Methods for the study of environmental changes using the structural and chemical information in molluscan shells

by

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ABSTRACT

In order to compare growth rates between different individuals and between different populations, standardized growth curves were constructed. These curves showed that there is a positive correlation of growth rates between individuals and populations. In environments less affected by human activity the shell growth rates show a significant similarity in populations at a distance of 1,200 km. The growth rates were positively correlated with annual- and summer mean temperatures. Methodology of elemental analysis of molluscan shells, carried out at low time resolution by neutron activation (INAA) and other methods is described. Results on applications of these methods are summarized.

Méthodes d'étude des modifications de l'environnement basées sur les informations structurelles et chimiques fournies par les coquilles de mollusques

RÉSUMÉ

Dans le but de comparer les taux de croissance entre divers individus et différentes populations, des courbes de croissance standardisées de coquilles de mollusques ont été établies, qui révèlent une corrélation positive des taux de croissance entre individus et populations. Ainsi, dans des environnements

peu affectés par les activités humaines, les taux de croissance de la coquille sont similaires pour des populations distantes de 1 200 km, et sont corrélés avec les températures moyennes annuelles et estivales. L'analyse des éléments dans les coquilles, par activation neutronique, est décrite de même que d'autres méthodes pour lesquelles on donne les résultats de leur application.

INTRODUCTION

In the Global Change Report No. 6, a summary was given on methods used for studies of environmental history. These methods are limited in number and have several drawbacks. For example, in dendrochronology, measurements of tree-ring widths give us information about temperature, soil moisture, nutrients, etc, several thousand years back in time. However, owing to the porosity of the wood, elemental distribution in tree-rings is still incompletely examined. Besides, trees are mainly preserved in the temperate zone of the continents. Studies of coral skeletons may serve as another example. They elucidate climatological and sea level changes in semi-tropical and tropical seas. However, chemical information is difficult to obtain due to a large scale destruction of coral skeletons by boring organisms.

Several studies have been published on growth rates and chemistry in molluscan shells. However, few such studies have been performed for reconstruction of environmental history.

Molluscs have several advantages for environmental studies: (1) they occur globally in marine, brackish, and freshwater environments; (2) their solid, impermeable shells of calcium carbonate and glycoproteins retain elements and allow analyses of their distribution; (3) shells in several bivalves show distinct annual growth increments, and (4) many bivalves have a long life-span.

Three Swedish research institutes: Department of Nuclear Chemistry, Royal Institute of Technology, Stockholm; Department of Radiation Sciences, Uppsala University; and Swedish Museum of Natural History, Stockholm, have since 1987 carried out joint studies on structure, growth, and elemental distribution in bivalve shells from northern Europe. Joint studies have also been initiated with the Institutes of Limnology and Zoology in St Petersburg, Russia, and with the Institute of Zoology and Botany, Tartu, Estonia, in the last two-three years.

In the present paper, methods and some results of our studies on shell growth rates and elemental distribution are presented. Elemental analyses with Nuclear Microscopy (μ-PIXE), and studies of human influence on shell growth rates and structure are dealt with in separate papers (NYSTRÖM and DUNCA, 1994; MUTVEI *et al.*, 1994; DUNCA and MUTVEI, 1994; DUNCA *et al.* 1994).

Study area and material:

Shell structure and elemental distribution were studied in the following molluscs (Figure 1):

MARINE AND BRACKISH WATER BIVALVES: (1) The ocean quahog *Arctica islandica* (Figure 2A) from Belt Sea, Germany; Skagerrak, W Sweden; Atlantic at Bergen, Norway; and Kandalaksha Bay, White Sea, Russia. (2) *Macoma baltica* (Figure 2B) from S coast of Gulf of Finland, Baltic, comprising Estonia and St Petersburg area, Russia.

FRESHWATER BIVALVES: (1) The pearl mussel *Margaritifera margaritifera* (Figures 3A,B) from Swedish and N Russian rivers. (2) *Unio crassus* from Estonian rivers. (3) *Unio tumidus* and *U. pictorum* in Estonian and N Russian lakes.

LAND GASTROPODS: Several species of land gastropods (pulmonates) from S. Sweden (GÄRDENFORS *et al.*, 1988; GÄRDENFORS *et al.*, 1994).

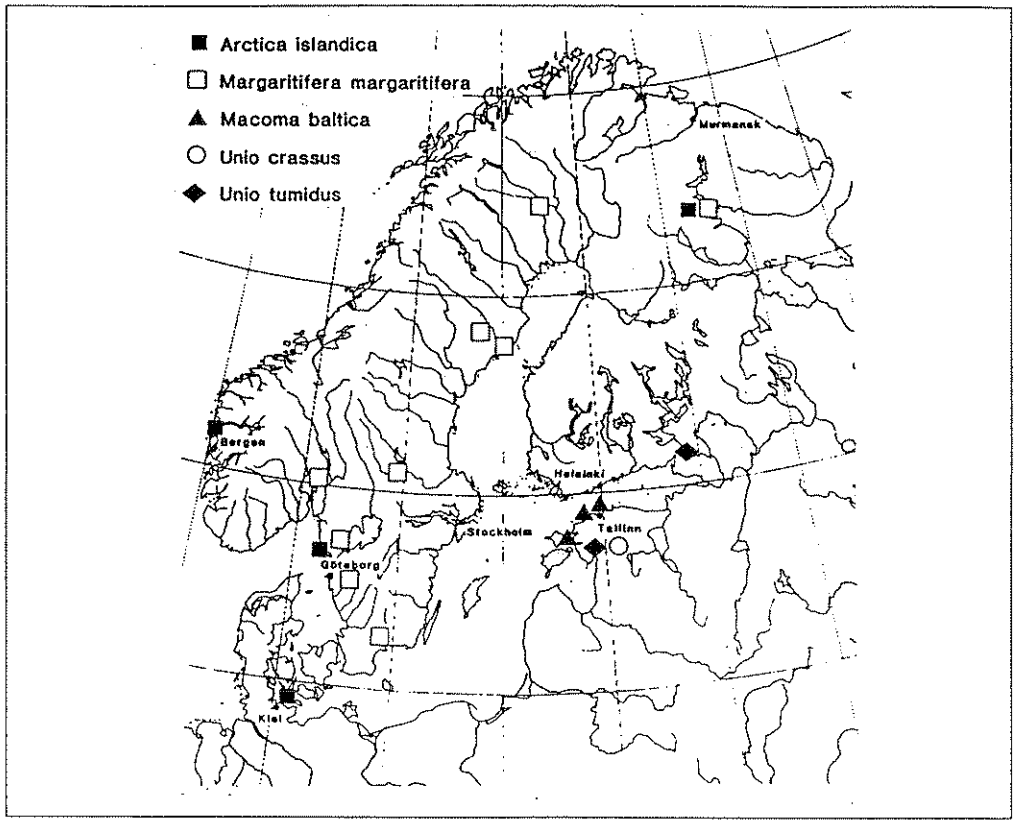

FIGURE 1 – Map of localities.

A. METHODS FOR STUDIES OF GROWTH RATES AND STRUCTURE IN MARINE, BRACKISH, AND FRESHWATER BIVALVE SHELLS

1. Measurements of widths of annual growth increments

Studies of shell growth rates have been carried out by several writers mainly on marine bivalves (e.g. OHNO, 1985; TANABE, 1987; CRISP, 1989; RICHARDSON and WALKER, 1991). In most studies, annual shell growth increments were counted on acetate peels from polished and etched cutting planes.

For our studies of annual growth increments in marine, brackish and freshwater bivalves, the following methods were used. Shell was cut with a diamond saw from umbonal region to ventral margin, and a thin section or a polished section was prepared from the cutting plane. The sections were treated for 30 min in a 1:1 mixture of 1% acetic acid and 25% glutaraldehyde to which various amounts of alcian blue were added. This treatment etched the calcium carbonate fraction of the shell, and simultaneously provided a fixation of the shell glycoproteins.

Widths of the annual growth increments (Figures 2 and 3) were measured in light microscope equipped with monitor, video camera and computer. Usually ten or more individuals of each population were measured.

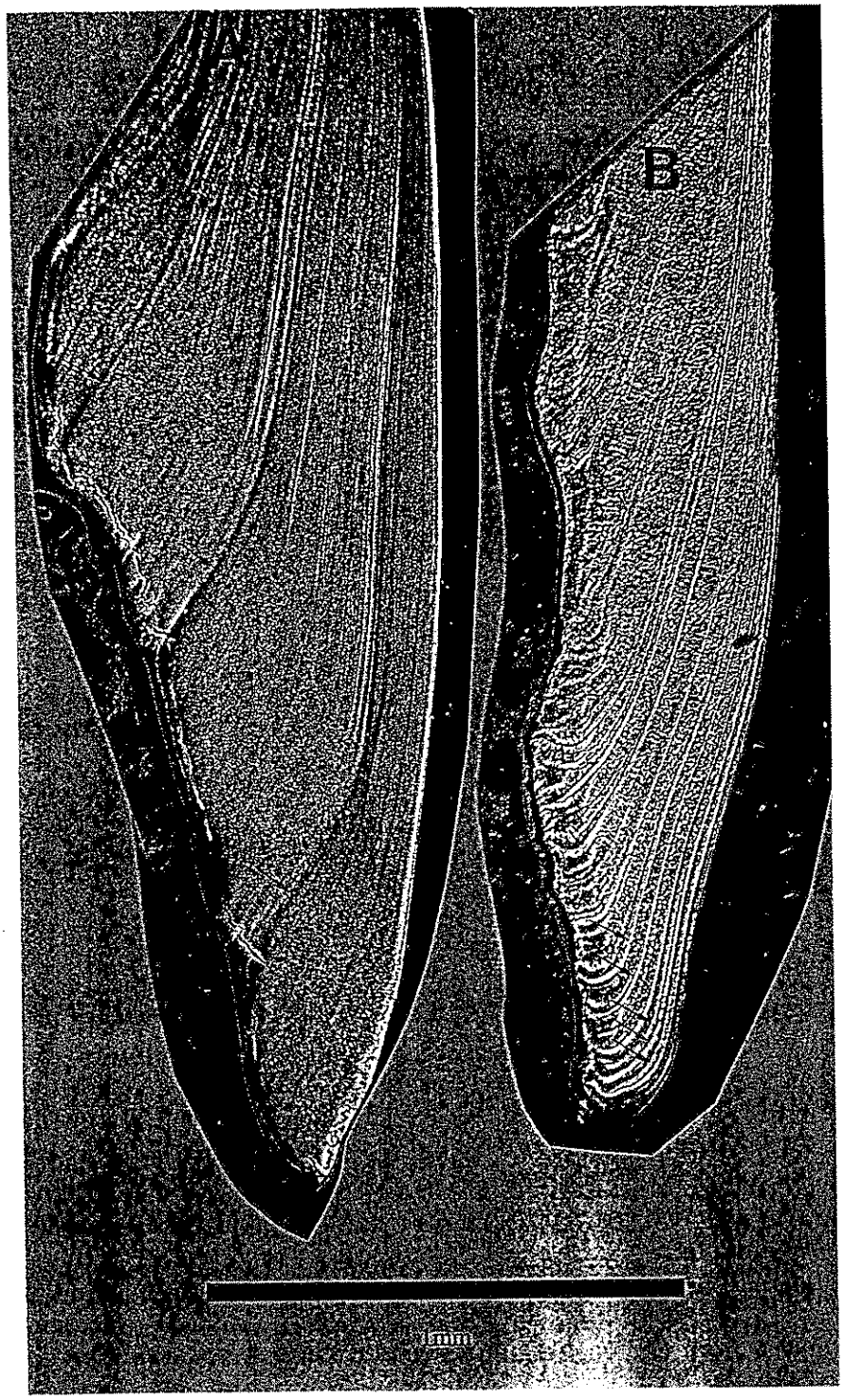

FIGURE 2 – Vertical polished and etched sections at shell margin, showing annual growth increments. A, *Arctica islandica*, Kockholmarna, Skagerrak, W Sweden; B, *Macoma baltica*, Askö, Baltic, Sweden. Arrows indicate winter lines.

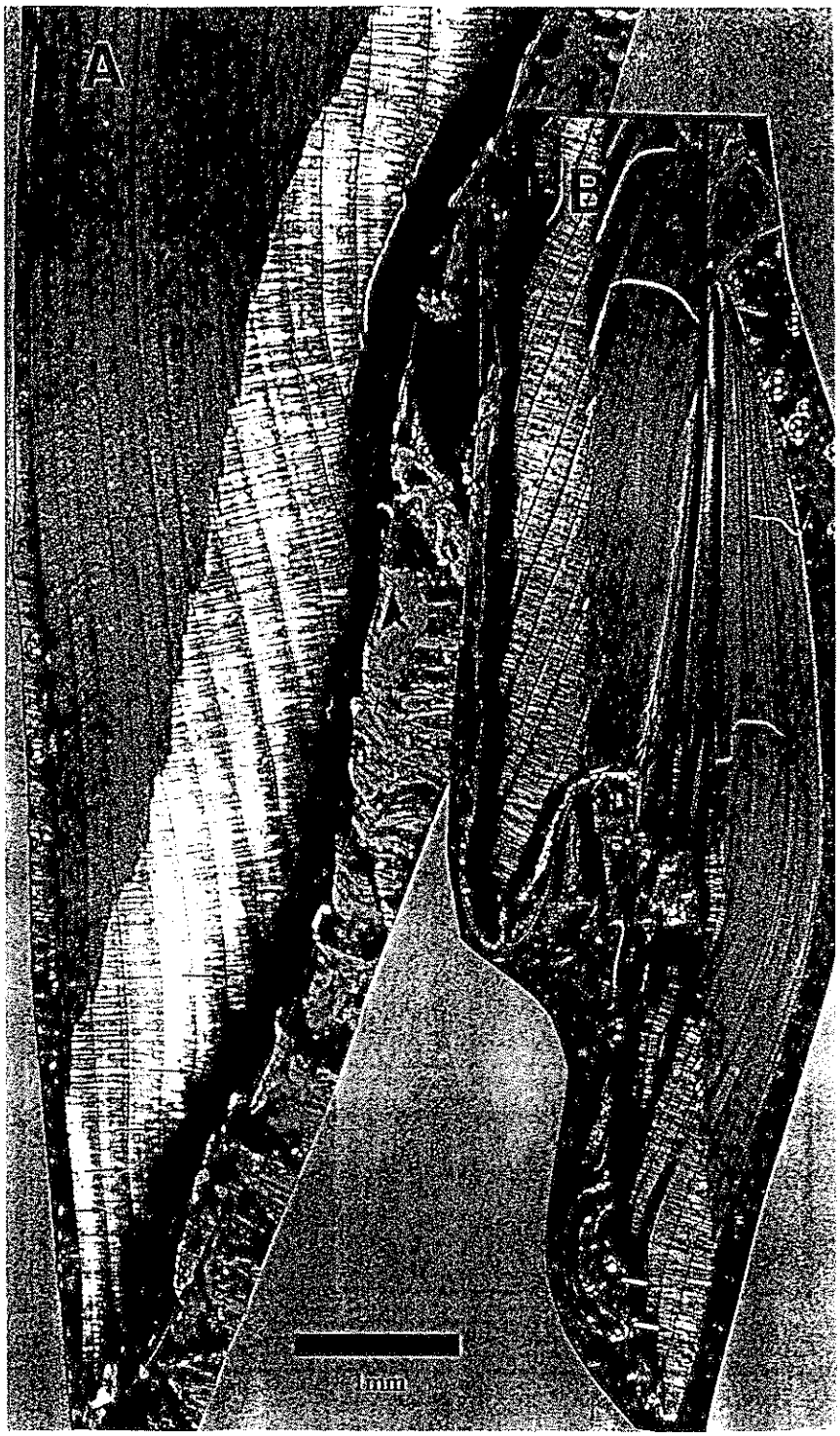

FIGURE 3 – *Margaritifera margaritifera*, vertical polished and etched sections at shell margin, showing annual growth increments. **A**, shell from the River Vattenån, N Sweden, unaffected by human activities; **B**, shell from the acidified River Slereboån, W Sweden. Arrows indicate winter lines.

When animals grow older, the marginal growth of the shell decreases. This causes a narrowing of the annual growth increments. In order to compare growth rates between young and old individuals, growth series from individual shells were standardized to eliminate ontogenetic differences. These series were then computed by averaging the standardized series. In this manner the generalized shell growth series of each population were determined (Figure 4).

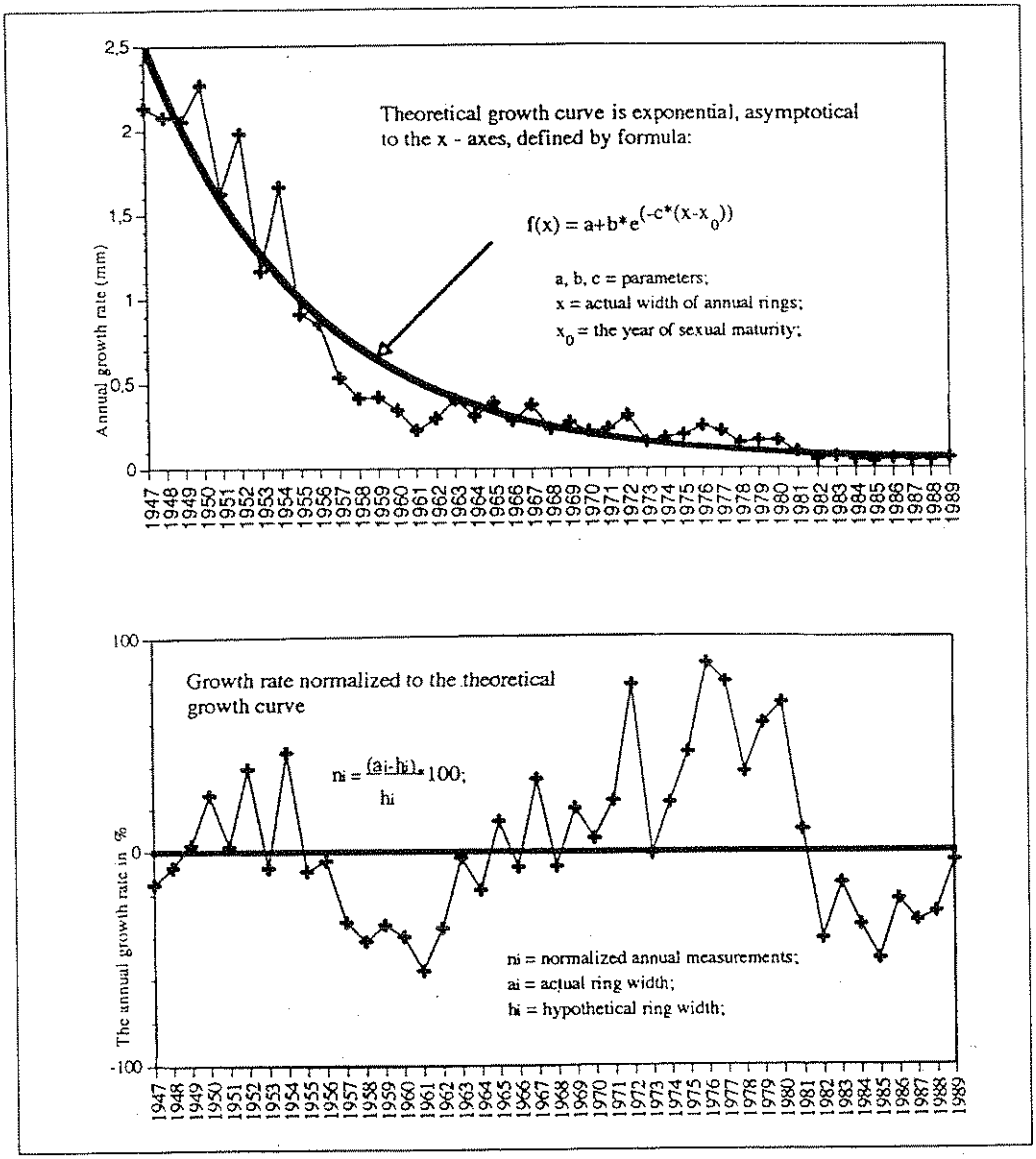

FIGURE 4 – Standardization of shell growth curve.

2. Variations of growth rates in a single population of freshwater *Margaritifera*

Individual variations of shell growth rates were studied in detail in thirty specimens from the River Vramsån (S Sweden, Scania, near the town Kristianstad), collected in 1989, 1991 and 1993. A standardized population

curve was constructed, based on growth rates in 25 shells which represent about 85% of the total number of shells from this locality (Figure 5). The population curve shows four peaks of the shell growth rates between 1930 and 1992.

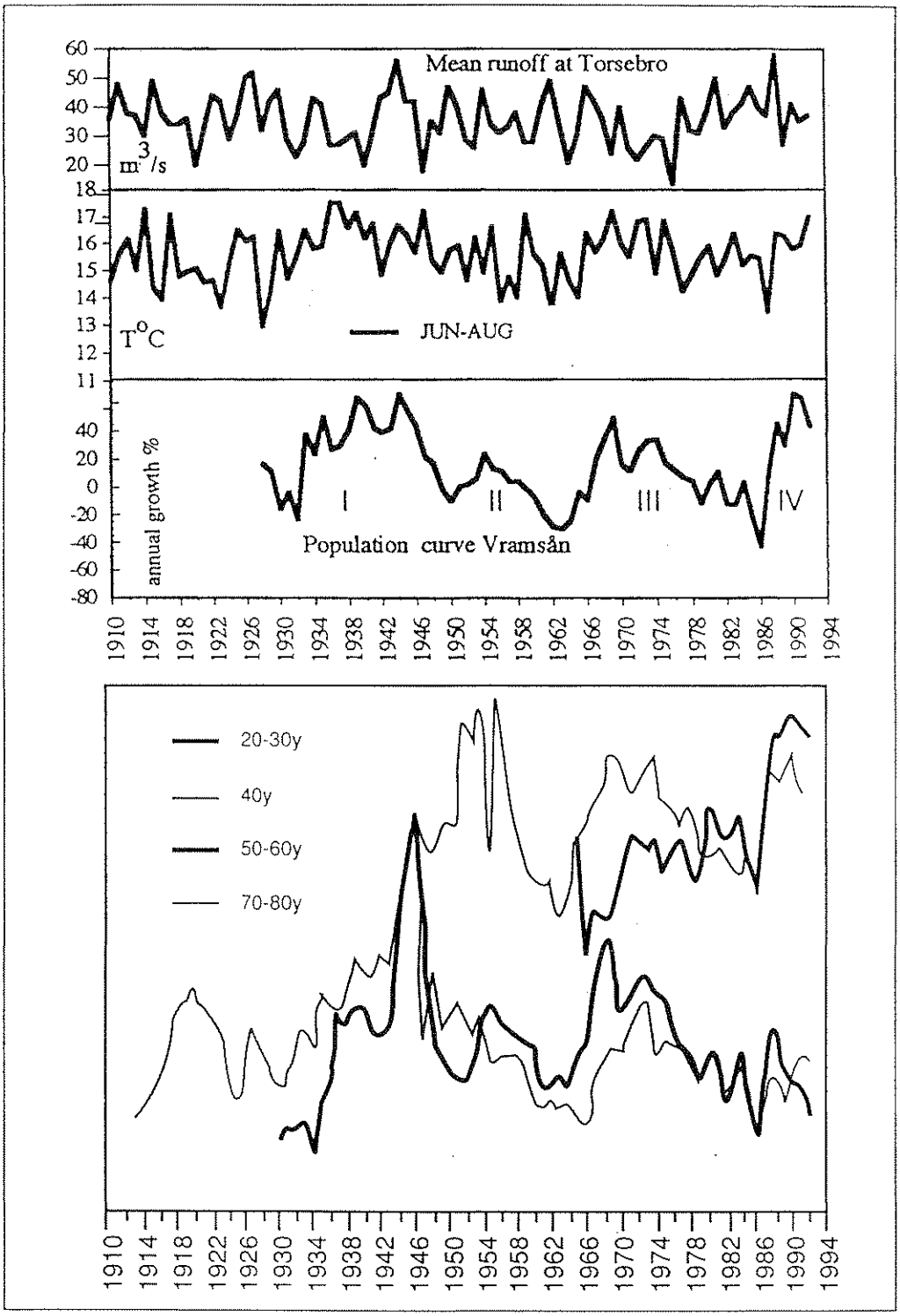

FIGURE 5 – *Margaritifera margaritifera*. Standardized population growth curve from the River Vramsån, S Sweden; growth curves of different age-groups; curves of mean summer temperatures and mean annual runoff.

The first peak (I) occurred between 1934 and 1946; the second, somewhat lower peak (II), between 1954 and 1958; the third peak (III) between 1968 and 1982; and the fourth peak (IV) between 1988 and 1992. A positive correlation was found between the peaks and periods of high average summer temperatures (Figure 5). The shells were arranged into four age-groups: 20-30 years, 40 years, 50-60 years, and 70-80 years. For each age-group a standardized growth curve was computed (Figure 5). Shells of the age-groups of 20-30 years and 40 years have three distinct growth maxima (II, III, IV) which correspond to those in the generalized population curve. The age-groups of 50-60 years and 70-80 years clearly show the three first growth maxima (I, II, III), but during the fourth maximum, between 1988-1992 (IV), the growth rate shows small fluctuations but not a distinct peak. Consequently, *Margaritifera* shells which are best adapted for environmental studies are between 20 and 60 years old.

Growth rates were also studied in shells collected between 1844-1848 by A. Malm (A. Malm's collection at the Natural History Museum, Göteborg). The standardized growth curves of four shells show a clear positive correlation with each other. For most years, the high shell growth rates are positively correlated with the high mean temperatures from April to October (Figure 6).

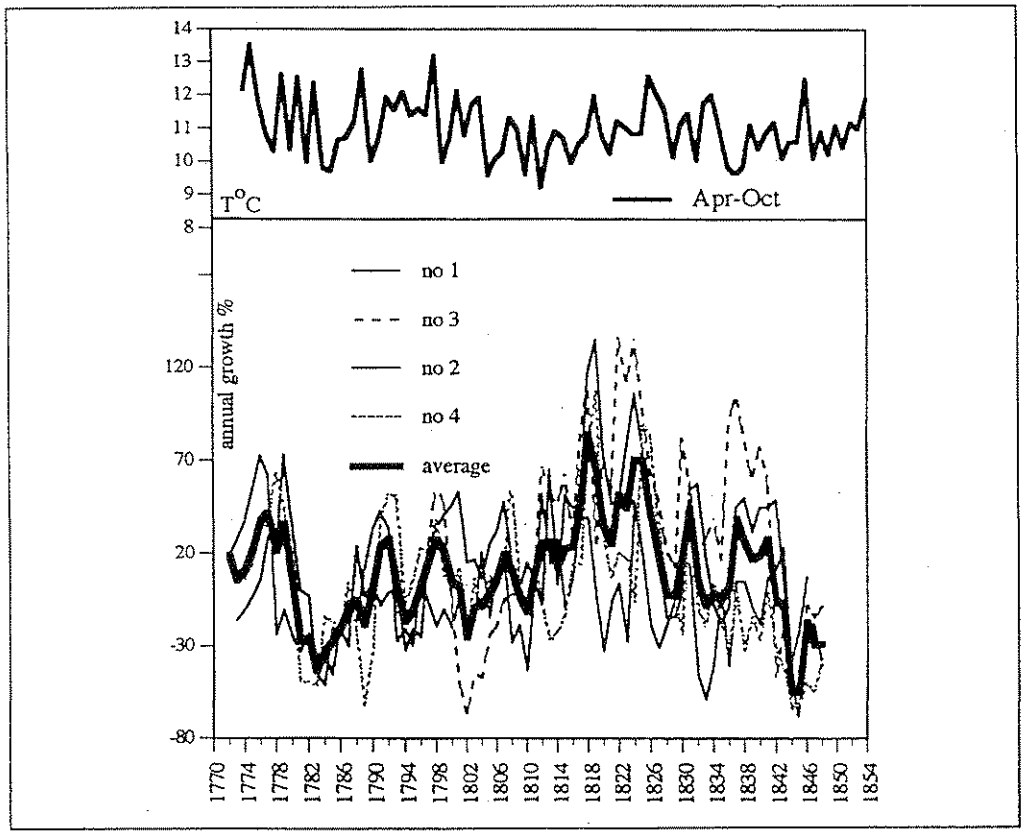

FIGURE 6 – *Margaritifera margaritifera*. Standardized population growth curve from the River Vramsån, based on shells collected between 1844 and 1848; for comparison, a curve of mean summer temperatures during the same period.

3. Variations of growth rates in different *Margaritifera* populations from climatologically different regions

Shell growth rates in three populations of *Margaritifera* were selected and compared with each other (Figure 7). These populations were only slightly

influenced by human activities, such as acidification, liming, industrial and agricultural pollution. The southern population was from the town Kristianstad, S. Sweden, at about 56° N lat., and the northern population at Jokkmokk, above the Polar Circle, at about 66° 5′ N lat. Standardized growth curves of the three populations show a distinct positive correlation with each other, despite the long distance between them.

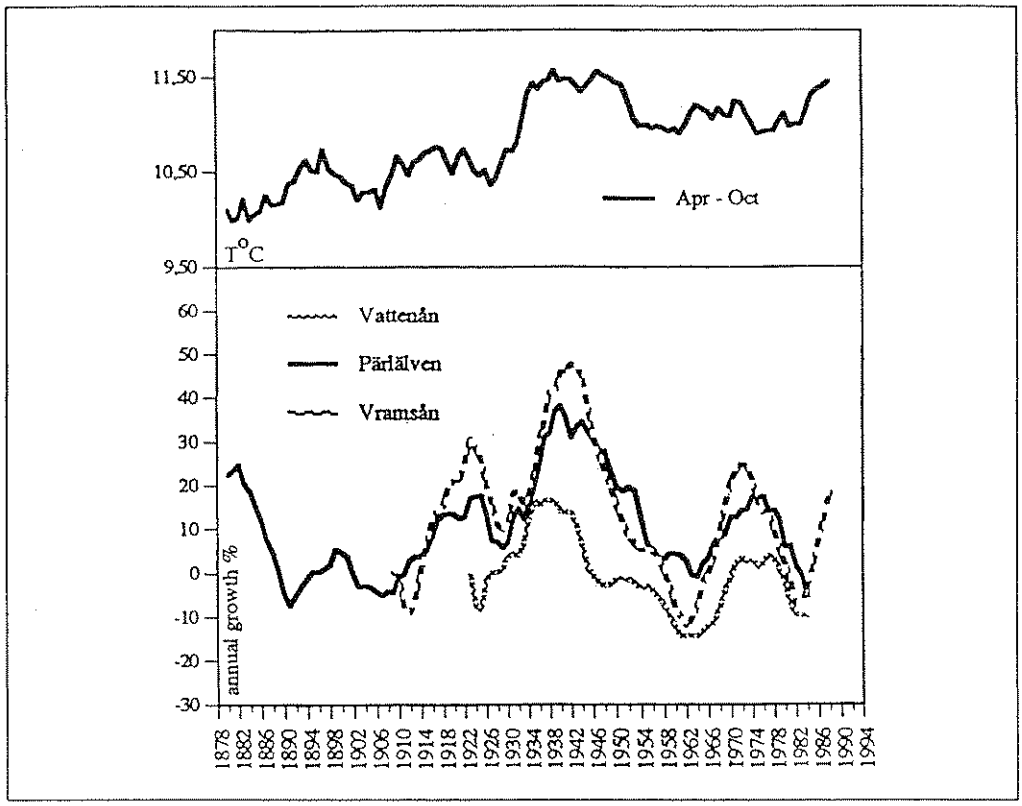

FIGURE 7 – *Margaritifera margaritifera*. Standardized shell growth curves from three populations: the River Vramsån, S Sweden, and the River Vattenån and Pärlälven, N Sweden; for comparison, a curve of mean summer temperatures.

The standardized growth curves were then compared with mean annual temperatures, mean summer temperatures, and mean annual precipitation. A significant positive correlation exists between annual shell growth rates and mean annual and summer temperatures, whereas there is a negative correlation between annual precipitation and growth rates.

BAUER (1992) studied growth rates and life-spans of *Margaritifera margaritifera* along the north-south distribution of this species, from N Spain to the Arctic. He noted that this bivalve has a high growth rate but short lifespan in the south, whereas northwards the growth rate decreases and the life-span increases. BAUER explained the high growth rates in the south as a result of higher metabolic activity due to higher annual temperatures. There also exists a positive correlation between higher water temperatures and growth rates in intertidal marine bivalves from the temperate zone (e.g. RICHARDSON *et al.*, 1981).

Thus, the results of our studies confirm the positive correlation between the temperature and shell growth rate. However, high temperature not only

affects the metabolic activity of the animal but it also makes possible a longer feeding season, better availability of food, etc. As stressed by RICHARDSON *et al.* (1990, p. 264) '*a major difficulty in correlating growth rate with environmental factors is that many of these factors co-vary; consequently, although a correlation may be apparent, the relationship may not necessarily be causal*'.

The optimal environment for freshwater pearl mussels is still insufficiently known. These mussels usually inhabit rivers with running cool water, poor in nutrients; pH is around 6.5, and eutrophication is low. However, as shown by MUTVEI *et al.* (1994), adult animals can survive at low pH levels. Better availability of food increases the shell growth rate considerably.

4. Studies of shell microgrowth pattern and its relationship to μ-PIXE analyses

As reported separately (DUNCA and MUTVEI, 1994), a distinct microgrowth pattern is seen in shells of freshwater unionids, notably in *Margaritifera* (Figure 8).

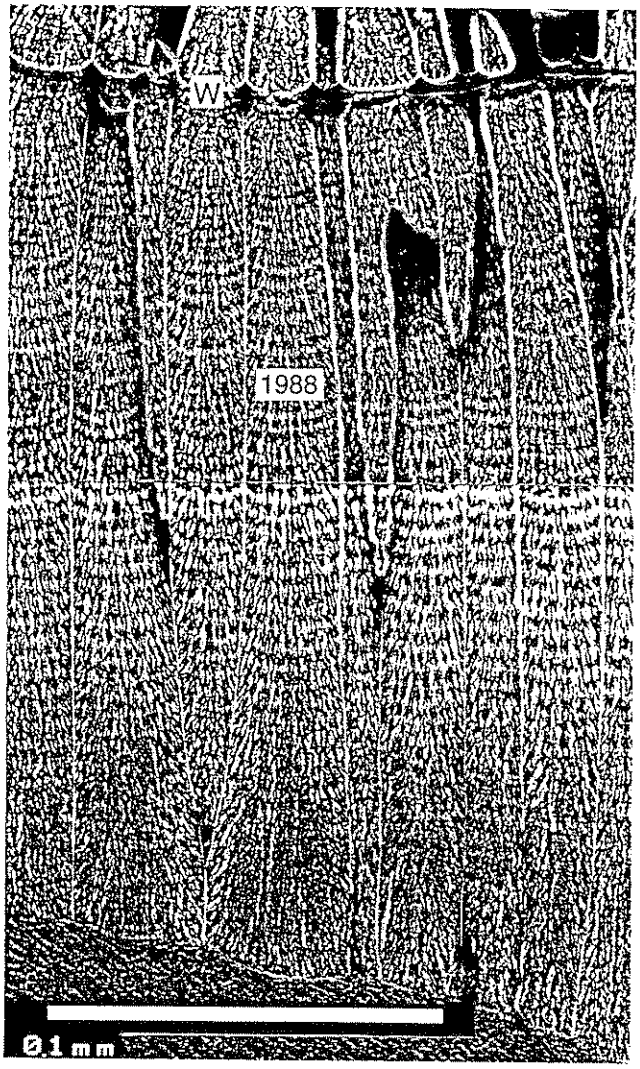

FIGURE 8 – *Margaritifera margaritifera*. Vertical polished and etched shell section to show microgrowth lamellae deposited during the growth season in 1988; the River Stommebäcken, W Sweden.

This pattern is similar to that in marine bivalves. However, several periods of increase and decrease of the widths of microgrowth lamellae during growing season has been noted. These periods mark rapid and slow growth, respectively (Figure 8). Studies of this kind are of great importance for evaluation of results of elemental analyses, carried out particularly with the Nuclear Microscope (μ-PIXE) (NYSTRÖM and DUNCA, 1994). Locations of points analyzed by μ-PIXE can be recognized as distinct pits on the polished shell sections, and these pits can be correlated with the shell microgrowth pattern (Figure 9).

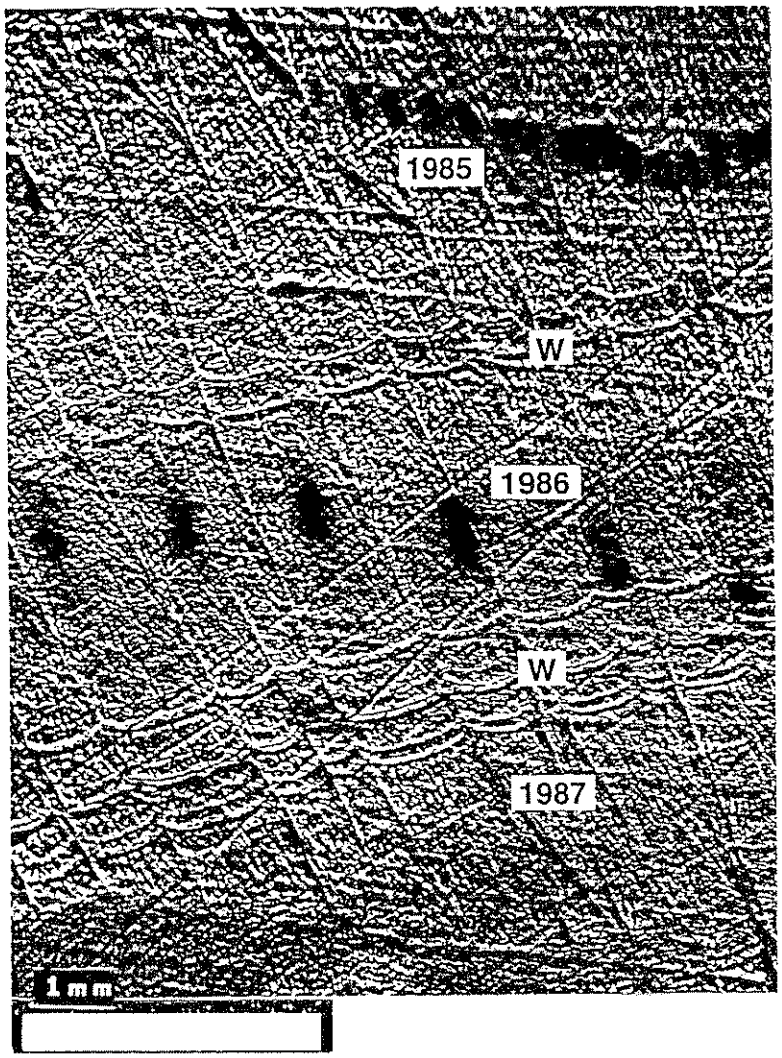

FIGURE 9 – *Margaritifera margaritifera*. Vertical polished and etched shell section to show sites of μ-PIXE analyses (dark spots) in 1985 and 1986; the River Vramsån, S Sweden.

B. METHODS FOR STUDIES OF ELEMENTAL CONCENTRATIONS IN MARINE, BRACKISH, AND FRESHWATER BIVALVE SHELLS

1. Elemental analysis

Several methods have been used for elemental analyses of molluscan shells, in addition to Nuclear Microscopy (μ-PIXE) (NYSTRÖM and DUNCA, 1994).

(1) Neutron activation is the basic analytical method. It begins with the instrumental, non-destructive mode (INAA), and is often followed by a radiochemical procedure for P. By scanning the gamma emission spectra from a semiconductor Ge detector, many elements are recorded. The sensitivity is often high, being ng/g.

Some nuclides, produced by so-called threshold nuclear reactions, have been detected. One is the 312 days half-life Mn-54 formed by a (n,2n)-reaction in Mn by fast reactor neutrons. As this nuclide is simultaneously formed in Fe by a (n,p)-reaction, it is necessary to correct it, using data from a Fe standard. If the Fe content in the sample is too high, this method cannot be used.

Another example is the detection of Ni by (n,p)-produced Co-58.

(2) Table I gives an account of gamma lines. Because P-32, formed from P, has no gamma radiation, a radiochemical method was used (RIESEL, 1990). Samples were dissolved 60-70 days after neutron irradiation when all gamma activities had been recorded.

The beta particle produced Cerenkov radiation pulses in the aqueous sample were detected. Corrections were made for P-32, formed from S, and for pulses from Sr-85 and Sr-89. Brackish water *Macoma* shells have a high Sr content (2500 µg/g) and Sr correction can amount to 50-60%.

TABLE I

Instrumental neutron activation analysis (INAA).
Several gamma emission lines were listed in CARELL et al. 1987.
The following additional gamma emission lines were used in the present paper.

Element	Nuclide	E(gamma) keV
Samarium	Sm-153	103.2
Terbium	Tb-160	879.4
Thorium	Th(Pa-233)	311.9
Uranium	U(Np-239)	228.2
Manganese	Mn-54[x]	834.8
Nickel	Ni(Co-58)[xx]	810.8
Antimony	Sb-124	1691.1
Tellurium	Te(I-131)	364.5

[x] – Produced by $^{55}Mn(n,2n)^{54}Mn$

[xx] – Produced by $^{58}Ni(n,p)^{58}Co$

(3) Chemical activation analysis (CNAA) were applied in certain cases when greater selectivity and sensitivity were required. An example is the removal of Na by sorbent HAP and a detection of K in *Arctica islandica* shells (WESTERMARK *et al.*, 1994).

For detection of low concentrations of Hg and Cd (ng/g) in molluscan shells, a chemical extraction procedure has been worked out, using tetrahexyl-ammonium jodide.

(4) For environmental studies, total N and S levels in shells are important. Analysis of these elements were carried out in a laboratory (Mikro Kemi Co., Uppsala), using special methods (KIRSTEN and HESSELIUS, 1983; KIRSTEN and NORDENMARK, 1987). For N analysis, samples of 10-25 mg were needed if N content was in the range of 100-1000 µg/g.The method was based on gas chrômatography. For S analysis, a sample size of 20-40 mg was required. This

element was detected by an EC-detector after burning the sample at high temperature and purifying the resulting gas.

(5) ICP-MS method, applied at the Swedish Institute for Metal Research, Stockholm (DATE and GRAY, 1989), was used for analysis of the elements: Mg, Cu, Mn, Ni, Pb and Al. Sample requirement is 50-100 mg. The sensitivity of this method was at µg/g level.

2. Analytical precision

Analytical precision was tested in a *Margaritifera* shell sample from the River Vramsån, S Sweden. The sample was crushed, sieved, homogenized and analyzed mainly by INAA and CNAA. Small amounts of contaminations were detected which resulted in slightly elevated levels of Fe, Co and Zn. The results are summarized in Table II. The variation coefficient was choosen as the measure of the precision of integral procedure. The precision was best for the following elements: P (± 1.5%, n=7), Ca (± 2.1%, n=9) and Na (± 2.8, n=8). Higher values were obtained for elements: La, Ce, Mn (10-30%), and Zn, Ag, Au (50-80%), for the latter elements probably as the 'nugget effect'.

Ideal CRM samples for accuracy tests in molluscan shells were not found. For P, milk powder has been used (BCR no. 63, Commission of European Communities, Bureau of References, Bruxelles) (Figure 10).

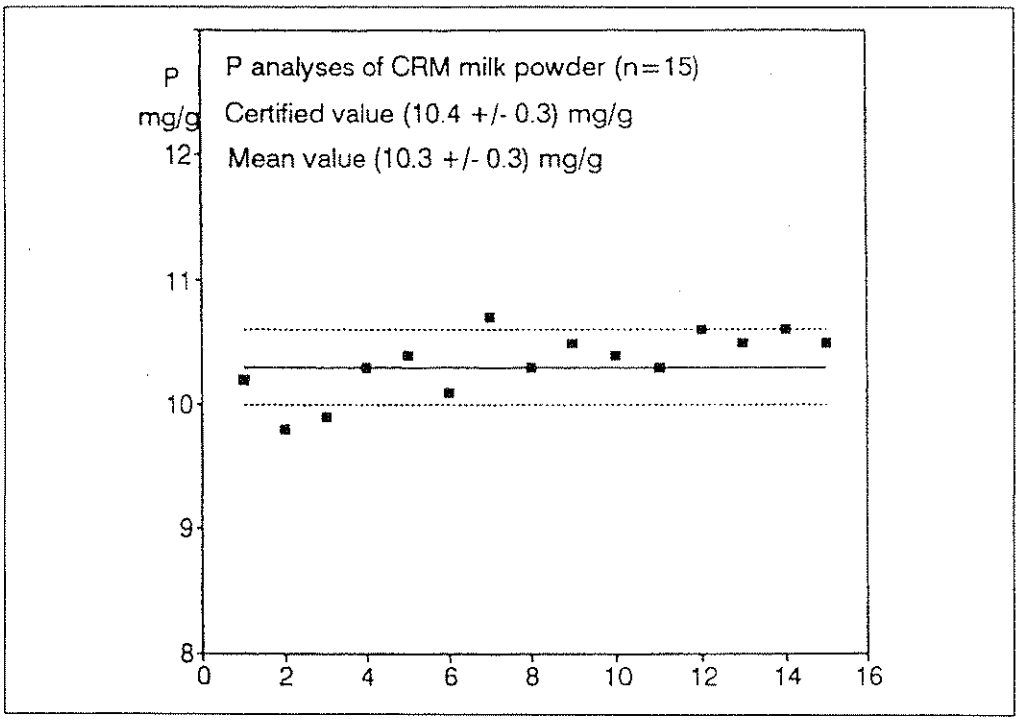

FIGURE 10 – Precision and accuracy of P analysis.

3. Elemental concentrations in soft tissues and shells

Molluscan soft tissues (homogenized, moisture checked) were studied in a few cases by techniques described above. It was observed that they contain higher levels of K, Fe, Hg and radioactive Cs-137 than the shells.

Periostracum, which covers the outer shell surface, has been analyzed for

organic compounds (HUTCHINSON *et al.*, 1993) and for Hg (Ott R., 1992, Personal communication). We carried out pilot studies of *Margaritifera* periostracum (rivers Lyckeboån and Vramsån, S Sweden) and compared the elemental concentrations with those in the shell. Due to high Fe content, Mn concentration could not be determined from Mn-54 by INAA. Therefore, ICP-MS method was used. Because P activity was covered by other beta radiations, Riesel's Cerenkov method (see above) could not be used. We found that Ca level is 1.3-1.9% in the periostracum as compared with 36-40% in the shell. The striking feature, however, was much higher levels of numerous elements in periostracum, several belonging to PS group no 3: Sc, La, Ce and other lanthanides ; U, Th, Fe, Co, Zn, Ta, Hf, Rb and Cs. Hg was found at levels 300-500 ng/g as compared with about one ng/g in shells. Also Br concentration in periostracum was high, 40-70 µg/g, but only 0.1-0.2 µg/g in shells. The high elemental levels suggest elemental binding to glycoproteins in periostracum.

TABLE II.

Lab. standard precision study (series VX).
n = number of analyses.

Element		Average	+/- prec.	Var. coeff. %	n	Pulse SD %
Ca	%	38.62	0.82	2.13	9	0.2
Na	µg/g	1848	52	2.79	8	0.3
Rb	ng/g	35	14	39	5	20
Cs	ng/g	1.32	0.30	22.4	8	10
Sr	µg/g	335	17.7	5.3	8	0.2
Ba	µg/g	49.4	2.9	5.8	8	3
Sc	ng/g	3.0	0.4	13.5	8	1
La	ng/g	21.0	3.7	17.8	6	15
Ce	ng/g	120.0	8.2	6.8	7	2
Eu	ng/g	0.57	0.09	15.5	8	20
Tb	ng/g	1.06	0.40	38	8	15
Th	ng/g	10.1	1.6	15.6	8	5
U	ng/g	29.6	3.7	12.6	7	23
Ta	ng/g	2.79	0.39	14	7	7
Cr	ng/g	1.50	0.12	8.1	8	0.5
Mn	µg/g	164.3	43.2	26.3	7	35
Fe	µg/g	17.1	2.3	13.6	8	2
Co	ng/g	23.6	1.3	5.5	8	1
Ag	ng/g	18.0	135	74.4	8	6
Au	ng/g	1.7	0.8	47.6	8	6
Zn	µg/g	1.59	1.22	76.9	8	1
Hg	ng/g	2.3	0.6	24.8	3	27
N	µg/g	2100			2	
P	µg/g	63.6	0.98	1.54	7	
Sb	ng/g	7.1	3.6	51.6	8	7
S	µg/g	113			2	
Se	ng/g	14.0	0.93	6.6	8	12
Br	µg/g	0.336	0.014	4.2	8	6

It is still uncertain whether elemental concentrations in periostracum can be used for studies of time related environmental changes or if they only mirror the present steady state of sorption-desorption phenomena.

Shells of several thousand years old subfossils of *Arctica islandica* from the North Sea were studied for their elemental concentrations (WESTERMARK

et al., 1994). Ca, Sr and Ba were present at similar levels as contemporary shells. Lanthanides, U, Th, Ta, Hf and Br had two magnitudes higher concentrations in subfossil shells, probably due to intrusion and sorption after death. These studies elucidate palaeo-ecological applications of shell analysis.

4. Differences in elemental concentrations in structurally different shell layers

Freshwater unionid shells consist of an outer prismatic layer (PRI) and an inner nacreous layer (NAC). As shown by μ-PIXE studies, these layers differ somewhat in elemental concentrations (NYSTRÖM and DUNCA, 1994).

In our INAA studies on *Margaritifera*, shell samples usually contained equal amounts of PRI and NAC. Larger deviations from this ratio may introduce analytical errors. A sensivity analysis indicated that for most elements a systematic error of less than 5% may be expected when the PRI and NAC ratio is 60:40 per cent.

Both shell layers in unionids are composed of a $CaCO_3$ fraction and a glycoprotein fraction (GP). Different elements in the shell are fixed to different fractions (POULICEK, 1985). In order to study this fixation, the following methods were applied on neutron-irradiated shell samples (Forberg and Mutvei, unpublished). (1) Shell samples were dissolved in a mixture of diluted acetic acid (about 1%) and 25% glutaraldehyde. The insoluble fraction of GP and the dissolved $CaCO_3$ were separated, and each was analyzed for gamma radioactivity. (2) Shell samples were treated with concentrated sodium hypochlorite solution which dissolved GP and left the solid phase of $CaCO_3$ behind. Dissolved GP and $CaCO_3$ were then analyzed for gamma radioactivity.

Lanthanides, Na, Sr and Ba followed the $CaCO_3$ fraction in the shell to a large extent. Br and Ag followed the GP fraction. Third group of elements: As, Au, Cr, Fe, Sb, Se, Sn, and probably also Co and Zn, were equally fixed to the two shell fractions.

With increasing knowledge of elemental concentration in shells, mass balance can be calculated, based on Ca level. e. g. 39.3% in *Macoma* shells. To this element, dominating cation elements are added in carbonates, such as Sr, Na and Mn. GP amount is estimated from the total N-level.

5. Variability

Different individuals of bivalves, belonging to one and same population, may differ with regard to uptake of elements from the water, and to deposit these elements in varying quantities into the shell. Malmberg (1992, 1993, personal communication) pointed out that these differences set a limit for how far we can interpret environmental changes. He suggested that variation coefficient for each element should be calculated on the basis of its concentrations in five or more shells from the same locality, collected at the same time.

These requirements were fullfilled in our studies on brackish water *Macoma* shells from Gulf of Finland, Baltic (SEIRE *et al.*, 1994). The variation coefficients in *Macoma* shells are as follows: 2-6% for Ca and Na; 10-20% for Sr and Ba; 30-50% for lanthanides, N, P, Mn and several other elements; about 100% for Au and Ag, the latter probably caused by the 'nugget' effect. No clear relationship has been found so far between the variation coefficients and the elemental concentrations in the water. One example of variability in freshwater bivalves could be upwelling of old groundwater (RODHE, 1987, 1989).

6. Dose-response

Elemental composition in aquatic molluscan shells is often assumed to mirror that in the water. In the simplest case, this relation is thought to be linear, and concentration factor water/shell can be calculated from concentration data.

Studies in this field are few. STURESSON (1976, 1978, 1984) carried out experiments in submerged aquaria with *Mytilus* shells, and found that uptake of Cu, Cd and Pb has a linear relationship. SIMKISS (1983) pointed out that increased elemental deposition at high loads causes a break down of the ion regulating mechanism of the animal. A linear relationship between Sr-90 in water and in molluscan shells was found at the Tschernobyl reactor accident site (FRANTSEVITJ *et al.*, 1993; see below).

In our studies, dose-response in aquatic molluscan shells was elucidated in the following cases. In several localities of Gulf of Finland, Baltic, P content in the water had an approximately linear relation with that in *Macoma* shells (SEIRE *et al.*, 1994). A study in the River Vramsån, S Sweden, gave a similar result. In this river, county authorities have measured P concentrations four times per year during a period of 10-12 years. Uptake of P in *Margaritifera* shells has here a linear relationship to P concentrations in the river (WESTERMARK *et al.*, 1994). Indirect evidence was found for N, S and several metals.

7. Principles of sampling for elemental analysis

Figure 11 elucidates schematically sampling procedure in shells of short-lived bivalves (Case 1; *Macoma*, several unionids) and long-lived bivalves (Cases 2a, 2b); *Arctica*, *Margaritifera*), including both museum and recently collected specimens.

In statistical analysis we followed the principles by BOX *et al.* (1978) and BIGNERT *et al.* (1993). Number of shell samples depended of the age of the bivalve. In *Margaritifera*, which attains an age of 100-200 years, the number of consecutive samples (time-windows) was at least six or more from each shell, whereas in *Macoma*, which has an age of 10-20 years, each shell was taken as one sample.

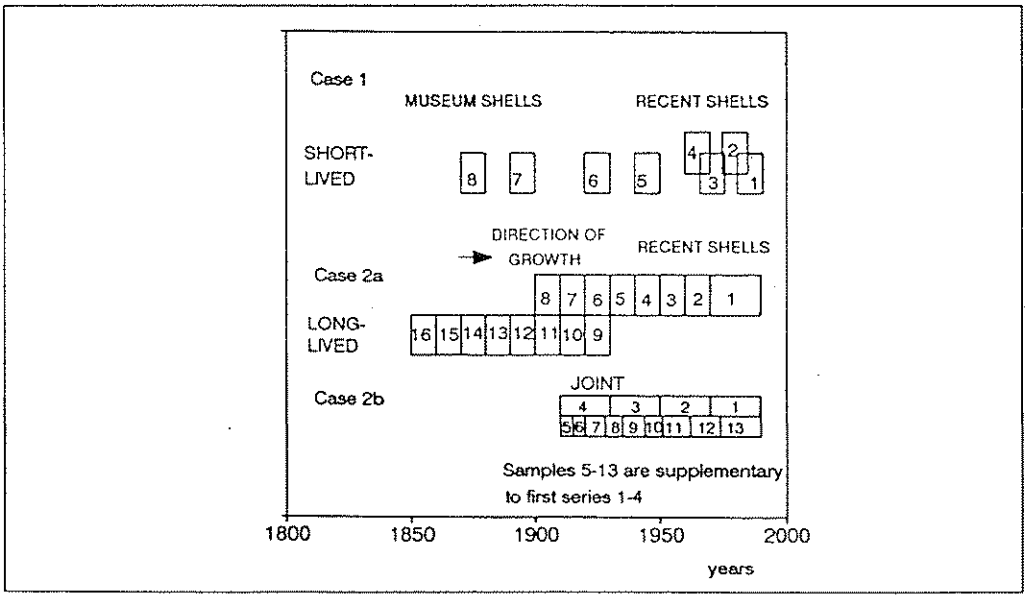

FIGURE 11 – Principles of organizing mussel shell environmental studies in time.

8. Radioactivity studies

Radioactivity has been detected in molluscan shells, e. g. in *Arctica islandica* from New York Bight (TUREKIAN *et al.*, 1982). Our methodology to detect radioactivity was: (1) alpha emission autoradiography (CARELL *et al.*, 1987, for periostracum), and (2) gamma emission spectroscopy. The latter method uses the same Ge detector and pulse height scanning equipment as the NAA method. Scanning of gamma emission peaks from a shell sample is unconditional, i. e. one can find natural radioactivities from U-Ra and Th series, and fallout fission products like Cs-137 from nuclear explosions and reactor accidents, or other nuclear spills.

Beta radiation was measured by FRANTSEVITJ *et al.* (1993) in molluscan shells collected close to the Tschernobyl reactor accident site. Figure 12 shows part of the gamma spectrum from a gastropod shell (*Planobarius purpura*) from the River Pripyat, near Tschernobyl (collected by Frantsevitj). The spectrum clearly shows Cs-137 and 134 from the fallout, taken up by the gastropod shell. The level is several thousands Bq per kg shell matter.

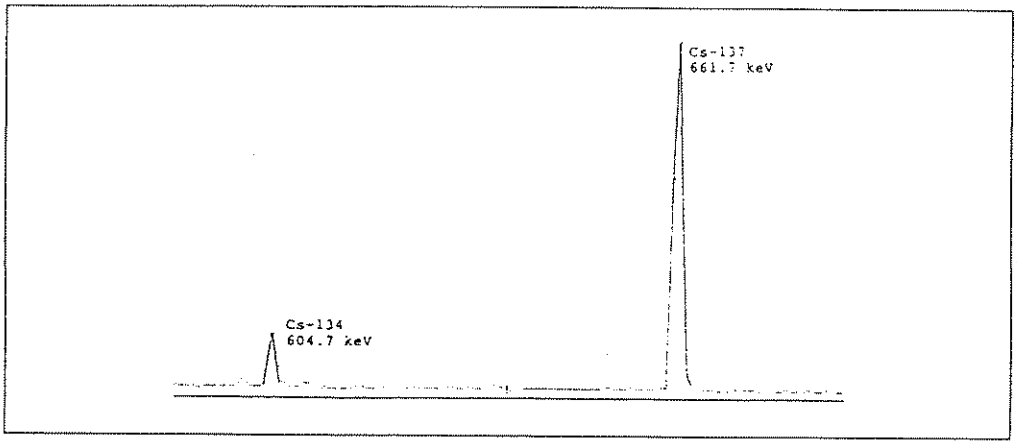

FIGURE 12 – Emission gamma spectrum of Tschernobyl fallout products in the shell of the gastropode, *Planobarius purpura*, collected close to the accident site.

FRANTSEVITJ *et al.* (1993) studied Sr-90 fission products in molluscan shells from Tschernobyl region. Correlation between this element in water and in shells was high, showing a dose-response.

Sweden received fallouts from the Tschernobyl accident in Västernorrlands County, N Sweden. Soft tissues and shell of *Margaritifera* from the River Bollstaån in this county were analyzed for gamma emission. As shown in Figure 13, both Cs-137 and 134 were recognized, and also the short-lived Ag110. Cs-137 level was about 50 Bq/kg in the soft tissues, and about 10 Bq/kg in the shell. Fresh fish meat (perch) from the vicinity showed about 2000-6000 Bq/kg, according to the health authorities. In comparison, the gastropod shell from the Tschernobyl region showed about two magnitude higher values.

The bivalve *Macoma* shells from the Gulf of Finland, Baltic, have been used for radioactive monitoring (SEIRE *et al.*, 1994). The Cs-137 peaks were not statistically significant. The sensitivity of the analysis was good enough to show members of Ra daughter products: Bi and Pb-214, and Th daughter products: Pb-212 and Ac-228. These products probably belong to the natural background of the region.

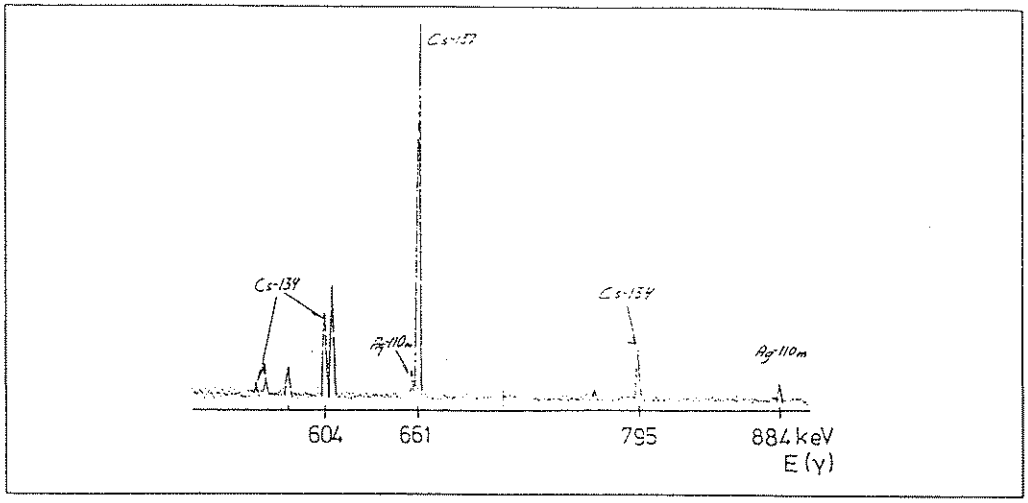

FIGURE 13 – Gamma emission spectrum from soft parts of *Margaritifera margaritifera*, the River Bollstaån, N Sweden (coll. H. Söderberg, Härnosand); the spectrum, recorded in 1989, shows Ag-110^m together with Cs-isotopes 137 and 134.

9. Methods under development

As pointed out (WESTERMARK *et al.*, 1994), some elements, notably Fe, Co and Zn, in *Margaritifera* shells from acidified W Swedish rivers, exhibit characteristic minima in series of samples taken of consecutive growth stages (for Fe, see WESTERMARK *et al.*, 1994, Fig. 11). On the other hand, these elements in *Margaritifera* shells from the River Kerjet, at the White Sea, NW Russia, either lack these minima or have only traces of them (Fig. 12, WESTERMARK *et al.*, 1994). These observations can be explained by the solubility constrained and pH controlled phenomena described by STUMM and MORGAN (1981). Fe is a typical example in this context, and other elements at lower concentrations may follow it by co-precipitation. Complexing agents like humic acids may complicate the picture derived from laboratory research and shift the pH minimum. Nevertheless, this model explains why elemental concentrations can decrease when acidification begins (Fig. 13, WESTERMARK *et al.*, 1994). As acidification increases, the solubility may be brought down to the minimum and even increase at severe acidification. After liming of the river or the source-lake, the solubility of the elements is reversed. Concentration of Mn offers a pH calibration since its solubility *versus* pH is known in the granitic bedrock areas of W Sweden.

The relationship between the solubility of Fe, Co and Zn, and pH can be used for estimations of pH levels in the past. The basis of this estimation is the minimum solubility of Fe at pH 6.5. According to STUMM and MORGAN (1981), a change of pH with one unit causes a change of Fe solubility ten times.

Figure 14 shows a model of solubility-constraint in logarithmic presentation. In Figure 15, relationship between Fe in a *Margaritifera* shell and pH in the acidified River Slereboån, W Sweden, is given. The pH levels on the alkaline side of the Fe minimum are still insufficiently known.

Fe concentrations in *Margaritifera* shells from the highly acidified River Slereboån, W Sweden, and the less acidified River Vramsån, S Sweden, were compared with Co and Zn concentrations in the same shells. The uptake of the latter two elements can be regarded as statistically independent from that of Fe.

Figure 16 shows Co concentrations in *Margaritifera* shells. In the shell from the River Slereboån, the concentration curve forms a straight line on the acid side of the Co minimum. On the alkaline side of the Co minimum, the slopes of the concentration curves are similar in shells from the River Slereboån and Vramsån.

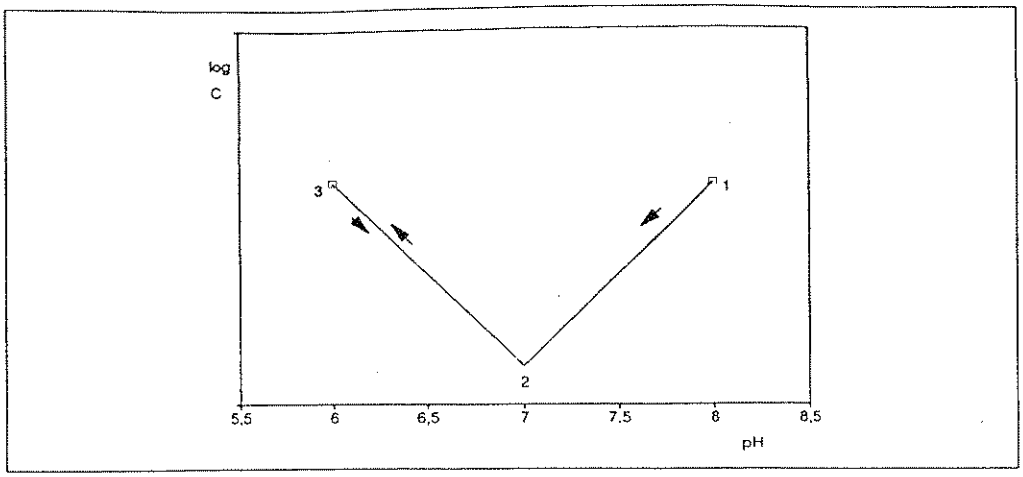

FIGURE 14 – A model of elemental concentrations for Fe, Co, Zn *versus* pH in fresh water and mussel shells. Acidification causes a decrease of these elements from point 1 to point 2; then the concentrations of the elements increase to point 3; liming of the water system reverses the course.

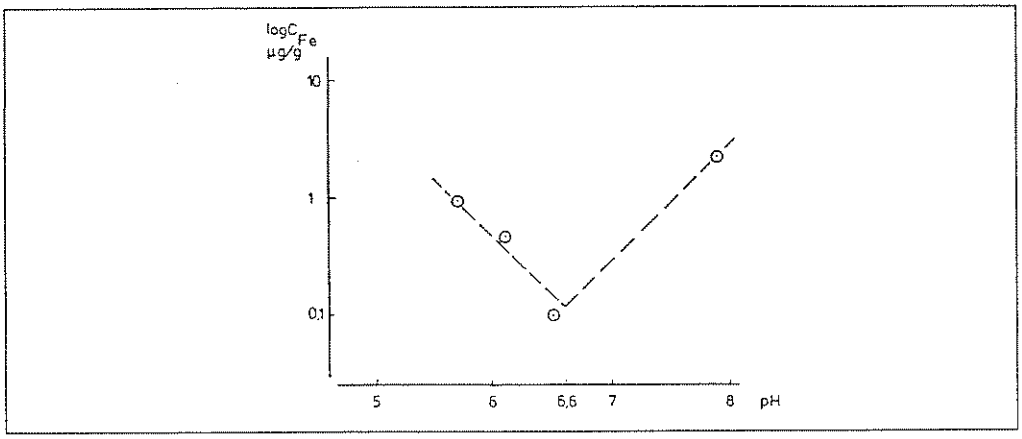

FIGURE 15 – Solubility of Fe *versus* pH in a shell of *Margaritifera margaritifera* (coll. L. Henrikson, 1990); note that the solubility minimum is much lower than in the case of pure systems, described by STUMM and MORGAN (1981).

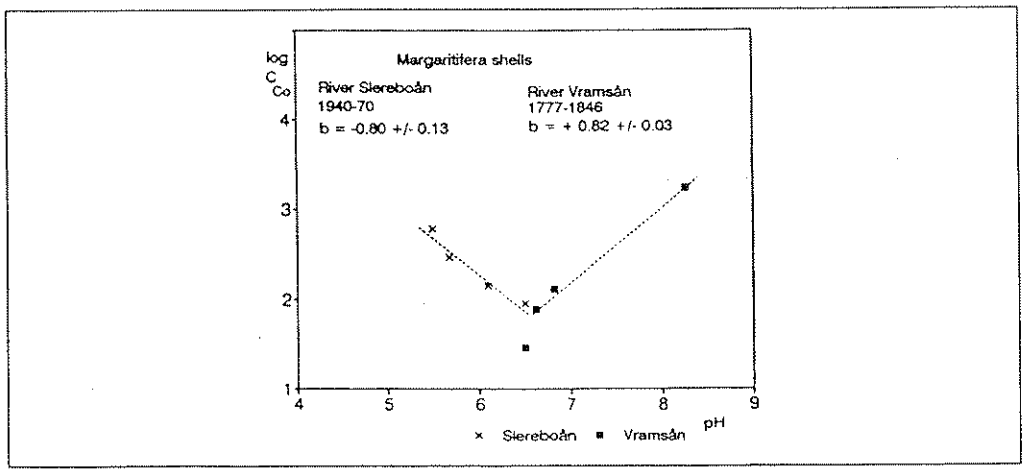

FIGURE 16 – Co concentrations *versus* pH in two shells of *Margaritifera margaritifera* ; crosses: the River Slereboån, W Sweden, 1940-1990; squares: the River Vramsån. S Sweden, 1777-1846 (coll. A. W. Malm).

Figure 17 shows Zn concentrations in *Margaritifera* shells. The Zn concentration curve on the acid side of the pH minimum is as steep (0.57 ± 0.14) in the River Slereboån as that (0.70 ± 0.35) in the River Vramsån. On the alkaline side the Zn curve in the River Vramsån has a slope of 0.54 ± 0.06.

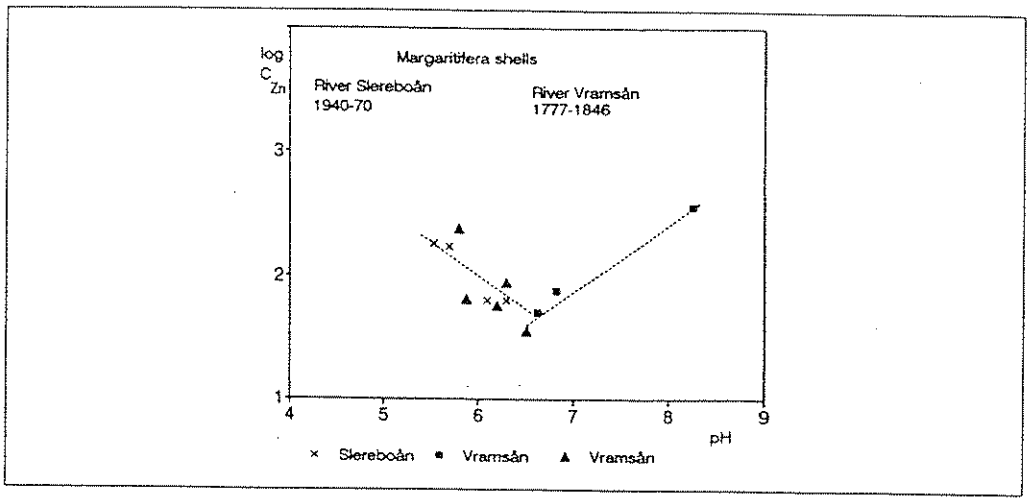

FIGURE 17 – Zn concentrations *versus* pH in two shells of *Margaritifera margaritifera*; crosses: the River Slereboån, W Sweden, 1940-1990; triangles: the River Vramsån, S Sweden, 1810-1846; squares: the River Vramsån, 1777-1810.

Figure 18 gives a preliminary pH history in the River Vramsån from 1770 to 1987. The reduced pH at the beginning of 19th century is assumed to have been caused by intense ditching and cultivation of new farming areas at that time (EMANUELSON and MÖLLER, 1990). Access of oxygen may have caused nitrification and mineralization of N and S, respectively, and release of H_3O^+ ions.

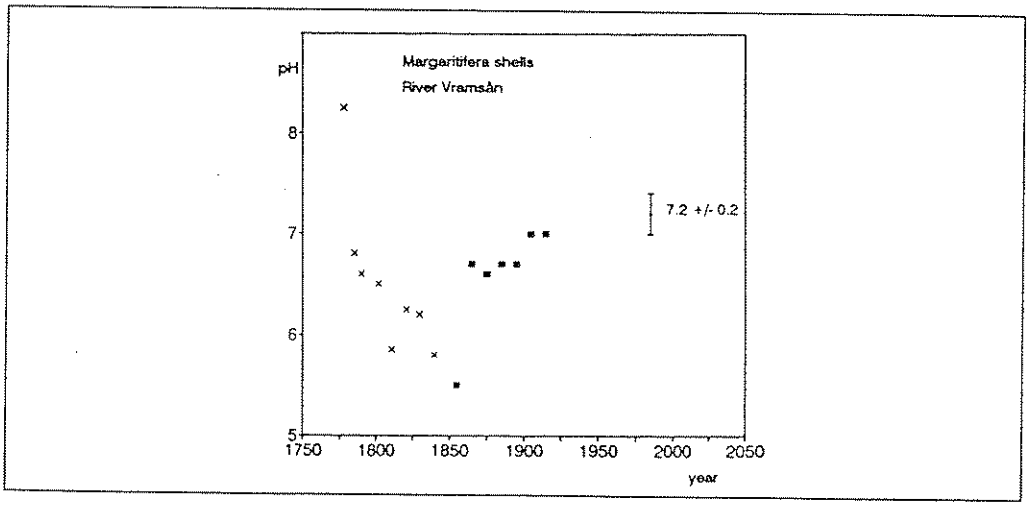

FIGURE 18 – Tentative pH history of the River Vramsån, S Sweden; data calculated from three shells of *Margaritifera margaritifera*.

To summarize, we believe that the methods of pH estimations based on solubility of Fe, Co and Zn will become increasingly important for reconstruction of environmental changes in the present and past.

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Métodos de estudio de las modificaciones del medio ambiente a partir de los datos químicos y estructurales de las conchas de los moluscos.

RESUMEN

Con el fin de comparar el índice de crecimiento entre varios indivíduos y varias poblaciones, se han establecido y estandardizadas curvas de crecimiento. Estas curvas muestran una correlación positiva de los coeficientes de crecimiento entre los indivíduos y las poblaciones. Asi, en un

medio ambiente poco afectado por las actividades humanas, los coeficientes de crecimiento de la concha son similares aun en poblaciones distantes 1200 km. Las tasas de crecimiento están relacionadas positivamente con las temperaturas medias anuales y veraniegas. El análysis de los elementos de las conchas por activación neutrónica, con periodes cortas de resolución (INAA) es descrita junto con otros métodos. Los resultados de las aplicaciones de estos métodos son compendiados.

PROPOSAL

Global environmental monitoring based on studies of structure, growth rates, and elemental distribution in molluscan shells

SUMMARY OF PROPOSAL

Molluscs comprise one of the largest invertebrate phyla. They live in all the climatic regions, and in multitude of environments: on land, in fresh-, brackish-, and marine-waters. The majority of molluscs have a hard, impermeable shell. By using advanced analytical methods, more than 40 elements have hitherto been determined in their shells. Several of these elements mirror the elemental concentrations in the environment. Life-span in bivalve molluscs is from 10 years to more than 200 years. In the long lived bivalves, it is threrefore possible to trace environmental changes 150-200 years back, before the beginning of the industrial and agricultural revolution. It has been shown that environmental factors govern the shell structure and growth rates. The magnitude of eutrophication, acidification, and agricultural, industrial and radioactive pollution can be estimated by analyzing structural changes, growth rates and elemental distribution in shells.

A global network of environmental monitoring is here proposed, based on studies on molluscan shells.

METHODS FOR STUDIES OF SHELL GROWTH RATES

Shell growth rates are mainly studied in bivalves. The growth rates are not uniform during the year but in temperate regions the growth ceases in the winter when the temperature is low. In regions with warmer climate the growth ceases when the temperature is too high, or when the animal has its reproductive cycle. Adjacent annual growth increments are separated by a thin line which marks the growth cessation. By measuring the widths of the annual growth increments, the growth rates can be established and standarized growth curves for individual shells and populations can be constructed.

The widths of the annual growth increments are measured by light microscope and scanning electron microscope on vertical polished and etched sections.

METHODS FOR STUDIES OF ELEMENTAL CONCENTRATIONS

1. Instrumental Neutron Activation Analysis (INAA). By using this method, concentrations of more than 40 elements can be measured with great accuracy. The age of the shell is determined by counting the annual growth increments. Then samples are cut out, each weighing 15-30 mg and representing a growth period of 5-15 years. The samples are neutron activated and the elemental concentrations are measured by gamma spectra.

2. Chemical Neutron Activation (CNAA). This method is sensitive for analyses of Na, K, Hg and Cd.

3. Hot Wire Detector. N and S are analyzed by this method. S is detected by an EC-detector after burning the sample at high temperature and purifying the resulting gas.

4. Inductively Coupled Plasma Mass Spectrometry (ICPMS). This method is used for detection of Mg, Cu, Mn, Ni and Pb.

5. Nuclear Microscopy (μ-PIXE). This method uses a proton beam of 1 μm to 5 μm diameter which interacts with atoms in polished surface of the shell section, producing X-ray photons characteristic to the elements in the sample.

MOLLUSCAN SHELLS USED FOR ENVIRONMENTAL MONITORING

Shells of marine bivalves: *Mytilus, Spisula, Mercenaria, Cerastoderma, Arctica* and others, have been used for environmental studies. Among these bivalves, the ocean quahog, *Arctica islandica,* has the longest life-span, from 100 to 200 years. In brackish water, *Macoma baltica* shells can be used for environmental monitoring. This bivalve can survive in highly polluted waters and survive salinities as low as 3‰.

Among fresh water unionid bivalves, shells of the pearl mussel, *Margaritifera margaritifera,* have been used for environmental studies. This species has a long life-span, 100-200 years, and commonly occurs in Scandinavian and N Russian rivers. Another unionid, *Unio crassus,* has a wide distribution in Estonian rivers which have an elevated pH due to calcareous bedrock. For environmental studies in lakes, three *Unio* species have been used: *U. tumidus*, *U. pictorum* and *U. conus,* which have a lifespan between 20 and 40 years.

Studies have been carried out to use elemental concentrations as environmental indicators in shells of land snails (e. g. *Cepea, Arianta, Cochlodina, Clausilia*).

ENVIRONMENTAL MONITORING

A summary is given below on possible applications to environmental monitoring, based on studies of growth rates and elemental distribution in molluscan shells:

1. Acidification of freshwaters in acid sensitive areas. Evaluation of measures against acidification: liming and/or reduction of acidifying agents.

2. Eutrophication of freshwaters by releases from densely populated areas. Evaluation of measures against eutrophication: water purification by mechanical, biological and chemical means.

3. Pollution by agricultural fertilizers.

4. Pollution of coastal and marine waters.

5. Pollution by mining and mining wastes.

6. Pollution by process industry; iron-ore processing and metal production; wood and pulp industry.

7. Pollution by energy producing units: coal, peat, oil, ash leaching, hypochlorite discharges from cooling water of power stations, etc.

8. Nuclear industry: mining and mine tailings; leachates; reactor stations; reprocessing; reactor or radioactive waste accidents; nuclear weapon tests.

9. Miscellaneous: blind cutting of forest; forest fertilization; volcanic ash emissions; construction of dams; pollution by motor vechicle exhaust gases.

In order to improve the monitoring program, it is necessary to study in detail molluscan biology, and carry out laboratory experiments on uptake of elements from the medium into the shell.

INTERNATIONAL ENVIRONMENTAL PROJECTS

Six years ago joint-studies on environmental monitoring and environmental history were established between three Swedish research institutes: Department of Nuclear Chemistry, Royal Institute of Technology, Stockholm; Department of Radiation Sciences, Uppsala University; and Swedish Museum of Natural History. These studies have been carried out on growth rates and elemental distribution in bivalve shells from Scandinavian rivers, coastal regions of the Baltic and North Sea; later, studies on shells of land snails .were also included. Three years ago a joint-study was initiated with the Botanical and Zoological Institute, Tartu, Estonia, on bivalve shells from Estonian rivers, lakes, and coastal regions of Gulf of Finland, Baltic. Another study was initiated in cooperation with Limnological and Zoological Institutes, St Petersburg, Russia, on bivalve shells from NW Russia (White Sea, Lakes Krasnoe and Ladoga, N Russian rivers). Estonian and Russian studies are particularly urgent because of extremely high industrial pollution in Gulf of Finland (Baltic), White Sea, and the Lake Ladoga, the latter of which is the fresh water reservoir for St Petersburg. These studies also include radioactive pollution.

Studies on British marine bivalves, including laboratory experiments, are planned in co-operation with the School of Ocean Sciences, Gwynedd, UK, and on Japanese fresh water and marine bivalves in co-operation with Universities of Kyoto, Aichi and Niigata.

Discussion

by

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Following the presentations of Dr H. Mutvei and Professor T. Westermark discussions ensued regarding the possibilities of using molluscan growth patterns as recorders of environmental change. Below are a selection of questions, comments and answers which were raised during questions.

PROFESSOR T. OHNO: Dendrochronological studies have been valuable in understanding the relationship between long-term climatic change and tree growth. Would it be possible to combine this data with information obtained from bivalve shell chronology to document atmospheric, climatic and environmental change?

DR MUTVEI: Yes, and we are writing a paper comparing shell chronology and dendrochronology. There have been papers written concerning bivalve chronology and tree dendrochronology, however both bivalves and trees have their own particular problems. For example, female freshwater mussels, *Margaritifera margaritifera* reproduce every 3 years so that the reproductive event might be recorded in the growth patterns, although in this species this is not the case. Howewer in other bivalve species such events might be recorded.

DR RICHARDSON: You have shown elegantly how annual growth lines contained within bivalve shells can be used to document changes in environmental conditions. An underlying assumption however is that growth lines are deposited with annual periodicity. My main concern is that many of the growth line patterns that have been used in previous studies might not be of truly annual origin. There is evidence to indicate that growth lines in *Artica islandica*, for example, might be biannual in origin. How can you be sure that the growth lines are annual one?

DR H. MUTVEI: This is a good point, but your studies have involved the examination of acetate peels rather than the actual surface in the scanning electron microscope. The S.E.M. provides more powerful resolution of the

patterns. We use different etching methods to make annual increments more clear. We have had some problems with individual shells from some regions such as the White sea. Our studies however use many individuals from large population and we accept 20% mismeasurement.

DR RICHARDSON: I feel it is important to study the relationship between formation of growth lines and environmental variables over known time scales to determine the cause and periodicity of growth lines.

PROFESSOR K. SIMKISS: Shells as biomarkers are regarded as obtaining a physiological response to environmental change. Bivalves are long-lived and they are a relatively good and simple system. The work you have described forms the basis for an exciting program.

DR BUDDEMEIER: Biomarkers might of course die during the period of study. Dendrochronological studies may be able to characterise chemical changes although extreme changes may be difficult to interpret. It is also important to distinguish between the two recording devices, bivalve shells and trees and also be able to recognise failures in the system such as only being able to produce qualitative data.

PROFESSOR EREZ: Have you considered using stable isotopes to determine the annual nature of the growth patterns. Margaret Deith in Cambridge has worked on scallop shells and has looked at seasonal patterns of growth. Also, some stable isotopes e.g. ^{31}S and ^{32}S produced during oil combustion might be recorded in the organic matrix of the shell. Another possibility exists to study growth patterns using, for example, staining markers.

DR H. MUTVEI: With regard to marking experiments and the validation of the growth patterns there are often problems associated with the lack of suitable aquariums and of course poor shell growth in the laboratory may not be representative of natural growth.

Further *ad hoc* discussion occurred amongst delegates at the workshop before Dr Mutvei thanked us all for attending and adjourned the meeting.