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OXIDATIVE STRESS IN MARINE ENVIRONMENTS: Biochemistry and Physiological Ecology

Michael P. Lesser
Department of Zoology and Center for Marine Biology, University of New Hampshire, Durham, NH 03824; email: mpl@unh.edu

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■ **Abstract** Oxidative stress—the production and accumulation of reduced oxygen intermediates such as superoxide radicals, singlet oxygen, hydrogen peroxide, and hydroxyl radicals—can damage lipids, proteins, and DNA. Many disease processes of clinical interest and the aging process involve oxidative stress in their underlying etiology. The production of reactive oxygen species is also prevalent in the world's oceans, and oxidative stress is an important component of the stress response in marine organisms exposed to a variety of insults as a result of changes in environmental conditions such as thermal stress, exposure to ultraviolet radiation, or exposure to pollution. As in the clinical setting, reactive oxygen species are also important signal transduction molecules and mediators of damage in cellular processes, such as apoptosis and cell necrosis, for marine organisms. This review brings together the voluminous literature on the biochemistry and physiology of oxidative stress from the clinical and plant physiology disciplines with the fast-increasing interest in oxidative stress in marine environments.

INTRODUCTION

Early History of Oxygen

The geological record provides convincing evidence of the long history of life on Earth, starting in the Archean as far back as 3.8 Gyr (1). The atmosphere of Earth was originally highly reduced and dominated by microbes (2), but by the mid-to-early Archean cyanobacteria capable of oxygenic photosynthesis had evolved (1, 2). With an abundance of carbon dioxide (CO_2), water (H_2O) as a reductant, and solar radiation, oxygenic photosynthesis by cyanobacteria spread and evolved into other taxa by serial endosymbioses (3). As a result, molecular oxygen, or dioxygen (O_2), appeared in significant amounts in the Earth's atmosphere ~2.5 Gyr and accumulated in the upper atmosphere. The accumulation of O_2 changed terrestrial and shallow oceanic habitats and provided strong selective pressures on anaerobic life forms existing at the end of the Archean. The evolution of aerobic respiration,

with its greater efficiency and higher yields of energy, is believed to have been critical to the development of complex multicellular eukaryotic organisms.

Theory of Oxygen Toxicity

It has only been fifty years since it was proposed that free radicals are responsible for the toxic effects of oxygen (4). Atmospheric O_2 in its ground state is distinctive among the elements because it has two unpaired electrons (and thus is known as a biradical) (5–8). This property makes O_2 paramagnetic, which significantly limits its ability to interact with organic molecules unless it is "activated." The univalent reduction of molecular oxygen produces reactive intermediates such as the superoxide radical (O_2^-), singlet oxygen ($^1O_2$), hydrogen peroxide (H_2O_2), hydroxyl radical ($HO^\bullet$), and finally water (H_2O) (5–8). Often biologists label all of the reduction products of oxygen as free radicals. As defined above, however, a free radical is an atom or molecule with an unpaired electron. It therefore is more appropriate to refer to the intermediate reduction products of oxygen as activated and not as free radicals, but for consistency I use reactive oxygen species (ROS) throughout and include H_2O_2 in that definition.

All photosynthetic and respiring cells produce ROS, including O_2^- via the univalent pathway; H_2O_2 is formed by the continued reduction of O_2^-; and eventually $HO^\bullet$ is formed and then reduced to the hydroxyl ion and water (5–8). For biological systems the production of ROS is directly and positively related to the concentration of O_2 (9). Oxidative stress, the production and accumulation of ROS beyond the capacity of an organism to quench these reactive species, can damage lipids, proteins, and DNA, but ROS can also act in signal transduction (5–8). The central purposes of antioxidant defenses in biological systems are to quench $^1O_2$ at the site of production and to quench or reduce the flux of reduced oxygen intermediates such as O_2^- and H_2O_2 to prevent the production of $HO^\bullet$, the most damaging of the ROS (5–8).

Reactive Oxygen Species

SINGLET OXYGEN In biological systems $^1O_2$ is produced through several photochemical and chemical pathways. Singlet oxygen is often produced by photosensitization reactions in which molecules absorb light of a specific wavelength and are raised to a higher energy state. The energy can then be passed to O_2 and forms $^1O_2$ while the sensitizing molecule returns to its ground state. The lifetime of $^1O_2$ is $\sim$3.7 μs in aqueous media. Its high reactivity with cellular components is controlled primarily by diffusion, whose mean distance has been estimated to be $\sim$82 nm; therefore, site-specific effects in biological systems are likely to occur with this ROS (5–8).

SUPEROXIDE RADICALS O_2^- can act as either an oxidant or a reductant in biological systems. The dismutation of O_2^-, leading to the formation of H_2O_2, occurs spontaneously or is catalyzed by the antioxidant enzyme superoxide dismutase with

a rate constant of 2×10^9 mol^{-1} s^{-1} (5). In its protonated state (pKa = 4.8) O_2^- forms the perhydroxyl radical ($^\bullet OOH$), which is a powerful oxidant (5–8), but its biological relevance is probably minor because of its low concentration at physiological pH. Within the aprotic interiors of biological membranes, such as mitochondrial or thylakoid membranes, O_2^- is stable (10, 11) and can diffuse across the membrane in a concentration-dependent manner but at extremely slow rates (2.1×10^{-6} cm s^{-1}) (5–8). Although SOD reduces the steady-state concentration of O_2^- by several orders of magnitude, O_2^- still has significant, and independent, damaging potential (12) with a lifetime of 50 μs and a diffusion distance of ~320 nm (5–8).

HYDROGEN PEROXIDE Because hydrogen peroxide is uncharged, it readily diffuses across biological membranes. H_2O_2 causes significant damage because it is not restricted to its point of synthesis in the cell and can enter into numerous other reactions. Exposure to H_2O_2 can damage many cellular constituents directly, such as DNA and enzymes involved in carbon fixation (5–8). H_2O_2 is also involved in pathways such as programmed cell death, or apoptosis (8). If H_2O_2 is further reduced, it can produce $HO^\bullet$. One source of electrons for that reduction in biological systems is transition metals via so-called Fenton chemistry, such as the conversion of Fe from its ferrous to ferric form (5–8).

HYDROXYL RADICAL The $HO^\bullet$ is the most reactive oxygen radical. It has tremendous potential for biological damage because it attacks all biological molecules in a diffusion-controlled fashion, with a lifetime of 10^{-7} s and mean diffusion distance of 4.5 nm. It also tends to initiate free radical chain reactions, can oxidize membrane lipids, and causes proteins and nucleic acids to denature (5–8). The production of $HO^\bullet$ in biological systems is regulated by the availability of ferrous iron. Any recycling of iron from the ferric to the ferrous form by a reducing agent can maintain an ongoing Fenton reaction, leading to the generation of $HO^\bullet$. One excellent reducing agent is O_2^-, which participates in the metal-catalyzed Haber-Weis reaction (6–8). Metals other than iron (e.g., copper) may also participate in these electron transfer reactions by cycling between the oxidized and reduced states.

REACTIVE NITROGEN SPECIES Many cells also produce nitric oxide, or nitrogen monoxide ($NO^\bullet$), a molecule implicated initially in neurotransmission but now a known participant in diverse processes involving oxidative stress (13). Nitric oxide synthase produces $NO^\bullet$, which can react with O_2^- to form the peroxynitrite anion ($ONOO^-$), a potent oxidant (12). Because the solubility of $NO^\bullet$ is similar to that of H_2O, the former can readily diffuse across biological membranes. It can then react at near-diffusion-limited rates with free radicals, especially O_2^-, to form $ONOO^-$, which can diffuse across biological membranes at rates 400 times greater than does O_2^- (14, 15). The half-life of $ONOO^-$ is <0.1 s at physiological pH, mostly because of its high reactivity with organic molecules, especially lipids.

The high concentrations of $NO^{\bullet}$ may create significant competition between $NO^{\bullet}$ and SOD for O_2^-. This balance between the competition for O_2^- may be a major determinant of oxidative stress in many organisms. Many investigators are now re-evaluating the role of O_2^- in oxidative stress because of these new insights and because many of the observed in vitro effects ascribed to O_2^- may in fact be mediated by $ONOO^-$ (8).

Cellular Sites of ROS Production

CHLOROPLASTS Chloroplasts, because of their photosynthetic nature, are hyperoxic, produce ROS, and are susceptible to oxidative stress. ROS in the chloroplast may damage photosystem (PS) II, primarily through oxidative degradation of the D1 protein (5, 16–19), and also inhibit the repair of damage to PS II (20). In addition to $^1O_2$ (21), O_2^- and $HO^{\bullet}$ also are produced in the PS II reaction center (22). The reducing side of PS I can reduce O_2 to O_2^- by the Mehler reaction and is the most significant site of O_2^- production in the chloroplast (5, 16, 23). The production of O_2^- increases under stressful conditions, such as exposure to xenobiotics or pollutants, high visible irradiances, exposure to ultraviolet radiation (UVR), and/or exposure to thermal stress. This elevated production can overwhelm antioxidant defenses to produce damage to both PS II and to the carbon fixation process (5, 16).

MITOCHONDRIA Two main sites of O_2^- generation in the inner mitochondrial membrane are (*a*) NADH dehydrogenase at complex I and (*b*) the interface between ubiquinone and complex III. Once generated, the O_2^- is then converted to H_2O_2 by spontaneous dismutation or by SOD (24). The integrity of the inner membrane and the associated complexes is essential to oxidative phosphorylation. The inner membrane is also permeable to H^+. Although it causes energy loss, this H^+ leakage can be beneficial because it reduces ROS production. The loss of energy and the production of ROS via H^+ leakage can also be regulated by specific uncoupling proteins, which themselves are upregulated by the production of ROS (25).

ENDOPLASMIC RETICULUM The endoplasmic reticulum of animals, plants, and some bacteria contain cytochromes collectively known as cytochrome P-450. Cytochrome P-450 is involved in several detoxification processes, including hydroxylations, dealkylations, deaminations, dehalogenations, and desaturations that involve the reduction of O_2 (8). These mixed-function oxygenase (MFO) reactions add an O_2 atom to an organic substrate using NADPH as the electron donor. Superoxide can be produced by microsomal NADPH-dependent electron transport involving cytochrome P-450.

MICROBODIES Peroxisomes and glyoxysomes are subcellular organelles that contain enzymes involved in the β-oxidation of fatty acids and in photorespiration, such as glycolate oxidase, catalase, and several peroxidases. Found in both animals and plants, these organelles were initially believed to be involved in detoxification

reactions and the quenching of H_2O_2. The H_2O_2 synthesized by these microbodies may also contribute to the pool of signal transduction molecules (26). In the glyoxisomes of plants, glycolate oxidase produces H_2O_2 in a two-electron transfer from glycolate to oxygen (26). In addition to H_2O_2, glyoxisomes produce O_2^- via a xanthine oxidase reaction with purines (26, 27). The dismutation of O_2^- to H_2O_2 also occurs via SOD in both peroxisomes and glyoxisomes (28).

THE DUAL ROLE OF REACTIVE OXYGEN SPECIES

Oxidative Damage

Reactive oxygen species are both agents of disease and cellular damage, but they are also participants in many normal cellular functions. Below I briefly describe some of the major sites of damage and pathways in which ROS plays an important regulatory role.

OXIDATIVE DAMAGE TO LIPIDS The reaction of ROS, especially of $HO^\bullet$, with lipids is one of the most prevalent mechanisms of cellular injury and is dependent on the degree of membrane fluidity, which in turn is a function of the saturation state of the lipid bilayer (8). The degradation products of lipid peroxidation are aldehydes, such as malondialdehyde, and hydrocarbons, such as ethane and ethylene (29, 30). Lipid peroxidation in mitochondria is particularly cytotoxic, with multiple effects on enzyme activity and ATP production as well as on the initiation of apoptosis (31).

OXIDATIVE DAMAGE TO PROTEINS Oxidative attack on proteins results in site-specific amino acid modifications, fragmentation of the peptide chain, aggregation of cross-linked reaction products, altered electrical charge, and increased susceptibility to removal and degradation. The amino acids in a peptide differ in their susceptibility to attack, and the various forms of ROS also differ in their potential reactivity. The primary, secondary, and tertiary structure of a protein determines the susceptibility of each amino acid to attack by ROS (8, 30). For many enzymes, the oxidation by O_2^- of iron-sulphur centers inactivates enzymatic function (30, 32), and other amino acids, such as histidine, lysine, proline, arginine, and serine, form carbonyl groups when oxidized (33). A wide range of proteins and their amino acid building blocks is damaged or degraded by ROS (34), and the accumulation of these proteins in cells has been hypothesized to be part of the aging process (35).

OXIDATIVE DAMAGE TO DNA The generation of ROS can induce numerous lesions in DNA that cause deletions, mutations, and other lethal genetic effects. Both the sugar and the base moieties are susceptible to oxidation, causing base degradation, single-strand breakage, and cross-linking to proteins (36, 37). In vitro,

H_2O_2 or O_2^- cannot by themselves cause strand breaks under normal physiological conditions, and therefore, their toxicity in vivo is most likely the result of Fenton reactions in the presence of a transition metal (36, 37). Both prokaryotic and eukaryotic cells have DNA repair enzymes; for a cell with DNA damage, it is the balance between damage and repair that determines the fate of that cell (38).

Signal Transduction

ROS are also produced for specific cellular functions, and it has been proposed that the antioxidant systems of cells regulate intracellular levels of ROS so that they can function as second messengers (39). ROS as second messengers are important for the expression of several transcription factors and other signal transduction molecules such as heat shock–inducing factor, nuclear factor, the cell-cycle gene *p*53, mitogen-activated protein kinase, and *oxy*R gene products (40, 41).

Oxidative stress also plays a role in apoptosis through several cell-cycle genes (42). Two apoptotic pathways, the death-receptor and the mitochondrial pathways, have been described. The mitochondrial pathway is commonly associated with DNA damage and upregulation or activation of *p*53 (43). Exposure to UVR also causes ROS production in the electron transport chain of mitochondria (44) and can lead to apoptosis through the activation of caspases (45). Both cellular necrosis and apoptosis, which have overlapping features, can result from oxidative stress and lead to cell death (42). Whereas high levels of oxidative stress cause cell necrosis, lower levels either cause DNA damage and cell-cycle arrest or initiate apoptosis (8, 41).

Exposure to ROS and subsequent apoptosis are also common in higher plants, and many caspases homologous to animal caspases have been identified (46, 47). ROS are also an important component of plant defense systems against pathogens (48); O_2^- is directly involved in the apoptotic hypersensitive reaction of higher plants against pathogens (49). Interestingly, caspases have also been identified in unicellular photoautotrophic eukaryotes (i.e., phytoplankton) as well as in simple metazoans, and when compared to more derived plant and metazoan caspases, they are regulated in a similar fashion during experimentally induced apoptosis (50, 51).

One of the most interesting signal transduction roles for ROS is the mediation of morphogenic events associated with the onset of mutualistic symbiotic associations. The symbiosis of the serpiolid squid (*Euprymna scolopes*) light organ and the bioluminescent bacterium *Vibrio fisheri* is one of the best understood systems in terms of the attraction, initiation, and ultimate establishment of a symbiotic association that involves dramatic changes in host morphology to accommodate the symbionts (52). When the *V. fisheri* cells enter the eventual light organ, they first encounter the hostile environment of the ducts and then the crypt space, which includes epithelial cells that line the crypt (52). Potential symbionts associate themselves with the microvilli of the crypt epithelial cells and induce changes in the light-organ crypt epithelial cells that help maintain this unique symbiosis (52). Macrophages are abundant in the light-organ crypt and apparently patrol this

space for nonspecific bacteria, while leaving symbiotic bacteria unharmed (53). Additionally, the epithelial cells apparently secrete a halide peroxidase that produces bacteriocidal hypohalous acid from H_2O_2, which presumably comes from the phagocytic activity of the macrophages (52, 53). Colonization of the crypt by *V. fisheri* in this hostile environment requires the removal of H_2O_2 using a catalase enzyme that is required for bacterial competency to successfully establish the symbiotic association (52–54). In addition, the luciferase enzyme of *V. fisheri* is a MFO that utilizes molecular oxygen (54). The luciferase enzyme is expressed in large quantities and, combined with bacterial respiration, can maintain a low pO_2 that subsequently results in lower ROS production (9) and an environment conducive to the successful maintenance of the symbiosis (54).

ANTIOXIDANT DEFENSES

Enzymatic Antioxidants

SUPEROXIDE DISMUTASE Superoxide dismutase (SOD) (EC 1.15.1.1), originally discovered by McCord & Fridovitch (55), occurs as different metalloproteins with different cellular distributions. The Cu/Zn SOD is principally a cytosolic enzyme in eukaryotes but is also found in chloroplasts, bacteria, and peroxisomes, and as an extracellular enzyme (8, 28). The Mn form of SOD is principally found in mitochondria and bacteria, and the Fe SOD is found in chloroplasts and bacteria (5–8). The prokaryotic Mn SOD and Fe SOD and the eukaryotic Cu/Zn SOD are dimers, whereas the Mn SODs of mitochondria are tetramers, with each subunit consisting of 151 amino acids (5–8). All forms of the SOD are nuclear-encoded and are targeted to their respective subcellular compartments by an amino-terminal targeting sequence (56). SOD is an efficient catalyst and can keep the steady-state concentration of O_2^- at 10^{-10} mol liter^{-1} (5–8). With SOD concentrations at 10^{-5} mol liter^{-1}, any molecule of O_2^- is more likely to encounter a molecule of SOD then another O_2^- (5–8). At a rate constant (k_2) of 2×10^9 mol liter^{-1} s^{-1}, the lifetime of O_2^- is significantly shortened by SOD (5–8).

Prokaryotic cells and many eukaryotic algae contain the Mn SOD and Fe SOD enzymes, which are believed to be more ancient forms of SOD, whereas some phytoplankton also contain a Ni metalloprotein (57). Protein sequence data clearly show two distinct evolutionary paths for the Cu/Zn and the Fe/Mn SODs, and within the Cu/Zn SOD clade there is a varying degree of conservation in the protein sequences (5–8). The evolution of the Mn and Fe forms of SOD, most likely from a common ancestral protein, is attributed to the availability of the Mn and Fe metal cofactors under conditions when O_2 was four orders of magnitude lower than it is today (58). The Cu/Zn form has been reported to be present only in higher plants and animals and in the Charophyacean alga, *Spirogyra* sp. (59). These data suggest that the divergence of the chloroplast and cytosolic forms of the Cu/Zn SOD occurred very early in the evolution of the protein (59). There is, however, evidence that the Cu/Zn SOD exists in unicellular eukaryotic algae,

specifically dinoflagellates (60–62), whose evolutionary history extends back to the early Jurassic and which contain plastids derived from an ancestral red alga by secondary symbiosis (3).

CATALASE Catalase (EC 1.11.1.6) is a heme-containing enzyme that catalyzes the conversion of H_2O_2 to H_2O and O_2. The enzyme is a tetramer with molecular weights in excess of 220 kD and has a high K_m for H_2O_2, which makes it most efficient at scavenging high concentrations of H_2O_2 (5–8). An unusual feature of catalase is its sensitivity to light and rapid turnover, which may result from light absorption by the heme group. Conditions that reduce the rate of protein turnover, such as osmotic, heat, or cold stress, can lower catalase activity (5–8, 63). For photoautotrophs, this feature of the catalase enzyme may affect their ability to tolerate oxidative stress when exposed to environmental perturbations. Phylogenetically, catalases from plants and animals are unique and divergent from those of bacteria and fungi, with bacteria containing several separate lineages (64).

PEROXIDASES Peroxidases, like catalase, catalyze the reduction of H_2O_2 to H_2O, but they require a source of electrons that subsequently becomes oxidized. Ascorbate peroxidase (EC 1.11.1.11) is a heme-containing monomeric enzyme with a molecular mass of 30 kD (5). It has a significantly lower K_m for H_2O_2 than does catalase and uses a large pool (10–20 mM) of ascorbate as its specific electron donor to reduce H_2O_2 to H_2O in the stroma and on the thylakoids of chloroplasts (5).

Glutathione peroxidase (EC 1.11.1.9) is a tetrameric enzyme with a molecular weight of 84 kD. The enzyme is found in both selenium-containing and selenium-independent forms in the cytosol and mitochondria of animal tissues, but not in plants (8). This enzyme catalyzes the oxidation of glutathione, a low-molecular-weight tripeptide thiol compound, with H_2O_2 (8). Glutathione is very abundant in animal tissues through the action of glutathione reductase, which regenerates reduced glutathione (8).

Nonenzymatic Antioxidants

ASCORBIC ACID L-ascorbic acid, or vitamin C, is an essential vitamin in animals and is abundant in plant tissues. All plants and animals, except humans, can synthesize ascorbate de novo; animals also can obtain vitamin C through their diet. Ascorbate functions as a reductant source for many ROS, thereby minimizing the damage caused by oxidative stress. Ascorbate scavenges not only H_2O_2 but also O_2^-, $HO^\bullet$, and lipid hydroperoxides without enzyme catalysts (5–8), and it can indirectly scavenge ROS by recycling α-tocopherol to its reduced form. Ascorbate has been found in plant cell chloroplasts and cytosol, where it also acts as a substrate for ascorbate peroxidase.

GLUTATHIONE Glutathione (GSH) is a tripeptide (Glu-Cys-Gly) found in animals and plants. It forms a thiyl radical that reacts with a second oxidized glutathione, forming a disulphide bond (GSSG) when oxidized (8). The ratio of GSH/GSSG is often used as an indicator of oxidative stress in cells, and glutathione functions as an antioxidant in many ways by reacting with $^1O_2$, O_2^-, and $HO^\bullet$. Glutathione can also act as a chain-breaker of free radical reactions and is an essential substrate for glutathione peroxidase (8). The maintenance of GSH levels, and therefore the reducing environment of cells, is crucial in preventing damage to cells exposed to conditions that promote oxidative stress.

TOCOPHEROL The tocopherols, specifically α-tocopherol (vitamin E), are lipid-soluble antioxidants that scavenge ROS (5–8). This phenolic antioxidant is found in both animals and plants. α-tocopherol, due to its hydrophobic nature, is located exclusively within the bilayers of cell membranes. α-tocopherol is generally considered to be the most active form of the tocols. Plants synthesize α-tocopherol in chloroplasts, with the aromatic ring formed by the shikimic acid pathway—the same pathway that produces UVR-absorbing compounds, the mycosporine-like amino acids (MAAs; see below), in many marine algae. By contrast, animals must acquire tocopherol through their diet. The antioxidant properties of tocopherol are the result of its ability to quench both $^1O_2$ and peroxides (5–8). A marine-derived tocopherol known as α-tocomonoenol has been isolated from salmon eggs and provides enhanced antioxidant protection because of its ability to diffuse in viscous lipids and prevent lipid peroxidation (65).

CAROTENOIDS Carotenoids are lipid-soluble molecules that protect both plants and animals against oxidative damage. Photoautotrophs produce carotenoids de novo, whereas animals must acquire carotenoids dietarily. In photosynthetic organisms, some carotenoids function as accessory pigments in light harvesting, whereas others specifically quench ROS produced as a result of overexcitation of the photosynthetic apparatus by light (5–8, 66). β-carotene can quench both excited triplet-state chlorophyll and $^1O_2$ because they have highly conjugated double bonds. Carotenoids can also dissipate excess excitation energy through the xanthophyll cycle (5–8, 67), a process, also known as dynamic photoinhibition, that prevents the overexcitation of the photosynthetic apparatus. Many carotenoids also serve as effective quenchers of ROS and can prevent lipid peroxidation in marine animals (68).

SMALL-MOLECULE ANTIOXIDANTS Uric acid, a product of purine metabolism, can quench both $^1O_2$ and $HO^\bullet$ (69). It is found in high concentrations in marine invertebrates, in which it can be a potent antioxidant (70). Another group of small-molecule antioxidants is compatible solutes (71). In particular, mannitol can quench $HO^\bullet$ and prevent damage to critical carbon-fixing enzymes in photoautotrophs (72). Dimethylsulfide (DMS) is an important component of global sulfur cycles and a significant contributor to aerosol fractions in the atmosphere

(73). Many species of marine macrophytes and phytoplankton produce DMS from dimethylsulphoniopropionate (DMSP), whose primary function had been assumed to be as an osmolyte (73). Both DMS and DMSP have been shown to quench $HO^{\bullet}$. DMS can diffuse through biological membranes and act as an effective antioxidant in any cellular compartment (74).

Mycosporine-like amino acids are UVR-absorbing compounds with broadband absorption from 310–360 nm. They have been extensively studied in a wide variety of marine organisms (75). Some MAAs have antioxidant activity (76–78). These compounds are synthesized de novo by the shikimic acid pathway in photoautotrophs but are acquired by animals through their diet (75). Mycosporine-glycine can quench $^1O_2$ (79), whereas other MAAs can quench O_2^- (78). In reef-forming corals, mycosporine-glycine concentrations decline significantly upon exposure to prolonged high-temperature stress, while antioxidant enzymes increase (80). The concentration of MAAs in corals declines with increasing depth in proportion to photooxidative potential caused by exposure to UVR and hyperoxia due to photosynthesis (75).

OXIDATIVE STRESS IN THE MARINE ENVIRONMENT

Reactive Oxygen Production in Seawater

In marine systems, the absorption of solar radiation, and especially of its UVR wavelengths, by dissolved organic matter in seawater leads to the photochemical production of diverse reactive transients, including ROS (81). These ROS may have deleterious effects on bacteria and phytoplankton by affecting cell membranes or inhibiting photosynthesis. Hydrogen peroxide has the longest lifetime in seawater and the highest steady-state concentrations (10^{-7} M) and can readily pass through biological membranes (5–8, 81).

Hydrothermal vents also produce ROS (82). The abundance of hydrogen sulfide (H_2S) and O_2 near vents leads to the oxidation of H_2S in seawater and the production of both oxygen- and sulfur-centered radicals (82). In particular, electron paramagnetic resonance spin-trapping has shown convincingly that sulfide oxidation produces O_2^- (82). High concentrations of O_2^- near vents probably leads to H_2O_2 production by the dismutation of O_2^- and to subsequent oxidative stress for vent fauna. As may be expected, vent worms (*Riftia pachyptila*), vent clams (*Calyptogena magnifica*), and their bacterial symbionts all express SOD and exhibit peroxidase activity (83).

Oxidative Stress in Marine Organisms

Marine organisms are exposed to and adjust to a wide variety of environmental factors on varying temporal and spatial scales, from polar to tropical and from hourly to seasonal, in order to maintain homeostasis and growth and to reproduce. It should be no surprise that, just like any other metabolic pathway, those processes

that lead to the production of ROS vary significantly over large gradients in many environmental factors, and adjustments in antioxidant defenses are required in order to maintain the steady-state concentration of ROS at low levels and thus prevent oxidative stress and cellular damage.

Although antioxidant protection is almost always associated with aerobic organisms, there exists a wide spectrum of oxygen tolerance in anaerobic organisms, principally prokaryotes (8, 37). Specialized anaerobic bacteria from hydrothermal vent environments have evolved novel enzymes to quench O_2^- without producing oxygen, which would also be toxic (84). *Pyrococcus furiosus*, a hyperthermophilic anaerobic bacterium, contains a superoxide reductase that reduces O_2^- to H_2O_2, which is then reduced to H_2O by peroxidases (84). The superoxide reductase maintains its activity at 25°C, which is far below the growth optimum of 100°C for this bacterium but may be adaptive, as these free-living bacteria in the hydrothermal fluids are mixed with the surrounding cold water (84).

Many bacteria contain Fe and Mn SOD, but several also contain Cu/Zn SOD (8). These Cu/Zn SODs are distinct from and may be the evolutionary precursor to eukaryotic Cu/Zn SODs (8). One unique example of a prokaryotic Cu/Zn SOD is from the bacterium *Photobacterium leiognathi*, a bioluminescent bacterium symbiotic with pony fish (85). Originally believed to arise from horizontal gene transfer from eukaryotes to prokaryotes, differences in the gene sequence of the *P. leiognathi* and other prokaryotic Cu/Zn SODs, as well as important differences in gene structure and function, actually support a prokaryotic origin for the Cu/Zn SODs (86). One of the important attributes of these bacterial symbioses is the use of bioluminescence as a mechanism of signaling between con-specific hosts. The luciferase enzyme that produces bioluminescence is a MFO that utilizes molecular oxygen. Several recent studies strongly support the hypothesis that the original selective pressure for the evolution of luciferase was to prevent oxidative stress and that it then was co-opted for its bioluminescent characteristic, originally a by-product of its antioxidant activities that can still be experimentally demonstrated in *V. harveyi* (87–90).

The production of ROS is a consistent feature of photoautotrophs, and marine algae are no exception. In unicellular eukaryotic algae, especially dinoflagellates, all three metalloproteins of SOD have been identified (60–62, 91). Many of these cells exhibit a daily cycling of maximum SOD activities and other antioxidant enzymes that are associated with peak midday irradiances and the production of ROS (61, 62, 91, 92). At least one study has demonstrated that this daily rhythm is under transcriptional control and that new SOD protein is produced on a daily basis (91). Other studies have reported distinct seasonal regulation of antioxidant enzymes based on total daily irradiance in addition to daily rhythms (62, 92). Some of these species are toxic to bacteria and fish owing to production of extracellular ROS (93, 94).

Green, brown, and red macrophytes are conspicuous components of many marine ecosystems, but especially of rocky intertidal systems, in which many species of attached seaweed are dominant members of the community. These algae

withstand some of the harshest environmental conditions known, including freezing, desiccation, carbon limitation, and heat stress. These environmental extremes are conducive to the formation of ROS and contribute to the photoinhibition of photosynthesis observed in these ecologically important marine algae. The production of ROS in the brown alga *Fucus evanescens* has been detected with fluorescent dyes (95) and is enhanced in freezing, high light, and desiccation stress (96–98). The increase in ROS production also causes an increase in lipid peroxidation and a decrease in the quantum yield of PSII fluorescence (96). Additionally, species vary in susceptibility to oxidative stress (97) and in seasonal acclimatization of antioxidant defenses to changes in temperature-induced oxidative stress (98). Two red algae, *Mastocarpus stellatus* and *Chondrus crispus*, exhibit zonational patterns in temperate rocky intertidal ecosystems that reflect their ability to resist freezing and the accompanying oxidative stress (99, 100). The activities of enzymatic and nonenzymatic antioxidants increase with tidal height, as does, therefore, daily exposure to air temperatures, for *M. stellatus*, which always has greater antioxidant capabilities than *C. crispus*, which is found in the lower intertidal zone (99). Seasonal acclimatization to irradiance, both its visible and ultraviolet components, also occurs in macrophytes at high latitudes, where the amplitude in the changes in seawater temperature is low (101). Macrophytes exposed to increased visible radiation and UVR during the breakup of sea ice increase SOD, catalase, and MAAs, all of which prevent oxidative stress and its subsequent effects on photosynthesis (101).

Many marine invertebrates produce ROS. Bivalve molluscs produce ROS in response to xenobiotics (102) and changes in temperature, especially heat stress (103). ROS are also important in the cell-mediated immune response of molluscs to both prokaryotic and eukaryotic pathogens (104). Interestingly, many bivalve molluscs are euryoxic and survive fluctuations between hypoxia/anoxia and normoxia with each tidal cycle. During anoxic-normoxic transitions, euryoxic species produce far less ROS and therefore avoid oxidative stress (105).

Sponges (Phylum: Porifera) with symbiotic cyanobacteria undergo elevated pO_2 in their tissues from photosynthetically produced O_2 (106, 107). Exposure to summertime highs in seawater temperature result in the highest values of total oxidative scavenging capacity and catalase, which is attributed to the production of H_2O_2 (107). Similar temperature-related increases in prooxidant pressure have been observed in the eurythermal lug worm, *Arenicola marina* (Annelida: Polychaeta) (108). The increase in ROS production is associated with an increase in mitochondrial substrate oxidation and higher rates of proton leakage in summer animals as compared to winter animals (108). Oxybiotic meiofauna also can experience photochemically generated ROS in intertidal pools (109). H_2O_2 can diffuse across the redoxcline, and annelid worms such as *Nereis diversicolor* respond by increasing their activities of catalase (109). Worms maintained in anoxic conditions also increase SOD activities, which is, again, a physiological adaptation to withstand the transition from anoxia to normoxia and the subsequent burst in the production of ROS (109). Thiobiotic meiofauna, including gastrotrichs and turbellarians, living in anoxia and exposed to H_2S also have higher activities of

antioxidant enzymes than their oxybiotic counterparts and may be exposed to oxygen- and sulfur-based radicals like their hydrothermal vent cousins (110).

Marine arthropods (i.e., crabs, lobsters, and shrimp) vary in their antioxidant defenses according to their level of aerobic metabolism, exposure to chronically cold environments, or exposure to UVR (111–113). Surprisingly, marine arthropods lack Cu/Zn SOD (114, 115). Instead, these animals use a copper-dependent hemocyanin for oxygen transport and have an unusual cytosolic Mn SOD lacking the signal transit peptide that would otherwise direct it to the mitochondrion (115). This occurs in all Crustacea that use a copper-dependent oxygen transport system and is believed to be evolutionarily linked to the fluctuation in copper metabolism induced by the use of copper-dependent oxygen transport systems (115).

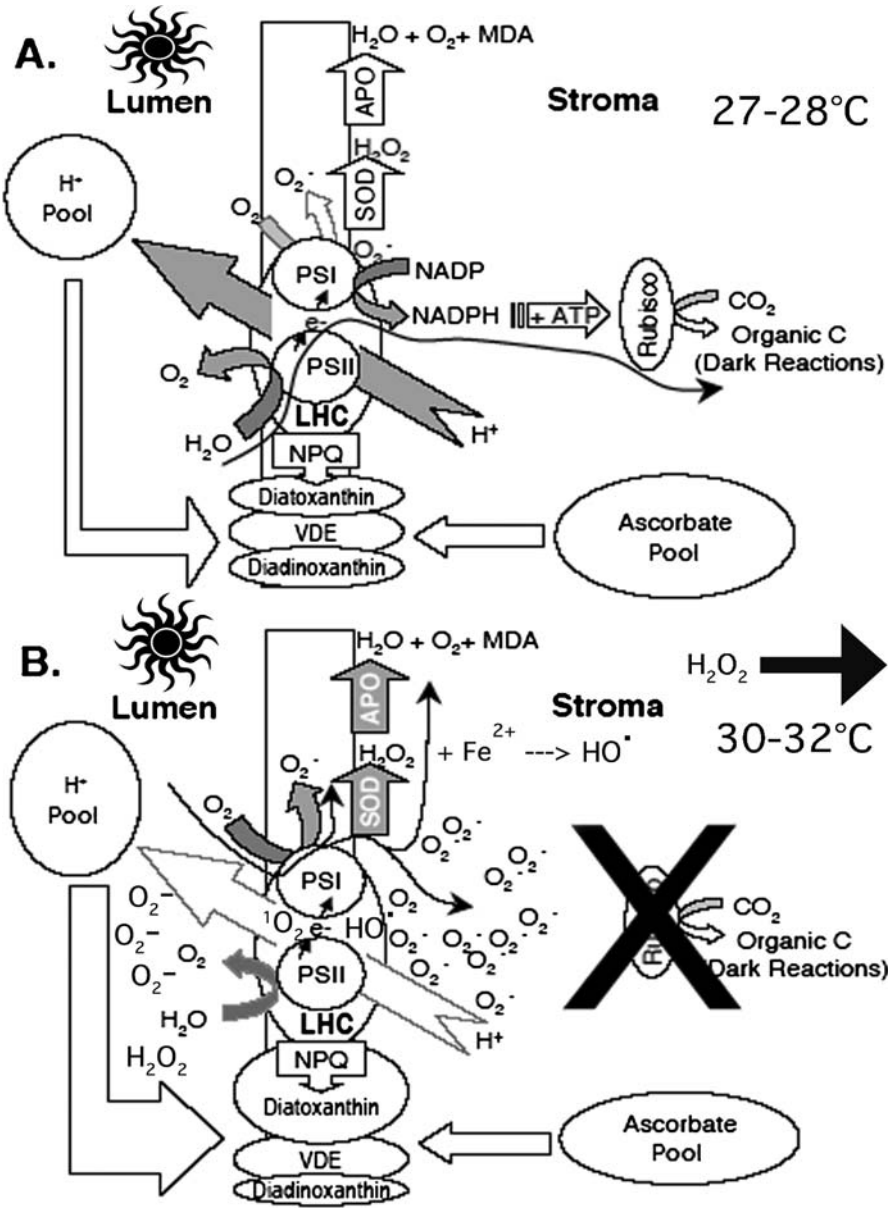
Studies on oxidative stress in echinoderms (i.e., sea stars, sea urchins, sea cucumbers, and crinoids) are few. To prevent polyspermy, sea urchins create a physical barrier to multiple fertilizations of a single ovum. They accomplish this by hardening the vitelline membrane to create the fertilization membrane, which raises and hardens as a result of covalently cross-linked products of the oxidation of tyrosyl residues released by the cortical granules. This reaction requires extracellular H_2O_2, which is formed by a membrane-bound NADPH oxidase during a respiratory burst upon fertilization (116). The excess H_2O_2 is quenched by a secreted peroxidase and ovothiol C, a nonenzymatic scavenger of H_2O_2 (116). Although the H_2O_2 produced is extracellular, this H_2O_2, if not scavenged in the extracelluar space, may diffuse back into the now newly fertilized zygote. Recently, major yolk proteins from sea urchin eggs, which were believed to be vitellogenin, have been identified as transferrin-like, iron-chelating proteins (117) that could potentially be very useful in preventing H_2O_2 from participating in Fenton chemistry within the zygote.

Many echinoderms are important broadcast-spawning members of benthic marine communities, and their planktonic embryos and larvae may therefore be susceptible to the detrimental effects of UVR. Although total exposure to UVR is dependent on the stability and optical properties of the water column, planktonic larvae can be easily advected into surface waters, where irradiances of UVR are higher. Sea urchin embryos exposed to UVR irradiances equivalent to shallow temperate coastal environments show symptoms of oxidative stress, as indicated by elevated concentrations of SOD protein, DNA damage, and apoptosis (118). Field exposures of embryos at fixed depths reveal similar results down to a depth of 8 m (M.P. Lesser, unpublished data). Both laboratory and field experiments also show abnormal embryonic morphology typical of that seen in apoptosis (118).

Marine vertebrates are not immune from oxidative stress. Fish in particular, including those from the Antarctic, have several well-characterized Cu/Zn SODs (119–121) that have evolved to maintain catalytic function over a wide range of temperatures (121–122). Fish also respond to prooxidant pressure due to differences in metabolic rates (123), pollution (124), and exposure to UVR (125). As in sea urchin embryos, the larvae of Atlantic cod (*Gadus morhua*) exposed to UVR show significant increases in SOD activity and in expression of the cell-cycle gene *p*53 (125).

Physiological Extremes

A relatively new area of investigation is oxidative stress in polar, especially Antarctic, environments (122). Because solubility of O_2 is high in the constant, $-1.8°C$, seawater temperatures of Antarctica, polar ectotherms potentially experience



increased prooxidant pressure and metabolic costs associated with antioxidant defenses. But low temperatures also reduce the conductance of O_2 because of changes in tissue viscosity. Increases in mitochondrial volume density and lipid stores may potentially compensate for this decreased conductance (122). The increased unsaturation of membranes in Antarctic ectotherms can also promote lipid peroxidation by ROS unless antioxidant defenses are available. Indeed, the Antarctic bivalve, *Laternula elliptica*, exhibits a greater potential for lipid peroxidation than does the temperate species, *Mya arenaria*, with similar total lipid concentrations (126). But *L. elliptica* also has higher concentrations of α-tocopherol and β-carotene, both lipid-soluble antioxidants known for their lipid peroxidation chain–breaking capabilities (5–8, 66). Polar invertebrates that may be predisposed to oxidative stress also contain higher activities of antioxidant enzymes. The Antarctic scallop, *Adamussium colbecki*, has significantly higher activities of SOD in its gills as compared to the Mediterranean scallop, *Pecten jacobaeus* (127). Consistent with the chronically cold environment in which it lives, *A. colbecki* also exhibits seasonally invariant antioxidant capacities except during the austral spring phytoplankton bloom or during reproduction (128). Studies to date, mostly involving measurements of enzyme activity, generally support that antioxidant enzymes compensate for exposure to chronically cold seawater temperatures (129, 130). Whether these compensation strategies are quantitative or qualitative, *sensu* Hochachka & Somero (131), is unknown.

One of the best-understood marine invertebrate systems, as relating to oxidative stress, is that of cnidarians (i.e., sea anemones, corals, and jellyfish) with symbiotic zooxanthellae. In particular, reef-forming corals are important members of

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Figure 1 Detail of events leading to oxidative stress on the thylakoid membrane of the chloroplast of zooxanthellae. (*A*) During normal temperatures and irradiances, light is absorbed by the light-harvesting complex (LHC) and photochemistry in PSI and PSII produce ATP and NADPH for the dark reactions, in which CO_2 is fixed by the enzyme Rubisco. The efficiency of photochemistry is regulated by the interconversion of the two pigments diatoxanthin and diadinoxanthin, which is part of the xanthophyll cycle used to protect the photosystems from overexcitation. Superoxide dismutase (SOD) and ascorbate peroxidase enzymes in the chloroplast degrade reactive oxygen species (ROS). (*B*) During heat stress, membrane fluidity changes (154) result in the production of ROS. Subsequently, the simultaneous overreduction of photosynthetic electron transport and the decreased fixation of CO_2 (i.e., sink limitation) result in the overexcitation of the photosystems and the flow of excitation energy primarily through PSI. The excess absorbed energy cannot be dissipated by the xanthophyll cycle (NPQ, nonphotochemical quenching). More ROS are formed than can be quenched by the available enzymatic and nonenzymatic antioxidants, and some species (e.g., H_2O_2) can be exported from the chloroplast (*bold horizontal arrow*). APO, ascorbate peroxidase; LHC, light-harvesting complex; VDE, violaxanthin de-epoxidase. Adapted from Jones et al. (150) and Hoegh-Guldberg (132).

this group. Global climate change, principally the emission of greenhouse gases (e.g., CO_2 and CH_4), and the subsequent effects on seawater temperature are the primary causes of "coral-bleaching" events around the world (132, 133). Seawater temperatures of 2–3°C above long-term average summer temperatures result in a stress response, known as bleaching in corals, in which they lose their zooxanthellae (132, 133). Both field and laboratory studies on bleaching in corals and other symbiotic cnidarians have established a causal link between temperature stress and bleaching (132, 133). The extent of coral-bleaching, the extent of subsequent mortality, and the underlying mechanism(s) that cause bleaching are related to the magnitude of temperature elevation and the duration of exposure for any individual event.

Although thermal stress is seen as the principal cause of coral bleaching, other environmental factors, including those that are affected by anthropogenic influences, act synergistically by effectively lowering the threshold temperature at which coral bleaching occurs. The abiotic factor that has the most significant influence on the severity of thermally induced coral bleaching is solar radiation, both its visible and ultraviolet components (UVB: 290–320 nm; UVA: 320–400 nm) (133, 134). Exposure to UVR is particularly important during the hyperoxic conditions (135, 136) that occur intracellularly in corals during photosynthesis, and leads to the photodynamic production of ROS (5–8). An important response of corals during exposure to UVR is the synthesis of MAAs and enzymes involved in the protection of both the host and symbiont from oxidative stress (60, 75, 137, 138).

Exposure to elevated temperatures alone (139), UVR alone (60), or in combination (137, 140) can result in photoinhibition of photosynthesis in zooxanthellae. Photoinhibition occurs as a result of the reduction in photosynthetic electron transport combined with the continued high absorption of excitation energy and the production of ROS. ROS have many cellular targets, including photosystem II and the primary carboxylating enzyme, Rubisco, in zooxanthellae (Figure 1) (60, 137). Elevated temperatures functionally lower the set point for light-induced photoinhibition. Enzymic defenses in the cnidarian host occur in proportion to the potential for photooxidative damage in symbiotic cnidarians (141, 142). However, high fluxes of ROS in the host (141, 143) or zooxanthellae (60, 137, 144) can overwhelm the protective enzymatic response and result in hydroxyl radical production via the Fenton reaction (5–8). Both the cnidarian host and zooxanthellae express Cu/Zn and Mn SODs (60, 138, 145), whereas zooxanthellae also express an Fe SOD (146).

Oxidative stress has been proposed as a unifying mechanism for several environmental insults that cause bleaching (137, 138). Oxidative stress can lead to bleaching of corals by zooxanthellae exocytosis from coral host cells (140, 147, 148) or by apoptosis (138, 147–149). A cellular model of bleaching in symbiotic cnidarians has been developed (Figure 2) that includes oxidative stress, PSII damage, sink limitation, DNA damage, and apoptosis as underlying processes (137, 138, 140, 147, 150, 151). This model is consistent with biomarker proteins expressed in corals during thermal stress (152, 153). Recent findings can also be included

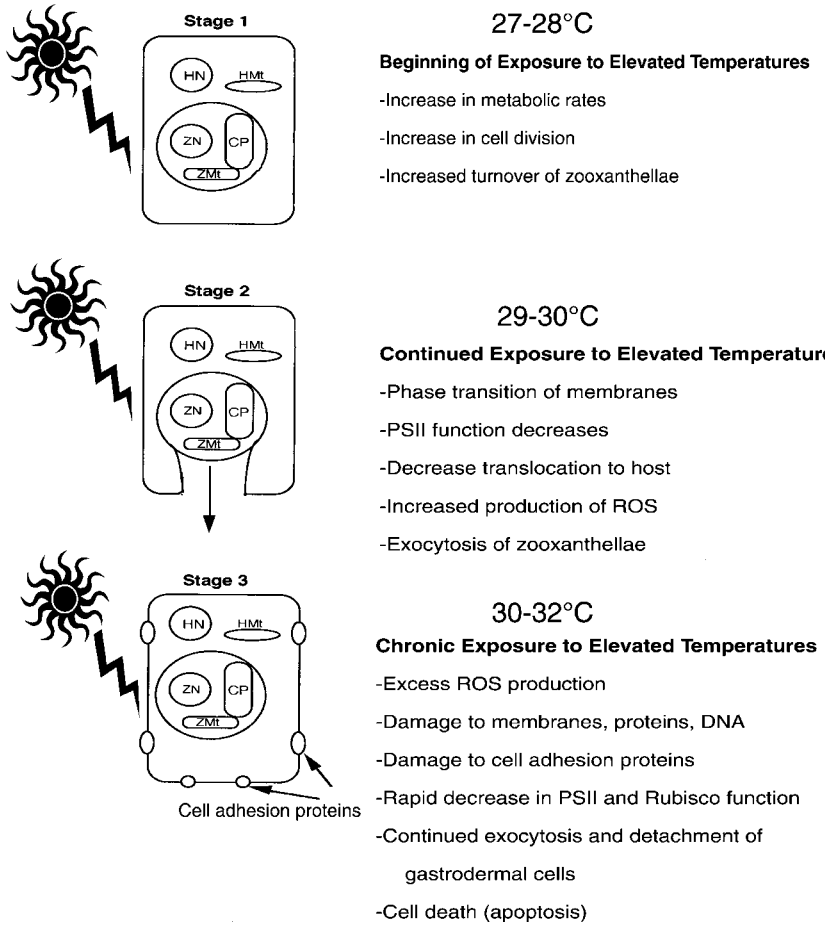

Figure 2 Model of coral bleaching caused by oxidative stress that incorporates photoinhibition (150) and apoptosis (137, 138, 147). The chloroplast and mitochondria (both host and zooxanthellae) are major sources of ROS. Continued exposure to elevated seawater temperatures, concurrent with the increase in ROS production, causes a progression from Stage 1 (27–28°C) to Stage 3 (30–32°C) and apoptosis or cell necrosis. HN, host nucleus; ZN, zooxanthellae nucleus; HMt, host mitochondria; CP, chloroplast; ZMt, zooxanthellae mitochondria.

in this model and include differential sensitivity of zooxanthellae thylakoid membranes to thermal stress (154) and the presence of nitric oxide synthase activity (155, 156), which produces $NO^{\bullet}$ and in turn reacts with O_2^- to form $ONOO^-$, whose ability to diffuse through membranes is much greater than that of O_2^-.

UVR and thermal stress also damage DNA in corals (169). DNA damage can also lead to apoptosis if not repaired. The expression pattern of a putative *p53*

protein in *Montastraea faveolata* after exposure to thermal stress and high irradiances of solar radiation is consistent with DNA damage (138). Morphological evidence indicates both apoptosis and necrosis in host and algal cells of thermally stressed symbiotic sea anemones. Similarly, in thermally stressed symbiotic cnidarians, ROS-mediated apoptosis and possibly necrosis are consistent with morphological evidence and the upregulation of a putative *p*53 protein. Apoptosis and cell necrosis are extremes in a range of cellular responses of corals to oxidative stress caused by thermal stress, with and without the synergistic effects of solar radiation (133).

CONCLUSIONS AND FUTURE DIRECTIONS

Increasingly, ecologists/physiologists are examining oxidative stress. Additionally, oxidative stress is emerging as a common theme in connection with the impact of global climate change (e.g., global warming and ozone depletion) on natural ecosystems at all trophic levels. Responses of various marine taxa to this impact both mitigate protein damage (e.g., heat shock proteins and ubiquination) and, by quenching ROS, limit damage to DNA, proteins, and lipids.

The future for integrated studies includes molecular genetics, microarrays, proteomics, RNAi assays, knockouts, and marine model organisms, combined with a quantitative organismal approach. Methods routinely used (e.g., electron paramagnetic resonance, enzyme assays, and fluorochromes) to assess the level of oxidative stress should be applied to a variety of marine taxa. These techniques should be combined with assessments of (*a*) costs to respond to and repair the damage from oxidative stress and (*b*) sublethal impacts on growth and reproduction. Biomarker development should also be integrated more extensively into an ecological setting. Few antioxidant genes have been sequenced for marine organisms or have had their expression quantified. Some research groups have established EST libraries, which will facilitate the development of microarrays for stress genes and genes of intermediate metabolism, which can assess stress and energetic costs for marine taxa under diverse environmental conditions.

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LITERATURE CITED

1. Nisbet EG, Sleeo NH. 2001. The habitat and nature of early life. *Nature* 409:1083–91
2. Kasting JF, Siefert JL. 2002. Life and the evolution of Earth's atmosphere. *Science* 296:1066–68
3. Falkowski PG, Katz ME, Knoll AH, Quigg A, Raven JA, et al. 2004. The evolution of modern eukaryotic phytoplankton. *Science* 305:354–60
4. Gerschman R, Gilbert DL, Nye SW, Dwyer P, Fenn WO. 1954. Oxygen

poisoning and X-irradiation: a mechanism in common. *Science* 119:4562–70
5. Asada K, Takahashi M. 1987. Production and scavenging of active oxygen in photosynthesis. In *Photoinhibition*, ed. DJ Kyle, CB Osmond, CJ Arntzen, pp. 228–87. Elsevier: Amsterdam
6. Cadenas E. 1989. Biochemistry of oxygen toxicity. *Annu. Rev. Biochem.* 58:79–110
7. Fridovich I. 1998. Oxygen toxicity: a radical explanation. *J. Exp. Biol.* 201:1203–9
8. Halliwell B, Gutteridge JMC. 1999. *Free Radicals in Biology and Medicine*. New York: Oxford Univ. Press. 936 pp.
9. Jamieson D, Chance B, Cadenas E, Boveris A. 1986. The relation of free radical production to hyperoxia. *Annu. Rev. Physiol*. 48:703–19
10. Takahashi M, Asada K. 1988. Superoxide production in aprotic interior of chloroplast thylakoids. *Arch. Biochem. Biophys*. 267:714–22
11. Shiraishi T, Takahashi M, Asada K. 1994. Generation of superoxide anion radicals and hydroxyl radicals in chloroplast thylakoids. In *Frontiers of Reactive Oxygen Species in Biology and Medicine*, ed. K Asada, T Yoshikawa, pp. 31–32. Amsterdam: Elsevier Sci.
12. Fridovich I. 1986. Biological effects of the superoxide radical. *Arch. Biochem. Biophys.* 247:1–11
13. Fang FC. 2004. Antimicrobial reactive oxygen and nitrogen species: concepts and controversies. *Nat. Rev. Microbiol.* 2: 820–32
14. Marla SS, Lee J, Groves JT. 1997. Peroxynitrite rapidly permeates phopholipid membranes. *Proc. Natl. Acad. Sci. USA* 94:14243–48
15. Denicola A, Souza JM, Radi R. 1998. Diffusion of peroxynitrite across erythrocyte membranes. *Proc. Natl. Acad. Sci. USA* 95:3566–71
16. Asada K. 1994. Mechanisms for scavenging reactive molecules generated in chloroplasts under light stress. In *Photoinhibition of Photosynthesis*, ed. NR Baker, JB Boyer, pp. 129–42. Oxford, UK: BIOS
17. Falkowski PG, Raven JA. 1997. *Aquatic Photosynthesis*. Malden, MA: Blackwell Sci. 375 pp.
18. Richter M, Rüle W, Wild A. 1990. Studies on the mechanism of photosystem II photoinhibition II. The involvement of toxic oxygen species. *Photosynth. Res.* 24:237–43
19. Lupínkova L, Komenda J. 2004. Oxidative modification of the photosystem II D1 protein by reactive oxtgen species: From isolated protein to cyanobacterial cells. *Photochem. Photobiol.* 79:152–62
20. Nishiyama Y, Yamamoto H, Allakhverdiev SI, Inaba M, Yokota A, et al. 2001. Oxidative stress inhibits the repair of photodamage to the photosynthetic machinery. *EMBO J.* 20:5587–94
21. Macpherson AN, Telfer A, Barber J, Truscott GT. 1993. Direct detection of singlet oxygen from isolated photosystem II reaction centers. *Biochem. Biophys. Acta* 1143:301–9
22. Liu K, Sun J, Song Y, Liu B, Xu Y, et al. 2004. Superoxide, hydrogen peroxide, and hydroxyl radical in D1/D2/cytochrome *b-559* photosystem II reaction center complex. *Photosynth. Res.* 81:41–47
23. Asada K. 1999. The water-water cycle in chloroplasts: scavenging of active oxygens and dissipation of excess photons. *Annu. Rev. Plant Physiol. Mol. Biol.* 50: 601–39
24. Brookes PS. 2005. Mitochondrial H^+ leak and ROS generation: An odd couple. *Free Radic. Biol. Med.* 38:12–23
25. Brand MD, Affouritit C, Esteves TC, Green K, Lamber AJ, et al. 2005. Mitochondrial superoxide: production, biological effects, and activation of uncoupling proteins. *Free Radic. Biol. Med.* 37:755–67
26. Corpas FJ, Barroso JB, del Rio LA. 2001. Peroxisomes as a source of reactive oxygen species and nitric oxide signal

molecules in plant cells. *Trends Plant Sci.* 6:145–50
27. Sandalio LM, Fernández VM, Rupérez FL, del Rio LA. 1988. Superoxide free radicals are produced in glyoxisomes. *Plant Physiol.* 87:1–7
28. Sandalio LM, del Rio LA. 1988. Intraorganellar distribution of superoxide dismutase in plant peroisomes (glyoxisomes and leaf peroxisomes). *Plant Physiol.* 88:1215–18
29. Gutteridge JMC, Halliwell B. 1990. The measurement and the mechanism of lipid peroxidation in biological systems. *Trends Biochem. Sci.* 15:129–35
30. Freeman BA, Crapo JD. 1982. Biology of disease, free radicals and tissue injury. *Lab. Invest.* 47:412–26
31. Green DR, Reed JC. 1998. Mitochondria and apoptosis. *Science* 281:1309–12
32. Hyslop PA, Hinshaw DB, Halsey WA Jr, Schraufsätter IU, Sauerheber RD, et al. 1988. Mechanisms of oxidant-mediated cell injury. *J. Biol. Chem.* 263:1665–75
33. Stadtman ER. 1986. Oxidation of proteins by mixed-function oxidation systems: implication in protein turnover, aging and neutrophil function. *Trends Biochem. Sci*. 11:11–12
34. Davies KJA. 1987. Protein damage and degradation by oxygen radicals. *J. Biol. Chem.* 262:9895–901
35. Dean RT, Gieseg S, Davies MJ. 1993. Reactive species and their accumulation on radical-damaged proteins. *Trends Biochem. Sci.* 18:437–41
36. Imlay JA, Linn S. 1988. DNA damage and oxygen radical toxicity. *Science* 240:1302–9
37. Imlay JA. 2003. Pathways of oxidative damage. *Annu. Rev. Microbiol.* 57:395–418
38. Beyer W, Imlay J, Fridovich I. 1991. Superoxide dismutases. *Prog. Nucleic Acid Res*. 40:221–53
39. Schrek R, Baeuerle PA. 1991. A role for oxygen radicals as second messengers. *Trends Cell Biol.* 1:39–42
40. Zheng M, Åslund F, Storz G. 1998. Activation of the OxyR transcription factor by reversible disulfide bond formation. *Science* 279:1718–21
41. Martindale JL, Holbrook NJ. 2002. Cellular response to oxidative stress: signaling for suicide and survival. *J. Cell. Physiol.* 192:1–15
42. Johnson TM, Yu Z, Ferrans VJ, Lowenstein RA, Finkel T. 1996. Reactive oxygen species are downstream mediators of p53-dependent apoptosis. *Proc. Natl. Acad. Sci. USA* 93:11848–52
43. Hengartner MO. 2000. The biochemistry of apoptosis. *Nature* 407:770–76
44. Gniadecki R, Thorn T, Vicanova J, Petersen A, Wulf HC. 2000. Role of mitochondria in ultraviolet-induced oxidative stress. *J. Cell. Biochem.* 80:216–22
45. Pourzand C, Tyrell RM. 1999. Apoptosis, the role of oxidative stress and the example of solar UV radiation. *Photochem. Photobiol.* 70:380–90
46. Korthout HAAJ, Berecki G, Bruin W, van Duijn B, Wang M. 2000. The presence and subcellular localization of caspase 3-like proteinases in plant cells. *FEBS Lett.* 475:139–44
47. Lam E, del Pozo O. 2000. Caspase-like protease involvement in the control of plant cell death. *Plant Mol. Biol.* 44:417–28
48. Mehdy MC. 1994. Active oxygen species in plant defense against pathogens. *Plant Physiol.* 105:467–72
49. Jabs T, Dietrich RA, Dang JL. 1996. Initiation of runaway cell death in an *Arabidopsis* mutant by extracellular superoxide. *Science* 273:1853–56
50. Cikala M, Wilm B, Hobmayer E, Böttger A, David CN. 1999. Identification of caspases and apoptosis in the simple metazoan *Hydra*. *Curr. Biol.* 9:959–62
51. Segovia M, Haramaty L, Berges JA, Falkowski PG. 2003. Cell death in the unicellular chlorophyte *Dunaliella tertiolecta*. A hypothesis on the evolution of

apoptosis in higher plants and metazoans. *Plant Physiol.* 132:99–105
52. McFall-Ngai MJ. 1999. Consequences of evolving with bacterial symbionts: insights from the squid-*Vibrio* associations. *Annu. Rev. Ecol. Syst.* 30:235–56
53. Visick KL, McFall-Ngai MJ. 2000. An exclusive contract: specificity in the *Vibrio fisheri-Euprymna scolopes* partnership. *J. Bacteriol.* 182:1779–87
54. Ruby EG, McFall-Ngai MJ. 1999. Oxygen-utilizing reactions and symbiotic colonization of the squid light organ by *Vibrio fisheri*. *Trends Microbiol.* 7:414–20
55. McCord JM, Fridovich I. 1969. Superoxide dismutase, an enzymatic function for erythrocuprein. *J. Biol. Chem.* 244:6049–55
56. Bannister JV, Bannister WH, Rotils G. 1987. Aspects of the structure, function and applications of superoxide dismutase. *CRC Crit. Rev. Biochem.* 22:110–80
57. Wolfe-Simon F, Grzebyk D, Schofield O, Falkowski PG. 2005. The role and evolution of superoxide dismutases in algae. *J. Phycol.* 41:453–65
58. Asada K, Kanematsu S, Okada S, Hayakawa T. 1980. Phylogenetic distribution of three types of superoxide dismutase in organisms and in cell organelles. In *Chemical and Biochemical Aspects of Superoxide and Superoxide Dismutase*, ed. JV Bannister, HAO Hill, pp. 136–53. Amsterdam: Elsevier Sci.
59. Kanematsu S, Asada K. 1989. CuZn-superoxide dismutases from the fern *Equisetum arvense* and the green alga *Spirogyra* sp.: occurrence of chloroplast and cytosol types of enzyme. *Plant Cell Physiol.* 30:717–27
60. Lesser MP, Shick JM. 1989. Effects of irradiance and ultraviolet radiation on photoadaptation in the zooxanthellae of *Aiptasia pallida*: primary production, photoinhibition, and enzymic defenses against oxygen toxicity. *Mar. Biol.* 102:243–55
61. Hollnagel HC, di Mascio P, Asano CS, Okamoto OK, Stringer CG, et al. 1996. The effect of light on the biosynthesis of β-carotene and superoxide dismutase activity in the photosynthetic alga *Gonyaulax polydra*. *Brazil. J. Med. Biol. Res.* 29:105–10
62. Butow BJ, Wynne D, Tel-Or E. 1997. Superoxide dismutase activity in *Peridinium gatunense* in Lake Kinneret: effect of light regime and carbon dioxide concentration. *J. Phycol.* 33:787–93
63. Hertwig B, Steb P, Feierabend J. 1992. Light dependence of catalase synthesis and degradation in leaves and the influence of interfering stress conditions. *Plant Physiol.* 100:1547–53
64. Klotz MG, Klassen GR, Loewen PC. 1997. Phylogenetic relationships among prokaryotic and eukaryotic catalases. *Mol. Biol. Evol.* 14:951–58
65. Yamamoto Y, Fujisawa A, Hara A, Dunlap WC. 2001. An unusual vitamin E constituent (α-tocomonoenol) provides enhanced antioxidant protection in marine organisms adapted to cold-water environments. *Proc. Natl. Acad. Sci. USA* 98: 13144–48
66. Demmig-Adams B, Adams WW. 1993. The xanthophyll cycle. In *Antioxidants in Higher Plants*, ed. RG Alscher, JL Hess, pp. 59–90. Boca Raton, FL: CRC Press
67. Krinsky NI. 1989. Antioxidant functions of carotenoids. *Free Radic. Biol. Med.* 7:617–35
68. Miki W, Otaki N, Shimidzu N, Yokoyama A. 1994. Carotenoids as free radical scavengers in marine animals. *J. Mar. Biotechnol.* 2:35–37
69. Ames BN, Cathcart R, Schwiers E, Hochstein P. 1981. Uric acid provides an antioxidant defense in humans against oxidant- and radical-caused aging and cancer: a hypothesis. *Proc. Natl. Acad. Sci. USA* 78:6858–62
70. Dunlap WC, Shick JM, Yamamoto Y. 2000. UV protection in marine organisms. I. Sunscreens, oxidative stress and

antioxidants. In *Free Radicals in Chemistry, Biology and Medicine*, ed. T Yoshikawa, S Toyokuni, Y Yamamoto, Y Naito, pp. 200–14. London: OICA Int.
71. Smirnoff N, Cumbes QJ. 1989. Hydroxyl radical scavenging activity of compatible solutes. *Phytochemistry* 28:1057–60
72. Shen B, Jensen RG, Bohnert HJ. 1997. Mannitol protects against oxidation by hydroxyl radicals. *Plant Physiol.* 115:527–32
73. Broadbent AD, Jones GB, Jones RJ. 2002. DMSP in corals and benthic algae from the Great Barrier Reef. *Estuar. Coast. Shelf. Sci.* 55:547–55
74. Sunda W, Kleber DJ, Klene RP, Huntsman S. 2002. An antioxidant function for DMSP and DMS in marine algae. *Nature* 418:317–20
75. Shick JM, Dunlap WC. 2000. Mycosporine-like amino acids and related gadusols: biosynthesis, accumulation, and UV-protective functions in aquatic organisms. *Annu. Rev. Physiol.* 64:223–62
76. Dunlap WC, Yamamoto Y. 1995. Small-molecule antioxidants in marine organisms: antioxidant activity of mycosproine-glycine. *Comp. Biochem. Physiol. B* 112:105–14
77. Conde FR, Churio MS, Previtali CM. 2000. The photoprotector mechanism of mycosporine-like amino acids. Excited-state properties and photostability of porphyra-334 in aqueous solution. *J. Photochem. Photobiol. B* 56:139–44
78. Kim CS, Lim WA, Cho YC. 2001. Mycosporine-like amino acids as the UV sunscreen with oxygen radical scavenging activity. *Bull. Natl. Fish. Res. Dev. Inst. Korea* 60:65–71
79. Suh H, Lee H, Jung J. 2003. Mycosporine glycine protects biological systems against photodynamic damage by quenching singlet oxygen with a high efficiency. *Photochem. Photobiol.* 78:109–13
80. Yakoleva I, Bhagooli R, Takemura A, Hidaka M. 2004. Differential susceptibility to oxidative stress of two scleractinian corals: antioxidant functioning of mycosporine-glycine. *Comp. Biochem. Physiol. B* 139:721–30
81. Mopper K, Kieber DJ. 2000. Marine photochemistry and its impact on carbon cycling. In *The Effects of UV Radiation in the Marine Environment*, ed. S De Mora, S Demers, M Vernet, pp. 101–30. Cambridge, UK: Cambridge Univ. Press
82. Tapley DW, Buettner GR, Shick JM. 1999. Free radicals and chemiluminescence as products of the spontaneous oxidation of sulfide in seawater, and their biological implications. *Biol. Bull.* 196:52–56
83. Blum J, Fridovich I. 1984. Enzymatic defenses against oxygen toxicity in the hydrothermal vent animals *Riftia pachyptila* and *Calyptogena magnifica*. *Arch. Biochem. Biophys.* 228:617–20
84. Jenny FE Jr, Verhagen MFJM, Cui X, Adams MWW. 1999. Anaerobic microbes: oxygen detoxification withot superoxide dismutase. *Science* 286:306–9
85. Bannister JV, Parker MW. 1985. The presence of a copper/zinc superoxide dismutase in the bacterium *Photobacterium leiognathi*: a likely case of gene transfer from eukaryotes to prokaryotes. *Proc. Natl. Acad. Sci. USA* 82:149–52
86. Bourne Y, Redford SM, Steinman HM, Lepock JR, Tainer JA, et al. 1996. Novel dimeric interface and electrostatic recognition in bacterial Cu, Zn superoxide dismutase. *Proc. Natl. Acad. Sci. USA* 93:12774–79
87. Timmins GS, Jackson SK, Swartz HM. 2001. The evolution of bioluminescent oxygen consumption as an ancient oxygen detoxification mechanism. *J. Mol. Evol.* 52:321–32
88. Rees J-F, de Wergifosse B, Noiset O, Dubuisson M, Janssens B, et al. 1998. The origins of marine bioluminescence: turning oxygen defense mechanisms into deep-sea communication tools. *J. Exp. Biol.* 201:1211–21

89. Szpilewska H, Czyz A, Wegrzyn G. 2003. Experimental evidence for the physiological role of bacterial luciferase in the protection of cells against oxidative stress. *Curr. Microbiol.* 47:379–82
90. Wergifosse B, Dubuisson M, Marchand-Brynaert J, Trout A, Rees J-F. 2004. Coelenterazine: a two stage antioxidant in lipid micells. *Free Radic. Biol. Med.* 36:278–87
91. Okamato OK, Robertson DL, Fagan TF, Hastings JW, Colepicolo P. 2001. Different regulatory mechanisms modulate the expression of a dinoflagellate iron-superoxide dismutase. *J. Biol. Chem.* 276:19989–93
92. Butow BJ, Wynne D, Tel-Or E. 1997. Antioxidative protection of *Peridinium gatunense* in Lake Kinnerret: seasonal and daily variation. *J. Phycol.* 33:780–86
93. Yang CZ, Albright LJ, Yousif AN. 1995. Oxygen-radical-mediated effects of the toxic phytoplankter *Heterosigma carterae* on juvenile rainbow trout *Oncorhynchus mykiss*. *Mar. Ecol. Prog. Ser.* 23:101–8
94. Oda T, Ishimatsu A, Shimada M, Takeshita S, Muramatsu T. 1992. Oxygen-radical-mediated toxic effects of the red tide flagellate *Chattonella marina* on *Vibrio alginolyticus*. *Mar. Biol.* 112:505–9
95. Collén J, Davison IR. 1997. *In vivo* measurement of active oxygen production in the brown alga *Fucus evanescens* using 2',7'-dichlorohydrofluorescein diacetate. *J. Phycol.* 33:643–48
96. Collén J, Davison IR. 1999. Reactive oxygen production and damage in intertidal *Fucus* spp. (Phaeophyceae). *J. Phycol.* 35:54–61
97. Collén J, Davison IR. 1999. Reactive oxygen metabolism in intertidal *Fucus* spp. (Phaeophyceae). *J. Phycol.* 35:62–69
98. Collén J, Davison IR. 2001. Seasonal and thermal acclimation of reactive oxygen metabolism in *Fucus vesiculosus* (Phaeophyceae). *J. Phycol.* 37:474–81
99. Collén J, Davison IR. 1999. Stress tolerance and reactive oxygen metabolism in the intertidal red seaweeds *Mastocarpus stellatus* and *Chondrus crispus*. *Plant Cell Environ.* 22:1143–51
100. Lohrmann NL, Logan BA, Johnson AS. 2004. Seasonal acclimatization of antioxidants and photosynthesis in *Chondrus crispus* and *Mastocarpus stellatus*, two co-occurring red algae with differing stress tolerances. *Biol. Bull.* 207:225–32
101. Aguilera J, Bischof K, Karsten U, Hanelt D, Wiencke C. 2002. Seasonal variation in ecophysiological patterns in macroalgae from an Arctic fjord. II. Pigment accumulation and biochemical defence systems against high light stress. *Mar. Biol.* 140:1087–95
102. Winston GW, Livingstone DR, Lips F. 1990. Oxygen reduction metabolism by the digestive gland of the common marine mussel, *Mytilus edulis* L. *J. Exp. Zool.* 255:296–308
103. Abele D, Heise K, Pörtner HO, Puntarulo S. 2002. Temperature-dependence of mitochondrial function and production of reactive oxygen species in the intertidal mud clam *Mya arenaria*. *J. Exp. Biol.* 205:1831–41
104. Adema CM, Van der Knaap WPW, Sminia T. 1991. Molluscan hemocyte-mediated cytotoxicity: the role of reactive oxygen intermediates. *Rev. Aquat. Sci.* 4: 201–23
105. Dykens JA, Shick JM. 1988. Relevance of purine catabolism to hypoxia and recovery in euryoxic and stenoxic marine invertebrates, particularly bivalve molluscs. *Comp. Biochem. Physiol. C* 91:35–41
106. Regoli F, Cerrano C, Chierici E, Bompadre S. 2000. Susceptibility to oxidative stress of the Mediterranean demosponge *Petrosia ficiformis*: role of endosymbionts and solar irradiance. *Mar. Biol.* 137:453–61
107. Regoli F, Cerrano C, Chierici E, Chiantore MC, Bavestrello G. 2004. Seasonal variability of prooxidant pressure and antioxidant adaptation to symbiosis in the Mediterranean demosponge *Petrosia*

ficiformis. *Mar. Ecol. Prog. Ser.* 275:129–37

108. Keller M, Sommer AM, Pörtner HO, Abele D. 2004. Seasonality of energetic functioning and production of reactive oxygen species by lugworm (*Arenicola marina*) mitochondria exposed to acute temperature changes. *J. Exp. Biol.* 207:2529–38
109. Abele-Oeschger D, Oeschger R, Theede H. 1994. Biochemical adaptations of *Nereis diversicolor* (Polychaeta) to temporarily increased hydrogen peroxide levels in intertidal sandflats. *Mar. Ecol. Prog. Ser.* 106:101–10
110. Morrill AC, Powell EN, Bidigare RR, Shick JM. 1988. Adaptations to life in the sulfide system: a comparison of oxygen detoxifying enzymes in thiobiotic and oxybiotic meiofauna (and freshwater planarians). *J. Comp. Physiol. B* 158:335–44
111. Maciel FE, Rosa CE, Santos EA, Monserrat JM, Nery LEM. 2004. Daily variations in oxygen consumption, antioxidant defenses, and lipid peroxidation in the gills and hepatopancreas of an estuarine crab. *Can. J. Zool.* 82:1871–77
112. Camus L, Gulliksen B. 2005. Antioxidant defense properties of Arctic amphipods: comparison between deep-, sublittoral and surface species. *Mar. Biol.* 146:355–62
113. Gouveia GR, Marques DS, Cruz BP, Geracitano LA, Nery LEM, et al. 2005. Antioxidant defenses and DNA damage induced by UV-A and UV-B radiation in the crab *Chasmagnathus granulata* (Decopoda, Brachyura). *Photochem. Photobiol.* 81:398–403
114. Brouwer M, Brouwer TH, Grater W, Enghild JJ, Thogersen IB. 1997. The paradigm that all oxygen-respiring eukaryotes have cytolosolic CuZn-suproxide dismutase and that Mn-superoxide dismutase is localized to mitochondria does not apply to a large group of marine arthropods. *Biochemistry* 36:13381–88
115. Brouwer M, Brouwer TH, Grater W, Brown-Peterson N. 2003. Replacement of a cytosolic copper/zinc superoxide dismutase by a novel cytosolic manganese superoxide dismutase in crustaceans that use copper (haemocyanin) for oxygen transport. *Biochem. J.* 374:219–28
116. Shapiro BM. 1991. The control of oxidant stress at fertilization. *Science* 252:533–36
117. Brooks JM, Wessel GM. 2002. The major yolk protein in sea urchins is a transferrin-like, iron binding protein. *Dev. Biol.* 245:1–12
118. Lesser MP, Kruse VA, Barry TM. 2003. Exposure to ultraviolet radiation causes apoptosis in developing sea urchin embryos. *J. Exp. Biol.* 206:4097–103
119. Ken C-F, Lin C-T, Shaw JF, Wu JL. 2003. Characterization of fish Cu/Zn-superoxide dismutase and its protection from oxidative stress. *Mol. Biotechnol.* 5:167–73
120. Natoli G, Calabrese L, Capo C, O'Neil P, di Prisco G. 1990. Icefish (*Chaenocephalus aceratus*) Cu,Zn superoxide dismutase conservation of the enzyme properties in extreme adaptation. *Comp. Biochem. Physiol. B* 95:29–33
121. Cassini A, Favero M, de Laureto P, Albergon V. 1997. Cu-Zn suproxide dismutase from *Pagothenia bernacchii*: catalytic and molecular properties. In *Antarctic Communities*, *Species, Structure, and Survival*, ed. B Battaglia, J Valencia, DWH Walton, pp. 266–71. Cambridge, UK: Cambridge Univ. Press
122. Abele D, Puntarulo S. 2004. Formation of reactive species and induction of antioxidant defence systems in polar and temperate marine invertebrates and fish. *Comp. Biochem. Physiol. A* 138:405–15
123. Janssens B, Childress JJ, Baguet F, Rees J-F. 2000. Reduced enzymatic antioxidative defenses in deep-sea fish. *J. Exp. Biol.* 203:3717–25
124. Bacanskas LR, Whitaker J, di Giulio RT. 2004. Oxidative stress in two populations

of killifish (*Fundulus heteroclitus*) with differing contaminant histories. *Mar. Environ. Res.* 58:597–601

125. Lesser MP, Farrell JH, Walker CW. 2001. Oxidative stress, DNA damage, and p53 expression in the larvae of Atlantic cod (*Gadus morhua*) exposed to ultraviolet (290–400 nm) radiation. *J. Exp. Biol.* 204:157–64
126. Estevez MS, Abele D, Puntarulo S. 2002. Lipid radical generation in polar (*Laternula elliptica*) and temperate (*Mya arenaria*) bivalves. *Comp. Biochem. Physiol. B* 132:729–37
127. Viarengo A, Canesi L, Martinez PG, Peters LD, Livingstone DR. 1995. Prooxidant processes and antioxidant defence systems in the tissues of the Antarctic scallop (*Adamussium colbecki*) compared with the Mediteranean scallop (*Pecten jacobaeus*). *Comp. Biochem. Physiol. B* 111:119–26
128. Regoli F, Nigro M, Chiantore M, Winston GM. 2002. Seasonal variations of susceptibility to oxidative stress in *Adamussium colbecki*, a key bioindicator species for the Antarctic marine environment. *Sci. Total Environ.* 289:205–11
129. Abele D, Tesch C, Wencke P, Pörtner HO. 2001. How does oxidative stress relate to thermal tolerance in the Antarctic bivalve *Yolida eightsi*. *Antarct. Sci.* 13:11–18
130. Viarengo A, Abele-Oeschger D, Burlando B. 1998. Effects of low temperature on prooxidant processes and antioxidant defence systems in marine organisms. In *Cold Ocean Physiology*, ed. HO Pörtner, RC Playle, pp. 212–35. Cambridge, NY: Cambridge Univ. Press
131. Hochachka PW, Somero GN. 2002. *Biochemical Adaptation: Mechanism and Process in Physiological Evolution*. New York: Oxford Univ. Press. 478 pp.
132. Hoegh-Guldberg O. 1999. Climate change, coral bleaching and the future of the world's coral reefs. *Mar. Freshw. Res.* 50:839–66
133. Lesser MP. 2004. Experimental coral reef biology. *J. Exp. Mar. Biol. Ecol.* 300:217–52
134. Shick JM, Lesser MP, Jokiel P. 1996. Effects of ultraviolet radiation on corals and other coral reef organisms. *Glob. Change Biol.* 2:527–45
135. Dykens JA, Shick JM. 1982. Oxygen production by endosymbiotic algae controls superoxide dismutase activity in their animal host. *Nature* 297:579–80
136. Kühl M, Cohen Y, Dalsgaard T, Jørgensen BB, Revsbech NP. 1995. Microenvironment and photosynthesis of zooxanthellae in scleractinian corals studied with microsensors for O_2, pH, and light. *Mar. Ecol. Prog. Ser.* 117:159–72
137. Lesser MP. 1996. Exposure of symbiotic dinoflagellates to elevated temperatures and ultraviolet radiation causes oxidative stress and inhibits photosynthesis. *Limnol. Oceanogr.* 41:271–83
138. Lesser MP, Farrell J. 2004. Solar radiation increases the damage to both host tissues and algal symbionts of corals exposed to thermal stress. *Coral Reefs* 23:367–77
139. Iglesias-Prieto R, Matta JL, Robins WA, Trench RK. 1992. Photosynthetic response to elevated temperature in the symbiotic dinoflagellate *Symbiodinium microadriaticum* in culture. *Proc. Natl. Acad. Sci. USA* 89:10302–5
140. Lesser MP. 1997. Oxidative stress causes coral bleaching during exposure to elevated temperatures. *Coral Reefs* 16:187–92
141. Dykens JA, Shick JM, Benoit C, Buettner GR, Winston GW. 1992. Oxygen radical production in the sea anemone *Anthopleura elegantissima*: and its symbiotic algae. *J. Exp. Biol.* 168:219–41
142. Richier S, Furla P, Plantivaux A, Merle P-L, Allemand D. 2005. Symbiosis-induced adaptation to oxidative stress. *J. Exp. Biol.* 208:277–85
143. Nii CM, Muscatine L. 1997. Oxidative stress in the symbiotic sea anemone *Aiptasia pulchella* (Calgren, 1943): Contribution of the animal to superoxide ion

production at elevated temperature. *Biol. Bull.* 192:444–56

144. Matta JL, Trench RK. 1991. The enzymatic response of the symbiotic dinoflagellate *Symbiodinium microadriaticum* (Freudenthal) to growth *in vitro* under varied oxygen tensions. *Symbiosis* 11:31–45
145. Plantivaux A, Furla P, Zoccola D, Garello G, Forcioli D, et al. 2004. Molecular characterization of two CuZn-superoxide dismutases in a sea anemone. *Free Radic. Biol. Med.* 37:1170–81
146. Matta JL, Govind NS, Trench RK. 1992. Ploy clonal antibodies against iron-superoxide dismutase from *Escherichia coli* B cross react with superoxide dismutases from *Symbiodinium microadriaticum* (Dinophyceae). *J. Phycol.* 28:343–46
147. Gates RD, Baghdasarian G, Muscatine L. 1992. Temperature stress causes host cell detachment in symbiotic cnidarians: implications for coral bleaching. *Biol. Bull.* 182:324–32
148. Franklin DJ, Hoegh-Guldberg O, Jones RJ, Berges JA. 2004. Cell death and degeneration in the symbiotic dinoflagellates of the coral *Stylophora pistillata* during bleaching. *Mar. Ecol. Prog. Ser.* 272: 117–30
149. Dunn SR, Bythell JC, Le Tessier DA, Burnett WJ, Thomason JC. 2002. Programmed cell death and necrosis activity during hyperthermic stress-induced bleaching of the symbiotic sea anemone *Aiptasia* sp. *J. Exp. Mar. Biol. Ecol.* 272: 29–53
150. Jones RJ, Hoegh-Guldberg O, Larkum AWD, Schreiber U. 1998. Temperature-induced bleaching of corals begins with impairment of the CO_2 fixation mechanism in zooxanthellae. *Plant Cell Environ.* 21:1219–30
151. Warner ME, Fitt WK, Schmidt GW. 1999. Damage to photosystem II in symbiotic dinoflagellates: a determinant of coral bleaching. *Proc. Natl. Acad. Sci. USA* 96: 8007–12
152. Downs CA, Mueller E, Phillips S, Fauth JE, Woodley CM. 2000. A molecular biomarker system for assessing the health of coral (*Montastraea faveolata*) during heat stress. *Mar. Biotechnol.* 2:533–44
153. Downs CA, Fauth JE, Halas JC, Dustan P, Bemiss J, Woodley CM. 2002. Oxidative stress and seasonal coral bleaching. *Free Radic. Biol. Med.* 33:533–43
154. Tchernov D, Gorbunov MY, de Vargas C, Yadav SN, Milligan AJ, et al. 2004. Membrane lipids of symbiotic algae are diagnostic of sensitivity to thermal bleaching in corals. *Proc. Natl. Acad. Sci. USA* 101:13531–35
155. Trapido-Rosenthal H, Zielke S, Owen R, Buxton L, Boeing B, et al. 2005. Increased zooxanthellae nitric oxide synthase activity is associated with coral bleaching. *Biol. Bull.* 208:3–6
156. Morrall CE, Galloway TS, Trapido-Rosenthal HG, Depledge MH. 2000. Characterization of nitric oxide synthase activity in the tropical sea anemone *Aiptasia pallida*. *Comp. Biochem. Physiol. B* 125:483–91

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