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Influence of age and size on pumping activity and stress resistance in the marine bivalve *Mytilus edulis* L.

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Abstract

We studied age and size dependence of pumping and respiration rates, mortality during prolonged air exposure and the changes in behavioural response to cyclic salinity fluctuations combined with elevated temperatures in the White Sea blue mussels Mytilus edulis L. Five age groups (2-9 years) and three size groups: small (S, mean wet tissue mass 0.12 g), medium (M, mean wet tissue mass 0.39 g) and large (L, mean wet tissue mass 0.78 g) were analysed. Respiration rate was measured in closed respirometers. Pumping rate was determined by a laser beam to monitor the changes in water level when the inhalant and exhalant siphons were in separate compartments of the aquarium. To study their response to environmental stress, the mussels were subjected to cyclic salinity fluctuations at normal (10 °C) and elevated (20 °C) temperatures. Two parameters were noted: (1) the salinity at which shell valves either opened or closed at both temperatures, and (2) intraindividual variation of the behavioural response to salinity changes at both temperatures. Size and age affect physiological processes in the blue mussel. The responses of M. edulis to environmental stress including low salinity, elevated temperature and air exposure as well as respiration rate are generally size-dependent. Effects of age on sensitivity to low salinity and survival in air are minor. Pumping rates of mussels are significantly affected by both size and age of the animals. The disproportional decrease of pumping rate is observed at the age of about 7-8 years old. © 2002 Elsevier Science B.V. All rights reserved.

Keywords: Age-dependent differences; Size effects; Respiration rate; Pumping rate; Low salinity stress; Behavioural response; Survival in air; Mussels; Mytilus edulis; White Sea

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1. Introduction

Blue mussels *Mytilus edulis* L. are long-lived molluscs with a maximal longevity of more than 15–20 years (Savilov, 1953; Theisen, 1973; Zolotarev, 1989; Heller, 1990). Maximal life span and mortality of mussels in natural populations depend upon both environmental factors like predation pressure (Seed and Suchanek, 1992; Hilgerloh, 1997; Nehls et al., 1997) and intrinsic factors such as an increasing unbalance between energy requirements and energy acquisition or genetic differences in suscept-ibility to non-specific stresses (Freeman and Dickie, 1979; Bayne and Newell, 1983; Hawkins et al., 1986; Mallet et al., 1990; Pellerin-Massicotte, 1993; Tremblay et al., 1998).

In the course of ageing, many animals including mussels become more susceptible to oxidative stress (Viarengo et al., 1991; Canesi and Viarengo, 1997), a tendency upon which "Free Radical" theory of ageing is based (Harman, 1956; Sohal, 1986; for review see Emerit and Chance, 1992). However, it is not clear if senescent animals are more sensitive than younger ones to such environmental stress factors as prolonged air exposure, low/high salinity and temperature. The "survival in air" response was successfully used in mussels as a general stress index to evaluate the impact of contamination in coastal areas (Smaal et al., 1991; Eertman et al., 1993; Viarengo et al., 1995). If senescence in mussels is accompanied by the general weakening of the organism, higher sensitivity to non-specific stress factors may be expected.

Intra-individual variation of morphological characters, usually determined by fluctuating asymmetry, is frequently used as a measure of fitness and stress of populations and individuals (Zakharov, 1989; Graham et al., 1993; Leung and Forbes, 1996). It is known that fluctuating asymmetry is normally increased under unusual or unfavourable environmental conditions. We have applied this principle to the variation of behavioural response of mussels to reductions in salinity and found that intra-individual variation increased in response to abrupt environmental changes (Lajus and Sukhotin, 1998). We might expect that salinity sensitivity is also associated with ageing.

The objective of the present study was to find out how the respiration, water pumping activity as well as the susceptibility of blue mussels to environmental stresses change in the course of ageing. Prolonged air exposure, low salinity and combined action of low salinity and elevated temperature were used as stress factors. The special aim was to distinguish mere age from size effects on the studied parameters basing on a great variability of growth rates in blue mussels from the White Sea natural populations.

2. Material and methods

2.1. Animals

Blue mussels, *M. edulis* L., were collected in August, 2000 from an intertidal mussel bed situated in the 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 about +0.7 m level, where the emersion period comprises ca. 20% of the tidal cycle. After sampling, mussels were sorted by size and age in accordance with the experimental design. The collected mussels were divided in three size groups: small (S, wet tissue mass = 0.12 ± 0.004 g, shell length 17–22 mm), medium (M, wet tissue mass = 0.39 ± 0.013 g, shell length 28–35 mm) and large (L, wet tissue mass = 0.78 ± 0.037 g, shell length 40–50 mm). Mussels in the S group were between 2 and 8 years old, in M group 2 and 9 years old and in L group 4 and 10 years old. The age of the mussels was determined by counting the rings of winter growth delays on the shells. This method has previously been validated in studies with White Sea mussels, which compared the number of internal rings and the seasonal growth of mussels marked individually (Chemodanov and Maximovich, 1983; Sirenko and Saranchova, 1985). Animals were kept for 3 days in 50-1 static aquaria in natural sea water at 25 % salinity and +10 °C, which corresponded to the ambient temperature and salinity values. No special food was added. All the experiments were performed at the White Sea Biological Station of the Zoological Institute of Russian Academy of Sciences.

2.2. Air exposure experiment

Mussels were placed in flat 25×35 cm trays filled with fine gravel (5–8 mm in diameter). Gravel was covered with cellulose tissue paper and the mussels were placed over it. Water was added to the trays so that the paper was always wet, maintaining constant humidity around the mussels. The experiment was performed at 10 °C. Each tray contained 30–50 mussels belonging to one age–size group. S group mussels were separated into four age classes: 2, 4, 6 and 8 years old, the M group contained 4-, 6- and 8-years-old mussels, while the L group had only 6- and 8-year-old animals. Mussels were checked once a day, dead animals were counted and removed from the trays. Mussels were considered dead if the shell was open and the animal did not respond when the foot or mantle edge was touched with a needle.

2.3. Response to salinity and temperature changes

The experiment on the assessment of mussels' reaction to salinity and temperature changes was carried out on 88 animals of the following age-size groups: S group-2, 4, 6 and 8 years old, M group 2, 4, 6 and 8 years old, and L group-4, 6 and 8 years old. Each age-size group contained 5–11 specimens. The basic idea of the experiment was to follow an organism's response to the gradual change of an environmental factor as proposed by Fry (1971) and modified for salinity by Ivanchenko and Lajus (1985). All 88 mussels were subjected simultaneously to stepwise decreases and increases of salinity as described in Lajus and Sukhotin (1998). The experiment started with the control salinity (25‰), where all animals were open. Salinity was then lowered by 1‰ per 10 min until all the mussels closed their valves (isolation response). A mollusc was considered closed if the edges of its mantle were not visible. Immediately after all the molluscs closed their valves, salinity was increased at the same rate until all animals

opened the shells. Each cycle contained two half-cycles of decreasing and increasing salinity, respectively, and lasted for approximately 1.5 h. Altogether, six cycles were conducted at the control temperature (+10 $^{\circ}$ C). The temperature was then quickly increased to +20 $^{\circ}$ C, and four further cycles were performed. Thus, for each animal we obtained:

- the mean values of salinity at which closing/opening of shell valves (threshold salinity) was recorded at +10 and +20 °C (SAL₁₀ and SAL₂₀);
- repeatability of the threshold salinity among cycles at +10 and +20 °C (VAR₁₀ and VAR₂₀). As the threshold salinity depended on cycle number (see Results section), repeatability was calculated as the variance of residuals of individual linear regression of threshold salinity on cycle number. This reflected intra-individual variation of behavioural response to salinity fluctuations;
- difference between mean salinity at +10 °C and salinity of opening/closing at first observation at +20 °C (SAL₂₀₋₁₀);
- difference between VAR₂₀ and VAR₁₀ (VAR₂₀₋₁₀).

 SAL_{20-10} and VAR_{20-10} were used as a measure of temperature effects on behavioural responses.

2.4. Respirometry

Oxygen consumption rates (RR') were measured in closed respirometers of 200-500 ml volume at +10 °C. After an exposure period of 60 to 90 min in the respirometer, water samples were carefully drained from the chamber into air-tight bottles. Oxygen concentration was determined according to Winkler as described in Strickland and Parsons (1968). Oxygen consumption was calculated from the difference between oxygen concentration in the experimental chambers and control chambers, which were similarly exposed but contained no animals. Mass-specific respiration rate was expressed as μ mol O₂ g⁻¹ tissue wet wt h⁻¹. Respiration rate was determined individually in 74 mussels of all three size groups including the following age classes: S—2, 4, 6, 8 years, M and L—4, 6, 8 and 9 years old.

2.5. Pumping rate

Pumping rate of mussels was measured using a system separating the exhalant and inhalant water currents according to Famme et al. (1986). The set-up consisted of a plexiglas 4-1 aquarium divided by a rubber membrane into two chambers. The mussel was inserted in a slit in the membrane in such a way that the exhalant and inhalant siphons were in the different chambers. As the mussels pumped water from one chamber to the other, the change of the water level was recorded using a reflected laser ray. Measurements of pumping rate of individual mussels started after reaching constant levels of pumping rate was determined in 10-12 replicates on the same mussel. Pumping rate was determined in 52 mussels of same age-size groups as for respiration rates. The accuracy of pumping rate determination by this method was 0.1 ml min⁻¹.

2.6. Calculations and statistics

Mortality curves from the air exposure experiment were linearized using the probit transformation as described in Bliss (1935a). The regression lines for all age-size groups were compared in pairs by sequential comparisons of slopes using Student's *t*-test. In case slopes did not differ, intercepts were compared (Bliss, 1935b; Glotov et al., 1982; Sokal and Rohlf, 1995).

Time when 50% (LT_{50}) and 99% (LT_{99}) mortality was reached was calculated using the regression lines to establish the abscissa when the ordinate was 5.0 and 7.3263 probits, respectively. Standard errors for LT_{50} and LT_{99} were calculated as the SE's for respective *Y* values on the regression lines (Bliss, 1935b; Glotov et al., 1982; Sokal and Rohlf, 1995). These parameters were compared for all age–size groups using Student's *t*-test with Bonferroni correction for multiple comparisons (Rice, 1989).

Given the negative size dependency of respiration (RR) and pumping (PR) rates were corrected for the corresponding mean mass values in each size group using the regression coefficients (*b*) -0.148 for RR (Sukhotin and Pörtner, 2001) and -0.234 for PR (see Results section), according to the equation: $R = R'(W_{\text{mean}}/W)^b$, where *R'* and *R* are observed and corrected rates, respectively, *W*—observed tissue wet mass, *W*_{mean}—mean group tissue wet mass. For comparisons among all the experimental animals, RR' and PR' were corrected for the overall mean mass of 0.432 g wet tissue using the same power coefficients.

One-way ANOVA was used for analysing the effects of age and size separately on the studied parameters. Post-hoc comparisons were made by Tukey's HSD Test for unequal N. Correlations were calculated using Spearman's non-parametric correlation coefficients. If not specially noted, mean values \pm SE's are presented.

3. Results

3.1. Mortality in air

The mortality experiment lasted for 27 days when all the mussels exposed to air had died. Regression lines of mortality probits vs. time did not differ at p < 0.05 for 4- and 6-year-old mussels from S group and in 6- and 8-year-old specimens from M group. The other lines were different (results of pairwise comparisons are not shown), however no consistent between-group patterns were found. LT₅₀ varied between 6 and 11 days, while LT₉₉ varied between 14.4 and 33.8 days.

Mortality of mussels in air was influenced by the size of the animals. L mussels demonstrated significantly lower LT_{50} and LT_{99} (p < 0.05, Student's *t*-test), than either S or M specimens (Fig. 1). Age effect on mortality in air was not clearly pronounced. However, in general, the change of both LT_{50} and LT_{99} with age was similar. The highest values were observed in mussels of intermediate ages, while in young and old specimens LT_{50} and LT_{99} somewhat decreased (Fig. 2). The exception was the unexpectedly high LT_{99} in 2-year-old S mussels.



Fig. 1. Mortality in air of mussels of different size groups (age groups are combined). X-axis: days from the beginning of the experiment; Y-axis: mortality.

3.2. Response to low salinity

Decrease of water salinity below the critical values caused the isolating response in mussels, e.g. closing the mantle cavity, retraction of siphons and closing the shell valves (see for review Ginetsinskiy, 1963). Gradual increases of salinity led the



Fig. 2. Age-dependence of LT₅₀ (left Y-axis) and LT₉₉ (right Y-axis) in mussels (size groups are combined).

mussels to open their shells and to resume respiration and pumping activity. During the experiment, the repeated cycles of decreasing and increasing salinity led to a gradual shift of the "critical" (threshold) salinity for each mussel to lower values. In average the "critical" salinity at +10 °C decreased by 1‰ from 11.3‰ to 10.3‰ (Fig. 3) over five cycles (8 h). According to the trend lines, after the increase of temperature to +20 °C, the "critical" salinity reached the value of 11.2‰ and then after 3.5 cycles (ca. 5 h) again decreased to 10.7‰. The "critical" salinity was highly individual-specific: correlation between an individual's "critical" salinities for two consecutive trials ranged from r=0.49 to r=0.80 (p<0.001 in all cases) with average value r equal to 0.61. Also, the individuals showed high positive correlations (r=+0.746, p<0.001, n=88) between the "critical" salinity values at 10 and 20°C.

Size of the mussels was a major factor influencing the response to low salinity and temperature. The effect of size on SAL₁₀, SAL₂₀ and SAL₂₀₋₁₀ was valid at p=0.069, p=0.023 and p=0.050, respectively (ANOVA). Neither of these parameters differed between mussels from M and S size groups while L specimens were characterised by significantly lower values of SAL₁₀, SAL₂₀ and SAL₂₀₋₁₀ (Fig. 4a). VAR₁₀ was not affected by size of the mussels (p<0.824, ANOVA) (Fig. 4b). In contrast, VAR₂₀ and, therefore, VAR₂₀₋₁₀ significantly increased with increasing size of mussels (p<0.029 and p<0.039, respectively) (Fig. 4b).

Age did not determine the "sensitivity" of mussels to low salinity and elevated temperature. Nevertheless, in all size groups, a decline of both SAL_{10} and SAL_{20} with age was observed except for 4-year-old mussels from L group, which displayed somewhat lower "critical" salinity values than 6-year-old ones.



Fig. 3. Threshold salinity of closing/opening the shells of mussels at +10 °C (filled symbols) and +20 °C (open symbols). All age-size groups are combined.



Fig. 4. Threshold salinity of closing/opening the shells (a) and variance of individual salinity values (b) of mussels of Small (S), Medium (M) and Large (L) size groups.

3.3. Respiration rate

Respiration rates of mussels varied from 2 to 17 μ mol O₂ h⁻¹ g⁻¹ wet wt. No significant age effect was observed within L (ANOVA, p < 0.782) and S (ANOVA, p < 0.277) size groups. In M group the effect of age was significant at p < 0.009 (ANOVA), because 6-year-old animals showed higher respiration rate, than 8-year-old ones (p < 0.005, Tukey's HSD). However, in the overall sample, respiration corrected for the mean size was not significantly affected by age of the mussels. (ANOVA, p < 0.360) (Fig. 5).



Fig. 5. Respiration and pumping rates of mussels of different ages corrected for the whole sample mean tissue mass.

3.4. Pumping rate

Mass specific pumping rate (PR) varied by nearly two orders of magnitude in the studied mussels and was between 0.07 and 6.2 1 $h^{-1} g^{-1}$ wet wt. This parameter decreased with increasing tissue mass (*W*, g) of the mussels in accordance with a power function

$$PR = 1.11W^{-0.482}, \ r = -0.436, \ n = 51.$$

This regression includes animals of different sizes and ages and therefore reflects both size and age effects on pumping rate of mussels. In order to divide these two factors, separate regressions of the pumping rate vs. mass were calculated for mussels of different age:

 $PR = 2.23W^{-0.196}$, r = -0.421, n = 15 for mussels 4 years old,

 $PR = 2.01W^{-0.234}$, r = -0.348, n = 16 for mussels 6 years old,

 $PR = 0.47W^{-0.879}$, r = -0.533, n = 15 for mussels 8 - 9 years old.

Regression lines for 4- and 6-year-old mussels did not differ at p < 0.001 while the line for the oldest animals was different (p < 0.05) (Fig. 6a). The power coefficient decreased with the age of animals.

The age effect on PR in separate size groups was not significant (ANOVA, p=0.57, p=0.20 and p=0.37 for S, M and L groups, respectively). However, in



Fig. 6. Pumping rate in mussels as a function of wet tissue mass (a) and age (b). Different age (a) and size (b) groups are given separately. N is shown close to the values.

the mussels in M and L groups, a 2–2.5-fold reduction of the pumping rate was observed with age, while in S group the youngest and the oldest specimens showed similar pumping activity (Fig. 6b). In the whole sample, age dependence of the mass-corrected pumping rate was significant at p < 0.020 (ANOVA). PR drastically decreased in mussels between 6 and 8 years of age, while 2-year-old animals demonstrated intermediate values (Fig. 5).

4. Discussion

4.1. Air exposure and decreasing salinity stresses

M. edulis like many other intertidal molluscs uses a complex of behavioural, physiological and biochemical mechanisms to withstand prolonged periods of unfav-

ourable environmental conditions such as air exposure or extreme salinity changes. Thus, blue mussels can survive in air for 10-14 days at temperature varying between 10 and 20 °C and longer at lower temperatures (Alyakrinskaya, 1972; Golikov and Smirnova, 1974; Hummel et al., 1989). In our experiment at 10 °C, the mean LT₉₉ was 23 days. Large mussels appeared to be more susceptible to air exposure, than medium and small ones, which may be due to the higher absolute values of metabolic rate in the former than in the latter mussels. On the other hand, the size effect on survival in air was not observed in M and S mussels despite more than 3-fold differences in tissue mass between these groups.

Age did not significantly affect mussels' survival in air. However, 6-year-old mussels demonstrated the highest values of LT_{50} and LT_{99} in the three size groups. Six years is an intermediate age for mussels from the studied population. According to the Pelseneer–Shelford rule (Shelford, 1915), in many species stress resistance increases with developmental stage of the animal (Berger and Lebsky, 1969; Hrs-Brenko, 1978; Chen and Richardson, 1987). Probably, resistance to air exposure increases further and reaches a maximum at an age when senscence slows or reverses this increase. Similarly, age did not influence survival of blue mussels *M. edulis* (Hole et al., 1993) and Antarctic scallop *Adamussium colbecki* (Viarengo et al., 1993) exposed to high concentrations of copper and cadmium, respectively. Hole et al. (1993) found that although the susceptibility to stress was age-independent in the blue mussels, their ability to recover declined with the age.

Mean annual salinity of the surface waters in the Kandalaksha Bay of the White Sea is about 24–25 ‰, which is much lower than open ocean values. An isolating response in the White Sea blue mussels is normally observed when salinity falls below 12-14‰ (Lukanin and Gurina, 1977; Berger and Lukanin, 1985; Lajus and Sukhotin, 1998). This threshold may be shifted by preliminary acclimation to higher or lower salinity. This well-documented shift in salinity tolerance is found in a great number of euryhaline species including M. edulis and is brought about by efficient cellular and whole-organism compensatory mechanisms allowing the animals to adjust to a persistent change in the environmental salinity (for review, see Kinne, 1971; Berger, 1986; Berger and Kharazova, 1997). The progressive decrease of the "critical" salinity at +10 °C and later at +20 °C during the repeating salinity cycles can be explained by at least two reasons. Firstly, it may reflect the acclimation of mussels to low salinity. For example, acclimation of *M. edulis* from the White Sea to 10% and to 40% for a month shifts the thresholds of the isolating response to less than 8% and to 20%, respectively (Lukanin and Gurina, 1977). It is unclear whether the 8-h acclimation used in our experiments was sufficient for shifting the "critical" threshold by ca. 1%. However, it is worth noting that acclimation can be especially quick at the initial stages (Berger, 1986). Second, there may be a progressive accumulation of an oxygen debt in the mussels. Being oxygen limited during shell closure, mussels may seek to minimise the inactive period and to start respiration as soon as possible. Thus, the steeper decline in the "critical" salinity threshold at the elevated temperature may reflect the increased metabolic requirements of the experimental animals.

Positive correlation between SAL_{10} and SAL_{20} means that we can consider individual response to salinity in wider terms: some animals tend to close (or open)

their shells at consistently higher (or lower) salinity than others, irrespective of the temperature. Large mussels were found to stay open and retain activity at lower salinities than M and S mussels at both the control and elevated temperatures. This may be due to a greater resistance of salinity stress or to higher metabolic requirements. We think the latter explanation is more plausible especially given the greater variance of critical salinities at elevated temperature in the L mussels compared to the M and S ones. The difference in variation of the behavioural response (VAR₂₀₋₁₀) reflects the difference in sensitivity to low salinity between the studied groups. Increase of intra-individual variation, like fluctuating asymmetry in morphological characters, can be generally interpreted as an indicator of stress (Zakharov, 1989; Graham et al., 1993; Leung and Forbes, 1996). Therefore, we assume that the decrease of "critical" salinity observed in L animals associated with increase of intra-individual variation of these animals at 20 °C, should be interpreted as indicator of some trouble rather than indicator of well-being. In addition, the higher metabolic demands and low resistance to hypoxia in the L mussels are in line with their greater mortality in air (see above).

In S and M mussels SAL_{10} and SAL_{20} gradually decreased with increasing age. This decrease was not significant (p=0.514 and p=0.385, respectively, ANOVA), however the above-discussed metabolic mechanisms may also explain this trend. VAR₂₀₋₁₀ showed nearly constant level in all age classes, except 6-year-old specimens where it declined approximately 3-fold. Six-year-old mussels of L group were characterised by somewhat higher values of SAL_{10} , SAL_{20} and SAL_{20-10} , than 4- and 8-year-old ones. Although the effect of age on survival in air and on the low salinity stress response was not significant, similar features can be noted. Maximal resistance was observed in 6-year-old specimens. One may speculate that stress resistance increases in ontogenesis until metabolic changes related to ageing appear at certain a "critical" age specific for the given population.

4.2. Respiration and pumping rates

Respiration and pumping rates are strongly size-dependent physiological processes, both rates being proportional to a power of the body mass. Respiration and pumping rates have been intensively studied for decades, and a large number of studies describe the relationship of respiration and pumping rates with body mass in bivalves, including *M. edulis*. The power coefficients of regressions of mass specific respiration and pumping rates on the body mass are usually about -0.3 to -0.2 and -0.4 to -0.3, respectively (for review, see Winter, 1978; Bayne and Newell, 1983; Jørgensen, 1990; Riisgård, 2001). In general, the power coefficients obtained in the present study are within the range of those published previously. Interestingly, the decrease of pumping rate with body mass of mussels appeared to differ between old (8–9 years old) and younger specimens (Fig. 6a). Because of this, the mass specific pumping rate was relatively low in mussels older than 6 years. This agrees with our previous finding showing the start of senescence in mussels from this population at the age of ca. 7 years (Sukhotin and Pörtner, 2001; Sukhotin et al., 2002). In contrast to the pumping rate, the respiration rate did not demonstrate such age-specific differences. So

far, the age effects on the pumping and respiration rates are very poorly studied in bivalves and in benthic species in general, because the pure age influence is usually masked by the much more pronounced size-dependence. For the most of species with infinite growth, it is difficult to distinguish between the effects of these two correlated factors. The results of studies on age-dependence of metabolic rate in aquatic ectotherms are controversial. Thus, decreases of respiration rate with age have been reported for the fish Cichlasoma nigrofasciatum (Fidhiany and Winckler, 1998) and the bivalves Crenomytilus gravanus (Zolotarev and Ryabushko, 1977) and M. edulis (Sukhotin and Pörtner, 2001). In contrast, in the other mytilid bivalve, Mytilus galloprovincialis, a minor increase in mass-specific respiration rates in older (22 months) compared to younger (10 months) mussels within one size group has been observed (Pérez Camacho et al., 2000). On the other hand, the clearance rate and ingestion rate were not affected by age in the same groups of animals. The latter may be due to the very narrow age range used. In contrast, our data covering much wider range of ages in M. edulis showed that the effect of age on the pumping rate only becomes detectable in M. edulis older than 6 years. In the present study, we found no pronounced age effect on respiration rate of the White Sea blue mussels over a wide range of ages. Similar data have been reported previously for the White Sea M. edulis (Sukhotin et al., 2002).

An extensive literature shows that the exponent in the allometric function $Y=aW^b$, connecting filtration (and pumping) rate and body mass in bivalves is slightly but consistently lower than exponent in the regression of respiration rate on body mass (for review, see Winter, 1978; Bayne and Newell, 1983; Riisgård, 2001). This difference has not been satisfactorily explained. One possible explanation suggested by Jørgensen (1990) is the increasing sensitivity towards adverse environmental conditions with increasing size of the animals. Our data showing higher mortality in air and higher sensitivity to low salinity and elevated temperature in the large mussels compared to the medium and the small ones support this statement. However, our findings give another explanation of the phenomenon. Our data suggest that the beginning of ageing process in mussels causes the disproportional decrease of mass specific pumping rate with size. Lowering of the feeding rate together with the constant or slowly decreasing metabolic demands would lead to a drastic reduction of somatic growth followed by the ultimate mass loss and death of the animal.

In conclusion, size, age and combination of these factors affect physiological processes in the blue mussels. In general, the responses of *M. edulis* to environmental stress including low salinity, temperature and air exposure are mostly size-dependent. Effect of age on sensitivity to the studied environmental factors is minor. The whole animal respiration rates are controlled mainly by size, while an age effect remains unclear. The pumping rate of mussels is significantly affected by both size and age of the animals. The disproportional decrease of pumping rate is observed at the "critical" age when other physiological signs of senescence, like rapid decrease of growth rate and net growth efficiency, lowering of ATP and phosphagens levels in tissues, decline of citrate synthase activity and intracellular pH as well as an exponential increase in the accumulation of lipid peroxidation products, are recorded (Sukhotin and Pörtner, 2001; Sukhotin et al., 2002).

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References

- Alyakrinskaya, I.O., 1972. Biochemical adaptations of water molluscs to air exposure. Zool. Ž. 51 (11), 1630–1636 (In Russian).
- Bayne, B.L., Newell, R.C., 1983. Physiological energetics in marine molluscs. The Mollusca. In: Saleuddin, A.S.M., Wilbur, K.M. (Eds.), Physiology, Part I, vol. 4. Academic Press, New York, pp. 407–515.
- Berger, V.Ja., 1986. Adaptations of Marine Molluscs to Changes of Environmental Salinity. Nauka, Leningrad. 216 pp., In Russian.
- Berger, V.Ja., Kharazova, A.D., 1997. Mechanisms of salinity adaptations in marine molluscs. Hydrobiologia 355, 115–126.
- Berger, V.Ja., Lebsky, V.K., 1969. Ontogenic changes of salinity resistance in polychaete *Eulalia viridis* (L.). Rep. Acad. Sci. USSR 186 (6), 1427–1428 (In Russian).
- Berger, V.Ja., Lukanin, V.V., 1985. Adaptive reactions of mussels from the White Sea to changes of the environmental salinity. In: Lukanin, V.V. (Ed.), Investigations of the White Sea Mussels. Zoological Institute, Leningrad, pp. 3–21. In Russian.
- Bliss, C.I., 1935a. The calculation of the dosage-mortality curve. Ann. Appl. Biol. 22, 134-167.
- Bliss, C.I., 1935b. The comparison of dosage-mortality data. Ann. Appl. Biol. 22, 307-339.
- Canesi, L., Viarengo, A., 1997. Age-related differences in glutathione metabolism in mussel tissue (*Mytilus edulis* L.). Comp. Biochem. Physiol. 116B, 217–221.
- Chemodanov, A.V., Maximovich, N.V., 1983. Internal structure of *Mytilus edulis* L. shells as an indication of their seasonal growth variation. Proc. of the 7th Malac. Conference. Nauka, Leningrad, pp. 178–180. In Russian.
- Chen, Y.S., Richardson, A.M.M., 1987. Factors affecting the size structure of two populations of the intertidal periwinkle, *Nodilittorina unifasciata* (Gray, 1839), in the Derwent River, Tasmania. J. Molluscan Stud. 53, 69–78.
- Eertman, R.H.M., Wagenvoort, A.J., Hummel, H., Smaal, A.C., 1993. "Survival in air" of the blue mussel *Mytilus edulis* L. as a sensitive response to pollution-induced environmental stress. J. Exp. Mar. Biol. Ecol. 170, 179–195.
- Emerit, I., Chance, B. (Eds.), 1992. Free Radicals and Aging. Birkhäuser Verlag, Basel, 440 pp.
- Famme, P., Riisgård, H.U., Jørgensen, C.B., 1986. On direct measurement of pumping rates in the mussel Mytilus edulis. Mar. Biol. 92, 323–327.
- Fidhiany, L., Winckler, K., 1998. Influence of body mass, age, and maturation on specific oxygen consumption in a freshwater cichlid fish, *Cichlasoma nigrofasciatum* (Guenther, 1869). Comp. Biochem. Physiol. 119A, 613–619.
- Freeman, K.R., Dickie, L.M., 1979. Growth and mortality of the blue mussel (*Mytilus edulis*) in relation to environmental indexing. J. Fish. Res. Board Can. 36, 1238–1249.
- Fry, F.E.J., 1971. The effect of environmental factors on the physiology of fish. In: Hoar, W.S., Randall, D.J. (Eds.), Fish Physiology. Environmental Relations and Behavior, vol. VI. Academic Press, New York, pp. 1–98.
- Ginetsinskiy, A.G., 1963. Physiological Mechanisms of Water-Salt Equilibrium. Leningrad, Moscow. 427 pp.
- Glotov, N.V., Zhivotovsky, L.A., Hovanov, N.V., Khromov-Borisov, N.N., 1982. Biometry. Leningrad University, Leningrad. In Russian.

- Golikov, A.N., Smirnova, N.F., 1974. Resistance of some gastropod and bivalve species from Chupa Inlet (White Sea) to extreme influences in relation to the problem of evolution of resistance. In: Bykhovsky, B.E., Khlebovich, V.V. (Eds.), Seasonal Phenomena in the Life of White and Barents Seas. Nauka, Leningrad, pp. 307–319. In Russian.
- Graham, J.H., Freeman, D.C., Emlen, J.M., 1993. Developmental stability: a sensitive indicator of populations under stress. In: Landis, W.G., Hughes, J.S., Lewis, M.A. (Eds.), Environmental Toxicology and Risk Assessment. American Society for Testing and Materials, Philadelphia, pp. 136–158.
- Harman, D., 1956. Aging: a theory based on free radical and radiation biology. J. Gerontol. 11, 298-300.
- Hawkins, A.J.S., Bayne, B.L., Day, A.J., 1986. Protein turnover, physiological energetics and heterozygosity in the blue mussel, *Mytilus edulis*: the basis of variable age-specific growth. Proc. R. Soc. Lond., B 229, 161–176.
- Heller, J., 1990. Longevity in molluscs. Malacologia 31, 259-295.
- Hilgerloh, G., 1997. Predation by birds on blue mussel *Mytilus edulis* beds of the tidal flats of Spiekeroog (southern North Sea). Mar. Ecol., Prog. Ser. 146, 61–72.
- Hole, L.M., Moore, M.N., Bellamy, D., 1993. Age-related cellular reactions to copper in the marine mussel *Mytilus edulis*. Mar. Ecol., Prog. Ser. 94, 175–179.
- Hrs-Brenko, M., 1978. The relationship of temperature and salinity to larval development in mussels (*Mytilus galloprovincialis* Lamarck). In: McLusky, D.S., Berry, A.J. (Eds.), Physiology and Behaviour of Marine Organisms. Pergamon, Oxford, pp. 359–365.
- Hummel, H., Fortuin, A.W., Bogaards, R.H., Wolf, L., de Meijboom, A., 1989. Changes in *Mytilus edulis* in relation to short-term disturbances of the tide boom. In: Klekowski, R., Styczynska-Jurewicz, E., Falkowski, L. (Eds.), Proc. 21st Eur. Mar. Biol. Symp., Zakland Narodowy im. Ossolinskich, Wroclaw, pp. 77–89.
- Ivanchenko, O.F., Lajus, D.L., 1985. The resistance of larvae and fry of the White Sea herrings to low salinity. In: Kulachkova, V.G. (Ed.), Ecological Investigations of the Possible Objects of the White Sea for the Mariculture. Zoological Institute, Leningrad, pp. 70–78. In Russian.
- Jørgensen, C.B., 1990. Bivalve Filter Feeding: Hydrodynamics, Bioenergetics, Physiology and Ecology. Olsen and Olsen, Fredensborg, Denmark. 144 pp.
- Kinne, O., 1971. Salinity: animals: invertebrates. In: Kinne, O. (Ed.), Marine Ecology. Environmental Factors, Part 2, vol. 1. Wiley-Interscience, London, pp. 821–996.
- Lajus, D.L., Sukhotin, A.A., 1998. A new approach for the assessment of stochastic variation: analysis of behavioural response in blue mussel (*Mytilus edulis* L.). Helgol. Meeresunters. 52, 141–145.
- Leung, B., Forbes, M., 1996. Fluctuating asymmetry in relation to stress and fitness: effect of trait type as revealed by meta-analysis. Ecoscience 3 (4), 400–413.
- Lukanin, V.V., Gurina, V.I., 1977. Adaptive reactions of mussels from the White Sea to changes of the environmental temperature and salinity. Biol. Morya 2, 46–50 (In Russian).
- Mallet, A.L., Carver, C.E.A., Freeman, K.R., 1990. Summer mortality of the blue mussel in the eastern Canada: spatial, temporal, stock and age variation. Mar. Ecol., Prog. Ser. 67, 35–41.
- Nehls, G., Hertzler, I., Scheiffarth, G., 1997. Stable mussel *Mytilus edulis* beds in the Wadden Sea—they're just for the birds. Helgol. Meeresunters. 51, 361–372.
- Pellerin-Massicotte, J., 1993. Variation de la condition physiologique de moules d'elevage aux Iles-de-la-Madeleine selon l'origine des stocks. In: Pirquet, K.T. (Ed.), Proc. 10th Ann. Meeting of the Aquaculture Assoc. of Canada, Charlottetown, PE (Canada), pp. 62–65. In French.
- 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.
- Rice, W.R., 1989. Analysing tables of statistical tests. Evolution 43, 223-225.
- Riisgård, H.U., 2001. On measurement of filtration rates in bivalves—the stony road to reliable data: review and interpretation. Mar. Ecol., Prog. Ser. 211, 275–291.
- Savilov, A.I., 1953. Growth variability in the White Sea invertebrates *Mytilus edulis*, *Mya arenaria* and *Balanus balanoides*. Tr. Inst. Okeanol. 7, 198–256 (In Russian).
- Seed, R., Suchanek, T.H., 1992. Population and community ecology of *Mytilus*. In: Gosling, E. (Ed.), The Mussel *Mytilus*. Ecology, Physiology, Genetics and Culture. Dev. Aquac. Fish. Sci., vol. 25. Elsevier, Amsterdam, pp. 87–169.
- Shelford, V.E., 1915. Principles and problems of ecology as illustrated by animals. J. Ecol. 3, 1-23.

- Sirenko, B.I., Saranchova, O.L., 1985. Two-year study of seasonal growth of mussels *Mytilus edulis* in cages in Chupa Inlet (the White Sea). In: Kulachkova, V.G. (Ed.), Ecological Investigations of the Possible Objects of the White Sea for the Mariculture. Zoological Institute, Leningrad, pp. 23–28. In Russian.
- Smaal, A.C., Wagenvoort, A., Hemelraad, J., Akkerman, I., 1991. Response to stress of mussels (*Mytilus edulis*) exposed in Dutch tidal waters. 12th Ann. Conf. of the European Society for Comparative Physiology and Biochemistry, Utrecht (Netherlands), 27–31 Aug. 1990. Comp. Biochem. Physiol., C 100, 197–200.
- Sohal, R.S., 1986. The rate of living theory: a contemporary interpretation. In: Collatz, K.G., Sohal, R.S. (Eds.), Insect Aging. Spring-Verlag, Berlin, pp. 23–44.
- Sokal, R.R., Rohlf, F.J., 1995. Biometry, 3rd ed. Freeman, New York.
- Strickland, J.D., Parsons, T.R., 1968. A practical handbook of seawater analysis. Bull. Fish. Res. Board Can. 167 (310 pp.).
- Sukhotin, A.A., Pörtner, H.-O., 2001. Age-dependence of metabolism in mussels *Mytilus edulis* L. from the White Sea. J. Exp. Mar. Biol. Ecol. 257, 53–72.
- Sukhotin, A.A., Abele, D., Pörtner, H.-O., 2002. Growth, metabolism and lipid peroxidation in *Mytilus edulis* L.: age and size effects. Mar. Ecol., Prog. Ser. 226, 223–234.
- Theisen, B.F., 1973. The growth of *Mytilus edulis* L. (Bivalvia) from Disco and Thule District, Greenland. Ophelia 12, 59–77.
- Tremblay, R., Myrand, B., Sevigny, J.-M., Blier, P., Guderley, H., 1998. Bioenergetic and genetic parameters in relation to susceptibility of blue mussels, *Mytilus edulis* (L.) to summer mortality. J. Exp. Mar. Biol. Ecol. 221, 27–58.
- Viarengo, A., Canesi, L., Petrica, M., Livingstone, D.R., Orunesu, M., 1991. Age-related lipid peroxidation in the digestive gland of mussels: the role of the antioxidant defense systems. Experientia 47, 454–457.
- Viarengo, A., Canesi, L., Mazzucotelli, A., Ponzano, E., 1993. Cu, Zn and Cd content in different tissues of the Antarctic scallop *Adamussium colbecki*: role of metallothionein in heavy metal homeostasis and detoxication. Mar. Ecol., Prog. Ser. 95 (1–2), 163–168.
- Viarengo, A., Canesi, L., Pertica, M., Mancinelli, G., Accomando, R., Smaal, A.C., Orunesu, M., Forlin, L., Andersson, T. (Eds.), 1995. Stress on stress response: a simple monitoring tool in the assessment of a general stress syndrome in mussels. 7th Int. Symp. on Responses of Marine Organisms to Pollutants (PRIMO 7), Goteborg (Sweden), 20 Apr. 1993. Mar. Environ. Res. 39, 245–248.
- Winter, J.E., 1978. A review of the knowledge of suspension-feeding in lamellibranchiate bivalves, with special reference to artificial aquaculture systems. Aquaculture 13, 1–33.
- Zakharov, V.M., 1989. Future prospects for population phenogenetics. Sov. Sci. Rev. (Sect. F) 4, 1–79 (In Russian).
- Zolotarev, V.N., 1989. Sclerochronology of Marine Bivalve Molluscs. Naukova Dumka, Kiev. 112 pp., In Russian.
- Zolotarev, V.N., Ryabushko, V.I., 1977. Age changes of energy metabolism in *Crenomytilus grayanus* Dunker. Z. Obs. Biol. 38, 923–928 (In Russian).