al., 2016).

Cultivation activities for redclaw crayfish typically aim to satisfy food consumption needs, targeting sizes between 6 to 8 inches in length. However, the high demand for juveniles has shifted focus toward breeding activities, which has resulted in discoveries of the species' natural populations in East Malaysia and southern Peninsular Malaysia (Johan *et al*., 2012). The presence of wild crayfish in these areas has caught the attention of local fishermen, who have experienced losses due to crayfish damaging their catches and fishing gear.

C. quadricarinatus is the only freshwater crayfish species known to have established a population in Malaysian freshwater habitats, despite the presence of other crayfish species like the highly invasive *Cherax destructor* and *P. clarkii* in the aquarium trade. This is likely attributed to *C. quadricarinatus* demonstrating superior characteristics in terms of growth, reproduction, and size compared to the other two species (Naqiuddin *et al*., 2016). Additionally, its desirable traits for the aquaculture industry suggest that this species has been introduced in significant numbers to Malaysia for use as broodstock. Consequently, it has successfully established a wild population, as non-native species are more likely to thrive when introduced in large numbers or repeatedly (Alpert, 2006; Yuliana *et al*., 2021). Furthermore, aquaculture has historically been Malaysia's primary avenue for introducing foreign fish, accounting for 64% of all non-native fish species brought into the country (Khairul Adha *et al*., 2013).

However, invasive species are recognized as the second-largest contributor to biodiversity loss, as they disrupt ecological balance by competing for resources and shelter, spreading diseases, directly preying on native species and their eggs, and altering habitats through burrowing and grazing on macrophytes (Holdich, 1988). The invasive potential of *C. quadricarinatus* to disrupt native ecosystems in the United States has already been observed in regions where it has been introduced (Morningstar et al., 2020; Haubrock *et al*., 2021; Sanjar *et al*., 2023). Thus, redclaw crayfish exhibit characteristics typical of successful invaders, such as a broad and adaptable diet and high reproductive capacity. Nevertheless, there are currently no documented instances of *C. quadricarinatus* causing environmental harm in Malaysia. This may be due to underappreciated bio-invasion threats and the time lag between observable impacts and the establishment of the species (Othman & Hashim, 2003).

Crustacean Diseases

In 2008, the EC Council Directive 2006/88/EC created a list of three significant crustacean diseases recognized globally. These diseases are white spot disease (WSD), yellow head disease (YHD) caused by the yellow head virus (YHV), and Taura syndrome (TS) caused by the Taura syndrome virus (TSV). WSD, which was previously regarded as a "non-exotic" disease in Europe due to its documented occurrence in penaeid shrimp farms in southern Europe, has since been reclassified (Stentiford *et al*., 2009). In contrast, YHD and TS are considered exotic diseases because they are not naturally present in Europe. Their inclusion is based on their potential to spread internationally via the trade of live animals and their products, as well as their considerable economic impact worldwide (Stentiford *et al*., 2009; Morningstar *et al*., 2020).

Additionally, the International Organisation for Animal Health (OIE) has listed the crayfish plague (A. astaci) and the infectious hypodermal and hematopoietic necrosis virus (IHHNV) as other crustacean diseases that require mandatory reporting. Diagnostic techniques for detecting these diseases include traditional methods such as gross pathology, histology, classical microbiology, animal bioassay, antibody-based approaches, and molecular techniques involving DNA probes and amplification. Since shrimp aquaculture became a major commercial enterprise in the 1970s, these diseases have had a significant impact on the industry. Major diseases affecting farmed shrimp include viruses, rickettsial-like bacteria, true bacteria, protozoa, and fungi (Walker & Mohan, 2009).

Today, modern medicine employs chemotherapeutics, routine sanitation practices, and improved culture methods to combat various bacterial, fungal, and protozoan diseases. Managing these illnesses has been challenging, posing a threat to the entire industry and representing some of the most financially burdensome epizootics (Walker & Mohan, 2009). For example, the Taura syndrome outbreak from 1991 to 1992 was deemed "notorious" in the context of viral epizootics when the disease emerged in Ecuador. Likewise, white spot disease pandemics had severe repercussions for the industry in Southeast Asia during the same period. The socioeconomic importance of shrimp farming has resulted in five out of nine crustacean diseases listed by the OIE being viral diseases of shrimp (OIE, 2012), although none are as damaging as the crayfish plague.

The industry has had to adapt its practices to become more sustainable following substantial losses incurred from the crayfish plague, while the adoption of technology has opened up new opportunities. These changes have allowed the industry to recover from serious viral pandemics and resume production, ushering in a new phase of rapid growth (FAO, 2006). However, despite the implementation of new shrimp farming strategies, protocols, and technologies as well as the elimination of 'high-risk' practices, the crayfish plague caused by *A. astaci* has continued to persist. The transition away from relying on wild stocks for production has not been sufficient, as *A. astaci* has now been linked to domesticated stocks (Lightner, 2005).

Crayfish Plague

The invasive oomycete *A. astaci* is responsible for crayfish plague, posing a significant threat to freshwater crayfish populations. This pathogen is highly virulent, leading to elevated mortality rates among crayfish in Europe, Asia, Australia, and South America (Koivu-Jolma *et al*., 2023). The plague stems from the oomycete parasite *A. astaci*, a fungal-like aquatic mold that inhabits the cuticle of crayfish throughout its vegetative life stage and subsequently infects other crayfish through zoospores. While oomycetes are commonly referred to as water molds and consist of various types, some have been identified while others remain undiscovered. Regardless, these organisms can be classified as either parasites or saprophytes (Kokko *et al*., 2018). Aphanomyces belongs to the Saprolegniales group, which also includes the notorious parasitic species Saprolegnia (Leclerc *et al*., 2000). The genus Aphanomyces is associated with serious fish diseases such as mycotic granulomatosis and epizootic ulcerative syndrome (EUS), directly resulting from infections by *Aphanomyces invadans* (Viljamma *et al*., 2011).

Fungal isolation occurs upon the observation of infection symptoms, which include brownish-red melanisation, whitening of the abdomen (Figure 2), and reduced overall mobility. The non-specific melanisation seen in crayfish serves as a defensive reaction to pathogen infections (Victor & Pahirulzaman, 2020). Additional indicators of infection include the whitening of the musculature in the ventral abdomen. In advanced stages of the infection, affected crayfish may display sluggish behaviour and limb deformities (Nicky, 2008; Victor & Pahirulzaman, 2020).

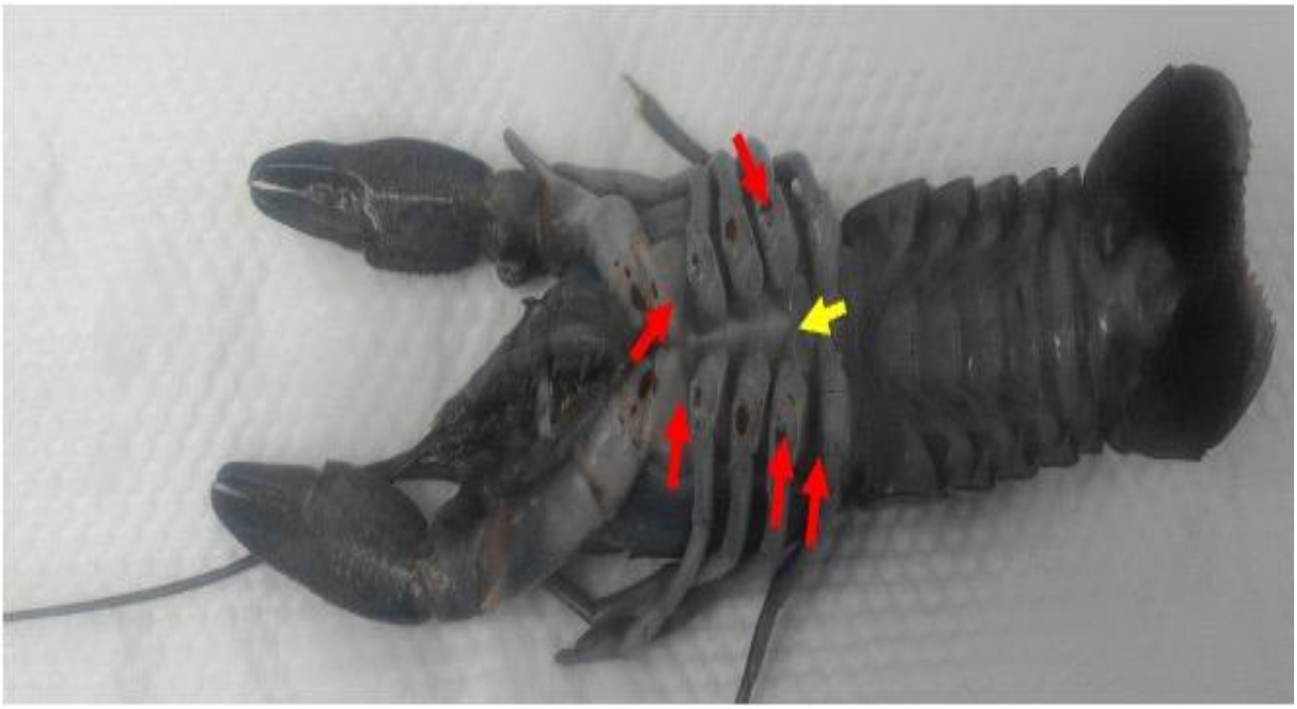

Figure 2: Signs of infection in *C. quadricarinatus* included the presence of brownish-red melanisation (red arrows) and the whitening of the abdomen (yellow arrow) (Victor & Pahirulzaman, 2020).

In addition to marine oomycetes linked to the pathology of fish and crustaceans, there are many undiscovered saprophytic species that thrive in freshwater environments, which have yet to be explored and properly classified (Vrålstad *et al*., 2009). *A. astaci*, in its somatic form, comprises a mycelium organized into structures resembling fungal hyphae (Viljamaa-Dirks & Heinikainen, 2019). These hyphae are aseptate, meaning they lack septa, and they appear turgid, colourless, and measure approximately 7.5 to 9.5 μm in width. As the life cycle progresses into the infectious stage, the spores are termed zoospores, formed in sporangia of similar size to the hyphae, separated by septa. The main spores within the sporangium aggregate into a spore ball that typically houses between 10 and 40 individual spores developed from the cytoplasm.

Following a hibernation-like phase, these cysts transform into swimming zoospores, which measure about 9-11 μm in diameter and possess two distinct flagella. The zoospores begin swimming towards nutrient sources, directing them toward crayfish (Viljamaa-Dirks, 2016). Upon contacting a crayfish, they attach to the exoskeleton, encyst, germinate, and initiate a new growth cycle. At this point, the rapidly growing hyphae invade the crayfish's tissues, and if unchecked, *A. astaci* can ultimately lead to the crustacean's death within a few weeks (Holdich, 2002; Belle & Yeo, 2010; Kokko *et al*., 2018).

The agent or agents responsible for crayfish plague are situated in the crevices of a crustacean's cuticle. The development of these agents is usually inhibited by the crustacean's immune system while being protected from competition with other organisms in the environment (Makkonen *et al*., 2013). The disease is transmitted between hosts by zoospores, which have a short lifespan but can swim for about three days, enhancing the fungi's chances of locating new hosts. The organism possesses a mechanism that allows a zoospore to encyst and produce new zoospores if the initial growth is insufficient, reinforcing the classification of these parasites as "highly specialized." While experimental replication of the survival strategy of parasitic oomycetes is possible, the exact process of generating new spores remains elusive; it is understood, however, that most spores are produced as the host is deteriorating (David *et al*., 2014).

Crayfish Plague Distribution

The crayfish plague was inadvertently introduced to Europe from North America around 1860, with *A. astaci* being recognized as a natural parasite of North American crayfish (Alderman, 1996). Despite the disease's existence since 1860, its origins remained a mystery for many years, resulting in ineffective measures to prevent its spread. The thriving crayfish trade during that time likely contributed to the propagation of the plague, which has remained a significant health issue for the

European noble crayfish, *A. astacus*. The rapid mortality caused by the disease made it virtually impossible to restore native crayfish populations to their previous levels.

The disease earned the alarming title "crayfish plague" due to its rapid and lethal nature, initially recognized through widespread crayfish die-offs. It has been shown that the native European crayfish population, which includes eastern and northern *Astacus spp*., as well as southern and western *Austropotamobius spp*., is highly susceptible to *A. astaci* infection. Laboratory studies in pathobiology indicated that these species could experience 100% mortality rates (Alderman & Polglase, 1986; Cerenius *et al*., 1988). The combination of infectious dosages of zoospores and water temperatures mimicking agricultural conditions played a crucial role in the disease's development, enhancing understanding of the mechanics of the crayfish plague.

In contrast, North American crayfish species were found to be resistant to the disease, often harbouring *A. astaci* as a latent infection without experiencing mortality unless under stress (Unestam & Weiss, 1970). Consequently, North American species were introduced to Europe to compensate for the declining native crayfish populations. The first North American crayfish, the spiny-cheek crayfish (Orconectes limosus), was introduced to Poland in 1890 (as cited in Viljamaa-Dirks, 2016). When populations of the noble crayfish, *A. astacus*, began to decline in Sweden due to the plague, the signal crayfish (*Pacifastacus leniusculus*) was introduced as a replacement. This species was considered suitable due to its size and adaptability (as cited in Viljamaa-Dirks, 2016). The signal crayfish was subsequently introduced in significant numbers into Swedish and Finnish waters during the 1960s. As predicted, the signal crayfish gradually replaced the declining noble crayfish and became a vital part of the European crayfish fishery. While the introduction of the signal crayfish has aided the recovery of European crayfish fisheries (Westman, 1991), it has complicated population management. The signal crayfish has emerged as a chronic carrier of the crayfish plague agent, spreading the disease to the already vulnerable populations of noble crayfish. Consequently, management strategies have shifted toward conserving the noble crayfish, the only native crayfish species in Europe.

Crayfish Plague in Asia

In Japan, China, Thailand, Malaysia, and Taiwan, red swamp crayfish have established populations, which are sometimes supported by ornamental fish outlets in the region (NOBANIS, 2011). This creates a risk of disease spreading across the area, either through contaminated water or via contact with live or deceased crayfish. Notably, between December 2013 and January 2014, four crayfish farms in Taiwan reported five outbreaks of an unidentified disease, resulting in moderate to high cumulative mortality among populations of freshwater redclaw crayfish, *C. quadricarinatus* (Hsieh

et al., 2016). A polymerase chain reaction (PCR) analysis identified *A. astaci* DNA in the deceased redclaw crayfish. The nucleotide sequence identities of these strains were found to be very similar to recognized *A. astaci* strains in Europe, exhibiting a sequence similarity of 99.8–100% in that genetic region. Furthermore, in situ hybridization using a digoxigenin-labelled DNA probe confirmed that *A. astaci* was responsible for the outbreaks. This represented the first documented case of a natural *A. astaci* infection in Asian freshwater redclaw crayfish. Hsieh et al. (2016) highlighted the high susceptibility of redclaw crayfish to this pathogen, noting that certain fungal strains related to crayfish plague could proliferate at temperatures as high as 29.5 °C. This raises concerns about the potential for the disease to spread throughout larger parts of Asia where it is already present, leading to devastating consequences.

Crayfish Immunology

Multicellular organisms have evolved immune systems to defend against 'non-self' substances that are foreign to them. There are two primary types of immune systems: innate immunity and adaptive immunity. Innate immunity is present in invertebrates, providing an effective defence against microbial symbionts, disease, wound repair, and responses to biotic and abiotic stimuli, while adaptive immunity is found only in vertebrates (Clark & Greenwood, 2016). The innate immune systems of invertebrates are sufficient to protect them from invasive microorganisms. Invertebrates respond to infectious pathogens through various immune cells that initially eliminate these invaders by enclosing them. The innate immune systems of invertebrates can mount both cellular responses (including phagocytosis, nodule formation, and encapsulation) and humoral responses when confronted with pathogens (Clark & Greenwood, 2016). These immune reactions are facilitated by blood cells known as hemocytes. When a disease or parasite is too large or numerous for a single phagocytic cell to handle, more complex processes, such as multicellular encapsulation or nodule formation, are required. Typically, phagocytosis is carried out by individual hemocytes (Lee, 2001). The enzyme phenoloxidase often melanises the nodules, which are aggregates of hemocytes connected by a sticky extracellular matrix. Encapsulation serves as a defensive mechanism against larger threats like fungi, nematodes, or eggs and larvae of parasitoids and is like nodule formation.

Moreover, the activation of the humoral immune system in invertebrates triggers a variety of responses, including blood clotting, melanin production, opsonization, and the temporary synthesis of potent antibacterial peptides (Lee, 2001). Therefore, extensive research on the crustacean immune system has been conducted to understand the influence of *A. astaci* on its host (Filipova *et al*., 2013). Crayfish, being invertebrates, do not possess antibodies for adaptive immunity, relying instead on innate immune response mechanisms for protection (Torrijos *et al*., 2021). The activation of the

prophenoloxidase system (proPO) in response to recognizing "non-self" patterns, such as lipopolysaccharides and peptidoglycans from bacteria, as well as -1,3-glucans from fungi, initiates a series of innate immune mechanisms, combining both humoral and cellular responses, including melanin production, cell adhesion, encapsulation, and phagocytosis (Filipova *et al*., 2013). ProPO is found in granules within the blood cells of crayfish and is released into the plasma via exocytosis triggered by the -1,3-glucan binding protein.

This response culminates in the production of melanin, which surrounds and inhibits the growth of invasive hyphae. In North American crayfish, a severe infection with A. astaci may lead to the appearance of dark brown melanized patches on the exoskeleton (Unestam & Weiss, 1970). Conversely, A. astaci can infect an individual or population without presenting obvious symptoms (Vralstad et al., 2011). Thus, both native European crayfish and their North American counterparts utilize the same crustacean immune system as their primary defense against intruders

Selecting reliable reference genes is critical for studying immune cell gene expression patterns, as various factors—including diet, changes in body size, and tissue composition—can influence the messenger ribonucleic acid (mRNA) levels of target genes, as well as gene expression control (Hibbeler *et al*., 2008). To address this, the mRNA levels of the target gene should be compared to those of a housekeeping gene, like 18S ribosomal RNA (rRNA), which is intended to reflect the health of the crayfish or a specific tissue under various conditions. One of the primary immunological effector cells in crustaceans is the hemoglobin cell. In freshwater crayfish, hemoglobin is produced from hemopoietic tissue (Hpt) located on the dorsal side of the stomach (Hai-peng *et al*., 2011). Research has shown that when noble crayfish are experimentally challenged with proPO-activating polysaccharides, there is an increase in proPO mRNA levels in the hemoglobin, indicating the crayfish's ability to respond to intruders (Cerenius *et al*., 2003)

In contrast, the signal crayfish exhibits a different response, as it was found that the proPO transcript was already maintained at a high level, and the experimental challenge did not result in any further increase. Although the crayfish plague agent has adapted to confront the effective defence mechanisms of its natural North American host, the European species has proven to be ill-prepared for this challenge. This inadequate defence response results in a critical mismatch between *A. astaci* and its new host species. Consequently, a species' ability to adapt and develop tolerance or resistance to emerging diseases caused by invasive parasites and pathogens is crucial for its survival. The genetic diversity of the host’s immune system can significantly influence the development of resistance within a population. As parasites and pathogens apply intense selection pressure, some hosts manage to withstand this pressure and reproduce, promoting the emergence of resistance (Gruber *et al*., 2014). Pauwels *et al*. (2010) demonstrated that resistance to pathogens could develop in Drosophila

melanogaster within less than ten generations in laboratory conditions, and immunological protection can arise in wild populations of Daphnia magna, a planktonic crustacean, in a similar timeframe. However, there is still uncertainty regarding whether wild hosts can adapt quickly enough to counter newly emerging diseases.

Additionally, a ferritin gene (PcFer), an iron storage protein, has been identified in *P. clarkii* (Liu *et al*., 2017). The increased expression of PcFer in the hepatopancreas of crayfish after exposure to various heavy metals and lipopolysaccharides suggests its role in immune defence and protection against heavy metal stress. Similarly, the laminin receptor has also been implicated in defence against bacterial and viral infections (Rusaini *et al*., 2013; Victor & Pahirulzaman, 2024). For instance, redclaw crayfish infected with White Spot Syndrome Virus (WSSV) demonstrated an up-regulation of the laminin receptor, indicating its protective role against viral infections by binding to viral proteins and preventing them from attaching to target host cells (Liu *et al*., 2018).

Molecular Analysis for Crayfish Plague Identification

By using random oligonucleotides as primers in the amplification of deoxyribonucleic acid (DNA) through polymerase chain reaction (PCR), researchers can identify genetic differences among various isolates of organisms. This approach is known as random amplification of polymorphic DNA-PCR (RAPD-PCR) (Welsh & McClelland, 1990). The RAPD-PCR technique has been applied to *A. astaci* isolates from different sources (Huang *et al*., 1994). The study revealed two distinct groupings and an additional strain. Despite the extensive geographic and temporal isolation of these isolates, a notable degree of genetic similarity was found among these groups, largely due to the absence of sexual reproduction in *A. astaci*. The first major group included a strain from the Turkish narrow-clawed crayfish *Astacus leptodactylus* and isolates from noble crayfish populations in Sweden. These *A. astaci* strains, known as Astacus strains or group A (As), were present in European waters prior to the introduction of the signal crayfish. Consequently, it is widely believed that the As genotype represents the original genotype of *A. astaci*, which was inadvertently introduced to Europe approximately 150 years ago. However, it remains unclear which North American crayfish species initially hosted this genotype.

While there is limited information regarding the role of different genotypes in previous outbreaks of crayfish plague, the first recorded mass mortalities in European crayfish in 1859 were likely attributed to strain As (Alderman, 1996). Research utilizing RAPD-PCR has confirmed the presence of the Ps1 genotype causing the disease in native crayfish species across Sweden, Finland, England, Spain, and Germany (Filipova *et al*., 2013), as well as the Pc genotype in Spain (as cited in Viljamaa-Dirks, 2016). The As genotype, in contrast, was found to be less common and was first

identified in Sweden, Finland, and Turkey (Filipova *et al*., 2013). Nonetheless, advancements in molecular techniques have begun to enhance our understanding of the distribution of various genotypes throughout Europe (Grandjean *et al*., 2014). It is anticipated that when North American crayfish species are present or nearby, *A. astaci* strains associated with those species will cause diseases in neighbouring native populations (Kozubikova-Balcarova *et al*., 2014).

Since the first study identifying the genotypes of *A. astaci* was published in the early 1990s, there have been very few efforts to investigate the potential variability among these genotypes (Huang et al., 1994). This scarcity of research stems from the limited number of isolates available from each genotype, which hinders comparative studies. It has been established that the Pc genotype can tolerate higher water temperatures than the other three known genotypes at that time (Dorret *et al*., 2006). Variations in the chitinase gene between the As and Ps1 genotypes have also been observed, suggesting a link between these differences and the pathogenicity of the strains (as cited in Viljamaa-Dirks, 2016). Other factors that may influence virulence include the ability to produce zoospores, recognize and adhere to hosts, germinate, and penetrate the cuticle (Cerenius & Saderhall, 1984; Cerenius *et al*., 1988), as well as the capability to repeatedly generate new zoospores to pursue hosts or to produce enzymes beyond chitinases (Viljamaa-Dirks, 2016). However, these variable traits can evolve over time.

Quantitative real-time PCR (qPCR) specific to a particular species allows for the rapid identification of the pathogen. However, the *A. astaci* qPCR assay, endorsed by the World Organization for Animal Health (WOAH), also detects the recently identified *Aphanomyces fennicus*, which may lead to false-positive results. Therefore, the existing species-specific *A. astaci* qPCR assay needs refinement to prevent the amplification of *A. fennicus* when screening for *A. astaci* (Strand *et al*., 2023).

CONCLUSION

C. quadricarinatus specimens in Malaysia have been shown to be vulnerable to most diseases that also affect native Australian crayfish. This is particularly true for the redclaw crayfish, which faces threats from crayfish plague caused by *A. astaci* and other fungal pathogen variants. It is crucial to actively pursue parasitic organisms capable of decimating entire populations of specific species, like the redclaw crayfish, to ensure their protection. Fungal plagues, such as those caused by *A. astaci*, often present minimal symptoms that can be easily overlooked, with mortality being one of the first signs of infection. The immune system is a complex structure that varies among species and remains incompletely understood. A deeper understanding of their roles could lead to therapeutic interventions

aimed at controlling inflammation in affected specimens. Although cross-examination and data sharing on crayfish specimens from other regions would have been beneficial to this study, research in this area involving crustaceans is still in its early stages, and data on the subject is limited.

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