



Minerals in the regulation of molt and other physiological functions of crustaceans

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Abstract

Minerals are essential for maintaining various physiological functions in crustaceans, including the process of molting. Molting (ecdysis) is a critical physiological process that enables growth and regeneration through the periodic shedding of the old exoskeleton and subsequent formation and hardening of a new exoskeleton. Calcium, magnesium, sodium, potassium, Phosphorus are among the most important minerals, playing a crucial role in regulating the molt cycle and other physiological activities. Trace elements, though required only in small amounts, are equally vital for maintaining physiological balance and ensuring successful molting. Iron, zinc, chromium, copper, manganese, and selenium support various biological functions, such as enzymatic activity, immune regulation, oxidative stress control, and energy metabolism. Both a deficiency and an excess of these minerals can disrupt molting, reduce survival rates and impair growth. This review highlights the influence of various minerals on the growth and physiological health of crustaceans.

Keywords: Crustaceans, molting, minerals, physiology, regulation

1 Introduction

Molting is regulated through interactions between internal endocrine signals (e.g., ecdysteroids, methyl farnesoate and molt-inhibiting hormone) (Ramachandra Reddy et al., 2006; S B Sainath, 2011) and external environmental factors such as temperature, salinity, Photoperiod, Water quality, and nutrient availability (Hosamani et al., 2017; Legrand et al., 2021; Shyamal et al., 2018; Techa & Chung, 2015). While it is known that Inorganic elements, or minerals, play a crucial role in the molting process, studies specifically detailing their regulatory mechanisms remain limited. Therefore, this review aims to compile and synthesize available research regarding minerals and their importance in regulating molting and other vital physiological activities in crustaceans.

2 Crustacean molting

Crustaceans belong to the phylum Arthropods, the largest group in the animal kingdom, which includes species such as insects and arachnids. Arthropods possess a cuticle system that forms the outermost boundary of the body- the exoskeleton- effectively separating the organism from its external environment (Jabbari et al., 2014). The Molting process, or ecdysis, is a complex biological phenomenon essential for growth, involving the periodic shedding of the hard, mineralized exoskeleton and the formation of a new one. This process is tightly regulated by various physiological and biochemical mechanisms; however, it remains highly sensitive to environmental factors such as temperature, chemical exposure and water quality parameters like PH and salinity (Legrand et al., 2021). The crustacean molt cycle is essentially unidirectional and typically divided into four phases: intermolt (growth), premolt (preparatory), ecdysis (shedding), and postmolt (recovery and hardening) (Table 1) (Mykles, 2021; Pamuru & Hosani, 2016; Truong et al., 2023). During intermolt, the exoskeleton remains functionally hardened; however, Ongoing mineral deposition, repair, and remodelling processes continue to maintain structural integrity (Huang et al., 2015). The transition into Premolt is triggered hormonally, initiating apolysis- the separation of the old cuticle from the epidermis -and the secretion of Epidermal cells synthesize and assemble new cuticular layers beneath the existing exoskeleton following apolysis (M. Zhang et al., 2021). Eventually, the exoskeleton cracks open along predetermined “molting lines,” and the crustacean emerges during ecdysis, rapidly expanding its soft new cuticle (Xu et al., 2020). In the postmolt stage, Post-ecdysial shell hardening involves both sclerotization (cross-linking of cuticular proteins) and mineralization through deposition of calcium carbonate and other minerals, and body tissues regain their fullness (Gao et al., 2015). While these stages occur in all crustaceans, their precise duration and timing vary significantly by species and environmental conditions (Vatcher et al., 2015). Morphologically, premolt is characterized by the formation of the new cuticle and preparatory rearrangements. For instance, many crustaceans reabsorb calcium from their old exoskeleton and store it internally – within tissues or specialized organs called gastroliths -to be reused during the formation of the new skeleton. Crucially, while the new cuticular layers are laid down during this stage, they remain unmineralized until the molt is complete. As noted by (Vatcher et al., 2015), the epi-and exocuticular layers of the new

decapod cuticle are elaborated during premolt but do not calcify until the old cuticle is shed (Vatcher et al., 2015). During ecdysis, the old exoskeleton splits open and the crustacean pulls itself free (often moving backward) immediately inflating its body with water or air to expand the soft, new cuticle. In the subsequent postmolt stage, hemolymph ecdysteroid levels drop significantly. This Hormonal shift permits the rapid calcification of the new cuticle and re-expansion of muscle tissue (Mykles, 2021). Following ecdysis, postmolt recovery continues as the newly formed exoskeleton undergoes sclerotization and mineralization until full mechanical strength is restored. Finally, the cycle resets, leading into a new-and often much longer -Intermolt period that lasts until the next hormonal trigger initiates the process again (Mykles, 2021). “While the fundamental pattern of the molting stage is conserved, crustaceans exhibit significant species-specific variations in both timing and physiological detail. For example, certain freshwater and terrestrial crustaceans store calcium in gastroliths; however, many marine crustaceans lack specialized calcium storage organs and rely predominantly on environmental calcium uptake and temporary storage in tissues. During the premolt phase, these dissolve postmolt rapidly stiffen the new cuticle. Additionally, the number and duration of premolt substages (typically categorized as D1–D4) vary, as both internal physiological cues and external environmental factors heavily influence timing. Consequently, Intermolt periods can range from a few days in fast-growing juveniles to several years in mature adults facing poor nutritional conditions. Behavioral adaptations also diverge: while many marine species bury themselves or reduce activity to avoid predation, terrestrial crabs often retreat to damp burrows to maintain hydration during the molt.”

Table 1. Role of elements at different growth stages of a typical crustacean.

Molt stage	Sub stage	Exoskeleton	Element required	Role of element	References
Postmolt	A1, A2	Soft, shiny soft	Ca, Zn, Cu, Mn, Se	Exoskeleton hardening begins, antioxidant and immune protection needed during soft-shell stage.	(Huang et al., 2015)
	B1, B2	Hardening	Ca, P, Mg, Cu	Mineralization and strengthening of cuticle. energy use via phosphate pathways.	(M. Zhang et al., 2021)
Intermolt	C1–C4	Fully hardened	Na, K, Mg, Cr, Zn	Ion balance, enzyme function, and maintenance metabolism. Cr for glucose metabolism during growth phase.	(Xu et al., 2020)
Premolt	D0–D4	Thinning old cuticle.	Mg, P, Fe, Se	Activation of digestive enzymes and preparation for molting. Fe and Se support energy and immune readiness.	(Gao et al., 2015)
Ecdysis	E	Shedding old cuticle.	Ca, Cu, Zn, Mn	Exoskeleton restructuring, chitin synthesis, and oxygen transport. (Cu as part of hemocyanin)	(Techa & Chung, 2015)

3 Minerals of shell and their role in crustacean molting

Minerals are fundamental to the crustacean molting process, primarily through their roles in the formation and maintenance of the exoskeleton. This structure, composed of a chitinous matrix reinforced with calcium salts, provides essential support and protection. During the molt cycle, crustaceans must efficiently manage mineral resources by resorbing elements from the old cuticle and rapidly mineralizing the new one—a process vital for restoring mechanical strength. Beyond structural rebuilding, the molting cycle requires the precise regulation of a diverse array of elements including calcium, magnesium, sodium, potassium, phosphorus, iron, zinc, chromium, copper, manganese, and selenium. These minerals not only support the integrity of the new shell but also activate critical biochemical pathways (Truong et al., 2023). The following sections detail the specific roles and mechanisms of these minerals in crustacean molting.

3.1 Calcium

Crustaceans predominantly inhabit aquatic environments, which serve as their primary exogenous source of calcium. While the calcium concentration in seawater is relatively high, it is significantly lower in freshwater and terrestrial habitats where calcium availability for ecdysis varies greatly. Despite these environmental differences, the fundamental process of ecdysis remains consistent across groups. To compensate for mineral-poor environments, terrestrial and freshwater species have developed specialized strategies for calcification—most notably the internal storage of calcium in tissues or gastroliths during the premolt period (Graf & Delbecque, 1987; Graf & Meyran, 1983, 1985; Graf & Michaut, 1980). Consequently, the primary source of calcium used for cuticle mineralization depends largely on the animal's habitat. While food can provide calcium, its contribution to the calcification process is relatively minor. The ability of crustaceans to calcify exoskeleton, and in certain species—utilize internal storage forms makes them biologically remarkable.

The role of calcium in promoting growth within the crab species *Oziotelphusa senex senex* was investigated by (Pamuru et al., 2022). Their findings revealed that crabs injected with methyl farnesoate (MF) (10^{-8} mol/crab) and reared in a medium of calcium hydroxide and ethylene diamine tetra acetic acid ($\text{Ca(OH)}_2 + \text{EDTA}$) exhibited

significantly shorter molt cycles compared to those injected with MF alone. Furthermore, the results indicated that a low availability of environmental calcium slows the molting process, underscoring the critical importance of calcium in facilitating crustacean ecdysis and overall growth.

The crustacean exoskeleton is primarily composed of calcium carbonate. To support the intensive calcification process, these organisms derive calcium from three main sources: dietary intake, absorption from the surrounding water, and mobilization of internal reserves. However, the calcium obtained through food alone is usually insufficient to meet the physiological demands of proecdysis and the subsequent formation of a new skeleton (GREENAWAY, 1985).

Consequently, supplementing the aquatic environment with calcium has become a standard practice in crustacean aquaculture to ensure successful molting and structural integrity.

Various organic and inorganic calcium-based compounds, including calcium citrate and quicklime, are frequently added to pond water to maintain optimal calcium concentrations in crustacean aquaculture (D. Zhang et al., 2016; Zhang Dan, 2020). While moderate supplementation is known to facilitate the molting process, the specific molecular mechanisms by which exogenous calcium influences the hormonal or cellular pathways of ecdysis remain largely poorly understood. Reports by indicate that calcium absorption, transport, and storage in crustaceans fluctuate significantly throughout the molting cycle (Cheng & Li, 2012). During the proecdysis (pre-molt) phase, calcium is reabsorbed from the existing exoskeleton and transported into the hemolymph via the epithelial cells. A portion of this calcium is sequestered within internal reserves, such as the hepatopancreas and gastric gill (often in the form of gastroliths), primarily as calcium phosphate ($\text{Ca}_3(\text{PO}_4)_2$), while excess ions are excreted through the gills. Following the molt, during the metecdysis (post-molt) stage, these stored reserves are mobilized back into the rapid calcification and hardening of the nascent exoskeleton (Figure 1) (Ahearn et al., 2004). Because crustaceans lose a significant amount of calcium during molting, they require a substantial intake during the post-molt stage to ensure successful exoskeleton hardening. This necessity creates a cyclical demand for calcium throughout the molting process (Neufeld & Cameron, 1993). To maintain calcium balance, specialized epithelial tissues, specifically the gills, hepatopancreas, and antennal glands - play vital crucial roles in transporting and temporarily storing calcium ions (Ca^{2+}). By managing these ions effectively, these tissues prevent the disruption of intracellular calcium homeostasis while facilitating the rapid, efficient calcification of the new exoskeleton (Wheatly et al., 2002).

During ecdysis, increased internal hydrostatic pressure generated by water uptake and muscular contractions causes rupture of the old exoskeleton along predetermined ecdysial sutures, allowing the animal to emerge.

Current research indicates that extracellular calcium in crustaceans is transported in a unidirectional manner, moving from the apical to the basal side of epithelial membranes (Figure 1). This process is tightly regulated by the X-organ-sinus gland complex, located at the base of the eyestalk. This complex secretes key neuropeptides, such as molt-inhibiting hormone (MIH) and crustacean hyperglycemic hormone (CHH), which play fundamental roles in the hormonal of the molting cycle (Figure 2). (Webster et al., 2012).

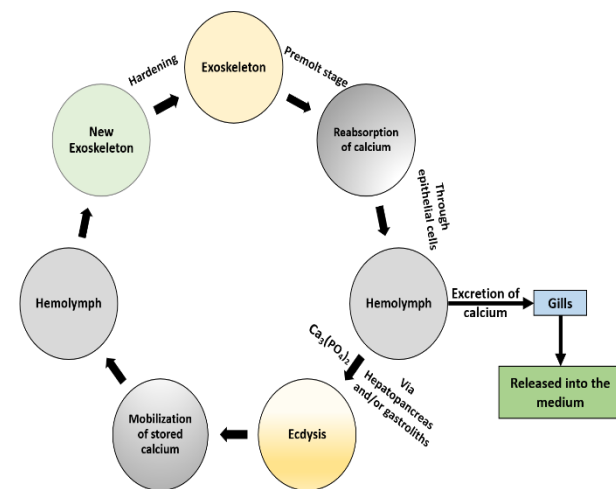


Figure 1. Calcium flow during molt cycle in crustaceans

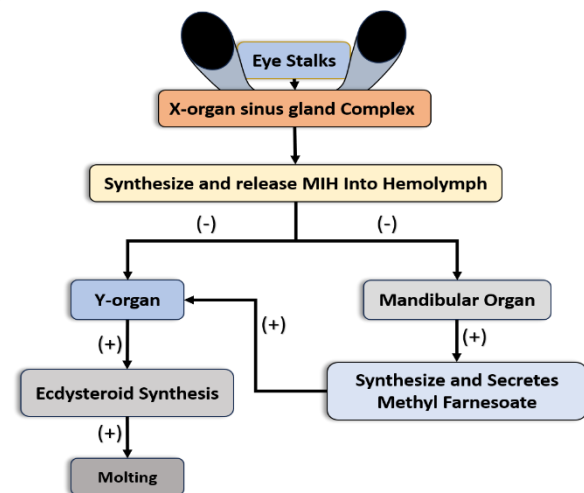


Figure 2. Role of molt-inhibiting hormone (MIH), methyl farnesoate (MF) and ecdysteroids on crustacean molting. X-organ sinus gland complex located in eyestalk neural ganglia of crustaceans synthesizes and release a peptide hormone called MIH into the hemolymph. In one way MIH reaches to Y-organ and influences the ecdysteroid synthesis and release, on the other hand MIH in mandibular organ restricts the synthesis and release of terpenoid hormone MF which is known promoter of ecdysteroid synthesis from Y-organs.

Calcium signalling pathways are also believed to interact directly with MIH (Molt-Inhibiting hormone) activity. For instance, when MIH binds to its membrane receptor, it can activate adenylate cyclase, triggering an increase in intracellular calcium levels. This elevated calcium then binds to calmodulin (CaM), which subsequently regulates various downstream enzymatic reactions (Kim et al., 2005).

Furthermore, research by (Nakagawa & Henrich, 2009) suggests that fluctuations in ecdysone levels may provide feedback that regulates MIH activity. Specifically, increased intracellular calcium levels appear to enhance phosphodiesterase (PDE) activity, which may inhibit MIH secretion through a negative feedback loop. While these insights underscore the critical role of calcium in crustaceans can molting, the precise cellular mechanisms by which calcium influences ecdysone synthesis and secretion are not yet fully understood Figure 3. *Macrobrachium rosenbergii*, commonly known as the giant freshwater prawn, is a valuable aquaculture species in China and Southeast Asia due to its favorable growth characteristics (Schumann et al., 2018). Throughout its molting cycle, the prawn requires a substantial intake of calcium from both dietary sources and the surrounding water. In high-density aquaculture systems, mineral deficiencies in the water are common; these often lead to physiological issues such as soft shells, molting failure, and increased cannibalism resulting from asynchronous molting. In a recent study, (Ding et al., 2024). investigated the effects of different calcium concentrations in the culture water of *M. rosenbergii*. They examined molting frequency and exoskeleton ultrastructure during the post-molt stage. Furthermore, they utilized RNA sequencing (RNA-seq) and qPCR to explore the molecular pathways influenced by exogenous calcium supplementation. Their findings provide valuable insights into how calcium availability regulates molting at the genetic level, offering practical guidance for enhancing crustacean health and productivity in aquatic systems.

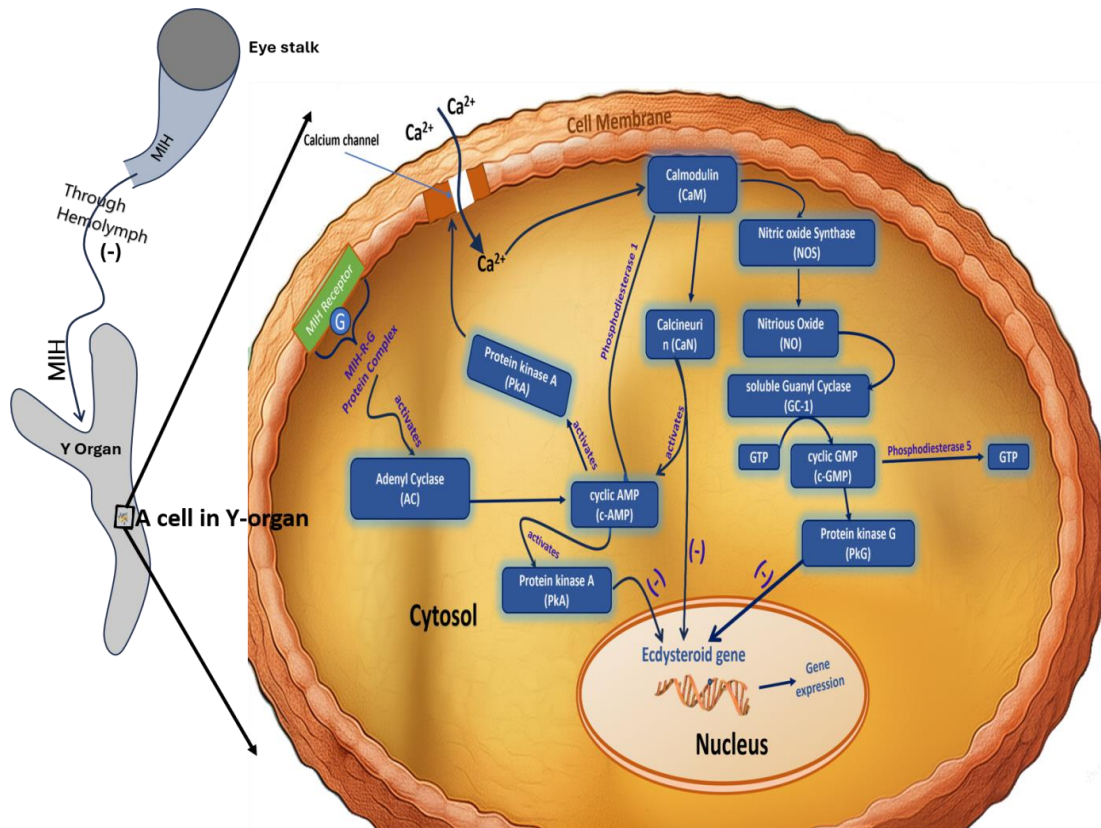


Figure 3. Mechanism of calcium mediated ecdysteroid synthesis in the cells of Y-organ in crustaceans.

Calcium is a crucial mineral for crustaceans, playing a vital role in exoskeleton formation, ecdysis (molting), muscle contraction, and cellular signaling. The diverse physiological functions of calcium within crustaceans are illustrated in Figure 4. Unlike vertebrates, crustaceans lack internal bones, and their exoskeleton (carapace) is heavily reliant on calcium carbonate for hardness and protection. Deficiency of calcium can therefore cause a range of serious physiological issues. Soft-shell syndrome, deformities, increased vulnerability to predation and disease occur due to calcium deficiency (Graf & Delbecq, 1987). (Wheatly et al., 2002) reported that deficiency causes delayed molting processes, incomplete shedding and mortality.

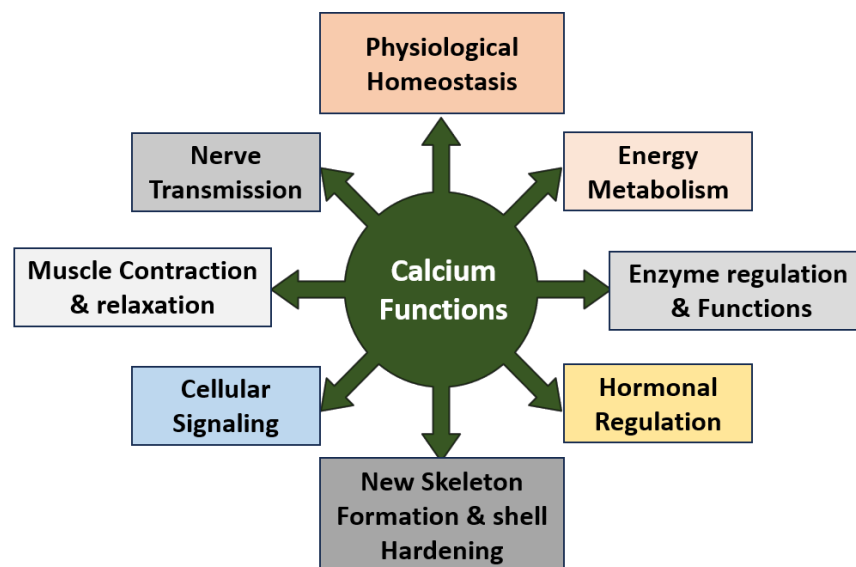


Figure 4. Calcium's role in the regulation of various physiological activities of crustaceans.

3.2 Magnesium

Magnesium, an alkaline earth metal plays a vital role in the physiological functions of crustaceans. As a critical intracellular divalent cation, it is an essential element involved in various biological processes, including protein synthesis, cell division, and the metabolism of carbohydrates and lipids. Magnesium also facilitates enzymatic reactions by stabilizing ATP and forming the Mg-ATP complex, which acts as a necessary cofactor.

Functioning both intracellularly and extracellularly, magnesium regulates ion channels, signaling molecules, nerve conduction, and muscle contraction, while also assisting in potassium transport and oxidative phosphorylation. Specifically, extracellular Mg^{2+} is indispensable for maintaining normal nerve impulses, muscle function and skeletal tissue metabolism.

Magnesium serves as a cofactor for numerous enzymes involved in energy metabolism, nucleic acid synthesis, and ion transport. In crustaceans, magnesium is crucial for ATPase function, which is essential for maintaining cellular energy levels and ion gradients (Florkin & Scheer, 1967). Additionally, magnesium plays a regulatory role in nerve impulse transmission and muscle contraction by acting as a natural calcium antagonist, which helps reduce excessive muscle excitation and spasms (Kirkland et al., 2018).

Marine crabs utilize magnesium channels to regulate internal ion concentrations in response to seawater, with magnesium ions playing a key role in modulating gill function and ion transporters (Pequeux, 1995). Furthermore, magnesium levels in crab hemolymph are carefully controlled to support physiological processes, including pH buffering and immune responses (Wheatly et al., 2002).

Magnesium is a key component of the crustacean exoskeleton, where it is often associated with calcium carbonate in the form of Mg-calcite. During the molting process, crustaceans rapidly absorb minerals to harden their newly formed exoskeleton (GREENAWAY, 1985). Beyond its structural role in molting, magnesium is integral to the regulation of enzymatic and metabolic reactions, muscle function, nerve transmission, and ionic and acid-base balance. Furthermore, it plays a vital role in strengthening immune function Figure 5.

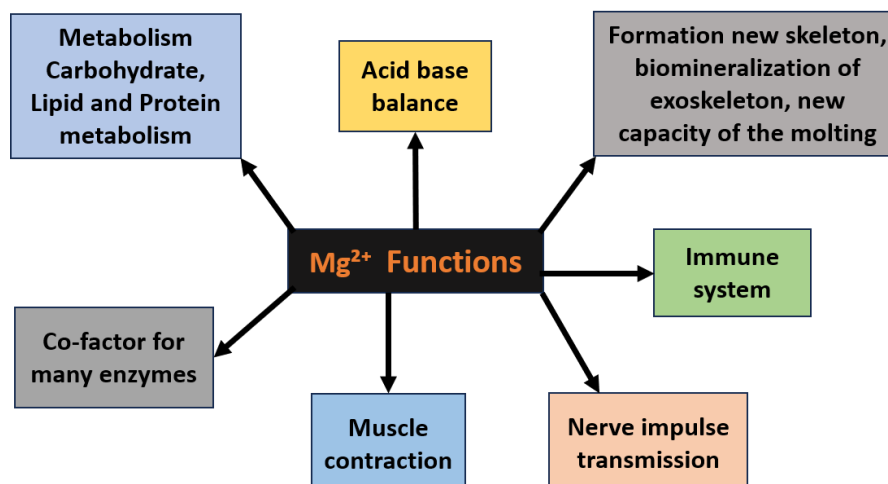


Figure 5. Different physiological functions of magnesium in crustaceans.

Magnesium deficiency in crustaceans, although less frequently studied than calcium or sodium imbalances, can have significant physiological and developmental effects. Because magnesium is vital for numerous biological functions, its deficiency can impair multiple systems simultaneously. For instance, a lack of magnesium may lead to poor calcification of the exoskeleton, resulting in softer or malformed shells during the post-molt stage. It can also cause delayed or incomplete hardening of the new carapace after molting (GREENAWAY, 1985).

Additionally, a deficiency of Mg^{2+} can result in over-excitation of nerves and muscles, causing spasms, paralysis, or uncoordinated movements (Florkin & Scheer, 1967). This can disruption often extends to ionic homeostasis, particularly in osmoregulatory organs like the gills, impairing the animal's ability to maintain internal salt and water balance. Such imbalances in the hemolymph can lead to mortality in hypo- or hyper-osmotic conditions. Furthermore, research (Kirkland et al., 2018) indicates that magnesium deficiency can interfere with the molting process itself, leading to molting delays or pauses and stunted growth. Overall, magnesium deficiency can severely reduce the survival, fitness, and reproductive success of crustaceans, especially in aquaculture or polluted environments where mineral imbalances are common (Atwood, 1976; Kirkland et al., 2018). The functional activities of Mg^{2+} in crustaceans are depicted in Figure 5.

Magnesium acts as a physiological calcium antagonist by competing for calcium-binding sites and modulating voltage-gated calcium channels. Elevated Mg^{2+} concentrations reduce calcium influx into neurons and muscle cells, thereby decreasing neurotransmitter release and muscle excitability. This antagonistic interaction contributes to neuromuscular stability and prevents excessive stimulation during physiological stress and molting.

3.3 Sodium (Na)

In crustaceans, the macro element sodium (Na^+) acts as an electrolyte, participates in osmoregulation (Kirkland et al., 2018), is involved in nerve impulse transmission and balances the acid- base conditions in the body. Because crustaceans live in environments with varying salinities (e.g., estuaries, intertidal zones), maintaining proper sodium levels is crucial for survival and physiological homeostasis.

Crustaceans regulate their internal ion concentration through specialized gill transport mechanisms. Sodium is absorbed or excreted through the gill epithelia depending on external salinity, which helps maintain hemolymph osmotic pressure and ionic balance (Pequeux, 1995). Furthermore, Sodium is essential in generating action potentials across nerve and muscle cell membranes; Similar to vertebrates, sodium channels initiate depolarization during nerve impulses in crustaceans (Atwood, 1976).

Sodium exchange (often with H^+ or NH_4^+) is a key component of acid-base regulation, particularly under stress or changing environmental conditions, allowing the animal to maintain hemolymph pH and buffering capacity (Goss et al., 1992). In euryhaline crabs (those that tolerate a wide salinity range), sodium transport is upregulated in low-salinity water to combat ion loss. This process occurs due to Na^+/K^+ -ATPase and Na^+/H^+ exchangers in gill tissues (Lucu & Towle, 2003). Sodium is a primary cation in the hemolymph (crustacean blood), contributing significantly to maintaining osmotic pressure and water balance. Therefore, appropriate sodium concentration avoids dehydration or overhydration in varying aquatic environments (El-Sayed et al., 2024).

Molting or ecdysis requires precise regulation of ions, water, and hormones in crustaceans. Sodium (Na^+) plays a pivotal role throughout the molting cycle-specifically in osmoregulation, water uptake, and volume expansion. During ecdysis, crustaceans rapidly absorb water to inflate their body size before the new exoskeleton hardens. Sodium ions are actively transported across the gills, creating an osmotic gradient that draws water into the hemolymph (GREENAWAY, 1985). Active sodium ion uptake by crustaceans causes an increase in hemolymph osmolarity, leading to water influx via osmosis. This process swells the body to expand the soft exoskeleton and aid in molting. The Na^+/K^+ -ATPase transporter is highly active during premolt and postmolt stages. It pumps Na^+ into the hemolymph from surrounding water while exporting K^+ , helping to regulate ion gradients and support water balance, driving ion and water movement necessary for successful molting Figure 6. Sodium ions prevent dehydration or overhydration during shell softening and re-hardening. Insufficient sodium levels in the environment (e.g., in low-salinity water) can impair ion transport and reduce hemolymph volume expansion, resulting in incomplete ecdysis (molting failure), soft-shell syndrome, and increased mortality (Kirschner, 1991). Sodium is essential for ion balance, osmoregulation, neural function, and water uptake in crustaceans. A deficiency or lack of available sodium can severely compromise crustacean physiology and overall survival. This vulnerability is particularly pronounced in Low-salinity environments or within aquaculture systems suffering from poor water quality, where the animal must expend significant energy to maintain internal homeostasis Figure 7. Since calcium makes up the majority of the hardened exoskeleton, it is the subject of the majority of research. On the other hand, sodium functions as a physiological regulator, primarily regulating calcium mobilization and deposition, osmotic balance, ion transport pathways and water uptake during ecdysis. As a result, sodium plays a crucial but frequently overlooked role in the physiology of crustacean molting. Comprehensive research on sodium-mediated mechanisms in crustaceans in crustacean molting is necessary because little is known about sodium dynamics during molt stages and its interaction with other vital minerals during exoskeleton production.

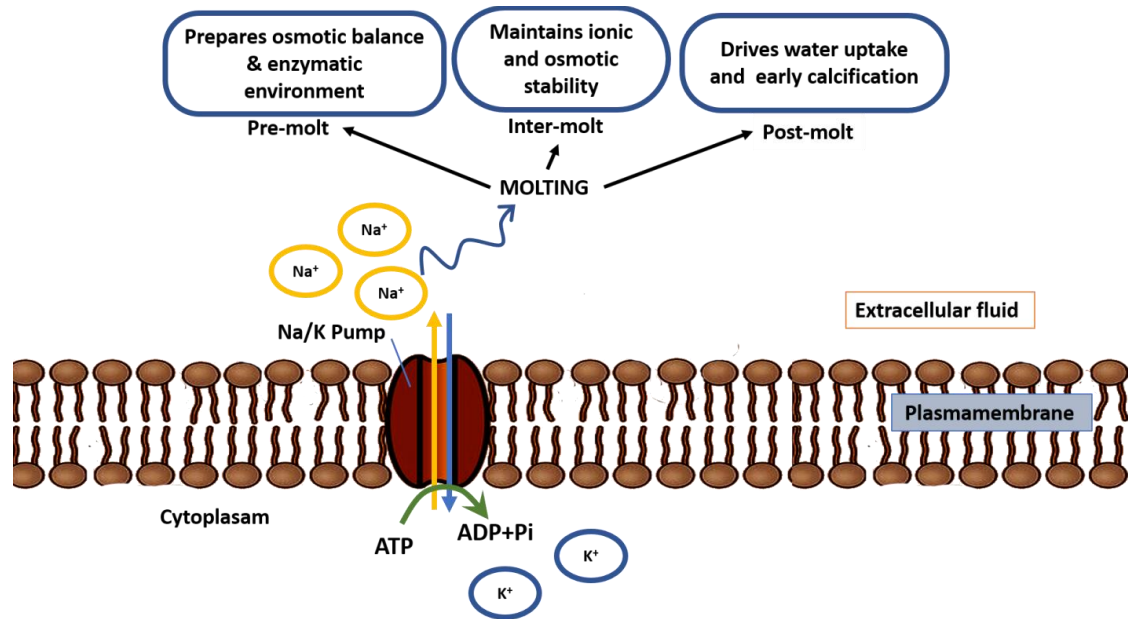


Figure 6. Sodium/ potassium pump with role of sodium in the regulation of molt in crustaceans.

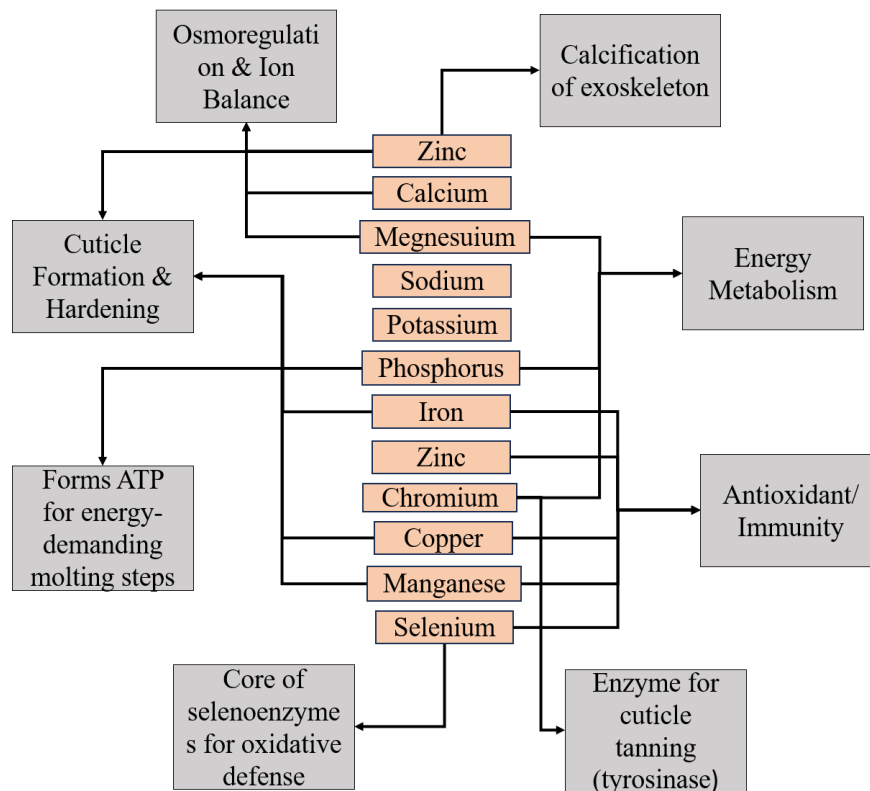


Figure 7. Different roles of various minerals in crustaceans.

3.4 Potassium (K)

As a vital macronutrient, potassium (K⁺) is essential for regulating a wide array of physiological processes in crustaceans. It serves as a critical cofactor for enzyme activation and is fundamental to nerve impulse transmission, Muscle contraction, and acid-base equilibrium. While K⁺ is significantly less abundant than sodium in extracellular

fluids (hemolymph), it is the primary cation required for maintaining intracellular osmotic balance and cellular homeostasis.

In nerve and muscle cells, potassium ions (K^+) are fundamental to maintaining the resting membrane potential and facilitating repolarization (Atwood, 1976). Proper muscle excitability and contraction in crustaceans depend heavily on precise K^+ concentrations; consequently, a potassium imbalance can lead to severe neuromuscular dysfunction, manifesting as either flaccid paralysis or tetany (muscle cramps) (Florkin & Scheer, 1967).

Alongside sodium, potassium (K^+) is essential for fine-tuning osmoregulatory responses within the gills and excretory organs, such as the antennal glands (Lucu & Towle, 2003). Intracellular pH regulation is primarily achieved through the exchange of K^+ for H^+ ions, a mechanism that stabilizes the internal environment during periods of metabolic change or environmental stress. While potassium is less prominent than calcium, magnesium, or sodium during the crustacean molting cycle, it works in tandem with sodium to facilitate cuticular formation and the water uptake process (Roy et al., 2010). Specifically, potassium assists in maintaining the necessary osmotic pressure required for successful ecdysis (Figure 7). Reduced feed intake in crustaceans is a primary indicator of potassium deficiency. A lack of this essential mineral can lead to significant molting abnormalities, including soft-shell syndrome, increased post-molt mortality, and incomplete molting (GREENAWAY, 1985). As a result, potassium shortage may indirectly impact molting by interfering with Na^+/K^+ -ATPase-mediated ion transport and postmolt water absorption. However, there is currently no experimental data throughout molt stages, which represents a substantial research gap. Crustaceans grown in freshwater or low-salinity habitats are most commonly found to be deficient in potassium.

3.5 Phosphorus (P)

Phosphorus, a macronutrient, plays an equally significant role in energy metabolism, exoskeleton development, nucleotide synthesis and enzyme activities. This role is often overlooked in favor of calcium regarding the development of hard tissues. As a fundamental component of adenosine triphosphate (ATP)- the primary energy currency of the cell- Phosphorus is essential for energy metabolism. It supports muscle contraction, active ion transport, and biosynthetic processes. For instance, Na^+/K^+ -ATPase requires ATP to function properly (Lall, 2003). Additionally, phosphate ions ($H_2PO_4^-$ and HPO_4^{2-}) act as buffers in hemolymph, helping to stabilize pH levels during stress or molting (Florkin & Scheer, 1967).

Unlike other invertebrates (such as Mollusca and Echinodermata), many crustaceans incorporate varying amounts of phosphorus within their carbonate skeletons (Elmasry et al., 2023; Vatcher et al., 2015). This phenomenon was observed by (Darwin, 1851) who noted that while crustacean calcareous shells show high variability in phosphate content, molluscan shells contain virtually none. The presence of phosphate in crustacean skeletons has been mainly attributed to co-precipitation with amorphous calcium carbonate (Hild et al., 2009). However, recent studies indicate that calcium phosphate mineralization is widespread in crustacean mandibles.

Specifically, calcium phosphate teeth often form in distinct locations in the mandibles of Malacostraca, the largest crustacean class, which includes lobsters, crayfish, prawns, crabs, and shrimp (Bentov et al., 2016). Furthermore, phosphorus is crucial for reproduction and egg development, significantly affecting embryonic growth and larval survival (Roy et al., 2010).

A phosphorus deficiency in crustaceans can lead to stunted growth, soft shells, and poor molting, as well as decreased reproductive output and lower survival rates-especially during early life stages. Crustaceans appear to have overcome certain limitations of amorphous calcium carbonate, such as high solubility and low hardness, by incorporating phosphate into their skeletal structures. Ultimately, the available phosphate ratio directly determines the structural integrity and strength of the crustacean shell (Bentov et al., 2016).

Although the dynamics of calcium during crustacean molting have been thoroughly researched, phosphorus's significance is still relatively unknown. ATP generation, nucleic acid metabolism, membrane construction and exoskeleton mineralization-all of which are crucial during molt progression-all depend on phosphorus. There is a substantial knowledge gap on the mineral physiology of crustacean molting, though, as little is known about phosphorus mobilization, storage and redistribution across molt stages.

3.6 Iron (Fe)

Iron is a trace element essential for oxygen transport, energy metabolism, cellular health and immune function in crustaceans. While required in small amounts, both iron deficiency and excess can significantly impact crustacean physiology and survival. Specifically, iron is critical for the mitochondrial electron transport chain and aerobic energy production; for instance, Cytochrome c oxidase- an iron-based enzyme catalyzes the reduction of oxygen within the electron transport chain (Davis & Gatlin, 1996). Furthermore, iron maintains Phagocytosis and oxidative killing mechanisms in crustacean hemocytes. Research by (AZAZA et al., 2009) found that iron-based enzymes generate reactive oxygen species (ROS) in immune cells, which effectively neutralize pathogens.

Iron is not directly involved in crustacean shell mineralization; it plays a vital role throughout the molting cycle. Iron-containing macromolecules mediate critical activities such as energy metabolism, enzymatic function, immunity, and post-molt tissue regeneration. Specifically, iron-based molecules facilitate the following processes:

Apolysis: the enzymatic breakdown of the old exoskeleton. Cuticular synthesis: The formation of new cuticular proteins and chitin matrix (Hossain et al., 2025)

Iron functions as a cofactor for various oxidoreductases and hydrolases, supporting cuticle softening and reabsorption, and the remodelling of proteins and collagen. Consequently, iron deficiency can result in incomplete molting, diminished muscle strength, reduced shell hardness, and immune suppression. Conversely, an excess of iron can trigger oxidative tissue damage, systemic inflammation or physiological stress during the vulnerable post-molt period (Roy et al., 2010). Although Iron is essential for enzyme activity and cellular respiration, little is known about iron redistribution and metabolic demand during the various stages of crustacean molting.

3.7 Zinc (Zn)

Zinc is an essential trace element that functions as a critical cofactor for over 300 enzymes. It is a structural and functional component of various metalloenzymes, including carboxypeptidases A and B, Carbonic anhydrase, alcohol dehydrogenase, glutamic dehydrogenase, D-glycerate-3-phosphate dehydrogenase, D-glyceraldehyde-3-phosphatase dehydrogenase, lactate dehydrogenase, malate dehydrogenase, alkaline phosphatase, aldolase, superoxide dismutase (SOD), ribonuclease, and DNA polymerases (National Research Council, 1980).

Zinc plays a pivotal role in the molting cycle and chitin metabolism. While its influence on calcium and phosphorus metabolism is indirect, it acts synergistically to regulate mineral homeostasis. Specifically, Zinc modulates gene expression and activates enzymes that oversee cuticle formation and mineral deposition. Following ecdysis (molting), zinc facilitates the cross-linking of cuticular proteins with carbohydrates; this biochemical reinforcement provides the necessary strength and rigidity to the new exoskeleton (Hossain et al., 2025).

Furthermore, Zinc is vital for maintaining a robust immune response in crustaceans. It supports the production of antioxidant enzymes and the activity of phagocytic hemocytes, which are essential for cellular defense. Additionally, Zinc is a key requirement for effective wound healing and tissue repair (Liu et al., 2020).

Zinc deficiency in crustaceans is characterized by reduced shell hardness (soft shell) or a prolonged molting cycle, both of which contribute to stunted growth. Furthermore, inadequate zinc levels result in a weakened immune system and significantly lower survival rates, particularly during the early life stage. Additionally, chronic deficiency has been linked to impaired reproductive performance and reduced fecundity (Roy et al., 2010). Although zinc is known to be an essential cofactor for enzymes involved in chitin formation and cellular development, little is known about the processes controlling Zinc mobilization and use during the course of subsequent molt stages.

Trace levels of chromium (Cr) play a significant role in the nutrition and health of crustaceans. Traditionally, chromium was considered a non-essential element for molting process when compared to calcium (Ca) and copper (Cu). However, recent studies have revealed that trivalent chromium (Cr^{3+}) in organic forms provide indirect benefits to the molting cycle (Liu et al., 2020).

Specifically, (Paul et al., 2005), observed that organic chromium enhances molting efficiency. Furthermore, research indicates that both organic supplementation (such as chromium picolinate or chromium yeast) and inorganic forms (such as chromium chloride) effectively promote animal growth and weight gain in crustaceans (Paul et al., 2005; Yildiz et al., 2004).

Dietary chromium sources significantly impact growth and glucose metabolism in shrimp, such as *Litopenaeus vannamei*. Research has demonstrated the beneficial effects of chromium on carbohydrate metabolism, lipid utilization, protein synthesis, and stress tolerance (Liu et al., 2020). Furthermore, chromium bolsters the immune system by enhancing phagocytosis, lysozyme activity, and phenoloxidase activity, while modulating antioxidant enzyme levels (Yildiz et al., 2004). Among the various forms, organic chromium (such as chromium picolinate) is particularly effective in promoting growth (Zhou et al., 2009).

From a toxicological perspective, hexavalent chromium (Cr^{6+}) is significantly more toxic than the trivalent (Cr^{3+}) form. While inorganic Cr^{3+} (such as CrCl_3) is used in studies, organic Cr^{3+} is generally more bioavailable and safer. Although chromium is not a primary structural element in the molting process, it is indirectly involved via the energy and metabolic pathways that support ecdysis. Consequently, either a deficiency or an overdose of chromium can lead to increased organ stress—particularly in the hepatopancreas and gills—as well as impaired respiration, mineral imbalances, and increased mortality.

3.8 Copper (Cu)

Crustaceans absorb copper from their surroundings, though their specific requirements remain a subject of debate (Bryan, 1968). Copper is essential to numerous physiological processes, including oxygen transport, molting, pigmentation, immune response, and enzyme activity. Most crustaceans rely on hemocyanin, a copper-based respiratory pigment, to transport oxygen to their tissues and cells. Within the hemocyanin molecule, two Cu^{2+} ions bind reversibly to a single oxygen molecule, facilitating efficient gas exchange throughout the organism (Mykles, 2021). Copper acts as a cofactor for several critical enzymes, including tyrosinase, superoxide dismutase (Cu/Zn-SOD), and cytochrome oxidase. These enzymes are essential for metabolic regulation, immune responses, and the molting cycle. As an essential element, Copper is particularly vital during ecdysis and postmolt recovery; it facilitates shell hardening via tyrosinase activity, regulates chitin synthesis, and supports protein cross-linking within the cuticle. Furthermore, copper strengthens the immune system by triggering hemocyte activation and the phenoloxidase (proPO) cascade. The processes are fundamental for pathogen encapsulation and the production of reactive oxygen species (ROS) used to neutralize invading pathogens (Rainbow, 2002).

A dietary deficiency of copper impairs oxygen delivery, resulting in lethargy, stunted growth, and high mortality in crustaceans. Conversely, an excess of copper-particularly in its ionic form (Cu^{2+})-is toxic in aquatic environments, with larval stages being particularly vulnerable to these toxic effects. Crustaceans hemocyanin is a large extracellular oxygen-transport protein typically organized as hexamers or multi-hexamer complexes. Each hemocyanin subunit contains a binuclear copper center composed of two copper atoms that reversibly bind a single oxygen molecule. Thus, oxygen transport depends on paired copper atoms located within each functional subunit rather than on free copper ions.

3.9 Manganese (Mn)

Manganese is an essential trace element for crustaceans, playing a significant role in enzymatic reactions, immune system activation, shell formation, antioxidant defences, and molt regulation (Roy et al., 2010). It serves as a vital cofactor or activator for several key enzymes, including arginase, Mn-superoxide dismutase (Mn-SOD)- a critical mitochondrial antioxidant that neutralizes reactive oxygen species, pyruvate carboxylase, which facilitates gluconeogenesis, and glycosyltransferase, which is essential for cuticle synthesis. Consequently, manganese-dependent pathways are fundamental to ammonium detoxification, mitochondrial protection, carbohydrate metabolism, and the formation of the new exoskeleton during the molting cycle (White et al., 1975).

In enzymes containing metal ions-primarily Mg^{2+} , Mn^{2+} , Zn^{2+} - the metal ion binds to specific groups within the substrate, acting as a strain-producing agent by forming a chelated intermediate. Simultaneously, the positive charge of the metal ion functions as an efficient electrophile, actively participating in the catalytic reaction (Lall, 2003). During the molting cycle, manganese is essential for the proper calcification of the exoskeleton. Furthermore, manganese bolsters the immune system; when reactive oxygen species (ROS) levels rise, it serves as a key component of antioxidant defences, mitigating cellular damage during periods of physiological stress or infection. Manganese is known to activate chitin metabolism-related enzymes, but its precise function in controlling molt-stage-dependent exoskeleton formation is still mostly known.

3.10 Selenium

In crustaceans, selenium is a vital trace element that plays a significant role in immune system, antioxidant defences, and reproductive health. The biological efficacy of selenium is highly dependent on its chemical speciation and concentration, because the margin between requirement and toxicity is narrow, both deficiency and excess can lead to adverse physiological effects.

Selenium is a crucial component of selenoproteins, most notably glutathione peroxidase (GPx), which shields tissues from oxidative damage during physiological stressors such as molting or fluctuating environmental conditions. Adequate selenium levels bolster antioxidant enzyme activity and effectively scavenge reactive oxygen species (ROS)(Lall, 2003). Furthermore, Selenium enhances non-specific immune responses by stimulating hemocyte activity, promoting phagocytosis, and upregulating the expression of immune-related genes, thereby improving resistance to infection (de Almeida Rodrigues et al., 2022). In crustaceans such as shrimp and crabs, selenium is vital for optimal growth rates, successful ecdysis, and subsequent shell hardening; it also supports metabolic regulation and maintains energy homeostasis during high-demand periods (Lall, 2003). Conversely, selenium toxicity induces oxidative stress and tissue damage, leading to reduced survival rates. Excessive levels can disrupt molting cycles and impair the function of the hepatopancreas, particularly when dietary or environmental concentrations are poorly regulated (Banace et al., 2024). Selenium is vital for crustacean antioxidant defences, immune function, and molting process; however, precise inclusion levels are essential to prevent toxicity. Its fundamental role in selenoprotein activity monitoring to ensure health and productivity. In crustaceans, little is known about the function of selenium-mediated anti-oxidant protection during exoskeleton formation and postmolt recovery.

Iron, Zinc, Manganese, and selenium are trace elements that are essential for the growth and molting of crustaceans. While selenium serves as a crucial antioxidant that shields tissues from oxidative stress brought on by aging, iron is mostly engaged in cellular respiration and energy consumption. As an enzymatic cofactor, manganese aids in the creation of chitin and the development of exoskeletons, whereas zinc promotes the synthesis of proteins, immunological defense, gene control, and chitin formation. Reduced development, delayed molting, worse shell quality, elevated oxidative stress, and increased susceptibility to illness are all possible outcomes of these element deficiencies. The dynamics of these trace elements during various molt stages are still poorly understood, despite their biological significance. This represents a major knowledge gap in crustacean mineral physiology.

4 Discussion

Minerals are essential for the successful molting process in crustaceans, as they support both structural and physiological transformations. The exoskeleton- primarily made of chitin reinforced with calcium carbonate and calcium phosphate-requires a carefully regulated supply of minerals such as calcium, magnesium, sodium, potassium, and phosphorus throughout the molting cycle. Calcium is particularly important for exoskeletal formation and hardening. Crustaceans have developed strategies-such as internal calcium storage and environmental uptake-to cope with fluctuating calcium levels, especially in freshwater or terrestrial environments. Calcium not only aids in cuticle calcification but also plays a role in endocrine pathways, including the synthesis of ecdysteroids mediated by molt-inhibiting hormone (MIH). The importance, deficiency and symptoms of overabundance of calcium, magnesium,

sodium, potassium and phosphorus in crustaceans are outlined in Table 2. Together, these minerals form an integrated physiological framework that enables crustaceans to shed and rebuild their exoskeletons efficiently during the growth cycle. Their bioavailability, regulation, and balance are especially critical in aquaculture, where deficiencies or excesses can lead to molting disorders, soft-shell syndrome, or increased mortality. Advancing our understanding of mineral metabolism and its molecular regulation in crustaceans can enhance aquaculture sustainability, improve animal welfare, and optimize growth and productivity.

Trace elements such as iron, zinc, chromium, copper, manganese, and selenium play a vital role in the regulation of crustacean physiological processes. Although required in minute quantities, these elements are indispensable for enzyme activation, cellular signalling, oxidative stress management, and the successful regeneration of the exoskeleton during molting. In summary, the proper balance and bioavailability of the above trace elements are critical to crustacean health Figure 7. In both natural habitats and aquaculture systems, understanding and managing trace mineral nutrition is essential to support optimal growth, immunity, and survival.

There are numerous gaps in our understanding of crustacean mineral nutrition, including their nutritional needs, physiological processes, gastrointestinal absorption, and the bioavailability of feed ingredients. The biochemical roles of several inorganic elements-such as their functions in skeletal tissue metabolism, cellular respiration, oxygen transport, and the regulation of acid-base equilibrium- have been confirmed through research in animal and human nutrition. Studies on livestock have consistently shown that mineral requirements fluctuate throughout the life cycle. Furthermore, trace elements are proven to be crucial for immune system function and disease prevention. While specific techniques can predict the bioavailability of minerals in feed, a challenge unique to aquatic species is the role of waterborne minerals from both an environmental and nutritional perspective.

Table 2. The role of different minerals in the regulation of crustacean physiology.

S. No	Name of the mineral	Role of mineral	Deficiency	Overdose	Reference
1	Calcium	To form an exoskeleton (as calcium carbonate, CaCO_3). Hardening shell in post-molt stage. played an important role muscle contraction and signaling during molting. Activates Ca^{2+} -ATPase for calcium transport. Interacts through signaling pathways with hormones (MIH, CHH). supports the post-ecdysis mineralization of shells.	Soft-shell syndrome, delayed or inadequate molting, and weak muscle contraction. A rise in cannibalism and mortality in aquaculture.	Reduced cuticle flexibility; impaired molting hormone control; abnormal shell hardening (excessively brittle exoskeleton)	(de Almeida Rodrigues et al., 2022; Wheatly et al., 2002)
2	Magnesium	Mg^{2+} levels in hemolymph, exoskeleton, and tissues increase significantly during premolt, peaking at $\sim 1.5 \text{ mmol} \cdot \text{L}^{-1}$ in hemolymph. Acts as a cofactor in ATPases crucial for Ca^{2+} transport during exoskeleton hardening. Elevated Mg^{2+} modulates Ca^{2+} currents and suppresses CHH (crustacean hyperglycemic hormone) release in neurosecretory neurons	Crustaceans like <i>Macrobrachium rosenbergii</i> suffer from ion regulation, molting, and growth impairments due to magnesium insufficiency. In aquaculture studies, low Mg^{2+} concentrations lead to reduced survival and delayed development in shrimp larvae.	Stress in crustaceans is indicated by elevated Mg^{2+} , which raises metabolic rate (e.g., heart rate). In muscle and gills, excess Mg^{2+} inhibits Na^+/K^+ -ATPase, which impacts neuromuscular performance and osmoregulation. Mg^{2+} at 75–100 mM blocks Ca^{2+} currents and CHH secretion, disrupting molting hormone pathways.	(Amaya et al., 2007; Kirschner, 1991; Ro et al., 2006; Roy et al., 2010; Schumann et al., 2018)
3	Sodium	Maintains hemolymph osmotic pressure during water uptake at ecdysis. - Facilitates body swelling for soft exoskeleton expansion	Reduced survival in low-salinity aquaculture; incomplete molting or soft-shell syndrome; impaired water absorption during ecdysis.	Osmotic gradients can be disrupted by hypernatraemia. Modifies neuromuscular coordination; Hypo-osmotic regulators may be under stress.	(Henry et al., 2012; Lucu & Towle, 2003; Pequeux, 1995; Wheatly et al., 2002)
4	Potassium	supports neuromuscular signaling during ecdysis and controls membrane potential, which is essential for body movement and muscle contraction during molting.	Lethargy or incomplete molting, muscle cramps and decreased contractility; poor osmoregulation; and hemolymph imbalance in low-salinity settings	Hyperkalaemia may cause paralysis and neurological impairment by altering membrane potential. Reduced ATPase-linked transport enzyme activity.	(Henry et al., 2012; Lall, 2003; Lucu & Towle, 2003; Pequeux, 1995; Roy et al., 2010; Wheatly et al., 2002)
5	Phosphorus	Participates in cuticle hardening and exoskeleton mineralization, especially in the mandibles; supports ATP-	Reduced growth, soft-shell syndrome, and delayed molting.	In aquaculture systems, too much phosphorus can cause algal blooms and deteriorate	(Bentov et al., 2016; Davis & Gatlin, 1996;

	dependent energy-demanding processes during ecdysis.	Poor reproduction and lower survival rates. particularly in the early phases of life	water quality by changing the calcium/phosphorus ratio.	Knight et al., 2010; Lall & Kaushik, 2021)
6 Iron	Iron facilitates cuticle synthesis and breakdown during molting and is necessary for energy consumption through the mitochondrial electron transport chain.	Reduced metabolic and enzymatic activity impairs molting, weak exoskeleton and muscular development; and a weakened immune system.	Reactive oxygen species (ROS) are created when there is an excess of iron, which results in oxidative stress, tissue damage, and inflammation.	(de Almeida Rodrigues et al., 2022; Roy et al., 2010)
7 Zinc	Essential for cuticle formation, chitin metabolism, and molting enzyme function - Supports post-molt exoskeleton hardening.	Reduced growth, delayed molting, and soft-shell syndrome Low antioxidant activity, compromised cuticle cross-linking, and weakened immunity.	Excess zinc causes oxidative stress, gill damage, and hepatopancreas problems, which hinder the absorption of other metals (Cu, Fe).	(de Almeida Rodrigues et al., 2022; Lall & Kaushik, 2021)
8 Chromium	Although chromium does not directly contribute to the production of cuticles, it does assist energy consumption during molting, protein synthesis, and glucose metabolism.	Reduced growth rate, frequency of molting, and immunological function; impaired energy metabolism; and decreased resistance to environmental stress.	Toxic hexavalent chromium (Cr ⁶⁺) reduces molting success by causing oxidative stress, DNA damage, and gill/hepatopancreas degeneration.	(Banace et al., 2024; de Almeida Rodrigues et al., 2022; Roy et al., 2010)
9 Copper	Crucial for molting enzyme activity, chitin metabolism, and cuticle production; promotes post-molt exoskeleton hardening.	Reduced growth, delayed molting, and soft-shell syndrome Low antioxidant activity, compromised cuticle cross-linking, and weakened immunity.	Excess Zn interferes with absorption of other metals (Cu, Fe) - Oxidative stress, gill damage, and hepatopancreas dysfunction.	(de Almeida Rodrigues et al., 2022; Lall & Kaushik, 2021)
10 Manganese	Essential for the production of chitin and the hardening of cuticles, it also activates enzymes that are involved in metabolism, molting, and antioxidant defense (e.g., Mn-SOD).	Weak antioxidant protection, limited growth, decreased energy metabolism, impaired molting, and a fragile exoskeleton.	Toxic accumulation affects neurological function, enzyme activity, and molting hormone balance Can cause oxidative stress and mortality.	(de Almeida Rodrigues et al., 2022; Lall & Kaushik, 2021; Roy et al., 2010)
11 Selenium	Essential to selenoproteins that guard against oxidative stress during molting, such as glutathione peroxidase, or GPx promotes immunological preparedness, metabolism, and cuticle repair.	Delays in molting, low-quality shells, and heightened vulnerability to illness are all consequences of decreased antioxidant enzyme activity.	Excess selenium leads to oxidative tissue damage, hepatopancreas dysfunction Disrupts molting hormone cycles and lowers survival.	(de Almeida Rodrigues et al., 2022; Lall & Kaushik, 2021)

5. Conclusion

In crustaceans, macro and trace minerals play a crucial role in regulating numerous physiological functions, including the molting process. This review emphasizes the significance of minerals across various aspects of crustacean physiological regulation. Specifically, Minerals such as calcium, magnesium, sodium, potassium, phosphorus, iron, zinc, chromium, copper, manganese and selenium are involved in molt regulation and other vital processes-all of which are thoroughly discussed herein. These data suggest the minerals are vital for maintaining growth in culture ponds; therefore, understanding mineral nutrition is essential when formulating crustacean feed. While feed manufacturers must ensure adequate mineral levels to regulate molting, existing information on these mechanisms remains insufficient. Consequently, further studies should focus on feed formulations with varying mineral concentrations to optimize growth. Additional research is needed to bridge these knowledge gaps and elucidate the detailed molecular mechanisms required for effective crustacean development through mineral regulation. The pharmaceutical sector placed a high value on biologically isolated trace elements. The shells of crustaceans, which are produced as waste in the aquaculture business, offer hope for the extraction of biological trace elements. Due to the widespread consumption of fast food, trace element deficiencies are growing daily. Since crustaceans-based food is tasty and many people choose to eat it, the trace elements will be replenished through diet.

Credit authorship contribution statement

Manasa N: Writing review & editing, Ideation, Software, Resources, writing-original draft, Formal analysis, P R Reddy: Review & editing, Ideation, Visualization, Funding acquisition, Resources, Methodology, Investigation, Formal analysis. Ramesh Koduru and Lakshmi Prasanna Thota: Writing review & editing, Ideation, Software, Characterization.

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