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Age, Diet, and Season Do Not Affect Longevity-Related Differences in Peroxidation Index Between *Spisula solidissima* and *Arctica islandica*

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The susceptibility of membrane lipids to peroxidation (peroxidation index [PI]) increases with the double bond content of fatty acids and is inversely correlated to longevity in mammals, birds, and bivalve molluscs. In molluscs, membrane polyunsaturated fatty acids content can be affected by temperature, nutrition, and the individual's age. In this study, we evaluated how these three parameters may alter correlations between PI and longevity. We determined the fatty acid and dimethyl acetal compositions of phospholipids from gill mitochondrial and nonmitochondrial preparations from the short-lived *Spisula solidissima* (maximum longevity = 37 years) and the long-lived *Arctica islandica* (maximum longevity = 507 years) exposed to diet abundance and temperature (season) treatments. We also evaluated the effect of individual age on PI in *S. solidissima* (from 6 to 23 years). The temperature increase from winter to summer (2 to 12°C) coincided with decreases in values of PI, proportions of eicosapentaenoic acid, and dimethyl acetals. Higher microalgae supplementation increased polyunsaturated fatty acids and PI and decreased dimethyl acetals; age did not affect the PI in *S. solidissima*. Our finding that the PI of *A. islandica* remained significantly lower than that of *S. solidissima* in corresponding fractions throughout treatments suggests that longevity-related differences in PI are resilient to environmental conditions.

Key Words: *Arctica islandica*—Membrane lipids—Mitochondria—Longevity—Polyunsaturated fatty acids.

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THE membrane pacemaker theory of aging (also referred to as the homeoviscous-longevity theory of aging) is a hypothesis that refines the general oxidative stress theory of aging by emphasizing the key role of membrane lipids in the processes of detrimental oxidation of macromolecules underlying cellular senescence. The hypothesis has gained strong support from comparative studies in vertebrates models (mostly mammals and birds) that shows a quasi-universal inverse correlation between longevity and the susceptibility to oxidation of membrane lipids (1,2). The susceptibility to oxidation of a fatty acids (FAs) increases with increasing number of double bonds (unsaturation) on the carbon chain. Polyunsaturated fatty acids (PUFAs) are therefore more susceptible to attack by reactive oxygen species and are known to undergo a chain reaction of autoxidation within membranes that ultimately releases reactive aldehydes. These reactive aldehydes are toxic secondary reactive oxygen species known to form adducts to protein, lipids, and DNA (advanced lipoxidation end products), causing losses of functionality and mutations that contribute to cellular aging (3). Because the individual susceptibility to oxidation of FAs has been determined empirically and assigned a peroxidation index (PI) (4), whole-membrane PI can be derived from the abundance of each class of FA following phospholipid (PL) analysis. This value has been found to negatively correlate with species' longevity for liver, skeletal muscle, and heart (reviewed in 1,2) and intraspecifically between strands of invertebrates of diverging longevity (5,6).

Long-lived bivalve molluscs are emerging biogerontology model system that allow assessing evolutionary conserved mechanisms of aging (7). Using the longest lived metazoan, the marine bivalve (*Arctica islandica*, maximum reported longevity [MRL] = 507 years) (8,9), and four related species of shorter longevity, we recently demonstrated the existence of this correlation in bivalve molluscs (10). The present study has been undertaken to verify three potential bias that arise when considering the susceptibility to oxidation of bivalve membranes.

1. The membrane FA composition and consequently the PI value can change over the course of an organisms' life. In vertebrates and invertebrates with finite growth, PUFAs (and hence PI) have either been shown to increase or remain constant with age (1,11), however, there are currently no information available for bivalves. Bivalves present infinite growth, following a von Bertalanffy function, that is, it is very rapid in the first years until the

curve reaches an inflexion point past which it is asymptotic and never ceases (12). If the PI keeps changing constantly during infinite growth, then comparative studies addressing the membrane pacemaker theory of aging in bivalve requires age corrections.

2. The availability of dietary PUFAs, and especially highly unsaturated FAs (HUFAs), may drastically alter membrane PI in bivalves. As opposed to mammals, bivalves cannot synthesize PUFAs de novo, and HUFAs such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which strongly impact PI, must be acquired from the microalgal diet (13).
3. Ectotherm invertebrates are well known to perform cold-induced lipid remodeling. Cold increases membrane lipid packing order and decreases membrane fluidity which results in a decrease in integral membrane protein activity and ion permeability and a decrease in the overall pace of biological processes. The majority of the cold-active ectotherm species investigated so far respond to cold by either increasing the ratio of unsaturated to saturated FAs (homeoviscous adaptation) or increasing membrane HUFAs (reviewed in 14,15). Cold-induced lipid remodeling may therefore alter membrane PI of bivalves.

Investigating membrane PI in bivalves is further complicated by the complex lipid biochemistry. Bivalves are capable of synthesizing non-methylene-interrupted FAs (NMI; mostly 20:2 and 22:2 FAs with four or six methyl groups between double bonds) (16) that are preferentially incorporated into membrane PLs (17). NMI are thought to be recruited to compensate HUFA dietary deficiencies as well as to maintain membrane fluidity in response to declining temperatures (17). These FAs have a very low class specific PI (18) and can be found in important proportions, for example, 18.8% in gills of *A. islandica* (10). A change in their proportion with acclimatization to temperature or feeding regime is highly susceptible to result in drastic changes in PI.

Plasmalogens (1-alkenyl-2-acyl-sn-glycero-3-phospholipids) are also abundant in all types of tissue in bivalves. Although, in vertebrates, they are only found in significant proportions in the nervous and cardiac tissue, they represent, respectively, 38% and 44% of PLs in the whole body of the blue mussel (*Mytilus edulis*) and the Pacific oyster (*Crassostrea gigas*) (19). The plasmalogen-specific vinyl ether bond is known to act as a reactive oxygen species scavenger and breaker of autoxidation; it is believed to confer in situ antioxidant protection in membranes (20–22), providing a potential mechanism to modulate resistance to lipoxidation. The plasmalogen content of a membrane therefore provides additional information about its susceptibility to undergo lipoxidation that complements the information given by the sole PI value. Plasmalogen content has been shown to increase with environmental temperature in other taxa of invertebrates (14,23), hence, the overall resistance to oxidation of membrane could also be modulated by temperature through adjustments in plasmalogens.

Branched FAs and branched alkenyl chains (dimethyl acetals [DMAs]) are also found in nonnegligible proportion in bivalves (10). These originate from the bacterial component of the diet, and very few information is available regarding their role in modulating lipoxidation and membrane viscosity.

The aim of this study is to provide a first assessment of the impact of diet abundance and season (temperature) as potential factors influencing the longevity-related difference in PI (and plasmalogens) between a short-lived *Spisula solidissima* (MRL = 37 years) and an extremely long-lived *A. islandica* bivalve species. The existence of an age–PI relationship was also investigated in *S solidissima*. All analysis included NMI FAs, plasmalogens, and branched FAs and were performed in parallel for mitochondrial membrane PLs and for PLs of other cell membranes as to contrast the response in each type of membrane.

Methods

Animal Care and Dissection Schedule

A first lot of *S solidissima* and *A. islandica* were collected during spring 2009 in the Îles-de-la-Madeleine, Québec (Canada) at the following locations and depths: *S. solidissima* 47° 32′N, 61° 37′W at 5 m; *A. islandica* 47° 21′N, 61° 45′W at 15 m. Specimens were air-shipped to the Station Aquicole in Pointe-au-Père, Québec. Upon arrival at the station, they were numbered and placed in an open flow-through, two-tank system containing 20 cm of sand. In addition to the natural phytoplankton remaining in the semi-filtered (50 µm) water of the station, bivalves were fed a mixed suspension of live microalgae (12% *Nannochloropsis* sp., 44% *Isochrysis galbana*, and 44% *Pavlova lutheri* in cell number) purchased at NutrOcéan Inc. (Québec, Canada). This supplementation was adjusted to reproduce the natural cycle of microalgae abundance, that is, it was given at 1% body mass/d at 8°C and adjusted following temperature changes between April and November according to a Q_{10} (for metabolism) of 2.5.

A first group was dissected in April following the mild starvation of winter that corresponds to the period of very low food abundance in the natural environment; this group is referred to as the natural cycle group with respect to the effect of food abundance. At this period of the year, water temperature had been at its minimum of 2°C for 4 months, which should have allowed the full extent of cold lipid remodeling. Hence, individuals collected at this period are referred to as the winter group with respect to temperature effect. A second group (summer group) was dissected in late August, when water temperature had been stable at its highest value (12°C) for 2 weeks, allowing the full extent

of warm lipid remodeling. Hence, the effect of season was assessed on bivalves fed following the natural cycle of food abundance. A second lot of *S solidissima* was collected in April 2010 at the same location in the Îles-de-la-Madeleine and a second lot of *A. islandica* was collected off the coast of Maine (USA) in early March 2010. These new clams were kept under identical conditions, with the exception that they were provided with the microalga diet earlier in spring and at full rate of feeding (corresponding to 8°C for the natural cycle group) even though water temperature was still between 2 and 6°C. These were subsequently sampled in late May and are referred to as the early spring supplemented group with respect to the effect of food abundance. Hence, the effect of food abundance was estimated at a low temperature, when metabolic requirements are still low and additional FA is more available for incorporation into membranes instead of being used for reproductive investment or fuel of the high metabolism associated with warm temperatures. *S. solidissima* of all groups had similar size distributions. Those of the spring supplementation were further aged to test for a possible relationship between age and membrane FA composition. All *A. islandica* were over the size of maturity according to fishery management reports (24); however, they were all below 1/5 of the species' MRL according to size and previous age determination of a subsample.

Isolation of Biological Fractions

Mitochondria were isolated as described in (10). Briefly, bivalves were opened by inserting a knife blade between valves and cutting the adductor muscles, gills were removed, minced, and homogenized using a Potter-Elvehjem. The homogenate was centrifuged at 1,250*g* for 10 minutes, and the supernatant was removed and conserved. The pellet was resuspended in 15 mL of homogenization buffer and centrifuged at 1,000*g* for 10 min; this second supernatant was removed and pooled with the first. The final pellet of cellular debris was conserved; this represented the biological fraction containing nonmitochondrial membranes. The two supernatants were then combined and a first centrifugation was run at 1,250*g* for 10 minutes to eliminate residual debris. The final supernatant was centrifuged at 10,500*g* for 15 minutes to recover the final mitochondrial pellet. All homogenization and centrifugation steps were carried out at 4°C. Both biological fractions were held at −80°C under nitrogen atmosphere until lipid extraction. The nonmitochondrial fraction was assayed for mitochondria-specific enzymes and was found essentially devoid of mitochondria (10).

Lipid Analysis

Lipids were analyzed as described in (10). Briefly, polar lipids of the mitochondrial or nonmitochondrial fractions were separated on silica gel (60, mesh 150–230) microcolumns. The total PLs acyl and alkenyl chains were then transmethylated in boron trifluoride-methanol (SUPELCO, Bellefonte, PA), and the resulting FA methyl esters (FAME) and DMAs were analyzed with GC-MS (GC: AGILENT 6850 series II; MS: AGILENT 5975 B) using a high polarity column (HP-88 60 m, 0.25 mm × 0.20 µm). Calibration of the system was performed using regular FA mix standards (SUPELCO 37 FAME), marine FA standards (SUPELCO), and DMA standards (SIGMA-Aldrich). Because triglycerides were eliminated from the analysis and only PLs were subjected to methylation, the percentage of plasmalogens among PLs is simply double the percentage of DMAs among the total of carbon chains. The chain fluidity index (CFI) is calculated according to equation (1), where "chains" refers to both FAs and DMAs. This equation aims to represent the relative contribution of monounsaturated and polyunsaturated FAs or DMAs to the overall membrane fluidity according to Coolbear and colleagues (25).

$$\begin{aligned}\text{CFI} = {} & (1 \times \% \text{ Monounsaturated chains}) \\ & + (1.5 \times \% \text{ Polyunsaturated FAs})\end{aligned} \quad (1)$$

The PI is calculated according to equation (2), see (1).

$$\begin{aligned}\text{PI} = {} & (0.025 \times \% \text{ Monoenoics}) + (0.258 \times \% \text{ 20:2 NMI}) \\ & + (0.32 \times \% \text{ 22:2 NMI}) + (1 \times \% \text{ Dienoics}) \\ & + (2 \times \% \text{ Trienoics}) + (4 \times \% \text{ Tetraenoics}) \\ & + (6 \times \% \text{ Pentaenoics}) \\ & + (8 \times \% \text{ Hexaenoics})\end{aligned} \quad (2)$$

Age Determination

The bivalve *S. solidissima* is characterized by a large chondrophore located adjacent to the hinge that has interannual growth lines visible to the naked eye for at least the first 10 years of growth (26). Hence, age can be estimated by using only a magnifying glass or laboratory binocular microscope. To ascertain of the validity of this method, individual ages were previously determined for 16 of our individuals with a classical method under the supervision of a bivalve sclerochronology expert (Dr. Julien Thébault, UBO, France). Briefly, a 1.6 mm thick section of the left valve of shells was cut perpendicularly to the growth lines in the chondrophore section using a low speed precision saw (Struers secotom-10) equipped with a diamond-coated blade (see Supplementary Figure S1). The section was mounted on a glass slide, manually ground with sequentially decreasing grades of sand paper, and polished with 3 µm Al_2O_3 powder. Interannual growth lines were resolved by taking high-resolution photographs (20×) using a Zeiss (AxioCam MRc5) camera attached to a Zeiss (Lumar V12) microscope (see Supplementary Figure S2). The right valve of the same individual was then read with a binocular

microscope to establish the correspondence. The binocular method does not resolve the first interannual growth line, but the subsequent lines are easily distinguished. Therefore, we aged individuals for this study with the binocular method and added 1 year to account for this bias.

Statistics

The factors age, wet weight, and length were included in the analysis of covariance as covariates to test for a possible correlation with PI, EPA, DHA, and DMA contents in parallel for both biological fractions. When no interaction between the biological fraction (mitochondria or nonmitochondrial) was found, the interaction term was eliminated to test the effect of the covariates alone. The effects of microalgae supplementation (supp.) and season were tested with a three-way analysis of variance including the factors species and biological fraction (mitochondrial or nonmitochondrial membrane) and all their interactions. Significant differences between groups ($\alpha = .05$; Tukey's post hoc) are presented in figures for significant interactions or factors. Homogeneity of variance was ascertained by visual examination of the residuals. All analyses were performed using the JMP v10.0 statistical package (SAS Institute Inc.). Results are presented as means ± standard error of the mean in Figures 2 and 3. A table listing *F*-ratio, *df*, and *p* value for the analysis of covariance and analysis of variance that preceded the Tukey–Kramer post hoc test is available online as Supplementary Table S3.

Results

S. solidissima individuals of the sampled population are reported to be mature at lengths between 75 and 92 mm (27). All individuals used in this study were more than 100 mm and should therefore be sexually mature. However, our specimens ranged in age from 6 to 23 years, which encompasses a large portion of the total longevity of this species (MRL = 37 years). A suspension of microalgae rich in HUFAs was provided for 1 month during early spring before dissection to allow the expression of age-specific membrane FA composition not affected by dietary restriction. We found no relationship between age and HUFAs, that is, there was no relationship between age and levels of total pentaene FAs (FAs with five double bonds), mostly EPA ($F_{1,35} = 0.005$; $p = .941$), and with levels of DHA ($F_{1,35} = 1.02$; $p = .320$) (data not shown). NMI levels were also not related to age ($F_{1,35} = 0.016$; $p = .901$) (data not shown). Similarly, no relationship was found between age and PI (Figure 1A). However, sizes are surprisingly variable

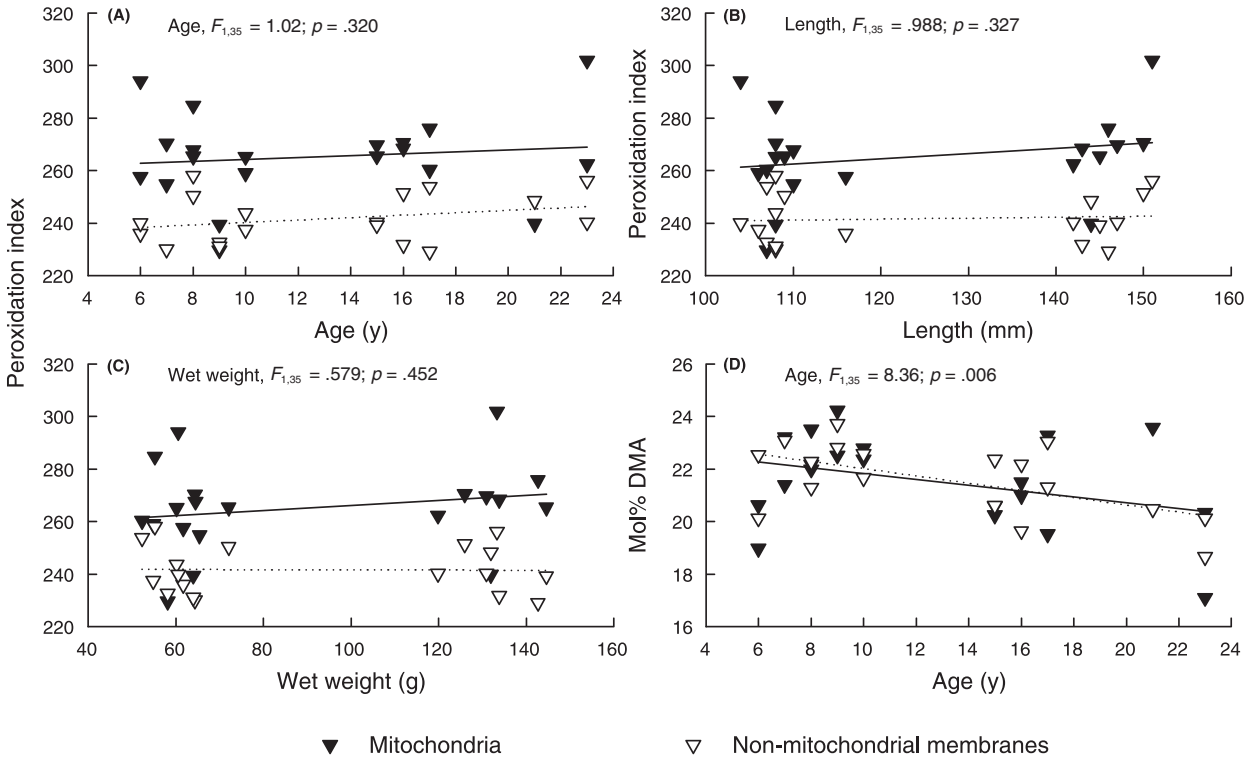

Figure 1. Relationship between the peroxidation index (PI) and age (**A**), length (**B**), and wet weight (**C**) for gill membranes in *Spisula solidissima*. (**D**) Relationship between age and the percent of dimethyl acetals (DMAs) among all carbon chains (fatty acids [FAs] + DMAs). The solid and dotted lines show the relationship for the mitochondrial and non-mitochondrial fractions, respectively. No interaction between the biological fraction and the covariates (age, length, or wet weight) were found in any case (analysis of covariance, Supplementary Table S3).

for bivalves of similar age. Because there is a known relationship between body size and membrane FA composition among mammal species (27), we further examined whether PI was correlated with length and wet weight and found that neither was (Figure 1B and C). Interestingly, however, DMA levels decreased with age for both biological fractions (Figure 1D). Supplementary Table S3 provides detailed results for the tests examining interactions between biological fractions and the covariates (age, length, wet weight), which were nonsignificant in all cases.

Because age had no effect on HUFAs or PI, the 20 *S solidissima* were pooled (early spring supplemented group, spring supp) and compared with individuals of similar sizes that were also sampled during spring, but for which feeding had not yet resumed after the mild winter starvation (natural cycle group). Furthermore, *A. islandica* individuals had also been maintained under the same two conditions of natural feeding cycle or early spring supplementation, which allows us to compare the effect of supplementation for a short-lived (fast growth rate) and a long-lived (slow growth rate) species (28). Spring supplementation increased the proportion of HUFAs incorporated into membranes (see Supplementary Material for tables with complete details of carbon chain composition of each membrane in each treatment). Pentaene FAs significantly increased in *S solidissima* (Figure 2A), and this increase was similar for both biological fractions (Supp. × Fraction, $F_{1,7}$ = 1.046; p = .310). DHA also increased in parallel for both species and fractions (Supp. × Species, $F_{1,71}$ = 0.271, p = .604; Supp. × Fraction, $F_{1,71}$ = 1.714, p = .195; Figure 2B). In accordance

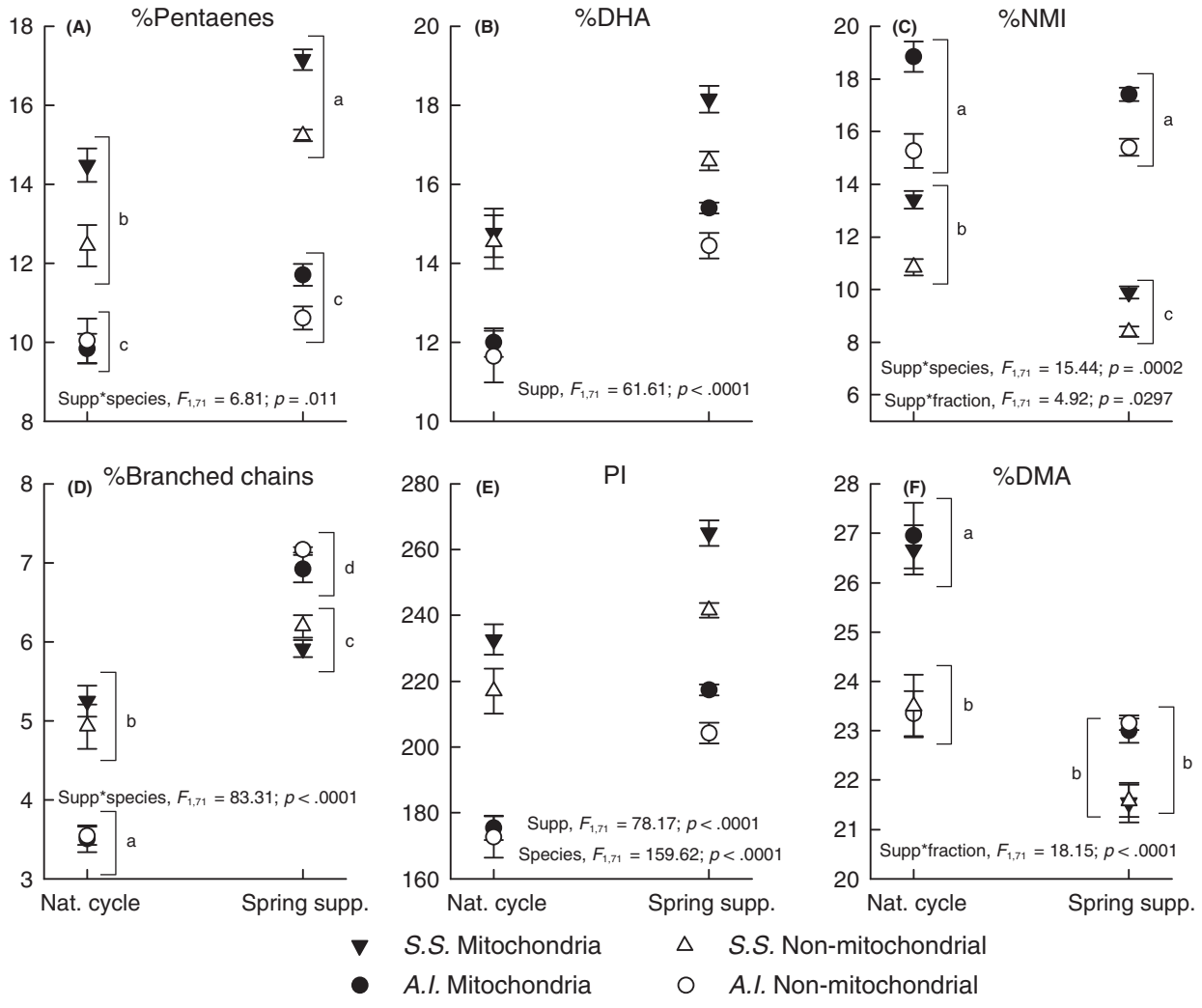

Figure 2. Key markers of membrane lipid composition in gills of *Spisula solidissima* and *Arctica islandica* sampled in spring after a natural cycle of low winter food abundance or after early spring microalgal supplementation. Significant interactions or effects are presented and different letters indicate differences among selected groups (three-way analysis of variance). (**A**) Percent pentaenes fatty acids (five double bonds); (**B**) percent docosahexaenoic acid; (**C**) percent of non-methylene-interrupted fatty acids; (**D**) percent of branched carbon chains (fatty acids + dimethyl acetals); (**E**) peroxidation index; (**F**) percent dimethyl acetals. Sample size for *A. islandica*: natural cycle mitochondrial = 9; natural cycle non-mitochondrial = 8; spring supp. = 4 pooled tissues of two individuals in each fraction and for *S. solidissima*: natural cycle = 8 in each fraction; spring supplemented mitochondrial = 20; non-mitochondrial = 18. Results are means ± standard error of the mean.

with a role for NMI to compensate for HUFA deficiencies, these FAs decreased with supplementation in *S solidissima* (Figure 2C), the species for which total HUFA increases were most pronounced. Supplementation equally increased branched chains (iso and anteiso FAs + DMAs) among fractions (Supp. × Fraction, $F_{1,71} = 2.094$, $p = .152$); among species however, the increase was more pronounced for *A. islandica* (Figure 2D). The difference between species was due to a large increase in branched DMAs that was only observed in *A. islandica* (Supp. × Species, $F_{1,71} = 103.24$, $p < .0001$; data not shown).

Overall, and mostly as a result of the substitution of NMI dienes for HUFAs, supplementation increased the PI. However, no significant interaction terms were found (Supplementary Table S3), and hence this increase was uniform between the two species and fractions. As a result, the differences in PI between the two species are maintained for both food groups (Figure 2E). Plasmalogens also affect membrane susceptibility to oxidation, and their amount can be evaluated by the levels of DMAs. Supplementation decreased DMAs in mitochondria of both species but not in the other cell membranes (Figure 2F).

Clams from the natural cycle group were sampled again in late summer (late August), when water temperature has been at its highest annual point (12°C) for 2 weeks. This allowed us to test the effect of season (mostly the effect of temperature) on membrane lipid composition by comparing these individuals with those sampled during early spring at 2°C, after winter acclimation. We found no effect of season on the abundance of branched carbon chains (data not shown). However, in accordance with expectations of cold-induced lipid remodeling, the levels of pentaenes decreased with the rewarming from winter to summer. This decrease was observed for both species, but significantly more pronounced for *S. solidissima* and for the mitochondrial membrane of both species; levels of pentaenes nonetheless remained higher for *S. solidissima* in corresponding fractions and season (Figure 3A). Rewarming tended to increase DHA in *S. solidissima* and decrease it in *A. islandica*, (Season × Species, $F_{1,57} = 3.31$, $p = .074$), maintaining a significant difference between species throughout seasons (Figure 3B). Rewarming tended to increase NMIs in *A. islandica* and decrease it in *S. solidissima*, which maintained significant difference between species (Figure 3C).

We calculated the CFI, which reflects the contribution of carbon chain unsaturation on the resulting overall membrane fluidity, allowing to appreciate a possible homeoviscous adaptation response to cold. Summer rewarming significantly decreased the CFI in *S. solidissima* but not in *A. islandica* (Figure 3D). Overall, temperature-induced lipid remodeling resulted in a decreasing trend in PI from winter to summer in the mitochondrial fractions but not in the nonmitochondrial fractions (Season × Fraction, $F_{1,57} = 3.38$, $p = .071$). Because the trends were similar in magnitude for both species, the significant differences in PI between them were maintained from winter to summer; the absolute difference was even slightly increased with summer rewarming in the case of mitochondria. Brackets in Figure 3E illustrate the maintenance of this significant difference between species at the level of membrane type (mitochondrial and nonmitochondrial). DMA levels (estimating membrane antioxidant plasmalogens) significantly decreased from winter to summer in *S. solidissima* but not in *A. islandica*, producing a significant difference between species for the summer that we have not observed before (10) (Figure 3F).

Discussion

The aim of this study was to assess the effect of microalgal diet abundance and season (temperature) on membrane lipid composition of the short-lived *S. solidissima* and the long-lived *A. islandica* and test if these effects can modify (or suppress) the longevity-related difference in PI (and plasmalogens) between them. We found that both diet and season (two major modulators of membrane composition) proved capable of modifying the PI to some extent, however, the magnitude of the change was similar for both species, maintaining differences between them across conditions.

Membrane PI in mammalian species is under homeostatic control that maintains constant values throughout a range of medium to high manipulation of dietary PUFAs (29). This is made possible in part because of their capacity to synthesize long-chain *n*-6 and *n*-3 PUFAs from the precursors (linoleic acid, 18:2*n*-6 and linolenic acid, 18:3*n*-3). Long-chain PUFAs are highly abundant in marine phytoplankton, and possibly as a result, many filter-feeding species do not have the capacity for their de novo synthesis. Furthermore, mammalian species can also exchange FAs between triacylglycerol reserves and membrane PLs through the action of the acyltransferase (30). Bivalves do not store large lipid reserves (31), which may impose a proximal limit in resorting to this mechanism to stabilize membrane PI under conditions of dietary deficiencies. Here, we found that a high abundance of dietary microalgae elevated membrane HUFA content, mostly DHA, leading to a high PI value in both biological fractions investigated (mitochondrial and nonmitochondrial membranes of the gills). However, no significant interaction was found between the treatment and the species, that is, the amplitude of the difference in PI between *S. solidissima* and *A. islandica* was maintained across diet abundance treatments.

Season was also expected to affect membrane lipid composition of our bivalve species mainly through a cold-induced lipid remodeling (14,32–34). Lipid remodeling does not necessarily involve modifications of the PI because homeoviscous adaptation solely requires a change in the ratio of saturated to monounsaturated FAs (both have negligible influence on PI) and not in the proportion of PUFAs or HUFAs (25). Many ectotherms, however, respond to cold

by specifically increasing HUFA levels (32,33,35). This second type of response is highly likely to increase the PI. Here, we found that the homeoviscous adaptation response to cold (measured by the CFI) was solely expressed by *S. solidissima* while the increase in HUFAs was observed in both species. Interestingly, the outcome of these changes was limited to a trend for a higher mitochondrial PI during winter that was similar in magnitude for both species. Hence, the nature of the cold-induced lipid remodeling was species specific, but the longevity-related difference in PI between *S. solidissima* and *A. islandica* was maintained across seasons.

The study also aimed at assessing changes in PI with age in *S. solidissima*. In contrast to diet abundance and season, individual age did not affect the PI of among mature *S. solidissima*. To our knowledge, this is the first study investigating this physiological trait in an infinitely growing species. In rats, PI is generally reported to increase with age in liver and heart, but some studies found no changes in these organs while a decrease has been reported for brain synaptosomes and cerebral cortex (reviewed in 1). In the exceptionally long-lived naked mole-rat (*Heterocephalus glaber*), age had no effect on PI levels in muscle, heart, kidney, liver, or brain, and the authors conclude that PI levels are rather a species-specific trait (11). In flies, membrane PI increased with age in drosophila (1), but not in honey bee queens or workers (head + thorax + abdomen) (5). In aquatic invertebrates, whole-body PUFAs in all lipid classes increased with age in *Daphnia magna* (36,37), whereas they decreased in *Gammarus locusta* (38) as well as in total lipids of the shrimp brain (*Aristeus antennatus*) (39). Growth never ceases completely in bivalves, it rather

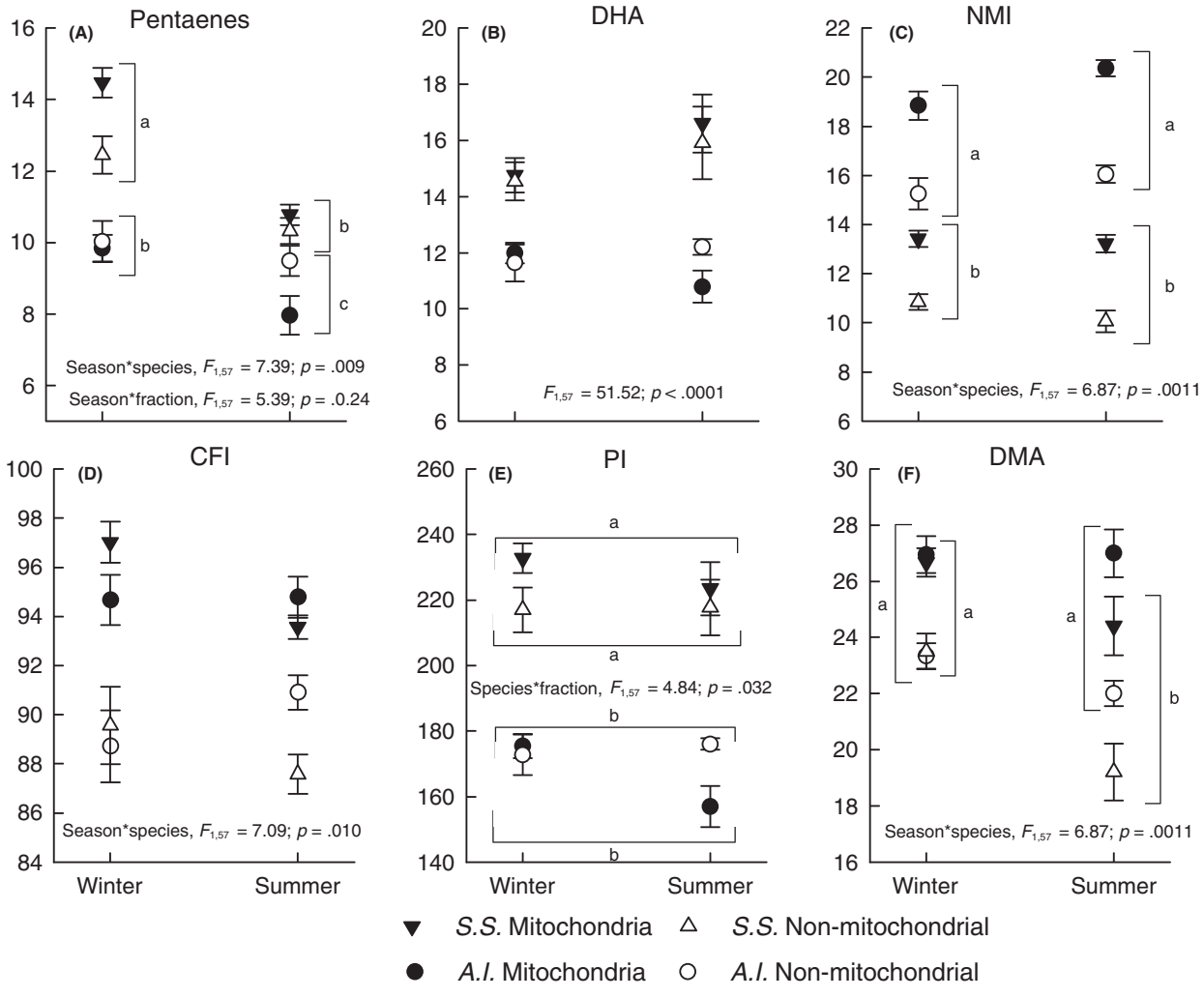

Figure 3. Key markers of membrane lipid composition in gills of *Spisula solidissima* and *Arctica islandica* sampled during winter (early April), when water temperatures had been stable at 2°C for 4 months, and summer (late August), when water temperatures had been stable at 12°C for 2 weeks. (**A**) Percent pentaenes fatty acids (five double bonds); (**B**) percent docosahexaenoic acid; (**C**) percent of non-methylene-interrupted fatty acids; (**D**) chain fluidity index; (**E**) peroxidation index; (**F**) percent dimethyl acetals. The chain fluidity index (CFI) is calculated as CFI = (1× monounsaturated FAs or DMAs) + (1.5× polyunsaturated FAs). Significant interactions or effects are presented and different letters indicate differences among selected groups (three-way analysis of variance). Sample sizes = 8, except for *A. islandica* winter mitochondria for which it is 9. Results are means ± standard error of the mean.

follows a von Bertalanffy function, that is, it is very rapid in early ages until it reaches an inflexion point in the curve after which it is asymptotic and infinite for a period of time that can be very long depending on the species (12,28). The impact of chronological age on a number of modulators of the redox state has recently been investigated in *A. islandica*. It has been found that some components of the antioxidant system (glutathione, catalase) and of all mitochondrial electron transport system complexes (and citrate synthase) and the expression of heat shock proteins expression decline over the course of the first 20–40 years of age (12,40). This represents 5%–20% of the maximum (investigated population) longevity and corresponds to the phase of rapid growth. However, the activity of the superoxide dismutase (SOD) and the proteasome did no change, and only the cytokine-like molecules were reported to change during the slow asymptotic growth phase (40). The author suggests that the rapid changes of some physiological traits early in life may rather pertains to biological stages and not senescence per se. Here, we investigated the influence of age in membrane lipids of the short-lived *S. solidissima* and found no changes in HUFAs, PUFAs, or PI among 20 mature individuals ranging from 6 to 23 years old (MRL = 37 years). This range of age covers the end of the rapid growth phase and most of the asymptotic growth phase of the species.

The antioxidant capacities of plasmalogen PLs are well established (21,22); research on aging diseases in human further shows that levels are decreased in several neurological disorders including Alzheimer's, ischemia, and spinal cord trauma (41). The contribution of plasmalogens to the resulting resistance to lipoxidation of biological membranes must therefore be considered in parallel of the PI. Here, we estimated the abundance of plasmalogens on the basis of DMA content (42) and found that they represent 53%–55% of PLs in *A. islandica* and *S. solidissima* mitochondria (Figures 2F and 3F), and a little less for other membranes. This exceeds by far the abundance found in the human brain 20% (41), suggesting a very important contribution to improving membrane resistance to lipoxidation in bivalves. Plasmalogens abundance of our two species decreased in the mitochondrial fraction as a result of supplementation, and for *S. solidissima* as a result of warmer temperature of the summer. Hence, plasmalogen content of the short-lived *S. solidissima* remained lower or equal to that of the long-lived *A. islandica* across all conditions tested. Higher PI combined with lower plasmalogens levels support that the overall resistance to lipoxidation is lower in the short-lived *S. solidissima* than in *A. islandica*. Interestingly, plasmalogen abundance decreased with age in both biological fractions of the short-lived *S. solidissima* during the phase of asymptotic shell growth. This contrast with the stability of the PI during the same life phase, however it is in line with observation of decreasing serum plasmalogen levels between young and elderly human subjects (43). At constant membrane PI, decreasing plasmalogen levels will foster higher lipoxidation rates in older individuals, impairing membrane functions such as permeability, physical state, etc., and increasing release of reactive aldehyde which together foster cellular senescence (3).

We previously demonstrated the existence of an inverse correlation between longevity and PI in gills of five species of veneroid bivalves maintained in common garden condition of diet and temperature (10). This first contribution supported that intrinsic resistance to oxidation of cell membranes is an evolutionary conserved modulator of longevity among animals of very diverse taxa, strongly supporting the membrane pacemaker theory of aging. There was however some questions left unanswered. Membrane PUFAs are known to change as a function of temperature and diet in bivalves which may disrupt the relationship between PI and longevity. Furthermore, longevity can be highly variable among populations of the same species bivalves (44–46), which requires commensurate changes in the PI for the hypothesis to remain valid. The present finding conciliates these concerns. We found that the environment does affect the PI which maintains the possibility that longevity of different populations within the same species is adjusted by different PI values. Further, the effect of environment seen here is to maintain absolute differences in PI between species of different longevity. This is in accordance with the general observations that changes in longevity among populations preserve the ordering of maximal longevity among species.

Higher resistance to lipoxidation of cell and mitochondrial membranes in long-lived bivalve provides a mechanistic explanation for the recent findings of their better resistance to oxidative challenges. When challenged with *tert*-butyl hydroxyperoxide, whole organismal survival was better for the extraordinarily long-lived *A. islandica* (MRL = 507 years) than for the long-lived *Mercenaria mercenaria* (MRL = 106 years); and caspase-3 activity was lower in the former indicating alleviated apoptotic activity (47). The long-lived pacific giant clam (*tridacna derasa*; MRL > 100 years) also presented lower mortality rates compared with the short-lived Atlantic bay scallop (*Argopecten irradians irradians*; MRL = 2 years) when similarly challenged with *tert*-butyl hydroxyperoxide. The pro-oxidant drugs paraquat and rotenone increases the mitochondrial reactive oxygen species production rate, challenging organisms with an increased oxidative stress of topologically relevant origin. When treated with these drugs, the survival rates among three species of bivalves were as follow: extraordinarily long-lived *A. islandica* > long-lived *M. mercenaria* > short-lived *A. irradians* (48). These studies did not find better antioxidant defenses/repair mechanisms for the long-lived species (hydroxyl radical antioxidant capacity, oxygen radical absorbance capacity, catalase, SOD, gluthation peroxydase, and proteasome activity), suggesting that the better resistance may be the result of intrinsic better resistance to oxidation of the

structural and functional macromolecules. Taken together, biogerontology studies on bivalve model system are in line with the more advanced research on vertebrates. The common pattern found is that long-lived species are characterized by better resistance to oxidative challenge which is not explained by accrued antioxidant defenses but rather by intrinsically more resistant macromolecules (protein, lipids, and DNA). Among changes in intrinsic resistance of macromolecules, the modulation of the resistance to oxidation of membrane lipid seems highly conserved in both cases.

Limitations of the Study

Our conclusions are based on the comparison of two species which highly increases the chances of finding a difference in the expected direction (49). Further, only one species was used to test the effect of individual age on the PI and plasmalogens. Further studies are highly desirable to confirm the results that have been found here.

Supplementary Material

Supplementary material can be found at: http://biomedgerontology.oxfordjournals.org/

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