26

OXIDATIVE CHALLENGE AND REDOX SENSING IN MOLLUSKS: EFFECTS OF NATURAL AND ANTHROPIC STRESSORS

Julie Letendre, François Leboulenger and Fabrice Durand
Laboratoire d'Ecotoxicologie—Milieux Aquatiques EA, Université du Havre, Le Havre Cedex, France

26.1 INTRODUCTION

Mollusks are exposed to a variety of stresses in relation to their habitat and biology or to human activities. In aquatic environments, O_2 concentration and speed of diffusion are admittedly much lower than those in the air, but infeoded animals are likely exposed to an important range of oxygen concentrations with potentially great changes over time and space. Hence, coastal or estuarine animals can be subjected to tidal rhythm and consequently to oxygen deprivation during air exposure followed by reoxygenation at recovery. In tidal pools, and likewise in lake or lagoon shallow waters, animals are exposed to nyctemeral pO_2 variations due to O_2 consumption and production by algae. Pelagic mollusks are also likely to cross differently oxygenated water masses during vertical migration. Benthic mollusks live in sediments or at the interface with the surface, both characterized by low but variable pO_2. Concerning terrestrial snails that are air breathers, oxygen availability is quite constant unless the climate impels them to isolate from their surroundings to avoid desiccation or to limit gel formation. In this case, they undergo oxygen deprivation similarly to intertidal mollusks but for much longer periods, in addition to extreme body temperature.

All these situations induce oxidative challenges that mollusks are able to face under certain limits; indeed, they have evolved efficient defense strategies that allow them to limit the harmful consequences of oxidative stress. Antioxidant defenses are close to those encountered in vertebrates, constituted by molecular and enzymatic actors and highly modulated as a function of environmental conditions. The antioxidant capacities and the relative contribution of the diverse antioxidant forces depends on the tissue, the species, or even the population for species living in heterogeneous habitats. For instance, high levels of antioxidant defenses are generally measured in animals living in permanent prooxidant conditions as in upper levels of the shore and in polar areas [1–3].

In addition to that ensuing from abiotic constraints, oxidative stress is also encountered in mollusks during microbial challenge, since their simple immunological responses consist in great part in triggering reactive oxygen species (ROS) surproduction by hemocytes to kill invaders with potential harmful side effects on host cells themselves. The last but not least aspect is the exposure of aquatic mollusks and, also terrestrial mollusks to a lesser extent, to environmental pollution due to industrial, agricultural, and domestic activities. A common denominator to a great part of these contaminants, notably organic compounds and metals, is the induction of oxidative stress due to direct toxicity or metabolization processes [4]. The discovery of the production of free radicals as a general feature of pollution exposure gave rise in the last two decades to a great interest in pro- and antioxidant processes in mollusks,

Oxidative Stress in Vertebrates and Invertebrates: Molecular Aspects of Cell Signaling, First Edition.
Edited by Tahira Farooqui and Akhlaq A. Farooqui.

increasing the related knowledge for this phylum usually poorly studied. Hence, most of the data on antioxidant processes in mollusks were provided by ecotoxicological studies, in which antioxidants are used as biomarkers of chemical stress [4, 5].

The mechanisms of ROS formation, their deleterious effects on cellular components, as well as cellular defense responses are now better understood in mollusks and have been reviewed in several papers dealing with natural oxidative stress linked to ecology [6, 7] or with anthropic stresses [5, 8, 9]. Some papers only evoke the consequences of immune oxidative burst on the host itself [10, 11]. The cellular signaling processes involved in the response to variations in cellular redox status represent a field of research whose interest has grown recently. Related data in mollusks are provided by a few studies that address directly or indirectly this question by miscellaneous approaches. They allow us to understand some general features of these processes, but efforts in investigations have to be maintained to complete the picture. The aim of this review is to summarize current knowledge in the understanding of the cellular signaling response to redox variations in mollusks, in the frameworks of changes in O_2 availability and exposure to pollutants or pathogens.

26.2 OXIDATIVE STRESS LINKED TO VARIATIONS IN OXYGEN AVAILABILITY

Changes in O_2 environmental concentration are a major source of oxidative challenge in mollusks, mainly because of their original respiratory metabolism. In particular, intertidal and terrestrial mollusks are subjected to hypoxia-reoxygenation succession and exhibit adaptations that consist overall in regulation of energetic metabolism and redox balance to face this situation. These processes are triggered when tissular oxygen declines but part of them, for example, regulation of antioxidant forces, are involved above all in the cellular protection against the harmful consequences of recovery. Hence, mechanisms allowing the mollusk to face reoxygenation-induced oxidative stress encompass those underlying low O_2-sensing and downstream regulations, which are therefore described in this section.

26.2.1 Oxyconformity and Ectothermy

Mollusks are subjected to variations of abiotic factors that can change oxygen concentrations and consequently are likely to generate a recurrent situation of redox imbalance. The important influence of environmental oxygen on their oxidative status is linked to the fact that most mollusks are ectotherms and oxyconformers.

Ectothermy implies that body temperature follows that of the external medium. Oxidase functions and subsequent ROS production are a priori proportional to the temperature [12]. Hence, despite the decrease of O_2 solubility at high temperature, mollusks experiencing thermal stress likely suffer from oxidative stress all the more if the critical temperature inactivating antioxidant enzymes is reached [13]. On the other hand, oxidative stress can also be encountered in cold environments. In polar organisms, the lower metabolic activity and weaker speed of diffusion of oxygen in viscous fluids is counteracted by the fact that dissolved oxygen is more soluble and consequently more concentrated in seawater and body fluids of such animals than that of temperate animals [6]. Moreover, the adaptations of polar animals (homeoviscous adaptation, area of mitochondria) to facilitate O_2 transport and capture imply higher lipid content and higher proportion of unsaturation in membranes [6] so that equivalent levels of free radicals are actually registered and greater levels of oxidative damages are observed in certain species [14, 15]. In compensation, antioxidant capacities are generally more important in such organisms than in temperate organisms [2, 16].

In oxyconformers, oxygen consumption varies with environmental pO_2 but in a greater or lesser range according to their habitat and biology. Generally this conformity occurs above the low critical pO_2 until hyperoxic conditions, whereas in microoxic animals such as mud clams, oxygen uptake is independent from the environmental pO_2 above low concentrations [7]. At the molecular level, it has been shown that this conformity exists for both respiratory states 4 (unphosphorylating resting state) and 3 (phosphorylating active state) in mantle mitochondria of *Arctica islandica* and would be allowed by the alternative oxidase pathway rather than the futile cycling of protons (see next paragraph). Reduced aerobic activity when environmental oxygen is decreased may be necessary to avoid or at least delay tissular hypoxia and consequently limit the formation of ROS by reduced ubiquinone [7].

26.2.2 Hypoxia and ROS Production

Oxygen depletion in cell generates a slowdown of mitochondrial respiration and electron transfer, which leads to an increase in membrane potential, in reduction of complex III ubiquinone, and ultimately in ROS production, mainly superoxide anion [17, 18]. In mollusks, this production of ROS during hypoxia but also at recovery could be limited because of H^+ leakage that can reduce membrane potential [7, 19]. This could be achieved by uncoupling proteins (UCPs) that evolved from the ancestral form of UCP2 and 3 known in

vertebrates, where they play among others a protective role against excessive ROS production [20]. In addition, it was shown that superoxide anions increase the expression of UCPs and activate them in rat muscle [21]. Another potential way to control ROS production when respiratory rate is low is the divergence of electron flow to an alternative oxidase (AOX) pathway by the influence of nitric oxide (NO). The latter interacts with cytochrome *c* oxidase of which it decreases the affinity for oxygen, leading to a shunt of electron flow into the AOX pathway [7]. Oxidases involved in the AOX pathway are expressed in diverse phyla of invertebrates as well as plants, fungi, and prokaryotes and can be transcriptionally induced by several kinds of stress (oxidative stress, hypothermia, pathogen infection, hypoxia) and their activity modulated at the posttranslational level [22, 23].

26.2.3 Hypoxia-Reoxygenation Challenge

Coastal as well as terrestrial mollusks are likely to encounter hypoxic stress in the way that the former undergo regular emersion and the latter isolate themselves from the surroundings when the aerial temperature reaches seasonal *extrema*, in order to limit water loss (aestivation) or gel formation (hibernation). In addition to the stress resulting from exposure to extreme temperatures [24], consequent deprivation of the medium of gas exchange implies that they are rapidly exposed to tissular hypoxia. To deal with these constraints that can occur frequently in intertidal species, anoxia-tolerant mollusks evolved adaptations of which the main adaptation is a metabolic depression up to 5% of the normal rate. This is possible because of a general breakdown of ATP-consuming processes and supply pathways in order that the ATP level remains constant [25–27]. Hence, the expression of almost all genes is suppressed, as well as translation, proteolysis, and part of the activity of ionic pumps with ATPase [28]. This situation already constitutes a challenge in itself, but the more harmful situation is the reoxygenation of tissues when animals are submerged or reawake. Indeed, when mollusks return to water, or emerge from lethargy in the case of land snails, the respiration rate increases suddenly and oxygen tension rises in tissues that were depleted and accumulated reducing equivalents. This situation is analogous to the ischemia/reperfusion phenomenon, which is known to lead to ROS overproduction and subsequent huge oxidative stress [29, 30]. Another situation comparable to ischemia/reperfusion is that generated by hyperthermia [7]. Indeed, rise in temperature of ambient water implies a parallel decrease of oxygen solubilty as well as an enhanced metabolic activity. The combination of low pO_2 and increased O_2 consumption leads to a functional hypoxia for animals exposed to such a stress. Thus, in addition to that ensuing from heat-induced ROS production, oxidative stress can occur when temperature declines and tissular pO_2 rises.

26.2.3.1 Triggering Hypoxic Response As in vertebrates, hypoxia triggers the activation of specific factors that regulate metabolism as well as the transcription of targeted genes whose products are involved in the cellular adaptation to low oxygen, but in a different manner to that of mammals. Few authors have taken interest in the understanding of low O_2 sensing in anoxia-tolerant mollusks, so that the mechanisms underlying these processes are still partially unclear, even if brand new findings help to complete the picture.

Low O_2 Sensing Most hypotheses concerning mechanisms of low O_2 sensing and regulation of gene expression are issued from mammal data that Larade and Storey reviewed in the context of anoxia-tolerant mollusks [26]. Hochachka proposed in the 1990s that O_2 itself could be a regulating molecule that directly controls the genic response to anoxia in anoxia-tolerant animals [28]. However, no evidence of the presence of a hypoxia-inducible transcription factor and associated regulators was provided in such organisms until recently [26]. Indeed, transcripts of hypoxia-inducible factor (HIF) α subunit as well as HIF-prolyl hydroxylase were newly isolated in *Crassostrea gigas* by Piontkivska and co-workers [31]. These transcripts share key functional domains and present a significant sequence similarity with invertebrate and vertebrate homolog. Exposure to 6-day anoxia did not significantly affect levels of these mRNAs, but they increased at recovery [32]. In fact, the α subunit of HIF-1 can be induced by hypoxia at the transcriptional level through a functional loop but the predominant regulation occurs at the posttranslational level [33], which may explain these results. Moreover, a 6-day air exposure is admittedly a challenge that oysters might encounter in wildlife. but it represents an extreme situation, and the consequent cellular response probably does not exactly reflect mechanisms involved in adaptation to cyclic tidal emersion. In any event, further experiments are required to understand the mode of action of this protein and the extent to which mechanisms are comparable with those of mammalian HIF-1 pathway. A priori, these findings allow us think that in anoxia-tolerant mollusks like oysters, HIF-α would be oxidatively inactivated during normoxia by a prolyl hydroxylase, which needs oxygen to perform the HIF proline hydroxylation [33]. When oxygen tension declines, prolyl hydroxylases become unable to catalyze the oxidation of HIF-α, which can be translocated into the nucleus, interact with other subunits, and induce the transcription of hypoxia response element.

The nature of the hypoxia response element triggered by HIF in anoxia-tolerant mollusks is probably partly specific to these organisms, but the relative similarity in sequence and functional domains of oyster HIF-α with those of mammals suggests that some comparable genic responses such as NO synthase (NOS) expression could also be expected [7].

Second Messengers NOS activity has been detected in several groups of mollusks and has been particularly studied in the nervous system, where NO acts as a neuromodulator [34]. As an ancient and general biological regulator, NO is nonetheless involved in several functions of which are mediation of O_2 sensing and redox balance [34, 35]. NO is able to inhibit mitochondrial functions, and regulation of NO production is an important feature of hypoxic response [26, 35, 36]. However, there is a paradox in the fact that NO synthesis by NOS requires molecular oxygen and is consequently limited in severe hypoxia [35]. NO is a radical that is alternatively considered as a pro- or antioxidant according to the reaction in which it is involved and consequent products [38]. Hence, it can react with superoxide anion to form peroxynitrite ($ONOO^-$), a highly cytotoxic radical, which is probably the major way of NO disappearance. This oxidation depends on O_2^- production and consequently on O_2 concentration. It has been shown that at low pO_2 NO half-life is longer than at physiological pO_2; hence, during hypoxia, NO degradation is lowered [36]. NO can also form nitrites NO_2^-/NO_3^-, which could constitute a form of storage of NO molecules. Mitochondria are sites of NO production, and this could be achieved by the reduction of nitrites when hypoxia occurs, probably thanks to cytochrome *c* oxidase [39, 40]. It is intriguing to note that cytochrome oxidase can both generate NO (at low pO_2) and have its oxidase activity inhibited by NO (particularly at phosphorylating and active state 3).

NO is also involved in hypoxia response through its role as activator of guanylyl cyclase and consequent influence on the production of cGMP [41], which most probably mediates O_2 sensing in anoxia-tolerant mollusks [26, 37]. Recent experiments suggest that guanylyl cyclases may actually not be activated by NO itself but rather by a complex of NO with glutathione, called *S*-nitrosoglutathione (GSNO). Indeed, it has been shown that GSNO, which has a hypoxia-mimetic activity, can activate guanylyl cyclase and that GSNO reductase, which reduces GSNO to restore reduced glutathione (GSH) and degrade NO in NH_4^+ form, affects significantly NO-mediated hypoxic response [35]. Regulation of the GSNO pool could constitute a link between cellular redox status and signal transduction insofar as GSNO can generate nitrosothiols by transnitrosylation of protein sulfhydryl groups that may participate in cell signaling. In addition, a relationship between the phosphorylation state of glycolytic enzymes and GSH content and synthesis was found in *Mytilus galloprovincialis* [42] that may be due to GSNO-induced cGMP production and subsequent activation of metabolic regulators. Indeed, the involvement of cGMP during hypoxia in mollusks seems to be linked to the cGMP-dependant protein kinase PKG, a key signaling protein that is known to mediate both phosphorylation of metabolic enzymes and anoxia genic response [26, 43]. Among numerous substrates, PKG activates the stress-activated kinase p38 MAPK that regulates the activity of transcription and translation factors, these functions being highly modulated in anoxia-tolerant animals [44]. In *Mytilus galloprovincialis*, p38 MAPK activation is apparently involved in response to different stresses (oxidative stress, osmotic stress, hyperthermia, exposure to diverse pollutants) [45–47]. Such stimuli also activate other stress-activated kinases (SAPK/JNK) at the same time as p38 MAPKs [47–48]; however, this is not the case for ischemia/reperfusion phenomenon in rat heart, where p38 MAPKs are activated during both anoxia and recovery but SAPKs only at reoxygenation [49]. This suggests that p38 MAPK could be a specific contributor to the response to anoxia-reoxygenation. Gaitanaki and co-workers found that anoxia exposure induced biphasic changes in phosphorylated rate of p38 MAPK in *M. galloprovincialis*: maximal values occurred at 1 h and 8 h of anoxia [45]. Interestingly, levels of phosphorylated p38 MAPK also increased transiently in the first 5 min of recovery after a 15-min air exposure. In *Littorina littorea*, an increase in phosphorylated p38 MAPK as well as phosphorylated 27-kDa stress protein (HSP27), which is regulated by the p38 MAPK downstream element MAPK-activated kinase 2 [51], was found in hepatopancreas after 12 h of aerial exposure [50]. HSP27 is a chaperone-like protein that increases cell resistance to heat shock or osmotic stress as well as short-term oxygen deprivation and myocardial ischemia-reperfusion, providing this protection in the latter situations by a role of the phosphorylated form in F-actin stabilization [52, 53]. Interestingly, 2-DE analysis on *Mytilus edulis* gills showed a downregulation of gelsolin, an efficient actin filament-severing factor, after 2 h of emersion on the shore [54], which is consistent with the idea of a reinforcement of the polymerization rate and stabilization of the cytoskeleton during hypoxic stress. However, activation of the p38 MAPK pathway is also involved in mediating apoptosis or necrosis during ischemia-reperfusion in myocardium and kidney [55, 56]; further experiments should help us to understand whether its role in the tolerance of mollusks to anoxia-reoxygenation is related to an enhanced cell resistance or an elimination of insulted cells.

26.2.3.2 Cellular Response to Oxygen Decline—Preparation for Oxidative Stress In addition to metabolic adjustments, signaling cascades and transcription factors activated by hypoxia most probably control the regulation of actors involved in the fight against oxidative stress. Evidence of such anticipated regulation is provided at the genomic level and through the modulation of antioxidant enzymatic activities during hypoxic stress.

Gene Transcription Contrary to mechanisms observed in anoxia-sensitive species, which consist mainly of compensatory mechanisms (expression of glycolytic enzymes and proteins involved in oxygen transport and vascularization), the genic response triggered in hypoxia-tolerant animals like certain mollusks generates products that are rather involved in altering metabolism, which allows them to withstand long-term oxygen deprivation [58]. Upregulation of certain transcripts has been shown in *Littorina littorea* during hypoxia, but most of them remain as latent mRNAs during this stressing period [37, 57]. These transcripts, which probably correspond to functions that are important for recovery, accumulate in the course of metabolic depression until normal conditions enable their translation. cDNA microarrays performed in hepatopancreas and foot of *L. littorea* allowed identification of a metallothionein [59] and a ferritin heavy chain [37] as upregulated genes. Both transcripts showed quick and important increase of expression and a maintenance of high levels throughout the hypoxia stress before declining at recovery. Resulting proteins are able to sequester metals and presumably participate in antioxidant defenses when free radical production is enhanced at recovery. Indeed, metallothioneins are shown to provide protection against oxidative stress in vertebrates [60, 61] and in invertebrates [62] due to metal binding but also to an inherent antioxidant function as ROS scavenger. Ferritin regulates Fe storage when its translation is not prevented by the binding to iron-regulatory proteins (IRP) that occurs when iron is low. This interaction is regulated by oxygen concentration [63]; in *L. littorea*, hypoxia probably triggers the dissociation of IRP-ferritin mRNA complexes, allowing ferritin translation. Management of the Fe pool and especially sequestration of free iron ions is a crucial aspect of coping with oxidative stress for mollusks [64]. In similar experiments in *C. gigas*, upregulation of glutathione peroxidase (GPx) and metallothionein transcripts were also observed in gills and mantle, which is consistent with the results described above [65]. At the proteic level, 2-DE experiments showed that a decrease of a thioredoxin peroxidase and Cu/Zn superoxide dimutase (Cu/Zn SOD) occurred between the end of emersion and 2 h of recovery in gills of *M. edulis* [54]. Both of these are antioxidants, and these decreases may indicate that they are partially degraded after having contributed to the protection against oxidative stress at immediate recovery. Surprisingly, measurement of Cu/Zn SOD activity in the same study showed that it remained stable during hypoxia and increased after 2 h of recovery [54]. This discrepancy between expression and activity underlines that enzymatic antioxidant activities are regulated to a great extent at the posttranslational level. In this way, specific revelation of mussel Cu/Zn SOD activity after isoelectrofocusing reveals that the total activity visible on gel results from the contribution of three isoforms with different isoelectric points. The relative contribution of isoforms to total activity is different according to the tidal height of animals and reversibly modulated during the tidal cycle and by the pollution status of the environment [66, 67]. Authors suggest that these changes, which are clearly linked to cellular redox status, could correspond to interconversion of isoforms through oxidative modifications, perhaps as a functional response.

Regulation of Antioxidant Activities Earlier studies on the antioxidant response in mollusks exposed to air showed that antioxidant activities are modulated during hypoxia as well as at recovery according to species and tissue [6]. Most observations suggest that a spread strategy is an increase of certain antioxidant forces during hypoxia as a preparation to recovery, considering the probable adaptation to cyclic emersion or aestivation of anoxia-tolerant mollusks [68-70]. In *L. littorea*, antioxidant activities diminished during a 6-day exposure to N_2 and increased at recovery in hepatopancreas and foot muscle, but total glutathione increased during anoxia [69]. These changes in molecular antioxidant that are attributed to an anticipated adaptation to ROS generation seem efficient, since a decrease in lipid hydroperoxides was observed in hepatopancreas throughout the experiment. Almeida and co-workers exposed *Perna perna* mussels to air for 24 h and found that only glutathione transferase activity increased at the end of the anoxia period and remained high after recovery; on the other hand, increase in oxidative damages on lipids and DNA was reported during air exposure, with a return to basal values at recovery [29]. This suggests that oxidative stress occurs also during emersion for aquatic mollusks. In addition to the ROS generation by mitochondria due to electron flow lowering, another possible cause is gaping behavior. This consists in opening briefly the shell in order to extract aerial oxygen and increase pO_2 in water of the palleal cavity [71]. This behavior that significantly participates in energy production during emersion implies a sudden exposure to air of tissues that are oxygen depleted and consequently the generation of oxidative stress [29, 30]. In another experiment where mussels were exposed to air

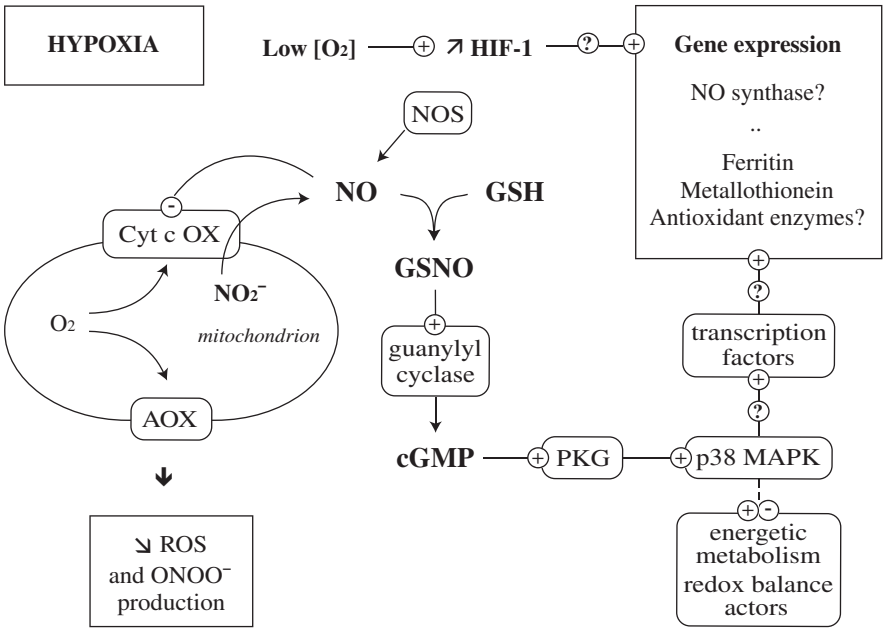

Fig. 26.1 A model describing modulation of mitochondrial functions and cellular signaling in response to hypoxia in anoxia-tolerant mollusks. Hypoxia triggers the production of NO, probably in part due to the mitochondrial reduction of nitrites. NO inhibits cytochrome *c* oxidase and causes a shunt of electron flow into an alternative oxidase pathway that allows minimization of ROS production. NO also associates with GSH to form *S*-nitrosoglutathione (GSNO), whose pool is regulated thanks to a GSNO reductase. GSNO complexes can then activate guanylyl cyclase to enhance cGMP production that probably helps to activate kinases like PKG. PKG in turn activates p38 MAPK, whose role could involve the activation or deactivation of metabolic and redox balancing actors. Also, it is possible that it initiates phosphorylation cascades until the induction of expression of targeted genes. In parallel, brand new information suggests that hypoxia genic response, at least in oysters, is triggered by the activation of HIF-α by a prolyl hydroxylase, but currently whether the mechanisms of transcription of responsive genes are comparable to those of mammals remains unknown. In this schematic hypothesis, signals triggered by anoxia generate a rapid metabolic response and a slower genic response that completes or reinforces the adjustment of energetic and redox processes.

for 4 h, which is more ecologically relevant, a short-term induction of SOD activity was observed in digestive gland at the end of emersion and considered as a preparation to reoxygenation [72]. In the pulmonate land snail *Helix aspersa*, a 20-day estivation led to an increase exclusively in GPx activity that the authors also attribute to a preparative mechanism, and 24-h recovery was characterized by a decrease in protein carbonylation and lipid peroxidation [73]. In an experiment where *M. edulis* was exposed to 6-h emersion, this strategy of anticipation appeared in gills, an interface tissue that is directly exposed to variations of environmental oxygen, but not in digestive gland, where levels of antioxidant activities seem to be dependant from metabolic state and consequently low at the end of emersion [54]. In field studies, tidal variations of antioxidant activities in *M. edulis* were admittedly significant but not drastic, suggesting that basal and sufficient levels of antioxidant capacities could be maintained despite the energically unfavorable situation [54]. In addition, comparison of antioxidant status of mussels issued from low shore and high shore showed that individuals regularly exposed to emersion (high shore) exhibit globally higher levels of antioxidant defenses than their subtidal counterparts [3], consistently with observations done on two close species of limpets living at different tidal heights [1]. Mussels that are regularly exposed to oxidative stress seem to be able to maintain constitutively high levels of defense as an acclimation, rather than adjusting them for any variation; such a strategy is also encountered in anoxia-tolerant turtles [74].

Even if gaps remain in our knowledge of cellular response to hypoxia in anoxia-tolerant mollusks and particularly of the involved transduction signals, the picture becomes clearer. Figure 26.1 presents a prospective scheme that summarizes the knowledge in this field.

26.3 POLLUTANT-INDUCED OXIDATIVE STRESS

Aquatic compartments constitute the ultimate receptacle of most environmental pollutants, so that organisms inhabiting such habitats are massively and chronically

exposed to a mixture of potentially toxic substances. Oxidative stress is a common consequence of xenobiotic presence responsible for biomolecule alteration. Some of these alterations constitute probable links with signaling processes that bring about cell death or, conversely, increase of resistance capacities.

26.3.1 Free Radical Production by Pollutants in Mollusks

As soft-bodied organisms, xenobiotics can enter in molluscan organisms through different ways (digestive tract by ingestion of water and food, respiratory surfaces, and across integumentary system) and accumulate because of low degradation capacities. Many compounds are implicated in the production of free radicals in mollusks, the main compounds being metals, polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), and other organohalogenated molecules [38]. This toxicity is either due to the metabolization of xenobiotics in the case of organic molecules, although such capacities are limited in invertebrates [4], or the catalysis of redox reactions in the case of transition metals and depletion of thiol-containing antioxidants for other metals [75]. The ROS produced as a result of Haber–Weiss reactions or incomplete redox cycle, as well as organic peroxides that can be generated by cytochromes, can interact with biomolecules such as lipids, proteins, and DNA and, as in other organisms, generate damage that can be irreversible. Measurement of this damage (mainly products of lipid peroxidation and, to a lesser extent, DNA oxidation and fragmentation and protein damage) as well as induction of defense and repair mechanisms are used as biomarkers of oxidative insult in ecotoxicology [76]. Among the diverse oxidative attacks, protein and glutathione oxidations as well as DNA alteration probably play a role in the activation of pathways controlling cell response.

26.3.2 Oxidation of Proteins: Sensing Redox Changes?

Free radicals induce different kinds of oxidative modifications on proteins that can be reversible—and possibly involved in regulatory functions—or not [77]. A dramatic modification is the carbonylation of proteins, which consists of irreversible modifications of lateral chains into ketones or aldehydes and leads to protein aggregation and consequent inactivation and degradation. Measurement of carbonyl groups in soluble proteins is a tool used in ecotoxicology for evaluating oxidative damages to proteins induced by contamination [78]; new approaches now allow study of the specific patterns of proteins that are affected by these modifications [79]. In 2-DE experiments coupled with blotting and specific immunorevelation of carbonylated groups, it was shown that levels of carbonylation increased in parallel to the intensity of oxidative stress in *M. edulis* and in *Ruditapes decussatus* but with tissue-specific and pollutant-specific patterns [80, 81]. In particular, these studies revealed that a major target of such oxidation in *M. edulis* is actin, which is known as one of the most sensitive cytoskeletal component to oxidant attacks [82]. Interestingly, revelation of ubiquitinated proteins in similar experiments done in *M. edulis* and *R. decussatus* showed that the print is different from that of carbonylated proteins, suggesting that the latter are degraded independently from ubiquitin-proteasome pathway [83–85]. Other modifications generally involve alteration of sulfhydryl groups, which are preferential targets of free radicals; oxidation of thiol functions gives rise to cleavage or formation of disulfide bonds that can be intra- or interproteins or occur between proteins and cysteines, glutathione, or even lipids. Disulfide bonds were thought to lead to the loss of protein function and inactivation of enzymes [86], but these bonds are reversible and could play regulatory roles in mild stress situations [87]. Glutathionylation of proteins, for instance, is a post-translational modification whose level increases in response to prooxidant treatment in diverse models. It allows the regulation of protein function and consequent modulation of cellular processes, protects cysteines during oxidative stress by preventing irreversible oxidation, and could play an essential role in diverse signaling pathways [88, 89]. It notably regulates the ubiquitin-conjugating activity and therefore controls the protease activity in mammalian cells [90]. An equivalent role of glutathionylation in the modulation of signal transduction can be expected in mollusks, but no evidence has been provided until now. Study of the glutathionylated proteome in gills and digestive gland of *M. edulis* showed that actin is again a preferential target of this modification [80]. This protein was slightly affected by inter- or intraprotein disulfide bond formation in redox proteomics experiments performed on tissues of mussels exposed to H_2O_2 [91]. A modified protocol helped to identify proteins touched by sulfhydryl oxidation in response to menadione treatment and revealed that proteic response to oxidative stress in *M. edulis* principally involves disulfide isomerases and cytoskeletal and chaperone proteins [92]. The role and mechanisms of these specific proteic modifications in cellular signaling and processes of mollusks have yet to be elucidated.

26.3.3 Pollutant-Induced Apoptosis

There is a correlation between apoptosis levels in mollusks and environmental stresses (salinity or temperature changes, pollution) whose common denominator is

oxidative stress. Stress-induced cell death, then, is probably linked to oxidative insult, and it is assumed that it is triggered by ROS excess and activation of the intrinsic pathway [93]. Genotoxic damages are a probable cause of the induction of cell death to prevent the threat of accumulation of DNA modifications. In mollusks, apoptosis is observed in response to chemicals in digestive gland, gills, mantle, as well as hemocytes and has to be distinguished for the latter from immune apoptosis occurring in case of pathogen attack. Exposure to sublethal concentrations of 4-nonylphenol gave rise to oxidative stress and hemocyte apoptosis in the clam *Tapes philippinarum*, with cell shrinkage and changes in morphology [94]. *Lymnaea stagnalis* exposed to a prooxidant pesticide showed an increase in hemocyte apoptosis with a time- and dose-dependent decrease in mitochondrial membrane potential [95]. Cd^{2+} induced hemocyte apoptosis in the oyster *Crassostrea virginica* in a dose-dependent manner with almost no effect at low concentration [96], and simultaneous exposure to elevated temperature significantly increased the occurrence of these events [97]. Curiously in oysters, Cd^{2+}-induced hemocyte apoptosis appears to involve a putatively original pathway different from the mitochondria/caspase pathway, since no decrease in mitochondrial membrane potential was recorded [96]. Cadmium introduced in the food also induced apoptosis in digestive gland of *Helix pomatia* [98]. The digestive gland is the major site of pollutant accumulation and metal detoxication; apoptosis probably allows the elimination of altered cells particularly in environments characterized by high heavy metal concentrations like hydrothermal vents, where such a phenomenon could be adaptative [99]. Apoptotic events are more rarely reported in gills, but DNA fragmentation followed by apoptosis has nevertheless been detected in gills of *M. galloprovincialis* exposed to single injections of different tri-*n*-butyltin (TBT) doses [100].

DNA fragmentation and changes in membrane potential strongly support the hypothesis of involvement of the intrinsic pathway in stress-induced molluscan apoptosis, but the exact mechanisms underlying this process are still obscure. In mammals, the key factor p53 is involved in both extrinsic and intrinsic apoptotic pathways. In the latter, redox changes activate p53, which promotes apoptosis either via nuclear translocation and gene transcription or by acting directly at the mitochondrial level, interacting with Bcl-2 proteins and inducing membrane permeabilization and release of cytochrome *c* [101]. In mollusks, there is evidence that equivalent pathways exist. Hence, human p53 homolog identified in leukemic *Mya arenaria* adductor muscle and hemocytes possesses highly conserved regions of functional domains, suggesting roles similar to those of mammalian p53 [102]. Leukemic hemocytes of *M. arenaria* overexpress mortalin, a p53-binding stress protein that is responsible for p53 cytoplasmic sequestration [103]. Stress-induced DNA fragmentation led to the reversion of p53 cytoplasmic sequestration and translocation into the nucleus [103, 104], where p53 certainly promotes gene expression. The mechanisms of redox sensing by p53 are not clear, but depletion in cellular GSH is generally observed prior to the induction of apoptosis by p53 [105, 106]. Impaired glutathione redox status in mussel *M. galloprovincialis* and scallop *Flexopecten flexosus* has been associated with decrease in survival, which might be explained by GSH depletion-induced cell death [107]. Nitric oxide is also involved in apoptosis triggering, but its influence depends on dose, cell type, and cellular physiological status [108]. The exact role of these two compounds in apoptosis is not clear, but both are indirectly involved in the regulation of signaling cascades as a function of redox status, and in this view, activation of p53 may be attributed to upstream effectors such as stress kinases [109]. In addition, an increase in global tyrosine phosphorylation levels is observed in mussels exposed to various prooxidant chemicals (oil, mixture of oil/PAHs/alkylphenols, copper) [110]. In particular, p38 MAPK activation can promote cell death either through p53 pathway [111] or independently from this key regulator, possibly by direct activation of caspases [112, 113]. Involvement of p38 MAPK pathway in stress-induced apoptosis has been shown in *M. galloprovincialis* exposed to Zn^{2+} or Cu^{2+} since transient or biphasic activation p38 MAPK and increased levels of activated caspase 3 as well as DNA fragmentation have been observed in mantle [46].

26.3.4 Activation of MAPK Pathways in Prosurvival Response to Pollutants

In stress response, p38 MAPK regulates diverse pathways that can promote either cell death or resistance, depending on the cell type and the kinase isoform activated [109]. For instance, exposure to Zn^{2+} or Cu^{2+} induced in *M. galloprovincialis* gills a strong and long-lasting phosphorylation of p38 MAPK, while no DNA fragmentation or caspase 3 cleavages were detected at the same time, which contrasts with the results obtained in the mantle of the same species [46]. This suggests that activation of p38 MAPK by such a treatment in gills actually rather involves antiapoptotic signaling cascades, with upstream or downstream substrates able to reactivate p38 MAPK that would explain the persistence of phosphorylation [48]. Moreover, an increase in the levels of HSP70, a stress protein considered as an anti-apoptotic factor [114], has also been registered in gills in

response to Cu^{2+} exposure [46]. Stress-induced HSP70 overexpression is generally observed at the same time as p38 MAPK activation in bivalves, probably because of induction of HSPs by MAPK prosurvival pathways in addition to protein damage, but it is notable that HSPs could themselves regulate signaling cascades [115].

Hence, tissues present a differential sensitivity to chemical stress, as suggested by the higher levels of chaperones in gills than in mantle [116], the diverse p38 MAPK pathways apparently activated by oxidant stimuli [46], and the differences in global phosphorylation levels observed in response to pollutant exposure [110]. This divergence of vulnerability could be due to a higher level of oxidative stress undergone by mantle cells, since mantle is more sensitive to lipid peroxidation [117] and gills possess higher levels of enzymatic antioxidant defenses [4]. The disparity in the response between tissues may also be due to a different "threshold" of stress level that activates proapoptotic pathways; indeed, it has been shown that MAPK activation involves different pathways and responses according to the severity of oxidative insult [118]. For instance, in human lymphoid cells, a high level of oxidative stress induced apoptosis, whereas a low level induced mitotic arrest and probable transcription of antioxidant actors to balance redox status. Gills are the first tissue exposed to adverse factors, to which they probably exhibit greater tolerance than other tissues because of differential sensitivity and capacities to cope with a higher level of insult. Effects of diverse pr-oxidant contaminants on MAPKs in *M. galloprovincialis* gills were studied recently [47]. Low concentrations of TBT activated p38 MAPK and JNK but not ERK, and higher doses activated only p38 MAPK; in both cases DNA damages decreased, suggesting that the concentrations used in this study induced protective pathways of MAPKs, which contrasts with previous results obtained with very high doses of TBT directly introduced in the palleal cavity of mussels [100]. p38 MAPK/JNK activation and reduction of DNA damage were also observed in gills of mussels exposed to soluble fraction of diesel oil, indicating a probable role of MAPK activation in DNA repair [47].

Figure 26.2 proposes a schematic view of the hypothetical induction of apoptotic or resistance responses to oxidative stress in mollusks according to the severity of the insult, with p38 MAPK as a central regulator.

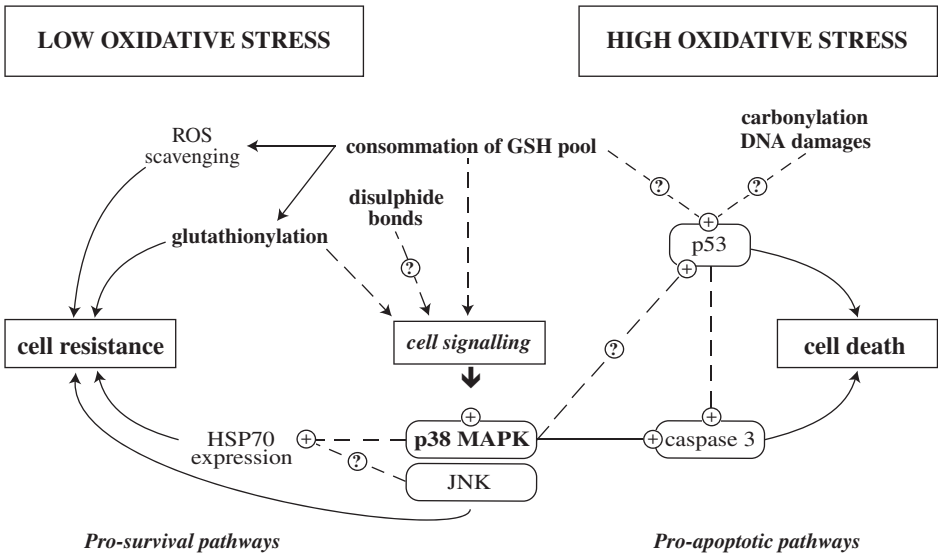

Fig. 26.2 A hypothetical model of the induction of apoptotic or resistance responses to oxidative stress according to the severity of insult. Oxidative stress induces the oxidation of thiol groups of proteins. Resulting disulfide bridges could play a role in signaling, while oxidation of free thiol is generally deleterious except in some cases (metal binding protein) where it allows scavenging of ROS. Glutathione is also oxidized or conjugates to proteins (glutathionylation), protecting them from irreversible modifications; in addition, glutathionylated proteins are also probably involved in signal transduction. Stress kinases, namely, p38 MAPK and to a lesser extent JNK, are activated probably in correlation with GSH levels. p38 MAPK induces the expression of HSP70 that protects proteins from damages and misfolding. These features allow enhancement of cell tolerance, which becomes capable of resisting oxidative stress if not too high. If oxidative stress is too severe, which is a relative notion depending on cell type sensitivity, p38 MAPK activates proapoptotic pathways by the cleavage of caspases, perhaps through p53 activation. Activation of p53 could also be regulated by GSH level more directly and by proteins or DNA damage. p38 MAPK plays a central role in the modulation of cell response to oxidative stress in mollusks.

26.4 IMMUNE SYSTEM

The molluscan immune system is less complex than that of mammals and involves humoral (lectins, hydrolytic enzymes, antimicrobial peptides) and cellular (hemocytes) mechanisms that recognize invaders and destroy them by various means [11]. Hemocytes are crucial components of this system and capable of migration, phagocytosis, aggregation, and secretion of antibacterial substances and ROS. One of the common features of molluscan host reaction against pathogen infection is the generation of an oxidative burst by hemocytes to kill microbes [119]. NADPH oxidase catalyzes the production of singlet oxygen, superoxide O_2^-, hydroxyl radical OH^-, as well as hydrogen peroxide H_2O_2 that forms hypochlorous acid in a reaction with chloride catalyzed by myeloperoxidase, then enhancing antibacterial activity. It is nonetheless proposed that H_2O_2 could also originate from the Fenton reaction and hydroxyls result from interaction of H_2O_2 and superoxides. In addition, the amount of the different ROS produced depends on the cellular subtype as well as the stimulation [119, 120]. Oxidative burst is triggered by exposure to pathogens [119] but also carbohydrates [121], bacterial extracellular products [122], and cytokines [123]. Then, whereas it was thought that the phagocytosis itself activates a ROS-forming system associated with NADPH oxidase [119], it seems probable that ROS production is mediated by cGMP-dependent protein kinase PKC [124] and stress-activated MAPKs like p38 MAPK, as observed in *M. galloprovincialis* challenged with strains of *Vibrio sp* [120]. However, pathways triggered by a bacterial challenge depend on the pathogen as well as the extent and duration of the stimulation [125].

ROS production by hemocytes as a defensive reaction has the disadvantage of potentially inducing cytotoxicity to the host itself, which exhibits antioxidant forces to counteract this deleterious aspect of microbial killing. In addition to cellular antioxidants, circulating antioxidants are detected that could help to protect host cells from ROS insults. For instance, an extracellular superoxide dismutase has been characterized in oyster plasma [126]. This Cu/Zn form is exclusively expressed by hemocytes and is able to bind lipopolysaccharides (LPS) exhibited by bacteria such as *Escherichia coli* as well as integrin-like receptors on the surface of the oyster's hemocytes. In addition to putative roles in oxidative burst modulation and host protection against oxidative injury, this extracellular SOD could thus be involved in the recognition of LPS and subsequent triggering of the immune response by interacting with integrin receptors [126]. In the bay scallop *Argopecten irradians*, an extracellular SOD that does not possess the LPS-binding motifs has been characterized, whose expression was enhanced in hemocytes when animals underwent *Vibrio anguillarum* challenge, probably as a consequence of ROS accumulation during oxidative burst [127].

If not balanced, ROS surproduced for bacterial killing may generate irreversible damage to host cells, even death. Apoptosis is observed as an important component of molluscan immune reaction to degrade infected or phagocytic cells [10, 128]. The mechanisms ruling this immunomodulatory process are still unclear, but oxidative burst probably participates in its activation, as observed for other environmental stress involving ROS formation [11, 95]. Hence, *C. gigas* hemocytes challenged in vitro with the Gram-positive marine bacterium *Planococcus citreus* phagocytized and killed invading cells within a few hours; then phagocytizing hemocytes, mainly hyalinocytes, underwent cell death that probably corresponds to apoptosis as suggested by preceding membrane blebbing, cell shrinkage, and chromatin condensation [11]. Besides, treatment with antioxidant agents suppressed phagocytizing-hyalinocyte death, whereas treatment of nonchallenged hemocytes with a prooxidant failed to induce apoptosis, suggesting that hyalinocyte apoptosis triggered by *P. citreus* exposure is actually induced by ROS produced within cells. Curiously, other experiments showed that granulocytes generally present higher levels of apoptosis than hyalinocytes, possibly due to higher phagocytic and respiratory burst activities [10].

In addition to ROS *sensu stricto* themselves, NO is also produced as a defense mechanism by molluscan hemocytes, where NO synthase activity has been detected, and different isoforms of NOS, some of them unique to mollusks, have been characterized [11]. NO synthesis occurs apparently after phagocytosis and would be involved in bacterial clumping [34, 123]. In the clam *R. decussatus*, NO synthesis seems to be independent from phagocytosis and constitutes an alternative method to kill pathogens [129]. In *C. virginica*, infection by the protozoan parasite *Perkinsus marinus* also induced NO production that was correlated with a decrease in parasite loads at early time points after infection [130]. In parallel, it has been shown that *P. marinus* increased hemocyte apoptosis at early and later stages of infection, with differential profiles according to the virulence of the strain [128]. Hence, the role of NO in the triggering of hemocyte apoptosis in mollusks is under question [10, 11]. Outside the immune system, the roles of NO in cell death are investigated in only one study that suggests an antiapoptotic role of NO in larval development [131]; on the other hand, data on mammalian macrophages show that stimulated NO production is associated with apoptosis of host cells [132, 133].

The putative role of ROS or NO in molluscan immune apoptosis intervenes certainly in addition to the direct stimulation of specific proapoptotic pathways by pathogen motifs and inflammatory or stress-induced endogenous molecules. In any case, the possible involvement of classical apoptotic actors like caspases is not elucidated, since divergent results are observed. In the abalone *Haliotis diversicolor*, bacterial challenge increased in a biphasic manner the expression of a specific caspase that is close to human caspases 8 and 10 [134]. On the other hand, recent work showed that *P. marinus*-induced apoptosis did not involve caspase activation in *C. virginica* [128]. Further experiments are required to understand the involvement of ROS and NO in immune apoptosis and the multiple pathways concerned with this process in mollusks.

26.5 CONCLUSION

Recent interest in signaling mechanisms involved in molluscan response to environmental stresses and study of oxidative modifications on the proteome allow us to gain insight into the mechanisms underlying redox sensing and cellular response to oxidative stress. Actors equivalent to those of mammals are generally concerned; in particular, MAPKs seem to play a key role and to control pathways and issues that vary according to the sensitivity of tissue or organisms and the severity of the insult. This provides interesting knowledge on molluscan stress response, which could be applied in the field of ecotoxicology and potentially used in biomonitoring studies. This also brings supplementary elements in the understanding of the mechanisms supporting anoxia tolerance in mollusks; however, a lot of information is lacking, and some considerations are speculative and require confirmation. Further investigations are necessary to elucidate remaining unknown mechanisms and give an integrated picture of the signaling processes involved in the anoxia-reoxygenation phenomenon. In addition, this review highlights the convergence of pathways involved in the response to diverse environmental stresses that could be undergone simultaneously by wild mollusks, therefore raising the potential interactive effects of multiple stresses.

ACKNOWLEDGMENTS

Julie Letendre is the recipient of a postdoctoral fellowship from the European Funds for Regional Development. The authors wish to thank Marie Gerbron for English revision of the manuscript.

REFERENCES

1. Malanga G, Estevez MS, Calvo J, Puntarulo S. Oxidative stress in limpets exposed to different environmental conditions in the Beagle Channel. *Aquatic Toxicol* 2004; 69(4): 299–309.
2. Camus L, Gulliksen B, Depledge MH, Jones MB. Polar bivalves are characterized by high antioxidant defences. *Polar Res* 2005; 24(1–2): 111–118.
3. Letendre J, Chouquet B, Manduzio M, Marin M, Bultelle F, Leboulenger F, Durand F. Tidal height influences the levels of enzymatic antioxidant defences in *Mytilus edulis*. *Marine Environ Res* 2009; 67(2): 69–74.
4. Livingstone DR, Martinez PG, Michel X, Narbonne JF, O'Hara S, Ribera R, Winston GW. Oxyradical production as a pollution-mediated mechanism of toxicity in the common mussel, *Mytilus edulis* L., and other molluscs. *Funct Ecol* 1990; 4: 415–424.
5. Winston GW, Di Giulio RT. Prooxidant and antioxidant mechanisms in aquatic organisms. *Aquatic Toxicol* 1991; 19(2): 137–161.
6. Abele D, Puntarulo S. Formation of reactive species and induction of antioxidant defence systems in polar and temperate marine invertebrates and fish. *Comp Biochem Physiol A Mol Integr Physiol* 2004; 138(4): 405–415.
7. Abele D, Philipp E, Gonzalez PM, Puntarulo S. Marine invertebrates mitochondria and oxidative stress. *Front Biosci* 2007; 12: 933–946.
8. Livingstone DR. Contaminant-stimulated reactive oxygen species production and oxidative damage in aquatic organisms. *Marine Pollution Bull* 2001; 42(8): 656–666.
9. Vasseur P, Leguille C. Defense systems of benthic invertebrates in response to environmental stressors. *Environ Toxicol* 2004; 19(4): 433–436.
10. Sokolova IM. Apoptosis in molluscan immune defense. *Invertebrate Survival J* 2009; 6: 49–58.
11. Terahara K, Takahashi K. Mechanisms and immunological roles of apoptosis in molluscs. *Curr Pharmaceut Des* 2009; 14: 131–137.
12. Newell RC, Walkey M. Oxidative activity of mammalian liver mitochondria as a function of temperature. *Nature* 1966; 212: 428–429.
13. Abele D, Heise K, Pörtner HO, Puntarulo S. Temperature-dependence of mitochondrial function and production of reactive oxygen species in the intertidal mud clam *Mya arenaria*. *J Exp Biol* 2002; 205: 1831–1841.
14. Viarengo A, Canesi L, Martinez PG, Peters LD, Livingstone DR. Pro-oxidant processes and antioxidant defence systems in the tissues of the Antarctic scallop (*Adamussium colbecki*) compared with the Mediterranean scallop (*Pecten jacobaeus*). *Comp Biochem Physiol B Biochem Mol Biol* 1995; 111(1): 119–126.
15. Estevez MS, Abele D, Puntarulo S. Lipid radical generation in polar (*Laternula elliptica*) and temperate (*Mya arenaria*) bivalves. *Comp Biochem Physiol B Biochem Mol Biol* 2002; 132(4): 729–737.

16. Regoli F, Principato GB, Bertoli E, Nigro M, Orlando E. Biochemical characterization of the antioxidant system in the scallop *Adamussium colbecki*, a sentinel organism for monitoring the Antarctic environment. *Polar Biol* 1997; 17(3): 251–258.
17. Chandel NS, Maltepe E, Goldwasser E, Mathieu CE, Simon MC, Schumacker PT. Mitochondrial reactive oxygen species trigger hypoxia-induced transcription. *Proc Natl Acad Sci USA* 1998; 95(20): 11715–11720.
18. Keller M, Sommer M, Pörtner HO, Abele D. Seasonality of energetic functioning and production of reactive oxygen species by lugworm (*Arenicola marina*) mitochondria exposed to acute temperature changes. *J Exp Biol* 2004; 207: 2529–2538.
19. Miwa S, Brand MD. Mitochondrial matrix reactive oxygen species production is very sensitive to mild uncoupling. *Biochem Soc Trans* 2003; 31(6): 1300–1301.
20. Sokolova IM, Sokolov EP. Evolution of mitochondrial uncoupling proteins: novel invertebrate UCP homologues suggest early evolutionary divergence of the UCP family. *FEBS Lett* 2005; 579(2): 313–317.
21. Echtay KS, Roussel D, Saint-Pierre M, Jekabsons B, Cadenas S, Stuart JA, Harper JA, Roebuck SJ, Morrison A, Pickering S, Clapham JC, Brand MD. Superoxide activates mitochondrial uncoupling proteins. *Nature* 2002; 415: 96–99.
22. Vanlerberghe GC, McIntosh L. Alternative oxidase: from gene to function. *Annu Rev Plant Physiol Plant Mol Biol* 1997; 48: 703–734.
23. McDonald AE, Vanlerberghe GC, Staples JF. Alternative oxidase in animals: unique characteristics and taxonomic distribution. *J Exp Biol* 2009; 212: 2627–2634.
24. Helmuth BS, Hofmann GE. Microhabitats, thermal heterogeneity, and patterns of physiological stress in the rocky intertidal zone. *Biol Bull* 2001; 201(3): 374–84.
25. Hochachka PW, Lutz PL. Mechanism, origin, and evolution of anoxia tolerance in animals. *Comp Biochem Physiol B Biochem Mol Biol* 2001; 130(4): 435–459.
26. Larade K, Storey KB. A profile of the metabolic response to anoxia in marine invertebrates. In: Storey KB, Storey JM, editors, *Cellular and Molecular Responses to Stress*. Amsterdam: Elsevier Press, 2002, p27–46.
27. Larade K, Storey KB. Living without oxygen: anoxia-responsive gene expression and regulation. *Curr Genomics* 2009; 10: 76–85.
28. Hochachka PW. Oxygen–a key regulatory metabolite in metabolic defense against hypoxia. *Am Zoologist* 1997; 37: 595–603.
29. Almeida EA, Bainy ACD, Dafre AL, Gomes OF, Medeiros MGH, Mascio PD. Oxidative stress in digestive gland and gill of the brown mussel (*Perna perna*) exposed to air and re-submersed. *J Exp Marine Biol Ecol* 2005; 318(1): 21–30.
30. Ferreira R, Milei J, Grana D. Oxidative stress and ischaemia-reperfusion injury in the heart. *Asia Pacific Heart J* 1999; 8(2): 97–101.
31. Piontkivska H, Chung JS, Ivanina AV, Sokolov EP, Techa S, Sokolova IM. Molecular characterization and mRNA expression of two key enzymes of hypoxia-sensing pathway in eastern oysters *Crassostrea gigas* (Gmelin): hypoxia-inducible factor a (HIF-a) and HIF-prolyl hydroxylase (PHD). *Comp Biochem Physiol D: Genomics Proteomics* 2011; 6(2): 103–114.
32. Ivanina AV, Sokolov EP, Sokolova IM. Effects of cadmium on anaerobic energy metabolism and mRNA expression during air exposure and recovery of an intertidal mollusk *Crassostrea gigas*. *Aquatic Toxicol* 2010; 99(3): 330–342.
33. Bruick RK. Oxygen sensing in the hypoxic response pathway: regulation of the hypoxia-inducible transcription factor. *Genes Dev* 2003; 17: 2614–2623.
34. Palumbo A. Nitric oxide in marine invertebrates: a comparative perspective. *Comp Biochem Physiol A Mol Integr Physiol* 2005; 142(2): 241–248.
35. Dijkers PF, O'Farrell PH. Dissection of a hypoxia-induced, nitric oxide-mediated signaling cascade. *Mol Biol Cell* 2009; 20: 4083–4090.
36. Okada S, Takehara Y, Yabuki M, Yoshioka T, Yasuda T, Inoue M, Utsumi K. Nitric oxide, a physiological modulator of mitochondrial function. *Physiol Chem Physiol Med* 1996; 28(2): 69–82.
37. Larade K, Storey KB. Accumulation and translation of ferritin heavy chain transcripts following anoxia exposure in a marine invertebrate. *J Exp Biol* 2004; 207: 1353–1360.
38. Manduzio H., Rocher B, Durand F, Galap C, Leboulenger F. The point about oxidative stress in molluscs. *Invertebrates Survival J* 2005; 2: 91–104.
39. Kozlov AV, Staniek K, Nohl H. Nitrite reductase activity is a novel function of mammalian mitochondria. *FEBS Lett* 1999; 454(1–2): 127–130.
40. Castello PR, David PS, McClure T, Crook Z, Poyton RO. Mitochondrial cytochrome c oxidase produces nitric oxide under hypoxic conditions: implications for oxygen sensing and hypoxic signaling. *Cell Metab* 2006; 3(4): 277–287.
41. Krumenacker JS, Hanafy KA, Murad F. Regulation of nitric oxide and soluble guanylyl cyclase. *Brain Res Bull* 2004; 62(6): 505–515.
42. Canesi L, Ciacci C, Betti M, Gallo G. Growth factor-mediated signal transduction and redox balance in isolated digestive gland cells from *Mytilus galloprovincialis* Lam. *Comp Biochem Physiol C Comp Pharmacol Toxicol* 2000; 125: 355–363.
43. Brooks SPJ, Storey KB. Glycolytic controls in estivation and anoxia: A comparison of metabolic arrest in land and marine molluscs. *Comp Biochem Physiol A Physiol* 1997; 118(4): 1103–1114.
44. Browning DD, McShane MP, Marty C, Ye RD. Nitric oxide activation of p38 mitogen-activated protein kinase in 293T fibroblasts requires cGMP-dependent protein kinase. *J Biol Chem* 2000; 275: 2811–2816.

45. Gaitanaki C, Kafeloyianni E, Marmari A, Beis I. Various stressors rapidly activate the p38-MAPK signaling pathway in *Mytilus galloprovincialis* (Lam.). *Mol Cell Biochem* 2004; 260: 119–127.
46. Kefaloyianni E, Gourgou E, Ferle V, Kotsakis E, Gaitanaki C, Beis I. Acute thermal stress and various heavy metals induce tissue-specific pro- or anti-apoptotic events *via* the p38-MAPK signal transduction pathway in *Mytilus galloprovincialis*. *J Exp Biol* 2005; 208: 4427–4436.
47. Châtel A, Hamer B, Talarmin H, Dorange G, Shröder HC, Müller WEG. Activation of MAP kinase signalling pathway in the mussel *Mytilus galloprovincialis* as biomarker of environmental pollution. *Aquatic Toxicol* 2010; 96(4): 247–255.
48. Kyriakis JM, Avruch J. Mammalian mitogen-activated protein kinase signal transduction pathways activated by stres and inflammation. *Physiol Rev* 2001; 81(2): 807–869.
49. Bogoyevitch MA, Gillespie-Brown J, Ketterman AJ, Fuller SJ, Ben-Levy R, Ashworth A, Marshall CJ, Sugden PH. p38/RK mitogen-activated protein kinases and c-Jun N-terminal kinases are activated by ischemia-reperfusion. *Circ Res* 1996; 79: 162–173.
50. Larade K, Storey KB. Analysis of signal transduction pathway during anoxia exposure in a marine snail: a role for p38 MAP kinase and downstream signaling cascades. *Comp Biochem Physiol B Biochem Mol Biol* 2006; 143(1): 85–91.
51. Larsen JK, Yamboliev IA, Weber LA, Gerthoffer WT. Phosphorylation of the 27-kDa heat shock protein via p38 MAP kinase and MAPKAP kinase in smooth muscle. *Am J Physiol* 1997; 273(5): L930–L940.
52. Guay J, Lambert H, Gingras-Breton G, Lavoie JN, Huot J, Landry J. Regulation of actin filaments dynamics by p38 MAP kinase-mediated phosphorylation of heat shock protein 27. *J Cell Sci* 1997; 110(3): 357–368.
53. Maulik N. Effect of p38 MAP kinase on cellular events during ischemia and reperfusion: possible therapy. *Am J Physiol Heart Circ Physiol* 2005; 289: H2302–H2303.
54. Letendre J. Effets combinés de la condition intertidale et de la contamination chimique chez *Mytilus edulis*: suivi des mécanismes enzymatiques anti-oxidants et approche protéomique [dissertation]. Le Havre (France): University of Le Havre, 2009. pp273.
55. Okada T, Otani H, Wu Y, Kyoi S, Enoki C, Fujiwara H, Sumida T, Hattori R, Imamura H. Role of F-actin organization in p38 kinase-mediated apoptosis and necrosis in neonatal rat cardiomyocytes subjected to simulated ischemia and reoxygenation. *Am J Physiol Heart Circ Physiol* 2005; 289(6): H2310–H2318.
56. Du J, Zhang L, YangY, Li W, Chen L, Ge Y, Sun C, Zhu Y, Gu L. ATP depletion-induced actin rearrangement reduces cell adhesion via p38 MAPK-HSP27 signaling in renal proximal tubule cells. *Cell Physiol Biochem* 2010; 25: 501–510.
57. Larade K, Storey KB. Characterization of a novel gene up-regulated during anoxia exposure in the marine snail, *Littorina littorea*. *Gene* 2002; 283(1–2): 145–154.
58. Larade K, Storey KB. Arrest of transcription following anoxic exposure in a marine mollusc. *Mol Cell Biochem* 2007; 303: 243–249.
59. English TE, Storey KB. Freezing and anoxia stresses induce expression of metallothionein in the foot muscle and hepatopancreas of the marine gastropod *Littorina littorea*. *J Exp Biol* 2003; 206(14): 2517–2524.
60. Lazo JS, Kondo Y, Dellapiazza D, Michalska AE, Choo KHA, Pitt BR. Enhanced sensitivity to oxidative stress in cultured embryonic cells from transgenic mice deficient in metallothionein I and II genes. *J Biol Chem* 1995; 270: 5506–5510.
61. Campagne MvL, Thibodeaux H, v. Bruggen N, Cairns B, Gerlai R, Palmer JT, Williams SP and Williams D. G. Lowe DG. Evidence for a protective role of metallothionein-1 in focal cerebral ischemia. *Proc Natl Acad Sci USA* 1999; 96 (22): 12870–12875.
62. Leung KMY, Furness RW. Metallothionein induction and condition index of dogwhelks *Nucella lapillus* (L.) exposed to cadmium and hydrogen peroxide. *Chemosphere* 2001; 44(3): 321–325.
63. Schneider BD, Leibold EA. Effects of iron regulatory protein regulation on iron homeostasis during hypoxia. *Blood* 2003; 102(9): 3404–3411.
64. Gonzalez PM, Puntarulo S. Iron and nitrosative metabolism in the Antarctic mollusc *Laternula elliptica*. *Comp Biochem Physiol C Comp Pharmacol Toxicol* 2011; 153(2): 243–250.
65. David E, Tanguy A, Pichavant K, Moraga D. Response of the Pacific oyster *Crassostrea gigas* to hypoxia exposure under experimental conditions. *FEBS J* 2005; 272: 5635–5652.
66. Manduzio H, Monsinjon T, Rocher B, Leboulenger B, Galap C. Characterization of an inducible isoform of the Cu/Zn superoxide dismutase in the blue mussel *Mytilus edulis*. *Aquatic Toxicol* 2003; 64(1): 73–83.
67. Letendre J, Chouquet B, Rocher B, Manduzio H, Leboulenger F, Durand F. Differential pattern of Cu/Zn superoxide dismutase isoforms in relation to tidal spatio-temporal changes in the blue mussel *Mytilus edulis*. *Comp Biochem Physiol C Toxicol Pharmacol* 2008; 148(3): 211–216.
68. Hermes-Lima M, Storey JM, Storey KB. Antioxidant defenses and metabolic depression. The hypothesis of preparation for oxidative stress in land snails. *Comp Biochem Physiol B Biochem Mol Biol* 1998; 120(3): 437–448.
69. Pannunzio TM, Storey KB. Antioxidant defenses and lipid peroxidation during anoxia stress and aerobic recovery in the marine gastropod *Littorina littorea*. *J Exp Marine Biol Ecol* 1998; 221(2): 277–292.
70. Gorr TA, Wichmann D, Hu J, Hermes-Lima M, Welker AF, Terwilliger N, Wren JF, Viney M, Morris S, Nilsson GE, Deten A, Soliz J, Gassmann M. Hypoxia tolerance in animals: biology and application. *Physiol Biochem Zool* 2010; 83(5): 733–752.

71. Guderley H, Demers A, Couture P. Acclimatization of blue mussel, (*Mytilus edulis* Linnaeus, 1758) to intertidal conditions: effects on mortality and gaping during air exposure. *J Shellfish Res* 1994; 13(2): 379–385.
72. Almeida EA, Bainy ACD. Effects of aerial exposure on antioxidant defenses in the brown mussel *Perna perna*. *Braz Arch Biol Technol* 2006; 49(2): 225–229.
73. Ramos-Vasconcelos GR, Hermes-Lima M. Hypometabolism, antioxidant defenses and free-radical metabolism in the pulmonate land snail *Helix aspersa*. *J Exp Biol* 2003; 206: 675–685.
74. Storey KB. Oxidative stress: animal adaptations in nature. *Braz J Med Biol Res* 1996; 29(12): 1715–33.
75. Ercal N, Gurer-Orhan H, Aykin-Burns N. Toxic metals and oxidative stress. Part I: mechanisms involved in metal-induced oxidative damages. *Curr Top Med Chem* 2001; 1(6): 529–539.
76. Valavanidis A, Vlahogianni T, Dassenakis M, Scoullos M. Molecular biomarkers of oxidative stress in aquatic organisms in relation to toxic environmental pollutants. *Ecotoxicol Environ Safety* 2006; 64(2): 178–189.
77. Sheehan D, McDonagh B. Oxidative stress and bivalves: a proteomic approach. *Invertebrate Survival J* 2008; 5: 110–123.
78. Manduzio H. Etude des modifications d'expression protéique sous l'effet d'un stress environnemental chez deux bivalves estuariens, la moule zébrée (*Dreissena polymorpha*) et la moule bleue (*Mytilus edulis*): suivi de marqueurs de défense cellulaire et approche protéomique. [dissertation]. Le Havre (France): University of Le Havre, 2004. pp337.
79. Sheehan D. Detection of redox-based modification in two-dimensional electrophoresis proteomic separations. *Biochem Biophys Res Commun* 2006; 349(2): 455–462.
80. McDonagh B, Tyther R, Sheehan D. Carbonylation and glutathionylation of proteins in the blue mussel *Mytilus edulis* detected by proteomic analysis and Western blotting: actin as a target for oxidative stress. *Aquatic Toxicol* 2005; 73(3): 315–326.
81. Dowling V, Hoarau PC, Roméo M, O'Halloran J, v. Pelt F, O'Brien N, Sheehan D. Protein carbonylation and heat shock response in *Ruditapes decussatus* following p,p′-dichlorodiphenyldichloroethylene (DDE) exposure: a proteomic approach reveals that DDE causes oxidative stress. *Aquatic Toxicol* 2006; 77(1): 11–18.
82. Dalle-Donne I, Rossi R, Milzani A, Simplicio PD, Colombo R. The actin cytoskeleton response to oxidants: from small heat shock protein phosphorylation to changes in the redox state of actin itself. *Free Radic Biol Med* 2001; 31(12): 1624–1632.
83. McDonagh B, Sheehan D. Redox proteomics in the blue mussel *Mytilus edulis*: carbonylation is not a pre-requisite for ubiquitination in acute free radical-mediated oxidative stress. *Aquatic Toxicol* 2006; 79(4): 325–333.
84. Chora S, McDonagh B, Sheehan D, Starita-Geribaldi M, Roméo M, Bebianno MJ. Ubiquitination and carbonylation as markers of oxidative-stress in *Ruditapes decussatus*. *Marine Environ Res* 2008; 66(1): 95–97.
85. Chora S, McDonagh B, Sheehan D, Starita-Geribaldi M, Roméo M, Bebianno MJ. Ubiquitination and carbonylation of proteins in the clam *Ruditapes decussatus*, exposed to nonylphenol using redox proteomics. *Chemosphere* 2010; 81: 1212–1217.
86. Dean RT, Fu S, Stocker R, Davies MJ. Biochem and pathology of radical-mediated protein oxidation. *Biochem J* 1997; 324: 1–18.
87. Biswas S, Chida AS, Rahman I. Redox modifications of protein-thiols: emerging roles in cell signalling. *Biochem Pharmacol* 2006; 71(5): 551–564.
88. Dalle-Donne I, Rossi R, Colombo G, Giustarini D, Milzani A. Protein S-glutathionylation: a regulatory device from bacteria to humans. *Trends Biochem Sci* 2009; 34(2): 85–96.
89. Xie Y, Kole S, Precht P, Pazin MJ, Bernier M. S-Glutathionylation impairs Signal Transducer and Activator of Transcription 3 activation and signaling. *Endocrinology* 2009; 150(3): 1122–1131.
90. Obin, M., Shang F, GongX, Handelman G, Blumberg J, Taylor A. Redox regulation of ubiquitin-conjugating enzymes: mechanistic insights using the thiol-specific oxidant diamide. *FASEB J* 1998; 12(7): 561–9.
91. McDonagh B, Tyther R, Sheehan D. Redox proteomics in the mussel, *Mytilus edulis*. *Marine Environ Res* 2006; 62(S1): S101–S104.
92. McDonagh B, Sheehan D. Effects of oxidative stress on protein thiols in the blue mussel *Mytilus edulis*: proteomic identification of target proteins. *Proteomics* 2007; 7(18): 3395–3403.
93. Kiss T. Apooptosis and its functional significance in molluscs. *Apoptosis* 2010; 15: 313–321.
94. Matozzo V, Marin MG. 4-Nonylphenol induces immunomodulation and apoptotic events in the clam *Tapes philippinarum*. *Marine Ecol Prog Ser* 2005; 285: 97–106.
95. Russo J, Madec L. Haemocyte apoptosis as a general cellular immune response of the snail, *Lymnaea stagnalis*, to a toxicant. *Cell Tissue Res* 2007; 328(2): 431–441.
96. Sokolova IM, Evans S, Hughes FM. Cadmium-induced apoptosis in oyster hemocytes involves disturbance of cellular energy balance but no mitochondrial permeability transition. *J Exp Biol* 2004; 207(19): 3369–3380.
97. Cherkasov AS, Grewal S, Sokolova IM. Combined effects of temperature and cadmium exposure on heamocyte apoptosis and cadmium accumulation in the eastern oyster *Crassostrea virginica* (Gmelin). *J Thermal Biol* 2007; 32(3): 162–170.
98. Chabicovsky M, Klepal W, Dallinger R. Mechanisms of cadmium toxicity in terrestrial pulmonates: programmed cell death and metallothionein overload. *Environ Toxicol Chem* 2004; 23(3): 648–655.
99. Cunha L, Amaral A, Medeiros V, Martins GM, Wallenstein FFMM, Couto RP, Neto AI, Rodrigues A. Bioavailable metals and cellular effects in the digestive gland of marine

limpets living close to shallow water hydrothermal vents. *Chemosphere* 2008; 71(7): 1356–1362.

100. Micic M, Bihari N, Labura Z, Muller WEG, Batel R. Induction of apoptosis in the blue mussel *Mytilus galloprovincialis* by tri-n-butyltin chloride. *Aquatic Toxicol* 2001; 55(1–2): 61–73.
101. Speidel D. Transcription-independent p53 apoptosis: an alternative route to death. *Trends Cell Biol* 2009; 20(1): 14–24.
102. Kelley ML, Winge P, Heaney JD, Stephens RE, Farell JH, Beneden RJV, Reinisch CL, Lesser MP, Walker CW. Expression of homologues for p53 and p73 in the softshell clam (*Mya arenaria*), a naturally-occuring model for human cancer. *Oncogene* 2001; 20(6): 748–758.
103. Walker CW, Böttger S, Low B. Mortalin-based cytoplasmic sequestration of p53 in a nonmammalian cancer model. *Am J Pathol* 2006; 168(5): 1526–1530.
104. Böttger S, Jerszyk E, Low B, Walker C. Genotoxic stress-induced expression of p53 and apoptosis in leukemic clam hemocytes with cytoplasmically sequestered p53. *Cancer Res* 2008; 68(3): 777–782.
105. Wang YJ, Ho YS, Chu SW, Lien HJ, Liu TH, Lin JK. Induction of glutathione depletion, p53 protein accumulation an cellular transformation by tetrachlorohydroquinone, toxic metabolite of pentachlorophenol. *Chemico-Biol Interactions* 1997; 105(1): 1–16.
106. Armstrong JS, Steinauer KK, Hornung B, Irish JM, Lecane P, Birrell GW, Peehl DM, Knox SJ. Role of glutathione depletion and reactive oxygen species generation in apoptotic signaling in a human B lymphoma cell line. *Cell Death Differ* 2002; 9(3): 252–263.
107. Pena-Llopis S, Ferrando MD, Pena JB. Impaired glutathione redox status is associated with decreased survival in two organophosphate-poisoned marine bivalves. *Chemosphere* 2001; 47(5): 485–497.
108. Brüne B, v. Knethen A, Sandau KB. Nitric oxide (NO): an effector of apoptosis. *Cell Death Differ* 1999; 6: 969–975.
109. Roux PP, Blenis J. ERK and p38 MAPK-activated protein kinases: a family of protein kinases with diverse biological functions. *Microbiol Mol Biol Rev* 2004; 68(2): 320–344.
110. Burlando B, Berti E, Viarengo A. Effects of seawater pollutants on protein tyrosine phosphorylation in mussel tissues. *Aquatic Toxicol* 2006; 78S: S79–S85.
111. Bulavin DV, Saito SI, Hollander MC, Sakaguchi K, Anderson CW, Appella E, Fornace Jr AJ. Phosphorylation of human p53 by p38 kinase coordinates N-terminal phosphorylation and apoptosis in response to UV radiations. *EMBO J* 1999; 18(23): 6845–6854.
112. Bacus SS, Gudkov AV, Lowe M, Lyass L, Yung Y, Komarov AP, Keyomarsi K, Yarden Y, Seger R. Taxol-induced apoptosis depends on MAP kinase pathway (ERK and p38) and is independent of p53. *Oncogene* 2001; 20(2): 147–155.
113. Wang Y, Sun L, Xia C, Ye L, Wang B. p38 MAPK regulates caspase-3 by binding to caspase-3 in the nucleus of human hepatoma Bel-7402 cells during anti-Fas antibody and actinomycin D-induced apoptosis. *Biomed Pharmacother* 2009; 63(5): 343–350.
114. Wei YQ, Zhao X, Kariya Y, Teshigawara K, Uchida A. Inhibition of proliferation and induction of apoptosis by abrogation of heat-shock protein (HSP) 70 expression in tumor cells. *Cancer Immunol Immunother* 1995; 40(2): 73–78.
115. Fabbri E, Valbonesi P, Franzellitti S. HSP expression in bivalves. *Invertebrate Survival J* 2008; 5: 135–161.
116. Sanders BM, Martin LS, Howe RR, Nelson WG, Hegre ES, Phelps DK. Tissue-specific differences in accumulation of stress proteins in *Mytilus edulis* exposed to a range of copper concentrations. *Toxicol Appl Pharmacol* 1994; 125(2): 206–213.
117. Ribera D, Narbonne JF, Daubeze M, Michel X. Characterization, tissue distribution and sexual differences of some parameters related to lipid peroxidation in mussels. *Marine Environ Res* 1989; 28(1–4): 279–283.
118. Kurata SI. Selective activation of p38 MAPK cascade and mitotic arrest caused by low level of oxidative stress. *J Biol Chem* 2000; 275(31): 23413–23416.
119. Takahashi KG, Mori K. Functional profiles of hemocytes in the bio-defense process of the Pacific oyster, *Crassostrea gigas*. *Tohoku J Agric Res* 2000; 51(1–2): 15–27.
120. Ciacci C, Betti M, Canonico B, Citterio B, Roch P, Canesi L. Specificity of anti-*Vibrio* immune response through p38 MAPK and PKC activation in the hemocytes of the mussel *Mytilus galloprovincialis*. *J Invertebrate Pathol* 2010; 105: 49–45.
121. Hahn UK, Bender RC, Bayne CJ. Production of reactive oxygen species by hemocytes of *Biomphalaria glabrata*: carbohydrate-specific stimulation. *Dev Comp Immunol* 2000; 24(6–7): 531–541.
122. Buggé DM, Hégaret H, Wikfors GH, Allam B. Oxidative burst in hard clam (*Merceneria merceneria*) heamocytes. *Fish Shellfish Immunol* 2007; 23: 188–196.
123. Barcia R, Ramos-Martinez JI. Effects of interleukin-2 on nitric oxide production in molluscan innate immunity. *Invertebrate Survival J* 2008; 5: 43–49.
124. Humphries JE, Yoshino TP. Cellular receptors and signal transduction in molluscan hemocytes: connections with the innate immune system of vertebrates. *Integr Comp Biol* 2003; 43: 305–312.
125. Canesi L, Betti M, Ciacci C, Lorusso LC, Pruzzo C, Gallo G. Cell signalling in the immune response of mussel hemocytes. *Invertebrate Survival J* 2006; 3: 40–49.
126. Gonzalez M, Romestand B, Fievet J, Huvet A, Lebart MC, Gueguen Y, Bachère E. Evidence in oyster of a plasma extracellular superoxide dismutase which binds LPS. *Biochem Biophys Res Commun* 2005; 338(2): 1089–1097.
127. Bao Y, Li L, Wu Q, Zhang G. Cloning, characterization and expression analysis of extracellular copper/zinc superoxide dismutase gene from bay scallop *Argopecten irradians*. *Fish Shellfish Immunol* 2009; 27(1): 17–25.

128. Hughes FM, Foster B, Grewal S, Sokolova IM. Apoptosis as host defense mechanism in *Crassostrea virginica* and its modulation by *Perkinsus marinus*. *Fish Shellfish Immunol* 2010; 29(2): 247–257.
129. Tafalla C, Gomez-Leon J, Novoa B, Figueras A. Nitric oxide production by carpet shell clam (*Ruditapes decussatus*) hemocytes. *Dev Comp Immunol* 2003; 27(3): 197–205.
130. Villamil L, Gomez-Leon J, Gomez-Chiarri M. Role of nitric oxide in the defenses of *Crassostrea virginica* to experimental infection with the protozoan parasite *Perkinsus marinus*. *Dev Comp Immunol* 2007; 31(10): 968–977.
131. Gifondorwa DJ, Leise EM. Programmed cell death in the apical ganglion during larval metamorphosis of the marine mollusc *Ilyanassa obsoleta*. *Biol Bull* 2006; 210: 109–120.
132. Messmer UK, Lapetina EG, Brüne B. Nitric oxide-induced apoptosis in RAW 264.7 macrophages is antagonized by protein kinase C- and protein kinase A-activating compounds. *Mol Pharmacol* 1995; 47(4): 757–765.
133. Chiapello LS, Baronetti JL, Garro AP, Spesso MF, Masih DT. Cryptococcus neoformans glucuronoxylomannan induces macrophage apoptosis mediated by nitric oxide in a caspase-independent pathway. *Int Immunol* 2008; 20(12): 1527–1541.
134. Huang WB, Ren HL, Gopalakrishnan S, Xu DD, Qiao K, Wang KJ. First molecular cloning of a molluscan caspase from variously colored abalone (*Haliotis diversicolor*) and gene expression analysis with bacterial challenge. *Fish Shellfish Immunol* 2010; 28(4): 587–595.