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Cell turnover in tissues of the long-lived ocean quahog *Arctica islandica* and the short-lived scallop *Aequipecten opercularis*

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Abstract Cell proliferation and apoptosis were investigated in tissues of two bivalve species, *Arctica islandica* from the German Bight (age of bivalves: 33–98 years) and Iceland (7–148 years) and *Aequipecten opercularis* from the English Channel (2–4 years). High proliferation rates (10% nuclei dividing) and apoptosis in tissues of *A. opercularis* were in line with high-energy throughput and reduced investment into antioxidant defence mechanisms in the scallop. In contrast, cell turnover was slow (<1% nuclei dividing) in *A. islandica* and similar in mantle, gill and adductor muscle between young and old individuals. In the heart, cell turnover rates decreased with age, which indicates less-efficient removal of damaged cells in ageing *A. islandica*. Cell turnover rates, mass specific respiration and antioxidant enzyme activities were similar in German Bight and Iceland ocean quahog. Variable maximum life expectancies in geographically separated *A. islandica* populations are determined by extrinsic factors rather than by fundamental physiological differences.

Introduction

Bivalve maximum life span potentials (MLSP) vary between just a few and several hundreds of years, and a large variety of different life history models can be found

among molluscs. Currently, bivalves are discussed as new models in ageing research that add to the understanding of the mechanistic basis of longevity with a focus on physiology and gene expression of long- and short-lived bivalve species (Abele et al. 2009; Bodnar 2009; Philipp and Abele 2009). The ocean quahog *Arctica islandica* with reported individual ages of >350 years in an Icelandic population (Schöne et al. 2005; Wanamaker et al. 2008) and the queen scallop *Aequipecten opercularis* with a MLSP of only 8–10 years, represent not only extremes in life spans but also life styles. *Arctica islandica* is rather inactive, burrowing beneath the sediment surface but remaining in contact with the overlying sea water through short siphons for oxygen uptake and feeding. At regular intervals, the clams burrow deeper into the sediment exposing themselves to hypoxia for periods of 1–7 days and inducing metabolic rate depression (MRD) (Taylor 1976). When experiencing prolonged hypoxia of >24 h, the ocean quahog reduces its metabolism to 10%, and after several days of experimental anoxia to as low as 1% of their aerobic rates (Oeschger and Storey 1993). *Arctica islandica* occurs on the continental shelves on both sides of the North Atlantic in Europe and North America (Nicol 1951; Thompson et al. 1980; Jones 1980; Murawski et al. 1982; Dahlgren et al. 2000), spanning an overall temperature range from 0 to 16°C (Mann 1982). In contrast, *A. opercularis* is an actively swimming epibenthic scallop with a wide geographical distribution on the European continental shelf from Northern Norway to Gibraltar and within the Mediterranean (Waller 1991), tolerating water temperatures of 6–24°C (Ansell et al. 1991). The scallops swim through jet propulsion reaching speeds up to 30 cm s⁻¹ (Moore and Trueman 1971).

Since the pioneer work of Pearl (1928) and Harman (1956), proposing the ‘Free Radical—Rate of Living theory’, which predicts a negative correlation between mass

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specific respiration (MSR) and MLSP of a species, studies of ageing focus on the link between mitochondrial production of reactive oxygen species (ROS) and MSR. Applying this theory to the molluscs, a variety of physiological parameters such as growth, metabolism, antioxidant defence and accumulation of oxidative damage products have been studied in different tissues of several bivalves and squids in an age-dependent manner (Philipp et al. 2005a, b, 2006; Strahl et al. 2007; Abele et al. 2008; Zielinski and Pörtner 2000). The long-lived Icelandic *A. islandica* has stable antioxidant capacities in mantle and gill over an age range of 30–200 years (Abele et al. 2008) and the fluorescent age pigment lipofuscin, an oxidative stress and ageing marker, accumulates in several tissues as they age (Strahl et al. 2007). The abundance of lipofuscin granules differs between highest densities in gills, less in mantle and hardly detectable accumulations in the adductor muscle of very old clams. The protein carbonyl concentration resulting from protein oxidation remains low in *A. islandica* up to 200 years compared to shorter-lived bivalves (Strahl et al. 2007). In contrast, mitochondrial volume density, as well as antioxidant protection decrease significantly in mantle tissue of ageing *A. opercularis*, within only a couple of years. Lipofuscin content in mantle tissue increases with age, and protein carbonyls are twice as concentrated as in 200-year-old *A. islandica* (Philipp et al. 2006; Strahl et al. 2007). These results indicate that the energy-saving life style of *A. islandica* reduces oxygen radical damage accumulation and maintains high antioxidant capacities into extremely old age. In contrast, high-energy throughput and the epibenthic lifestyle of *A. opercularis* are correlated with a short life expectancy, due to an elevated risk of predation compared to burrowing bivalves, and a restricted investment in cellular protection and mitochondrial maintenance in mature specimens.

In the present work, we investigated tissue maintenance in terms of cell proliferation and apoptosis in mantle, gill, adductor muscle, and heart of both bivalve model species *A. islandica* and *A. opercularis*. Cell division and programmed cell death are essential processes in multicellular organisms, which eliminate and replace damaged, infected and senescent cells. Apoptosis is a highly regulated energy-consuming process in which intracellular compounds are degraded and disposed of in an orderly manner, without the induction of inflammation. Caspases, a family of proteases involved in the apoptotic program, have recently been described in bivalves (Sokolova et al. 2004; Sokolova 2009). Both, proliferation and apoptosis of fatally damaged cells may dilute and eliminate waste products such as lipofuscin and protein carbonyls, as well as damaged mitochondria in bivalve tissues, which thereby preserve function over an individual's lifetime. Young (7–13 years), middle-aged (27–33 years) and old (93–148 years)

Icelandic *A. islandica* were studied to test for age-dependent capacity differences in proliferation and apoptosis in a long-lived organism. We included a second *A. islandica* population from the German Bight, to test for possible intra-specific variability, and compared cell turnover rates as well as antioxidant enzyme activities and mass specific respiration rates between individuals from both populations. Ocean quahog from the German Bight have shorter life expectancy than the Iceland population and reported problems with new recruitment, which is possibly induced by environmental conditions and results in missing age cohorts <30 and >100 years (Begum et al. 2009).

Materials and methods

Bivalve collection and maintenance

Arctica islandica were collected at Helgoland “Tiefe Rinne” in the German Bight (54°09.05'N, 07°52.06'E) at 40–45 m water depth, using a hydraulic dredge in May 2008. Surface water temperature on the sampling date was 12°C. In August 2008, *A. islandica* were collected north-east of Iceland (66°01.44'N, 14°50.91'W) at 8–15 m water depth and water surface temperatures of 9°C. Clams were transported in a cooled container to the Sandgerdi Marine Centre, University of Iceland, where they were kept for 2 weeks at constant temperature (9°C) and salinity (34 PSU) in 400-l tanks. *Aequipecten opercularis* were dredged in the English Channel (48°43.07'N, 03°59.02'E) in August 2008 at about 60-m water depth and 16°C surface temperature. *Arctica islandica* from the German Bight and Iceland and *A. opercularis* were transported in cooled containers to the Alfred-Wegener Institute for Polar and Marine Research in Bremerhaven. Bivalves were acclimated for 2 months at 10°C and 33 PSU in 60-l aquaria with recirculating sea water containing 10 cm of pea gravel sediment. All bivalves were fed once a week with DT-live marine plankton (DT, USA, 3 ml bivalve⁻¹ week⁻¹).

Measurement of proliferation rates

The measurement of proliferation rates in bivalve tissues was modified after Moore et al. (1994). For labelling with proliferation reagents, 5 German Bight *A. islandica*, 15 Iceland *A. islandica* of different age classes, and 5 *A. opercularis* were exposed individually in 3-l flasks filled with natural seawater (33 PSU) at constant water temperatures of 10°C. Three days prior to incubation, experimental bivalves were not fed any more to avoid eutrophication through faeces and microbial contamination of the incubation water. 5-Bromo-2-deoxyuridine (BrdU, Fluka, 30 mg l⁻¹) and 5-Fluoro-2-deoxyuridine (FdU, Fluka,

3 mg l⁻¹) were dissolved in the experimental water and taken up by the bivalves with the inhaled medium. 5-Fluoro-2-deoxyuridine reduces competition with endogenous thymidine and increases BrdU incorporation. Duration of exposure of *A. opercularis* was 24 h, and because of slower proliferation rates incubation time of Iceland and German Bight *A. islandica* was prolonged to 5 days. During the 5-days *A. islandica*-incubation, 1.5 l of the experimental medium was exchanged with an equal amount of freshly prepared BrdU and FdU seawater every 24 h. Control bivalves were held in seawater without proliferation reagents. Bivalves were dissected and blocks of gill, mantle, adductor muscle and heart tissue transferred to cryovials and snap frozen in liquid nitrogen for both proliferation and apoptosis measurements. The shells of all bivalves were cleaned and numbered for individual age determination. Tissue blocks were embedded on chucks using frozen section medium (Neg-50, Thermo Fisher Scientific, Waltham, USA) and cut with a cryostat HM 500 OM (MICROTOM GmbH, Walldorf, Germany) at -20°C in 1-µm-thick cryo-sections, which were placed on Bio-Bond (BBInternational, West Chester, USA) coated slides. Cryosections were stained with anti-BrdU and diaminobenzidine (DAB) using an Amersham cell proliferation kit (GE Healthcare, Buckinghamshire, UK) with three changes to the standard protocol: (1) before rehydration, sections were fixed for 30 s in 100% acetone, washed 3 times in phosphate-buffered saline (PBS) and incubated 3 min in 3% H₂O₂, (2) 50 mM Tris-HCl buffer (50-mM Tris, 146-mM NaCl, 200-mM HCl) was used instead of PBS before staining with DAB, (3) eosin-phloxin solution was used to counterstain the chromogen for 30 s, this was followed by 6 dips in 80% ethanol, 10-min dehydration and embedding of the sections in Euparal (CHROMA-GESELLSCHAFT, 3C 239). Method controls were prepared from tissues of control bivalves and not labelled with BrdU, and tissues labelled with BrdU but using PBS to replace the primary antibody. A second cryosection of each sample was stained in haematoxylin and eosin-phloxin (H&E) to visualise all nuclei in the tissue section. Cryosections were analysed with a Zeiss Axioscope light microscope equipped with a KS300 image analysis software to count both nuclei stained by H&E ($N_{\text{H\&E}}$) and dividing nuclei stained with the Amersham cell proliferation kit (N_{p}). Per cent cell proliferation day⁻¹ was calculated as:

$$\% \text{ proliferation } 24 \text{ h}^{-1} = N_{\text{p}}/N_{\text{H\&E}} \times 100 \\ \times \text{day of incubation}^{-1}$$

Measurement of caspase-3 and -7 activities

The measurement of apoptosis via caspase-3 and -7 activity was modified after Liu et al. (2004). Frozen samples of

mantle, gill and adductor muscle of 5 German Bight *A. islandica*, 15 Iceland *A. islandica* of different age classes, and 5 *A. opercularis* were ground in liquid nitrogen and homogenised with a glass homogeniser (Nalgene, USA) in lysis buffer (100-mM HEPES, pH 7.5, 5-mM MgCl₂, 1-mM EGTA, and 1 µg ml⁻¹ each leupeptin, pepstatin and aprotinin) 1:100 (w/v). Homogenates were centrifuged for 15 min at 15 000g and 4°C, and apoptosis intensities in supernatants were determined as caspase-3 and -7 activities using a Caspase-Glo 3/7 Assay kit (Promega, Madison, USA). The assay provides a luminogenic caspase-3/7 substrate, diluted in a reagent optimised for caspase activity, luciferase activity and cell lysis. Equal volumes of reagents and supernatant were added to a white-walled 96-well plate and incubated at 25°C for 1 h. The pro-luminescent substrate was cleaved by caspases and extracted into the supernatant, where it formed a substrate for luciferase. The resulting luminescence signal is proportional to the amount of caspase activity present in the supernatant. Samples were analysed using a Multilabel Reader LB 941 TriStar (Berthold Technologies GmbH & Co.KG, Bad Wildbad, Germany) which gave luminescence readings as relative light units (RLU). Subsequently, protein concentration was determined in the supernatant according to Bradford (1976) and apoptosis intensities expressed as RLU mg⁻¹ protein.

Measurement of superoxide dismutase activity

German Bight and Iceland *A. islandica* were dissected and gill and mantle tissues snap frozen in liquid nitrogen for enzyme activity measurements. Shells of all bivalves were collected for individual age determination. Superoxide dismutase (SOD) activity was determined after Livingstone et al. (1992). 100–180 mg of frozen mantle and gill tissue were ground in liquid nitrogen and homogenised with a micropistill in Tris-HCl buffer (20-mM TRIS, 1-mM EDTA, 20-mM HCl, pH 7.6) 1:10 (w/v). Samples were centrifuged for 3 min at 18 000g and 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. One unit SOD causes 50% inhibition under assay conditions. Mitochondrial and cytosolic SOD isoforms were not distinguished.

Measurement of catalase activity

Catalase (CAT) activity was determined after Aebi (1984). In phosphate buffer (50-mM KH₂PO₄, 50-mM Na₂HPO₄, pH 7.0) with 0.1% Triton x-100 at 1:30 (w/v), 20–50 mg of frozen mantle and gill tissue were ground in liquid nitrogen

and homogenised with a micropistill. Samples were centrifuged for 15 min at 13 000g and 4°C, and CAT activity was determined by recording the period required for H₂O₂ decomposition, resulting in a decrease in absorption from 0.45 to 0.4 at 240 nm (1 unit).

Calculation of respiration and metabolic rate

Respiration rates of German Bight and Iceland *A. islandica* were measured at 10°C in a multichannel intermittent flow system as described by Begum et al. (2009) and Heilmayer and Brey (2003). To eliminate the impact of specific dynamic action (SDA) on respiration, bivalves were maintained without food for three days (Bayne et al. 1976), and measured separately after one night in the respiration chamber. Oxygen concentration was monitored continuously with fluorescein-coated oxygen microoptodes (PSt1-L5-TF, PreSens, Neuweiler, Germany) connected to a MICROX TR3 array (PreSens, Neuweiler, Germany). After the measurement, clams were dissected and soft tissue dried at 68°C for at least 48 h to determine dry mass (DM). To obtain ash-free dry mass (AFDM = DM—ash), dried tissues of *A. islandica* were combusted for 24 h at 500°C. Oxygen consumption rates (VO₂, μmol O₂ h⁻¹ ind⁻¹) and mass specific respiration rates (MSR, μmol O₂ h⁻¹ ind⁻¹ AFDM⁻¹) were calculated according to Begum et al. (2009). Shells of all bivalves were cleaned and numbered for individual age determination.

Age determination

Age determination for *A. islandica* was carried out as described by Schöne et al. (2004) and Strahl et al. (2007). Briefly, the right shell was embedded in epoxy resin and sectioned along the axis of maximum shell growth (height *H*) with a Buehler low-speed diamond saw. Cross-sections were ground and polished and annual shell growth bands were counted using a stereomicroscope at 10- to 80-fold magnification. In *A. opercularis*, individual age was inferred from shell height and a von Bertalanffy growth model (VBGM) established by Heilmayer et al. (2004) and based on height-at-age-data of the same scallop population from the English Channel. The von Bertalanffy growth function (VBGF):

$$H_t = H_\infty \times (1 - e^{-k \times (t-t_0)})$$

was used to model the relation between shell height and age, where *H_t* is height at age *t*, *H_∞* is height at infinite age, *k* is the growth constant and *t₀* is age at which size would be zero. To estimate age *t* from height of our experimental clams, we fitted the inverse VBGF:

$$t = \ln(1 - H_t/H_\infty) / -k + t_0$$

Statistical analysis

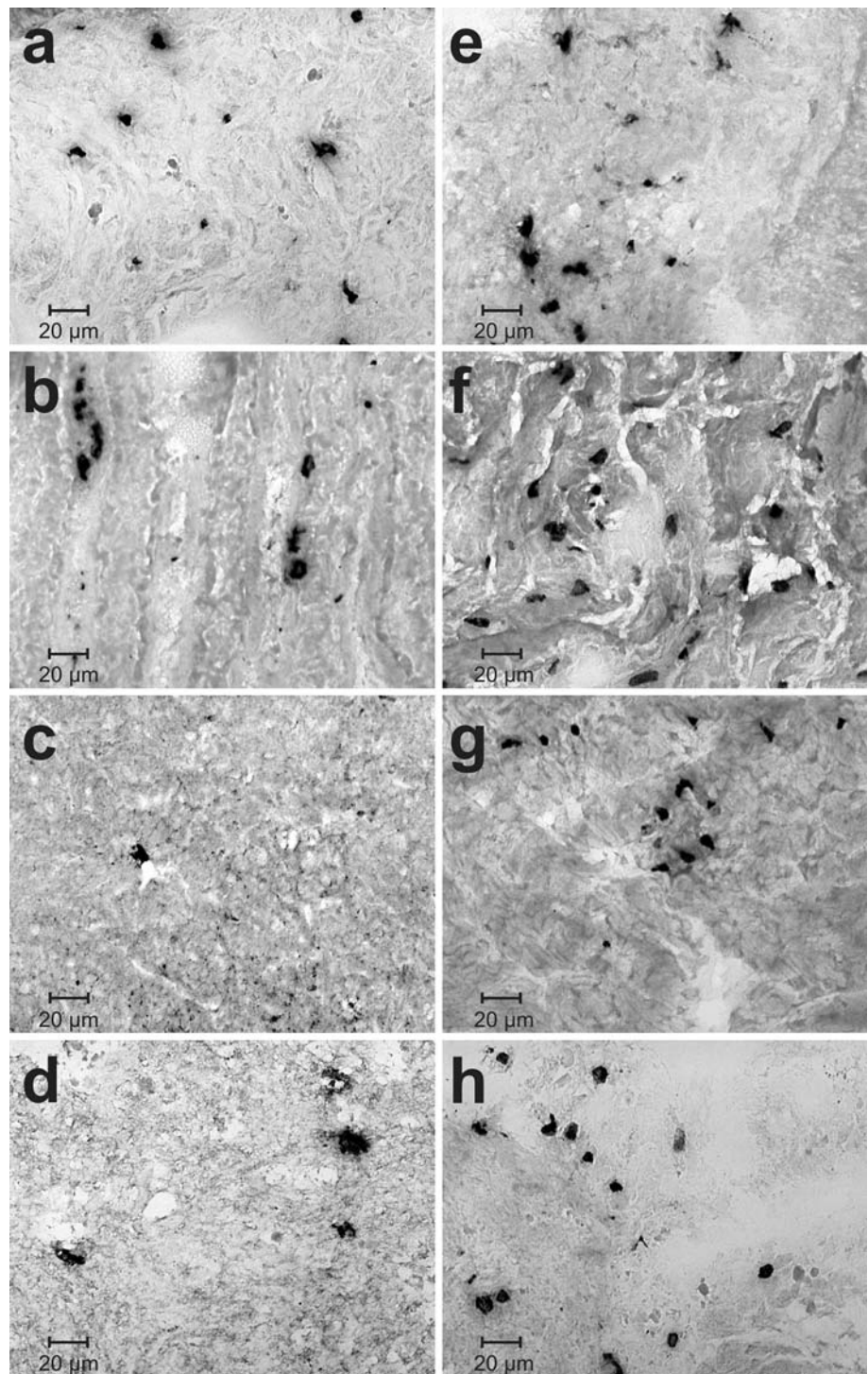
Proliferation rates were transformed according to: arcsin √% proliferating cells 24 h⁻¹100⁻¹. Statistical analyses were performed with GraphPad Prism 5 Software (La Jolla, California, USA). Mass specific respiration rates, superoxide dismutase and catalase activity, and proliferation rates and apoptosis intensities of all three populations were tested for normality (Kolmogorov–Smirnov test) and homogeneity of variances (Bartlett’s test). One-way ANOVA (Kruskal–Wallis test for non-Gaussian distributed data) with post-hoc test Tukey (Dunn’s) was used to test for differences in mass specific respiration and superoxide dismutase and catalase activity in mantle and gill within the three populations. One-way ANOVA (Kruskal–Wallis test for no Gaussian distributed data) with post-hoc test Tukey (Dunn’s) was used to test for differences in proliferation rates and apoptosis intensities between tissues within one population and between three age groups within one tissue type. Two-way ANOVA and Bonferroni post-hoc test were used to test whether tissue type, species/population and age differentially affects proliferation rates or apoptosis intensities in different populations. Slopes of linear regressions were tested for deviation from zero with an *F*-test (proliferation rates and apoptosis intensities versus age for each tissue of all three populations).

Results

Proliferation rates and apoptotic intensity in tissues of *Arctica islandica* and *Aequipecten opercularis*

Proliferating cells were detected in mantle, gill, adductor muscle and heart of the two investigated species (Fig. 1). Proliferation rates and ratios between tissues were similar in German Bight (GB) and Iceland (IC) *Arctica islandica*, with slightly higher proliferation values in IC clams. GB *A. islandica* had significantly higher proliferation rates in gill tissue (0.8 ± 0.6% cell division in 24 h) compared to values <0.1% cell division in 24 h in mantle and heart muscle (One-way ANOVA *P* < 0.0001, Tukey *P* < 0.01). Proliferation rates in GB *A. islandica* adductor muscle were below detection limit (=0%) after 5 days of BrdU incubation. IC *A. islandica* mantle and gills had highest proliferation rates with around 1% of nuclei dividing in 24 h, followed by the heart muscle and significantly lower rates in adductor muscle with 0.22 ± 0.30% cell division in 24 h (Kruskal–Wallis *P* < 0.001, Dunn’s *P* < 0.01, Fig. 2, arcsin transformed data are shown). Proliferation rates were significantly higher in all tissues of the scallop *Aequipecten opercularis* compared to GB and IC *A. islandica* (Two-way

Fig. 1 Proliferating cell nuclei in cryo slices of mantle (**a, e**), gill (**b, f**), adductor muscle (**c, g**) and heart (**d, h**) of Icelandic *Arctica islandica* (*left*) after 5 days of BrdU incubation vs. *Aequipecten opercularis* from the English Channel (*right*) after 24 h of BrdU incubation. BrdU-labelled nuclei appear *black*



ANOVA $P < 0.0001$; arcsin transformed data are given in Fig. 2). In gill tissue of *A. opercularis*, $12.3 \pm 7.9\%$ of nuclei were dividing within 24 h, whereas mantle, adductor muscle and heart were less actively proliferating without significant differences between scallop tissues. Apoptosis intensities within one tissue type were higher in *A. opercularis* compared to either GB or IC *A. islandica*, but only in adductor inter-specific differences were

significant, with tenfold higher values in *A. opercularis* (One-way ANOVA $P < 0.0001$, Tukey $P < 0.001$, Fig. 3). Mantle tissue had 3-times and gill 1.5-times higher apoptosis rates in *A. opercularis* compared to *A. islandica*. In the tissue comparison, highest apoptotic activity was observed in scallop gills, followed by adductor muscle and mantle. Again, differences were not statistically significant. Apoptosis intensity in gills of both GB and IC *A. islandica*

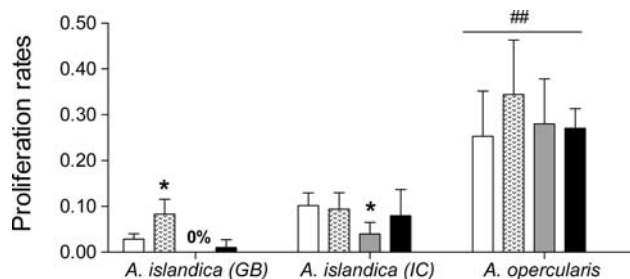


Fig. 2 Proliferation rates in $\arcsin\sqrt{(\% \text{ proliferating cells } 24 \text{ h}^{-1} 100^{-1})}$ in mantle (open bars), gill (dotted bars), adductor muscle (shaded bars) and heart (filled bars) of German Bight (GB) *Arctica islandica* (means \pm SD, $n = 5$ per tissue type), Icelandic (IC) *A. islandica* (means \pm SD, $n = 15$ per tissue type) and *Aequipecten opercularis* from the English Channel (means \pm SD, $n = 5$ per tissue type). ## Significant differences in tissues of *A. opercularis* compared to GB and IC *A. islandica* (Two-way ANOVA $P < 0.0001$). * Gill differs significantly from all other tissues in GB *A. islandica* (One-way ANOVA $P < 0.0001$; Tukey $P < 0.01$) and adductor muscle differs significantly from all other tissues in IC *A. islandica* (Kruskal–Wallis $P < 0.001$; Dunn's $P < 0.01$). 0%: cell proliferation rate is zero in the adductor muscle of GB *A. islandica*

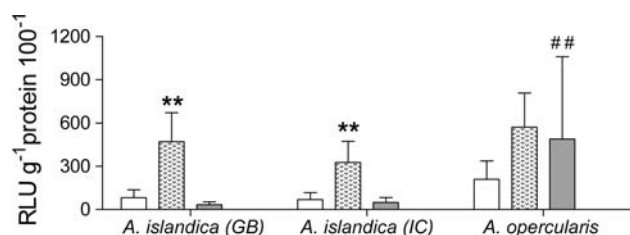


Fig. 3 Apoptosis intensities ($\text{RLU g}^{-1} \text{ protein } 100^{-1}$) in mantle (open bars), gill (dotted bars) and adductor muscle (shaded bars) of German Bight (GB) *Arctica islandica* (means \pm SD, $n = 5$ per tissue type), Icelandic (IC) *A. islandica* (means \pm SD, $n = 15$ per tissue type) and *Aequipecten opercularis* from the English Channel (means \pm SD, $n = 5$ per tissue type). ## Significant differences in adductor muscle between *A. opercularis* vs both *A. islandica* populations (One-way ANOVA $P < 0.0001$, Tukey $P < 0.001$). ** Significant differences in gill compared to other tissues of IC and GB *A. islandica* (One-way ANOVA $P < 0.0001$; Tukey $P < 0.001$)

ranged significantly higher than in mantle and adductor muscle, with eightfold lower values in adductor muscle and fivefold lower values in mantle (One-way ANOVA $P < 0.0001$, Tukey $P < 0.001$, Fig. 3).

Proliferation rates and apoptosis intensities in tissues of Icelandic *Arctica islandica* over an age range of 7–148 years

Proliferation rates decreased significantly with increasing age in the heart muscle of IC *A. islandica* (proliferation: One-way ANOVA $P < 0.01$; Tukey $P < 0.05$; Fig. 4, arcsin transformed data are shown). Proliferation in the heart was 20 times lower in 118–145 years old clams than in young individuals of 7–10 years. Average rates in young clams were $1.93 \pm 1.22\%$ of proliferating heart cells in 24 h

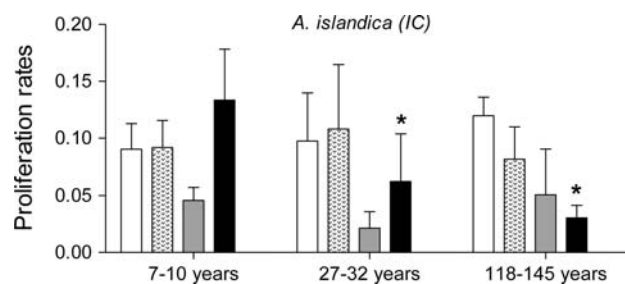


Fig. 4 Proliferation rates in $\arcsin\sqrt{(\% \text{ proliferating cells } 24 \text{ h}^{-1} 100^{-1})}$ in mantle (open bars), gill (dotted bars), adductor muscle (shaded bars) and heart (filled bars) of young (7–10 years), middle-aged (27–32 years) and old (118–145 years) Icelandic (IC) *Arctica islandica* (means \pm SD, $n = 5$ per tissue type and age class). * Significant differences between heart of middle aged and old compared to young individuals (One-way ANOVA $P < 0.01$; Tukey $P < 0.05$)

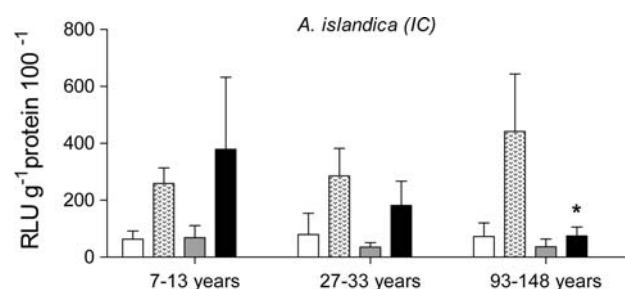


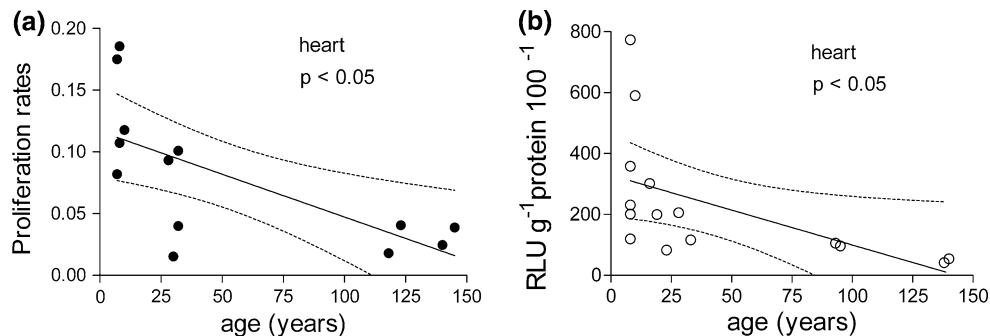
Fig. 5 Apoptosis intensities ($\text{RLU g}^{-1} \text{ protein } 100^{-1}$) in mantle (open bars), gill (dotted bars), adductor muscle (shaded bars) and heart (filled bars) of young (7–10 years), middle-aged (27–32 years) and old (118–145 years) Icelandic (IC) *Arctica islandica* (means \pm SD, $n = 5$ per tissue type and age class). * Significant difference between heart of old compared to young individuals (One-way ANOVA $P < 0.05$; Tukey $P < 0.05$)

compared to $0.52 \pm 0.49\%$ in middle aged (27–32 years) and $0.10 \pm 0.07\%$ in old individuals (118–145 years) (linear regression $P < 0.05$ Fig. 6a, arcsin transformed data are shown). Also, apoptosis intensities decreased significantly with age in the heart muscle of IC *A. islandica* (apoptosis: One-way ANOVA $P < 0.05$; Tukey $P < 0.05$; Fig. 5). Apoptotic intensity ranged highest in heart tissue of young ocean quahog and was five-times lower in bivalves >100 years old (linear regression $P < 0.05$, Fig. 6b). In contrast to the heart values, proliferation rates and apoptotic activity in mantle, gill and adductor muscle of *A. islandica* were the same over an age range of 7–148 years (Figs. 4, 5).

Respiration rates and antioxidant enzyme activities in tissues of *Arctica islandica* and *Aequipecten opercularis*

Mass specific respiration rate (MSR) was significantly lower in GB and IC *A. islandica* compared to *A. opercularis* from the English Channel (Kruskal–Wallis $P < 0.001$, Dunn's $P < 0.005$), which showed twice as high MSR. IC

Fig. 6 a Proliferation rates in $\arcsin\sqrt{(\% \text{ proliferating cells } 24 \text{ h}^{-1} 100^{-1})}$ (filled circles) ($y = -0.0007x + 0.117$; $r^2 = 0.462$; $n = 13$) and **b** Apoptosis intensities in $\text{RLU g}^{-1}\text{protein } 100^{-1}$ (open circles) ($y = -2.295x + 329.1$; $r^2 = 0.2907$; $n = 15$) in heart of Icelandic *Arctica islandica*



A. islandica had slightly higher MSR than GB clams, but differences were not statistically significant (Table 1). In contrast, both superoxide dismutase (SOD) and catalase (CAT) activity were significantly higher in mantle tissue of GB and IC *A. islandica* compared to *A. opercularis* (Kruskal–Wallis $P < 0.0001$, Dunn's $P < 0.05$). SOD activity was twice as high in both *A. islandica* populations compared to *A. opercularis*. CAT activity was six-times higher in GB *A. islandica* and seven-times higher in IC *A. islandica* than in *A. opercularis*. No significant differences were detected in mantle or gill between the two *A. islandica* populations for either enzyme (Table 1).

Discussion

We compared two bivalve model species, the long-lived and slow-growing ocean quahog *Arctica islandica* and the short-lived and fast-growing scallop *Aequipecten opercularis*. In the case of the Icelandic ocean quahog, we could distinguish between age groups, whereas our entire sample of *A. opercularis* comprised 2–4 years old mature individuals still in the phase of highest growth. Von Bertalanffy growth models for *A. opercularis* from the English Channel

demonstrate highest shell growth rates in conjunction with highest somatic production between 0 and 2 years of age, whereas from the 5th year on growth rates become asymptotic (Heilmayer et al. 2004). Rapid growth in young and mature *A. opercularis* of up to 4 years is in agreement with higher proliferation and cell turnover rates in tissues of the scallops compared to the ocean quahog. Rapid proliferation, especially in tissues such as the voluminous adductor muscle, cannot represent net-growth efficiency, because a muscle growth of $10\% \text{ day}^{-1}$ would exceed the slower shell growth within days. Indeed, high proliferation is in part counterbalanced by a high intensity of programmed cell death. Next to early sexual maturation, starting at the age of <1 year (Román et al. 1996), short-lived *A. opercularis* are characterised by rapid cell turnover in somatic tissues, which explains the higher basic energy demand indicated by high mass specific respiration (MSR) compared to more slowly growing *A. islandica*. According to the 'Free-Radical-Rate-of-living' theory of Pearl (1928) and Harman (1956), high oxygen turnover would suggest a high formation rate of reactive oxygen species (ROS) in the mitochondria. This could cause accelerated ageing and therefore be one reason why life span is limited to 10 years in *A. opercularis* (Ansell et al. 1991). However,

Table 1 Investigated age range in year, mass specific respiration rate (MSR) in $\mu\text{mol O}_2 \text{ h}^{-1} \text{ ind}^{-1} \text{ AFDM}^{-1}$ and antioxidant enzyme activity of superoxide dismutase (SOD) and catalase (CAT) in $\text{U g}^{-1}\text{WM}$ in tissues of German Bight (GB) and Iceland (IC) *Arctica*

	<i>A. islandica</i> (GB)	<i>A. islandica</i> (IC)	<i>A. opercularis</i> ^a
Age	33–98	29–141	1–6
MSR	2.46 ± 0.40	4.05 ± 1.83	$8.32 \pm 3.91^{**}$
SOD gill	728.62 ± 226.10	809.97 ± 406.53	– ^b
SOD mantle	790.04 ± 430.61	658.22 ± 364.18	$379 \pm 169^*$
CAT gill	$2,944.35 \pm 1,016.08$	$3,526.23 \pm 740.30$	– ^b
CAT mantle	$2,939.07 \pm 633.21$	$2,330.51 \pm 452.43$	$384 \pm 69^{**}$

** Significant differences in MSR and CAT in mantle of *A. opercularis* compared to GB and IC *A. islandica* (Kruskal–Wallis $P < 0.0001$, Dunn's $P < 0.001$)

* Significant differences in SOD in mantle of *A. opercularis* compared to GB and IC *A. islandica* (Kruskal–Wallis $P < 0.0001$, Dunn's $P < 0.05$)

^a Data for *A. opercularis* from Philipp et al. (2006), SOD and CAT activity recalculated in U g WM^{-1}

^b No data available

mitochondrial production of ROS is hardly measurable in *A. opercularis* and ranged tenfold higher under the same assay conditions in *A. islandica* (Philipp et al. 2006, 2008; S. Hardenberg, D. Abele, unpublished data). Thus, scallops have higher mitochondrial respiration, but control ROS generation in tissues at extremely low levels, which presumably protects them from severe oxidative damage during frequent phases of exhaustive swimming. To conserve these low ROS levels over lifetime, *A. opercularis* eliminate damaged mitochondria from adductor muscle cells during ageing (Philipp et al. 2008). Permanent elimination of damaged mitochondria through intensive apoptosis is in keeping with absence of mitochondrial ageing in *A. opercularis*, and mitochondrial respiration and energetic coupling indeed do not change with age (Philipp et al. 2006).

Proliferation and apoptosis also eliminate oxidative waste products such as the fluorescent age pigment lipofuscin and protein carbonyls. Highest tissue cell turnover rates occur in gills of *A. islandica* and *A. opercularis*, possibly triggered by oxidative damage. The gill is one of the most active tissues and ROS production in this organ is assumed to be high. Accordingly, studies of lipofuscin accumulation in different tissues of the ocean quahog indicated better tissue protection and maintenance in adductor muscle and mantle compared to gills, where the age pigments were most highly concentrated (Strahl et al. 2007).

Arctica islandica is not only the longest lived bivalve known today, but moreover one of the slowest growing bivalves worldwide (Strahl et al. 2007). In addition to low MSR, slow gonad ripening and late onset of maturation at ages of 10–14 years in the ocean quahog (Thompson et al. 1980), slow cell proliferation and growth are additional criteria characterising long-lived species (Bauer 1992; Mangel 2003). These slow rates of cell division enable individual cells in ocean quahog tissues to grow to large diameters. Muscle fibre diameter increased fourfold in old (>100 years) compared to young clams (<10 years, Strahl et al. 2007). At the same time, low proliferation and cell turnover rates suggest that *A. islandica* cells should be endowed with an efficient system of waste removal through proteasomal and autophagic processes, and a well-established antioxidant defence system, to offset accumulation of cellular oxidative damage. Indeed, we found high and stable antioxidant protection in tissues of the ocean quahog between 30 and 200 years (Abele et al. 2008), whereas *A. opercularis* has a low investment in cellular protection and maintenance at advanced age (Philipp et al. 2006). Similarly, in the oyster *Crassostrea virginica* with a maximal live span (MLSP) of only 6 years, not only antioxidant enzyme activity was lower but, the density of apoptotic and mitotic cells was also diminished in gill, adductor muscle and heart of mature compared to young oysters (Ivanina

et al. 2008; Sunila and LaBanca 2003). It would be interesting to see whether in short-lived scallops the reported shift in energy allocation from somatic maintenance towards reproduction at the end of life (see also Heilmayer et al. 2004; Abele et al. 2009) leads to reductions of cell proliferation in somatic tissues, and whether or not this is accompanied by a decline of apoptotic activity. On the other hand, low metabolic activity and sustained protection from oxidative damage over age, in combination with slow but continued cell turnover, presumably slow senescent decline in *A. islandica*. Long-lived molluscs are known to budget lifetime energy investments equally between somatic maintenance and persistent but slow rates of gametogenesis (Arntz et al. 1994) and the lifelong continued damage repair and cell turnover explains negligible senescence in long-lived species (Kirkwood 2005; Ebert 2008).

The present study indicates negligible evidence of senescence in the mantle, gill and adductor muscle, whereas proliferation and apoptosis in the heart of *A. islandica* decreased dramatically with age. Since studies in the 1920s reported difficulties in detecting dividing cells or mitosis in the human heart (Karsner et al. 1925), the adult heart was regarded as postmitotic tissue. In contrast, we found proliferating heart cells in mature scallops and ocean quahog of all age classes, which is in line with more recent studies on vertebrates and suggests that heart cells of vertebrates and invertebrates can proliferate until old age and thus regenerate heart functions to some extent (Anversa et al. 1991; Kajstura et al. 1998; Poss et al. 2002). However, decreasing cell turnover rates over a lifetime of 140 years in the ocean quahog heart were indeed the only symptoms of old age indicating less-active cell renewal through proliferation and less-efficient removal of damaged cells. This in turn may indicate higher damage levels and possibly ongoing loss of function in the heart of ageing *A. islandica*. Impaired cardiac function has been suggested to limit life span in mammals (Leri et al. 2003) and perhaps ‘heart failure’ could finally cause natural death in old ocean quahog.

Our second question was whether geographically distant populations of *A. islandica* from the German Bight and from Iceland, that differ by at least 100 years in the recorded maximum age (Begum et al. 2009), are also physiologically distinct. Our measurements documented no significant differences between the two populations for proliferation, apoptosis, respiration rates and antioxidant protection. Thus, a difference of 5°C in bottom habitat temperature between the southern North Sea and the North Iceland fishing grounds was not accompanied by differences in ocean quahog physiology that would explain the shorter life span of *A. islandica* in warmer North Sea waters. This contrasts with other studies which, either by comparing latitudinal populations of the same species (Ziuganov et al. 2000) or bivalves from similar habitats in temperate and polar habitats (Philipp

et al. 2005a, b, 2006), document longer life span and slower change of physiological parameters with age in the cold-adapted populations/species. Similar physiological capacities are, however, consistent with observations of a low level of genetic differentiation between climatically distinct *A. islandica* populations throughout the NE Atlantic (Dahlgren et al. 2000; Begum et al. in prep).

Age-dependent measurements of physiological parameters in German Bight *A. islandica* would have allowed a comparison of trends with age with the Icelandic population, but were impossible, because individuals <30 years old are missing, and older individuals >100 years are rarely encountered in the German Bight. Instead, our sample was limited to 32–98 years old clams with an average shell height of 74 ± 5 mm. The narrow age range was earlier reported by Witbaard and Bergman (2003) who studied populations in the south-eastern North Sea between 1990 and 2000 and found them dominated by adults of 50–85 mm shell height, whereas young clams of smaller size were extremely rare. The relative absence of young individuals either indicates problems with larval settlement and recruitment, or high mortality rates of very young *A. islandica* during the past 35 years in the southern North Sea (Witbaard and Bergman 2003). An important impact comes from beam trawl fishery which caused a general decline in the number of benthic bivalves in the past 100 years in the southern North Sea (Rumohr and Kujawski 2000) and mainly affects young *A. islandica* (Witbaard and Klein 1994). Moreover, environmental change in the German Bight area, including strong eutrophication in the 1960s–1990s (Rijnsdorp and van Leeuwen 1996) and slow but steadily increasing water temperatures around Helgoland (Wiltshire and Manly 2004), could cause thinning of the *A. islandica* population and the reduction of individual life expectancy. Indeed, conditions around Iceland are more favourable in many respects including food availability and absence of severe fishery impacts (Witbaard et al. 1999; Kaiser et al. 2006), which explains the higher densities of Iceland *A. islandica* (Ragnarsson and Thórarinsdottir 2002; Witbaard and Bergman 2003).

Our study suggests that maximum life expectancy in long-lived ocean quahog populations is mainly constrained by environmental impacts in different areas and does not reflect differences in the basic physiology and cellular renewal capacities between North Sea and Iceland *A. islandica*. Low metabolic rates and slow but stable cell turnover in tissues of Iceland *A. islandica* over 140 years, combined with high antioxidant protection with age, characterised the long-lived and slow-growing ocean quahog in both regions. In contrast, the short-lived and fast-growing scallop *A. opercularis* is characterised by high-energy throughput, reduced investment in cellular protection and high proliferation and apoptosis intensities. Low rates of cell proliferation and

apoptosis in the heart of Iceland *A. islandica* were the only symptoms of old age which may indicate higher damage levels and ongoing loss of function. Future studies of ageing in molluscs should look into cellular viability and oxidative stress parameters in the heart.

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