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# Growth and chemical analyses of freshwater pearl mussel, *Margaritifera margaritifera,* shells from Haukåselva river, Norway

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Foto: Sølve Sondbø, Hordaland Fylkeskommune. Elevemuslingen i Haukåselva.

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# Report No 10/2014:

# Growth and chemical analyses of freshwater pearl mussel, *Margaritifera margaritifera,* shells from Haukåselva river, Norway

by

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## *Foreword*

Freshwater bivalves build their shells of calcium carbonate during the warm period of the year when the temperature becomes higher than 5°C. During the cold period of the year the shells do not grow much and a denser and more etch resistant thin zone is build up delimitating two annual increments. In trans-section the shells show annual rings that are distinctly delimitated by winter lines similar with tree-rings. The width of annual increment or ring varies depending on environmental conditions such as temperature, pH, food availability etc. Sometimes during the growth season in extreme conditions such as dry-outs or long-term pollutions when the bivalves are forced to keep close under a longer period of time the shells cease to grow forming growth disturbance lines as result, which may look like a winter line. A special method that makes winter lines more visible in trans-sections has been developed at the Swedish Museum of Natural History in Stockholm. The special etching and colouring technique enhances the winter lines and enables to distinguish them from growth disturbance lines facilitating a more accurate age determination of the shells.
Furthermore, the dens structure of the shells preserves the imprint of the water chemistry at the time of shell deposition, which makes them reliable environmental indicators.

The aim of the present study is to analyse growth and elemental distribution in shells of *M. margaritifera* collected in Haukåselva river.

The study has been carried out by Elena Dunca, Bivalvia Company, in collaboration with Palaeobiology Department at the Swedish Museum of Natural History in Stockholm and is ordered and financed by Håvard Bjordal, miljøsjef i Bergen kommune, Byrådsavdeling for byutvikling, klima og miljø, Klimaseksjonen.

## *Abstract*


Dunca, E. 2014. Growth and chemical analyses of freshwater pearl mussel, *Margaritifera margaritifera*, shells from Haukåselva river, Norway. Bivalvia report no 10. 21 p.

Age determination and annual growth analyses of two *M. margaritifera* shells from river Haukåselva, Norway, was carried on thin transversal sections prepared using the method developed at the Swedish Museum of Natural History in Stockholm, Sweden. In one of the shells (the oldest) elemental distribution was analysed using ICP-MS.
The growth analyses showed that mussel B is younger and had higher growth rate than mussel A indicating that the two locations where the shells were found had different growth conditions. As such, the annual growth pattern of the two shells had a poor match but in both shells the growth was found decreasing after 2008, which indicated that some environmental factors had caused distress in both locations in the last living years.
Variations in annual growth showed lower growth in the periods 1961-1978 (except 1967) and 1996-2013. In the same periods of time an increased amount of Fe, Mn, P, Ba and Sr was measured in the shell, while the levels of Cu, Co and Pb were lower. This together with the absence of heavy metals (such as Al, As, Cd and Cr) in the shell indicated that the freshwater pearl mussels in Haukåselva are not affected by the pH condition of the water, but highly affected by low O levels possibly due to eutrophication.

## *Introduction*

The status report from 2004 on *M. margaritifera* in Haukåselva river (figure 1) estimated a very small population of adult mussels with around 400 individuals (0.06 individuals/$m^2$) and only few young mussels. It was also found that the density of hosting fish was low with low infestation rate of mussel larvae on the gills indicating that even if reproduction did take place the mussel population would face extinction in a near future unless measures were taken. The water quality presented a potential threat due to higher particle and nutrient loads (Hobæk *et al*. 2004).

However, the latest status report from 2013 found 738 living mussels with many juveniles, which indicates that the population is slowly recovering (Bjordal 2013).

In order to bring more clarity on the environmental impact on the naiad population in Haukåselva river two shells from dead collected individuals were analysed with respect to their growth and elemental distribution.

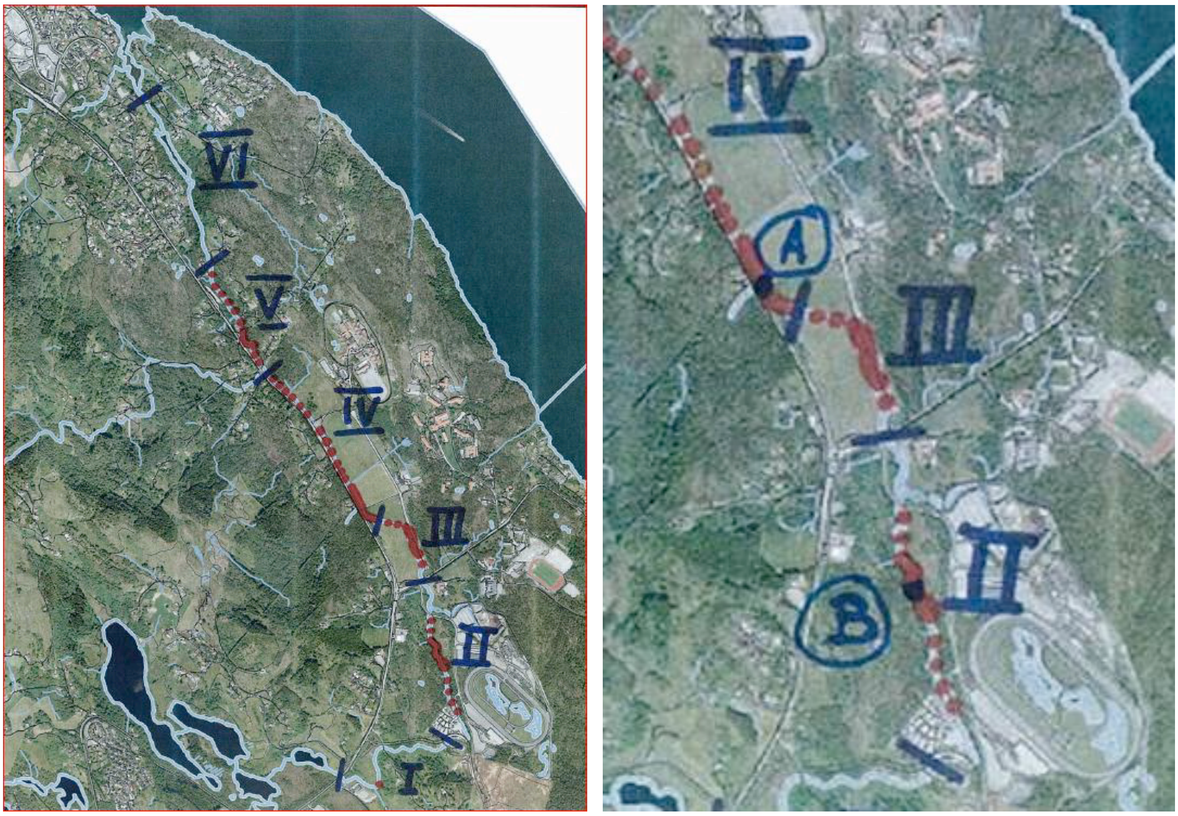


Figure 1. Maps showing Haukåselva river with the sites where the naiad shells A and B were collected (from Bjordal 2013).

## *Material and methods*

Shells halves from two *M. margaritifera* specimens dead collected from Haukåselva river (figure 1) were chosen for analyses. Shell A had soft parts inside when collected in 25th July 2013 while mussel B was found dry on land near the river bed upstream on 31st July same year (figure 1). The shells were prepared for age determination using the method developed at the Swedish Museum of Natural History in Stockholm and described in previous work on freshwater pearl mussel (Dunca 1999, Dunca & Mutvei 2001, Dunca *et al*. 2005, Dunca & Larsen 2012).

Thin transverse sections were manufactured from each shell and then polished with tin oxide, coloured and etched with Mutvei's solution (Schöne *et al*. 2005). This solution etched the calcite crystals and coloured the organic sheets between crystals in blue enhancing in this way the visibility of winter lines (figure 2). Annual increments were counted from the ventral edge to the umbonal part. Missing years of the eroded umbonal part were estimated by general growth curves of freshwater pearl mussel in Sweden (Dunca *et al*. 2011).

For the purpose of growth analyses the thin sections were photographed in light microscope. Each annual increment was measured on digital pictures as the shortest distance between two winter lines in the shell portion at the border between the prismatic layer and the nacreous layer (Fig. 2) using Panopea image processing software (developed by Peinl & Schöne, University of Frankfurt).
The ontogenetic trend within measured values (exponentially decreasing) was removed in order to compare individuals at different growth stages (Dunca 1999; Dunca et al. 2005, 2011). Consequently, the measurements were standardized using similar methods to those of dendrochronologists (Cook & Kairiukstis 1990) and described in previous work (Dunca 1999; Dunca et al. 2005, 2011). The obtained values, i. e. the standardized growth indices (SGI), show higher (if positive) or lower (if negative) annual growth than expected (theoretical growth) offering the possibility to compare growth with series of environmental parameters, such as annual temperatures, pH, precipitation, etc.

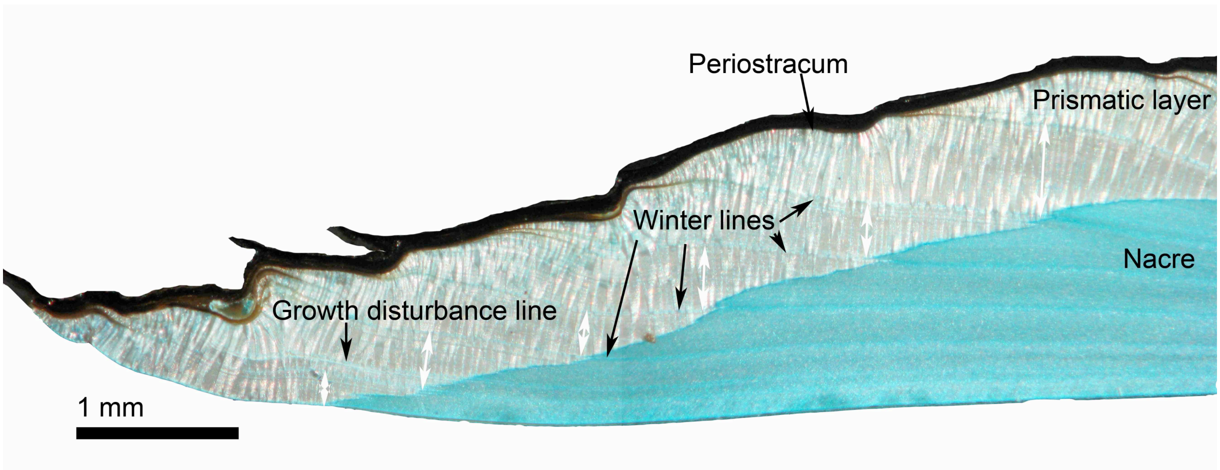


Figure 2. Thin section of mussel B etched and coloured with Mutvei's solution viewed in light microscope. The white arrows mark annual increments between two winter lines.

Chemical analyses were made on bivalve A only. For this purpose periostracum and the inner shell layer of secondary growth were removed so that the growth increments were free from contamination. A vertical section of 3-4 mm was cut perpendicularly to the growth lines and then divided into 7 portions with a weight between 15 and 40 mg and with a well-defined number of annual increments (for details see appendix 1 and 2). For cutting and cleaning the shell, a 0.1 mm thin diamond blade attached to a dental drill Taro Fc-82 was used. The samples were rinsed with ethanol 95% in ultrasonic bath and thereafter, dissolved in 60% $HNO_3$. The obtained solutions were analysed with an ICP-OES, Varian Vista Ax (inductively coupled plasma using optical emission) in collaboration with Prof. Carl Magnus Mörth at the Department of Geochemistry, Stockholm University.

## Results

### *Growth analyses*

Shell length in relation to the age was different in the two shells (table 1, figure 3). The growth of shell A was near the normal growth curve while the growth of shell B was above the high growth curve for *M. margaritifera* species (Dunca *et al*. 2011). The age included estimation of the eroded part at the umbo.

Table 1. Shell length and age of the two shells.

| | Age estimation umbo (mm) | Age estimation section (mm) | Age (years) | Length (mm) |
|---|---|---|---|---|
| Mussel A | 11 | 53 | 64 | 110,4 |
| Mussel B | 9 | 23 | 32 | 110,8 |

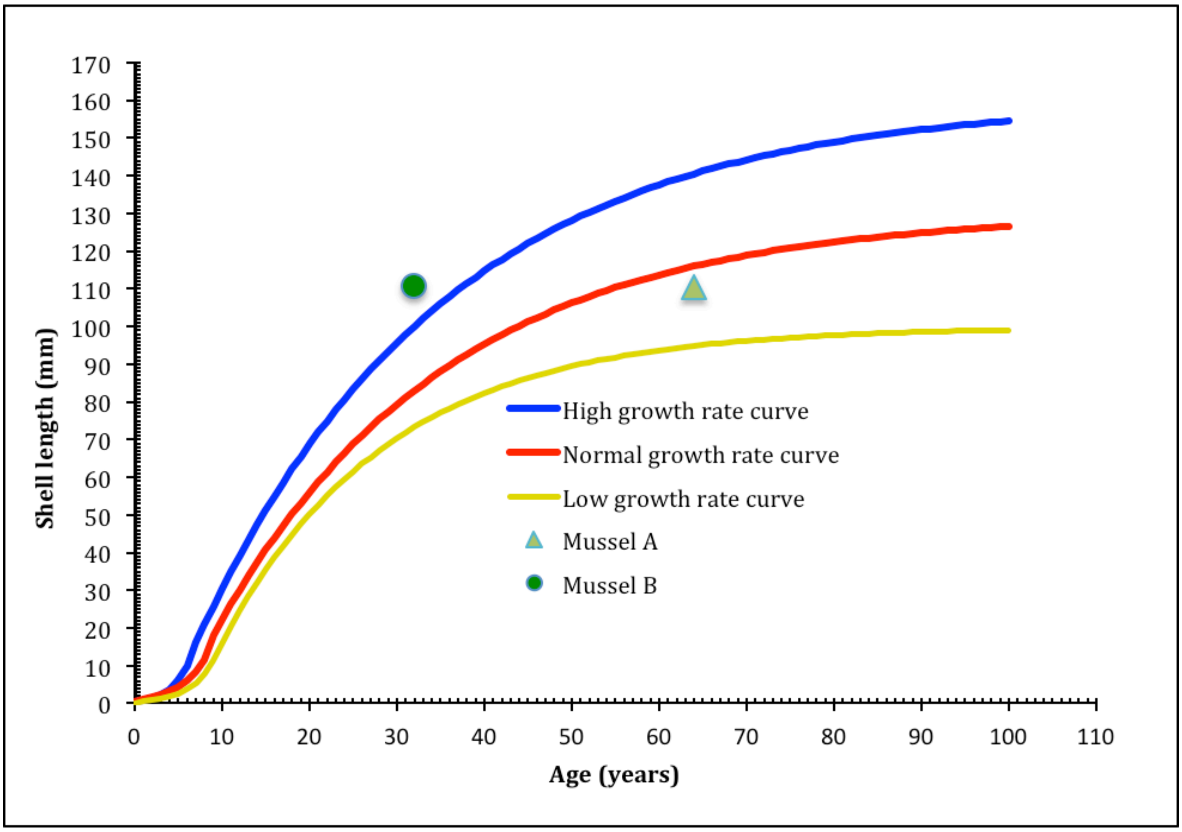


Figure 3. Diagram showing the relationship between age (from thin sections including estimations of the eroded umbonal part) and shell length of both shell A and B and their placement on the general growth curves for *M. margaritifera* species (Dunca et al. 2011).

The last annual increment in shell A showed that the naiad died in the middle of growth season, which corresponded with July, the same month that it was collected. In shell B the last annual increment ended with winter line, which indicated that the naiad died during wintertime. To establish which year it died both annual growth patterns and intra annual growth patterns (microgrowth) were compared in the two shells. The annual growth of year 2011 in shell A showed a strong growth disturbance line (figure 4) and the year after (2012) had lower growth. A strong growth disturbance line was visible even in bivalve B and the year after that was smaller and the last living year, which suggested that naiad B died in the winter 2012-2013.

The pattern of annual growth in the two shells did not match well. In bivalve A the annual growth rate decreased after 1996 having lower values until the last living year (figure 5 and appendix table 2). In bivalve B the annual growth rate decreased in the last living years beginning with 2008 (figure 5 and appendix table 2). Between 1992 and 1995 the growth rate was higher in bivalve A (figure 5 and appendix table 2).

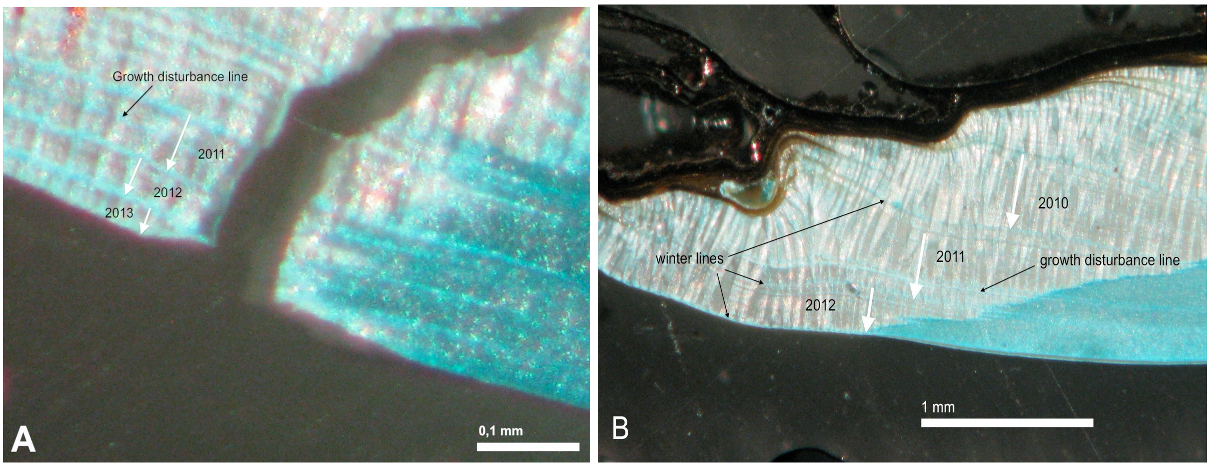


Figure 4. Last annual increments in both shells.

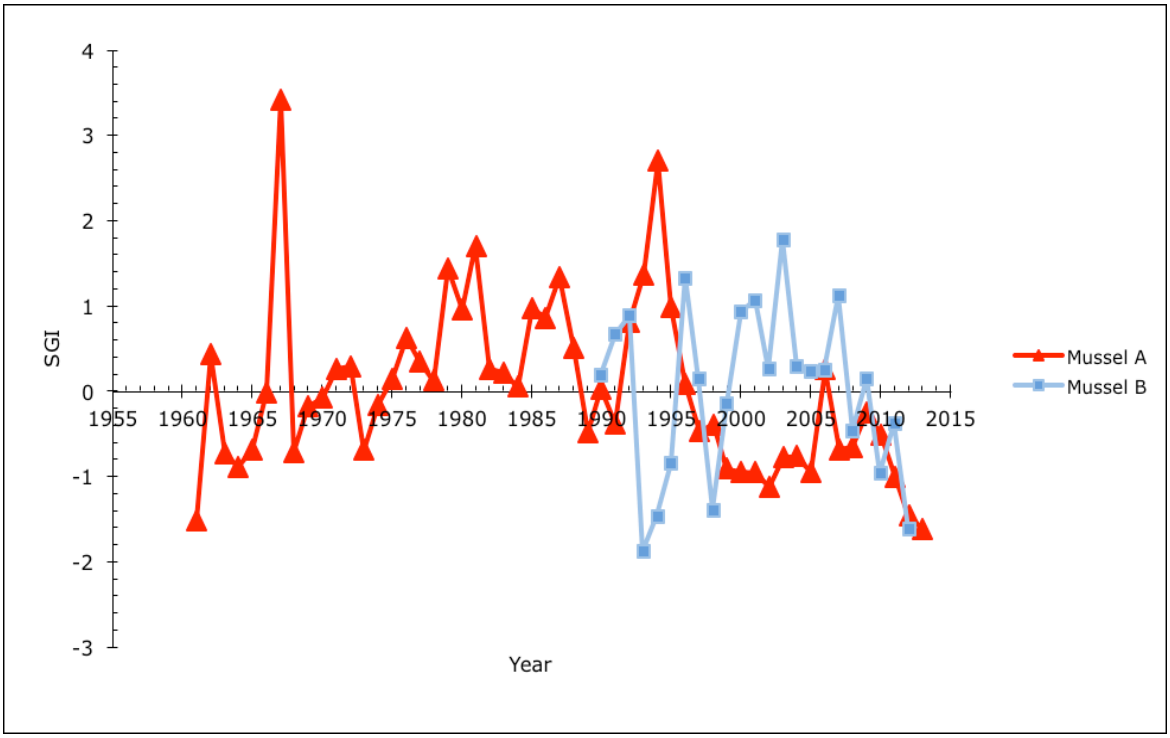


Figure 5. Diagram showing the growth curves (expressed in SGI values) for both mussels A and B.

## *Chemical analyses*

Results of chemical analyses are presented in appendix 1.
Some elements such as calcium (Ca) and lithium (Li) showed a constant distribution in the shell with only slightly higher values between 1979 and 1986 (figure 6).

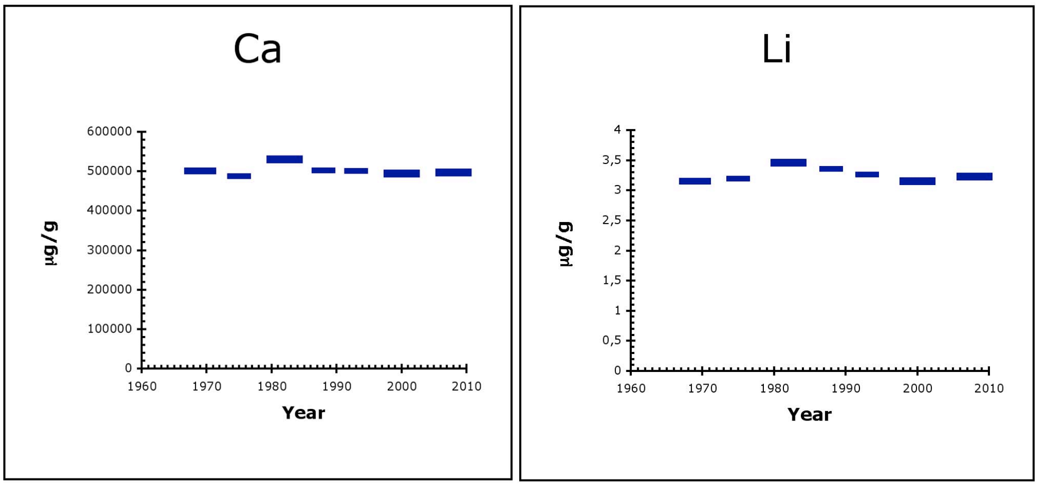


Figure 6. Diagrams with elemental distribution in mussel A for Ca and Li.

Other elements such as sodium (Na), sulphur (S) and strontium (Sr) had descending trends or ascending trends such as silica (Si; figure 7).

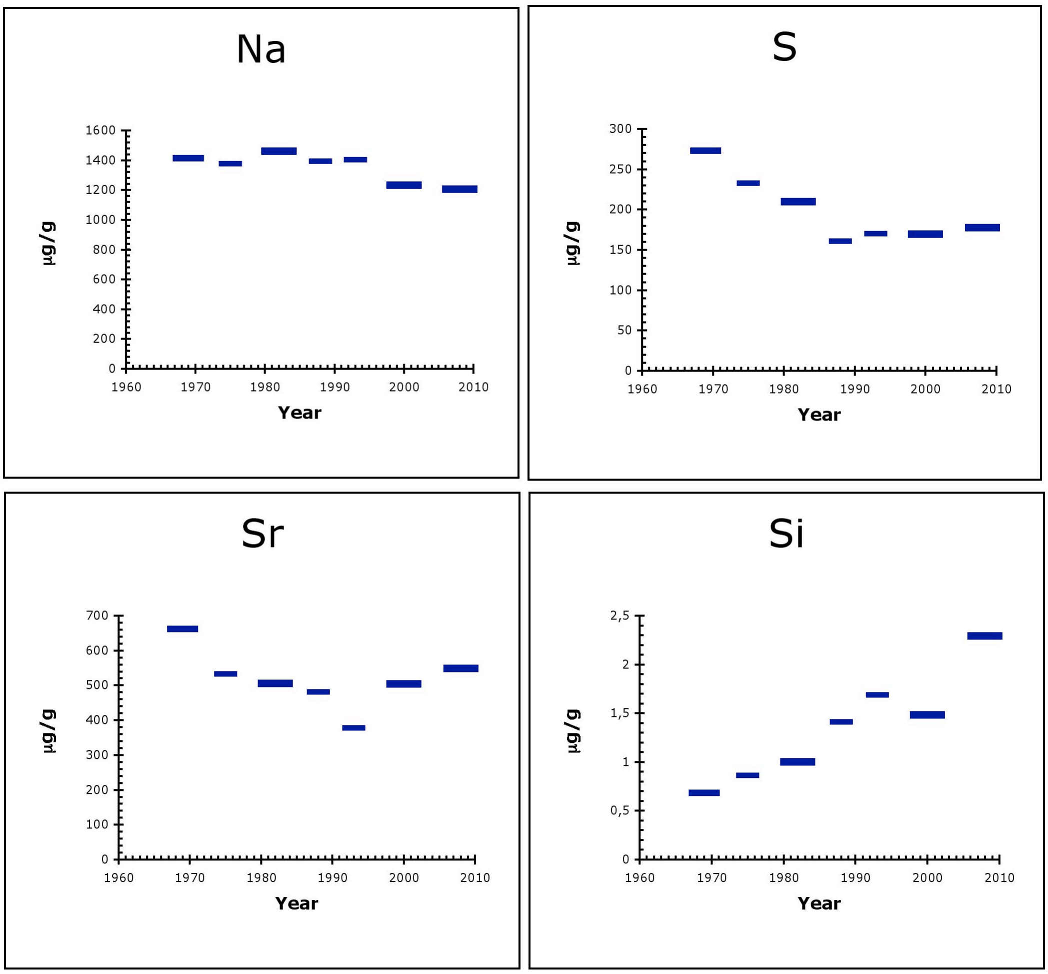


Figure 7. Diagrams with elemental distribution in mussel A for Na, S, Sr and Si.

Sr and S had lower values in sample 4, the period between 1987 and 1991.
The distribution of metals such as copper (Cu), cobalt (Co), magnesium (Mg) and potassium (K) varied through time with higher levels in 1970-s. The amount of Cu decreased towards 2013 while K decreased towards 2006 and then slightly increased until 2013 (figure 8).

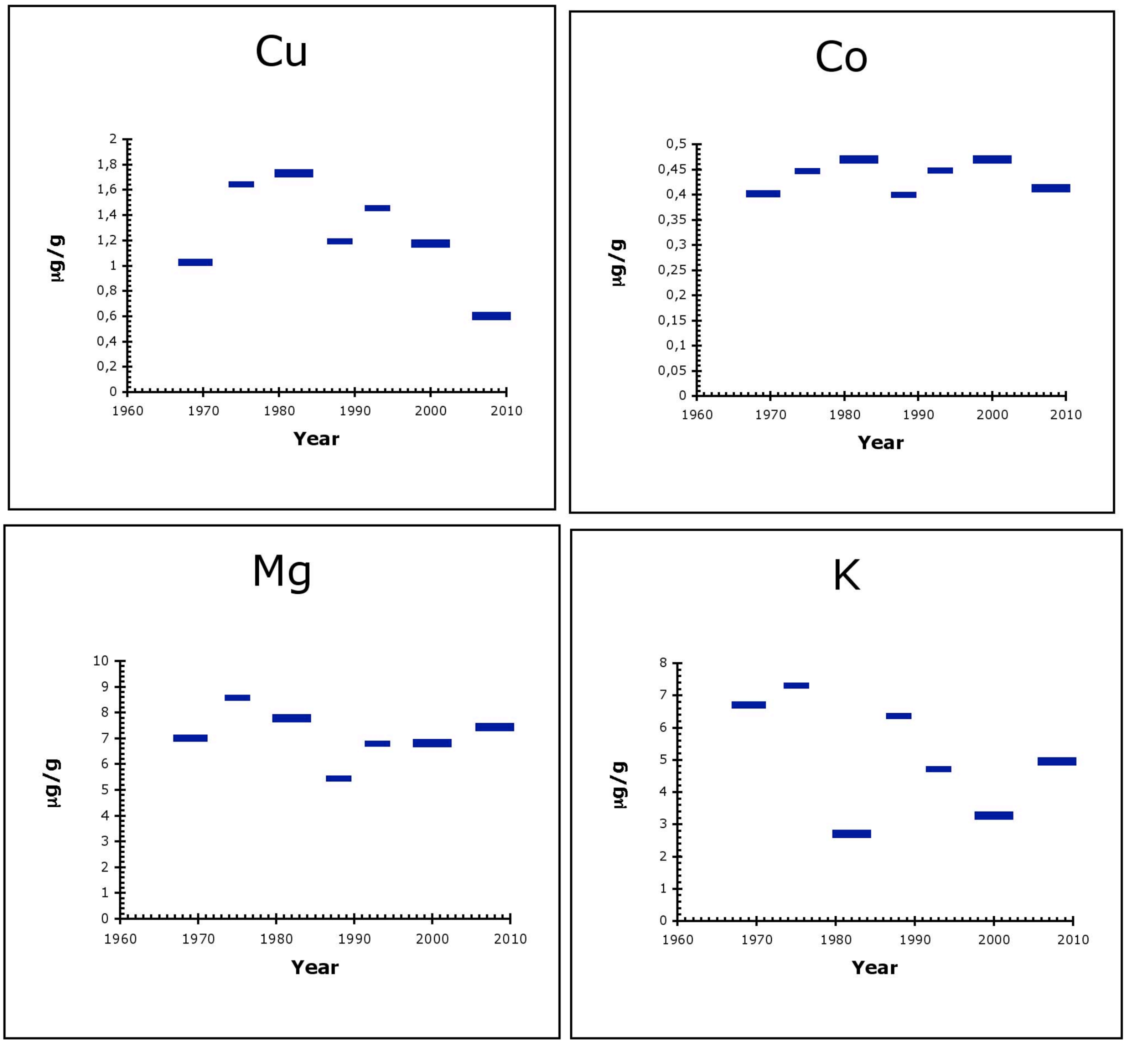


Figure 8. Diagrams with elemental distribution in mussel A for Cu, Co, Mg and K.

The levels of iron (Fe), manganese (Mn), barium (Ba) and phosphorus (P) had similar trends with higher levels in time intervals 1967-1973 and 2005-2013 and lower levels in between. Sample 4 representing period 1987-1991, showed slightly higher levels of Mn, Ba and P (figure 9).

Titanium (Ti) and molybdenum (Mo) amounts were higher during late 60's and 70's (figure 10) while zinc (Zn), vanadium (V) and nickel (Ni) was present only sporadically (figure 11).
Lead (Pb) was present in the shell in higher amount during the period 1987-1996.

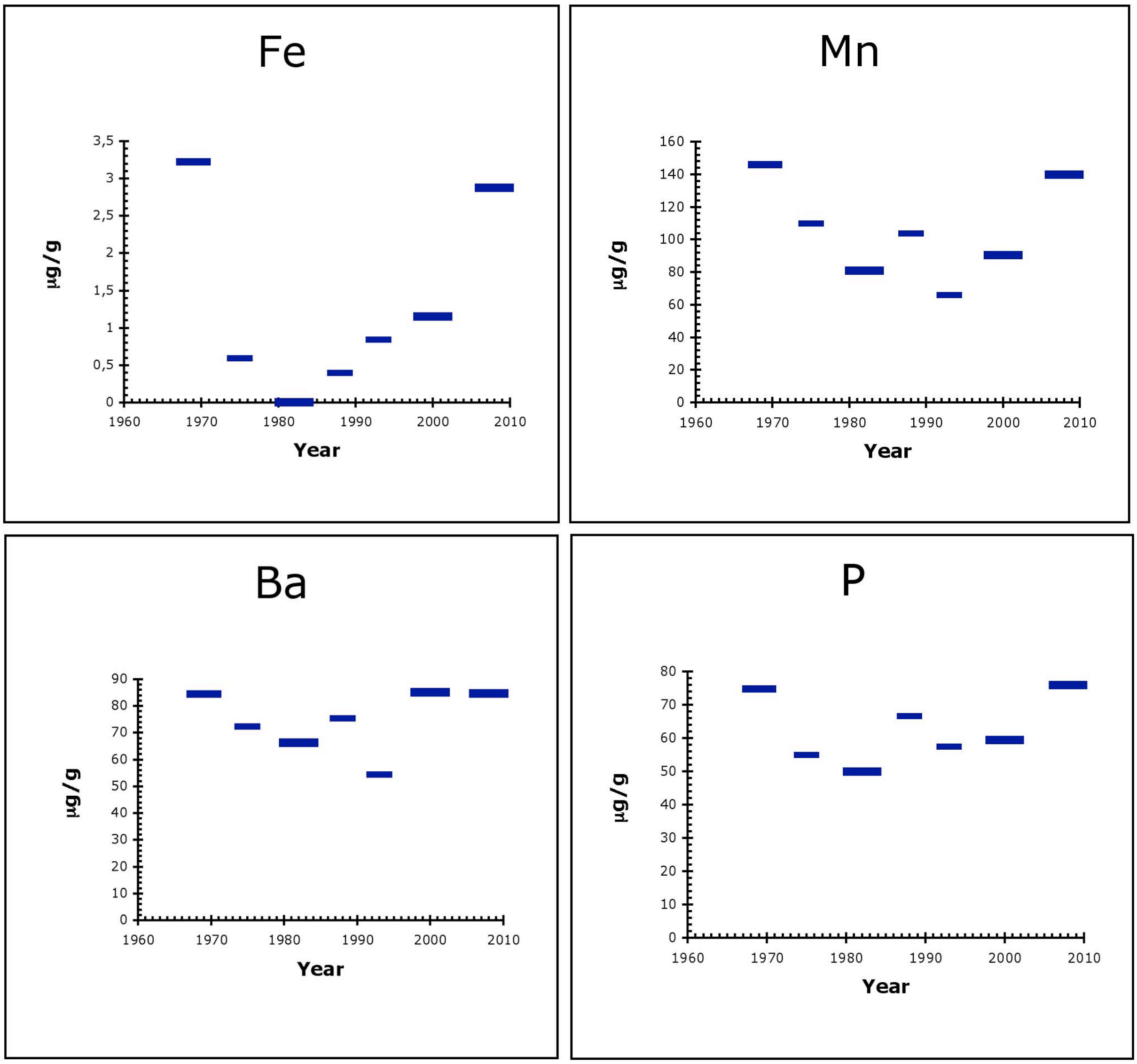


Figure 9. Diagrams with elemental distribution in mussel A for Fe, Mn, Ba and P.

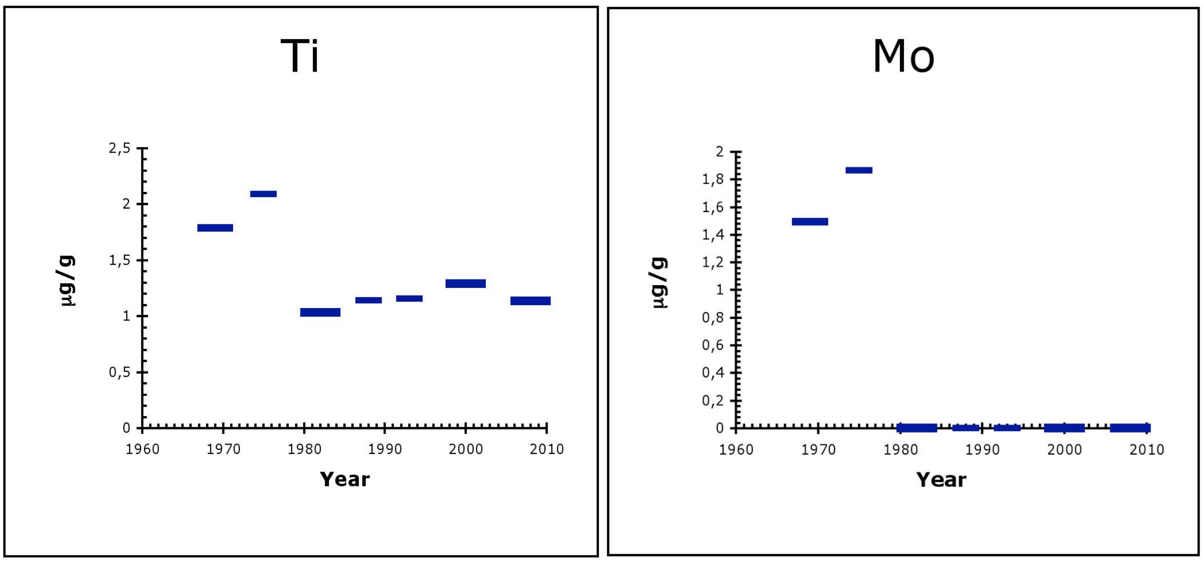


Figure 10. Diagrams with elemental distribution in mussel A for Ti and Mo.

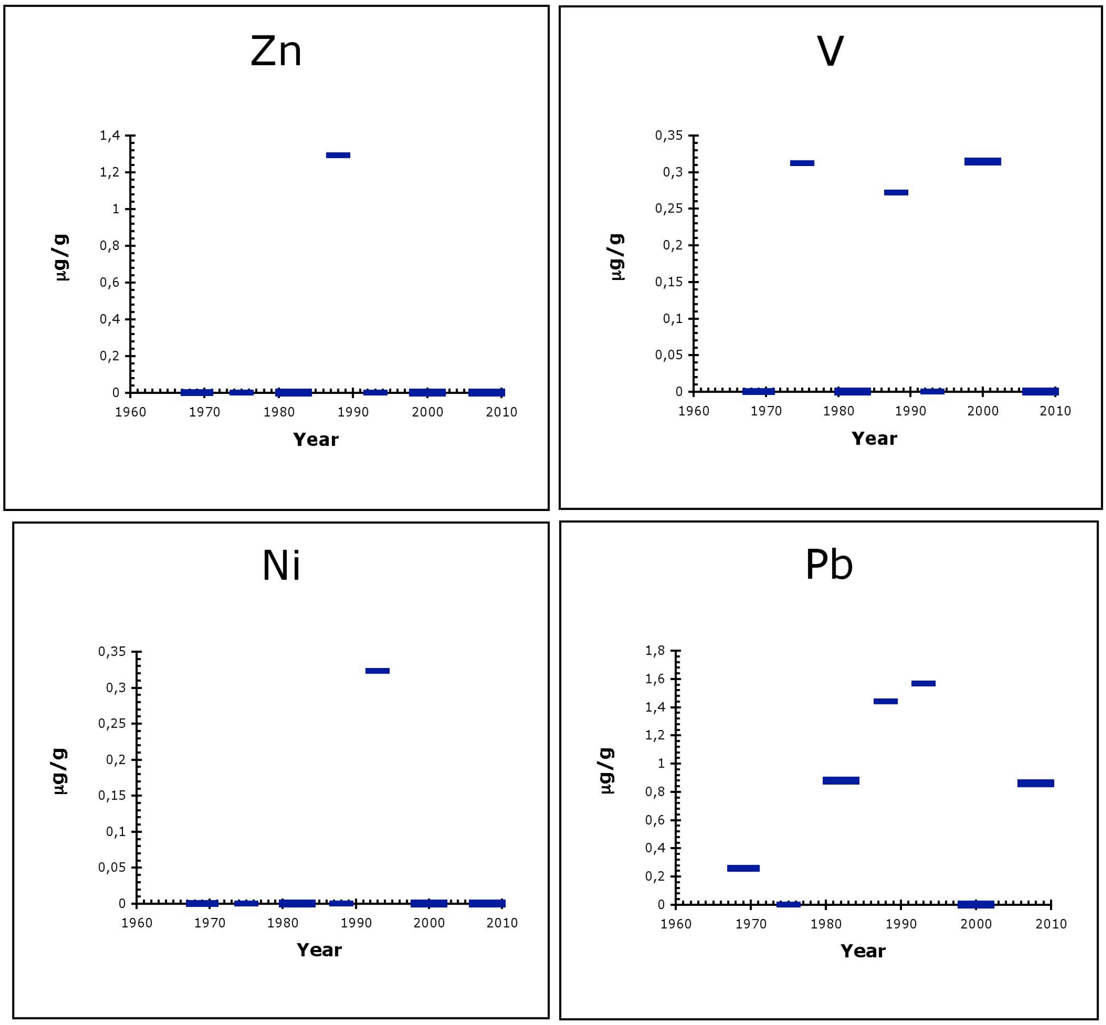


Figure 11. Diagrams with elemental distribution in mussel A for Zn, V, Ni and Pb.

## *Discussion*

The observed growth differences and the poor match in annual growth pattern between the two studied shells could be caused by different living conditions, as the shells were found at different locations, and/or by age difference. Previous studies showed that in younger bivalves the growth patterns could slightly differ in the same population (Mutvei *et al*. 1994, Dunca & Mutvei 2001). In order to elaborate more on the growth rate and annual growth patterns of this population at least 20 shells of different length are needed to be analysed from several locations on Haukåselva river. However, in both shells the growth rate was decreasing after 2008, which could indicate that environmental factors may be involved causing distress in both locations.

Variations in annual growth showed lower growth rate between 1961 and 1978 (except 1967) as well as, between 1996 and 2013. In the same period of time an increased amount of Fe, Mn, P, Ba and Sr was measured. The amount of Cu, Co and Pb, on the other hand, were lower during these periods of time. Variations in the content of metals has been shown to be dependent of the pH levels in water, where both lower and higher pH increase the solubility of the metals from the substrate and make available higher amount of metals in the water column, which explain the enrichment of the metals in the shells (Mutvei *et al*. 1994). However, Fe and Mn amounts are also subject to the redox effect in the water. Lower oxygen (O) levels increase the amount of Fe and Mn available in the water (Nyström *et al*. 1996). This may be the case for the bivalves from Haukåselva as Zn, Cu, Pb showed low levels while Mn and Fe showed higher levels during periods with low growth rate.

Heavy metals such as alumina (Al), arsenic (As), cadmium (Cd) and chromium (Cr) were not detected in the shell. These metals were present *in M. margaritifera* shells from other rivers in Norway as Hunnselva (Dunca *et al.* 2009), Ogna and Figga (Dunca *et al.* 2010) that had waters with lower pH. This strongly indicates that the shells in Haukåselva river were not subjected to low pH but rather to low O levels.

The amount of Si was rising in the shell, but the levels were much lower than in shells from other rivers such as Ogna and Figga from Norway (Dunca *et al.* 2009, 2010) and Imälven (Dunca 2009) and Lärjeån in Sweden. The amount of Si in the water, and ultimately in the shell, is substrate related (Dunca *et al.* 2009) and may indicate an increase of particle inflow from late 60's until present time.

Variations in Sr amount is known to be related to the density of winter lines and it is expected that higher levels of Sr are present in portions of lower growth rate in the shells (Nyström *et al.* 1996), which could be the case for shell A.

Levels of S and Sr were higher in Haukåselva shell in comparison with the shells from Hunnselva, Ogna and Figga rivers. The observed descending trend of S in Haukåselva shell could be ontogenetically as shown in the shells from Hunnselva river (Dunca *et al.* 2009) but the higher levels of S in general could be explained as an effect of organic decay processes at low O levels possibly due to eutrophication. Water quality studies of this river support these indications (Anders *et al.* 2013).

## *Conclusions*

Growth and chemical analyses on shells from Haukåselva river showed that lower growth rates during 1961-1978 (except 1967) and 1996-2013 correlated with higher levels of Mn and Fe. This together with the absence of heavy metals such as Al, As, Cd and Cr, and higher amounts of S indicated that the freshwater pearl mussels in Haukåselva were affected by low O levels in the water.

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## *Appendix*

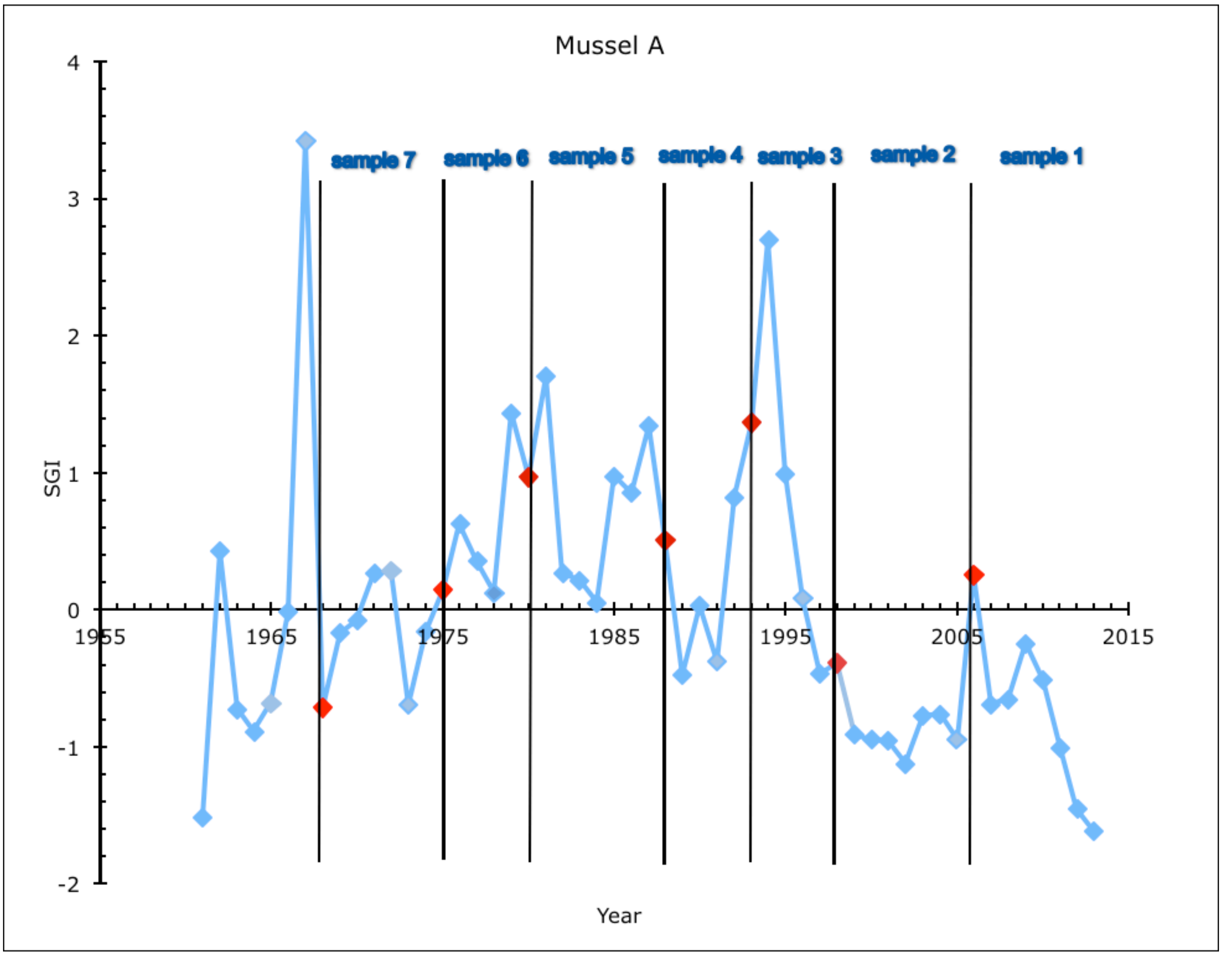


Figure 1. Diagram showing how the partition of the shell for chemical analyses related to the annual growth curve.

Table 1. Results of the chemical analyses made on *M. margaritifera* shell A from Haukåselva river, Norway. Values are expressed in µg/g (<DL = less than detection limit).

| Sample no | No of annual increments | Years | Al | As | Ba | Ca | Cd | Co | Cr | Cu | Fe | K | Li | Mg |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 8 | 2005-2013 | <DL | <DL | 84,53 | 495715 | <DL | 0,41 | <DL | 0,60 | 2,87 | 5,0 | 3,22 | 7,44 |
| 2 | 8 | 1997-2004 | <DL | <DL | 85,00 | 494010 | <DL | 0,47 | <DL | 1,17 | 1,15 | 3,3 | 3,15 | 6,80 |
| 3 | 5 | 1992-1996 | <DL | <DL | 54,38 | 500280 | <DL | 0,45 | <DL | 1,45 | 0,84 | 4,7 | 3,26 | 6,79 |
| 4 | 5 | 1987-1991 | <DL | <DL | 75,23 | 501160 | <DL | 0,40 | <DL | 1,19 | 0,39 | 6,4 | 3,35 | 5,43 |
| 5 | 8 | 1979-1986 | <DL | <DL | 66,23 | 529540 | <DL | 0,47 | <DL | 1,73 | <DL | 2,7 | 3,46 | 7,76 |
| 6 | 5 | 1974-1978 | <DL | <DL | 72,28 | 486585 | <DL | 0,45 | <DL | 1,64 | 0,59 | 7,3 | 3,19 | 8,57 |
| 7 | 7 | 1967-1973 | <DL | <DL | 84,43 | 500720 | <DL | 0,40 | <DL | 1,02 | 3,22 | 6,7 | 3,15 | 6,99 |

| Sample no | No of annual increments | Years | Mn | Mo | Na | Ni | P | Pb | S | Si | Sr | Ti | V | Zn |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 8 | 2005-2013 | 139,6 | <DL | 1205 | <DL | 75,8 | 0,86 | 177 | 2,29 | 548 | 1,1 | <DL | <DL |
| 2 | 8 | 1997-2004 | 90,4 | <DL | 1232 | <DL | 59,3 | <DL | 169 | 1,48 | 504 | 1,3 | 0,31 | <DL |
| 3 | 5 | 1992-1996 | 65,8 | <DL | 1403 | 0,323 | 57,3 | 1,57 | 170 | 1,68 | 377 | 1,2 | <DL | <DL |
| 4 | 5 | 1987-1991 | 103,6 | <DL | 1392 | <DL | 66,6 | 1,44 | 161 | 1,41 | 481 | 1,1 | 0,27 | 1,29 |
| 5 | 8 | 1979-1986 | 80,7 | <DL | 1459 | <DL | 49,8 | 0,88 | 210 | 1,00 | 506 | 1,0 | <DL | <DL |
| 6 | 5 | 1974-1978 | 109,5 | 1,86625 | 1377 | <DL | 54,8 | <DL | 233 | 0,86 | 533 | 2,1 | 0,31 | <DL |
| 7 | 7 | 1967-1973 | 145,8 | 1,49375 | 1411 | <DL | 74,7 | 0,26 | 273 | 0,68 | 662 | 1,8 | <DL | <DL |

Table 2. SGI values for the two analysed shells of *M. margaritifera* from Haukåselva river, Norway.

| Year | Naiad A | Naiad B |
|---|---|---|
| 2013 | -1,621 | |
| 2012 | -1,457 | -1,627 |
| 2011 | -1,013 | -0,384 |
| 2010 | -0,520 | -0,969 |
| 2009 | -0,249 | 0,138 |
| 2008 | -0,657 | -0,466 |
| 2007 | -0,693 | 1,113 |
| 2006 | 0,249 | 0,240 |
| 2005 | -0,951 | 0,222 |
| 2004 | -0,768 | 0,281 |
| 2003 | -0,779 | 1,765 |
| 2002 | -1,129 | 0,256 |
| 2001 | -0,959 | 1,063 |
| 2000 | -0,952 | 0,928 |
| 1999 | -0,915 | -0,157 |
| 1998 | -0,394 | -1,396 |
| 1997 | -0,473 | 0,141 |
| 1996 | 0,080 | 1,321 |
| 1995 | 0,989 | -0,852 |
| 1994 | 2,694 | -1,470 |
| 1993 | 1,359 | -1,884 |
| 1992 | 0,815 | 0,883 |
| 1991 | -0,379 | 0,665 |
| 1990 | 0,028 | 0,189 |
| 1989 | -0,481 | |
| 1988 | 0,504 | |
| 1987 | 1,335 | |
| 1986 | 0,848 | |
| 1985 | 0,972 | |
| 1984 | 0,047 | |
| 1983 | 0,210 | |
| 1982 | 0,261 | |
| 1981 | 1,700 | |
| 1980 | 0,960 | |
| 1979 | 1,431 | |
| 1978 | 0,119 | |
| 1977 | 0,350 | |
| 1976 | 0,619 | |
| 1975 | 0,138 | |
| 1974 | -0,165 | |
| 1973 | -0,696 | |
| 1972 | 0,281 | |
| 1971 | 0,260 | |
| 1970 | -0,085 | |
| 1969 | -0,176 | |
| 1968 | -0,718 | |
| 1967 | 3,415 | |
| 1966 | -0,018 | |
| 1965 | -0,686 | |
| 1964 | -0,892 | |
| 1963 | -0,731 | |
| 1962 | 0,423 | |
| 1961 | -1,519 | |

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