Experimental Gerontology xxx (2009) xxx–xxx

Contents lists available at ScienceDirect

Experimental Gerontology

journal homepage: www.elsevier.com/locate/expgero

Review

Bivalve models of aging and the determination of molluscan lifespans

Doris Abele [a,*], Thomas Brey [a], Eva Philipp [b]

[a] Alfred-Wegener-Institute for Polar and Marine Research, Am Handelshafen 12, 27570 Bremerhaven, Germany
[b] Institute for Clinical Molecular Biology, Schittenhelmstrasse 12, 24105 Kiel, Germany

ARTICLE INFO

Article history:
Received 17 October 2008
Received in revised form 21 February 2009
Accepted 23 February 2009
Available online xxxx

Keywords:
Bivalve
Longevity
Aging
Stress tolerance
Heterozygosity
Antarctic
Genes
Metabolic rate depression

ABSTRACT

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

1. Bivalves: a diverse set of models for aging studies and environmental recorders

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

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

Within the Bivalvia, we find species firmly attached to the substratum (e.g., Ostreidae, oysters, and Mytilidae, mussels), others that crawl with the help of their muscular foot (most species), and even facultative swimmers (e.g., Pectinidae, scallops). Infaunal species burrow in soft (e.g., Tellinidae, clams) and hard substrates (e.g., Teredinidae, shipworms) and can live at the sediment-water interface (e.g., Unioinidae, freshwater mussels), or burrow down to 100 cm of sediment depth (e.g., Myidae, soft shell clams, or Hiatellidae, geoducks). Epifaunal species are encountered on all kinds of surfaces, from rocks to such exotic places as the spines of sea urchins (e.g., *Lissarca notorcadensis* on Antarctic cidaroids, Brey and Hain, 1992). Bivalves inhabit a pressure range from 1 atm in the littoral to about 1000 atm in the deep sea; a temperature range from −1.8 °C in polar waters (Antarctic clams) to >30 °C in tropical

* Corresponding author. Tel.: +49 471 4831 1567; fax: +49 471 4831 1149.
E-mail address: Doris.abele@awi.de (D. Abele).

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doi:10.1016/j.exger.2009.02.012

seas and around deep sea hot vents (e.g., Vesicomyidae), and a salinity range from fresh water (e.g., Unionidae) to hypersaline waters (e.g., the cockle *Fragum erratum* at ±60‰, Morton, 2000).

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

The distinct advantage of bivalves compared to the classical aging models, however, is their carbonate shell. The shell can serve the determination of individual age and, at the same time, it can archive changes of environmental conditions throughout the animal's lifetime. Both the instantaneous shell growth rate and the composition of newly forming shell material are subject to extrinsic change, meaning they can be modified by environmental factors. Shell growth rate is largely governed by temperature and food availability (Richardson, 2001). Hence, bivalves, coming from systems with a distinct seasonality with regard to either one or both parameters, exhibit seasonal shell growth patterns that produce an annual growth banding, similar to trees. Once the annual shell band patterns are calibrated for a population, animals can be taken from the environment and individually aged. This enables the study of the impact of environmental drivers on the ecology and physiology of bivalves over time in natural populations from different climatic or ecological settings. Moreover, lunar, daily, or even tidal cycles are distinguishable in some fast growing species, at least a posteriori (e.g., Chauvaud et al., 2005; Kanazawa and Sato, 2008). Further, archived banding patterns allow reconstruction of lifetime growth history, including long-term trends, such as climate driven decadal patterns (e.g., Schöne et al., 2005), as well as unique physical disturbance events caused by storms, predator attack, extreme temperatures or even incidents of low oxygen concentrations (Witbaard and Klein, 1994; Sejr et al., 2002).

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

2. ROS formation and antioxidant strategies in bivalves

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

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

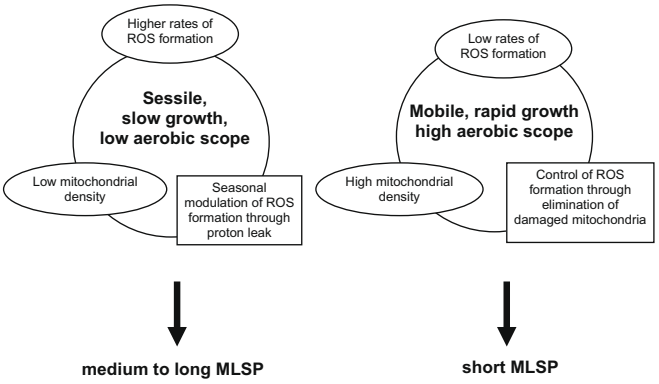

Fig. 1. Principle of ROS control in low PO_2 (left) and high PO_2 (right) strategic lifestyles in bivalves.

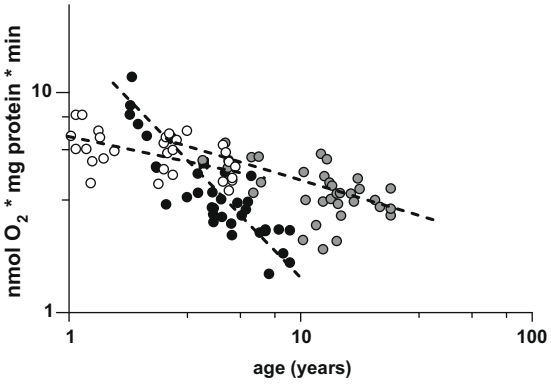

Fig. 2. Change in respiration rate of isolated mitochondria with age of the bivalves *Mya arenaria* (black dots), *Aequipecten opercularis* (white dots) and *Laternula elliptica* (grey dots) measured at mean in situ temperature of each species (10 °C for *M. arenaria* and *A. opercularis* and 0 °C for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

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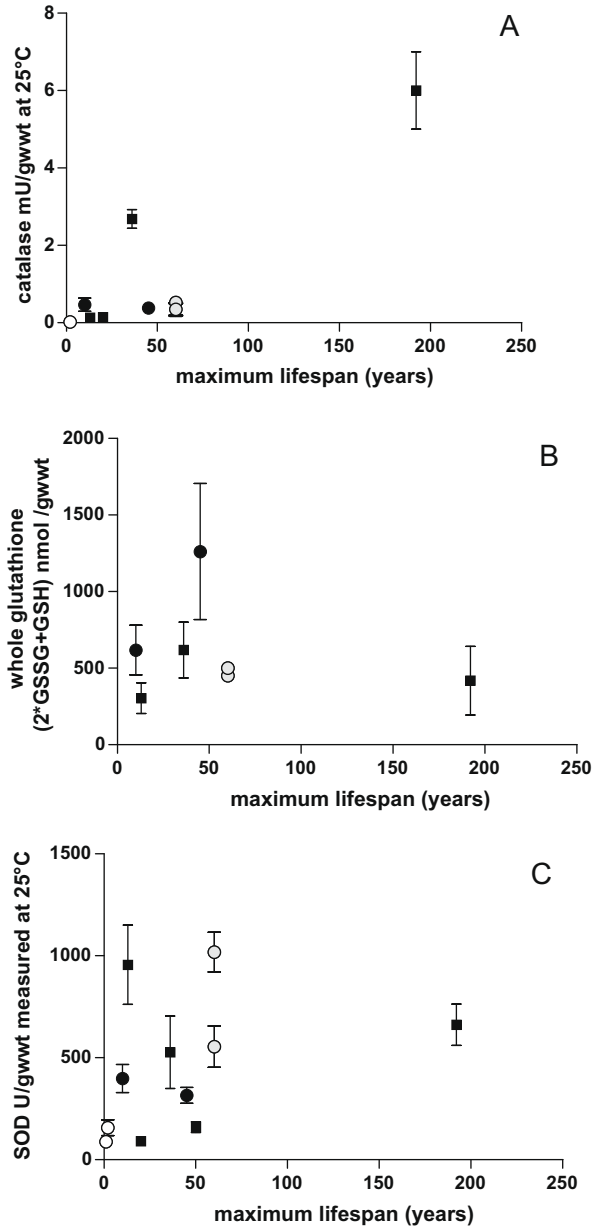

Fig. 3. Antioxidant capacity of molluscs (black dots: scallops, black squares: clams, grey dots: limpets, white dots: cephalopods) with known maximum lifespans. Shown are means and SD. Data from Zielinski and Pörtner (2000); Philipp et al. (2005a, 2006); Abele et al. (2008) and the Antarctic limpet *Nacella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

MLSPs as in the ocean quahog seem to further require high antioxidant potential.

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

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

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

Arctica islandica displays a characteristic behaviour in spontaneously burrowing into the sediment, which is not regulated by external triggers and therefore occurs at irregular and individually determined intervals. The normal position of *A. islandica* is just below the sediment surface, so that the very short siphons reach into the water column. Occasionally, the animals burrow a couple of cm deeper into the sediment and, by closing their siphons and shell valves, expose their tissues to hypoxic and even completely anoxic conditions for 1–5 days (Taylor, 1976 and our own observations). During these periods, the animals enter a metabolically depressed state (“metabolic rate depression”, MRD) down to 10% of their normoxic metabolic activity (Taylor, 1976: heart beat; Oeschger and Storey, 1993: down regulation of glycolytic enzyme activity) which allows them to survive on anaerobic energy production alone. Similarly, Ziuganov and colleagues reported pearl shell clams to be capable of “altering metabolic rate independently of temperature” and thereby “enter and exit diapause” (Ziuganov et al., 2000; Ziuganov, 2004). This energy saving behaviour could

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theoretically slow senescence in several ways in the bivalves. Firstly, it involves a slow down of metabolic ROS production in the depressed state, which, in oxyconforming species not prone to oxidative stress during hypoxia-reoxygenation cycles, could be beneficial (Abele et al., 2007). On the contrary, frequent hypoxia-reoxygenation cycles in ectotherms may trigger antioxidant protection (Hermes-Lima and Zenteno-Savin, 2002). Another important mechanism is the induction of a "famine-like" state (Ziuganov et al., 2000) during shell closure, bound to involve up-regulation of autophagic processes of cellular self-digestion. So far, molluscan autophagic genes are unspecified, and the function of pro- and anti-autophagic factors, like glucagon and insulin still wait to be investigated in these animals.

Metabolic rate depression (MRD) in bivalves resembles the state of caloric restriction (CR), recognized as life prolonging intervention in several metazoans. It is important to note that both, depression of metabolic rate in hypoxia tolerant animals as much as CR, which after an initial transition phase is completely aerobic (Lin et al., 2008), could result in prolongation of life expectancy. Buick and Ivany (2004) proposed that one of the processes conferring an extension of lifespan in bivalves from high latitudes could be the seasonal limitations of light and food availability. Periodic times of low food intake might induce autophagic activity and possibly apoptosis in these animals, clean their cellular biochemical background from oxidized intermediates, and eliminate damaged cells and mitochondria. Regional and seasonal cold climates obviously favour frequent metabolic rate depression in bivalves like *M. margaritifera* (Ziuganov, 2004), *L. elliptica* (Morley et al., 2007) or *A. islandica* (Taylor, 1976). Slower growth, increased stress hardiness and shell repair in the Arctic and subarctic compared to warm adapted populations in Spain (*M. margaritifera*) or in the North Sea (*A. islandica*) support this hypothesis. Specifically, Ziuganov and colleagues (2000) found shell repair to function 30-times faster in Arctic pearl shells than in specimens collected in Catalonian rivers. Survival of specimens with severe injury of the anterior adductor muscle was 4-times better in the Arctic than in Spain. Thus, temporary depression of metabolic rate, self-induced or through an environmental signal, may shift resources from growth to damage repair and cellular renewal in long-lived Arctic specimens, allowing for a marked extension of lifespan in the cold.

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

Whereas the determination of long lifespans is always biased by the impracticality to sample the oldest of all specimens in a population, short bivalve lifespans under 2 y are relatively easily determined by mark-and-recapture field studies. Another possibility is to detect onset of senescent mass mortality as cohorts of known age approach death (Bricelj and Krause, 1992). As in longer lived bivalves, lifespan in short lived species can differ between geographical and climatic zones (Bricelj et al., 1987; ; Cardoso and Veloso; 2003). Powell and Cummins (1985) listed MLSPs of 53 bivalves and 48 gastropod snails. Their compilation indicates two families with conspicuously short life expectancy: the scallops, Pectinidae, particularly species within the genus *Argopecten* and surf or wedge clams, Donacidae.

Donacidae colonize tropical and warm temperate sandy beaches, deserted by most macrofauna, where they then achieve very high densities (12.000 ind/m^2 Wilson, 1999). They are characterized by high growth rates, high production to biomass ratios (P/B: 6–7:1, see Wilson, 1999), short lifespans of 1–2 y (Cardoso and Veloso, 2003) and semelparous reproduction. Moreover, surf clams are characterized by a "supreme ability as rapid burrowers" (Wilson, 1999), which perform seasonal as well as daily migrations up and down the beach with the tides. *Donax* species from tropical beaches are shorter lived and display higher spontaneous mortality rates under experimental conditions than temperate *Donax* species (Cardoso and Veloso, 2003).

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

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

Decreased metabolic rates in the second year may reflect reduced mitochondrial densities in the cells of aged animals. This was recently shown to happen in queen scallops, *Aequipecten opercularis*, from the Irish Sea, with an MLSP of 8 y. Between 2 ± 0.5 y and 4 ± 0.5 y of age, the investigated *A. opercularis* lost 30% of their muscle fibre mitochondria and also a consistent 30% of citrate synthase activity per unit wet weight. This loss of mitochondria in *A. opercularis* corresponded to the age dependent decline of ATP concentration and whole adenylate pool in this longer lived, iteroparous scallop (Philipp et al., 2008). Interestingly the respiratory capacity of mitochondria isolated from young and older queen scallops revealed only minor loss of function with size and age, as compared to longer lived mud clams (Philipp et al.,

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2006, 2008). We termed this phenomenon the "*superman effect*" and proposed that it might be their strategy to maintain the remaining mitochondria in good shape, rather than to retain high numbers of poorly functioning mitochondria per cell in the swimming muscle over their entire life.

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

5. Age effects on metabolic and antioxidant capacities in bivalves from different climate regions

Several studies of moderately long-lived bivalves (up to 20 y) document a decline of growth rates as well as respiratory and filtration capacities with age, albeit independent of changes of body mass (see data compilation in Table 1). Sukhotin et al. (2003) and Sukhotin and Pörtner (2001) analyzed the blue mussel, *M. edulis*, from temperate, subarctic and fully Arctic habitats. They documented decreasing respiration and filtration rates in a subarctic White Sea population above 6 y of animal age, indicating that blue mussels either lose or reduce respiratory capacities and functional activity over lifetime. The decline of specific respiration (μmol O_2 g^{-1} tissue wet weight h^{-1}) was even more pronounced when data were normalized to a standard size in all age groups. This makes sense, because growth rates in mussel settlements are highly variable, and each age cluster encompasses a wide range of animal sizes. However, it also enables size independent evaluation of age effects on respiration and other parameters in the *M. edulis* model and excludes the possibility that any age-dependent change is merely an allometric effect, where larger animals have lower specific parameter activity. Sukhotin and Pörtner (2001) and Sukhotin et al. (2002) analyzed citrate synthase activity in gills and whole body tissue of subarctic *M. edulis* from the White Sea and found no clear indication for an age-dependent change of the mitochondrial marker enzyme in this species. However, direct measurement of respiratory capacity in mitochondrial isolates of mud clams and a scallop (Fig. 3, Table 1) document loss of respiratory efficiency and of mitochondrial energetic coupling (RCR: respiratory control rate which indicates the intensity of mitochondrial energetic coupling) over animal lifetime (Philipp et al., 2005b, 2006). The intensity of the decrease of mitochondrial respiration and phosphorylation differed significantly between the species and was most pronounced in the North Sea soft shell clam *M. arenaria* over an age range from 2 to 10 y. Comparison of temperate and Antarctic mud clam mitochondria revealed more rapid "quality loss" in mitochondria of the North Sea clam: RCR and mitochondrial membrane potential declined 6-fold more rapidly, whereas proton leak increased 5-times faster with age than in *L. elliptica* from the Antarctic. The percent rate of state four (uncoupled respiration without ADP) and state three (coupled respiration in the presence of ADP) H_2O_2 formation by the isolated mitochondria increased only in the temperate mud clam, whereas it remained low and constant over 25 y of lifespan in the Antarctic species (Philipp et al., 2005b). Thus, the superior longevity in the polar clam, which otherwise resembles the North Sea clam in lifestyle (sedentary benthic infauna), feeding mode (particle filtration), and shell size (max ±100 mm), seems to be based on its potential to control the intensity of mitochondrial ROS formation, presumably through a higher proton leak which exerts a mild uncoupling effect on the mitochondrial membranes (Brand, 2000).

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

The temperate soft shell clam *M. arenaria* was the only bivalve in which we found GSSG to accumulate with age, the concentration doubling in mantle tissue within 6 y of lifetime from 30 to over 80 nmol GSSG g^{-1} wet weight. Within the same period, the overall glutathione concentration (GSH + 2GSSG) increased from 200 to 400 nmol g^{-1} wet weight in *M. arenaria* mantle tissue. Together with the decrease in mitochondrial respiration and coupling, increased conversion of oxygen to ROS and accumulation of GSSG with age, increases in the overall glutathione concentration in mantle tissue suggest that prooxidant processes intensify, and higher antioxidant protection is needed in aging soft shell clams.

Both burrowing mud clams, *M. arenaria* from the North Sea and *A. islandica* from the Iceland population had very low glutathione levels around 300 nmol g^{-1} wet weight in mantle tissue. In *A. islandica* the concentration remained constant between 36 and 196 y of age, following maturation of the animals (Abele et al., 2008). Thus, these burrowing mud clams preserve glutathione at constant (or even increasing) levels over lifetime, as opposed to epibenthic scallops or blue mussels (Table 1), in which glutathione levels decrease over lifetime in mantle and adductor muscle.

Another interesting question regarding polar and temperate ectotherm longevity is whether or not changes involved in physiological cold adaptation may directly impinge on the process of aging. According to Porter et al. (1996), proton leak increases as a function of inner mitochondrial membrane surface area (cristae density) and can be modulated by the degree of unsaturated fatty acids in the membrane. Higher numbers of mitochondria and higher cristae densities are found in cold adapted ectotherms, helping them to overcome diffusion problems and compensate for the decline in metabolism at cold temperatures. A comparison of the ratio of cytochrome oxidase/citrate synthase activity (COX/CS) in temperate and polar mud clams indeed suggests that polar species have higher mitochondrial cristae densities (Philipp et al., 2005b). Thus, on the one hand, higher proton leak which, theoretically,

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Table 1
Changes of metabolic and oxidative stress parameters over lifetime in marine bivalves from different climatic environments. Data from 1: (Hopkins, 1930); 2: (Sukhotin and Pörtner, 2001); 3: (Philipp et al., 2005b); 4: (Philipp et al., 2006); 5: (Philipp et al., 2008); 6: (Abele et al., 2008); 7: (Viarengo et al., 1991); 8: (Philipp et al., 2005a); 9: (Sukhotin et al., 2002); 10 (Canesi and Viarengo, 1997); 11: (Hole et al., 1995); 12: (Strahl et al., 2007); 13: (Lomovasky et al., 2002); 14: (Viarengo et al., 1989).

Species	Tissue	Climate regime	Parameter	Effect	Ref
Metabolism					
Venus mercenaria	Adductor muscle	Temperate	Oxygen consumption	–	(1)
Pecten irridians	Adductor muscle	Temperate	Oxygen consumption	–	(1)
Mytilus edulis	Whole animal	Cold temperate	Oxygen consumption	–	(2)
Mitochondrial parameters					
Laternula elliptica	Mantle	Cold	Mitochondrial respiration	–	(3)
Mya arenaria	Mantle	Temperate	Mitochondrial respiration	–	(3)
Aequipecten opercularis	Mantle, add. muscle	Cold temperate	Mitochondrial respiration	–	(4)
Laternula elliptica	Mantle	Cold	COX	0	(3)
Mya arenaria	Mantle	Temperate	COX	0	(3)
Aequipecten opercularis	Mantle	Cold temperate	COX	–	(4)
Adamussium colbecki	Mantle	Cold	COX	0	(4)
Laternula elliptica	Mantle	Cold	CS	0	(3)
Mya arenaria	Mantle	Temperate	CS	0	(3)
Aequipecten opercularis	Mantle, add. muscle	Cold temperate	CS	–	(4,5)
Arctica islandica	Mantle, gill	Cold	CS	–	(6)
Adamussium colbecki	Mantle	Cold	CS	0	(4)
Mytilus edulis	Whole body	Cold temperate	CS	–	(2)
ROS generation rate					
Laternula elliptica	Mantle	Cold	ROS/mg mitochondrial protein	Low and decrease	(3)
Mya arenaria	Mantle	Temperate	ROS/mg mitochondrial protein	High and no change	(3)
Aequipecten opercularis	Mantle, add. muscle	Cold temperate	ROS/mg mitochondrial protein	Very low and no change	(4, 5)
Antioxidant enzymes					
Mytilus edulis	Digestive gland	Temperate	CAT	–	(7)
Laternula elliptica	Mantle	Cold	CAT	0	(8)
Mya arenaria	Mantle	Temperate	CAT	0	(8)
Aequipecten opercularis	Mantle, add. muscle	Cold temperate	CAT	-	(4,5)
Adamussium colbecki	Mantle	Cold	CAT	0	(4)
Arctica islandica (>36 y)	Mantle, gill	Cold	CAT	0	(6)
Mytilus edulis	Whole body	Cold temperate	CAT	0	(9)
Mytilus edulis	Whole body	Cold temperate	SOD	0	(9)
Mytilus edulis	Digestive gland	Temperate	SOD	0	(7)
Laternula elliptica	Mantle	Cold	SOD	0	(8)
Mya arenaria	Mantle	Temperate	SOD	0	(8)
Arctica islandica	Mantle, gill	Cold	SOD	0	(6)
Aequipecten opercularis	Mantle	Cold temperate	SOD	0	(4)
Mytilus edulis	Digestive gland	Temperate	GPX	–	(7)
Non-enzymatic antioxidants					
Mytilus edulis	Digestive gland	Temperate	Glutathione	–	(7, 10)
Mytilus edulis	Gills	Temperate	Glutathione	–	(10)
Laternula elliptica	Mantle	Cold	Glutathione	–	(8)
Mya arenaria	Mantle	Temperate	Glutathione	+	(8)
Aequipecten opercularis	Mantle, add. muscle	Cold temperate	Glutathione	–	(4,5)
Adamussium colbecki	Mantle	cold	Glutathione	–	(4)
Arctica islandica (>36 y)	Mantle, gill	Cold	Glutathione	0	(6)
Laternula elliptica	Mantle	Cold	GSSG: GSH	0	(8)
Mya arenaria	Mantle	Temperate	GSSG: GSH	+	(8)
Aequipecten opercularis	Adductor muscle	Cold temperate	GSSG: GSH	0	(5)
Aequipecten opercularis	Mantle	Cold temperate	GSSG: GSH	–	(4)
Arctica islandica	Mantle, gill	Cold	GSSG: GSH	0	(6)
Adamussium colbecki	Mantle	Cold	GSSG: GSH	–	(4)
Cell damage parameters					
Mytilus edulis	Whole body	Cold temperate	Lipofuscin	+	(9)
Mytilus edulis	Digestive gland	Temperate	Lipofuscin	–	(11)
Laternula elliptica	Mantle	Cold	Lipofuscin	+	(8)
Mya arenaria	Mantle	Temperate	Lipofuscin	first – then +	(8)
Aequipecten opercularis	Mantle	Cold temperate	Lipofuscin	+	(4)
Arctica islandica	Mantle, gill, muscle	Cold	Lipofuscin	+	(12)
Adamussium colbecki	Mantle	Cold	Lipofuscin	+	(4)
Eurhomalea exalbida	Connective tissue	Cold	Lipofuscin	+	(13)
Mytilus edulis	Whole body	Cold temperate	MDA	0	(9)
Aequipecten opercularis	Adductor muscle	Temperate	MDA	0	(5)
Mytilus edulis	Digestive gland	Temperate	MDA	+	(7,14)
Laternula elliptica	Mantle	Cold	Protein carbonyls	+	(8)
Mya arenaria	Mantle	Temperate	Protein carbonyls	–	(8)
Aequipecten opercularis	Mantle	Cold temperate	Protein carbonyls	0	(4)
Arctica islandica	Mantle, gill	Cold	Protein carbonyls	0	(6)
Adamussium colbecki	Mantle	Cold	Protein carbonyls	0	(4)

Parameter: COX, cytochrome oxidase activity; CS, citrate synthase activity; CAT, catalase activity; SOD, superoxide dismustase activity; GPX, glutathione peroxidase activity. Effect: decrease (–), increase (+), no change (0) with age.

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mitigates ROS formation and slows aging, may indeed be a consequence of cold adaptation in polar and especially Antarctic ectotherms with low aerobic scopes. This brings up the question whether mild uncoupling of the mitochondria and high glutathione levels in polar compared to temperate mud clams are primarily a consequence of physiological cold adaptation. Alternatively *L. elliptica* may have evolved higher proton permeability of the inner membrane and store more glutathione in order to prolong lifespan as a feature supporting sustainable "population management" in the cold. In other words, do polar clams need longer life time to insure survival of the stock, or does a different lipid composition render polar clam membranes leaky as a "by-effect of cold adaptation"? The long lifespan may be adaptive for survival of ectotherms in polar habitats, where temperature slows not only adult growth and development, but moreover protracts molluscan gametogenesis and embryonic development, and possibly delays hatching of mature trochophora larvae from the eggs (Peck et al., 2007).

6. Genes and lifespan in bivalves

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

Rather than a small number of specific "old age genes" that, just by being present as wild or mutated alleles confer extreme species longevity, it seems that certain combinations of genes involved in choreographing the relevant processes of life-long metabolic performance, are important in determining the velocity of aging. In order to be selected, these genetic "choreographers" need to confer fitness advantage to the animals at a young age (see Williams, 1957; Zwaan, 1999). Along this line of thinking, the "gene cluster hypothesis of aging" has been put forward by Barja (2008), who proposes central *homeotic regulator genes* (transcription factors like *hox* genes) to coordinate and optimize lifetime gene transcription for an optimal exploitation of environmental resources and adaptation to environmental constraints (such as times of low food availability in nature). Bivalves are of low complexity in their organizational hierarchies and ontogeny when compared to insects or mammals. This might make it easier for them to rapidly adjust their metabolism in order to meet abruptly changing environmental conditions. The regulatory cascades involved in these adaptive changes may be shorter and less complex, and could potentially contribute to the understanding of the genetic basis of longevity in higher organisms.

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

Tumour formation would range among "late acting deleterious mutations" and has not yet been recognized as a major cause of death in aged bivalves. Blood cancer, as well as different malignant neoplasms have been identified in bivalves and are listed by Cheng (1993) for several species. Experiments to induce tumours in bivalves using carcinogenic hydrocarbons generally fail, but application of aromatic amines can cause basophilic cell neoplasms and hematopoetic cancer (200–400 ppm dimethylnitrosamines causes cancer in painters shell *Unio pictorum*, Khudolei and Sirenko, 1977), as these compounds are being converted to mutagens in bivalve digestive glands. The effect of tumour formation on animal life expectancy is unclear. Thus, mass mortality in the hard shell clam *Mercenaria* spp. could not be related to germ cell tumours (germinoma) occurring massively in this species. Instead, increased tumour incidence is detected during times of rapid environmental change (Hesselmann et al., 1988). Germinal tumours were also seasonally increased and more abundant at warm summer water temperatures. Hard shell clams from Florida generally displayed higher tumour incidence than clams from colder Rhode Island waters (Hesselmann et al., 1988). Thus, higher susceptibility to malignant neoplasmic diseases may be involved in shortening population specific maximum lifespan in bivalves with a geographical distribution between different climate zones. Tumour promoters discussed in the literature are infestations by herpes-type viruses which are more common at warmer water temperatures. On the other hand, there might also be a general predisposition through certain proto-oncogenes (genes that by mutation are converted into tumour inducing oncogenes) in warm water bivalves. Information on proto-oncogene signalling cascades in molluscan species is largely missing, however, Degnan and collaborators confirmed conservation of the *ets*-multigene family, homolog to the human oncogenes, throughout nearly all marine metazoan phyla (Degnan et al., 1993), including molluscs and sponges. The proteins encoded by these *ets* proto-oncogenes are involved in the regulation of multicellular development and cell proliferation, but their specific function in protostome metazoans, or their role in tumour formation, are far from clear. However, higher tumour incidence in warmer waters underlines temperature is a prime factor in control of bivalve life expectancy.

Another interesting question is whether minor variations in maximum lifespans between different populations of one species within a climatic zone can have a genetic or genomic basis. These variations are reported from the long-lived pearl clam, which in the Russian Arctic may attain population Amax between 190 (Varzuga) and 114 (Keret) (Ziuganov et al., 2000: Table 1). In our investigation of *A. islandica*, the most conspicuous discrepancy occurred between three populations with the same habitat temperature range (0–10 °C) but from different locations and with very different Amax of 370 (Iceland), 93 (North Norway) and 53 (White Sea) (Begum and Basova unpubl. data). Is this due to environmental effects that limit lifespan in one environment and not in the other? This would be in line with the symmorphosis concept proposed by Weibel and Taylor (1991), which states that biological systems are designed to meet, but not exceed, their natural requirements. Pleiotropic trade-offs are largely a function of environmental forcing of the budgeting of energy between fitness and fecundity during species lifetime. Minor variations of MLSP between populations by the 1.3 to 2-fold therefore seem to be primarily a consequence of environmental adaptations and ecological adjustments see also Barja (2008).

Heterozygosity (heterosis) may be the link between the genetic and adaptive physiological determinators of population MLSP. It is an important criterion for animal fitness, as heterozygous alleles for functional proteins theoretically increase adaptability. Bivalves

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with multiple allozyme heterozygosity display lower metabolic rates (Koehn and Shumway, 1982) and lower rates of protein turnover, which enables them to invest more energy into growth, reproduction and presumably predator avoidance (Hawkins et al., 1986; Myrand et al., 2002). Meanwhile, an extensive body of literature documents better resistance to stress to be due to elevate levels of heterozygosity in bivalves, gastropods and fish (cited in Myrand et al., 2002). The authors speculate that heterozygous bivalves may be more apt to enter hypometabolic states, but this is not yet backed by experimental evidence. To the contrary, correlation of allozyme heterozygosity with either growth, reproduction, or survival (individual lifespan) is not found in pectinids (Volkaert and Zouros, 1989; Bricelj and Krause, 1992; Fevolden, 1992). This speaks against heterozygote superiority supporting predator avoidance in motile bivalves, and, indeed, from the marine ecologist's point of view, predator avoidance is a behavioural trait and presumably involves a multitude of genetic and physiological traits and cascades. Moreover, hypometabolism is not an option for scallops given their active energy consuming life style. A more radical reduction of genetic variability may be the consequence of important losses of genetic material including key telomeric sequences, possibly occurring in the evolution of the bay scallop, as proposed by Estabrooks (2007) and referred above. This could indeed be the radical way of shortening life expectancy in scallops.

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

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

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

7. Conclusions and further research directions

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

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

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Please cite this article in press as: Abele, D., et al. Bivalve models of aging and the determination of molluscan lifespans. Exp. Gerontol. (2009), doi:10.1016/j.exger.2009.02.012