TRACE ELEMENTS IN THE PELAGIC COELENTERATE, *VELELLA LATA*

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ABSTRACT

Twelve elements (Cu, Ni, Mn, Ti, Si, Cr, Sr, V, Ba, Ag, Pb, and Mo), not previously reported from the genus *Velella*, were found in the ash of *V. lata*; zirconium was also searched for but was not found. Cu, Ni, Mn, Ti, Mg, Ca, and Al were determined quantitatively. The concentration of trace elements in the whole animal and in five different tissues of *V. lata* was determined in specimens ranging from 14.4 to 84.6 mm in length. The Mg:Ca ratio in *Velella* is lower than that in sea water but higher than that in Scyphozoa. Presumably this is due to concentration of magnesium in the chlorophyll of commensal zooxanthellae.

INTRODUCTION

Although the distribution and function of trace elements in plants and animals has received widespread attention in the past 50 years, especially with regard to agriculture, the influence of trace elements on marine plankton is still relatively unknown. One of the many difficulties that has delayed advances in this field is the extreme variability of trace element concentration, not only among species but also among individuals of the same species (Vinogradov, 1953). When a study of the trace element content of plankton from different water masses was thwarted because of the great variation in results between different parts of the same plankton sample, the present study of variability in a single species, *Velella lata*, was undertaken. An attempt has been made to eliminate the following variables: collection time, collection locality, individual variation, and variation due to undigested food.

METHODS

The animals used in this study were dip-netted at Lat. 32°41′N, Long. 121°04′W on 10 May 1950. Immediately after capture they were preserved in a 5% buffered formalin solution. The body parts were dissected and all particles identifiable as food were removed from the gastrozooids and gonozooids.

Wet weights of the body parts were determined volumetrically or gravimetrically, depending on the amount of material. After drying at 100°C, the body parts were ashed in a muffle furnace that was raised slowly to 800°C. Dry and ash weights of the parts were determined before the samples cooled to room temperature.

The ash residue of each kind of body part was mixed with a pure graphite powder matrix in a ratio of 1:1, after which the mixture was ground to a fine homogeneous powder in a silica mortar. The graphite matrix was prepared by adding potassium sulfate to graphite in a ratio of 1:9 and then by adding cobalt oxide to this mixture in the ratio of 0.001:1. The K_2SO_4 was used to promote uniform volatilization of the ash. The cobalt served as an internal standard of known concentration for correction of such variables as the amount of sample packed in each electrode or length of burning time, local variation in plate sensitivity, and amperage variation during burning. Preliminary analysis of ash from whole animals revealed less than 20 ppm cobalt.

Standards for calcium and magnesium were prepared by mixing appropriate amounts of dolomite (analyzed by U. S. National Bureau of Standards) with NaCl and combining this with the cobalt-graphite matrix. Standards for heavy metals were made up from their salts mixed in suitable concentrations with dolomite and the matrix. Duplicate quantitative analyses were run in all cases, and only those that agreed within $\pm 12\%$ were averaged and are reported here.

Details of equipment and technique used are as follows: Spectrograph—Jarrel-Ash, 3.4 m plane grating. Excitation—17 amp, D.C. Exposure—complete burning time of sample averaged about one minute. Rotating sector—seven steps with 1:2 step sector. Gap—4μ. Slit—10μ. Electrodes—$\frac{1}{8}$ inch (3 mm) diameter graphite drilled to 10 mm depth with a $^3/_{32}$ inch drill; counter electrode $\frac{1}{8}$ inch graphite, pointed. Film—spectrum analysis No. 1, Eastman. Development—D-19 developer, three minutes at 20°C, stop bath one minute, fixer three minutes, wash 10 minutes, air dried. CO_2 blower—O. Joensuu modification of the Stallwood (1954) carbon dioxide blower used to provide uniform burning and to reduce drift of working curves; pressure controlled with the aid of a mercury manometer. Emission lines (Å)—Ca 3181, Mg 3336, Al 3082, Cu 3274, Ti 3088, Ni 3050, Mn 2576, Fe 3021, Si 2882, Cr 2843, Sr 3464, V 3102, Ba 4554, Ag 3383, Pb 2833, Mo 3170, Zr 3391.

RESULTS

In the sun-dried remains of 100 kg of wet *Velella*, Haurowitz and Waelsch (1926) found nine elements: Na, K, Mg, Ca, Al, Fe, Cl, S, and P. In addition to these, the present study shows the following to be present in *V. lata*: Cu, Ni, Mn, Ti, Si, Cr, Sr, V, Ba, Ag, Pb, and Mo. Elements measured qualitatively in this study were present in concentrations greater than the following limiting sensitivities: Fe 10 ppm, Si 10 ppm, Cr 100 ppm, Sr 200 ppm, V 70 ppm, Ba 100 ppm, Ag 3 ppm, Pb 10 ppm, and Mo 10 ppm. One element, zirconium, was below the limit of detectability (150 ppm).

Table I lists the concentrations of Mg, Ca, Al, Cu, Mn, Ni, and Ti which were determined quantitatively in the ash of *V. lata* at five sizes. In order to facilitate conversion of the data in Table I to percent of dry or wet weight, the ratios of wet weight to dry weight and of dry weight to ash weight are given in Table II; also given are total wet weights. At a length of 85 mm, 61% of the wet weight of

TABLE I. Trace elements in *Velella lata*, percent of ash (mean of 2 analyses)

	$\bar{X}$ length (mm) and number of specimens					
	14.4	24.4	34.9	53.8	84.6	*Wtd. Mean*
	39	31	15	8	6	
Tissue	Magnesium					
P	2.6	4.8	7.8	5.5	12.0	7.7
SL	—	—	—	—	—	5.2
G	5.4	7.0	8.8	4.0	7.4	6.9
M	—	1.9	2.5	5.0	6.0	4.7
D	(2.9)		6.7	—	3.1	4.3
W	5.1	5.7	7.1	5.0	7.1	—
	Calcium					
P	7.5	—	4.6	9.0	9.8	6.0
SL	—	—	—	—	—	4.8
G	0.54	0.64	0.78	0.76	1.4	0.71
M	—	0.30	0.32	0.99	1.7	1.1
D	(0.53)		2.2	6.4	6.5	5.2
W	1.5	0.56	2.4	5.2	5.6	—
	Aluminum					
P	—	—	—	—	—	—
SL	—	—	—	—	—	—
G	0.37	0.25	0.32	0.17	0.26	0.29
M	—	0.31	0.20	0.32	0.28	0.28
D	(0.29)		0.12	—	0.018	0.17
W	0.36	0.29	0.23	0.28	0.16	—

TABLE I.—*Continued*

Tissue						
	Copper					
P	0.078	0.060	0.26	0.27	0.52	0.28
SL	—	—	—	—	—	0.36
G	0.070	0.16	0.15	0.04	0.16	0.13
M	—	0.09	0.13	0.12	0.10	0.11
D	(0.024)		0.02	0.057	0.049	0.042
W	0.071	0.12	0.16	0.13	0.22	—
	Manganese					
P	0.0031	0.0019	0.0010	0.0034	0.0055	0.0029
SL	—	—	—	—	—	0.0026
G	0.0004	0.0004	0.0008	0.0010	0.0027	0.0007
M	—	0.0004	0.0004	0.0027	0.0030	0.0023
D	(0.0006)		0.0004	0.0047	trace	0.0016
W	0.0010	0.0009	0.0007	0.0036	0.0041	—
	Nickel					
P	0.0084	—	0.0085	0.026	0.025	0.018
SL	—	—	—	—	—	0.011
G	0.0054	0.0026	0.011	0.019	0.0052	0.0060
M	—	0.0026	0.0038	0.019	0.0093	0.013
D	(0.0085)		0.0050	—	—	0.0019
W	0.0059	0.0033	0.0079	0.022	0.016	—
	Titanium					
P	0.14	—	0.034	0.061	0.032	0.044
SL	—	—	—	—	—	—
G	0.046	0.017	0.062	0.014	0.014	0.034
M	—	0.070	0.10	0.14	0.082	0.11
D	(0.058)		0.015	—	trace	0.027
W	0.059	0.14	0.046	0.088	0.051	—

P = Tissues covering the skeleton, SL = Skeleton, G = Gonozooids, M = Medusae, D = Dactyls, W = Whole Organism.

Samples in parentheses were combined because of insufficient ash. Size group analyses were not made on skeletons due to insufficient ash.

Dashes indicate analysis not available.

$$\text{Weighted mean} = \frac{\Sigma \text{ weights of the element in each size group}}{\Sigma \text{ ash weight of each size group}}.$$

TABLE II.

$\bar{X}$ length mm	14.4	24.4	34.9	53.8	84.6
Wet weight / Dry weight	22.4	19.0	20.0	20.5	23.5
Dry weight / Ash weight	3.0	4.1	4.5	6.5	10.1
Total wet weight per specimen (mg).	128	370	1050	2670	8310

whole *V. lata* was tissue covering the skeleton, 15% dactyls, 12% skeleton; the gonozoids and medusae each made up 6%.

Magnesium and Calcium. These occur in sea water at concentrations of 0.13 and 0.04% respectively. Strictly speaking they are not trace elements. In *V. lata* magnesium made up 6.0% of the total ash and calcium 3.4%. Thus the Mg:Ca ratio is 1.8:1 in *Velella* compared to 3.7:1 in sea water, indicating that calcium is concentrated over magnesium in *Velella.* The Mg:Ca ratio in *Aurelia* has been reported as 0.92:1 and in *Cyanea* and *Aequorea* as 1:1 (Prosser, 1950), but since these are ratios of ions in the body fluids, they are not strictly comparable to the present data on the whole animal. However, it seems reasonable to expect *V. lata* to have a higher Mg:Ca ratio than these medusae, because *V. lata* contains large amounts of commensal zooxanthellae. Presumably these organisms would add to the concentration of magnesium since they contain chlorophyll.

Calcium is important in the control of permeability and hence of osmoregulation in estuarine animals. It is also essential for normal neuromuscular activity, for amoeboid and ciliary movement and for stabilization of intercellular matrices and mucus coverings. Thus it has widespread and vital physiological functions. Magnesium, on the other hand, though an essential constituent of chlorophyll, is not as important to animals as calcium and generally is not taken up as readily by marine animals. The Mg:Ca ratio varies widely in different tissues of *V. lata.* It is highest in the gonozooids and medusae, 9.7 and 4.3 respectively, is intermediate in the skeleton and in the tissue covering the skeleton, 1.1 and 1.2, and is lowest in the dactyls, 0.83. This distribution agrees well with the occurrence of the zooxanthellae, which are most abundant in the medusae and gonozooids and less common in the dactyls and tissue covering the skeleton. The ratio decreases in the gonozooids, medusae and dactyls as the size of *V. lata* increases, but it remains relatively constant or perhaps increases slightly in the tissue covering the skeleton. The relative concentration of calcium in the ash of the gonozooids, medusae, and dactyls appears to increase with size of *V. lata.* In the whole animal, from 14 to 85 mm total length, calcium concentration increases by a factor of about 5.

Aluminum. Although this is an essential element in terrestrial plant nutrition and is widespread in its occurrence in the animal kingdom, its function or functions are not known. It appears to be an essential constituent of the succinic oxidase system (Prosser, 1950). That Haurowitz and Waelsch (1926) found aluminum in *Velella* in a preliminary qualitative analysis of its ash indicates that it is unusually common there. In the present study it was concentrated by a factor of 2000 over its average concentration in sea water and appears to remain relatively constant in the gonozooids and medusae as *V. lata* becomes larger. Aluminum appears to decrease in concentration in the dactyls with increase in size of *V. lata*. In the whole animal its concentration appears to decrease by a factor of about 2. Its concentration varies rather widely in the sea, Sverdrup, *et al.* (1946) reporting a range of 0.16 to 1.8 ppm. If aluminum should prove to be vital to the metabolism of *V. lata*, variations in the concentration of this element in the sea could be important in the ecology of the species.

Copper. This element is generally considered to be an essential constituent of protoplasm. It is necessary in the synthesis of haemocyanin, haemoglobin, and cytochrome a, and it occurs (like molybdenum, iron, and manganese) in certain metalloflavoproteins (Mahler, 1956).

The skeleton and the tissue covering the skeleton had relatively more copper than the gonozooids and medusae, which contained about three times more than the dactyls. The percent of copper in the ash of all tissues remained fairly constant as *V. lata* increased in size, excepting the tissue that covers the skeleton, where it increased by a factor of about seven. The only other comparable increase in concentration with increase in age of *V. lata* is manganese in the gonozooids and medusae. In the whole animal the copper concentration increases by a factor of about three.

Using the value of 0.003 ppm of copper in sea water (Goldberg, 1957), it appears that its concentration in *V. lata* tissues is about 5000 times greater than its abundance in sea water. This agrees well with its approximate concentration factor by soft marine invertebrates (Krumholz, *et al.*, 1957).

Manganese. This element was found in small amounts, making up from 0.0016 to 0.0029% of the total ash in all tissues except the gastrozooids, where it averaged about 0.0007%. In general the proportion of manganese in the ash increased in all tissues as *V. lata* increased in size. In the gonozooids and medusae the concentration of manganese increased by a factor of about seven, and in the whole animal by a factor of four.

It seems possible that manganese in *V. lata* is primarily tied up with the chlorophyll of the zooxanthellae, because it is essential for the synthesis of chlorophyll. However, there is a less pronounced correlation of manganese with the occurrence of the zooxanthellae than there is in the case of magnesium. On the average, *V. lata* appears to concentrate manganese by a factor of about 100 over its concentration in sea water.

Nickel. Nickel makes up between 0.01 and 0.02% of the total ash except in the gonozooids and dactyls, where it is 0.006 and 0.002% respectively. In the largest specimens nickel is about three times more concentrated than in the smallest *Velella* examined. On the average, *V. lata* concentrates nickel about 2000 times over its concentration in the sea. This is considerably greater than the nickel concentration factors of 170–970:1 reported by Laevastu and Thompson (1956) for fresh plankton. They reported a greater concentration of nickel by plankton at the surface than by plankton in deeper water.

Titanium. This element, found in all tissues except the skeleton, forms 0.02 to 0.04% of the ash in the skin, gonozooids, and dactyls, and 0.1% in the medusae. In the whole animal the concentration may vary by a factor of 4; however there is no trend with increase in size. Goldberg (1957) has recently reported titanium in sea water at a concentration of 0.001 ppm. Thus it appears that titanium is concentrated in *Velella* by a factor of about 5000, which is about five times the approximate concentration in most other soft marine invertebrates studied so far (Goldberg, 1957).

Griel and Robinson (1952) have reported that ash of marine diatoms is between 0.003 and 0.2% titanium, and that this is about $^1/_{20}$ of the range reported by Webb and Fearon (1937) for living organisms in general. The titanium content of *V. lata* falls within the range reported by Griel and Robinson. Some recent workers, Goldberg and Arrhenius (1958) for example, have suggested that titanium in the marine environment is primarily of terrigenous origin. Note that these *Velella* as well as the diatoms analysed by Griel and Robinson were collected relatively near shore, in water that could well be characterized as neritic. Thus organisms collected in truly oceanic water might show considerably lower titanium concentrations than these.

CONCLUSION

This study has demonstrated the presence of 12 elements not previously reported from *Velella*. Those analysed quantitatively are concentrated from 100 to 5000 times over their concentrations in the sea. The problem of uptake, distribution, and utilization of these elements in *V. lata* is complicated by the fact that each individual contains large quantities of commensal zooxanthellae, hence both plant and animal have been analyzed simultaneously. The plant factor could possibly be eliminated by keeping *Velella* in darkness long enough to bleach or kill the zooxanthellae. It seems reasonable to postulate that the higher Mg:Ca ratio in *V. lata* compared to that reported in scyphozoans is due to the presence of the zooxanthellae.

Between the lengths of 14 and 85 mm, manganese and calcium increase by factors of 4 and 5 respectively in whole *V. lata*. Nickel and copper increase by a factor of about 3, while aluminum and magnesium increase by factors of 2 and 1.5. Titanium showed no consistent change in concentration with change in size of *Velella*. The greatest changes noted in any tissues were those of copper in the skin covering the skeleton, a 7-fold increase, and of manganese in the gonozooids and medusae, a 7- and 7.5-fold increase.

ACKNOWLEDGEMENTS

Preliminary analyses were carried out at the University of Chicago with the assistance of Mr. O. Joensuu. Final determinations, made in the geochemistry section of the Lamont Geological Observatory, were partially supported by a grant from the Rockefeller Foundation. The material was collected by the Scripps Institution of Oceanography in connection with the Cooperative Oceanic Fisheries Investigations. Miss Marla Bottesch assisted with part of the dissection of *V. lata*. The assistance of these persons and organizations is gratefully acknowledged.

REFERENCES

Goldberg, E. D.
1957. Biogeochemistry of trace metals, in: Treatise on marine ecology and paleoecology. Chap. 12. Mem. Geochem. Soc. Amer., 67, *1*: 345–357.

Goldberg, E. D. and G. O. S. Arrhenius
1958. Chemistry of Pacific pelagic sediments. Geochim. Cosmochim. Acta, *13*: 153–212.

Griel, J. V. and R. J. Robinson
1952. Titanium in sea water. J. Mar. Res., *11*(2): 173–179.

Haurowitz, F. and H. Waelsch
1926. Ueber die chemische Zusammensetzung der Qualle *Velella spirans*. Hoppe-Seyl Z., *161*(6): 300–317.

Krumholz, L. A., E. D. Goldberg and H. Boroughs
1957. Ecological factors involved in the uptake, accumulation, and loss of radionuclides by aquatic organisms, in: Effects of atomic radiation on oceanography and fisheries. Chap. 7. Publ. nat. Res. Coun. Wash., No. 551: 69–79.

LAEVASTU, TAIVO AND T. G. THOMPSON
1956. The determination and occurrence of nickel in sea water, marine organisms, and sediments. J. Cons. int. Explor. Mer, *21*(2): 125–143.

MAHLER, H. R.
1956. Nature and abundance of metalloflavoproteins, in: Advances in enzymology and related subjects of biochemistry, by F. F. Nord, *17*, Interscience Publishers, N. Y. 556 pp.

PROSSER, C. L. (EDIT.)
1950. Comparative animal physiology. Saunders Co., Philadelphia. 888 pp.

STALLWOOD, B. J.
1954. Air cooled electrodes for spectrochemical analysis of powders. J. opt. Soc. Amer., *44*(2): 171–176.

SVERDRUP, H. U., M. W. JOHNSON AND R. H. FLEMING
1946. The Oceans. Prentice Hall, Inc., New York. 1087 pp.

VINOGRADOV, A. P.
1953. The elementary chemical composition of marine organisms. Mem. Sears Found. Mar. Res., II; 647 pp.

WEBB, D. A. AND W. R. FEARON
1937. Studies on the ultimate composition of biological material I. Sci. Proc. R. Dublin Soc., *21:* 484–504.