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Metal concentrations in *Unio pictorum mancus* (Mollusca, Lamellibranchia) from of 12 Northern Italian lakes in relation to their trophic level

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ABSTRACT

This research aims to test the reliability of environmental monitoring by bioaccumulators of pollutants; that is to establish a positive relationship between the pollutant concentrations in the bioaccumulator and those in the water in which it lives. To this end we analysed the contents of Al, Cu, Zn, Fe, Mn and Ca in the soft tissues and shell of Unio pictorum mancus*. The filtered water samples from the mussel habitat were analysed for the same metals. The mussels were collected from 15 stations settled in 12 Northern Italian lakes during the first two weeks of July 2001. These results were obtained: a) metal concentrations varied widely with mussel size and among stations; b) a significant positive correlation between the concentration of calcium in the water and in the mussel tissues, but no relationship emerged for the other metals; c) no relationship between the metal concentrations in the tissues and those in the shell was found; d) there was a certain tendency for Mn, Fe and Zn concentrations in the soft tissues to increase with shell size; e) the sequence of the decreasing metal concentrations arranged for the tissues was similar to that of the shell, but rather different from that in the water; and f) the concentration factor values of the trace metals were high for the shell and soft tissues. In highly productive lakes large size mussels dominated, whereas small mussels were more abundant in low productive lakes. Although the metal concentrations in the water of productive lakes were greater than in low productive ones, the metal concentrations in the tissues of the mussels from the latter were generally higher than those in the mussels from the former. We propose some hypotheses to explain this paradox. Finally, our results show that the metal concentrations in the mussels do not reflect the metal concentrations in the water in which they live. It follows that this commonly used but oversimplified monitoring system cannot be recommended. On the other hand mussels may be very useful for other purposes, such as identifying new pollutants or pollutants present in such low concentrations that they cannot be measured with the commonly used methods. The pollutant content of mussels may enable the variations in time of the pollutant level of an environment to be monitored. In addition, the transplantation of mussels from a clean site to a polluted one may be a useful tool for identify the pollutants of the receiving environment.*

Key words: Unionidae, biomonitoring, shell, soft tissues, size distribution, concentration factor, calcium, heavy metals

1. INTRODUCTION

Mussels have several characteristics which appear to indicate their use as biomonitors for estimating the environmental pollution level and the bioavailability of various types of pollutants (e.g. Crawford & Luoma 1993; Metcalfe-Smith *et al*. 1996; Ravera 2001).

The most important example of the monitoring by mussels is the "Mussel Watch Programme" run by the NOAA (National Oceanic and Atmospheric Administration). Under the framework of this programme the Atlantic and Pacific coastal zones and many estuaries of the United States are monitored by analysing the pollutants accumulating in the soft tissues of sea mussels (e.g. Ferrington *et al*. 1983; O'Connor *et al*. 1994). Freshwater mussels are also used to evaluate the distribution and availability of trace metals, their radioisotopes and organic pollutants (e.g. Merlini *et al*. 1965; Czarnezki 1987; Riccardi & Ravera 1989; Doherty *et al*. 1993; Oertel 1998; Ravera 2001), although biomonitoring by freshwater mussels has received far less attention than biomonitoring by marine species (Philipps & Rainbow 1993). Several authors (e.g. Metcalfe-Smith *et al*. 1996) have discussed the possible causes of this difference and underlined the need for more research on freshwater mussels to be used as bioindicators (e.g. Metcalfe-Smith 1994; Metcalfe-Smith *et al*. 1996).

An earlier research compared the accumulation capacity of 15 trace elements by eight species of freshwater macrophytes and three species of mussels (Ravera *et al*. 2003). To exclude any influence either of the season or the physical environment, the materials were sampled at the same time in one habitat (Ranco Bay, Lago Maggiore, Northern Italy). In addition, to compare possible relationships between the trace element concentrations in macrophytes and mussels with those in their habitat the same elements were analized in water and surface sediment samples contemporarely collected at the same time from the same bay.

The results of this study, designed to compare the accumulation capacity of trace elements by various species, highlight the most suitable indicators for monitoring. In addition, by analysing the metals in mussels of different sizes, it was possible to evaluate the influence of age on metal accumulation.

Tab. 1. Lake characteristics. The source of the data listed in the table are: Quaderni IRSA-CNR n° 72. Catasto dei laghi italiani. Volume I - Italia Settentrionale (Gaggino & Cappelletti 1984) and Caratteristiche limnologiche dei laghi del Trentino – Rapporto 1998 (Corradini & Flaim 1998) Istituto Agrario S. Michele all'Adige (Trento). [a)] The water quality of Lake Levico, Lake Caldonazzo and Lake Segrino has improved since the 70s and these lakes are currently regarded as mesotrophic. Station 3 – Ranco; Station 4 – Sabbie d'Oro (Ispra); Station 7 - Parco; Station 8 – 200 m from Station 7; Station 11 – 1 km from the town of Mergozzo; Station 12 – Mergozzo camping site. E = eutrophic; M = mesotrophic; O = oligotrophic; O-M = oligotrophic and oligo-mesotrophic.

Station	Lake	Altitude (m)	Surface (km^2)	Watershed (km^2)	Volume (m^3 10^6)	Max. depth (m)	Mean depth (m)	Trophic level	pH		
									mean	min	Max
1	Varese	238	14.80	111.50	100.00	26.0	10.7	E	7.8	7.2	9.8
2	Comabbio	243	3.58	15.30	16.50	8.0	4.6	E	8.2	7.2	8.3
3-4	Maggiore	193.8	212.00	6599.00	37500.00	370.0	177.0	O – M	7.4	7.2	8.8
5	Pusiano	259	4.95	94.30	69.20	24.3	14.0	E	7.8	7.3	8.5
6	Annone Est	224	3.81	28.08	24.04	11.3	6.3	E	7.7	7.2	8.9
7-8	Segrino[a]	374	0.38	3.38	1.20	8.6	3.2	E	8.1	7.2	8.4
9	Montorfano	397	0.46	1.90	1.90	6.8	4.2	O – M	7.8	7.2	8.8
10	Viverone	230	5.58	25.70	125.00	50.0	22.5	E	7.5	7.3	8.7
11–12	Mergozzo	194	1.83	10.40	82.90	73.0	45.4	O	6.8	6.5	9.1
13	Caldonazzo[a]	450	5.60	47.90	149.00	49.0	26.5	E	7.6	-	-
14	Levico[a]	440	1.16	27.00	12.90	38.0	11.0	E	-	6.9	8.5
15	Candia	226	1.35	7.50	8.10	8.0	5.9	E	7.7	7.2	8.9

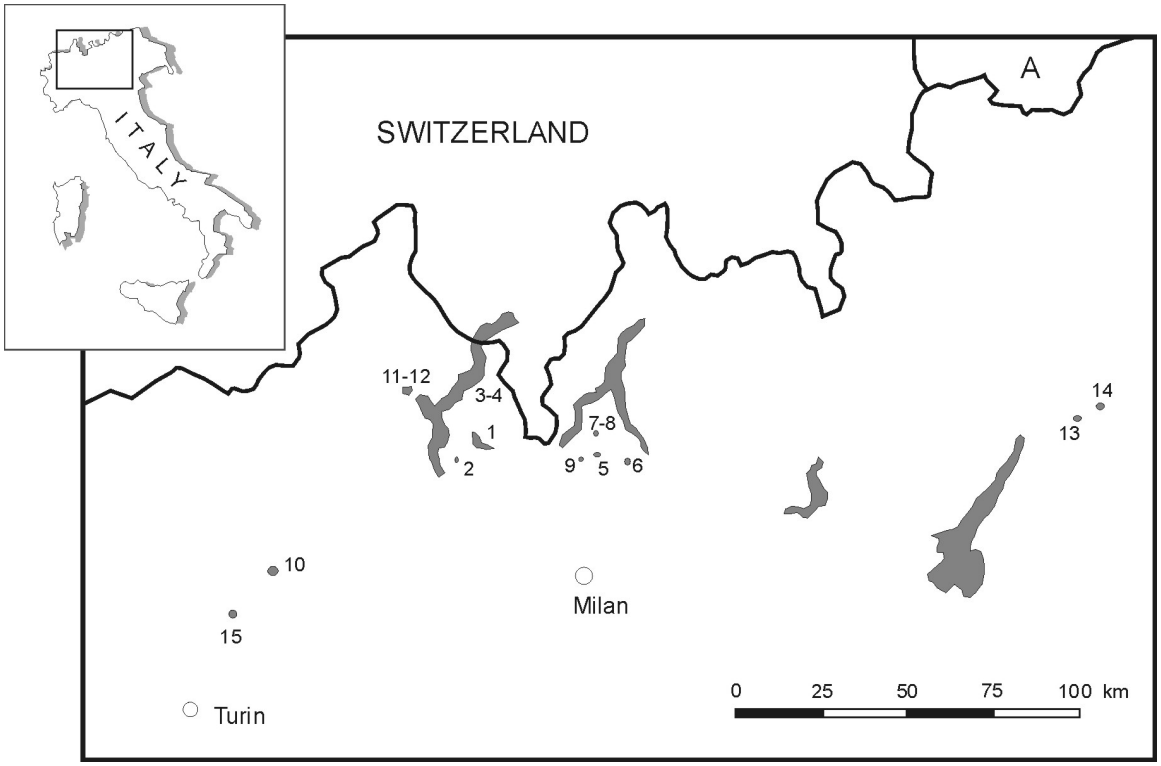

Fig. 1. Map of the sampled lakes.

On the other hand, the study did not yield any information on variations in the trace element concentrations in populations of the same species living in environments with various concentrations of the same elements.

2. MATERIAL AND METHODS

Samples of *Unio pictorum mancus* were collected from 12 lakes, with different morphometric and trophic characteristics distributed over a wide area of Northern Italy: Piedmont, Lombardy and Trentino (Tab. 1, Fig. 1). All the material was collected during the first two weeks of July 2001.

Six elements (Al, Cu, Zn, Fe, Mn, Ca) were analysed in samples of *Unio* shells and soft tissues from each population and water collected from their habitat.

The main reasons for our choosing these metals are as follows. Aluminium was analysed because little information on its distribution in mussel shell and soft tissues is available. Moreover, its physiological role is not

known. The soil and sediments are the main sources of this metal. Its co-precipitating power is well known and this property makes it one of most effective substances for abating phosphorus in waste water treatment.

At low concentrations, copper is an essential element for organisms, but is toxic at high concentrations, so that its accumulation must be strictly regulated. At low concentrations zinc also plays an important role in physiology; it too is toxic at high concentrations, but to a lesser extent than copper.

The capacity of mussels (especially Unionidae) to concentrate manganese has been known for about a century (Bradley 1907a; 1907b). The highest concentrations were measured in the gills and mantle (e.g. Ravera & Gaglione 1962; Gaglione & Ravera 1964;). In the shell most of the manganese is tied up in organic matter encircling aragonite crystals of the nacreous layer (Nyström *et al*. 1996). Dissolved oxygen transforms the Mn oxide to bioxide, which in precipitating decreases the manganese concentration in the water, enriching the sediments with the metal. As for the high concentration of manganese in the soft tissues, Unionidae seem to accumulate an amount of manganese in excess of their needs.

Iron is another essential metal, generally abundant in any environment, and has several properties similar to those of manganese; for example, its partitioning between water and sediments is largely controlled by the oxygen concentration in the water.

Apart from calcium's major role in organism physiology, it is the most abundant metal in the shell and in the soft tissues. Its low affinity with chelating substances increases its availability to the organism. According to some authors (e.g. Markich & Jeffree 1994), accumulation by the mussels of non-essential and even toxic metals my be explained by the Ca-influence on the uptake-rate of certain divalent, and trivalent metals.

At each station mussels were collected by hand from the littoral zone at a maximum depth of about 1 meter. Samples of water were taken from the same area, filtered on 0.45 µm (pore size) Millipore filter and preserved in plastic bottles with the addition of some drops of nitric acid. The film of sediments and attached algae, coating the periostracum of the mussel shell was scrubbed off with a nylon nailbrush. The water bottles and the cleaned mussels, preserved in plastic bags, were placed on ice and rushed to the laboratory where they were refrigerated at –20 °C until analysed. The mussels were frozen, without being allowed to clear their digestive tract, which would have eliminated undigested material and its content of elements. The advantages of this practice were discussed in a previous paper (Ravera *et al*. 2003). The mussels from each station were partially thawed and divided into six classes according to the length of their shell in: B (35-44 mm); C (45-54 mm); D (55-64 mm); E (65-74 mm); F (75-84 mm) and G (85-94 mm). 2 to 6 classes were found at each station. No mussel shorter than 35 mm was collected. According to Patzner & Müller (2001) it is very difficult to find juvenile mussels because of their small size and their burrowing way of life. For each station the soft tissues of the individuals of each length class, removed from their shells were pooled. The shells of the same mussels were also pooled, so that for each station the number of samples for analysis was twice the number of the length classes.

The soft tissues of the mussels were then freeze-dried and kept at 40 °C for 24 hours. The samples were pulverised by a Planetary Micro Mill in agate as well as the balls. The shells were broken up before pulverisation. The resulting powder was selected using a plastic sieve with 0.2 mm opening size. The shell and soft tissue samples were mineralised by a Micro Wave Digestion System CEM (Mattews, NC, USA) MDS-2000 model using HNO_3 (65% m/v) and H_2O_2 (30% m/v) at 180 °C and a pressure of 1300 psi. The reagents were "Suprapur" (E. Merck, Darmstadt) High purity water was produced starting from distilled water using a Mill. QTM deionizing system (Millipore, Bedford, MA. USA). The solution obtained was filtered onto paper and analyzed for Ca, Al, Cu, Zn, Fe and Mn by ICP-OESJY (Jobin Yvon Emission Horiba Group, Long Jumeau, Cedex, France) JY 24 model. These analyses were performed by G.M. Beone and co-workers (Istituto di Chimica Agraria e Ambientale, Università Cattolica del Sacro Cuore, Piacenza, Italy). The water samples were analyzed for the same metals by the Inductive Coupled Plasma – Mass Spectrometry (ICP-MS) by R.M. Cenci and co-workers (Institute of the Environment, Joint Research Center, E.C.Ispra, VA, Italy). The higher sensitivity of ICP-MS instrument allows measurement of the very low concentrations of metal (except calcium) in lake waters. Both instruments (ICP-AES and ICP-MS) are suitable for analysing the other properties.

A certified mussel tissue reference sample, CRM 278 (*Mytilus edulis*), prepared by the Community Bureau of Reference (BCR), was used to check the accuracy of the analytical procedure. The reliability of the analytical data is also demonstrated by the fact that concentrations of Cu, Zn, Mn and Ca in soft tissues and shell of *U. pictorum mancus* collected from station 3 (Ranco, Lago Maggiore) in 2001 were very similar to those of the same metals in the same species from the same station in 2000 (see tables 2a, 2b, 2d, 2e, 3a, 3b, 3d and 3e in this paper and table 4 in Ravera *et al*. 2003).

3. RESULTS

The metal concentrations calculated for each station and length class in the soft tissues and shells are reported in tables 2, 2a, 2b, 2c, 2d, 2e and **3**, 3a, 3b, 3c, 3d, 3e.

Tab. 2. Aluminium concentration (µg g^{-1}dw) in the soft tissues. B, C, D, E, F, G = size classes. The range of each class is given in the text.

Station	B	C	D	E	F	G	mean	SD
9	233	334	97				221.33	118.93
8	466	204	97				255.67	189.85
6		69	49	74			64.00	13.23
1		3	3		1		2.33	1.15
4		475	420	417	246		389.50	99.31
3		363	472				417.50	
15	296		154	206	60	30	149.20	108.36
2		13		51	42		35.33	19.86
10				373	356	446	391.67	47.82
14				3	361		182.00	
13				491	420	539	483.33	59.87
12		708	404	494			535.33	156.16
11			204	316			260.00	
5			187	173	336	246	235.50	74.09
7	487		83	135			235.00	219.78
mean	370.50	271.13	197.27	248.45	227.75	315.25	mean	SD
SD	125.36	247.47	162.09	178.22	167.87	226.07	252.98	±183.77

Tab. 2a. Copper concentration (µg g^{-1}dw) in the soft tissues.

Station	B	C	D	E	F	G	mean	SD
9	11	10	7				9.33	2.08
8	16	9	12				12.33	3.51
6		17	8	10			11.67	4.73
1		9	12		7		9.33	2.52
4		82	86	90	38		74.00	24.22
3		12	14				13.00	1.41
15	9	6	63	58	4	11	25.17	27.52
2		7		8	10		8.33	1.53
10				11	9	13	11.00	2.00
14					25		25.00	
13				17	11	11	13.00	3.46
12		21	20	19			20.00	1.00
11			18	21			19.50	2.12
5			13	10	9	7	9.75	2.50
7	14		7	9			10.00	3.61
mean	12.50	19.22	23.64	25.30	14.13	10.50	mean	SD
SD	3.11	24.03	26.00	27.12	11.47	2.52	19.37	±21.21

Tab. 2b. Zinc concentration (µg g^{-1}dw) in the soft tissues.

Station	B	C	D	E	F	G	mean	SD
9	189	176	205				190.00	14.53
8	214	231	422				289.00	115.49
6		346	307	559			404.00	135.64
1		176	192		423		263.67	138.22
4		632	949	791	1030		850.50	176.26
3		343	374				358.50	
15	136	142	182		426	1012	379.60	373.14
2		174		376	481		343.67	156.03
10				297	293	411	333.67	67.00
14				1118	925		1021.50	
13				317	232	193	247.33	63.41
12		373	287	319			326.33	43.47
11			268	267			267.50	
5			205	327	515	465	378.00	140.08
7	228		289	377			298.00	74.91
mean	191.75	288.11	334.55	474.80	540.63	520.25	mean	SD
SD	40.52	155.96	217.59	276.07	286.60	348.28	395.52	±253.95

Tab. 2c. Iron concentration (µg g^{-1}dw) in the soft tissues.

Station	B	C	D	E	F	G	mean	SD
9	863	6946	6465				4758.00	3381.73
8	4452	2088	3554				3364.67	1193.32
6		907	1556	11236			4566.33	5785.21
1		16666	1532		1568		6588.67	8727.25
4		7436	4581	1715	2586		4079.50	2538.96
3		2515	2157				2336.00	
15	1750	1771	3689	4121	5233	4206	3461.67	1411.82
2		447	166	1537	2315		1125.25	999.36
10				2799	2193	3167	2719.67	491.82
14				6064	9445		7754.50	
13				1788	2499	5043	3110.00	1711.36
12		1818	1856	4413			2695.67	1487.38
11			1421	2860			2140.50	
5			1517	2065	2588	2113	2070.75	438.18
7	808		1513	2561			1627.33	882.08
mean	1968.25	4510.44	2500.58	3745.00	3553.38	3632.25	mean	SD
SD	1711.18	5200.34	1744.84	2839.91	2614.19	1270.69	3388.02	±2998.82

Tab. 2d. Manganese concentration (µg g^{-1}dw) in the soft tissues.

Station	B	C	D	E	F	G	mean	SD
9	2697	22289	20797				15261.00	10906.29
8	10874	7861	11344				10026.33	1889.90
6		3569	5901	31958			13809.33	15760.40
1		4509	3865		6590		4988.00	1424.25
4		24816	16579	4358	6831		13146.00	9400.05
3		4979	4957				4968.00	
15	2874	4147	8942	6337	9752	6345	6399.50	2653.80
2		1294	111	10486	13522		6353.25	6659.14
10				4664	6090	7385	6046.33	1361.03
14				4998	7359		6178.50	
13				5554	5864	6004	5807.33	230.29
12		2508	1820	3516			2614.67	853.02
11			2057	2412			2234.50	
5			3292	6797	9431	8240	6940.00	2659.79
7	2300		5128	7855			5094.33	2777.65
mean	4686.25	8441.33	7066.08	8085.00	8179.88	6993.50	mean	SD
SD	4132.14	8776.00	6294.96	8216.08	2600.19	1017.63	7538.71	±6322.96

Tab. 2e. Calcium concentration (µg g^{-1}dw) in the soft tissues.

Station	B	C	D	E	F	G	mean	SD
9	29291	148008	98024				91774.33	59604.74
8	95825	54138	67992				72651.67	21230.54
6		32095	44903	265062			114020.00	130962.88
1		40588	38483		45767		41612.67	3748.55
4		211141	155094	56640	74495		124342.50	71990.82
3		42086	39649				40867.50	
15	15634	21193	36765	24552	38253	32454	28141.83	9086.35
2		6060	989	53624	71907		33145.00	35068.92
10				40147	48072	57999	48739.33	8944.69
14				426	39619		20022.50	
13				42812	39355	34988	39051.67	3920.81
12		23787	20737	36243			26922.33	8214.73
11			24003	2333			23668.00	
5			33624	55877	74796	67216	57878.25	17941.36
7	21117		32130	49770			34339.00	14453.66
mean	40466.75	64344.00	49366.08	58953.27	54033.00	48164.25	mean	SD
SD	37329.66	68608.36	41156.72	70438.64	16670.55	17128.12	54307.56	±49522.47

Tab. 3. Aluminium concentration (µg g^{-1}dw) in the shell. B, C, D, E, F, G = size classes. The range of each class is given in the text.

Station	B	C	D	E	F	G	mean	SD
9	94	99	2	2			49.25	54.60
8	32	19	446				165.67	242.86
6		61	24	158			81.00	69.20
1		33	15	39	7		23.50	15.00
4		415	62	150	21		162.00	177.04
3		50	46				48.00	
15	81	102	44	13	17	14	45.17	38.25
2		63	23	25	9		30.00	23.12
10				21	13	7	13.67	7.02
14				121	305		213.00	
13				4		103	53.50	
12			100				100.00	
11			120				120.00	
5			218	168		33	139.67	95.70
7	145			34			89.50	
mean	88.00	105.25	100.00	66.82	62.00	39.25	mean	SD
SD	46.44	128.42	130.41	67.19	119.16	43.90	80.86	±100.48

Tab. 3a. Copper concentration (µg g^{-1}dw) in the shell.

Station	B	C	D	E	F	G	mean	SD
9	2.8	2.8	0.8	0.3			1.675	1.31
8	0.8	2.4	3.6				2.27	1.40
6		3.1	1.3	2.9			2.43	0.99
1		0.9	1.4	0.8	2.6		1.43	0.83
4		3.6	3.3	2.4	2.5		2.95	0.59
3		4.7	2.8				3.75	
15	1.1	1.3	14.7	1.6	0.9	1.6	3.53	5.48
2		1.7	1.5	1.9	2.2		1.83	0.30
10				4.6	3.7	6.6	4.97	1.48
14				6.3	16.9		11.60	
13				3.9		3.6	3.75	
12			7.8				7.80	
11			9.1				9.10	
5			3.4	3.6		3.6	3.53	0.12
7	3.6			4.1			3.85	
mean	2.08	2.56	4.52	2.95	4.80	3.85	mean	SD
SD	1.35	1.26	4.30	1.77	6.00	2.06	3.53	±3.29

Tab. 3b. Zinc concentration (µg g^{-1}dw) in the shell.

Station	B	C	D	E	F	G	mean	SD
9	22	27	21	25			23.75	2.75
8	29	28	27				28.00	1.00
6		18	20	20			19.33	1.15
1		19	23	31	22		23.75	5.12
4		23	19	20	20		20.50	1.73
3		24	21				22.50	
15	30	19	20	22	30	22	23.83	4.92
2		21	22	23	21		21.75	0.96
10				7	26	14	15.67	9.61
14				38			38.00	
13				14			14.00	
12			7				7.00	
11								
5			5	4		21	10.00	9.54
7	90			69			79.50	
mean	42.75	22.38	18.50	24.82	23.80	19.00	mean	SD
SD	31.70	3.78	6.96	17.58	4.15	4.36	24.00	±14.63

Tab. 3c. Iron concentration (µg g^{-1}dw) in the shell.

Station	B	C	D	E	F	G	mean	SD
9	63	75	65	91			73.5	12.79
8	129	62	376				189.00	165.38
6		147	66	147			120.00	46.77
1		72	66	50	46		58.50	12.48
4		227	62	110	47		111.50	81.55
3		91	104				97.50	
15	120	127	319	396	498	558	336.33	184.26
2		93	57	58	74		70.50	16.90
10				258	138	332	242.67	97.90
14				541	1492		1016.50	
13				343		1029	686.00	
12			95				95.00	
11			87				87.00	
5			143	146		136	141.67	5.13
7	91			66			78.50	
mean	100.75	111.75	130.91	200.55	382.50	513.75	mean	SD
SD	29.94	54.70	110.69	162.74	570.10	384.35	211.20	± 273.71

Tab. 3d. Manganese concentration (µg g^{-1}dw) in the shell.

Station	B	C	D	E	F	G	mean	SD
9	542	579	860	916			724.25	191.06
8	382	428	607				472.33	118.87
6		275	416	422			371.00	83.19
1		299	232	298	378		301.75	59.72
4		158	176	172	206		178.00	20.20
3		203	242				222.50	
15	557	457	789	1138	1110	1006	842.83	289.34
2		379	428	540	605		488.00	103.80
10				380	396	395	390.33	8.96
14				528	723		625.50	
13				362		576	469.00	
12			200				200.00	
11			168				168.00	
5			206	279		361	282.00	77.54
7	322			611			466.50	
mean	450.75	347.25	393.09	513.27	569.67	584.50	mean	SD
SD	116.79	140.13	253.20	288.03	321.21	296.42	461.52	±252.67

Tab. 3e. Calcium concentration (mg g^{-1}dw) in the shell.

Station	B	C	D	E	F	G	mean	SD
9	413	403	399	392			401.75	8.77
8	397	403	383				394.33	10.26
6		368	389	380			379.00	10.54
1		408	379	402	389		394.50	13.03
4		384	402	404	396		396.50	9.00
3		394	396				395.00	
15	405	381	398	406	396	405	398.50	9.52
2		406	391	405	369		392.75	17.25
10				364	340	322	342.00	21.07
14				349	352		350.50	
13				323		348	335.50	
12			321				321.00	
11			341				341.00	
5			375	368		363	368.67	6.03
7	378			457			417.50	
mean	398.25	393.38	379.45	386.36	373.67	359.50	mean	SD
SD	15.00	14.36	25.81	35.49	23.91	34.74	382.82	±27.55

Tab. 4. Mean metal concentration and standard deviation (SD), variation coefficient (% VC) and number of analysed samples (N°). Mean values are expressed in µg g^{-1}dw, except that of Ca in the shell expressed in mg g^{-1}dw. The data in this table were calculated on all the values listed in tables 2 and 3.

	soft tissues			shell		
	mean & SD	% VC	N°	mean & SD	% VC	N°
Al	252.98 ± 183.77	73	46	80.86 ± 100.48	124	44
Cu	19.37 ± 21.21	109	46	3.53 ± 3.29	93	44
Zn	395.52 ± 253.95	64	46	24.00 ± 14.63	61	42
Fe	3388.02 ± 2998.82	88	48	211.20 ± 273.71	130	44
Mn	7538.71 ± 6322.96	84	48	461.52 ± 252.67	55	44
Ca	54307.56 ± 49522.47	91	48	382.82 ± 27.55	7	44

Tab. 5. Metal concentration in the filtered water (0.45 µm pore size) concentrations are expressed in µg l^{-1}, except for calcium which is expressed in mg l^{-1}.

Station	Al	Cu	Zn	Fe	Mn	Ca
1	5.70	1.03	7.03	94.0	2.19	20.27
2	4.60	0.99	10.50	80.0	6.18	9.48
3	11.10	1.06	17.60	78.0	1.47	13.99
4	24.90	1.50	35.30	61.0	10.40	13.41
5	2.30	0.94	10.50	15.0	4.18	14.25
6	3.00	0.60	7.50	33.0	2.48	33.28
7	1.60	0.45	31.50	61.4	1.14	28.11
8	6.30	0.60	4.68	1.7	1.42	34.45
9	11.60	0.70	3.63	8.2	2.28	21.39
10	734	1.58	14.60	12.4	2.29	14.49
11	259	0.91	10.80	16.1	0.92	4.31
12	44.60	0.85	6.97	24.7	0.53	3.43
13	184	1.56	12.21	75.3	9.88	16.98
14	24.10	1.01	10.90	26.7	2.20	21.15
15	166	0.76	86.85	25.5	7.84	10.21

Table 4 lists the mean concentrations of metals in the soft tissues and shells calculated for all stations and length classes. These values highlight the fact that the Ca concentration in the shell is seven times that calculated for the soft tissues, whereas the concentrations of the other metals are higher in the tissues than those in the shells: three times higher for Al, five times for Cu and sixteen times for Zn, Fe and Mn. Because of the high values of the standard deviation (SD) compared to those of the mean, it seemed interesting to calculate the variation coefficient (% V.C. = SD/mean ×·100) for each metal in the soft tissues and shell. The VC values, except for those of Ca in the shell, are very high and for Cu in the tissues and Al and Fe in the shell the values are higher than 100, i.e. the standard deviation is greater than the mean (Tab. 4).

The sequences of the mean trace metal concentrations arranged in order of decreasing concentration for filtered water (Tab. 5), soft tissues and shells from the 15 stations are the following:

- For water: Al(45%)>Fe(35%)>Zn(15%)>Mn(3%)> >Cu(0.83%)
- For shell: Mn(59%)>Fe(25%)>Al(12%)>Zn(3%)> >Cu(0.54%)
- For soft tissues: Mn(65%)>Fe(28%)>Zn(3%)> >Al(2%)>Cu(0.18%).

The figures in brackets are the mean percentages of the trace metals. To get more homogenous samples, we used the data referring to the mussels of length classes D and E (55-74 mm), which are those with the highest number of specimens. Because the macroelement calcium is always the most abundant metal the water, in the shell and soft tissues, it was not taken into consideration in the sequences of trace metals. After calcium, aluminium is the most abundant metal in the water, with manganese most abundant in the shell and soft tissues.

Interestingly, the sequence in the shell is very similar to that in the soft tissues, except for aluminium which is concentrated more in the shell than in the tissues. The sequence in the water is rather different from the sequences both in the shell and in the tissues, suggesting that mussels have an effective capacity to select metals.

In addition, the sequences of metals in water, shell and tissues were drawn up for each station. The concentration of aluminium in the water as well as in the tissues and shells shows great variability, so that in the sequences for the same compartment (e.g. shell, tissues, water) of the different stations it may fill different places in the sequence. Because of this, and because this metal does not play a physiological role, aluminium was not taken into account in the sequences of the stations. If the Al is excluded, the sequences for the water of 11

stations, those for the shells of 12 stations and those for tissues of 13 stations were identical to the sequences corresponding to the water, shell and tissues based on the mean values of the 15 stations reported above. These sequences were observed in most of the stations, but in a few of them the sequences were different due to the exceedingly high concentrations of one metal. For the water, in stations 8, 10 and 15 Zn concentration was higher than that of Fe and in station 12 Cu was higher than Mn. For the shell, in the station 14 Fe concentration was higher than that of Mn, in station 12 Cu was higher than Zn and in station 7 Zn was higher than Fe. For the soft tissues, in stations 12 and 14 Fe concentration was higher than that of Mn.

A positive relationship between Ca concentrations in the water of the lakes and those in the soft tissues from the same environments was evident (Fig. 2). Conversely, no negative relationship between trace metal concentrations in the tissues and Ca concentration in the water was observed. No relationship for Mn, Fe and Zn was noted and only a tendency for Cu and Al concentrations to increase in the tissues at low Ca concentrations in the water was found.

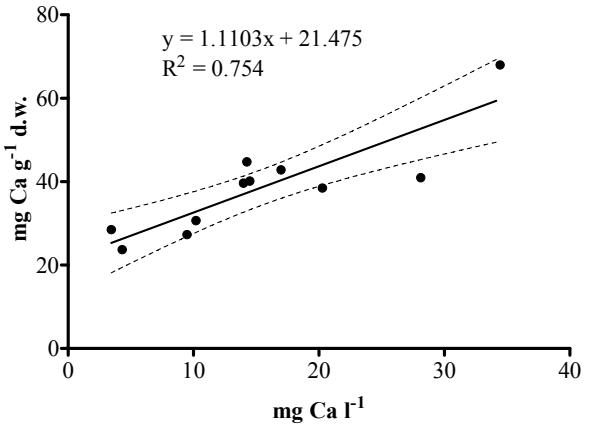

Fig. 2. Relationship between calcium concentrations in filtered lake water and in mussel soft tissues.

For each station the percentage of mussels belonging to each length class showed that in 7 stations (3, 4, 6, 7, 8, 9, 12) the smaller specimens (belonging to length classes B, C and D) were more abundant than those in the other 8 stations (1, 2, 5, 10, 11, 13, 14, 15), where larger individuals (E, F and G classes) were more frequent (Fig. 3). In fact, in the 7 stations more than 60% of the specimens were <65 mm and less than 40% greater than 65 mm; conversely in the other 8 stations more than 60% of the mussels were >65 mm and less than 40% <65 mm.

Because shell length increases with age, the demographic structure of the populations of the 7 stations (group A) is different from that of the populations of the 8 stations (group B). With this in mind it seemed interesting to ascertain if there was a relationship between the demographical structure of a population (directly or indirectly influenced by the physical environment) and the mean concentration of metals in the soft tissues of the mussels of the two groups of populations (A and B).

Because the results may be influenced by the different capacity for accumulating metals by individuals of different size (e.g. Boyden 1977; Strong & Luoma 1981; Popham & D'Auria 1983; Brix & Lyngby 1985); the homogeneity of the samples is essential. Consequently, only the metal concentrations in the mussels belonging to classes D and E (55-74 mm) were considered.

The results (Tab. 6) showed clearly that the mean concentration of metals in the tissues of the mussels of group A are always higher than those of B group; the greatest differences were observed for Al, Fe and Mn and the smallest for Cu and Zn. The mean concentration of metals in the water of the stations in group A is always lower than that of group B, except for the Ca concentration, which in the water of the group A is higher than that of group B. Therefore, the metal concentrations in the tissues of mussels do not reflect the concentrations of the metal in the water. For example, Mn concentration in the tissues of mussels of group A is more than twice that calculated for group B, although the concentration of the same metal in the water of the group B is 1.6 times than that of group A. Aluminium concentration in the tissues of the group A is greater than that of the B group, but the Al concentration in the water of the group B is 16 times higher than that in the water of group A.

To obtain a better information on this relationship, for each station the metal concentrations in the tissues were compared with those in the water. No relationship for Al, Cu, Fe and Zn was found for the stations of groups A and B and only a weak relationship for Ca and Mn for the stations of the B group. The mean concentrations of Al, Zn, Mn and Ca in the shell of group A are higher than those of the group B, while concentrations of Cu, and particularly of Fe, are higher in group B than those calculated for group A. As stated above for the tissues, no relationship emerged between metal concentrations in the shell and those in the water.

Of the seven stations of the group A one was located in a eutrophic lake (Lake Annone), two in a mesotrophic lake (Lake Segrino), four in oligo-mesotrophic lakes (Lago Maggiore and Lake Montorfano) and one in the oligotrophic Lake Mergozzo. Of the eight stations of the group B five were located in eutrophic lakes (Lake Varese, Lake Comabbio, Lake Pusiano, Lake Viverone, Lake Candia), two in lakes (Lake Levico and Lake Caldonazzo) which two decades ago were eutrophic and now are considered mesotrophic and only one (Lake Mergozzo) is oligotrophic (Tab. 1).

In conclusion, the group A stations are generally in lakes with low production, those of group B in lakes with middle and high trophic levels.

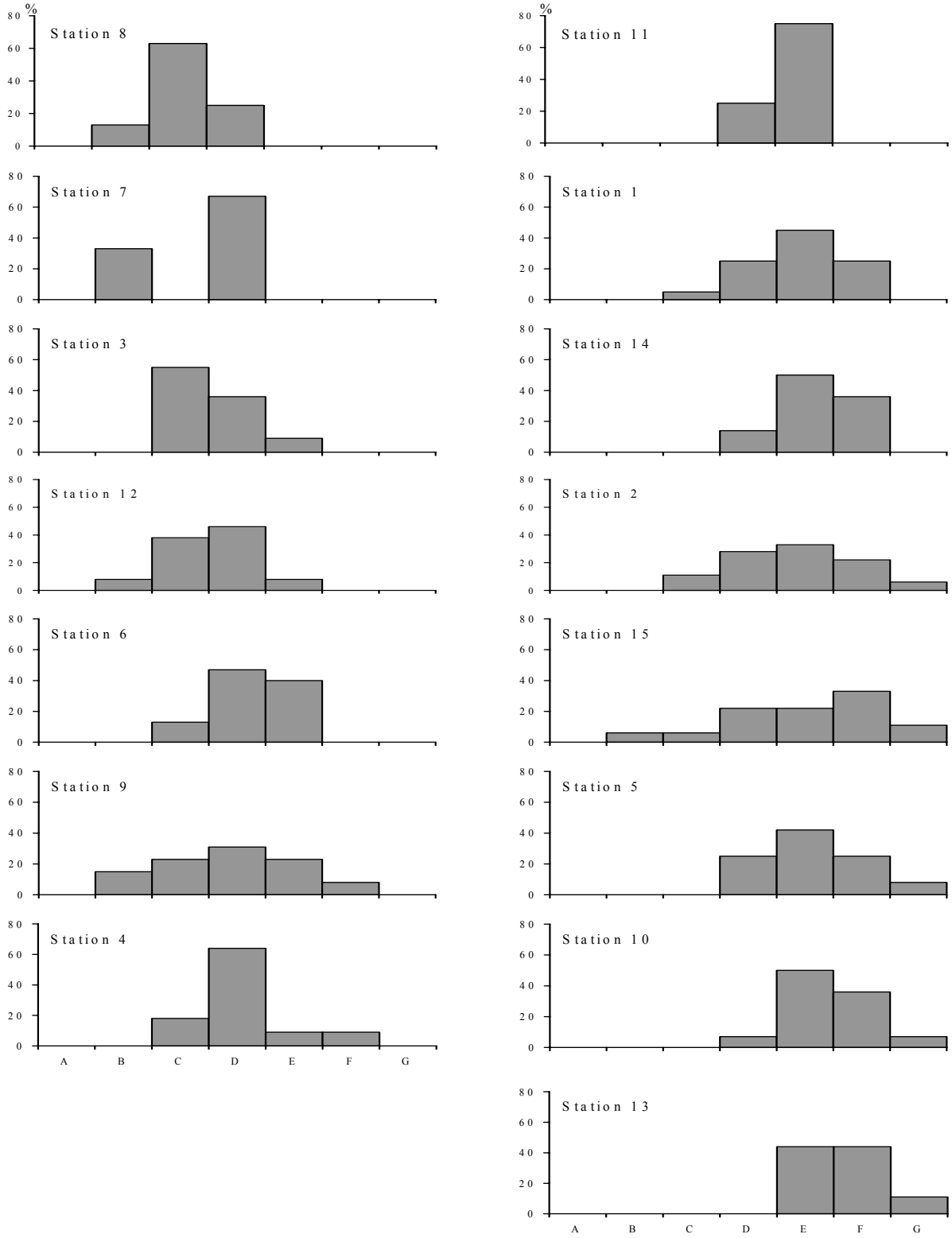

Fig. 3. Percent size distribution of the mussels in the size classes (shell length) from the 15 stations.

Tab. 6. Mean metal concentrations in the soft tissues, shell and water from 7 stations of the group A (N° 3, 4, 6, 7, 8, 9, 12) and 8 of the group B (N° 1, 2, 5, 10, 11, 13, 14, 15). The concentrations in the soft tissues and shell are expressed in μg g^{-1}dw, except those of calcium in the shell (mg g^{-1}dw). The concentrations in the water are expressed in μg·l^{-1}. Only the mussels belonging to the size-classes D and E were considered.

Group		Al	Cu	Zn	Fe	Mn	Ca
A	tissues	244	23	420	3842	10808	76565
	shell	118	3	27	130	449	391
	water	15	1	15	38	3	21
B	tissues	199	20	405	2611	4912	31032
	shell	67	5	19	231	424	367
	water	172	1	20	43	4	14

Tab. 7. Mean metal concentrations in the soft tissues, shell and water from 5 stations (N° 3, 4, 9, 11, 12) settled in oligo- and oligo-mesotrophic lakes (O), 4 stations (N° 7, 8, 13, 14) in mesotrophic lakes (M) and 6 stations (N° 1, 2, 5, 6, 10, 15) in eutrophic lakes (E). The concentrations in the soft tissues and shell are expressed in μg g^{-1}dw, except those of calcium in the shell (mg g^{-1}dw). The concentrations in the water are expressed in μg l^{-1}. Only the mussels belonging to the size-classes D and E were considered.

		Al	Cu	Zn	Fe	Mn	Ca
O	tissues	339	30	404	3409	8225	59140
	shell	75	5	17	90	335	371
	water	70	1	15	38	3	11
M	tissues	175	12	547	3361	7094	38045
	shell	151	4	37	331	527	378
	water	54	1	15	41	4	25
E	tissues	141	19	313	2882	7574	56055
	shell	64	3	17	163	459	385
	water	153	1	23	43	4	17

The considerations outlined above suggest that the trophic level of the lake may (albeit indirectly) influence the demographic structure of the mussel populations as well as the capacity of the mussels to accumulate metals in their tissues. Moreover, it cannot be ruled out that demographic characteristics, influenced by the trophic level of the environment, may in their turn effect the mussels' capacity for accumulating metals.

To ascertain the relationship between metal concentrations in the mussels and the trophic level of the lake in which they live, the 15 stations were divided into three groups reflecting the trophic level of the lake where the station was located: a) oligotrophic and oligo-mesotrophic (stations: 3, 4, 9, 11, 12); b) mesotrophic (stations: 7, 8, 13, 14) and c) eutrophic (stations: 1, 2, 5, 6, 10, 15). Interesting, the genus *Anodonta* was present only in the mesotrophic and eutrophic stations.

Table 7 shows the mean metal concentrations in the soft tissues and the shells of the mussels (length classes D and E) collected from the stations of the three groups, a), b), and c). The same table lists the mean metal concentrations in the waters of the three groups. Apart from zinc, which has a higher concentration in the tissues of mussels from mesotrophic lakes, the other five metals are more concentrated in the tissues of mussels from oligotrophic and oligo-mesotrophic lakes than in those from eutrophic and mesotrophic lakes.

The metals enrichment in the tissues of mussels from low productive lakes cannot be due to an abundance of metals in the water, because the metal concentrations in the oligotrophic and oligo-mesotrophic lakes are lower than those measured in the mesotrophic and eutrophic ones. Copper is the sole exception, with the same mean concentration in the water of the three groups of lakes (0.9 μg l^{-1}). A comparison between high productive and low productive lakes shows clearly that in the latter the metal concentrations are lower in the water and higher in the mussel tissues.

There is no clear relationship between metal concentrations in the tissues and those in the water of the three groups of lakes. The differences between the mean concentrations of copper in the shell of the three groups of lakes are negligible, while the concentrations of the other five metals are higher in the shells from the mesotrophic and eutrophic lakes than those from the oligotrophic and oligo-mesotrophic lakes.

In conclusion, the pattern of metal concentrations in the shell is very different from that of the tissues; this is true for populations with a different demographic structure (groups A and B) as well as for populations

Tab. 8. Trace metal concentrations (µg g^{-1}dw) and Ca concentrations (µg g^{-1}dw for tissues and mg g^{-1}dw in for shell) in mussels belonging to different size classes (B, C, D, E, F, G). The mussels were collected from Lake Candia.

		Al	Cu	Zn	Fe	Mn	Ca
soft tissues	B	296	9	136	1750	2874	15634
	C	10608	6	142	1771	4147	21193
	D	154	63	182	3689	8942	36765
	E	206	58	-	4121	6337	24552
	F	60	4	426	5233	9752	38253
	G	30	11	1012	4206	6345	32454
shell	B	81	1	30	120	557	405
	C	102	1	19	127	457	381
	D	44	15	20	319	789	398
	E	13	2	22	396	1138	406
	F	17	1	30	498	1110	396
	G	14	2	22	558	1006	405

living in lakes with different trophic levels. These differences are due to both the metabolism and the pathway of metals in the shell, which are very different from those in the soft tissues.

In the same population, individuals of larger size are likely to be older than those of smaller size. In this connection, we compared the metal concentrations in the tissues of mussels with their shell length to ascertain if there is a relationship between the age of the mussel and the metal accumulation in its tissues. We used the mussels from station 15 (Lake Candia), which had the only population with sufficient mussel biomass for each length class to permit chemical analyses.

The data reported in table 8 reveal a certain tendency of Mn, Fe, Zn and Ca concentrations in the soft tissues to increase with the size of the mussel, but the correlation was significative only for Fe (Fig. 4). No relationship emerged for Cu and Al; the former probably due to its low concentration and Al because it cannot be metabolized. In the shell Fe and Mn concentrations were significatively correlated with mussel size, a tendency which is not evident for Ca, Cu and Al.

To evaluate the capacity of the mussel to concentrate in its tissues metals from a diluted solution of lake water the concentration factors (C.F.) were calculated. C.F. is the value of the ratio between the concentration of a metal in the tissues, expressed in terms of the wet weight, and the concentration of the same metal in filtered water. The concentrations in the soft tissues calculated on the dry weight must therefore be transformed into concentrations on the wet weight by dividing the value by 5.38 (Ravera *et al*. 2003). To minimise the influence of mussel size and to obtain more homogeneous values, only the concentrations in mussel tissue from length classes D and E were considered.

The mean values of the C.F. for each metal and the 15 stations are the following: Al = 3615; Cu = 4143; Zn = 7444; Fe = 51,634; Mn = 651,065 and Ca = 709. The high C.F. for Mn is the result of the low concentration of this metal in the water and the great capacity of the Unionidae to concentrate Mn in their tissues.

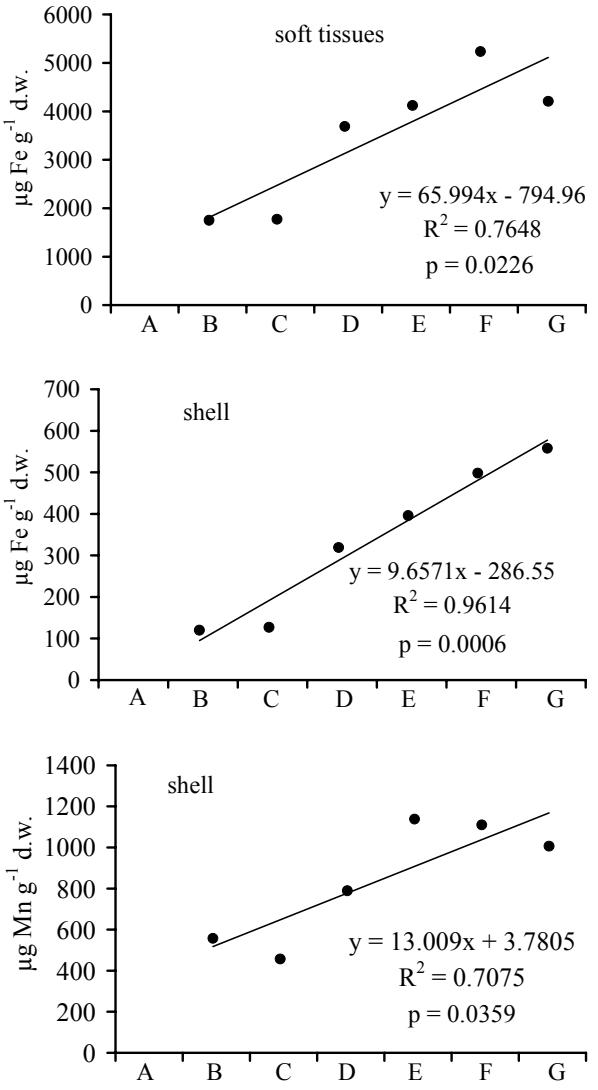

Fig. 4. Significative correlation between Fe concentrations in the soft tissues of mussels and their size classes and between Fe and Mn concentrations in the shell of mussels and their size classes. B = 35-44 mm; C = 45-54 mm; D = 55-64 mm; E = 65-74 mm; F = 75-84 mm; G = 85-94 mm.

Tab. 9. Concentration factors (C.F.) of metals in mussels belonging to the size classes D and E. Upper C.F. of mussels (soft tissues) from the stations of the group A (N° station: 3, 4, 7, 8, 9, 12) and the group B (N° station: 1, 2, 5, 10, 11, 13, 14, 15). Down – C.F. of mussels (soft tissues) from stations (N° 3, 4, 9, 11, 12) settled in oligotrophic-oligo-mesotrophic lakes (O) from stations (N° 7, 8, 13, 14) in mesotrophic lakes (M) and from stations (N° 1, 2, 5, 6, 10, 15) in eutrophic lakes (E).

Group	Al	Cu	Zn	Fe	Mn	Ca
A	5216	4259	9461	86486	1072140	842
B	2215	4027	6491	21141	282625	577
O	3464	4817	8272	41593	788715	1082
M	4013	3018	10661	108195	782944	369
E	3476	4144	5533	22297	448438	569

The mean values of the C.F. calculated for the stations of group A are higher than those of group B (Tab. 9). If the metal concentration in the tissues is fairly constant and the concentration of the same metal in the water is low, the C.F. value increases. This may explain the high C.F. values in group A for Al, Cu and Zn, but not for Mn, Fe and Ca. In fact, the C.F. values for Fe and Mn in A are 4 times those calculated for B, whereas the Fe concentrations in the water of B are about the same as those in the water of A; that of Mn in B is 1.5 times that of A. Ca concentration in the water of A is very high compared with that of B and in the tissues of A the concentration is 2.5 times that in B. The C.F. values for Fe, Mn and Ca, higher in A than in B, are probably not the consequence of the different concentrations of these metals in the water, but are probably due to the fact that the mussels of group A have a greater capacity for accumulating metals than the mussels of group B, or because of the greater availability of metal forms in the lakes of group A.

The influence of the trophic level of the lakes on the C.F. values varies with the metal (Tab. 9). In fact, the highest values for Cu, Mn and Ca were calculated in the stations of the oligotrophic and oligo-mesotrophic lakes and for Al, Zn and Fe in the mesotrophic lakes, whereas the values for the eutrophic lakes are generally low.

4. DISCUSSION AND CONCLUSIONS

The primary aim of this research was to compare the concentrations of some metals in soft tissues of *Unio pictorum mancus* (Unionidae) from 12 different lakes with the concentrations of the same metals in the lake waters. This comparison forms the basis of the biomonitoring method which uses species accumulators of pollutants.

The metal concentrations in the soft tissues should reflect the present level of the water contamination by the same metals, while those in the shell the time-integrated metal contamination of the environment. This difference is due to the metabolic turnover time, which is very slow for the shell and relatively rapid for the soft tissues.

Time integration is commonly considered an advantage by the advocates of this type of monitoring. It is undoubtedly an advantage if the pollution history of the environment can be reconstructed by analysing the pollutants in the layers of the shell. Unfortunately, studies on this subject are rather scarce because of the difficulties of the methods (e.g. Nelson 1964; Clark 1980; McCuaig & Green 1983; Day 1984; Carell *et al*. 1987; Nyström & Dunca 1996; Westermark *et al*. 1996; Mutvei & Westermark 2001). If, on the other hand, the shell is analysed *in toto* time integration cannot be an advantage because the pollutants accumulated in the past together with the more recent ones cannot reflect either the present environmental situation or that of the past. For this reason our main aim in analysing the shell was not to establish a relationship between the metal concentrations in the shell and those in the water, but to compare the metal concentrations in the shell with those in the soft tissues.

Mussels take up elements from the water and with food, and a fraction of these is accumulated in the soft tissues. A part of the metabolised elements is transferred from the mantle to the shell. A certain amount of the elements present in the shell is adsorbed from the water onto the periostracum, which is colonized by a film of bacteria, algae, protozoa and other small organisms. These organisms may play an important role on the shell; for example, according Chipman & Schommers (1968) the bacterial activity on the periostracum controls the manganese concentration in the shell.

Mussels were collected over two weeks during the growing season (Summer) from 12 lakes located in the same ranges of latitude and altitude during two weeks to reduce to a minimum the seasonal influence on the metal concentrations in the mussels. In fact, this influence, the combined result of the biological cycle and the seasonal variations of the physical environment, may be considerable (Bryan 1973; Metcalfe-Smith 1994; Nyström *et al*. 1996).

From the measurement of the shell length of all the specimens it was clear that the small mussels were more frequent in some stations, while large ones were relatively more abundant in others. The maximum size at-

tained by mussels is determined by the resources of their environment; over this limit growth is very slow or negligible (Seed 1968). In fact, the stations with a greater number of small individuals were those in less productive lakes, while large individuals were commoner in more productive lakes. This difference may be due to the different abundance and availability of food, which consists of suspended organic particles (e.g. algae), which are generally more abundant in productive than in low productive lakes. The higher mean concentrations of metal in the mussels from oligotrophic and oligo-mesotrophic lakes than in those from mesotrophic and eutrophic lakes are more difficult to explain.

In fact, this difference was due neither to mussel size, because only the medium-sized individuals (55-74 mm) were considered nor to the metal concentrations in the water, because these were higher in productive than low productive lakes. Bryan (1973) observed a decrease of metal concentrations in the tissues of marine bivalves during periods of high phytoplankton density, and an increase when the phytoplankton density was low. The rapid reproduction of the phytoplankton caused a decrease of the metal concentrations in the water and a low concentration of metals in the algal cells due to biological dilution. As a result the bivalves feed on phytoplankton poor in metals so that the metal concentrations in their tissues were low. In contrast, the low density of phytoplankton richer in metals was the cause of the high concentrations of metals in the bivalve tissues.

Another possible cause may be the more abundant metals in available forms in the oligotrophic and oligo-mesotrophic lakes than in mesotrophic and eutrophic lakes. Unfortunately, this hypothesis cannot be tested because our analyses are of the total metals and not their physical and chemical species (Zamuda & Sunda 1982).

There may be another cause. In an low productive lake, the low concentration of suspended particles (e.g. phytoplankton), which constitute the mussels' food, may reduce the growth-rate of the molluscs. As a result, mussels of the same size might be older in low productive than in productive lakes, and so have had more time to accumulate metals in their tissues. To test this hypothesis the absolute age of the mussels must be known. Dating mussels which live at Southern European latitudes is more difficult than it is for those from the Northern areas (e.g. Scandinavia), where the long cold winter suspends shell growth, producing a well-defined dark growth line on the external surface of the shell. Another cause of the high concentration of metal in mussels from low productive lakes may be their greater capacity to accumulate metals in their bodies.

In conclusion, the trophic level of the environment appears to have a considerable impact on the biometrical structure of the population as well as on metal accumulation in mussel tissues.

These hypotheses, or interactions between them, may explain the quantitative differences of metal accumulation in mussels from lakes with different trophic levels; unfortunately, the reliability of these hypotheses cannot be demonstrated by this study. Although on the basis of our data, the importance of the lake's trophic level seems to be clear, the variables responsible for the different accumulation of metals in mussels from lakes with different trophic levels cannot be identified. This is the usual difficulty with field studies, which have to cope with the complexity of the ecosystem. For example, eutrophic lakes always have high concentrations of suspended particles, which on their surface rapidly adsorb ionic metals, the metal most available form to aquatic organisms (Spry & Wiener 1991). By metal adsorption onto the particles, metal sedimentation is accelerated and concentrations in the water decrease. On the other hand, if a lower concentration of ionic metals in the water is available, mussels take up a greater amount of metals with the particles ingested as a food. In addition, a fairly important influence on metal concentrations in the water is constituted by iron and manganese co-precipitating with other metals in the presence of oxygen (e.g. Markich & Joffree 1994).

The detoxification processes studied in several species of mussels work through the production of calcium phosphate granules (Jeffree et al. 1993; Naimo 1995; Adams & Shorey 1998; Langston et al. 1998; Byrne 2000) and/or thioneins (es. Roesijadi 1992; High *et al*. 1997). The granules and thioneins sequester the excess of metals uptaken by the mussel and abolish their potential toxic effects, which may explain the relatively high concentrations of toxic metals in the mussels.

Although mussels acquire a certain percentage of their calcium in food, most of the content of this metal in their tissues, and then in the shell, is uptaken through the gills from the water (e.g. Pynnönen 1991). Calcium, with its relatively low affinity for chelating substances, is generally present in the water in forms available to the organisms (Förstner & Wittmann 1983).

In their laboratory experiments, on mussels Markich & Jeffree (1994) observed two very interesting effects deriving from the increase of the Ca concentration in the water: a) the Ca uptake-rate increases and b) there is a considerable reduction of divalent trace metals in the tissues through competition for sites at the calcium channel. Result b) means that the divalent (and also some trivalent) trace metals follow the same metabolic pathway as calcium from water to the mussel. This may explain why mussels also uptake and accumulate non-essential and toxic elements in their bodies.

Our data show clear evidence of a relationship between the Ca concentrations in the water of the various lakes and those in the tissues of mussels from the same lakes (Fig. 2). Conversely, no relationship was observed for Mn, Zn and Fe, and there was a certain tendency for the Cu and Al concentrations in the tissues to increase at

low concentrations of Ca in the water. This partial contrast with Markich and Joffree's results is probably due to the different media in which the studies were carried out. These authors' research was short-term and was carried out in the laboratory with "artificial water" contaminated by prefixed amounts of trace metals, mostly in ionic form. Our research was carried out in natural waters containing suspended particles, colloids and chelating substances (Winner 1986), in addition to the metals in various physical-chemical forms.

In conclusion, the metal body burden of a mussel is controlled by the metal concentrations in the water and food, the amount of available forms of the metal, the selective capacity of the mussel, and its metabolic rate, which in turn, is influenced by the physical environment. The combined influence of these variables controls the relationship between the metal concentration in the mussel tissues and that in the water in which it lives.

In our case, establishing this relationship is very difficult because the influence of the physical environment on the metal concentration in the mussels varies with the lake. In addition, the populations of the same mussel species living in various lakes may have developed different capacities for selecting and accumulating metals by adaptation and selection processes.

At the same depth, the pelagic environment is fairly homogeneous compared to the littoral zone. The littoral may show more or less important differences along the lake perimeter, which is directly exposed to the various influences from the watershed. These differences increase with the sinuosity of the lake perimeter. This may explain the differences in metal concentrations in the tissues of mussels collected from different stations in the same lake. For example, in the oligotrophic Lake Mergozzo (stations 11 and 12) the differences between stations, except in the case of aluminium, are smaller than those in the oligo-mesotrophic Lago Maggiore (stations 3 and 4) and the mesotrophic Lake Segrino (stations 7 and 8) for iron, manganese and aluminium.

The metal concentration in an organism reflects that in the water in which it lives if the ratio between the metal concentration in the organism and that in the water is constant or at least similar. This means that the organism has very little or no capacity to select the metals to be uptaken. Such an ideal indicator species does not exist. A simple approach to test discrimination capacity consists in comparing the sequence of the metals arranged in order of decreasing concentration for the water with the sequence for mussel soft tissues and shell.

Our data highlight the similarity of the sequences of the soft tissues with those of the shell and the wide difference between both of these and the sequence of the metals in the water. This comparison shows that it is very difficult to evaluate the metal contamination in the water from the concentration of the metals in the mussels. Other authors have encountered the same difficulties (e.g. Johnson *et al*. 1993). Moreover, the capacity of mussels to discriminate between metals is quite clear, although this capacity is less effective than that developed by other, more evolved taxa, such as fish (Metcalfe-Smith *et al*. 1996).

A major drawback of using mussels as biomonitors of pollutants is their wide variability in concentrating the pollutants, even in individuals of the same size from the same population (e.g. Bryan 1973; Millington & Walker 1983; Metcalfe-Smith *et al*. 1996).

These are the most important conclusions drawn from this study:

a) small sized mussels dominate in low productive lakes, whereas in productive lakes large sized mussels are dominant. According to some authors (e.g. Buddensiek *et al*. 1993; Patzner & Müller 2001), only the oldest mussels may survive in very trophic environments, as they are less sensitive than the juveniles. It is obvious that these populations are fated to the extinction;
b) in spite of the lower concentrations of trace metals in the water, the metal concentrations in the tissues of mussels living in low productive lakes are higher than those in the mussels (belonging to the same size class) living in productive lakes, which have higher metal concentrations in the water;
c) a direct, positive relationship between calcium concentration in the water and in the tissues of mussels has been established;
d) no relationship between trace metal concentrations in the soft tissues and in the shell has been found.

The implications of b) are that the trace metal concentrations in the soft tissues do not reflect those measured in the water of the environment in which the mussels live. As an example, figure 5 is a schematic representation of the relationship between the manganese concentrations in the soft tissues of mussels from the 15 stations and those measured in the water form the same stations. The result, at least in our study, is that mussels cannot be regarded as reliable bioindicators for predicting the contamination level of their environment; which is the essential aim of routine biomonitoring. This may be due to the fact that, in our study, there were no lake with extreme differences in metal pollution levels. As is noted above, other authors (e.g. Johnson *et al*. 1993; Metcalfe-Smith *et al*. 1996) have found a similar difficulty with using mussels as a pollutant indicator.

On the other hand, if differences among physical environments are taken into account (e.g. available forms of metal concentrations, the quality and quantity of mussel food and its concentrations of available metals), and the different reactions from the various populations of a species to the same environmental variables are considered, a clear relationship between the metal concentrations in the filtered water and those in the mussel tissues might be difficulty expected.

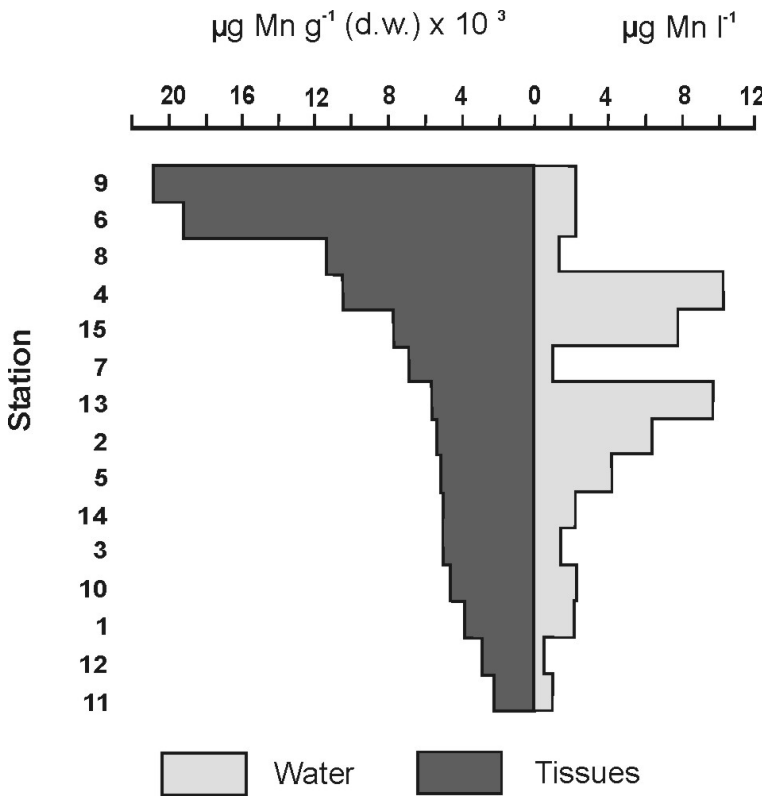

Fig. 5. Manganese concentrations in the tissues of mussels (µg g^{-1}dw) and in the water (µg l^{-1}) of the 15 stations. To minimize the possible influence of mussel size, all the specimens taken into account ranged from 55 mm to 74 mm length (size classes: D and E).

In addition, the most commonly used mussel monitoring technique consist in analysing samples of mussel tissues and water collected at the same time from the same stations. This means that the water analyses reflect the situation at the moment of sampling, whereas those of the soft tissues is the result of integrating present and past situations, which depends on the biological turnover time of the metals in the tissues in relation to variations of the metal concentrations in the water. Although the turnover time of metals in the tissues is very short compared with that in the shell, it undoubtedly has an influence on the comparison between metal concentrations in the water and the tissues.

These considerations, which refer to the oversimplified type of monitoring commonly used, do not mean that the mussels are not excellent material for monitoring focusing on well-identified problems. First of all, in addition to the biological factors influencing the uptake and accumulation of pollutants in the organism, the environmental variables acting on the abundance and availability of the pollutants must be taken into account.

Some examples of the use of mussels for pollution monitoring are given below.

The transplantation of mussels from a clean site to a polluted one may be a useful tool for identifying the pollutants present in the polluted site and for following the kinetics of the pollutant uptake (e.g. Andres *et al*. 1999; Baudrimont *et al.* 1999; Furely & Oliveira Filho 2000). In addition, transferring the contaminated mussels back to their original, clean site can be used to follow the pollutant loss over time.

Mussel analyses may be used to identify new pollutants in the environment and to follow their load variations over time. This was the method first used to detect Mn-54 from the fall-out of nuclear tests in the Pacific area in Europe in 1960, when mussels from two lakes in Northern Italy, Lago Maggiore and Lake Varese, were analysed (Ravera & Vido 1961). At that time, the Mn-54 activity was so low that it could not be measured in any aquatic organism (e.g. aquatic plants, gastropods, fish) except mussels (*Unio* and *Anodonta*). Variations in Mn-54 activity from September 1960 to December 1963 were also monitored by analysing its activity in mussels from Lago Maggiore (Gaglione & Ravera 1964). The distribution in lakes and rivers of Northern Italy of the fission products (Cs-134 and Cs-137) from the fall-out of the Chernobyl accident was also studied through analyses on mussels (Riccardi & Ravera 1986).

Since shell chemistry roughly reflects that of the water in which the mussels live, important information on past environmental conditions may by acquired by studying the shell (e.g. Carell *et al*. 1987; Westermark *et al*. 1996). For the same purpose, the chemical composition of shells of living mussels was compared to that of shells from museum collections. The results of this comparison were very interesting and involved a variety

of topics such as acidification, eutrophication and metal pollution (Mutvei & Westermark 2001).

Long term variations of pollutant concentrations in an environment may be monitoring by analysing the tissues of a mussel population, taking into account both the variations with the season and mussel size. To establish a relationship between the pollutant concentrations in the mussels and those in their environment, the pollutants in the water, sediment and suspended particles must be also analysed. This long term monitoring may produce better and more useful results than oversimplified short term monitoring.

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