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Masters of Longevity: Lessons from Long-Lived Bivalves – A Mini-Review

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Abstract
The individual ages of bivalve molluscs can be inferred from the age rings laid down every year in the shell, especially in species inhabiting areas with seasonal variability in environmental factors such as food supply and temperature. Animals obtained from different environmental settings can therefore be used to investigate how specific environmental factors shape the process of ageing in this animal class. Some bivalves have extraordinary long life spans. Species like the ocean quahog *Arctica islandica* and the freshwater pearl mussel *Margaritifera margaritifera* live for over hundreds of years. Few studies so far have attempted to study the process of ageing, either specifically in long-lived bivalves or generally in very long-lived species. This review summarizes the current knowledge of cellular ageing in bivalves with a focus on the antioxidant system, as well as tissue repair and metabolic capacities of extremely long-lived species. We discuss the applicability of these animals as models for different ageing theories. We recommend a focus of future research on the molecular mechanisms potentially involved in supporting longevity in these species, including evolutionary old cellular mechanisms such as autophagy and apoptosis, as well as diverse cellular repair pathways.

Introduction

The questions of why and how animals age, and why some species reach Methuselah ages whereas others dwindle away within days or months, have fascinated researchers of all times. Why do cells stop dividing and start growing old or even degenerate while, as an individual, we are still longing for a more active and prosperous life? Especially nowadays, in the light of a rising proportion of senior citizens in the human population and an increasing societal burden through age-related disorders, concerns of how to sustain good health in later life are increasing.

To achieve 'healthy ageing' and prevent or even off-set age-related diseases, such as cardiovascular and Alzheimer disease, type II diabetes and cancer in humans, it is necessary to identify the primary causes and understand the molecular patterns of cellular degeneration and dysfunction. A promising approach seems to follow the evolutionary biology of aging alongside a comparative inter-species approach. Model organisms can be used 'as Rosetta stones for deciphering biological complexity' [1]; this implies that comparative studies of such diverse organisms as yeast, fruit flies and birds, including their ecology and physiological functions, as well as genomic and proteomic approaches, can be used to define the nature of regulatory networks and biochemical processes.

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Species	MLSP (years)
Yeast *(Saccharomyces cerevisiae)*	0.04
Nematode *(Caenorhabditis elegans)*	0.16
Fruit fly *(Drosophila melanogaster)*	0.3
Bay scallop *(Argopecten irradians irradians)*	**1–2**
Pink salmon *(Oncorhynchus gorbuscha)*	3
Mouse *(Mus musculus)*	4
Short-tailed field vole *(Microtus agrestis)*	4.8
Queen scallop *(Aequipecten opercularis)*	**8–10**
American hookworm *(Necator americanus)*	17–18
Antarctic mud clam *(Laternula elliptica)*	**36**
Herring gull *(Larus argentatus)*	49
Gorilla *(Gorilla gorilla)*	55.4
Humans (Jeanne Calment)	122.5
Warty oreo *(Allocyttus verrucosus)*	140
Tortoise *(Geochelone gigantean)*	152
Sturgeon *(Acipenser fulvescens)*	152
Geodruck clam *(Panopea abrupta)*	**163**
Freshwater pearl shell *(Margaritifera margaritifera)*	**190**
Red sea urchin *(Strongylocentrotus franciscanus)*	200
Rockfish *(Sebastes aleutianus)*	205
Ocean quahog *(Arctica islandica)*	**375**
Hexactinellid sponge *(Scolymastra joubini)*	15,000
Hydra	non-ageing?

Fig. 1. Different life spans of animals throughout the animal kingdom and selected bivalve species (in bold). Life spans from bivalves range from 1–2 years up to 400 years. Life spans of bivalve species from: *Argopecten irradians irradians* [59], *Aequipecten opercularis* [22], *Laternula elliptica* [20], *Panopea abrupta* [13], *Margaritifera margaritifera* [7] and *Arctica islandica* [8]; other life spans are from the AnAge database, except for Hydra [40] and *Necator americanus* [82].

Bivalves as Model Organisms for Ageing

Despite the differences in physiology and lifestyle, simpler model organisms (e.g. fruit flies or worms) of different evolutionary characteristics and ageing rates have recently contributed considerably to our understanding of human ageing [2, 3]. Evolutionary early marine and freshwater bivalves are ectothermal models of low complexity, and useful for understanding cellular functioning under diverse and sometimes extreme environmental conditions [4, 5]. Furthermore, bivalves are the record holders within the long-lived animal species reaching more than 150 years, including turtles, tortoises, whales, fish and sea urchins (fig. 1).

Reports of long-lived organisms are always met with curiosity as they may help to unravel the mystery of longevity, and 2 bivalve species have been found to live for extraordinarily long times: the freshwater pearl mussel *Margaritifera margaritifera* (maximum life span, MLSP, 190 years [7]) and the ocean quahog *Arctica islandica* (reported maximum age, 375 years [8]). Although not yet fully appreciated as model organisms for ageing studies, bivalves have been recognized as extremely long lived in several reviews on aging and, sure enough, the Bivalvia represent the longest-lived metazoan class (AnAge database [6, 9]). Their shell's hard structure records each individual's chronological age in the form of yearly age rings [10], so the age of each specimen can be inferred. In contrast to the classical models of ageing, *Drosophila, C. elegans* or mice, which must be reared in the laboratory to determine their chronological age, bivalves can be taken from their natural environment and the chronological age of each individual can be seen from the shell, which allows us to study the ageing process in wild populations.

Bivalves have vastly differing MLSP ranging from 1–2 years *(Donax* spp., *Argopecten irradians irradians)* to hundreds of years *(Tindaria callistiformis, A. islandica, M. margaritifera)*. Turekian et al. [11] reported a life span of over 100 years for the deep sea clam *Tindaria callistiformis,* as determined by radium-228 concentrations in shell ring patterns. By mark-and-recapture experiments in shell growth, Anthony et al. [12] determined life spans of way over 150 years in freshwater species like *Elliptio complanata* and *Lampsilis siliquoidea* (family Unionidae), and a 163-year-old geoduck clam specimen *(Panopea abrupta)* was reported by Strom et al. [13]. MLSP in populations of freshwater pearl mussels, *M. margaritifera,* from different latitudes determined by shell ring counts were found to range between 30 and 200 years [7]. Individuals from a population in southern Spain reach 30–40 years, whereas specimens from the Arctic can live for 200 years, indicating species' MLSP may vary between climatic zones. The same holds true for the longest-lived bivalve, the ocean quahog *A. islandica*. Animals from the warmer Baltic Sea have a shorter lifespan of up to 35 years [14], whereas a maximum age of 375 years has been recorded in an Icelandic population [8]. Some bivalves are extremely good in slowing down the rates of aging and cellular senescence, and attain extremely long life spans. These may represent valuable models also for understanding human ageing [6].

Bivalve Ageing

According to the 'free radical theory of ageing' by Harman [15], oxygen free radicals generated during a lifetime of metabolic activity are responsible for the pro-

gressive oxidation of cellular compounds, as well as for the decline in cellular function in organisms with age. So far, the process of cellular ageing has been studied in only a few long- and short-lived bivalves of differing ecotypes (clams, scallops) and in different climate zones (polar, temperate). Mitochondrial formation of oxygen free radicals, oxidative damage and antioxidant capacity were studied in bivalves with different metabolic scopes and maximum life spans to determine whether and how reactive oxygen species (ROS) release is involved in the cellular aging process.

Bivalves belong to different ecotypes with different modes of living that may influence the ageing process. Some bivalve species live burrowed in the sediment. They share reduced mobility, low metabolic rates and avoid too high tissue oxygenation and respiration rates by keeping P_{O_2} in their shell water at low and protective levels [16]. These bivalves often show declining mitochondrial function (respiration, citrate synthase activity) as well as increased accumulation of cellular damage (protein carbonyls, malondialdehyde, lipofuscin) with age [17–20]. In contrast, pectinids or scallops are epibenthic (i.e. live above the sediment) active swimmers of comparatively higher metabolic activity and also higher mantle water P_{O_2} [16]. They belong to a 'high activity – higher shell water oxygenation' category of bivalves [16]. In these animals, the mitochondrial function seems to be conserved, whereas mitochondrial volume density and citrate synthase activity diminish over lifetime [21, 22].

As a common feature, the decline in antioxidant defense (catalase, glutathione) and protective chaperones (mitochondrial HSP60) was detected in bivalve models like oysters (HSP60 and antioxidants) [23] and clams and scallops (antioxidants) [20–22] over age. Shorter-lived species showed a faster increase in damage parameters and decrease in antioxidants compared to longer-lived species of similar lifestyles [20, 22, 24]. Comparing different endobenthic (i.e. living within the sediment) bivalve species, shorter lifespan correlated with higher mitochondrial ROS production, a harmful process which accelerates as aging proceeds. In contrast, longer-lived bivalve models seem to maintain mitochondrial ROS production at low and stable levels throughout their life [24]. The observed age-related molecular changes appear to go hand in hand with a change in cellular function and stress response over age. A swimming experiment of young and old scallops demonstrated how the capacity for exercise and the stress response changes with age in these active swimmers. In young animals, exhaustive swimming results in a faster decrease in muscle pH and glutathione as well as diminished glycogen concentration compared to older scallops under the same exercise conditions. Young animals also closed their shells more often and for longer times upon predator attack, whereas older animals kept the shells open, which matches the lower aerobic (mitochondrial volume density and citrate synthase activity) and anaerobic (glycogen concentration) energetic capacity of old compared to young individuals [21, 25].

In summary, although different ecotype models may differ in their strategy of oxidative damage mitigation, the general ageing process in bivalves involves a loss of energetic function and higher susceptibility to oxidative stress. Similar to mammals, ageing in bivalves proceeds with a progressive deterioration of cells, tissues and organs, associated with a decline in physiological function over the lifetime.

Arctica islandica – Negligible Ageing?

Recent records of the ocean quahog *A. islandica* at Bangor University [unpublished data], with individual shells indicating 400-year life spans, have strengthened scientific interest in both the physiological implications and the ecological importance of such life spans in bivalves. Species' reported maximum life spans in wild populations must be viewed with caution, as the determination depends largely on contingency with respect to the chance of sampling the 'oldest' individual in a population. This is especially true at sea, where sampling is mechanical (dredges, nets and drags) and random, making it difficult to reliably determine mean or maximum life spans of wild populations. Further, the question arises of whether the 400-year-old quahog represents the exception or the rule for the population? However, as outlined by Hedrick [9], the occurrence of old individuals is indicative of population longevity, and even though *A. islandica* of 405 and 410 years might be exceptional, selected quahog populations can be anticipated to be extremely long lived. The question then arises as to the cellular mechanisms allowing for such a long lifespan. Only few studies have been undertaken so far to investigate the physiological and cellular ageing process in these long-lived species [7, 17, 18]. Notwithstanding the general similarities between the bivalve and mammalian ageing process, the only study of physiological ageing in an animal living regularly in units of centuries shows that the aging process can be slowed to negligible observable rates in the ocean quahog *A. islandica* [18]. In *A. islandica,* a decrease in tissue glutathione, as well as citrate synthase

and catalase activities, was observed only in the first 30 years, compared to high initial levels in juveniles. Following maturation, these parameters remain stable for over 150 years in adult animals. Superoxide dismutase (SOD) activities did not even change at all over 192 years of recorded life span, an interesting finding, especially in the light of other studies on yeast and flies, indicating SOD to be important for longevity [26]. The time pattern of cellular changes in Icelandic *A. islandica* could be nicely fitted with other life history patterns in the population. The animals mature at around 33 years of age [27], which coincides with the stabilization of cellular biochemistry on lower adult levels and the beginning of the asymptotic phase of shell growth [see fig. 3 in 18]. The observed downfall in cellular antioxidant and energetic parameters in young animals (<33 years) must therefore not be confounded with senescent loss of these capacities, and rather reflects physiological changes the animals' experience during the early phase of rapid growth and sexual maturation. During the remaining 150 years of the studied adult lifespan, no further senescent decline in the antioxidant and energy parameters could be observed.

This first investigation of an extremely long-lived organism corroborates the concept of Finch and Austad [6] that long-lived species should belong to a 'negligible-ageing type'.

The authors identified 2 criteria that indicate lack of senescence in a species: (1) 'no observable age-related decline in physiological capacity or disease resistance' and (2) 'no observable increase in age-specific mortality rate or decrease in reproduction rate after sexual maturity' [6].

Soma Maintenance for Longevity

The question of how much available energy an organism allocates to reproduction, somatic maintenance or growth forms the basis of the 'disposable soma theory' [83]. An organism's lifetime energy budget is principally divided between population sustainment (i.e. reproduction) and individual sustainment (i.e. individual body maintenance). Rapid growth is often supportive to population and individual sustainability as larger body size normally decreases individual mortality risk at increased reproductive output. It can be assumed that long-lived species and individuals from populations with a lifespan of over hundreds of years must either invest more into somatic maintenance or exhibit amazing cell protection and repair capacities.

Ziuganov et al. [7] tested the regenerative capacity upon experimental injury in populations of different maximum life spans (40 and 200 years) of the freshwater pearl mussel *M. margaritifera*. Animals were mutilated by cracking the shell, cutting into the foot, siphon or mantle, or injecting microbeads to produce sublethal injury. The results showed that an Arctic population with a longer MLSP had better wound healing and survival capacity as compared to a shorter-lived population from Spain, implying more energy allocation to injury repair in the cold. However, there is also the possibility that the Arctic animals have specialized and highly efficient cold-adapted repair programs, in which case these would urgently need to be investigated. Furthermore, the Arctic population was able to accelerate shell growth 130-fold during the repair phase, compared with the regular undisturbed shell growth rate (0.03–0.05 mm/year), which exceeded basal (non-injured) shell growth rates in the southern population by about 30 times. The experiments indicate that the higher longevity of the Arctic population may in part result from a lifelong reduced expenditure on growth and a higher investment of the population into somatic maintenance when compared to the Spanish pearl clam population.

Reproduction

It is difficult to asses age-related changes within the reproductive success or the mortality after maturation in long-lived bivalves. Thorarinsdottir and Steingrimsson [27] investigated sexual maturity in Icelandic *A. islandica*, and found that sexually mature animals over 100 years of age (maximum age of males: 104 years, females: 120 years) had slightly smaller follicles than younger adults, interpretable as an indication of the onset of senility and a decrease in reproductive success. They further observed a minor shift of sex ratio from males to females in older age classes. This may indicate somewhat longer life expectancy of female than male *A. islandica*, a pattern also found in many mammalian species [28]. However, Thompson et al. [29] found similar sized gonads in *A. islandica* individuals up to 100 years of age, and thus no indication of senility in older animals, which is in line with findings from shorter-lived species such as *Mercenaria mercenaria* (gonad size/mass data recorded up to age 19 years, MLSP >32 years) [30], *Chlamys islandica* (age-specific reproductive effort model up to 26 years of age, MLSP >20 years [31]) and *Panopea zelandica* (histological measurements of gonads up to 24 years of age,

MLSP >34 years, [32]). While investigating age-related changes in reproduction and mortality in long-lived *M. margaritifera*, Bauer [33] also observed undiminished reproduction into old age in the pearl clam, along with constant low mortality of mature clams. The overall impression from these data is that absence of reproductive senescence may be a general feature in bivalves rather than a criteria marking extreme species longevity.

Waste Product Accumulation Theory

Aerobic metabolism implies the formation of oxidized and damaged cell structures (e.g. membranes, proteasomes, mitochondria) [34] as well as the accumulation of cellular waste within the lysosomal cellular garbage bins. Indigestible highly oxidized waste products that exaggerate lysosomal autodigestive capacity accumulate in the form of the cellular age pigment lipofuscin, an inert fluorescent substance easily detectable in aging cells. Other known age products, the advanced glycation end products, form in reactions of sugars with the amino groups of proteins and induce protein-protein cross-linking and DNA mutations [35]. Whereas in many bivalves under hypoxic or anoxic conditions anaerobic fermentation leads to clearly defined end products, e.g. short-chained organic acids or alcohols that can be reused or easily removed from the cells [36, 37], waste products from oxidative metabolism are often indigestible for the cellular autophagosome. Accumulation of this indigestible material can start a vicious cycle. Oxidized biomolecules like protein carbonyls or lipofuscin have been found to interfere with cellular functioning (e.g. proteolytic activity [38]), leading to further accumulation of waste products. We hypothesize that stable low oxygen and frequent anoxic conditions, as found in bivalves and especially in endobenthic clams like *A. islandica* [16, 39], may favor a long lifespan by preventing oxidation of biomolecules and reducing cellular waste accumulation.

In the long-lived *A. islandica,* the age-dependent accumulation of oxidized proteins (protein carbonyls) and lipofuscin in different tissues of Icelandic individuals from 8–192 years of age was investigated [17]. Interestingly, the mean protein carbonyl concentrations in mantle tissue had a lower range than in shorter-living and faster-growing soft shell clams, such as *Mya arenaria* and *Laternula elliptica,* or scallops, such as *Aequipecten opercularis* and *Adamussium colbecki* [for more details, see compilation 17]. Moreover, no significant change in carbonyl concentration with age was found in either the gill or mantle tissue of the ocean quahog. However, lipofuscin accumulated in mantle, gill and adductor muscle. Values were lowest in the adductor muscle, compared to mantle tissue and gill. This order corresponds to the metabolic activity of the tissues themselves. Gills are highly active tissues due to ciliary epithelia, involved in maintaining the flow of oxygen-rich seawater, and thus higher ROS generation and lipofuscin accumulation can be anticipated. This trend could also be seen for the carbonyl content, with slightly higher carbonyl levels in the gill compared to mantle tissues. Lipofuscin concentration in *A. islandica* can be compared with an analysis of the South American bivalve *Eurhomalea exalbida* from Patagonia, which lives at least 70 years [40] and where the same (histological) technique of lipofuscin detection was applied. Age pigment accumulation rates in mantle, gill and adductor muscle were far lower in *A. islandica* (up to max. 5% lipofuscin granula per investigated area) [pers. commun. Strahl et al. 17] compared to *E. exalbida* connective tissue around the intestine (up to 12% [40]). Although different tissues were used in the 2 studies, the overall picture implies that *A. islandica* effectively keeps damage accumulation at a low level.

Bivalves represent applicable models that show how lipofuscin accumulation with age relates to tissue and cellular metabolism [17, 19, 20, 22]. Endobenthic clams actually seem to tolerate higher levels of cellular damage and accumulation of waste products, compared to more active scallops [20, 22]. It is presently not clear whether the lower damage concentration in scallops is due to faster cellular proliferation and apoptosis resulting in dilution and removal of age pigment, which might be fundamental for the active scallop lifestyle.

Autophagy and apoptosis, cellular autodigestion and self-killing programs, help to remove fatally damaged compounds from the tissues. The mechanisms underlying these processes are ancient and highly conserved in evolution. They are functional in early metazoans like hydra [41] and sponges [42] where they serve to reduce body mass in times of low food supply, control the number of algal endosymbionts per host cell through digestion [43] and help to get rid of bacterial pathogens [44]. Hydra is assumed to be immortal partly due to an intense proliferation and apoptotic activity in its tissue, supposed to prevent accumulation of waste products and thereby accounting for a constant rejuvenation of the animals [45]. Likewise, in bivalves, high proliferation rates and autophagic processes are assumed to rejuvenate digestive gland tissue after lipofuscin accumulation has been propelled by environmental toxins [46]. In some bivalve tis-

sues, lipofuscin concentrations decline after an initially high accumulation in fast-growing young animals, which may also involve autophagic and apoptotic removal, without any secure evidence yet available [20, 47]. Although a lot of work has already been undertaken in the field of the accumulation of lipofuscin and other cellular damage markers, the mechanisms of apoptosis and autophagy are still an unexplored field in bivalves and open for future investigations. Kefaloyianni et al. [48] could show activation of the apoptotic markers capsase-3 and p38-MAPK in *Mytilus edulis* when exposed to different environmental stressors (heavy metals, temperature); molluscan autophagic genes are so far unspecified.

DNA Repair

In contrast to protein and lipid oxidative damage, damage of nuclear and also of mitochondrial DNA is quickly repaired, as it would otherwise inflict rapid loss of function on cells and organisms. Oxidation of bases or strand breaks and lesions can lead to different damage scenarios. The transcription of genes can be disabled or the transcription product becomes false and mutated. Sublethal gene defects passed on to the daughter cells will reduce their viability. If not repaired, a large amount of DNA damage will accumulate and can lead to programmed cell death (apoptosis) or initiate tumor formation. As mitochondrial DNA is far more abundant than nuclear DNA in animal cells and further exposed to ROS action in the vicinity of the mitochondrial respiratory chain, damage to mitochondrial DNA is much more likely to happen. Each mitochondrion carries multiple copies of its ring chromosome; thus, certain levels of damage may be tolerable. However, progressive damage of mitochondrial DNA leads to defective mitochondria with higher ROS production rates which starts a vicious cycle summarized as the 'mitochondrial theory of aging' [49]. The final consequence of this is a deficit of cellular energy production which eventually restricts cell division and growth and leads to apoptosis.

Only few papers so far have investigated DNA damage and repair in bivalves with respect to age-related changes, whereas the majority used DNA damage as biomarker for environmental pollution [50, 51]. The existing data indicate DNA damage intensity to differ between species, and DNA repair capacity in bivalves to correlate inversely with age [52, 53]. Accomando et al. [52] investigated DNA repair activity and damage in different age classes of *Mytilus edulis* and found higher DNA damage and lower DNA polymerase activity in the older age classes. In line with this, Pruski and Dixon [54] found a higher DNA repair capacity in young hydrothermal vent bivalves *(Bathymodiolus azoricus)* compared to older individuals. Currently, no information on mtDNA damage progression in aging bivalves is available and data for long-lived bivalve species are completely lacking.

Telomeres and Telomerase Activity

Most animals exhibiting a long lifespan or belonging to the negligible type of ageing are species with indeterminate growth, like turtles, fish, lobsters and bivalves [55, 56]. These animals grow past sexual maturity and although body growth slows down with size and age, it never stops completely. Even bivalves with 'multi-centennial' life spans, like *A. islandica*, keep on growing at late age, albeit at very slow rates [17]. Based on experiments with human fibroblast cultures, Leonard Hayflick [84] postulated cells to be able to perform only a limited number of cellular divisions (the Hayflick limit) before replicative senescence starts and division finally stops. While the applicability of the Hayflick limit to the in vivo system is still under debate, it is well accepted that telomere shortening is a counting mechanisms for cell cycle arrest [49]. Thus, animals with extremely long life spans would be assumed to either stretch the time between subsequent cell passages or else to have evolved strategies to prevent the shortening of the telomeres.

The enzyme telomerase is known to re-elongate telomeres after cell division. In humans, telomerase is normally repressed in adult somatic tissue [57] leading to the shortening of the telomeres with increasing age. In several investigated aquatic invertebrates and vertebrates, however, telomerase activity has been found to be active throughout the lifetime, also in somatic cells [55, 56, 58, 59]. Klapper et al. [55] suggested that continuous cell proliferation, as found in animals with indeterminate growth, must require high telomerase activities, and indeed they found high telomerase activities in the different tissues of rainbow trout *(Oncorhynchus mykiss)* and lobsters. In bivalves, telomerase activity was detected in different tissues of adult *Euvola ziczac* scallops [58] indicating the possibility of preservation of telomere length in pectinids. The only study so far investigating telomere length in bivalves was undertaken in the rather short-lived pectinids *Argopecten irradians* (MLSP < 2 years) and *Argopecten purpuratus* (MLSP 7–10 years). In *A. irradians,* a decrease in telomere length was found in gill and kidney tissue and

most strongly in digestive glands, a tissue known for its lifelong high proliferation rate. In muscles and the heart, no change in telomere length with age was found, which is in line with low cell proliferation in both tissues. Comparisons of the short-and the slightly longer-lived scallop are suggestive of faster telomere loss in very short-lived species; however, the currently available data do not allow final conclusions [59].

Telomere shortening has also been reported to happen independently of telomerase activity, through ROS (especially $OH^{\bullet}$)-induced telomere damage [49]. It is therefore interesting to note that ROS production in long-lived bivalves is found to be comparably low [18, 60], and supposed to be even less under the low natural tissue oxygenation in sediment dwelling organisms. Approaches using primary cell isolates, involving measurements of ROS formation and studies of telomere length in short- and long-lived bivalves, seem promising ways to answer the question of the connection between telomerase, telomere length, ROS and a long lifespan.

Bivalves and Cancer

Repression of telomerase activity as found in somatic tissue in humans is assumed to have a tumor protective function and prevent immortalization of cells. Hence, there is a trade-off between lifetime replicative capacity through preservation of telomere length and cancer suppression. This is corroborated by the finding that 90% of tumors were telomerase positive, and implies that cells that do not repress telomerase are more likely to be immortalized [57]. In future studies, the issue of cell proliferation and apoptosis rates in different bivalve tissues needs to be addressed in combination with measurements of telomere length and telomerase activities in long-lived species as well, as no data are presently available. Do these animals really keep up proliferation over hundreds of years? And is telomerase activity high or repressed in some tissues, as seen in humans to prevent tumor formation and allow a long and healthy lifespan? Neoplasm is common in some species of bivalves [61], the causes not yet being completely clear. In some cases, cross-infection studies with hemolymphatic cells and serum indicated the involvement of a species-specific infective agent [62, 63]. Moreover, the involvement of viruses has been discussed [64]. Some bivalves, like scallops or the oyster *Crassostrea gigas* and the blue mussel *Mytilus edulis,* seem to be more resistant to disseminated neoplasia than others, a finding which might point to a genetic background [61]. In the long-lived bivalves, like the ocean quahog *A. islandica*, tumors are rarely observed [65] and, so far, neoplasms have never been observed in *M. margaritifera*, which may point out another feature contributing to the extreme longevity of these species.

Metabolic Rate Depression

Life span prolongation by metabolic downregulation has been reported for several organisms, and is assumed to be an important mechanism to reach extreme longevity in bivalves [66]. Taylor [39] described the burrowing behavior of *A. islandica* which involves self-induction of metabolic reduction (MRD). In irregular intervals, individuals burrow into the sediment, close their shells and reduce the metabolic rate to 10% of normoxic levels. The animals remain in this state for several days before surfacing again. Whereas on the one hand this behavior is certainly not unique in *A. islandica*, it may, within the species, contribute to the higher longevity in the Northern Atlantic high-salinity Arctica populations (North Sea, Irish Sea and around Iceland) which all exhibit distinct MRD behavior, and are the populations with reported extraordinary life expectancies [17; own unpubl. obs.]. Similar behavior was reported by Ziuganov et al. [7] for the northern pearl mussel population. Within the animal kingdom, dauer larvae of nematodes and crustaceans in particular are known to survive over long times in a metabolically reduced state. The metabolism of the cysts of the brine shrimp *Artemia franciscana* is close to zero [67] and accordingly cysts can last for over 300 years [68], whereas the adult shrimp lives less than 6 months. Less drastic examples of metabolic rate depression can be found in bats, snails, frogs and hamsters, all of which undergo daily or seasonal metabolic reduction; and here a life-prolonging effect is also sometimes reported. In warm-blooded animals, hibernation goes hand in hand not only with a reduction in metabolism but moreover with body temperature [69]. Furthermore, the inactive state during metabolic reduction also represents a phase of calorie restriction, known as the only life-prolonging intervention in the vast majority of tested animals. Thus, 3 factors (cold temperatures, low metabolic rates and calorie restriction) act during periods of MRD, and possibly synergistically enhance longevity in vertebrates and invertebrates.

The life-prolonging action of low metabolism, temperature and calorie restriction is certainly based in part on the reduced generation of ROS in such conditions. Nev-

ertheless, accelerated generation of ROS is thought to occur as normal respiration is resumed following the shut down phase, as reduced respiratory chain intermediates in the mitochondria autoxidize during tissue re-oxygenation. The fact that respiration in hypoxia-tolerant endobenthic bivalves slows as tissue and cellular oxygen levels decline [60] could have a protective function in ameliorating mitochondrial chain reduction as electron transport slows. The possible antioxidant mechanism of this so-called 'oxyconforming' respiration needs to be clarified. Other changes, like an increase in antioxidant capacity, heat shock protein concentration and induction of autophagic processes have been reported during calorie restriction, as well as during periods of metabolic depression [70, 71]. As a side effect, this re-enforcement of antioxidant and stress protein levels may contribute to the extreme longevity and enhance cellular waste removal (autophagy) in MRD species like the long-lived *A. islandica* [4]. Evidence for a protective effect of transient metabolic depression may be obtained from the comparison of the ageing process and MLSP of bivalve populations with differing MRD behavior.

In this context, the shorter life span of *M. margaritifera* was reported under elevated nutrient concentrations [cited in 7] which points towards a possible relationship between longevity and calorie restriction in these bivalves. The difference in life span between different populations of long-lived bivalves, such as *M. margaritifera* and *A. islandica,* on geographical north-south gradients [14, 72, 73] has mainly been attributed to an effect of temperature. However, by itself temperature can explain only 50% of latitudinal difference in life span in *M. margaritifera* [73], and we conjecture that more intense metabolic reduction behavior and the involved cellular maintenance processes may explain another part of the longer life span in Northern populations.

Polar Longevity

Constant low temperatures and 'seasonal calorie restriction' during the polar night are experienced by Antarctic and Arctic bivalves and, as expected, long-lived species are found in these regions [20]. It is an interesting question, when dealing with polar and temperate ectotherm longevity, whether or not changes involved in physiological cold adaptation may directly impinge on the process of ageing and, next to low temperature and calorie restriction, contribute to the observed longer life span of the polar species. A comparison of a polar *(Laternula elliptica)* and a temperate *(Mya arenaria)* endobenthic clam with similar living mode and life strategy indicates lower ROS generation rates, possibly due to a higher proton leak which leads to 'mild uncoupling' of mitochondria and high glutathione levels, in the polar compared to the temperate clams [20, 24]. According to Porter et al. [74], 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, and these help to overcome diffusion problems and compensate for the decline in metabolism at cold temperatures [75]. A comparison of the cytochrome-*c* oxidase/citrate synthase activity ratio in temperate and polar mud clams indeed suggests the polar species to have higher mitochondrial cristae densities [24]. Thus, on the one hand, a higher proton leak which theoretically mitigates ROS formation and slows aging, may well be a consequence of cold adaptation in polar and especially Antarctic ectotherms with low aerobic scope. Alternatively, *L. elliptica* may have evolved higher proton permeability of the inner membrane and store more glutathione in order to prolong life span as a feature supporting sustainable 'population management' in the cold. A long life span may be favorable for survival of ectotherms in polar habitats, where temperature slows not only adult growth and development, but moreover protracts mollusk gametogenesis and embryonic development, and possibly delays hatching of mature trochophora larvae from the eggs [76]. It remains open whether polar clams need a longer lifetime to insure survival of the stock, or whether different lipid composition renders polar clam membranes more leaky so that life span prolongation is achieved as 'by-product accompanying cold adaptation'?

Involvement of Extrinsic Mortality in Bivalve Longevity

It has been proposed that environments with low predation pressure or the animal's enhanced ability to escape predation, as with birds, both lead to a slowing of the ageing process compared to animals with high environmental mortality risk [77]. The rationale behind this is that high extrinsic mortality (hazardous environment) a priori limits life expectancy and acts against selection of somatic maintenance genes. Conversely, in an environment with low extrinsic mortality (safe), it pays to invest

more energy into somatic maintenance. Safe environments may be geographically isolated like islands, but the same effect can be achieved by the acquisition of wings or protective shells, which will increase the chances for escape and lower extrinsic mortality [77]. Animals with wings or suits of armor, like bats, birds, turtles, lobsters and bivalves have been found to live to extraordinary ages. Also, bivalve shells not only protect against predators, but moreover may allow the animals to endure and control the physiological conditions in their closest environment, the mantle cavity water (e.g. oxygen concentration [78]), which may be beneficial for the evolution of long life spans.

Bivalve Genomics – The Best Has Yet to Come

The comparative interspecies approach of identifying ageing- and longevity-related genes and pathways is currently gaining attention due to the availability of high-throughput sequencing techniques (GS-FLX/Roche, SOLiD/ABI). Novel technologies do not only allow the introduction of whole-genome transcriptional profiles of non-model organisms into the field of ageing. They moreover provide the basis to screen genomes for pathways of DNA and tissue repair, defense mechanisms against pathogens, and diseases and ageing-associated genes and pathways instead of focusing only on specific pathways [1, 3]. Such a comparative approach has the potential to clarify whether genes and pathways associated with ageing are 'public' and present in the majority of organisms, or 'private', meaning restricted to single species with special capacities, as for example extremely long-lived bivalves.

Genomic sequence information on bivalves is still very limited. Currently, efforts are underway to sequence the genome of the pacific oyster *Crassostrea gigas* [79] and *Mytilus californianus* (Joint Genome Institute). First, information of the pacific oyster shows a high rate of single nucleotide polymorphisms, with approximately 1 every 60 bp in coding regions and every 40 bp in non-coding regions [80]. If this finding also holds true for all other bivalves, it would make this animal class especially suitable for the investigation of the link between genetic diversity and gene function.

Conclusion

Systematic investigations of the process of ageing in extremely long-lived bivalves are only just beginning. First results indicate that these animals belong to the negligible-ageing type apt to preserve cellular function over centuries. The longest-lived bivalves are species which live burrowed in the sediment and have only low metabolic rates. The P_{O_2} is tightly controlled on low and protective levels in the shell cavity water, and frequent phases of metabolic rate depression presumably support low lifelong ROS formation in these animals. Cellular damage accumulation proceeds slowly in these long-lived species, and wound healing as well as anoxia tolerance and general defense mechanisms against diseases (cancer, microorganisms) seem to be extraordinarily high, especially in populations from cold climates. Changes in telomere length with age in extremely long-lived bivalves are still waiting to be investigated; however, it seems likely that telomere length will remain stable or may even increase with age, as found in long-lived avian species [81]. Ageing research involving the molecular (genetic) level in these species has to be addressed in future research to clarify whether and how extreme longevity in bivalves is genetically programmed, and which genes and processes, such as DNA repair, autophagy and apoptosis or immune protection, are important in this context.

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