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Chapter 15

Trace elements, functions, sinks and replenishment in reef aquaria

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ABSTRACT

Trace elements in closed or semiclosed marine aquaria are a matter of permanent discussion.

As the name states the concentrations of trace elements are extremely low and quantitative analyses are difficult. They play important roles as parts of enzymes in metabolism and enrichment in organisms is achieved often under high expenditures. For corals, trace elements besides physiological roles also have an important role in the skeleton. Also zooxanthellae are in need of many trace elements. Due to consumption and precipitation within aquarium systems addition of trace elements is necessary. This can be done via food, water changes or chemical addition. With published data about the concentrations and distributions of trace elements in the ocean, additions can be calculated.

This paper will describe the biological and physiological functions of the different trace elements. A recipe for the supplementation of essential trace elements calculated from the most important sinks in reef aquaria will be given. A summary of addition experiences in public aquaria and institutions will be presented and discussed.

Bioavailability of trace elements as well as bioindication of trace element intoxication is discussed. At last some recommendations for first aid measures at suspected trace element intoxications are given.

INTRODUCTION

Elements that are present in a medium in very low concentrations, in concentrations of mg.kg^{-1} (ppm) or lower, are generally named trace elements. This article will take a closer look only on those elements that have a function in metabolism or calcification of marine organisms. Trace metals in this interpretation are strontium, barium, vanadium, chrome, molybdenum, manganese, iron, cobalt, nickel, copper, zinc, cadmium, boron, silicon, selenium, fluorine, bromine and iodine. Table 1 gives a short overview over the functions of these trace elements.

TRACE ELEMENTS IN CORALS

Trace elements in corals – functions, bioinorganic chemistry

Some of the most important functions of trace elements, in particular of transition metals, are related to the regulation of metabolism by enzyms. Enzyms are proteins that speed up chemical reactions in biological processes or make them possible at all under physiological conditions. Typical mechanisms are multi-electron transformations as photosynthesis, respiration and nitrogen fixation. These enzyms contain transition metals that can exist in multiple oxidation states like for example iron.

In hydrolytic transformations like proteolysis and the equilibration of carbonic dioxide and bicarbonate ion transition metals do not change oxidation state but function as Lewis acid-type catalysts. An example for a hydrolytic enzyme is the carbonic anhydrase shown in Figure 1. This enzyme contains zinc as a cofactor, a non-protein component that activates the enzyme. The biochemical reaction catalysed by carbonic anhydrase is the hydrolysis of carbonic dioxide as shown in Figure 2. This hydrolytic reaction is speeded up by a factor of 10^7 by the enzyme.

Sometimes the same or very similar processes are catalysed by different enzymes, so called isoenzymes, under different conditions. Obviously the symbiosis of anemones and corals with zooxanthellae makes diverse superoxide dismutases (SODs) necessary to survive the change from hypoxia (oxygen deficiency) to hyperoxia (over-supply of oxygen) within minutes without damage (Richier *et al*., 2003). These SODs work with different transition metals. In the symbiotic Mediterranean anemone *Anemonia viridis* and its zooxanthellae, SODs with copper and zinc (CuZnSOD) in ectoderm and endoderm, with manganese (MnSOD) in all compartments and with iron (FeSOD) in zooxanthellae and endoderm have been found. This means in this anemone four different transition metals are involved in the detoxification of reactive oxygen species. These are just few examples on how different trace elements play central roles in the metabolism of all organisms including corals.

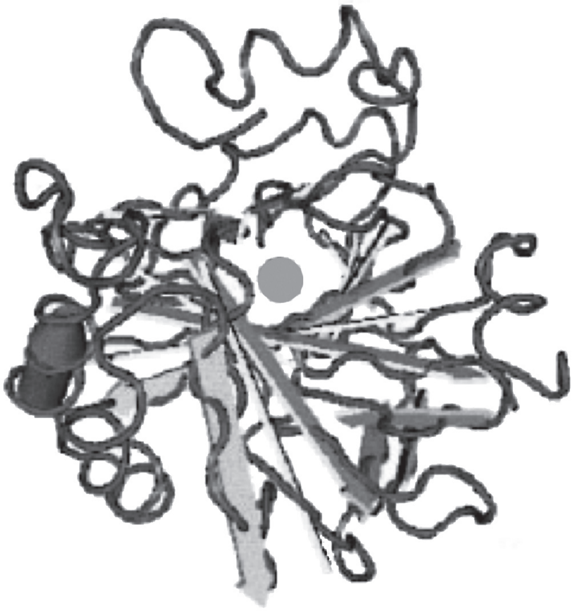

Figure 1: Human carbonic anhydrase. The zinc cofactor is shown as a grey sphere in the middle (De Boer et al.*, 2006).*

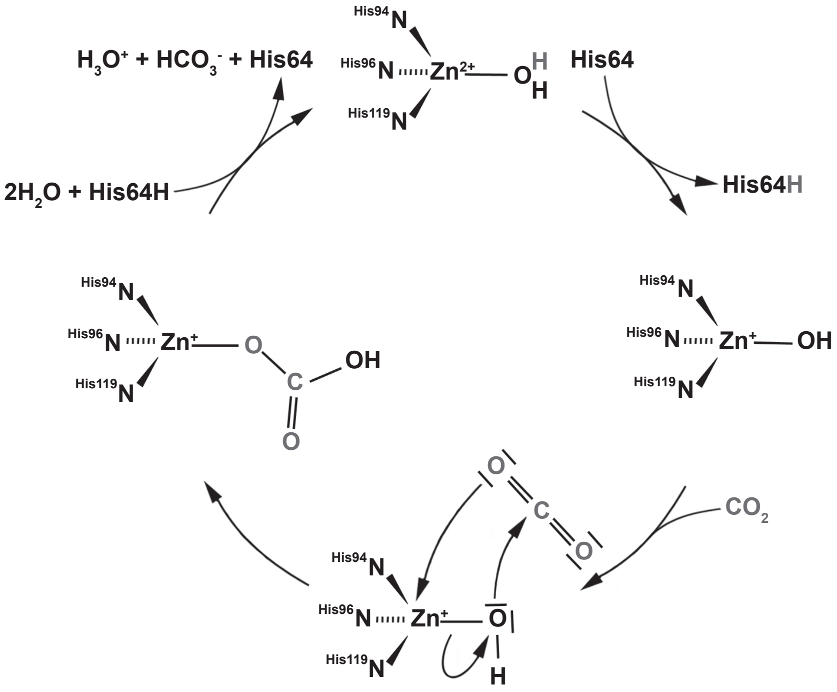

Figure 2: Hydrolysis of CO_2 by Carbonic Anhydrase (www1)

Table 1: Functions of trace elements

Element	**General function**	**Enzymes (examples)**	**citations**
Strontium	Gets incorporated into the aragonite skeletons of scleractinian corals, stabilizes the aragonite modification of calcium carbonate and supports skeletal growth. No further biological functions known.	-	1
Barium	Similar to strontium but less abundant.	-	1
Vanadium	High concentrations in ascidians. Production of bromoform in algae to repel epiphyts. Alternative nitrogenase for nitrogen-fixation.	Bromoperoxidase of algae, nitrogenase	2
Chrome	Glucose-tolerance-factor for the proper function of insulin.	-	2
Molybdenum	Electron transfer	Nitrogenase, nitrate reductase, sulfit reductase	3
Manganese	Electron transport, hydrogen transfer, protein metabolism, citrate cycle, RNA and DNA synthesis, detoxification of reactive oxygen species.	Oxygen evolving complex of photosystem II, superoxide dismutase, phosphatases.	4
Iron	Redox systems, electron transport chain, oxygen transport, detoxification of reactive oxygen species.	FeS of photosystem I, cytochrome oxidase, katalase, peroxidase, superoxide dismutase.	4
Cobalt	Vitamin B_{12}, redox reactions, methylations, nutrient for algae.	Coenzyme B_{12} of many enzymes like mutases.	2
Nickel	Hydrolysis of urea, bacterial methane and acetate production.	Urease, CO dehydrogenase.	2
Copper	Oxygen transport, respiration chain, electron transfer, oxidations (preferably), reductions, competing and displacing against iron and manganese.	Hemocyanin, plastocyanin, ascorbate oxidase, tyrosinase, cytochrome c oxidase, Cu-Zn superoxide dismutase, nitrite reductase, N_2O reductase.	4
Zink	Hydrolysis of bicarbonate, hydrolysis of proteins in digestion, hydrolysis of phosphate esters, energy metabolism, carbohydrate metabolism, oxidation of alcohols, detoxification of reactive oxygen species.	Carbonic anhydrase, carboxypeptidase, alkalinic phosphatase, alcohol dehydrogenase, Cu-Zn superoxide dismutase	4
Cadmium	Biological function only in alternative carbonic anhydrase of diatom *Thalassiosira weissflogi*, only toxic for other organisms.	Carbonic anhydrase	5
Boron	Stabilization of cell membrane, multiple functions and regulations in metabolism but mechanisms are not well understood.	-	6
Selenium	Antioxidans, quenching of radicals.	Peroxidase	2

Table 1 (continued): Functions of trace elements

Element	General function	Enzymes (examples)	citations
Fluorine	Hardening of biominerals, heavy metal complexation.	-	2
Bromine	Bromoform is produced by algae as repelling agent against epiphytes.	-	
Iodine	Similar to bromine, constituent of hormones, hardening of exoskeletons of sponges, crustaceans and gorgonians, highly enriched in algae, especially in brown algae. Iodate (IO_3^-), which is the stable form under surface conditions, is reduced to iodide by nitrate reductase in algae, protecting the photosynthetic apparatus under high irradiation in this way.	-	2 7

1 Milliman, (1974), Balling (1995, 1996)
2 Kaim and Schwederski (1995)
3 Mengel (1991)
4 Kaim and Schwederski (1995), Amberger (1996)
5 Lane (2005)
6 Mengel (1991), Amberger (1996)
7 Kaim and Schwederski (1995), Balling (1995, 1996)

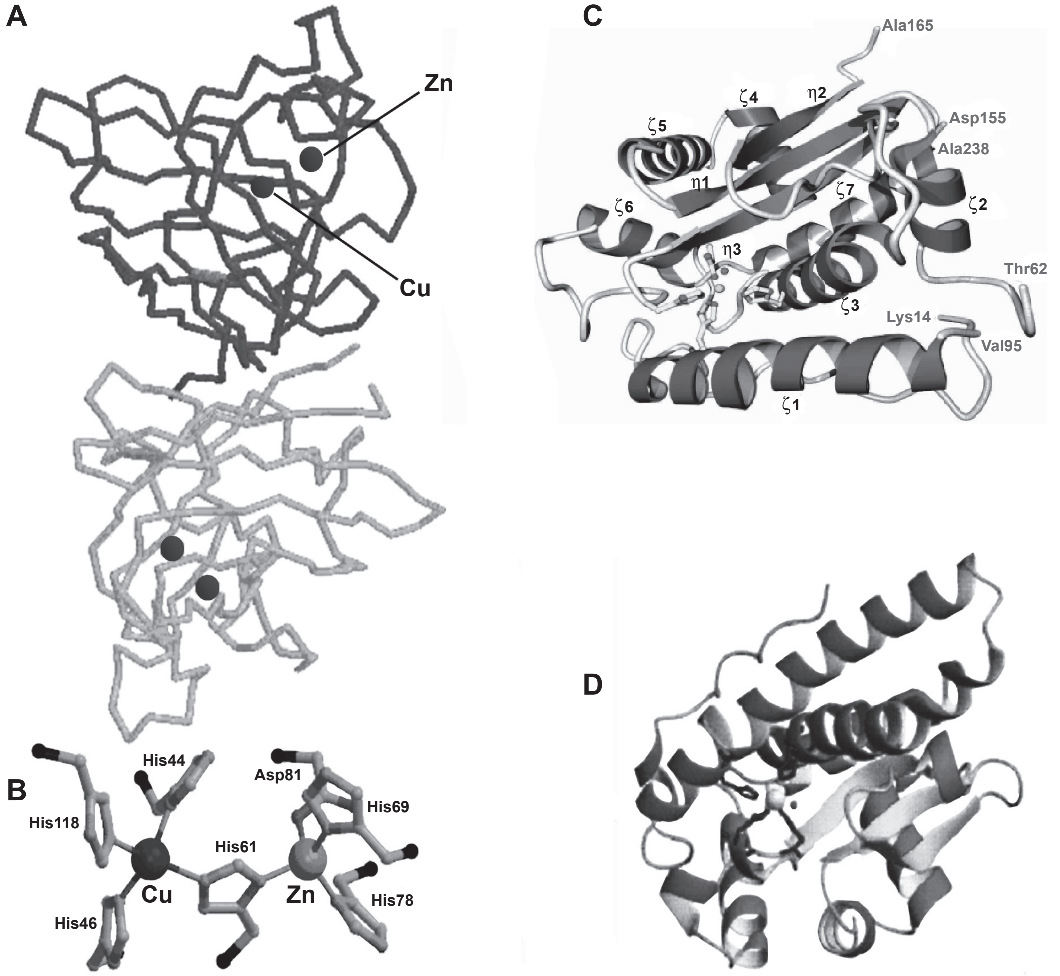

Figure 3: Dimer of CuZnSOD (A) with detail of active site (B, both www2), monomer of FeSOD (C, www3) and of MnSOD (D, www4).

Trace elements in corals – concentrations

Table 2 shows the trace metal concentrations of the components of the scleractinian coral *Acropora tenuis*. The skeleton reflects the concentrations of trace elements in the surrounding water (Druffe, 1997; Mokhtar *et al.,* 2002; Fichez *et al.,* 2005; Madkour, 2005; Al Ouran, 2005). Further data on the trace element concentrations of coral skeletons are given in table 3. An extensive overview with data on the heavy metal concentrations of hard coral skeletons is also given in Reichelt-Brushet and Orist (2003). Skeletons show the least enrichment of trace elements of all parts, which will be important for our later calculations.

A closer study of the concentrations shows that coral tissue shows the smallest variability in trace metal concentration. This confirms the finding (Fisher, 2002) that essential trace metals are more or less under homeostatic control.

Surprisingly the trace metals in the zooxanthellae are highly variable and of high concentration. This may be the cause why the zooxanthellae show signs of stress while the polyp of the coral remains nearly unaffected by moderately elevated trace metal concentrations.

Trace elements in corals – stress and intoxication

While an elevated concentration of iron increases number of zooxanthellae in corals but decreases growth rate (Ferrier-Pages *et al.*, 2001), enrichment with copper decreases zooxanthellae density and can partially bleach corals (Jones, 1997). No significant change was detected in any other function after exposure to copper between 0.01 and 1.0 mg.L^{-1} (Howard *et al.*, 1986).

The mechanism of bleaching is competition of heavy metal ions with iron for uptake (De Haan, 1984; Pätsikkä *et al.,* 2002; Hirose, 2006), inhibition of photosystem II by copper outcompeting iron (Burda *et al.*, 2002) and by induction of oxidative stress by various heavy metals (Pinto *et al.*, 2003)

Experiments with symbiotic (containing zooxanthellae) and aposymbiotic (without zooxanthellae) specimens of *Anthopleura elegantissima* conducted by Mitchelmore *et al.*,

Table 2: Trace element concentrations found in Acropora tenuis *zooxanthellae, tissue and skeleton (µg.g^{-1}) from Magnetic Island and One Tree Island.(Reichelt-Brushett and Orist (2003), altered, except * from Millero (1996))*

Acropora tenuis	Br [a] σg.g^{-1}	I [a] σg.g^{-1}	Ba [a] σg.g^{-1}	Fe [b] σg.g^{-1}	Mn [b] σg.g^{-1}	Ni [b] σg.g^{-1}	Cu [b] σg.g^{-1}	Zn [b] σg.g^{-1}
Magnetic Island								
Zooxanthellae								
a	33 ∂ 7	4 ∂ 3	45 ∂ 20	379.8	3.52	14.4	15.7	128.1
b	25 ∂ 9	6 ∂ 4	89 ∂ 17	292.4	1.99	7.5	11.5	42.6
Tissue								
a	105 ∂ 9	< 5	690 ∂ 70	23.3	1.38	1.8	1.0	19.5
b	227 ∂ 24	< 9	930 ∂ 150	32.4	1.67	3.8	1.1	17.1
Skeleton								
a	1.3 ∂ 0.4	6 ∂ 4	116 ∂ 23	16.3	0.15	1.1	0.06	1.8
b	1.1 ∂ 0.3	8 ∂ 5	55 ∂ 16	ND	0.09	0.7	0.10	0.5
One Tree Island								
Zooxanthellae								
a	75 ∂ 22	12 ∂ 8	470 ∂ 50	301.3	1.86	5.4	0.73	25.1
b	36 ∂ 3	6 ∂ 4	105 ∂ 40	298.2	1.57	11.5	0.62	32.3
Tissue								
a	161 ∂ 14	< 10	660 ∂ 80	22.2	1.55	2.3	1.2	18.6
b	7 ∂ 3	<1	-	7.2	0.11	0.7	0.4	17.3
Skeleton								
a	< 1	6 ∂ 3	80 ∂ 17	ND	0.02	0.6	0.03	0.3
b	1 ∂ 0.3	7 ∂ 3	64 ∂ 21	ND	0.01	0.3	0.08	0.2
Seawater, average*	67.1	58.4 * 10^{-3}	13.7 * 10^{-3}	0.055 * 10^{-3}	0.027 * 10^{-3}	0.47 * 10^{-3}	0.25 * 10^{-3}	0.39 * 10^{-3}

a NAA
b ICPMS

Table 3: Trace element concentrations of scleractinian coral skeletons

Species	**Ca** %	**Sr** %	**Ba** σg.g^{-1}	**Fe** σg.g^{-1}	**Mn** σg.g^{-1}	**Ni** σg.g^{-1}	**Cu** σg.g^{-1}	**Zn** σg.g^{-1}	**Cr** σg.g^{-1}	**Co** σg.g^{-1}	**B** σg.g^{-1}
Acropora sp.[1]	38.8	0.81		65	6		1				21
Acropora humilis[2]				49	2.5	4.4	3.7	8.5		0.5	
Acropora hemprichii[2]				72.6	2.9	0.4	0.7	7.3		0.7	
Acropora hyazinthus[2]				250.7	7.0	0.5	0.8	15.8		0.5	
Madracis sp.[1]	39.1	0.85	24	35	4	2	3	< 2	13	0.1	85
Meandrina sp.[1]	38.1	0.85	42	13	2	2	5	< 2	< 1	0.05	78
Meandrina areolata[3]	38.5	0.83	32	30	< 5	< 2	1	2	< 1	0.04	110
Meandrina braziliensis[3]	37.6	0.87	85	< 5	< 2	< 2	8	2	1.1	0.058	70
Meandrina braziliensis[3]	38.1	0.85	10	8	< 2	< 2	5	2	< 2	0.067	55
Madracis cf. *pharensis*[3]	38.1	0.83	38	10	4	2	< 2	2	23	0.077	80
Madracis mirabilis[3]	40.0	0.86	11	< 5	< 2						80
Montastrea sp.[1]	39.0	0.68	17	12	3	2	6	< 2	5	0.1	57
Montastrea annularis[3]	39.6	0.80	10	5	< 2	3	2	< 2	10	0.064	50
Phyllangia americana[3]	38.1	0.77	27	290	130	2	20	7	< 2	< 2	60
Pocillopora damicornis[2]				51.0	7.6	0.6	2.4	2.0		0.4	
Porites sp.[1]	39.4	0.79	15	80	6		4	< 2			21
Porites compressa[2]				62.4	13.1	0.4	4.2	3.5		0.3	
Porites lutea[2]				179.2	3.7	0.6	3.1	7.5		0.2	
Porites porites[3]	36.8	0.71	15	45	4	< 2	2	< 2	< 2	2	15
Scolymia ubensis[3]	38.6	0.75	9	13	3	3	< 2	< 2	2	0.058	80
Scolymia cubensis[3]	36.0	0.75	43	880	30	3	16	3	5	0.47	60
Stylophora wellesi[2]				33.6	2.5	2.0	0.6	6.5		0.5	

1 Milliman (1974);
2 Madkour (2005);
3 Livingston and Thompson (1971)

(2003) showed that the expulsion of zooxanthellae may be a detoxification mechanism for copper. Obviously elevated copper concentrations are taken up by the zooxanthellae and get disposed when zooxanthellae are released, moderating copper accumulation in this way. Symbiotic anemones accumulated more cadmium, zinc and nickel than aposymbiotic ones. Symbiotic anemones returned to pre-exposure levels after nickel exposure.

Exposed to copper, symbiotic *A. elegantissima* also produced more mucus than aposymbiotic ones, wrapping them in a thick layer of mucus. Algal cell density in *A. elegantissima* was reduced by nickel and high zinc levels.

Similar results where achieved with *Anemonia viridis* in similar experiments by Harland and Nganro (1989).

Multi-element analysis in seawater

Analyzing trace and minor elements in seawater is complicated, the disturbance of high halogenide concentrations against the sometimes very low element concentrations is immense. Most regular analytical laboratory procedures are not suitable. Inductive coupled plasma optical emissions spectroscopy (ICP-OES) formerly called inductive coupled plasma atomic emission spectroscopy (ICP-AES) and inductive coupled plasma-mass spectrometry (ICP-MS) are the most useful methods for determination even very low quantities of elements.

These spectroscopic techniques exploiting the fact that under certain circumstances excited electrons of elements emit energy at a specific wavelength peculiar to their chemical character. This happens in a gaseous state (the plasma). The intensity of the energy emitted is proportional to the amount (concentration) of the analyzed element. By determining which wavelengths are emitted and determining the intensities, the elemental composition of a sample can be quantified relative to a reference standard. ICP-OES is useful to determine Na, K, Ca, Mg, B, Sr, S and P, while ICP-MS is suitable for determination Sb, Co, Mn, Zn, Cu, As, Se, Al, Ti, Fe, Cr, Si, Mo, Li, Ti, Co, Ni, Br and Ba.

Some major elements such as calcium and magnesium can easily be measured by titrimetry, spectroscopical analitycal methods (Anonymous, 1999, 2000), or commercial available suitable testkits.

Not all elements can be analyzed by the ICP-OES and ICP-MS methods, because of its complexity behaviour. Iodide (Luther and Cole, 1988) and iodate (Herring and Liss, 1974) can best be measured by a polarographic method, the voltametric method in which the current is measured as a function of applied potential, using a dropping mercury electrode.

The specific method for iodide is called cathodic stripping square wave voltammetry (CSSWV) and for iodate differential pulse polarography (DPP). Measuring trace elements can best be carried out by specialized laboratories.

The most convenient way of reaching the best environment circumstances for coral husbandry is to measure besides calcium, magnesium and strontium the following elements at a regular basis K, Fe, Si, Mo, Mn, I, Li, Al, Ti, Cr, Co, Ni, Cu, Zn, Br, Ba.

Comparing such results with references of natural seawater prevents surprises, table 4 gives just an example.

Analyzing trace elements in institutions

To obtain a view what kind of analyzing efforts public aquariums perform and which element additions are being used, a questionnaire was send out on the aquaticinfo list server, 15 institutions responded.

Natural seawater was used in 53% of the institutions the rest used artificial seawater. Many aquariums (53%) measure trace elements on weekly, monthly or yearly basis, with spectrophotometry, ICP-MS and ICP-OES.

67% of the institutions are adding some kind of trace element mixtures into their systems, half of them used commercial available solutions, others had mixtures composed by their own.

Control of the effects of these additions was performed in 90% by bio monitoring and in some cases completed with measurements.

Positive effects were mentioned but also doubts if these effects were related to the additions only. As references Spotte (1992), Delbeek and Sprung (1994) and Nilsen and Fossa (2002) were mentioned.

The importance of analyzing water quality is understood, but only 50 % of the institutions is measuring in some way. However the number of answered questionnaires was limited.

From the institutions that are adding mixtures into their systems little is known what really happens. Measuring shortness or overdimensioned additions of elements (Sondervan, 2001) is not carried out on a regular basis. This seems to be a small basis for proper husbandry when handling is almost only related on bio monitoring.

Conclusions of the above results are limited to low respondents on the questionnaire, but

Table 4: Three references of important major, minor and trace elements in seawater (S=35 ‰)

Element		**Species**	**A**[1] $mg.L^{-1}$	**B**[2] $mg.L^{-1}$	**C**[3] $mg.L^{-1}$
Hydrogen	H	H_2O	10,700	10,800	
Chlorine	Cl	Cl^-	19,870	19,000	
Sodium	Na	Na^+	11,050	10,500	10,880
Magnesium	Mg	Mg^{2+}	1,326	1,350	1,320
Sulphur	S	SO_4^{2-}, $NaSO_4^-$	928	885	903
Calcium	Ca	Ca^{2+}	422	400	433
Potassium	K	K^+	416	380	407
Bromine	Br	Br^-	68	65	69
Carbon	C	HCO_3^-, CO_3^{2-}, CO_2	28	28	
Strontium	Sr	Sr^{2+}	8.5	8	7.17
Boron	B	$B(OH)_3$, $B(OH)_4^-$	4.5	4.6	
Silicon	Si	$Si(OH)_4$	1*	3	
Fluorine	F	F^-, MgF^+	1.4	1.2	
Nitrogen	N	N_2, NO_3^-, NO_2^-, NH_4^+, NH_3	0.5 - 15	0.5 - 15	
Lithium	Li	Li^+	0.18	0.17	0.176
Rubidium	Rb	Rb^+	0.12	0.12	
Phosphorus	P	$H_2PO_4^-$, HPO_4^{2-}, PO_4^{3-}	0.07	0.07	0.04
Iodine	I	IO_3^-, I^-	0.06	0.06	0.082
Barium	Ba	Ba^{2+}	0.03	0.03	0.008
Aluminium	Al	$Al(OH)_4^-$	0.005*	0.01	0.029
Iron	Fe	$Fe(OH)_2^+$, $Fe(OH)_4^-$	0.003*	0.01	< 0.01
Molybdenum	Mo	MoO_4^{2-}	0.010	0.01	0.001
Nickel	Ni	Ni^{2+}	0.002*	0.007	0.0032
Arsenic	As	$HAsO_4^{2-}$, $H_2AsO_4^-$	0.0023	0.003	
Zinc	Zn	$ZnOH^+$, Zn^{2+}, $ZnCO_3$	0.0050	0.01	0.131
Copper	Cu	Cu^{2+}, $CuOH^+$, $CuCO_3$	0.0030	0.003	0.0101
Tin	Sn	$SnO(OH)_3^-$	0.00001*	0.0008	0.0005
Uranium	U	$UO_2(CO_3)_2^{4-}$	0.0033	0.003	
Chromium	Cr	$Cr(OH)_3$, CrO_4^{2-}	0.0006*	0.00005	0.001
Manganese	Mn	$MnCl^+$, Mn^{2+}	0.0020	0.002	0.0394
Vanadium	V	$H_2VO_4^-$, HVO_4^{2-}, VO^{3-}	0.0015	0.002	
Titanium	Ti	$Ti(OH)_4$	0.0010	0.001	0.002
Cesium	Cs	Cs^+	0.0005	0.0003	0.003
Antimonium	Sb	$Sb(OH)^{6-}$	0.0002	0.0003	<0.001
Silver	Ag	$AgCl_2^-$, $AgCl_3^{2-}$	0.00010	0.0003	
Yttium	Y	$Y(OH)_3$	0.00001	0.00001	< 0.001
Cobalt	Co	Co^{2+}	0.00008*	0.0004	0.001
Neon	Ne	Ne(gas)	0.00012	0.0001	
Cadmium	Cd	$CdCL_2$, $CdCl^+$, Cd^{2+}	0.00005	0.00011	0.0002
Tungsten	W	WO_4^{2-}	0.00012	0.0001	
Selenium	Se	SeO_3^{2-}	0.00045	0.00009	

* Considerable variations occur

[1] Riley and Chester (1971)

[2] Spotte (1992)according to - Bidwell and Spotte (1985), Mactyre (1976),Goldberg(1980) and Brewer (1975)

[3] Natural Seawater Gulf of Biskaje, N 045 º,49,40’. W 007º,30,80’ (Sondervan, unpubl. results)

should not be ignored.

Another questionnaire (Sondervan and Causer, in press) showed that 76.2 % of the European aquariums measured their water quality daily, 88.1 % weekly and 42.9 % monthly or in combination.

Table 5 gives a view which parameters are measured in the European aquariums, only 7.1 % of the aquariums were measuring trace or minor elements.

The similarity in both questionnaires is that still less efforts have been made in measuring trace elements. It is important to come to a standard procedure that describes which elements should be analyzed at aquariums which have invertebrates and life corals in their collections. When these results are completed with information of present life support systems, better knowledge will be obtained what really is necessary for coral husbandry.

It's advised to measure in any case the following macro and trace elements in coral systems: K, Ca, Mg, Sr, Fe, Si, Mo, Mn, I, Li, Al, Ti, Cr, Co, Ni, Cu, Zn, Br and Ba on a regularly interval.

Trace elements in reef aquaria - supplementation

In the natural environment corals, especially scleractinian corals, meet their demand for energy and nutrients from various sources with a substantial portion that is met by the capture of prey. In reef aquaria in most cases the portion of heterotrophic nutrition of symbiotic scleractinian corals is much lower and especially in regards of trace elements an additional supplementation may be indicated.

In the Jura-Museum, Eichstätt, where one of the authors (Balling) worked from 1987 until end of 2000 the first *Acropora* corals where kept in the early 90s. The problem of effective calcium hydrogencarbonate supplementation was already overcome by a three part calcium additive published in 1994 (Balling, 1994). However the growth of the *Acropora* corals seemed to be not optimum since the new grown branches where quite pale and thin. Considering the accepted importance of trace elements in plant nutrition the author started first experiments on the supplementation of trace elements for corals.

Especially the calcareous coral skeletons seemed to be a major sink for trace elements.

The data published by Milliman (1974) showed up the trace element concentrations of the diverse marine carbonates. From these data the amounts of trace elements that have to be supplied to the aquarium to compensate for the loss of trace elements that have been trapped in the calcium carbonate skeletons of the corals and corallinaceous algae have been calculated. The recipe has been published together with first experiences with this kind of trace element addition in 1996 (Balling, 1996 b).

Calculated from data from Milliman (1974) one of the authors published the following trace element recipe (Balling, 1996 b):

Solution 1

243.45 g $SrCl_2.6H_2O$; 356 mg $BaCl_2.2H_2O$;
make up with water to 1 L solution.

Solution 2

4 g $FeSO_4.7H_2O$; 185 mg $MnSO_4.H_2O$;
98 mg $CuSO_4.5H_2O$; 88 mg $ZnSO_4.6H_2O$; 89 mg $NiSO_4.6H_2O$; 324 mg $CrCl_3.6H_2O$;
4 mg $CoCl_2.6H_2O$;
make up with water to 1 L solution.
(10 ml of each solution is added to 2 L solution of 143 g $CaCl_2.2H_2O$ in water)

Solution 3

2.5 g KI; 13.3 g NaF;
make up with water to 1 L solution.
(10 ml of this solution is added to 2 L solution of 168 g $NaHCO_3$ in water)

Table 5. Percentages of used analysis in European Aquariums

Parameter	%	Parameter	%	Parameter	%
Salinity	95.2	Oxygen	66.7	Calcium	59.5
Temperature	97.6	Chloride	23.8	Magnesium	19.0
pH	97.6	Ammonia	83.3	Iodide	21.4
Redox potential	95.5	Nitrite	81.0	Bromine	14.3
Turbidity	14.3	Nitrate	90.5	Strontium	23.8
		Phosphate	59.5	Trace/Minor elements	7.1

The application of trace elements according to the recipe above led to better opening, good continuous growth of corals and better colours. The corals especially enhanced the green fluorescent colours. The corals returned to natural more sturdy growth shapes.

After the onset of trace element supplementation repeated expellings of zooxanthellae have been observed which may be interpreted as attempts of the corals to regulate internal trace metal concentration but which may more probable be attributed to enhanced algal cell division.

Shimek (2002) expects high mortality of corals at elevated trace metal concentrations during acclimation. In the time of the regular trace element application, from 1994 until the end of 2000, no problems with the acclimation of freshly imported corals have been observed.

General biology of the aquaria supplemented with trace elements was rich and diverse with a good abundance of planktonic organisms like copepods and polychaets. Occasionally nocturnally captured polychaets could be observed on large polyped stony corals in the morning.

The green brittle star (*Ophiarachna incrassata*) propagated repeatedly.

The proportions of trace elements (and nutrients in general) seem to be more important than the absolute concentration in a certain extent. An advantage of a balanced trace element supplementation with an elevated concentration of all essential trace metals may be a higher stability against imbalances caused by minor accidental additions of just one trace element, for example with contaminants.

The recipe given above has been republished by Renke (1999) complemented with additional molybdenum, strontium, iron and manganese for better growth of corallinaceous algae and again in 2001 (Renke, 2001) with additional calculations.

This repeated publication of the recipe in German aquariophilic newspapers and homepages initiated the development of several commercial trace element mixtures with widespread use. The different commercial trace element mixtures competed for the induction of brighter colouring of corals which finally led to a mode of trace element addition that induces a controlled bleaching through temporary trace metal stress.

Another technique to add trace elements is via the medium in calcium reactors. Besides lime ($CaCO_3$) also coral sand can be used to dissolve within a calcium reactor. Measurements of the influent and effluent at different pHs show there is a consistant addition of strontium when using 1 to 2 cm large coral rubble as medium in a calcium reactor (Figure 4) (Janse, unpublished results). No conclusions can be taken from the ICP-MS readings of the other trace elements data. Possibly not all elements will dissolve with the same ease as calcium and strontium. The calcium and strontium ratio of average 45, is in agreement with the ratio found in stony coral skeleton.

Long term changes in strontium levels, measured with ICP-OES in three coral systems at Burgers' Zoo, Arnhem, The Netherlands are

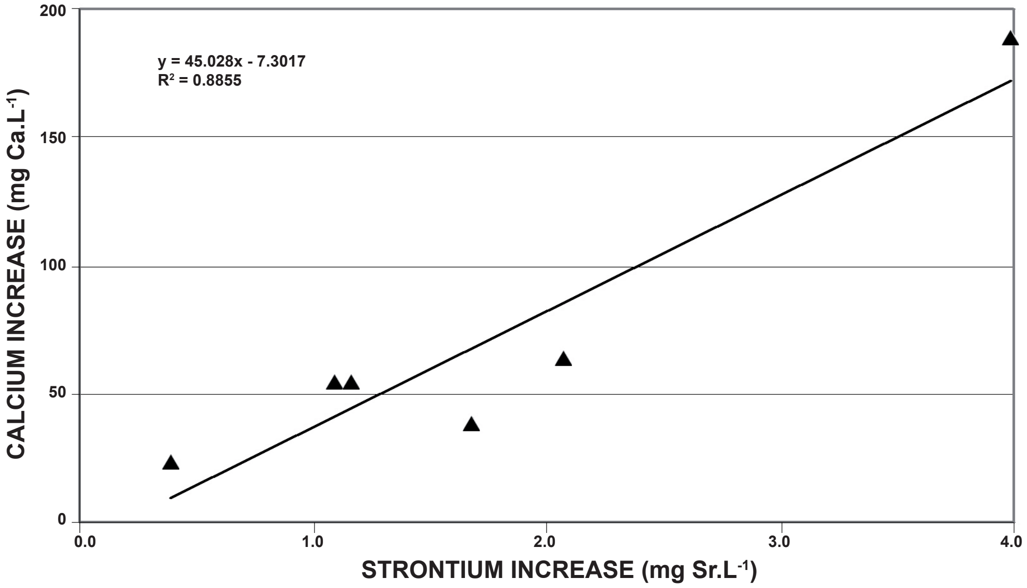

Figure 4: Differences in calcium and strontium concentration between the influent and effluent of a calcium reactor at Burgers' Ocean, Arnhem, measured with an ICP-OES

displayed in Figure 5. Due to changes of the artificial salt from 7.2 mg $Sr^{2+}.L^{-1}$ in the first year to 8.5 mg $Sr^{2+}.L^{-1}$ in the later years the strontium level increased towards a maximum of 8.5 $mg.L^{-1}$. Addition of a calcium rector (with coral sand as medium) increases the strontium level further. From year three the two smaller systems had received twice a week extra additions of a $SrCl_2.6H_2O$ solution, which was an addition of approximately 0.01 mg $Sr^{2+}.L^{-1}.d^{-1}$. The largest system received addition of the same solution from year 5 on at an average rate of 0.02 mg $Sr^{2+}.L^{-1}.d^{-1}$.

Even though the water management and stocking densities of corals differed between the systems it's clear that strontium addition via a calcium reactor and chemical addition will keep the strontium level between a predefined range of 10 to 12 mg $Sr^{2+}.L^{-1}$. When possible the addition management can be changed when the trace elements are measured on a regular basis.

Trace elements in reef aquaria – chemical speciation and toxicity

The chemical speciation is of central importance for bioavailability and toxicity of trace metals (De Haan, 1984).

Organic ligands that bind to the trace metals alter the toxicity. Most trace metal complexes are less toxic but also less bioavailable.

In need for iron and under competition for iron the diverse organisms excrete different specific ligands, so called siderophores that reduce availability of complexed trace metal to other organisms. Siderophore complexes of copper differ from the siderophore complexes of iron and the organisms can discriminate against the copper complexes.

But organic substances can not only have a moderating but also an enhancing effect on trace metal toxicity. For example it was observed that complexes of copper with citrate and with nitrilotriacetate are more toxic to phytoplankton than ionic copper.

The apparently unregulated way in which the zooxanthellae take up copper in corals may be attributed to such a mechanism.

Trace elements in reef aquaria – bioindication

The most important bioindicators for the trace element and nutrient status in the reef aquarium are the corals. Thoroughly observed they are good indicators of a healthy environment and good growing conditions.

Deep brown opaque tissue where the zooxanthellae are not covered by fluorescent pigments indicate high nutrients and moderate light. Light brown colours and transparent tissue indicate low nutrients nitrogen and phosphate and/or strong illumination. Weak fluorescent pigmentation can indicate low trace element concentration. Bleaching and excessive mucus secretion can indicate trace metal intoxication. Since different species and different clones

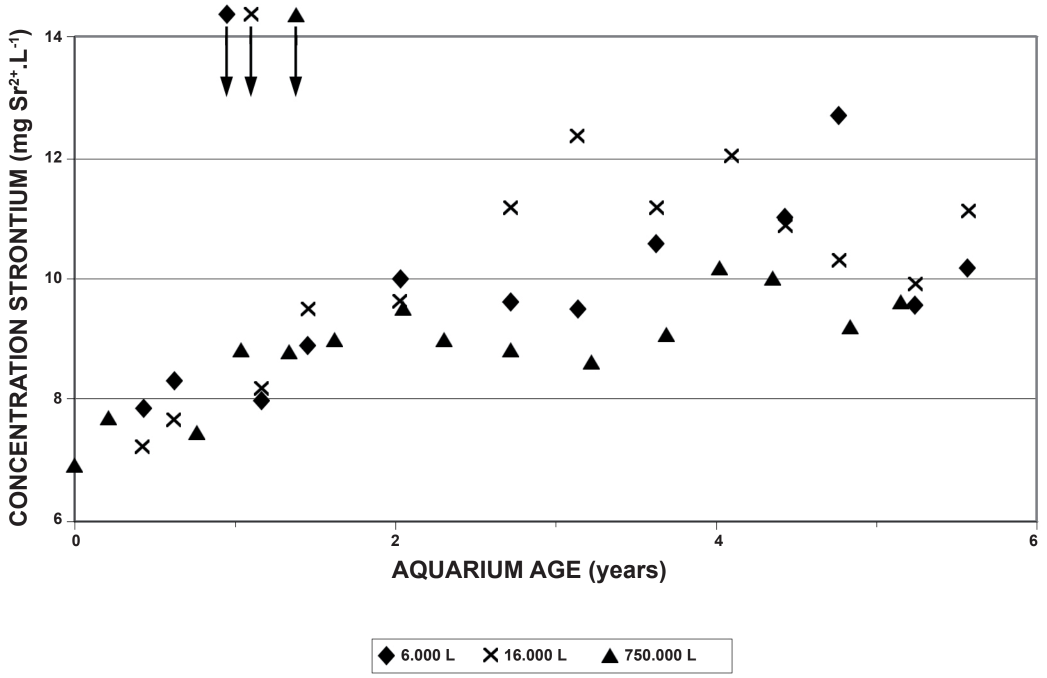

Figure 5: Strontium conentrations changes in three coral reef systems at Burgers' Zoo, Arnhem, Netherlands. The arrow indicates the addition of a calcium reactor to the specific aquarium system

of one species show different colours, it is necessary to know the normal appearance of a coral under the given culture conditions. With the necessary sensibility for changes in the appearance of the corals it is possible to react to negative changes and developments in the aquarium milieu.

The skeletons of corals can be used for the monitoring of the trace metal concentration in reef aquaria. Branches of colonies can easily be broken by hand, protected with disposable gloves. Different parts of the branches can be taken as record of the trace metal concentrations at the time they grew.

Fragments of corals grown in the Jura-Museum under the trace elements supplementation described above where analysed by the Geological Institute in Munich. They showed comparable trace element concentration as wild caught corals which died during import. However the variations in trace element concentrations between different parts of the same colony frequently showed the same variation as the different colonies amongst each other.

Also other invertebrate animals in the reef aquarium can be good bioindicators. Sea urchins, especially the species *Diadema antillarum* is highly sensitive to heavy metal pollution of the environment but also *Diadema setosum* is a good bioindicator.

Intoxication by copper results in changes in the behaviour of the sea urchin. *D. antillarum* positions itself on the bottom of the tank followed by spine closure at concentrations below 10 µg.L^{-1} and 96-h LC 50 was 25 µg.L^{-1} (Bielmeier *et al*., 2005).

High concentrations of zinc (approximate 1,000 µg.L^{-1}) caused death of brittle stars (*Ophiuroidea*), feather stars (*Comatuloidea*) and gammarids in a invertebrate system of the Artis Aquarium, Amsterdam (Sondervan, 2004). Scleractinian corals, sea urchins and other invertebrates showed signs of intoxication but were affected less severely.

Echinoderms show a high sensitivity to contaminants due to the extensive contact to the surrounding water via their ambulacrale system.

Trace elements in reef aquaria – first aid measures and depletion at suspected trace element intoxication

If corals show signs of trace element intoxication the safest but not always practicable method to lower the concentrations of toxic substances are water changes. With salt and tempered reverse osmosis water, water changes of 50 or 60 % are no problem for the inhabiting corals and *Tridacna* clams. Other invertebrates should tolerate such water changes too, but in some like sponges or feather stars contact of the animal to air should be avoided. Big water changes are always beneficial and always a good first aid measure for any problem related to water quality. Regular water changes are an important measure for continuous and good water quality.

Trace metals are adsorbed to ferric oxides and hydroxides. The addition of dissolved iron salts like iron(II)-sulphate to the reef tank or filtration through granular ferric oxide hydroxide is another first aid measure against trace metal intoxications.

Many aquatic and marine organisms, like cnidarians, bacteria, cyanobacteria and algae react to copper intoxication by the excretion of chelating substances (Clarke *et al*., 1987, Mitchelmore *et al*., 2003, Kazy *et al*., 2002, Gledhill *et al*., 1999), exopolysaccharides with acidic groups like carboxyl groups. These exopolysaccharides adsorb to air bubbles (Zhou and Mopper, 1998) and can be removed from the tank which is also an important export mechanism in the regular running of a reef tank. As can be expected from this conclusion Shimek (2002) found high concentrations of copper in skimmate. To support this kind of export, exopolysaccharides and substances with similar binding behaviour through carboxyl groups can be added to the tank. Good candidates are cellulose and cellulose derivates, xanthan gum, carrageenan and alginate. They are easily available in food grade quality as thickening food additive. The most promising one is alginate since its copper binding activity has many times been tested and described (Klimmek, 2003; Jang *et al*., 2006; Cheng *et al*., 1992; Vieira and Volesky, 2000; Hameed, 2006) and one of the authors (Balling) has tried the application of sodium alginate as fine powder in reef aquaria himself and considers this natural substance as safe. Applied as powder the alginate does not completely dissolve but partially settles down on the bottom and can be removed from there with a hose. If a sodium alginate solution is used it must be thinned down to low concentrations (<1 g.L^{-1}) since thick sodium alginate solution forms beads and lumps when poured into the reef tank. This may be found out by trials at the individual system.

Trace elements in reef aquaria – outlook

This article has tried to give some instructions for the useful application and control of trace elements in reef aquaria. With further experiments, investigations and articles it will be possible to control all nutrients in the reef aquarium and adjust them at optimum levels in the next years. This will be an important step forward to keeping conditions as close to nature as possible. This will make propagation of corals by cuttings and fragments more economic and perhaps sexual propagation in captivity possible.

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