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# Physiological ageing in a temperate and a polar swimming scallop

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ABSTRACT: We compared physiological ageing parameters in 2 scallops, the temperate *Aequipecten opercularis* and the Antarctic *Adamussium colbecki*. These 2 species are phylogenetically closely related and display a similar lifestyle but have distinctly different maximum lifespans (MLSP). *A. opercularis* does not live longer than 8 to 10 yr, whereas *A. colbecki* lives over 18 yr. The development of several physiological ageing parameters over time, chosen according to the 'free radical theory of ageing', was compared in the 2 species to identify differences in the ageing process. In the shorter-lived *A. opercularis*, activities of the mitochondrial enzymes citrate synthase and cytochrome *c* oxidase and of the antioxidant enzyme catalase showed a more pronounced decrease with increasing age than in the longer-lived *A. colbecki*. In line with this finding, lipofuscin accumulation increased more distinctly in *A. opercularis* than in *A. colbecki*, while tissue protein content decreased in *A. opercularis* but increased in *A. colbecki*. Its better preservation of mitochondrial and antioxidant enzyme activities and the avoidance of waste accumulation may enable *A. colbecki* to live longer than *A. opercularis*. Mitochondrial function investigated in *A. opercularis* showed only minor changes with age, and mitochondrial $H_2O_2$ generation rates were low at all ages. We relate our findings to the 'free radical–rate of living' theory, to the 'uncoupling to survive' hypothesis, and to the particular lifestyle of these scallops.

KEY WORDS: Ageing · scallops · Reactive oxygen species · ROS · Mitochondria



## INTRODUCTION

There is much empirical evidence for higher maximum lifespans (MLSP) in cold-adapted ectotherms compared to their close relatives from temperate waters (Brey 1991, Brey et al. 1995, Ziuganov et al. 2000, Cailliet et al. 2001, La Mesa & Vacchi 2001, Philipp et al. 2005a). We aim to explore the physiological principles underlying those differences in MLSP, especially with respect to the accelerating effect of temperature on aerobic metabolic rates and reactive oxygen species (ROS) formation.

Two ageing theories link ageing and MLSP to mitochondrial ROS formation: (1) The 'free radical–rate of living' theory (Pearl 1928, Harman 1956) predicts a negative correlation between standard metabolic rate (SMR) and MLSP due to increased mitochondrial production of ROS at higher SMR (Ku et al. 1993); in some species, however, this strict relationship between MLSP and SMR is not maintained, especially in primates and birds, which live longer than expected from their SMR (Pérez-Campo et al. 1998). (2) The 'uncoupling to survive' hypothesis by Brand (2000) attempts to explain this contradiction by proposing that mitochondrial uncoupling modulates ROS production, thus altering the strict dependency of ROS formation on SMR.

A recent comparison of sessile infaunal soft-shell clams from temperate regions (*Mya arenaria*) and from Antarctica (*Laternula elliptica*) (Philipp et al. 2005a,b), showed that lower metabolic rates of the Antarctic *L. elliptica*, along with the maintenance of a reduced tissue redox-state, lower mitochondrial $H_2O_2$ generation, and a less pronounced decline in mitochondrial func-

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tions (aerobic capacity, respiratory control ratio [RCR], proton leak) could form the basis for the extended lifespan of the Antarctic species compared to the temperate *M. arenaria*.

The present study extends our approach to actively swimming bivalves, to see whether there are unifying principles of physiological ageing in bivalves or whether an active and energy-consuming lifestyle leads to different strategies regarding maintenance of physiological fitness. The temperate queen scallop *Aequipecten opercularis* is found along the European coast from Norway to the Mediterranean and from the Adriatic to the west of Ireland, spanning an overall temperature range from 6 to 24°C (Ansell et al. 1991). The Antarctic scallop *Adamussium colbecki* is a common member of Antarctic coastal communities, where it experiences a habitat temperature range of –1.8 to 2.5°C (Berkman 1990, Berkman et al. 2004). Both pectinids are epibenthic with a well-developed capacity of jet–propulsion for swimming (Brand 1991, Ansell et al. 1998). Despite their close phylogenetic relationship (Canapa et al. 2000) and principal similarity in their lifestyle, the 2 species differ distinctly in maximum lifespan. *A. opercularis* has a relatively short MLSP which does not exceed 8 to 10 yr (Ansell et al. 1991). *A. colbecki* is a slow-growing, long-lived species, but its exact MLSP is, however, still unknown. Chiantore et al. (2003) reported an individual of 18 yr of age, inferred from a von Bertalanffy growth function. Berkman et al. (2004) recaptured scallops after a 12 yr period, which had been already large adults when released. According to these and other studies (Berkman 1990, Cattaneo-Vietti et al. 1997, Heilmayer et al. 2003) a MLSP of 30 to 40 yr for *A. colbecki* is most likely.

## MATERIALS AND METHODS

**Sampling and maintenance.** ***Aequipecten opercularis:*** *A. opercularis* were dredged from the Irish Sea 15 nautical miles south of Port St. Mary, Isle of Man, in July-August 2003 at about 64 m water depth. Scallops were transported to the Port Erin Marine Laboratory and kept in natural seawater flow-through aquaria at ~10°C and 34 PSU for several days prior to experimentation. Experiments with isolated mitochondria were carried out at the Port Erin Marine Laboratory. Specimens for whole animal respiration were transported in thermo boxes to the Alfred Wegener Institute in Bremerhaven, Germany. For all other analyses, samples were freeze-clamped immediately and stored in liquid nitrogen for transportation to the Alfred-Wegener-Institute, Bremerhaven.

***Adamussium colbecki:*** Antarctic *A. colbecki* were dredged at Terra Nova Bay ('Road Bay', 74°43' S, 164°13' E) in February 2004. Scallops were either sacrificed after collection and the mantle tissue stored in liquid nitrogen, or frozen whole in liquid nitrogen. Samples were transported in liquid nitrogen from Antarctica to Genova, Italy, and at –80°C from Genova to the Alfred Wegener Institute in Bremerhaven.

**Age determination.** External annual shell growth checks were used to establish relations between shell height and age in both species. For *Aequipecten opercularis* we aged 76 individuals and took an additional 200 height-at-age data from Allison (1993). For *Adamussium colbecki*, 185 height-at-age data pairs of the Terra Nova population were taken from Heilmayer et al. (2003). 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 $t$, $H_\infty$ is height at infinite age, $k$ is the growth constant, and $t_0$ is age at which size would be zero (for details see Brey 2001 http://www.awi-bremerhaven.de/Benthic/Ecosystem/FoodWb/Handbook/main.html Alfred Wegener Institute for Polar and Marine Research, Germany). To estimate Age $t$ from size $H_t$ of our experimental scallops, we fitted the inverse VBGF

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

to the height-at-age data sets using the non-linear, iterative Newton algorithm.

**Metabolic rate.** The SMR of *Aequipecten opercularis* (N = 31, mass range 219 to 3411 mg ash-free dry mass [AFDM], size range 30 to 80 mm, age range 1 to 6 yr) was measured at 10°C and 34 PSU in a multichannel, modified, intermittent-flow system with oxygen microoptodes connected to a Microx TX 2–array (PreSens), as previously described in Gatti et al. (2002). Microoptodes were calibrated to 100% oxygen solubility in air-saturated seawater and to 0% in $N_2$-saturated seawater at the experimental temperature of 10°C. Prior to respiration measurements, individuals of *A. opercularis* were maintained without food for 3 d to eliminate effects of specific dynamic action (SDA).

The respiration chambers (200 to 650 ml) were placed in a plastic tub with filtered seawater; a peristaltic pump (Ismatec) ensured continuous water circulation. To reduce handling stress, scallops were allowed to accommodate to the respiration chambers overnight. After closing the respiration chambers, a decrease in oxygen content from 100 to about 70% oxygen over a time of 2 to 3 h was recorded. Individuals were measured 2 to 3 times. After measurement, the scallops were dissected and soft tissue wet mass and dry mass (3 d at 60°C) were determined. Tissues were combusted at 500°C for 5 h to determine the AFDM. SMRs were de-

termined after subtraction of the microbial oxygen demand of the system, determined in a parallel blank chamber. Percent $O_2$ saturation was transformed to micromoles of dissolved oxygen in seawater, using known values of oxygen solubility (according to Benson & Krause 1984) and converted to µg $O_2$.

**Mitochondrial measurements.** ***Isolation:*** Mitochondria were isolated from the mantle tissue of freshly sacrificed bivalves. Tissues of up to 3 individual *Aequipecten opercularis* specimens were pooled for 1 experiment. About 3 g of mantle tissue were finely chopped in 10 ml ice-cold homogenisation buffer, modified after Moyes et al. (1985) (400 mM sucrose, 70 mM HEPES, 100 mM KCl, 3 mM ethylenediaminetetraacetic acid [EDTA], 6 mM ethelyneglycol-bis-tetraacetic acid [EGTA], 1% bovine serum albumine, 1 µl $ml^{-1}$ aprotinine, pH 7.3).

Briefly, the tissue was homogenised in a pre-cooled glass/teflon-homogeniser, centrifuged at 1300 ×*g* for 15 min at 2°C, and the supernatant collected. The pellet was resuspended and homogenised, and again mitochondria were separated at the same speed. The 2 supernatants were combined and centrifuged at 10500 ×*g* for 10 min to sediment the mitochondria. The resulting mitochondrial pellet was resuspended in 1.5 to 2 ml assay medium (560 mM sucrose, 100 mM KCl, 10 mM $KH_2PO_4$, 70 mM HEPES, 5 mM glutamate, 1 µg $ml^{-1}$ aprotinine and 1% bovine serum albumine, pH 7.3).

***Respiration:*** Mitochondrial respiration measurements were carried out at 10°C. Respiration rate was recorded with oxygen microoptodes (PreSens). Oxygen concentrations were calculated using oxygen solubility (ß$O_2$) according to Johnston et al. (1994) and the atmospheric pressure on the relevant day. We used 5 mM succinate, with 5 µM rotenone to prevent respiration of endogenous NAD-linked substrates (Brand 1995), as substrate, and State 3 respiration was induced by addition of 0.15 mM ADP. Non-phosphorylating respiration, oxygen consumption due to proton leakage and ROS formation (State 4+) were recorded after adding 2 µg $ml^{-1}$ of the $F_0F_1$-ATPase inhibitor oligomycin. The percentage of proton leak of State 3 oxygen consumption was calculated as (State 4+/State 3) ×100. The respiratory control ratio (RCR), which describes how effectively the respiratory chain is coupled to the ATPase, was calculated according to Estabrook (1967), using State 4+ respiration. The ADP:O ratio was determined by recording the time used for the consumption of the given ADP and the corresponding oxygen consumption.

***Hydrogen peroxide ($H_2O_2$) production:*** Mitochondrial hydrogen peroxide production was measured fluorimetrically ($\lambda_{exitation}$ = 312 nm and $\lambda_{emission}$ = 420 nm) by recording the reaction of $H_2O_2$ with homovanilic acid (HVA) in the presence of horse radish peroxidase (HRP), using a procedure modified after Miwa et al. (2003). Measurements of the $H_2O_2$ generation rate of *Aequipecten opercularis* mitochondria were performed with a Shimadzu (RF-1501) fluorometer at the Port Erin Marine Laboratory, as described in detail by Philipp et al. (2005b) *for Laternula elliptica*. Both $H_2O_2$ generation rates and oxygen consumption rates were measured in parallel and related to mitochondrial protein content (for details see Keller et al. 2004).

**Enzyme assays.** Enzyme activity was measured in mantle tissue of *Aequipecten opercularis* and *Adamussium colbecki*. All samples were measured at a reference temperature of 20°C. To investigate temperature sensitivity of the different enzymes ($Q_{10}$ values), enzyme activities were also determined at 0 to 5°C for *A. colbecki* and at 10°C for *A. opercularis*. The range of experimental temperatures given for *A. colbecki* data arose from the difficulty in achieving stable thermal conditions in the low temperature range. We therefore recorded the exact temperature in each assay and variations in temperature were taken into account for $Q_{10}$ calculations. Data are expressed as international units (µmol of substrate converted to product $min^{-1}$) per mg protein. Protein content of the extracts was determined by the Biuret method (Kresze 1988).

***Superoxide-dismutase (SOD, EC 1.15.1.1).*** Superoxide-dismutase (SOD) was determined after Livingstone et al. (1992). Frozen mantle tissue was ground in liquid nitrogen and homogenised in Tris buffer (20 mM TRIS-HCl, 1 mM EDTA, pH 7.6) 1:8 (w/v). Samples were centrifuged for 3 min at 18000 ×*g* and at 2°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. 1 U SOD causes 50% inhibition under assay conditions.

***Catalase (EC 1.11.1.6):*** Catalase activity was determined after Aebi (1984). Frozen mantle tissue was ground in liquid nitrogen and homogenised in 50 mM phosphate buffer (50 mM $KH_2PO_4$, 50 mM $Na_2HPO_4$, pH 7.0) with 0.1% Triton x-100 at 1:5 (w/v). Samples were centrifuged at 13 000 ×*g* for 15 min at 2°C. The activity was determined by recording the period required for $H_2O_2$ decomposition, resulting in a decrease of absorption from 0.45 to 0.4 at 240 nm.

***Citrate synthase (CS, EC 4.1.3.7) and cytochrome c oxidase (COX, EC 1.9.3.1):*** Frozen mantle tissue was ground in liquid nitrogen and homogenised with a glass homogeniser (Nalgene) in Tris-HCl buffer (20 mM Tris-HCl, 1 mM EDTA, 0.1% (v/v) Tween® 20, pH 7.4), 1:4 (w/v) for CS and 1:3 (w/v) for COX.

Homogenates for CS activity 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 2°C. CS activity was measured

after Sidell et al. (1987) by recording the absorbance increase of 0.25 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 oxaloacetate at 412 nm. Activity was calculated using the extinction coefficient $\varepsilon_{412}$mM of 13.61 $mM^{-1}$ $cm^{-1}$.

For COX measurements, homogenates were centrifuged for 10 min at 1000 × *g* and 2°C. COX activity was determined after Moyes et al. (1997) by measuring the oxidation rate of cytochrome *c* at 550 nm in 20 mM Tris HCl buffer with 0.5% Tween 20, pH 8.0. Activity was calculated using the extinction coefficient $\varepsilon_{550}$mM 19.1 $mM^{-1}$ $cm^{-1}$ after Hardewig et al. (1999).

**Glutathione content.** The concentrations of the oxidised (GSSG) and the reduced form (GSH) of glutathione were measured using high-performance liquid chromatography (HPLC) with a method modified after Fariss & Reed (1987) (for more details see Philipp et al. 2005a). The measurement is based on the derivatisation of the thiols with dinitrofluobenzene (DNFB). GSH oxidation during extraction is prevented by iodoacetic acid (IAA) binding of GSH. The HPLC system consisted of an $NH_2$-spherisorb column (240 × 4 mm, 5 µm particles) at 39°C and a binary solvent system of 80% methanol/water (Solvent A) and 80% Solvent A/20% acetate stock (272 g Na-acetate-trihydrate diluted in 122 ml water plus 378 ml glacial acetic acid) (Solvent B). Flow rate was 1.2 ml $min^{-1}$ at a maximum backpressure of 2500 pounds per square inch (psi). The gradient programme was 85% Solvent A/15% Solvent B for 8 min, followed by 20 min of linear gradient elution to 45% Solvent A/55% Solvent B and a subsequent 8 min hold. Thereafter the system was returned to the initial conditions within 5 min and re-equilibrated for 15 min.

Protein oxidation. Detection of protein carbonyl groups as a measure of protein oxidative modifications was carried out after Levine et al. (1990). Samples were homogenised in 50 mM HEPES (125 mM KCl, 0.5 mg $ml^{-1}$ leupeptine, 0.7 µg $ml^{-1}$ pepstatine, 40 µg $ml^{-1}$ phenylmethylsulfonyl fluoride, 0.5 µg $ml^{-1}$ aprotinine, 1.1 mM EDTA, 0.6 mM $MgSO_4$) and centrifuged at 31 000 × *g* for 15 min. Supernatants were incubated for 1 h with 10 mM DNTP (2,4-dinitrophenylhydrazine) in 2 M HCL. Blanks were run without DNTP. After incubation, 100% trichloroacetic acid (TCA) was added to the sample to precipitate the protein, followed by centrifugation for 10 min at 11 000 × *g*. The protein pellet was washed 3 times with ethanol: ethylacetate (1:1) and resuspended in 6 M guanidine hydrochloride in 20 mM potassium phosphate (pH = 2.3). Samples were measured photometrically at 360 nm using guanidine hydrochloride as a reference. The protein content of the samples was determined according to Bradford (1976), using bovine serum albumin as a standard.

**Fluorescent age pigment, lipofuscin.** Lipofuscin contents were determined by an extraction method modified after Vernet et al. (1988), as described in Philipp et al. (2005a). The fluorescence intensity of each sample was determined at an emission maximum of 450 nm for *Aequipecten opercularis* and 435 nm for *Adamussium colbecki*. Following Hill & Womersley (1991), lipofuscin concentrations were expressed as relative fluorescent intensity (RFI) using 0.1 µg quinine sulphate $ml^{-1}$ 1 N $H_2SO_4$ as standard.

**Protein content.** Protein content was determined as described by Philipp et al. (2005a). Lyophilised mantle tissue of both species was hydrolysed with 3% NaOH. Samples were centrifuged for 20 min at 10 000 × *g* to remove cell debris, and cooled on ice to facilitate protein precipitation. We added 60% TCA to precipitate the protein. After 20 min at 10 000 × *g*, the supernatant was discharged and the pellet dissolved in 3% NaOH using 20 ml per initial g tissue dry mass. Protein concentration was determined spectrophotometrically with the biuret method.

**Statistical analysis.** Analysis of variance (ANOVA) and analysis of covariance (ANCOVA) were used to analyse the relationship between individual parameters and age and to identify differences between species after testing the data for normality. Differences in $Q_{10}$ values between species were analysed with a Student's *t*-test.

## RESULTS

Relations between shell height and age were well-described by the von Bertalanffy growth model in both species (Fig. 1). Age can be inferred from shell height by Age $t = \ln(1 - H_t/108.74)/-0.199 + (-1.115)$, N = 276, mean squared error (MSE) = 0.370 in *Aequipecten opercularis*, and by Age $t = \ln(1 - H_t/122.61)/-0.083 + 0.237$, N = 185, MSE = 0.178. in *Adamussium colbecki*.

### Metabolism

Respiration rates ($R$ = µg $O_2$ $ind.^{-1}$ $h^{-1}$) of *Aequipecten opercularis* correlated well with body mass, $M$ (mg AFDM) (Fig. 2): *Aequipecten opercularis* (10°C): $\log(R) = 0.647 \times \log(M) + 0.447$ (N = 31, $r^2 = 0.864$, $p < 0.001$).

### Age-related changes in mitochondrial functions of *Aequipecten opercularis*

Oxygen consumption of mantle tissue mitochondria ($R_M$, nmol $O_2$ $mg^{-1}$ protein $min^{-1}$) at *in situ* temperature

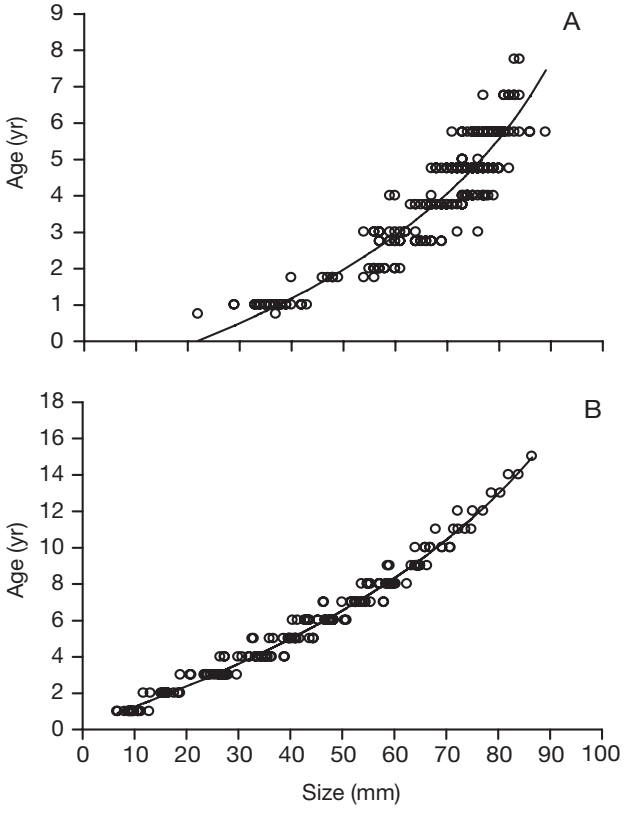


Fig. 1. (A) *Aequipecten opercularis* from Isle of Man: Age $t$ = ln (1 – $H_t$/108.74)/–0.199 + (–1.115), N = 276, mean squared error (MSE) = 0.370; (B) *Adamussium colbecki* from Terra Nova Bay: Age $t$ = ln (1 – $H_t$/122.61)/–0.083 + 0.237, N = 185, MSE = 0.178. Inverse Von Bertalanffy growth models, where $H_t$ = height at Age $t$ estimating size from age: $H_t = 84.47 \times (1 - e^{-0.427 \times (t + 0.347)})$, N = 276, MSE = 15.569 for *A. opercularis*, and $H_t = 126.30 \times (1 - e^{-0.078 \times (t - 0.103)})$, N = 185, MSE = 7.189 for *A. colbecki*

(10°C) declined significantly with increasing age (Fig. 3): $R_M = -0.385 \times \text{Age} + 6.460$ (N = 29, $r^2 = 0.245$, p = 0.0063, age range = 1 to 5 yr). The respiratory control ratio (RCR) and ADP:O ratio declined with age too, but the relationship was much weaker: RCR = –0.074 × Age + 3.274 (N = 34, $r^2 = 0.111$, p = 0.054, age range = 1 to 5 yr); ADP:O = –0.033 × Age + 1.535 (N = 33, $r^2$ = 0.118, p = 0.05, Age range = 1 to 5 yr).

Proton leak showed a slightly positive but non-significant relationship with age (p = 0.120). $H_2O_2$ generation (nmol $H_2O_2$ $mg^{-1}$ protein $min^{-1}$) of isolated mitochondria of *Aequipecten opercularis* was extremely low and followed no significant trend with age. Average values across the whole age range (1 to 5 yr) were RCR = 3.070 (SD ± 0.312, N = 34), ADP:O = 1.443 (SD ± 0.133, N = 33), proton leak (%) = 32.84 (SD ± 3.87, N = 34), $H_2O_2$ generation (nmol $mg^{-1}$ protein $min^{-1}$) = 0.0025 (SD ± 0.0012, N = 30).

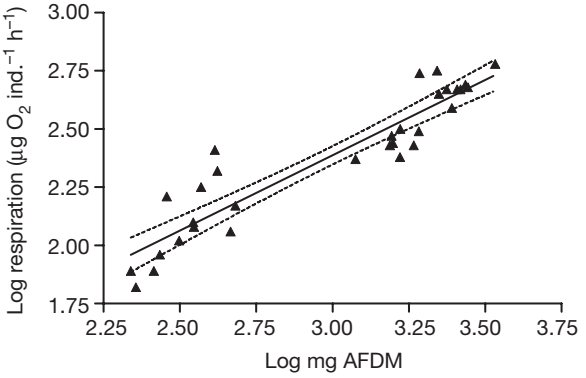


Fig. 2. *Aequipecten opercularis*. Standard metabolic rate. Respiration rates at 10°C: log($R$) = 0.646 × log(ash-free dry mass, AFDM) + 0.447; $r^2 = 0.864$, $p < 0.001$. N = 31; age range = 1 to 6 yr. Here and in following figures, continuous line is regression line and dashed lines are upper and lower 95% confidence limits

## Mitochondrial enzyme activities in mantle tissue of ageing *Aequipecten opercularis* and *Adamussium colbecki*

Activities of mitochondrial enzymes CS and COX decreased significantly with increasing age in *Aequipecten opercularis* but not in *Adamussium colbecki* (Fig. 4). CS values measured at *in situ* temperatures (data not shown) did not differ significantly between both species as a function of age. At the reference temperature of 20°C (Fig. 4A), values were in the same range in young individuals of both species. With

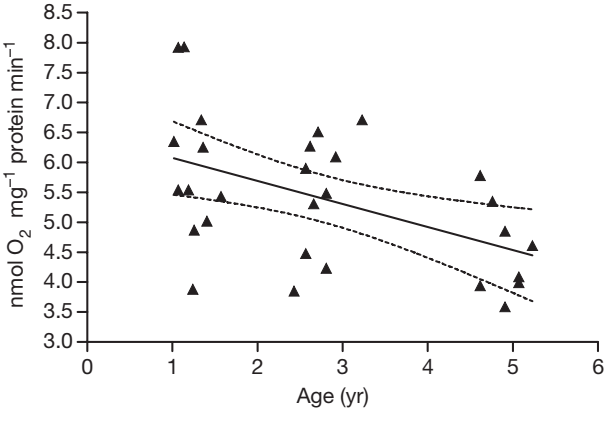


Fig. 3. *Aequipecten opercularis*. Decline in State 3 respiration of isolated mitochondria from mantle tissue at mean *in situ* temperature (10°C) with increasing age. Data points represent 1 to 3 replicate measurements per mitochondrial isolation. $O_2$ consumption of mantle tissue mitochondria, $R_M$ = 0.385 × Age + 6.46, N = 29, $r^2 = 0.245$, p = 0.0063, Age range = 1 to 5 yr

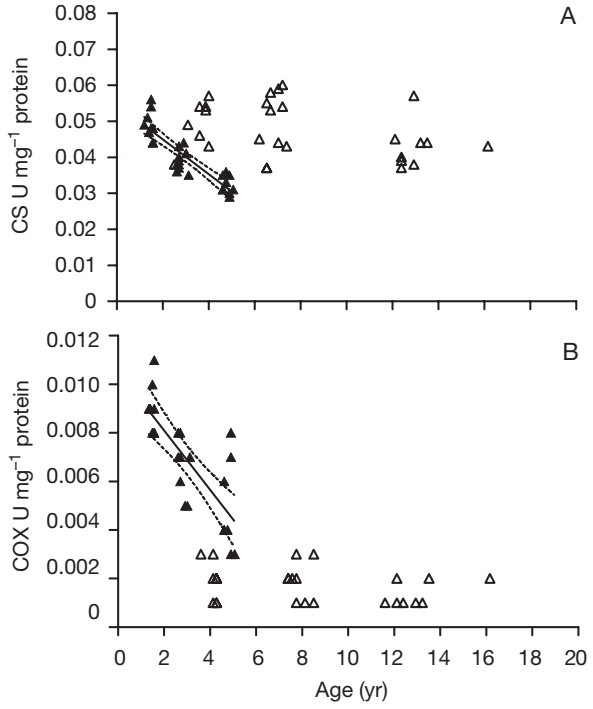


Fig. 4. *Aequipecten opercularis* (▲) and *Adamussium colbecki* (△). Activities of (A) citrate synthase (CS) and (B) cytochrome *c* oxidase (COX) at 20°C in mantle tissue versus age. Data are means of 2 to 3 replicate measurements. Slopes differed significantly between species ($p < 0.001$, ANCOVA). CS: *A. opercularis*: CS = –0.0048 × Age + 0.055, N = 28, $r^2 = 0.786$, $p < 0.001$, age range = 1 to 5 yr; *A. colbecki*: no significant relationship, N = 28, age range = 2 to 16 yr. COX: *A. opercularis*: COX = –0.00122 × Age + 0.010, N = 24, $r^2 = 0.601$, $p < 0.001$, age range = 1 to 5 yr; *A. colbecki*: no significant relationship, N = 26, age range = 2 to 16 yr

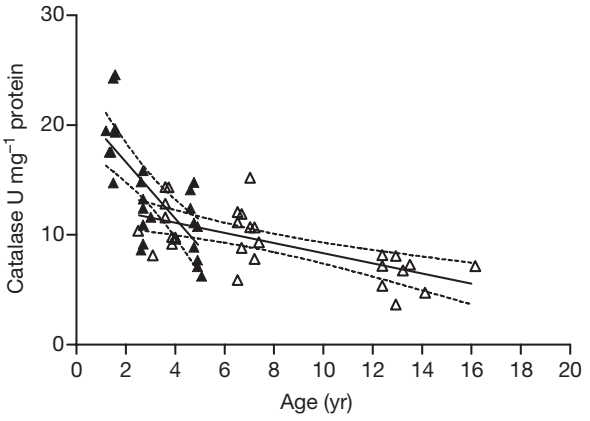


Fig. 5. *Aequipecten opercularis* (▲) and *Adamussium colbecki* (△). Activity of catalase at 20°C in mantle tissue versus age. Each symbol is mean of 2 to 3 replicate measurements. Slopes differed significantly between species ($p < 0.001$, ANCOVA). *A. opercularis*: catalase activity = –2.564 × Age + 21.75, N = 25, $r^2 = 0.540$, $p < 0.001$, age range = 1 to 5 yr; *A. colbecki*: catalase activity = –0.463 × Age + 12.97, N = 29, $r^2 = 0.438$, $p < 0.001$, age range = 2 to 16 yr

advanced age (>5 yr), *A. colbecki* showed significantly higher values than *A. opercularis*. COX values were significantly higher in *A. opercularis* than in *A. colbecki* at both *in situ* and reference temperatures (Fig. 4B).

### Age-dependent changes in antioxidant defence mechanisms

Activity of the antioxidant enzyme catalase (U mg$^{-1}$ protein) decreased significantly with increasing age in both species, but with a 5-fold faster decrement in *Aequipecten opercularis* compared to *Adamussium colbecki*. (Fig. 5). In young individuals, *A. colbecki* had lower catalase activities than *A. opercularis*. In old *A. colbecki*, however, activities were in the same range as in old *A. opercularis*. SOD activity (U mg$^{-1}$ protein) was not affected by age in *A. opercularis* (N = 27, p = 0.443, age range = 1 to 5 yr) and was assumed to be independent of age in *A. colbecki* also (N = 5, age range = 4 to 8 yr). Mean (±SD) SOD activities at 20°C were significantly lower in *A. colbecki* than in *A. opercularis*: 10.19 (±0.89, N = 5) versus 12.77 (±2.63, N = 27) U mg$^{-1}$ protein (p = 0.040, Student's *t*-test), respectively.

$Q_{10}$ values of COX, SOD and catalase did not differ significantly between species (Students *t*-test, data not shown), but the $Q_{10}$ of CS was significantly lower in *Adamussium colbecki* (1.54 ± 0.11, N = 15) than in *Aequipecten opercularis* (1.74 ± 0.15, N = 30) at $p < 0.001$.

In both *Aequipecten opercularis* and *Adamussium colbecki*, glutathione concentrations (GSH, GSSG,

Table 1. *Aequipecten opercularis* and *Adamussium colbecki*. Regression equations for whole (tGSH), reduced (GSH) and oxidised (GSSG) glutathione (nmol g$^{-1}$ wet mass) and ratio of oxidised to reduced (GSSG:GSH) glutathione versus age (in years) in mantle tissue of *A. opercularis* and *A. colbecki*; $p < 0.001$ for all equations

| Species<br>Equation | $r^2$ |
|---|---|
| *A. opercularis* (Age 1 to 5 yr; N = 28) | |
| tGSH = –94.74 × Age + 904.1 | 0.727 |
| GSH = –76.56 × Age + 788.0 | 0.664 |
| GSSG = –9.092 × Age + 58.08 | 0.631 |
| GSSG:GSH = –0.0098 × Age + 0.0824 | 0.399 |
| *A. colbecki* (Age 3 to 14 yr; N = 26) | |
| tGSH = –91.82 × Age + 1951 | 0.676 |
| GSH = –60.56 × Age + 1448 | 0.653 |
| GSSG = –14.61 × Age + 240.0 | 0.651 |
| GSSG:GSH = –0.0081 × Age + 0.1861 | 0.524 |

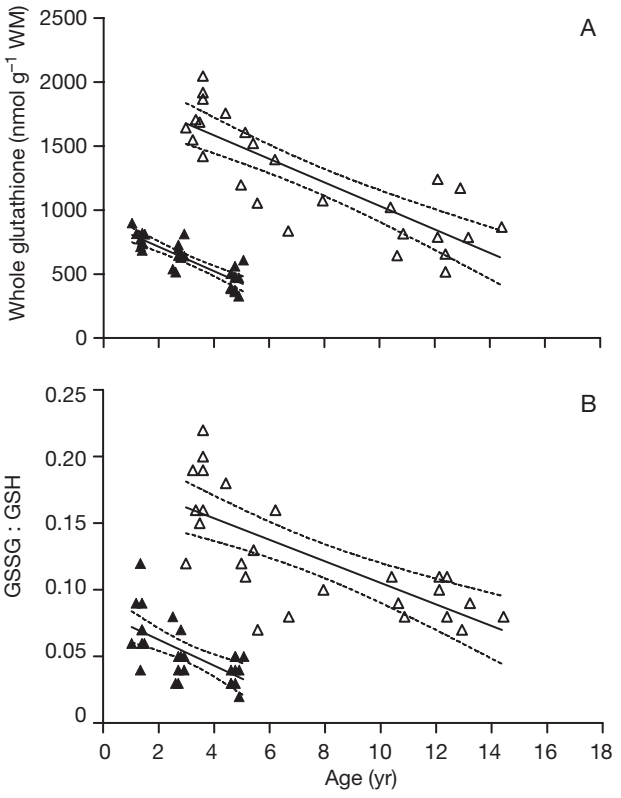


Fig. 6. *Aequipecten opercularis* (▲, N = 28) and *Adamussium colbecki* (△, N = 26). Decrease in (A) total glutathione (tGSH = 2 × GSSG+GSH) and (B) ratio of oxidised to reduced glutathione (GSSG:GSH) in mantle tissue with age. Each symbol is mean of duplicate measurements of 1 or 3 to 4 pooled scallops. Slopes were not significantly different between species. *A. opercularis*: tGSH = –94.74 × Age + 904.1, $r^2$ = 0.727; GSSG:GSH= –0.0098 × Age + 0.0824, $r^2$ = 0.399; *A. colbecki*: tGSH = –91.82 × Age + 1951, $r^2$ = 0.676; GSSG:GSH = –0.0081 × Age + 0.1861, $r^2$ = 0.524. p > 0.001 for all equations. WM : wet body mass

tGSH (total GSH) = 2 × GSSG + GSH) as well as the ratio of oxidised to reduced glutathione (GSSG:GSH) decreased significantly with increasing age (p < 0.001). Slopes did not differ significantly between the 2 species with respect to any parameter, but the intercepts did; i.e. all concentrations (ratios) were higher in *A. colbecki* than in *A. opercularis* across the whole age range (Table 1; Fig. 6: graph only shown for tGSH and GSSG:GSH ratio).

### Age-dependent changes in oxidative damage

Lipofuscin accumulated with age in both species (Fig. 7). Lipofuscin concentrations were in the same range in both species, but accumulation occurred at a 3 times faster rate in *Aequipecten opercularis.* Protein carbonyl content, a marker for protein oxidation, did not vary with age in either species, but was significantly (p < 0.001) higher in *Adamussium colbecki* (1.319 nmol carbonyls mg$^{-1}$ protein ± 0.181, N = 24) than in *A. opercularis* (1.016 nmol carbonyls mg$^{-1}$ protein ± 0.072, N = 24).

### Age-related changes in protein content of mantle tissue

Mantle tissue protein content showed opposing trends with age in the 2 species (Fig. 8). In *Aequipecten opercularis*, protein concentrations decreased significantly from around 309 to 267 mg protein g$^{-1}$ dry mass between 1 and 5 yr of age, whereas in *Adamussium colbecki* they increased from around 193 to 290 mg protein g$^{-1}$ dry mass between individuals aged 4 to 16 yr.

## DISCUSSION

### Metabolic rate

According to the predictions of the 'free radical–rate of living' theory (Pearl 1928, Harman 1956) low SMR of marine ectotherms living at low temperatures might be a prime reason for higher maximum lifespans in these animals compared to their temperate counterparts. Recent studies of pectinid SMR are in line with this hypothesis and revealed a positive relation of metabolic rates and ambient temperature across a wide latitudinal gradi-

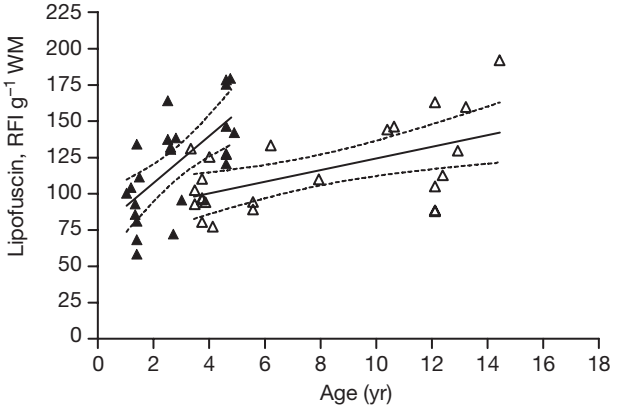


Fig. 7. *Aequipecten opercularis* (▲) and *Adamussium colbecki* (△). Lipofuscin content (relative fluorescence intensity, RFI, g$^{-1}$ wet mass, WM) in the mantle tissue versus age. Slopes differed significantly between species (p = 0.003, ANCOVA). *A. opercularis*: lipofuscin = 16.24 × Age + 74.88; N = 24, $r^2$ = 0.453, p < 0.001, age range = 1 to 5 yr; *A. colbecki*: lipofuscin = 4.009 × Age + 84.27; N = 24, $r^2$ = 0.312; p = 0.005, age range = 3 to 14 yr

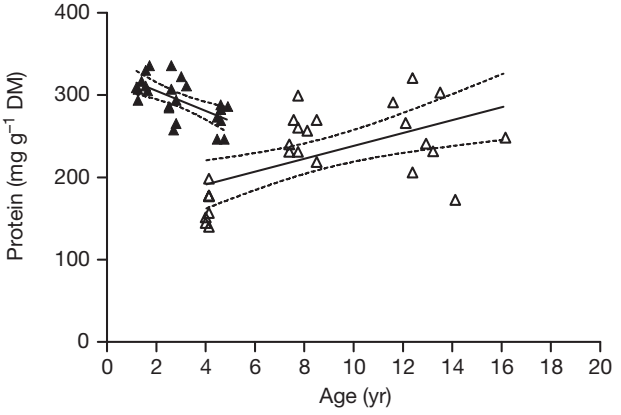


Fig. 8. *Aequipecten opercularis* (▲, N = 25, age range = 1 to 5 yr) and *Adamussium colbecki* (△, N = 25, age range = 4 to 16 yr) protein content (mg$^{-1}$ protein g$^{-1}$ dry mass, DM) in mantle tissue of versus age. Data are means of duplicate measurements. Slopes differed significantly between species ($p < 0.001$, ANCOVA). *A. opercularis*: protein content = −12.17 × Age + 329.7, N = 25, $r^2 = 0.396$, $p < 0.001$; *A. colbecki*: protein content = 7.821 × Age + 160.1, N = 25, $r^2 = 0.324$, $p = 0.003$

ent (Heilmayer & Brey 2003, Heilmayer et al. 2004a). In our study, the SMR of the temperate *Aequipecten opercularis* from the Isle of Man were measured. As no living individuals of the Antarctic *Adamussium colbecki* were available for SMR measurements, we used respiration and AFDM data of Heilmayer & Brey (2003 and pers. comm.) (N = 85, mass range 104 to 3911 mg AFDM, size range 27 to 74 mm, age range 3 to 10 yr) from a Terra Nova Bay population, measured in austral summer during January and February 2000. *A. opercularis* reaches maturity at the end of its first year (Aravindakshan 1955), while *A. colbecki* is reported to reach maturity at 6 to 7 yr (Cattaneo-Vietti et al. 1997). Therefore, in both data sets a similar proportion of immature and mature individuals were present. Whole animal specific metabolic rates of temperate and Antarctic scallop species were lower in small (young) Antarctic *A. colbecki* individuals than in temperate *A. opercularis* of the same body size (200 to 500 mg AFDM), which is in line with the pattern reported by Heilmayer et al. (2004a). This difference, however, diminished with increasing body mass and became negligible above 1200 mg AFDM, corresponding to individual ages of >2 yr in *A. opercularis* and >6 yr in *A. colbecki* (95% confidence range of the predicted values overlap completely after 1200 mg body massis attained: Fig. 9). The similar SMR in the older individuals of both species may be related to the fact that in *A. opercularis* individuals up to 6 yr of age were studied, representing a significant proportion of the whole age range, whereas *A. colbecki* were measured only up to 10 yr of age, presumably covering only a small fraction of their overall lifespan. In *A. opercularis*, respiration of old molluscs could already be depressed by age effects (Sukhotin & Pörtner 2001), thus reducing the scaling exponent of the overall SMR versus body mass relation. In *A. colbecki*, individuals of 10 yr can be classified as 'young', and presumably do not experience an age-related depression in respiration. Adding more data sets on the respiration of *A. opercularis* obtained by other authors did not alter the overall picture: the scaling exponent remained significantly smaller in *A. opercularis*, regardless of the combination of analysed data sets. Nevertheless, in the smaller and middle size classes, respiration rates of *A. opercularis* were distinctly higher than those of *A. colbecki* (Fig. 9). Obviously, this is a preliminary conclusion based on incomplete evidence. For a more reliable comparison, year-round measurements of respiration for both species, and over a wider age range for *A. colbecki*, are required in order to take age as well as environmental effects into account, since metabolic rates can be affected heavily by somatic growth, gonad development and environmental parameters such as food availability and increased temperature (Peck et al. 1987, Clarke 1988, Shumway et al. 1988, Peck 1998, Brockington 2001, Lesser & Kruse 2004). Hence the question arises as to whether other parameters such as mitochondrial ageing, antioxidant defence and oxidative damage are more distinctly different, and whether these parameters show different trends with age in both species that might explain the higher MLSP of the polar scallop.

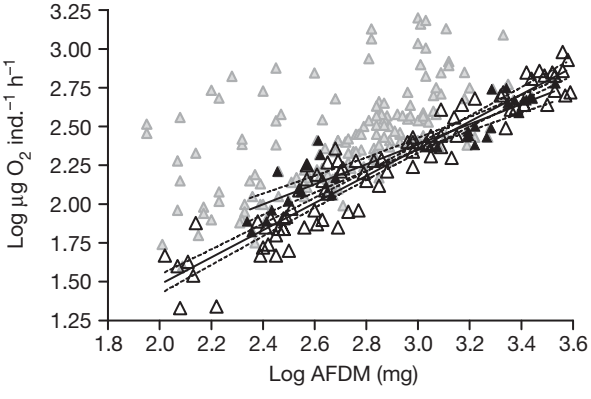


Fig. 9. *Aequipecten opercularis* and *Adamussium colbecki*. Metabolic rates (µg $O_2$ h$^{-1}$ ind. $^{-1}$). Temperate *A. opercularis* from (▲) present study ($T$ = 10°C) and (▲) from Heilmayer et al. (2004b) ($T$ = 10.2, 12.4, 14.4°C), McLusky (1973) ($T$ = 5, 10, 15°C) and Vahl (1972) ($T$ = 12°C). (△) Antarctic *A. colbecki* from Heilmayer & Brey (2003) ($T$ = 0°C). For equation details for linear regression of *A. opercularis* from present study see Fig. 2; for *A. colbecki*, $\log(R) = 0.876 \times \log(\text{AFDM}) - 0.269$; $r^2 = 0.899$, $p < 0.001$. N = 85, age range = 3 to 10 yr

### Mitochondrial ageing

Age-related changes in mitochondrial function (respiration, RCR, ADP:O and proton leak) in mantle tissue of *Aequipecten opercularis* were small (Fig. 3), but followed a pattern already known for humans (Trounce et al. 1989, Cooper et al. 1992, Boffoli et al. 1994), other terrestrial vertebrates (Goodell & Cortopassi 1998, Kokoszka et al. 2001, Hagen et al. 2002, Lopez-Torres et al. 2002, Ventura et al. 2002, Grattagliano et al. 2004), and invertebrates (Sohal et al. 1995) including marine bivalves (Philipp et al. 2005b). Protein-specific activities of the mitochondrial enzymes CS and COX declined significantly with increasing age in *A. opercularis*, as found in humans, rats and mice (Paradies et al. 1993, 1997, Rooyackers et al. 1996, Kokoszka et al. 2001). This change is held responsible for the decline in aerobic capacity and muscle performance associated with age in most organisms (Rooyackers et al. 1996). In contrast to *A. opercularis*, mitochondrial enzyme activities were independent of age in *A. colbecki* (Fig. 4). The relevance of stable CS and COX activities for delayed mitochondrial senescence remains questionable, as mitochondrial functions could not be measured directly in *A. colbecki.* In the infaunal soft-shell clams *Mya arenaria* (MLSP 13 yr) and *Laternula elliptica* (MLSP 36 yr) CS and COX activities were stable, but the decline in mitochondrial functions in *M. arenaria* was even more pronounced than in *A. opercularis* (Philipp et al. 2005b). Regarding energy conservation, however, stable CS and COX activities in *A. colbecki* might play a role in lifespan extension, as they indicate better preservation of tissue aerobic capacity with age.

The very minor age-related decline in mitochondrial functioning of *Aequipecten opercularis* came as a complete surprise, as we would have expected a more rigid onset of mitochondrial deterioration in this short-lived and actively swimming species, especially when compared to the sessile mud clams. Moreover, $H_2O_2$ generation rates with either succinate plus rotenone, or pyruvate and malate (without rotenone), or at higher assay temperatures (20°C) were close to zero in *A. opercularis*, despite intense mitochondrial respiration and a lower proton leak (33%). In contrast, the longer-lived temperate soft-shell clam *Mya arenaria* showed far higher $H_2O_2$ generation rates $mg^{-1}$ mitochondrial protein than *A. opercularis*, despite lower State 3 respiration rates (individuals >5 yr) and a generally higher mitochondrial proton leak (Philipp et al. 2005b).

The lower proton leak of *Aequipecten opercularis* mitochondria compared to mitochondria of the less active infaunal bivalves *Mya arenaria* and *Laternula elliptica* (Philipp et al. 2005b), corresponds to the more active lifestyle of the scallop. Following Brand's (2000) rationale of mild uncoupling, less proton leakage would, however, cause higher rather than lower ROS generation. This raises the question as to whether some special mechanism may prevent ROS generation in mitochondria of *A. opercularis*, or even of scallops in general. Preliminary data point in this direction, as $H_2O_2$ generation rates (at 0°C) of mitochondria isolated from 3 available live *Adamussium colbecki* individuals were below detection limits in States 3 and 4, whereas in the polar mud clam *L. elliptica* $H_2O_2$ generation of isolated mitochondria amounted to 0.03 nmol $H_2O_2$ $min^{-1}$ $mg^{-1}$ protein at 0°C (Philipp et al. 2005b).

Higher glutathione concentrations were found in the tissues of *Aequipecten opercularis* and *Adamussium colbecki* than in tissue of mud clams from comparable temperature regimes (Philipp et al. 2005a), but GSSG concentrations and tissue redox-states (GSSG:GSH) were lower in the scallops than in temperate and polar mud clams (Philipp et al. 2005a). This indicates lower ROS scavenging activity of the glutathione system in scallops and would coincide with lower rates of mitochondrial ROS production. Moreover, comparable or lower catalase and SOD activity levels in scallops than in mud clams indicate that the more active scallop lifestyle does not imply vastly higher mitochondrial ROS formation.

In both scallop species, the concentration of tissue glutathione decreased linearly with increasing age (Fig. 6A, Table 1), which is in line with findings for humans, rats, insects and marine bivalves (Sohal et al. 1987, Sanz et al. 1996, Canesi & Viarengo 1997, Hernanz et al. 2000, Philipp et al. 2005a). Likewise, the ratio of GSSG:GSH decreased with increasing age in both species (Fig. 6B). Thus, we suggest that the scallops adjust their glutathione content to match the mass and age-related decline in mass-specific respiration. Whereas in *Adamussium colbecki* the decline in mass-specific respiration might be a mere effect of the increase in size and not related to age, this could be the case for *Aequipecten opercularis*, and has previously been reported for *Mytilus edulis* (Sukhotin & Pörtner 2001). As reported for the polar clam *Laternula elliptica*, for which a similar development of GSH concentration and tissue redox-ratio was found, the glutathione level should suffice to maintain low tissue oxidation in older individuals (Philipp et al. 2005a).

In contrast to the uniform decrease in glutathione, the decrease in catalase activity was much more pronounced in *Aequipecten opercularis* than in *Adamussium colbecki*, indicating a more rapid loss of antioxidant capacity with advancing age in the temperate scallop. Accordingly, the increase in lipofuscin concentrations was more pronounced in the temperate scallop, indicating faster oxidation of lipids and proteins in *A. opercularis* with increasing age, as antioxidative capacity declined.

The comparatively slow increase in lipofuscin levels (Fig. 7) and the preservation of mantle protein content (Fig. 8) with increasing age in *Adamussium colbecki* support the idea that avoidance of waste accumulation and maintenance of cellular integrity are prerequisites of longevity in the polar scallop. The higher protein carbonyl content in *A. colbecki* than in *Aequipecten opercularis* might therefore not necessarily reflect higher protein oxidation rates, but might result from thermally reduced proteolytic activity in *A. colbecki*, leading to higher steady-state levels of oxidized protein at an ambient water temperature of 0°C. Further studies are necessary to prove this hypothesis.

### Importance of maintenance of physiological performance in active animals throughout their lifetime

Mitochondria from mantle tissue of their temperate scallop *Aequipecten opercularis*, exhibit a slower senescence than mitochondria from the temperate mud clam *Mya arenaria* (Philipp et al. 2005b), most probably due to a suppression of mitochondrial ROS production in the scallop. CS and COX activities, however, decrease with increasing age in the mantle of *A. opercularis*, whereas they remained constant in *M. arenaria*. This could indicate an age-related decrease in mitochondrial numbers in the mantle tissue of *A. opercularis* and, therefore, a loss in tissue aerobic capacity with increasing age. So far, age-related changes in mitochondrial numbers per cell have only been reported for human liver (Tauchi & Sato 1968).

A prerequisite for an active lifestyle is the maintenance of cellular functions and fitness on a high level to insure maximal scope for activity. *Aequipecten opercularis* is an active swimmer, with fast escape reactions when disturbed (see e.g. Chapman 1981). The Antarctic *Adamussium colbecki* features the same active lifestyle in order to escape scouring icebergs and predators such as the fish *Trematomus bernacchii* and invertebrates (e.g. *Neobuccinum eatoni*, *Paraborlasia corrugata*) (Ansell et al. 1998, Vacchi et al. 2000, Peck et al. 2004). A comparison of mature and immature *Chlamys islandica* showed that reduced CS activity and mitochondrial capacity caused a decrease in the aerobic capacity of adductor muscle in mature specimens and subsequently a delay in recovery after exhaustive swimming, which requires aerobic metabolism (Livingstone et al. 1981, Brokordt et al. 2000). Hence, a decrease in mitochondrial capacity and a decline in CS and COX activity with increasing age, as found in *A. opercularis*, may significantly reduce fitness in older specimens. Corresponding observations were reported by Brand (1991), who found a higher threshold stimulus to elicit swimming and less frequent swimming in larger (older) *A. opercularis*. This in turn could cause an increase in extrinsic mortality (= elevated susceptibility to predation) and thus a comparatively shorter MLSP in such highly active animals. On an evolutionary scale, these scallops may have adjusted their life history and physiological ageing strategies to an optimal functioning until a threshold after which maintenance of physiological competitive capacities requires more energy than justified by the reproductive gain. Theoretical studies by Abrams (1993) and Williams & Day (2003) presented the rationale that an increase in interactive extrinsic sources of mortality (e.g. predation) may select for slower senescence deterioration early in life. A recent study by Reznick et al. (2004) on guppies indicates such a relation between predation pressure and senescence. Although scientific proof for the validity of this concept for *A. opercularis* has still to be put forward, these ideas offer an explanation for the combination of short MLSP and minor decrements in mitochondrial functions with increasing age in *A. opercularis*. In the Antarctic *A. colbecki*, CS and COX activities remained stable over a lifespan of 16 yr; hence loss of fitness and corresponding increase in extrinsic mortality may be delayed until a higher age. However, individuals across the full age-range must be studied to discover whether physiological fitness in *A. colbecki* is also preserved until close to its MSLP, as in *A. opercularis*.

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