


Hemolymph as a biomarker to access the health of decapod crustaceans: a review

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Abstract The aquaculture of decapod crustaceans such as shrimp, crab and crayfish has emerged as a significant contributor to the income of tropical countries. This cultivation is being driven by the growing human population, demand for aquatic animal nutrition, and the diversification of fisheries products. However, the emergence of diseases in decapod aquaculture presents a significant challenge, especially those caused by bacteria and viruses. Decapod crustaceans lack an adaptive immune system and rely on an innate immune system that responds to the recognition of molecular patterns in pathogens. Activation of this response triggers cellular or humoral defense mechanisms to combat pathogens. Hemocytes act as mediators of cellular immune components, while hemolymph contains free-cellular components that contribute to humoral responses. This review discussed an understanding of the characteristics and roles of the hemolymph and hemocytes to decapod health. We also describe the innate immunity systems (i.e., cellular and humoral) in decapod crustaceans and the factors affecting their immunity systems. Common tests used to quickly access decapod health were also highlighted to benefit aquaculturists and seafood processors in accessing their stock health status. All these topics are necessary to know before an outbreak happens, as some communities rely on various decapod species, especially shrimp, for their economic well-being.

Keywords Aquatic diseases . Immunology . Challenge

Introduction

Decapoda is an order within the class Malacostraca and subphylum Crustacea, consisting of approximately 14 400 species inhabiting freshwater and marine habitats (Nunomura et al. 2023). Some species such as crab, lobster, shrimp, crayfish have been the focus of significant research due to their importance as human food sources and their major economic influence (Albalat et al. 2022). According to the FAO (2022), around 17 million tons of decapods were produced and caught globally, accounting for 10 % of global fisheries production. Nonetheless, decapoda in both controlled and natural environments continue to face disease outbreaks, particularly from pathogens including bacteria, parasites, fungi, and viruses (Lin et al. 2024; Alimin et al. 2023), that endanger their lives and cause serious economic losses to global seafood production (Bondad-Reantaso et al. 2012). Pathogens such as Acute Hepatopancreatic Necrosis Disease (AHPND), *Enterocytozoon hepatopenaei* (EHP), and White Spot Syndrome Virus (WSSV) have been the subject of previous reports on their significant economic impacts (Patil et al. 2021; Thitamadee et al. 2016;

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Tangprasittipap et al. 2013).

Decapod crustaceans lack an adaptive immune system and rely entirely on their innate immune system, which consists of cellular immunity (i.e., phagocytosis, apoptosis, nodulation, and encapsulation) and humoral immunity (i.e., prophenoloxidase cascade, lectins, and antimicrobial proteins) for protection. Both cellular and humoral immunities are mediated by hemocytes and free-cell components found in the hemolymph and act as biomarkers reacting to external disturbances pathogens (Patnaik et al. 2023). However, our understanding of the role of hemolymph as an indicator of immune responses to illness is incomplete. Thus, a review of the characteristics of hemolymph and hemocytes and an overview of the innate immune systems in decapod crustaceans is warranted. Factors that influence the hemolymph level and immunity of decapods (i.e., biotic and abiotic) and the effects of pathogen perturbations on decapod crustaceans will also be covered in this review. Additionally, recent methodology and rapid tests for assessing the health of decapods will also be highlighted, with emphasis on significant gaps in understanding of the use of hemolymph and hemocytes as biomarkers of decapod health.

Hemolymph and hemocyte: characteristics and functions in decapods

Decapoda have an open circulatory system that allows hemolymph to leave vascular structures (e.g., heart, arteries) and permeate interorgan sinuses, thus facilitating lymphatic drainage (McGaw and Whiteley 2024; Sun et al. 2023). Hemolymph serves as the primary fluid for transport within the body of decapods, circulating freely through the hemocoel or body cavity with limited use of arteries and veins (Gianazza et al. 2021). Hemolymph contains hemocyanin with two copper atoms that reversibly bind to oxygen, facilitating its transport to all body tissues (Dilna et al. 2023; Bautista-Covarrubias et al. 2022; Gianazza et al. 2021). Hemolymph does not contain iron, and its protein components are 90 to 95 % of the total protein content (Terwilliger and Ryan 2015). Hemolymph is colorless in the deoxygenated Cu (I) form, while it appears blue in the oxidized Cu (II) form. Decapod hemolymph comprises many physiological compounds (i.e., proteins, carbohydrates, lipids, amino acids, and electrolytes) and plays an important role in oxygen and nutrient distribution, metabolism, osmotic regulation, innate immune, and inflammatory responses (Ren et al. 2023; Sui et al. 2023; Cuenca et al. 2021; Gianazza et al. 2021). Immune-active substances include phenoloxidase (PO), complement factors, lectins, and antimicrobial peptides that respond and kill pathogens (Wu et al. 2023). Research indicates that hemolymph exhibits viscosity properties that are influenced by high protein and lipoprotein content, which are important for lipid transport, blood coagulation, and immunological responses (McGaw and Whiteley 2024; Arnold et al. 2022). For example, Davies et al. (2020) and Meza-Buendia et al. (2024) reveal that infected hosts exhibit significantly higher hemolymph viscosity and a more turbid appearance compared to healthy individuals, thus affecting circulation capacity and reducing oxygen transport, which may in turn increase mortality rates.

Hematopoiesis refers to the process of hemocyte cell synthesis produced by hematopoietic tissue (HPT) (Xin and Zhang 2023; Söderhäll and Söderhäll 2022;). The HPT demonstrates a complex network of tightly packed lobules, with the dorsal and dorsolateral aspects of the stomach encircling the antennal artery, and a basal position near the maxillipeds, bordering the epigastric region (Liu et al. 2021), as shown in Figure 1. The hemocyte cells are released into the hemolymph circulation (Sun et al. 2023; Tong et al. 2022). Hemocytes will flow into the smallest arterioles in the brain, penetrate the surrounding sinus tissue and function as immune defense mediators, with functions that including detoxification, pathogen invasion, and regulating cellular metabolism (Sun et al. 2023; Tran et al. 2023). The classification of hemocytes is based on morphological characteristics observed through microscopes, as depicted in Table 1. Hemocyte cells are generally divided into three types: hyaline cells (HC), semi-granular cells (SGC), and granular cells (GC). The variation in hemocyte types can be identified through morphological characteristics encompassing both quantitative and qualitative aspects, such as total cell area, the ratio of nuclear area to total cell area (nucleocytoplasmic ratio [N/C]), the number of cellular granules, and surface corrugation (Edwards and Beltz 2024; Liu et al. 2021). Generally, HC are small in size, have few or no discernible granules, are oval or irregularly shaped like raisins, and have a centrally located nucleus (Hannan et al. 2024). Meanwhile, SGC are larger than HC, ranging from oval to spindle-shaped and contain small granules visible within the cytoplasm; and GC are the largest hemocytes, containing the highest number of granules per cell (Hannan et al.



2024). The details of the classification, characteristics, and role of hemocyte cells are depicted in Figure 2.

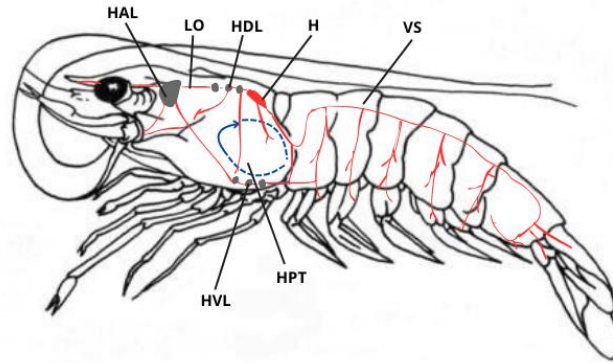
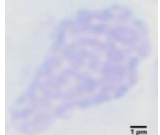
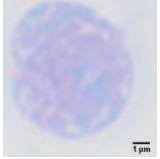
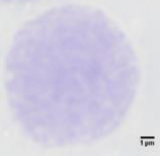


Fig. 1 Diagram of the hemopoietic tissue location (HPT). HAL: hematopoietic antennal lobules, LO: lymphoid organ, HDL: hematopoietic dorsal lobules, H: heart, HP: hepatopancreas, VS: vascular system, HVL: hematopoietic ventral lobules, HPT: hepatopancreas.

Table 1 A summary of three hemocyte subunits in crustaceans, and staining of hemocytes from the shrimp decapods, *Penaeus monodon*

Hemocyte subpopulations	Stained hemocytes	Size (µm)	Characteristics	Process
Hyaline cells (HC)		10-15	Smallest cell size, highest N/C ratios, round to oval, nucleus located in the middle, and with none or few cytoplasmic granules.	Phagocytosis (Mapanao et al. 2022); hemolymph coagulation, wound repair (Lei et al. 2022).
Semi-granular cells (SGC)		10-20	Medium cell size, smaller N/C ratio, oval to the spindle, the nucleus is usually central.	Encapsulation (Rahardjdo et al. 2022); phagocytosis limited (Ray et al. 2013); storage and release of the proPO system (Ekblom et al. 2024); cytotoxicity (Söderhäll et al. 1985); phagocytosis and wound repair (Lei et al. 2022); hemolymph coagulation (Hideaki et al. 1993).
Granular (GC)		15-20	Largest cell size, lowest N/C ratio, oval or irregular shape, and has larger cytoplasmic granularity in the hemocytes.	Cytotoxicity (Johansson et al. 2000); storage and release of the proPO system (Lei et al. 2022).

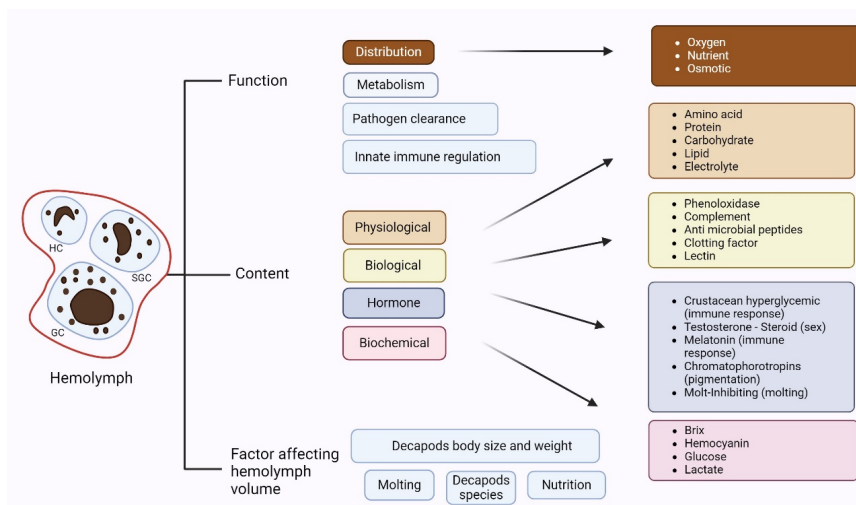


Fig. 2 Decapod hemolymph profile schematic. HC: hyaline cell, SGC: semi-granular cell, GC: granular cell



The innate immune system in decapods: cellular and humoral immunity

Decapods appear to rely completely on innate (natural) immunity to recognize and respond to attacking pathogens. Immune regulators in decapods have been reported to occur in the hemocytes, hepatopancreas, intestine, and gills (Tong et al. 2022). They are aided by various structural barriers (e.g., cuticular exoskeleton) and oral route defense (e.g., gut enzymes and acids and peritrophic membrane, which protect the intestinal epithelium tract) to prevent pathogen entry and hemolymph loss, called first-line defense immunity (Kulkarni et al. 2021; Amparyup et al. 2013). However, when invading pathogens are able to breach this first-line defense system, particularly through damaged cuticle and/or during the moulting stage, the extracellular signal molecules will bind to the receptor on the cell surface to induce a signal in the cytoplasm labelled pattern recognition receptors (PRRs), followed by a subsequent innate immunity response in the host cells (i.e., cellular and humoral immunity) (Kulkarni et al. 2021). The host innate immunity will activate the NF- κ B signaling pathways for the secretion of antimicrobial peptides and other immune proteins to eradicate the pathogen invasion (Keawthong et al. 2023). Both cellular and humoral immunity are mainly produced by hemolymph and hemocytes and are mutually complementary to mount a concerted response to pathogen invasion (Tassanakajon et al. 2013). The cellular immune response is mediated by the hemocytes, whereas the humoral immune response involves free-cell components found in the hemolymph (Gu et al. 2023). The scheme of the immune response pathway for both cellular and humoral immunity is depicted in Figure 3.

Cellular immunity

The immune response involving cellular activation includes conserved innate immune cells, which resemble macrophages in appearance and function with similar phagocytic capabilities (Yang et al. 2020). These cells play a key role in distinguishing between self and non-self entities, enabling them to bind to, ingest, and ultimately eliminate pathogens (Rathinam et al. 2024). As primary mediators in the systemic immune response, hemocytes function as essential effector cells in various cellular processes, including phagocytosis, encapsulation, nodule formation, and apoptosis. These processes are carried out by the different types of hemocytes (i.e., hyaline cells (HC), granular cells (GC), and semigranular cells (SGC)) and are responsible for recognizing microorganisms and playing roles in encapsulation and phagocytosis (Tribamrung et al. 2023; Duan et al. 2023; Kulkarni et al. 2021).

Phagocytosis is a non-specific cellular defense mechanism that plays a crucial role in combating pathogen attacks in invertebrate animals (Patnaik et al. 2023). The phagocytosis process is started by hemocytes recognizing foreign antigens or pathogens through pathogen-associated molecular patterns (PAMP), called pattern recognition receptors (PRRs), which include Toll-like receptors (TLRs), C-type lectin receptors (CRLs), RIG-I-like receptors, and NOD-like receptors (NLRs) (Patnaik et al. 2023; Kulkarni et al. 2021).

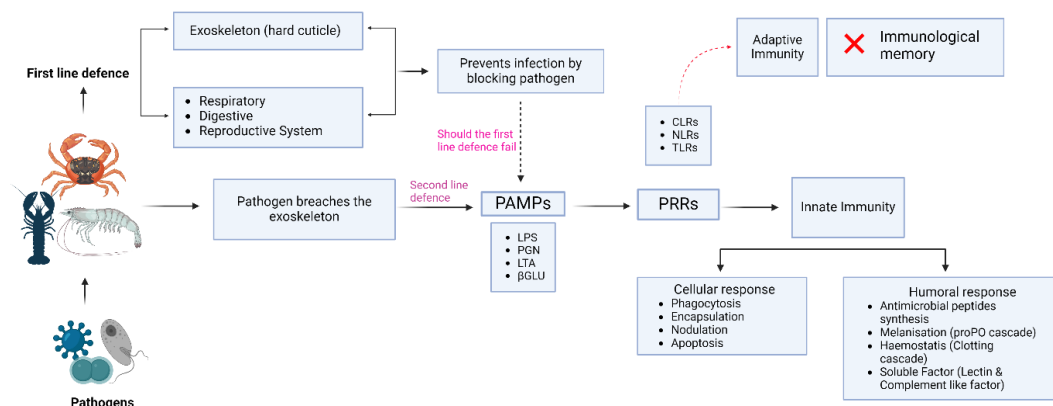


Fig. 3 Immune response pathway in response to pathogens. PAMPs: pathogen-associated molecular patterns, LPS: lipopolysaccharides, PGN: peptidoglycan, LTA: lipoteichoic acid, β -GLU: beta-glucans, PRRs: pattern-recognition receptors, CLRs: C-type lectin receptors, NLRs: NOD-like receptors, TLRs: Toll-like receptors.



Then, a process of particle binding to the phagocytic cell occurs, which then activates the mechanism of phagocytosis by remodelling the actin cytoskeleton and the formation of membrane projections to surround the pathogen particles. This formation is accompanied by pseudopodia that fully engulf the particle, creating a phagosome inside the cell. The phagosome then fuses with a lysosome, forming a phagolysosome responsible for degrading the particle to become harmless or can either be recycled by the cell or expelled (Mengal et al. 2023a). Hemocytes at all developmental stages play a role as immune cells and are actively involved in phagocytosis, though this may be species specific. A study conducted by Giulianini et al. (2007) reported the presence of HC, SGC, and GC in the phagocytic activity of crayfish, *Astacus leptodactylus*, whereas only SGC and GC were involved in the phagocytic activity of *Penaeus monodon* (Sung and Sun 2002). Zhu et al. (2022) discovered the presence of GC in the phagocytic activity of the crayfish *Cherax quadricarinatus*. Also, in the *Procambarus virginalis* post-limb amputation, SGC are the main hemocytes that are responsible for engulfing necrotic materials resulting from muscle degeneration (Mengal et al. 2023a). In *Penaeus monodon*, phagocytosis is dominated by GC, while HC contributes the least (Xian et al. 2021). These differences may be influenced by various factors, including the type of pathogen involved and the presence of hemocytes. Zuo et al. (2022) revealed that a significant reduction in hyaline cells (HCs) may lead to greater SGC involvement in recognizing pathogens and initiating degranulation, followed by phagocytosis. Moreover, hemocyte subpopulations also show specific preferences for phagocytosis of particular bacteria or viruses (Liu et al. 2020).

Pathogens and parasites that are too big for phagocytosis seemed to be killed in similar ways by encapsulation and nodulation, but against different targets (Mengal et al. 2023a). Encapsulation is when the hemocytes progressively surround a foreign intruder in multiple layers, to prevent the growth and development of the parasites (e.g., cestodes, nematodes, trematodes) and fungi, whereas nodulation targets smaller targets such as bacteria (Mengal et al. 2023a). Then, both capsule and nodule will end with the melanization derived from the activation of the prophenoloxidase (proPO) system, and trapped pathogens will be killed and dismantled (Mengal et al. 2023b). During encapsulation, hemocytes like SGC and GC were increased to initiate melanization, produce reactive oxygen species (ROS), and cytotoxic compounds to limit the spread and kill the pathogens (Sánchez-Salgado et al. 2021). The SGC and GC also play roles in various cellular responses to lipopolysaccharide (LPS) stimulation. According to Xian et al. (2017), GC is more sensitive as their numbers decline after lipopolysaccharides (LPS) injection treatment. The cellular mechanisms of the immune responses for phagocytosis, encapsulation, and nodulation are illustrated in Figure 4.

Apoptosis serves as a kind of cellular innate immunity in shrimp that involves the antiviral process. Once the pathogen invades the host cells, the cells initiate apoptosis, a programmed cell death, to eliminate virus-infected cells thereby hampering virus proliferation and spreading (Cui et al. 2020). This process involves a series of complex interactions that are essential for maintaining cellular homeostasis and supporting the immune system. Caspases, which include both initiator and effector caspases, are very important for starting the morphological changes that happen during apoptosis (Huang and Ren 2021). Signs of apoptosis can be observed through ultrastructural analysis, revealing chromatin condensation and aggregation within the nucleus, along with the release of large cellular fragments, as seen in shrimp (Xu et al. 2024). Apoptosis

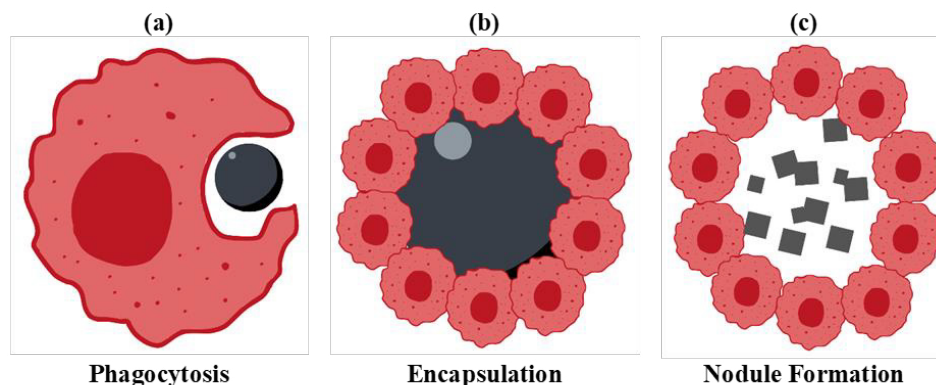


Fig. 4 Different cellular defense mechanism. Red colour: hemocytes, grey colour: foreign invader



is known to be mediated by two pathways: the extrinsic pathway, which is activated by death receptors, and the intrinsic pathway, which is triggered by mitochondrial and intracellular stimuli. The extrinsic pathway is initiated when ligands such as tumour necrosis factor (TNF), TNF-related apoptosis-inducing ligand (TRAIL), and fatty acid synthetase ligand (FasL) bind to death receptors (e.g., Fas receptor FASRs, tumour necrosis factor receptor TNFR1) (Lin et al. 2020). This interaction triggers a cascade of signals that ultimately lead to apoptosis, with caspases acting as the key molecules in this pathway. Caspase-3, a conserved family of cysteine proteases, plays a crucial role in cellular apoptosis (Lin et al. 2022; Cheng et al. 2021). This pathway activates caspases-8 and -10, which in turn activate caspase-3 and -7, causing the degradation of essential cellular proteins and inducing apoptosis (Xiong et al. 2024). The intrinsic pathway is mediated by caspase-9 and -2, which are activated by the release of cytochrome c from mitochondria, indicating the initiation of the caspase cascade and ultimately leading to apoptosis through increasing caspase-3 activity (Kong et al. 2023).

Beside abiotic factors, biotic stressors, such as pathogens, have been widely reported to affect decapod crustaceans. For instance, an increase in caspase activity in *S. paramamosain* and *Macrobrachium nipponense* shrimp when infected with viruses has been demonstrated (Gao et al. 2024; Li et al. 2020). According to Alinejad et al. (2020), viruses use different methods to stop the hosts from going through apoptosis, including attempts to delay the host's initiation of apoptosis so that they can make enough viral offspring and use the apoptosis pathway to spread their offspring to other cells. Other studies show parasites such as Microsporidia have also developed strategies to suppress host cell apoptosis to secure their dormancy and support their own reproduction (Ding et al. 2024). In addition, *in vivo* experiments show that bacteria can also knock down caspase-3, thereby reducing cell apoptosis and increasing crustaceans' mortality (Cheng et al. 2021).

Humoral immunity

The humoral response is a mechanism that fights invasive pathogens through the secretion of proteins from the hemocytes into the hemolymph circulatory system (Sánchez-Salgado et al. 2021). This is categorized into three main parts (i.e., the prophenoloxidase (proPO) system that synthesizes melanin, the blood clotting system, and circulating antimicrobial peptides (AMPs)) (Cervera et al. 2023; Laohawutthichai et al. 2023). The activation of genes crucial to host defense is a result of signalling pathways such as two nuclear factor- κ B (NF- κ B), Toll, and immune deficiency (IMD), which are all components of the innate signalling cascade involved in humoral immunity (Tran et al. 2023). Briefly, when PRRs recognize PAMPs, viral protein antigens subsequently trigger immune defense responses to induce NF- κ B signalling pathway activation and stimulate the secretion of AMPs and other immune proteins in response to infection (Gu et al. 2023; Tassanakajon et al. 2018). The Toll and IMD signalling pathways are two primary immune signalling pathways that directly influence this response, where the Toll pathway responds to Gram-positive bacteria with Lys-type peptidoglycan, fungi, and certain viruses (e.g., WSSV) (Tassanakajon et al. 2018; Wang et al. 2011); meanwhile, the IMD pathway functions against Gram-negative bacteria, a few Gram-positive bacteria with meso-diaminopimelic acid (DAP) type peptidoglycan, and RNA viruses (e.g., Yellow Head Virus (YHV)) (Kulkarni et al. 2021; Jearaphunt et al. 2015; Visetnan et al. 2015). The humoral immunities were illustrated in Figure 3.

In detail, the recognition by PRPs triggers a serine protease cascade to activate the pro-phenoloxidase activating enzyme (PPAE), which performs proteolytic cleavage of inactive proPO enzymes into active PO enzymes (Kumar et al. 2023). The activity of PO oxidizes phenols into quinones, resulting in melanin, which is subsequently secreted to coat particles produced by cell damage and pathogenic attacks (Romo-Quiñonez et al. 2020). Additionally, the melanization process involves cytotoxic products such as reactive oxygen and nitrogen species (ROS/RNS), which play a role in combating foreign microbes. The proPO cascade is triggered by picogram levels of bacterial lipopolysaccharides, or peptidoglycans and beta-1,3-glucans from bacteria and fungi (Söderhäll and Cerenius 1998; Söderhäll and Ajaxon 1982; Nyhlén and Unestam 1980). Recent research by Sukonthamarn et al. (2024) demonstrates that products of the melanization are cytotoxic to *Enterocytozoon hepatopenaei* (EHP) spores, causing significant morphological damage and making them look wrinkled and severely degraded. Serine protease cascades are one of the most prominent enzyme families in the animal kingdom, characterized by three catalytic residues (i.e.,



His, Asp, and Ser) (Shankar et al. 2023). Proteases are often involved in many physiological processes in the body through the proteolytic cleavage of specific proteins (Rojo-Arreola et al. 2020); meanwhile, serine proteases are involved in digestion, embryonic development, hemolymph coagulation, and melanization (Huang et al. 2013). The importance of the proPO system in arthropods is that they play a role in several physiological processes, including sclerotization, defense response, and wound healing (Wanvimonsuk et al. 2023; Aloui et al. 2022), and the expression of proPO is known to occur in the hemolymph and in different parts of shrimp (e.g., gills, heart, lymphoid organ, and stomach) (Phan et al. 2023; Kewcharoen and Srisapoom 2022). Wang et al. (2010) reported the increased proPO activation mainly caused by *Vibrio harveyi* infection in *P. vannamei* shrimp. Meanwhile, Charoensapsri et al. (2011) discovered a new class of crustacean prophenoloxidase (proPO)-activating enzyme (PmPPAE2) in the hemocytes of *P. monodon*, where it plays an essential role in immune defense against *V. harveyi* infection. The process of melanization produces compounds that can damage and inhibit the growth of pathogens, indicating the active role of proPO in the defense mechanisms once the host gets infected (Sukonthamarn et al. 2024).

Apart from being related to pathogens, proPO levels are also associated with stressors originating from abiotic factors (e.g., temperature, pH, and chemical contamination) (Leadprathom et al. 2012), which can be used as a biomarker for early warning signs in an ecosystem. According to Wanvimonsuk et al. (2023), the increase in proPO activity is part of the immune response preparation against pathogens; however, different crustacean species exhibit distinct proPO activities. For example, proPO activity in *Scylla olivacea* is abundant in the GC in large quantities and sizes, while it is not found in other types of hemocytes (Mangkalan et al. 2014). In *Farfantepenaeus californiensis* and *Litopenaeus stylirostris*, proPO activity is mainly located in GC (75 %), SGC (25 %), while it is absent in HC (Vargas-Albores et al. 2005). Similar observations were made in the freshwater crab *Sinopotamon henanense* (Liang et al. 2023) and *P. vannamei* (Chang and Chang 2022), where proPO activity was only detected in SG and GC. However, extreme proPO activity was reported in GC and was weak or absent in SGC in *C. quadricarinatus* (Li et al. 2019). Interestingly, in spiny lobster *Panulirus interruptus*, proPO activity was not found in hemocytes like other crustaceans but was detected in plasma (Hernandez-Lopez et al. 2003), while in Caribbean spiny lobster *Panulirus argus*, proPO was found in both plasma and hemocytes (Perdomo-Morales et al. 2007). The presence of proPO was also influenced by the different species of decapod crustaceans when infected and given treatments. As for the example, Ai et al. (2008) reported the fluctuations in the total hemocyte count (THC) quantity along with proPO activity when *P. vannamei* gets infected with WSSV. A similar finding was also reported by Fagutao et al. (2009), where *Marsupenaeus japonicus* exhibited lower THC levels when lacking proPO in the hemolymph. Table 2 shows the presence of PO activity, which is known to be associated with the quantity and differential hemocytes of various decapod species.

A relation of THC to decapods health

Hemocytes are an essential component of the decapods innate immune system for combating pathogens (Tassakka et al. 2024). Transcriptional immune responses are predominantly carried out in hemocytes such as GC and SGC, which are involved in proPO system activation, and HC with their role in phagocytosis (Sun et al. 2023; Mapanao et al. 2022). These three types of hemocytes are present in different proportions, with SGC occupying approximately 75 % of the hemocyte composition (Martin and Graves 1985). This viewpoint is reinforced by Xian et al. (2021) stating that in *P. monodon*, SGC is the most abundant cell type circulating in hemocytes (42–73 %), while GC (13–36 %) and HC contribute the least (2–18 %). The latest report by Su et al. (2024) reveals that crabs from the species *E. sinensis* and *S. paramamosain* possess a similar GC content proportion of approximately 20 %. Meanwhile, shrimp from the species *Litopenaeus vannamei* and *P. monodon* also demonstrate a relatively consistent GC content proportion of around 10 %. However, contrasting findings are observed in lobsters; the species *C. quadricarinatus* and *P. clarkii* exhibit significantly different GC proportions of 31 % and 15 %, respectively. These findings indicate that the differential hemocyte count (DHC) does not reflect a clear consensus, even among the same species or closely related ones.

Albalat et al. (2022) emphasize that total protein, lactate, and hemocyte counts are key indicators of decapod health. Additionally, low levels of hemolymph may indicate trauma, stress, or poor nutritional status in decapods (Zeinert et al. 2021). A different study also reported that the activity of phagocytosis is



Table 2 The impact of various factors on changes in THC values and PO activity in decapods

Factor(s)	Treatment	Species	THC subsets			References			
			HC	SGC	GC				
Handling pre- and post infection	Injecting WSSV post-feeding with pure ulvan extract mixed feed Use of poly-β-hydroxybutyrate/biosurfactant on WSSV-infected hosts <i>V. alginolyticus</i> infection post octopamine injection Use cacao pod husk pectin on <i>V. alginolyticus</i> -infected hosts <i>Eucheuma cottonii</i> and <i>Gracillaria verrucosa</i> extracts challenged with WSSV WSSV and <i>V. harveyi</i> coinfection post <i>Pseudalteromonas piscicida</i> IUB & <i>fructooligosaccharide</i> treatment <i>V. parahaemolyticus</i> challenged <i>Bacillus subtilis</i> <i>Sonneratia alba</i> extract for WSSV-infected hosts <i>Musa acuminata</i> challenged with <i>Lactococcus garvieae</i> <i>Musa acuminata</i> extract <i>Morinda citrifolia</i> extract <i>Gracillaria tenuistipitata</i> extract <i>Limosilactobacillus fermentum</i> <i>Scutellaria radix</i> water extract <i>Panax notoginseng</i> extract <i>Lactobacillus plantarum</i> Dopamine <i>V. harveyi</i> WSSV <i>V. harveyi</i> <i>V. parahaemolyticus</i> Lipopolysaccharide (LPS) <i>V. parahaemolyticus</i> <i>V. parahaemolyticus</i> <i>V. parahaemolyticus</i> <i>V. parahaemolyticus</i> <i>V. harveyi</i> <i>Vibrio</i> sp.	<i>P. monodon</i>	n.a	n.a	n.a	↑	Declarador et al. (2014)		
		<i>P. monodon</i>	n.a	n.a	n.a	↑	↑	Monica et al. (2017)	
		<i>L. vannamei</i>	n.a	↑	↓	↓	↑	↑	Kuo et al. (2019)
		<i>L. vannamei</i>	↑	↑	↑	↑	↑	↑	Lee et al. (2020)
		<i>L. vannamei</i>	↑	↓	↑	↑	↑	↑	Kilawati et al. (2021)
		<i>L. vannamei</i>	n.a	n.a	n.a	n.a	↑	↑	Nababan et al. (2022)
		<i>L. vannamei</i>	↓	↑	↑	↑	↑	↑	Aribah et al. (2022)
		<i>P. monodon</i>	n.a	n.a	n.a	n.a	↑	↑	Muliani et al. (2022)
		<i>M. rosenbergii</i>	↑	↓	↓	↓	↑	↑	Mapanao et al. (2022)
		<i>M. rosenbergii</i>	↑	↑	↑	↑	↑	↑	Rattanavichai and Cheng (2015)
Immunomodulator	<i>Morinda citrifolia</i> extract <i>Gracillaria tenuistipitata</i> extract <i>Limosilactobacillus fermentum</i> <i>Scutellaria radix</i> water extract <i>Panax notoginseng</i> extract <i>Lactobacillus plantarum</i> Dopamine <i>V. harveyi</i> WSSV <i>V. harveyi</i> <i>V. parahaemolyticus</i> Lipopolysaccharide (LPS) <i>V. parahaemolyticus</i> <i>V. parahaemolyticus</i> <i>V. parahaemolyticus</i> <i>V. parahaemolyticus</i> <i>V. harveyi</i> <i>Vibrio</i> sp.	<i>M. rosenbergii</i>	↑	↑	↑	↑	↑	Mariisa Halim et al. (2017)	
		<i>M. rosenbergii</i>	↑	↑	↑	↑	↑	↑	Liu et al. (2019)
		<i>L. vannamei</i>	↑	↑	↑	↑	↑	↑	Lee et al. (2021)
		<i>M. rosenbergii</i>	n.a	n.a	n.a	n.a	↑	↑	Andriawan et al. (2023)
		<i>L. vannamei</i>	↓	↑	↑	↑	↑	↑	Chen et al. (2023)
		<i>L. vannamei</i>	n.a	n.a	n.a	n.a	↑	↑	Tseng et al. (2023)
		<i>P. monodon</i>	n.a	↓	n.a	n.a	↓	↓	Chang et al. (2007)
		<i>P. monodon</i>	↓	↓	↓	↓	↓	↓	Harikrishnan et al. (2011)
		<i>P. monodon</i>	n.a	n.a	n.a	n.a	↓	↓	Immanuel et al. (2012)
		<i>P. monodon</i>	n.a	↑	↑	↑	↑	↑	Maftuch et al. (2013)
Antigen and stressor	<i>V. parahaemolyticus</i> Lipopolysaccharide (LPS) <i>V. parahaemolyticus</i> <i>V. parahaemolyticus</i> <i>V. parahaemolyticus</i> <i>V. parahaemolyticus</i> <i>V. harveyi</i> <i>Vibrio</i> sp.	<i>P. monodon</i>	n.a	n.a	n.a	↓	↓	Sivaganavelmurugan et al. (2014)	
		<i>P. monodon</i>	n.a	↓	↓	↓	↓	↓	Xian et al. (2016)
		<i>L. vannamei</i>	↓	↓	↓	↓	↓	↓	Ananda Raja et al. (2017)
		<i>L. vannamei</i>	n.a	n.a	n.a	n.a	↓	↓	Zhai et al. (2019)
		<i>L. vannamei</i>	↑	↓	↓	↓	↓	↓	Munaeni et al. (2020)
		<i>L. vannamei</i>	↓	↓	↓	↓	↓	↓	Mani et al. (2021)
		<i>L. vannamei</i>	n.a	n.a	n.a	n.a	↓	↓	Bautista-Covarrubias et al. (2022)
		<i>L. vannamei</i>	↓	↓	↓	↓	↓	↓	Shakweer et al. (2023)
		<i>L. vannamei</i>	↓	↓	↓	↓	↓	↓	
		<i>L. vannamei</i>	↓	↓	↓	↓	↓	↓	

Note: n.a.: not available, ↓=decrease, ↑=increase, ⇕=fluctuate



influenced by the number of HC (Chang and Chang 2022). Similar findings were also found in *Neohelice granulata* and *Scylla paramamosain* when these decapod species become infected by pathogens (Ayres et al. 2024; Wei et al. 2022). Ekblom et al. (2021) suggest that the phagocytic role of different hemocyte types may vary in closely related species. Moreover, THC heavily relies on the host condition and how it reacts to things inside and outside the body (Kuo et al. 2023; Mengal et al. 2023a) and it is heavily influenced by handling stress (Skafar et al. 2023; Powell et al. 2017). In general, higher hemocyte levels indicate better health status, while a decrease in hemocyte count can be caused by various factors (i.e., lysis due to pathogen infection, tissue regeneration, and wound healing) (Jones et al. 2024). For instance, previous studies reported a significant increase in THC in *Macrobrachium rosenbergii* after treatment with plant extract (Andriawan et al. 2023; Hamdan et al. 2023), where this plant administration is believed to trigger the proliferation of shrimp immune cells to enhance cytokine secretion, thereby stimulating increased hemocyte production in the host hematopoietic tissue. Meanwhile, Rahi et al. (2022) reported a significant decrease in THC in *V. parahemolyticus* and WSSV-infected *P. monodon*. A decrease in hemocyte count was also observed in *P. vannamei* when infected with *Enterocytozoon hepatopenaei* (EHP) through injection (Subash et al. 2022). The decrease in THC implies hemocyte lysis as a response to pathogenic substances or environmental pressure, or due to the migration of the hemocytes to the EHP infection site for phagocytosis or to heal wounds (Shakweer et al. 2023). A decrease in THC was also observed in Mediterranean green crabs, *Carcinus aestuari*, when exposed to copper and their eyestalks removed (Qyli et al. 2020). Shen et al. (2021) reported that the sensitivity of decapods to pathogen infections differs based on the life stage of the host, *Penaeus vannamei*. Meanwhile, Berry et al. (2019) argues that the hemolymph biochemistry (i.e., brix, lactate, hemocyanin, protein, triglycerides, lactate, and glucose) is strongly correlated with the nutritional and physiological condition of *P. monodon*. There could be other factors affecting hemolymph volume in decapods, such as the moulting and nutritional conditions, even though individuals have the same body size (Landman et al. 2020; Berry et al. 2019).

Table 2 indicates a correlation between the quantity of each hemocyte type, the presence of invasive pathogens, and various other treatments. Generally, the result indicates that healthy shrimp usually exhibit a higher THC level compared to unhealthy shrimp. On the other hand, SGC is associated with the number of HC, as SGC is formed from the further development of HC. When these cells fail to develop into SGC, it leads to a decrease in the number of SGC. SGC plays a more dominant role in the encapsulation process by responding to large amounts of polysaccharides found in the pathogen cell wall (Risjani et al. 2021). Moreover, SG is a further development of HC and SGC cells, which are involved in storing and releasing phenoloxidase and cytotoxins, and it serves as one of the cellular elements between HC and SGC (Rahardjo et al. 2022).

A common test to quick access decapods health, with the recent studies

Ongoing stressors are known to impact the decapod immune system, where prolonged exposure to them can be fatal. Most of the time, weight and visual assessment are non-invasive methods used for accessing decapod crustacean conditions in production ponds (Pedrazzani et al. 2023); but they are insufficient to fully capture the internal conditions of decapods. Therefore, hemolymph plays a valuable indicator for health assessment, particularly to determine feeding and starvation status, moulting stage, nutritional level, and immunity level (Hammel et al. 2024; Xu et al. 2024; Berry et al. 2019). The impact of these stressors can be seen in various hemolymph parameters, including enzyme activity, ammonia, glucose, lactate, total protein, lysosomal stability, urea, and hemocyte counts. Parameters such as THC and DHC focus more on immune response, with a decrease in hemocyte counts often attributed to biotic and abiotic factors like pathogen infections (Zheng et al. 2021). An increase in THC usually indicates an improvement in immunological status (Silva et al. 2023). Additionally, the activity of ProPO, activated by hemocytes in the presence of Ca^{2+} , plays a vital role in immune response by triggering melanization processes and pathogen elimination (Kumar et al. 2022; Kulkarni et al. 2021).

Understanding hemolymph is crucial for aquaculturists to monitor the health of cultured animals, ensuring sustainable farming practices. For seafood processors, hemolymph provides an indication of decapod stock quality, where a higher hemolymph level suggests a good physiological condition, impacting the final product quality. Scientists also use hemolymph to study immune responses, metabolism, and reactions to



environmental factors, as well as to detect pathogens or oxidative stress. These analyses support the development of strategies to improve decapod welfare and minimize disease-related losses. This can be achieved through simple and field-applicable methods, such as using the brix index as a proxy for hemolymph protein measurement, which can be conducted quickly and accurately using a digital pocket refractometer. The brix index is widely applied in the lobster and crab industries to assess health (Mdaini et al. 2023; Wilson et al. 2022). It takes less than 60 seconds from sample collection to measurement. However, its sensitivity in detecting the effects of oil exposure appears to be limited (Berthod et al. 2021); nonetheless, this method provides a cost-effective and highly accurate assessment to determine shrimp health (Berry et al. 2019).

Besides the aforementioned above, THC can also be used to determine the health condition of decapods, usually by using an automatic cell counter and a flow cytometer cell sorter (Mauro et al. 2022). If the method struggles to differentiate subpopulations by size and granularity, forward-scatter and side-scatter parameters offer an alternative (Su et al. 2024). Moreover, visual observation of melanosis and melanin pigment formation at the cephalothorax and tail is feasible, particularly after shrimp are removed from water (Pedrazzani et al. 2023; Albalat et al. 2022). Recently, it was discovered that PO activity from hemocyanin induces red coloration in crustaceans, influencing consumer preferences (Ji et al. 2024); therefore, strong visual observation by seafood processors is necessary to ensure animal welfare and consumer satisfaction. However, more detailed analysis of PO activity can also be used to determine decapods health; however, it requires specialized laboratory equipment. Fluctuations in hemolymph parameters as a response to various stressors during sampling also become a concern; therefore, pre-transport cooling to reduce stress and enhance metabolic recovery, particularly during long-distance transport, is essential for crustacean welfare and reducing mortality during transportation (Lorenzo et al. 2020).

Conclusion and future perspective

This review provides an up-to-date overview of hemolymph components and their implications for immunological responses, focusing on the defense mechanisms of decapod crustaceans. This knowledge could help design strategies against pathogen invasion by optimizing immune responses mediated by hemocytes in the hemolymph. Furthermore, it is essential to consider that biotic and abiotic factors often manifest immunosuppressive effects; therefore, detailed knowledge of how the combined interactions between abiotic factors and pathogens influence immunological parameters is needed. Few studies state that anthropogenic factors directly affect the performance of hemocyte-mediated immune responses (Qyli et al. 2020), so further research is needed to provide a deeper understanding. The implementation of an ‘omics’ approach is also necessary to clarify the molecular basis of pathogenicity against hemocyte-mediated immune responses. This is crucial because it has been observed that few pathogens can inhibit the activity of immune components, such as proPO, which can affect PO levels. Then, the understanding of the interactions and influences of apoptosis pathways and their connections to the immune system is crucial for developing therapeutic strategies. All of these strategies are meant to help us learn more about how the active cell parts of decapods’ hemolymph and hemocytes help the immune system. This will eventually help fisheries science, animal health, and biotechnology.

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Author contributions All authors have contributed to the final manuscript and approved the submission. Ade Wahyul Fajri Alimin: data curation, formal analysis, writing-original draft, writing-review and editing. Nor Asma Husna Yusoff: data curation, formal analysis, writing-original draft, writing-review and editing. Ince Ayu Khairana Kadriah, Muharijadi Atmomarsono, Andi Parenrengi, Nurhidayah Nurhidayah, and Endang Susianingsih: writing-review and editing, validation, and investigation. Marina Hassan: conceptualization, writing-original draft, writing-review and editing, validation, and investigation.

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