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## Variations of Mercury and Selenium Concentrations in Adamussium Colbecki and Pagothenia Bernacchii from Terra Nova Bay (ANTARCTICA) During a Five Years Period

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# VARIATIONS OF MERCURY AND SELENIUM CONCENTRATIONS IN ADAMUSSIUM COLBECKI AND PAGOTHENIA BERNACCHII FROM TERRA NOVA BAY (ANTARCTICA) DURING A FIVE YEARS PERIOD

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Pagothenia bernacchi and Adamussium colbecki specimens have been collected in Terra Nova Bay, Ross Sea, Antarctica, from 1987 to 1992. Total and organic mercury and selenium concentrations were measured in the muscular tissue of *P. bernacchii* and in the soft parts of *A. colbecki*. Sampling, samples preparation, and analysis were the same for all the specimens, and the temporal variation in concentrations are discussed. Large variations for total and organic mercury contents are found in *A. colbecki*, while *P. bernacchii* shows significant interannual differences in selenium concentration. However, none of these differences reflect a consistent temporal trend. Medians obtained during the five years in 109 specimens of *P. bernacchii* are: 0.60 µg/g dry weight for total mercury, 78% for the percentage of organic mercury, and 3.0 µg/g dry weight for selenium. Values found in 117 specimens of *A. colbecki* are: 0.17 µg/g dry weight for total mercury, 28% for the percentage of organic mercury, and 9.1 µg/g dry weight for selenium.

KEY WORDS: Antarctica, marine organisms, mercury, selenium.

#### INTRODUCTION

The scallop Adamussium colbecki and the bony fish Pagothenia bernacchii are two widely distributed Circum-Antarctic species, and they both can be easily sampled in the neighbourhood of the Italian Base in Terra Nova Bay, Ross Sea.

A. colbecki is a filter feeding bivalve able to move, in some degree, on the substratum<sup>1</sup>. It is usually found on soft and mixed bottoms, typically between 8 and 75 meters water depth in the area in study<sup>2</sup>. P. bernacchii is a predator, feeding on small benthic invertebrates such as A. colbecki<sup>3</sup>. It represents the most abundant fish in the area, and it is typically caught between 0 and 100 meters<sup>4</sup>. The two species belong to quite different trophic levels and they both have the features of the typical bioindicator<sup>5</sup>. More information on biology and detailed descriptions of these species are reported by Fisher and Hureau<sup>6</sup>.

During five Expeditions carried out in the framework of the Italian National Program of Researches in Antarctica (PNRA), between 1987 and 1992, 117 specimens of *A. colbecki* and 109 of *P. bernacchii* were collected and analysed for organic mercury, total mercury, and selenium concentrations. The amount of data acquired, which spans over five years, permitted to evaluate temporal variations of these elements in the Antarctica.

#### EXPERIMENTAL

Samples of *P. bernacchii* and *A. colbecki* were collected in the Terra Nova Bay, Ross Sea (Antarctica) during the Antarctic Expeditions organised by Italy during the five austral summers: 1987–88 to 1991–92.

After sampling, the specimens were stored at  $-25^{\circ}$ C until analysis in the laboratory. The A. colbecki specimens were measured (length of the shell along the dorso-ventral axis from the umbone region), weighed as whole and soft parts and sexed. The P. bernacchii individuals were measured, weighed and sexed.

The soft parts of scallops and the muscular tissue of fishes were freeze-dried and homogenised. An aliquot (0.2-0.5 g dry weight) of homogenate was mineralized by 3-8 ml of 65% nitric acid (Merck Suprapur) in Pyrex volumetric flasks, equipped with air cooled condersers.

Total mercury was determined by cold vapour atomic absorption spectrometry (CVAAS, Perkin-Elmer Mod. 560) by reduction with 10% (w/v) tin(II) chloride dihydrate in 10% (v/v) sulphuric acid, using the preconcentration over gold technique, using an accessory built in our laboratory.

Selenium was determined with the hydride generation (HGAAS) method by reduction with sodium borohydride. A Perkin Elmer 1100B AAS equipped with the MHS-20 accessory was used.

Organic mercury was determined on a second aliquot of the homogenate, after extraction in toluene and back-extraction in a cysteine solution. The sample (0.2–0.5 g dry weight) was mixed with 2 ml 47% hydrobromic acid and 12 ml of toluene, shaken manually for 5 minutes and centrifuged. The toluene (10 ml) was transferred into another polyethylene tube and 5 ml of 1% (w/v) L-cysteine solution were added. After shaking for 5 minutes (an antifoam agent can be used if any foam appears), the samples are centrifuged and the cysteine solution is recovered. Aliquots of the aqueous phase were analysed with the same equipment utilized for the total mercury determination. The reducing solution was 1 ml of 50% (w/v) tin (II) chloride-di-hydrate and 10% (w/v) cadmium chloride monohydrate solution and 4 ml of 10% (w/v) sodium hydroxide solution. Reduction is carried out at 70°C.

Calibration is carried out by the standard additions method. "Blank" samples are processed together with real samples.

Accuracy of analytical determinations was verified using Standard Reference Materails obtained from the IAEA/Monaco Laboratory or from the National Research Council, Canada. A summary of results obtained during a period of about four years (42 months) is reported in Figure 1. Fluctuations are evident but there are no trends which can explain the differences found.

#### **RESULTS AND DISCUSSION**

Results concerning mercury (total and organic) and selenium concentrations in the muscular tissue of *P. bernacchii* collected during 1987–88<sup>7</sup>, 1988–89<sup>8</sup>, and 1989–90<sup>9</sup>,



Figure 1 Quality control for mercury (total and organic) and selenium determinations from January 1990 to July 1993. Standard Reference Materials used are: IAEA/Monaco Laboratory MA-M-2 (Mussel tissue), and National Research Council, Canada, TORT-1 (Lobster hepatopancreas). Results are shown as percentage of certified or reference values.

and in the soft parts of A. colbecki collected during  $1987-88^7$ , and  $1988-89^{10}$  have been already published. Latest unpublished data are reported in Tables 1–5. Distributions of data obtained, for all the five years, are summarised as "Box and Whisker" plots<sup>11</sup> in Figures 2–4. Differences in concentrations between the years are tested using one-way analysis of variance<sup>12</sup>.

The differences for total mercury concentration among the years examined for A. colbecki, shown in Figure 2, are statistically significant (F = 11.06, p < 0.001). In 1987-88 and 1989-90, concentrations are lower (mean 0.14  $\pm$  0.04 µg/g dry weight, n = 41) than in all other years (mean 0.20  $\pm$  0.06 µg/g dry weight, n = 76).

It has to be noted that mercury concentration depends on the size of the specimen, as shown in Figure 5. The correlation coefficients (r) between total mercury concentration and size are 0.536 (n = 111, p < 0.001) and 0.553 (n = 109, p < 0.001) for A. colbecki and P. bernacchii, respectively. However, the differences in mercury concentration described for A. colbecki are not due to the size of specimens analysed. In fact, the same findings are observed by comparing specimens homogeneous in size. P. bernacchii shows no significant differences among the years, and the mean value, for all samples, is  $0.64 \pm 0.31 \mu g/g dry weight (n = 109)$ .

Samp.	Length cm	Weight 8	Sex	FW/DW	Hg-t µg/g DW	Hg-o µg/g DW	Hg-0%	Se µg/g DW
PABE22	22.0	160.0	F	5.16	0.476	0.408	85.7	3.30
PABE23	22.5	160.0	F	5.25	0.535	0.496	92.7	3.24
PABE24	23.0	163.0	U	5.31	0.515	0.517	100.0	2.98
PABE25	24.5	211.0	F	5.26	0.600	0.495	82.5	3.52
PABE26	25.5	228.0	F	4.85	0.394	0.330	83.8	2.81
PABE27	19.5	105.0	F	6.36	0.541	0.541	100.0	3.96
PABE28	22.0	149.0	F	5.27	0.310	0.214	69.0	3.28
PABE29	28.0	280.0	F	6.02	1.210	0.961	79.4	3.01
PABE30	22.5	166.0	F	5.30	0.480	0.421	87.7	2.86
PABE31	24.5	216.0	F	5.09	0.660	0.510	77.3	4.16
PABE32	23.0	189.0	F	5.28	0.485	0.386	79.6	3.01
PABE33	27.0	235.0	F	6.12	1.080	0.820	75.9	4.11
PABE34	20.5	123.0	F	4.84	0.310	0.170	54.8	2.76
PABE35	24.5	239.0	F	5.17	0.990	0.680	68.7	3.00
PABE36	23.5	185.0	М	5.47	0.975	0.901	92.4	3.37
PABE37	25.5	249.0	F	5.54	0.600	0.236	39.3	3.44
PABE38	25.5	240.0	F	5.39	0.920	0.790	85.9	3.96
PABE39	26.5	263.0	F	5.61	0.870	0.790	90.8	2.59
PABE40	24.5	226.0	F	5.44	0.740	0.594	80.3	4.38
PABE41	25.5	241.0	F	5.37	0.420	0.370	88.1	2.72
PABE42	21.0	146.0	М	5.41	0.420	0.392	93.3	2.40
PABE43	22.0	139.0	Μ	5.11	0.500	0.433	86.6	3.28
PABE44	30.5	430.0	F	5.87	1.620	1.434	88.5	2.25
PABE45	22.5	178.0	F	5.19	0.660	0.518	78.5	3.92
PABE46	25.0	202.0	Μ	5.75	1.100	0.942	85.6	3.10
PABE47	22.0	166.0	F	5.06	0.520	0.350	67.3	3.20
PABE48	21.5	136.0	М	5.23	0.590	0.419	71.0	2.70
PABE49	20.0	101.0	F	5.19	0.370	0.264	71.4	2.52
PABE50	21.0	136.0	U	5.48	0.340	0.205	60.3	2.24

 Table 1 Results obtained for muscular tissue of Pagothenia bernacchii collected during the 1990-91 Expedition.

The following is reported for each sample: sample code (Samp.), total length in cm; weight in g; sex (F = Female, M = Male, U = Undetermined); fresh weight/dry weight ratio (FW/DW); concentration of total mercury (Hg-t), organic mercury (Hg-o), and selenium (Se) in  $\mu g/g$  DW; percentage of organic mercury (Hg-o%).

 Table 2 Results obtained for muscular tissue of Pagothenia bernacchii collected during the 1991–92 Expedition.

Samp.	Length cm	Weight g	Sex	FW/DW	Hg-t μg/g DW	Hg-o μg/g DW	Hg-0%	Se µg/g DW
PABE51	21.0	116.0	F	5.21	0.879	0.452	51.4	2.59
PABE52	21.2	163.7	F	5.11	0.322	0.307	95.3	2.90
PBAE53	22.1	137.6	М	4.31	0.342	0.341	99.7	3.40
PABE54	20.0	124.0	М	5.06	0.186	0.162	87.1	3.24
PABE55	20,4	119.3	М	5.09	0.199	0.154	77.4	2.59
PABE56	22.0	146.3	F	4.98	0.461	0.425	92.9	2.50
PABE57	16.2	45.6	М	4.76	0.242	0.242	100.0	2.75
PABE58	22.6	168.3	Μ	5.58	0.879	0.826	94.0	1.82
PABE59	23.2	291.0	F	5.40	0.806	0.643	79.8	2.63
PABE60	22,5	237.5	F	5.13	0.389	0.335	86.1	3.23
PABE61	28.6	403.8	F	4.60	1.344	1.344	100.0	2.07
PABE62	27.5	332.2	U	7.48	0.838	0.824	98.3	2.07

The following is reported for each sample: sample code (Samp.), total length in cm; weight in g; sex (F = Female, M = Male, U = Undetermined); fresh weight/dry weight ratio (FW/DW); concentration of total mercury (Hg-t), organic mercury (Hg-o), and selenium (Se) in  $\mu g/g$  DW; percentage of organic mercury (Hg-o%).

Samp.	Length mm	Weight 8	Sex	FW/DW	Hg-t µg/g DW	Hg-o µg/g DW	Hg-0%	Se µg/g DW
AC1/7	67.0	12.1	M	7.18	0.250	0.079	31.6	7.46
AC1/8	82.0	20.3	F	6.33	0.180	0.078	43.3	5.28
AC1/9	56.0	9.2	Μ	6.40	0.148	0.064	43.2	7.00
AC1/10	69.0	12.4	F	5.99	0.172	0.064	37.2	10.88
AC1/11	73.0	14.5	U	5.62	0.132	0.100	75.8	11.21
AC1/12	76.0	14.7	F	6.87	0.158	0.121	76.6	9.74
AC1/13	59.0	7.5	F	6.06	0.087	0.043	75.4	10.70
AC1/14	68.0	9.9	U	6.81	0.148	0.086	58.1	9.86
AC1/16	82.0	17.0	F	6.57	0.178	0.098	55.1	12.96
AC1/17	58.0	7.9	F	5.11	0.073	0.029	39.7	8.67
AC1/18	67.0	17.8	U	5.19	0.060	0.046	76.7	9.17
AC1/20	64.0	7.3	F	5.30	0.095	0.046	48.4	13.15

Table 3 Results obtained for soft parts of Adamussium colbecki collected during the 1989-90 Expedition.

The following is reported for each sample: sample code (Samp.), length of the shell in mm; weight of the soft parts in g; sex (F = Female, M = Male, U = Undetermined); fresh weight/dry weight ratio (FW/DW); concentration of total mercury (Hg-t), organic mercury (Hg-o), and selenium (Se) in  $\mu g/g$  DW; percentage of organic mercury (Hg-o%).

Table 4 Results obtained for soft parts of Adamussium colbecki collected during the 1990-91 Expedition.

Samp.	Length mm	Weight g	Sex	FW/DW	Hg-t µg/g DW	Hg-o µg/g DW	Hg-0%	Se µg/g DW
AC20	71.6	25.6	М	5.14	0.168	0.047	28.0	13.86
AC21	76.0	26.4	F	4.87	0.205	0.064	31.2	7.96
AC22	77.7	23.5	Μ	5.53	0.310	0.078	25.2	11.96
AC23	72.4	21.9	F	5.32	0.187	0.050	26.7	10.86
AC24	81.4	26.9	F	5.33	0.230	0.070	30.4	13.30
AC25	76.5	23.6	Μ	4.07	0.372	0.120	32.3	14.90
AC26	67.3	19.3	F	4.80	0.106	0.030	28.3	9.53
AC27	72.0	23.0	F	4.91	0.185	0.057	30.8	9.10
AC28	66.0	19.9	F	4.54	0.166	0.061	36.7	10.80
AC29	71.0	22.9	Μ	4.79	0.160	0.044	27.5	9.05
AC30	71.1	23.8	М	5.05	0.176	0.057	32.4	11.12
AC31	71.6	22.0	F	5.03	0.154	0.055	35.7	11.32
AC32	70.1	24.0	Μ	5.19	0.205	0.068	33.2	9.60
AC33	72.2	25.2	Μ	5.54	0.198	0.067	33.8	11.96
AC34	72.3	22.6	F	5.19	0.166	0.047	28.3	8.48
AC35	70.6	21.6	F	5.13	0.214	0.077	36.0	14.61
AČ36	78.5	27.9	F	4.64	0.177	0.045	25.4	10.65
AC37	72.0	19.5	F	5.31	0.276	0.105	38.0	10.39
AC38	68.0	20.6	F	5.13	0.202	0.056	27.7	8.81
AC39		25.2	F	5.01	0.164	0.052	31.7	8.38
AC40	67.3	19.6	F	4.81	0.127	0.038	29.9	6.78
AC41	75.3	24.1	F	5.05	0.188	0.070	37.2	8.44
AC42	83.0	33.1	F	5.35	0.157	0.066	42.0	8.44
AC43	79.0	24.3	F	5.15	0.242	0.078	32.2	7.95
AC44	74.2	25.5	F	4.73	0.140	0.054	38.6	9.28

The following is reported for each sample: sample code (Samp.), length of the shell in mm; weight of the soft parts in g; sex (F = Female, M = Male); fresh weight/dry weight ratio (FW/DW); concentration of total mercury (Hg-t), organic mercury (Hg-o), and selenium (Se) in  $\mu g/g$  DW; percentage of organic mercury (Hg-o%).

Percentages of organic mercury are reported in Figure 3. A. colbecki shows significant differences among the years considered, with a maximum of  $55 \pm 17\%$  (n = 12) in

Samp.	Length mm	Weight 8	Sex	FW/DW	Hg-t µg/g DW	Hg-o µg/g DW	Hg-0%	Se µg/g DW
AC75	74.0	17.5	F	6.84	0.280	0.089	31.8	11.31
AC76	71.0	18.0	F	7.04	0.272	0.074	27.2	7.54
AC77	72.0	17.3	F	7.24	0.180	0.048	26.7	8.07
AC78		18.3	Μ	6.43	0.126	0.038	30.2	6.35
AC79	76.0	21.2	F	7.03	0.362	0.084	23.2	6.41
AC80	84.0	21.5	F	7.14	0.238	0.069	29.0	6.85
AC81	84.0	23.0	F	7.35	0.290	0.070	24.1	6.94
AC82	70.0	20.4	Μ	6.53	0.202	0.074	36.6	7.91
AC83	75.0	16.4	F	6.97	0.307	0.090	29.3	8.59
AC84	79.0	24.8	F	6.70	0.186	0.032	17.2	4.91
AC85	76.0	19.8	F	7.08	0.217	0.067	30.9	3.67
AC86	77.0	15.8	F	7.31	0.292	0.101	34.6	7.78
AC87	75.0	18.9	F	6.95	0.212	0.038	17.9	6.83
AC88	79.0	18.9	F	7.20	0.234	0.056	23.9	7.25
AC89	68.0	11.8	F	6.24	0.175	0.042	24.0	6.89
AC90	74.0	19.6	М	6.34	0.132	0.035	25.6	5.85
AC91		17.9	М	6.70	0.169	0.035	20.7	6.83
AC92	72.0	18.2	F	6.69	0.236	0.082	34.8	9.43
AC93	66.0	18.4	М	7.00	0.248	0.055	22.2	7.74
AC94	81.0	20.0	F	6.86	0.210	0.067	31.9	4.83
AC95	81.0	30.1	М	6.70	0.146	0.034	23.3	6.78
AC96	75.0	17.1	F	6.27	0.210	0.058	27.6	7.69
AC97	72.0	19.1	U	6.75	0.174	0.058	33.3	8.92
AC98	75.0	17.9	F	6.94	0.183	0.066	36.1	6.94
AC99	_	18.4	М	6.08	0.181	0.082	45.3	8.52
AC100	—	15.5	F	5.89	0.145	0.038	26.2	6.10

Table 5 Results obtained for soft parts of Adamussium colbecki collected during the 1991-92 Expedition.

The following is reported for each sample: sample code (Samp.), length of the shell in mm; weight of the soft parts in g; sex (F = Female, M = Male, U = Undetermined); fresh weight/dry weight ratio (FW/DW); concentration of total mercury (Hg-t), organic mercury (Hg-o), and selenium (Se) in  $\mu g/g$  DW; percentage of organic mercury (Hg-o%).

1989–90, and a minimum of  $19 \pm 16\%$  (n = 29) in 1987–88. For 1988–89, 1990–91, and 1991–92 no significant differences are observed, and the mean value is  $30 \pm 6\%$  (n = 76). *P. bernacchii*, shows in 1991–92 a percentage of organic mercury (mean  $88 \pm 14\%$ , n = 12) higher than those in other years (mean  $75 \pm 16\%$ , n = 97).

Mercury, as total concentration and as organic fraction, is higher in *P. bernacchii* than in *A. colbeckii*. This is in agreement with the already known magnification of mercury in the aquatic environment<sup>13-15</sup>, being the *P. bernacchii* at a higher trophic level than *A. colbecki*. Considering all the differences among the sampling years observed, it can be noted that the two species respond differently. It is not surprising that larger variations occur in *A. colbecki*, since this species is more sensitive to environmental changes<sup>13,16</sup>.

Selenium too, reported in Figure 4, exhibits significant differences, both for A. colbecki (F = 11.74, p < 0.001) and for P. bernacchii (F = 18.60, p < 0.001). For A. colbecki, selenium concentrations in 1991-92 (mean 7.19  $\pm$  1.54 µg/g dry weight, n = 26) are lower than in the other years (mean 10.31  $\pm$  2.27 µg/g dry weight, n = 91). For P. bernacchii the higher concentration is detected in 1987-88 (mean 4.13  $\pm$  0.95 µg/g dry weight, n = 25), followed by 1990-91 (mean 3.17  $\pm$  0.58 µg/g dry weight, n = 29). In 1988-89, 1989-90, and 1991-92 no differences are evident and the mean content is 2.57  $\pm$  0.67 µg/g dry weight (n = 46).



**Figure 2** Comparison of mercury concentrations, expressed as  $\mu g/g$  dry weight, in muscular tissue of *P. bernacchii* (top), and in soft parts of *A. colbecki* (bottom), observed from 1987 to 1992. Outliers have been plotted as \* (> 1.5 times the interquartile range), o (> 3 times the interquartile range).



Figure 3 Comparison of percentages of organic mercury, in muscular tissue of *P. bernacchii* (top), and in soft parts of *A. colbecki* (bottom), observed from 1987 to 1992. Outliers have been plotted as \* (> 1.5 times the interquartile range), o (> 3 times the interquartile range).



Figure 4 Comparison of selenium concentrations, expressed as  $\mu g/g$  dry weight, in muscular tissue of *P. bernacchii* (top), and in soft parts of *A. colbecki* (bottom), observed from 1987 to 1992. Outliers have been plotted as \* (> 1.5 times the interquartile range).



**Figure 5** Mercury concentration vs. size in *P. bernacchii* (left) and *A. colbecki* (right). Concentrations are expressed in  $\mu g/g$  dry weight, size for *P. bernacchii* in cm, and for *A. colbecki* in mm.

Selenium concentrations are higher in *A. colbecki*, and this is consistent with what was found by Thibaud and Noel<sup>17</sup> in the Mediterranean for two species (*Mytilus galloprovincialis* and *Sparus aurata*) of analogous trophic levels. Again, the two species differ in variations, *P. bernacchii* showing the larger difference in concentrations.

#### CONCLUSIONS

The annual differences observed in the concentrations of the trace elements considered, were not the same for the two species, thus making difficult to identify the cause for such variations.

Linear trend analysis for temporal variations was done on the variables measured, using the median values for each year. For *P. bernachii* the percentage of organic mercury shows a positive trend (r = 0.935), while for *A. colbecki* the selenium decreases with the time (r = -0.882). Obviously more data are needed to support the trends found, and research should be extended for several consecutive years, if any variation for trace elements in the environment have to be detected.

Data available in literature<sup>18,19</sup>, for total mercury, concerning blue mussel (*Mytilus* edulis), flounder (*Platichthyus flesus*), and plaice (*Pleuronectes platessa*) collected in areas of the Northern emisphere far from any point source of contamination, show that no evident temporal trend can be detected.

The results obtained, despite the large number of specimens analysed, probably reflect variations in the biological conditions of the organisms rather than the environmental concentrations.

Data reported form, therefore, the basis for the knowledge of existing levels for mercury and selenium in two species widely distributed in the Antarctic seas. Medians obtained during the five years in *P. bernacchii* are: 0.60  $\mu$ g/g dry weight for total mercury, 78% for the percentage of organic mercury, and 3.0  $\mu$ g/g dry weight for selenium. Values found in *A. colbecki* are: 0.17  $\mu$ g/g dry weight for total mercury, 28% for the percentage of organic mercury, and 9.1  $\mu$ g/g dry weight for selenium.

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