

## Imperceptible senescence: Ageing in the ocean quahog *Arctica islandica*

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### Abstract

The ocean quahog *Arctica islandica* is the longest-lived of all bivalve and molluscan species on earth. Animals over 100 years are common and reported maximum live span around Iceland is close to 400 years. High and stable antioxidant capacities are a possible strategy to slow senescence and extend lifespan and this study has investigated several antioxidant parameters and a mitochondrial marker enzyme in a lifetime range spanning from 4–200 years in the Iceland quahog. In gill and mantle tissues of 4–192 year old *A. islandica*, catalase, citrate synthase activity and glutathione concentration declined rapidly within the first 25 years, covering the transitional phase of rapid somatic growth and sexual maturation to the outgrown mature stages (~32 years). Thereafter all three parameters kept rather stable levels for >150 years. In contrast, superoxide dismutase activities maintained high levels throughout life time. These findings support the 'Free Radical-Rate of Living theory', antioxidant capacities of *A. islandica* are extraordinarily high and thus may explain the species long life span.

**Keywords:** *Arctica islandica*, ageing, antioxidant capacity, glutathione, life history model

### Introduction

The Ocean quahog, *Arctica islandica*, is known for its extraordinary longevity [1,2], even among the rather long lived class of bivalve molluscs and can live close to 400 years. Oxygen free radical formation is one major cause of physiological ageing [3] and elevated antioxidant protection may be instrumental to extend lifespan through mitigating and retarding oxidative damage. We have studied a variety of physiological and morphometric parameters characterizing animal growth and metabolism, as well as the status of tissue oxidation and antioxidant defence over age and in different tissues in a North Iceland *A. islandica* population, to see how the animals achieve such long lifespan. In a first paper [2] we described the change of oxidative damage marker levels over a 200 year age span. Lipofuscin, the final waste product of

cellular oxidative damage, accumulated in gill, mantle and adductor muscle over age. The absolute amount of oxidative stress marker, however, differed between tissues with gill > mantle > muscle tissue. In the adductor muscle, age pigment granules accumulated only outside the myofibrils within the accompanying interstitial cells. The concentration of protein carbonyls, representing an estimate of oxidative damage in the protein fraction over lifetime, was extremely low compared to other bivalve species and remained constant with age.

The fact that lifetime oxidative damage seems so low in the ocean quahog speaks either for very low natural levels of ROS formation or for an extremely well developed antioxidant defense system, including repair of cellular damage and autodigestion of severely damaged macromolecules and cellular structures in this species. Spontaneous and self-induced

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metabolic rate depression (MRD) by burrowing into the sediment and exposing itself to hypoxia for between 1–5 days [4,5] may be involved in the mitigation and slow down of metabolic ROS production in this oxyconforming bivalve. Moreover, we hypothesized that frequent hypoxia-reoxygenation cycles, inherent to the mode of living in this burrowing species, trigger the antioxidant protection [6,7]. This phenomenon has been reported in several hypoxia tolerant ectotherms [8] where hypoxic triggering of antioxidant activities appears to assure control of oxidative stress on reoxygenation and, in so doing, preserves the beneficial effect of MRD with respect to tissue oxidative damage.

The present paper complements the data set presented in our first paper in that here we describe the change with age of the activity of the mitochondrial marker citrate synthase, key enzyme of the citric acid cycle and of the enzymatic antioxidants, catalase and superoxide dismutase, as well as parameters of the non-enzymatic antioxidant glutathione system in gill and mantle tissue of the Iceland ocean quahog. The glutathione system comprises the oxidized and reduced form of glutathione and the enzymes catalysing oxidation and reduction. It is centrally involved in intracellular scavenging of radicals and buffering of the cellular redox state. Our approach was to model changes of oxidative stress parameters over animal lifetime and see how they fit species growth parameters and life history. The aim was to understand whether or not invertebrates with such outstanding longevity owe this, at least in part, to a very well established antioxidant protection.

## Materials and methods

### *Animal and tissue sampling*

*Arctica islandica* were collected in August 2004 and in May 2005 northeast of Iceland (N 66°1.50' – W 14°50.90') between 14–22 m water depth with a hydraulic dredge. Water temperature on the sampling date was 9°C in August 2004 and 4°C in May 2005. In the first year only young (min 6 years) and middle aged specimens (max 80 years) were sampled, whereas in the second year, care was taken to sample very young (min 4 years) and old (max 192 years) specimens. The animals were transported in cooling boxes to the Sandgerdi Marine Station, University of Iceland, where they were kept at constant temperature of 9°C and at permille (34.9 permille salinity) in 400 l tanks for 7 days. On day 7, animals were dissected and gill and mantle snap frozen in liquid nitrogen for biochemical analysis. Age analysis of individual specimens (see Figure 1) was carried out by counting annual shell growth rings as described in Strahl et al. [2] and Schöne [9].

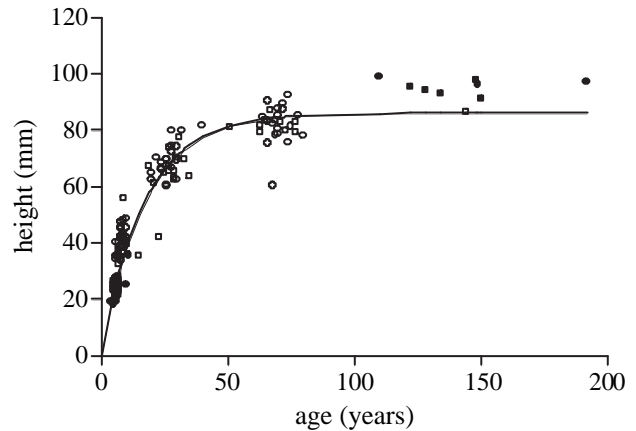


Figure 1. Von Bertalanffy growth model of the northern Iceland population of *Arctica islandica* based on samples from August 2004 (circles) and May 2005 (black dots).  $H_t = 86.00 * (1 - e^{-0.057 * (t+0.399)})$ ,  $n = 179$ ,  $R^2 = 0.933$ . Data and graph from Strahl et al. [2] courtesy of Inter-Research Science Center, Nordbueente, Germany.

### *Measurements of enzyme activities*

For measurements of the mitochondrial key enzyme *citrate synthase* (CS: EC 4.1.3.7, key enzyme of the citric acid cycle), frozen samples of mantle and gills were ground in liquid nitrogen and homogenized with a glass homogenizer (Nalgene, USA) in Tris-HCl buffer (20 mM Tris-HCL, 1 mM EDTA, 0.1% (v/v) Tween® 20, pH 7.4) 1:8 (w/v). Homogenates were sonicated for 15 min in a Branson Sonifier 450 (output control 8, Duty cycle 50%) cooled to 0°C and centrifuged at 7400 g for 5 min at 0°C. CS activity was measured after Sidell et al. [10] recording the absorbance increase of 5 mM DTNB (5,5'-dithiobis(2-nitrobenzoic acid)) in 75 mM Tris-HCL (pH 8.0), 0.4 mM Acetyl-CoA and 0.4 mM Oxaloacetat at 412 nm. Activity was calculated using the mmolar extinction coefficient  $\epsilon_{412}$  of 13.61  $\text{mM}^{-1} \text{cm}^{-1}$ . Catalase (CAT) activity was determined after Aebi [11]. Aliquods of 20–50 mg of frozen mantle and gill tissue were ground in liquid nitrogen and homogenized with a micropistill in 50 mM phosphate buffer (50 mM  $\text{KH}_2\text{PO}_4$ , 50 mM  $\text{Na}_2\text{HPO}_4$ , pH 7.0) with 0.1% Triton x-100 at 1:30 (w/v). Samples were centrifuged at 13 000 g for 15 min at 4°C. The activity was determined by recording the time of  $\text{H}_2\text{O}_2$  decomposition, resulting in a decrease of absorption from 0.45 to 0.4 at 240 nm (1 unit).

Superoxide dismutase (SOD) activity was measured in aliquods of 25–60 mg of frozen mantle and gill tissue that were ground in liquid nitrogen and homogenized with a micropistill in Tris buffer (20 mM TRIS-HCL, 1 mM EDTA, pH 7.6) 1:30 (w/v). Samples were centrifuged for 3 min at 18 000  $\times$  g and at 4°C. SOD activity was measured as degree of inhibition of the reduction of cytochrome c by superoxides generated by a xanthine oxidase/xanthine system at 550 nm in 43 mM potassium buffer with 0.1 mM EDTA, pH 7.8 according to

Livingstone et al. [12]. One unit SOD causes a 50% inhibition under the assay conditions. Mitochondrial and cytosolic SOD isoforms were not distinguished.

#### Determination of reduced (GSH) and oxidized (GSSG) glutathione by HPLC

The glutathione status represents the most important determinant for the cellular redox environment. The content of GSH and GSSG was determined according to Fariss and Reed [13]. Frozen tissue was ground in liquid nitrogen and the resulting powder homogenized in 1:10 (w:v) pre-cooled PCA (10% containing 2 mM bathophenanthroline-disulphonic acid) bubbled with nitrogen. After centrifugation at 15 000 g for 5 min at 4°C, 500 µl of the supernatant were mixed with 10 µl pH-indicator (1 mM m-cresol purple sodium salt containing 0.5 M iodoacetic acid (IAA)); 50 µl 1 mM  $\gamma$ -glutamyl-glutamate (in 0.3% PCA) was added as internal standard. The pH was adjusted to 8.5 with 5 mM KOH (containing 0.3 mM N-morpholine-propanesulphonic acid). The mixture was incubated at room temperature for 45 min, to allow IAA to bind GSH. Subsequently samples were centrifuged for 5 min at 15 000 g and 4°C; 300 µl of the supernatant were added to the double amount of 1% 1-fluor-2,4-dinitrobenzene (diluted in 100% ethanol, HPLC grade) and derivatized in dark vials at room temperature over 24 h. Samples were stored in dark HPLC vials at -20°C. Prior to measurement, thawed samples were centrifuged at 7500 g for 1 min at 4°C and filtered through 0.2 µm nylon membrane filters.

HPLC determination was carried out on a Beckmann Coulter HPLC System using a NH<sub>2</sub>-spherisorp column, 5 µm 240 × 4 mm (Waters, Germany). Solvent A: 80% methanol and solvent B: 20% sodium acetate stock and 80% solvent A. Sodium acetate stock was prepared by dissolving 272 g sodium acetate trihydrate in 122 ml Milli-Q water and 378 ml of concentrated HPLC-grade acetic acid. The gradient programme was as follows: 3 min hold at 92% A followed by a 28 min linear gradient to 40% A at a flow rate of 1.2 ml \* min<sup>-1</sup> and 2.3 to 2.8 psi backpressure and 15 min re-equilibration. Peaks were recorded with a photodiode array detector at 365 nm.

#### Tissue pH (pHi)

Tissue pH was determined using the homogenate technique [14] in a system thermostated at 3°C, the maintenance temperature of the mud clams. Prior to measurements the pH electrode (SenTix Mic, WTW, Germany) was calibrated at *A. islandica in situ* temperature 3°C with precise calibration solutions (AppliChem Darmstadt; pH 6.865-A1259; pH 7.413-A1260). Readings of pH were recorded on a Kipp & Zonen chart recorder. For tissue measurements, mantle and gill tissue (100–120 mg) was

ground in liquid nitrogen and the powder added to a 0.5 ml eppendorf cup containing 0.15 ml of medium composed of 160 mM potassium fluoride, 2 mM nitrilotriacetic acid. The cup was closed after layering with air bubble free medium and the tissue homogenized by ultrasound (Brandson sonifier 450, duty cycle 40%, output control 8) at 0°C and centrifuged at 20 000 g at 3°C for 30 s. The pH in the supernatant was determined in the system thermostated at 3°C. Glutathione concentrations and corresponding pHi values of each sample were used to calculate the tissue redox potential after Schafer and Büttner [15].

#### Statistics

Enzyme activity (U/g wet mass WM; CS, SOD, CAT) and total glutathione concentration (GSH, nmol/g WM) may be affected by age, tissue type and sampling year. We tested for differences between both sampling years (2004 and 2005) by means of 2-way ANOVA (dependent variable vs year and tissue) in the age range < 7 years, where animals from both years were present (see Figure 1). Subsequently we analysed the effect of tissue and age on GSH<sub>tot</sub>, GSSG:GSH ratio, CAT, CS and SOD by full interaction linear as well as non-linear, Arrhenius-type models:

$$Y = a + b_1 * \text{Age} + b_2 * \text{Tissue} + b_3 * (\text{Age} * \text{Tissue})$$

$$\log_e(Y + 1) = a + b_1 * 1/\text{Age} + b_2 * \text{Tissue} + b_3 * (1/\text{Age} * \text{Tissue})$$

where 'Tissue' is a dummy variable with mantle = -1 and gill = +1.

#### Results

The sampling year had no significant effect ( $p > 0.05$ ) on enzyme activity (U/g WM; CS, SOD, CAT) in young animals < 7 years. Glutathione (GSH) concentrations could not be compared, because GSH was not determined in animals of the same age in both years. CAT, SOD, GSH<sub>tot</sub> and GSSG:GSH were significantly higher in gill than in mantle tissue, whereas CS showed the opposite relation. Except for SOD, all parameters declined significantly with age in both tissues (see Figure 2). There is, however, no significant interaction between age and tissue, meaning that the rates of the age dependent decline do not differ between tissues. In GSSG:GSH a linear model describes this relationship best (in terms of overall goodness of fit and of linearity):

#### GSSG:GSH

$$= 0.5493 - 0.0009 * \text{Age} + 0.2381 * \text{Tissue};$$

$$n = 65; R^2 = 0.0624; p < 0.001,$$

i.e. GSSG:GSH declines with a rate of ~ 1% per year during 200 years of life.

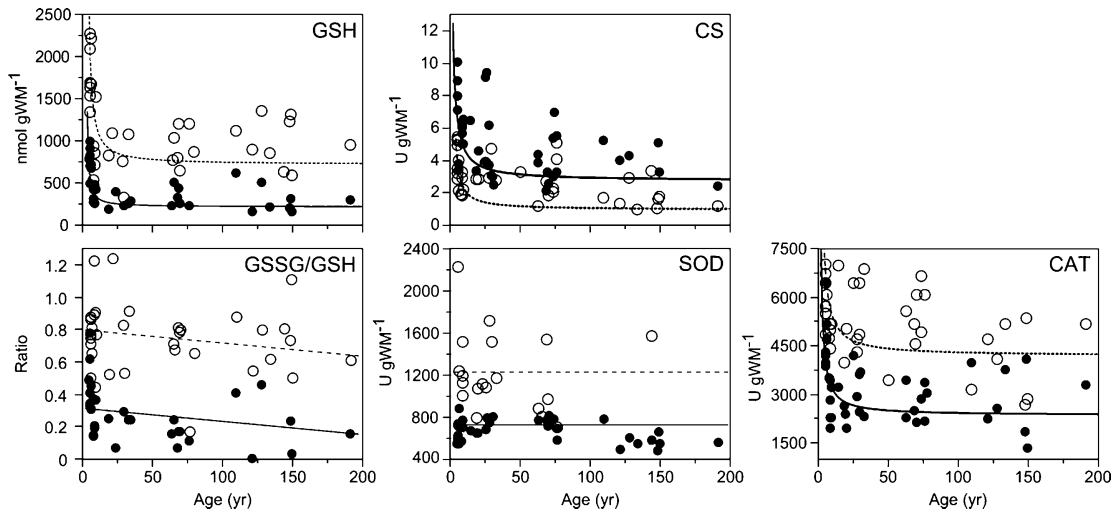


Figure 2. Relationship between enzyme activity (CS, SOD, CAT), total GSH concentration and GSSG:GSH ratio and bivalve age in mantle (dots, solid line) and gill tissue (circles, stippled line) of *Arctica islandica*. Lines indicate best fitting model. ‘Tissue’ = 1 for gill and ‘Tissue’ = -1 for mantle.  $\ln(\text{GSH}_{\text{tot}}) = 6.1304 + 4.3802/\text{Age} + 0.5232 * \text{Tissue}$ ;  $n = 65$ ;  $R^2 = 0.698$ ;  $p < 0.001$ .  $\text{GSSG}/\text{GSH} = 0.5493 - 0.0009 * \text{Age} + 0.2381 * \text{Tissue}$ ;  $n = 65$ ;  $R^2 = 0.6239$ ;  $p < 0.001$ .  $\ln(\text{CS}) = 1.0019 + 3.7830/\text{Age} - 0.3072 * \text{Tissue}$ ;  $n = 77$ ;  $R^2 = 0.4923$ ;  $p < 0.001$ .  $\ln(\text{CAT}) = 8.1507 + 1.9931/\text{Age} + 0.2632 * \text{Tissue}$ ;  $n = 81$ ;  $R^2 = 0.574$ ;  $p < 0.001$ . SOD shows no relation to age, mean SOD is 637.63 U/g WM in mantle and 1217.92 U/g WM in gill.

In  $\text{GSH}_{\text{tot}}$ , CS and CAT the Arrhenius model fits the data distinctly better than the linear model.

$$\ln(\text{GSH}_{\text{tot}}) = 6.1304 + 4.3802/\text{Age} + 0.5232 * \text{Tissue}; n = 65; R^2 = 0.698; p < 0.001$$

$$\ln(\text{CS}) = 1.0019 + 3.7830/\text{Age} - 0.3072 * \text{Tissue}; n = 44; R^2 = 0.4923; p < 0.001$$

$$\ln(\text{CAT}) = 8.1507 + 1.9931/\text{Age} + 0.2632 * \text{Tissue}; n = 81; R^2 = 0.574; p < 0.001$$

The models indicate a steep initial decrease during the early life after which the year-to-year rate of change falls below 0.01 (as fraction of previous years value) at age 22 years ( $\text{GSH}_{\text{tot}}$ ), 25 years (CS) and 15 years (CAT), respectively.

Measurements of the internal pH (pHi) in gill and mantle tissues for calculation of the redox potential were carried out in very young (5–8 years) and very old (110–192 years) animals sampled in 2005, and the tissue redox potential was accordingly calculated only for these samples. In mantle tissue pHi declined significantly with age from pH 6.92 in young to 6.69 in old animals. In gill tissue pHi was 6.90 in younger and 6.77 in older specimens (see Table I). Effectively, older animals had significantly lower overall glu-

tathione content, as well as lower pHi than the young animals in both tissues, resulting in a more oxidized redox state compared to younger animals in both tissues. Redox potential did not differ between tissues (see Table I).

## Discussion

It is a paradigm in ageing physiology that reactive oxygen species, produced in respiring tissues and not detoxified by antioxidant systems, are one, if not *the* major cause of cellular senescence and tumour formation [16]. In recent studies we confirmed that ageing in marine bivalves indeed involves increased ROS production per oxygen respired in the mitochondria and that this is accompanied by an age-dependent decline of mitochondrial respiratory efficiency in bivalve model species [17]. Further, low overall antioxidant capacities or a fast decline with age in antioxidant capacities appears to be associated with shorter maximum life spans (MLSP) [17–19]. Based on current knowledge, it looks like the observed decrease of mitochondrial capacities, meaning the product of mitochondrial numbers and

Table I. Redox potential (Rp) related parameters in tissues of young and old *A. islandica* from Iceland sampled in 2005.  $\text{GSH}_{\text{tot}}$ :  $[\text{GSH}] + 2[\text{GSSG}]$ . Redox potential was calculated using Nernst’s equation after Schafer and Büttner [15]. All parameters differed significantly between age groups in both tissues (Anova  $p < 0.001$ , Tukey  $p < 0.05$ ). Data shown are mean  $\pm$  SD,  $n = 8$  in all groups.

Tissue	Age (y)	$\text{GSH}_{\text{tot}}$ (nmol* $\text{g}^{-1}$ fwt)	GSH (nmol* $\text{g}^{-1}$ fwt)	GSSG (nmol* $\text{g}^{-1}$ fwt)	GSSG:GSH	pHi	Rp (mV)
mantle	5–8	754.3 $\pm$ 152.3	402.6 $\pm$ 78.8	179.2 $\pm$ 47.4	0.43 $\pm$ 0.19	6.92 $\pm$ 0.004	-316.5 $\pm$ 5.6
	110–192	304.3 $\pm$ 169.4	214.1 $\pm$ 66.1	49.1 $\pm$ 52.7	0.17 $\pm$ 0.18	6.69 $\pm$ 0.027	-306.7 $\pm$ 9.9
gill	5–8	1798.9 $\pm$ 341.6	730.6 $\pm$ 108.8	534.2 $\pm$ 130.5	0.73 $\pm$ 0.12	6.90 $\pm$ 0.035	-316.0 $\pm$ 2.8
	110–192	1027.5 $\pm$ 262	420.2 $\pm$ 70.4	303.7 $\pm$ 104.4	0.71 $\pm$ 0.20	6.77 $\pm$ 0.037	-303.6 $\pm$ 3.0

respiratory efficacy, and ROS generation rate over age, together with a decline in antioxidant capacities, could be the main determinants of the maximum lifespan in marine invertebrates. High antioxidant capacities inside mitochondria and cells may therefore limit oxidative damage and can perhaps slow down mitochondrial deterioration.

Indeed, especially in the gills, the antioxidant capacities measured in old *Arctica islandica* were higher than in shorter lived bivalves. Gill enzyme activities exceeded the activities in other clams by 4-fold (catalase) and 10-fold (SOD) and the 2–10-fold concentration (see below) of glutathione was measured in Icelandic *A. islandica* gills. Enzyme activities in bivalve gills were summarized by Abele and Puntarulo [20] and can be compared on a tissue protein basis to our results, which relate to protein in the following manner: catalase<sub>gills</sub> 342 U/mg protein, SOD<sub>gills</sub> 93 U/mg protein. In the mantle tissue, catalase and SOD values ranged among but not above the highest values in less long lived species [18,19]. The concentration of glutathione in mantle tissue ranged between 200–1700 nmol/g WM in different bivalves: *Mya arenaria* (MLSP: ~13) 420 nmol/g WM; *Laternula elliptica* (MLSP: ~36) 850 nmol/g WM; *Aequipecten opercularis* (MLSP: ~10): 800 nmol/mg WM, *Adamussium colbecki* (MLSP: ~45): 1750, Baltic Sea *Arctica islandica* (MLSP: < 50 years, own observations and Brey et al. [21]) 250 nmol/g WM, and thus the concentration of the glutathione redox buffer does not appear extraordinarily high in Icelandic *A. islandica*: 300–750 nmol/g WM. Thus overall antioxidant capacity itself certainly supports retarded senescence in the Icelandic quahog, but might not exclusively account for the extraordinary lifespan.

The secret in the longevity of *A. islandica* rather appears to lie in slowing down the ageing process after the first 20–25 years, an early phase of rapid physiological changes due to active growth and maturation, after which growth and somatic production cease [22]. Citrate synthase and catalase activities, as well as glutathione concentrations remained stable over 150 years, following this initial maturation period, and also stable superoxide dismutase activities were recorded throughout 192 years of lifetime. The model synchronizing morphometrical and physiological parameters (see Figure 3: exemplified for glutathione) indicates a sharp decline in the physiological parameter (dotted line) which coincides with the phase of ‘deceleration’ in growth (dashed line) and somatic production (solid line). The rapid change in the lifetime physiology occurs between both inflexion points of the production curve at 5.2 and 33.3 years of age. Interestingly, 33 years coincides with the oldest age of immaturity in the Iceland *A. islandica* population reported by Thorarinsdottir and Steingrimsdottir [22]. Out of 200 analysed ocean

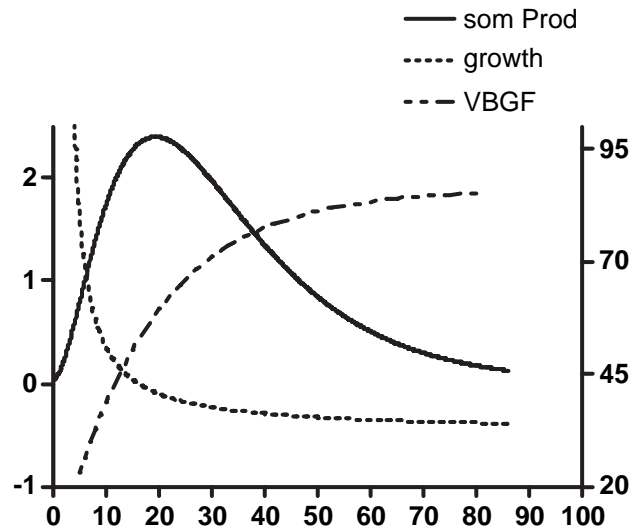


Figure 3. Development of growth in shell height (from Figure 1), individual somatic production (gWM/y) calculated from shell growth and size-mass relation  $WM = 0.59606 \cdot H^{3.059}$  (own data) by the mass specific growth rate method [26] and the development of GSH content (normalized to 100% at age 4) with age. Note that CAT and CS activity show almost identical patterns over lifetime and GSH was chosen as an exemplary physiological parameter.

quahog specimens in their study, 17 animals covering an age range between 7–32 years (24–60 mm shell size) were still not sexually differentiated. Thus, our model indicates respiratory capacity, catalase and the redox buffer glutathione to be of exaggerated importance during this initial phase of rapid growth and highest weight specific metabolic rate. Contrarily, older animals with less active growth and, hence, less active metabolism maintain aerobic metabolism and antioxidants in balance on low albeit on astonishingly constant (> 150 years) levels. Aside from being a major cellular antioxidant, glutathione further holds important metabolic functions for the cellular uptake of amino acids which may be important in rapidly growing animals. Moreover, glutathione is a cofactor for the synthesis of prostaglandin E2 from arachidonic acid [23], which may also be more active in young animals. However, in combination with the higher activities of catalase and superoxide dismutase the elevated glutathione levels will presumably serve to maintain the antioxidant defence in young animals.

The more reduced redox potential maintained in mantle and gills of young animals (see Table I) supports the idea that a high antioxidant protection is already needed and perhaps specifically important in the very young stages, to sustain the extraordinary species longevity. Also, it is the first time that such a pronounced decrease in tissue glutathione over age is recorded and directly relatable to growth and sexual maturity of a mollusc species. In contrast, in short lived mice with a life expectancy of just 4 years, only a very minor decline of the glutathione concentration

by ~0% was observed in the heart, kidney, testis and in the eye of 2 years compared to 4 months old animals and liver and brain GSH concentrations remained stable [24]. Interestingly, in the mouse study the GSH/GSSG ratio changed dramatically in all tissues except for the brain. The effect was most pronounced with GSSG concentrations, even doubling in some of the tissues. A similar development of glutathione parameters over age was observed in frogs (*Rana perezi*) with a maximum life span of 6 years [25] and shows that the rapid change in glutathione oxidation in short lived species is not limited to mammals. Although none of the measured parameters compared in young (2 years) and old (4–5 years) frogs reached significance, stable GSH and increasing GSSG concentration over age caused an oxidation of the GSSG:GSH ratio in the liver over lifetime (GSSG/GSH young  $0.07 \pm 0.2$ ; old  $0.13 \pm 0.05$ ). This effect was completely reversed in the long lived quahog where the amount of GSSG was lower in the old than in the young animals (Table I). Further, the quahog data suggest that it is not so much the GSSG/GSH ratio itself, but the tissue redox potential which is important for longevity. Thus, both gill and mantle had relatively high GSSG per GSH molecule. However, because of the extremely high overall glutathione concentration, which is a decisive parameter for the redox milieu when applying Nernst's equation, the redox milieu was extremely reduced in the quahog ( $< -300$  mV), whereas mouse tissues ranged from as high (oxidized) as  $-250$  mV (heart) to  $-290$  mV (in testis, eye and liver). Only mouse brain maintained a redox potential lower than  $-300$  mV [24].

Glutathione parameters were more variable in the gills than mantle, reflecting perhaps more influence of the animals' acute oxygenation state at the time of killing. However, the variability of the glutathione data was astonishingly similar between young and old animals in Table I. The model suggests that late survivors can indeed be assumed to have had high antioxidant potential in their youth. Hypothetically, an individual that for some physiological or genetic deficiency is not capable of establishing sufficiently high antioxidant protection as young animals will not be found any more in the group of late survivors. This would strongly support the idea that a well developed antioxidant protection in a young animal secures sufficiently stabilized protection at old age and would thus be one important pre-condition for reaching long lifespan.

As stated above, the preservation of mitochondrial densities and respiratory capacity seems to be an important fitness parameter in aged bivalves. Here we measured only CS activity as a mitochondrial density marker and found comparably high activities in both tissues when compared to other bivalve species. Especially in mantle tissue where we measured a

mean activity of  $> 6$  U/g WM other bivalves range between 1–1.5 U/g WM (includes two scallops and two mud clams and all data measured in our laboratory at 20°C, see below). The high CS activity in *Arctica islandica* remained moreover constant throughout the last 150 years of species lifetime, speaking for a negligible reduction in mitochondrial numbers at old age. Constant CS activities/mitochondrial densities in tissue over lifetime seem characteristic for long lived bivalves (*Mya arenaria* and *Laternula elliptica* [17]; *Adamussium colbecki* [19]). In contrast, the short lived scallop *Aequipecten opercularis* from the Irish Sea, with a life expectancy of only 10 years rapidly loses CS and cytochrome-oxidase (COX) activities in mantle tissue within the first 7 years of life [19].

In summary, we found young Icelandic *Arctica islandica* to exhibit high antioxidant capacity (catalase, SOD, glutathione) and CS activity in mantle and gill tissue compared to other bivalves. Between 30 and close to 200 years of life, the mitochondrial marker enzyme CS as well as the antioxidants catalase and glutathione stabilized on post-maturation levels and showed no tendency for further decline with age including the oldest individuals of 192 years. The sharp decline in these cellular maintenance parameters in early life ( $< 25$  years) is tightly coupled to somatic production and growth (see Figure 3) and, therefore, not related to cellular senescence. Minimized senescence after maturation and peak somatic production seems to be a pre-condition for attaining such a long life span and cellular antioxidants play a major role in this context. Perhaps the constant sediment burrowing and surfacing behaviour in the quahog may not only induce metabolic rate depression, but also help to freshen up antioxidant capacities as the animals go through repeated hypoxia-reoxygenation stress, thereby additionally supporting the long life span of up to 400 years in *A. islandica*.

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