Evaluating chemical signatures in a coastal upwelling region to reconstruct water mass associations of settlement-stage rockfishes

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Supplementary information

Text S1: Analysis of the relationship between freshwater discharge into the marine environment and elemental ratios in seawater.

One of the primary goals of this study was to explore whether coastal waters differ in their elemental signatures. However, freshwater input from nearby rivers and watersheds may influence coastal seawater chemistry, particularly for elements like Ba known to attach to estuary sediments (Carroll et al. 1993, Coffey et al. 1997, Walther et al. 2013). We were unable to use salinity measurements collected near the Bodega Marine Laboratory because the mooring was not deployed during a significant period of the study. In order to determine whether freshwater input contributes substantially to the variability in elemental ratios of seawater samples collected in this study, we correlated daily estimates of freshwater discharge with seawater Ba/Ca, Sr/Ca, and Mg/Ca in each year. Measurements of mean daily discharge (cubic feet per second) from the Russian River watershed was accessed through the United States Geological Survey database (http://waterdata.usgs.gov/ca/nwis/) and used to index freshwater input associated with each water sample. We found elemental ratios to Ca were not correlated to rates of discharge (Table S1), indicating that variation in freshwater flow over short time-scales has a minimal impact on elemental ratios over similar time-scales. For this reason, we believe it is reasonable to exclude rates of discharge from the cluster analysis to group water samples into water mass types associated with upwelling in each year. Additionally, we believe this approach is appropriate given that the primary objective of the analysis is to identify signatures associated with upwelling strength and evaluate their potential application to otolith microchemistry research.

Text S2: Seawater sample analysis protocol

Water samples were analyzed for Ca, Sr, Mg, and Ba by the Interdisciplinary Center for Plasma Mass Spectrometry at the University of California, Davis (ICPMS.UCDavis.edu) using an Agilent 7500CE inductively coupled plasma mass spectrometer (Agilent Technologies, Palo Alto, CA). The samples were diluted into 2% Optima nitric acid by 1/25 and introduced using a MicroMist Nebulizer (Glass Expansion 4 Barlow's Landing Rd., Unit 2A Pocasset, MA 02559) into a temperature-controlled spray chamber with He as the collision cell gas. Instrument standards were diluted from Certiprep ME 2A Standard (SPEX CertiPrep Claritas, 203 Norcross Avenue, Metuchen, NJ 08840) to 0.1 ppb, 0.5 ppb, 1 ppb, 5 ppb, 10 ppb, 50 ppb, 100 ppb, 500 ppb, 1000 ppb and CertiPrep ICS3 Standard to 1 ppm, 5 ppm, 10 ppm, 50 ppm, and 100 ppm respectively in a 3% Trace Element grade HNO_3 (Fisher Scientific) solution prepared with 18.2 Mohm/cm water. The accuracy of the analyses was determined using the NIST 1643E Standard (National Institute of Standards and

Technology, 100 Bureau Drive, Stop 2300, Gaithersburg, MD 20899-2300), which was analyzed at the beginning of each run, along with quality control standards CertiPrep ME 2A (10 ppb) and CertiPrep ICS3 (5 ppm), which were subsequently analyzed every 10th sample. The Internal Standard (IS) was prepared using a CertiPrep Sc Standard (diluted to 10 ppm) and Inorganic Ventures (Inorganic Ventures, 300 Technology Drive, Christiansburg, VA 24073) Ge, Y, In, and Bi Standards diluted to 30 ppm in 3% HNO_3 and introduced using a peristaltic pump at a 1:30 IS/Sample ratio. Limits of Detection (LOD) were calculated automatically using Chemstation G1834B, B.03.04 software. The LOD is defined here as 3σB/a, where 'σB' is the standard deviation of the replicate analyses of the analyte/IS (cps/cps) ratio of the calibration blank, and 'a' is the coefficient from the IS corrected calibration curve's regression equation: $y = ax + blank$. Raw data were corrected for drift using a single sample re-analyzed multiple times over the course of the run. The drift-corrected elemental concentrations of these re-analyzed samples also provided a measure of within-run precision (expressed as RSD, % of mean). Within-run precision was <2% (for Mg, Ca, and Sr) over four runs and 10% for Ba over three runs (Table S2). On the last run, Ba precision was 18%. Sample-to-sample precision of elemental ratios (also expressed as RSD) was determined using a subset of samples (17 out of 125 days) for which 3 replicates were collected on the same day. The mean precision of sample replicates was <3% (Mg/Ca and Sr/Ca) and <9% for Ba/Ca. The range of precision was 0.53 to 5.49 for Mg/Ca, 0.06 to 7.74 for Sr/Ca and 1.93 to 16.86 for Ba/Ca.

Text S3: Details of seawater sample analysis and evaluation of a subset samples within 15% of salinity-predicted values.

Salinity-normalized Mg, Ca, and Sr concentrations in the open ocean are nearly constant (within 2%) because of their high oceanic inventory relative to riverine input (residence time) (de Villiers 1999, de Villiers and Nelson 1999). In our data, the salinity-normalized Ca, Sr, and Mg concentrations (mmol/kg) of some seawater samples were approximately 33% lower than expected based on concentrations reported for S = 35 (see Table 4.1 in Pilson 2013). However, the elemental ratios (to Ca) and their standard deviations were consistent with those measured in a subset of samples with seawater Ca concentrations within 15% of the salinity-predicted value, as well as with measurements reported in Woodson et al. (2013) (Table S3). We believe that the low concentrations measured in this subset of samples were probably caused by dilution during sample processing, given the magnitude of the concentration depletion and the lack of any correlation between concentration and salinity, river discharge, or year. To test for potential bias introduced by using the Sr/Ca and Mg/Ca ratios from these samples, we compared the results generated using the full dataset to those generated using the subset of water samples within 15% of expected seawater elemental concentrations.

A subset of 118 out of the 254 water samples collected were within 15% of expected Ca concentrations (average = 0.965 mmol/kg, range = 8.21-11.05 mmol/kg). The subset comprises 35 replicates from three years (Table S4), although statistical analyses were conducted for samples in 2011 and 2012 only because one replicate existed in 2009. A two-factor PERMANOVA was conducted with year and water mass type as factors, using Mg/Ca, Sr/Ca and Ba/Ca ratios as predictor variables. Statistical analyses used the average value of replicate water samples and employed PRIMER 6 and PERMANOVA+ statistical software (PREMIER Enterprises, PRIMER 6 ver. 6.1.13 and PERMANOVA+ ver. 1.0.3). Results of the PERMANOVA indicated a significant interaction between year and water mass type (Table S5). Pairwise comparisons were made among water masses within each level of the factor year. In 2011, there was no difference in the elemental chemistry among water masses ($P > 0.05$). In 2012, strong upwelling and relaxation differed from weak upwelling (t = 2.895, $P = 0.001$ and t = 3.057, $P = 0.002$, respectively) and there was no difference in

strong upwelling and relaxation (t = 1.902, *P* = 0.053). The ratio of Ba to Ca exhibited elevated concentrations during weak upwelling, which is consistent with results generated by the full dataset.

The consistency of the results generated using the subset compared to the full dataset suggests that inadequate sample mixing before subsampling likely caused the observed low Ca and metal concentrations. Since this did not appear to affect the elemental ratios to Ca or bias in our statistical analyses, we are confident that the analysis of the full dataset is valid.

Text S4: Hierarchical cluster analysis

The hierarchical cluster analyses generated three primary groups in each year (Fig. S1), which were subsequently characterized as strong upwelling, weak upwelling and relaxation conditions.

Table S1. Correlation (*r*) between Mg/Ca, Sr/Ca and Ba/Ca in seawater samples and rates of discharge associated with the day of water sample collection.

Year	Mg/Ca	Sr/Ca	Ba/Ca
2009	0.022	0.257	0.190
2010	-0.084	-0.084	-0.097
2011	-0.146	-0.146	-0.195
2012	0.288	0.288	-0.045

Table S2. Analytical precision of seawater sample runs, measured as the mean concentration and relative standard deviation (RSD: relative standard deviation, as % of mean) of elements (Mg, Ca, and Sr in mmol/kg and Ba in µmol/kg) and elemental ratios to calcium (Mg/Ca mol/mol, Sr/Ca mmol/mol and Ba/Ca in µmol/mol).

Sample run	n	Mg [RSD]	Ca [RSD]	Sr [RSD]	Ba [RSD]	Mg/Ca [RSD]	Sr/Ca [RSD]	Ba/Ca [RSD]
1	5	47.62	9.49	0.0741	3041	5.02	7.81	5.84
		[1.63]	[2.34]	[0.65]	[8.63]	[3.14]	[2.21]	[9.92]
2	5	48.23	9.03	0.0747	314	5.34	8.27	6.32
		[1.85]	[1.55]	[1.62]	[12.08]	[0.92]	[0.24]	[11.06]
3	6	46.59	9.33	0.0749	282	5.71	8.70	5.43
		[1.63]	[1.97]	[1.66]	[9.16]	[0.95]	[0.95]	[8.15]
4	3	23.62	4.18	0.0363	123	5.65	8.70	5.36
		[0.66]	[1.14]	[0.79]	[16.51]	[0.95]	[0.39]	[17.53]

Table S3. The range of standard deviations (SD) of elemental ratios (Mg/Ca mol/mol, Sr/Ca mmol mol^{-1}, and Ba/Ca µmol mol^{-1}) measured in water masses within each year for all replicated samples, the subset of replicated samples, and by Woodson et al. 2013.

Dataset	Mg/Ca SD range	Sr/Ca SD range	Ba/Ca SD range
all samples	0.075-0.589	0.176-0.735	0.480-1.092
subset	0.059-0.243	0.121-0.615	0.309-1.174
Woodson et al.	0.03-0.22	0.11-0.59	0.14-0.41

Table S4. Number of replicate seawater samples collected from oceanographic water masses in each year.

Year	Strong upwelling	Weak upwelling	Relaxation
2011	5	7	3
2012	5	10	4
total	10	17	7

Table S5. PERMANOVA main test (a) and pair-wise comparisons in 2011 (b) and 2012 compare seawater chemistry among water masses.

(a)

Sources of variation	df	SS	MS	*Pseudo-F*	P_{PERM}
water-mass type	2	4.7968	2.3984	0.94676	0.414
year	1	7.9027	7.9027	3.1196	0.061
water mass type X year	2	13.108	6.5541	2.5872	0.046
residual	28	70.931	2.5333		
total	33	101.53			

(b) Pair wise test within level 2012 of factor year

Groups	t	P_{PERM}
upwelling, relaxation	1.9016	0.053
strong upwelling, weak upwelling	2.8951	0.001
relaxation, weak upwelling	3.0573	0.002

(b) Pair wise test within level 2011 of factor year

Groups	t	P_{PERM}
strong upwelling, relaxation	0.95924	0.434
strong upwelling, weak upwelling	0.67707	0.538
relaxation, weak upwelling	0.44316	0.598
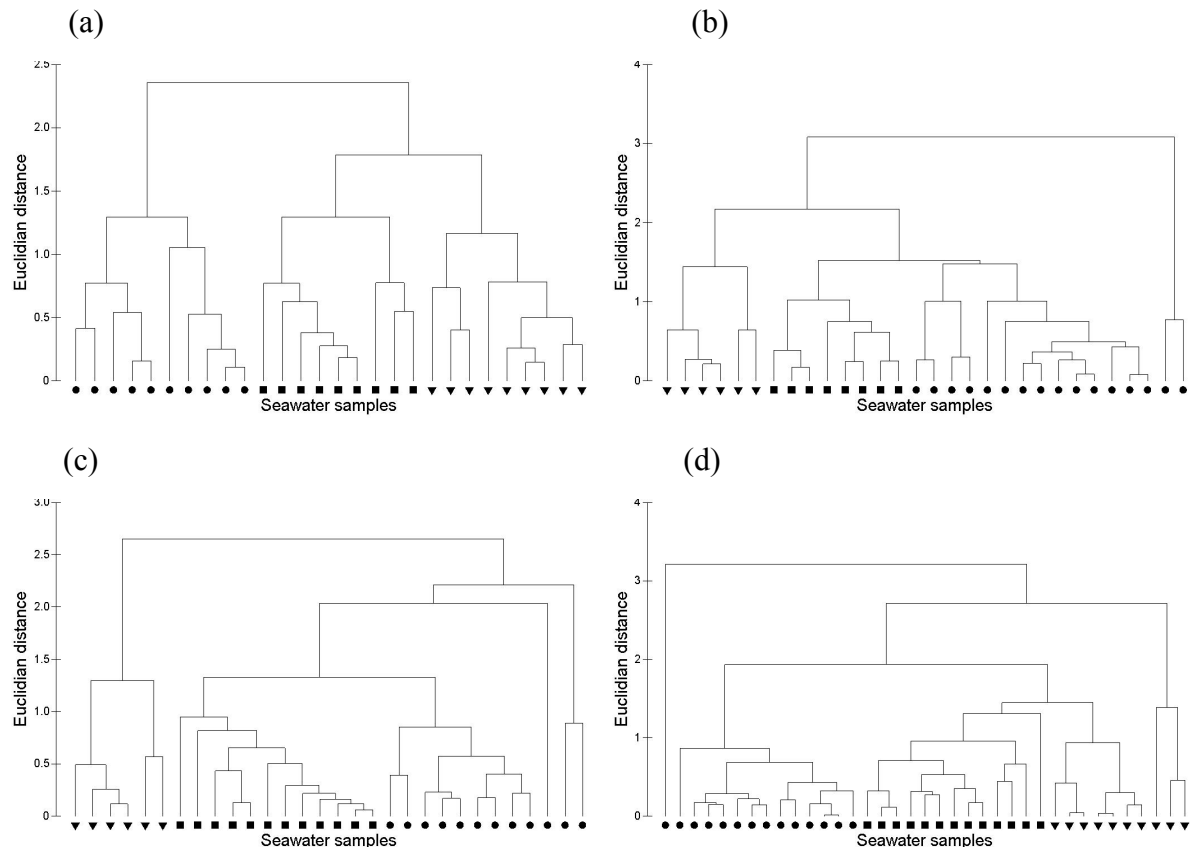
Fig. S1. Clusters produced by Ward's hierarchical cluster method in 2009-2012 (a-d). The distance scale on the y-axis reflects the Euclidian distance between samples using sea surface temperature and alongshore wind speed as predictor variables. Samples subsequently characterized as strong upwelling, weak upwelling and relaxation are indicated by circles, squares, and triangles, respectively.

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