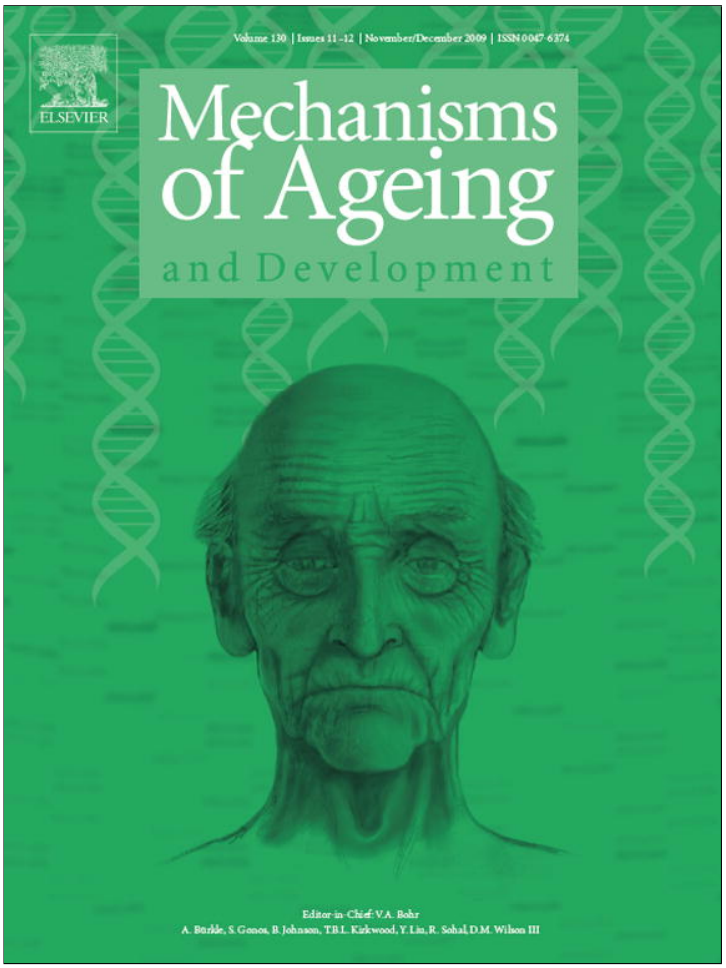

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# Aging reduces reproductive success in mussels *Mytilus edulis*

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ABSTRACT

The present study was aimed to determine whether reproductive success constantly increases with age in a relatively short-lived invertebrate with continuous growth – the bivalve mollusc *Mytilus edulis* or there is an age-related decline such as observed in species with finite growth (mammals, insects, nematodes, etc.). We studied the reproductive output and viability of the offspring during early embryogenesis in females of different sizes and ages, and used allometric approaches to correct for the effects of the body size and to discern pure age-specific effects on these reproductive traits. We have also determined contributions of females of different age and size classes to the total larval pool of a population. Both gonadosomatic index and individual fecundity significantly decreased in the course of aging if the size of the animals was accounted for. The proportion of normally developing embryos declined from almost 100% to 60% in females of 2–10-year-old. We suggest that animals with infinite growth and "slow aging", such as molluscs, undergo senescence, the physiological manifestations of which can be masked by a more pronounced effect of continuously increasing size.



## 1. Introduction

The rate of aging differs greatly between species from extremely high in semelparous organisms to almost undetectable in some trees, sponges, cnidarians and other modular organisms. Finch and Austad (2001) suggested two criteria for the absence of senescence: (1) no observable decline in reproduction rate or increased mortality after maturation and (2) no age-related decline in physiological performance or disease resistance. Therefore, the reduction of reproductive success is considered an inherent trait of aging and senescence. In animals with finite growth such as mammals and insects the senescence-related reproductive deterioration has been well documented. Indeed, species with a pronounced aging after sexual maturation show marked decrease in fecundity or overall spawning performance at the advanced ages (e.g., in mammals, Packer et al., 1998; Kirkwood and Austad, 2000, in polychaetes, Qian and Chia, 1992; in planktonic crustaceans, Hoang et al., 2002; Dudycha, 2003, and insects, Collatz, 2003 and references therein). To the contrary, it is generally accepted that animals with so-called infinite (or continuous) growth (such as fish, amphibians, reptiles and many invertebrates) increase their reproductive output throughout their life until they die, due to age-related changes in energy allocation. Several models of energy allocation in species with infinite growth have been suggested (Calow, 1981; Sebens, 1982; Bayne, 1984; Charnov et al., 2001) which proposed that after animals reach maturity, an increasing portion of energy that was originally allocated to somatic growth, is gradually but constantly redirected to energy investments in reproduction. Physiological hypotheses based on such models predict a constant increase of reproductive success in the course of life after maturation in infinitely growing animals (see Congdon et al., 2001). Alternative hypothesis proposes that reproductive success declines in the advanced age even in infinitely growing organisms. Distinguishing between these two alternative hypotheses critically depends on the ability to experimentally disentangle the confounding effects of the body size and age of the organism on reproductive traits. Indeed, many physiological functions, such as standard metabolic rate, feeding rate, growth rate, fecundity and others strongly depend on the size of an organism. In infinitely growing animals, the body size constantly increases with age, and thus, the effect of size may overlap or mask the influence of age (if there is any) on a trait of interest, making it difficult to disentangle the effects of these two correlated factors on vital functions and processes. A few studies have shown evidence of reproductive senility (manifested in a decrease in fecundity or viability of the offspring in old individuals) if specimens of similar size are compared or if size is accounted for (in molluscs, Vahl, 1985, MacDonald and Bayne, 1993; in fish, Reznick et al., 2001; in turtles, Congdon et al., 2001). At the same time, there are studies reporting absence of reproductive senescence in some invertebrate species (such as mollusc *Mercenaria mercenaria*, Peterson, 1986; or sea urchin *Strongylocentrotus franciscanus*, Ebert, 2008). Thus, our knowledge of the senescence-related changes in reproduction of infinitely growing

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organisms is still very limited, and for some important invertebrate groups is virtually absent.

The aim of the present study was to test the two above-described alternative hypotheses to determine whether reproductive success, i.e. the ability to produce numerous and viable offspring, constantly increases with age in a relatively short-lived invertebrate with continuous growth – the bivalve mollusc *Mytilus edulis* or there is an age-related decline such as also observed in species with finite growth (mammals, insects, nematodes, etc.). To this end, we studied the reproductive output (individual fecundity, i.e. total number of eggs produced by a female during one reproduction period) and viability of the offspring during early embryogenesis in mussels of different sizes and ages, and used allometric approaches to correct for the effects of the body size and to discern pure age-specific effects on these key reproductive traits. We have also determined contributions of females of different age and size classes to the total larval pool of a population to find out whether individual senescence has an impact on reproduction potential at the population level.

## 2. Material and methods

### 2.1. Animals

Blue mussels *M. edulis* L. were collected in early June 2002, from an intertidal mussel bed in the Kandalaksha Bay of the White Sea (66°20′N:33°40′E). The population lies between −0.2 and +1.2 m above 0 tidal level. Animals were sampled 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 age of mussels was determined by counting rings of winter growth delays on shells. The reliability of annual external rings as age estimate has previously been verified in studies carried out at the White Sea by comparing the number of internal rings and seasonal growth of individually marked mussels of different ages and sizes (Chemodanov and Maximovich, 1983; Sirenko and Sarantchova, 1985). Growth history of each mussel was reconstructed by measuring the maximal distance between umbo and the most distant point of every winter mark on the shell. At the sampling time sea water temperature was +5.2 °C. Collected mussels were kept in aquaria for 2 days at 25 ppt salinity and +5 °C, and under constant light without feeding.

### 2.2. Fecundity and gonadosomatic index

Spawning of *M. edulis* in the White Sea is triggered by the water temperature increase to +9 to +10 °C (Maximovich, 1985). In the shallow waters of Kandalaksha Bay these temperature values are typically recorded in late June–July (Berger et al., 2001). By early June gonads in mussels are fully developed; however, gametes are not yet released because of temperature constraints. Therefore, samples collected in early June (as in this study) allow one to estimate the potential maximal gamete production in mussels. We determined individual fecundity in mussels of different sizes and ages (Table 1) as a measure of potential egg production by females as follows. Mussel was cut open and mantle was visually examined for a presence of mature oocytes (60–65 μm in diameter) and absence of signs of the preliminary spawning such as an empty gonad branch. In sexually mature mussels, a small sample of gonad and extrapallial fluid was taken with a pipette and examined under a stereomicroscope at a magnification of 40× for oocytes or spermatozoa. Males were discarded; in females a small piece (2–30 mg) of gonad tissue was sampled, weighed and torn apart under a stereomicroscope with a needle in a drop of water to release the oocytes. The oocyte suspension was diluted with 10–30 ml (in some exceptional cases in 5 ml) of filtered (2 μm) sea water and thoroughly mixed; three replicates of 40 μl were sampled and all oocytes were counted in these subsamples under a microscope (magnification 20×). The gonad was carefully excised, blotted dry on a tissue paper and weighed. The residual tissues were also extracted and weighed. The gonad and other tissues were then lyophilized and re-weighed. The individual fecundity of a mussel (*AF*, eggs/female) was calculated as

$$AF = \bar{N}\frac{W_g}{W_s}\frac{V}{0.04}\frac{100}{95},$$

where $\bar{N}$ is an average number of oocytes in three 0.04 ml subsamples; $W_g$ is wet mass of gonad (g); $W_s$ is wet mass of a sample of gonad taken for oocytes extraction (g). $V$ is a volume of water in which oocytes were suspended (ml); 0.04 ml is a volume of subsample of oocytes suspension. For estimation of dry tissue $W_s$ was multiplied by 0.25 in according to our preliminary measurement of gonad tissue water content. Microscopic observations showed that some egg cells remained in the tissue after the oocyte extraction (on average, 5% of the total number of eggs were not extracted; our unpublished data). Therefore, 100/95 in the formula for *AF* is a correction factor for 95% efficiency of oocytes extraction from gonad tissue. Relative or mass-specific fecundity (*RF*, eggs/g of gonad) was calculated as $AF/W_g$.

Gonadosomatic index reflecting the fraction of gonad tissue mass in the total soft tissue mass (*GI*, %) was estimated in mussels of different ages and sizes to compare their relative investment in reproductive tissues.

### 2.3. Early embryonic development

Effect of female age on the quality of eggs was tested by determining fertilization success of the eggs and their ability to develop normally. Mussels of different ages were placed individually in dishes with filtered sea water. Spawning was triggered by gradual temperature increase to +15 °C. Sperm produced by 10 males of different ages (5–7 years old) was collected, mixed in equal proportions and used for subsequent fertilization of eggs. Eggs produced by individual females of different ages were collected in separate 5 ml Petri dishes (ca. 500 oocytes per Petri dish), fertilized by adding the 0.2 ml of sperm suspension and left for 4–5 h at +15 °C. Pilot studies showed that embryos of *M. edulis* reach the stage of 16 blastomeres under these time/temperature conditions. After this time period, the suspension was thoroughly mixed and three 0.25 ml samples were taken with a pipette. Samples were immediately examined under the microscope (400×), and all embryos and unfertilized eggs (Fig. 1B) were counted. Normal embryos (Fig. 1A) and those with visible deviations from the normal development (Fig. 1C) were counted separately. Number of eggs/embryos counted in one sample ranged from 50 to 300. Age, length, wet and dry tissue mass of the females used in the experiment was recorded. Proportions of normal embryos, abnormal embryos and unfertilized eggs to the total number of eggs and embryos were calculated for each female as an average from three replicate samples.

### 2.4. Population sampling

In order to estimate the relative contribution of females of different ages and sizes to the total reproductive potential of the population, quantitative random sampling of mussels was performed on June 17 in the studied mussel population. Animals were sampled from 12 squares of 0.006 $m^2$ each at +0.5 to 0.8 m level, where the emersion period comprises about 15–30% of the tidal cycle. Samples were washed through the set of soil sieves (minimal mesh size 0.5 mm), mussels were cleaned from epibionts, and algae, sand, gravel and dead shells were removed. All mussels were measured to the nearest 0.1 mm with callipers or under a stereoscopic microscope. Age was determined for all mussels by counting the winter growth checks on the shells. All mussels above 10 mm size were cut open and their sex was determined as described above. Sexually immature juveniles were recorded. In some specimens of advanced ages, gender could not be determined because gametogenic tissue while present, did not contain any gametes. The most probable reason was the infestation of these specimens with parasitic unicellular green algae *Chorocystis* sp. which can be located in mantle, adductor muscle or gonad tissues of mussels causing significant morphological and functional changes including reproductive failure in heavily infected individuals (Migunova et al., 2000; Rodríguez et al., 2008). These animals were counted separately.

**Table 1**
Age and size of mussels used for the determination of gonadosomatic index and fecundity.

| Age (years) | Length range (mm) | Wet tissue mass range (g) | Mean $W_{wet} \pm$ S.E. (g) | Dry tissue mass range (g) | Mean $W_{dry} \pm$ S.E. (g) | Number |
|---|---|---|---|---|---|---|
| 2 | 18.4 – 19.1 | 0.106 – 0.122 | 0.114 ± 0.008 | 0.0262 – 0.0275 | 0.0269 ± 0.0006 | 2 |
| 3 | 15.0 – 26.2 | 0.056 – 0.321 | 0.159 ± 0.023 | 0.0137 – 0.0785 | 0.0384 ± 0.0057 | 11 |
| 4 | 14.7 – 38.6 | 0.061 – 1.129 | 0.381 ± 0.082 | 0.0137 – 0.2262 | 0.0825 ± 0.0171 | 15 |
| 5 | 14.1 – 39.9 | 0.060 – 1.224 | 0.480 ± 0.103 | 0.0145 – 0.2712 | 0.0991 ± 0.0238 | 16 |
| 6 | 17.1 – 39.9 | 0.087 – 1.385 | 0.439 ± 0.097 | 0.0192 – 0.2395 | 0.0733 ± 0.0177 | 15 |
| 7 | 14.0 – 44.0 | 0.054 – 2.093 | 0.601 ± 0.166 | 0.0122 – 0.4802 | 0.1295 ± 0.0376 | 15 |
| 8 | 20.0 – 52.0 | 0.138 – 2.213 | 0.946 ± 0.171 | 0.0227 – 0.4714 | 0.1969 ± 0.0369 | 16 |
| 9 | 26.6 – 54.4 | 0.373 – 3.022 | 1.568 ± 0.305 | 0.0665 – 0.6415 | 0.3123 ± 0.0630 | 10 |
| 10 | 36.7 – 62.9 | 0.774 – 3.270 | 1.829 ± 0.250 | 0.1340 – 0.6127 | 0.3607 ± 0.0521 | 11 |

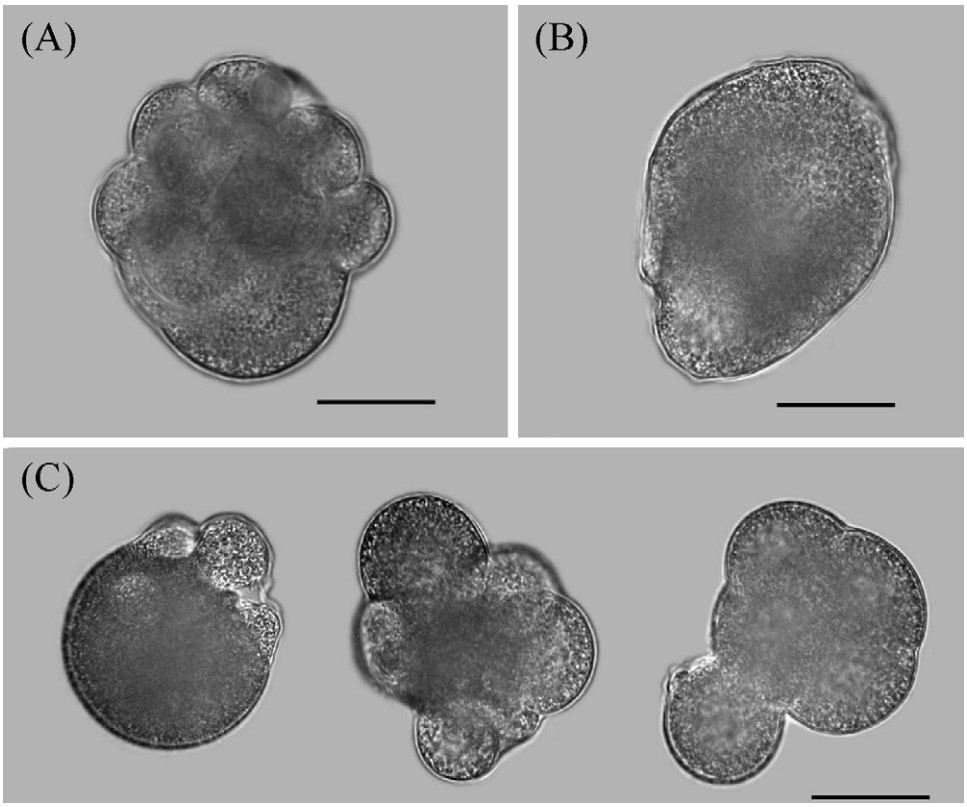


**Fig. 1.** Examples of a normal embryo (A), unfertilized oocyte (B) and defective embryos (C). Scale bar = 25 μm.

*2.5. Calculations and statistics*

Effects of the mussel size on gonadosomatic index, absolute and relative fecundity and proportions of normal/abnormal developing embryos and eggs were estimated by standard algorithms of regression and correlation analyses. The coefficients for power functions were obtained using linear regressions of the log transformed original data. Statistical significance of age effects on gonadosomatic index and proportions of developing embryos and eggs was determined using 1-way ANOVA after φ-transformation of the dependent variables. Age effect on fecundity was tested using Kruskal–Wallis rank ANOVA. Post-hoc comparisons of all the studied parameters in mussels of different ages were performed with Tukey's test for unequal N. Bivariate linear regressions with lg*GI* and lg*AF* as dependent variables and lg*W* and age as predictors were created. All data are presented as mean values ± standard errors of means (S.E.) unless specified otherwise.

## 3. Results

### 3.1. Gonadosomatic index

Gonadosomatic index in mussels varied from 5.5% to 38%. *GI* increased with size of molluscs according to a power function. The regression of *GI* on tissue mass was highly significant within the whole data set ($GI = 32.1 \times W^{0.271}$, $R^2 = 0.383$, $n = 91$, $p < 0.0001$) and within many separate age classes (Table 2). The average *GI* of a standard 1 g mussel tended to decrease with the age of molluscs. The simultaneous effect of dry mass (g) and age (years) on *GI* (%) was best fit by the equation:

$$\mathrm{Lg}GI = 1.763 + 0.346 \times \mathrm{Lg}W - 0.027 \times \mathrm{Age};$$

$$N = 91,\ R^2 = 0.402,\ p < 0.001.$$

The model describes the whole data set and allows prediction of *GI* values within studied ranges of mass and age of mussels. Plus and minus in front of the coefficients at lg*W* and Age in the equation indicate enhancing and inhibiting effects of mass and age on the *GI*, respectively. However, because of the correlation between predictor variables – mass and age, the separate effects of the predictors cannot be estimated precisely using this model. In order to estimate the pure effect of age on the relative gonad weight (*GI*), the original data were corrected for the influence of size as follows. The *GI* values were size-corrected by standardizing all values to the mean dry mass of 0.150 g, using a formula $GI_{\mathrm{corr}} = GI_{\mathrm{original}} \times (\text{mean weight/original weight})^b$, where *b* is

**Table 2**
Gonadosomatic index (*GI*, %) and absolute fecundity (*AF*, $10^6$ eggs per female) as a function of dry tissue mass (*W*, g) in mussels of different age. *a* and *b* are constants in the respective power equations, *R* – correlation coefficient of the respective linear regressions of log transformed data, *p* – significance level.

| Age (years) | $GI = aW^b$ | | | | $AF = aW^b$ | | | |
|---|---|---|---|---|---|---|---|---|
| | *a* | *b* | *R* | *p* | *a* | *b* | *R* | *p* |
| 2 and 3 | 150.89 | 0.757 | 0.686 | <0.05 | 43.65 | 2.202 | 0.756 | <0.05 |
| 4 | 49.85 | 0.383 | 0.824 | <0.001 | 11.22 | 1.678 | 0.954 | <0.001 |
| 5 | 33.02 | 0.222 | 0.426 | n.s. | 2.09 | 1.030 | 0.724 | <0.01 |
| 6 | 40.26 | 0.286 | 0.498 | n.s. | 2.40 | 1.221 | 0.727 | <0.01 |
| 7 | 35.34 | 0.325 | 0.601 | n.s. | 7.08 | 1.751 | 0.969 | <0.001 |
| 8 | 41.92 | 0.426 | 0.905 | <0.001 | 3.55 | 1.341 | 0.961 | <0.001 |
| 9 | 32.64 | 0.432 | 0.768 | <0.01 | 2.88 | 1.606 | 0.882 | <0.001 |
| 10 | 34.25 | 0.456 | 0.519 | n.s. | 3.63 | 1.510 | 0.824 | <0.01 |

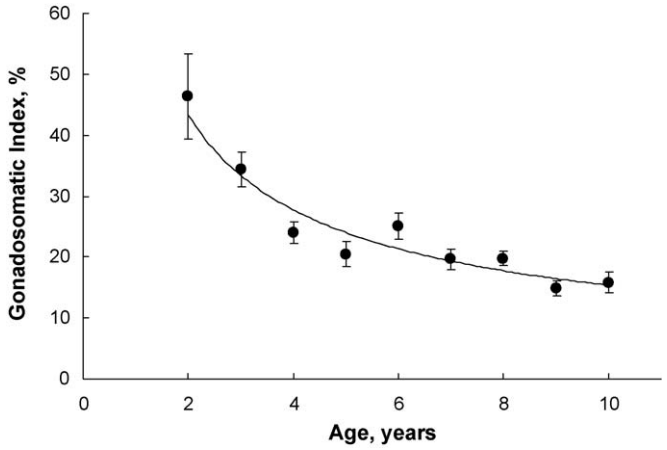


**Fig. 2.** Size-corrected gonadosomatic index (*GI*, %) as a function of age (years) in mussels. Trendline: $GI = 67.8 \times \text{Age}^{-0.644}$, $R^2 = 0.904$, $n = 9$.

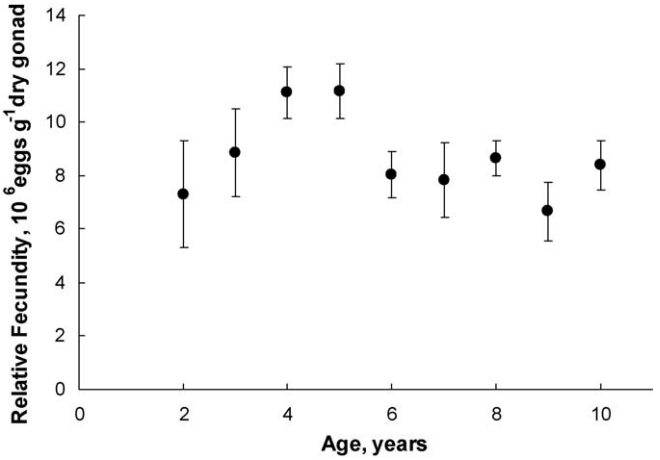


**Fig. 4.** Relative fecundity (*RF*, mln eggs $g^{-1}$ dry gonad) as a function of age (years) in mussels.

regression coefficient of a power function of $GI_{corr}$ *vs* tissue mass for the respective age class (Table 2). The size-corrected $GI_{corr}$ was significantly affected by mussel age (ANOVA, $F_{8,99} = 10.06$, $p < 0.0001$) (Fig. 2). The maximal proportion of gonad tissue in the whole body mass, 35–45%, was recorded in the youngest animals followed by a progressive decrease to about 15% in the 9–10-year-old molluscs.

### 3.2. Fecundity

Absolute individual fecundity (*AF*, $10^6$ eggs per female) of mussels depended on both size and age of the molluscs. The linear regression describing this relationship was highly significant:

$$\text{Lg}AF = 6.831 + 1.459 \times \text{Lg}W - 0.032 \times \text{Age};$$

$$N = 107, \quad R^2 = 0.791, \quad p < 0.001.$$

Within each age class the number of eggs per female increased with the mass of the soft tissues of mussels; the relationship followed a power function with power coefficients ranging from 1.03 to 2.20 (Table 2). Correction of the original values of *AF* for the mussel size was performed in order to disentangle the pure age effect, similar to the above-described size correction for *GI*. $AF_{corr}$ standardized by the average dry soft body mass ($W = 0.150$ g) progressively decreased with increasing age (ANOVA, $F_{8,101} = 258.7$, $p < 0.001$) (Fig. 3) from

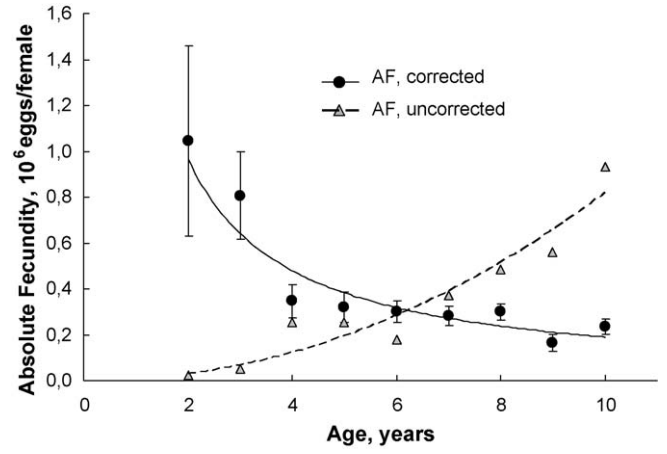


**Fig. 3.** Size-corrected absolute fecundity (*AF*, mln eggs/female) as a function of age (years) in mussels. Trendline: $AF_{corr} = 3.56 \times \text{Age}^{-1.276}$, $R^2 = 0.943$, $n = 9$. Initial (uncorrected) values of *AF* are presented (triangles).

$>1 \times 10^6$ eggs per 2 years old female to about $0.2 \times 10^6$ eggs per female of 9–10 years of age.

Number of eggs per gram dry gonad (*RF*) varied strongly from 1.0 to 23.5 million (average $8.94 \times 10^6$) eggs without any consistent size-related pattern either within the complete data set (regression analysis, $R^2 = 0.01$, $n = 110$, $p = 0.709$) or within separate age classes (data not shown). Age influence on *RF* was marginally significant (ANOVA, $F_{8,101} = 1.91$, $p < 0.067$) (Fig. 4). *RF* values, estimated for dry tissues in 4- and 5-year-old mussels were somewhat higher than in animals of other ages, although this was not statistically significant (Tukey's HSD test, $p > 0.05$).

### 3.3. Early embryonic development

During the exposure time on average 96% of eggs were fertilized and started embryonic development. The remaining eggs (on average, 4%) consisted of unfertilized eggs. In general development was normal and in 4–5 h most of the embryos reached the stage of 16 blastomeres. Relative proportions of normal embryos, defective embryos and eggs in the samples did not depend on the size of females within each age class (data not shown). In contrast, age of females had a pronounced effect on the proportions of the normal and defective embryos and non-developing eggs (ANOVA: normal embryos $F_{8,54} = 2.66$, $p = 0.016$; defective embryos $F_{8,54} = 2.51$, $p = 0.021$; eggs $F_{8,54} = 3.37$, $p = 0.003$). Females of 3–7 years of age produced about 88% of normally developing embryos, 10.5% of embryos with deviations in development and about 1.5% of non-developing eggs, which did not start cell division (Fig. 5). In the offspring of the females older than 7 years, a progressively lower proportion of normal embryos was observed which was mirrored by the correspondent increase of the number of deviants. The maximal proportions of non-developing eggs (8.3% and 12.1%) were recorded in the samples from 8- and 9-year-old mussels, respectively. Offspring of a single 2 years old mussel, which was used in the experiment, contained more than 98% of normal embryos, few deviants and no non-developing eggs.

### 3.4. Population

Total mussel density in the studied intertidal population was $16{,}200 \pm 1755$ ind $m^{-2}$ ($n = 12$). In this population, mussels became sexually mature starting from the size of approximately 11–12 mm and irrespective of age when this size was reached. At 14 mm size about 50% of mussels reached sexual maturity, while at the size 18 mm immature juveniles comprised less than 1% of this size group. Overall, juveniles dominated in the studied population comprising

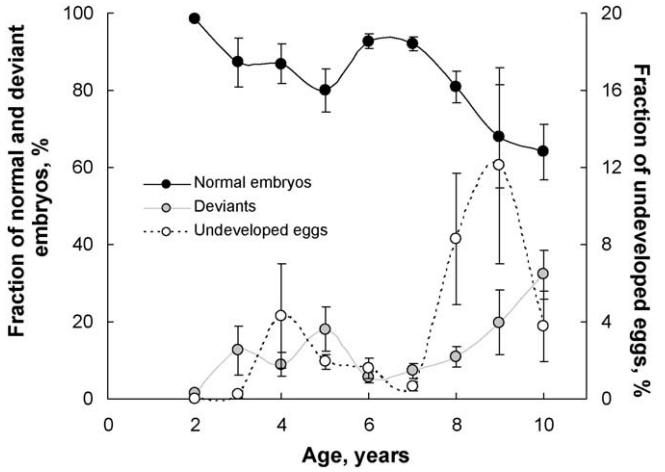


**Fig. 5.** Relative success of embryonic development of the offspring as a function of the female age.

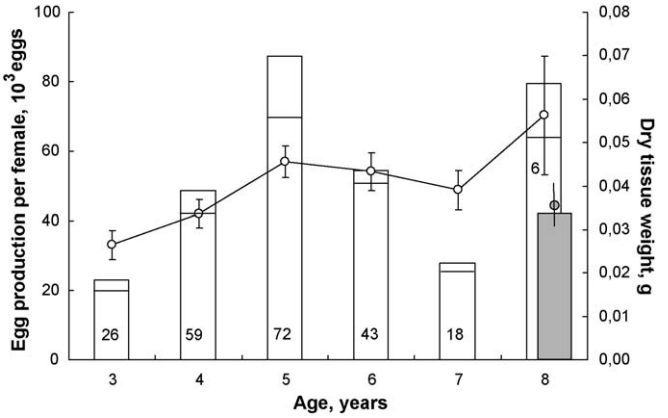


**Fig. 6.** Average egg production (bars, thousand eggs) and dry tissue weight (white circles, g) of females of different ages. Horizontal lines in the bars indicate the number of potential larvae when the eggs production is corrected for the success of the embryonic development (number of produced eggs are multiplied by the percent of normally developing embryos for each age class). Eggs production and dry weight of 8-year-old females are represented by white bar and circle when all six females are considered and grey bar and circle when the two big females are excluded (see text). *n* values are given as figures in the bars.

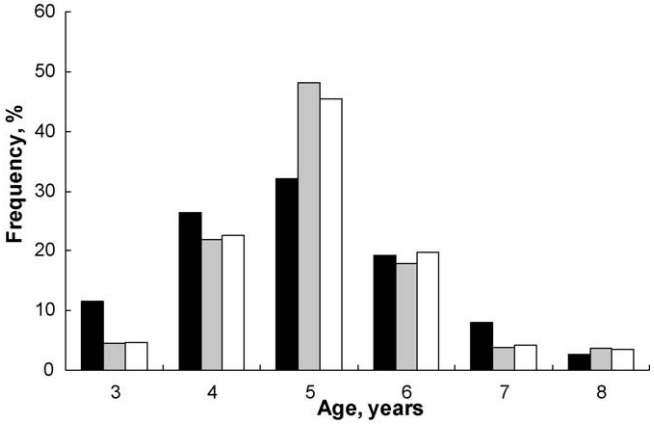


**Fig. 7.** Relative impact of females of different ages in total number of adult mussels (black bars), total number of eggs produced by all females (grey bars) and the total number of potential larvae when the eggs production is corrected for the success of the embryonic development (white bars) as a function of female age (years).

56.6 ± 2.6% of the total number of mussels. Mature males and females were represented almost equally with a minor prevalence of females, 18.6 ± 1.4% and 21.0 ± 2.0%, respectively. About 3.8 ± 0.8% of population were represented by large mussels of unidentifiable gender.

In general, the population was characterized by a unimodal age distribution with the highest frequency (20%) of 3-year-old animals. The oldest mussel found in the samples was a single 9 years old specimen of unidentified gender (see Section 2). The youngest adults were two 2-year-old male molluscs, while 3–8-year-old age classes were represented by both males and females. The average proportion of females to males was 51.7:48.3.

Individual *AF* of all females in the population was calculated from their dry tissue weights using the regressions obtained earlier for each separate age class (Table 2). Total egg production by all females was estimated as $1.91 \pm 0.38 \times 10^8$ eggs $m^{-2}$, which corresponded to 9.04 g dry mass $m^{-2}$, assuming 47.3 mg $10^{-6}$ dry mussel eggs (Thompson, 1979). Therefore, on average, sexually mature females from the studied population released 4.04% of their dry tissue biomass as eggs with the maximum value (5.84%) recorded in the 5-year-old molluscs. Egg production per female in different age classes reflected both the size-related increase and age-associated decline of *AF* (Fig. 6). A rapid increase of average mussel fecundity between 3 and 5 years of age was mostly due to the growth of animals by nearly two-fold (in terms of body mass) during this period. After reaching the age of 5 years, the average size of females leveled off, while average egg production dropped dramatically indicating the inhibiting effect of age on reproductive abilities of mussels. The average *AF* of the oldest females (8-year-old) was approximately 80,000 eggs per mussel, which is about 3 times higher than in 7-year-old animals. The reason of such a dramatic rise in relative impact of the oldest mussels was that they were too few in the sample (six specimens were encountered) and heterogeneous in terms of their size. Four of them were of the size (0.036 ± 0.004 g dry weight) similar to 5–7-year-old ones with a calculated average *AF* about 40,000 eggs per female (Fig. 6). The rest two old females were 2.5 and 3 times bigger than the others in this age class with fecundity values of 134 and 180 thousand eggs per mussel, respectively. These two mussels being in a small group significantly pulled up the average fecundity of the oldest age class.

The age-specific contributions of mussels to the total larval pool produced by the population were determined by the individual fecundity of mussels and by the proportion of females of different age classes in the population. Almost 50% of eggs in the population were produced by 5-year-old mussels, which is disproportionally high, compared to their relative number (32%) among the mature mussels (Fig. 7). In contrast, proportion of the eggs produced by 3- and 7-year-old mussels was about 2 times lower than it could be expected accounting to their relative abundance in the population. The number of the potential offspring by mussels of different ages was estimated from the number of produced eggs by a certain age group and their viability (measured as a percent of normally developing embryos obtained in the experiment, see above). The correction for embryos viability lowered the absolute numbers of the viable offspring produced by females by 10–20%, but had little effect on the distribution of proportional contributions of different age classes to the larval pool (Fig. 7).

## 4. Discussion

Our data clearly demonstrate the age-related decline in reproductive abilities of mussels, which becomes apparent if size and age effects are separated. The proportion of gonad to the total tissue weight in pre-spawning mussels progressively increases with mussel size although this increase is slower at advanced ages. In the largest specimens dry gonad tissue takes about 35% of total

dry body weight. These values are in a good agreement with the data obtained for *Mytilus* from Atlantic Canada (Thompson, 1979) and NE USA (Rodhouse et al., 1986) where in similarly sized mussels the gonad mass constituted 29–39% of total dry tissue mass.

The overall individual fecundity of mussel is determined by the total mass of gonad and the number of eggs per gram of gonad tissue. While the latter (*RF*) does not show any consistent size- or age-related pattern, the total gonad mass depends on both size and age of the mussels, which explains the variation of absolute individual fecundity (*AF*). On average, *AF* in the studied mussel population is at the low range of fecundity observed in *Mytilus* species from the other regions. Thus the intertidal White Sea *M. edulis* of 200 mg dry soft tissue (about 35–37 mm shell length) produces on average $0.38 \times 10^6$ eggs (ranging from 0.75 to $0.22 \times 10^6$ eggs at the age 4–9 years old, respectively). Within the area of distribution, the individual fecundity of females of the respective size in intertidal populations of *M. edulis* ranges from $0.2 \times 10^6$ to $1.4 \times 10^6$ eggs per female (Thompson, 1979; Bayne et al., 1983; Sprung, 1983; Rodhouse et al., 1984). Females from subtidal populations of *M. edulis* are often significantly more fecund than their intertidal counterparts.

The same way as the *GI*, the *AF* in mussels was affected by both size and age of the female. While in growing mussels age and size (dry tissue mass) were closely positively correlated (Spearman $R = 0.589$), their influence on *AF* had opposite directions: fecundity significantly increased with animal size within the same age group (Table 2) and at the same time tended to decrease with age (Fig. 3).

Several explanations of the age-related decline in absolute fecundity in mussels can be suggested. One of them would be the age-related decrease in number of eggs per unit of gonad mass (*RF*); however, this was not observed. There is a somewhat decrease in *RF* in older mussels compared to 4- and 5-year-old ones, but it was not the case in younger specimens. Alternatively, this could be a result of age-related changes in shell morphology and space constraints inside the shell as well as disproportional growth of different parts of the body. We are not aware of any studies of age effect on morphological variation in soft tissues in Mytilids.

The other explanation of age-associated decline in relative gonad weight could be the effect of parasitic infestation. Mussels in the White Sea and other areas serve as final or intermediate hosts for numerous parasites including several trematode species (Kulachkova, 1987; Galaktionov, 2001). Trematodes accumulate in the course of their hosts' life (e.g., Lauckner, 1983; Galaktionov and Dobrovolskij, 2003) often impeding or disrupting vital functions of the hosts including reproduction (Sousa, 1983; Hall et al., 2007). Our earlier study (Nikolaev et al., 2006) showed that intertidal mussels in the research area may become infected with trematodes at the age of 1–2 years, before maturation. An age-associated exponential increase in infection intensity has been observed in 2–8-year-old specimens. Parasites inhabiting mantle and gonad tissues and accumulating with the age of the host may progressively reduce gonad mass and therefore may cause age-related decline in the fecundity of molluscs (see also Schallig et al., 1991).

Finally, the strategy of age-related energy allocation in infinitely growing animals may differ depending on the food availability, as suggested by MacDonald and Bayne (1993). They found the significant decrease of gonad mass in senescent *Placopecten magellanicus* living in deeper habitats with potential food deprivation, while such a decrease was not recorded in molluscs from shallower waters. In the habitats with limited resources such as intertidal zone, energy invested in reproduction and somatic growth in large old individuals can be sacrificed for maintenance. However, our data show that age-dependent decrease of *GI* and *AF* occurs not only in large (old), but in all mussels starting from the age of maturity until the maximal age recorded in the population. Similar decline of reproductive function have been observed by Vahl (1985) in Iceland scallop *Chlamys islandica* that could be attributed to age. In this species, an increase of reproductive effort, i.e. the proportion of energy allocated in gametes production at the expense of somatic growth with body size was highest in the smaller (=younger) molluscs and lowest in the larger (older) specimens (Vahl, 1985). The age-associated leveling off or even decline in gonad mass was recorded at advanced age in *P. magellanicus* (MacDonald and Bayne, 1993) but not in the younger molluscs. The absence of correction for size effects could be responsible for an inability to detect the age-related effects among young *P. magellanicus*.

The inhibiting effect of age on reproductive abilities in mussels is also reflected in the reduced success of embryonic development of the offspring produced by old females. The proportion of normally developing embryos was relatively stable at about 90% in the females 3–7-year-old and declined to less than 70% in 9–10-year-old females. The data available in the literature data on the effect of parental age on the offspring survival are controversial. Thus, the embryonic survival in the ocean quahog *M. mercenaria* within 48 h did not depend on the age of females (Walker and Heffernan, 1996). In the seahorse *Hippocampus kuda* growth rate and survival of offspring was positively correlated with the parental size (Dzyuba et al., 2006). Males brood the fertilized eggs in a brood pouch, and bigger males produced more viable and faster growing offspring, than the smaller ones. This was explained by the fact that the bigger males possess bigger pouch and relatively smaller number of eggs, which may lead to better conditions inside the pouch during gestation and accordingly to the fittest offspring. However, the authors of these studies could not disentangle the effects of female and male sizes or the effects of size and age, because these were closely correlated. The tendency to produce bigger eggs and larvae thus increasing survival of the progeny by larger females is typical for many fish species (see also Trippel et al., 1997; Raventos and Planes, 2008). To the contrary, in guppies *Poecilia reticulata* the offspring quality (offspring weight and to a lesser extent fat content) gradually declined with female age while the individual fecundity was strictly size-dependent, i.e. increased with increasing size and leveled off after cessation of growth rate (Reznick et al., 2001). Similar pattern, which can be interpreted as reproductive senescence was also observed in a long-lived turtles *Emydoidea blandingii* (Congdon et al., 2001). In these reptiles the mortality of embryos due to developmental problems progressively increased with the age of females partially offsetting the benefits of the larger clutch sizes of oldest females compared to the younger age groups.

In the studied mussel population molluscs reach maturity at a size of 11–12 mm (about 6–8 mg dry tissue mass) while the age of maturity varies from 2 to 6 years due to the high variability of growth rate. Our data suggest that at least in this population, size rather than the age of mussels determines the onset of maturation (see also Kautsky, 1982). However, the size at maturity may significantly vary when molluscs from different habitats or different *Mytilus* species are compared. Thus, *M. edulis* from the upper subtidal (<10 m depth) populations in the White Sea become mature at 20–26 mm shell length (Maximovich, 1985), and in suspended culture in N. Spain maturation occurs during the first year at about 35 mm size (Pilar-Aguirre, 1979). At the rocky intertidal in Ireland mussels as small as 2–7 mm may be sexually mature (Seed, 1969). *M. trossulus* from the Baltic reach maturation at 2–10 mm size (Sunila, 1981; Kautsky, 1982). Such comparisons lead to a conclusion that age and growth rate, but not the size *per se* determine the onset of sexual maturation in mussels (Seed, 1969; Sprung, 1983; Maximovich, 1985). It can be suggested that in populations living under stressful conditions (e.g., low salinity,

prolonged air exposure at the intertidal zone, strong wave action) sexual maturation occurs when mussels attain smaller size than in the more optimal environment. This agrees with the model of optimal energy allocation in animals with indeterminate growth, predicting a decrease of size at maturity at limited resources (Calow, 1981).

The dry weight loss on spawning (4–6% of biomass in mussels of 50–60 mg dry tissue weight) in the intertidal White Sea population was between the values observed in mussels of the same size from estuaries in South England (1–4%, Bayne and Worrall, 1980) and those obtained in intertidal populations from Helgoland, North Sea (6–9%, Sprung, 1983). The contribution of mussels of different age classes to the total larval pool of the population is determined by the abundance of mussels of the certain age group, their size and to a lesser extent by their age *per se*. The sexually mature females were observed in 2–8-year-old age classes. The highest contribution (of about 50% of eggs produced in the population) belonged to 5-year-old females. Although 3-year-old mussels were the most abundant in the population, they contributed 10 times less to the egg pool than 5-year-old females due to their smaller size. Beyond 5 years of age, mussels in the studied population almost stop growing, and their abundance significantly decreased; therefore their egg production progressively declined. The pure effect of age also contributed to this process which can be seen from the fact that relative abundance of 6- and 7-year-old mussels was considerably higher than their relative reproductive output. The group of 8-year-old mussels was highly heterogeneous. It contained the "average" individuals with low contribution to the overall egg pool, and two relatively large specimens, characterized by high fecundity. It is unclear whether these two highly reproductive mussels represented very healthy, possibly free of parasites late survivors typical in a small proportion in the population or they migrated from subtidal zone or other places.

The age effects on *GI* and *AF* may have important implications for future qualitative population-level studies or the efforts to model reproductive capacity and output of molluscan populations. Age influence pulls down the curve of *GI* or *AF vs* size and this effect is higher in the bigger animals. Therefore, the estimated power coefficient of *GI* or *AF* as a function of size in a sample of mussels from a natural population will depend on the relative proportions of animals of different ages. This should be taken into account when estimation of population-level fecundity is needed.

As a corollary, our data show a clear effect of aging on reproductive abilities of mussels (reduced absolute individual fecundity and viability of offspring in old females). Besides the reproductive abilities other physiological functions show the age-associated changes in animals with infinite growth (for molluscs see review: Abele et al., 2009). Thus, age-related decline in respiration rate (in fish, Fidhiany and Winckler, 1998; in molluscs, Sukhotin and Pörtner, 2001; Sukhotin et al., 2006), decrease in mitochondrial function (Philipp et al., 2005, 2008), reduction in pumping rate (Sukhotin et al., 2003), decrease in expression of chaperones (Ivanina et al., 2008) were observed. We suggest that animals with infinite growth, such as molluscs as well as many other invertebrate and vertebrate species including fishes, reptiles and amphibians, undergo senescence, the physiological manifestations of which can be masked by a more pronounced effect of continuously increasing size. In other words, there can be relatively rapid aging, but we are not able to detect it. Therefore, in order to understand aging in these organisms it is important to separate the effects of "growing older" from those of "getting bigger".

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