

Physiological, Immunological, Genotoxic and Histopathological Biomarker Responses of Molluscs to Heavy Metal and Water-Quality Parameter Exposures: A Critical Review

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Abstract

Aquatic molluscs are ideal invertebrate model systems for monitoring environmental quality and toxicology. However, they are subjected to a wide variety of stressors including the heavy metals, Cadmium, Arsenic, and adverse water-quality conditions, thermal, hypoxia that can have significant effects on host physiology, biochemical and histology. When acting singly or in combination, both stressors represent a serious threat to the health of the invertebrate communities by enhancing the production of reactive oxygen species, ROS, which can cause oxidative alterations. They have been proposed and reported to cause significant ecological damage to the mollusc population through a reduction in scope of growth leading to overall small size of individuals, mutation or DNA damage, immunosuppression, tissue deformities and abnormalities, disease outbreaks, mass mortalities, amongst others.

This article reviews some of the stress evidences or biomarker responses of the molluscs to trace metals and water-quality parameters especially thermal stress.

Keywords: Molluscs; Heavy metals; Water-quality conditions; Stress; Biomarker responses

Introduction and Justification of Study

Seismic, anthropogenic and climate change activities, especially those associated with earthquake, cyclones, hurricanes, typhoons, volcanic eruptions, flooding, industrial, agricultural and domestic activities, chemical contaminations of the environment, increased temperature, hypoxia, ocean acidification, have increased the potential impacts of stresses of heavy metals, pharmaceuticals, pesticides, polycyclic aromatic hydrocarbons, thermal, on seafood, especially molluscs and crustaceans, in aquaculture farms and coastal areas [1-4]. Trace metals have been considered as the most important contaminants in the estuary and marine ecosystems after organic matter and hydrocarbons, oil and can impact their lethal and sub-lethal toxicity and deterioration on molluscs [5,6]. Over the years, researchers have measured the levels of contaminants, such as trace metals, in the water, sediments and aquatic biota and evaluated their toxicological effects on a variety of marine organisms at the organismal, tissue and cellular level [7-9]. It has been reported that trace elements even at low concentrations can have adverse effects on the ecological, bio-chemical, physiological and immunological functions of the shellfish populations [10,11]. They can affect their metabolic activities and behavioral patterns, such as; growth, reproduction, endocrine, immunity or survival, valve-movement, increase their susceptibility to infectious diseases, mortalities and in severe cases linked to local extinction [12-14]. In addition to this, extreme climatic conditions, increased temperature, hypoxia, reduced pH, can facilitate or enhance the absorption and accumulation of trace metals in the tissues of the molluscs and can co-jointly result in oxidative stress, reduced growth, DNA or protein denaturation, immunosuppression, tissue hypoxia, disease outbreak, mass mortality, histopathological abnormalities and other impaired metabolic activities [15,16]. Wang and Overgaard [17] reported that a key issue in global warming effects is the thermal limitation of whole organism performance. Britz et al. [18] and Hooper et al. [19] reported that high temperature of over 20°C has been shown to cause immunosuppression, reduced growth, heat shock to protein in abalone during summer season and could lead to serious disease

outbreak. Tomanek et al. [20] demonstrated that pH of 7.5 can cause oxidative stress in *Crassostrea virginica*. This is supported by Matozzo et al. [21] who showed that decreased pH and high temperature can strongly affect the immune-parameters of clam, *Chamelea gallina* and mussel, *Mytilus galloprovincialis*.

In the context of global warming, ocean acidification and prevalent marine pollution, the use of biomarkers has become a common tool for environmental assessment of metallic pollution and to predict their effects, such as cell damage and dysfunction, on aquatic organisms for quick intervention and adoption of effective monitoring program [22-26]. Histopathological analyses could be carried out on the gills, guts, gonads and interstitial tissues of the molluscs accompanying with various observations such as; lesions, atrophy, oedema, hyperplasia, epithelial cell damage [27,28]. Histology is regarded as the easiest method of assessing short and long term toxic effects of metals on organisms in the field [29]. Valko et al. [30] and Viarengo et al. [31] reported that toxicity of trace metals depends on their capacity to produce and increase the cellular levels of Reactive Oxygen Species (ROS), although certain antioxidant defending enzymes, catalase, glutathione-s-transferase, are secreted in response to this, but if suppressed, can cause oxidative stress and which can result in DNA damage, lipid peroxidation and depletion of protein sulphhydryls. In most recent finding, Chandurvelan et al. reported that the physiological and biochemical responses of biomarkers of clearance rate, absorption efficiency, respiration rate, Metallothionein-Like Protein (MTLP) content, catalase activity and alkaline phosphatase activity, to heavy

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metals in green-lipped mussel, *Perna canaliculus*, were negatively affected following seismic disturbance of earthquake in Canterbury region of New Zealand.

Marine molluscs, such as mussels, oysters, clams, scallops, cats-eye, are sessile, mobile, plankton or algae, deposit or suspended feeders and of commercial importance on a global scale due to their cosmopolitan distribution, abundance, availability, accessibility, and constitute the diet of large number of people and have been regarded as ideal bio-indicators as a result of their accumulation and sensitivity to environmental pollutants [32-35]. The gills, digestive gland and haemolymph of molluscs are regarded as excellent candidates for assessing toxic impacts of metal exposure simply because of their contact with the environment (water medium), centre of metabolic regulations and transferring pathways of metals to detoxifying tissues [7,36].

Coastal water quality (especially in reference to heavy metals) is of paramount importance to marine farmers who rely on it for the growth and production of high quality and healthy seafood products that are desirable, valuable and command good market price [6,37,38]. Biomarkers have been defined as a measurable alteration of a physiological, biochemical, behavioral steady state induced by an environmental change which renders individual or population more susceptible to further environmental changes [39]. Biomarkers such as feeding rate, absorption efficiency, oxygen-consumption, excretion rate, defence-enzyme activities (immunology) and other metabolic activities of the molluscs, are sensitive to environmental variables and all form the basis of this review. Investigating the levels and various toxicological effects of trace metals on the bivalve and gastropod molluscs following a seismic event and from human influence point of view, provides relevant information on the health and quality status of the marine ecosystems, help to identify acute, sub-lethal and lethal dosage of exposure to contaminants, early warning signals and understanding metabolic performance and the occurrence of infectious diseases even to human [26,40,41]. For instance, cadmium has been reported to increase the risk of prostate disease in human, following shellfish consumption noted as the exposure route [42].

Surprisingly, there is dearth of reports on the effects of environmental stressors of trace metals, high temperature, pH and hypoxia on the physiology and histology of molluscs [43], thus hampering updating knowledge on the risk evaluation, thereby necessitates this study. This paper reviews some of the few published work on the biochemical and physiological impacts of trace metals on bivalve molluscs.

Physiological Biomarkers

A drastic reduction (by 50%) in filtration rate was observed for *Mytilus edulis* on sublethal exposure to 0.04 ppm, 0.15 ppm copper and 1.6 ppm zinc [44,45] while the rate of oxygen consumption of excised gill of *Crassostrea virginica* increased when exposed to sublethal concentrations of 50 and 100 ppb copper for 14 days [46]. Swinehart and Crowe [47] reported that mercury has a dramatic effect on amino-acid efflux in the gills of *Mytilus californianus* whereas copper, iron and mercury inhibit the influx of glycine into *Mytilus* gills. By deviating from the normal tolerable temperature of 8°C for pacific oysters, *Crassostrea gigas*, Shpigel et al. [48] observed that when thermal stress of 30°C was imposed, the molluscs experienced accelerated rates of oxygen consumption and ammonia excretion. Wang et al. [49] reported a reduced clearance rate of green-mussels collected from and associated with contaminated sediments and surface water. Based on the work of Boldina-Cosqueric et al. [12], they observed a reduction

in the energy reserved contents (glycogen, lipids and proteins) in the digestive glands and gonads of clam, *Scrobicularia plana*, in metal polluted estuaries (Goyen, Loire and Seine) of France. Although, in most cases, no significant difference was observed between the mussels collected in the experimental and reference sites. Anestis et al. [15] affirmed that there was severe impairment in the clearance rate of mussels, *Mytilus galloprovincialis*, by 50% and scope for growth lowered by 46% at 24-26°C compared to 18°C but no significant change was observed for excretion rate between the experimental and control. Although, the scope of growth was initially positive at 26°C but later became negative at higher temperature indicating the inability of the mollusk to regain energy from the ingested food. Al-Subiai et al. [50] showed that exposure of *Mytilus edulis* to Copper, Cu, and concentrations of 18-56 µg L⁻¹ caused a significant reduction (94-96%) in the clearance rate of the mollusc. Chandurvelan et al. [51] reported that exposure of green-lipped mussel, *Perna canaliculus*, to acute and sub-chronic concentrations (2000-4000 µg L⁻¹) of Cadmium, Cd, resulted in drastic decline and inhibition in the clearance rate, absorption efficiency, respiration rate and higher ammonia excretion rate than the control mussels, which were all statistically significant. In addition, they concluded that a negative scope of growth was recorded for this species after Cadmium exposure on 10-20 days. Chandurvelan et al. [7] reported a significant reduction in the levels of glycogen found in the digestive gland of green-lipped mussel at 2000-4000 µg L⁻¹ during a 4-28 day bio-assay exposure to acute and subchronic Cadmium concentrations. According to the experiment set up by Sanders et al. [52], they subjected mussels, *Mytilus edulis*, to hypoxic condition, 2.11 mg/L of dissolved oxygen, for 7 days and found out a significant reduction in the clearance rate, assimilation efficiency, respiration rate, ammonia excretion and scope for growth of the mollusc. In their investigation, Chandurvelan et al. showed that mussel, *Perna canaliculus*, from contaminated sites (affected by both anthropogenic and seismic events) exhibited reduced or lower clearance rate, absorption efficiency, impaired respiration rate and a large negative scope for growth [32]. Yeung et al. [32] reported that glycogen, total lipid content and computed total energy reserve index of mussel, *Perna viridis*, were affected by copper at 5 and 10 µg/L and cadmium 0.01 µg/L treatments.

Biochemical Biomarkers

Immunotoxic/Genotoxic biomarkers

Wang et al. [53] recorded a number of nuclear abnormalities in mussels exposed to arsenic and linked these symptoms to metal levels in the biological tissues due to accumulation. Canesi et al. [54] reported a reduction in glutathione-S-hydroxylase, GSH, after exposing *Mytilus* spp. to copper concentration for a day. In contrast to this, Regoli and Principato [55] showed an increase in this antioxidant enzyme activity when exposing the species to copper for three weeks. Brousseau et al. [56] measured the adverse effects of a number of trace metal contaminants on the phagocytic disturbance in clam, *Mya arenaria*, in conjunction with haemocyte viability. They observed that at high concentrations (10⁻⁶ -10⁻⁴ M) of mercuric chloride, silver nitrate, Zinc and Cadmium exposures, could result in reduced or impaired haemocyte phagocytic activity and cell viability. Gagnaire et al. and Sokolova et al. [57,58] investigated the adverse effects of cadmium, Cd and mercury, Hg, exposures on the defence mechanisms of Pacific oysters, *Crassostrea gigas*. They reported that both metals caused high levels of hemocyte apoptosis, necrosis and mortality after a 24 h *in vitro* incubation at certain concentrations ranging from 10-1000 µmol L⁻¹. Both metals adversely affected the immune-parameters

(phenoloxidase, reactive oxygen species generation, cyclooxygenase, COX, activity, cell viability, cell-adherence to polystyrene microwells, phagocytosis) of the species. In a similar work by Gagnaire et al. [57], by exposing haemocytes collected from *Crassostrea gigas*, they found no significant influence on the haemocyte of cadmium concentrations ranging from 3×10^{-11} M to 3×10^{-4} M but on exposure to 2×10^{-4} M of mercury, mass mortality (80%) was recorded at 4-24 h assay. Choi et al. [59] documented the effects of cadmium doses and duration on the heat shock protein 90 (HSP90) messenger RNA, mRNA, glutamate pyruvate transaminase, GPT, and glutamate oxaloacetate transaminase, GOT, in the gills, digestive glands and haemolymph of pacific oysters, *Crassostrea gigas*. They found out that the levels of GPT, GOT and mRNA increased rapidly at Cd concentrations of 0.05 and 0.1 ppm in 7 and 11 days of exposures. These expressions or responses take place to maintain the homeostasis and protect the cell from cadmium damage stress and toxicity. Boldina-Cosqueric et al. [12] showed that cellulase and amylase activities in the digestive glands and styles of clams, *Scrobicularia plana*, collected from metal contaminated estuaries (Loire and Seine) had lower values than the reference counterpart. On the other hand, there was significant increase in the antioxidant metallothionein-like protein in the soft tissues of the clams from the contaminated than the reference site as well as both glutathione-S-transferase (GST) and lactate dehydrogenase activities. Similarly, Funes et al. [60] reported significant higher antioxidant enzymatic activities of catalase, superoxide dismutase, taurine, metallothionein and total glutathione peroxidase, of oysters, *Crassostrea angulata*, and mussels, *Mytilus galloprovincialis*, collected from polluted sites than the control site. They reported a serious oxidative damage to DNA of mussel collected from contaminated site (Punta Umbria) which could explain the absence (extinction) of the species in most polluted site of Mazagón. Al-Subiai et al. [50] observed a distinct DNA damage in haemocytes from mussel, *Mytilus edulis*, exposed to different Copper, Cu, and concentrations, ranged from 18 to $56 \mu\text{g L}^{-1}$. Ahmad et al. [61] investigated the effects of mercury on the immunity of bivalve, *Scrobicularia plana*, inhabiting a contaminated area. They reported that there was higher concentration of the metal in the haemolymph of the animal which had some significant effects on the hemocyte density and oxidative burst activity. Ciacci et al. [62] also reported a decreased phagocytic activity and total hemocyte counts after exposing *Mytilus galloprovincialis* to Chromium concentrations ranging from 0.1-10 $\mu\text{g L}^{-1}$. They also observed changes in the transcription of immune related genes (defensin, mytilin C, myticin B and lysosome and serotonin receptor). Dimitriadis et al. [24] reported a significant reduction in the activity of acetyl-cholinesterase, AchE, of digestive gland of mussels, *Modiolus barbatus*, after long exposure of 30 days at 28° and 30°C respectively. Vosloo et al. [16] reported on the combined effects of high temperature and dissolved oxygen on abalone. They found out that antioxidant enzyme (superoxide dismutase, glutathione peroxidase and catalase) activities were significantly higher and affected in thermal (19°C) and oxygen-stressed abalone than the control counterpart. They reported more DNA damage in abalone kept in high oxygen, 7.7 mg/L at 19°C than 14°C and control, 16°C sections. According to Hooper et al. [8], after subjecting hybrid Australian abalone to a thermal stress (26°C) from its normal 16°C, they observed a significant decline in antibacterial, phagocytic, phenoloxidase activities but high total haemocyte counts [62]. Leucine aminopeptidase activity between the experimental and control treatments was not significantly different. After exposing three species of mussels, *Mytilus edulis*, *M. trossulus* and *M. galloprovincialis*, to sub-lethal and acute concentrations of copper; 10, 100 and 500 $\mu\text{g/L}$, Brooks et al. [63] recorded significant increase in the glutathione-S-hydroxylase, GSH, activity of digestive gland at

4 days of exposure of *M. edulis* and *M. trossulus* to 500 $\mu\text{g/L}$ Cu. Hu et al. [25] demonstrated increased activities of superoxide dismutase, SOD, glutathione peroxidase, GPX, glutathione-S-hydroxylase, GSH and glutamic-pyruvic transaminase when thick-shell mussel, *Mytilus coruscus*, was treated at high temperature of 25 and 30°C and reduced pH, 7.3, 7.7 and 8.1 for 14 days. Chandurvelan et al. [14] showed that mussels, *Perna canaliculus*, from four contaminated sites had a lower or reduced metallothionein like protein, MLTP, alkaline phosphatase activity and reduced lipid peroxidation, in the gill and digestive gland. This is contrary to what was obtained by Chandurvelan et al. [26] who reported significant elevated activities for catalase, lipid peroxidation and alkaline phosphatase in the digestive glands of the mollusc. Chandurvelan et al. [14,28] reported a numerical decline of haemocytes due to metal exposure in contaminated ecosystems but contradict what was obtained by Chandurvelan et al. [26]. Most recently, Peric et al. [64] observed a gradual decrease of metallothionein level with increasing copper concentrations of 10 $\mu\text{g/L}$ and higher after *M. galloprovincialis* was exposed to the metal.

Histological Assessments

Mussels collected from polluted sites indicated structural alterations in the digestive gland epithelium which might be due to increased lipid accumulation associated with enlarged secondary lysosomes [65]. Weis et al. [66] observed atrophy of digestive diverticular epithelium of oysters, *Crassostrea virginica*, exposed to wood preservative, chromated copper arsenate. The same report was recorded for American oysters, *Crassostrea virginica*, sampled from metal contaminated sites showed damage in the digestive diverticula and gills of the mollusc [27]. Porte et al. [67] and Nasci et al. [68] recorded a severe, heavy haemocytic infiltration and enlargement of lysosome in mussels along Mediterranean Coast and in the connective tissues of clams transplanted in a highly metal contaminated estuary located in urban and industrialized area of Tampa Bay. Increased vacuolation and granulocytomas of digestive tubules was observed in mussels, *Mytilus edulis*, exposed to trace metal contamination [69]. Prolonged sublethal heat stress has been shown to cause digestive gland atrophy and replacement of glandular tissue with fibrosis [70]. Zorita et al. [71] observed a significant higher destabilized lysosomal membrane in mussels, *Mytilus edulis*, collected near a copper mine in Norway than the reference site but found a reversal for its volume and surface densities between the two sites. They also reported digestive tubule atrophy (in the digestive gland) of the copper contaminated site. Aarab et al. [72] identified tubular dilation and large lipid vacuoles in the digestive gland of mussel, *Mytilus edulis*, obtained from an aluminium-contaminated site in Norway. In their study, Al-Subiai et al. [50] reported that the posterior adductor muscles, gills and digestive gland of *Mytilus edulis*, observed under Olympus microscope after exposure to different concentrations (18-56 $\mu\text{g L}^{-1}$) of Copper showed various histological abnormalities, deformities and alterations ranging from increased myocytes, loss of myocyte bundles, hypo and hyperplasia, necrosis and haemocyte infiltration. After exposing green-lipped mussel, *Perna canaliculus*, to subchronic and acute concentrations ranging from 200-4000 $\mu\text{g L}^{-1}$ Cadmium, Chandurvelan et al. [7] reported an increased number of micro-nuclei, nuclear bud formation and fragmented-apoptotic cells in the gills of the mollusc and these nuclear aberrations were strongly significantly ($p < 0.05$) correlated with metal accumulation in the gill. Hooper et al. [8] observed a decreased goblet cell numbers and damage to the gill epithelial cells and infiltration of the haemocytes in the digestive gland of heat stressed abalone. Brooks et al. [63] reported an increase in the frequency of

micro-nuclei formation in the haemocytes of three species of mussels; *Mytilus edulis*, *galloprovincialis* and *Mytilus trossulus*, after being subjected to copper concentrations of 10, 100 and 500 µg/L for 4 days. They also measured a reduction in lipofuscin contents of the lysosomes in the three *Mytilus* species which could be repaired and explained by cell type replacement and tissue renewal processes after a long time exposure to copper for 21 days. Lipofuscins are pigments regarded as the end products of lipid peroxidation [73,74]. Chandurvelan et al. [14] showed that there were nuclear buds, micronuclei and fragmented apoptotic cells in the gills of mussels from contaminated sites of two regions in the South Island of New Zealand. Chandurvelan et al. [26] further explained that there were significantly more micronuclei and fragmented apoptotic cells in the gill haemolymphs of the mussels from contaminated site than reference site but no difference was found for binuclei and nuclear buds between these two sites. Martinez-Gomez et al. [75] did a comprehensive research by using some biomarkers in mussel, *Mytilus edulis*, in conducting a regional biomonitoring program of North and Mediterranean Seas. They found lowest values of Lysosomal Membrane Stability (LMS) in mussels, that is, impaired membrane integrity, from metal polluted sites which were significantly different from two reference sites.

Conclusion

Physiology, biochemical and histopathology are essential analytical tools and warning signals of disease outbreaks in shellfish. It has been reported that change in enzymatic activities could be a general mechanism by which different biological tissues try to compensate to the actions of environmental pollutant and stressors [76]. The most widely used and reliable physiological biomarker is scope of growth. A decline or negative scope of growth under condition of constant ration indicates an impaired growth efficiency which lowers the fitness of the individual by indirectly reducing fecundity [77]. Although, a number of studies have used a multiple biomarker approach to examine the pollution status of coastal waters [78], this review updates and clearly supports the utility of stress indices of mollusc cells and tissues' responses in environmental risk assessment on which further studies could be based to evaluate mollusc health status, recovery following exposure to specific metal stressor and finally enforce monitoring.

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