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Growth, metabolism and lipid peroxidation in *Mytilus edulis*: age and size effects

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ABSTRACT: The age dependence of growth, metabolic rate, the degree of lipid peroxidation and antioxidative defence was studied in 3 different size groups of White Sea (Russia) blue mussels *Mytilus edulis* L.: small (S, wet tissue weight = 0.23 g, length 20–25 mm), medium (M, wet tissue weight = 0.52 g, length 30–35 mm) and large (L, wet tissue weight = 1.05 g, length 40–50 mm). S group mussels were 2–8 yr old, M group mussels 2–9 yr old, and L group mussels 3–10 yr old. Absolute and weight-specific growth rates were determined from the beginning of the growth season (May) to the time of sampling (August). Respiration rates were measured in individuals from all size groups. Net growth efficiency coefficients were calculated. Malondialdehyde (MDA) and fluorescent age pigment (FAP) concentrations were determined as a measure of lipid peroxidation. The level of antioxidant defence was estimated from superoxide dismutase (SOD) and catalase (CAT) activities in whole body homogenates. Metabolic performance was found to be a function of both age and body size. Weight-specific growth rates and net growth efficiencies decreased with age until mussels reached a 'critical' age, beyond which growth virtually stopped. Respiration rates were size dependent, but did not show a clear correlation with age. MDA levels remained unrelated to both age and body size, whereas FAP accumulation increased exponentially with age. However, FAP levels correlated negatively with size (growth rate). At constant body size, SOD and CAT activities did not display clear age-related changes; however, CAT activity decreased significantly with increasing body size. As a corollary, size effects potentially mimic age effects on key physiological functions of continuously growing species, and this needs to be considered in physiological studies of the ageing process.

KEY WORDS: Ageing · Mussels · *Mytilus edulis* · Growth · Respiration rate · White Sea · Antioxidants · Lipid peroxidation

INTRODUCTION

The process of ageing has been studied extensively for many years, leading to several theories on the mechanisms of ageing (Timiras 1994, Masoro 1995). The great majority of these studies focus on humans, laboratory rodents and insects. All of these animals are characterised by the fact that there is a limit to the size the adult animal attains. By contrast, numerous invertebrate species, including many marine benthic organisms, continue to grow throughout their entire lives. Therefore, the process of ageing in these animals is accompanied by a continuous increase in body size. Since many physiological rates in the organism such as metabolic rate, feeding rate, assimilation rate, activities of many metabolic enzymes, are size dependent, the effect of age on each of these processes still remains unclear.

There are 2 major theories of ageing, namely the 'rate of living' theory and the 'free radical' theory, which link metabolic rate and ageing in animals. The

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former is based on the assumption that an increased rate of damage occurs over time and is related to metabolic rate (Pearl 1928, Sohal 1986). The latter theory states that oxidative damage caused by oxygen free radicals plays a significant role in limiting the life span of an organism (Harman 1956, Sohal 1986, for a review see Emerit & Chance 1992). The rate of living theory is criticised as it is not supported by many current studies (McCarter 1995). The theories may appear as 2 sides of the same problem, since metabolic rate and rate of oxyradical production are closely related (Emerit & Chance 1992). According to existing knowledge, damage by free radicals accumulates as a function of life span (McCarter 1995) and individual stress levels. According to the free radical theory deleterious actions of oxygen-derived free radicals are responsible for the decline of cellular functioning with age. Among the most important targets for free radical attack are membrane lipids, which are oxidized to lipid radicals and lipid hydroperoxides. Lipid peroxides are known to decompose and produce a variety of substances, including ethane, penthane and aldehydes, the most important of which is malondialdehyde (MDA; Leibovitz & Siegel 1980). MDA is incorporated into various large fluorescent biomolecules which accumulate in the cells. These fluorescent age pigments (FAP) are regarded as good biomarkers of age and of the degree of oxidative stress in animals (see Sohal 1981 for a review). Since oxygen radicals are generated continuously, all organisms have developed antioxidant defence systems composed of both enzymatic and non-enzymatic components. These substances detoxify free radicals, including their reactive products, thereby protecting the cell from oxidative stress effects (for a review see Williams et al. 1993).

The objective of this study was to find out how changes in growth and metabolic rate as well as in oxidative stress parameters and antioxidative defence develop with age in the blue mussel *Mytilus edulis* L. To achieve this purpose we had to distinguish mere age from size effects on metabolism and oxidative stress parameters in animals with infinite growth. The intermediate and final products of lipid peroxidation—MDA and FAP—were chosen as indicators of oxidative stress. The level of enzymatic antioxidant defence was determined by measuring the activities of superoxide dismutase (SOD), which metabolizes $O_2^{\bullet}$ by the reaction $2O_2^{\bullet} + 2H^+ = H_2O_2 + O_2$, and of catalase (CAT) which further eliminates H_2O_2 via $2H_2O_2 = 2H_2O + O_2$.

MATERIALS AND METHODS

Mussels. Blue mussels *Mytilus edulis* L. were collected on August 16, 1999, from one of the intertidal mussel beds situated in Kandalaksha Bay of the White Sea, Russia (66° 20′ N, 33° 40′ E). The settlement lies between −0.2 and +1.2 m above 0 tidal level. Mussels were sampled from the shore at about +0.7 m, where the emersion period comprises approximately 20% of the tidal cycle. For comparison mussels were also sampled from suspended mussel aquaculture rafts situated in the same area. After sampling, mussels were sorted by size and age in accordance with the experimental design. The collected mussels were divided into 3 size groups: small (S, wet tissue weight = 0.23 ± 0.01 g, n = 58, length 20–25 mm), medium (M, wet tissue weight = 0.52 ± 0.01 g, n = 62, length 30–35 mm) and large (L, wet tissue weight = 1.05 ± 0.03 g, n = 60, length 40–50 mm). Mussels in the S group were 2–8 yr old, whereas the M group mussels were 2–9 yr old and the L group mussels were 3–10 yr old. Mussels from aquaculture rafts formed a separate group (A), the individuals in which were 6 yr old, had a mean wet tissue weight of 4.28 ± 0.55 g (n = 12) and were 43–77 mm in length. The age of the mussels was determined by counting the rings of winter growth delays on the shells. This method has previously been justified in studies carried out in the White Sea which compared the number of internal rings and seasonal growth of mussels marked individually (Chemodanov & Maximovich 1983, Sirenko & Saranchova 1985). Mussels were kept in aquaria at 25‰ salinity and +10°C and under constant light without feeding. After 2 wk of acclimation, respiration rates were determined. They were weighed, placed in net cages and transferred to the sea. In November they were transported to the Alfred Wegener Institute (AWI, Bremerhaven, Germany) and kept without feeding in aquaria at 25‰ salinity and +2°C for 1 wk before the measurements started.

Growth rate determination. Growth rate was determined in individual mussels as absolute (*AI*) and relative (*RI*) tissue wet weight increments from the beginning of the growth season in 1999 to the time of sampling, according to the formulas:

$$AI = W_f - W_i \quad \text{and} \quad RI = (W_f - W_i)/W_i$$

where W_i and W_f are the initial and final wet tissue weights (g) of the mussels, respectively. W_i was calculated using mussel length at the beginning of the growth season, which was taken as the length from the umbo of the shell up to the most distant edge of the last ring marking the winter growth delay. W_f was calculated using the mussel lengths before the experiments. Active growth in mussels in the area of investigation usually starts in late May (Sukhotin & Maximovich 1994). Assuming that about 100 d passed from the beginning of growth until collection, *AI* can be expressed in g wet tissue per 100 d. The relationships between length (*L*, mm) and tissue wet weight (*W*, g)

differed between age classes. For the calculations we used the following equations, which were derived from the obtained mussel samples:

$W = 0.00007 \cdot L^{2.619}$, r = 0.947, n = 12
for mussels 2–4 yr old,

$W = 0.0002 \cdot L^{2.341}$, r = 0.940, n = 28
for mussels 5–6 yr old,

$W = 0.0002 \cdot L^{2.227}$, r = 0.958, n = 36
for mussels 7–10 yr old.

$W = 0.0001 \cdot L^{2.546}$, r = 0.992, n = 5
for mussels from aquaculture rafts.

Respirometry. Oxygen consumption rates (R) were measured in closed 200–500 ml respirometers at 10°C. After an exposure period of 60 to 90 min in the respirometer, water samples were sucked carefully from the chamber into air-tight bottles. Oxygen concentration was determined according to the Winkler method as described in Strickland & Parsons (1968). Oxygen consumption was calculated from the difference from control chambers without mussels. Respiration rate was expressed in µmol O_2 specimen^{-1} h^{-1}or as a weight-specific rate in µmol O_2 g^{-1} tissue wet wt h^{-1}.

Analyses. All tissue analyses were carried out at the AWI. Mussels were opened rapidly, all tissues were removed from the shells, weighed and frozen by freeze-clamping in liquid nitrogen (Wollenberger et al. 1960). Frozen tissues of the whole body were ground in liquid nitrogen and then homogenised using the respective buffers.

SOD (EC 1.15.1.1) activity was determined using the pyrogallol autooxidation method (Marklund & Marklund 1974) after extraction (1:3 w/v) in 0.05 M Tris-succinate buffer (pH 8.2). Extracts obtained after centrifugation for 10 min at 13 000 rpm (14 000 × g) were used within 2 h. Inhibition of air-saturated 8 mM pyrogallol auto-oxidation was measured by monitoring the change in absorbance at 420 nm using a Beckman DU 7400 spectrophotometer at 25°C. Non-enzymatic inhibition of pyrogallol auto-oxidation was determined in a fraction of each sample heated for 5 min in a water bath at 100°C. Non-enzymatic inhibition comprised ca 15 to 20% of the total inhibition.

CAT (EC 1.11.1.6) activity was measured using 0.05 M potassium phosphate as a homogenisation buffer (pH = 7.0) and 10.5 mM H_2O_2 as a substrate (Aebi 1984, as described in Abele-Oeschger et al. 1994). The degradation of peroxide was monitored at 240 nm and 25°C. One unit of CAT decomposes 1 µmol H_2O_2 min^{-1}.

MDA concentrations were determined after Uchiyama & Mihara (1978). Deep frozen tissues were ground and homogenised in 1:5 (w/v) 0.2% H_3PO_4. Then 0.4 ml of homogenate was mixed with 0.4 ml of 1% thiobarbituric acid (TBA). Individual blanks were prepared by replacing the TBA solution with 3 mM HCl. pH was adjusted to 1.6. The samples were heated to 100°C for at least 15 min. After cooling to room temperature, 1.5 ml butanol were added to samples and blanks. After mixing the butanol phase was separated by centrifugation, and the absorbance of the MDA-TBA acid complex was measured as the difference between emissions at 532 and 600 nm. The concentration was quantified using a previously obtained calibration curve.

FAPs were determined by an extraction method modified after Nicol (1987) and Vernet et al. (1988). Mussels were ground under liquid nitrogen and homogenised (1:20 w/v) in a chloroform-methanol solution (2:1 v/v). After 10 min of centrifugation at 2000 × g the FAP levels were analysed in the chloroform phase using a Perkin Elmer LS 50B luminescence spectrometer. An emission spectrum between 350 and 550 nm was obtained at an excitation wavelength of 350 nm. The luminescence of the sample was determined at an emission maximum of 445 nm. FAP concentrations were expressed as relative fluorescence intensity (RFI) according to Hill & Womersley (1991), using 0.1 µg quinine sulphate per 1 ml N H_2SO_4 as a standard.

Calculations and statistics. Since the data on individual growth and respiration rate were obtained for each experimental mussel, it was possible to approximate the net growth efficiency coefficients (K_2) for all mussels on the basis of the following assumptions: (1) the measured respiration rate reflects the average respiration rate of mussels in nature during the summer (growth) period or (2) the average summer growth rate corresponds to that at the end of August, when respiration rate was measured.

These K_2 estimates suffice to compare the experimental groups. In the literature the term 'net growth efficiency' has 2 meanings: (1) $G/(G + ME)$, i.e. the proportion of energy used for growth (G) in all assimilated energy ($G + ME$) (Ivlev 1938, Winberg 1966, Jørgensen 1976, Calow 1977, Winberg 1986), and (2) $G/(G + ME_G)$, i.e. the proportion of energy used for growth in the total assimilated energy, minus the energy expenditure for maintenance (Wieser 1994), where G is the energy utilised for growth, ME is the total metabolisable energy and ME_G corresponds to the part of ME supporting growth. The term net growth efficiency (K_2) in the present paper is understood as described in (1). Hence, K_2 was calculated as follows:

$$K_2 = G/(G + ME)$$
$$G = (AI \cdot 2386)/100$$
$$ME = R \cdot 0.454 \cdot 24$$

where G (J mussel^{-1} d^{-1}) is the energy required for growth of the mussel d^{-1} within the period from the

beginning of growth in 1999 (last ring on the shell) until the time of collection; *ME* (J mussel^{-1} d^{-1}) is the energy required for total mussel metabolism per day at the time of respiration measurements; *AI* is the absolute growth increment of a certain specimen (see above) (g wet tissue per mussel); 2386 (J per g wet tissue) is the energy value of wet tissue of the White Sea *Mytilus edulis* (Sukhotin 1992); 100 (d) is the approximate period of growth from the beginning of growth in 1999 until the time of collection; *R* is the rate of mussel oxygen consumption (µmol O_2 mussel^{-1} h^{-1}); 0.454 (J µmol O_2^{-1}) is the oxycaloric coefficient; and 24 is the day length in hours.

One-way ANOVA was used to analyse the effects of the factors age and size. Post-hoc comparisons were made by Tukey's HSD test for unequal N. Calculations of linear regression parameters were performed according to a standard algorithm (Glotov et al. 1982). Correlations were calculated using Spearman's nonparametric correlation coefficients. If not specially noted mean values ± SEs are presented.

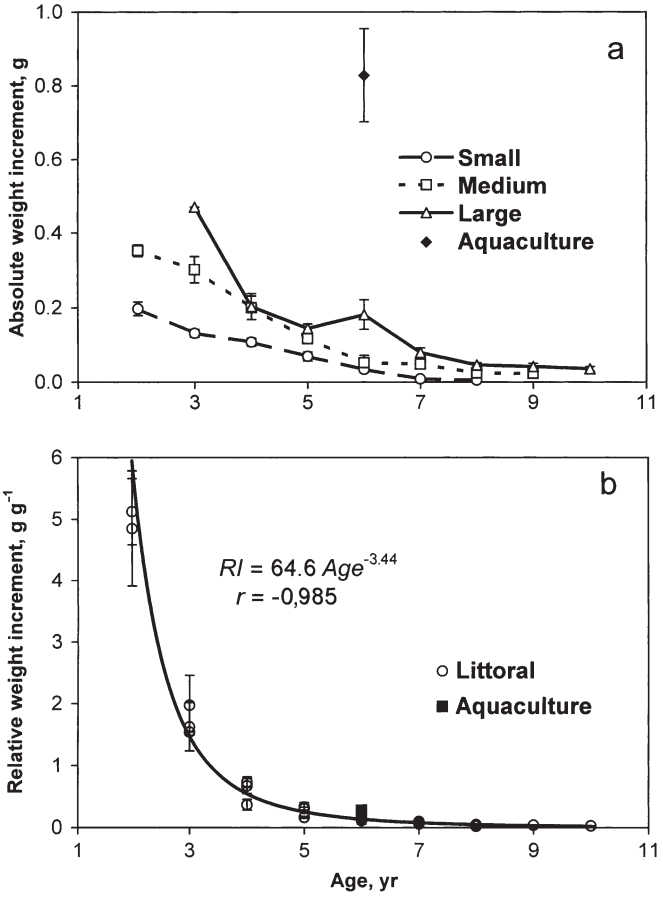

Fig. 1. *Mytilus edulis*. Changes in (a) absolute and (b) relative growth increments of mussels with age. N varies from 4 to 14, except for the group of 3 yr old L mussels, which is represented by only 1 specimen

RESULTS

Growth

The absolute 100 d growth increment (*AI*) was strongly dependent on both size (by definition) and age (Fig. 1a) (ANOVA, $F = 2.78$, df = 8, 90, $p < 0.001$). In the littoral population it varied in the range between 0.005 and 0.47 g per 100 d. A maximal *AI* was observed in L mussels while minimal values were characteristic for S ones. The absolute growth increment in mussels from aquaculture rafts was about 2 times higher (0.83 g) than the highest values recorded for littoral mussels. The age dependence of growth in the mussels was typical of asymptotic growth. The highest values of *AI* were reached at young stages, followed by a gradual decrease in *AI* with increasing age. In L mussels a minor increase in *AI* was observed for 6 yr old specimens but it was not statistically different from those that were 5 (Tukey's HSD, $p = 0.99$) and 7 yr old (Tukey's HSD, $p = 0.32$).

The weight-specific (*RI*) growth increment was independent of size (ANOVA, $F = 0.92$, df = 2, 55, $p = 0.67$) (Fig. 1b). It decreased rapidly with increasing age between 2 and 4 yr and did not differ statistically in older individuals. The age dependence of *RI* can be perfectly approximated by the power function:

$$RI = 64.6 \cdot \mathrm{Age}^{-3.44}, \quad r = -0.985, \; n = 23.$$

Mussels from the aquaculture demonstrated an *RI* = 0.28, which was close to the values typical for 6 yr old littoral specimens.

Respiration rates

Respiration rates (*R*', µmol h^{-1} g^{-1} wet wt) of the mussels studied demonstrated the well-known negative size dependency:

$$R' = 3.88\, W^{-0.248}, \; r = -0.411, \; n = 178$$

where *W* is tissue wet weight (g).

For comparisons between mussels of different ages within each size group, *R*' was corrected for the corresponding mean weights according to the formula $R = R' (W_{mean}/W)^{-0.248}$, where *R*' and *R* are observed and corrected oxygen consumption rates, respectively, *W* is the observed tissue wet weight, and W_{mean} is the mean group tissue wet weight (see 'Materials and methods'). For comparison within the whole batch, including mussels from aquaculture, *R*' was corrected for the overall mean weight of 0.506 g wet tissue using the same power coefficient.

No significant age effect was observed in each size group (ANOVA, $F = 2.19$, df = 6,50, $p = 0.059$ for S;

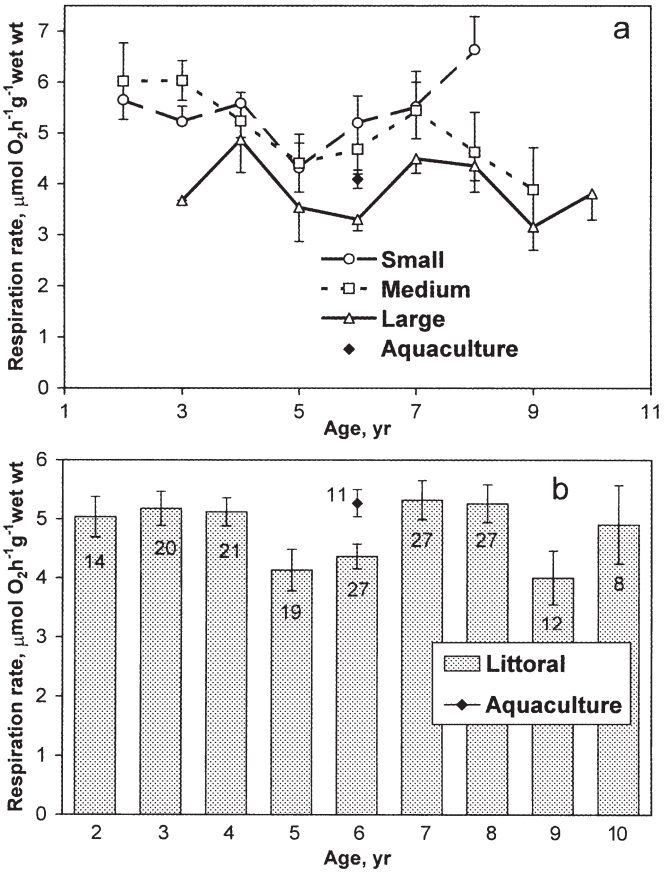

Fig. 2. *Mytilus edulis*. Respiration rate (a) of mussels of different age/size groups and (b) corrected for mean weight (0.506 g wet tissue). N varies from 4 to 14 in (a) and is presented inside the bars in (b)

$F = 1.32$, df = 7, 52, p = 0.260 for M; $F = 1.99$, df = 7, 50, p = 0.075 for L) (Fig. 2a). However, when the whole batch is considered, a significant influence of age becomes evident (ANOVA, $F = 2.32$, df = 8, 166, p = 0.021) (Fig. 2b) due to low values for 5, 6 and 9 yr old individuals. Respiration rates of aquaculture mussels were somewhat higher ($p < 0.044$, Tukey's HSD) than those of 6 yr old littoral ones, but similar to those recorded for mussels of the other ages (Fig. 2b).

Net growth efficiency

K_2 was strongly dependent on age (ANOVA, $F = 60.34$, df = 8, 166, $p < 0.001$) and did not depend on size within each age class (ANOVA, $p > 0.08$) (Fig. 3a). Cultured mussels displayed higher K_2 values than littoral ones of the same age (Tukey's HSD, p = 0.017, 0.004 and 0.076 for comparisons with S, M and L groups, respectively). K_2 was highest at young age (2–3 yr) and decreased linearly until 7 yr of age, after which it stayed nearly constant. *R/RI* ratios, showing the amount of energy dissipated per unit of energy stored as body mass, increased exponentially with age in all size groups (Fig. 3b). Mussels from the M and L groups did not differ with respect to *R/RI* ratio (ANOVA, $p > 0.08$), whereas small mussels from the S group which were older than 6 yr displayed much higher *R/RI* ratio than the M and L mussels.

Superoxide dismutase

SOD activities in whole mussel homogenates displayed a large variability between 35 and 160 U g^{-1} wet tissue. Animal size (tissue wet weight) did not modulate SOD activity neither in the whole data set nor in any specific age class (p = 0.85) (Fig. 4a), reflecting no significant age or size dependence of SOD activity (ANOVA, $F = 1.38$, df = 8, 78, p = 0.22) (Fig. 4b). Nei-

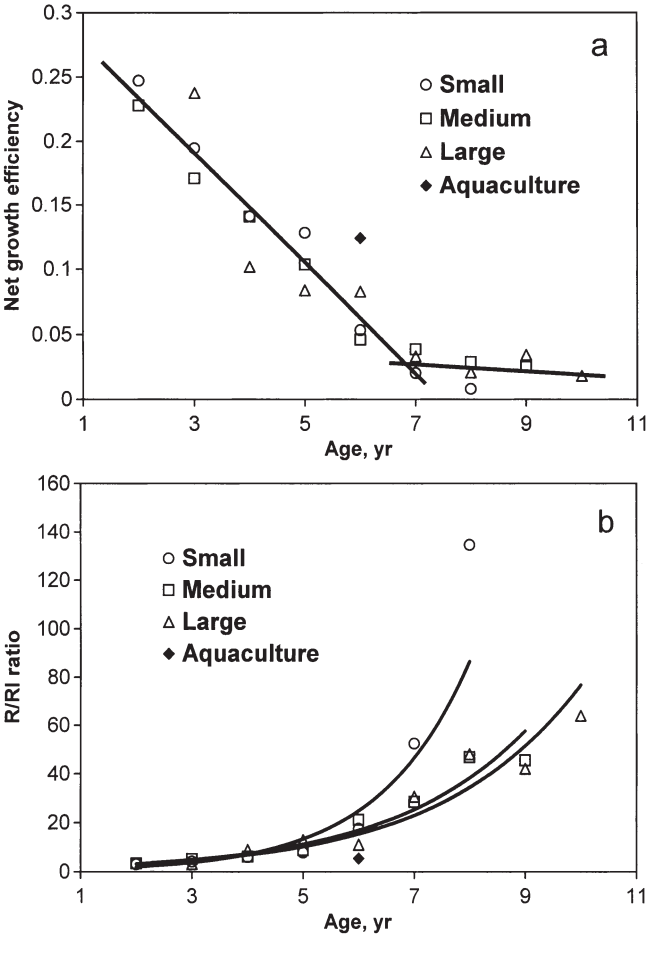

Fig. 3. *Mytilus edulis*. (a) Net growth efficiency and (b) the amount of energy dissipated as respiratory metabolism (*R*) over the energy stored as body mass of mussels of different age/size groups

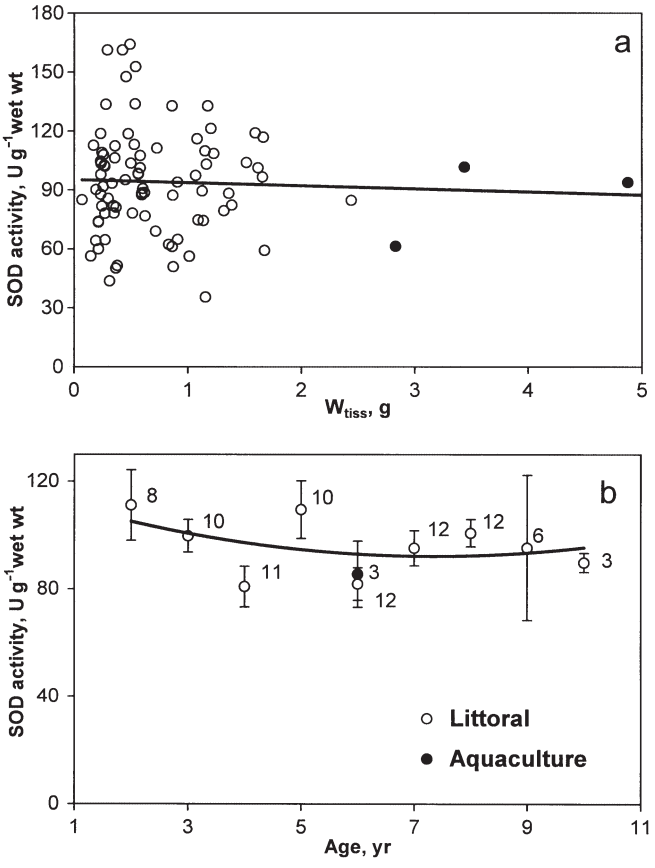

Fig. 4. *Mytilus edulis*. (a) Size and (b) age dependence of superoxide dismutase (SOD) activity in littoral (○) and cultured (●) mussels. N is shown close to the values in (b)

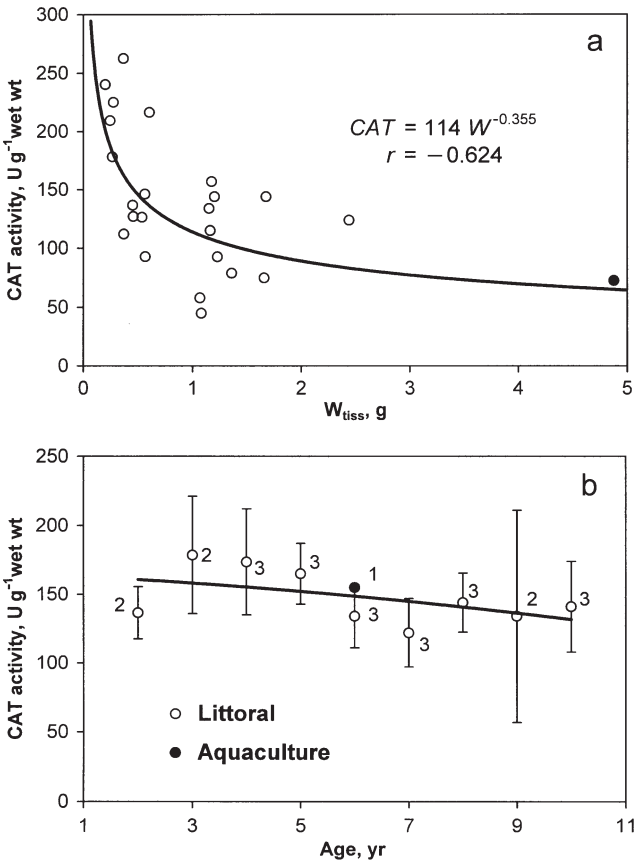

Fig. 5. *Mytilus edulis*. (a) Size and (b) age dependence of catalase (CAT) activity (corrected for mean weight, 0.506 g wet tissue) in littoral (○) and cultured (●) mussels. N is shown close to the values in (b)

ther growth rates (r = 0.198, p = 0.353) nor respiration rates (r = 0.104, p = 0.628) correlated significantly with SOD.

Catalase

CAT activity varied in the range between 45 and 260 relative units g^{-1} wet tissue. This parameter demonstrated a pronounced decrease with increasing animal size within the age classes of 2 and 4–7 yr. Within the whole data set the size dependence of CAT can be expressed as (Fig. 5a)

$$CAT = 114\,W^{-0.355}, \quad r = -0.624, n = 24$$

This regression is highly significant ($p < 0.01$).

No correlation of CAT activity and age of mussels was found either in each of the 3 studied size groups (regression analysis) or within the whole batch of mussels (ANOVA, $F = 0.27$, df = 8,15, p = 0.965) (Fig. 5b), corrected for mean body size. The aquaculture sample was equivalent to 6 yr old littoral animals.

Malondialdehyde

MDA levels were in the range of 0.05 to 0.5 µmol g^{-1} wet tissue. Animal size (wet tissue weight) had no statistical effect on MDA concentration neither in the whole data set (Fig. 6a) nor in any specific age class. Further, no significant influence of age was found (ANOVA, $F = 1.16$, df = 8,73, p = 0.33) (Fig. 6b). Mussels from the aquaculture showed 30% lower levels of MDA than littoral mussels of the same age, but this difference was not significant. Neither growth nor respiration rates were significantly correlated with MDA levels.

Fluorescent age pigments

The content of FAP appeared to strongly depend on the summed effects of both size and age, resulting in a significant correlation of FAP content with tissue wet weight. In order to distinguish between these effects and to obtain the true coefficient for the weight correc-

Table 1. Parameters of the regression $\log FAP = a + b \cdot \log W_{tiss}$ for each age class. R: correlation coefficient. n: number of measurements

Age	*a*	*b*	*R*	p	n
2	−0.099	−0.434	−0.415	0.306	8
3	−0.177	−0.77	−0.74	0.023	9
4	−0.272	−0.777	−0.844	0.001	11
5	−0.123	−0.695	−0.8	0.005	10
6	0.083	−0.572	−0.889	0	16
7	0.122	−0.332	−0.58	0.048	12
8	0.139	−0.653	−0.736	0.004	13
9+10	0.326	−0.333	−0.355	0.349	9
All data	0.078	−0.338	−0.488	0	88

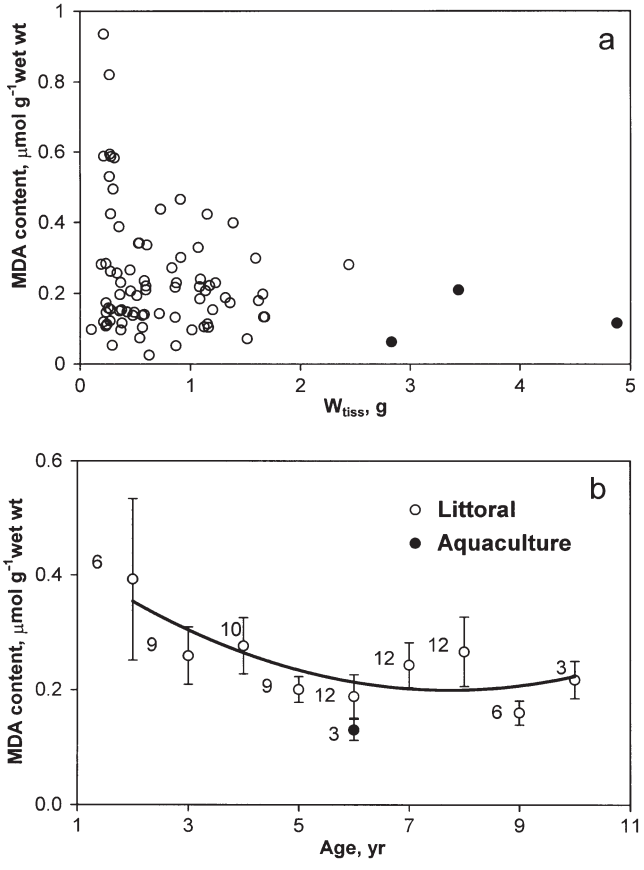

Fig. 6. *Mytilus edulis*. (a) Size and (b) age dependence of malondialdehyde (MDA) concentration in littoral (○) and cultured (●) mussels. N is shown close to the values in (b)

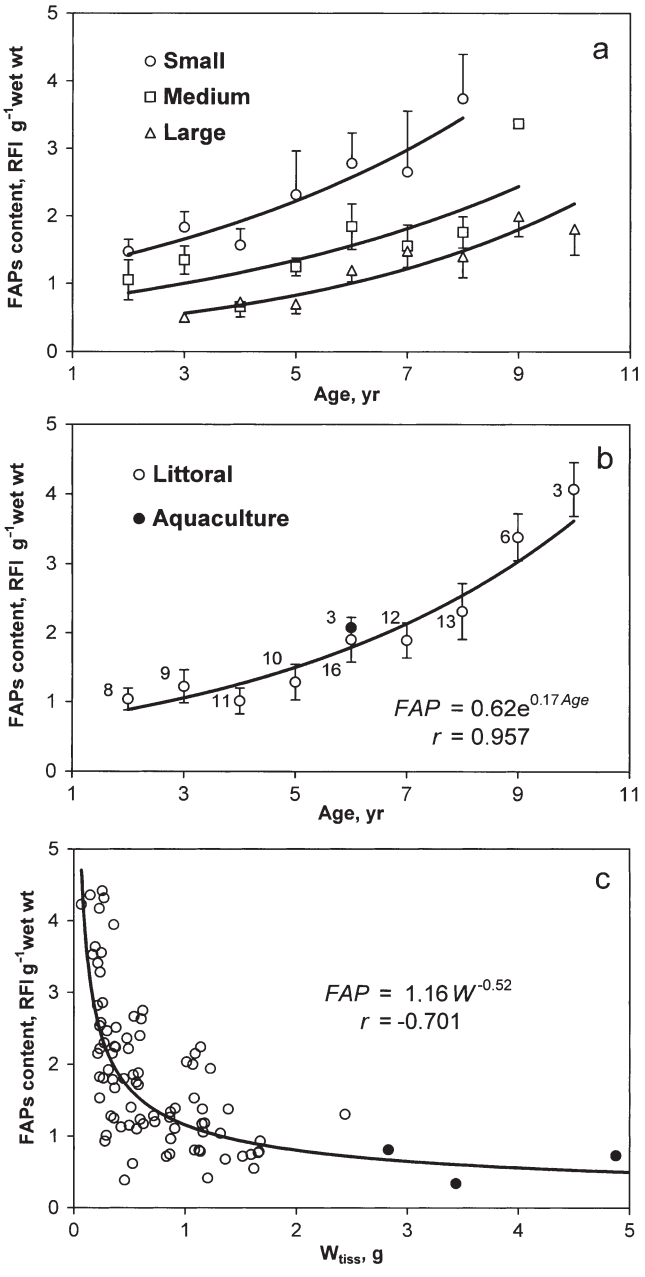

Fig. 7. *Mytilus edulis*. (a,b) Age and (c) size dependence of fluorescent age pigment (FAP) content in littoral (○) and cultured (●) mussels. (a) Weight-specific FAP content as a function of age. (b,c) FAP content corrected (b) for mean weight (0.506 g) and (c) for mean age (6 yr). N is indicated close to the values in (b) and is 3 to 6 in (a) except for 3 yr old L and 9 yr old M mussels, which were represented by only 1 specimen each

tion, the effect of weight was checked for each individual age class. Size dependence was statistically confirmed for most of the separate age classes (Table 1), except the 2 and 9–10 yr olds, owing to relatively low sample numbers (n). In all age groups larger mussels contained less FAP per g wet weight than smaller specimens. The mean power coefficient of FAP-weight regressions for all age levels was −0.57.

Age had a significant effect on both whole-animal and weight-specific FAP content in the studied size

groups ($p < 0.003$ and $p < 0.013$ for S; $p < 0.004$ and $p < 0.016$ for M; and $p < 0.027$ and $p < 0.066$ for L; Fig. 7a). S-group mussels demonstrated the highest levels of weight-specific FAP content, and L ones the lowest, in accordance with the general size effect described above. In order to correct for the influence of size, the FAP contents were calculated for a mean weight of 0.506 g using a power function with the regression coefficient –0.57 (see above). The size-corrected FAP content was significantly (ANOVA, $F = 11.59$, df = 8, 79, $p < 0.001$) affected by mussel age (Fig. 7b).

Post-hoc comparisons (Tukey HSD test) show that the weight-corrected FAP content does not differ between 2–7 yr old mussels, and 9 and 10 yr old ones. Eight yr old ones differ significantly only from those 2 and 4 yr of age. However, the main trend is an increasing FAP content with age. This increase is more pronounced in older specimens and can be approximated well by an exponential function with a regression coefficient of 0.17 (Fig. 7b). Using the formula $FAP_6 = FAP \cdot \exp[0.17\ (6 - Age)]$, it became possible to correct all primary data on FAP content for a mean age of 6 yr. Thereby a specific FAP-weight regression was obtained (Fig. 7c). The power coefficient (–0.52) appeared to be very close to the one (–0.57) used for the elimination of weight effects (see above). Mussels from aquaculture did not differ from littoral ones with respect to FAP content.

FAP decreased significantly with increasing absolute growth increments in mussels ($p < 0.001$) and showed a lower change with relative growth rate; however, this relationship was still significant at $p < 0.01$. K_2 displayed a significant negative correlation with FAP level (Spearman's $r = -0.587$, $p < 0.003$). Respiration rate was not correlated with FAP content.

DISCUSSION

Absolute weight increments in the studied littoral mussels varied greatly depending on both age and initial size of the specimens. Size dependence of growth was determined by the sampling design, where the achieved size of the animals of one age class was a direct function of absolute growth rate. Therefore, the bigger mussels displayed higher weight increments. Much higher absolute growth rates were observed in mussels from suspended aquaculture habitats compared to intertidal ones. This well-documented phenomenon (Rodhouse et al. 1984, Fréchette et al. 1989, Sukhotin & Kulakowski 1992) occurs as a result of differences in hydrodynamics resulting in better trophic conditions in raft cultures and the elimination of air-exposure periods. A gradual decrease in absolute weight increments with increasing age mirrors the pattern characteristic of asymptotic growth. The relative growth rate was nearly identical in mussels of different sizes within one age class and demonstrated a strong age-dependence. In our recent paper a similar equation connecting *RI* and age was obtained for mussels 3–7 yr old from the same population: $RI = 73.7\ Age^{-3.336}$ (Sukhotin & Pörtner 2001). The equation obtained in the present study does not differ significantly from the previous one. It is remarkable that the *RI* of mussels from the aquaculture settlement coincides with that of intertidal specimens of the same age (Fig. 1b). This indicates that the obtained relationship is valid for *Mytilus edulis* in extremely different habitats at least in the area of investigation.

The age dependence of metabolic rate has been studied intensely in homeotherms and insects with respect to the mechanisms of ageing (for a review see McCarter 1995). In fishes and benthic invertebrates, most of which are characterised by continuous growth throughout life, the size of the animals increases with age. Since the respiration rate and many other physiological parameters (ingestion rate, filtration rate, excretion rate and others) depend strongly on body weight, it is difficult to distinguish allometric effects from age effects. Studies of mere age effects on metabolic rates in these species are very rare, and the results are controversial. It has been shown that oxygen consumption rates in *Crenomytilus grayanus* from the Japan Sea were lower in older specimens when individuals of only 1 size class were compared (Zolotarev & Ryabushko 1977). Respiration rate in freshwater fish *Cichlasoma nigrofasciatum* was found to be dependent on both size and age of the specimens (Fidhiany & Winckler 1998). Sukhotin & Pörtner (2001) reported a 30% decrease in weight-corrected respiration rate in *Mytilus edulis* from the White Sea between 3 and 7 yr old. By contrast, cultured *Mytilus galloprovincialis* displayed a minor increase in mass-specific respiration rates in older (22 mo) compared to younger mussels (10 mo) within 1 size group (Pérez Camacho et al. 2000). In the present study, which is complementary to our previous paper, we did not observe a long-term decrease in mass-specific respiration rates over a wider age range between 2 and 10 yr. Although a decrease was observed again between 5 and 7 yr old, specimens even older than 7 yr appeared to display a rise rather than a further decrease in metabolic rate (Fig. 2b). Whole-animal respiration might be influenced both by endogenous factors such as stage of sexual cycle, degree of parasitic infestation and individual genotype and by environmental or experimental conditions exerting variable stress levels on individuals and populations. Clear data showing an effect of age on metabolism might be obtained by using cell cultures or by study-

ing the respiratory capacities of isolated mitochondria.

Net growth efficiency, K_2, in ageing mussels decreased progressively in all size groups until mussels reached an age of 7 yr. During further ageing K_2 remained more or less constant, indicating that active growth in this mussel population becomes very slow beyond this age (Fig. 3a). The present data are in line with our previous study (Sukhotin & Pörtner 2001), where we documented an onset of physiological changes in mussels from the same population at exactly the same age. These changes comprised a decrease in citrate synthase activity or in the concentrations of high-energy phosphates, as well as a lowering of intracellular pH. An exponential decrease in K_2 with increasing age is well documented for bivalves (Fuji & Hashizume 1974, Alimov 1981, Shafee & Conan 1984, Iglesias & Navarro 1991, Urrutia et al. 1999). In some cases (Rodhouse 1978, Vahl 1981) K_2 declines rapidly until maturity is reached and then tends to level off. This is explained by an increased allocation of energy to the production of gametes at the expense of somatic growth. The age dependence of K_2 in the present study showed a similar pattern; however, it was not associated with maturity. *Mytilus edulis* in the White Sea matures when they reach 15–20 mm body length, independent of age (Maximovich 1985). Therefore, all mussels in the sample were mature. Jørgensen (1976) calculated similar K_2 values for mussels of 2 age groups (7–8 and 14–15 yr old). The reported data support our finding that K_2 becomes relatively age-independent in senescent specimens. It is remarkable that within age classes K_2 did not depend on size and therefore on growth rate itself. The only exception concerns the very fast growth of cultured mussels, with a higher K_2 at the same age and size than in their intertidal conspecifics. Higher nutrient availability and reduced stress levels are likely to influence net growth efficiencies more than initial body size.

The MDA concentrations in *Mytilus edulis* from the White Sea varied mainly between 0.1 and 0.4 µmol g^{-1} wet tissue, values which were somewhat higher than those reported for the same species from Britain (0.07 to 0.13 µmol g^{-1} wet tissue; Viarengo et al. 1991) and *M. galloprovincialis* (0.05–0.07 µmol g^{-1} wet tissue; Viarengo et al. 1990). This might be related either to tissue-specific analyses carried out in the cited studies compared to the use of whole body homogenates in the present study or to higher metabolic rates in the White Sea compared to temperate-zone populations, as found for some benthic organisms (Tschischka et al. 2000, A. Sommer & H.-O. Pörtner unpubl.). In the present study, MDA levels were independent of size or age of the mussels. Similarly, no age-related trends of MDA concentrations were found in the marine shrimp *Aristeus antennatus* (Mourente & Diaz-Salvago 1999). By contrast, *M. edulis* from the British Isles displayed higher MDA concentrations in older specimens (>10 yr old) compared to younger ones (2–4 yr old) (Viarengo et al. 1989, 1991). In the cephalopod *Sepia officinalis* reared in the laboratory, low levels of MDA were observed until the specimens reached an age of 10 mo, when a significant increase in MDA was recorded (Zielinski & Pörtner 2000). Since MDA is a precursor of FAP (e.g. lipofuscin) and, as a small molecule, may be released to the water, its accumulation is transient and concentrations might be expected to vary over time. Thus, MDA levels might reflect recently experienced ecological stress rather than age-dependent accumulation.

FAP are extensively studied as end products of lipid peroxidation, which makes them promising markers of both the degree of oxidative stress and the ageing process in animals (for a review see Sohal 1981, Rikans & Hornbrook 1997, Terman & Brunk 1998). Our data give evidence that FAP levels in mussels depend strongly on both age and size and therefore on either growth rate or weight-specific metabolic rate. Regardless of body size, FAP concentrations increased exponentially with age between 2 and 10 yr. In some other species the FAP-age relationship is also reported to be exponential (Ju et al. 1999, Zielinski & Pörtner 2000). However, there are publications where either no age-related changes in FAP weight-specific content in molluscs are recorded (Clarke et al. 1990, Hole et al. 1993) or they are even negative depending on the tissue studied (Clarke et al. 1990).

The negative correlation of FAP content with absolute growth rate of mussels reflects the size dependence mentioned above. Age- and weight-corrected data on FAP content did not show any significant correlation with *AI*. FAP content corrected for mean age (6 yr) and plotted against weight (Fig. 7c) demonstrates a strong dependence on growth rate; however, it is not dependent on the absolute value (*AI*), which changes with age, but on the 'average' growth, i.e. body size, which mirrors the whole life growth history (averaged for an age of 6 yr). Mussels which reach a large size possess less FAP than the slow-growing ones, which remain smaller and therefore metabolically more active. Nonetheless, the relationship between FAP content and respiration rate displayed no significant trend. The high FAP amounts in the small slow-growing mussels may have accumulated also because they encountered less-favourable conditions, i.e. higher stress levels throughout life, than the fast-growing ones. S mussels older than 8 yr were not found in the studied population and probably have a shorter lifespan than M and L specimens. This fact may also indicate the differences in conditions of the microhabitats where mussels live.

An extensive body of publications is devoted to the role of various substances with antioxidative properties in the ageing process in animals (see Williams et al. 1993 and Warner & Starke-Reed 1997 for a review). The argument has been put forward that the age-related decrease in antioxidant defence causes accumulation of lipid peroxidation products in the organism (Viarengo et al. 1991, Canesi & Viarengo 1997). Despite the fact that SOD and CAT are key enzymes in antioxidant protection, direct links between lifespan and an up-regulation of antioxidant defence systems have been established only in model systems such as laboratory insects (fruitflies) and nematodes (Larsen 1993, Orr & Sohal 1994). The studies of the effect of ageing on the activities of SOD and CAT have produced conflicting results, since this effect appears to be specific to species, strain, sex and tissue. Most authors observed different patterns in SOD and CAT activities with respect to age. A minor increase in or constant levels of SOD and a decrease in CAT activities with age have been reported for *Mytilus edulis* (Viarengo et al. 1991), the marine shrimp *Aristeus antennatus* (Mourente & Diaz-Salvago 1999) and the cephalopod *Sepia officinalis* (Zielinski & Pörtner 2000). For some other species—the frog *Rana perezi* (López-Torres et al. 1991), the polychaete *Arenicola marina* (Buchner et al. 1996), laboratory rats (Cand & Verdetti 1989)—contrasting age-related changes in these enzymes (a decrease in SOD and an increase or no change in CAT) have been recorded. Significant increases in both enzyme activities in the muscles of older rats have also been observed (Leeuwenburgh et al. 1994).

No age effect on SOD and CAT activity was found in ageing *Mytilus edulis* in the present study. At the same time an age-related exponential rise in FAP content indicates that the intensity of net lipid peroxidation increases in ageing mussels. Further effort should be devoted to the analysis of other components of the antioxidative system, including changes of the cellular redox state with ageing.

The size dependence of CAT, shown in the present study, is a well-known phenomenon for many, especially metabolic, enzymes in between-species comparisons. A similar scaling of citrate synthase was recorded for skeletal muscles of mammals (Hochachka et al. 1988) and fish (Somero & Childress 1980, Burness et al. 1999). Different size classes of the same age in blue mussels provide the unique opportunity to test for the effect of size versus age with respect to metabolic parameters. The weight-specific decrease in CAT activity which has been found within separate age classes of *M. edulis* in our study illustrates that in those papers where an age-related decrease in CAT has been reported (Viarengo et al. 1991, Mourente & Diaz-Salvago 1999, Zielinski & Pörtner 2000) it might be due to the increasing size of the ageing mussels and not to the effect of age per se.

In conclusion, the physiological functions in the blue mussels are influenced by both age and size. Weight-specific growth rate and net growth efficiency depend on age until the 'critical' age is reached, beyond which growth virtually stops in the senescent mussel. The effect of age on respiration rate at the whole-animal level is not yet clear and may be masked by the influence of so-far-neglected factors. The process of lipid peroxidation which leads to the accumulation of fluorescent age pigments increases exponentially with age, but also shows a significant negative correlation with mussel size (lifetime growth rate), in accordance with an increased rate of oxidative metabolism in smaller mussels. This occurs in the absence of age-related changes in the activity of the 2 main antioxidant enzymes (SOD and CAT). The age and size effects on the physiological functions of continuously growing species can act in opposite directions and mask each other. Thus, ignoring the interaction of age and size in physiological studies in marine ectotherms may lead to inadequate and conflicting conclusions.

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LITERATURE CITED

Abele-Oeschger D, Oeschger R, Theede H (1994) Biochemical adaptations of *Nereis diversicolor* (Polychaeta) to temporarily increased hydrogen peroxide levels in intertidal sandflats. Mar Ecol Prog Ser 106:101–110

Aebi H (1984) Catalase. In: Bergmeyer HU (ed) Methods in enzymatic analysis, Vol II. Academic Press, New York, p 673–683

Alimov AF (1981) Functional ecology in freshwater bivalve molluscs. Nauka, Leningrad (in Russian)

Buchner T, Abele-Oeschger D, Theede H (1996) Aspects of antioxidant status in the polychaete *Arenicola marina*: tissue and subcellular distribution, and reaction to environmental hydrogen peroxide and elevated temperatures. Mar Ecol Prog Ser 143:141–150

Burness GP, Leary SC, Hochachka PW, Moyes CD (1999) Allometric scaling of RNA, DNA, and enzyme levels: an intraspecific studies. Am J Physiol 277: R1164-R1170

Calow P (1977) Conversion efficiencies in heterotrophic organisms. Biol Rev 52:385–409

Cand F, Verdetti J (1989) Superoxide dismutase, glutathion peroxidase, catalase, and lipid peroxidation in the major organs of the ageing rats. Free Radical Biol Med 7:59–63

Canesi L, Viarengo A (1997) Age-related differences in glutathione metabolism in mussel tissue (*Mytilus edulis* L.). Comp Biochem Physiol 116B:217–221

Chemodanov AV, Maximovich NV (1983) Internal structure of *Mytilus edulis* L. shells as an indication of their seasonal

growth variation. In: Proc 7th Malacology Conference. Nauka, Leningrad, p 178–180 (in Russian)
Clarke A, Kendall MA, Gore DJ (1990) The accumulation of fluorescent age pigments in the trochid gastropod *Monodonta lineata*. J Exp Mar Biol Ecol 144:185–204
Emerit I, Chance B (eds) (1992) Free radicals and aging. Birkhäuser Verlag, Basel
Fidhiany L, Winckler K (1998) Influence of body mass, age, and maturation on specific oxygen consumption in a freshwater cichlid fish, *Cichlasoma nigrofasciatum* (Günther, 1869). Comp Biochem Physiol 119A: 613–619
Fréchette M, Butman CA, Geyer WR (1989) The importance of boundary-layer flows in supplying phytoplankton to the benthic suspension feeder, *Mytilus edulis* L. Limnol Oceanogr 34:19–36
Fuji A, Hashisume M (1974) Energy budget of a Japanese common scallop *Patinopecten yessoensis* (Jay) in Mutsu Bay. Bull Fac Fish Hokkaido Univ 25:7–19
Glotov NV, Zhivotowski LA, Hovanov NV, Khromov-Borisov NN (1982) Biometry. Leningrad University, Leningrad
Harman D (1956) Aging: a theory based on free radical and radiation biology. J Geronthol 11:298–300
Hill KT, Womersley C (1991) Critical aspects of fluorescent age-pigment methodologies: modification for accurate analysis and age assessments in aquatic organisms. Mar Biol 109:1–11
Hochachka PW, Emmett B, Suarez RK (1988) Limits and constraints in the scaling of oxidative and glycolytic enzymes in homeotherms. Can J Zool 66:1128–1138
Hole LM, Moore MN, Bellamy D (1993) Age-related cellular reactions to copper in the marine mussel *Mytilus edulis*. Mar Ecol Prog Ser 94:175–179
Iglesias JIP, Navarro E (1991) Energetics of growth and reproduction in cockles (*Cerastoderma edule*): seasonal and age-dependent variations. Mar Biol 111:359–368
Ivlev VS (1938) Energy transformations in growing invertebrates. Bull Moskovskogo Obschestva Ispytateley Priody (MOIP) 47 (4):267–277 (in Russian)
Jørgensen CB (1976) Growth efficiencies and factors controlling size in some mytilid bivalves, especially *Mytilus edulis* L.: a review and interpretation. Ophelia 15:175–192
Ju SJ, Secor DH, Harvey HR (1999) Use of extractable lipofuscin for age determination of blue crab *Callinectes sapidus*. Mar Ecol Prog Ser 185:171–179
Larsen PL (1993) Aging and resistance to oxidative damage in *Caenorhabditis elegance*. Proc Natl Acad Sci USA 90: 8905–8909
Leeuwenburgh C, Fiebig R, Chandwaney R, Li Li Ji (1994) Aging and exercise training in skeletal muscle: responses of glutathione and antioxidant enzyme systems. Am J Physiol 267:R439–R445
Leibovitz BE, Siegel BV (1980) Aspects of free radical reactions in biological systems: aging. J Gerontol 35:45–56
López-Torres M, Pérez-Campo R, Barja de Quiroga G (1991) Effect of natural aging and antioxidant inhibition on liver antioxidant enzymes, glutathione system, peroxidation, and oxygen consumption in *Rana perezi*. J Comp Physiol 160B:655–661
Marklund S, Marklund G (1974) Involvement of the superoxide anion radical in the antioxidation of pyrogallol and a convenient assay for superoxide dismutase. Eur J Biochem 47:469–479
Masoro EJ (ed) (1995) Handbook of physiology, Section 11, Aging. Oxford University Press, New York
Maximovich NV (1985) Reproductive cycle of *Mytilus edulis* L. in Chupa Inlet. In: Lukanin VV (ed) Investigations on mussels from the White Sea. Zoological Institute, Leningrad, p 22–35 (in Russian)
McCarter RJM (1995) Energy utilization. In: Masoro EJ (ed) Handbook of physiology, Section 11, Aging. Oxford University Press, New York, p 95–118
Mourente G, Diaz-Salvago E (1999) Characterisation of antioxidant systems, oxidation status and lipids in brain of wild-caught size-class distributed *Aristeus antennatus* (Risso, 1816) Crustacea, Decapoda. Comp Biochem Physiol 124B:405–416
Nicol S (1987) Some limitations on the use of the lipofuscin aging techniques. Mar Biol 93:609–614
Orr WC, Sohal RS (1994) Extension of life-span by overexpression of superoxide dismutase and catalase in *Drosophila melanogaster*. Science 263:1128–1130
Pearl L (1928) The rate of living. Alfred Knopf, New York
Pérez Camacho A, Labarta U, Navarro E (2000) Energy balance of mussels *Mytilus galloprovincialis*: the effect of length and age. Mar Ecol Prog Ser 199:149–158
Rikans LE, Hornbrook KR (1997) Lipid peroxidation, antioxidant protection and aging. Biochim Biophys Acta 1362: 116–127
Rodhouse PG (1978) Energy transformations by the oyster *Ostrea edulis* L. in a temperate estuary. J Exp Mar Biol Ecol 34:1–22
Rodhouse PG, Roden CM, Hensey MP, Ryan TH (1984) Resource allocation in *Mytilus edulis* on the shore and in suspended culture. Mar Biol 84:27–34
Shafee MS, Conan G (1984) Energetic parameters of a population of *Chlamys varia* (Bivalvia, Pectinidae). Mar Ecol Prog Ser 18:253–262
Sirenko BI, Saranchova OL (1985) Two-years study of seasonal growth of mussels *Mytilus edulis* in cages in Chupa Inlet (the White Sea). In: Ecological investigations of perspective aquaculture species in the White Sea. ZIN AN SSSR, Leningrad, p 23–28 (in Russian)
Sohal RS (1981) Metabolic rate, aging and lipofuscin accumulation. In: Sohal RS (ed) Age pigments. Elsevier/North-Holland Biomedical Press, Amsterdam, p 303–316
Sohal RS (1986) The rate of living theory: a contemporary interpretation. In: Collatz KG, Sohal RS (eds) Insect aging. Springer-Verlag, Berlin, p 23–44
Somero GN, Childress JJ (1980) A violation of the metabolism-size scaling paradigm: activities in glycolytic enzymes in muscle increase in larger-size fish. Physiol Zool 53:322–337
Strickland JD, Parsons TR (1968) A practical handbook of seawater analysis. Bull Fish Res Board Can 167
Sukhotin AA (1992) Respiration and energetics in mussels (*Mytilus edulis* L.) cultured in the White Sea. Aquaculture 101:41–57
Sukhotin AA, Kulakowski EE (1992) Growth and population dynamics in mussels (*Mytilus edulis* L.) cultured in the White Sea. Aquaculture 101:59–73
Sukhotin AA, Maximovich NV (1994) Variability of growth rate in *Mytilus edulis* L. from the Chupa Inlet (the White Sea). J Exp Mar Biol Ecol 176:15–26
Sukhotin AA, Pörtner HO (2001) Age-dependence of metabolism in mussels *Mytilus edulis* L. from the White Sea. J Exp Mar Biol Ecol 257:53–72
Terman A, Brunk UT (1998) Lipofuscin: mechanisms of formation and increase with age. APMIS 106:265–276
Timiras PS (ed) (1994) Physiological basis of aging and geriatrics. CRC Press Inc, Boca Raton
Tschischka K, Abele D, Pörtner HO (2000) Mitochondrial oxyconformity and cold adaptation in the polychaete *Nereis pelagica* and the bivalve *Arctica islandica* from Baltic and White Sea. J Exp Biol 203:3355–3368

Uchiyama M, Mihara M (1978) Determination of malonaldehyde precursor in tissues by thiobarbituric acid test. Anal Biochem 86:271–278

Urrutia MB, Ibarrola I, Iglesias JIP, Navarro E (1999) Energetics of growth and reproduction in a high-tidal population of the clam *Ruditapes decussatus* from Urdaibai Estuary (Basque Country, N. Spain). J Sea Res 42:35–48

Vahl O (1981) Energy transformations by the Iceland scallop, *Chlamys islandica* (O.F. Müller), from 70° N. I. The age-specific energy budget and net growth efficiency. J Exp Mar Biol Ecol 53:281–296

Vernet M, Hunter JR, Vetter RD (1988) Accumulation of age-pigments (lipofuscin) in two cold-water fishes. Fish Bull 86:401–407

Viarengo A, Pertica M, Canesi L, Accomando R, Mancinelli G, Orunesu M (1989) Lipid peroxidation and level of antioxidant compounds (GSH, vitamin E) in the digestive glands of mussels of three different age groups exposed to anaerobic and aerobic conditions. Mar Environ Res 28:291–295

Viarengo A, Canesi L, Pertica M, Poli G, Moore MN, Orunesu M (1990) Heavy metal effects on lipid peroxidation in the tissues of *Mytilus galloprovincialis* Lam. Comp Biochem Physiol 97C:37–42

Viarengo A, Canesi L, Petrica M, Livingstone DR, Orunesu M (1991) Age-related lipid peroxidation in the digestive gland of mussels: the role of the antioxidant defence systems. Experentia 47:454–457

Warner HR, Starke-Reed P (1997) Oxidative stress and aging. In: Clerch LB, Massaro DJ (eds) Oxygen, gene expression and cellular function. Marcel Dekker, Inc, New York, p 139–168

Wieser W (1994) Cost of growth in cells and organisms: general rules and comparative aspects. Biol Rev 68:1–33

Williams GM, Sies H, Baker GT, Erdman JW, Henry CJ (eds) (1993) Antioxidants: chemical, physical, nutritional and toxicological aspects. Princeton Scientific Publishing Co, Princeton, NJ

Winberg GG (1966) Growth and metabolic rates in animals. Uspekhi Sovremennoy Biologii 6:107–126 (in Russian)

Winberg GG (1986) Growth efficiency and production in aquatic animals. In: Khmeleva NN (ed) Growth efficiency in aquatic animals. Gomel University Publishers, Gomel, p 20–61 (in Russian)

Wollenberger A, Ristau, D, Schoffa G (1960) Eine einfache Technik der extrem schnellen Abkühlung grösserer Gewebestücke. Pflügers Arch 270:399–412

Zielinski S, Pörtner HO (2000) Oxidative stress and antioxidative defence in cephalopods: a function of metabolic rate or age? Comp Biochem Physiol 125B:147–160

Zolotarev VN, Ryabushko VI (1977) Age changes of energy metabolism in *Crenomytilus grayanus* Dunker. Zhurnal Obschey Biologii 38:923–928 (in Russian)

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