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Environmental History as Reflected by Freshwater Pearl Mussels in the River Vramsån, Southern Sweden

The distribution of elements in shells of the freshwater pearl mussel, *Margaritifera margaritifera*, from the River Vramsån, southern Sweden, was analyzed with high temporal resolution with the nuclear microscope SLIM-UP. The results show: (i) annual variations of strontium and manganese. The variation of Sr is anticorrelated to that of Mn with high Sr concentration on the winter lines, and high Mn in the summer. The high Mn concentration in the summer correlates to a depletion in oxygen in the surrounding water; (ii) high Sr concentrations on growth disturbance lines; (iii) an ontogenetical variation of Sr with high concentrations in younger stages (before sexual maturation); and (iv) a long-term decrease of manganese in the shells. The long-term decrease of Mn is also consistent with results from Neutron Activation Analysis.

INTRODUCTION

Bivalve shells have proven to have advantages in environmental studies, as their solid and impermeable shells of calcium carbonate and glycoproteins retain elements from the water without any transport after incorporation. The shells show distinct annual growth increments which allow analysis of elemental distribution over time. Our aim has been to exploit the possibilities of a powerful method to unveil environmental history by analysis of the mussel shells. Two analytical methods, Nuclear Microscopy (NM) and Instrumental Neutron Activation Analysis (INAA), have proven to be a fruitful combination for this purpose. Results of this co-operation have been presented in earlier publications (1, 2).

In this paper we focus on freshwater pearl mussels (*Margaritifera margaritifera*) from the River Vramsån, Scania, S. Sweden. We measured elemental distributions within mussel shells with high temporal resolution, using a nuclear microscope. The results were compared with the chemical and temperature data of the water as well as INAA measurements.

MATERIAL

Eight specimens of *M. margaritifera* were used. They were all collected alive from the River Vramsån at different dates. No. 46-3, came from the Museum of Natural History in Göteborg. It was collected by the Swedish biologist A.W. Malm in 1846 (3). No. 11-1 was collected by an unknown person in 1911, and made available to us by the Kristianstads Vattenrike Ecological Museum with help from S.-E. Magnusson. The rest were collected upstream from Tollarp village (Fig. 1) by H. Mutvei and E. Dunca during 1989 to 1993 (Table 1).

The pearl mussel *M. margaritifera* is a freshwater bivalve belonging to the Unionidae family. This species usually inhabits rivers with fairly high velocity and low eutrophication (4). For these mussels the optimal water pH is around 6.5, but they can survive lower (pH 4.5) as well as higher pH (pH > 8). The maximum life-span of individuals is high in the North (5), usually

over 100 years, but even 200-year old individuals have been reported (6).

The pearl mussel shell consists of two components: the crystalline calcium carbonate in the form of aragonite, and the organic matrix consisting of glycoprotein, which acts as cement between the crystals (7). There are three distinctive layers in the shell: the organic outer layer, periostracum, and two aragonitic inner layers, the prismatic and nacreous layers which have been analyzed previously for their elemental distribution using the INAA method (6). The present study focused on the aragonitic part of the shell.

Locality

The River Vramsån was chosen as a reference for the studies of pearl mussels in Sweden, as its agricultural history has been monitored for a very long time (8), and also because we had access to A.W. Malm's mussel shell collections from the 19th century for this location (3).

Catchment Area

The River Vramsån has a catchment area of 383 km². It is the second important tributary to the Helgeå River, the most important watercourse system in Kristianstad County. The topography of Kristianstad County is flatter compared to surrounding areas because of its different geological background. The plain of Kristianstad, called Kristianstad Downs, is composed of depositions of Cretaceous sea on granite and gneiss bedrock as well as sand of both glacialfluvial and morainian origin from the last glaciation.

The geology of the River Vramsån catchment area is represented mostly by sedimentary rocks, cretaceous limestone, calcareous sandstone and slightly consolidated glauconite sandstone. These rocks are the most important suppliers of groundwater due to their porosity and permeability (9). These properties have a great practical significance for the possible risks of pollution. Notably, downstream of Tollarp, the river flows through agricultural areas, thereby exposing it to the effects of farming, such as leakage of fertilizers and pesticides.

The water source of the River Vramsån is the Lake Bosarpa and the Store Mosse peat, which rests on granite bedrock. In Store Mosse, peat was exploited from the 19th century until 1967.

Water Chemistry

The water analyses in the Kristianstad Downs area (including the River Vramsån) show that both the ground and surface water have low concentrations of Fe, Mn, ammonium and nitrite (with some local variations), but a high nitrate level, due to extensive use of fertilizers in farming during the 1970s (Table 2).

The water quality of the River Vramsån has been systematically monitored since 1975, with water samples being taken monthly (10). During this period no dramatic events were registered in River Vramsån water. The pH is about 7.5 due to the calcareous bedrock, with seasonal variations generally between 6.5 and 8.1 (lower pH in the spring and autumn due to snow-melting and rainfall, and higher in the summer).

Figure 1. Looking for shells in the River Vramsån. Photo: E. Dunca.



The interest for this river is growing because of its exceptional fauna composition including rare species of fish such as *Noemacheilus barbatulus*, *Gobio gobio*, *Pelecus cultratus*, *Salmo trutta trutta*, *Salmo salar* and *Leuciscus idus*, and pearl mussels growing together with other mussel species (*Unio crassus*, *Unio tumidus*, *Anodonta cygnea*, and *Anodonta complanata*) (3, 11).

The annual rainfall in Kristianstad Downs is generally less than 600 mm and the annual evapotranspiration less than 500 mm. An increase in evapotranspiration has been estimated at 2% over a period of 100 yrs (1869–1969), due to enlargement of the forest area from 14.6 to 27.4% (9).

Cultural-technical activities, such as drainage and irrigation, seem to have had little effect on the water equilibrium, but some negative influence on the mussel population. A census of pearl-mussel populations from Kristianstad County made in 1989 showed that of seven, known populations in 1959, two were extinct. Three major causes for this are suggested (12):

- Water regulation. Less water in the rivers during summer and winter cause the shells to dry out and freeze, respectively.
- Artificial fertilizers were extensively used in the area during the 1970s.
- Pearl fishing. Statistically, every one thousand mussels contain one pearl, so fishing may cause severe damage to a single population.

METHODS

Material Preparation

From each shell, a four mm thick section was cut from umbo to the ventral edge, perpendicular to the winter lines, using a diamond saw (13). The section was polished with diamond paste of one mm grain size. Thereafter, a 30 mm long section was cut from the ventral edge (Fig. 2) to be used in Nuclear Microscopy (NM). In specimen 93–5 we used two sequential segments which covered almost the whole life-span.

Each section was washed for two minutes in an ultrasonic bath with 95% ethanol, and then carbon coated using a carbon evapo-

Table 1. The shell samples and their age.

No.	Specimen Id	Collected in	Legato	Age
1	46–3	1846	A.W. Malm	56
2	11–1	1911	unknown	91
3	89–5	1989	H. Mutvei	60
4	89–7	1989	H. Mutvei	56
5	91–2	1991	E. Dunca	35
6	93–1	1993	E. Dunca	57
7	93–5	1993	E. Dunca	30
8	93–11	1993	E. Dunca	84

Table 2. Water chemistry of Kristianstads Downs area (9).

Element	Surface water		Groundwater	
	Highest value (mg L ⁻¹)	Lowest value (mg L ⁻¹)	Highest value (mg L ⁻¹)	Lowest value (mg L ⁻¹)
Manganese	0.15	<0.05	0.3	<0.05
Iron	1.8	0.18	3.4	<0.1
Calcium	91	5	175	47
Ammonium	1.6	<0.1	0.5	<0.1
Nitrite	0.3	0.004	0.64	<0.001
Nitrate	17	<1	170	<1

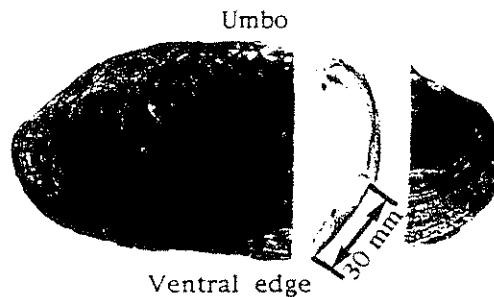


Figure 2. Shell showing how sectioning is carried out. Photo: U. Samuelsson.

ration equipment until 90% absorption of transmitted light through a glass slide was reached.

Measuring Growth

The shells of *M. margaritifera* from the River Vramsån grow mainly between May and September. In the winter, when the water temperature is below 4°C, the two valves of the mussel are closed and growth virtually ceases. This cessation of growth is manifested by an etch-resistant ridge that appears as a distinct dark (winter) line in a light microscope.

Dating the annual growth period of the shells is based on observations made on the last year increment of several mussels collected at different periods of the year. The last year increment was measured and expressed in percentage of the increment formed in the previous year. All individuals that are between 20 and 80 yrs old have similar growth period from May to September. Older specimens begin to grow later, around July, and start to build their winter line sooner, in September.

For measurements of shell growth rates, the widths of the annual growth increments were measured perpendicularly to the winter lines in the prismatic layer, close to the boundary to the nacreous layer. The umbonal region is almost always damaged and, for this reason, annual growth rates are difficult to measure before the mussel has attained sexual maturity (13).

Growth Curves

Each mussel has a characteristic growth pattern, which generally decreases with age. In order to compare the growth rates between individuals of different age, the ontogenetic growth dif-

ferences have to be eliminated by standardising the growth series (13). The population curve of the River Vramsån is the mean curve of 46 such standardized growth series.

The Nuclear Microscope

The Nuclear Microscope (NM) is set up at the Tandem van de Graaff accelerator at the Tandem Laboratory in Uppsala and is named SLIM-UP (Scanning Light Ion Microprobe in Uppsala). The accelerator supports nine permanent experiments, which means that the time allotted for NM measurements is about 24 hrs per week. The microscope (14) is typically used with a 200–500 pA proton beam of 3 MeV energy, focused to an approximate spot size of $5 \times 15 \mu\text{m}$ with the probe-forming lenses configured as a coupled triplet. The system is constantly kept at a vacuum of 10^{-7} torr. Beam positioning on a shell target is done by observation of the fluorescent light that is emitted from the area covered by the beam. The light is observed through a stereo microscope mounted at a 45° angle to the target surface normal (Fig. 3).

The start and stop point and the number of points of each line measurement were manually defined at the beginning of each run by moving the target with stepper motors. Thereafter, the control computer managed the data acquisition and target movement until the end of each line. We have a 30 mm^2 PGT Si(Li)-detector with a FWHM resolution of about 160 eV at 5.9 keV X-ray energy. To reduce the predominant Ca peaks in the spectrum, a $200 \mu\text{m}$ mica (muscovite) film with 10% potassium content was used as an X-ray filter. By coating the shell surface with carbon (*vide supra*) the induced charge could effectively be transported off the shell, thus, reducing to a minimum the Bremsstrahlung radiation from discharge electrons. The count rate was typically in the range of 1–2 kHz. The data acquisition system used was a PC-based multiparameter system from FAST ComTec GmbH, Germany (15).

Each sample was measured with at least one line scan in the aragonitic layer near the ventral margin (Fig. 4). The actual scan positions varied for each measurement which caused some scan points to cross the border between the prismatic and the nacreous layers. The distance between two consecutive points was also varied between 4–200 mm as we tried to adjust it to the thickness of the annual increments of each specimen.

The resulting spectra were analyzed with GUPIX, a PIXE analysis software package from University of Guelph, Canada (16), and as normalisation, the very stable 39% ($\pm 1\%$) concentration of calcium was used.

Scanning Electron Microscopy

After NM analysis, the samples were polished and etched (13). The irradiated points were observed in SEM, and correlated to the season of the year, and the shell growth rate. The proton beam heated the aragonite crystals in the irradiated point so that the crystals evaporated, thereby producing a crater-like pit on the surface.

RESULTS

Measurements

The measurements performed on each specimen are given in Table 3.

Most mussels were at least 50 yrs old at the time of collection. Thus, due to sample preparation, the measurements on these mussels were located to areas representing the last 20 yrs of life (*vide supra*). In one mussel shell, No. 93–5, it was possible to cover areas corresponding to an age of 9–12 years, i.e. before sexual maturity, as well as an age of 23–30 years.

As we did not calibrate the instrument against a known certified standard, a certain measure of precaution should be taken

with our absolute values for the manganese and strontium levels.

Strontium

The measurements showed strontium levels between 40–180 mg kg^{-1} . In those measurements, where the number of points per annual increment was high enough (at least four), a seasonal variation of strontium was found. This variation is characterized by a high peak during the winter compared to the spring and summer concentrations (Fig. 5). The height of the peaks varies between different years and in different shells, being between 10

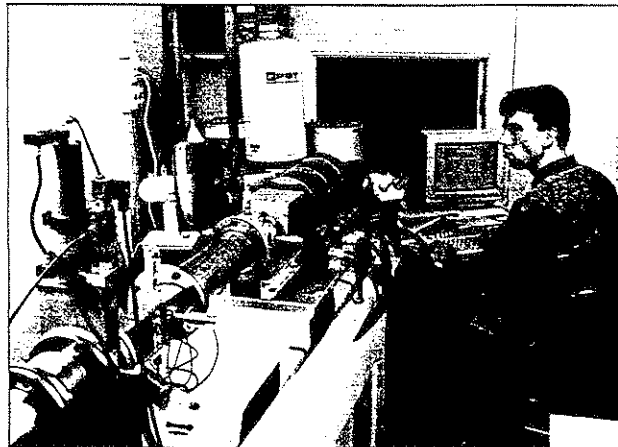


Figure 3. Optimizing the beam spot size. The target chamber is at the end of the beam line behind the three quadrupoles. Photo: T. Thömlund.

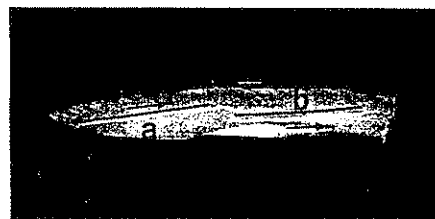


Figure 4. Shell No. 11–1 after NM analysis. The two scribed marks are made with a diamond knife to mark out the area where the line scans are to be made. Two actual scans are marked a and b, respectively. Photo: T. Thömlund.

Table 3. Measurements performed on each shell with number of points, the covered time period, and type of layer covered.

Mussel Id	Measurement	Points	Time	Layer
46–3	a	36	1836–41	prisma
	b	25	1826–36	nacre
	c	41	1838–41	nacre
11–1	a	32	1853–73	nacre
	b	30	1894–1903	prisma
89–5	a	18	1985	prisma
	b	39	1984–88	prisma/nacre
89–7	a	38	1981–88	nacre
	b	31	1969–80	nacre
91–2	a	34	1985–89	prisma
93–1	a	50	1980–91	prisma
	b	45	1965–82	prisma
	c	90	1954–58	prisma
	d	15	1974–80	prisma
	e	25	1986–91	nacre
93–5	a	25	1986–91	nacre
	b	30	1989–93	nacre
	c	90	1972–73	prisma/nacre
	d	56	1970–72	nacre
	e	25	1972–73	prisma
	f	28	1972–73	prisma
93–11	a	36	1944–60	nacre
	b	25	1961–93	prisma/nacre

and 100% higher than the corresponding summer values. The higher values are obtained when the beam hits the winter line exactly.

High peaks were also found on lines which mark growth disturbances. These lines are probably formed during temporary growth decrease.

In specimen No. 93-5, where we measured the whole shell profile, an ontogenetical variation in Sr was found. In the juvenile stage of the mussel, about 9-12 years of age, there is three times higher Sr concentration, ca 180 mg kg⁻¹, compared with about 100 mg kg⁻¹ when it is 23-30 yrs old.

The other mussels generally showed a concentration of 100 mg kg⁻¹ independent of age, except for two; No. 89-7 and No. 93-1 having 40 mg kg⁻¹ and 180 mg kg⁻¹, respectively. During the last 200 yrs, no variation in Sr has been found. The mussels collected in 1846, 1911 and recently all have the same average Sr levels, about 100 mg kg⁻¹.

Manganese

The measurements showed large differences in manganese concentrations between the two aragonitic layers of *M. margaritifera* shells. Higher concentrations and variations, about 100-300 mg kg⁻¹, were found in the nacreous layer, and 10 times lower concentrations, about 10-80 mg kg⁻¹, in the prismatic layer. No ontogenetical variation was observed (Fig. 6).

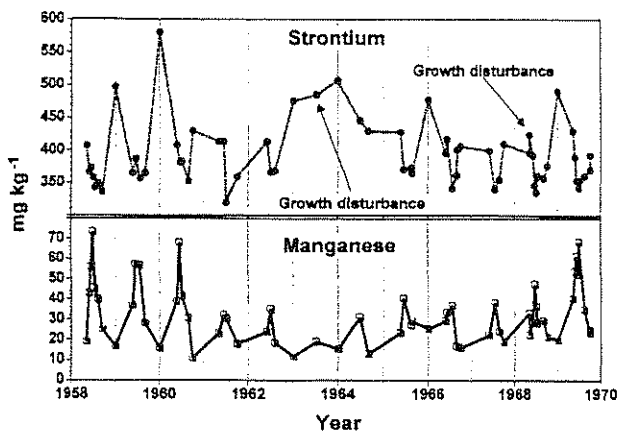


Figure 5. Sr and Mn annual variation in the nacreous layer of No. 93-1. Sr has peaks on winter lines and growth disturbance lines.

We separated the points in each line measurement into two sets, one for each layer, thereby eliminating the variation due to different layer concentrations.

Manganese also shows an annual variation, with peaks in the period May-June-July, correlating with high water temperature. These peaks also correlate to the oxygen concentration minima in the water (Fig. 7). The Sr variation is in opposite phase to the Mn variation with Sr maxima co-located with Mn minima, and vice versa (Fig. 5).

The amount of manganese in the nacreous layer of the shells decreases during the last 200 yrs from about 600 mg kg⁻¹ to 100 mg kg⁻¹. In the shell collected in 1846, the concentration of Mn found was about 570 mg kg⁻¹ between 1826 and 1835 and 435 mg kg⁻¹ between 1838 and 1841. The mussel shell No. 11-1 contains lower Mn concentration, about 330 mg kg⁻¹ for the period 1852-1870. These results can be compared with the results of INAA analyses, which show a similar trend, but with higher concentrations (Fig. 8).

Comparison with Neutron Activation Analysis (INAA)

We have observed good agreement with results of INAA analysis. The problem with comparison of data is that in INAA, the whole shell profile is analyzed by cutting the shell in relatively large pieces. Thereby, each piece represents both a large time span (typically 10 yrs each) and also contains both prismatic and

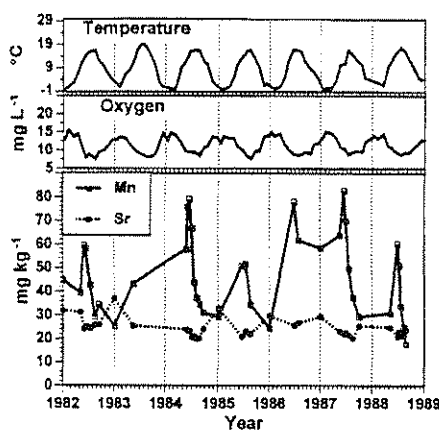


Figure 7. Mn and Sr variation in No. 89-7 compared with water temperature and oxygen level in the water.

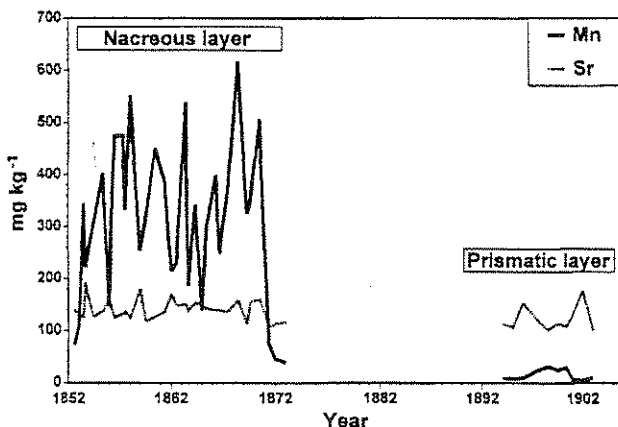


Figure 6. Mn and Sr in the two aragonitic layers of the shell No. 11-1.

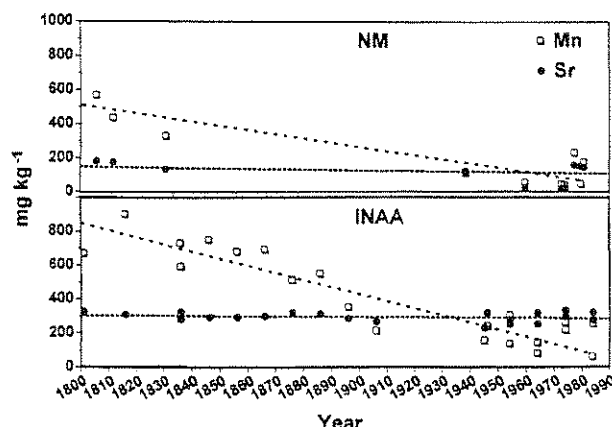


Figure 8. Comparison of NM and INAA analysis data from the same samples.

nacreous layers in equal proportions. This is in contrast to NM analysis where the time resolution is of the order of weeks (or even days) and the measurement can be made in both layers separately. In order to compare the results, some adaptations had to be made on the NM data. The median value for each scan and each layer was used to represent the value for that time interval. These points are plotted in Figure 8 together with corresponding INAA data. It can be seen that there is a good trend agreement between the two data sets.

The normalization of measured spectra was done under the assumption that calcium is homogeneously distributed in the shell. Thus, we set the calcium value to the mean of the INAA calcium measurements, i.e. 39%. Since this value varies within two or three percent, and the accuracy of the NM analysis is in the range of 5%, the possible error introduced is not large

DISCUSSION

Seasonal variations in uptake of several elements into the shell, notably sodium and magnesium, have been reported (17). Also, seasonal variations of ^{18}O , ^{13}C and ^{14}C have been demonstrated in several molluscan shells (18). However, variations of this kind can only be studied in fast growing shells in which the annual growth increments are exceptionally wide. In such shells, samples for elemental analysis can be taken by using a dental drill or similar device. In the vast majority of bivalves the annual increments are narrow, particularly during the later growth stages. In *M. margaritifera*, the width of annual growth increments is about 500 μm , which makes NM the only method available today to assess elemental distribution within a single annual growth increment, with its spatial resolution combined with adequate detection limits. X-ray microanalysis in a scanning electron microscope presents higher spatial resolution, but the detection limits are high because of the intrinsic electron Bremsstrahlung radiation background.

Strontium Variation

Strontium is one of the rare elements in the environment (35 mg kg^{-1} in the earth's crust and less than 0.1 mg kg^{-1} in surface water) and an important trace element for building of calcium carbonate shells. This element is termed as an "A-class" hard metal (19), and it is assumed to exist in natural waters and aerobic conditions in the form of Sr^{2+} with no redox activity (20). Sr is also well known for accompanying calcium as an elemental analogue. It replaces the Ca atoms in aragonite crystals and it is required for the crystal nucleation and alignment of the bivalve embryonic shell. Gallager et al. suggest that Sr acts as "mortar between an organic component and mineral portion of the embryonic shell" (21). The typical levels of Sr in freshwater bivalve shells is about 200 mg kg^{-1} (22).

Hallam and Price found differences in Sr content between outer and inner shell layers (aragonitic) in marine bivalves, caused presumably by biochemically controlled processes. They also found a correlation between Sr content and water temperature, which was explained by an increase in the metabolic rates with higher temperature leading to an efficient exclusion of trace element impurities (23). Swann et al. noticed a rise in the Sr/Ca ratio in the nacreous (aragonitic) layer of marine oysters during the winter with a peaking in May, but no such variation in the prismatic (calcitic) layer. They suggested that in the late winter and early spring a dissolution of calcium carbonate occurs in the aragonitic inner shell layer (24).

We found that for *M. margaritifera*, strontium forms a sharp peak (about 20% higher concentration than the mean concentration) in the winter line and growth disturbance lines, both in prismatic and nacreous layer. This seasonal variation of Sr can be related to the growth rate and/or the Sr concentration in the water. Two theories that connect the variation of Sr to the growth

rate are: *the anaerobiosis theory*, which sustains that during the period when valves are closed, shell dissolution occurs, causing an enrichment of organic matrix, which binds Sr (25), and *the high density theory*, which says that during the period of growth cessation, mussels build "etch resistant-zones", which are organic-poor deposits with higher density (26). If strontium is located only in the aragonitic crystal lattice, then higher concentrations in the winter line may suggest organic-poor depositions and support the high density theory.

It has been shown that the Sr concentration in the shell is directly proportional to the Sr concentration in the water in which deposition takes place (27). The low concentration of Sr found in the shell during the summer period might be caused by a dilution effect due to aquatic plant growth, i.e. eutrophication (28). The algae blooming simply decreases the amount of Sr available per individual.

We also found an ontogenetical variation in Sr, in which the concentration decreases with age. This variation may be controlled by the growth rate, which in turn is controlled by the calcium carbonate metabolism of the shell (high metabolism at a young age and lower in old age) (29). Earlier observations show an increase of Sr with age in some species, and a decrease in others, both in marine and freshwater bivalves (30). In some species of freshwater bivalves, Nelson observed an increase in Sr content with age, however, he measured very young individuals (< 13 yrs old). He explained the increase in Sr as being related to a decreasing surface to volume ratio. In bivalves with high surface to volume ratio, the exchange between the depositional tissues and the external environment is rapid and the Sr excluded from the crystal deposition escapes more readily (22).

Manganese Variation

Manganese is termed an intermediate first transition metal (19) and is found as Mn^{2+} under aerobic conditions in natural waters (20).

The manganese concentration may be related to the shell growth processes as well as to the water chemistry. There is evidence that Mn binds to the organic matrix between the aragonite crystals (31), but also that it substitutes Ca^{2+} ions in the lattice during biomineralization (32). If Mn binds mostly to the organic part, then the summer peaks, also observed in *Anodonta* by Lindh et al. (2), may support the observations made by Koike (26) that a more organic matrix implies a high growth rate. The water temperature is the main factor influencing shell growth. This explains the positive correlation between the amount of Mn in the shell and the water temperature. The amount of manganese varies in the two layers of the shell, with higher levels in the inner nacreous layer. A similar observation was made by Yoshioka and Terai on another freshwater bivalve *Anodonta woodiana*. This variation may suggest a difference between the two layers in the mode of calcium carbonate secretion (33).

The oxygen level in the water may influence the concentration of soluble Mn, since the reduced state of Mn(II) then can be oxidized to Mn(IV). The Mn(IV) binds to oxygen and forms MnO_2 , which is insoluble in water and precipitates (34). If so, our observations lend support to the redox effect; the Mn reaches maximum levels in the mussel shells when the oxygen has its minima in the surrounding water (Fig. 7). Analyses of water chemistry from lakes in Örebro County show a seasonal variation of Mn concentration (35), similar to the seasonal variation of Mn in mussel shells with peaks in the spring. We have no such chemical analysis for the River Vramsån and, therefore, are not in the position to discuss this in detail.

The level of Mn is correlated to the degree of acidification in the water (36). Earlier measurements on pearl mussels from areas with high acidification pressure (River Slereboån, western Sweden) show a decrease of Mn in the nacreous layer in the or-

der of 60%, after liming of the source lake in 1984. Strontium followed Mn in this decrease with a 50% reduction which probably is a suppression effect due to the increase of Ca^{2+} ions in the water. Phosphorus showed a similar reduction (unpubl. data). This cannot be seen in the River Vramsån, as the pH level has been roughly constant, around pH 7, since the measurements started.

Regarding longer periods of time, Mn decreases from the beginning of the 19th century until now, and the same trend is shown by measurements with Neutron Activation Analysis (Fig. 8). However, during this long time interval there are large periods which have not been measured using the NM. The solubility in water of Mn, as well as Fe, Co and Zn, is controlled by the pH (36). The concentration of these elements can, therefore, be used to estimate the pH levels in the past. In the River Vramsån a reduced pH at the beginning of 19th century was estimated (6). This is assumed to have been caused by introducing a new farming system characterized by intensive ditching, cultivation of large areas and introduction of artificial fertilizers (6). It is assumed that nitrogen leaks into the water system due to ditching of large wetland areas. The result is a pH decrease in the water, thereby causing release of formerly bound cations of trace elements. In the 1850s, the pH suddenly in-

creased again to normal values; an effect supposedly due to improvement of the soil with marl (6).

CONCLUSIONS

The two methods, INAA and NM, complement each other in extracting information about the chemistry of the mussel shell. With INAA it is possible to detect amounts at $\mu\text{g kg}^{-1}$ level from a large spectrum of elements over a long time interval, and with NM, the annual variations of some elements can be detected.

We have found a long-term decrease in Mn level, from the early 19th century until today, which is consistent with INAA data.

With the high resolution of NM, we have also been able to detect a seasonal variation in Mn and Sr. The variation in concentration of the two elements are anti-phased. High levels of Sr were found both in winter lines, and in growth disturbance lines. Also, a decrease in Sr has been found after sexual maturity.

For Mn, the concentration maxima in the summer are correlated to minima in oxygen levels. This makes it possible to detect earlier oxygen depletion periods, and, in the future, use it for environmental history applications.

References and Notes

- Carell, B., Forberg, S., Grundelius, E., Henrikson, L., Johnels, A., Lindh, U., Mutvei, H., Olsson, M., Svärdsström, K. and Westermark, T. 1987. Can mussel shells reveal environmental history? *Ambio* 16, 1-10.
- Lindh, U., Mutvei, H., Sunde, T. and Westermark, T. 1988. Environmental history told by mussel shells. *Nuc. Instrum. Methods B30*, 388-392.
- Malm, A.W. 1855. Om svenska land och sötvattenmollusker. *Göteborgs Museums Handlingar*, 75-151. (In Swedish).
- Björk, S. 1962. Investigations on *Margaritifera margaritifera* and *Unio crassus*. *Acta Limnol.* 4. PAGES???
- Bauer, G. 1992. Variation in the life span and size of the freshwater pearl mussel. *J. Anim. Ecol.* 61, 425-436.
- Mutvei, H., Westermark, T., Dunca, E., Carell, B., Forberg, S. and Bignert, A. 1993. Methods for the study of environmental changes using the structural and chemical information in molluscan shells. *Bull. Inst. Oceanogr. n° spécial 13*, 163-186.
- Taylor, J.D. and Layman, M. 1972. The mechanical properties of bivalve (Mollusca) shell structures. *Palaeontology* 15 part 1, 73-87.
- Emanuelsson, U. and Möller, J. 1990. Flooding in Scania: A method to overcome the deficiency of nutrients in agriculture during the nineteenth century. *Agric. Hist. Rev.* 38, 127-148.
- Wejman-Hane, G. and Hörberg, I. 1969. *Samarbetskommittén för Kristianstadslätens Hydrologi-Slutrapport*. (Mimeographed report from the County Administration of Kristianstad, Sweden). (In Swedish).
- Anonymous, 1989. *Helgedin - Vattenkvalitet och Transport 1977-1986*. Mimeographed report from the County Administration of Kristianstad, Sweden. (In Swedish).
- Wagnström, J., Magnusson, S.-E., Söderlund, K. and Vägren, G. 1992. Fiskar i Kristianstads Vattenrike. Mimeographed report from the County Administration of Kristianstad, Sweden. (In Swedish).
- Henrikson, L. and Oscarson, H.G. 1987. *Flodpärlmusslan i Kristianstads Län 1986*. Mimeographed report from the County Administration of Kristianstad, Sweden. (In Swedish).
- Nyström, J., Lindh, U., Dunca, E. and Mutvei, H. 1994. A study of *M. margaritifera* shells from the River Paulistörnsån, S. Sweden. *Nuc. Instrum. Methods B104*, 612-618.
- Sunde, T., Nyström, J. and Lindh, U. 1991. The new scanning nuclear microprobe in Uppsala. *Nuc. Instrum. Methods B54*, 80-83.
- FAST ComTec GmbH, Grünwalder Weg 28a, D-820 41 Oberhaching, Germany.
- Maxwell, J.A., Campbell, J.L. and Teesdale, W.J. 1989. The Gueiph PIXE software package. *Nuc. Instrum. Methods B43*, 218-230.
- Masuda, F. 1981. Variation of Mg and Na contents with growth in the outer shell layer of *Mizuhopecten yessoensis* from Mutsu Bay, northern Japan. *Ann. Rep. Inst. Geosci. Univ. Tsukuba* 7, 49-51.
- Witbaard, R., Jenness, M.I., van der Borg, K. and Ganssen, G. 1994. Verification of annual growth increments in *Arctica islandica* from the North Sea by means of oxygen and carbon isotopes. *Neth. J. Sea Res.* 33, 91-101.
- Frausto da Silva, J.J.R. and Williams, R.J.P. 1991. *In: The Biological Chemistry of the Elements - the Inorganic Chemistry of Life*. Clarendon Press, Oxford.
- Stumm, W. and Morgan, J.J. 1981. *In: Aquatic Chemistry - An Introduction Emphasizing Chemical Equilibria in Natural Waters*. Second edition. John Wiley, New York.
- Gallager, S.M., Bidwell, J.P. and Kuzirian, M. 1989. Strontium is required in artificial seawater for embryonic shell formation in two species of bivalve molluscs. *In: Origin, Evolution, and Modern Aspects of Biomineralization in Plants and Animals*. Crick, R.E. (ed.). Plenum Press, New York.
- Nelson, D.J. 1961. The strontium and calcium relationships in Clinch and Tennessee River mollusks. *In: Radioecology*. Schulz, V. and Klement, A.W. Jr. (eds). Reinhold, New York, p. 203-211.
- Hallam, A. and Price, B. 1968. Environmental and biochemical control of Sr in shells of *Cardium edule*. *Geochim. Cosmochim. Acta* 32, 319-328.
- Swann, C.P., Mueller-Hansen, K., Price, K. and Lutz, R. 1991. Application of PIXE in the study of shellfish. *Nuc. Instrum. Methods B56-57*, 683-686.
- Wülbur, K.M. 1972. Shell formation in mollusks. *In: Chemical Zoology vol. VII*. Florin, M. and Scheer, B.T. (eds). Academic Press, New York and London.
- Koike, H. 1986. Microstructure of growth increments in the shell of *Mercenaria mercenaria*. *Trans. Proc. Palaeont. Soc. Japan* 141, 289-295.
- Goldberg, E.D. 1957. Biogeochemistry of trace metals. *Geol. Soc. Am. Mem.* 67 Vol. 1, 345-358.
- Olsson, M. 1977. *Mercury, DDT and PCB in Aquatic Test Organisms*. Report from the Swedish Environment Protection Board, SNV PM 900.
- Masuda, F. 1981. Chemical composition in marine carbonates as an indicator of paleoenvironment. *Report, Grand-in-Aid. Sci. Res. (C)*, Project No. 454262, pp. 1-44.
- Rosenberg, G.D. 1980. An ontogenetic approach to the environmental significance of bivalve shell chemistry. *In: Skeletal Growth of Aquatic Organisms vol. 1*. Rhoads, D.C. and Lutz, R.A. (eds). Plenum Press, New York, pp. 133-168.
- Poulicek, M. 1986. Chitin diagenesis in deep-water sediments. *In: Chitin in Nature and Technology*. Muzzarelli, R.A.A., Gooday, G. and Jeuniaux, C. (eds.). Plenum Press, New York, pp. 523-530.
- Babutky, Y. and Chacko, J. 1992. Trace metals in an estuarine bivalve from the south-west coast of India. *Ambio* 16, 292-296.
- Yoshioka, S. and Terai, M. 1993. Manganese accumulation in freshwater bivalves. *Biomineralization '93, L'Institut Oceanographique, Monaco*. (Abstract).
- Jenne, E.A. 1968. Controls on Mn, Fe, Co, Ni, Cu, and Zn concentrations in soils and water: the significant role of hydrous Mn and Fe oxides. *In: Trace Inorganics in Water*. American Chemical Society VII, 337-384.
- Andersson, P., Gråhn, P., Hörnsström, E., Nyberg, P. and Dahlquist, K. 1989. *Kalkning och Gåsling i Rammsjön och Amien, Örebro Län 1980-1987*. Mimeographed report from the Swedish Environmental Protection Agency, report 3584. (In Swedish).
- Dickson, W. 1980. Properties of acidified waters. *Proc. Int. Conf. Ecol. Impact Acid Precip., Norway*. SNSF project, 75-83.
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