Journal of Trace Elements in Medicine and Biology 27 (2013) 273–285

Contents lists available at ScienceDirect

Journal of Trace Elements in Medicine and Biology

journal homepage: www.elsevier.de/jtemb

ANALYTICAL METHODOLOGY

Analysis methods and reference concentrations of 12 minor and trace elements in fish blood plasma

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ARTICLE INFO

Article history:
Received 24 August 2012
Accepted 4 March 2013

Keywords:
Fish
Multi-elemental
Blood
Plasma
Microchemistry

ABSTRACT

A comprehensive review of the analytical literature revealed substantial under-representation of trace element concentrations in fish blood, particularly for marine species. We describe a simple dilution procedure to measure Li, Mg, K, Ca, Mn, Cu, Zn, Se, Rb, Sr, Ba and Pb concentrations in low volumes of blood plasma of adult plaice (*Pleuronectes platessa*) using high resolution-inductively coupled plasma-mass spectrometry (HR-ICP-MS). Captive male and female plaice ($n = 18$) were serially sampled for one year and samples collected outside of the spawning season ($n = 157$) used to estimate reference ranges for this species. Method accuracy was deemed satisfactory, based on its application to the analysis of a certified reference material. Precision was generally <3%, with the most conservative measure of precision being ≤10% for all elements except Pb (~20%). This is the first study to analyse fish blood plasma by ICP-MS and includes some of the first reference ranges for trace element concentrations in fish blood.

Introduction

Essential trace elements are, by definition, vital to the health and survival of all animals. Concentrations of elements in body fluids need to be tightly controlled, given their requirement in many metabolic reactions and their potential toxicity at elevated levels [1]. Precise and accurate reference concentrations are crucial to establish bioindicators of population health and to understand the functionality of elements in biological processes [2]. Variations may indicate differences in physiological status among individuals, impaired body function, and/or differences in environmental exposure. All of these are of interest for monitoring wild animal populations, particularly those exploited for food. Despite global importance as a protein resource, both from wild fisheries and increasingly from aquaculture, fish blood trace elemental chemistry has received relatively little attention compared with their mammalian counterparts [3]. A review of the analytical literature indicated strong representation of Na^+, Cl^+, K^+ and Ca^{2+} concentrations in whole blood (henceforth described simply as 'blood'), serum and plasma [reviewed in 4–8], but serious under-representation of many trace elements (Table 1). This, along with the lack of suitable reference materials and the large (often >2sd) differences in concentrations among species, studies and blood fractions (Table 1), hamper studies requiring a robust baseline to examine elemental uptake, distributions and processing mechanisms.

Accurate and precise measurement of trace and ultra-trace elements in biological fluids presents a number of challenges due to the complex nature of the medium and low detection limits necessary for analysis [9]. Inductively coupled plasma mass spectrometry (ICP-MS) has proven to be the most reliable method for rapid multi-element screening of a variety of complex solutions, but spectral and non-spectral interferences can hinder analyses, particularly for elements close to detection limits. High resolution ICP-MS (HR-ICP-MS) offers greater sensitivity and can reduce or even eliminate mass interference problems [10]. Direct analysis of biological fluids can be problematic because the high protein and salt content can block the nebuliser and torch tubes [9]. This can be overcome through microwave or acid digestion [11], but such processes can introduce contamination or cause loss of volatile elements [12]. To reduce contamination risk and processing time, simple dilution methods are generally favoured [9,13,14].

While a number of studies have examined optimal methods for the analysis of human biological fluids [e.g. 11], none have focused on determination of trace elements in fish blood. Marine fish present additional challenges as they are hypo-osmotic and continually drink seawater to maintain ionic homeostasis [15]. Despite highly evolved excretory mechanisms to remove excess salts, their bloods are characterised by elevated Na^+ and Cl^- concentrations, typically 25–30% higher than freshwater species (Table 1) and 20–70% higher than humans [16]. Salt loading could have

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http://dx.doi.org/10.1016/j.jtemb.2013.03.001

Table 1
Concentrations (in ppm) of 14 elements in serum (S), plasma (P) or whole blood (B) collected from fish inhabiting freshwater (FW) or seawater (SW). Study details provided where relevant, such as when male (M), female (F) or juvenile (J) subject animals were specified. Concentrations include averages (*X*) and values extrapolated from graphs. Table updated from S. Campana (pers. comm.). Trace elements were the primary focus of the review, but examples of major ion concentrations were included to illustrate coarse differences in blood composition between freshwater and marine species.

Taxonomic group	Fish species	Reference	Fraction	Water	Li	Na	Mg	Cl	K	Ca	Sr	Details
Class Actinopterygii												
Order Acipenseriformes	*Acipenser fulvescens*	[26]	S	FW		2888		4141	94.0	48.0		
Family Acipenseridae												
	Acipenser naccarii	[27]	S	FW[a]		3065		3712		106.0		7 yrs, cannulated
	Acipenser transmontanus	[28]	B[b]	FW[a]								*X* (J, time zero). Non PB[b] fraction = 0.031 μg/g
	Ameiurus nebulosus	[29]	S	FW		2943	46.0	3900	78.2	107.0		
Order Siluriformes	*Campostoma oligolepis*	[30]	B[c]	FW								*X* (2 ref sites)
Family Ictaluridae												
	Ictalurus punctatus	[31]	B[c]	FW								*X* (2 ref sites)
	I. punctatus	[32]	P	FW		3150		4396	101.7			
Order Cypriniformes	*Catostomus commersoni*	[33]	S	FW						175.3		*X* (F, 4 sites, ΔpH)
Family Catostromidae												
	Hypentelium spp.	[34]	B[c]	FW								
	Hypentelium nigricans	[30]	B[c]	FW								
	H. nigricans	[35]	B	FW								*X* (protected sites)
Family Cyprinidae	*Cyprinus carpio*	[36]	P	FW			20.0					Anaethetised, 'Initial concs'
	C. carpio	[31]	B[c]	FW								
	C. carpio	[37]	B	FW					1695			
	C. carpio	[37]	S	FW		3000	33.2	4010	246.0	115.0		*X*
	C. carpio	[38]	P	FW		3166	30.9	3921	162.6	81.6		*X*
	C. carpio	[39]	P	FW		3104	30.0	3470	235.0	85.0		Controls
	Pimephales promelas	[40]	B	FW								*X* (J, time zero)
Order Salmoniformes	*Oncorhynchus mykiss*	[41]	P	FW		2457			117.1	76.7		Controls
Family Salmonidae												
	O. mykiss	[38]	P	FW		3529	17.5	4634	79.4	96.8		M
	O. mykiss	[42]	P	FW		4370		4056	170.1			*X* (stunned MS222 treated)
	O. mykiss	[43]	S	FW								
	O. mykiss	[44]	S	FW			117.2					*X*
	O. mykiss	[45]	S	FW		3438	22.5	4294	50.0	103.0		*X*
	O. mykiss	[46]	S	FW			17.0			99.0		*X* (Jan–Dec)
	O. mykiss	[47]	P	FW						111.0		pH 6.62
	O. mykiss	[48]	B	FW		2941	72.0		812.0	109.0	0.17	
	O. mykiss	[49]	P	FW		3564	17.0	4786	105.6	98.2		
	O. mykiss	[50]	P	FW								
	O. mykiss	[51]	P	FW				3829				*X*
	Oncorhynchus mykiss	[52]	P	FW		3420	41.0		41.0	256.0		*X* (Controls)
	Oncorhynchus nerka	[53]	P	FW[a]								*X* (M, F, spawning)
	O. nerka	[53]	P	SW[a]								*X* (M, F)
	Oncorhynchus tshawytscha	[54]	P	FW		3862		4680	75.9	107.4		J, caudal puncture
	Salmo salar	[55]	P	FW[a]								J, Mn < LOD
	S. salar	[56]	S	FW[a]								*X* (4 diets, ΔZn)
	Salmo trutta	[57]	P	FW			41.8	5353		192.4		Controls
	S. trutta	[58]	S	FW		3580	23.0	4240	201.0	125.0		*X*
	Salvelinus namaycush	[59]	S	FW			28.0			115.0		
	S. namaycush	[60]	B	FW		2620	67.8		1990	102.0	0.14	
Order Lophiiformes	*Lophius piscatorius*	[61]	P	SW		3816		5859	249.1			*X* (J, F, M)
Family Lophiidae												
Order Gadiformes	*Gadus morhua*	[62]	S	SW	0.048	3931	23.8		127.9	139.5		
Family Gadidae												
	G. morhua	[63]	P	SW						171.9		*X* (M, F, 1 yr)
Family Lotidae	*Lota lota*	[60]	B[c]	FW		2670	66.4		1970	80.2	0.21	
Family Moridae	*Antimora rostrata*	[64]	P	SW		4115	34.8	5602	89.5	101.8		
	Pseudophysis barbatus	[20]	P	SW		4213			279.3	71.6	1.10	*X* (M, F, 8 mo)
Order Mugiliformes	*Mugil cephalus*	[65]	P	FW[a]			33.8			101.0		*X*
Family Mugilidae												
	M. cephalus	[2]	S	SW[a]		3862	55.0	4963	97.7	120.0		*X* (all months)
	M. cephalus	[65]	P	SW[a]			47.4			95.8		*X*
	M. cephalus	[66]	P	SW[a]						134.7		Controls

Table 1 (*continued*)

Taxonomic group	Fish species	Reference	Fraction	Water	Li	Na	Mg	Cl	K	Ca	Sr	Details
Order Beryciformes	*Holocentrus adscensionis*	[67]	P	SW								*X* (F)
Family Holocentridae												
Order Pleuronectiformes	*Platichthys flesus*	[68]	P	FW[a,d]		3472	14.6	4680	140.8	96.2		
Family Pleuronectidae												
	P. flesus	[69]	P	FW[a,d]		3518		4485	82.1			
	Kareius bicoloratus	[46]	S	SW[a]			19.0			93.0		*X* (1 yr)
	Parophrys vetulus	[70]	P	SW[a]			25.4			113.4		*X* (F, all stages)
	Pleuronectes platessa	[71]	S	SW		4012	14.0	5619	162.0	102.0		
	P. platessa	[72]	S	SW								
	P. platessa	[73]	B	SW								
	P. platessa	[74]	S	SW								*X* (F, all months)
	Pseudopleuronectes americanus	[75]	P	SW[a]		5081		6807	43.0			
Family Scophthalmidae	*Scophthalmus aquosus*	[76]	P	SW		3851			185.7	79.8		*X* (field, 4 seasons)
Order Scorpaeniformes	*Myoxocephalus scorpius*	[62]	S	SW	0.076							
Family Cottidae												
	Myoxocephalus quadricornis	[77]	P	SW[a]		3678	10.9	4396	125.1	97.0		
Family Scorpaenidae	*Scorpaena porcus*	[78]	P	SW	0.051	3747						
Order Perciformes	*Lepomis macrochirus*	[33]	S	FW						200.0		*X* (M, F)
Family Centarchidae												
	Lepomis megalotis	[79]	B	FW								
	L. megalotis	[30]	B	FW								*X* (2 ref sites)
	Lepomis sp.	[80]	S	FW		1825		2365	88.0	178.5		*X* (ref site, 2 yr)
	Micropterus salmoides	[31]	B[c]	FW								
Family Centracanthidae	*Spicara smaris*	[78]	P	SW	0.056	3839						
Family Cichlidae	*Oreochromis mossambicus*	[81]	P	FW[a]			24.1					Control (>8 wk)
	O. mossambicus	[82]	P	FW[a]		3674		4804	106.3			Control
	O. mossambicus	[83]	S	FW[a]						440.9		F
	O. mossambicus	[83]	S	FW[a]		3720	34.0	5038	129.0	128.2		M, F (Ca = M)
	O. mossambicus	[83]	S	SW[a]						384.7		F
	O. mossambicus	[83]	S	SW[a]		4028	29.2	6180	129.0	144.3		M, F (Ca = M)
Family Istiophoridae	*Makaira mazara*	[84]	B	SW								*X*
	Tetrapturus audax	[84]	B	SW								*X*
Family Moronidae	*Dicentrarchus labrax*	[85]	S	SW[a]		4198		6044	134.1	172.6		*X* (ref value)
	Morone saxatilis	[86]	P	SW[a]		4483			258.0	90.2		
Family Mullidae	*Mullus barbatus ponticus*	[78]	P	SW	0.045	3678						
Family Percidae	*Perca fluviatilis*	[87]	P	FW			42.5			142.8		*X* (M, F, 2 sites, monthly)
Family Scombridae	*Thunnus alalunga*	[88]	B	SW	0.022	2650			2270			
	Thunnus albacares	[89]	B	SW								
	T. albacares	[90]	B	SW								*X* (M, F)
	T. albacares	[84]	B	SW								*X*
	Thunnus obesus	[89]	B	SW								
	T. obesus	[90]	B	SW								*X* (M, F)
	T. obesus	[84]	B	SW								*X*
Family Sparidae	*Diplodus annularis*	[78]	P	SW	0.053	3770						
	Lagodon rhomboides	[2]	S	SW		4023	42.0	5424	117.3	130.0		*X* (all months)
	Pagrus auratus	[91]	S	SW		4690	41.3	6311	234.6	124.2		Sedated
	P. auratus	[92]	P	SW		4616		6736	168.1	100.2		Rested, cannulated
Family Zoarcidae	*Lycodes esmarkii*	[64]	P	SW		4621	67.6	6559	211.1	110.6		
Grand means (± SEM)				**FW**	-	3216 *(112)*	38.2 *(5.2)*	4240 *(145)*	381.3 *(129)*	131.3 *(14.2)*	0.17 *(0.02)*	
				SW	0.050 *(0.006)*	4043 *(109)*	34.2 *(4.9)*	5875 *(209)*	287.2 *(125)*	128.9 *(14.8)*	1.10 *(-)*	

Table 1 (*continued*)

Taxonomic group	Fish species	Reference	Fraction	Water	Mn	Cu	Zn	Se	Rb	Ba	Pb	Details
Class Actinopterygii												
Order Acipenseriformes	*Acipenser fulvescens*	[26]	S	FW								
Family Acipenseridae												
	Acipenser naccarii	[27]	S	FW[a]								7 yrs, cannulated
	Acipenser transmontanus	[28]	B[b]	FW[a]				0.64				*X* (J, time zero). Non PB[b] fraction = 0.031 μg/g
	Ameiurus nebulosus	[29]	S	FW								
Order Siluriformes	*Campostoma oligolepis*	[30]	B[c]	FW			56.6				0.197	*X* (2 ref sites)
Family Ictaluridae												
	Ictalurus punctatus	[31]	B[c]	FW			114.0				0.095	*X* (2 ref sites)
	I. punctatus	[32]	P	FW								
Order Cypriniformes	*Catostomus commersoni*	[33]	S	FW								*X* (F, 4 sites, ΔpH)
Family Catostromidae												
	Hypentelium spp.	[34]	B[c]	FW			69.0				0.630	
	Hypentelium nigricans	[30]	B[c]	FW			50.5				0.287	
	H. nigricans	[35]	B	FW			49.4				0.090	*X* (protected sites)
Family Cyprinidae	*Cyprinus carpio*	[36]	P	FW	0.001	0.84	0.7					Anaethetised, 'Initial concs'
	C. carpio	[31]	B[c]	FW			52.0				0.290	
	C. carpio	[37]	B	FW	0.058							
	C. carpio	[37]	S	FW								*X*
	C. carpio	[38]	P	FW								*X*
	C. carpio	[39]	P	FW		0.10	4.60					Controls
	Pimephales promelas	[40]	B	FW				0.37				*X* (J, time zero)
Order Salmoniformes	*Oncorhynchus mykiss*	[41]	P	FW		0.69						Controls
Family Salmonidae												
	O. mykiss	[38]	P	FW								M
	O. mykiss	[42]	P	FW								*X* (stunned MS222 treated)
	O. mykiss	[43]	S	FW	0.070	1.30	10.2					
	O. mykiss	[44]	S	FW								*X*
	O. mykiss	[45]	S	FW								*X*
	O. mykiss	[46]	S	FW								*X* (Jan–Dec)
	O. mykiss	[47]	P	FW								pH 6.62
	O. mykiss	[48]	B	FW	0.220	0.94	13.0					
	O. mykiss	[49]	P	FW								
	O. mykiss	[50]	P	FW								
	O. mykiss	[51]	P	FW								*X*
	Oncorhynchus mykiss	[52]	P	FW		1.25	18.3					*X* (Controls)
	Oncorhynchus nerka	[53]	P	FW[a]		0.85	8.5					*X* (M, F, spawn)
	O. nerka	[53]	P	SW[a]		1.39	23.4					*X* (M, F)
	Oncorhynchus tshawytscha	[54]	P	FW								J, caudal puncture
	Salmo salar	[55]	P	FW[a]		1.60	12.8					J, Mn < LOD
	S. salar	[56]	S	FW[a]			22.9					*X* (4 diets, ΔZn)
	Salmo trutta	[57]	P	FW								Controls
	S. trutta	[58]	S	FW		2.80						*X*
	Salvelinus namaycush	[59]	S	FW								
	S. namaycush	[60]	B	FW	0.103		8.8		2.42	0.25	0.161	
Order Lophiiformes	*Lophius piscatorius*	[61]	P	SW		0.72						*X* (J, F, M)
Family Lophiidae												
Order Gadiformes	*Gadus morhua*	[62]	S	SW								
Family Gadidae												
	G. morhua	[63]	P	SW								*X* (M, F, 1 yr)
Family Lotidae	*Lota lota*	[60]	B[c]	FW	0.084		5.78		1.96	0.37	0.054	
Family Moridae	*Antimora rostrata*	[64]	P	SW								
	Pseudophysis barbatus	[20]	P	SW								*X* (M, F, 8 mo)
Order Mugiliformes	*Mugil cephalus*	[65]	P	FW[a]								*X*
Family Mugilidae												
	M. cephalus	[2]	S	SW[a]								*X* (all months)
	M. cephalus	[65]	P	SW[a]								*X*
	M. cephalus	[66]	P	SW[a]		2.70						Controls

Table 1 (*continued*)

Taxonomic group	Fish species	Reference	Fraction	Water	Mn	Cu	Zn	Se	Rb	Ba	Pb	Details
Order Beryciformes	*Holocentrus adscensionis*	[67]	P	SW			6.54					*X* (F)
Family Holocentridae												
Order Pleuronectiformes	*Platichthys flesus*	[68]	P	FW[a,d]								
Family Pleuronectidae												
	P. flesus	[69]	P	FW[a,d]								
	Kareius bicoloratus	[46]	S	SW[a]								*X* (1 yr)
	Parophrys vetulus	[70]	P	SW[a]								*X* (F, all stages)
	Pleuronectes platessa	[71]	S	SW								
	P. platessa	[72]	S	SW		0.57						
	P. platessa	[73]	B	SW		0.67						
	P. platessa	[74]	S	SW			9.93					*X* (F, all months)
	Pseudopleuronectes americanus	[75]	P	SW[a]								
Family Scophthalmidae	*Scophthalmus aquosus*	[76]	P	SW								*X* (field, 4 seasons)
Order Scorpaeniformes	*Myoxocephalus scorpius*	[62]	S	SW								
Family Cottidae												
	Myoxocephalus quadricornis	[77]	P	SW[a]								
Family Scorpaenidae	*Scorpaena porcus*	[78]	P	SW								
Order Perciformes	*Lepomis macrochirus*	[33]	S	FW								*X* (M, F)
Family Centarchidae												
	Lepomis megalotis	[79]	B	FW							0.030	
	L. megalotis	[30]	B	FW			51.7				0.194	*X* (2 ref sites)
	Lepomis sp.	[80]	S	FW								*X* (ref site, 2 yr)
	Micropterus salmoides	[31]	B[c]	FW			53.9				0.100	
Family Centracanthidae	*Spicara smaris*	[78]	P	SW								
Family Cichlidae	*Oreochromis mossambicus*	[81]	P	FW[a]								Control (>8 wk)
	O. mossambicus	[82]	P	FW[a]								Control
	O. mossambicus	[83]	S	FW[a]								F
	O. mossambicus	[83]	S	FW[a]								M, F (Ca = M)
	O. mossambicus	[83]	S	SW[a]								F
	O. mossambicus	[83]	S	SW[a]								M, F (Ca = M)
Family Istiophoridae	*Makaira mazara*	[84]	B	SW				0.96				*X*
	Tetrapturus audax	[84]	B	SW				0.80				*X*
Family Moronidae	*Dicentrarchus labrax*	[85]	S	SW[a]								*X* (ref value)
	Morone saxatilis	[86]	P	SW[a]								
Family Mullidae	*Mullus barbatus ponticus*	[78]	P	SW								
Family Percidae	*Perca fluviatilis*	[87]	P	FW								*X* (M, F, 2 sites, monthly)
Family Scombridae	*Thunnus alalunga*	[88]	B	SW					0.44			
	Thunnus albacares	[89]	B	SW				42.5				
	T. albacares	[90]	B	SW				38.6				*X* (M, F)
	T. albacares	[84]	B	SW				40. 8				*X*
	Thunnus obesus	[89]	B	SW				41.4				
	T. obesus	[90]	B	SW				44.8				*X* (M, F)
	T. obesus	[84]	B	SW				45.2				*X*
Family Sparidae	*Diplodus annularis*	[78]	P	SW								
	Lagodon rhomboides	[2]	S	SW								*X* (all months)
	Pagrus auratus	[91]	S	SW								Sedated
	P. auratus	[92]	P	SW								Rested, cannulated
Family Zoarcidae	*Lycodes esmarkii*	[64]	P	SW								
Grand means (± SEM)				**FW**	0.089 *(0.03)*	1.15*(0.8)*	33.5 *(7.1)*	0.505 *(0.14)*	2.19 *(0.2)*	0.312 *(0.06)*	0.193 *(0.05)*	
				SW	-	1.21 *(0.9)*	13.28 *(8.9)*	31.9 *(6.8)*	0.443 *(-)*	-	-	

[a] Euryhaline species.
[b] Protein bound (PB) blood fraction.
[c] Analyses carried out by ICP-MS.
[d] Brackish water (salinities of 7–7.6).

important implications for trace metal analyses and would be augmented in studies (such as this) using sodium-based anticoagulants. Otherwise, blood composition is broadly conserved among vertebrates, particularly for essential trace elements such as Zn, Mn and Cu (Table 1, Ref. [17]).

Biomineral microchemistry represents a growing field that would benefit greatly from an improved understanding of elemental uptake and transport mechanisms in fish [15]. Otoliths are acellular calcium carbonate 'earstones' that are common to all bony fish and grow incrementally, incorporating chemical markers from the environment to produce a time-resolved natural tag that can infer stock identity and individual movements [15]. The elements selected for the current study (Li, Mg, K, Ca, Mn, Cu, Zn, Se, Rb, Sr, Ba and Pb) have all been reported as useful (or potentially useful) otolith markers, but their relationships with the environment are not always clear [e.g. 18], and many are of minor interest in human biomedical fields and under-represented in the analytical literature (Table 1). Otolith Sr/Ca and Ba/Ca ratios are often used to monitor fish movements across salinity gradients [19], however physiological processes may significantly influence Sr behaviour, particularly in marine fishes [18,20]. Otolith Li/Ca, Mg/Ca, K/Ca, Mn/Ca, Cu/Ca and Zn/Ca ratios have been used to infer population structuring of wild marine fishes [21,22], while correlations between water chemistry and otolith Se/Ca [23], Rb/Ca [24] and Pb/Ca [25] ratios have indicated potential utility as additional geographic markers. Despite increasing prominence of otolith microchemistry in the scientific literature [18], little is known about element sources, uptake mechanisms or transport pathways prior to their incorporation into the otolith, nor the relative importance of intrinsic and extrinsic factors.

The objectives of this study were to (1) develop a simple procedure for simultaneous quantification of 12 trace, minor and major elements in the blood plasma of plaice (*Pleuronectes platessa*) using low sample volumes, (2) provide a baseline of reference values for future studies, and (3) explore possible reasons for differences among studies.

Methods

Animals

Adult plaice (8 females, 10 males; mean total length = 26.2 cm) were collected by beam trawl from the Irish Sea in February 2009 and maintained in a large outdoor tank at CEFAS Lowestoft fed with continuously flowing coastal seawater. They were acclimatised for three months prior to blood sampling and fed twice weekly with live lugworms (*Arenicola marina*) from a single, local beach. Sampling was carried out between 8 and 10 am on days prior to feeding to avoid diurnal or food-related artefacts.

Sample collection

Plasma was collected instead of serum, as metabolism of blood components during clotting can result in serum being a less reliable recorder of *in vivo* elemental concentrations [93], particularly in animals with nucleated red blood cells such as fish [93]. Also, clotting time of fish blood is particularly rapid [94] and needles frequently blocked during trials attempting to sample without an anticoagulant. A stock of heparin solution was prepared (500 IU/ml) using MQ and high purity heparin sodium salt from porcine intestinal mucosa (Sigma–Aldrich). Aliquots were stored at –20 °C. Before each sampling session an aliquot was thawed at 4 °C and 0.1 ml drawn up into each needle (25 mm, 25 gauge) and syringe (1 ml BD Plastipak) then fully vented. This left 25 ± 2.76 mg (mean ± SE, $n = 17$) heparin in each syringe.

Table 2
Concentration ranges (in ng/g) for external calibration of fish plasma and Seronorm at 150-fold dilution.

	Fish plasma		Seronorm	
	Min	Max	Min	Max
Li	0.012	1.396	17.38	83.76
Mg	5.92	698.3	60.51	291.6
K	10.34	1220.1	375.3	1808.6
Ca	24.78	2925.1	317.4	1529.3
Mn	0.009	1.112	0.030	0.145
Cu	0.221	26.09	4.036	19.45
Zn	2.329	274.9	4.088	19.70
Se	0.099	11.65	0.199	0.961
Rb	0.002	0.205	0.011	0.055
Sr	0.198	23.35	0.086	0.416
Ba	0.0024	0.2806	0.4207	2.0274
Pb	0.0001	0.0078	0.0099	0.0478

Monthly blood samples were collected from each fish for one year (02/06/09–28/05/10) by caudal venipuncture and stored on ice blocks until processing. Bloods were centrifuged within 3 h (15 min, 2500 rpm, 4 °C) and the plasma stored at –20 °C. Procedural blanks were collected in the same way, using MQ in place of blood. For quality control purposes, clotted or haemolysed samples were discarded. All processes apart from the initial blood collection were carried out using acid cleaned consumables [95] in a dedicated Class 100 clean room or Heraeus Class II Safety Cabinet lined with clean plastic sheeting. To avoid blood contamination, subject animals were not sedated prior to sampling, but handling time and sample volume were minimised (usually <3 min and 0.3 ml, respectively) in compliance with UK Home Office guidelines (Project License no. 80/2260).

Reagents

Multi-element stock solutions were prepared using 2% nitric acid (HNO_3) and single element standards (Inorganic Ventures, Lakewood, USA, and Romil, Cambridge, UK). These were diluted to produce five-point calibration curves in physiologically relevant ranges (Table 2). MilliQ water (MQ, >18.2 MΩ) and trace metal grade HNO_3 (Fisher Scientific, UK), further purified by sub-boiling (sb.) distillation in a Savillex Teflon still, were used throughout. Seronorm Human Trace Elements Serum L-1 (Batch 0608414, Nycomed, Norway) was used as the certified reference material (CRM), but required separate calibration due to differences in its composition (Table 2). Samples and calibration standards were diluted using two mixtures previously developed for human biological fluids spiked with internal standards (Be at 20 μg/L; Rh and Re at 10 μg/L). Both diluents were weakly acidic and contained Triton-X 100 (Sigma–Aldrich, UK) to form a stable emulsion and avoid build-up of organic residues, and butan-1-ol (≥99.5, Fisher Scientific, UK) to ensure efficient ionisation of Se [96]. 'Mix 1' [14,96] comprised 0.05% HNO_3, 0.1% Triton X-100 (replacing 'TAMA SC') and 3% butanol, while 'Mix 2' [13] comprised 1% HNO_3, 0.01% Triton X-100 and 0.5% butanol.

Method optimisation

To determine optimal diluent mixture and dilution factor (DF), plasma and Seronorm samples were serially diluted 10, 20, 30, 50, 100, 250, 600 and 1000-fold. Exact DF was determined by weight. Samples were analysed sequentially from the least to the most concentrated. Internal standard-spiked 'acid blanks,' sb. 2% HNO_3 and 'diluent blanks' were analysed at the start and end of each batch to correct for instrument drift and to calculate detection limits (LOD, mean + 3 sd of diluent blanks). The optimal preparation was

defined as the preparation that minimised signal suppression while producing concentrations >LOD.

Memory effects were informally assessed by analysing replicate 2% HNO_3 washes after plasma and Seronorm samples. Generally, counts had returned to background levels by the second wash (equivalent to ~3.5 min), so wash times were set to 4 min. Even with this extended wash time, Seronorm Li (almost 100× higher than fish plasma Li) remained elevated in subsequent samples, so Seronorm-based samples and calibration standards were analysed only at the end of runs.

Sample preparation

All solutions were weighed to 5 d.p. and concentrations calculated by weight, given the potential for pipetting error when using low volumes and viscous solutions. The following steps were taken to improve matrix matching among blanks, standards and samples:

1. Volumes of Be-, Rh- and Re-spiked diluent mixture were matched among all tubes (2.5 ml).
2. MQ was used in place of plasma or Seronorm in blank and calibration tubes (20 μl).
3. All tubes were spiked with an equal volume of 2% HNO_3 or 2% HNO_3 based standard (480 μl).

'Procedural standards' were prepared in the same manner, scaled up to larger volumes to allow repeat-sampling within and across runs. Also, while sample order was randomised, blanks, calibrations and Seronorm were analysed only at the start or end of runs. Both 'procedural' and 'laboratory' blanks were analysed, their only difference being the source of the MQ added (via heparinised syringe and multiple tubes, or direct from the lab, respectively).

Sample analysis

Analyses were carried out on a Thermo Fisher Scientific Element 2 XR HR-ICP-MS. Low-resolution (LRM, $m/\Delta m$ 300), medium-resolution (MRM, $m/\Delta m$ 3000) and high-resolution (HRM, $m/\Delta m$ 7500) modes were utilised to avoid spectral interferences (Table 3). Sample introduction was achieved via autosampler (ESI SC2, Omaha, US) using a pumped micro PFA nebuliser into a peltier cooled PFA cyclonic spray chamber (ESI PC3). Additional Ar add gas was ported into the spray chamber to improve sensitivity and signal stability. The instrument used the standard torch, sapphire injector and guard electrode. Using this configuration and adjusting ion lens settings to maximise sensitivity, the instrument was tuned to achieve sensitivity of around 1×10^6 cps in LRM 1 ng/g ^{115}In. Oxide formation was minimised using the $^{238}U^{16}O^+/^{238}U^+$ ratio. The instrument was 'conditioned' at the start of each run by repeat-analysis of diluent mixture for 30–45 min.

Data analysis

Raw counts were blank and drift-corrected offline, and concentrations quantified by direct calibration (sample calibration curves $r^2 > 0.999$ except Pb, which ranged from 0.954 to >0.999). Differences between procedural and laboratory blanks were tested by oneway ANOVA. Log transformation was applied if data were heterogeneous (Brown-Forsythe test, JMP 8.0). Concentrations <LOD (3 SD of repeat blanks) were excluded, as were samples taken during the spawning season (January–March), as 'resting' basal values can be significantly altered during this period [53,67].

Internal precision (instrument error) was assessed using within-analysis relative standard deviation (%RSD) of plasma samples analysed across all runs. External precision was assessed using %RSD for repeat analysis of both 'procedural standards' and Seronorm CRM. To identify the sources of error in the method (i.e. preparation, instrument and/or calibration error), %RSD was calculated using replicate analyses of single and multiple preparations of a given sample, analysed within and across days. As no CRM for fish serum or plasma is currently available, accuracy was gauged by comparing measured Seronorm concentrations with its 'acceptable ranges' and through comparisons with previously published concentrations (Table 1).

Results and discussion

Dilution method

In all cases, ion yield for the internal standards was greater using Mix 2 as the diluent (Fig. 1), probably due to signal suppression from the butanol, so Mix 1 was excluded from further investigation. Signal suppression of Be, Rh and Re was also greatest in the most concentrated samples, but was negligible at 100 to 250-fold dilution (Fig. 1). However at 250-fold dilution, Mn, Ba and Pb concentrations were close to or <LOD. As such, 150 was chosen as the DF for future analyses. The relatively high DF resulted in almost no cases of nebuliser blockage, even during long runs of >100 analyses. It also helps to circumvent issues relating to low sample volume, which is crucial in serial sampling experiments such as this to improve animal welfare and ensure blood samples are representative of a healthy population.

Table 3
Isotopes, resolutions and operating conditions for HR-ICP-MS.

RF power (W)	1200		
Sample uptake rate (ml/min)	0.15, pumped		
Ar gas flow rates (L/min)			
Coolant	15.0	Nebuliser	0.7 (via nebuliser)
Auxiliary	0.95	Add gas	0.3 (ported into spray chamber)
Nebuliser	ESI PFA ST		
Spray chamber	ESI PFA cyclonic, peltier cooled		
Resolution	LRM	^7Li, ^9Be[a], ^{85}Rb, ^{88}Sr, ^{103}Rh[a], ^{137}Ba, ^{185}Re[a], ^{208}Pb	
	MRM	^9Be[a], ^{24}Mg, ^{44}Ca, ^{55}Mn, ^{63}Cu, ^{66}Zn, ^{103}Rh[a]	
	HRM	^{39}K, ^{77}Se, ^{78}Se, ^{103}Rh[a]	
Acquisition mode	E-scan, Mode 1, 12 scans per resolution		
Mass window (%)[b]	50 (LRM), 125 (MRM and HRM)		
Search window (%)[b]	25 (LRM), 60 (MRM and HRM)		
Integration window (%)[b]	20 (LRM), 60 (MRM and HRM)		
No. of samples per peak	50 (LRM), 20 (MRM and HRM)		

[a] Internal standards.
[b] Percent of peak width.

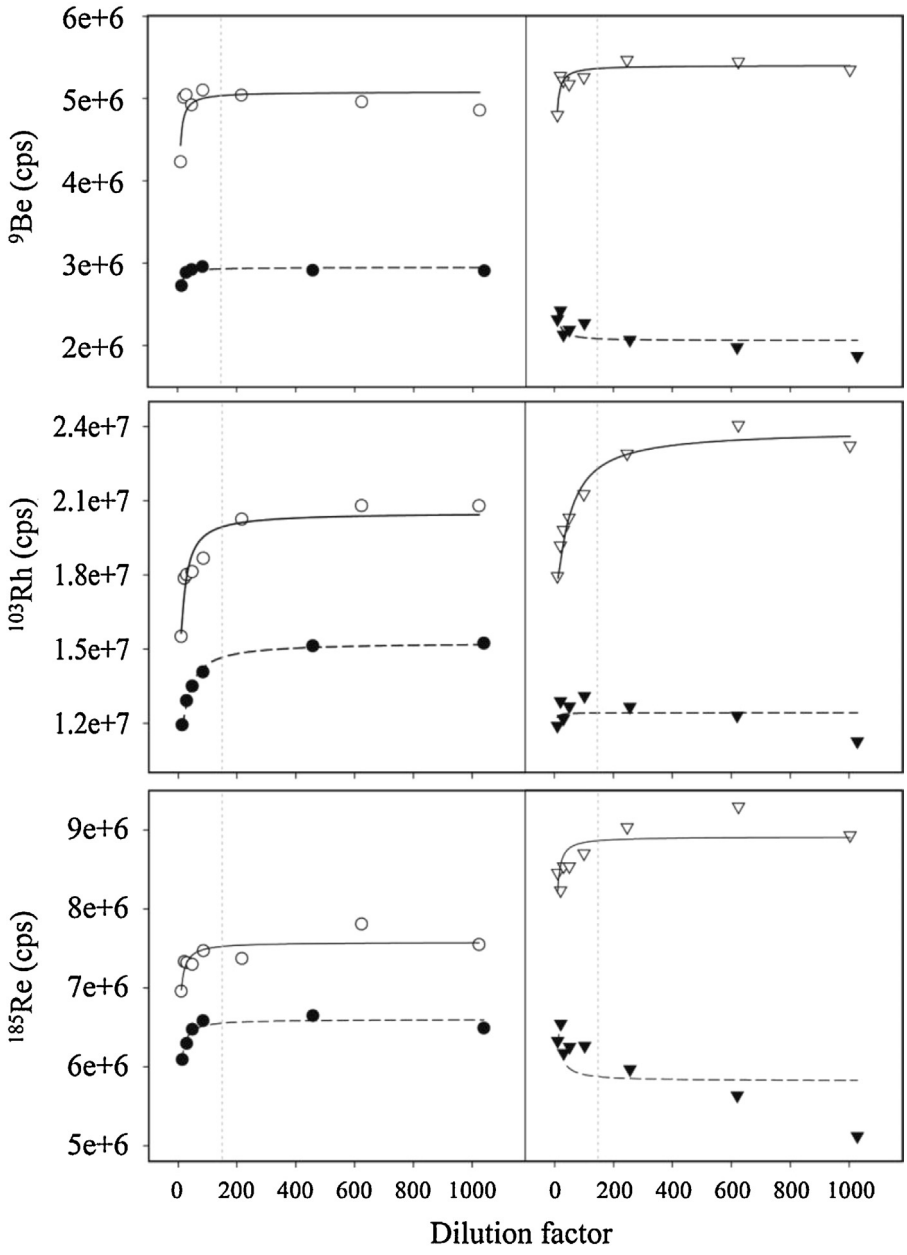

Fig. 1. Internal standard counts (Be, Rh, and Re) in fish plasma (circles) and Seronorm (triangles) diluted with Mix 1 (solid symbols, dashed line) and Mix 2 (open symbols, solid line). The vertical grey lines show the dilution factor chosen for subsequent analyses. Unexpected patterns were observed for the 'Seronorm + Mix 1' combination, but these analyses occurred at the start of the sequence and coincided with a shift in instrument performance, so should be treated with caution. Remaining samples were drift-corrected using subsequent, unaffected blanks.

Blanks

Blank subtractions were carried out using laboratory blanks due to inconsistent patterns in Ba. Raw counts did not differ between procedural and laboratory blanks for any other elements ($p > 0.05$, $n = 36$), but procedural blanks contained significantly higher Ba ($F_{1,34} = 73.2$, $p < 0.001$), and concentrations almost an order of magnitude higher than associated plasma samples (averages of 0.5 and 0.07 ng/g, respectively). Given identical treatment of plaice blood and procedural blank MQ, this appears to indicate a Ba source in the heparin, syringe or needle that was not transferred to (or was highly suppressed in) the plasma, possibly remaining in the pellet following centrifugation. Whatever the reason for the discrepancy, the final Ba concentrations should be taken with some caution.

Precision

With the exception of Pb and Ba, internal precision was consistently better than 3% and similar for estimates of external precision based on single tubes repeatedly analysed within a run (Table 4). Error relating to sample preparation resulted in higher %RSD for within-run replicates of multiple sample preparations, but precision was still generally around 3%, except for Ba (6.6%) and Pb (13.6%). The most conservative measure of external precision (inter-assay replicates of multiple preparations, encompassing preparation, calibration and instrument error) was poor for Pb measurements (23%), but ≥10% for all other elements. The elements closest to detection limits, Ba and Pb, consistently exhibited the lowest precision, although the former appeared primarily hindered by handling error and the latter by calibration issues.

Table 4
Detection limits (LOD, 3SD of repeat blanks, adjusted by 150× dilution factor) and precision estimates (%RSD) for plaice plasma and Seronorm analyses. Sample numbers are given in parentheses. Internal precision (instrument error) is represented by the mean within-analysis %RSD for all plasma samples. External precision is represented by the mean %RSD for repeat analyses of single (S) or multiple (M) preparations of a given sample analysed within or across runs.

Element	LOD (μg/g)	Internal precision	External precision				
			Plaice plasma			Seronorm	
			Within		Across	Within	Across
	(27)	(380)	S (8)	M (25)	M (12)	M (3)	M (6)
Li	0.00343	1.8	2.7	3	9.9	0.9	2.5
Mg	0.0503	0.9	1.4	2.7	5.2	0.8	1.9
K	0.311	1	1.1	2	5.4	1.2	2.2
Ca	1.66	0.9	2.1	3.6	5.1	0.7	2.8
Mn	0.00146	1.3	1	3.2	5.1	2	3.5
Cu	0.0111	1	0.7	3	4.5	0.2	1.3
Zn	0.0827	0.8	0.9	3.1	6	0.1	1.5
Se	0.149	2.6	2.7	3.2	8.1	5.4	5.9
Rb	0.00011	2.1	2.2	3.5	5.6	1	3.6
Sr	0.00287	1.5	0.5	2.8	4.1	4.7	2.3
Ba	0.00374	3.1	2.3	6.6	10	1	1.2
Pb	0.00030	5.5	5.1	13.6	23.3	5.2	21.2

On the whole, precision was superior for Seronorm analyses (generally <5%). This can partly be explained by differences in fluid composition. Concentrations of Li, K and Ba were higher in Seronorm, sometimes by orders of magnitude (Table 5), which resulted in greater measurement reliability. That said, Mn, Zn, Se and Sr were higher in fish plasma, but measurements were rarely accompanied by higher precision. Overall lower precision for plasma analyses was most likely related to sample matrix and treatment. Seronorm is a serum and thus contains no clotting factors; while visibly clotted and heterogeneous plasma samples were rejected, any protein precipitation could affect matrix viscosity and behaviour. Also, once prepared, Seronorm aliquots were stored <1 month prior to analysis, while plasma samples were stored for up to 24 months. Inter-assay sample degradation may have reduced precision to a certain extent, however for repeat plasma preparations made >3 months apart, precision was still generally better than 10%, implying that 'storage effects' were relatively minor.

Accuracy

Owing to the lack of commercially available fish plasma reference material, external accuracy was assessed using Seronorm, a human serum. While differences in elemental concentrations were observed, particularly for Li and Sr (Table 5), internal standard behaviour was similar for both blood types (Fig. 1). If one assumes broadly similar behaviour among blood types, accuracy was deemed satisfactory, as measured Seronorm concentrations were generally within certified ranges. Se was the only exception, for which four of the eight measurements were slightly out of range, producing an average concentration 0.2 ng/g higher than the maximum accepted value. Ba was also at the upper end of the range, although individual measurements were all within certified limits.

Agreement between reference ranges and previously published concentrations (Tables 1 and 5) provides some additional corroboration for the methods and values presented herein. Generally, where available, measured concentrations were within the ranges reported in the literature (e.g. Li, Mg, Ca, Cu), particularly when comparing analogous blood fractions. However, some elements exhibited considerable heterogeneity among studies, so inconsistencies and patterns are discussed below. Note again that whole blood is referred to simply as 'blood'.

Potassium

All studies reporting concentrations of K above 800 μg/g (Table 1) used blood as the analysis medium. Concentrations in the current study were in keeping with those determined in serum and plasma of other species, albeit at the lower end of the spectrum. It has been suggested that the release of K^+ from cells and platelets during blood coagulation results in unnaturally elevated concentrations in serum [93]. On the other hand, in a study examining optimal treatment of salmon blood, plasma K was significantly

Table 5
Summary of elemental concentrations measured in male and female plaice plasma and Seronorm CRM. All concentrations in ppm and reference ranges indicated by 5th–95th percentiles.

Element	All plasma analyses (n = 157[a])			Plaice plasma				Seronorm Batch No. 0608414 (n = 8)	
				Males (n = 85)		Females (n = 72)			
	Median	%RSD	% above LOD	Median	Reference range	Median	Reference range	Mean	'Certified acceptable range'
Li	0.0653	10.9	100	0.0632	0.0509–0.0740	0.0665	0.0549–0.0770	5.36	4.780–5.620
Mg	26.61	26.2	100	26.80	19.38–40.68	26.50	21.28–45.30	18.48	17.10–19.50
K	42.60	62.1	100	44.80	13.97–110.0	40.94	12.87–101.6	116.9	100.0–124.0
Ca	126.8	16.2	100	123.9	107.8–148.6	136.7	112.3–190.4	98.24	85.10–100.0
Mn	0.021	49.6	100	0.0166	0.0089–0.0413	0.0242	0.0112–0.0495	0.0095	0.0071–0.0107
Cu	0.736	24.8	100	0.7664	0.4887–1.093	0.7124	0.4522–1.023	1.116	1.010–1.330
Zn	12.50	21.2	100	13.10	9.094–16.56	11.54	7.109–16.89	1.320	1.100–1.340
Se	0.368	22.9	100	0.3747	0.2344–0.5158	0.3612	0.2317–0.4753	0.0650	0.0536–0.0648
Rb	0.0064	64.0	100	0.0070	0.0019–0.0155	0.0063	0.0021–0.0151	0.0030	0.0027–0.0039
Sr	1.030	16.0	100	0.9981	0.7643–1.284	1.078	0.8584–1.398	0.0261	0.0228–0.0280
Ba	0.0081	69.8	90	0.0082	0.0029–0.0245	0.0073	0.0029–0.0258	0.1549	0.0920–0.1560
Pb	0.00033	87.3	76	0.00027	0.0001–0.0007	0.00044	0.0001–0.0015	0.0026	0.0025–0.0033

[a] 157 blood samples from 18 fish (8F, 10M), taken monthly for one year, excluding the spawning period (January–March).

reduced in samples stored for extended periods (8.5 h) prior to centrifugation, possibly due to movement of K^+ into erythrocytes [97]. While this may help to explain our lower K concentrations, the pre-centrifugation periods in this study were less than half those used in the latter experiment, and our median concentration was almost identical to the value reported for a closely related flatfish [75] (42.6 cf. 43 μg/g), improving confidence in the reported reference range.

Manganese

Measured Mn concentrations in the current study were approximately five times lower than those reported for blood from four freshwater species (Table 1). This implies an effect of blood fraction and an association between Mn and erythrocytes, particularly as the same pattern has also been observed among analogous human blood fractions [13]. However, there is considerable variation in Mn concentrations reported by a very limited number of studies (Table 1), making it difficult to draw useful conclusions from these comparisons.

Zinc

Zn concentrations overlapped values reported for both marine and freshwater species, but were on average, approximately four times lower than those measured in blood (Table 1). Similarly, Zn concentrations were about six times lower in human serum than in blood, implying some erythrocyte binding [98]. However, given overlap in concentrations among the three blood fractions reported in Table 1, some of the more elevated concentrations [e.g. 31] are more likely to be the result of sample contamination [1], physiological status [67] and/or environmental exposure, with waterborne Zn tending to follow a nutrient type distribution and contained in many pollutants [99].

Selenium

Se concentrations were similar to those reported for two freshwater and two marlin species, but an order of magnitude lower than levels in tuna blood [84]. The discrepancy could be related to tuna-specific elemental processing as a result of its unique metabolism [100].

Rubidium

Rb concentrations in the blood of two freshwater species and one marine species were approximately 400 and 70 times higher than those measured in the current study, respectively (Table 1). Some of this variation could be due to differences among blood fractions, with human blood exhibiting concentrations approximately 12 times higher than equivalent serum samples [98]. However, given the magnitude of the differences and the fact that Rb is generally conserved with salinity [99], precluding an obvious 'environmental' explanation, the differences are more likely due to sample treatment.

Strontium

Measured Sr concentrations were almost identical to those previously reported in the plasma of another marine species [20], but almost an order of magnitude higher than those reported for blood from three freshwater species [48,60]. Sr is a conservative element [99] and concentrations are similarly elevated in the biomineralised tissues of marine *vs.* freshwater species [15], implying an effect of environmental availability rather than blood fraction, sample treatment or analysis method.

Barium

Even with the concerns for Ba contamination in the present study, measured concentrations were approximately 30 times lower than those reported in the blood of two freshwater fish species [60]. The discrepancy is unlikely due to fluid type as human plasma Ba levels were, if anything, higher than equivalent blood samples [13]. The differences are more likely the result of sample treatment or ambient concentrations, with Ba generally more abundant and bioavailable in freshwater systems [99,101].

Lead

Pb concentrations determined in the present study were approximately 500 times lower than those reported for blood samples from a number of freshwater species. This may not simply be due to sample contamination, although Pb contamination is notoriously difficult to avoid [1]. In humans, Pb has been found to be 56 and 420 times higher in blood than serum [98] and plasma [13], respectively. Such results imply that the majority of blood Pb exists bound to erythrocytes and other components, such as platelets.

Reference ranges

An investigation into the intrinsic and extrinsic influences on blood composition of plaice is beyond the scope of the current paper. Reproductive events in teleost fish are coupled with major changes in blood chemistry [20], so samples collected during the spawning period were excluded from the reference ranges. Even with this step in place, there was considerable variation in measured concentrations, as indicated by the high %RSD values across all blood samples (Table 5). Plaice are sexually dimorphic; males and females exhibiting significant differences in life history traits such as reproductive investment and growth rate [102]. These traits will almost certainly affect blood composition, so reference ranges were calculated for males and females separately. Indeed, the results indicate that plasma Ca, Mn, Sr and Pb concentrations tended to be higher in the females, while Cu, Zn and Se concentrations tended to be higher in the males. While these differences were not formally investigated, their directionality tended to corroborate trends reported in the literature. For example, the relationship between blood hypercalcaemia and reproductive investment in teleosts is well recognised, with females from oviparous species mobilising large quantities of Ca^{2+} for egg yolk production [103]. While Sr is thought to be a largely 'nonessential' element [104], blood concentrations were positively correlated with blood Ca concentrations and gonadosomatic index of *Pseudophycis barbata* [20] and *Oncorhynchus mykiss* [48] females, suggesting potential for a functional requirement during ovary development. The utilisation of blood Cu and Zn for oocyte production appears to be broadly conserved among vertebrates [1,67,75,105–107], while the importance of Se for reproduction has been reported for humans but not fish [1,107]. Thus, while samples collected during the active spawning period were excluded from the dataset, some effects of gonad development are still likely present within the results, so care should be taken if directly comparing them with samples from immature fish.

Conclusions

This study shows that a suite of trace, minor and major elements can be simultaneously quantified in small volumes of fish plasma using a simple dilution method. To date, most studies examining fish blood elemental chemistry have focused on major elements, freshwater species and/or whole blood. To our knowledge, no previous study has measured Mn, Ba or Pb in any blood fraction of a

marine fish species nor determined Rb or Pb in serum or plasma fractions of any fish species, and none have previously analysed fish plasma or serum by ICP-MS or HR-ICP-MS. Also, no previous study has investigated analysis methods for this unique biological fluid, characterised by elevated protein and salt concentrations. For serial sampling experiments such as this, it is crucial to minimise sample volumes to reduce stress in the subject animals. The lower yield obtained using serum over plasma [108], and the potential for reworking of blood components during coagulation [93], make plasma the preferred biofluid for such experiments. By heparinising syringes using a standardised, low volume procedure and a high purity preparation, we found that procedural blanks were not significantly higher than laboratory blanks in any of the measured elements except Ba. Whatever the source of the Ba contamination, it did not appear to transfer significantly into the blood samples, however, the reference ranges for this element should be noted with caution.

While HR-ICP-MS is an excellent tool for multi-element analyses, combining high sensitivity with a capability to separate analyte peaks from spectral interferences, it is susceptible to salt loading and matrix changes. By carefully tuning the instrument, using the highest possible DF and matrix matching among samples, blanks and standards where possible, such effects were minimised in this study. The dilution method used in the current study produced precise and accurate results for almost all elements in question; however Pb and Ba concentrations were close to detection limits. Studies prioritising these metals should use a slightly lower DF and analyse samples within a single run or across adjacent days to maximise accuracy and precision. Addition of methane to the plasma gas can also improve sensitivity and matrix tolerance (potentially eliminating the need for butanol) [109], however the current method utilises a standard instrument without additional accessories.

As discussed above, otoliths represent a hugely valuable resource in fish ecology and management, but the mechanisms underpinning elemental fractionation from water to blood, blood to endolymph, and endolymph to otolith, are poorly understood, hindering progress within this field [15]. Accurate and precise determination of element concentrations in multiple biological tissues, including plasma, is key to understanding the behaviour of ions in metabolic reactions and their movements across biological membranes.

It is also crucial that reliable blood reference ranges are determined across a variety of species and systems. These ranges can improve clinical diagnosis and provide crucial biomarkers of population health, both of which are increasingly important for the fast-growing aquaculture sector. Currently, few studies have attempted multi-elemental analysis of fish blood, while some of the variation among published concentrations implies contamination issues and/or incompatibilities among biofluids and analytical techniques. Accurate and precise measurements of trace element concentrations in a range of fish species, blood fractions and physiological states will allow a better understanding of both natural and anthropogenic variations. A key objective in the field of fish trace elemental chemistry should be the development of a CRM for fish blood and plasma, and inter-study standardisation of sample treatment and analytical techniques.

Acknowledgements

The research was funded (partially) by Defra contract M1102 MEMFISH (macroecology of marine finfish in UK waters) and an FSBI studentship to AMS. Particular thanks go to CEFAS staff for their help with animal husbandry and sample collection, and to S. Campana for his help with reference sourcing. We are also grateful to the anonymous reviewers for their constructive comments and suggestions.

References

[1] Versieck J, Cornelis R. Trace elements in human plasma or serum. Boca Raton, Florida, U.S.A.: CRC Press Inc.; 1989.
[2] Folmar LC, Moody T, Bonomelli S, Gibson J. Annual cycle of blood chemistry parameters in striped mullet (*Mugil cephalus* L.) and pinfish (*Lagodon rhomboides* L.) from the Gulf of Mexico. J Fish Biol 1992;41(6):999–1011.
[3] Watanabe T, Kiron V, Satoh S. Trace minerals in fish nutrition. Aquaculture 1997;151(1–4):185–207.
[4] Hille S. A literature review of the blood chemistry of rainbow trout, *Salmo gairdneri* Rich. J Fish Biol 1982;20(5):535–69.
[5] Folmar LC. Effects of chemical contaminants on blood chemistry of teleost fish: a bibliography and synopsis of selected effects. Environ Toxicol Chem 1993;12(2):337–75.
[6] Wolf K. Physiological salines for fresh-water teleosts. Prog Fish Cult 1963;25(3):135–40.
[7] Evans DH. The physiology of fishes. Boca Raton, FL: CRC Press, Inc.; 1993.
[8] Byrne JM, Beamish FWH, Saunders RL. Influence of salinity, temperature, and exercise on plasma osmolality and ionic concentration in Atlantic Salmon (*Salmo salar*). J Fish Res Board Can 1972;29(8):1217–20.
[9] Subramanian KS. Determination of metals in biofluids and tissues: sample preparation methods for atomic spectroscopic techniques. Spectrochim Acta B 1996;51(3):291–319.
[10] Bocca B, Alimonti A, Petrucci F, Violante N, Sancesario G, Forte G, et al. Quantification of trace elements by sector field inductively coupled plasma mass spectrometry in urine, serum, blood and cerebrospinal fluid of patients with Parkinson's disease. Spectrochim Acta B 2004;59(4):559–66.
[11] Rodushkin I, Odman F, Olofsson R, Axelsson MD. Determination of 60 elements in whole blood by sector field inductively coupled plasma mass spectrometry. J Anal At Spectrom 2000;15(8):937–44.
[12] Vanhoe H, Dams R. Use of inductively coupled plasma mass spectrometry for the determination of ultra-trace elements in human serum. J Anal At Spectrom 1994;9:23–31.
[13] Goulle J-P, Mahieu L, Castermant J, Neveu N, Bonneau L, Laine G, et al. Metal and metalloid multi-elementary ICP-MS validation in whole blood, plasma, urine and hair. Reference values. Forensic Sci Int 2005;153:39–44.
[14] Thermo. Note 5: trace element quantification in blood and serum in a single analytical run. X Series ICP-MS Clinical Applications. 2009; AN_E0649.
[15] Campana SE. Chemistry and composition of fish otoliths: pathways, mechanisms and applications. Mar Ecol Prog Ser 1999;188:263–97.
[16] Merck. Common Medical Tests. Merck Manuals, www.merckmanuals.com/home/appendixes/common_medical_tests/common_medical_tests.html; 2011 [cited 15.11.11].
[17] Versieck J, Cornelis R. Normal levels of trace elements in human blood plasma or serum. Anal Chim Acta 1980;116:217–54.
[18] Sturrock AM, Trueman CN, Darnaude AM, Hunter E. Can otolith elemental chemistry retrospectively track migrations in fully marine fishes? J Fish Biol 2012;81(2):766–95.
[19] Gillanders BM. Otolith chemistry to determine movements of diadromous and freshwater fish. Aquat Living Resour 2005;18(3):291–300.
[20] Kalish J. Determinants of otolith chemistry: seasonal variation in the composition of blood plasma, endolymph and otoliths of bearded rock cod *Pseudophysis barbatus*. Mar Ecol Prog Ser 1991;74:137–59.
[21] Edmonds J, Caputi N, Morita M. Stock discrimination by trace-element analysis of otoliths of orange roughy (*Hoplostethus atlanticus*), a deep-water marine teleost. Mar Freshw Res 1991;42(4):383–9.
[22] Longmore C, Trueman C, Neat F, O'Gorman E, Milton J, Mariani S. Otolith geochemistry indicates life-long spatial population structuring in a deep-sea fish, *Coryphaenoides rupestris*. Mar Ecol Prog Ser 2011;435:209–24.
[23] Lochet A, Limburg KE, Rudstam L, Montesdeoca M. Selenium incorporation in fish otoliths: effects of selenium and mercury from the water. Can J Fish Aquat Sci 2010;67(9):1388–97.
[24] Hicks AS, Closs GP, Swearer SE. Otolith microchemistry of two amphidromous galaxiids across an experimental salinity gradient: a multi-element approach for tracking diadromous migrations. J Exp Mar Biol Ecol 2010;394(1–2):86–97.
[25] Ranaldi M, Gagnon M. Trace metal incorporation in otoliths of black bream (*Acanthopagrus butcheri* Munro), an indicator of exposure to metal contamination. Water Air Soil Pollut 2008;194(1):31–43.
[26] LeBreton GTO, Beamish FWH. The influence of salinity on ionic concentrations and osmolarity of blood serum in lake sturgeon, *Acipenser fulvescens*. Environ Biol Fishes 1998;52(4):477–82.
[27] Di Marco P, McKenzie DJ, Mandich A, Bronzi P, Cataldi E, Cataudella S. Influence of sampling conditions on blood chemistry values of Adriatic sturgeon Acipenser naccarii (Bonaparte, 1836). J Appl Ichthyol 1999;15(4–5):73–7.
[28] Huang SS-Y, Strathe AB, Wang W-F, Deng D-F, Fadel JG, Hung SSO. Selenocompounds in juvenile white sturgeon: evaluating blood, tissue, and urine selenium concentrations after a single oral dose. Aquat Toxicol 2012;109(0):158–65.
[29] Folmar L, Gardner G, Hickey J, Bonomelli S, Moody T. Serum chemistry and histopathological evaluations of brown bullheads (*Ameiurus nebulosus*) from

the Buffalo and Niagara Rivers, New York. Arch Environ Contam Toxicol 1993;25(3):298–303.
[30] Schmitt CJ, Whyte JJ, Roberts AP, Annis ML, May TW, Tillitt DE. Biomarkers of metals exposure in fish from lead-zinc mining areas of Southeastern Missouri, USA. Ecotoxicol Environ Saf 2007;67(1):31–47.
[31] Brumbaugh WG, Schmitt CJ, May TW. Concentrations of cadmium, lead, and zinc in fish from mining-influenced waters of Northeastern Oklahoma: sampling of blood, carcass, and liver for aquatic biomonitoring. Arch Environ Contam Toxicol 2005;49(1):76–88.
[32] Kirk WL. The effects of hypoxia on certain blood and tissue electrolytes of channel catfish, *Ictalurus punctatus* (Rafinesque). Trans Am Fish Soc 1974;103(3):593–600.
[33] Wiener JG, Jacobson RA, Schmidt PS, Heine PR. Serum calcium concentrations in white sucker, *Catostomus commersoni* Lacepède, and bluegill, *Lepomis macrochirus* Rafinesque, in northern Wisconsin lakes: relation to pH and waterborne calcium. J Fish Biol 1985;27(6):699–709.
[34] Schmitt CJ, Dwyer FJ, Finger SE. Bioavailability of Pb and Zn from mine tailings as indicated by erythrocyte Î'-aminolevulinic acid dehydratase (ALA-D) activity in suckers (Pisces: Catostomidae). Can J Fish Aquat Sci 1984;41(7):1030–40.
[35] Schmitt C, Brumbaugh W, May T. Concentrations of cadmium, cobalt, lead, nickel, and zinc in blood and fillets of northern hog sucker (*Hypentelium nigricans*) from streams contaminated by lead–zinc mining: implications for monitoring. Arch Environ Contam Toxicol 2009;56(3):509–24.
[36] Ayik O, Hisar O, Gulcin I, Aras-Hisar S, Yanik T. Effects of light on trace elements and triiodothyronine levels in plasma of mirror carp (*Cyprinus carpio*). Biol Trace Elem Res 2005;108(1–3):147–53.
[37] Field JB, Elvehjem CA, Juday C. A study of the blood constituents of carp and trout. J Biol Chem 1943;148(2):261–9.
[38] Houston AH, Smeda JS. Thermoacclimatory changes in the ionic microenvironment of haemoglobin in the stenothermal rainbow trout (*Salmo Gairdneri*) and eurythermal carp (*Cyprinus Carpio*). J Exp Biol 1979;80(1):317–40.
[39] Kakuta I, Ishii K, Murachi S. Effects of diluted sewage on biochemical parameters of carp, *Cyprinus carpio*. Comp Biochem Physiol C 1994;107(2):289–94.
[40] Kleinow KM, Brooks AS. Selenium compounds in the fathead minnow (Pimephales promelas)—I. Uptake, distribution, and elimination of orally administered selenate, selenite and l-selenomethionine. Comp Biochem Physiol C 1986;83(1):61–9.
[41] Daglish RW, Nowak BF, Lewis TW. Copper/metal ratios in the gills of rainbow trout (*Oncorhynchus mykiss*) provide evidence of copper exposure under conditions of mixed-metal exposure. Arch Environ Contam Toxicol 2004;47(1):110–6.
[42] Korcock DE, Houston AH, Gray JD. Effects of sampling conditions on selected blood variables of rainbow trout, *Salmo gairdneri* Richardson. J Fish Biol 1988;33(2):319–30.
[43] Kucukbay Z, Yazlak H, Sahin N, Tuzcu M, Nuri Cakmak M, Gurdogan F, et al. Zinc picolinate supplementation decreases oxidative stress in rainbow trout (*Oncorhynchus mykiss*). Aquaculture 2006;257(1/4):465–9.
[44] Miller III WR, Hendricks AC, Cairns Jr J. Normal ranges for diagnostically important hematological and blood chemistry characteristics of rainbow trout (*Salmo gairdneri*). Can J Fish Aquat Sci 1983;40(4):420–5.
[45] Mugiya Y, Takahashi K. Chemical properties of the saccular endolymph in the Rainbow trout, *Salmo gairdneri*. Bull Fac Fish Hokkaido Univ 1985;36:57–63.
[46] Mugiya Y. Calcification in fish and shellfish, Seasonal change in calcium and magnesium concentration of the otolith fluid in some fish with special reference to the zone formation of their otolith. Bull Jap Soc Scient Fish 1966;32:549–57.
[47] Parker DB, McKeown BA, Macdonald JS. The effect of pH and/or calcium-enriched freshwater on gill Ca^{2+}-ATPase activity and osmotic water inflow in rainbow trout (*Salmo gairdneri*). Comp Biochem Physiol A 1985;81(1):149–56.
[48] Shearer KD. Changes in elemental composition of hatchery-reared rainbow trout, *Salmo gairdneri*, associated with growth and reproduction. Can J Fish Aquat Sci 1984;41(11):1592–600.
[49] Stormer J, Jensen FB, Rankin JC. Uptake of nitrite, nitrate, and bromide in rainbow trout (*Oncorhynchus mykiss*): effects on ionic balance. Can J Fish Aquat Sci 1996;53(9):1943–50.
[50] Walker RL, Fromm PO. Metabolism of iron by normal and iron deficient rainbow trout. Comp Biochem Physiol A 1976;55(4):311–8.
[51] Wedemeyer G, Chatterton K. Some blood chemistry values for the rainbow trout (*Salmo gairdneri*). J Fish Res Board Can 1970;27(6):1162–4.
[52] Zeitoun IH, Hughes LD, Ullrey DE. Effect of shock exposures of chlorine on the plasma electrolyte concentrations of adult rainbow trout (*Salmo gairdneri*). J Fish Res Board Can 1977;34(7):1034–9.
[53] Fletcher GL, Watts EG, King MJ. Copper, zinc, and total protein levels in the plasma of sockeye salmon (*Oncorhynchus nerka*) during their spawning migration. J Fish Res Board Can 1975;32(1):78–82.
[54] Congleton JL, LaVoie WJ. Comparison of blood chemistry values for samples collected from juvenile chinook salmon by three methods. J Aquat Anim Health 2001;13(2):168–72.
[55] Lorentzen M. Trace element status of juvenile Atlantic salmon *Salmo salar* L. fed a fish-meal based diet with or without supplementation of zinc, iron, manganese and copper from first feeding. Aquac Nutr 1999;5(3):163–71.
[56] Maage A, Julshamn K, Berge GE. Zinc gluconate and zinc sulphate as dietary zinc sources for Atlantic salmon. Aquac Nutr 2001;7(3):183–7.
[57] Laitinen M, Valtonen T. Cardiovascular, ventilatory and haematological responses of brown trout (*Salmo trutta* L.), to the combined effects of acidity and aluminium in humic water at winter temperatures. Aquat Toxicol 1995;31(2):99–112.
[58] Phillips AM, Brockway DR. The inorganic composition of brown trout blood. Prog Fish Cult 1958;20(2):58–61.
[59] Edsall CC. A blood chemistry profile for Lake Trout. J Aquat Anim Health 1999;11:81–6.
[60] Melancon S, Fryer BJ, Markham JL. Chemical analysis of endolymph and the growing otolith: fractionation of metals in freshwater fish species. Environ Toxicol Chem 2009;28(6):1279–87.
[61] Palacios L, Rubio M, Planas J. Plasma chemical composition in the goosefish (*Lophius piscatorius*, L). J Fish Biol 1972;4(1):99–102.
[62] Natochin YV, Shakhmatova EI. Lithium transport in the chicken and marine teleost kidney. Comp Biochem Physiol A 1996;115(1):73–9.
[63] Woodhead PMJ. Seasonal changes in the calcium content of the blood of arctic cod. J Mar Biol Assoc UK 1968;48(01):81–91.
[64] Graham MS, Haedrich RL, Fletcher GL. Hematology of three deep-sea fishes: a reflection of low metabolic rates. Comp Biochem Physiol A 1985;80(1):79–84.
[65] Nordlie FG, Whittier J. Influence of ambient salinity on plasma Ca^{2+} and Mg^{2+} levels in juvenile *Mugil cephalus* L. Comp Biochem Physiol A 1983;76(2):335–8.
[66] Peterson GL, Shehadeh ZH. Changes in blood components of the mullet, *Mugil cephalus* L., following treatment with salmon gonadotropin and methyltestosterone. Comp Biochem Physiol B 1971;38(2):451–7.
[67] Thompson ED, Mayer GD, Walsh PJ, Hogstrand C. Sexual maturation and reproductive zinc physiology in the female squirrelfish. J Exp Biol 2002;205(21):3367–76.
[68] Larsson Å, Bengtsson BE, Haux C. Disturbed ion balance in flounder, *Platichthys flesus* L. exposed to sublethal levels of cadmium. Aquat Toxicol 1981;1(1):19–35.
[69] Larsson Å, Lehtinen K-J, Haux C. Biochemical and hematological effects of a titanium dioxide industrial effluent on fish. Bull Environ Contam Toxicol 1980;25(1):427–35.
[70] Johnson LL, Casillas E. The use of plasma parameters to predict ovarian maturation stage in English sole *Parophrys vetulus* Girard. J Exp Mar Biol Ecol 1991;151(2):257–70.
[71] Cobb JLS, Fox NC, Santer RM. A specific ringer solution for the plaice (*Pleuronectes platessa* L.). J Fish Biol 1973;5(5):587–91.
[72] Harvey BR. Spark source mass spectrometric procedure employing stable isotopes to study the uptake of copper by fish from seawater and food. Anal Chem 1978;50(13):1866–70.
[73] Syed MA, Coombs TL. Copper metabolism in the plaice, *Pleuronectes platessa* (L.). J Exp Mar Biol Ecol 1982;63(3):281–96.
[74] Overnell J, McIntosh R, Fletcher TC. The levels of liver metallothionein and zinc in plaice, *Pleuronectes platessa* L., during the breeding season, and the effect of oestradiol injection. J Fish Biol 1987;30(5):539–46.
[75] Fletcher GL. The effects of capture, "stress," and storage of whole blood on the red blood cells, plasma proteins, glucose, and electrolytes of the winter flounder (*Pseudopleuronectes americanus*). Can J Fish Aquat Sci 1975;53(2):197–206.
[76] Dawson AS. Blood chemistry of the windowpane flounder *Scophthalmus aquosus* in Long Island sound: geographical, seasonal, and experimental variation. Fish Bull (Seattle) 1990;88:429–37.
[77] Bengtsson B-E, Larsson Ãk. Vertebral deformities and physiological effects in fourhorn sculpin (*Myoxocephalus quadricornis*) after long-term exposure to a simulated heavy metal-containing effluent. Aquat Toxicol 1986;9(4–5):215–29.
[78] Fleishman DG, Saulus AA, Vasilieva VF. Lithium in marine elasmobranchs as a natural marker of rectal gland contribution in sodium balance. Comp Biochem Physiol A 1986;84(4):643–8.
[79] Dwyer FJ, Schmitt CJ, Finger SE, Mehrle PM. Biochemical changes in longear sunfish, *Lepomis megalotis*, associated with lead, cadmium and zinc from mine tailings. J Fish Biol 1988;33(2):307–17.
[80] Lohner TW, Reash RJ, Ellen Willet V, Fletcher J. Assessment of tolerant sunfish populations (*Lepomis sp.*) inhabiting selenium-laden coal ash effluents: 3. Serum chemistry and fish health indicators. Ecotoxicol Environ Saf 2001;50(3):225–32.
[81] Bijvelds M, Flik G, Kolar Z, Wendelaar Bonga S. Uptake, distribution and excretion of magnesium in *Oreochromis mossambicus*: dependence on magnesium in diet and water. Fish Physiol Biochem 1996;15(4):287–98.
[82] Subash Peter MC, Lock RAC, Wendelaar Bonga SE. Evidence for an osmoregulatory role of thyroid hormones in the freshwater Mozambique *Tilapia Oreochromis mossambicus*. Gen Comp Endocrinol 2000;120(2):157–67.
[83] Vonck APMA, Bonga SEW, Flik G. Sodium and calcium balance in Mozambique tilapia, *Oreochromis mossambicus*, raised at different salinities. Comp Biochem Physiol A 1998;119(2):441–9.
[84] Kai N, Ueda T, Takeda Y. The state of oxidation and its distribution of selenium in the blood of tuna and marlins. Nippon Suisan Gakkaishi 1992;58(10):1883–6.
[85] Marino G, Di Marco P, Mandich A, Finoia MG, Cataudella S. Changes in serum cortisol, metabolites, osmotic pressure and electrolytes in response to different blood sampling procedures in cultured sea bass (*Dicentrarchus labrax* L.). J Appl Ichthyol 2001;17(3):115–20.
[86] Dawson MA. Effects of long-term mercury exposure on hematology of striped bass, *Morone saxatilis*. Fish Bull (Seattle) 1982;80:389–92.

[87] Valtonen T, Laitinen M. Acid stress in respect to calcium and magnesium concentrations in the plasma of perch during maturation and spawning. Environ Biol Fishes 1988;22(2):147–54.
[88] Hansen N, Folsom TR, Weitz Jr WE. Determination of alkali metals in blood from North Pacific albacore. Comp Biochem Physiol A 1978;60(4):491–5.
[89] Kai N, Ueda T, Takeda Y, Kataoka A. The levels of mercury and selenium in blood of tunas. Nippon Suisan Gakkaishi 1988;54(11):1981–5.
[90] Kai N, Ueda T, Nagatomo K. On mercury and selenium in fish blood, 3: comparison between blood and muscle or several organs in the levels of mercury and selenium in big-eye. J Shimonoseki Univ Fish 1989;38(1):11–6.
[91] Canfield PJ, Quartararo N, Griffin DL, Tsoukalas GN, Cocaro SE. Haematological and biochemical reference values for captive Australian snapper, *Pagrus auratus*. J Fish Biol 1994;44(5):849–56.
[92] Wells RMG, McIntyre RH, Morgan AK, Davie PS. Physiological stress responses in big gamefish after capture: observations on plasma chemistry and blood factors. Comp Biochem Physiol A 1986;84(3):565–71.
[93] Hrubec TC, Smith SA. Differences between plasma and serum samples for the evaluation of blood chemistry values in rainbow trout, channel catfish, hybrid tilapias, and hybrid striped bass. J Aquat Anim Health 1999;11:116–22.
[94] Wolf KEN. Plasmoptysis and gelation of erythrocytes in coagulation of blood of freshwater bony fishes. Blood 1959;14(12):1339–44.
[95] Kremling K. Determination of trace metals. In: Grasshoff K, Kremling K, Ehrhardt M, editors. Methods of seawater analysis. 3rd ed. Weinheim: Wiley-VCH; 1999. p. 253–364.
[96] Thermo. Note 1: Rapid and accurate measurement of As and Cr in urine. X Series ICP-MS Clinical Applications. 2009; AN_0601.
[97] Clark TD, Donaldson MR, Drenner SM, Hinch SG, Patterson DA, Hills J, et al. The efficacy of field techniques for obtaining and storing blood samples from fishes. J Fish Biol 2011;79(5):1322–33.
[98] Barany E, Bergdahl IA, Bratteby L, Lundhd T, Samuelsonc G, Schutz A, et al. Trace element levels in whole blood and serum from Swedish adolescents. Sci Total Environ 2002;286:129–41.
[99] Bruland KW, Lohan MC. 6.02 – controls of trace metals in seawater. In: Elderfield H, editor. Treatise on geochemistry, vol. 6. The oceans and marine geochemistry. Elsevier; 2003. p. 23–47.
[100] Dickson KA. Unique adaptations of the metabolic biochemistry of tunas and billfishes for life in the pelagic environment. Environ Biol Fishes 1995;42(1):65–97.
[101] Elsdon TS, Gillanders BM. Alternative life-history patterns of estuarine fish: barium in otoliths elucidates freshwater residency. Can J Fish Aquat Sci 2005;62(5):1143–52.
[102] Rijnsdorp AD. Maturation of male and female North Sea plaice (*Pleuronectes platessa* L.). J du Conseil International pour l'Exploration de la Mer 1989;46:35–51.
[103] Urist MR, Schjeide AO. The partition of calcium and protein in the blood of oviparous vertebrates during estrus. J Gen Physiol 1961;44(4):743–56.
[104] Underwood EJ. Trace elements in human and animal nutrition. 4th ed. New York: Academic Press; 1977.
[105] Fletcher PE, Fletcher GL. Zinc- and copper-binding proteins in the plasma of winter flounder (*Pseudopleuronectes americanus*). Can J Zool 1980;58(4):609–13.
[106] Tamura T, Goldenberg RL, Johnston KE, DuBard M. Maternal plasma zinc concentrations and pregnancy outcome. Am J Clin Nutr 2000;71(1):109–13.
[107] Bedwal RS, Bahuguna A. Zinc, copper and selenium in reproduction. Cell Mol Life Sci 1994;50(7):626–40.
[108] Uges DRA. Plasma or serum in therapeutic drug monitoring and clinical toxicology. Pharmacy World. Science 1988;10(5):185–8.
[109] Rodushkin I, Nordlund P, Engstrom E, Baxter DC. Improved multi-elemental analyses by inductively coupled plasma-sector field mass spectrometry through methane addition to the plasma. J Anal At Spectrom 2005;20(11):1250–5.