

The oldest recorded animal in Finland: ontogenetic age and growth in *Margaritifera margaritifera* (L. 1758) based on internal shell increments

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Three large and robust shells of freshwater pearl mussel (*Margaritifera margaritifera*) from Lapland were studied. Internal shell-growth increments, showing annual periodicity, provided information about the ontogenetic age and growth of the mussels. Increment widths generally ranged between 0.4 mm and 30 μ m and showed exponential growth trends. Moreover, it was shown that the increment series of different specimens could be temporally synchronized by their common growth signal. This enabled crossdating of the increment series, after which they were averaged into one sclerochronology. One of the shells showed past growth disturbance due to breakage of the shell. Subsequent growth increase, not observed in the other shells, had occurred over the next 5 to 8 years. The oldest specimen exhibited 162 annual increments. Considering the width of the umbonal corrosion and the widths of the increments next to the corrosion, the loss of 17 young increments could be estimated. Moreover, a number of four years was added to the age estimate due to presumably minimal shell growth during the early post-parasitic years. Our results indicate that, if not anthropogenically disturbed, super-centenarian ages may not be a rarity in the region and the oldest mussels may reach an age of even two centuries.

1. Introduction

Longevity is the length of the life span of a long-lived organism. While the longevity of long-living species or individuals is often a subject of interest and even myths, without documentary information the estimation of it is by no means an unambiguous task. The ontogenetic age of an animal or plant can be, in some cases, estimated retrospectively. Woody plants increase their stem diameter by annual increments, the number of tree-rings thus providing an accessible source of information about tree age (e.g. Schulman & Ferguson 1956; Schulman 1958). In the animal kingdom the retro-

spective estimation of an ontogenetic age is more difficult but not necessarily an unachievable goal. Annual growth increments in the hard parts of certain species, for example amphibians and reptiles (Schroeder & Baskett 1968, Halliday & Verrell 1988, Zug et al. 1997), fishes (Campana 2001) and molluscs (Thompson et al. 1980; Ropes et al. 1984; Bauer 1992) are known to provide age estimates.

The method of counting and measuring growth increments is generally termed either skeletochronology or sclerochronology. Literature review shows that the former term is more commonly used by herpetologists and the latter by ich-

thyologists and conchologists. The basic procedure is however identical regardless of taxon. Growth increments of known periodicity are counted and simultaneously measured from the sampled tissues, providing the estimate and reconstruction of ontogenetic age and life-trait history, respectively. A multitude of samples enables comparisons between the individuals. This is especially important for temporal synchronisation, sclerochronological crossdating (Marchitto et al 2000; Helama et al. 2006) of the growth series, and enables the detection of growth structures that may falsely mimic the appearance of the periodic tissue changes. Alternatively, a periodic growth layer may be missing due, for example, to transient stress. Detection of both falsely added or missing increments is naturally of importance for age estimations and life-trait history reconstructions.

The freshwater pearl mussel has been protected in Finland since 1955. Although protection shielded mainly the mussel from hunting it did nothing to prevent destruction of its habitat. In 1978 the WWF-Finland and the Finnish Museum of Natural History established a “*Margaritifera* working group” to study the distribution, ecology, morphology, and protection of this species (Valovirta 1980, 1995a). The sub-aqua work is very prominent in the research of this group. During the last 30 years the freshwater pearl populations have been assessed in more than 70 rivers in different parts of Finland and in some rivers in Russian Karelia, Estonia and Latvia (Valovirta 1998b, 2001, Valovirta et al. 2003).

Here we study the growth increments of three anomalously robust shells of freshwater pearl mussel from the collections of the Finnish Museum of Natural History. These shells originate from north-eastern Finnish Lapland and were included in a previous study aimed at revealing regional-scale characteristics in the life-trait histories of the species (Helama & Valovirta 2007). In the present account we are using only the largest (presumably oldest) shell specimens in the previously studied regional set. The annual shell growth increments of these large specimens were counted and the widths of the increments measured and cross-dated. Time-series of annual shell growth widths were used to characterise the growth of the mussels from their early life-stage until death. The ontogenetic growth trends of the three individuals

were determined and their growth histories compared. The umbo of each specimen was corroded. Consequently, the ontogenetic age of the mussels at the time of death was estimated by adding to the number of counted annual increments the estimated number of corroded umbonal increments. As an end result, ages and growths of the oldest known individual animals in Finland were recorded in detail.

2. Material

2.1. Shell material

The studied material comprises three shells of the freshwater pearl mussel (*Margaritifera margaritifera* (Linnaeus 1758)), collected from beside the River Kolmosjoki (municipality of Inari, Lapland, approx. 68°35'N) in 1980. The specimens were found lying on the surface sediments along the river margin. It is likely that these were the result of illegal pearl hunting (Helama et al. 2007). The samples now belong to the collections of the Finnish Museum of Natural History (Invertebrates Division), University of Helsinki (Valovirta 1990, 1998a). The exact year of death of the animals is unknown but from previous taphonomical analysis it can be inferred that the shells reached the museum collections not longer after death (Helama et al. 2007).

2.2. Preparing the cross-sections

Growth lines could be observed directly on the external surfaces of young shells (cf. Valovirta 1984), but shell cross-sections provide much better resolution. Following the methods described by Dunca and Mutvei (2001), one valve of each *Margaritifera margaritifera* specimen was cut from the umbo to the ventral margin, perpendicular to the winter lines and along the axis of minimum growth. Complete growth records for most species are found along the axis of maximum growth. However, Dunca and Mutvei (2001) counted exactly the same number of increments on the axes of minimum and maximum growth sections, with better visibility in the minimum growth section. The sections were ground (800- and 1,200-grit metallographic grinding paper), pol-

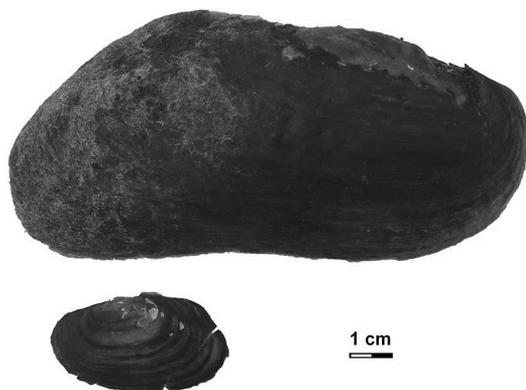


Fig. 1. Right valve of one of the studied large and robust freshwater pearl mussel shells from Lapland (Sample C). The smaller shell (left valve) belongs to a previously studied set of shells (southern Lapland) and was estimated as being at the time of death 10 years old (Helama & Valovirta 2007). Both shells show corrosion on the umbo.

ished (3- μm diamond paste) and then etched in Mutvei's solution at 37 to 40 °C for approx. 25 min, carefully rinsed in de-ionised water and allowed to air-dry (Mutvei et al. 1994, 1996). While the acetic acid dissolved the carbonate, glutaraldehyde fixed the organic matrix and alcian blue stained and fixed the mucopolysaccharids and glucosamids in the shell. This treatment resulted in an excellent three dimensional preservation of the growth structures with distinct, etch-resistant, blue-coloured winter lines. Finally, annual growth increments were viewed under a reflective-light binocular microscope and digitally photographed. The increment widths between successive annual growth lines were measured from photographic enlargements of known scale from the outer shell layer. Measurements were performed perpendicular to winter-lines, to the nearest 1 μm (micron), and compiled into time series of shell growth increment widths. This procedure has been used with *M. margaritifera* shells in several recent studies (Mutvei et al. 1994, 1996, Dunca 1999, Dunca and Mutvei 1996, 2001, Mutvei and Westermarck 2001, Dunca et al. 2005, Helama et al. 2007). The last increment of each specimen without identifiable winter-line was excluded, since these were likely to be incomplete due either to post-mortem corrosion of the ventral margin or unfinished shell growth due to death occurring during the growing season.

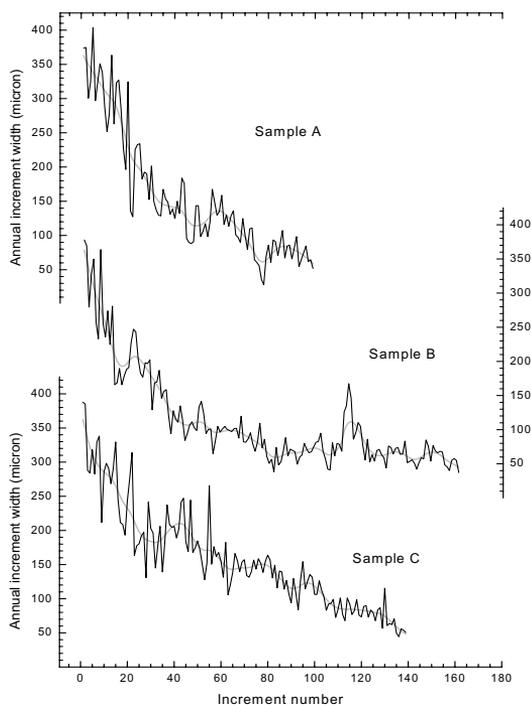


Fig. 2. Annual growth increments (thin black line) of the three sampled shells, shown here as a function of their ontogenetic age. The thick gray line depicts the long-term growth changes after filtering by spline function.

3. Methods

3.1. Time-series analyses

Bivalve shell growth increments exhibit variation at various time-scales (Mutvei *et al.* 1994, 1996, Dunca & Mutvei 1996, 2001, Dunca 1999, Marchitto *et al.* 2000, Mutvei & Westermarck 2001, Dunca et al. 2005, Epl e *et al.* 2006, Helama *et al.* 2006). Growth variations at shortest time-scales occur on a year-to-year basis, whereas the changes occurring as a function of ontogeny are referred to as growth trends. In order to emphasize the long-term growth variations, the time-series of annual increment widths were filtered using cubic smoothing splines (Cook & Peters 1981). That is, the spline function served as a model for variations in the growth at long time-scales. In order to emphasize the short-term growth variations, the yearly width values of the increments were divided by the values of the spline function. The resulting ratio-based growth index series thus

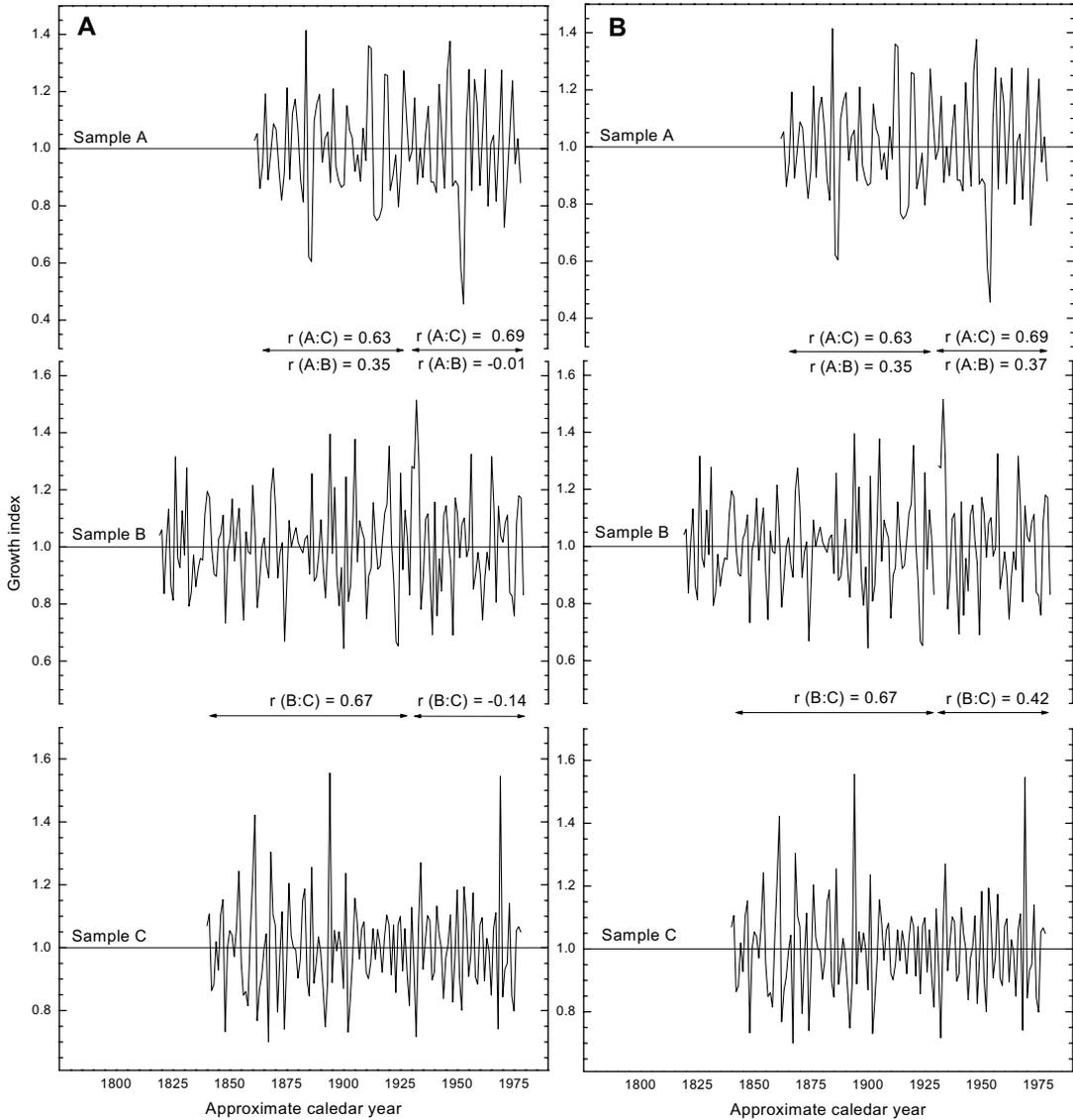


Fig. 3. Sclerochronological crossdating in process. Visual comparison was quantified using Pearson correlations (r) between the growth index series A, B and C. Correlations were markedly high between the three shells before approx. 1930 and after that date between the samples A and C. After 1930, a distinct drop in sub-segment correlations was observed between B and the other series (A). Reduction in correlations occurred due to growth disturbance (fig. 4) that caused a missing increment in the cross-section of the disturbed shell. Ascending correlations were observed subsequent to the final step of crossdating (B).

showed no long-term variations. Previously, the 16-year spline with 50 percent cut-off was found to be most effective for extraction of growth variations at short time-scales within the *M. margaritifera* population of southern Sweden (Helama et al. 2006). In consequence, this function was employed in the present study.

Bivalve shell growth records are generally correlated within populations. As a rule of thumb, growth variations that are common to a population occur mostly due to external factors, for example climatic influence. As a consequence, wide and narrow increments would be expected to occur in temporal synchrony between specimens. This syn-

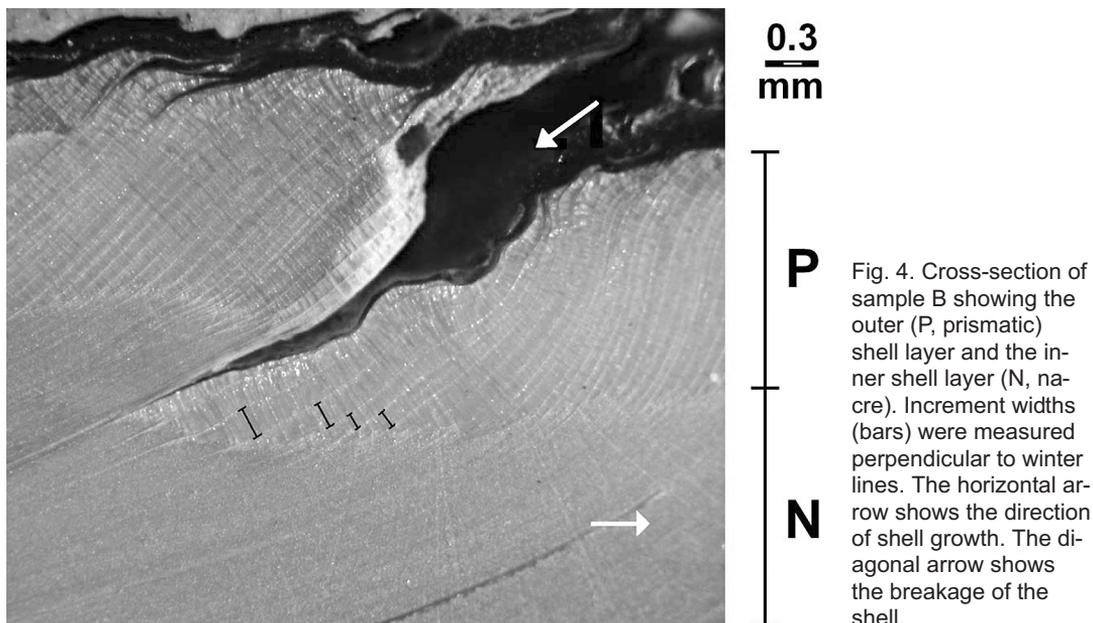


Fig. 4. Cross-section of sample B showing the outer (P, prismatic) shell layer and the inner shell layer (N, nacre). Increment widths (bars) were measured perpendicular to winter lines. The horizontal arrow shows the direction of shell growth. The diagonal arrow shows the breakage of the shell.

chrony, in turn, can be used as a basis for cross-dating of the sclerochronological record (Hudson et al. 1976, Marchitto et al. 2000, Helama et al. 2006).

In crossdating, the growth variations in each shell-specific series were compared with other series; each sample was lagged forward and backward in time to determine whether offsetting the series would yield higher visual and statistical correlation. If a higher correlation was found, then the number of years lagged was taken as an indication of the number of offsetting growth increments. As an additional step, similar lag-analysis was applied to all sub-segments of the series. Identifying a segment in which a (visual and statistical) correlation is noticeably reduced was used to locate the year of a dating error. When such a drop in sub-segment correlations was found, that part of the particular time-series, was re-examined for potential measuring errors (that is, misinterpretation of intra- and inter-annual growth structures of the prismatic layer as missed or falsely added increments).

Once a sample series was successfully cross-dated, it was accepted into the mean chronology. The strength of correlation was measured statistically using the Pearson test, whereby each of the increment series was evaluated on the strength of agreement with the other two series, as well as the mean of the other two series. This approach is

common among dendrochronological studies (e.g. Holmes 1983, Grissino-Mayer 2001, Helama et al. 2005) and its feasibility for bivalve shell growth increments has been demonstrated (Helama et al., 2006).

4. Results

Width of annual increments varied between 28 and 404 μm . In addition, increment widths exhibited growth trends that could be approximated by taking negative exponential decrease as a function of ontogeny (fig. 2). These trends were removed prior to growth comparison between the series in order to emphasise the year-to-year variation in increments.

Sample series A and C correlated well, regardless of the time interval used in crossdating (fig. 3A). Series B also correlated markedly well with other series but only prior to approx. 1930. After that date, correlations with series B dropped significantly. Re-examination of the cross-sections showed that shell breakage had affected sample B but left the other two shells undisturbed. Consequently, there was one year's growth that could not be observed in sample B (fig. 4). Accepting a one year forward temporal shift in the post-disturbance sub-segment of sample B (due to missing incre-

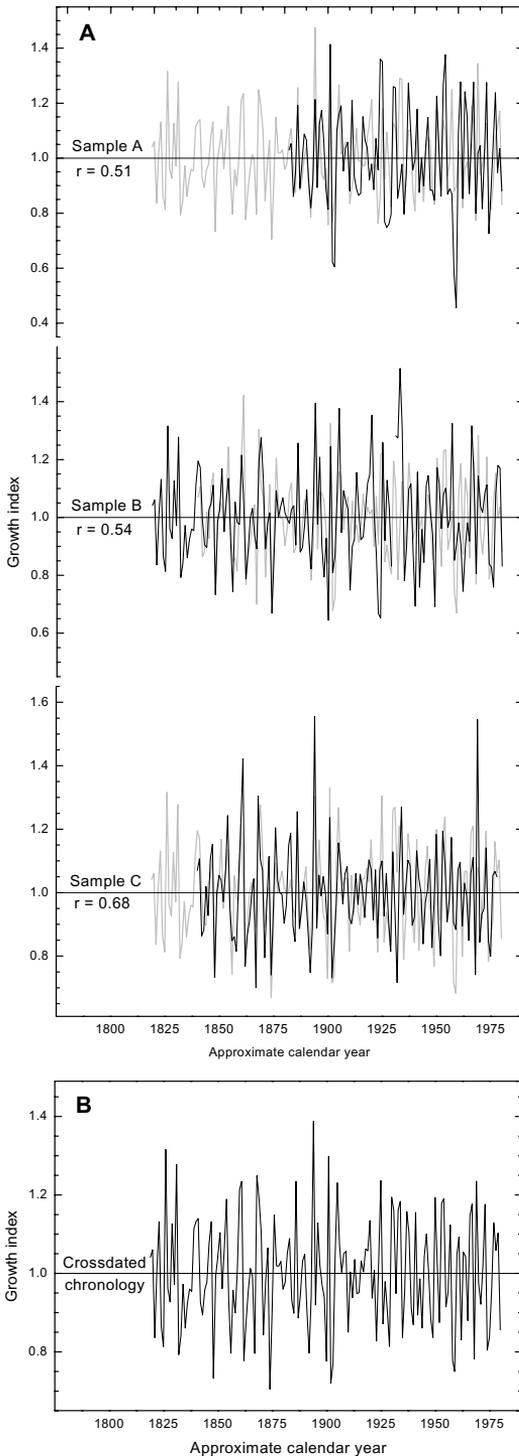


Fig. 5. Final crossdating between the sample series and the mean of other two series (A). Final crossdated sclerochronology (B).

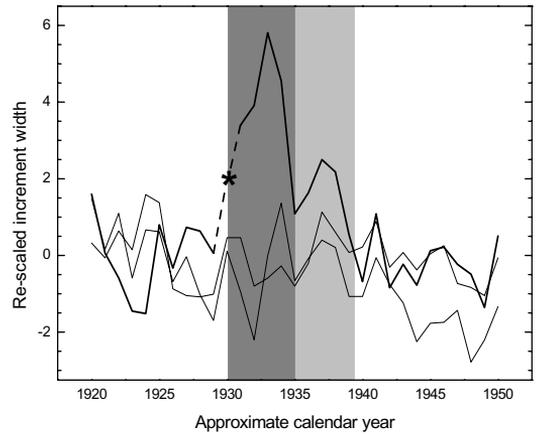


Fig. 6. After the growth disturbance (asterisk), the disturbed shell (thick black line) exhibited anomalous increment widths that were first markedly (gray area), and then moderately (light gray area), wider than the increments in the other two shells (thin black lines). Increment widths are shown here after re-scaling of the initial measurement series (from fig. 2) to the same mean ($X=0$) and standard deviation ($\sigma = 1$) over the pre-disturbance interval and applying the re-scaling to the post-disturbance increments.

ment in the same series) synchronised the series markedly, over both pre- and post-disturbance periods (figs. 3B, 5A). Resulting mean chronology (fig. 5B) was expected to show growth variations more reliably than any single series.

Closer examination showed that the disturbed shell had experienced anomalously wide increments subsequent to the aforementioned disturbance event. This post-disturbance period of anomalous growth was manifest in the form of markedly and moderately wider increments, that continued for 5 and 8 years, respectively (fig. 6).

After crossdating, the numbers of annual increments that could be assessed in the three shells showed that the mussels were all very old at the time of death (Table 1).

5. Discussion and conclusions

5.1. Estimating the age of mussels

Shell increments of *Margaritifera margaritifera* are an excellent source of detailed information about the age and growth of the species at the indi-

Table 1. Progressive estimates of the ontogenetic age as the number of observed annual increments subsequent to crossdating (A_{obs}), the estimated number of corroded annuli (A_{cor}) and the expected number of exceptionally narrow increments due to juvenile life-stage of the mussels (A_{jvn}), the latter based on the study of Buddensiek (1995). Length of the shells was measured using vernier callipers.

Sample	A_{obs}	$A_{\text{obs}} + A_{\text{cor}}$	$A_{\text{obs}} + A_{\text{cor}} + A_{\text{jvn}}$	Length (mm)
A	99	111	115	120.4
B	162	179	183	124.9
C	137	156	160	125.8

vidual to population level (figs. 2–3, 5–6). Documentation of annual growth increments of the species in several publications (Mutvei et al. 1994, 1996, Dunca & Mutvei 1996, 2001, Dunca 1999, Mutvei & Westermark 2001, Dunca et al. 2005, Helama et al. 2007) makes these increments a definite temporal framework of known periodicity. Halliday & Verrell (1988) compared several different methods for determining age for amphibians and reptiles and concluded that only skeletochronology (sclerochronology) and mark-recapture were reliable. The former was applied in this study, the latter cannot in practise be used for determining the entire lifespan of such an extreme long-lived species as *Margaritifera margaritifera*.

Importantly, the chronological control of skeletal diaries can be validated and consequently improved if the procedure of crossdating is applied to data. In our example, crossdating of the increment series verified the contemporaneity of the observed shell growth events at annual resolution. In addition, crossdating enabled us to detect an instance of growth disturbance in one shell and to date and compare that event relative to growth of undisturbed shells (fig. 6). The concepts and numerical approaches of sclerochronological crossdating are to a great extent inherited from dendrochronological studies. Dendrochronology has applied the technique to tree-rings for a much longer time (e.g. Douglass 1941, Fritts 1976). As a matter of fact, dendrochronologists restrict the term dendrochronology to refer only to material or studies to which careful crossdating has been successfully applied (e.g. Fritts 1976, Fritts & Swetnam 1989). Following this dendrochronological concept, it has been suggested that the use of the term ‘sclerochronology’ should similarly be applied only to crossdated skeletal diaries (Helama et al. 2006). Using this more precise terminology, our

study material fulfils the criteria for high-quality sclerochronology.

Corrosion had caused loss of the umbo in the *M. margaritifera* valves studied here (fig. 1). This is known to be typical of *M. Margaritifera*, indicated as early as in the writings of Linné (1806: p. 176). The loss of the ontogenetically youngest increments bears implications for age determination. As a consequence, the number of counted annual increments has to be taken as a minimum estimate of the lifespan of each mussel, the actual ontogenetic age at death being somewhat older. The number of corroded increments is a function of the width of the corroded area and the expected width of the corroded increments. In the direction of sampled cross-section, the width of the corroded area was 22.8, 29.8 and 27.6 mm, respectively, for samples 1, 2 and 3, causing concomitant loss of 12, 17 and 19 annuli (Helama & Valovirta 2007). Using this information, we can confirm estimates of supercentenarian age for the studied shells (Table 1). However, the ecology of juvenile *M. margaritifera* may have further implications to the aforementioned age estimates: subsequent to its larval parasite life-stage, *M. margaritifera* spends a number of years burrowed into the river sediment, where its increments may be exceptionally narrow. According to Buddensiek (1995) this may have caused a general underestimation of approx. 4 post-parasitic years in age determinations (see fig. 5 in Buddensiek 1995). Taking this into account the estimated age of the oldest mussel in our sample would thus have been more than 180 years (Table 1).

5.2. Longevity of freshwater pearl mussels

The supercentenarian age estimates for the northern European shells forming the basis of our study

reflect current biogeographical theory on *M. margaritifera* longevity. Bauer (1992) studied 48 populations from across the geographical range of this species in Europe and North America and concluded that the lifespan of the species tends to increase towards the high latitudes. Similar conclusions have been made by Björk (1962) in Sweden, and by Ziuganov et al. (2000), who recorded a 190-year old specimen from Arctic Russia, where still are many large and old freshwater pearl mussel rivers (Valovirta 1998b). The oldest Finnish mussels would thus be expected to occur exactly where they now have been found, in the northernmost part of the country. Even so, markedly old mussels can also be discovered at somewhat lower latitudes. Timm (1994) determined a maximum age of 132 years for *M. margaritifera* found in Estonia and Mutvei et al. (1996) recorded a 100-year old specimen of *M. margaritifera* from southwestern Sweden. It is thus likely that rather old individuals can be found almost irrespective of latitude, although the prediction would be for a general tendency that the oldest populations dwell at the northernmost habitats. For example, San Miguel et al. (2004) found a maximum lifespan of only 65 years among 16 populations in northwestern Spain.

According to Bauer (1992), the maximum biogeographical gradient in lifespan is probably linked to temperature-dependent variation in metabolism. Moreover, anthropogenic pollution is likely to increase mortality and thus decrease longevity (Bauer 1992). Considering anthropogenic factors further, it is noteworthy that the studied shells were probably from mussels killed by illegal pearl hunting (Helama et al. 2007). How much longer they might have lived without man's interference is a matter for conjecture.

5.3. Shells growth variations

All three shells exhibited clear decline in the increment widths through their ontogeny (fig. 2). The trends were not linear but slightly concave. The results are parallel to previous study where *M. margaritifera* internal shell growth increments were studied in material from two small river in southern Lapland and Northern Ostrobothnia. Moreover, *M. margaritifera* from Swedish rivers

showed similar growth trends (Mutvei et al. 1994, Dunca 1999, Helama et al. 2006) that could be mathematically modelled using negative exponential functions (Dunca 1999).

Apart from the ontogenetic trend, growth variations at shorter time-scales were evident throughout the life-span of the mussels. As discussed above, this was the variability that actually enabled the time-series of annual increment widths to be crossdated (figs. 3, 5) and averaged into one sclerochronology (fig. 5). Because the growth variations occurred in temporal synchrony, common forcing factors might be expected. Growth of shell in *M. margaritifera* has previously been shown to correlate with summer temperatures in Swedish rivers (Mutvei et al. 1994, Dunca 1999, Mutvei & Westermark 2001, Dunca et al. 2005). In parallel, Dunca & Mutvei (2001) demonstrated that the sub-annual *M. margaritifera* shell micro-lamellae corresponded to the number of days of the growing season (days with water temperature higher than 5 °C) in different Swedish populations. According to Bauer (1992), the major factor influencing growth rates would be temperature, through its potential influence on the metabolism of mussels. According to Dunca & Mutvei (2001), temperature may influence shell growth directly by regulating metabolism of the bivalve, an effect potentially amplified by temperature effects on regulation of food supply. The same climatic forcing – summer temperature – could be expected to influence shell growth variability of *M. margaritifera* in the study region.

5.4. Shell damage

In addition to ontogenetic and synchronous growth variations, we observed one case of obvious shell damage that caused growth anomaly (fig. 6). Facilitated by crossdating, the single-shell anomaly could be compared with the undisturbed growth shown by the other shells (fig. 6). Ziuganov et al. (2000) studied shell repair in *M. margaritifera* in the Thorma River (Kola Peninsula, North-West Russia). Examining three shells of ages greater than 90 years, they observed complete shell repair from shell damage in two years, with a rate of shell production to repair the damage up to even 130 times faster than expected growth

(Ziuganov et al. 2000). The damaged mussel included in our study was, at the time of damage, somewhat older (approx. 130–140 years) than the shells studied by Ziuganov et al. (2000), and repaired its shell at a slower rate, the repair process continuing for a longer time, up to 8 years (fig. 6). It is noteworthy that we examined the shell axis of minimum growth. Considering shell geometry, a higher rate of shell repair could have been expected in the direction of maximum growth.

According to Mutvei & Westermark (2001), there is increased occurrence of *M. margaritifera* growth disturbances that make recognition of annual growth periodicity difficult, in anthropogenetically disturbed rivers (Valovirta 1990, 1995b, Moorkens et al. 2000). In Finland, an increased number of growth disturbances could thus be expected to occur in southern populations, making sclerochronological studies somewhat more difficult there.

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Suomen vanhin eläin: ontogeneettinen ikä ja kasvu kuoren sisäisiin vuosilustoihin perustuen

Jokihelmisimpukan eli raakun (*Margaritifera margaritifera*) pitkäikäisyydestä on aikojen kuluessa esitetty erilaisia arvioita. Tässä tutkimuksessa raakun ikää selvitettiin sen kuoren vuosikasvuihin perustuen, tutkimusmateriaali sisälsi kolme suurehkoa ja rotevaa raakunkuorta, jotka oli kerätty Inarista ja talletettu Luonnontieteellisen keskusmuseon (Helsingin yliopisto) kokoelmiin.

Vuosikasvujen eli lustojen leveydet vaihtelivat 0.4 mm ja 30 µm:n välillä, yleisesti ottaen kaventuen raakun ikääntyessä. Vuosien välinen kasvunvaihtelu oli hyvin samantahtista kolmen eri simpukkayksilön välillä. Tämä osoitti lustoissa havaittavan vaihtelun tapahtuneen samojen ulkoisten ärsykkeiden vaikutuksesta. Kasvunvaihtelun samanaikaisuus mahdollisti lustonleveysaikaasarjojen ristiinajoituksen, jonka ansiosta yksittäiset

lustoleveysarjat voitiin laskea yhdeksi keskiarvoaikaasarjaksi eli simpukkoiden lustokronologiaksi.

Yksi raakun kuori oli osittain rikkoonut simpukan elinkaaren keskivaiheilla. Kuorivahingon jälkeen sen kasvussa havaittiin 5–8 vuoden äkillinen kasvunlisäys ilman että samanaikaista kasvunvaihtelua olisi voitu havaita muissa raakku-yksilöissä. Vanhimman yksilön kuori sisälsi yhteensä 162 vuosilustoa. Umbon ja sitä lähellä sijaitsevien lustojen leveyskasvun korroosio vanhimmassa raakussa, otettiin huomioon lisäämällä havaittujen lustojen määrään yhteensä 17 vuosilustoa. Tähän ikään lisättiin vielä neljä vuosilustoa perustuen aikaisempaan tutkimukseen raakun nuoruusvaiheen kehityksestä. Tulosten perusteella raakku voi hyvinkin elää alueella yleisesti ylitavutuotiaaksi. Jopa yli kaksisataavuotiaitten yksilöiden olemassaolo on hyvin mahdollista siellä, missä ihmistoiminnan aiheuttamia häiriötekijöitä ei ilmene.

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