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Age-dependence of metabolism in mussels *Mytilus edulis* (L.) from the White Sea

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Abstract

Age structure, natural mortality and growth, as well as age- and size-dependent changes in parameters of energy metabolism were studied in blue mussels *Mytilus edulis* (L.) from the White Sea. Mussels were sampled in August (Summer sample, SS) and October (Autumn sample, AS) and contained animals of three size groups, 2–9 years old. Field data showed an increase of mortality of mussels and strong decrease in growth rates after 6 years of age. Absolute tissue growth increment (AI) reconstructed from winter growth marks on the shells decreased with age and was strongly size-dependent, while relative tissue growth increment (RI) did not depend on size of the animals. Respiration rates and citrate synthase activity demonstrated power regression versus tissue weight with regression coefficients -0.231 and -0.170, respectively. After weight correction both parameters showed a decrease with increasing age. ATP and phosphagen levels also showed a pronounced decrease in animals older than 5–6 years despite considerable differences in the absolute values of both parameters in SS and AS. pH_i in mussels was also age-dependent and decreased with increasing age after 5 years. In air exposed mussels, pH_i was reduced only at young age such that pH_i was low and constant within the whole age range. Our data give evidence that aerobic metabolic rate in *M. edulis* from the studied population declines when animals reach an age of about 6 years. The decrease in oxygen consumption reflects the drop in mitochondrial respiration, which is mirrored by the decrease in CS activity. A concomitant fall in ATP turnover may include a downregulation of the mechanisms of acid–base regulation. pH_i will then approach equilibrium indicated by lower pH_i values in older animals. Our data suggest that intrapopulational comparisons of physiological parameters in mussels should take into account age composition of compared samples.

Keywords: Ageing; Citrate synthase; Growth; High energy phosphates; Intracellular pH; *Mytilus edulis*

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1. Introduction

The influences of various exogenous and endogenous factors on the rates of metabolism in marine invertebrates have been widely studied for decades. One of the main endogenous factors determining the rate of energy expenditure is body size (Zeuthen, 1953; Hemmingsen, 1960). The size of animals with unlimited growth is closely correlated with their age, however this correlation becomes weaker in older specimens when growth rate is low. Age effects on metabolism were intensively investigated mainly in animals with limited growth like humans and other homeotherms and insects. These investigations give evidence of a general decrease in basal metabolic rate with age (for review, see McCarter, 1995) related to a drop in the rate of cellular metabolism (for review, see Lints, 1985). However, nearly no data are obtained for species with infinite growth like many marine benthic invertebrates including such an extensive group as mollusks (Zolotarev and Ryabushko, 1977). The metabolic changes during the time course of ageing in animals with infinite growth may differ from those shown for homeotherms and insects. In order to obtain a comprehensive theory linking metabolic rate and ageing, infinitely growing species should be carefully studied. In the few available publications on this issue it is documented that ageing in molluscs is accompanied by changes in energy partitioning. Somatic growth decreases while reproductive output increases (Rodhouse, 1978; Vahl, 1981a,b; Peterson, 1983; Aldridge et al., 1986; Iglesias and Navarro, 1991). Net and gross growth efficiencies decrease as well (Bayne and Newell, 1983). This occurs due to the change in weight exponents of the main physiological rates — metabolic rate and feeding rate. Metabolic rate declines less rapidly in older (larger) specimens, than ingestion rate, so that per unit of ingested material larger animals face greater metabolic expenditure (Hawkins and Bayne, 1992). This model, as well as the others, does not deal with age-, but with size-dependent functions and does not explain the ageing influence. However, age-dependent changes in metabolism may be a significant part of the ageing process and may contribute to determine the life span of the animals. With the high variability in growth rate between individuals of some species it should be possible to distinguish between the effects of size and age in infinitely growing species.

Sedentary benthic animals from subtidal and intertidal zones as well as from different levels of the intertidal zone are characterised by pronounced differences in various physiological parameters (Moon and Pritchard, 1970; de Vooys and de Zwaan, 1978; Demers and Guderley, 1994; Sukhotin and Pörtner, 1999). The most variable parameter correlated with height on the shore is growth rate (Dickie et al., 1984; Sukhotin and Maximovich, 1994). In the intertidal zone animals regularly undergo hypoxic exposure during low tide. The length of the exposure period depends on the height on the shore. Since metabolic rate is largely reduced during hypoxia this process will have a significant influence on growth and possibly also on the ageing process. If no active migrations occur then animals of the same age reach different sizes at different tidal levels. Therefore, using animals of similar tissue weight from the various habitats for physiological experiments may lead to a significant age difference in groups from the investigated habitats. The question arises whether physiological differences between these are due to the heterogeneity of the samples with respect to age and show an age

effect on physiology. Marine mussels are excellent objects for carrying out studies on ageing because: (1) they are characterised by a long maximal life span; (2) their great variability in growth rates gives the opportunity to organise material in such a matrix that allometric and age effects can be distinguished; (3) age determination in mussels is relatively simple especially in animals living in the White Sea where the warm growing season alternates with a prolonged winter when growth stops because of negative water temperatures. This leads to clear growth layer formation in the shells and also allows to reconstruct the growth history of the individuals.

The main goal of the present work was to find out which parameters of energy metabolism are age-related in mussels *Mytilus edulis*. Another question addressed in this study was whether the physiological response of mussels to environmental hypoxia is age-dependent and to find out about the differences in this response. Therefore, mussels of different age from the same population and location in the intertidal zone were compared with respect to their metabolic response to hypoxia.

2. Material and methods

2.1. Animals

Mussels *Mytilus edulis* L. were collected on August 19, 1997 (Summer sample) and on October 12, 1997 (Autumn sample) from an intertidal mussel bed situated in Kandalaksha Bay of the White Sea (66°20′N: 33°40′E). The settlement lies between -0.2 and $+1.2$ m above 0 tidal level. Animals were sampled from the shore at $+0.7$ m level, where the emersion period comprises about 20% of the tidal cycle. Mean temperature of the surface water layer in the region is 13.8 ± 0.18°C in August and 4.8 ± 0.18°C in October (Babkov, 1982). After sampling mussels were sorted by size and age in accordance with the experimental design. Age was determined by counting the rings of winter growth delays on the shells. Animals were kept in aquaria for 2 days at 25‰ salinity, $+10$°C temperature and constant light. No food was added. Animals from the Summer sample were subjected to experimentation at the White Sea Biological Station of the Zoological Institute (St. Petersburg, Russia). Tissues were frozen in liquid nitrogen and transported to the Alfred-Wegener Institute (AWI, Bremerhaven, Germany). Mussels from the Autumn sample were brought to the AWI alive. There they were kept in aquaria with aerated sea-water at the above mentioned conditions.

Quantitative samples in the mussel settlement were taken on May 25 and October 1, 1997 to analyse the age structure of the studied population and growth rates of mussels at different age. Both spring and autumn samplings included 15 squares of 0.01 m^2 intertidal area. All mussels were measured to the nearest 0.1 mm with callipers or under the stereoscopic microscope. Age was determined as mentioned above.

2.2. Experimental procedure

The mussels collected in summer were divided in three size groups: small (S, wet tissue weight = 0.057 ± 0.001 g), medium (M, wet tissue weight = 0.356 ± 0.007 g) and

large (L, wet tissue weight = 1.085±0.047 g). Each group contained mussels of three ages: S — 3, 4 and 5 years old, M — 4, 5 and 6 and L — 5, 6 and 7 years old.

Growth rate of mussels was determined individually as absolute (*AI*) and relative (*RI*) tissue wet weight increment from the beginning of growth season in 1997 to the time of sampling and experiment 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 initial and final wet tissue weights (g) of the mussels used in the experiment, respectively. W_f was measured directly by weighing while W_i was calculated using the length of mussel at the beginning of growth season. The latter was taken as the length from the umbo of the shell up to the most distant edge of the last ring marking winter growth delay. Active growth in mussels in the region of the study usually starts in late May (Sukhotin and Maximovich, 1994). The length (L, mm)–tissue weight (W, g) relationship was expressed as $W = 0.00005L^{2.706}$, $r = 0.994$, $n = 126$.

Oxygen consumption rate was measured in closed 200–500 ml respirometers at +10°C. All of nine age/size groups contained 14 subsamples (ten, three and one specimen in each subsample of S, M, and L groups, respectively). Mussels in one subsample were treated together, tissues were pooled thereafter. Each respirometer contained one subsample. After 60–90 min in the respirometer, water was siphoned into air-tight bottles. Oxygen concentration was determined by the Winkler method (Strickland and Parsons, 1968). Oxygen consumption was evaluated from the difference to controls (chambers without animals). Respiration rate was expressed as a weight-specific rate (μmol O_2 g^{-1} tissue wet wt h^{-1}).

Half of the subsamples from each age/size group were exposed to air for 60 h at +10°C. During air exposure mussels were kept tightly closed by ribbon bands to prevent gaping. Control animals remained emersed in aquaria at the same temperature. After air exposure both control and exposed mussels were quickly opened, all tissues were withdrawn from shells, weighed and frozen by freeze-clamping in liquid nitrogen (Wollenberger et al., 1960).

The mussels collected in autumn were divided into two size groups: medium (M, wet tissue weight = 0.189±0.014 g) and large (L, wet tissue weight = 0.947±0.041 g). The M group contained mussels 2–8 years old, and the L group animals 4–9 years old. After 2 weeks of exposure to aquarium conditions at the AWI (see above) these animals were cut open and tissues were frozen as described above.

2.3. Analyses

All the measurements were performed using the whole soft body of the mussels. Intracellular pH (pH_i) was determined using the homogenate technique (Pörtner et al., 1990). Samples were powdered under liquid nitrogen and resuspended in ice-cold medium containing 160 mM KF and 1 mM nitrilotriacetic acid. pH of the medium was 6.5. After centrifugation pH of the supernatant was determined with a capillary pH electrode (Radiometer, Copenhagen E5021) thermostatted to the temperature of the experiment (+10°C).

Weighed tissue samples ground under liquid nitrogen were added to five times the volume of ice-cold 0.6 M perchloric acid. After homogenisation and centrifugation, the supernatant pH was neutralised by use of 5 M KOH. After final centrifugation the supernatant was stored at −80°C. The amounts of ATP, L-arginine (LA) and phospho-L-arginine (PLA) were determined spectrophotometrically using enzymatic tests according to Grieshaber et al. (1978) and Pörtner et al. (1990).

Citrate synthase activity was measured spectrophotometrically at 412 nm using an enzymatic test (Sidell et al., 1987) in 75 mM Tris + 1 mM EDTA homogenates (pH 7.6). Measurements were performed immediately after extraction at a temperature of +10°C.

2.4. Calculations and statistics

The ratio of PLA over PLA + LA levels (R_{PLA}) was calculated as a measure of L-arginine phosphorylation. Two-way ANOVA was used for analysing the effect of the factors age and exposure versus controls. 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 non-parametric correlation coefficients. All calculations were performed with the package Statistica for Windows, release 4.3 (StatSoft, Inc.).

3. Results

3.1. Population age structure

The investigated settlement is characterised by a bi-modal age structure (Fig. 1). In

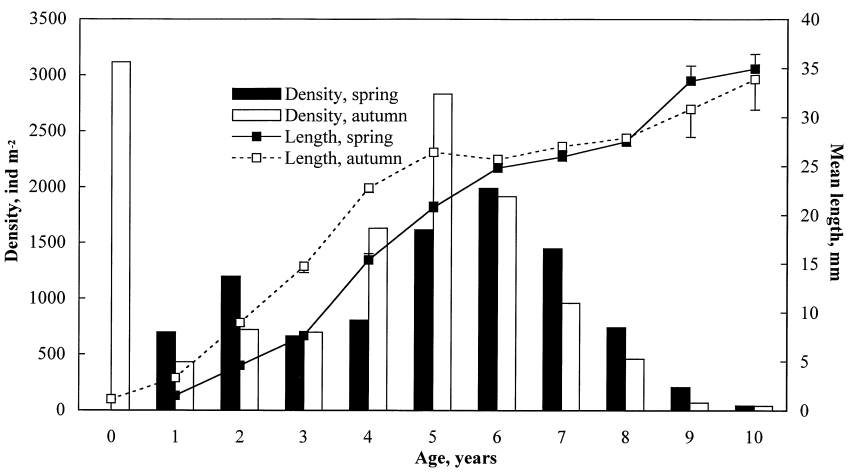

Fig. 1. Seasonal changes of mean length and density of different age classes in intertidal *M. edulis* population. Vertical bars represent standard errors.

spring the population had two predominant age classes — one at 2 and a more pronounced class at 6 years (Fig. 1). During summer, two processes influence the age structure of the population — elimination and recruitment. By autumn a considerable decrease in younger (1 and 2 years) and older (over 7 years) specimens is observed. By contrast, animals of intermediate age significantly increase in abundance. Besides that the new settling spat appear in the population at about one quarter of the total number of individuals.

The increase in mean mussel length within age classes from spring to autumn indicate the average growth increment of specimens. Mussels under 6 years old show 4.5–7.5 mm length increments during the summer season (Fig. 1). At an age of 6 years, growth rate decreases strongly and mean increments do not exceed 1 mm per summer season. Mean animal size at an age of 9 and 10 years in spring appeared to be somewhat larger than in autumn. This suggests that a decrease in density during the warm season which is more than 3-fold in 9-year-old mussels and somewhat lower in 10-year-old ones occurred mainly at the expense of bigger individuals.

3.2. Growth rate

In accordance with the structure of the sampled material the collected mussels demonstrated high variation in absolute tissue weight increment (*AI*). This parameter varied in the wide range from −0.202 to 0.934 g per growing period (May–August) (Fig. 2a). Although all the negative *AI* values were observed in L size group the lowest mean *AI* (0.01–0.03 g) was characteristic for the animals from S group. There was no statistical difference between ages within the group, however S mussels strongly differed ($P<0.05$) from those of other sizes by *AI* values. Seven years old mussels from L group and 5 and 6 years old ones from M group had similar *AI* of about 0.07–0.10 g. Six-years-old L specimens together with 4-years old M ones form another group with intermediate *AI* (0.13–0.14 g), while the highest absolute weight increment (0.27 g) was observed in the 'youngest' (5 years old) mussels of the L size group. *AI* declined linearly with age in each size group (Fig. 2a). The bigger the size of the animal the more pronounced was the decline in *AI* observed per year.

Relative weight increment (*RI*) appeared to be strongly age-dependent and gradually decreased from 1.5 (150%) in 3-year-old mussels to 0.1 (10%) at 7 years. The relationship between age (years) and *RI* is well described by the power function (Fig. 2b):

$$RI = 73.7 \cdot \mathrm{Age}^{-3.336}, \quad n = 9, \quad r = -0.979$$

According to Tukey's HSD test *RI* in mussels of 3 and 4 years differed significantly from the values for all other ages ($P<0.05$). It is important that the relative weight increment was not affected by size group.

3.3. Respiration rate

Oxygen consumption rate varied between 1.7 and 16.0 μmol O_2 g^{-1} tissue wet wt

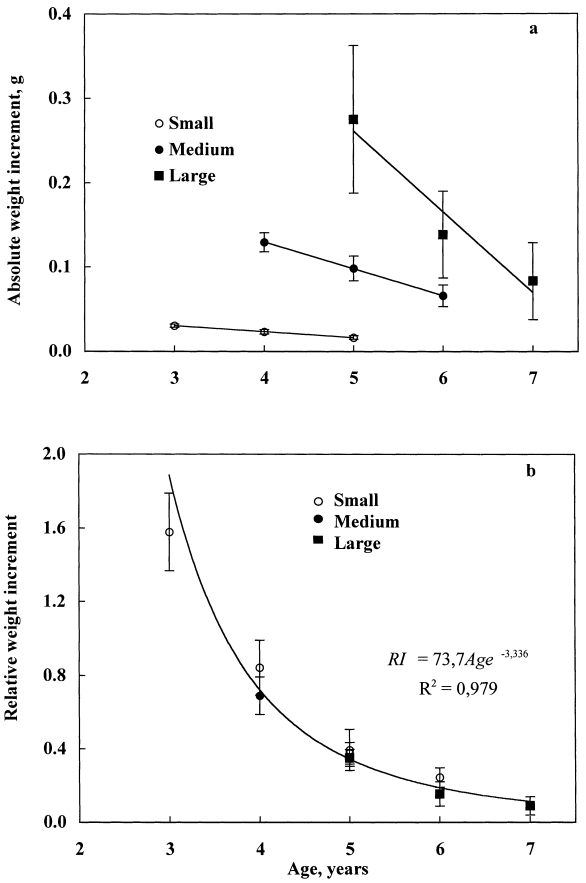

Fig. 2. Absolute (a) and relative (b) tissue wet weight increment in mussels of different age and size groups. Vertical bars represent standard errors, $n = 13–15$.

h^{-1} and showed a strong weight dependence (Fig. 3a), which was described as a power function:

$$R' = 5.51 \cdot W^{-0.231}, \quad r = -0.775, \quad n = 126,$$

where R is the weight specific oxygen consumption rate (μmol O_2 g^{-1} wet wt h^{-1}), W is tissue wet weight (g), r is correlation coefficient, n is number of specimens. In order to remove probable age effect from respiration/weight relationship, the equation was calculated for 5 years old mussels, which contained all three studied size classes:

$$R' = 6.42 \cdot W^{-0.148}, \quad r = -0.686, \quad n = 42,$$

For further analyses R was corrected for weight differences either in S, M, L size groups or in the whole weight range according to the formula $R = R'(W_{mean}/W)^{-0.148}$, where R' and R are observed and corrected oxygen consumption rates, respectively, W is observed

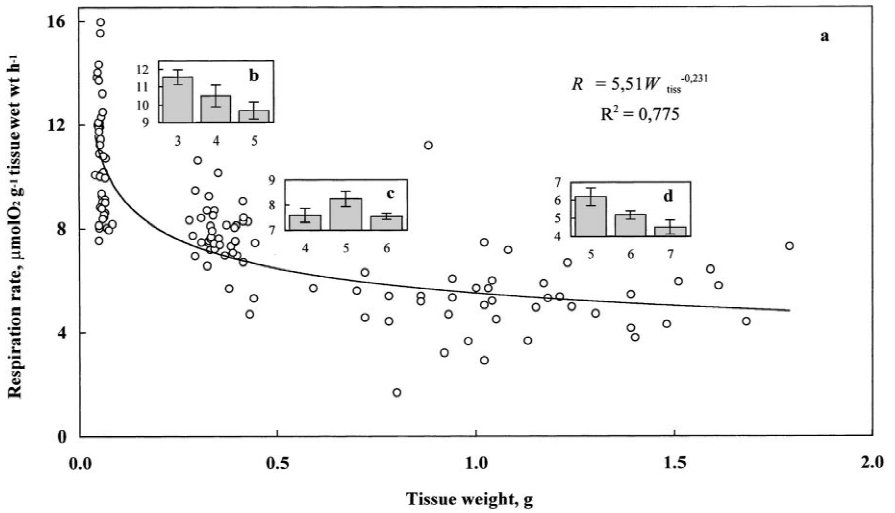

Fig. 3. Respiration rate as a function of weight of mussels (a). Age-specific respiration rates in Small (b), Medium (c), and Large (d) size groups. In (b–d): *x*-axis is age of mussels (years), *y*-axis is respiration rate (μmol g^{-1} tissue wet wt h^{-1}) corrected for mean tissue weight of each size group (see text). Vertical bars represent standard errors, $n=14$.

tissue wet weight, W_{mean} is mean group tissue wet weight, equal to 0.057 g for S, 0.356 g for M, 1.085 g for L groups and 0.272 g for the whole experimental stock of mussels.

Age appeared to be a significant factor influencing weight corrected respiration rate of mussels in S and L groups (Fig. 3b,d) ($P<0.05$) but was not statistically important at $P=0.05$ for the M group (Fig. 3c). In both S and L groups, a decrease of respiration rate is observed with increasing age. Respiration rate corrected for the whole weight range showed a pronounced decrease with increasing age of the animals ($P<0.001$, ANOVA) (Fig. 4a).

Respiration rate without weight correction correlated positively with *RI* (Spearman $R=0.537$, $n=125$, $P<0.001$) (Fig. 4b). After weight correction (see above), the correlation became weaker, however, remained highly significant (Spearman $R=0.437$, $n=125$, $P<0.001$). Respiration rate and CS activity also demonstrated positive correlation (Spearman $R=0.279$, $n=90$, $P<0.01$).

3.4. Citrate synthase activity

The activity of one of the key mitochondrial respiratory enzymes citrate synthase (CS) varied in White Sea mussels between 0.50 and 1.85 U g^{-1} tissue wet wt. CS activity displayed a pattern of weight dependence very similar to that observed for respiration rate (Fig. 5a), quantified by the formula:

$$CS = 0.755W^{-0.170}, \quad r = -0.708, \quad n = 90,$$

where *CS* is citrate synthase activity (U g^{-1} wet wt), *W* is wet tissue weight (g), *r* is

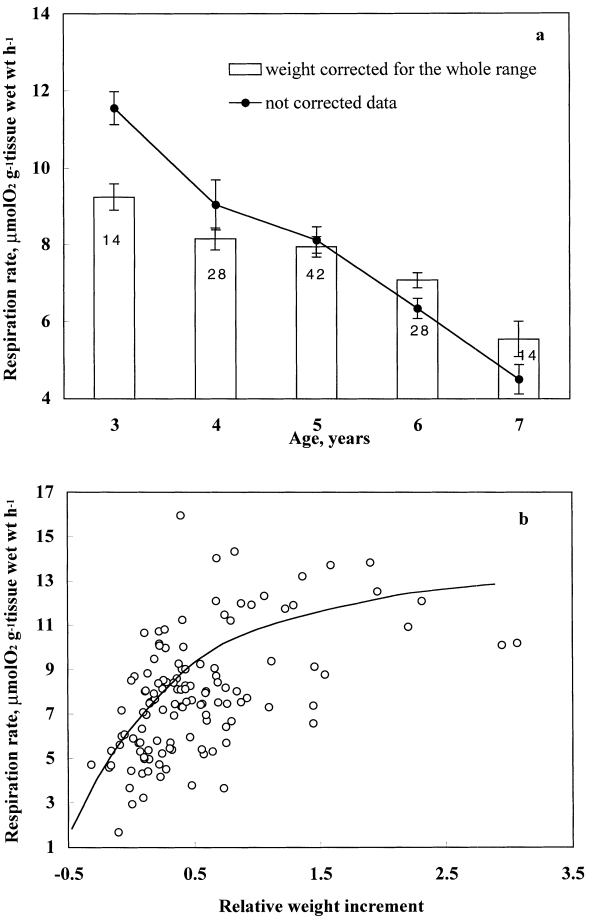

Fig. 4. Weight corrected respiration rate in mussels of different age (a). Weight correction was performed for mean tissue weight for the whole experimental stock of mussels (see text). Not corrected data are also shown. *N* is shown by figures in the columns. Vertical bars represent standard errors. Respiration rate without weight correction as a function of relative weight increment (b).

correlation coefficient, *n* is number of specimens. This regression calculated for 5 years old mussels has the following equation:

$$CS = 0.815W^{-0.121}, \quad r = -0.556, \quad n = 33.$$

Further calculations were made using weight corrected CS activity values. Corrections were performed in the same way as with respiration rate using the power coefficient -0.121.

No significant age-dependence of weight-corrected CS activity was found in Summer sample (Fig. 5b–d). This may be because only five age classes represent the Summer sample. Taking together all data from both summer and autumn sample sets to increase the power of analysis a two-step decrease of CS activity with increasing age can be seen

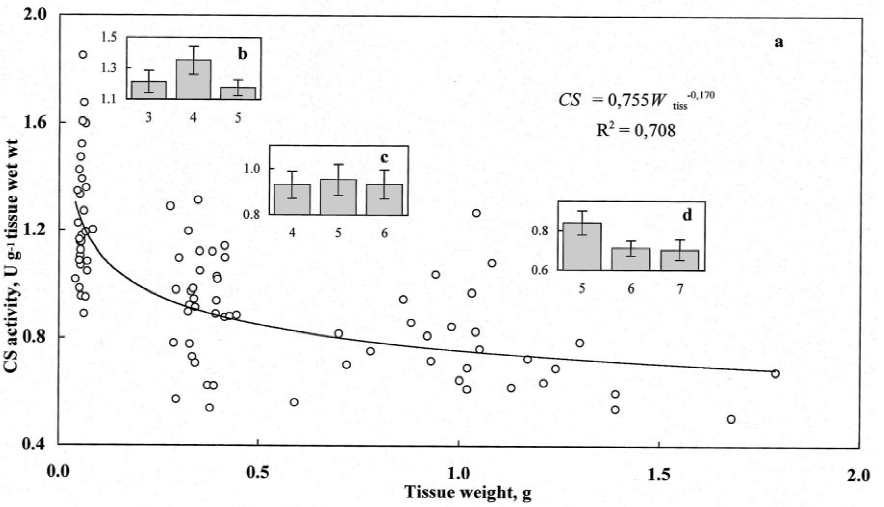

Fig. 5. Activity of citrate synthase in mussels related to tissue weight (a). Age-specific CS activities in Small (b), Medium (c), and Large (d) size groups. In (b–d): *x*-axis is age of mussels (years), *y*-axis is activity of citrate synthase (U g^{-1} tissue wet wt) corrected for mean tissue weight of each size group (see text). Vertical bars represent standard errors, $n=7–10$.

(Fig. 6a). The first drop occurs between ages 2 and 3 and then after 6–7 years. Mussels from Summer sample cover the interval with constant CS activities. Post-hoc comparisons show a significant difference ($P<0.03$) between CS activities in mussels 2 and 8 years old. Not weight corrected data decrease gradually with age reflecting not age- but mainly size-dependence of CS activity.

Like respiration rate CS activity without weight correction also correlated with *RI* (Spearman $R=0.471$, $n=90$, $P<0.001$) (Fig. 6b). After weight correction the correlation remained significant ($R=0.267$, $n=90$, $P<0.05$).

3.5. ATP concentration

Mean ATP concentrations in mussels were different in the Summer sample (mean = 0.92 ± 0.034, S.E., $n=37$) and the Autumn sample (mean $=1.98\pm0.16$, S.E., $n=32$) which prevented merging the data. Data on ATP levels were tested for size effects which were non-significant (ANOVA, regression analysis). ANOVA did not show a significant age dependence of [ATP] in mussels from the Summer sample (Fig. 7). In the Autumn sample, ATP levels were about two times higher than in summer. In contrast to the Summer sample, the age effect in Autumn was statistically significant at $P<0.05$ (ANOVA). ATP levels were between 2 and 3 μmol g^{-1} tissue wet wt in mussels from 2 to 5 years old and decreased gradually to 0.72 μmol g^{-1} tissue wet wt in 9 year old specimens (Fig. 7).

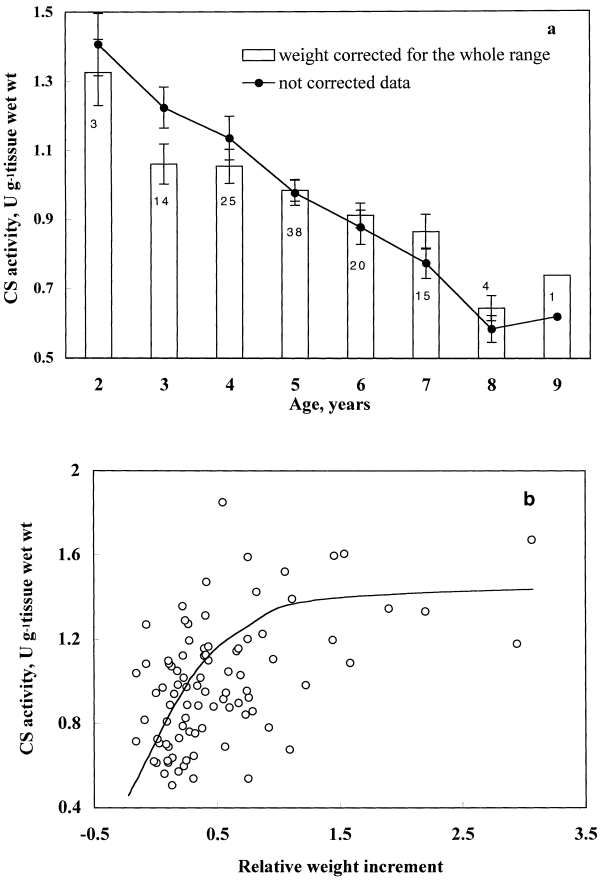

Fig. 6. Citrate synthase activity as a function of age (a) and relative weight increment (b). Weight correction was performed for mean tissue weight for the whole experimental stock of mussels (see text). Not corrected data are also shown. The number of animals included in the sample (*N*) is displayed inside the columns. Vertical bars represent standard errors.

3.6. Phosphagen concentrations

Average concentrations of phospho-L-arginine (PLA) and L-arginine (LA) in summer mussels were 2–3 times lower than those recorded for autumn animals (Table 1). Therefore the data were treated separately. In the Summer sample, the mean concentration of PLA was about 2.5 μmol g^{-1} tissue wet wt., LA 2 μmol g^{-1} tissue wet wt and R_{PLA} 0.55 (Table 1). No significant size- and age-dependence was found in the Summer sample (ANOVA) (Fig. 8a).

Phosphagen concentrations in mussels from the Autumn sample were extremely variable between 1 and 11–12 μmol g^{-1} tissue wet wt and strongly exceeded phosphagen levels in summer. The relative amount of PLA (R_{PLA}) was about 0.49

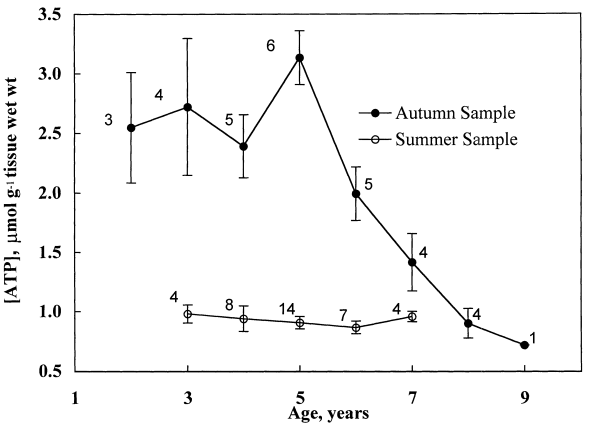

Fig. 7. ATP concentrations in mussels of different age compared between Summer and Autumn samples (see text). Vertical bars represent standard errors. *N* is given close to the symbols.

which did not differ significantly from the value 0.55 recorded for summer animals (Table 1). PLA concentration varied in narrow range (around 7 μmol g^{-1} tissue wet wt) in mussels between 2 and 6 years old and was reduced to 1 μmol g^{-1} tissue wet wt in 9 years old animals (Fig. 8a). Age dependence of [PLA] was statistically significant at $P<0.020$ (ANOVA). R_{PLA} values in L mussels were slightly (ANOVA, $P<0.01$) higher than in M ones. In both size groups R_{PLA} decreased with age (Fig. 8b).

3.7. Intracellular pH

pH_i measured by the homogenate technique was 6.80 ± 0.013 (S.E., $n=26$) in control mussels and varied in a narrow range between 6.68 and 6.97. Mean pH_i decreased significantly ($P<0.001$) during air exposure. For a clear picture of the age dependence of pH_i we combined all our data on control animals from summer and autumn samples. These data sets did not differ at $P>0.05$ (Tukey's HSD). No size effect was recorded for pH_i values. The analysis shows a significant decrease in the control values of pH_i with increasing age (Fig. 9). The figure gives evidence that pH_i values are similar in animals younger than 6 years, whereas a drastic fall in pH_i occurs thereafter. pH_i after

Table 1
Mean concentrations of phosphagens (μmol g^{-1} tissue wet wt). and relative amount of PLA in mussels from Small, Medium and Large size groups (S.E. of mean values are in parentheses)

	Summer sample			Autumn sample	
	S	M	L	M	L
PLA	2.92 (0.17)	2.47 (0.27)	2.37 (0.22)	6.71 (0.12)	5.18 (0.11)
LA	1.68 (0.14)	2.01 (0.15)	1.32 (0.15)	8.01 (0.18)	4.53 (0.19)
R_{PLA}	0.64 (0.03)	0.45 (0.02)	0.64 (0.02)	0.46 (0.004)	0.51 (0.005)

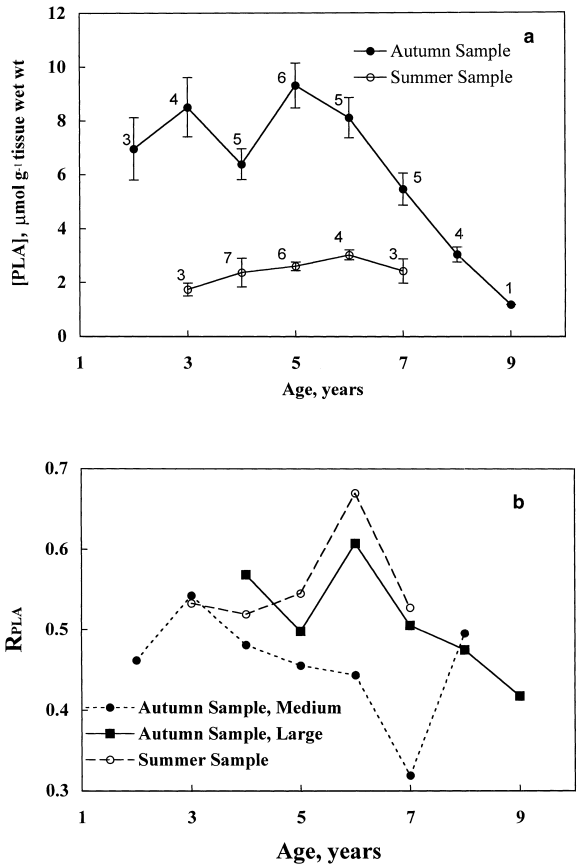

Fig. 8. Concentrations of phospho-L-arginine (a) and the relative amount of PLA (R_{PLA}) (b) in mussels of different age from Summer and Autumn samples (see text). Vertical bars represent standard errors. *N* is given near the symbols.

air exposure did not reveal a dependence on age in the range investigated, however, the pH_i drop seen during air exposure was significantly larger in young than in old animals.

4. Discussion

High variability of growth rate in natural mussel settlements gave a possibility to collect the material, allowing to separate size- and age-effects on the ecophysiology of the animals. The results obtained in the present study demonstrate a pronounced age-dependence in nearly all studied parameters, except growth rate. The most prominent feature of this relationship was that the effect became clear only when the animals reached a certain 'critical' age, which in the studied population was about 7 years.

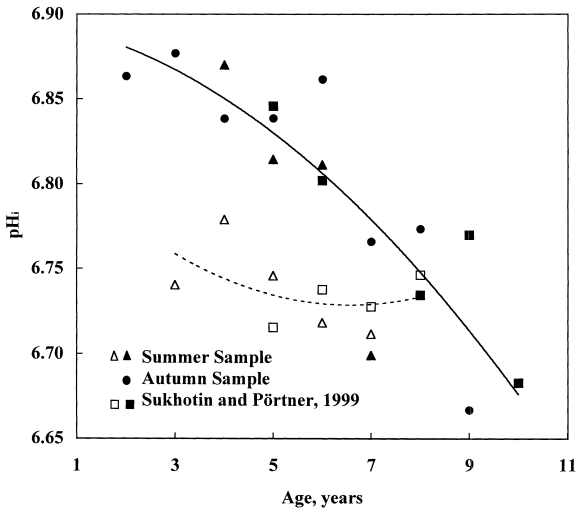

Fig. 9. Intracellular pH in mussels of different age. Filled symbols, solid line: control mussels; open symbols, dashed line: mussels after air exposure.

Asymptotic growth is typical for *M. edulis* like for the majority of bivalve species (for review, see Seed and Suchanek, 1992). In consequence a decrease of absolute and relative growth rates is observed in ageing mussels. In the present study the relative growth increment *RI* was not affected by body size. For example, 5 years old mussels increased their flesh weight during their 5th growth season by 40% independent of absolute individual growth rates and size reached at this time. This gives evidence for the 'pure' age dependence of the measured parameter. Unlike the other measured parameters *RI* decreased rapidly in younger specimens and did not change significantly in those older than 5 years.

The allometric equation describing the weight-dependence of respiration rate in the White Sea *Mytilus edulis* does not differ significantly from those published previously (Krüger, 1961; Bayne et al., 1978; Hamburger et al., 1983; Sukhotin, 1992). Weight corrected respiration rates demonstrated a statistically significant gradual decrease with increasing age in S and L groups. In the M group, this parameter was maximal at 5 years and decreased by about 9% between age 5 and 6 (Fig. 3c). Where observed the relative drop in respiration between age classes occurred by between 8–9% (S) and 13–16% (L) per year. Respiration rate corrected for the whole range of tissue wet weight decreased steadily by $\approx$40% between ages of 3 and 7 years. The most considerable drop was observed after 6 years of age. Some authors argue that age influences respiration rate in ectotherms only indirectly, due to the age-related gain in body size (Alimov, 1981). However, our data demonstrate that age can have an effect on respiration regardless of animal size. Similar results were obtained by Zolotarev and Ryabushko (1977) in *Crenomytilus grayanus* from the Japan Sea. In that study, the oxygen consumption rate was higher in specimens 0.5 years old than in mussels 1–2 years old and of the same weight. In *C. grayanus*, an age increment from about 20 to 60–70 years caused a

progressive 2–3-fold reduction in respiration rate. Golubev (1975) reported a decreased respiration rate in the brachiopod *Streptocephalis forvicornis* when the animals had reached their final size. Oxygen consumption of an older cohort of bay scallops *Argopecten irradians irradians* was by 30% lower than that of a younger cohort (Bricelj et al., 1987).

Relatively low metabolic rates in small mussels 5 years old compared to the same age class in M and L groups (Fig. 3b) may be due to reduced growth rates. The average length of 5 years old mussels in the studied population in summer 1997 was 25–27 mm (Fig. 1) and only 6% of all specimens of this age fell in the size range of the S group (12.5–15.2 mm). Therefore, S animals fell strongly behind the others in growth rate. Reduced metabolism and growth may be caused by the individual genotype (for review, see Hawkins and Bayne, 1992; Hawkins and Day, 1996).

At first sight citrate synthase activity in *M. edulis* is among the lowest found in marine invertebrates (Sugden and Newsholme, 1975; Michaelidis and Beis, 1990; Dahlhoff and Menge, 1996; Thuesen et al., 1998). However, enzyme activities are traditionally analysed at temperatures between 20 and 25°C. Considering a Q_{10} between 2 and 3 CS activity in White Sea mussels amounts to between 2 and 3 U g^{-1} tissue wet wt which is in the range reported for some marine benthic species.

The regression of CS activity versus tissue weight was very similar to that obtained for respiration rate. Allometric exponents for both regressions are close however somewhat smaller for CS at $P=0.05$. A similar scaling of CS was recorded for skeletal muscles of mammals (Hochachka et al., 1988) and fish (Somero and Childress, 1980). In a recent paper, Burness et al. (1999) reported a highly significant negative relationship between CS activity and body size in yearlings of rainbow trout. However, inclusion of five fishes in the analysis, which possessed high enzyme activity but were older than 1 year, made the whole correlation insignificant. Training effects might be involved in this case. In our study, CS activity decreased with body size over the whole size range studied. Also older animals in our study were characterised by a somewhat lower activity of citrate synthase. However, we did not use mussels younger than 2 years in the present study, therefore, we cannot extrapolate the obtained relationships to these early stages. Hochachka and co-workers (1981, 1988) argued that enzymes scaling with exponents less than those obtained for the change in respiration with body size cannot determine the latter because as body mass increases the catalytic potential is in excess of the one that is needed to pace flux. The decrease in the activity of citrate synthase in older specimens, however, coincides with a pronounced decrease in respiration in 7 years old mussels. This likely indicates a decrease in mitochondrial density and/or oxidative capacity causing a decrease in aerobic metabolism after the animal reached a certain age.

The change in absolute and relative amounts of high energy compounds in the tissues reflects not only an energetic stress of the organism but also their dependence on temperature and metabolic rate. The ratio of [PLA]/[LA]+[PLA] remained constant throughout and indicates that stress was minimal in summer specimens. ATP levels observed in mussels from the Summer sample were about two times lower than those published for *Mytilus edulis* (Addink and Veenhof, 1975; Wijsman, 1976; Ansell, 1977) and obtained for animals from the Autumn sample. Both LA and PLA concentrations

also appeared to be 2.5–3 times higher in autumn than in summer. We suggest that this difference might be caused by the seasonal changes in energetic state of the animals, possibly related to the thermal history of the animals. Seasonal fluctuations in both aerobic (Iglesias and Navarro, 1991; Hatcher et al., 1997) and anaerobic (Ahmad and Chaplin, 1979; Kluytmans et al., 1980; Pörtner et al., 1986; Moal et al., 1989) metabolism in marine benthos have been widely published. The amounts of high energy phosphates in tissues may also be related to the reproductive cycle and to nutritive variations (Ebberink et al., 1979; Zurburg and Ebberink, 1981; Moal et al., 1987).

ATP levels fell with increasing age above 5 years in the Autumn sample. The reduction of the total phosphagen/aphosphagen pool and of R_{PLA} with increasing age after 6 years was shown only for autumn specimens and was statistically valid in large animals possessing high growth rate. Since the amounts of ATP and phosphagens were strongly variable depending on both environmental conditions and reproductive state of the organism it is likely that in late summer season size and age differences are disguised by a total decrease of energy reserves. The age dependence of [ATP] and [phosphagens] rises in autumn based on higher absolute levels of these substances. This may be related to the onset of slow gametogenesis (Chipperfield, 1953; Maximovich, 1985) at reduced ambient water temperatures in the fall. The obvious depletion of high energy phosphates in senescent individuals goes hand in hand with a decreased rate of energy metabolism. General slowing of cell metabolism with increasing age has been shown for ageing humans, rats, some insects (Farooqui et al., 1987; for review, see McCarter, 1995). A decrease in glutathione synthesis was discussed to be related to the lower capacity of older cells to cope with increased energy requirements for the regeneration of glutathione or the replacement of oxidized cellular components in *Mytilus edulis* (Canesi and Viarengo, 1997).

Intracellular pH is considered to be one of the important factors reflecting or even controlling metabolic rate. The decrease of pH_i values during air exposure indicates the onset of anaerobiosis, depression of metabolic rate and accumulation of acidic end products (for review, see Pörtner, 1993; Grieshaber et al., 1994). In control animals whole body mean pH_i varied between 6.66 and 6.97, at lower values than published elsewhere (Wijsman, 1975; Walsh et al., 1984; Zange et al., 1990). Possible reasons are discussed elsewhere (Sukhotin and Pörtner, 1999). The lowest values were characteristic for animals older than 6 years. During emersion pH_i decreased on average by 0.1 units in mussels 3–6 years old, by 0.05 at the age of 7 years and not at all in animals 8 years old (Fig. 9). The lowest pH_i values in control mussels were 6.65, which are probably close to the lower limit. For comparison, pH in the foot muscle of *M. edulis* decreased rapidly from 6.95 to 6.65 during the first 5 h of air exposure and reached a value of about 6.5 within 7 days (Wijsman, 1975). Eight hours of air exposure led to a fall of pH_i in the whole body of mussels by about 0.35 units and in the mantle tissue by nearly 0.9 units reaching a low value of 6.27 (Walsh et al., 1984).

The present data give evidence that aerobic metabolic rate in *M. edulis* from the studied population declines when the animals reach an age of about 7 years (Fig. 3a). The decrease in oxygen consumption reflects the drop in mitochondrial respiration, which is mirrored by the decrease in CS activity (Fig. 4b). A concomitant fall in ATP turnover may include a downregulation of the mechanisms of acid–base regulation. pH_i

will then approach the equilibrium indicated by lower pH_i values in older animals. Furthermore, lower rates of anaerobic metabolism may explain why pH_i remains constant during air exposure in older specimens. Field data support the conclusion that the obvious decrease in aerobic metabolic rate, which starts at a certain age of the mussels, indicates the onset of the final phase of the ageing process. The density reduction of 7–9 years old mussels from spring to autumn (Fig. 1) reflects the increased natural mortality in these age classes. Growth rates in mussels from the studied population dropped drastically at the age of 6 years (Fig. 1). The age-related decrease of growth, ingestion and assimilation rates are well-described phenomena in molluscs (for review, see Bayne and Newell, 1983). Usually, both gross and net growth efficiencies decrease progressively with age (Aldridge et al., 1986; Iglesias and Navarro, 1991). In contrast, the obtained results demonstrate that the decrease in metabolic rate sets in only after 6–7 years of age. The longevity of mussels is a highly variable parameter, which depends on both environmental conditions and genetic peculiarities (for review, see Seed and Suchanek, 1992; Gosling, 1992). In the White Sea the maximal lifespan of *M. edulis* varies between 5 and about 20 years (Savilov, 1953). In the studied population, mussels start to get old at 6–7 years with a maximum age of about 10 years. The age at which the onset of metabolic depression can be recorded and the duration of the ageing period may be different between populations depending on local environmental conditions. The mechanisms starting the process of age related metabolic depression are still unclear and require further investigation.

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