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ORIGINAL PAPER

Dietary effects on multi-element composition of European eel (*Anguilla anguilla*) otoliths

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Abstract Otolith microchemistry is widely used as a tool to track individual migration pathways of diadromous fish under the assumption that the elemental composition of fish otoliths is directly influenced by the physicochemical properties of the surrounding water. Nevertheless, several endogenous factors are reported to affect element incorporation into fish otoliths and might lead to misinterpretations of migration studies. This study experimentally examined the influence of eight different diets on the microchemical composition of European eel (*Anguilla anguilla*) otoliths using laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS). Seven natural prey types and one artificial diet were fed during 8 weeks in freshwater circuits. Results show for the first time that food has no significant influence on the incorporation of Na, Sr, Ba, Mg, Mn, Cu and Y into European eel otoliths. This indicates that the incorporation of elements usually chosen for migration studies is not affected by diet and that individual feeding behaviour of *A. anguilla* will not lead to any misinterpretation of migration pathways.

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Introduction

Otoliths, the calcified earstones of bony fish, are mainly composed of aragonite, otolin and different minor and trace elements. The aragonite structure can be locally substituted by vaterite (Strong et al. 1986; Tzeng et al. 2007). In recent years, microchemical analyses primarily focusing on strontium concentrations were frequently used to track individual migration pathways of wild caught eels (e.g. Tzeng et al. 1997; Tsukamoto et al. 1998; Limburg et al. 2003; Arai et al. 2004; Daverat et al. 2005; Jessop et al. 2006; Shiao et al. 2006). The correlation between Sr/Ca ratios and ambient salinities is well established and the strontium content in otoliths is comparatively high and therefore easy to measure. Element composition of biogenic aragonites is thought to depend mainly on physicochemical properties of the surrounding water (Campana 1999). Nevertheless, it has been repeatedly reported that endogenous factors such as diet might have an effect on the element compositions of fish otoliths (Limburg 1995; Farrell and Campana 1996; Gallahar and Kingsford 1996; Buckel et al. 2004) as well as cephalopod statoliths (Zumholz et al. 2006), since cations entering the inner ear endolymph via the blood circuit can originate either from branchial or intestinal uptake (Campana 1999). Previous investigations on the importance of food on element incorporation into otoliths were highly ambiguous. Walther and Thorrold (2006) concluded that Sr and Ba contents in marine fish otoliths clearly reflect ambient Sr and Ba concentrations, while Kennedy et al. (2000)

reported 70% of the Sr isotopic signature in freshwater Atlantic salmon (*Salmo salar*) otoliths to originate from food. Significant dietary effects on strontium incorporation have been demonstrated for American shad (*Alosa sapidissima*) (Limburg 1995) and Black bream (*Girella elevata*) (Gallahar and Kingsford 1996) and for strontium and barium incorporation in bluefish (*Pomatomus saltatrix*) (Buckel et al. 2004). No effects were detected for the uptake of magnesium, potassium, strontium, sodium, and calcium in Red drum (*Sciaenops ocellatus*) (Hoff and Fuiman 1995), for strontium, copper and lead in barramundi (Milton and Chenery 2001), for sodium, magnesium, potassium, calcium and manganese in bluefish (*P. saltatrix*) (Buckel et al. 2004) and for strontium in Japanese eel (*Anguilla japonica*) (Lin et al. 2007). Lin et al. (2007) examined the influence of two different diets on strontium contents of *A. japonica* otoliths. It was shown that neither formulated feed nor tubifex had a measurable effect on Sr incorporation. This study performed a multi-element analysis to investigate the influence of a broad range of diets on the chemical composition of European eel (*Anguilla anguilla*) otoliths under fully controlled experimental conditions. In order to embrace the wide scope of eel nutrition, seven different limnic, brackish and marine species known as potential prey for eels in their natural environment and one artificial aquafeed were chosen as diets.

Different analytical methods are commonly used for microchemical studies of fish otoliths: Synchrotron X-ray fluorescence analysis (Tsukamoto et al. 1998), particle induced X-ray emission (PIXE; Elfman et al. 1999), electron probe microanalysis (Hoff and Fuiman 1995), solution based inductively coupled plasma mass spectrometry (ICP-MS; Buckel et al. 2004) and laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS; Walther and Thorrold 2006). In the present study, LA-ICP-MS was chosen to measure feed-dependency on the incorporation of 16 elements because of its high precision in multi-element analysis and good spatial resolution.

Materials and methods

Animal husbandry and experimental design

Ninety six pigmented juvenile eels (*A. anguilla*) were obtained from a commercial fish farm (Fischzucht Reese; Sarlhusen, Germany). Before the start of the experiment, eels were acclimated to water conditions for 8 weeks during which they were fed with commercial pellets (A 0.7 Perle Eel; Skretting) every second day. They were kept in a freshwater recirculation system in a temperature-controlled room at 20°C with a 12 h/12 h light regime. The system consisted of 32 plastic tanks (8 l each) connected to a biofilter with a total volume of 60 l. Inflow rates were adjusted to a three- to fourfold water exchange per day. Water lost by evaporation was refilled with freshwater every day. Shelter was provided in form of plastic tubes. Eels were divided into 32 groups of three individuals each. With eight different diets allocated to the 32 tanks, each treatment had four replicates. The experimental feeding period was set to 56 days. To document individual growth, each eel was marked with an individual code (visible implant elastomer system (Northwest Marine Technology, Inc., Shaw Island, WA, USA)).

Experimental diets

Considering the facultative catadromous lifecycle of the European eel, fresh-, brackish and saltwater organisms were chosen as experimental diets (Table 1). All food organisms were stored at −20°C and fed to the eels ad libitum in bite-sized pieces or as whole organisms once a day. Remaining food was removed every 24 h.

As a piscine freshwater diet we chose roach (*Rutilus rutilus*) caught in Lake Dörp near Kiel, Germany, of which small pieces (app. 0.5 cm^3) of filet were fed. Freshwater amphipods (*Gammarus pulex*) were chosen to represent a freshwater crustacean. Gammarids are considered a major contribution to the diet of small eels in freshwater habitats (Mann and Blackburn 1991). *G. pulex* was caught in River Eider (Kiel, Germany) and fed as a whole. As an insect diet Chironomid larvae, obtained frozen (Claudia Erdmann GmbH, Germany), were fed as a whole. Herring (*Clupea harengus)* represented a potential marine piscine diet of European eel. *C. harengus* were obtained frozen from a local commercial fishery (Wiese Eduard & Kruse Ivens GmbH, Germany) and fed as small pieces (app. 0.5 cm^3) of filet. Marine crustaceans were represented by Pacific krill *Euphausia superba* and mysids, both obtained frozen from an aquarium food manufacturer (Claudia Erdmann GmbH, Germany) and fed as whole organisms. Brown shrimp (*Crangon crangon*), caught in the Kiel Bight, was chosen as a potential marine/brackish crustacean prey, due to the hydrological properties of the Western Baltic. Small pieces (app. 0.5 cm^3) of *C. crangon* were fed. Commercial pellets were fed as an artificial reference diet. Pellets (A 0.7 Perle Eel) were obtained from a fish feed producer (Skretting Global) and stored at 4°C.

Prey and water analyses

Prey analyses were made by solution ICP-MS at the Institute of Geosciences, University of Bremen, using a Thermo Element2™. Organisms and fish pieces used for feeding were freeze-dried and pulverised. Between 100 and 200 mg of dry samples were mixed with 10 ml of concentrated

Table 1 Mean element concentrations ± 1 standard deviation (SD) of different diets and their origin

Food	*Clupea harengus*[b]	*Mysis* sp.[b]	*Euphausia superba*[b]	*Crangon. crangon*[a]	*Rutilus rutilus*[a]	Chironom. larvae[b]	*Gammarus pulex*[a]	Pellets[c]	*N*
Origin	Saltwater	Saltwater	Saltwater	Brackish	Freshwater	Freshwater	Freshwater	Undefined	
Ca (ppt)	7.2 ± 6.5	29.6 ± 29.1	24.0 ± 3.4	81.8 ± 11.0	27.2 ± 23.4	*2.0* ± 22.0	*112.6* ± 30.0	31.5 ± 0.5	3
Na (ppt)	*1.9* ± 0.8	4.9 ± 1.2	9.7 ± 7.1	*13.2* ± 2.6	3.6 ± 0.5	12.4 ± 0.2	4.5 ± 0.2	11.7 ± 0.2	3
Mg (ppt)	*1.6* ± 0.1	3.1 ± 0.1	*4.8* ± 0.7	3.1 ± 0.2	2.2 ± 0.2	2.1 ± 0.0	2.8 ± 1.9	2.6 ± 0.1	3
Sr (ppm)	13.9 ± 9.0	344.8 ± 24.2	349.7 ± 76.5	*918.1* ± 63.4	21.4 ± 18.1	*4.7* ± 0.1	331.5 ± 265.6	95.4 ± 1.2	3
Zn (ppm)	*43.1* ± 14.5	88.3 ± 1.5	68.4 ± 24.1	147.7 ± 72.4	94.6 ± 27.0	96.1 ± 0.1	81.3 ± 6.7	*268.1* ± 5.6	3
Mn (ppm)	*4.5* ± 3.5	90.2 ± 8.9	11.7 ± 1.0	45.9 ± 13.7	5.2 ± 2.8	64.3 ± 0.8	*140.7* ± 55.0	52.6 ± 2.3	3
Ba (ppm)	*1.0* ± 0.6	42.7 ± 4.1	8.4 ± 1.1	17.6 ± 5.0	5.0 ± 2.1	12.8 ± 0.3	*85.9* ± 42.5	11.4 ± 0.9	3
Cu (ppm)	4.8 ± 1.9	29.5 ± 4.3	59.2 ± 9.4	47.9 ± 9.3	*2.3* ± 0.5	12.8 ± 0.3	*72.4* ± 10.3	13.0 ± 0.6	3
Rb (ppm)	*1.1* ± 0.7	3.6 ± 1.2	2.2 ± 1.5	6.6 ± 0.4	*20.1* ± 4.7	14.0 ± 0.2	4.2 ± 1.2	2.5 ± 0.1	3
Y (ppb)	6 ± 4	170 ± 4	163 ± 29	128 ± 74	*3* ± 1	48 ± 6	*612* ± 83	81 ± 5	3

Concentrations were determined by solution ICP-MS and represent weight fractions of the freeze-dried samples prior to dissolution

[a] Caught and immediately frozen

[b] Bought frozen

[c] A 0.7 Perle Eel, Skretting

Lowest and highest element concentrations are italicized

ultrapure-grade nitric acid in PTFE digestion vessels. Samples were heated to 200°C within 10 min in a MLS Ethos™ microwave and kept at 200°C for another 20 min prior to pressure digestion. After cooling, the sample solutions were filled with deionized water to 35 ml. For ICP-MS analyses the solutions were diluted (1:20) and spiked with indium used as internal standard. A PEEK cyclonic spray chamber with a micro-flow nebulizer operating in self-aspirating mode was used for sample introduction. Mass interferences were avoided by measuring ^{23}Na, ^{25}Mg, ^{55}Mn, ^{63}Cu and ^{66}Zn at medium resolution (4,000) and all other elements (^{7}Li, ^{43}Ca, ^{85}Rb, ^{86}Sr, ^{89}Y, ^{90}Zr, ^{93}Nb, ^{111}Cd, ^{138}Ba, ^{208}Pb, ^{238}U) at low (300) resolution. Internal precision as expressed by the relative standard deviation of nine analytical passes was typically less than 4% for concentrations above 0.1 μg g^{-1} and increased to 13% for lower concentrations.

Water analyses were carried out at the beginning and at the end of the experiment by ICP-AES (inductively coupled plasma atomic emission spectroscopy; Institute of Geosciences, University of Bremen) for ^{43}Ca, ^{23}Na, ^{25}Mg and ^{86}Sr and by ICP-MS for all other elements (^{7}Li, ^{55}Mn, ^{63}Cu, ^{66}Zn, ^{85}Rb, ^{89}Y, ^{90}Zr, ^{93}Nb, ^{111}Cd, ^{133}Cs, ^{138}Ba, ^{208}Pb, ^{238}U).

Otolith processing and elemental analyses

Sagittal otoliths were dried in air and embedded in thermo-epoxy (Buehler; Düsseldorf, Germany). Embedded otoliths were polished from the proximal side until the core was exposed.

LA-ICP-MS analyses were performed with a NewWave UP193 solid-state laser coupled to a ThermoFinnigan Element2™ at the Institute of Geosciences, University of Bremen.

The analytical setup provided the determination of 16 isotopes (^{7}Li, ^{23}Na, ^{25}Mg, ^{43}Ca, ^{55}Mn, ^{65}Cu, ^{66}Zn, ^{85}Rb, ^{88}Sr, ^{89}Y, ^{90}Zr, ^{93}Nb, ^{111}Cd, ^{138}Ba, ^{208}Pb, ^{238}U) recorded along a transect of 600 μm length at the anterior edge of the proximal side of the otolith (Fig. 1). Prior to measurement, the transect was pre-ablated at 100 μm s^{-1} scan speed and a spot size of 120 μm in order to clean the surface. The analyses were performed at 3 μm s^{-1} scan speed and a spot size of 75 μm with a pulse rate of 10 Hz and an irradiance of approximately 1 GW cm^{-2}. Helium was used as sample gas (0.4 l min^{-1}) and Argon was subsequently added (0.8 l min^{-1}) to the gas flow.

Following every second transect a glass reference material (NIST612) was measured as external calibration standard. For quantification the concentrations of Pearce et al. (1997) were selected.

GeoPro™ software was used for quantification. Prior to ablation a blank of 20 s duration was measured and subtracted from a signal period of approximately 200 s. As internal standard we used calcium with an assumed concentration of 38.8 wt% (similar to the NIES22 otolith standard; National Institute for Environmental Studies, 2000). Our data indicate a precision of better than 3% for concentrations above 0.5–1 μg g^{-1} and up to 13% for concentrations between 0.01 and 0.5 μg g^{-1}.

As recently reported, the local formation of vaterite instead of aragonite can affect element incorporation into

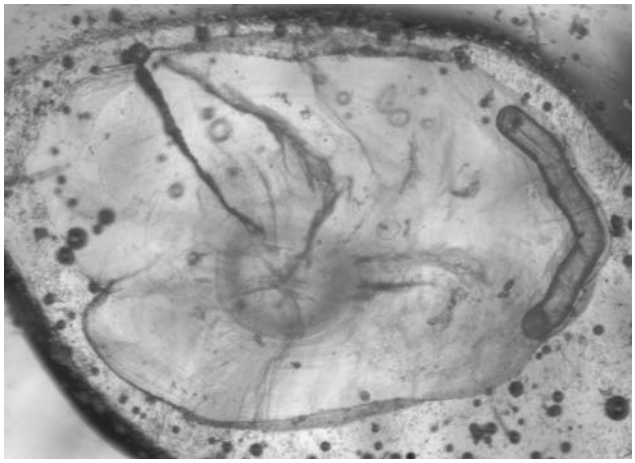

Fig. 1 Photograph of a polished eel otolith after LA-ICP-MS. The laser groove is located at the outer edge of the anterior side of the otolith

Table 2 Water element/calcium ratios at beginning and end of the feeding period (means ± 1 SD (mol)) determined by solution ICP-MS or ICP-AES

Element	Beginning	End	*N*	Method
Na/Ca (mol mol^{-1})	0.714 (±0.005)	0.334 (±0.002)	5	AES
Mg/Ca (mol mol^{-1})	0.385 (±0.005)	0.182 (±0.002)	5	AES
Sr/Ca (mmol mol^{-1})	4.604 (±0.026)	2.498 (±0.015)	5	AES
Ba/Ca (mmol mol^{-1})	0.399 (±0.002)	0.224 (±0.001)	5	AES
Cu/Ca (µmol mol^{-1})	34.277 (±0.524)	68.929 (±0.565)	5	ICP-MS
Zn/Ca (µmol mol^{-1})	5.161 (±0.092)	255.73 (±1.43)	5	ICP-MS
Rb/Ca (µmol mol^{-1})	18.548 (±0.177)	9.367 (±0.082)	5	ICP-MS
Mn/Ca (µmol mol^{-1})	6.374 (±0.02)	4.430 (±0.297)	5	ICP-MS
Y/Ca (nmol mol^{-1})	74.227 (±1.418)	43.307 (±0.466)	5	ICP-MS

eel otoliths (Tzeng et al. 2007). To avoid the use of data collected in vaterite structures, examined concentrations of Sr, Na, Ba, Mg and Mn were checked for values characteristically found in vaterite as described in Tzeng et al. (2007).

Statistical analyses

Element/Ca ratios of otoliths were calculated and values were averaged for each specimen. We compared otolith element/Ca ratios using a nested ANOVA to test for the hypotheses of no overall effect of diet on element incorporation into otolith aragonite followed by Tukey′s HSD multiple comparison tests. In case variances were not distributed homogeneously among the factor levels, a Kruskal–Wallis *H* test was used. The possible influence of growth rate on element incorporation was tested with a regression analysis. A significance level of $P \leq 0.05$ was used for all tests.

Results

Prey composition and water chemistry

Element concentrations differed largely among different prey types. Results showed a wide range of element concentrations covered by the different preys. Lowest and highest element concentrations are highlighted in Table 1.

Element concentrations in rearing water were quantified at the beginning and at the end of the experiment. All analysed elements except niobium and cadmium could be measured in water samples (^{7}Li, ^{23}Na, ^{25} Mg, ^{43}Ca, ^{55}Mn, ^{63}Cu, ^{66}Zn, ^{85}Rb, ^{86}Sr, ^{89}Y, ^{90}Zr ^{133}Cs, ^{138}Ba, ^{238}U). No significant changes in the concentrations of most elements were detected, except for Ca concentration which had doubled throughout the experiment and Zn concentrations which had changed strongly. Most of the differences found in Table 2 are caused by the increase in calcium content, since values are expressed as element/calcium ratios.

Otolith analyses

Eels fed with *G. pulex* and *E. superba* did not grow at all or even lost weight during the 8 weeks of feeding and were therefore excluded from further analyses.

We were able to quantify the following elements in the eel otoliths: ^{23}Na, ^{25} Mg, ^{55}Mn, ^{65}Cu, ^{66}Zn, ^{85}Rb, ^{88}Sr, ^{89}Y and ^{138}Ba. For some unknown reason Rb values scattered over a much wider range than other elements and had to be removed from further statistical analyses. Zn had to be excluded as well, since Zn concentrations differed widely along single transects, altering between high and low content regions without any hint of a biological cause.

^{23}Na, ^{25} Mg, ^{55}Mn, ^{65}Cu, ^{88}Sr, ^{89}Y and ^{138}Ba concentrations did not differ between feeding groups (*P* ranged from 0.14 for Mn/Ca to 0.92 for Y/CA) (Tables 3, 4; Fig. 2).

Effects of growth rate on element incorporation could not be observed for any of the analysed elements (*P* ranged from 0.251 for Na/Ca to 0.857 for Sr/Ca) (Table 5).

Discussion

Our results demonstrate that diet has no significant effect on the incorporation of trace and minor elements into European eel otoliths. Although element composition of food items differed widely (Table 1) no significant food effect on otolith microchemistry was detected. Na, Sr, Mg, Mn, Ba,

Table 3 Mean element/calcium ratios ± 1 SD (mol) in otoliths of different feeding groups determined by LA-ICP-MS

Food	*Clupea* harengus	*Mysis* sp.	*Crangon crangon*	*Rutilus rutilus*	Chironomidae larvae	Pellets	*N*
Na/Ca (mmol mol^{-1})	11.26 ± 1.21	11.11 ± 0.79	10.93 ± 1.15	10.99 ± 1.10	11.38 ± 1.16	10.81 ± 1.32	12
Sr/Ca (mmol mol^{-1})	0.65 ± 0.14	0.63 ± 0.17	0.66 ± 0.13	0.60 ± 0.16	0.69 ± 0.32	0.59 ± 0.13	12
Mg/Ca (μmol mol^{-1})	44.71 ± 23.01	44.23 ± 16.07	37.24 ± 16.89	69.20 ± 83.25	60.28 ± 35.58	48.35 ± 17.20	12
Ba/Ca (μmol mol^{-1})	1.26 ± 0.44	1.42 ± 0.40	1.07 ± 0.31	1.15 ± 0.36	1.30 ± 0.54	1.45 ± 0.46	12
Mn/Ca (μmol mol^{-1})	1.70 ± 0.57	2.29 ± 0.74	1.82 ± 0.48	1.45 ± 0.41	2.25 ± 1.32	2.21 ± 1.31	12
Cu/Ca (μmol mol^{-1})	0.12 ± 0.06	0.08 ± 0.05	0.15 ± 0.09	0.09 ± 0.05	0.11 ± 0.08	0.16 ± 0.14	12
Y/Ca (nmol mol^{-1})	3.33 ± 2.00	3.74 ± 1.79	3.79 ± 1.33	3.12 ± 1.95	3.60 ± 2.01	3.29 ± 1.18	12

Table 4 Results of analysis of variance (ANOVA) and Kruskal–Wallis *H* test summarising the effect of six different diets on otolith element/calcium ratios

	df	*F*	*H*	*p*
Na/Ca	5	0.39	–	0.856
Sr/Ca	5	–	5.49	0.359
Mg/Ca	5	–	6.74	0.241
Ba/Ca	5	1.52	–	0.196
Mn/Ca	5	–	8.34	0.138
Cu/Ca	5	–	7.22	0.205
Y/Ca	5	0.29	–	0.914

Cu and Y maintained rather constant concentrations across all feeding groups (Table 3; Fig. 2).

Significant effects on strontium and barium uptake into otoliths were found in bluefish, *P. saltatrix* (Buckel et al. 2004) fed on fish versus shrimp diets. Contents of Sr and Ba reached values from 110.4 ppm Sr and 0.996 ppm Ba in fish to 421.6 ppm Sr and 3.523 ppm Ba in shrimp diet. In our study Sr contents in the selected prey organisms ranged from 4.7 ppm in chironomidae larvae to 918.1 ppm in *C. crangon*, Ba contents from 1.0 ppm in herring to 42.7 ppm in mysids (Table 1). Surprisingly, except for bluefish (Buckel et al. 2004), no other published study that successfully detected dietary effects also compared element content of diets.

The absence of any significant dietary effect in our experiment and the conflicting results from similar investigations on the influence of natural and artificial prey on otolith microchemistry in salt- and freshwater suggest species specific physiological processes to be responsible for interspecies differences of element uptake into otoliths. The uptake of elements into otoliths strictly depends on the element composition of the endolymph, an acellular medium, which is secreted by the inner ear epithelium (Payan et al. 2004). Active element and ion discrimination at several barriers along the way from the environment to the endolymph causes a decoupling of otolith increment from ambient conditions or food composition (Payan et al. 2004; Campana 1999). Food-carried elements are selected at intestine/blood, blood/inner ear epithelium as well as inner ear epithelium/endolymph barriers (Payan et al. 2004). Selective processes like cellular transport and crystallisation additionally modify element concentrations (Campana 1999). Precise species specific knowledge about physiological processes at these barriers is required to fully understand the interspecific differences of element uptake into otoliths as recently reported by Hamer and Jenkins (2007) for the seabream *Pagrus auratus* and the sand flathead *Platycephalus bassensis*.

Our experimental setup intended to exclude every potential influence on otolith chemistry except feeding and we assume food to be the only variable between feeding groups. A laser diameter of 75 μm was chosen to ensure measurements within the otolith increments grown during the experiment. Umezawa and Tsukamoto (1991) reported a daily growth rate of about 1.97 μm (±0.433) day^{-1} in Japanese eel (*A. japonica*) elver otoliths. Assuming a similar growth rate in *A. anguilla,* otolith increments would amount to 86 and 135 μm during the experiment. Water temperature and chemistry were equal for all treatments and growth rate had no effect on element incorporation (Table 5). The circulation system and the high water exchange rate of three to four per day provided constant and equal water conditions for each treatment. Concentrations of most elements in water remained largely constant during the experiment, except for Ca and Zn (Table 2). The use of tap water to replace evaporation loss could have caused a notable enrichment of these elements, but can not fully explain the extreme increase of Zn. Nevertheless, we consider the changes of these two elements to have no impact on overall results since all treatments were carried out in the same circuit. Changes in water composition affect all treatments in the same way and food still remains the only variable between feeding groups. Therefore, Zn was removed from otolith analyses due to strong variations of concentrations within the same otolith increments.

We conclude feeding behaviour not to substantially contribute to otolith element composition in *A. anguilla*

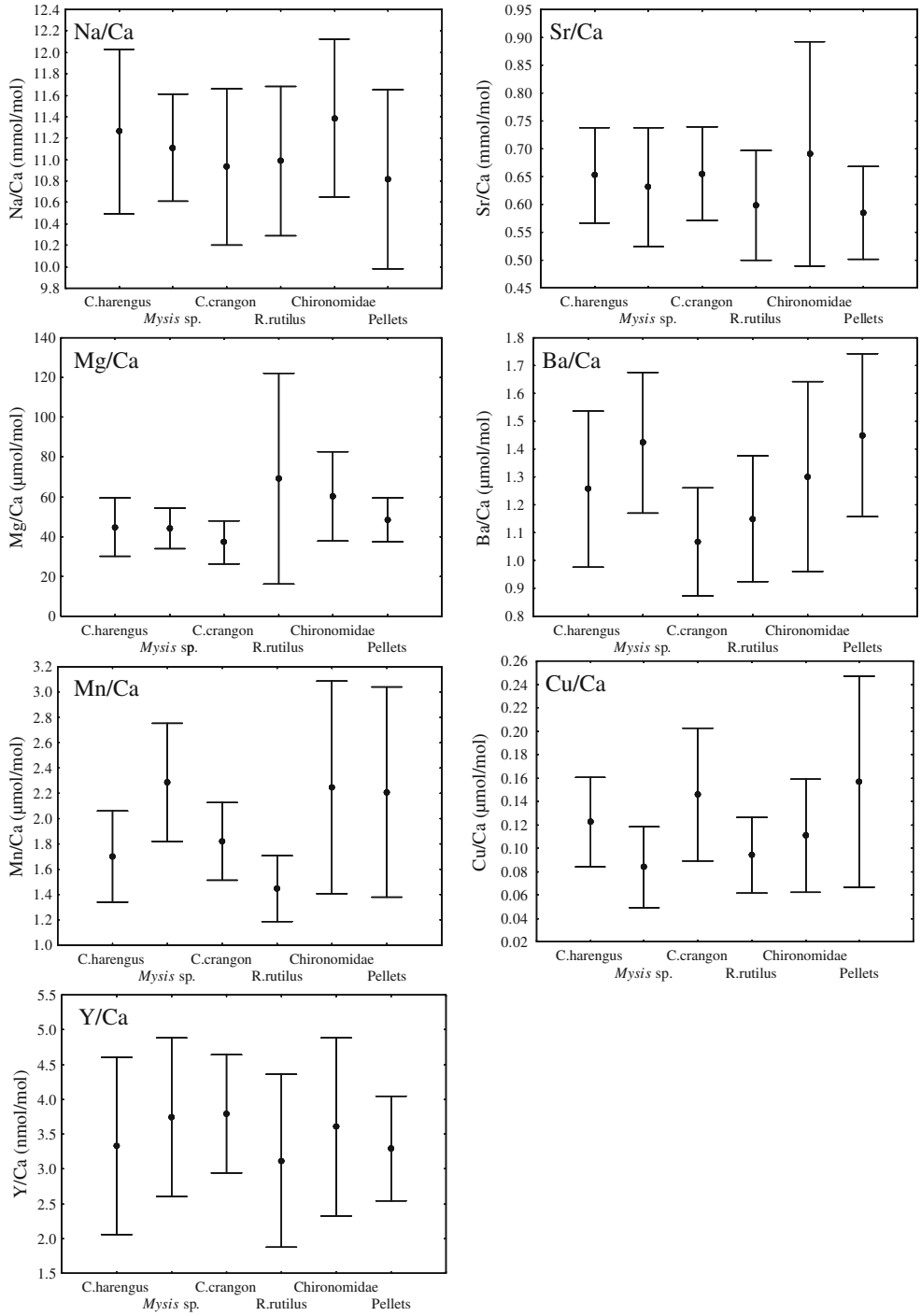

Fig. 2 Element/calcium ratios of seven minor and trace elements in eel otoliths after feeding six different diets (mol). *Points* and *error bars* represent means and 95% CI of 12 specimens

and consequently to have no disturbing effect on tracking migration through microchemical analyses, since widely used proxy elements like strontium or barium remained unaffected by diet even at high resolution analytics ensured through LA-ICP-MS measurements. Nevertheless, additional investigations are required to further unambiguously exclude effects on element incorporation into eel otoliths caused by exogenous and endogenous variables like temperature, stress and growth. The continuous enhancement of analytic methods like e.g. the detection of isotopic signatures with multi collector LA-ICP-MS (Fietzke et al. 2008) might

Table 5 Results of regression analysis summarising the effect of specific growth rate (SGR) (%weight day^{-1}) on otolith element/calcium ratios

	R^2	p	N
Sr/Ca vs. SGR	0.0005	0.857	72
Ba/Ca vs. SGR	0.0020	0.707	72
Mn/Ca vs. SGR	0.0102	0.399	72
Mg/Ca vs. SGR	0.0079	0.457	72
Na/Ca vs. SGR	0.0188	0.251	72
Cu/Ca vs. SGR	0.0142	0.320	72
Y/Ca vs. SGR	0.0148	0.308	72

help to gain further information about environmental influences on otolith microchemistry.

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