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(*Rangifer tarandus*) from northern and central Lapland,  
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**Swedish monitoring programme in terrestrial biota**

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# **SAKRAPPORT**

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**2005-05-17**

# **TIME TRENDS OF METALS IN LIVER AND MUSCLE OF REINDEER (*Rangifer tarandus*) FROM NORTHERN AND CENTRAL LAPLAND, SWEDEN, 1983-2003**

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## **INTRODUCTION**

The long-term monitoring of bio-accumulating contaminants in biota from terrestrial environments in Sweden as part of the Swedish National Environmental Monitoring Programme is based on analysis of organs and tissues of different animal species collected in certain pristine areas of the Swedish mainland (Odsjö and Olsson 1979 a,b). In the mountainous area of north-western Sweden, reindeer (*Rangifer tarandus*) is chosen as a representative indicator for the fauna living in that part of the country. Samples of reindeer have continuously been collected in three districts since the early 1980s. Later, the *Declaration on the Protection of the Arctic environment* established an *Arctic Monitoring and Assessment Programme (AMAP)* to monitor levels and assess effects of anthropogenic pollutants in components of the Arctic environment. The Programme recommends that collection of baseline data for heavy metals and radionuclides in caribou/reindeer should be mandatory for participating states due to the importance of that species in the diets of northern native people (AMAP 1993). The current material of reindeer from northern and central Lapland partly satisfies the Swedish association in the AMAP programme.

The herbivorous reindeer spend the summer time in the westernmost part of the high mountain areas. Summer diets include grasses, sedges, twigs, leaves and mushrooms. During autumn they migrate eastwards to winter grounds in the central coniferous forest areas of the country, where they primarily feed on lichens, which are noted for their ability to accumulate nutrients and contaminants from the air. Winter diets also include sedges and twigs.

The actual material of reindeer have earlier been utilised for analyses of e.g. radiocesium in a study of effects of the fallout of Cs-132 from the Chernobyl accident in 1986 (Forberg *et al.* 1992) and for studies of time trends of levels of HCHs and HCB (Odsjö *et al.* 1998).

## **AIM**

The aims of the study are to present long-term trend series of bio-accumulated concentrations of metals for the period 1983-2003 and to summarise results from statistical analyses. The analysed elements are Al, Ca, Cd, Co, Cr, Cu, Fe, Mg, Mn, Ni, V, Zn, Hg, Pb and Mo.

The present paper reports on trend studies of concentrations of metals in muscle and liver of reindeer collected from Gabna, Lævas and Girjas Saami Villages, the northernmost of the three collection districts presented below. The report also includes presentation of concentrations of metals in muscle and liver of reindeer collected in 1996-2003 in Ran and Gran Saami Villages, the central of the three collection districts presented below. Reindeer

from this district have so far been analysed from eight years only. For aluminium there are data only from two years why the extended statistics are not relevant and thus reduced. Tissues of reindeer collected 1983-1995 in Ran and Gran, not yet analysed, are stored frozen in the Environmental Specimen Bank at the Swedish Museum of Natural History and are available for retrospective analyses and trend studies. So are specimens of reindeer from the southernmost district, but have not yet been chemically analysed.

## **MATERIAL AND METHODS**

### **Sampling areas**

Collection of specimens of reindeer aimed at chemical analyses of bio-accumulating noxious substances is annually carried out in three districts along the Swedish, easternmost mountain chain.

- \* The northernmost district, Gabna, Lævas and Girjas Saami Villages, reaches from the Swedish/Norwegian border eastwards to the central forest areas in the northern part of Lapland. (Figure 1). Collection of specimens started in Rensjön and Aitejåkk in 1981.
- \* The central district, Ran and Gran Saami Villages, is situated in the mountain area in central Lapland and reaches from the Swedish/Norwegian border eastwards to the central forest areas (Figure 1). Collection of specimens started in Ammarnäs in 1983.
- \* The southernmost district, Handölsdalens and Mittådalens Saami Villages, covers the border areas between the provinces of Jämtland and Härjedalen from the Swedish/Norwegian border eastwards to the forest areas in the central part of the country. (Figure 1). Collection of specimens started in Ottsjölägret in 1982 and was moved over to Ljungris in 1989 due to changed slaughter schedules and artificial feeding prior to slaughter as a consequence of deposition of cesium polluted from Chernobyl, the Ukraine in 1986.

Since the start of the monitoring programme, collection of specimens of muscle, liver, kidney and left under jaw with teeth (for age determination) from at least 50 male reindeer has been carried out annually at regular slaughtering in each of the three districts. In 1983-1986, the muscle samples were taken from the mandibles. After that, routines were changed and muscle samples were taken from the front leg tibia. The change of muscle samples convinced us to exclude analytical results from mandible muscle in the presentation below since they were not comparable.

The slaughtering was carried out between the end of August and mid-October, mostly in mid of September. The age of the males from which samples were taken ranged mainly between 2 and 4 years. Most specimens were 3 years old. In 1998, the ordinary slaughter in the northernmost district was postponed from early September to early November, which should be noted when evaluating the time trends of levels. The fact that reindeer move from summer areas in the mountains to wintering areas in the forest means a change of diet, which might influence upon the exposition of metals via food. A later date for slaughtering means a longer foraging on winter diets that might contain higher concentrations of metals.

### **Sample preparation**

Ten male reindeer, three years old, were selected for chemical analyses annually. Multi-element analyses of metals were carried out on muscle and liver on an individual basis.

Approximately 15-20 g tissue was prepared of both muscle and liver. To avoid irrelevant contamination, the surface layer of each tissue sample was removed by ceramic knives at preparation for analyses. For extra quality assurance, the surface layer was removed once again by use of knives made of titanium (Ti) when the tissues were finally prepared at the analytical laboratory.

### Chemical analyses

For determination of Hg, continuous hydride generation by flow injection analysis and atomic absorption spectrometry was used (Galgan & Frank 1988). For pre-treatment of the samples, see: Frank 1976, 1983, 1988. Simultaneous analysis of the other 13 elements mentioned above was performed by use of a direct-current plasma-atomic emission spectrometer, DCP-AES (SpectraSpan IIIA, Applied Research Laboratories Inc., Valencia, CA, USA). For pre-treatment of the samples, see: Frank and Petersson 1983, 1985. The chemical analyses were carried out by the Department of Chemistry, National Veterinary Institute, Uppsala.

Table 1.

Number (n) of individual samples of reindeer from Gabna, Lævas and Girjas Saami Villages out of the total, found with concentrations below detection limit (D.L.). (m)=muscle (l)=liver,

Element	n below D.L.	% below D.L.	Total
Al (m)	10	6	122
Al (l)	3	2	160
Cd (m)	18	9	210
Co (m)	2	1	210
Cr (m)	25	12	210
Cr (l)	28	13	209
Ni (m)	41	20	210
Ni (l)	51	24	210
Pb (m)	64	30	210
Pb (l)	2	1	210
V (m)	90	43	209
V (l)	81	39	210
Hg (m)	24	12	200
Mo (m)	18	26	70

### Statistical treatment and graphical presentation

Statistical treatment and graphical presentation have been carried out according to Bignert (1998).

### Trend detection

One of the main purposes of the monitoring programme is to detect trends. The trend detection is carried out in three steps.

#### 1. Log-linear regression analyses

Log-linear regression analyses is performed both for the entire investigated time period and for time series longer than ten years, also for the recent ten years. The slope of the line describes the yearly percentage change. A slope of 5% implies that the concentration is halved in 14 years whereas 10% corresponds to a similar reduction in 7 years and 2% in 35 years. See Table 2, below.

Table 2. The approximate number of years required to double or half the initial concentration assuming a continuous annual change of 1, 2, 3, 4, 5, 7, 10, 12, 15 or 20% a year.

	1%	2%	3%	4%	5%	7%	10%	12%	15%	20%
Increase	70	35	24	18	14	10	7	6	5	4
Decrease	69	35	23	17	14	10	7	6	4	3

## 2. Non-parametric trend test

The regression analyses presuppose, among other things, that the regression line gives a good description of the trend. The leverage effect of points in the end of the line is also a well-known fact. An exaggerated slope, caused 'by chance' by a single or a few points in the end of the line, increases the risk of a false significant result when no real trend exists. A non-parametric alternative to the regression analysis is the Mann-Kendall trend test (Gilbert, 1987, Helsel & Hirsch, 1995, Swertz, 1995). This test has generally lower power than the regression analysis and does not take differences in magnitude of the concentrations into account, it only counts the number of consecutive years where the concentration increases or decreases compared with the year before. If the regression analysis yields a significant result but not the Mann-Kendall test, the explanation could be either that the latter test has lower power or that the influence of endpoints in the time series has become unwarrantably great on the slope. Hence, the eighth line reports Kendall's ' $\tau$ ', and the corresponding p-value. The Kendall's ' $\tau$ ' ranges from 0 to 1 like the traditional correlation coefficient ' $r$ ' but will generally be lower. 'Strong' linear correlation of 0.9 or above corresponds to  $\tau$ -values of about 0.7 or above (Helsel and Hirsch, 1995, p. 212). EPA recommended this test for use in water quality monitoring programmes with annual samples, in an evaluation comparing several other trend tests (Loftis *et al.* 1989).

## 3. Non-linear trend components

An alternative to the regression line in order to describe the development over time would be some kind of smoothed line. The smoother applied here is a simple 3-point running mean smoother fitted to the annual geometric mean values. In cases where the regression line is badly fitted the smoothed line may be more appropriate. The significance of this line is tested by means of an Analysis of Variance where the variance explained by the smoother and by the regression line is compared with the total variance. This procedure is used at assessments at ICES and is described by Nicholson *et al.*, 1995.

## Outliers and values below the detection limit

Observations too far from the regression line considering from what could be expected from the residual variance around the line is subjected to special concern. These deviations may be caused by an atypical occurrence of something in the physical environment, a changed pollution load or errors in the sampling or analytical procedure. The procedure to detect suspected outliers in this presentation is described by Hoaglin and Welsh (1978). It makes use of the *leverage coefficients* and the *standardised residuals*. The standardised residuals are tested against a  $t_{0.05}$  distribution with  $n-2$  degrees of freedom. When calculating the  $i$ th standardised residual the current observation is left out implying that the  $i$ th observation does not influence the slope nor the variance around the regression line. The suspected outliers are merely indicated in the figures and are included in the statistical calculations except in a few cases, pointed out in the figures.

Values reported below the detection limit is substituted using the 'robust' method suggested by Helsel & Hirsch (1995) p 362, assuming a lognormal distribution within a year. N.B. a

minimum of three values above LOQ (Limit of quantification) are required for this substitution, years with fewer results above LOQ are not represented in the figures.

### Legend to the plots

The analytical results from each of the investigated elements are displayed in figures. A separate plot represents each site.

The plot displays the geometric mean concentration of each year (circles) together with the individual analyses (small dots) and the 95% confidence intervals of the geometric means.

The overall geometric mean value for the time series is depicted as a horizontal, thin, dashed line.

The trend is presented by one regression line (plotted if  $p < 0.10$ , two-sided regression analysis). Ten years is often too short a period to statistically detect a trend unless it is of considerable magnitude. Furthermore, the residual variance around the line compared to the residual variance for the entire period will indicate if the sensitivity have increased as a result of e.g. an improved sampling technique or that problems in the chemical analysis have disappeared.

A smoother is applied to test for non-linear trend components. The smoothed line is plotted if  $p < 0.10$ . A broken line or a dashed line segment indicates a gap in the time series with a missing year.

The log-linear regression lines fitted through the geometric mean concentrations follow smooth exponential functions.

A cross inside a circle, indicates a suspected outlier, see above. The suspected outliers are merely indicated in the figures and are included in the statistical calculations except in a few cases, pointed out in the figures.

Each plot has a header with element, species name, tissue and sampling locality. Below the header of each plot the results from several statistical calculations are reported:

**n(tot)** = The first line reports the total number of analyses included together with the number of years ( **n(yrs)** = ).

**m** = The overall geometric mean value together with its 95% confidence interval is reported on the second line of the plot (N.B. d.f.= n of years - 1).

**slope** = reports the slope, expressed as the yearly percentage change together with its 95% confidence interval.

**SD(lr)** = reports the square root of the residual variance around the regression line, as a measure of between-year variation, together with the *lowest detectable change* in the current time series with a power of 80%, one-sided test,  $\alpha=0.05$ . The last figure on this line is the estimated *number of years* required to detect an annual change of 5% with a power of 80%, one-sided test,  $\alpha=0.05$ .

**power** = reports the power to detect a log-linear trend in the time series (Nicholson & Fryer, 1991). The first figure represents the power to detect an annual change of 5% with the number of years in the current time series. The second figure is the power estimated as if the slope were 5% a year and the number of years were ten. The third figure is the *lowest detectable change* for a ten-year period with the current between year variation at a power of 80%.

**y(03)** = reports the concentration estimated from the regression line for the last year together with a 95% confidence interval, e.g.  $y(03)=40.6(36.6,45.0)$  is the estimated concentration of



year 2003 where the residual variance around the regression line is used to calculate the confidence interval. Provided that the regression line is relevant to describe the trend, the residual variance might be more appropriate than the within-year variance in this respect.

$r^2$  = reports the coefficient of determination ( $r^2$ ) together with a p-value for a two-sided test ( $H_0$ : slope = 0) i.e. a significant value is interpreted as a true change, provided that the assumptions of the regression analysis is fulfilled.

$\tau$  = reports Kendall's ' $\tau$ ', and the corresponding p-value.

$SD(sm)$  = reports the square root of the residual variance around the smoothed line. The significance of this line could be tested by means of an Analysis of Variance. The p-value is reported for this test. A significant result will indicate a non-linear trend component.

## RESULT

### Long-term trends of metals in liver and muscle of reindeer

The analytical and statistical results from Gabna, Lævas and Girjas Saami Villages are displayed in Figure 2-8, which visualise the trends of metal concentration in muscle and liver of reindeer from the period 1983-2003. The data of metal concentrations in muscle of reindeer from Gabna, Lævas and Girjas Saami Villages, collected in 1983-1986, was excluded from the statistical calculations and is not visualised in the graphs. The reason is that muscle samples during the early part of the period were taken from the mandibles. From 1987 onwards, muscle samples were taken from the front leg tibia and we have reason to believe that concentrations in the both tissues are not fully comparable.

The analytical results from Ran and Gran Saami Villages from 1996-2003 are displayed in Figure 9-15. The time series is continuously updated once a year when new material is collected and analysed.

## Gabna, Lævas and Girjas Saami Villages

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### Aluminium (Al) *(Figure 2)*

Residue levels of aluminium have been analysed in muscle of reindeer from the period 1987-1998, and in liver from the period 1983-1998.

The aluminium concentrations in muscle and liver of reindeer show no significant log-linear trend during the period (parametric test).

The number of years required to detect an annual change of 5% was 30 years for both muscle and liver with a power to detect a 5% annual change varying between 0.10 and 0.18 for the full period.

The ANOVA test showed that the smoothed lines for concentrations of aluminium in both muscle and liver indicate a significant non-linear trend component ( $p < 0.077$ , muscle;  $p < 0.002$ , liver).

The overall geometric mean value of aluminium in muscle and liver was 0.143 and 0.332  $\mu\text{g/g}$  (fresh weight), respectively for the period 1987-1998 and 1983-1998.

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### **Calcium (Ca)** (Figure 2)

The calcium concentrations in muscle and liver of reindeer show no significant log-linear trend during the period (parametric test).

The number of years required to detect an annual change of 5% was 14 and 9 years for muscle and liver respectively, with a power to detect a 5% annual change varying between 0.99 and 1.0 for the full period.

The overall geometric mean value of calcium in muscle and liver was 39.1 and 42.6 µg/g (fresh weight) respectively, for the period 1987-2003 and 1983-2003.

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### **Cadmium (Cd)** (Figure 3)

The cadmium concentrations in muscle of reindeer show a significant log-linear change during the period ( $p < 0.063$ ; parametric test), while the concentrations in liver show no significant change during the period (parametric test). The average annual decrease in muscle was 6.8 %.

The number of years required to detect an annual change of 5% was 27 and 13 years for muscle and liver respectively, with a power to detect a 5% annual change varying between 0.20 and 1.0 for the full period.

The ANOVA test showed that the smoothed lines for concentrations of cadmium in both muscle and liver indicate a significant non-linear trend component ( $p < 0.097$ , muscle;  $p < 0.023$ , liver).

The overall geometric mean value of cadmium in muscle and liver was 0.012 and 0.434 µg/g (fresh weight) respectively, for the periods 1987-2003 and 1983-2003.

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### **Cobalt (Co)** (Figure 3)

The cobalt concentrations in liver of reindeer show a significant log-linear change during the period ( $p < 0.050$ ; parametric test), while the concentrations in muscle show no significant change during the period (parametric test). The average annual decrease in liver was 1.6%.

The number of years required to detect an annual change of 5% was 44 and 13 years for muscle and liver respectively, with a power to detect a 5% annual change varying between 0.09 and 1.0 for the full period.

The ANOVA test showed that the smoothed line for concentrations of cobalt in liver indicates a significant non-linear trend component ( $p < 0.011$ ).

The overall geometric mean value of cobalt in muscle and liver was 0.007 and 0.146 µg/g (fresh weight) respectively, for the periods 1987-2003 and 1983-2003.

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### **Chromium (Cr)** (Figure 4)

The chromium concentrations in muscle and liver of reindeer show no significant log-linear change during the period (parametric test).

The number of years required to detect an annual change of 5% was 38 and 28 years for muscle and liver respectively, with a power to detect a 5% annual change varying between 0.11 and 0.13 for the full period.

The ANOVA test showed that the smoothed line for concentrations of chromium in liver indicates a significant non-linear trend component ( $p < 0.001$ ).

The overall geometric mean value of chromium in muscle and liver was 0.020 and 0.014  $\mu\text{g/g}$  (fresh weight) respectively, for the periods 1987-2003 and 1983-2003.

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### **Copper (Cu)** (Figure 4)

The copper concentrations in liver of reindeer show a significant log-linear change during the period ( $p < 0.006$ ; parametric test), while the concentrations in muscle show no significant change during the period (parametric test). The average annual increase in liver was 2.7 %.

The number of years required to detect an annual change of 5% was 8 and 15 years for muscle and liver respectively, with a power of 1.0 to detect a 5% annual change in both tissues for the full period.

The overall geometric mean value of copper in muscle and liver was 1.33 and 68.2  $\mu\text{g/g}$  (fresh weight) respectively, for the periods 1987-2003 and 1983-2003.

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### **Iron (Fe)** (Figure 5)

The iron concentrations in muscle and liver of reindeer show no significant log-linear change during the period (parametric test).

The number of years required to detect an annual change of 5% was 9 and 18 years for muscle and liver respectively, with a power to detect a 5% annual change varying between 1.0 and 0.96 for the full period.

The ANOVA test showed that the smoothed line for concentrations of iron in muscle indicates a significant non-linear trend component ( $p < 0.048$ ).

The overall geometric mean value of iron in muscle and liver was 29.3 and 143  $\mu\text{g/g}$  (fresh weight) respectively, for the periods 1987-2003 and 1983-2003.

It should be noted that concentration of iron might vary with amount of blood and red blood capsules in the analysed samples.

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### **Magnesium (Mg)** (Figure 5)

The magnesium concentrations in muscle and liver of reindeer show no significant log-linear change during the period (parametric test).

The number of years required to detect an annual change of 5% was 10 and 7 years for muscle and liver respectively, with a power of 1.0 for both tissues to detect a 5% annual change for the full period.

The ANOVA test showed that the smoothed lines for concentrations of magnesium in both muscle and liver indicate a significant non-linear trend component ( $p < 0.023$ , muscle;  $p < 0.037$ , liver).

The overall geometric mean value of magnesium in muscle and liver was 201 and 184  $\mu\text{g/g}$  (fresh weight) respectively, for the periods 1987-2003 and 1983-2003.

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### **Manganese (Mn)** (Figure 6)

The manganese concentrations in liver of reindeer show a significant log-linear change during the period ( $p < 0.051$ ; parametric test), while the concentrations in muscle show no significant log-linear trends during the period (parametric test). The average annual decrease was 2.0%.

The number of years required to detect an annual change of 5% was 11 and 15 years for muscle and liver respectively, with a power of 1.0 in both muscle and liver series to detect a 5% annual change for the full period.

The ANOVA test showed that the smoothed lines for concentrations of manganese in both muscle and liver indicate a significant non-linear trend component ( $p < 0.075$ , muscle;  $p < 0.001$ , liver).

The overall geometric mean value of manganese in muscle and liver was 0.148 and 2.80  $\mu\text{g/g}$  (fresh weight) respectively, for the periods 1987-2003 and 1983-2003.

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### **Nickel (Ni)** (Figure 6)

The nickel concentrations in muscle and liver of reindeer show no significant log-linear change during the period (parametric test).

The number of years required to detect an annual change of 5% was 38 and 26 years for muscle and liver respectively, with a power to detect a 5% annual change of 0.08 and 0.12 for the full period.

The ANOVA test showed that the smoothed lines for concentrations of nickel in both muscle and liver indicate a significant non-linear trend component ( $p < 0.047$ , muscle;  $p < 0.019$ , liver).

The overall geometric mean value of nickel in muscle and liver was 0.012 and 0.015  $\mu\text{g/g}$  (fresh weight) respectively, for the periods 1987-2003 and 1983-2003.

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### **Vanadium (V)** (Figure 7)

During the period 1994-2003, except for 1998, the concentrations of vanadium in all analysed muscle samples were below the detection limit. As there were so many, values under the detection limit have been excluded from calculations of any mean values. In 1994-2003, concentrations of vanadium in all analysed liver samples, except for 1999-2001 were also below the detection limit. Also these values were excluded when calculating any mean values.

The vanadium concentrations in liver of reindeer show a significant log-linear change during the period ( $p < 0.036$ , parametric test). The average annual decrease was 8.2 %.

The ANOVA test showed that the smoothed line for concentrations of vanadium in liver indicates a significant non-linear trend component ( $p < 0.003$ ).

The number of years required to detect an annual change of 5% was 25 and 29 years for muscle and liver respectively, with a power to detect a 5% annual change varying between 0.06 and 0.14 for the full period. However, considering the large number of years missing from this data set, calculations of power are a blunt tool.

The overall geometric mean value of vanadium in muscle and liver was 0.006 and 0.007  $\mu\text{g/g}$  (fresh weight) respectively, for the periods 1987-1998 and 1983-2001.

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### **Zinc (Zn)** (Figure 7)

The zinc concentrations in muscle and liver of reindeer show no significant log-linear change during the period (parametric test).

The number of years required to detect an annual change of 5% was 8 and 12 years for muscle and liver respectively, with a power of 1.0 to detect a 5% annual change in both tissues for the full period.

The overall geometric mean value of zinc in muscle and liver was 80.4 and 33.6 µg/g (fresh weight) respectively, for the period 1987-2003 and 1983-2003.

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### **Mercury (Hg)** (Figure 8)

The mercury concentrations in muscle of reindeer show a significant log-linear change during the period ( $p < 0.003$ ; parametric test), while the concentrations in liver show no significant log-linear trends during the period (parametric test). The average annual increase was 9.6 %.

The number of years required to detect an annual change of 5% was 23 and 21 years for muscle and liver respectively, with a power to detect a 5% annual change varying between 0.29 and 0.78 for the full period.

The overall geometric mean value of mercury in muscle and liver was 0.001 and 0.041 µg/g (fresh weight) respectively, for the period 1987-2003 and 1983-2003.

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### **Lead (Pb)** (Figure 8)

The lead concentrations in liver of reindeer show a significant log-linear change during the period ( $p < 0.049$ , parametric test), while the concentrations in muscle show no significant log-linear trend during the period (parametric test). The average annual decrease was 3.5 %.

The number of years required to detect an annual change of 5% was 41 and 21 years for muscle and liver respectively, with a power to detect a 5% annual change varying between 0.07 and 0.81 for the full period.

The overall geometric mean value of lead in muscle and liver was 0.013 and 0.117 µg/g (fresh weight) respectively, for the period 1987-2003 and 1983-2003.

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### **Molybdenum (Mo)** (Figure 9)

The molybdenum concentrations in muscle of reindeer show a significant log-linear change during the period ( $p < 0.079$ , parametric test), while the concentrations in liver show no significant log-linear trend during the period (parametric test). The average annual decrease was 26 %.

The ANOVA test showed that the smoothed line for concentrations of molybdenum in muscle indicates a significant non-linear trend component ( $p < 0.004$ ).

The number of years required to detect an annual change of 5% was 26 and 11 years for muscle and liver respectively, with a power to detect a 5% annual change varying between 0.06 and 0.74 for the full period.

The overall geometric mean value of molybdenum in muscle and liver was 0.003 and 0.626 µg/g (fresh weight) respectively, for the period 1997-2003 and 1994-2003.

## Ran and Gran Saami Villages

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### **Aluminium (Al)** (Figure 10)

Aluminium have been analysed in muscle and liver sampled only in 1996 and 1997. Thus no statistics are reported

The geometric mean value of aluminium in muscle and liver was 0.035 and 0.042  $\mu\text{g/g}$  (fresh weight) respectively, for the two only years 1996-1997.

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### **Calcium (Ca)** (Figure 10)

The calcium concentrations in liver of reindeer show a significant log-linear change during the period ( $p < 0.075$ ; parametric test), while no significant log-linear change in muscle was shown during the period (parametric test). The average annual decrease in liver was 3.4 %.

The number of years required to detect an annual change of 5% was 9 years for both muscle and liver, with a power to detect a 5% annual change varying between 0.70 and 0.72 for the full period.

The overall geometric mean value of calcium in muscle and liver was 43.0 and 38.7  $\mu\text{g/g}$  (fresh weight) respectively, for the period 1996-2003.

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### **Cadmium (Cd)** (Figure 11)

The levels of cadmium in muscle from 1996 were all below the detection limit and were excluded from any calculation of a mean value and statistical calculations.

The cadmium concentrations in muscle and liver of reindeer show no significant log-linear change during the period (parametric test).

The number of years required to detect an annual change of 5% was 22 and 12 years for muscle and liver respectively, with a power to detect a 5% annual change varying between 0.07 and 0.32 for the full period.

The overall geometric mean value of cadmium in muscle and liver was 0.007 and 0.479  $\mu\text{g/g}$  (fresh weight) respectively, for the period 1997-2003 and 1996-2003.

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### **Cobalt (Co)** (Figure 11)

The cobalt concentrations in muscle and liver of reindeer show no significant log-linear change during the period (parametric test).

The ANOVA test shows a significant non-linear trend component for the concentrations of cobalt in liver ( $p < 0.010$ ).

The number of years required to detect an annual change of 5% was 13 years for both muscle and liver, with a power to detect a 5 % annual change varying between 0.24 and 0.25 for the full period.

The overall geometric mean value of cobalt in muscle and liver was 0.005 and 0.103  $\mu\text{g/g}$  (fresh weight) respectively, for the period 1996-2003.

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### **Chromium (Cr)** (Figure 12)

The levels of chromium in muscle and liver from 1996 were all below the detection limit and were excluded from any calculation of mean values and statistical calculations.

The chromium concentrations in muscle and liver of reindeer show no significant log-linear change during the period (parametric test).

The number of years required to detect an annual change of 5% was 21 and 16 years for muscle and liver respectively, with a power varying between 0.08 and 0.11 to detect a 5% annual change in both tissues for the full period.

The overall geometric mean value of chromium in muscle and liver was 0.028 and 0.013  $\mu\text{g/g}$  (fresh weight) respectively, for the period 1997-2003.

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### **Copper (Cu)** (Figure 12)

The copper concentrations in liver of reindeer show a significant log-linear change during the period ( $p < 0.049$ ; parametric test), while the concentrations in muscle show no significant log-linear trends during the period (parametric test). The average annual increase in liver was 7.4%.

The number of years required to detect an annual change of 5% was 8 and 13 years for muscle and liver respectively, with a power to detect a 5% annual change varying between 0.91 and 0.26 for the full period.

The overall geometric mean value of copper in muscle and liver was 1.31 and 57.3  $\mu\text{g/g}$  (fresh weight) respectively, for the period 1996-2003.

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### **Iron (Fe)** (Figure 13)

The iron concentrations in muscle of reindeer show a significant log-linear increase during the period ( $p < 0.010$ ; parametric test), while the concentrations in liver show no significant change during the period (parametric test). The average annual increase in muscle was 4.1%.

The number of years required to detect an annual change of 5% was 7 and 9 years for muscle and liver respectively, with a power to detect a 5% annual change varying between 0.97 and 0.72 for the full period.

The ANOVA test showed that the smoothed lines for concentrations of iron in both muscle and liver indicate a significant non-linear trend component ( $p < 0.013$ , muscle;  $p < 0.018$ , liver).

The overall geometric mean value of iron in muscle and liver was 29.4 and 92.2  $\mu\text{g/g}$  (fresh weight) respectively, for the period 1996-2003.

It should be noted that concentration of iron might vary with amount of blood and red blood capsules in the analysed samples.

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### **Magnesium (Mg)** (Figure 13)

The magnesium concentrations in muscle and liver of reindeer show no significant log-linear change during the period (parametric test).

The number of years required to detect an annual change of 5% was 10 and 7 years for muscle and liver respectively, with a power to detect a 5% annual change varying between 0.61 and 1.0 for the full period.

The overall geometric mean value of magnesium in muscle and liver was 193 and 178  $\mu\text{g/g}$  (fresh weight) respectively, for the period 1996-2003.

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### **Manganese (Mn)** (Figure 14)

The manganese concentrations in muscle and liver of reindeer show no significant log-linear change during the period (parametric test).

The number of years required to detect an annual change of 5% was 11 and 16 years for muscle and liver respectively, with a power to detect a 5% annual change varying between 0.41 and 0.14 for the full period.

The ANOVA test showed that the smoothed line for concentrations of manganese in liver indicates a significant non-linear trend component ( $p < 0.016$ ).

The overall geometric mean value of manganese in muscle and liver was 0.150 and 2.61  $\mu\text{g/g}$  (fresh weight), respectively for the period 1996-2003.

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### **Nickel (Ni)** (Figure 14)

The levels of nickel in muscle from 1996, 2002 and 2003 were all below the detection limit and were excluded from any calculation of mean values and statistical calculations, as well as levels in liver from 1996 and 2001-2003.

The nickel concentrations in muscle and liver of reindeer show a significant log-linear change during the period 1997-2001 and 1997-2000, respectively (average annual decrease 32%,  $p < 0.067$ , muscle; 25%,  $p < 0.051$ , liver; parametric test).

The number of years required to detect an annual change of 5% was 18 and 10 years for muscle and liver respectively, with a power of 0.06 and 0.09 to detect a 5% annual change in both tissues for the full period.

The overall geometric mean value of nickel in muscle and liver was 0.015 and 0.017  $\mu\text{g/g}$  (fresh weight), respectively for the period 1997-2001, and 1997-2000.

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### **Vanadium (V)** (Figure 15)

All levels of vanadium in muscle from the period were below the detection limit except for 2001. These levels were all excluded of any calculations of mean values and statistical calculations. The levels in liver from 1996-1997 and 2002 were all below the detection limit and were also excluded from any calculation of mean values and statistical calculations.



The vanadium concentrations in muscle and liver of reindeer show no significant log-linear change during the period (parametric test).

The number of years required to detect an annual change of 5% was 27 years for liver, with a power to detect a 5% annual change of 0.06 for the full period.

The overall geometric mean value of vanadium in liver was 0.002 µg/g (fresh weight), for the period 1998-2003.

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### **Zinc (Zn)** (Figure 15)

The zinc concentrations in muscle of reindeer show a significant log-linear change during the period ( $p < 0.049$ ; parametric test). The average annual increase was 2.3 %.

The number of years required to detect an annual change of 5% was 7 and 14 years for muscle and liver respectively, with a power to detect a 5% annual change varying between 0.99 and 0.20 for the full period.

The overall geometric mean value of zinc in muscle and liver was 81.2 and 29.2 µg/g (fresh weight) respectively, for the period 1996-2003.

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### **Mercury (Hg)** (Figure 16)

The mercury concentrations in muscle and liver of reindeer show no significant log-linear change during the period (parametric test).

The number of years required to detect an annual change of 5% was 25 and 21 years for muscle and liver respectively, with a power to detect a 5% annual change varying between 0.07 and 0.09 for the full period.

The ANOVA test shows that the smoothed lines for the concentrations of mercury in muscle and liver of reindeer indicate a non-linear trend component ( $p < 0.029$ , muscle;  $p < 0.048$ , liver).

The overall geometric mean value of mercury in muscle and liver was 0.001 and 0.032 µg/g (fresh weight) respectively, for the period 1996-.

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### **Lead (Pb)** (Figure 16)

The levels of lead in muscle from 1996-1998 and 2002 were all below the detection limit and were excluded from any calculation of a mean value and statistical calculations.

The lead concentrations in muscle and liver of reindeer show no significant log-linear change during the period (parametric test).

The number of years required to detect an annual change of 5% was 30 and 20 years for muscle and liver respectively, with a power between 0.06 and 0.10 to detect a 5% annual change in both tissues for the full period.

The overall geometric mean value of lead in muscle and liver was 0.005 and 0.132 µg/g (fresh weight) respectively, for the period 1999-2003 and 1996-2003.

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Figure 1. Sampling areas for reindeer in Sweden

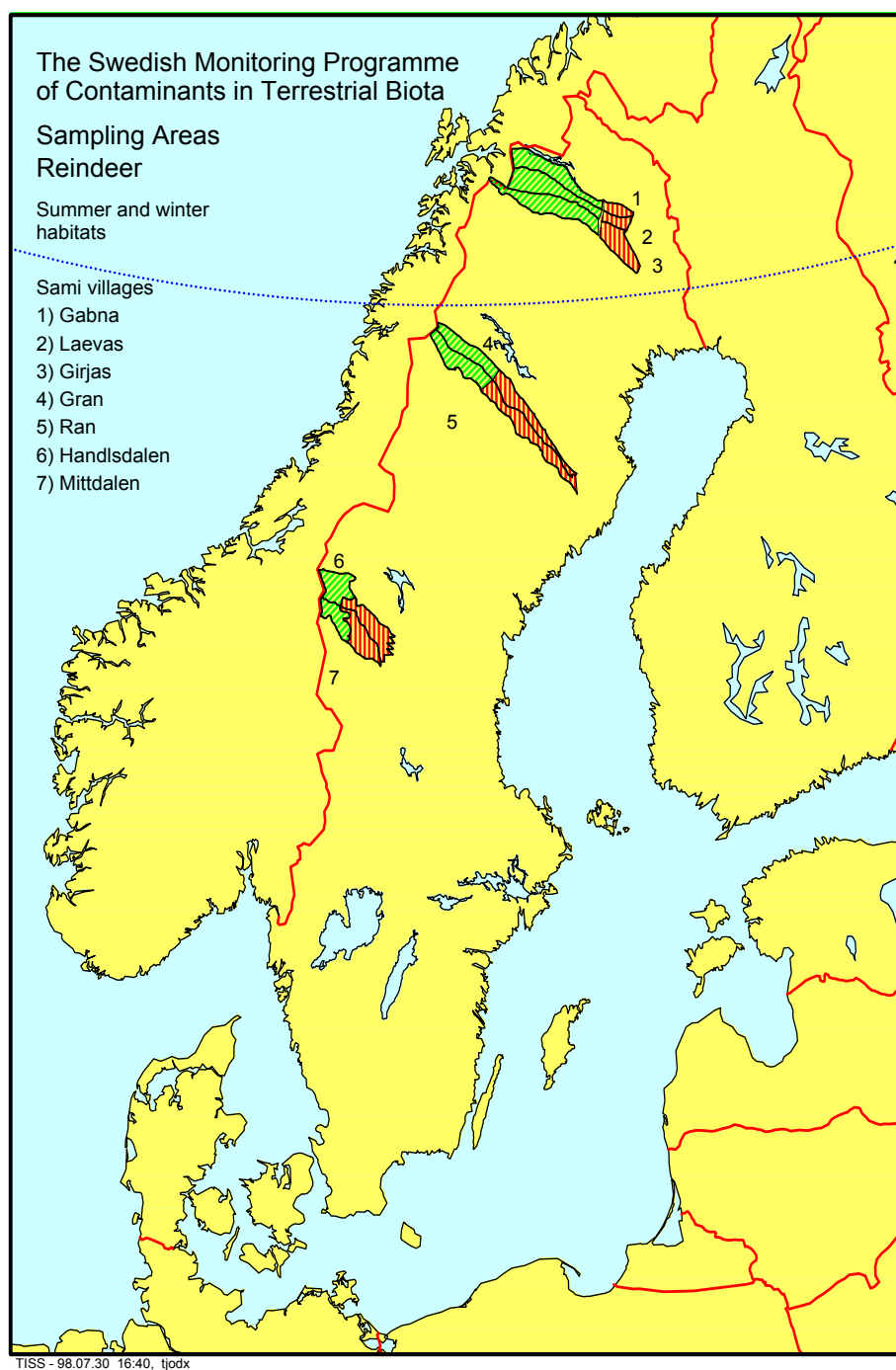


Figure 2. Al and Ca in reindeer from Gabna, Lævas and Girjas Saami Villages, N. Lapland. ( $\mu\text{g/g}$  fresh weight).

Log-linear regression on geometric means, suspected outliers indicated. Smoother: 3-point running mean, unweighted.

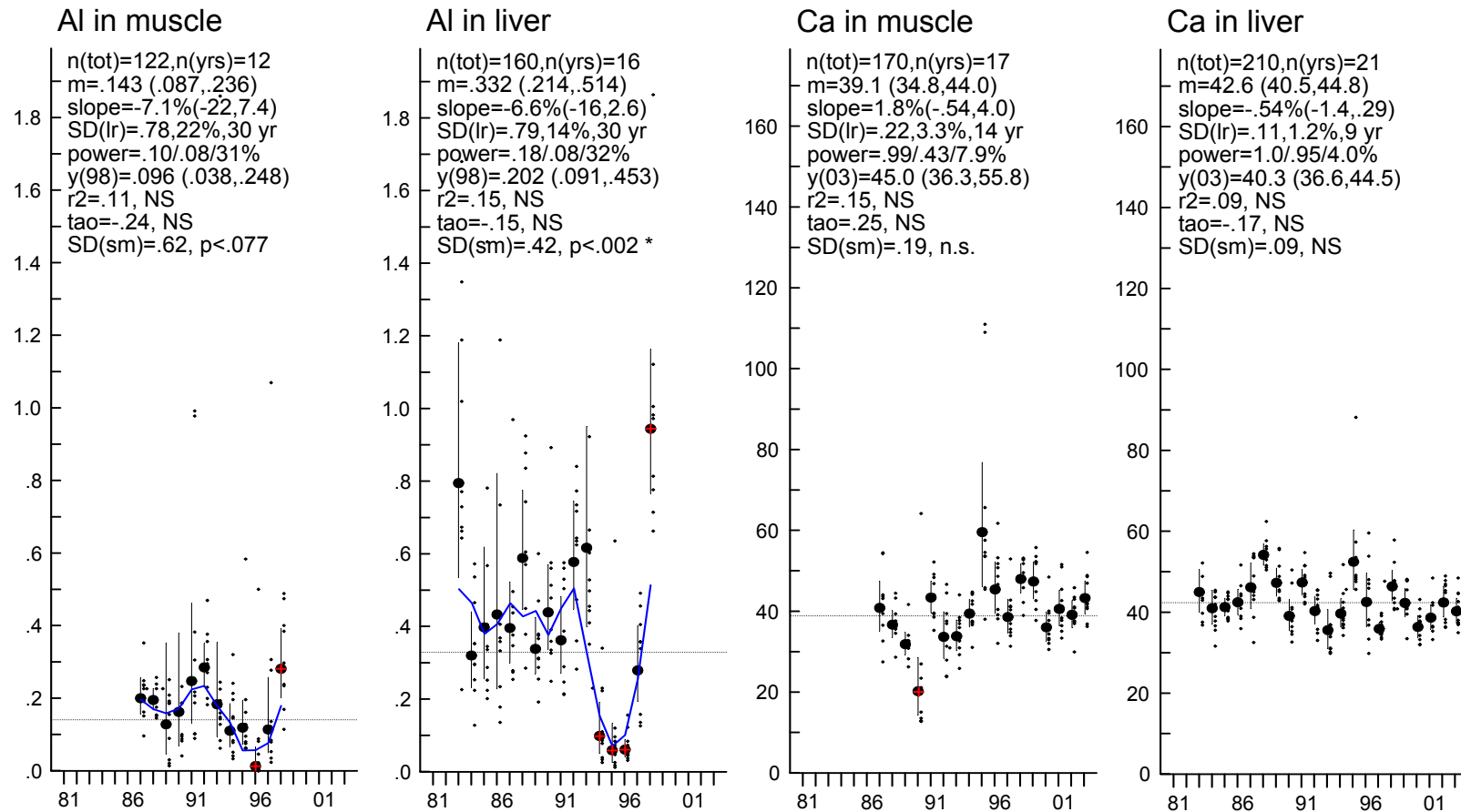
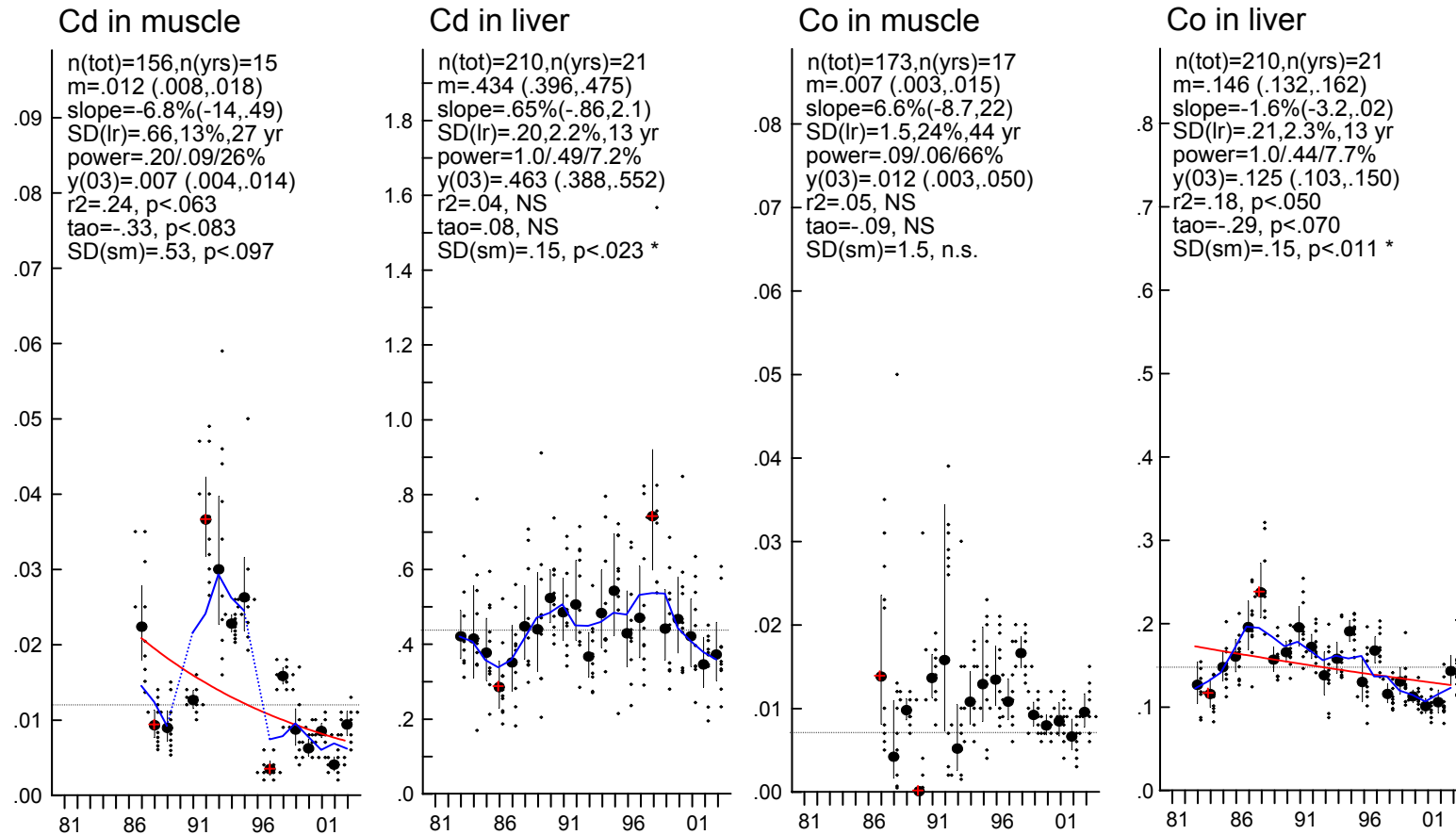
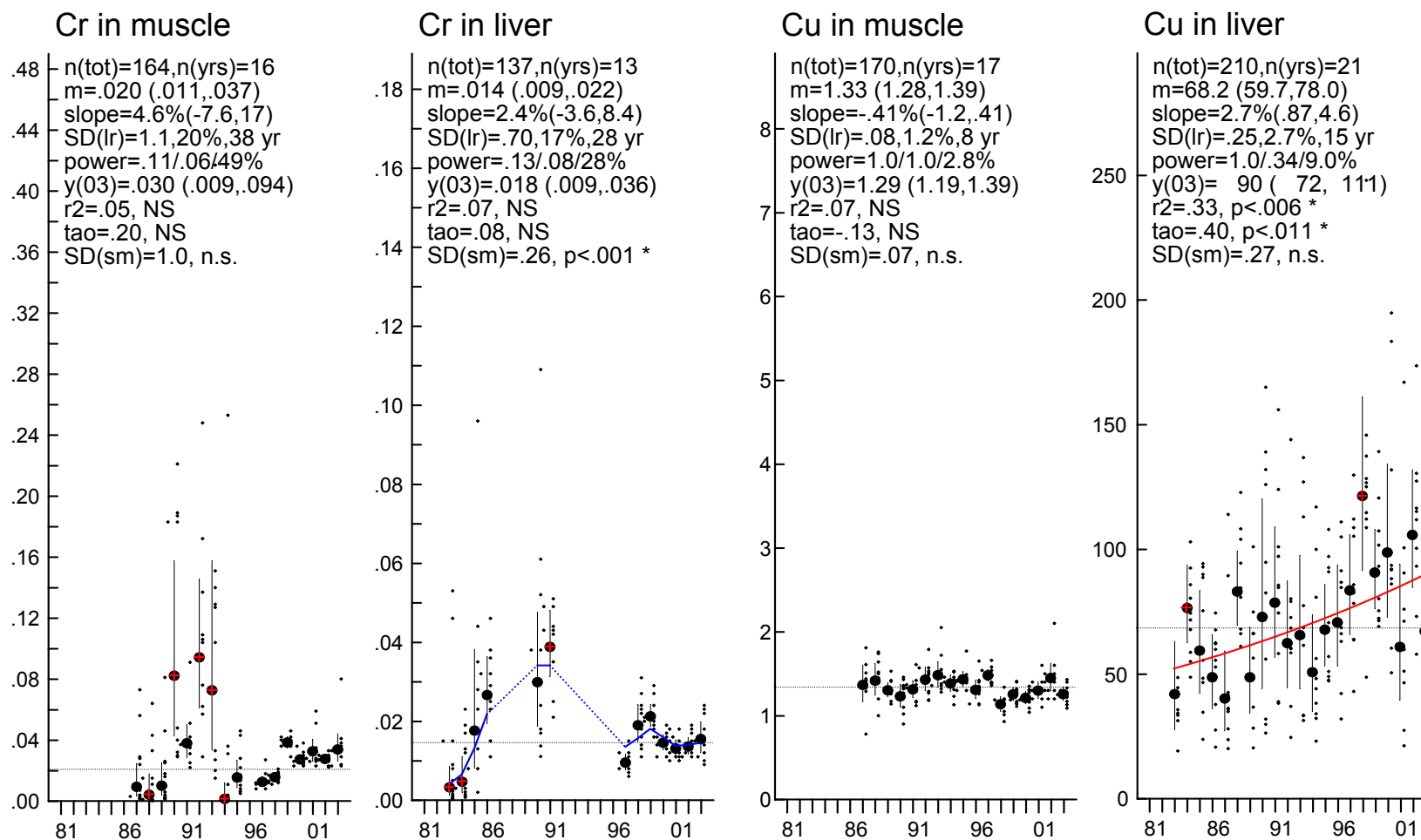


Figure 3. Cd and Co in reindeer from Gabna, Lævas and Girjas Saami Villages, N. Lapland. ( $\mu\text{g/g}$  fresh weight).  
Log-linear regression on geometric means, suspected outliers indicated. Smoother: 3-point running mean, unweighted.



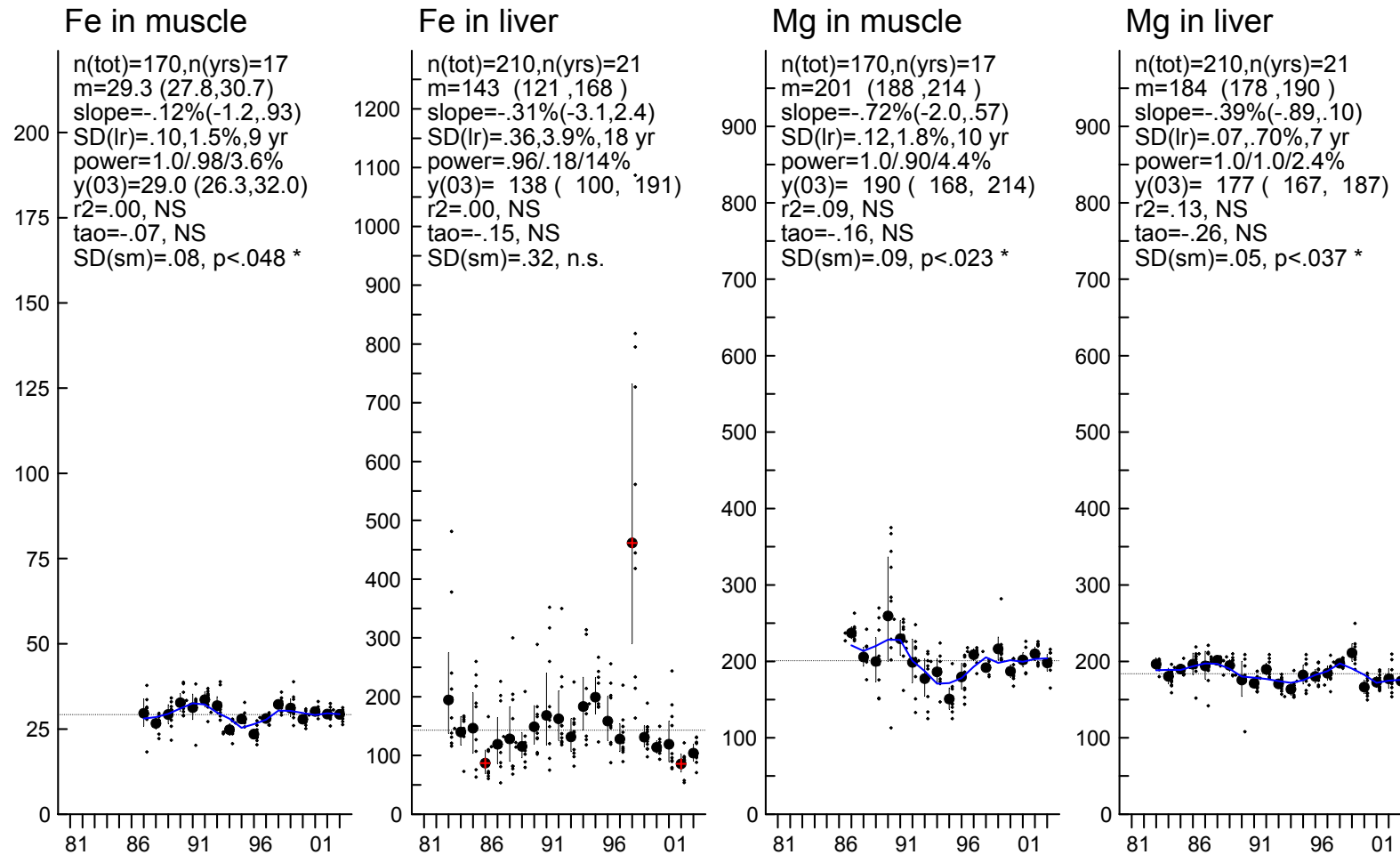
pia - 05.05.10 16:09, 1NYCdCo

Figure 4. Cr and Cu in reindeer from Gabna, Løevas and Girjas Saami Villages, N. Lapland. ( $\mu\text{g/g}$  fresh weight).  
Log-linear regression on geometric means, suspected outliers indicated. Smoother: 3-point running mean, unweighted.



pia - 05.05.10 16:09, 1NYCrCu

Figure 5. Fe and Mg in reindeer from Gabna, Lævas and Girjas Saami Villages, N. Lapland. ( $\mu\text{g/g}$  fresh weight).  
Log-linear regression on geometric means, suspected outliers indicated. Smoother: 3-point running mean, unweighted.



pia - 05.05.10 16:10, 1NYFeMg



Figure 6. Mn and Ni in reindeer from Gabna, Lævas and Girjas Saami Villages, N. Lapland. ( $\mu\text{g/g}$  fresh weight).  
Log-linear regression on geometric means, suspected outliers indicated. Smoother: 3-point running mean, unweighted.

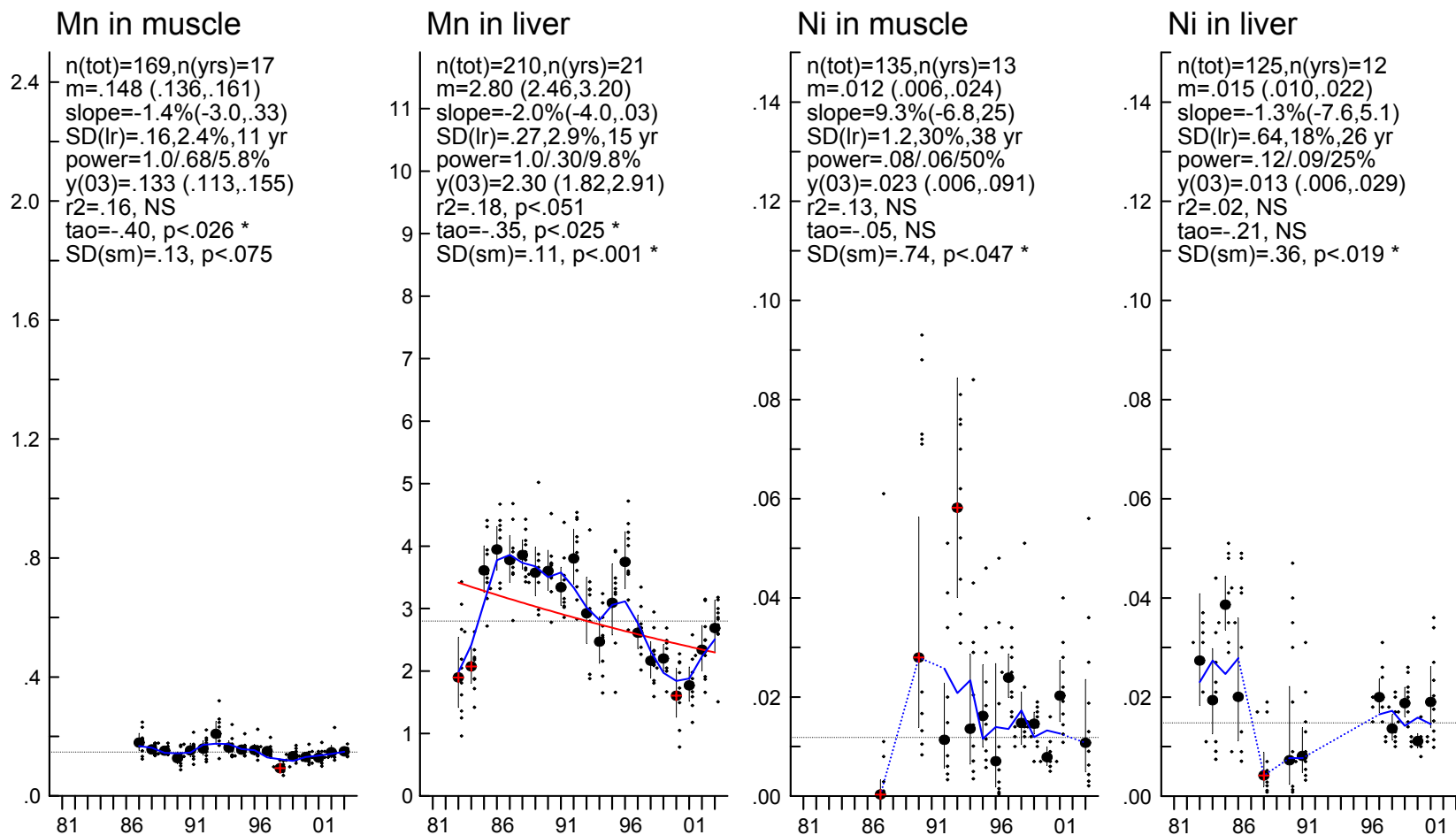
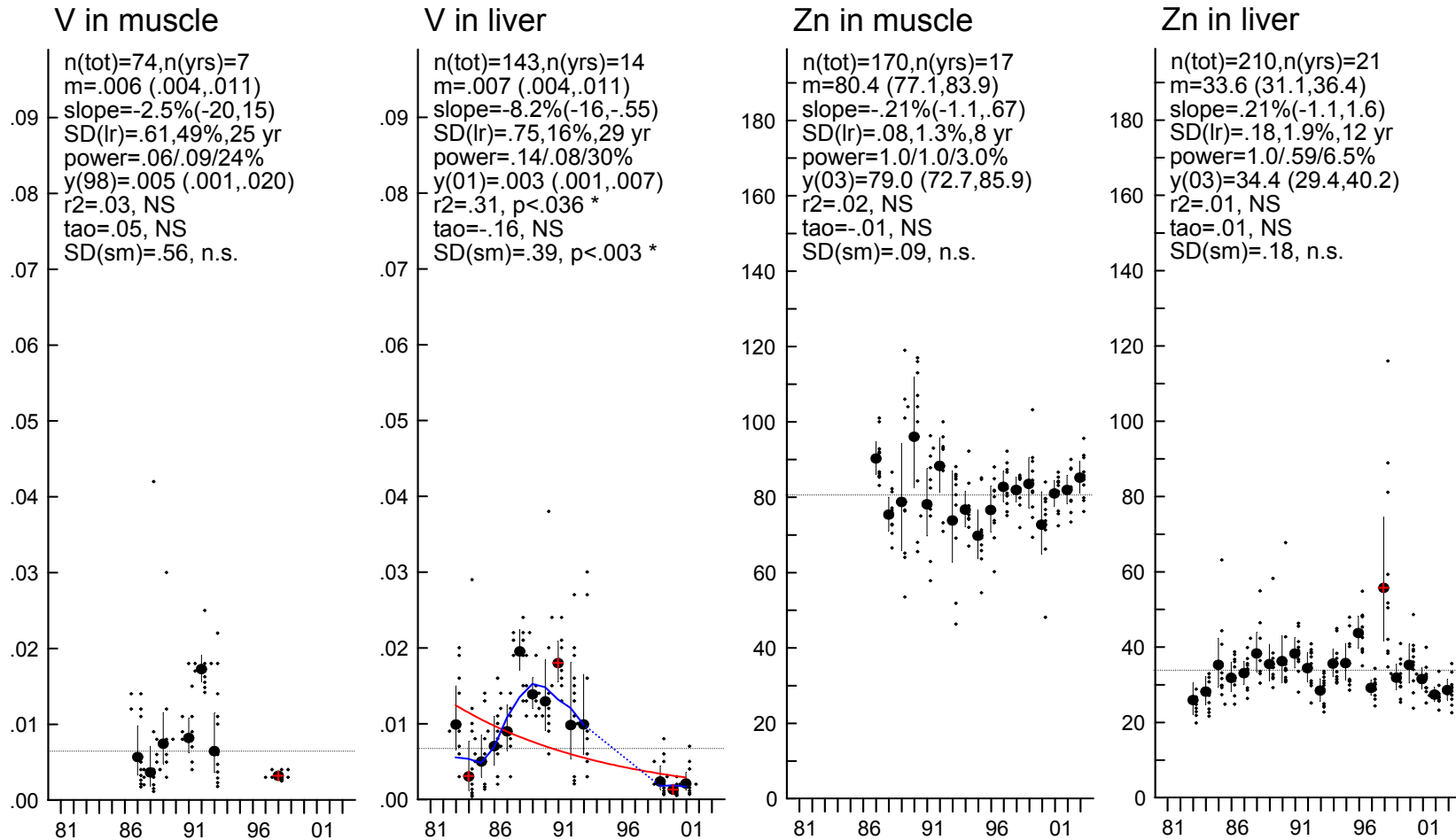
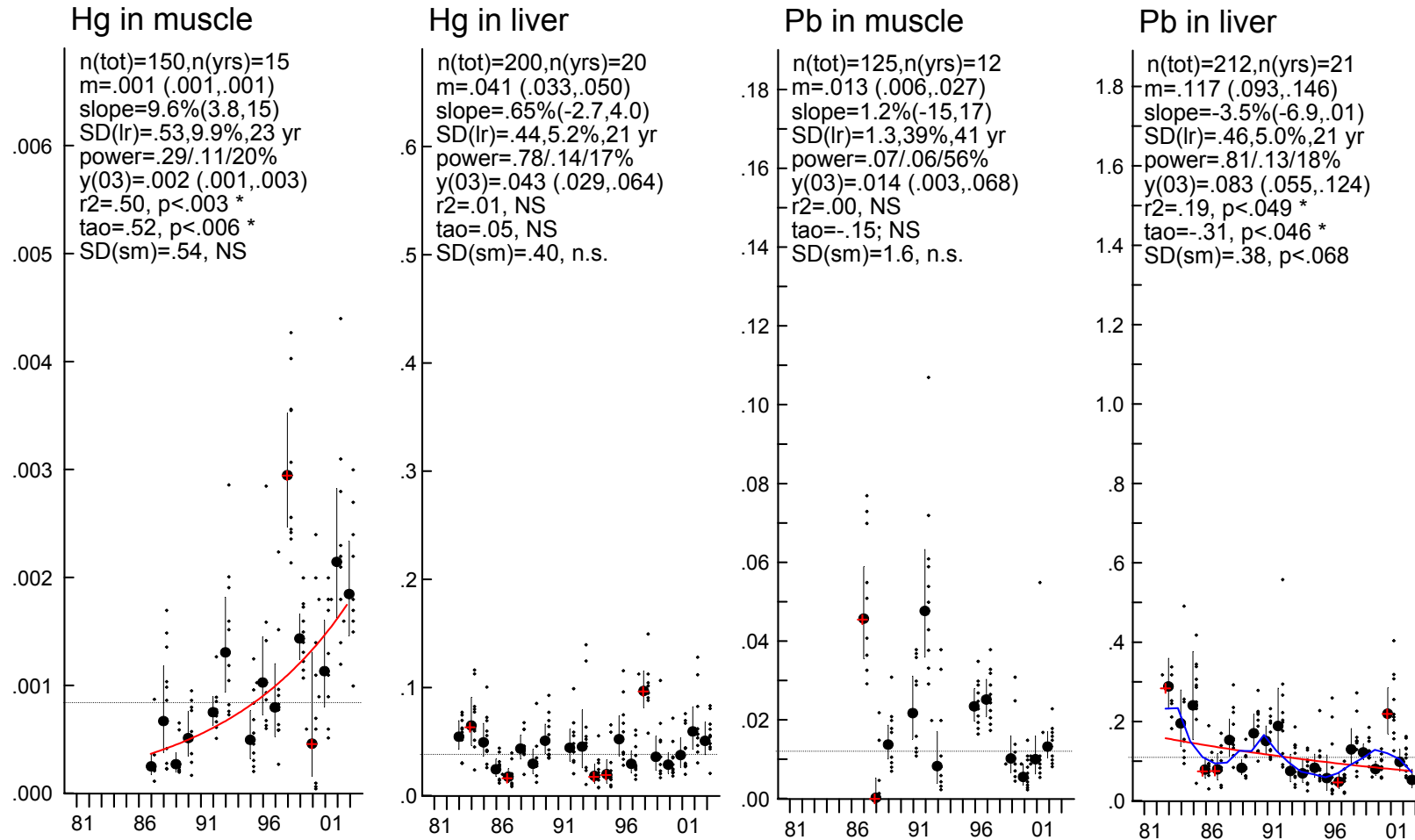


Figure 7. V and Zn in reindeer from Gabna, Løvas and Girjas Saami Villages, N. Lapland. ( $\mu\text{g/g}$  fresh weight).  
Log-linear regression on geometric means, suspected outliers indicated. Smoother: 3-point running mean, unweighted.



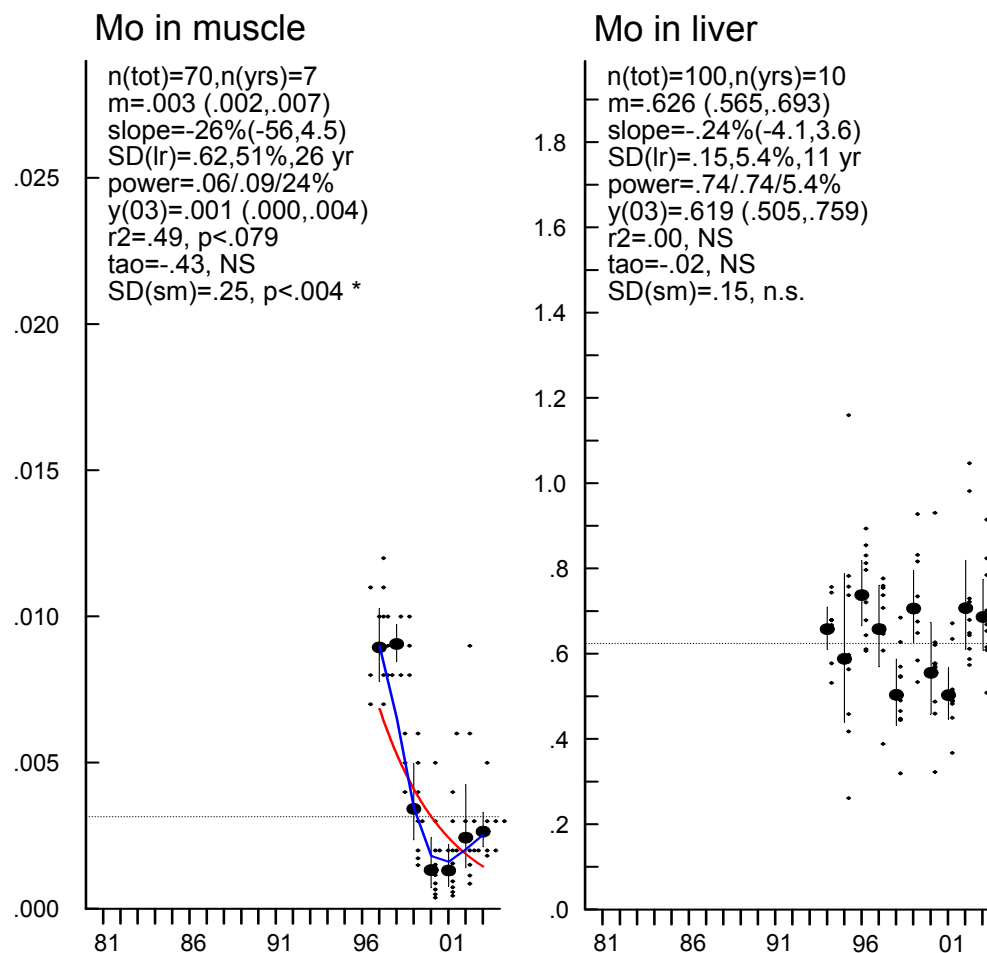
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Figure 8. Hg and Pb in reindeer from Gabna, Lævas and Girjas Saami Villages, N. Lapland. ( $\mu\text{g/g}$  fresh weight).  
Log-linear regression on geometric means, suspected outliers indicated. Smoother: 3-point running mean, unweighted.



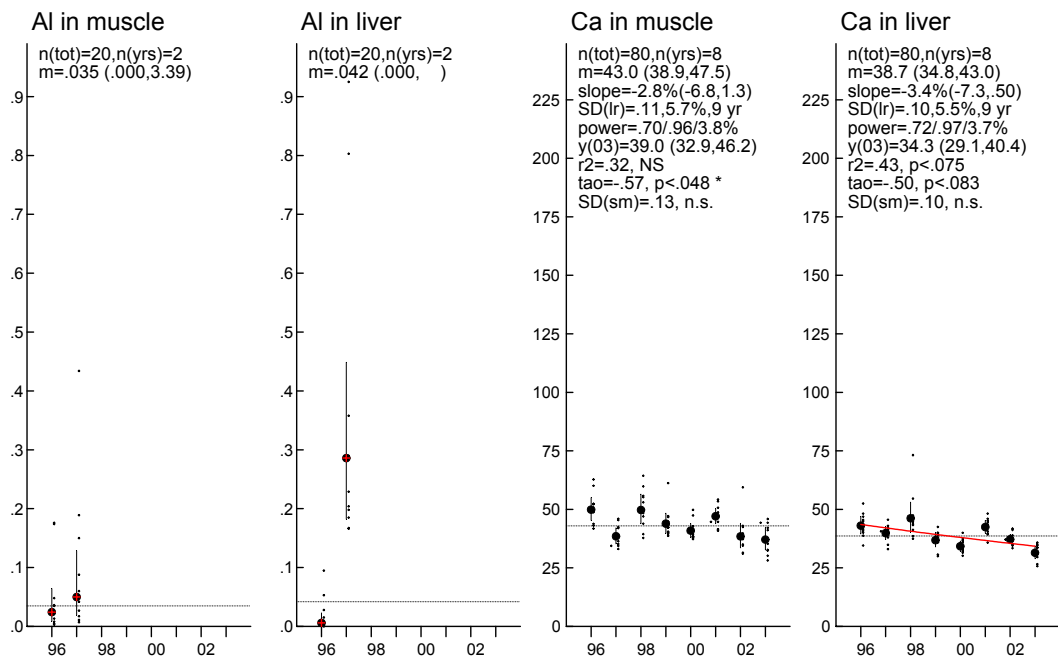
pia - 05.05.11 12:02, 1NYHgPb

Figure 9. Mo in reindeer from Gabna, Lævas and Girjas Saami Villages, N. Lapland. ( $\mu\text{g/g}$  fresh weight).  
Log-linear regression on geometric means, suspected outliers indicated. Smoother: 3-point running mean, unweighted.



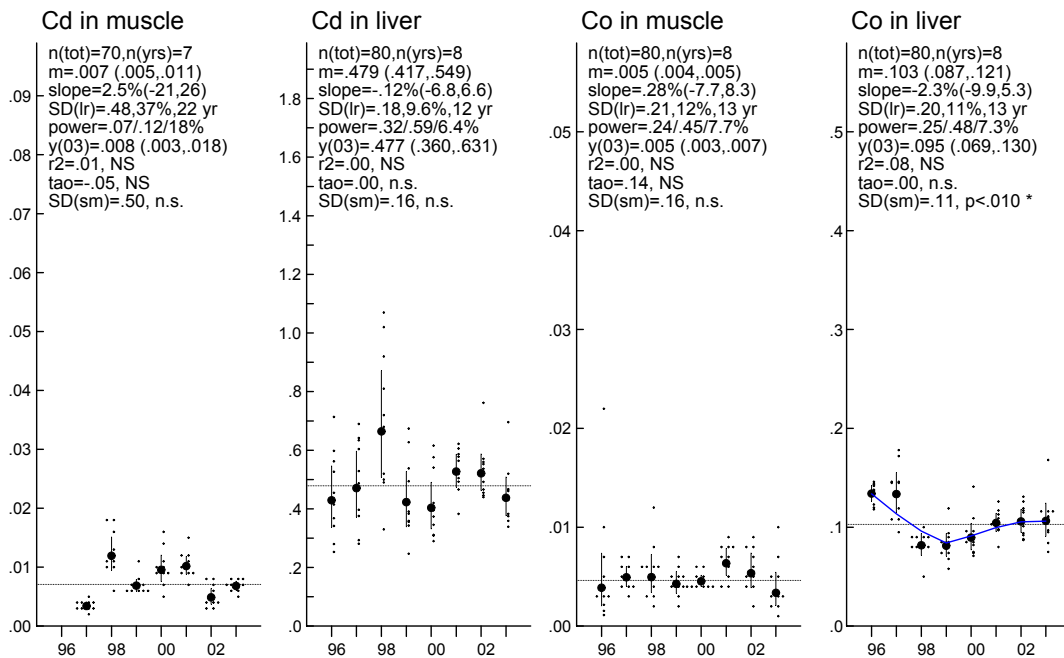
pia - 05.05.17 12:20, 1NYMo

Figure 10. Al and Ca in reindeer from Ran and Gran Saami Villages, ( $\mu\text{g/g}$  fresh weight).  
Log-linear regression on geometric means, suspected outliers indicated.  
Smoother: 3-point running mean, unweighted.



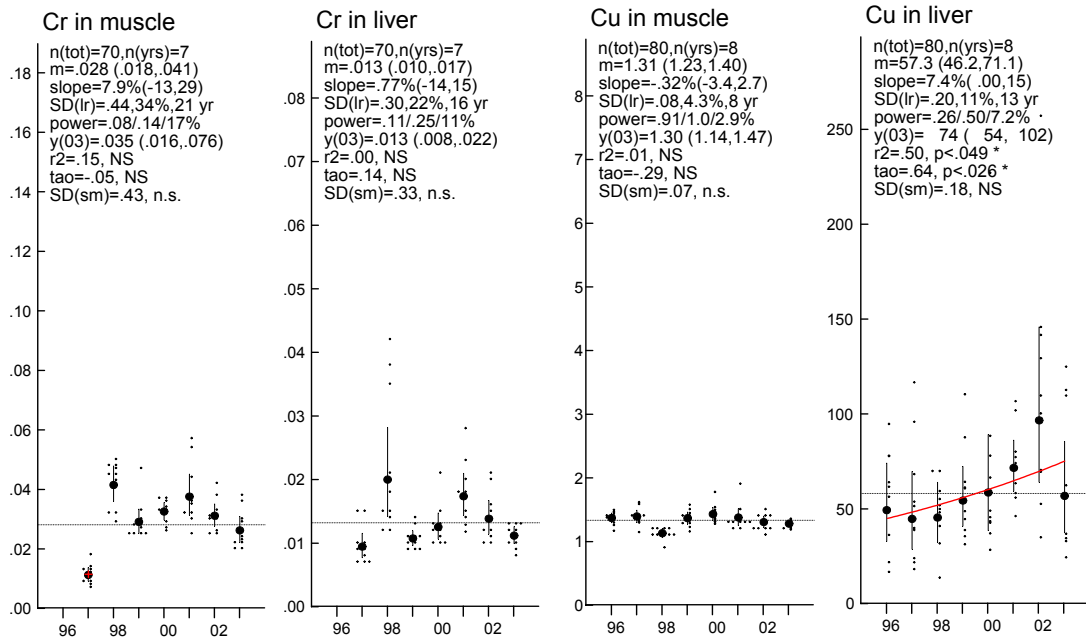
pia - 05.05.11 12:50, RGalca

Figure 11. Cd and Co in reindeer from Ran and Gran Saami Villages, ( $\mu\text{g/g}$  fresh weight).  
Log-linear regression on geometric means, suspected outliers indicated.  
Smoother: 3-point running mean, unweighted.



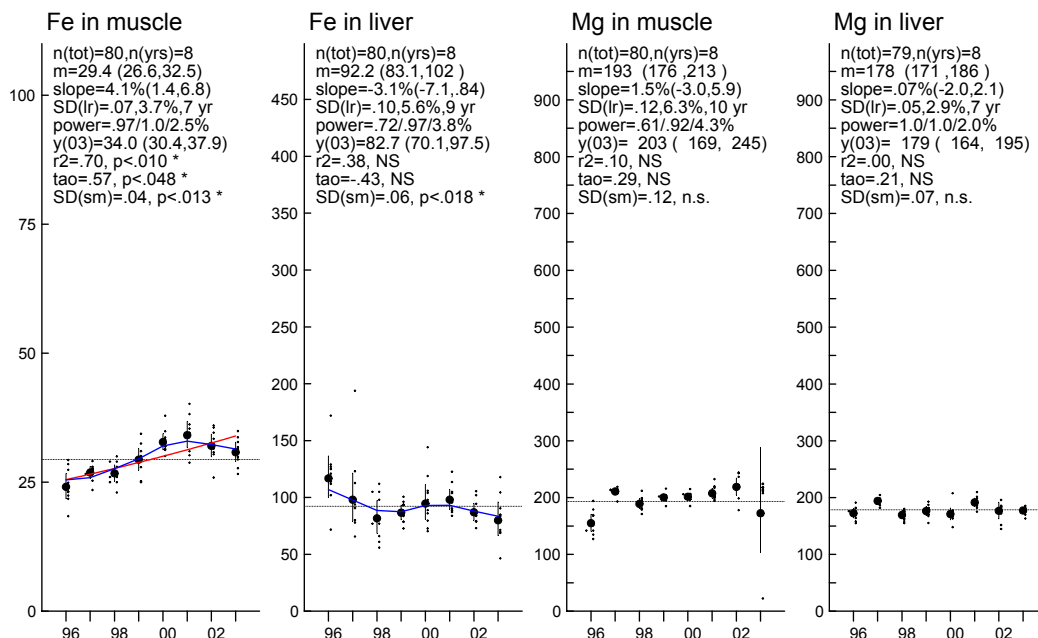
pia - 05.05.11 12:50, RGCdCo

Figure 12. Cr and Cu in reindeer from Ran and Gran Saami Villages, ( $\mu\text{g/g}$  fresh weight).  
Log-linear regression on geometric means, suspected outliers indicated.  
Smoother: 3-point running mean, unweighted.



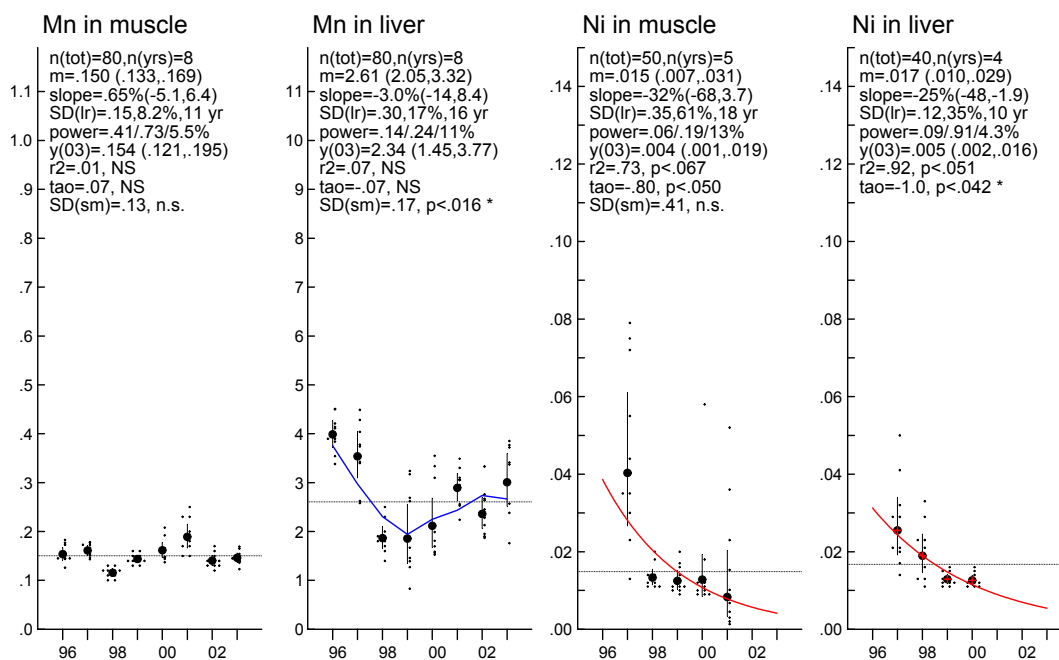
pia - 05.05.11 12:51, RGR/Cu

Figure 13. Fe and Mg in reindeer from Ran and Gran Saami Villages, ( $\mu\text{g/g}$  fresh weight).  
Log-linear regression on geometric means, suspected outliers indicated.  
Smoother: 3-point running mean, unweighted.



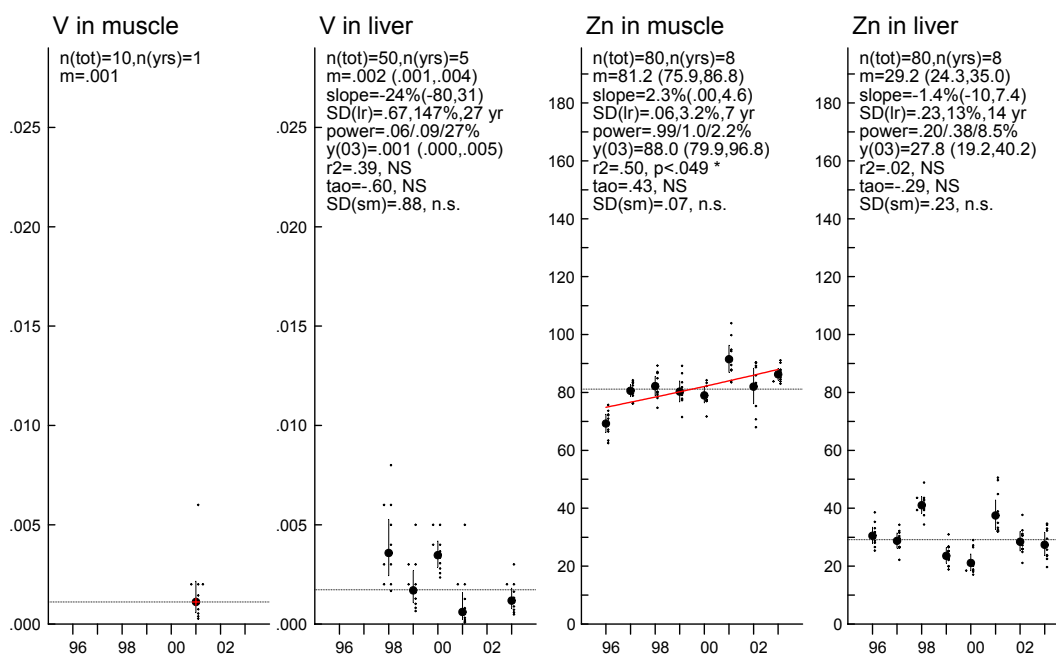
pia - 05.05.16 13:48, RGF/FeMg

Figure 14. Mn and Ni in reindeer from Ran and Gran Saami Villages, ( $\mu\text{g/g}$  fresh weight).  
Log-linear regression on geometric means, suspected outliers indicated.  
Smoother: 3-point running mean, unweighted.



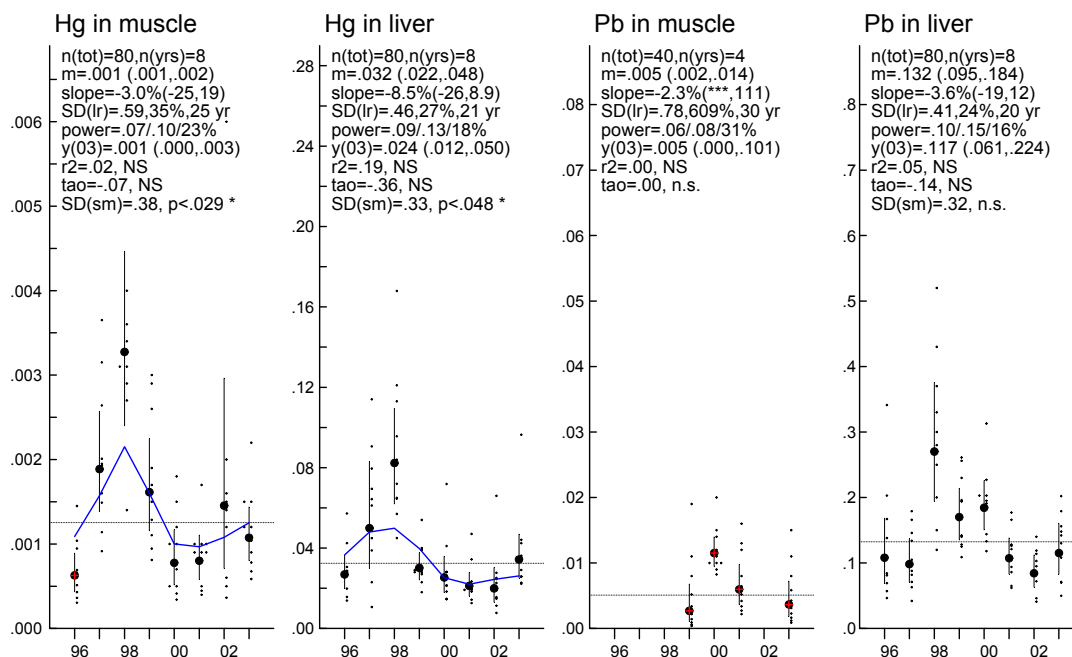
pia - 05.05.11 12:51, RGMnNi

Figure 15. V and Zn in reindeer from Ran and Gran Saami Villages, ( $\mu\text{g/g}$  fresh weight).  
Log-linear regression on geometric means, suspected outliers indicated.  
Smoother: 3-point running mean, unweighted.



pia - 05.05.11 12:52, RGVZn

Figure 16. Hg and Pb in reindeer from Ran and Gran Saami Villages, ( $\mu\text{g/g}$  fresh weight).  
 Log-linear regression on geometric means, suspected outliers indicated.  
 Smoother: 3-point running mean, unweighted.



plia - 05.05.11 12:52, RGHgPb

Contaminant Research Group/ Swedish Museum of Natural History in Stockholm and  
 Department of Chemistry at the National Veterinary Institute in Uppsala