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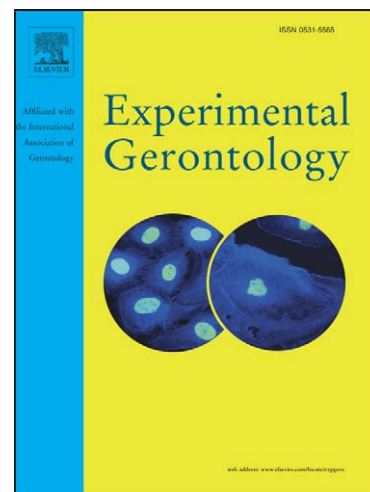
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1 **Bivalve models of aging and the determination of molluscan lifespans**

2

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21 **Abstract:**

22 Bivalves are newly discovered models of natural aging. This invertebrate group includes  
23 species with the longest metazoan lifespan approaching 400 years, as well as species of  
24 swimming and sessile lifestyles that live just for 1 year. Bivalves from natural populations  
25 can be aged by shell growth bands formed at regular intervals of time. This enables the  
26 study of abiotic and biotic environment factors (temperature, salinity, predator and physical  
27 disturbance) on senescence and fitness in natural populations, and distinguishes the impact of  
28 extrinsic effectors from intrinsic (genetic) determinantes of animal aging. Extreme longevity  
29 of some bivalve models may help to analyze general metabolic strategies thought to be life  
30 prolonging, like the transient depression of metabolism, which forms part of natural  
31 behaviour in these species. Thus, seasonal food shortage experienced by benthic filter  
32 feeding bivalves in polar and temperate seas may mimic caloric restriction in  
33 vertebrates. Incidence of malignant neoplasms in bivalves needs to be investigated,  
34 to determine the implication of late acting mutations for bivalve longevity. Finally, bivalves  
35 are applicable models for testing the implication of heterozygosity of multiple genes for  
36 physiological tolerance, adaptability (heterozygote superiority), and life expectancy.

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42 **Keywords:** Bivalve, longevity, aging, stress tolerance, heterozygosity, Antarctic, genes,

43 metabolic rate depression

44

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46

47 ***1. Bivalves: a diverse set of models for ageing studies and environmental recorders***

48 Commonly used animal models for cellular and molecular mechanisms underlying the  
49 process of aging, such as *Drosophila melanogaster*, *Caenorhabditis elegans* or small  
50 rodents, are bred and reared under controlled laboratory conditions. Most of these aging  
51 models are short lived (days in *C. elegans*, weeks in *D. melanogaster*, <5 y in rodents) and,  
52 hence, may not show all the age-dependent changes of long lived species (Reznik 1993;  
53 Kirkwood 2002). Especially, short lived models are not representative of the negligible aging  
54 type (Finch 1990; Terzibasi et al. 2007). Further, aging under controlled laboratory  
55 conditions may differ significantly from aging in the wild, and the applicability of the results  
56 to natural animal populations is often questionable. Good reasons to establish animal models  
57 that can be sampled from a range of environmental settings and scenarios, but at the same  
58 time provide information on their individual life history, including their chronological age  
59 (Austad 1996).

60 Such new models are found among bivalve molluscs. Worldwide, approximately 20,000  
61 species (Pearse et al. 1987) of marine bivalves exist so that this class offers a rich diversity of  
62 lifestyles, adaptations to specific environmental conditions and corresponding strategies of  
63 ageing, albeit based on the same filter feeder blueprint in the majority of species. Some  
64 bivalve species, like the long lived ocean quahog, *Arctica islandica* or the blue mussel  
65 *Mytilus edulis* exhibit broad geographical distribution, with habitats spanning large  
66 latitudinal ranges and covering different climate zones. Other species like the hydrothermal  
67 vent mussel *Bathymodiolus azoricus* are restricted to a rather narrow environmental window  
68 and have specified their physiology for adaptation to one ecological setting. Mainly filter and  
69 deposit feeders, bivalves are of central importance for carbon flux in many ecosystems.  
70 Often they have key species function with respect to habitat structuring and energy flow,  
71 such as the burrowing mud clams *Laternula elliptica* in the Antarctic, *Hiattella arctica* in the  
72 Arctic region, or bank forming species (mussels, oysters) in general.

73 Within the Bivalvia, we find species firmly attached to the substratum (e.g. Ostreidae,  
74 oysters, and Mytilidae, mussels), others that crawl with the help of their muscular foot (most  
75 species), and even facultative swimmers (e.g. Pectinidae, scallops). Infaunal species burrow  
76 in soft (e.g. Tellinidae, clams) and hard substrates (e.g. Teredinidae, shipworms) and can live  
77 at the sediment-water interface (e.g. Unionidae, freshwater mussels), or burrow down to  
78 100cm of sediment depth (e.g. Myidae, soft shell clams, or Hiatellidae, geoducks). Epifaunal  
79 species are encountered on all kinds of surfaces, from rocks to such exotic places as the  
80 spines of sea urchins (e.g. *Lissarca notorcadensis* on Antarctic cidaroids, Brey and Hain,  
81 1992). Bivalves inhabit a pressure range from 1 atm in the littoral to about 1000 atm in the  
82 deep sea; a temperature range from -1.8°C in polar waters (Antarctic clams) to >30°C in  
83 tropical seas and around deep sea hot vents (e.g. Vesicomidae), and a salinity range from  
84 fresh water (e.g. Unionidae) to hypersaline waters (e.g. the cockle *Fragum erratum* at ±60  
85 ‰, Morton 2000).

86 Adult bivalve size ranges across several orders of magnitude, from micro-bivalves of a  
87 few mm and mg in size such as the Neoleptonidae to the giant clam *Tridacna gigas* with a  
88 length of 120 cm and weighing 220 kg. Bivalve maximum lifespans (MLSPs) range from  
89 ≈ 1y in small warm water clams (Powell and Cummins 1985) to ± 400y in the ocean quahog  
90 *Arctica islandica* (Schöne et al. 2005), the longest lived invertebrate known. Thus, bivalves  
91 offer a diversity of ageing models, from which we can select those that best suit a particular  
92 question or hypothesis.

93 The distinct advantage of bivalves compared to the classical aging-models, however, is their  
94 carbonate shell. The shell can serve the determination of individual age and, at the same  
95 time, it can archive changes of environmental conditions throughout the animal's lifetime.  
96 Both the instantaneous shell growth rate and the composition of newly forming shell material  
97 are subject to extrinsic change, meaning they can be modified by environmental factors.  
98 Shell growth rate is largely governed by temperature and food availability (Richardson

99 2001). Hence, bivalves, coming from systems with a distinct seasonality with regard to either  
100 one or both parameters, exhibit seasonal shell growth patterns that produce an annual growth  
101 banding, similar to trees. Once the annual shell band patterns are calibrated for a population,  
102 animals can be taken from the environment and individually aged. This enables the study of  
103 the impact of environmental drivers on the ecology and physiology of bivalves over time in  
104 natural populations from different climatic or ecological settings. Moreover, lunar, daily, or  
105 even tidal cycles are distinguishable in some fast growing species, at least a posteriori (e.g.  
106 Chauvaud et al. 2005.; Kanazawa and Sato 2008). Further, archived banding patterns allow  
107 reconstruction of lifetime growth history, including long-term trends, such as climate driven  
108 decadal patterns (e.g. Schöne et al. 2005), as well as unique physical disturbance events  
109 caused by storms, predator attack, extreme temperatures or even incidents of low oxygen  
110 concentrations (Witbaard and Klein 1994; Sejr et al. 2002).

111 Thus, bivalve models of aging not only reveal their chronological age at capture, but also  
112 record environmental parameters important for the reconstruction of animal and population  
113 life history. This opens the field for a new ecophysiological approach to study principles of  
114 aging in marine and freshwater systems, and will perhaps enable a clearer distinction of the  
115 effects of intrinsic (genetically fixed) and extrinsic (environmental) factors on organismal  
116 life expectancy and fitness.

117

## 118 ***2. ROS formation and antioxidant strategies in bivalves***

119 Reactive oxygen species (ROS) released by electron transport systems of the mitochondria  
120 and the endoplasmic reticulum are well established to be important drivers of aging in  
121 senescent, post-mitotic animal cells and tissues (Harman 1968; Barja 2004). If not controlled  
122 on low levels by antioxidant systems (enzymes and low molecular antioxidants), ROS cause  
123 damage to cellular lipids and proteins, as well as to mitochondrial and nuclear DNA,  
124 resulting in accelerated senescence.

125 Bivalves are excellent models showing how different lifestyles modulate the balance  
126 between ROS production and antioxidant defence involved in the determination of the  
127 maximum lifespan (Fig 1): Pectinids have a high scope for activity, adaptive for burst  
128 swimming. To reduce oxidative stress during frequent exhaustive swimming, they need  
129 many energetically well coupled mitochondria with low oxygen radical output per  
130 mitochondrion. Indeed, pectinids have the lowest rates of mitochondrial *in-vitro* ROS  
131 production measured so far in any molluscan species (Abele et al. 2007). It appears that  
132 control of ROS formation on low levels is extremely important in these actively swimming  
133 animals, respiring fully oxygenated seawater. Mobile pectinids are still relatively short lived  
134 compared to sessile molluscs, indicating that even these low ROS amounts per  
135 mitochondrion produce enough wear and tear in active swimmers. We found a significant  
136 decrease in mitochondrial number with age in queen scallops, which could relate to the fact  
137 that damaged mitochondria are removed from their tissues, in this contrasting the mud clams  
138 like *Mya arenaria*, where respiratory efficiency in mitochondrial isolates declined  
139 dramatically with animal age (Philipp et al. 2006 and see Fig. 2). Short lifetime of scallops  
140 may also reduce the need for life-protecting antioxidant activity, and scallops range rather  
141 low in antioxidants compared to other molluscs (black dots in Fig. 3). The opposite is found  
142 in the ocean quahog, *Arctica islandica*, the longest-lived of sessile mud clams. The animals  
143 exhibit low aerobic scope and moderate ROS production rates per mitochondrion, but have  
144 the double to 10-fold catalase activity (depending on tissue) than other bivalves. MLSP is,  
145 however, 20-30 times longer in *A. islandica* than other mud clams, indicating moderate ROS  
146 formation and higher levels of antioxidants may be only a part of the long life program of the  
147 ocean quahog. Based on the current data, a sessile benthic lifestyle (Fig. 1, left) would  
148 involve moderate to high ROS formation rates, seasonal adjustment of the proton leak (=  
149 non-phosphorylating proton flux through inner mitochondrial membrane) with higher leak  
150 for mild uncoupling in summer in order to prevent thermally induced ROS formation and



151 oxidative damage. Extremely long MLSPs as in the ocean quahog seem to further require  
152 high antioxidant potential.

153

### 154 ***3. The ocean quahog and the pearl clam: models for extreme lifespan and negligible aging***

155 Life expectancy of more than 100 years are known for only a dozen out of the total 1.5  
156 millions of animal species on earth (Ziuganov et al. 2000). Among endotherms, only humans  
157 and presumably some whales reach such venerable ages (see Finch and Austad 2001).  
158 Astonishingly enough, the aquatic ectotherms hold all age records (Mangel 2003), even if we  
159 exclude colonial organisms with asexual reproduction from our considerations, such as the  
160 polyp *Hydra* which seemingly does not age at all (Martinez 1998). Although an attractive  
161 hypothesis (Cailliet et al. 2001), extreme longevity is neither an exclusive, nor a  
162 characteristic trait of deep sea fauna and the longest life expectancies are found among  
163 shallow water species (Mangel 2003). Two bivalves are the actual record holders: the ocean  
164 quahog *Arctica islandica* with a reported maximum lifespan (MLSP) of 374y around Iceland  
165 (Schöne et al. 2005) and the freshwater pearl shell clam *Margaritifera margaritifera* with an  
166 MLSP of 190 y in the Arctic (Ziuganov et al. 2000). Other long lived shallow water species  
167 like the hard clam *Eurhomalea exalbida* from the South Patagonian shelf (Lomovasky et al.  
168 2002), the Pacific mussel *Crenomytilus grayanus* (Zolotarev 1980), and the fossil *Cucullea*  
169 *raea* from Eocene Antarctic (Buick and Ivany 2004) have centenarian lifespans, and indicate  
170 that cold water temperatures and presumably food limitations during winter at high latitudes  
171 may be favourable for longevity (Buick and Ivany 2004). Extremely long-lived bivalves  
172 share common and characteristic life history features. The first is an extremely slow and  
173 seemingly indeterminate growth (Finch and Austad 2001) in Arctic and Subarctic climates,  
174 compared to sympatric species from warmer habitats (Ziuganov et al. 2000). A second is the  
175 late onset of reproduction which then continues into old age without a post-reproductive  
176 phase (Thorarinsdottir and Steingrimsson 2000; Mangel 2003; Ebert 2008).

177 Icelandic *A. islandica* can clearly be regarded as a prototype example for imperceptible/  
178 negligible senescence. The animals mature between 10 and 32y of age, and mature  
179 specimens maintain constant levels of several antioxidant factors, including the enzymes  
180 SOD and catalase, as well as the glutathione concentration and redox state in gill and mantle  
181 tissues over at least 150y. Higher antioxidant activities and glutathione (GSH) levels are seen  
182 during an initial phase of active growth in young animals, whereupon values decline rapidly  
183 until the age of maturation and then remain stable (Abele et al. 2008). Post maturation  
184 antioxidant defence levels are up to 10-fold above those found in other bivalve species with  
185 shorter life. As a consequence, oxidative damage accumulation is extremely low with protein  
186 carbonyl accumulation amounting to only 50% of the lowest values measured in shorter lived  
187 bivalves (Strahl et al. 2007). A recent comparison of fluorescent age pigment (lipofuscin) in  
188 mantle tissue of *A. islandica* populations from different geographic regions, differing  
189 distinctly with respect to longevity, showed that individuals of the extremely long lived  
190 Icelandic population, indeed, have the lowest lipofuscin accumulation rates (unpublished  
191 data of L. Basova). Thus, aside from its well developed antioxidant defence system, low  
192 natural levels of ROS formation and presumably a high capacity of cellular damage repair  
193 and autodigestion of severely damaged macromolecules might be important life prolonging  
194 factors in the ocean quahog. However, autophagy and apoptosis in these animals and their  
195 role in bivalve aging still wait to be addressed.

196 *Arctica islandica* displays a characteristic behaviour in spontaneously burrowing into  
197 the sediment, which is not regulated by external triggers and therefore occurs at irregular and  
198 individually determined intervals. The normal position of *A. islandica* is just below the  
199 sediment surface, so that the very short siphons reach into the water column. Occasionally,  
200 the animals burrow a couple of cm deeper into the sediment and, by closing their siphons and  
201 shell valves, expose their tissues to hypoxic and even completely anoxic conditions for 1 to 5  
202 days (Taylor 1976 and our own observations). During these periods, the animals enter a

203 metabolically depressed state (“metabolic rate depression”, MRD) down to 10% of their  
204 normoxic metabolic activity (Taylor 1976: heart beat; Oeschger and Storey 1993: down  
205 regulation of glycolytic enzyme activity) which allows them to survive on anaerobic energy  
206 production alone. Similarly, Ziuganov and colleagues reported pearl shell clams to be  
207 capable of “altering metabolic rate independently of temperature” and thereby “enter and exit  
208 diapause” (Ziuganov et al. 2000; Ziuganov 2004). This energy saving behaviour could  
209 theoretically slow senescence in several ways in the bivalves. Firstly, it involves a slow  
210 down of metabolic ROS production in the depressed state, which, in oxyconforming species  
211 not prone to oxidative stress during hypoxia-reoxygenation cycles, could be beneficial  
212 (Abele et al. 2007). On the contrary, frequent hypoxia-reoxygenation cycles in ectotherms  
213 may trigger antioxidant protection (Hermes-Lima and Zenteno-Savin 2002). Another  
214 important mechanism is the induction of a “famine-like” state (Ziuganov et al. 2000) during  
215 shell closure, bound to involve up-regulation of autophagic processes of cellular self-  
216 digestion. So far, molluscan autophagic genes are unspecified, and the function of pro- and  
217 antiautophagic factors, like glucagon and insulin still wait to be investigated in these animals.

218 Metabolic rate depression (MRD) in bivalves resembles the state of caloric restriction  
219 (CR), recognized as life prolonging intervention in several metazoans. It is important to note  
220 that both, depression of metabolic rate in hypoxia tolerant animals as much as CR, which  
221 after an initial transition phase is completely aerobic (Lin et al. 2008), could result in  
222 prolongation of life expectancy. Buick and Ivany (2004) proposed that one of the processes  
223 conferring an extension of lifespan in bivalves from high latitudes could be the seasonal  
224 limitations of light and food availability. Periodic times of low food intake might induce  
225 autophagic activity and possibly apoptosis in these animals, clean their cellular biochemical  
226 background from oxidized intermediates, and eliminate damaged cells and mitochondria.  
227 Regional and seasonal cold climates obviously favour frequent metabolic rate depression in  
228 bivalves like *Margaritifera margaritifera* (Ziuganov 2004), *Laternula elliptica* (Morley et al.

229 2007) or *Arctica islandica* (Taylor 1976). Slower growth, increased stress hardiness and shell  
230 repair in the Arctic and subarctic compared to warm adapted populations in Spain (*M.*  
231 *margaritifera*) or in the North Sea (*A. islandica*) support this hypothesis. Specifically,  
232 Ziuganov and colleagues (2000) found shell repair to function 30-times faster in Arctic pearl  
233 shells than in specimens collected in Catalonian rivers. Survival of specimens with severe  
234 injury of the anterior adductor muscle was 4-times better in the Arctic than in Spain. Thus,  
235 temporary depression of metabolic rate, self-induced or through an environmental signal,  
236 may shift resources from growth to damage repair and cellular renewal in long-lived Arctic  
237 specimens, allowing for a marked extension of lifespan in the cold.

238

#### 239 ***4. Surf clams and bay scallops: models for short lifespan and semelparous reproduction***

240 Whereas the determination of long lifespans is always biased by the impracticality to  
241 sample the oldest of all specimens in a population, short bivalve lifespans under 2y are  
242 relatively easily determined by mark-and-recapture field studies. Another possibility is to  
243 detect onset of senescent mass mortality as cohorts of known age approach death (Bricelj and  
244 Krause 1992). As in longer lived bivalves, lifespan in short lived species can differ between  
245 geographical and climatic zones (Bricelj et al. 1987; Cardoso and Veloso). Powell and  
246 Cummins (1985) listed MLSPs of 53 bivalves and 48 gastropod snails. Their compilation  
247 indicates two families with conspicuously short life expectancy: the scallops, Pectinidae,  
248 particularly species within the genus *Argopecten* and surf or wedge clams, Donacidae.

249 Donacidae colonize tropical and warm temperate sandy beaches, deserted by most  
250 macrofauna, where they then achieve very high densities (12.000 ind/m<sup>2</sup> Wilson 1999). They  
251 are characterized by high growth rates, high production to biomass ratios (P/B: 6-7:1, see  
252 Wilson 1999), short lifespans of 1-2 y (Cardoso and Veloso 2003) and semelparous  
253 reproduction. Moreover, surf clams are characterized by a “supreme ability as rapid  
254 burrowers” (Wilson 1999), which perform seasonal as well as daily migrations up and down

255 the beach with the tides. *Donax* species from tropical beaches are shorter lived and display  
256 higher spontaneous mortality rates under experimental conditions than temperate *Donax*  
257 species (Cardoso and Veloso 2003).

258 Published data on *Donax* physiology and biochemistry are limited. Wilson (1999)  
259 measured temperature dependent respiration (10 – 45°C at 5°C intervals) in *Donax variabilis*  
260 from South Carolina. His data document very efficient seasonal temperature compensation  
261 between 10 and 20°C in winter and suggest that the animals maintain high year round  
262 metabolic rates. A compilation of aquatic invertebrate respiration by T. Brey (unpubl. data)  
263 shows that Donacidae and Pectinidae (see next paragraph) have significantly higher  
264 metabolic activity than long lived Arcticidae (*A. islandica*). High standard metabolic rates  
265 correspond to high scopes for growth and activity in short lived clams, and altogether this  
266 indicates relatively higher mitochondrial density in *Donax* cells. However, neither  
267 mitochondrial densities nor mitochondrial respiration or marker enzyme activities have so far  
268 been measured. Antioxidant enzyme activities reported for Mediterranean *Donax trunculus*  
269 mid gut gland amounted to 2-12 U SOD mg<sup>-1</sup> protein and between 0.05 and 0.15 U catalase  
270 mg<sup>-1</sup> protein (Angel et al. 1999). Whereas the SOD activities are in the same range as in  
271 other bivalves (Abele and Puntarulo 2004: Tab. 2; Philipp et al. 2006), catalase activity is  
272 extremely low in *Donax trunculus* digestive gland. Low ROS detoxification and whole year  
273 high metabolic rates speak for low investments into cellular protection mechanisms and,  
274 according to Williams (1957), are typical for semelparous animals, which invest into  
275 reproduction rather than into prolonged somatic fitness. This means that after spawning,  
276 animals survive for limited periods of days and weeks with high probability for predation.  
277 Both burrowing infauna bivalves, *Donax* and *Arctica*, belong to the same ecotype and  
278 elegantly document adjustment of physiological and biochemical antioxidant parameters to  
279 different life history and reproductive strategies (see also chapter 2, Fig. 1 of this review).

280 A second group of short lived, semelparous bivalves, scallops of the *Argopecten*  
281 family, have lifespans of less than 2 years, and only few specimens survive for a second  
282 annual breeding cycle (Williams and Dredge 1981; Bricelj et al. 1987). Scallops belong to a  
283 different ecotype, namely that of motile swimming bivalves and populations are often under  
284 strong pressure from predation and commercial exploitation. In populations of the bay  
285 scallop *Argopecten irradians* only few specimens survive through the first winter (Bricelj et  
286 al. 1987; Bricelj and Krause 1992). Bricelj and coworkers (1987) investigated traits of  
287 senescence in caged *A. irradians* at Long Island by comparing young and old cohorts before  
288 and following spawning in late spring and summer. Mass occurrence of natural mortality in  
289 caged specimens shortly after spawning was not a consequence of reproductive efforts, and  
290 animals which survived through the winter continued somatic growth, although at lower rates  
291 and at significantly diminished oxygen uptake ( $\sim 30\%$  less  $MO_2$ ) during the second year.  
292 None of these senescent animals survived longer than 23 months and instead succumbed to a  
293 second year mass mortality event, concurrent with mass mortality in the younger cohort.

294 Decreased metabolic rates in the second year may reflect reduced mitochondrial densities  
295 in the cells of aged animals. This was recently shown to happen in queen scallops,  
296 *Aequipecten opercularis*, from the Irish Sea, with an MLSP of 8y. Between  $2 \pm 0.5y$  and  $4 \pm$   
297  $0.5y$  of age, the investigated *A. opercularis* lost 30 % of their muscle fibre mitochondria and  
298 also a consistent 30% of citrate synthase activity per unit wet weight. This loss of  
299 mitochondria in *A. opercularis* corresponded to the age dependent decline of ATP  
300 concentration and whole adenylate pool in this longer lived, iteroparous scallop (Philipp et  
301 al. 2008). Interestingly the respiratory capacity of mitochondria isolated from young and  
302 older queen scallops revealed only minor loss of function with size and age, as compared to  
303 longer lived mud clams (Philipp et al. 2006; Philipp et al. 2008). We termed this  
304 phenomenon the “*superman effect*” and proposed that it might be their strategy to maintain

305 the remaining mitochondria in good shape, than to retain high numbers of poorly functioning  
306 mitochondria per cell in the swimming muscle over their entire life.

307 Similar patterns of physiological aging in short lived *A. irradians* and 3-times longer lived  
308 queen scallops raise doubts about the trueness of semelparity in short lived bay scallops. As  
309 pointed out by Estabrooks (2007), there seems to be no selective advantage, justifying such  
310 early death in bay scallops, as, in contrast to the Pacific salmon, young scallops do not  
311 benefit from enrichment of nutrients and improved feeding conditions from dying older  
312 cohorts. To the contrary, Estabrooks, conducting investigations of telomere frequency in  
313 short lived bay scallops (*A. irradians*, MLSP 2y) and longer lived oston scallops (*A.*  
314 *purpuratus*, MLSP 6-8y) reached the conclusion that bay scallops represent a case of  
315 “interrupted iteroparity”, caused by an evolutionary loss of genomic material resulting in  
316 fewer telomeres perhaps related to loss of key chromosomal material in this species over  
317 evolutionary time scales.

318

#### 319 ***5. Age effects on metabolic and antioxidant capacities in bivalves from different climate*** 320 ***regions***

321 Several studies of moderately long-lived bivalves (up to 20 years) document a decline of  
322 growth rates as well as respiratory and filtration capacities with age, albeit independent of  
323 changes of body mass (see data compilation in [Table 1](#)). Sukhotin *et al.* (2003) and Sukhotin  
324 and Pörtner (2001) analyzed the blue mussel, *Mytilus edulis*, from temperate, sub-arctic and  
325 fully Arctic habitats. They documented decreasing respiration and filtration rates in a sub-  
326 arctic White Sea population above 6 years of animal age, indicating that blue mussels either  
327 lose or reduce respiratory capacities and functional activity over lifetime. The decline of  
328 specific respiration ( $\mu\text{mol O}_2 \text{ g}^{-1} \text{ tissue wet weight h}^{-1}$ ) was even more pronounced when data  
329 were normalized to a standard size in all age groups. This makes sense, because growth rates  
330 in mussel settlements are highly variable, and each age cluster encompasses a wide range of

331 animal sizes. However, it also enables size independent evaluation of age effects on  
332 respiration and other parameters in the *M. edulis* model and excludes the possibility that any  
333 age dependent change is merely an allometric effect, where larger animals have lower  
334 specific parameter activity. Sukhotin and Pörtner (2001) and Sukhotin *et al.* (2002) analyzed  
335 citrate synthase activity in gills and whole body tissue of sub-arctic *M. edulis* from the White  
336 Sea and found no clear indication for an age dependent change of the mitochondrial marker  
337 enzyme in this species. However, direct measurement of respiratory capacity in  
338 mitochondrial isolates of mud clams and a scallop (Fig. 3, Tab. 1) document loss of  
339 respiratory efficiency and of mitochondrial energetic coupling (RCR: respiratory control rate  
340 which indicates the intensity of mitochondrial energetic coupling) over animal lifetime  
341 (Philipp *et al.* 2005b; Philipp *et al.* 2006). The intensity of the decrease of mitochondrial  
342 respiration and phosphorylation differed significantly between the species and was most  
343 pronounced in the North Sea soft shell clam *Mya arenaria* over an age range from 2 to 10y.  
344 Comparison of temperate and Antarctic mud clam mitochondria revealed more rapid “quality  
345 loss” in mitochondria of the North Sea clam: RCR and mitochondrial membrane potential  
346 declined 6-fold more rapidly, whereas proton leak increased 5-times faster with age than in  
347 *L. elliptica* from the Antarctic. The percent rate of state 4 (uncoupled respiration without  
348 ADP) and state 3 (coupled respiration in the presence of ADP)  $H_2O_2$  formation by the  
349 isolated mitochondria increased only in the temperate mud clam, whereas it remained low  
350 and constant over 25 years of lifespan in the Antarctic species (Philipp *et al.* 2005b). Thus,  
351 the superior longevity in the polar clam, which otherwise resembles the North Sea clam in  
352 lifestyle (sedentary benthic infauna), feeding mode (particle filtration), and shell size (max  
353  $\pm 100$ mm), seems to be based on its potential to control the intensity of mitochondrial ROS  
354 formation, presumably through a higher proton leak which exerts a mild uncoupling effect on  
355 the mitochondrial membranes (Brand 2000).



356 Another important antioxidant protection regarding the mitochondrial ROS formation is  
357 the radical scavenging glutathione system (Table 1). An age dependent diminishment of  
358 parameters of the glutathione system was recorded in temperate *Mytilus edulis* from the  
359 Plymouth area (Canesi and Viarengo 1997: performed measurements in digestive gland and  
360 gills of total glutathione, glutathione reductase, glutathione transferase and  $\gamma$ -glu-cys  
361 synthetase activity). In a similar approach, our team recorded reduction of the glutathione  
362 tissue concentration over lifetime in temperate and polar scallops (Philipp et al. 2006 in  
363 mantle tissue) and in the Antarctic mud clam *Laternula elliptica* (Philipp et al. 2005a in  
364 mantle tissue). The GSSG:GSH ratio, however, decreased only in scallops, the temperate  
365 *Aequipecten opercularis* and the polar *Adamussium colbecki* over age, whereas it remained  
366 constant in *L. elliptica*. Scallops maintained generally less oxidized GSSG:GSH ratios  
367 compared to the mud clams, and it may be adaptive for these active species to preserve high  
368 GSH levels for quenching ROS during burst swimming. Philipp *et al.* (2008) showed that  
369 during exercise [GSH] and pH rapidly decline in the adductor muscle of queen scallops, *A.*  
370 *opercularis*, which markedly affects the cellular redox ratio. Further, the decrease in tissue  
371 GSSG:GSH ratios over lifetime in scallops suggests that the age dependent net loss of  
372 glutathione was not due to progressive oxidation, but rather to an effective removal of  
373 oxidized GSSG from the tissues. A high GSSG:GSH ratio is problematic as it can impact on  
374 signal transduction, DNA and RNA synthesis, protein synthesis, enzyme activation, and even  
375 regulation of the cell cycle.

376 The temperate soft shell clam *Mya arenaria* was the only bivalve in which we found  
377 GSSG to accumulate with age, the concentration doubling in mantle tissue within 6 years of  
378 lifetime from 30 to over 80 nmol GSSG g<sup>-1</sup> wet weight. Within the same period, the overall  
379 glutathione concentration (GSH + 2GSSG) increased from 200 to 400 nmol g<sup>-1</sup> wet weight in  
380 *M. arenaria* mantle tissue. Together with the decrease in mitochondrial respiration and  
381 coupling, increased conversion of oxygen to ROS and accumulation of GSSG with age,

382 increases in the overall glutathione concentration in mantle tissue suggest that prooxidant  
383 processes intensify, and higher antioxidant protection is needed in aging soft shell clams.

384 Both burrowing mud clams, *Mya arenaria* from the North Sea and *Arctica islandica* from  
385 the Iceland population had very low glutathione levels around 300 nmol g<sup>-1</sup> wet weight in  
386 mantle tissue. In *A. islandica* the concentration remained constant between 36-196 years of  
387 age, following maturation of the animals (Abele et al. 2008). Thus, these burrowing mud  
388 clams preserve glutathione at constant (or even increasing) levels over lifetime, as opposed  
389 to epibenthic scallops or blue mussels (Table 1), in which glutathione levels decrease over  
390 lifetime in mantle and adductor muscle.

391 Another interesting question regarding polar and temperate ectotherm longevity is whether  
392 or not changes involved in physiological cold adaptation may directly impinge on the process  
393 of ageing. According to Porter *et al.* (1996), proton leak increases as a function of inner  
394 mitochondrial membrane surface area (cristae density) and can be modulated by the degree  
395 of unsaturated fatty acids in the membrane. Higher numbers of mitochondria and higher  
396 cristae densities are found in cold adapted ectotherms, helping them to overcome diffusion  
397 problems and compensate for the decline in metabolism at cold temperatures. A comparison  
398 of the ratio of cytochrome oxidase/citrate synthase activity (COX/CS) in temperate and polar  
399 mud clams indeed suggests that polar species have higher mitochondrial cristae densities  
400 (Philipp et al. 2005b). Thus, on the one hand, higher proton leak which, theoretically,  
401 mitigates ROS formation and slows aging, may indeed be a consequence of cold adaptation  
402 in polar and especially Antarctic ectotherms with low aerobic scopes. This brings up the  
403 question whether mild uncoupling of the mitochondria and high glutathione levels in polar  
404 compared to temperate mud clams are primarily a consequence of physiological cold  
405 adaptation. Alternatively *L. elliptica* may have evolved higher proton permeability of the  
406 inner membrane and store more glutathione in order to prolong lifespan as a feature  
407 supporting sustainable “population management” in the cold. In other words, do polar clams

408 need longer life time to insure survival of the stock, or does a different lipid composition  
409 render polar clam membranes leaky as a “by-effect of cold adaptation”? The long lifespan  
410 may be adaptive for survival of ectotherms in polar habitats, where temperature slows not  
411 only adult growth and development, but moreover protracts molluscan gametogenesis and  
412 embryonic development, and possibly delays hatching of mature trochophora larvae from the  
413 eggs (Peck et al. 2007).

414

#### 415 **6. Genes and lifespan in bivalves**

416 Within the metazoan kingdom, extreme differences of species longevity, from a few days  
417 to a hundreds of years reflect the genetic basis of MLSP biodiversity. However, if life  
418 expectancy is partly in our genes, this raises the question of which genetic traits exactly  
419 support longevity, and whether or not the same genes are essentially involved in all  
420 metazoans (see also Austad 2001)? Furthermore, what renders a species eligible for the  
421 selection of longevity supporting genes in evolution?

422 Rather than a small number of specific “old age genes” that, just by being present as wild  
423 or mutated alleles confer extreme species longevity, it seems that certain combinations of  
424 genes involved in choreographing the relevant processes of life-long metabolic performance,  
425 are important in determining the velocity of aging. In order to be selected, these genetic  
426 “choreographers” need to confer fitness advantage to the animals at a young age (see  
427 Williams 1957; Zwaan 1999). Along this line of thinking, the “gene cluster hypothesis of  
428 aging” has been put forward by G. Barja (2008), who proposes central *homeotic regulator*  
429 *genes* (transcription factors like *hox* genes) to coordinate and optimize lifetime gene  
430 transcription for an optimal exploitation of environmental resources and adaptation to  
431 environmental constraints (such as times of low food availability in nature). Bivalves are of  
432 low complexity in their organizational hierarchies and ontogeny when compared to insects or  
433 mammals. This might make it easier for them to rapidly adjust their metabolism in order to

434 meet abruptly changing environmental conditions. The regulatory cascades involved in these  
435 adaptive changes may be shorter and less complex, and could potentially contribute to the  
436 understanding of the genetic basis of longevity in higher organisms.

437 Lower complexity and high adaptability may, moreover, render long lived bivalves more  
438 tolerant of non-lethal deleterious mutations, acting late in life and resulting in a graded loss  
439 of individual fitness that accelerates aging (Dall and Cuthill 1999). This may mean a higher  
440 probability of individuals surviving to ages closer to MLSP, once predation pressure (and  
441 other impact immediately killing the animals) has been ruled out. It may also make possible  
442 to sample individuals closer to maximum age in bivalve populations. In agreement with this,  
443 large infaunal bivalves with heavy shells, which experience relatively low pressure from  
444 predation, have the highest recorded MLSPs. They have presumably optimized their genomic  
445 life history program for increased longevity with undiminished or even increasing  
446 reproductive output at old age (Williams 1966). Important candidate traits include late  
447 maturation, low (less than yearly) reproductive frequency, self-programmed  
448 hypometabolism, as well as mitochondrial oxyconformity and low basal ROS formation  
449 rates.

450 Tumour formation would range among “late acting deleterious mutations” and has not yet  
451 been recognized as a major cause of death in aged bivalves. Blood cancer, as well as  
452 different malignant neoplasms have been identified in bivalves and are listed by Cheng  
453 (1993) for several species. Experiments to induce tumours in bivalves using carcinogenic  
454 hydrocarbons generally fail, but application of aromatic amines can cause basophilic cell  
455 neoplasms and hematopoietic cancer (200-400 ppm diethylnitrosamines causes cancer in  
456 painters shell *Unio pictorum*, Khudolei and Sirenko 1977), as these compounds are being  
457 converted to mutagens in bivalve digestive glands. The effect of tumour formation on animal  
458 life expectancy is unclear. Thus, mass mortality in the hard shell clam *Mercenaria spp.* could  
459 not be related to germ cell tumours (germinoma) occurring massively in this species. Instead,

460 increased tumour incidence is detected during times of rapid environmental change  
461 (Hesselmann et al. 1988). Germinal tumours were also seasonally increased and more  
462 abundant at warm summer water temperatures. Hard shell clams from Florida generally  
463 displayed higher tumour incidence than clams from colder Rhode Island waters (Hesselmann  
464 et al. 1988). Thus, higher susceptibility to malignant neoplastic diseases may be involved in  
465 shortening population specific maximum lifespan in bivalves with a geographical  
466 distribution between different climate zones. Tumour promoters discussed in the literature  
467 are infestations by herpes-type viruses which are more common at warmer water  
468 temperatures. On the other hand, there might also be a general predisposition through certain  
469 proto-oncogenes (genes that by mutation are converted into tumour inducing oncogenes) in  
470 warm water bivalves. Information on proto-oncogene signalling cascades in molluscan  
471 species is largely missing, however, Degnan and collaborators confirmed conservation of the  
472 *ets*-multigene family, homolog to the human oncogenes, throughout nearly all marine  
473 metazoan phyla (Degnan et al. 1993), including molluscs and sponges. The proteins encoded  
474 by these *ets* proto-oncogenes are involved in the regulation of multicellular development and  
475 cell proliferation, but their specific function in protostome metazoans, or their role in tumour  
476 formation, are far from clear. However, higher tumour incidence in warmer waters  
477 underlines temperature is a prime factor in control of bivalve life expectancy.

478 Another interesting question is whether minor variations in maximum lifespans between  
479 different populations of one species within a climatic zone can have a genetic or genomic  
480 basis. These variations are reported from the long lived pearl clam, which in the Russian  
481 Arctic may attain population Amax between 190 (Varzuga) and 114 (Keret) (Ziuganov et al  
482 2000: Tab.1). In our investigation of *Arctica islandica*, the most conspicuous discrepancy  
483 occurred between 3 populations with the same habitat temperature range (0 and 10°C) but  
484 from different locations and with very different Amax of 370 (Iceland), 93 (North Norway)  
485 and 53 (White Sea) (Begum and Basova unpubl. data). Is this due to environmental effects

486 that limit lifespan in one environment and not in the other? This would be in line with the  
487 symmorphosis concept proposed by Weibel and Taylor (1991), which states that biological  
488 systems are designed to meet, but not exceed, their natural requirements. Pleiotropic trade-  
489 offs are largely a function of environmental forcing of the budgeting of energy between  
490 fitness and fecundity during species lifetime. Minor variations of MLSP between populations  
491 by the 1.3 to 2 fold therefore seem to be primarily a consequence of environmental  
492 adaptations and ecological adjustments see also Barja (2008).

493 Heterozygosity (heterosis) may be the link between the genetic and adaptive physiological  
494 determinators of population MLSP. It is an important criterion for animal fitness, as  
495 heterozygous alleles for functional proteins theoretically increase adaptability. Bivalves with  
496 multiple allozyme heterozygosity display lower metabolic rates (Koehn and Shumway 1982)  
497 and lower rates of protein turnover, which enables them to invest more energy into growth,  
498 reproduction and presumably predator avoidance (Hawkins et al. 1986; Myrand et al. 2002).  
499 Meanwhile, an extensive body of literature documents better resistance to stress to be due to  
500 elevate levels of heterozygosity in bivalves, gastropods and fish (cited in Myrand et al.  
501 2002). The authors speculate that heterozygous bivalves may be more apt to enter  
502 hypometabolic states, but this is not yet backed by experimental evidence. To the contrary,  
503 correlation of allozyme heterozygosity with either growth, reproduction, or survival  
504 (individual lifespan) is not found in pectinids (Volkaert and Zouros 1989; Bricelj and Krause  
505 1992; Fevolden 1992). This speaks against heterozygote superiority supporting predator  
506 avoidance in motile bivalves, and, indeed, from the marine ecologist's point of view, predator  
507 avoidance is a behavioural trait and presumably involves a multitude of genetic and  
508 physiological traits and cascades. Moreover, hypometabolism is not an option for scallops  
509 given their active energy consuming life style. A more radical reduction of genetic variability  
510 may be the consequence of important losses of genetic material including key telomeric  
511 sequences, possibly occurring in the evolution of the bay scallop, as proposed by Estabrooks

512 (2007) and referred above. This could indeed be the radical way of shortening life  
513 expectancy in scallops.

514 In the context of the possible effect of gene flow on allele frequencies and aging in  
515 bivalves, a noticeable discrepancy exists between North and South polar regions. Supposing  
516 heterozygosity depends on gene flow (which is not entirely true, C. Held pers.  
517 communication) and further supposing that longevity is supported by heterozygosity in  
518 Arctic and Antarctic sessile molluscs: Can this help in explaining why extremely long lived  
519 bivalves such as *Arctica islandica*, *Hiatella arctica* and *Margaritifera margaritifera* are  
520 known from Arctic and Subarctic and not from Antarctic regions? The longest lived  
521 Antarctic bivalve so far reported in the literature is the infaunal nuculanid *Yoldia eightsi*  
522 which can live well over 50y. This is slightly more than the recorded Amax of the Antarctic  
523 mud clam, *Laternula elliptica* with a certified maximum age of 36y and the Antarctic scallop  
524 *Adamussium colbecki* (>40y). Other invertebrates in the Antarctic have been aged to over 50  
525 years including the sea urchins *Sterechinus antarcticus* and *Sterechinus neumayeri* (Brey et  
526 al. 1995) and 2 Antarctic brachiopods live over 55 years (Peck and Brey 1996), but none  
527 reach ages over 100 y comparable to the long-lived Arctic molluscs.

528 The Antarctic circumpolar current forms a barrier that reduces gene flow and genetically  
529 isolates the stenothermal Antarctic molluscs (Patarnello et al. 1990). Many Antarctic species  
530 are endemic and have optimized their ecology and physiology at constant low water  
531 temperature, with low flexibility and tolerance to environmental fluctuation. Genes/alleles  
532 selected during 10-15 millions of years of Antarctic evolution guarantee optimal thermal  
533 adaptation. The trade-off for this specialization may be found in the relatively short  
534 reproductive lifespan of Antarctic bivalves, between late maturation on the one hand, as  
535 development of Antarctic bivalve trochophora larvae takes 3-15 times longer than in  
536 temperate species, and moderate longevity on the other (Peck et al. 2006).

537 Data on heterozygosity in Arctic and Antarctic invertebrates are limited: The Iceland scallop,  
538 *Chlamys islandica*, is an Arctic and subarctic species with “exceptional variation at several  
539 gene loci” including stress genes like superoxide dismutase and glycolytic genes (Fevolden  
540 1992). In contrast, 3 species of lipid fish in Spitsbergen showed extremely low allelic  
541 heterozygosity (Fevolden et al. 1989). This led Fevolden to conclude that Iceland scallops  
542 represent an exception of high heterozygosity in cold polar environments. In the Antarctic,  
543 Patarnello *et al.* (1990) found very low average heterozygosity of 0.6 and 0.7% in 22  
544 investigated gene loci of 2 amphipods, supporting the idea that homozygous “specialists”  
545 with higher metabolic efficiency through cold adapted proteins/genes/alleles are selected  
546 under *Antarctic* conditions. However, given the scarcity of data in the Antarctic, as well as  
547 the variability in methods, numbers and nature of genes in the available data sets, it seems  
548 that any conclusion would be preliminary and, again, opens the field for future research.

549

#### 550 **7. Conclusions and further research directions.**

551 Bivalve molluscs are promising models for age research because they allow very clear  
552 distinctions between internal and environmental factors that control the velocity of  
553 senescence in cells and tissues. Similar ecotypes, or even the same species can be obtained  
554 from different climatic backgrounds and aged by hard structure analysis, yielding  
555 information on the role of climatic adaptation on population MLSP, and the ecological and  
556 physiological strategies that stabilize the population under the given conditions. As different  
557 bivalve life time strategies involve early and late onset of maturation with semelparous or  
558 iteroparous reproduction, we can analyze the trade-offs in both systems. Some species are  
559 able to maintain their population based on very short lifespans, and the genes dispensable in  
560 those species following early maturation may be just as informative for ageing studies, as the  
561 analysis of genes that are most actively transcribed in old individuals of the longest lived  
562 bivalve species.



563 In the future, a major interest will be to analyse the importance of gene heterozygosity in  
 564 relation to species adapting to stress, exploiting a niche and extending lifespan. Specifically,  
 565 we need to understand more about the roles of hypometabolism in bivalve ageing, as it seems  
 566 that the longest lived species employ intermittent dormancy as a basic behaviour, and that  
 567 seasonal low food conditions could indeed be life prolonging. Studies must include the  
 568 analysis of gene transcription before, during and after self-induced and seasonal dormancy,  
 569 to understand which genes and pathways are important for cellular maintenance.

570

571

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573

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772 Figure legends

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774 Figure 1: Principle of ROS control in low PO<sub>2</sub> (left) and high PO<sub>2</sub> (right) strategic lifestyles  
775 in bivalves.

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777 Figure 2: Change in respiration rate of isolated mitochondria with age of the bivalves *Mya*  
778 *arenaria* (black dots), *Aequipecten opercularis* (white dots) and *Laternula elliptica* (grey  
779 dots) measured at mean in situ temperature of each species (10°C for *M. arenaria* and *A.*  
780 *opercularis* and 0°C for *L. elliptica*) (data from Philipp et al. 2005b; data from Philipp et al.  
781 2006).

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783 Figure 3: Antioxidant capacity of molluscs (black dots: scallops, black squares: clams, grey  
784 dots: limpets, white dots: cephalopods) with known maximum lifespans. Shown are means  
785 and SD. Data from (Zielinski and Pörtner 2000; Philipp et al. 2005a; Philipp et al. 2006;  
786 Abele et al. 2008) and the Antarctic limpet *Nacella concinna* (unpublished data D. Abele, E.  
787 Weihe).

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790 **Table 1:** Changes of metabolic and oxidative stress parameters over lifetime in marine  
 791 bivalves from different climatic environments. Data from 1: (Hopkins 1930), 2: (Sukhotin  
 792 and Pörtner 2001), 3: (Philipp et al. 2005b), 4: (Philipp et al. 2006), 5: (Philipp et al. 2008),  
 793 6: (Abele et al. 2008), 7: (Viarengo et al. 1991), 8: (Philipp et al. 2005a), 9: (Sukhotin et al.  
 794 2002), 10 (Canesi and Viarengo 1997), 11: (Hole et al. 1995), 12: (Strahl et al. 2007), 13:  
 795 (Lomovasky et al. 2002), 14: (Viarengo et al. 1989)  
 796 Parameter: COX: cytochrome oxidase activity, CS: citrate synthase activity, CAT: catalase  
 797 activity, SOD: superoxide dismutase activity, GPX: glutathione peroxidase activity. Effect:  
 798 decrease (-), increase (+), no change (0) with age

Species	Tissue	Climate regime	Parameter	Effect	Ref
<b>Metabolism</b>					
<i>Venus mercenaria</i>	adductor muscle	temperate	oxygen consumption	-	(1)
<i>Pecten irradians</i>	adductor muscle	temperate	oxygen consumption	-	(1)
<i>Mytilus edulis</i>	whole animal	cold temperate	oxygen consumption	-	(2)
<b>Mitochondrial parameters</b>					
<i>Laternula elliptica</i>	mantle	Cold	mitochondrial respiration	-	(3)
<i>Mya arenaria</i>	mantle	temperate	mitochondrial respiration	-	(3)
<i>Aequipecten opercularis</i>	mantle, add. muscle	cold temperate	mitochondrial respiration	-	(4)
<i>Laternula elliptica</i>	mantle	Cold	COX	0	(3)
<i>Mya arenaria</i>	mantle	temperate	COX	0	(3)
<i>Aequipecten opercularis</i>	mantle	cold temperate	COX	-	(4)
<i>Adamussium colbecki</i>	mantle	Cold	COX	0	(4)
<i>Laternula elliptica</i>	mantle	Cold	CS	0	(3)
<i>Mya arenaria</i>	mantle	temperate	CS	0	(3)
<i>Aequipecten opercularis</i>	mantle, add. muscle	cold temperate	CS	-	(4,5)
<i>Arctica islandica</i>	mantle, gill	cold	CS	-	(6)
<i>Adamussium colbecki</i>	mantle	cold	CS	0	(4)
<i>Mytilus edulis</i>	whole body	cold temperate	CS	-	(2)
<b>ROS generation rate</b>					
<i>Laternula elliptica</i>	mantle	cold	ROS/mg mitochondrial protein	low and decrease	(3)
<i>Mya arenaria</i>	mantle	temperate	ROS/mg mitochondrial protein	high and no change	(3)
<i>Aequipecten opercularis</i>	mantle, add. muscle	cold temperate	ROS/mg mitochondrial protein	very low and no change	(4,5)

**Antioxidant enzymes**

<i>Mytilus edulis</i>	digestive gland	temperate	CAT	-	(7)
<i>Laternula elliptica</i>	mantle	cold	CAT	0	(8)
<i>Mya arenaria</i>	mantle	temperate	CAT	0	(8)
<i>Aequipecten opercularis</i>	mantle, add. muscle	cold temperate	CAT	-	(4,5)
<i>Adamussium colbecki</i>	mantle	cold	CAT	0	(4)
<i>Arctica islandica (&gt;36y)</i>	mantle, gill	cold	CAT	0	(6)
<i>Mytilus edulis</i>	whole body	cold temperate	CAT	0	(9)
<i>Mytilus edulis</i>	whole body	cold temperate	SOD	0	(9)
<i>Mytilus edulis</i>	digestive gland	temperate	SOD	0	(7)
<i>Laternula elliptica</i>	mantle	cold	SOD	0	(8)
<i>Mya arenaria</i>	mantle	temperate	SOD	0	(8)
<i>Arctica islandica</i>	mantle, gill	cold	SOD	0	(6)
<i>Aequipecten opercularis</i>	mantle	cold temperate	SOD	0	(4)
<i>Mytilus edulis</i>	digestive gland	temperate	GPX	-	(7)
<b>Non-enzymatic antioxidants</b>					
<i>Mytilus edulis</i>	digestive gland	temperate	Glutathione	-	(7, 10)
<i>Mytilus edulis</i>	gills	temperate	Glutathione	-	(10)
<i>Laternula elliptica</i>	mantle	cold	Glutathione	-	(8)
<i>Mya arenaria</i>	mantle	temperate	Glutathione	+	(8)
<i>Aequipecten opercularis</i>	mantle, add.muscle	cold temperate	Glutathione	-	(4,5)
<i>Adamussium colbecki</i>	mantle	cold	Glutathione	-	(4)
<i>Arctica islandica (&gt;36y)</i>	mantle, gill	cold	Glutathione	0	(6)
<i>Laternula elliptica</i>	mantle	cold	GSSG: GSH	0	(8)
<i>Mya arenaria</i>	mantle	temperate	GSSG: GSH	+	(8)
<i>Aequipecten opercularis</i>	adductor muscle	cold temperate	GSSG: GSH	0	(5)
<i>Aequipecten opercularis</i>	mantle	cold temperate	GSSG: GSH	-	(4)
<i>Arctica islandica</i>	mantle, gill	cold	GSSG: GSH	0	(6)
<i>Adamussium colbecki</i>	mantle	cold	GSSG: GSH	-	(4)

**Cell damage parameters**

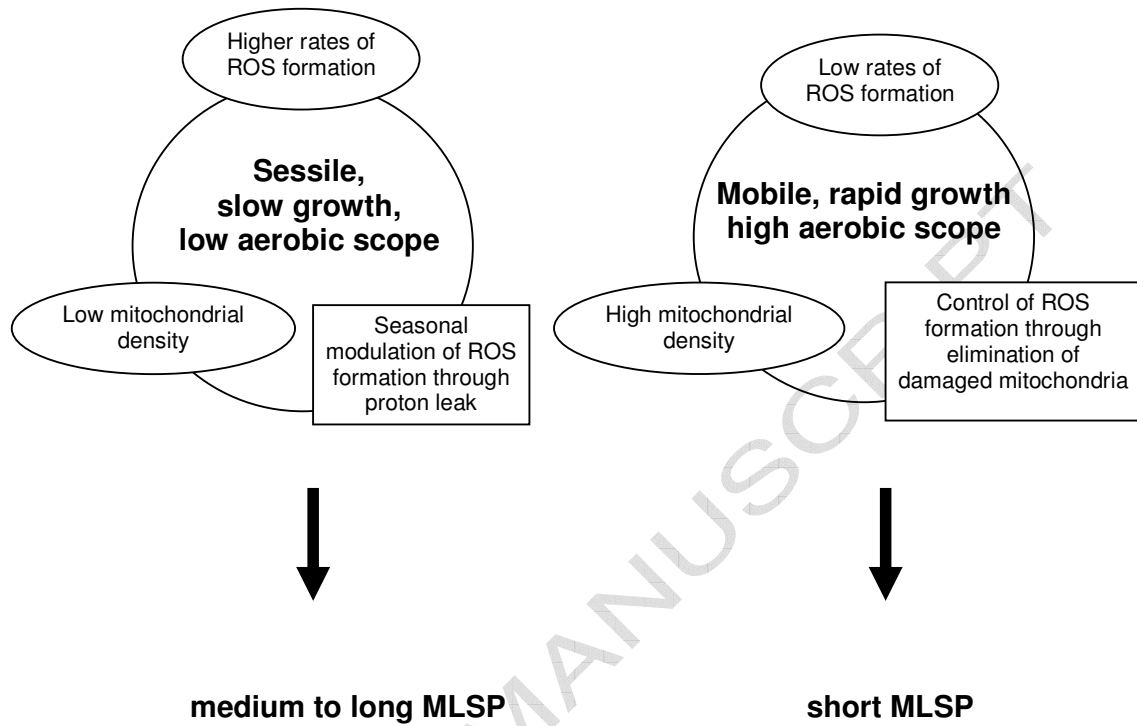
<i>Mytilus edulis</i>	whole body	cold temperate	lipofuscin	+	(9)
<i>Mytilus edulis</i>	digestive gland	temperate	lipofuscin	-	(11)
<i>Laternula elliptica</i>	mantle	cold	lipofuscin	+	(8)
<i>Mya arenaria</i>	mantle	temperate	lipofuscin	first - then +	(8)
<i>Aequipecten opercularis</i>	mantle	cold temperate	lipofuscin	+	(4)
<i>Arctica islandica</i>	mantle, gill, muscle	cold	lipofuscin	+	(12)
<i>Adamussium colbecki</i>	mantle	cold	lipofuscin	+	(4)
<i>Eurhomalea exalbida</i>	connective tissue	cold	lipofuscin	+	(13)
<i>Mytilus edulis</i>	whole body	cold temperate	MDA	0	(9)
<i>Aequipecten opercularis</i>	adductor muscle	temperate	MDA	0	(5)
<i>Mytilus edulis</i>	digestive gland	temperate	MDA	+	(7,14)
<i>Laternula elliptica</i>	mantle	cold	protein carbonyls	+	(8)
<i>Mya arenaria</i>	mantle	temperate	protein carbonyls	-	(8)
<i>Aequipecten opercularis</i>	mantle	cold temperate	protein carbonyls	0	(4)
<i>Arctica islandica</i>	mantle, gill	cold	protein carbonyls	0	(6)
<i>Adamussium colbecki</i>	mantle	cold	protein carbonyls	0	(4)



800 Fig. 1:

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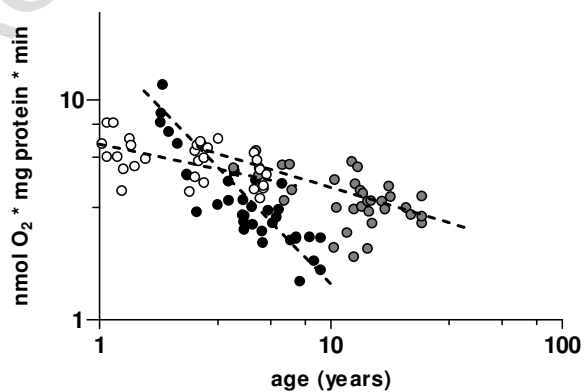
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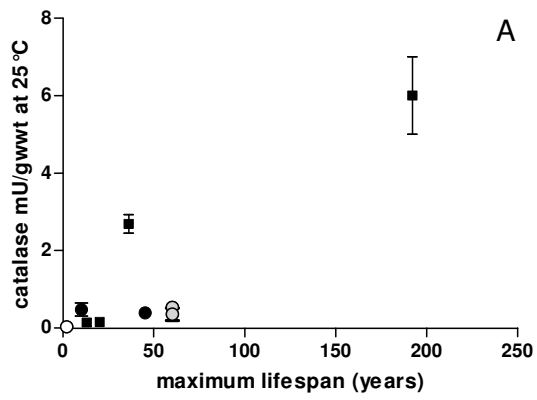


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815 Fig. 2: Change in respiration rate of isolated mitochondria with age of the bivalves *Mya*  
816 *arenaria* (black dots), *Aequipecten opercularis* (white dots) and *Laternula elliptica* (grey  
817 dots) measured at mean in situ temperature of each species (10°C for *M. arenaria* and *A.*  
818 *opercularis* and 0°C for *L. elliptica*). Data compiled from Philipp et al. 2005b and 2006.  
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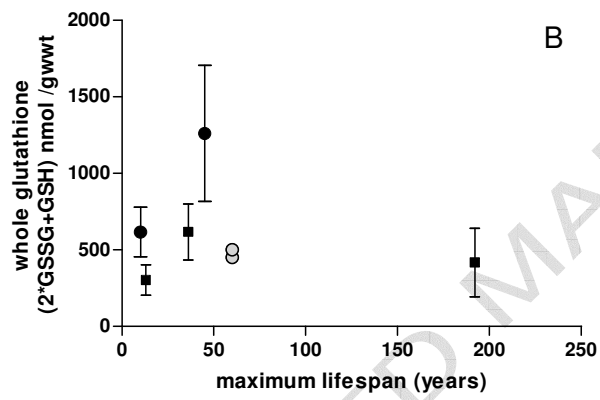
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822 Fig. 3

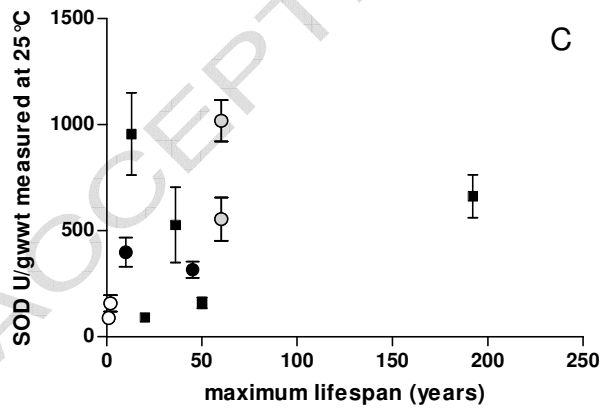


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