

# Extreme Longevity Is Associated With Increased Resistance to Oxidative Stress in *Arctica islandica*, the Longest-Living Non-Colonial Animal

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We assess whether reactive oxygen species production and resistance to oxidative stress might be causally involved in the exceptional longevity exhibited by the ocean quahog *Arctica islandica*. We tested this hypothesis by comparing reactive oxygen species production, resistance to oxidative stress, antioxidant defenses, and protein damage elimination processes in long-lived *A islandica* with the shorter-lived hard clam, *Mercuria mercenaria*. We compared baseline biochemical profiles, age-related changes, and responses to exposure to the oxidative stressor *tert*-butyl hydroperoxide (TBHP). Our data support the premise that extreme longevity in *A islandica* is associated with an attenuated cellular reactive oxygen species production. The observation of reduced protein carbonyl concentration in *A islandica* gill tissue compared with *M mercenaria* suggests that reduced reactive oxygen species production in long-living bivalves is associated with lower levels of accumulated macromolecular damage, suggesting cellular redox homeostasis may determine life span. Resistance to aging at the organismal level is often reflected in resistance to oxidative stressors at the cellular level. Following TBHP exposure, we observed not only an association between longevity and resistance to oxidative stress-induced mortality but also marked resistance to oxidative stress-induced cell death in the longer-living bivalves. Contrary to some expectations from the oxidative stress hypothesis, we observed that *A islandica* exhibited neither greater antioxidant capacities nor specific activities than in *M mercenaria* nor a more pronounced homeostatic antioxidant response following TBHP exposure. The study also failed to provide support for the exceptional longevity of *A islandica* being associated with enhanced protein recycling. Our findings demonstrate an association between longevity and resistance to oxidative stress-induced cell death in *A islandica*, consistent with the oxidative stress hypothesis of aging and provide justification for detailed evaluation of pathways involving repair of free radical-mediated macromolecular damage and regulation of apoptosis in the world's longest-living non-colonial animal.

**Key Words:** Comparative biology—Free radical—Oxidative stress.

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**H**ARMAN (1) developed the theory that organismal aging results from reactive oxygen species (ROS), generated as by-products of mitochondrial respiration, damaging macromolecules, and impairing the function of cellular

organelles (2). The oxidative stress hypothesis of aging predicts that long-lived animals utilize a combination of strategies to limit oxidative stress-induced cellular damage. One would expect that cells of successfully aging animals exhibit

lower generation of ROS per se. On the basis of the oxidative stress hypothesis of aging, it also predicted that successfully aging species have increased tolerance for oxidative stress–induced cellular injury through superior cellular antioxidant defense mechanisms or increased elimination/repair of damaged macromolecules. Although the oxidative stress hypothesis of aging continues to be among the most commonly adduced mechanistic hypotheses to explain variation in aging rate, it is also a subject of ongoing debate (3–9). Perhaps, the strongest support for the oxidative stress hypothesis of aging comes from previous studies comparing ROS production and resistance with oxidative stress in phylogenetically diverse, shorter-living, and longer-living mammalian (10–12) and avian (13,14) species.

Longevity evolved independently many times in various phyla, and it remains to be proven that mechanisms of aging, including the role of ROS and oxidative stress resistance, are conserved among these various groups. The present study was designed to test predictions of the oxidative stress hypothesis of aging by contrasting two bivalve mollusk species of dramatically differing longevity. We focused on the burrowing clam *Arctica islandica* (ocean quahog), which is the longest lived of all non-colonial animal species on earth (15,16). Animals more than 100 years old are common and reported maximum species life span is more than 400 years (15–17). Recent studies have characterized several aspects of *A. islandica* physiology, which renders this species a useful model for aging research (18–20). The taxonomically related burrowing clam, *Merccenaria mercenaria* (northern quahog) lives in a similar environment, has similar physiology but has a substantially shorter life span (Table 1). Despite an increasing biogerontological focus on bivalve models (23,25), the role of oxidative stress and antioxidant mechanisms in regulation of life span in bivalves (16–18,26–29) is not as comprehensively understood as those in vertebrate models of aging. In the present study, we compared ROS production, resistance to oxidative stress, antioxidant defenses, and protein damage elimination processes (proteasome activities) in *A. islandica* and the much shorter-lived *M. mercenaria*.

## METHODS

### Clam Collection and Maintenance

The extremely long-lived ocean quahog (*A. islandica*) and the shorter-lived northern quahog (*M. mercenaria*) were used in this study (Figure 1A). Maximum species life span and physiological characteristics for each species are shown in Table 1. All clams used in the present study were collected in July 2010 in the coastal waters of New England. The clams were transported to the Marine Aquatic Resources Center of the Marine Biological Laboratory (Woods Hole, MA), where they were kept at constant temperature (12°C for *A. islandica* and 20°C for *M. mercenaria*, which typically lives in warmer water than *A. islandica*) in 500-L tanks for more than 1 week

prior to the studies. On the day of the experiments, the quahogs were dissected. Gill, heart, and adductor muscle were isolated using microsurgery instruments and a stereo operating microscope. Fresh tissue samples were obtained for measurements of ROS production *ex vivo*. Additional samples from the gill, heart, and mantle were frozen in liquid nitrogen for subsequent biochemical analysis.

### Determination of Individual Age

Individual age of the clams used was determined from internal shell growth increments as previously described (16). For the purpose of this study, we classified less than 30-year-old *A. islandica* and less than 15-year-old *M. mercenaria*, respectively, as “young” and more than 80-year-old *A. islandica* and more than 40-year-old *M. mercenaria*, respectively, as “aged” and analyzed age-related changes in ROS production and antioxidant enzyme activities in both species.

### Measurement of Tissue $H_2O_2$ and $O_2^{\cdot -}$ Production

$H_2O_2$  production in gill, heart, and adductor muscle tissue samples was measured fluorometrically using the Amplex Red/horseradish peroxidase assay as described (30). The rate of  $H_2O_2$  generation was assessed by measuring resorufin fluorescence for 60 minutes by a Tecan Infinite M200 plate reader. Each experiment was run in triplicate. A calibration curve was constructed using  $H_2O_2$ , and the production of  $H_2O_2$  in the samples was expressed as picomole  $H_2O_2$  released per minute, normalized to tissue wet weight.

Production of  $O_2^{\cdot -}$  in the gill and in the heart was determined using dihydroethidium (DHE), an oxidative fluorescent dye, as we previously reported (31,32). In brief, small tissue pieces were incubated with DHE ( $3 \times 10^{-6}$  mol/L; at room temperature, for 30 minutes). The tissues were then washed three times, embedded in optimal cutting temperature medium, and cryosectioned. Optical sections were obtained and the red fluorescent images, captured at 20× magnification, were analyzed using the AutoMeasure function of the Axiovision (Carl Zeiss, Gottingen, Germany) imaging software (33). Four entire fields per tissue were analyzed. The mean fluorescence intensities of DHE-stained nuclei were calculated for each tissue. Thereafter, the intensity values for each animal in the group were averaged.

### Determination of Protein Carbonylation

Protein carbonyl content was assessed in the gill tissues of *A. islandica* and *M. mercenaria* using the OxiSelect Protein Carbonyl ELISA Kit (Cell Biolabs Inc., San Diego, CA), according to the manufacturer’s guidelines.

### Studies on Oxidative Stress Resistance

To assess resistance to oxidative stress, *A. islandica* and *M. mercenaria* were exposed to various concentrations of *tert*-butyl hydroperoxide (TBHP) in the sea water. TBHP is

Table 1. Chronological Age, Maximum Reported Life Span, and Physiological Characteristics of the Marine Bivalve Species Used in This Study

Species	Common Name	Average Chronological Age (y)	Maximum Life Span (y)	Maximum Size (mm)	Growth Rate (K (VBGF))	Mortality Rate (Z)	Age at Maturity (y)	Lifestyle	References
<i>Arctica islandica</i>	Ocean quahog, mahogany clam	~22 ("young") ~100 ("aged")	405	118	0.02	0.03	7–14	Infraunal burrower	(15,21)
<i>Mercentaria mercenaria</i>	Northern quahog, hard clam	~8 ("young") ~68 ("aged")	106	150	0.210	1.32	2–5	Infraunal burrower	(22,23); Iain Ridgway, unpublished data, 2010

Notes: Ridgway laboratory has recently identified a 106-year-old *M. mercenaria*, which is greater than the maximum life span for this species (~50 years) previously recorded in the literature. Thus, the new longevity record for this species is given in this table. Although recent analysis of data from 56 species of bivalves revealed a statistically significant positive impact of shell size maximum longevity (24), it is of note that *A. islandica* has a smaller maximum shell size as compared with *M. mercenaria*. VBGF = von Bertalanffy growth function.

an organic peroxide that is highly stable in aqueous solutions. It is known to induce apoptosis in a wide variety of eukaryotic cells by damaging DNA, lipids, and proteins and is a useful tool to assess cellular oxidative stress resistance. To study organismal resistance to oxidative stress, the survival of *A. islandica* and *M. mercenaria* exposed to  $10^{-3}$  mol/L to  $6 \times 10^{-3}$  mol/L TBHP was recorded for 10 days.

#### Apoptotic Cell Death

To compare cellular resistance with oxidative stress in *A. islandica* and *M. mercenaria*, increases in the rate of apoptosis in response to TBHP ( $10^{-4}$  mol/L, for 24 hours) was assessed. In pilot studies, we found that  $10^{-4}$  mol/L TBHP for 24 hours did not affect mortality in quahogs; therefore, this concentration was used to contrast oxidative stress-induced biochemical alterations in the gill of *A. islandica* and *M. mercenaria*. Gill segments were homogenized in lysis buffer, and caspase 3 activity, a useful measure of apoptosis, was measured as we reported (11,27,34,35), using the Caspase-Glo 3/7 assay system (Promega, Madison, WI). We chose to study the gill since this organ is directly exposed to the TBHP in the sea water. In contrast, preliminary studies showed that treatment with  $10^{-4}$  mol/L TBHP does not elicit significant caspase 3 activation in tissue samples obtained from the center of the adductor muscle, which we attribute to the limited diffusion of TBHP from the sea water into the muscle tissue. Luminescent intensity was measured using an Infinite M200 plate reader and were normalized to the sample protein concentration. As an additional measure, cytoplasmic histone-associated DNA fragments, which also indicate apoptotic cell death, were quantified by the Cell Death Detection ELISA<sup>Plus</sup> Kit (Roche Diagnostics Corporation, Indianapolis, IN) as described (31,34).

#### Cellular Antioxidant Capacity

To compare the capacity of cellular antioxidant enzymes and other redox molecules to counterbalance the deleterious effects of oxidative stress in tissues of *A. islandica* and *M. mercenaria*, we assessed the Hydroxyl Radical Antioxidant Capacity (HORAC) and Oxygen Radical Absorbance Capacity (ORAC) using the OxiSelect HORAC Activity Assay (Cell Biolabs Inc.) and the OxiSelect ORAC Activity

Assay (Cell Biolabs Inc), according to the manufacturer's guidelines. The HORAC Activity Assay is based on the oxidation-mediated quenching of a fluorescent probe by hydroxyl radicals produced by a hydroxyl radical initiator and Fenton reagent. The ORAC Activity Assay is based on the oxidation of a fluorescent probe by peroxy radicals produced by a free radical initiator. Antioxidants present in the tissues delay the quenching of the fluorescent probe until the antioxidant activity in the sample is depleted. The antioxidant capacity of the tissues was calculated on the basis of the area under the fluorescence decay curve compared with an antioxidant standard curve obtained with gallic acid (for HORAC) or the water-soluble vitamin E analog Trolox (for ORAC), respectively. Sample protein concentration was used for normalization purposes.

#### Antioxidant Enzyme Activities

Activity of antioxidant enzymes in gill homogenates was measured using the OxiSelect Superoxide Dismutase Activity Assay Kit, the OxiSelect Catalase Activity Assay Kit (Cell Biolabs Inc.), and the Glutathione Peroxidase Assay Kit (Cayman Chemical Company, Ann Arbor, MI), according to the manufacturers' guidelines. Sample protein concentration was used for normalization purposes.

#### Determination of Proteasome Activity

To compare protein recycling activities in tissues of *A. islandica* and *M. mercenaria*, we assessed three types of protease activities associated with the proteasome complex in gill and adductor muscle samples using the Proteasome-Glo Chymotrypsin-Like, Trypsin-Like, and Caspase-Like Assays (Promega), according to the manufacturer's guidelines.

#### Data Analysis

Statistical analyses of data were performed by Student's *t* test or by analysis of variance followed by the Tukey post hoc test, as appropriate. Survival curves were compared using the log-rank test, using GraphPad Prism 4.0 software.  $p < .05$  was considered statistically significant. Data are expressed as means  $\pm$  SEM, unless otherwise indicated.

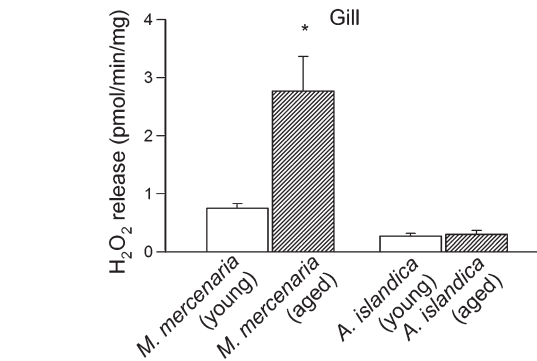
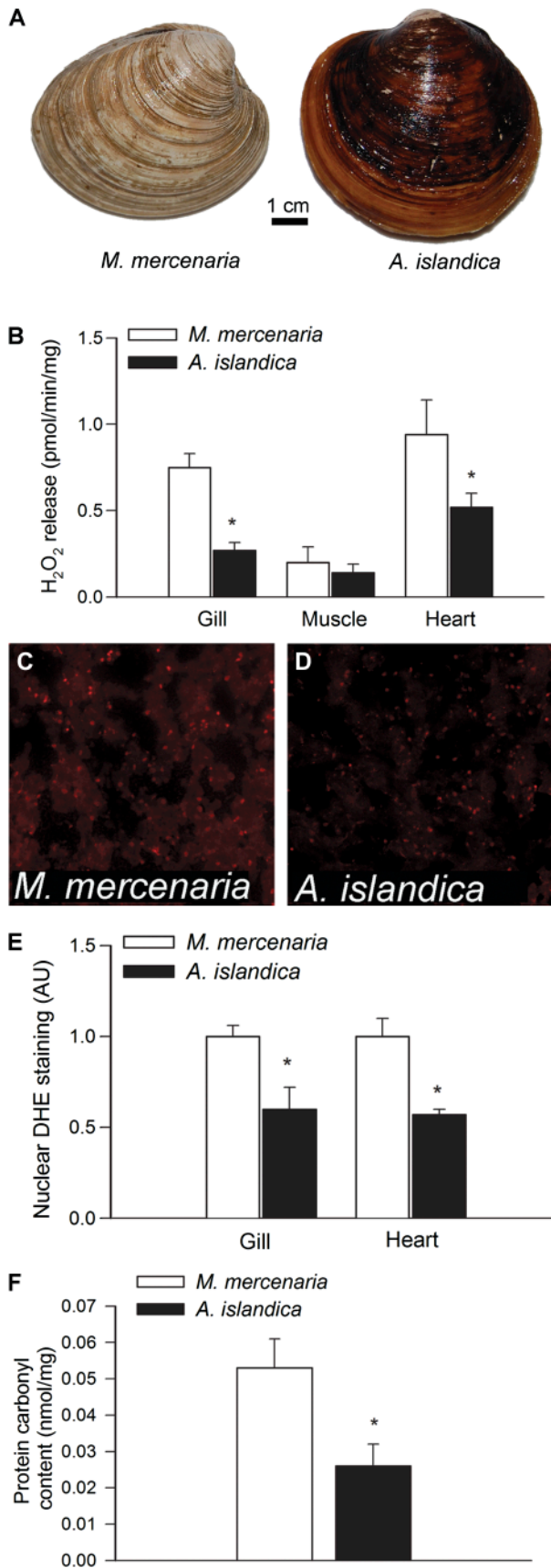


Figure 2. Production of H<sub>2</sub>O<sub>2</sub> in the gill of young and aged *Mercuria mercenaria* and *Arctica islandica*, as assessed by the Amplex Red/HRP assay (for the mean chronological ages of each group, see the Methods). Data are mean  $\pm$  SEM ( $n = 3$ –8 animals for each group). \* $p < .05$  versus respective young controls.

## RESULTS

### Cellular Production of ROS

Production of H<sub>2</sub>O<sub>2</sub> in gill and heart of *A. islandica* was significantly less than those of *M. mercenaria* (Figure 1B). Production of H<sub>2</sub>O<sub>2</sub> in the adductor muscle did not differ significantly between the two species (Figure 1B). Analysis of nuclear DHE fluorescence intensities (Figure 1C and D) showed that cellular superoxide production was also decreased in the gill and in the heart of *A. islandica* as compared with *M. mercenaria* tissues (Figure 1E). Lower cellular ROS production was associated with reduced protein carbonyl content in the gill of *A. islandica* as compared with *M. mercenaria* tissues (Figure 1F). There was a significant age-related increase in H<sub>2</sub>O<sub>2</sub> production in the gill of *M. mercenaria* (Figure 2) but not in that of *A. islandica* (Figure 2). There were no significant age-related increases in H<sub>2</sub>O<sub>2</sub> production in the adductor muscle and the heart of *A. islandica* and *M. mercenaria* (data not shown).

### Survival

To assess resistance to oxidative stress, we obtained survival curves of the clams in the presence of TBHP. Analysis of the survival curves revealed that *A. islandica* survived significantly longer than *M. mercenaria* both in 1 mmol/L TBHP ( $p = .002$ ; Figure 3A) and 6 mmol/L TBHP ( $p = .012$ ; Figure 3B).

Figure 1. (A) Photographs of the marine bivalves *Mercuria mercenaria* (left) and *Arctica islandica* (right). (B) Production of H<sub>2</sub>O<sub>2</sub> in gill, muscle, and heart tissues isolated from *M. mercenaria* and *A. islandica*, as assessed by the Amplex Red/HRP assay. Data are mean  $\pm$  SEM ( $n = 8$  animals for each group). \* $p < .05$  versus *M. mercenaria*. (C and D) Representative images showing red nuclear dihydroethidium (DHE) fluorescence, representing cellular O<sub>2</sub><sup>-</sup> production, in sections of the heart of *M. mercenaria* (C) and *A. islandica* (D). Original magnification: 20 $\times$ . (E) Summary data for average nuclear DHE fluorescence intensities in sections of the gill and heart of *M. mercenaria* and *A. islandica*. Data are mean  $\pm$  SEM ( $n = 5$  animals for each group). \* $p < .05$  versus *M. mercenaria*. (F) Carbonyl content of cellular proteins isolated from *M. mercenaria* and *A. islandica*. Data are mean  $\pm$  SEM ( $n = 8$  animals for each group). \* $p < .05$  versus *M. mercenaria*.



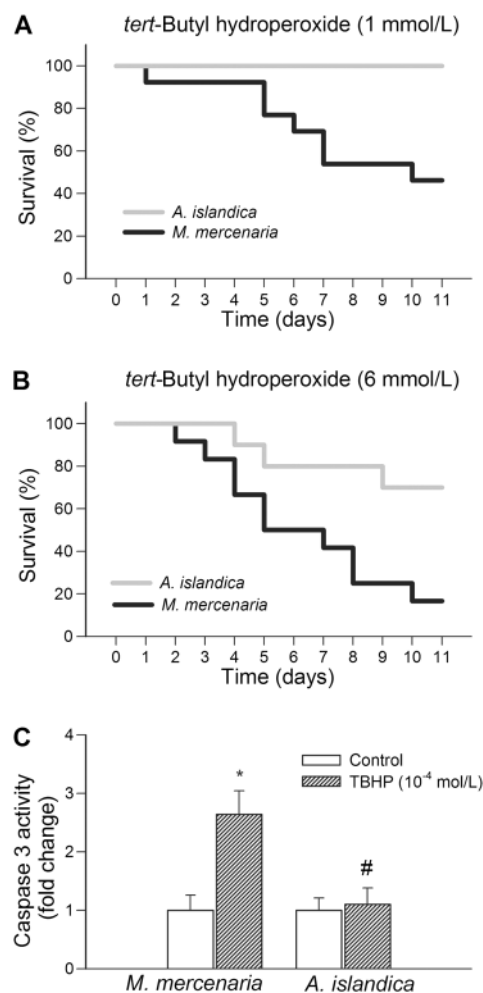


Figure 3. (A and B) Survival analysis of *Mercenaria mercenaria* and *Arctica islandica* under exposure to  $10^{-3}$  mol/L (A) or  $6 \times 10^{-3}$  mol/L (B) *tert*-butyl hydroperoxide (TBHP). (C) TBHP ( $10^{-4}$  mol/L, for 24 hours)-induced changes in caspase 3/7 activity in gills of *M. mercenaria* and *A. islandica*. Data are mean  $\pm$  SEM ( $n = 8$  for each group). \* $p < .05$  versus untreated control, # $p < .05$  versus *M. mercenaria*.

#### Apoptotic Cell Death

Exposure to TBHP ( $10^{-4}$  mol/L, for 24 hours) significantly ( $p < .05$ ) increased caspase 3 activity in gill tissues of *M. mercenaria* (Figure 3C) but not in the gill of *A. islandica* (Figure 3C). The interspecies difference that exists between the magnitude of TBHP-induced caspase activation in *M. mercenaria* and *A. islandica* tissues was statistically significant ( $p < .05$ ). Analysis of cytoplasmic histone-associated DNA fragments, which also indicate apoptotic cell death, yielded identical results (data not shown).

#### Cellular Antioxidant Capacity

There was no interspecies difference between ORAC (Figure 4A) and HORAC (Figure 4C) in gills of *A. islandica* and *M. mercenaria* under baseline conditions or after exposure to TBHP ( $10^{-4}$  mol/L, for 24 hours). There was an age-related decline in ORAC in *M. mercenaria*, whereas ORAC

did not change with age in the gills of *A. islandica* (Figure 4B). No age-related change in HORAC was detected in either species (Figure 4D).

#### Antioxidant Enzyme Activities

In young animals under baseline conditions, there were no interspecies differences between catalase activity (Figure 5A), SOD activity (Figure 5C), and GPX activity (Figure 5E) in gills of *A. islandica* and *M. mercenaria*. After exposure to TBHP ( $10^{-4}$  mol/L, for 24 hours), there were no interspecies differences between catalase activity (Figure 5A) and GPX activity (Figure 5E) in gills of *A. islandica* and *M. mercenaria*. SOD activity (Figure 5C) was significantly lower in gills of TBHP-exposed *A. islandica* than in tissues of *M. mercenaria*. There was an age-related decline in catalase (Figure 5B) and GPX activities (Figure 5F) both in *M. mercenaria* and in *A. islandica*, whereas SOD activity did not change with age in the gills of either species (Figure 5D).

#### Proteasome Activity

To test the hypothesis that enhanced protein recycling activities may underlie longevity and increased stress resistance in *A. islandica*, we measured three types of peptidase activities of the 20S/26S proteasome. Under baseline conditions, there were no interspecies differences between trypsin-like activity (Figure 6A) and chymotrypsin-like activity (Figure 6B) in gills of *A. islandica* and *M. mercenaria*, whereas caspase-like activity was significantly lower in gills of *A. islandica* than in those of *M. mercenaria* (Figure 6C). After exposure to TBHP ( $10^{-4}$  mol/L, for 24 hours), there were no interspecies differences between trypsin-like activity (Figure 6A) in gills of *A. islandica* and *M. mercenaria*, whereas chymotrypsin-like activity (Figure 6B) and caspase-like activity (Figure 6C) were significantly lower in gills of TBHP-exposed *A. islandica* than in tissues of *M. mercenaria*. In the adductor muscle, baseline trypsin-like proteasome activity (AU, M.m.:  $1 \pm 0.17$ , A.i.:  $0.76 \pm 0.08$ , n.s.), chymotrypsin-like proteasome activity (AU, M.m.:  $1 \pm 0.17$ , A.i.:  $0.7 \pm 0.25$ , n.s.), and caspase-like proteasome activity (AU, M.m.:  $1 \pm 0.17$ , A.i.:  $1.21 \pm 0.35$ , n.s.) did not differ significantly between *M. mercenaria* and *A. islandica*.

#### DISCUSSION

If cellular ROS production is a determinant in the rate of aging, then cells of long-lived animals should produce less ROS than shorter-lived ones. The data from this study demonstrating that extreme longevity in *A. islandica* is associated with an attenuated cellular  $H_2O_2$  production and superoxide generation in the heart and gill are consistent with this hypothesis (Figure 1B–E). These observations are also consistent with recent data obtained in mitochondria isolated from shorter- and longer-living burrowing clams

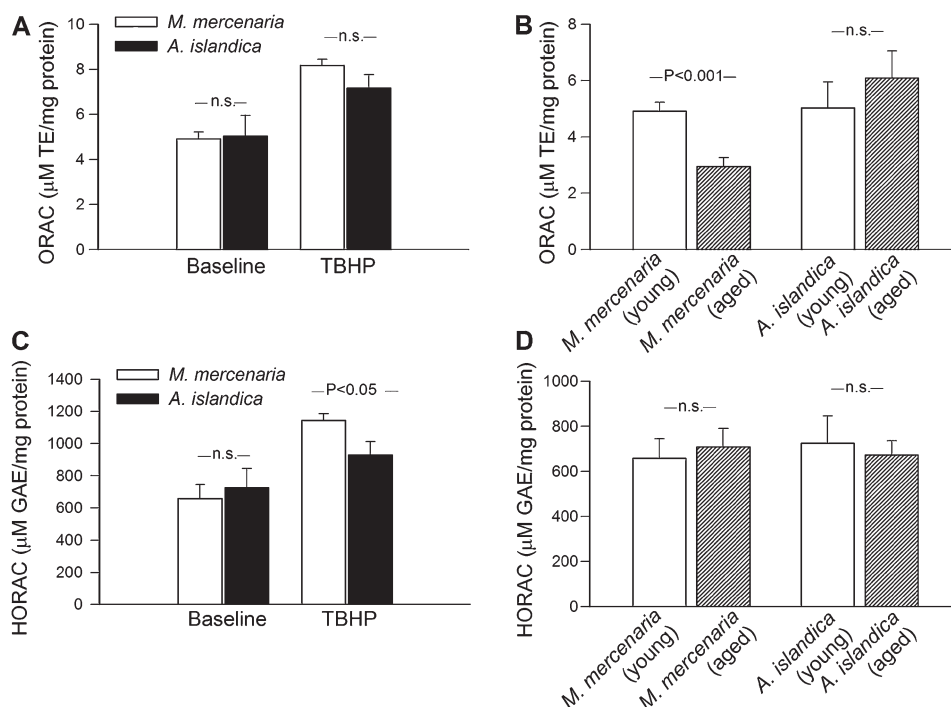


Figure 4. Oxygen radical absorbance capacity (ORAC, **A**) and hydroxyl radical antioxidant capacity (HORAC, **C**) in homogenates of gill tissues from young *Mercenaria mercenaria* and *Arctica islandica* maintained under control conditions ("baseline") or exposed to *tert*-butyl hydroperoxide (TBHP;  $10^{-4}$  mol/L, for 24 hours). Data are mean  $\pm$  SEM ( $n = 8$  in each group). ORAC (**B**) and HORAC (**D**) were also compared in young and aged *M. mercenaria* and *A. islandica* (for the mean chronological ages of each group, see the Methods). Data are mean  $\pm$  SEM ( $n = 3$ –8 animals for each group).

(29). Yet, contrary to our expectation, we did not find the same pattern in adductor muscle (Figure 1B). These data suggest that important tissue-specific differences exist in cellular ROS production in clams, perhaps due to differences in cellular mitochondrial content (mitochondria-rich cell types, such as gill cells, are expected to release more  $\text{H}_2\text{O}_2$  than cells with lower mitochondria density). The findings that mean protein carbonyl concentration in gill tissue was lower in *A. islandica* than in *M. mercenaria* (Figure 1F) suggest that reduced ROS production in long-living bivalves is associated with a lower level of accumulated macromolecular damage as compared with shorter-living animals. Similar conclusions were reached also by previous studies on *A. islandica* (17). Interestingly, these findings in marine bivalves agree with the recent findings of Lambert and colleagues (10) and others (30,36–40) indicating that in a range of vertebrate homeotherms, there exists an inverse correlation between cellular- and mitochondrial-free radical production, oxidative protein damage, and maximum life span. Our studies further demonstrate that cellular ROS production substantially increases with age in the gill of *M. mercenaria*, whereas in tissues from *A. islandica*, aging did not result in significant oxidative stress (Figure 2). Our results accord with that of Strahl and colleagues (17) showing that in the gill and mantle tissue of aged *A. islandica* (up to 190-year-old specimens), there is no significant age-related increase in protein carbonyl content. In that regard, it is significant that in mammals an association between longer life

span with a slower rate of age-related changes in cellular ROS production has also been documented (30). Taken together, the aforementioned findings are consistent with predictions based on the oxidative stress hypothesis of aging and suggest that cellular redox homeostasis may be an important contributing factor in life span in evolutionarily distant phyla.

At present, the mechanisms underlying the differences in mitochondrial and cellular ROS production between short-lived and long-lived species are not well understood. The mechanisms may include differences in the efficiency of the mitochondrial electron transport chain, uncoupling proteins, mitochondrial membrane composition, mitochondrial thiol redox state, amounts of coenzyme Q associated with mitochondrial membrane proteins, differential regulation of the entry of electrons into the cytochrome chain, as well as differential role of cytoplasmic and plasma membrane-associated oxidase systems. Further research in the regulation of mitochondrial function will be necessary. Insulin-like signaling is thought to play an important role in regulation of longevity in diverse vertebrate and nonvertebrate species. Interestingly, there are data suggesting that insulin-like signaling is important in regulating cellular metabolism in certain clam species (23); however, the role of insulin-like signaling in regulation of mitochondrial oxidative stress and longevity in bivalves is completely unexplored. Mitochondrial function in clams is also likely affected by the water temperature. Accordingly, recent studies demonstrated temperature-dependent

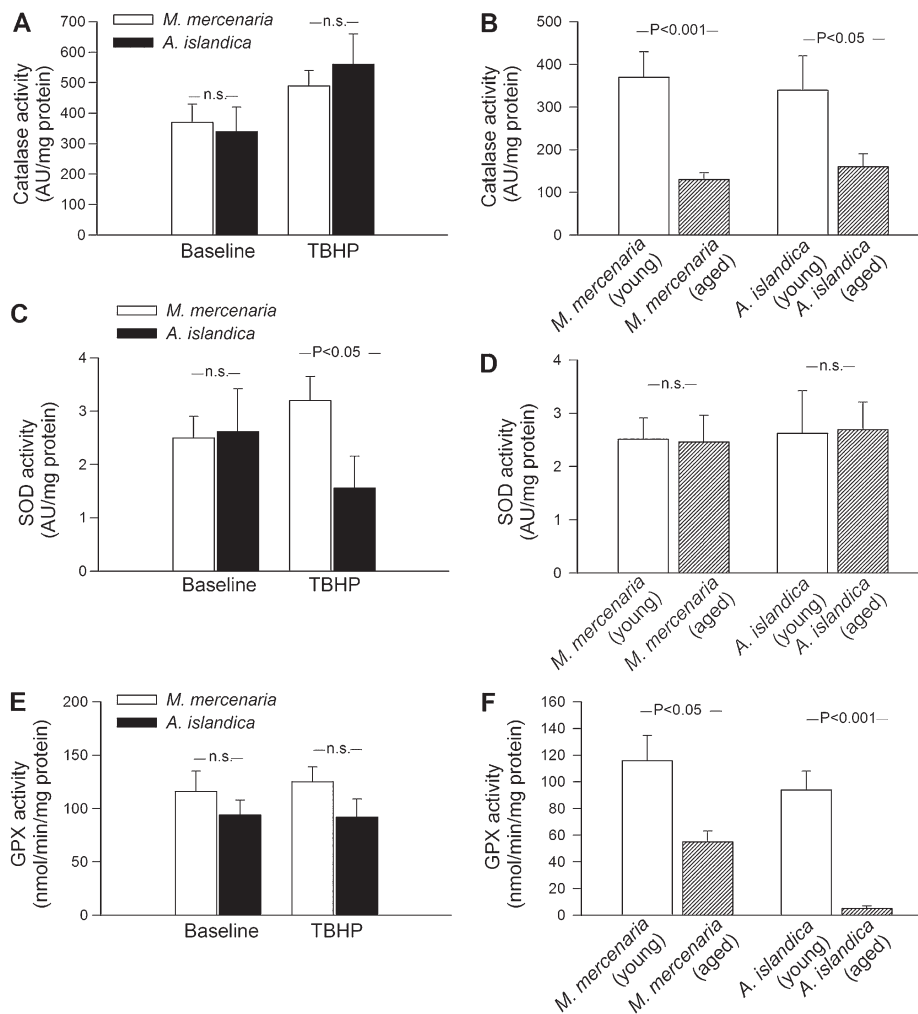


Figure 5. Antioxidant enzyme activities in *Mercenaria mercenaria* and *Arctica islandica*. Catalase activity (A), superoxide dismutase (SOD) activity (C), and glutathione peroxidase (GPX) activity (E) were assessed in homogenates of gill tissues from *M. mercenaria* and *A. islandica* maintained under control conditions ("baseline") or exposed to *tert*-butyl hydroperoxide (TBHP;  $10^{-4}$  mol/L, for 24 hours). Data are mean  $\pm$  SEM ( $n = 8$  in each group). Catalase activity (B), SOD activity (D), and GPX activity (F) were also compared between young and aged *M. mercenaria* and *A. islandica* (for the mean chronological ages of each group, see the Methods). Data are mean  $\pm$  SEM ( $n = 3$ –8 animals for each group).

protein phosphorylation responses in the mitochondria, which may regulate mitochondrial efficiency and perhaps ROS production in bivalves (41,42).

Accumulating empirical data obtained in diverse vertebrates and invertebrate model systems suggest that resistance to the aging process at the organismal level is often reflected in resistance to oxidative stressors at the cellular level (43,44). In the present study using two clam species exposed to organic peroxide treatment as a model system, we found that there was an association between longevity and resistance to oxidative stress-induced mortality (Figure 3A and B). We also found that longer-living *A. islandica* exhibited marked resistance to oxidative stress-induced cell death as compared with shorter-living *M. mercenaria* (Figure 3C). Our findings extend previous observations in a wide variety of experimental settings, ranging from model organisms to rodent models and primate fibroblasts (43–46).

The striking correlation between the increased oxidative stress resistance of longer-lived animals in evolutionarily distant phyla is consistent with the existence of evolutionary highly conserved pathways involved in both cellular stress resistance and lifespan regulation.

The mechanisms underlying the quantitative differences in cellular oxidative stress resistance between short-lived and long-lived bivalves are likely multifaceted. Here, we tested the hypothesis that differences in the efficiency of cellular antioxidant systems may explain the superior oxidative stress resistance of *A. islandica* as compared with that of *M. mercenaria*. Contrary to our predictions based on the oxidative stress hypothesis of aging, we found that in *A. islandica* neither basal antioxidant capacities (Figure 4A–B) nor specific antioxidant enzyme activities (Figure 5A, C, and E) were greater than in *M. mercenaria*. These results extend previous findings of Abele and colleagues (18). In

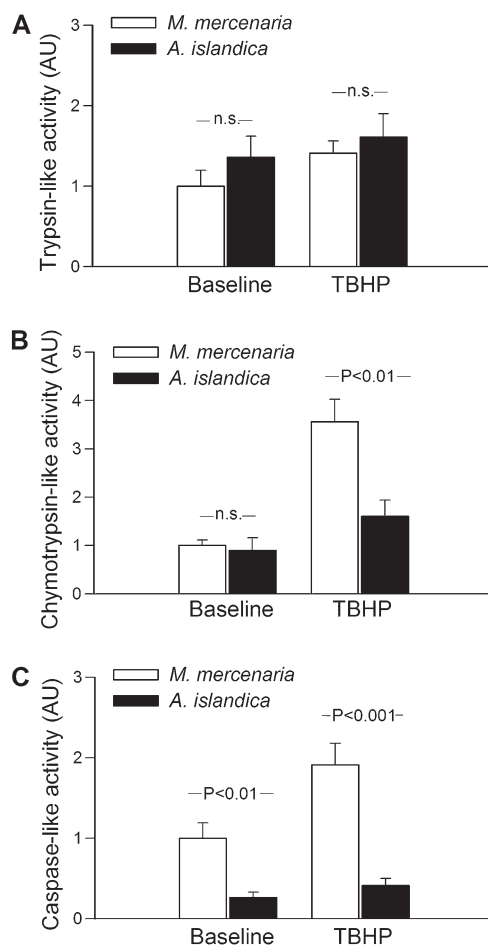


Figure 6. Proteasome activity in *Mercenaria mercenaria* and *Arctica islandica*. Trypsin-like activity (A), chymotrypsin-like activity (B), and caspase-like activity (C) were assessed in homogenates of gill tissues from *M. mercenaria* and *A. islandica* maintained under control conditions ("baseline") or exposed to *tert*-butyl hydroperoxide (TBHP;  $10^{-4}$  mol/L, for 24 hours). Data are mean  $\pm$  SEM ( $n = 8$  in each group).

that context, it is interesting to note that in mice with over-expression or genetic knockout of major antioxidant enzymes (including MnSOD, Cu,ZnSOD, catalase, and glutathione peroxidase), there is no correlation between alterations of cellular antioxidant capacity and life span(3,4). Because in response to oxidative stressors in eukaryotic cells an evolutionarily conserved antioxidant response can be manifest, we also analyzed these antioxidant systems in tissues of clams exposed to TBHP. In contrast to our expectation, we found that *A. islandica* did not exhibit a more pronounced homeostatic antioxidant response than *M. mercenaria* (Figures 4A and B and 5A, C, and E). On the basis of the oxidative stress hypothesis of aging, it is predicted that short-lived animals exhibit a greater age-related decline in antioxidant defenses than successfully aging species. Although the ORAC data were in agreement with this prediction (Figure 4B), in other experiments, we did not observe a consistent pattern for maintenance of superior antioxidant defenses in aged *A. islandica* (Figures 4D and 5B,

D, and F). Our results accord with the available evidence showing that age-related changes in antioxidant capacity can vary between antioxidants and between tissues of an organism and also depend on species-specific lifestyle (47). In conclusion, the aforementioned data do not support a predominant role of superior free radical detoxification systems in the extreme longevity and increased oxidative stress resistance of *A. islandica*.

Maintenance of protein homeostasis is also thought to be a critical determinant of both cellular stress resistance and life span (36,48). Previous studies reported that in various species, proteasome activity declines with age (49–52), and this aging-induced proteasome dysfunction was proposed to be involved in the etiology and/or progression of various age-related diseases (53,54). The findings that in tissues of *A. islandica*, proteasome activities are not increased as compared with those in that of *M. mercenaria* (Figure 6) suggest that in bivalves extreme longevity and resistance to oxidative stressors are not associated with enhanced protein recycling activities. Further studies are evidently needed to compare additional mechanisms involved in maintenance of protein stability and integrity (48,55) and to investigate whether interspecies differences in age-related changes in protein repair or proteasomal degradation capacities contribute to the divergent aging profiles observed in *A. islandica* and *M. mercenaria*. Mitochondrial heat-shock proteins were shown to decrease with age in mollusks, suggesting an age-related decline in mitochondrial chaperone protection (56). Thus, it will be interesting to compare the expression of proteins that are responsible for folding/refolding of newly synthesized and damaged proteins in bivalve models of exceptional longevity.

## Conclusions

To our knowledge, this is the first study comparing oxidative stress resistance in a longevity contrast pair of bivalve mollusk species. Our findings demonstrating an association between longevity and resistance to oxidative stress-induced cell death in *A. islandica* are in accordance with predictions based on the oxidative stress hypothesis of aging and provide justification for evaluation of pathways involved in repair of free radical-mediated macromolecular damage and regulation of apoptosis in the longest-living non-colonial animal.

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