Methods in Ecology and Evolution

British Ecological Society

Methods in Ecology and Evolution 2015, **6,** 806–816 doi: 10.1111/2041-210X.12381

Quantifying physiological influences on otolith microchemistry

Anna M. Sturrock[1, 2]*, Ewan Hunter[3], J. Andrew Milton[1], EIMF[4], Rachel C. Johnson[5], Colin P. Waring[6] and Clive N. Trueman[1]*

[1]*Ocean and Earth Science, National Oceanography Centre Southampton, University of Southampton, Waterfront Campus, European Way, Southampton SO14 3ZH, UK;* [2]*Department of Environmental Science, Policy & Management, University of California, Berkeley, 130 Mulford Hall #3114, Berkeley, CA 94720, USA;* [3]*Centre for Environment, Fisheries and Aquaculture Science, Pakefield Road, Lowestoft, Suffolk NR33 0HT, UK;* [4]*Edinburgh Ion Microprobe Facility, School of GeoSciences, The University of Edinburgh, Grant Institute, The King's Buildings, James Hutton Road, Edinburgh EH9 3FE, UK;* [5]*National Marine Fisheries Service, Southwest Fisheries Science Center, 110 Shaffer Road, Santa Cruz, CA 95060, USA;* [6]*Institute of Marine Sciences, University of Portsmouth, Ferry Road, Eastney, Hampshire PO4 9LY, UK*

Summary

1. Trace element concentrations in fish earstones ('otoliths') are widely used to discriminate spatially discrete populations or individuals of marine fish, based on a commonly held assumption that physiological influences on otolith composition are minor, and thus variations in otolith elemental chemistry primarily reflect changes in ambient water chemistry.

2. We carried out a long-term (1-year) experiment, serially sampling seawater, blood plasma and otoliths of mature and immature European plaice (*Pleuronectes platessa* L.) to test relationships between otolith chemistry and environmental and physiological variables.

3. Seasonal variations in otolith elemental composition did not track seawater concentrations, but instead reflected physiological controls on metal transport and biokinetics, which are likely moderated by ambient temperature. The influence of physiological factors on otolith composition was particularly evident in Sr/Ca ratios, the most widely used elemental marker in applied otolith microchemistry studies. Reproduction also triggered specific variations in otolith and blood plasma metal chemistry, especially Zn/Ca ratios in female fish, which could potentially serve as retrospective spawning indicators.

4. The influence of physiology on the trace metal composition of otoliths may explain the success of microchemical stock discrimination in relatively homogenous marine environments, but could complicate alternative uses for trace element compositions in biominerals of higher organisms.

Key-words: biochemistry, fisheries management, migration, oxygen isotopes, population structure, reproductive cycle, trace metal, vital effect

Introduction

Biominerals are formed by many taxa and have received attention across a variety of disciplines, including materials science, chemistry, palaeontology and ecology. Biomineral microchemistry is widely applied to reconstruct environmental histories (McCulloch *et al.* 2003; Corrège 2006), diet (Schutkowski *et al.* 1999), pollution exposure (Kierdorf & Kierdorf 2002), population structure (Edmonds *et al.* 1995), individual movements and habitat use (Secor *et al.* 2001). These applications broadly assume predictable elemental incorporation by the mineral relative to the ambient environment – that is, that physiological influences on metal fractionation are constant. Physiological variables such as metabolic rate, growth and reproduction can, however, complicate this relationship (de Villiers, Nelson & Chivas 1995). Determining the extent to which the trace element composition of biominerals reflects environmental or physiological factors ('vital effects') is an ongoing challenge. Our current understanding of biomineral microchemistry has mainly been acquired from organisms such as foraminifera, coccolithophores and corals (Cusack & Freer 2008). In these lower organisms, the relationship between biomineral and ambient trace element concentrations can often be accurately predicted from geochemical functions, implying minor or consistent physiological influences on metal fractionation. In more complex organisms however, physiological processes can significantly influence ion transport, binding and availability for incorporation into calcifying structures.

Otoliths (earstones produced by teleost fish) are the most intensively studied carbonate biomineral produced by higher

*Correspondence authors. E-mails: a.sturrock@berkeley.edu; trueman@noc.soton.ac.uk

organisms. Fish exposed to contrasting physicochemical conditions often develop distinct otolith trace element compositions (e.g. Edmonds *et al*. 1995; Thorrold *et al*. 1998; Gillanders & Kingsford 2003; Campana 2005; Walther, Thorrold & Olney 2008). As a comparative biochemical marker, otolith microchemistry thus represents a powerful natural tag of population structure and connectivity, complimentary to inferences based on molecular data (Campana 1999; Miller *et al*. 2005; Sturrock *et al*. 2012). However, elemental pathways from ambient water to the otolith involve complex ion–protein interactions and multiple physiological barriers (Campana 1999). Ultimately, the relative uptake and excretion rates between the environment and different body pools control elemental concentrations in biological tissues (Luoma & Rainbow 2005). Therefore, processes influencing metal transport (e.g. protein binding) have the potential to influence otolith composition (Kalish 1991; Brown & Severin 2009) and compromise ecological inferences if physiological differences between individuals (e.g. growth rate and reproductive state) induce chemical differences in the otolith. Conversely, in relatively homogeneous environments, chemical differences among groups could be augmented by stock-specific physiological influences on element fractionation (Clarke, Thorrold & Conover 2011).

Validation studies on fully marine species or adult fish are scarce. Most experimental work has focused on establishing distribution coefficients between water and otolith mineral across extreme salinity gradients, producing complex and conflicting results (Elsdon & Gillanders 2002; Miller 2011). It is thus currently unclear whether otolith elemental composition directly tracks the ambient environment (i.e. geochemical control with constant physiological influences) or reflects physiological processes that may, or may not, coincide with environmental change (i.e. biokinetic control). We predict biokinetic influences on element distribution coefficients to be stronger when physiological differences are pronounced (e.g. between life-history stages, or males vs. females during reproduction), and more problematic when variations in ambient concentrations are small (e.g. in open marine waters). Understanding the relative importance of geochemical and physiological controls on otolith composition would establish the correct framework for modelling element uptake and transport pathways, and improve the design and interpretation of applied biomineral microchemistry studies.

Here, we describe a long-term, mensurative (*sensu* Hurlbert 1984) experiment to identify the main controls on otolith microchemistry in European plaice (*Pleuronectes platessa* L.). For the first time, element concentrations were serially tracked in seawater, blood plasma and otoliths across a full reproductive cycle using fish from multiple life stages and stocks. We selected eight of the most commonly used elements in otolith microchemistry applications (Li, Mg, K, Mn, Cu, Zn, Sr and Ba) that were expected to be under varying levels of physiological control (Sturrock *et al*. 2012). We tested two null hypotheses: (i) water and otolith elemental concentrations are positively correlated; (ii) environmental variables explain most of the variation in otolith microchemistry. Rejection of either hypothesis implies significant physiological influence on otolith element composition, which would have implications for using otolith microchemistry to record individual migrations or environmental histories.

Materials and methods

Male and female plaice were maintained in near-natural conditions for 7–12 months and physiological [total length, weight, Fulton's condition factor, growth rate, female gonadosomatic index (GSI), blood plasma protein and elemental concentrations] and environmental (salinity, temperature, seawater elemental concentrations) measurements were collected at least monthly. After the spawning season, otolith measurements were quantified retrospectively. Throughout the text, concentrations and element/calcium ratios are denoted as El_X and El/Ca_X for concentrations of element ('El') in medium 'X', where 'X' is either seawater (W), blood plasma (B) or otolith (O). Similarly, distribution coefficients are denoted $D_{El(X1/X2)}$ and $D_{El/Ca(X1/X2)}$, respectively, and are referenced in the Supporting Information.

The experimental design and methods used to collect physiological and environmental measurements are described in Sturrock *et al*. (2013, 2014). In brief, most subject animals comprised mature adults from the Irish Sea (IS). Blood was sampled approximately monthly for 361 days (June 2 2009 to May 28 2010). Additional females (largely immature) were sourced from the English Channel (EC) and introduced to the same tank and sampling regime in October 2009. All fish were injected with oxytetracycline (OTC) on their first sampling day to visibly mark the otolith. In January 2010, half the fish were treated with gonadotropin-releasing hormone (GnRH) to encourage spawning. While stress can modify fish tissue chemistry (Kalish 1992; Waring, Stagg & Poxton 1996), physiological parameters of the subject animals (growth, condition, GSI, El_B) were broadly similar to those reported for wild fish (Sturrock *et al*. 2013, 2014). On experiment completion, sagittal otoliths were removed and sectioned for chemical analysis (see Supporting Information). Otolith oxygen isotope ratios ($\delta^{18}O$) and elemental concentrations (^{7}Li, ^{26}Mg, ^{41}K, ^{48}Ca, ^{88}Sr, ^{138}Ba) were measured at ~20 μm (Cameca-1270) and 10 μm (Cameca-4f) resolution, respectively, by secondary ion mass spectrometry (SIMS). Six isotopes (^{44}Ca, ^{55}Mn, ^{63}Cu, ^{66}Zn, ^{86}Sr, ^{137}Ba) were also quantified at ~20 μm resolution (ELEMENT XR) using laser-ablation high-resolution-inductively coupled plasma mass spectrometry (LA-HR-ICPMS; Fig. 1). Six individuals in the companion study (n = 25, Sturrock *et al*. 2014) exhibited limited otolith growth so were excluded from the current study (n = 19). This did not modify overall physiological trends (Table S2).

To match monthly physiological and environmental observations with otolith analyses, high-resolution $\delta^{18}O_{otolith}$ measurements were used to retrospectively assign calendar dates to individual otolith ablations. This allowed for decoupling of somatic and otolith growth rates, and variability in the timing of otolith increment deposition; both known to occur in mature fish (Van Neer *et al*. 2004; Høie & Folkvord 2006). Ambient salinity and temperature were used to calculate expected $\delta^{18}O_{otolith}$ profiles using the temperature-dependent fractionation equation developed by Hoie, Otterlei & Folkvord (2004). Measured profiles were compared with expected profiles and adjusted using ANALYSERIES 2.0 (Paillard, Labeyrie & Yiou 1996) so that inflection points corresponded with known calendar dates. Following 'temporal correction', the mean correlation coefficient between expected and measured profiles was 0·87 ± 0·08 SD (n = 19, Fig. S1). The correction was essential to match otolith distance with calendar age. For example, in an individual exhibiting elevated growth during the feeding season,

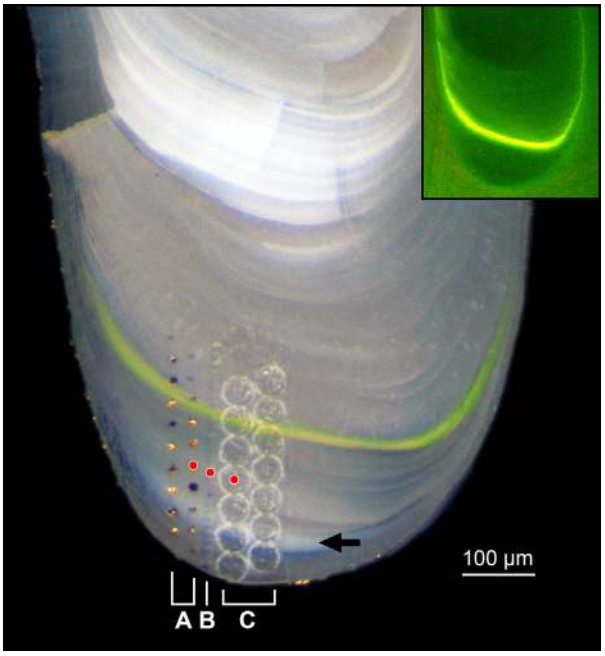

Fig. 1. Frontal otolith section under reflected light demonstrating the analyses used in the current study: (A) offset lines of SIMS $\delta^{18}O$ analyses (Cameca 1270), (B) SIMS elemental analyses (Cameca 4f) and (C) offset lines of HR-LA-ICPMS elemental analyses (ELEMENT XR). The OTC mark (inset image; 450-nm filter) has been superimposed on the main image to indicate the experiment start. The arrow indicates otolith material classified as 'opaque', and the red spots provide an example of temporally matched analyses from each instrument.

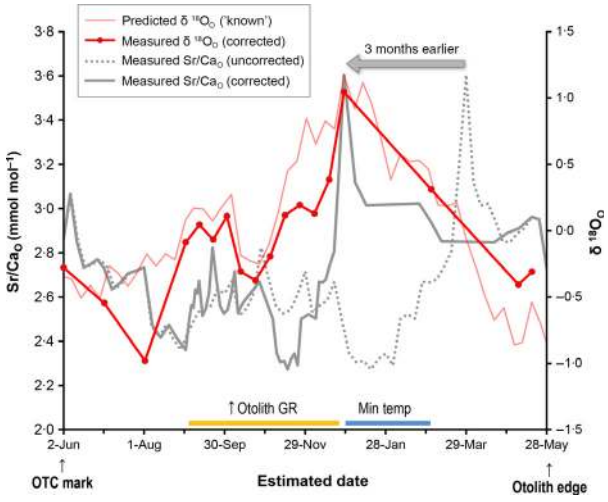

Fig. 2. Predicted otolith $\delta^{18}O$ values ($\delta^{18}O_O$) based on measured temperature and salinity were used to temporally match measured $\delta^{18}O_O$ values and otolith elemental ratios such as Sr/Ca_O. The otolith presented here exhibited elevated growth rates (GR) during the first half of the study (orange bar), prior to spawning and the period of thermal minima (blue bar). Temporal corrections resulted in the maximum Sr/Ca_O value being assigned a calendar date 3 months earlier than would have been estimated had constant growth rates been assumed.

an assumption of constant otolith growth would have resulted in temporal error in excess of 3 months (Fig. 2).

Elemental otolith analyses were assigned calendar dates by referencing them to the centre of date-assigned $\delta^{18}O$ ablations (e.g. Fig. 1) or the OTC mark, resulting in each transect being assigned a start date and multiple temporal anchor points. Between anchor points, ablations were assigned dates using linear interpolation, then 'local polynomial regression fitting' (LOESS) with minimal smoothing allowed prediction of elemental concentrations on specific blood sampling days.

Otolith growth rates were estimated for each blood sampling day as the distance between the flanking pair of Cameca-4f ablations (10 µm) divided by the estimated number of days between them. 'Mean' otolith growth rates (total growth from the OTC mark to the edge/no. days) were also estimated. Otolith opacity was scored visually under reflective light for each Cameca-4f spot as one (opaque) or zero (translucent). Only fully reflective white bands were scored as one (Fig. 1); anything intermediary was scored zero. Blood sampling days were assigned the mean score of the flanking Cameca-4f spots. Scores of 0·5 (representing sampling days during transition periods) were excluded when calculating the proportion of opaque to translucent growth zones.

To explore the main controls on otolith element concentrations (El/Ca_O), linear mixed effects models were built in R (lme4 package); the random effect (fish identity) allowing intercepts to vary by individual. Models were built using all individuals and females only (the latter including GSI). Akaike information criterion corrected for small sample sizes (AICc), r^2 values (Nakagawa & Schielzeth 2013) and 'dredge' (MuMIn package) terms were used for model selection, as detailed in Appendix S3. To further investigate element uptake mechanisms, we also modelled $D_{El(o/b)}$ values for those elements whose behaviour was explained by more complex models. Individual *P*-values were estimated using analysis of deviance (car package), and elements were grouped by behaviour using pairwise correlations adjusted for temporal autocorrelation (Pyper & Peterman 1998). The performance of the final selected models was compared to two environmental 'base' models (temperature + salinity and temperature + El/Ca_W) to measure the additional explanatory power provided by incorporating physiological variables in a model of otolith element chemistry (Appendix S3).

Results

MAIN OBSERVED TRENDS

Environmental variables (temperature, salinity and El_W values) tended to covary seasonally, with the lowest values occurring during winter. Salinity and temperature were correlated (r = 0·66), but lagged by 1–2 months (Sturrock *et al.* 2014). Many physiological measures (condition, GSI, plasma protein concentrations and El_B) also varied on a seasonal basis, but exhibited considerable differences among individuals and groups (Sturrock *et al.* 2014). There was limited seasonality in otolith growth rates or opacity (Figs S2 and S3), but most individuals exhibited faster growth in October and towards the end of the experiment.

(1) Were water and otolith elemental concentrations positively correlated?

Seawater, blood plasma and otolith elemental concentrations and El/Ca ratios (Figs 3 and 4; Sturrock *et al.* 2014) tended to vary seasonally, with the largest changes occurring around the winter spawning season. Conversely, El/Ca_W ratios for elements typically conserved with salinity (Li, K, Mg, Sr) remained near-constant (Fig. 4). Mn (all fish) and Zn (females only) were the only metals to exhibit consistent positive relationships between otolith and seawa-

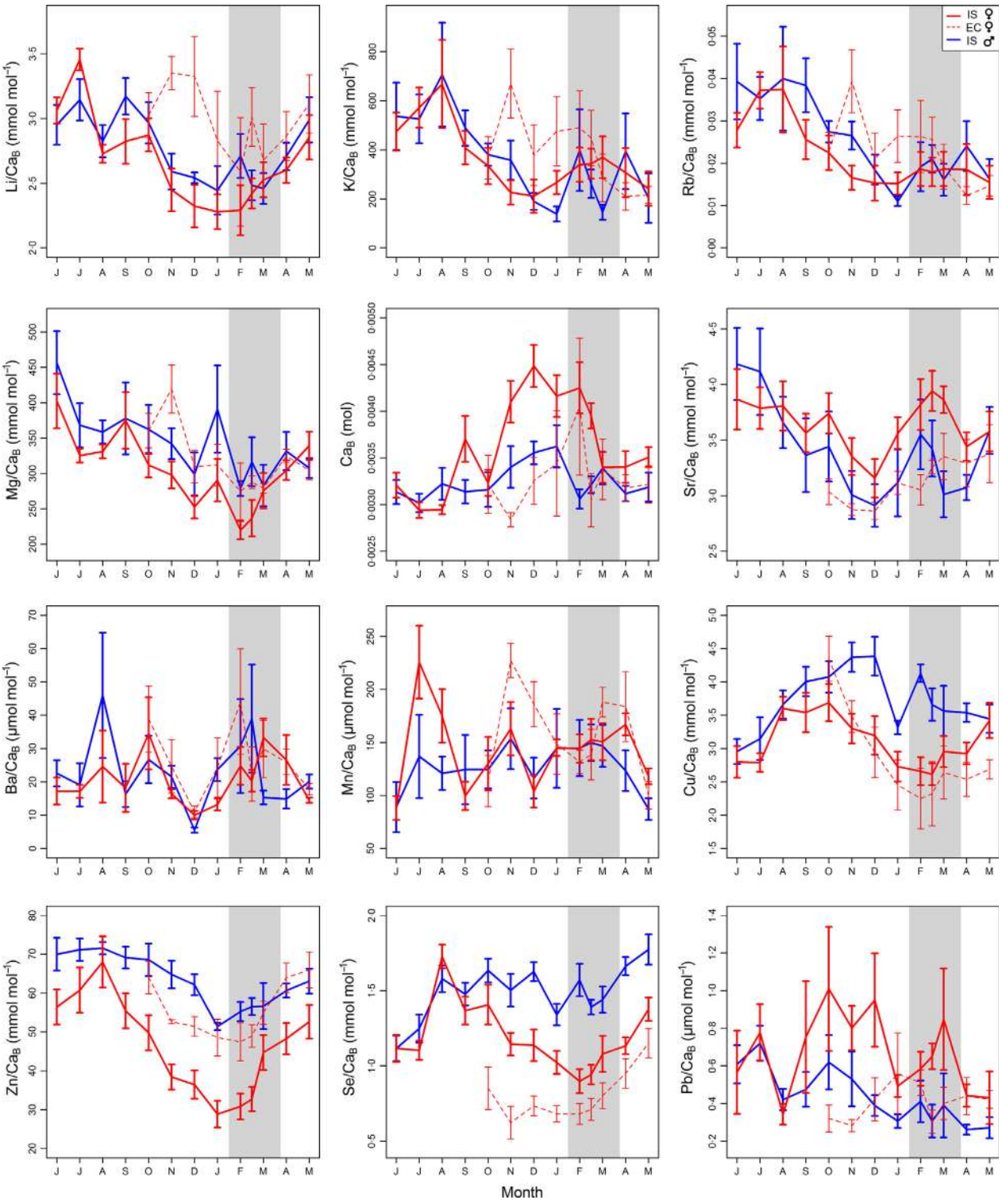

Fig. 3. Average (±SE) blood plasma El/Ca ratios (El/Ca$_B$) in plaice from the Irish Sea (IS) and English Channel (EC) serially sampled for 7–12 months. Note that sampling was carried out twice in February and Ca concentrations are displayed for reference. Note that Rb, Se and Pb were not quantified in the otoliths. Shading = peak spawning period. ♀ = female, ♂ = male.

ter concentrations, while Sr (all fish) and Zn (females only) were the only metals to exhibit consistent positive correlations between otolith and blood plasma concentrations (Table 1).

(2) What were the main controls on otolith element concentrations?

Model fits explained 40% (Zn/Ca$_{O(FEMALE)}$) to 83% (K/Ca$_O$) of the variation in otolith El/Ca ratios, with the fixed

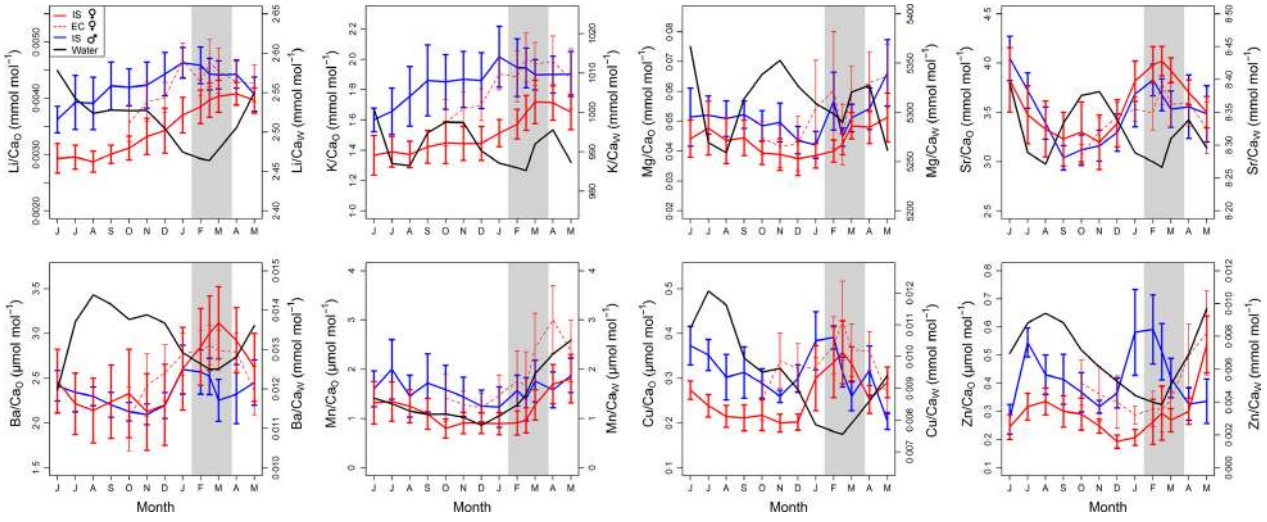

Fig. 4. Average (±SE) otolith element/calcium ratios (El/Ca$_O$) in 19 plaice from the Irish Sea (IS) and English Channel (EC) serially sampled for 7–12 months. Note that sampling was carried out twice in February. Shading = peak spawning period. ♀ = female, ♂ = male.

Table 1. Correlation coefficients relating element concentrations (and El/Ca ratios in parentheses) in otoliths vs. seawater, and otoliths vs. blood plasma for 19 plaice from the Irish Sea (IS) and the English Channel (EC) serially sampled for 7–12 months. To help visualize patterns, positive ($r \geq 0{\cdot}2$) and negative ($r \leq -0{\cdot}2$) correlations are displayed in red and blue, respectively, and significant correlations ($P < 0{\cdot}05$ after accounting for temporal autocorrelation, Pyper & Peterman 1998) are in bold

	Otolith vs. seawater			Otolith vs. blood plasma		
	IS males	IS females	EC females	IS males	IS females	EC females
Li	−0·22 (**−0·32**)	−0·49 (−0·38)	−0·37 (−0·27)	−0·04 (0·06)	−0·06 (−0·17)	−0·21 (−0·05)
K	−0·15 (−0·10)	−0·32 (−0·09)	−0·38 (−0·23)	−0·19 (−0·16)	−0·15 (−0·17)	0·17 (0·23)
Mg	−0·02 (−0·13)	−0·04 (−0·11)	−0·22 (−0·20)	0·04 (0·01)	0·08 (0·08)	0·03 (**−0·42**)
Sr	−0·33 (−0·12)	**−0·42** (−0·17)	−0·39 (−0·33)	**0·46 (0·58)**	**0·44 (0·36)**	0·11 (**0·60**)
Ba	−0·23 (−0·19)	−0·29 (−0·25)	**−0·37 (−0·35)**	−0·14 (−0·10)	0·04 (−0·02)	−0·16 (−0·20)
Mn	0·13 (0·12)	**0·32 (0·30)**	0·40 (0·42)	0·11 (0·13)	0·09 (0·15)	0·26 (0·12)
Cu	−0·04 (0·03)	−0·33 (−0·29)	−0·30 (−0·28)	−0·25 (−0·18)	0·01 (−0·05)	−0·27 (−0·31)
Zn	−0·15 (−0·17)	0·27 (**0·31**)	**0·36 (0·40)**	−0·20 (−0·01)	0·18 (0·25)	0·27 (0·17)

effects accounting for 22% (Ba/Ca$_O$) to 68% (Mg/Ca$_O$) of the explained variance (Table 2). The fixed effects in the final models consistently explained more variance than the environmental 'base' models and (apart from the Ba/Ca$_O$ model) performed significantly better (Table S5). Elements were putatively organized into 'functional groups' based on similar behaviours. Li/Ca$_O$ and K/Ca$_O$ ($r = 0{\cdot}82$) were largely explained by otolith opacity, exhibiting higher concentrations in opaque growth zones (Fig. 5). Mg/Ca$_O$ and Mn/Ca$_O$ ($r = 0{\cdot}67$) were primarily explained by growth rate (Fig. 5), but differed in their relationship with ambient concentrations, with Mg/Ca$_O$ negatively related to salinity and Mg$_W$, and Mn/Ca$_O$ positively related to both Mn$_W$ and Mn/Ca$_W$. Sr/Ca$_O$ and Ba/Ca$_O$ ($r = 0{\cdot}72$) were negatively related to growth rate (Fig. 5), as well as temperature, salinity and ambient concentrations. The negative relationships with ambient concentrations were assumed to be indirect, reflecting collinearity between temperature and water chemistry. Importantly, Sr/Ca$_O$ was positively related to Sr/Ca$_B$ (Fig. 5), which were, in turn, primarily influenced by negative relationships with somatic growth rate and body condition. While not included in the final model, Sr/Ca$_O$ also appeared to be modified by processes relating to reproduction, being positively correlated with GSI in mature females only (Fig. 6). Cu/Ca$_O$ behaved most similarly to Sr/Ca$_O$ and Zn/Ca$_O$ ($r = 0{\cdot}43$ and $0{\cdot}41$, respectively), with all three elements exhibiting negative correlations with condition. To better understand uptake mechanisms, we also modelled $D_{Cu\ (O/B)}$ and $D_{Sr\ (O/B)}$; both were negatively related to plasma protein concentrations in the females, but exhibited differing responses in the males (Fig. 6). Zn/Ca$_O$ was positively correlated with Zn/Ca$_W$ and temperature, although this was primarily driven by Zn/Ca$_O$ ratios in the females, which were also significantly correlated with GSI in individuals that successfully spawned (Fig. 6).

Discussion

In this study, variations in otolith element concentrations were generally unrelated or negatively related to those in the

Table 2. Final model outputs explaining otolith element/calcium ratios (El/Ca_O) in 19 plaice sampled for 7–12 months ($n = 211$). Fixed effects include somatic and otolith growth rate (GR), and GnRH 'Treatment'. The female-only model for Zn/Ca_O is displayed as it was the only case where gonadosomatic index (GSI) was included in the final model. R^2 values are indicated alongside the proportion of explained variance (full model r^2) attributed to the fixed effects

Response	Fixed effect	Directionality of effect	χ^2 statistic	*P*-value	Model fit (r^2)		Proportion explained by fixed effects
					Full model	Fixed effects	
Li/Ca_O	Otolith opacity	+ve	45·06	<0·0001	0·80	0·37	0·46
	Temperature†	−ve	14·83	0·0001			
	Length	−ve	37·89	<0·0001			
	Sex	M>F	17·19	0·0001			
K/Ca_O	Otolith opacity	+ve	40·66	<0·0001	0·83	0·24	0·29
	Salinity†	−ve	21·52	<0·0001			
	Length	−ve	16·50	<0·0001			
	Sex	M>F	8·177	0·0042			
Mg/Ca_O	Mean otolith GR	+ve	26·47	<0·0001	0·74	0·50	0·68
	Somatic GR	+ve	6·113	0·0134			
	Salinity	−ve	59·49	<0·0001			
	Temperature	+ve	31·27	<0·0001			
Sr/Ca_O	Sr/Ca_B	+ve	15·72	<0·0001	0·64	0·38	0·59
	Otolith GR	−ve	8·771	0·0031			
	Sr_W	−ve	80·04	<0·0001			
	Length	−ve	9·179	0·0024			
	Sex	M<F	4·004	0·0454			
Ba/Ca_O	Temperature†	−ve	35·74	<0·0001	0·73	0·16	0·22
	Somatic GR	−ve	5·832	0·0157			
	Treatment	Treated<untreated (ns)	2·809	0·0937			
Mn/Ca_O	Mn_W	+ve	78·54	<0·0001	0·77	0·45	0·58
	Somatic GR	+ve	8·994	0·0027			
	Mean otolith GR	+ve	17·83	<0·0001			
Cu/Ca_O	Condition	−ve	15·84	<0·0001	0·45	0·20	0·44
	Temperature	−ve	32·43	<0·0001			
	Treatment	ns	0·415	0·520			
	Temperature* Treatment	−ve (treated), ns (untreated)	18·88	<0·0001			
Zn/Ca_O	Zn/Ca_W	+ve	18·53	<0·0001	0·45	0·21	0·47
	Temperature	+ve	12·12	0·0005			
	Spawner	Spawners < non-spawners	4·987	0·0255			
	Sex	M>F	6·282	0·0122			
	Temperature*Sex	+ve (F), −ve (M)	18·58	<0·0001			
$Zn/Ca_{O\ (FEMALE)}$	Zn/Ca_W	+ve	13·78	0·0002	0·40	0·24	0·60
	Temperature	+ve	8·171	0·0043			
	Spawner	ns	3·080	0·079			
	GSI	ns	1·970	0·1605			
	GSI*Spawner	−ve (spawners), ns (non-spawners)	4·864	0·0274			

F, female; M, male; ns, not significant; +ve, positive; −ve, negative; *, Interaction.
†Near identical results produced using salinity or temperature (i.e. could not distinguish between them).

ambient seawater. We observed seasonal changes in otolith composition that were often correlated to, but lagged with, ambient temperature cycles. Our data emphasize the importance of environmentally-mediated physiological processes in regulating element uptake. Reassuringly, the large sex-specific differences observed in blood plasma total element concentrations (Sturrock *et al*. 2014) were generally absent from the otolith. This was particularly apparent for Sr/Ca ratios, reflecting similar Sr and Ca patterns in the blood, and positive correlations between otolith and plasma Sr/Ca ratios. Significant differences between males and females were, however, observed for otolith concentrations of Sr, Zn, Li and K, despite exposure to identical conditions throughout the study.

DOES OTOLITH MICROCHEMISTRY REFLECT AMBIENT WATER CHEMISTRY?

A fundamental assumption of most otolith microchemistry applications is that the relationship between the trace element composition of the otolith and ambient water is largely unaffected by physiology. However, the similar explanatory power attributed to the fixed and random effects (Table 2) reflects sig-

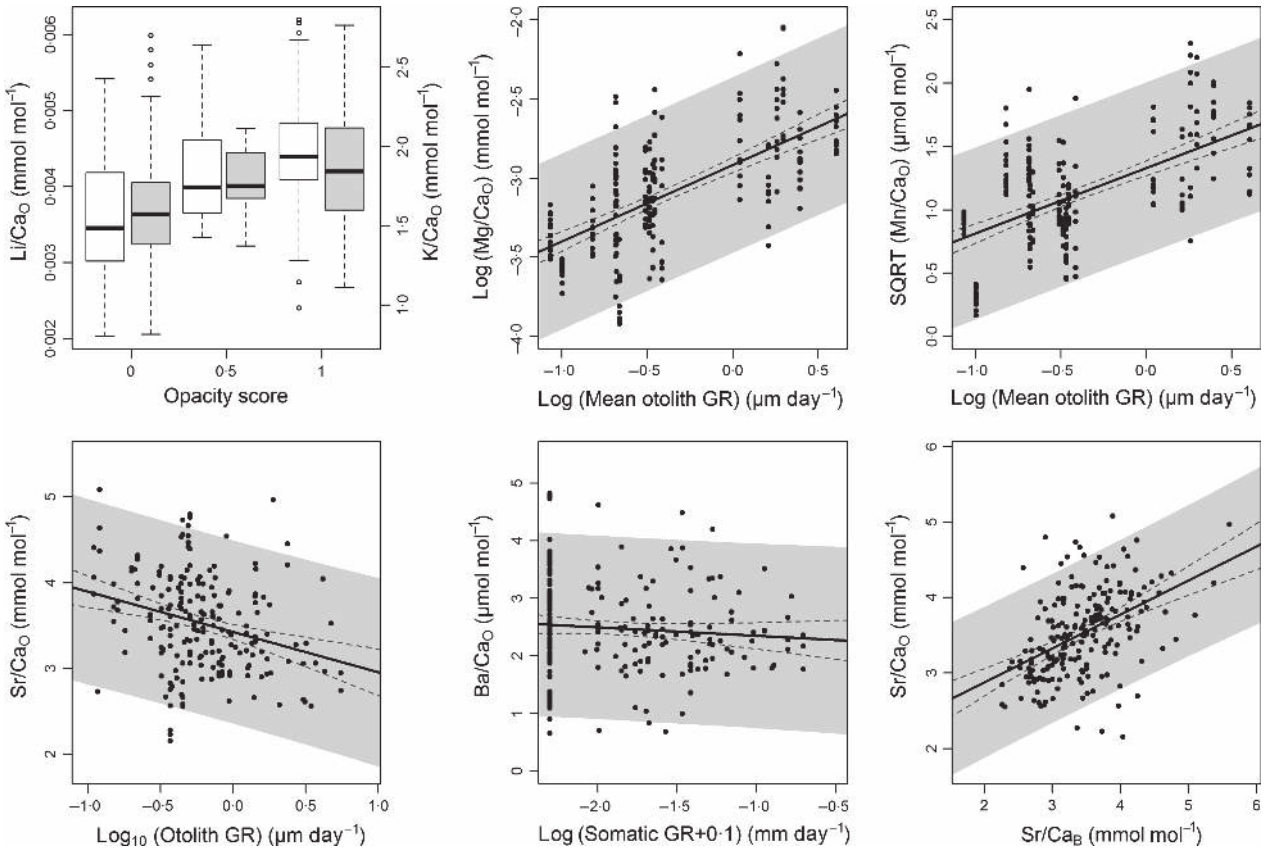

Fig. 5. The main relationships between otolith element/calcium ratios (El/Ca$_O$) and growth-related variables in 19 plaice serially sampled for 7–12 months. Variables include otolith opacity (1 = opaque, 0 = translucent) and growth rates (GR). Li/Ca$_O$ (open) and K/Ca$_O$ (filled) boxplots are shown on a single plot. Blood Sr/Ca ratios (Sr/Ca$_B$) are included because they were strongly correlated with growth rate. Regression lines (solid), 95% confidence intervals (dashed) and prediction intervals (shaded) are displayed. Some variables were transformed to meet model assumptions of homogeneity and normally distributed residuals.

nificant among-individual variation in otolith concentrations, which could have implications for characterizing unique geographic 'fingerprints'. Furthermore, the temporal instability of otolith to seawater distribution coefficients (Fig. S4) indicates that physiological variations between coexisting individuals can influence elemental biokinetics and modify otolith compositions, even in homogenous environments. Any measured distribution coefficient is therefore specific to a given set of physiological and environmental conditions operating over a particular period within an individual's life history (Walther *et al.* 2010), and can only accurately predict otolith (or water) element concentrations when such conditions are known (see also Darnaude *et al.* 2014).

Currently, otolith Sr/Ca and Ba/Ca ratios are the most frequently applied 'geographic markers' in the field of otolith microchemistry, based on typically inverse distributions across salinity gradients (Walther & Limburg 2012), and positive correlations between incorporation rates, ambient concentrations and/or temperature (Secor, Henderson-Arzapalo & Piccoli 1995; Elsdon & Gillanders 2004; Reis-Santos *et al.* 2013). However, seasonal cycles and ontogenetic trends in otolith Sr/Ca have been reported for many marine species that cannot be explained by ambient concentrations (Fuiman & Hoff 1995; Clarke & Friedland 2004; Brown & Severin 2009). Here, otolith Sr/Ca and Ba/Ca ratios were negatively correlated with ambient concentrations, salinity and temperature. The negative relationships between otolith and water concentrations contradict a number of laboratory studies using much larger concentration gradients (e.g. Elsdon & Gillanders 2003), and are clearly inconsistent with extrinsic control on element partitioning, implying seasonal covariance between environmental conditions and physiological factors.

The only elements exhibiting positive correlations between water and otolith concentrations were Mn (all fish) and Zn (females only). We assumed the latter was an artefact of temporally-correlated reproductive processes (see below); however, the use of Mn/Ca$_O$ as an environmental marker is gaining increasing traction (Limburg *et al.* 2015), with positive relationships between otolith and ambient concentrations observed here and in the field (Mohan *et al.* 2012). However, we advise some caution, as laboratory validation has proven elusive (Miller 2009; Limburg *et al.* 2015), the role of dietary Mn could be significant (Pentreath 1973, 1976), and we found no relationship between water and plasma Mn concentrations (Sturrock *et al.* 2014).

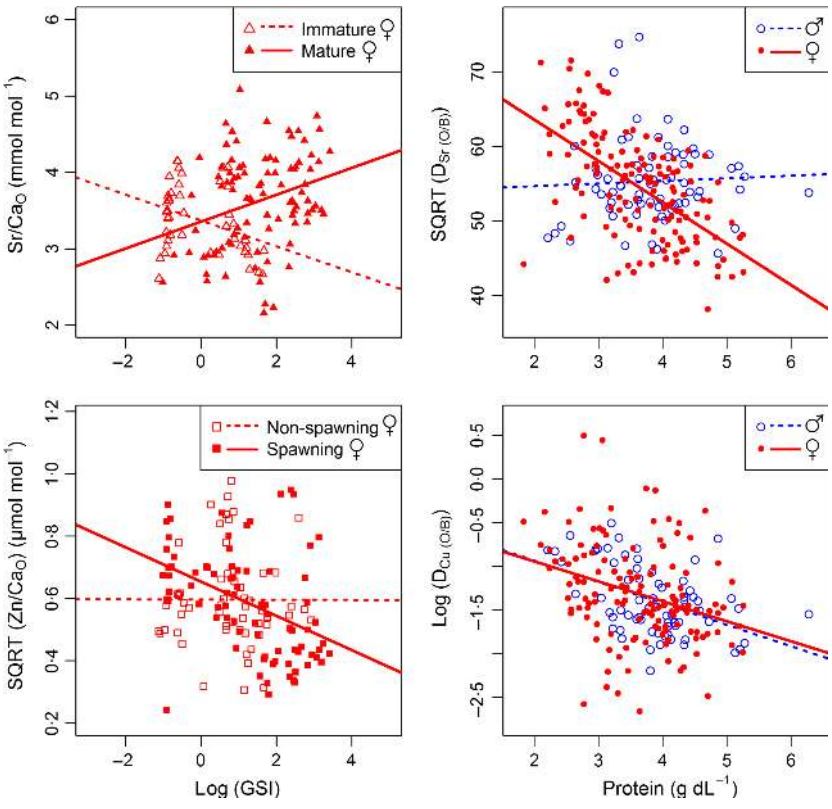

Fig. 6. The strongest relationships between gonadosomatic index (GSI) and otolith element/calcium ratios (El/Ca$_O$) in mature vs. immature and spawning vs. non-spawning female plaice, and between blood plasma-otolith distribution coefficients (D$_{El(O/B)}$) and plasma protein concentrations of male (♂) and female (♀) plaice serially sampled for 7–12 months. Some variables were transformed to meet model assumptions of homogeneity and normally distributed residuals.

IS OTOLITH MICROCHEMISTRY AFFECTED BY TEMPERATURE, MINERALOGY OR GROWTH RATE?

Without independent manipulation of temperature, calcification rates and otolith mineralogy, it was not possible to fully disentangle their effects on ion incorporation into the otolith. Temperature may influence the relationship between water and otolith element concentrations through modification of equilibrium partitioning from solution to otolith aragonite (Kinsman & Holland 1969), or indirectly, via temperature-mediated biokinetic processes. In previous studies, the effect of temperature on Sr and Ba partitioning between otolith and water has been inconsistent. Examples of positive, negative and non-significant relationships (Townsend *et al.* 1992; Elsdon & Gillanders 2002; DiMaria, Miller & Hurst 2010; Reis-Santos *et al.* 2013) provide a compelling argument against a direct, systematic temperature control on equilibrium partitioning of Sr and Ba into otolith aragonite. Furthermore, Sr/Ca$_O$ and temperature cycles are often temporally mismatched (e.g. Clarke & Friedland 2004) and exhibit ontogenetic increases in amplitude despite decreasing variability in thermal regime (e.g. Fuiman & Hoff 1995). We also often observed temporal mismatching between temperature minima and Sr maxima (e.g. Fig. 2). While artificial lags can be induced by ablating multiple layers of otolith material (Hoover & Jones 2013), here, they were observed with a pit depth <5 µm. We therefore hypothesize that the role of temperature and otolith opacity on Sr and Ba partitioning into the otolith crystal is minor compared with element biokinetics, such as growth- or reproduction-related differences in blood protein composition leading to differential element residence times. As temperature directly influences the rates of all biochemical processes, an effect of temperature on metal transport and distributions is not surprising.

Despite the relatively crude system used to score opacity, the monovalent ions (Li^+ and K^+) were consistently enriched in the opaque portions of the otolith, as seen in other studies (Fuiman & Hoff 1995; Tomás *et al.* 2006). The similar behaviour of these ions over the course of the experiment further implies common pathways and an affinity for the organic matrix (Hüssy, Mosegaard & Jessen 2004; Tomás *et al.* 2004). Here, correlation between salinity and temperature meant we were generally unable to resolve their relative influence; however, a negative effect of temperature on Li incorporation into foraminifera calcite has been reported (Marriott *et al.* 2004), and may partially explain the observed patterns.

Mn and Mg were the only elements whose otolith concentrations did not peak during the winter spawning season and were positively correlated with otolith and somatic growth rates, consistent with the growth model proposed by Limburg *et al.* (2011). Both were also weakly positively correlated with temperature. Positive temperature and growth rate effects on Mg/Ca$_O$ have been observed elsewhere (Miller 2011), but not in all studies (Martin & Thorrold 2005; Martin & Wuenschel 2006; DiMaria, Miller & Hurst 2010). Isolating their effects remains one of the key challenges in biomineral research, but here, growth rate appeared to be the primary driver of the observed patterns in otolith Mg/Ca and Mn/Ca.

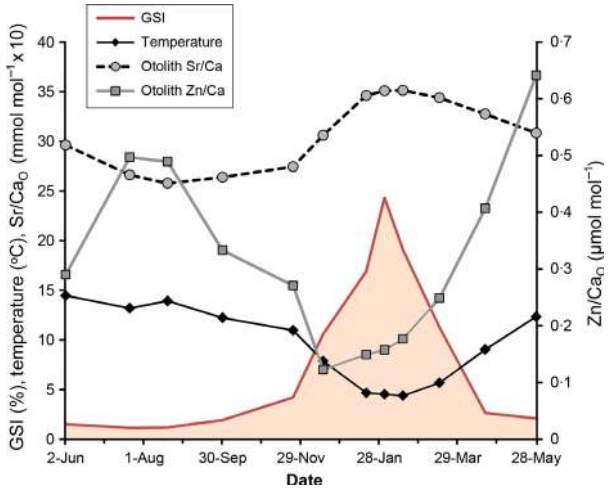

Fig. 7. Changes in otolith Sr/Ca and Zn/Ca ratios relative to gonadosomatic index (GSI) and temperature in a single mature female plaice serially sampled for 12 months.

IS OTOLITH MICROCHEMISTRY AFFECTED BY PHYSIOLOGY AND BLOOD CHEMISTRY?

Importantly, otolith Sr/Ca ratios were positively related to blood plasma Sr/Ca ratios ($Sr/Ca_O = Sr/Ca_B \times 0{\cdot}45$ [±0·06 SE] + 1·97 [±0·21 SE], $n = 209$, $r^2 = 0{\cdot}22$) (Fig. 5). Sr/Ca_B ratios were, in turn, most strongly correlated with somatic growth rate, condition and plasma protein concentrations, implying that Sr/Ca variations in marine fish tissues are primarily controlled by internal processes. Blood protein and Ca concentrations exert major influences on ion transport and availability to the otolith (Kalish 1991; Campana 1999). Here, plasma protein concentrations were often negatively related to $D_{El(O/B)}$ values, implying that when protein concentrations are low, a greater proportion of total plasma ions exist as free ions and are more readily available for rapid transport to the endolymph. It is likely that processes regulating blood protein and Ca concentrations in marine fishes (e.g. feeding and gonad maturation) will also modify blood Sr concentrations and other Ca homologues, and in turn, their El/Ca ratios in all tissues (Kalish 1991; Walther *et al.* 2010).

Blood plasma Ca concentrations peaked approximately 1–2 months earlier than Sr_B concentrations (Sturrock *et al.* 2014), resulting in seasonality in plasma and otolith Sr/Ca ratios, despite relatively constant seawater Sr/Ca ratios. We hypothesize that these Sr/Ca cycles are caused by differences in ion kinetics between Sr and Ca during reproduction. The lagged peak in Sr_B concentrations suggests increased residence time for Sr in body tissues compared with Ca, potentially caused by preferential removal of Ca into gametes (especially eggs), which are then expelled from the body.

Sex-specific differences in otolith element concentrations were observed for Li/Ca, K/Ca Sr/Ca and Zn/Ca. For the monovalent ions, this likely reflected sex-specific patterns in otolith opacity, although these elements may play a role during early ovarian maturation (Shearer 1984; Fuiman & Hoff 1995). The reduced Zn/Ca_O ratios observed in the females and the negative relationship with GSI replicate the patterns observed in the blood plasma (Sturrock *et al.* 2014), and likely reflect the rerouting of Zn from blood plasma to the ovaries bound to the yolk precursor protein, vitellogenin (Fletcher & King 1978; Fletcher & Fletcher 1980; Mitchell & Carlisle 1991). Consequently, the seasonal changes in female otolith Zn/Ca ratios may flag past spawning events, a previously unrealized application in this field. Because vitellogenesis does not normally commence in females that skip spawning (Horwood, Walker & Witthames 1989; Kennedy *et al.* 2008), their plasma and otolith chemistry should mimic pre-maturation patterns. Multi-elemental otolith spawning markers (Zn, Sr and possibly Cu, Se and Pb; Fig. 7) could help identify shifts in size- and age-at-maturity, and reconstruct skipped spawning events in marine fishes, improving estimates of spawning stock biomass (Engelhard & Heino 2005; Jørgensen *et al.* 2006; Skjæraasen *et al.* 2012).

Conclusions

Understanding element partitioning in higher organisms using geochemical concepts like partition coefficients can be impractical or even misleading, given the complexity in ion transport chemistry and individual variation in physiological state. Consequently, accurate prediction of otolith composition from water composition, or vice versa, is difficult or impossible. Our results suggest that in marine fish, otolith element concentrations can vary systematically within a population due to physiological biokinetic factors, even when ambient element/calcium ratios are constant. Physiological processes (e.g. growth and gonad development) can modify blood and otolith composition, and are often cued by changes in temperature and photoperiod, such that otolith chemistry responds both directly and indirectly to changes in the ambient environment. The success of otolith chemistry as a stock discriminator reflects the influence of physiology, as population-specific differences in otolith metal composition are often observed, even in chemically homogeneous conditions. To successfully record individual migration pathways however, environmental signals would need to outweigh physiological 'noise', which is unlikely in most marine settings. Sex-specific differences in otolith element processing offer potential applications for using otolith chemistry to reconstruct individual spawning histories; however, further research into the underlying mechanisms of metal ion transport in fishes is clearly required.

Acknowledgements

This research was funded by a Fisheries Society of the British Isles PhD studentship to AMS, and Defra contract M1102 Macroecology of marine finfish in UK waters and conducted in compliance with Home Office guidelines (Project License no. 80/2260). Additional financial support for AMS was provided by the Delta Stewardship Council and Delta Science Program. The Edinburgh Ion Microprobe facility (EIMF) is supported by NERC. We are extremely grateful for the

analytical and logistical support provided by J. Craven, R. Hinton and C. De Hoog (EIMF); M. Cooper (NOCS); C. Stewart, S. Hetherington, M. Eade, M. Smith, A. Pliru, J. Pettigrew, L. Privitera and S. Milligan (CEFAS). We also appreciate the statistical support provided by H. Sturrock (UCSF), R. Inger (University of Exeter) and P. Challenor (University of Southampton).

Data accessibility

All experimental data (physiological, environmental and elemental) available from the Dryad Digital Repository (Sturrock *et al*. 2015).

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Received 30 October 2014; accepted 10 March 2015
Handling Editor: Erica Leder

Supporting Information

Additional Supporting Information may be found in the online version of this article.

Appendices S1 and S2. Otolith preparation and chemical analyses.

Appendix S3. Linear mixed effects models.

Appendix S4. Distribution coefficients.

Table S1. Standard details, external precision and limits of detection.

Table S2. Details of the subject animals.

Tables S3 and S4. Distribution coefficients (ours vs. previously published).

Table S5. Comparison of final vs. 'base' models.

Fig. S1. Expected vs. predicted otolith δ^{18}O measurements.

Fig. S2. Otolith growth rates over time.

Fig. S3. Otolith opacity over time.

Fig. S4. Otolith:water distribution coefficients over time.