

Original Article

Heavy Metal Accumulation Effects on Marine Molluscs

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Abstract

Marine molluscs, particularly bivalves, are extensively used as sentinel organisms for monitoring heavy metal pollution in coastal and estuarine environments due to their sessile nature, filter-feeding behavior, and capacity to bioaccumulate contaminants. This review synthesizes current knowledge on the physiological, biochemical, and ecological impacts of heavy metal accumulation in marine molluscs, with emphasis on cadmium (Cd), copper (Cu), zinc (Zn), lead (Pb), and mercury (Hg). Heavy metal exposure induces multifaceted responses in molluscs, including altered cardiac function, reduced filtration and respiration rates, compromised energy reserves, and decreased condition indices. At the biochemical level, metals trigger oxidative stress responses characterized by elevated lipid peroxidation, induction of metallothioneins, modulation of antioxidant enzyme activities, and lysosomal destabilization. Species-specific differences in metal accumulation patterns, detoxification capacity, and tolerance mechanisms significantly influence biomarker responses and ecological outcomes. Chronic metal contamination can impair ecosystem services provided by bivalve populations, including water filtration and nutrient cycling, while also posing risks to human health through seafood consumption. This review highlights the importance of integrated biomarker approaches combining physiological, biochemical, and molecular endpoints for comprehensive assessment of metal pollution impacts. Future research should focus on understanding metal mixture effects, climate change interactions with metal toxicity, and the development of standardized biomonitoring protocols across diverse marine ecosystems.

Keywords: Heavy metals, marine molluscs, bivalves, bioaccumulation, metallothionein, oxidative stress, biomarkers, biomonitoring

Introduction

Heavy metal pollution represents one of the most persistent and widespread environmental challenges facing marine ecosystems worldwide. Unlike organic pollutants, heavy metals are non-biodegradable and can accumulate in sediments and biota over extended periods, posing long-term risks to ecosystem health and human populations dependent on marine resources. Coastal and estuarine environments are particularly vulnerable to metal contamination due to their proximity to anthropogenic sources including industrial effluents, mining activities, urban runoff, agricultural drainage, and maritime operations.

Marine molluscs, especially bivalves such as mussels, oysters, clams, and cockles, have emerged as invaluable sentinel organisms for assessing heavy metal pollution in aquatic environments. Their widespread distribution, sessile or limited mobility lifestyle, filter-feeding behaviour, and remarkable capacity to bioaccumulate contaminants make them ideal candidates for biomonitoring programs. Bivalves can concentrate metals from both the dissolved phase and particulate matter to levels several orders of magnitude higher than ambient water concentrations, providing an integrated measure of bioavailable metal contamination over time [5], [19].

The biological responses of molluscs to heavy metal exposure span multiple levels of organization, from molecular and cellular alterations to whole-organism physiological changes and population-level effects. Understanding these multifaceted responses is crucial for developing effective biomonitoring strategies, establishing water quality criteria, and predicting ecological consequences of metal pollution.

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Metals such as cadmium (Cd), copper (Cu), zinc (Zn), lead (Pb), and mercury (Hg) have received particular attention due to their prevalence in contaminated sites and their known toxicity to marine organisms [2], [7], [10].

While some metals like copper and zinc are essential micronutrients required for various physiological processes, they become toxic at elevated concentrations. Non-essential metals such as cadmium, lead, and mercury exert toxic effects even at low exposure levels. The toxicity of metals depends not only on their concentration but also on their chemical speciation, bioavailability, exposure duration, and the physiological state of the organism [3], [16]. Furthermore, molluscs exhibit species-specific differences in metal accumulation patterns, detoxification mechanisms, and tolerance thresholds, which must be considered when interpreting biomonitoring data [6], [18].

This review synthesizes current knowledge on the effects of heavy metal accumulation in marine molluscs, with emphasis on physiological, biochemical, and ecological impacts. We examine the mechanisms underlying metal bioaccumulation, the cascade of biological responses from molecular to population levels, and the implications for ecosystem health and biomonitoring applications. By integrating findings from laboratory experiments and field studies across diverse species and geographic regions, this review aims to provide a comprehensive framework for understanding and assessing heavy metal impacts on marine mollusc populations.

Heavy Metals in Marine Environments and Mollusc Bioaccumulation

• Sources and Distribution of Heavy Metals

Heavy metals enter marine environments through multiple pathways, both natural and anthropogenic. Natural sources include weathering of metal-bearing rocks, volcanic activity, and atmospheric deposition. However, anthropogenic inputs have dramatically increased metal loads in coastal waters over the past century. Industrial discharges, mining operations, agricultural runoff containing metal-based pesticides and fertilizers, urban wastewater, and atmospheric deposition from combustion processes represent major anthropogenic sources [7], [9].

The distribution and fate of metals in marine environments are influenced by various physicochemical factors including salinity, pH, temperature, dissolved organic matter, and sediment characteristics. Metals can exist in dissolved forms, be adsorbed onto particulate matter, or accumulate in sediments, which serve as both sinks and potential sources of contamination.

Estuarine environments, characterized by complex hydrodynamics and mixing of freshwater and seawater, often exhibit elevated metal concentrations and serve as important transition zones where metals can be remobilized or sequestered [2], [11].

• Bioaccumulation Mechanisms in Molluscs

Bivalve molluscs accumulate metals through multiple routes: direct uptake from the dissolved phase across gill and mantle epithelia, ingestion of contaminated particulate matter and phytoplankton, and sediment contact. The filter-feeding behavior of bivalves, which can process large volumes of water daily, facilitates efficient uptake of both dissolved and particulate-bound metals [1], [5].

Metal bioaccumulation in molluscs is governed by the balance between uptake and elimination processes. The biodynamic model of metal accumulation considers uptake rate constants from both dissolved and dietary sources, assimilation efficiency from ingested food, and efflux rate constants. Species-specific differences in these kinetic parameters result in varying bioaccumulation patterns and steady-state tissue concentrations [5], [15].

Once internalized, metals are distributed among different organs and tissues based on their chemical properties and the physiological functions of target organs. The digestive gland (hepatopancreas) typically accumulates the highest metal concentrations due to its role in digestion, absorption, and detoxification. Gills, which are in direct contact with the surrounding water, also accumulate significant metal burdens and serve as important sites for metal uptake and initial detoxification responses [1], [2], [8].

Direct contamination through waterborne exposure leads to rapid metal accumulation in gills, digestive gland, and mantle, with bioaccumulation patterns varying by metal type. Essential metals like copper and zinc may initially be regulated, whereas non-essential metals like cadmium and mercury bioaccumulate more readily [1]. Trophic transfer through contaminated food represents an additional exposure route, with metals accumulating primarily in the digestive gland following dietary exposure [1], [16].

• Species-Specific Accumulation Patterns

Significant interspecific variation exists in metal accumulation capacity and tissue distribution patterns among mollusc species. Studies comparing different bivalve species under similar exposure conditions have revealed that arctic clams (e.g., *Anadara* species) often exhibit higher cadmium accumulation than other bivalve families [3]. Oysters, particularly species in the

genus *Crassostrea*, are recognized as hyper-accumulators of metals such as cadmium, copper, and zinc, achieving tissue concentrations far exceeding those in the surrounding environment [15], [26].

Comparative studies between *Ruditapes decussatus* and *Ruditapes philippinarum* exposed to cadmium revealed that *R. decussatus* required a two-times higher cadmium concentration to achieve 50% mortality compared to *R. philippinarum*, indicating species-specific differences in tolerance [18]. However, *R. philippinarum* exhibited higher oxidative stress responses, while *R. decussatus* demonstrated greater capacity to increase metallothionein expression, suggesting different detoxification strategies between closely related species [18].

The green mussel *Perna viridis* and blood cockle *Tegillarca granosa*, both common in Indo-Pacific waters, function as macroconcentrators of cadmium but exhibit different oxidative response profiles, with *P. viridis* showing higher levels of lipid peroxidation markers, catalase activity, and reduced glutathione compared to *T. granosa* [10]. These species-specific differences in accumulation and response patterns have important implications for biomonitoring program design and interpretation of field data.

Physiological Impacts of Heavy Metal Accumulation

• Cardiac Function and Heart Rate Alterations

Cardiac function represents a sensitive physiological endpoint for assessing metal toxicity in bivalves. Heart rate measurements provide a non-invasive indicator of metabolic stress and overall organismal condition. Field studies on the oyster *Crassostrea hongkongensis* in the multimetal-contaminated Pearl River estuary revealed significant correlations between tissue metal burdens and cardiac performance [2]. Specifically, heart rate showed positive correlations with cadmium accumulation in gills but negative correlations with copper and zinc concentrations [2].

These metal-specific effects on cardiac function suggest different mechanisms of action. Cadmium may stimulate cardiac activity as a stress response, potentially increasing metabolic costs, while copper and zinc at elevated concentrations appear to depress cardiac function, possibly through interference with calcium-dependent contractile mechanisms or direct cardiotoxic effects. The differential cardiac responses to various metals highlight the complexity of multimetal contamination scenarios and the importance of considering metal-specific effects rather than treating all metals as equivalent stressors.

• Filtration and Respiration Rate Changes

Filtration and respiration rates are fundamental physiological processes in bivalves that directly influence energy acquisition, growth, and survival. These rates are highly sensitive to environmental stressors, including heavy metal exposure. Studies on tropical lamellibranchs exposed to cadmium revealed complex temporal patterns in filtration responses [3]. In *Anadara granosa*, cadmium initially stimulated filtration rate by 28% over 24 hours, followed by a dramatic 50% decrease after 96 hours of exposure [3].

This biphasic response pattern suggests an initial compensatory mechanism that becomes overwhelmed with prolonged exposure, leading to functional impairment. Copper exposure produced more consistent inhibitory effects, progressively reducing respiratory activity by up to 80% after 96 hours [3]. The severe depression of respiration under copper stress indicates compromised aerobic metabolism and reduced capacity for energy production, which can cascade into effects on growth, reproduction, and survival.

Prolonged exposures to cadmium, copper, and zinc demonstrated that initial synergistic toxic effects shifted to antagonistic interactions over time, with filtration declines up to 50% reported in some clams and mussels under extended exposure [3]. These temporal dynamics in physiological responses emphasize the importance of exposure duration in determining toxicity outcomes and suggest that chronic low-level exposures may produce different effect patterns than acute high-dose exposures.

• Condition Index and Energy Reserve Depletion

The condition index, typically calculated as the ratio of soft tissue dry weight to shell weight, provides an integrated measure of organismal health and nutritional status. Metal-exposed bivalves consistently exhibit reduced condition indices compared to reference populations, reflecting impaired energy balance and compromised physiological condition [2], [6], [8].

Laboratory exposures of *Anadara trapezia* to zinc, copper, lead, cadmium, and selenium resulted in significantly lower condition indices in exposed organisms compared to in situ resident organisms and reference populations [6]. This reduction in condition reflects the energetic costs of detoxification processes, repair of metal-induced damage, and reduced feeding efficiency under metal stress.

Energy reserves, particularly glycogen stored in the digestive gland, are depleted under metal stress as organisms mobilize energy to support detoxification and stress response

mechanisms. Field studies on *Crassostrea hongkongensis* revealed significant correlations between digestive gland metal accumulation (Cu, Zn, Ni) and reduced glycogen reserves [2]. The depletion of energy reserves can have cascading effects on growth rates, reproductive output, and overwinter survival, potentially affecting population dynamics in contaminated environments.

The condition index also showed significant differences in *Cerastoderma glaucum* collected from sites with varying cadmium contamination levels, with lower indices at more contaminated sites [8]. These field observations corroborate laboratory findings and demonstrate that metal-induced physiological impairment occurs in natural populations exposed to realistic contamination scenarios.

Biochemical and Molecular Responses

• Metallothionein Induction and Metal Detoxification

Metallothioneins (MTs) are low molecular weight, cysteine-rich proteins that play a central role in metal detoxification and homeostasis in molluscs. These proteins bind metals through their abundant cysteine residues, sequestering potentially toxic metals and reducing their availability to interact with sensitive cellular targets. MT induction represents one of the most widely studied and reliable biomarkers of metal exposure in bivalves [1], [8], [14].

Cadmium is consistently identified as the strongest inducer of metallothionein synthesis across multiple mollusc species and tissues [1], [8]. Studies on oysters and mussels exposed to silver, cadmium, copper, mercury, and zinc demonstrated that direct waterborne contamination induced metallothionein synthesis in gills for all metals tested, while trophic (dietary) contamination induced MT primarily in the digestive gland, particularly for cadmium [1]. This tissue-specific induction pattern reflects the different exposure routes and sites of metal accumulation.

Field studies on *Cerastoderma glaucum* in the Gulf of Gabes revealed positive correlations between metallothionein-like protein (MTLP) concentrations and cadmium levels in both digestive gland and gills, with the digestive gland showing stronger correlations and being more suitable for biomonitoring purposes [8]. However, at the most heavily contaminated site, MTLP capacity appeared insufficient to cope with the metal burden, and MTLP was no longer a useful biomarker [8]. This observation highlights an important limitation: metallothionein induction has a finite capacity, and at extreme contamination

levels, this detoxification mechanism can become saturated.

Recent molecular studies have identified specific genes involved in metallothionein synthesis and metal homeostasis. In the mangrove oyster *Crassostrea gasar*, exposure to cadmium and zinc for 48 hours led to significant upregulation of metallothionein-like genes, along with genes involved in glutathione biosynthesis (GCLC, CHAC1) and zinc transport (ZnT2) [13]. The coordinated upregulation of these metal-related genes indicates that oysters mobilize multiple molecular mechanisms to mitigate metal-induced toxicity [13].

Comparative studies between *Ruditapes decussatus* and *R. philippinarum* revealed that *R. decussatus* exhibited a higher capacity to increase MT expression when exposed to cadmium, which may contribute to its greater tolerance compared to *R. philippinarum* [18]. These species-specific differences in MT induction capacity help explain variation in metal sensitivity among closely related species.

• Oxidative Stress and Lipid Peroxidation

Heavy metals, particularly redox-active metals like copper and iron, can catalyze the formation of reactive oxygen species (ROS) through Fenton and Haber-Weiss reactions. Even non-redox-active metals like cadmium can induce oxidative stress indirectly by depleting antioxidant defenses, disrupting electron transport chains, or displacing essential metals from metalloproteins. The resulting oxidative stress leads to damage of cellular macromolecules, including lipids, proteins, and DNA [7], [10], [11].

Lipid peroxidation, measured as malondialdehyde (MDA) or conjugated dienes, represents a key indicator of oxidative damage to cellular membranes. Multiple studies have documented elevated lipid peroxidation in metal-exposed molluscs across diverse species and contamination scenarios. In *Tegillarca granosa* from Yueqing Bay, MDA content in the visceral mass was significantly higher at contaminated sites and showed positive correlations with tissue concentrations of cadmium, chromium, mercury, and copper [7]. Seasonal analysis revealed that MDA levels were significantly higher in July compared to January, suggesting interactions between metal stress and environmental factors such as temperature [7].

The green mussel *Perna viridis* and blood cockle *Tegillarca granosa* from Pattani Bay, Thailand, both exhibited strong relationships between cadmium accumulation and lipid peroxidation activation [10]. Importantly, *P. viridis* demonstrated a higher oxidative response than *T. granosa*, as

indicated by elevated malondialdehyde, catalase, and reduced glutathione levels [10]. This species-specific difference in oxidative response magnitude may reflect different antioxidant capacities or detoxification strategies.

Field studies on the mussel *Crenomytilus grayanus* at a contaminated site showed significantly increased levels of malondialdehyde, conjugated dienes, and lipofuscin (an age pigment formed from oxidized proteins and lipids) compared to a reference site [9]. Three years after environmental remediation, these lipid peroxidation indicators decreased to levels not significantly different from reference values, demonstrating the reversibility of oxidative damage when metal exposure is reduced [9].

In *Cerastoderma glaucum*, MDA levels were elevated at industrially and urban-effluent-exposed sites compared to reference locations [8]. The ark clam *Barbatia decussate* showed seasonal patterns in MDA levels, with elevated concentrations throughout spring and summer when metabolic rates are typically higher, followed by declines in autumn and winter [12]. These seasonal dynamics highlight the importance of considering temporal variability when interpreting oxidative stress biomarkers in field populations.

- **Antioxidant Enzyme Modulation**

To counteract metal-induced oxidative stress, molluscs possess an array of antioxidant enzymes including superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and glutathione S-transferase (GST). The activities of these enzymes are modulated in response to metal exposure, although the direction and magnitude of changes can vary depending on metal type, exposure level, duration, and species [7], [11], [12], [17].

Superoxide dismutase catalyzes the dismutation of superoxide radicals to hydrogen peroxide and oxygen, representing the first line of enzymatic defense against ROS. Catalase and glutathione peroxidase subsequently detoxify hydrogen peroxide to water. Studies on *Tegillarca granosa* in Yueqing Bay revealed that CAT and SOD activities in the visceral mass were significantly higher in July than January, and were elevated at contaminated sites [7]. This upregulation of antioxidant enzymes represents an adaptive response to increased oxidative stress.

However, the relationship between metal exposure and antioxidant enzyme activities is not always straightforward. In *Ruditapes decussatus* from Abo Quir Bay, a heavily polluted site, SOD and GPx activities were significantly decreased compared to less contaminated locations [17]. This apparent contradiction—

decreased rather than increased antioxidant enzyme activities at contaminated sites—may reflect enzyme inhibition at very high metal concentrations or depletion of enzyme synthesis capacity under chronic severe stress.

The ark clam *Barbatia decussate* exhibited seasonal patterns in antioxidant enzyme activities, with SOD, CAT, and GPx activities elevated throughout spring and summer, then declining in autumn and winter [12]. These seasonal fluctuations paralleled patterns in tissue metal accumulation, which increased significantly in late autumn and winter for cadmium, lead, and mercury [12]. The temporal offset between peak metal accumulation and peak enzyme activities suggests complex regulatory mechanisms and potential lag times in biochemical responses.

Glutathione S-transferase, which conjugates glutathione to various electrophilic compounds including metal-induced oxidative damage products, showed increased activity in *Ruditapes decussatus* from the metal-contaminated Tunis lagoon [11]. This elevation in GST activity, along with increased catalase activity, indicates activation of multiple antioxidant pathways in response to multimetal contamination [11].

The integrated biomarker response version 2 (IBRv2), which combines multiple biomarker measurements into a single index, has been applied to assess overall stress levels in metal-exposed molluscs. In *Tegillarca granosa* from Yueqing Bay, IBRv2 values ranked sites according to contamination levels, with the most contaminated site showing the highest integrated biomarker response [7]. This approach provides a holistic assessment of biochemical stress that may be more informative than individual biomarkers alone.

- **Lysosomal Destabilization and Cellular Homeostasis**

Lysosomes play critical roles in intracellular digestion, autophagy, and sequestration of toxic metals. Lysosomal membrane stability represents a sensitive indicator of cellular health and has been widely used as a biomarker of metal stress in bivalves. Metal accumulation can destabilize lysosomal membranes, leading to release of hydrolytic enzymes into the cytoplasm and cellular damage [2], [6].

Field studies on *Crassostrea hongkongensis* in the Pearl River estuary demonstrated that lysosomal membrane stability was significantly inhibited at metal-contaminated sites compared to reference locations [2]. This lysosomal destabilization occurred concurrently with elevated tissue metal burdens and other indicators of cellular stress, suggesting compromised cellular homeostasis.

Laboratory and field exposures of *Anadara trapezia* to zinc, copper, lead, cadmium, and selenium revealed significant increases in lysosomal destabilization in all metal-exposed organisms, regardless of exposure scenario [6]. The consistency of this response across different exposure conditions and metal types indicates that lysosomal destabilization represents a general cellular stress response to metal toxicity rather than a metal-specific effect.

Alkaline phosphatase activity, an indicator of cellular homeostatic regulation, showed significant correlations with digestive gland metal accumulation (Cu, Zn, Ni) in *Crassostrea hongkongensis* [2]. Alterations in enzyme activities involved in cellular homeostasis reflect disruption of normal metabolic processes and can have cascading effects on organismal performance.

• Gene Expression and Transcriptomic Responses

Modern molecular techniques have revealed complex transcriptomic responses to metal exposure in molluscs, providing insights into the molecular mechanisms underlying metal toxicity and tolerance. Transcriptomic studies on *Ruditapes decussatus* from the metal-contaminated Tunis lagoon revealed significant overexpression of multiple stress-responsive genes, including cytochrome c oxidase subunit I, ribosomal RNA 16S, Cu/Zn superoxide dismutase, heat shock protein 70, and metallothioneins [11]. This coordinated upregulation of genes involved in energy metabolism, protein synthesis, antioxidant defense, protein chaperoning, and metal detoxification indicates a comprehensive molecular stress response.

Heat shock proteins (HSPs), particularly Hsp70, function as molecular chaperones that assist in protein folding and protect against protein denaturation under stress conditions. Field studies on the bearded horse mussel *Modiolus barbatus* in Thermaikos Gulf demonstrated that inducible Hsp70 plays a cytoprotective role against metal toxicity [20]. Seasonal changes in cadmium and lead accumulation in gills were associated with modulation of heat shock response, suggesting that HSP induction represents an important component of the cellular defense against metal stress [20].

In *Crassostrea gasar*, acute exposure to cadmium and zinc led to upregulation of genes involved in glutathione biosynthesis (GCLC, CHAC1) and zinc transport (ZnT2), in addition to metallothionein-like genes [13]. The CHAC1 gene encodes a glutathione-specific gamma-glutamylcyclotransferase involved in glutathione degradation, while GCLC encodes the catalytic subunit of glutamate-cysteine ligase, the rate-

limiting enzyme in glutathione synthesis. The coordinated regulation of these genes suggests complex modulation of glutathione homeostasis in response to metal exposure [13].

Oysters possess higher numbers of stress-responsive genes compared to many other marine invertebrates, potentially due to gene duplication events during evolution [15]. This expanded stress gene repertoire may contribute to the remarkable metal tolerance and hyper-accumulation capacity observed in oysters, allowing them to thrive in contaminated environments that would be lethal to other species [15].

Ecological and Population-Level Consequences

• Ecosystem Services Impairment

Bivalve molluscs provide critical ecosystem services in coastal and estuarine environments, including water filtration, nutrient cycling, habitat provision, and serving as prey for higher trophic levels. The filtration capacity of bivalve populations can significantly influence water clarity, phytoplankton dynamics, and benthic-pelagic coupling. Metal-induced reductions in filtration and respiration rates at the individual level can scale up to affect these ecosystem functions when large populations are impacted [3].

Depressed filtration rates in metal-contaminated populations reduce the capacity of bivalve assemblages to filter particulate matter from the water column, potentially leading to increased turbidity and altered phytoplankton community structure. Similarly, reduced respiration rates indicate compromised metabolic capacity and energy flow through the ecosystem. When these physiological impairments affect substantial portions of bivalve populations, the cumulative effect can alter ecosystem-level processes [3].

The role of bivalves in nutrient cycling may also be affected by metal contamination. Bivalves influence nitrogen and phosphorus cycling through their feeding, excretion, and biodeposition activities. Metal-induced changes in feeding rates, metabolic processes, and tissue composition can alter the quantity and quality of nutrients released or sequestered by bivalve populations, with potential cascading effects on primary productivity and ecosystem trophodynamics.

• Population Health and Reproductive Effects

Chronic metal contamination can affect population health through multiple pathways, including direct mortality, reduced growth rates, impaired reproduction, and increased susceptibility to other stressors such as disease and predation. Histopathological studies have documented severe tissue damage in metal-exposed molluscs, including

alterations in gill structure, digestive gland pathology, and damage to foot tissue [10], [11].

In *Perna viridis* and *Tegillarca granosa* from contaminated sites in Pattani Bay, high metal accumulation led to deformed and damaged tissues in gills, digestive glands, intestines, and feet [10]. Gill damage is particularly concerning as it can impair both respiration and feeding, while digestive gland pathology affects nutrient absorption and detoxification capacity. Such tissue-level damage can reduce individual fitness and, when widespread, affect population demographics.

The clam *Ruditapes decussatus* from the metal-contaminated Tunis lagoon exhibited severe and diverse histopathological alterations in gills, with the most contaminated region showing the greatest damage [11]. These structural alterations to critical organs can compromise physiological function and reduce survival probability, particularly under additional environmental stressors.

Reduced condition indices and depleted energy reserves in metal-exposed populations have implications for reproductive success. Energy allocation to reproduction may be reduced when organisms must divert resources to detoxification and repair processes. Lower glycogen reserves can affect gamete production and quality, potentially reducing reproductive output and recruitment success in contaminated populations [2], [6].

Long-term field studies have documented population-level effects of chronic chemical contamination, including altered population structure, reduced abundance, and changes in community composition. While disentangling the specific effects of metals from other co-occurring stressors in field populations remains challenging, the weight of evidence from laboratory and field studies indicates that chronic metal contamination can have significant population-level consequences [24].

• Seasonal and Environmental Modulation

The effects of metal contamination on molluscs are modulated by seasonal and environmental factors, adding complexity to impact assessment and biomonitoring efforts. Seasonal variations in metal accumulation, biomarker responses, and physiological condition have been documented in multiple species and locations [7], [12], [19], [20].

In *Barbatia decussate*, tissue accumulation of cadmium, lead, and mercury increased significantly

in late autumn and winter, while nickel and chromium concentrations remained unchanged [12]. Concurrently, malondialdehyde levels and antioxidant enzyme activities (SOD, CAT, GPx) were elevated throughout spring and summer, then declined in autumn and winter [12]. These seasonal patterns likely reflect interactions between metal bioavailability, metabolic rates, reproductive cycles, and environmental conditions such as temperature and food availability.

Temperature represents a particularly important modulating factor. Studies on *Modiolus barbatus* suggest that bivalve tolerance to heavy metals may decrease during warming periods, raising concerns about the interactive effects of metal pollution and climate change [20]. As ocean temperatures rise due to global warming, metal-contaminated populations may face increased stress from the combined effects of elevated temperatures and metal toxicity.

Immunological parameters also show seasonal variation in metal-exposed populations. In *Barbatia decussate*, phagocytosis activity decreased from December to February and increased from March to September, while total haemocyte counts decreased from October to March and elevated until April [12]. These fluctuations in immune function may affect disease resistance and overall population health, particularly in contaminated environments where organisms are already physiologically compromised.

Metal concentrations in molluscs often vary seasonally, with some studies reporting higher levels in winter and lower levels in summer, although patterns can vary by metal, species, and location [19]. These seasonal variations may reflect changes in metal bioavailability, differences in uptake and elimination rates at different temperatures, or dilution effects during periods of rapid growth. Understanding these temporal dynamics is essential for designing effective biomonitoring programs and interpreting field data.

Comparative Analysis of Metal Effects Across Species

Comparative studies across multiple mollusc species have revealed important patterns in metal accumulation, tolerance, and response mechanisms. Table 1 summarizes key findings from studies examining different species under similar metal exposure conditions.

Table 1. Comparative metal accumulation and responses in selected mollusc species

Species	Metals Studied	Key Accumulation Patterns	Primary Biomarker Responses	Reference
<i>Crassostrea hongkongensis</i>	Cu, Zn, Cd, Cr,	High accumulation in digestive gland; gill accumulation correlated	Heart rate alterations, lysosomal destabilization,	[2]

	Ni	with heart rate	glycogen depletion	
<i>Anadara granosa</i>	Cd, Zn, Cu	Highest Cd accumulation among arcid clams; species-specific uptake	Biphasic filtration response, respiratory depression	[3]
<i>Tegillarca granosa</i>	Cd, Cr, Hg, Cu	Macroconcentrator of Cd; visceral mass primary accumulation site	Elevated CAT, SOD, MDA, MT mRNA; tissue damage	[7], [10]
<i>Perna viridis</i>	Cd, Co, Cu, Cr, Ni, Mn, Fe, Zn, Pb	Macroconcentrator of Cd; higher oxidative response than <i>T. granosa</i>	High MDA, catalase, reduced glutathione; severe tissue damage	[10]
<i>Cerastoderma glaucum</i>	Cd	Digestive gland and gill accumulation; MTLP saturation at high contamination	MTLP induction, MDA elevation, reduced condition index	[8]
<i>Ruditapes decussatus</i>	Cd, Pb, Hg, Cu, Zn	Higher Cd tolerance than <i>R. philippinarum</i> ; greater MT induction capacity	Strong MT response, transcriptomic changes, histopathology	[11], [18]
<i>Ruditapes philippinarum</i>	Cd	Lower Cd tolerance; higher oxidative stress response	Elevated oxidative stress, CAT activity	[18]
<i>Crassostrea gasar</i>	Cd, Zn	Gill accumulation; gene expression modulation	Upregulation of MT, GCLC, CHAC1, ZnT2 genes	[13]
<i>Modiolus barbatus</i>	Cd, Pb	Seasonal accumulation patterns; temperature-dependent tolerance	Hsp70 induction, metabolic stress responses	[20]
<i>Anadara trapezia</i>	Zn, Cu, Pb, Cd, Se	Clear dose-response relationships; biologically active and detoxified fractions	Reduced antioxidant capacity, increased lipid peroxidation, lysosomal destabilization	[6]

Oysters, particularly *Crassostrea* species, consistently demonstrate exceptional metal accumulation capacity and tolerance, functioning as hyper-accumulators [2], [13], [15]. This characteristic makes them valuable biomonitors but also raises concerns about human health risks from consumption of contaminated oysters. The molecular basis for oyster metal tolerance appears to involve expanded stress gene repertoires and efficient detoxification mechanisms [15].

Mussels (*Mytilus*, *Perna*, *Modiolus* species) show variable metal accumulation and response patterns depending on species and environmental context. *Perna viridis* exhibits particularly strong oxidative stress responses to cadmium exposure compared to co-occurring species like *Tegillarca granosa* [10]. This difference may reflect species-specific antioxidant capacities or different strategies for managing metal-induced oxidative stress.

Clams, including *Ruditapes*, *Anadara*, and *Cerastoderma* species, display diverse accumulation patterns and tolerance levels. Comparative studies between *Ruditapes decussatus* and *R. philippinarum* revealed that closely related species can differ substantially in metal tolerance and detoxification mechanisms, with *R. decussatus* showing greater cadmium tolerance and stronger metallothionein induction, while *R. philippinarum* exhibited higher oxidative stress responses [18]. These differences have practical

implications for species selection in biomonitoring programs and aquaculture in contaminated areas.

Arcid clams, particularly *Anadara* species, often show high metal accumulation capacity, especially for cadmium [3]. The blood cockle *Tegillarca granosa* (also an arcid) functions as a macroconcentrator of cadmium and has been extensively studied in Asian waters where it is commercially important [7], [10].

Biomonitoring Applications and Methodological Considerations

• Selection of Sentinel Species

The selection of appropriate sentinel species is critical for effective biomonitoring programs. Ideal sentinel species should possess several characteristics: wide geographic distribution, abundance sufficient for repeated sampling, sessile or limited mobility to reflect local contamination, tolerance to a range of environmental conditions, significant bioaccumulation capacity, and measurable biomarker responses [5], [6], [19].

Mussels of the genus *Mytilus* have been extensively used in biomonitoring programs worldwide, including the international "Mussel Watch" programs. Their widespread distribution, well-characterized biology, and reliable bioaccumulation make them valuable sentinels in temperate waters [14], [19]. However, in tropical

and subtropical regions, other species such as *Perna viridis*, *Crassostrea* species, and various clams may be more appropriate [10], [19].

The development of sentinel species for specific regions requires validation of metal exposure-dose-response relationships under both laboratory and field conditions. Studies on *Anadara trapezia* demonstrated clear dose-response relationships for multiple metals in laboratory exposures, in situ caging experiments, and resident populations, supporting its use as a sentinel species in Australian waters [6]. Such validation studies are essential for establishing the reliability and interpretability of biomonitoring data.

Species-specific differences in metal accumulation patterns, detoxification capacity, and biomarker responses must be considered when comparing data across species or regions. Standardization of sampling protocols, tissue selection, and analytical methods is essential for generating comparable data across biomonitoring programs [5], [19].

- **Integrated Biomarker Approaches**

Single biomarkers, while informative, may not capture the full complexity of metal impacts on mollusc populations. Integrated biomarker approaches that combine multiple endpoints across different levels of biological organization provide more comprehensive assessments of organism health and contamination effects [6], [7], [11].

The integrated biomarker response (IBR) approach combines multiple biomarker measurements into a single index, facilitating comparison across sites or treatments. Application of IBRv2 to *Tegillarca granosa* from sites with varying metal contamination successfully ranked locations according to overall stress levels [7]. This integrative approach can be particularly valuable for assessing multimetal contamination scenarios where individual metals may have different effects.

Combining biomarkers from different biological levels—molecular (gene expression, metallothionein), biochemical (enzyme activities, lipid peroxidation), cellular (lysosomal stability, histopathology), and physiological (condition index, heart rate)—provides a more complete picture of organism status than any single endpoint [6], [11]. This multi-level approach can also help distinguish between adaptive responses (e.g., metallothionein induction) and adverse effects (e.g., tissue damage).

The selection of appropriate biomarkers should consider the specific objectives of the monitoring program, the metals of concern, the species being studied, and practical constraints such as sample size requirements and analytical costs. Core biomarker suites typically include

metallothionein or metallothionein-like proteins, oxidative stress indicators (MDA, antioxidant enzymes), and a physiological endpoint such as condition index [8], [11], [12].

- **Laboratory versus Field Studies**

Both laboratory and field studies contribute essential but complementary information for understanding metal effects on molluscs. Laboratory studies allow controlled exposures to single metals or defined mixtures, enabling determination of dose-response relationships, identification of mechanisms of action, and comparison of species sensitivities under standardized conditions [1], [3], [13], [18]. However, laboratory conditions may not fully replicate the complexity of natural environments, including multimetal mixtures, fluctuating exposure levels, and interactions with other environmental stressors.

Field studies provide ecologically relevant assessments of metal impacts under realistic exposure scenarios but face challenges in attributing observed effects to specific contaminants and controlling for confounding variables [2], [7], [8], [10], [11], [12].

Spatial comparisons across contamination gradients and temporal monitoring at fixed sites can help establish relationships between metal exposure and biological effects in natural populations.

Comparative studies employing laboratory exposures, in situ caging experiments, and sampling of resident populations provide particularly valuable insights. Research on *Anadara trapezia* using all three approaches demonstrated that laboratory-exposed organisms accumulated higher total metal concentrations and showed stronger biomarker responses than in situ caged or resident organisms, despite similar exposure concentrations [6]. These differences may reflect factors such as stress from laboratory conditions, differences in metal bioavailability, or acclimation in resident populations [6].

In situ caging experiments, where organisms from reference sites are transplanted to contaminated locations, offer a middle ground between laboratory and field studies. This approach controls for genetic and prior exposure history while exposing organisms to realistic contamination scenarios. Comparison of caged and resident organisms can reveal effects of long-term acclimation or adaptation in resident populations [6], [14].

- **Future Directions and Research Needs**

Despite substantial progress in understanding heavy metal effects on marine molluscs, several important research gaps and emerging challenges require attention. The

interactive effects of metal mixtures remain poorly understood, yet natural contamination scenarios typically involve multiple metals with potentially synergistic, antagonistic, or additive effects [2], [11]. More research is needed on the toxicity of realistic metal mixtures and the mechanisms underlying metal interactions.

Climate change introduces additional complexity to metal pollution impacts. Rising ocean temperatures, ocean acidification, and changes in salinity and oxygen levels may alter metal bioavailability, uptake rates, and toxicity [20]. Studies on *Modiolus barbatus* suggest that metal tolerance may decrease under warming conditions, but systematic investigation of climate-metal interactions across diverse species and metals is needed [20]. Understanding these interactions is critical for predicting future impacts and developing adaptive management strategies.

The application of advanced molecular techniques, including transcriptomics, proteomics, metabolomics, and epigenomics, offers opportunities to elucidate mechanisms of metal toxicity and tolerance at unprecedented resolution [11], [13], [15]. Integration of multi-omics data with traditional physiological and biochemical endpoints can provide comprehensive mechanistic understanding of metal effects. Nanoscale imaging techniques are revealing new insights into subcellular metal localization and sequestration mechanisms [15].

Transgenerational effects of metal exposure represent an emerging area of concern. Parental exposure to metals may affect offspring through epigenetic mechanisms, potentially influencing population resilience and adaptation capacity. Research on transgenerational effects in molluscs is limited but represents an important frontier for understanding long-term population consequences of metal contamination.

The development of adverse outcome pathways (AOPs) for metal toxicity in molluscs would provide structured frameworks linking molecular initiating events to adverse outcomes at individual and population levels. AOPs can facilitate extrapolation from laboratory studies to field populations and support risk assessment and regulatory decision-making.

Standardization of biomonitoring protocols across regions and programs would enhance data comparability and enable large-scale assessments of metal pollution trends. International coordination in method development, quality assurance, and data sharing could significantly advance the field [5], [19].

Finally, the implications of metal accumulation in commercially harvested molluscs

for human health require continued attention. While molluscs serve as valuable sentinels for environmental contamination, they also represent important seafood resources. Establishing safe consumption guidelines based on tissue metal concentrations and understanding the bioavailability of accumulated metals to human consumers remain important public health priorities [16], [19].

Conclusion

Heavy metal accumulation in marine molluscs triggers a cascade of biological responses spanning molecular, biochemical, physiological, and ecological levels of organization. The extensive body of research reviewed here demonstrates that molluscs, particularly bivalves, serve as both sensitive indicators of metal contamination and important models for understanding metal toxicity mechanisms in marine environments.

At the physiological level, metal exposure alters cardiac function, depresses filtration and respiration rates, and depletes energy reserves, leading to reduced condition indices and compromised organismal performance. These whole-organism effects reflect underlying biochemical and molecular perturbations, including oxidative stress, lipid peroxidation, modulation of antioxidant enzyme activities, and lysosomal destabilization. Metallothionein induction represents a key detoxification mechanism, though this capacity can be overwhelmed at high contamination levels.

Modern molecular techniques have revealed complex transcriptomic responses involving coordinated regulation of genes related to metal detoxification, oxidative stress defence, protein chaperoning, and cellular homeostasis. The expanded stress gene repertoires in some species, particularly oysters, may contribute to their remarkable metal tolerance and hyper-accumulation capacity.

Species-specific differences in metal accumulation patterns, detoxification mechanisms, and tolerance thresholds are substantial and must be considered in biomonitoring program design and data interpretation. Comparative studies across species have identified distinct strategies for managing metal stress, from strong metallothionein induction in some species to robust antioxidant responses in others.

The ecological consequences of metal contamination extend beyond individual-level effects to impact population health, reproductive success, and ecosystem services. Impaired filtration capacity in contaminated bivalve populations can affect water quality and nutrient cycling, while

tissue damage and reduced condition may affect population demographics and community structure. Seasonal and environmental factors modulate metal effects, adding complexity to impact assessment and raising concerns about interactive effects with climate change.

Integrated biomarker approaches combining multiple endpoints across biological levels provide comprehensive assessments of metal impacts and are increasingly recognized as superior to single-biomarker strategies. The complementary use of laboratory experiments, in situ caging studies, and field surveys of resident populations offers the most complete understanding of metal effects under both controlled and ecologically relevant conditions.

Looking forward, research priorities include elucidating metal mixture effects, understanding climate-metal interactions, applying advanced molecular techniques to reveal toxicity mechanisms, investigating transgenerational effects, and developing standardized biomonitoring protocols. As anthropogenic pressures on coastal ecosystems intensify and climate change progresses, understanding and monitoring heavy metal impacts on marine molluscs will remain critical for protecting ecosystem health and ensuring the sustainability of valuable marine resources.

The remarkable capacity of molluscs to accumulate metals while exhibiting measurable biological responses at sublethal concentrations ensures their continued importance as sentinel organisms. By integrating knowledge across disciplines—from molecular biology to ecosystem ecology—and across approaches—from laboratory toxicology to field biomonitoring—the scientific community can provide the evidence base needed for effective management of metal pollution in marine environments.

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Conflicts of interest

The authors declare that there are no conflicts of interest regarding the publication of this paper

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