Electronic Supplementary Material (ESI) for Analytical Methods.

Supplementary Information for

Origin determination of the Eastern oyster (*Crassostrea virginica*) using a combination of whole-body compound-specific isotope analysis and heavy metal analysis

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Tables S1-S6

Table S1. One-way ANOVA of bulk $\delta^{13}C$ and $\delta^{15}N$ values of oyster samples from five different harvest areas. n = 9 or 10 for each region.

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
$\delta^{13}C$	Between Groups	46.414	4	11.604	6.011	.001
	Within Groups	84.931	44	1.930		
	Total	131.345	48			
$\delta^{15}N$	Between Groups	282.358	4	70.589	18.443	6.84e-9
	Within Groups	164.579	43	3.827		
	Total	446.937	47			

Table S2. One-way ANOVA followed by Tukey's HSD post-hoc test of of bulk $\delta^{13}C$ and $\delta^{15}N$ values of oyster samples revealed which samples could be statistically discriminated from one region to another. Values statistically significant above 90% confidence level are highlighted in bold letters with an asterisk symbol. n = 9 or 10 for each region.

Multiple Comparisons

Tukey HSD

Dependent Variable	(I) Harvest Area	(J) Harvest Area	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound
$\delta^{13}C$	FL 1642	FL 1662	1.73	.62	**.059***	-.041	3.49
		LA 28	2.60*	.62	**.001***	.83	4.36
		Galveston Bay	.52	.62	.927	-1.27	2.27
		Copano Bay	.39	.64	.972	-1.42	2.21
	FL 1662	FL 1642	-1.73	.62	**.059***	-3.49	.041
		LA 28	.87	.62	.630	-.90	2.64
		Galveston Bay	-1.22	.62	.297	-2.99	.54
		Copano Bay	-1.33	.64	.242	-3.15	.48
	LA 28	FL 1642	-2.60*	.62	**.001***	-4.36	-.83
		FL 1662	-.87	.62	.630	-2.64	.90
		Galveston Bay	-2.10*	.62	**.013***	-3.86	-.33
		Copano Bay	-2.21*	.64	**.010***	-4.02	-.39
	Galveston Bay	FL 1642	-.50	.62	.927	-2.27	1.27
		FL 1662	1.22	.62	.297	-.53	2.99
		LA 28	2.09*	.62	**.013***	.33	3.90
		Copano Bay	-.11	.64	1.000	-1.93	1.70
	Copano Bay	FL 1642	-.39	.64	.972	-2.21	1.42
		FL 1662	1.33	.64	.242	-.48	3.15
		LA 28	2.21*	.64	**.010***	.39	4.02
		Galveston Bay	.11	.64	1.000	-1.70	1.93

$\delta^{15}N$	FL 1642	FL 1662	-.19	.90	1.000	-2.75	2.37
		LA 28	-.98	.87	.795	-3.47	1.51
		Galveston Bay	-6.24*	.87	**.000***	-8.73	-3.75
		Copano Bay	-.12	.904	1.000	-2.68	2.43
	FL 1662	FL 1642	.19	.90	1.000	-2.37	2.74
		LA 28	-.79	.90	.902	-3.35	1.77
		Galveston Bay	-6.05*	.90	**.000***	-8.61	-3.49
		Copano Bay	.06	.92	1.000	-2.56	2.69
	LA 28	FL 1642	.98	.87	.795	-1.51	3.47
		FL 1662	.79	.90	.902	-1.77	3.35
		Galveston Bay	-5.26*	.87	**.000***	-7.75	-2.77
		Copano Bay	.86	.90	.874	-1.70	3.42
	Galveston Bay	FL 1642	6.24*	.87	**.000***	3.75	8.73
		FL 1662	6.05*	.90	**.000***	3.49	8.61
		LA 28	5.26*	.87	**.000***	2.77	7.75
		Copano Bay	6.117522*	.90	**.000***	3.56	8.68
	Copano Bay	FL 1642	.12	.90	1.000	-2.43	2.68
		FL 1662	-.06	.92	1.000	-2.69	2.56
		LA 28	-.857	.90	.874	-3.42	1.70
		Galveston Bay	-6.12*	.90	**.000***	-8.68	-3.56

Table S3. Linear discriminant analysis (classification) according to geographic origin using bulk $\delta^{13}C$ and $\delta^{15}N$ values from whole-body oyster samples collected in five different harvest areas. n = 9 or 10 for each region.

			Predicted Group Membership					
		Harvest Area	FL 1642	FL 1662	LA 28	Galveston Bay	Copano Bay	Total
Original	Count	FL 1642	8	0	0	0	2	10
		FL 1662	0	2	4	0	3	9
		LA 28	0	3	7	0	0	10
		Galveston Bay	0	1	1	8	0	10
		Copano Bay	5	1	1	0	2	9
	%	FL 1642	80.0	.0	.0	.0	20.0	100.0
		FL 1662	.0	22.2	44.4	.0	33.3	100.0
		LA 28	.0	30.0	70.0	.0	.0	100.0
		Galveston Bay	.0	10.0	10.0	80.0	.0	100.0
		Copano Bay	55.6	11.1	11.1	.0	22.2	100.0
Cross-validated[b]	Count	FL 1642	8	0	0	0	2	10
		FL 1662	0	2	4	0	3	9
		LA 28	0	3	6	0	1	10
		Galveston Bay	0	1	1	8	0	10
		Copano Bay	5	1	1	0	2	9
	%	FL 1642	80.0	.0	.0	.0	20.0	100.0
		FL 1662	.0	22.2	44.4	.0	33.3	100.0
		LA 28	.0	30.0	60.0	.0	10.0	100.0
		Galveston Bay	.0	10.0	10.0	80.0	.0	100.0
		Copano Bay	55.6	11.1	11.1	.0	22.2	100.0

a. 56.3% of original grouped cases correctly classified.

b. Cross validation is done only for those cases in the analysis. In cross validation, each case is classified by the functions derived from all cases other than that case.

c. 54.2% of cross-validated grouped cases correctly classified.

Table S4. Multivariate analysis of variance (MANOVA) followed by Games-Howell post-hoc test of $\delta^{13}C$ values of Asx, Glx, Ser and Gly and metal concentrations from oyster samples. The comparisons revealed which samples could be statistically discriminated from one region to another. Values statistically significant above 90% confidence level are highlighted in bold letters with an asterisk symbol. n = 9 or 10 for each region.

Multiple Comparisons

Games-Howell

Dependent Variable	(I) Region_code	(J) Region_code	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound
$\delta^{13}C$ ASX	FL 1642	FL 1662	.52188	1.970201	.999	-5.67631	6.72007
		LA 28	.87654	2.116076	.993	-5.82711	7.58018
		Galveston Bay	2.97837	1.073853	**.086***	-.31019	6.26693
		Copano Bay	3.27063*	1.024258	**.041***	.10612	6.43513
	FL 1662	FL 1642	-.52188	1.970201	.999	-6.72007	5.67631
		LA 28	.35465	2.619062	1.000	-7.57112	8.28042
		Galveston Bay	2.45649	1.880124	.693	-3.59976	8.51273
		Copano Bay	2.74874	1.852245	.592	-3.27171	8.76919
	LA 28	FL 1642	-.87654	2.116076	.993	-7.58018	5.82711
		FL 1662	-.35465	2.619062	1.000	-8.28042	7.57112
		Galveston Bay	2.10183	2.032474	.835	-4.48086	8.68453
		Copano Bay	2.39409	2.006713	.755	-4.15830	8.94648
	Galveston Bay	FL 1642	-2.97837	1.073853	**.086***	-6.26693	.31019
		FL 1662	-2.45649	1.880124	.693	-8.51273	3.59976
		LA 28	-2.10183	2.032474	.835	-8.68453	4.48086
		Copano Bay	.29226	.838021	.996	-2.28184	2.86635
	Copano Bay	FL 1642	-3.27063*	1.024258	**.041***	-6.43513	-.10612
		FL 1662	-2.74874	1.852245	.592	-8.76919	3.27171
		LA 28	-2.39409	2.006713	.755	-8.94648	4.15830

		Galveston Bay	-.29226	.838021	.996	-2.86635	2.28184
δ^{13}C GLX	FL 1642	FL 1662	1.61398	.766909	.273	-.78742	4.01538
		LA 28	1.42252	1.385051	.838	-3.11490	5.95995
		Galveston Bay	.27317	.627749	.992	-1.67595	2.22228
		Copano Bay	-1.19566	.511625	.182	-2.75425	.36293
	FL 1662	FL 1642	-1.61398	.766909	.273	-4.01538	.78742
		LA 28	-.19146	1.502449	1.000	-4.90353	4.52062
		Galveston Bay	-1.34081	.856187	.538	-3.95808	1.27646
		Copano Bay	-2.80964*	.775079	**.020***	-5.23048	-.38879
	LA 28	FL 1642	-1.42252	1.385051	.838	-5.95995	3.11490
		FL 1662	.19146	1.502449	1.000	-4.52062	4.90353
		Galveston Bay	-1.14936	1.436410	.925	-5.75224	3.45352
		Copano Bay	-2.61818	1.389591	.382	-7.16084	1.92448
	Galveston Bay	FL 1642	-.27317	.627749	.992	-2.22228	1.67595
		FL 1662	1.34081	.856187	.538	-1.27646	3.95808
		LA 28	1.14936	1.436410	.925	-3.45352	5.75224
		Copano Bay	-1.46883	.637706	.199	-3.44718	.50952
	Copano Bay	FL 1642	1.19566	.511625	.182	-.36293	2.75425
		FL 1662	2.80964*	.775079	**.020***	.38879	5.23048
		LA 28	2.61818	1.389591	.382	-1.92448	7.16084
		Galveston Bay	1.46883	.637706	.199	-.50952	3.44718
δ^{13}C SER	FL 1642	FL 1662	4.21699*	1.203442	**.021***	.52954	7.90443
		LA 28	1.99016	1.044047	.350	-1.17363	5.15396
		Galveston Bay	6.52686	2.116339	**.070***	-.45848	13.51221
		Copano Bay	4.83173*	.911405	**.000***	2.05749	7.60597
	FL 1662	FL 1642	-4.21699*	1.203442	**.021***	-7.90443	-.52954
		LA 28	-2.22682	1.266758	.427	-6.07728	1.62363

		Galveston Bay	2.30987	2.234621	.835	-4.83536	9.45511
		Copano Bay	.61474	1.159871	.983	-2.97783	4.20730
	LA 28	FL 1642	-1.99016	1.044047	.350	-5.15396	1.17363
		FL 1662	2.22682	1.266758	.427	-1.62363	6.07728
		Galveston Bay	4.53670	2.152973	.286	-2.48986	11.56326
		Copano Bay	2.84156	.993510	**.073***	-.19521	5.87834
	Galveston Bay	FL 1642	-6.52686	2.116339	**.070***	-13.51221	.45848
		FL 1662	-2.30987	2.234621	.835	-9.45511	4.83536
		LA 28	-4.53670	2.152973	.286	-11.56326	2.48986
		Copano Bay	-1.69514	2.091870	.921	-8.65912	5.26885
	Copano Bay	FL 1642	-4.83173*	.911405	**.000***	-7.60597	-2.05749
		FL 1662	-.61474	1.159871	.983	-4.20730	2.97783
		LA 28	-2.84156	.993510	**.073***	-5.87834	.19521
		Galveston Bay	1.69514	2.091870	.921	-5.26885	8.65912
δ^{13}C GLY	FL 1642	FL 1662	2.72509	.947539	**.067***	-.14124	5.59142
		LA 28	1.89280	.807182	.185	-.60370	4.38931
		Galveston Bay	-.05377	1.201490	1.000	-3.77453	3.66699
		Copano Bay	-.65616	1.324504	.986	-4.80731	3.49499
	FL 1662	FL 1642	-2.72509	.947539	**.067***	-5.59142	.14124
		LA 28	-.83228	.771743	.815	-3.20811	1.54354
		Galveston Bay	-2.77886	1.177975	.183	-6.44560	.88789
		Copano Bay	-3.38125	1.303210	.129	-7.48948	.72698
	LA 28	FL 1642	-1.89280	.807182	.185	-4.38931	.60370
		FL 1662	.83228	.771743	.815	-1.54354	3.20811
		Galveston Bay	-1.94657	1.068334	.410	-5.41065	1.51750
		Copano Bay	-2.54896	1.205018	.284	-6.50216	1.40423
	Galveston Bay	FL 1642	.05377	1.201490	1.000	-3.66699	3.77453

		FL 1662	2.77886	1.177975	.183	-.88789	6.44560
		LA 28	1.94657	1.068334	.410	-1.51750	5.41065
		Copano Bay	-.60239	1.498034	.994	-5.20229	3.99751
	Copano Bay	FL 1642	.65616	1.324504	.986	-3.49499	4.80731
		FL 1662	3.38125	1.303210	.129	-.72698	7.48948
		LA 28	2.54896	1.205018	.284	-1.40423	6.50216
		Galveston Bay	.60239	1.498034	.994	-3.99751	5.20229
Pb concentration	FL 1642	FL 1662	-.07550	.057002	.682	-.25385	.10285
		LA 28	-.06576	.038411	.452	-.18194	.05042
		Galveston Bay	-.00243	.031470	1.000	-.09952	.09465
		Copano Bay	-.01010	.032568	.998	-.10993	.08973
	FL 1662	FL 1642	.07550	.057002	.682	-.10285	.25385
		LA 28	.00974	.057680	1.000	-.16996	.18944
		Galveston Bay	.07307	.053310	.657	-.09930	.24544
		Copano Bay	.06540	.053965	.745	-.10787	.23867
	LA 28	FL 1642	.06576	.038411	.452	-.05042	.18194
		FL 1662	-.00974	.057680	1.000	-.18944	.16996
		Galveston Bay	.06333	.032682	.341	-.03786	.16451
		Copano Bay	.05566	.033740	.490	-.04807	.15939
	Galveston Bay	FL 1642	.00243	.031470	1.000	-.09465	.09952
		FL 1662	-.07307	.053310	.657	-.24544	.09930
		LA 28	-.06333	.032682	.341	-.16451	.03786
		Copano Bay	-.00767	.025560	.998	-.08608	.07075
	Copano Bay	FL 1642	.01010	.032568	.998	-.08973	.10993
		FL 1662	-.06540	.053965	.745	-.23867	.10787
		LA 28	-.05566	.033740	.490	-.15939	.04807
		Galveston Bay	.00767	.025560	.998	-.07075	.08608

Cd concentration	FL 1642	FL 1662	-.00180	.066226	1.000	-.20235	.19875
		LA 28	-.57660*	.119715	**.003***	-.95953	-.19367
		Galveston Bay	-.22139	.100460	.244	-.54309	.10031
		Copano Bay	-.52372	.254868	.319	-1.39189	.34445
	FL 1662	FL 1642	.00180	.066226	1.000	-.19875	.20235
		LA 28	-.57480*	.121862	**.003***	-.96111	-.18849
		Galveston Bay	-.21959	.103009	.266	-.54573	.10656
		Copano Bay	-.52192	.255884	.324	-1.39051	.34666
	LA 28	FL 1642	.57660*	.119715	**.003***	.19367	.95953
		FL 1662	.57480*	.121862	**.003***	.18849	.96111
		Galveston Bay	.35521	.143376	.143	-.08206	.79249
		Copano Bay	.05288	.274631	1.000	-.83422	.93998
	Galveston Bay	FL 1642	.22139	.100460	.244	-.10031	.54309
		FL 1662	.21959	.103009	.266	-.10656	.54573
		LA 28	-.35521	.143376	.143	-.79249	.08206
		Copano Bay	-.30233	.266801	.786	-1.17977	.57510
	Copano Bay	FL 1642	.52372	.254868	.319	-.34445	1.39189
		FL 1662	.52192	.255884	.324	-.34666	1.39051
		LA 28	-.05288	.274631	1.000	-.93998	.83422
		Galveston Bay	.30233	.266801	.786	-.57510	1.17977

Based on observed means.

The error term is Mean Square (Error) = 0.154.

Table S5. Regression analysis model using oyster harvesting regions as dependent variable and the $\delta^{13}C$ values of Asx, Glx, Ser, Gly and metal concentrations as predictors. The BCa confidence intervals excluding the value zero indicate which variables are significantly different between regions at the 95% confidence interval.

Bootstrap for Coefficients

Model		B	Bootstrap[a] Bias	Std. Error	Sig. (2-tailed)	BCa 95% Confidence Interval Lower	Upper
1	(Constant)	1.173	-.223	2.010	.545	-2.727	4.647
	Pb	.450	.205	1.521	.730	-2.607	4.873
	Cd	1.166	.054	.433	.007	**.454**	**2.277**
	$\delta^{13}C$ ASX	-.056	-.010	.048	.169	**-.159**	**-.002**
	$\delta^{13}C$ GLX	.088	-.004	.087	.264	-.098	.253
	$\delta^{13}C$ SER	-.157	.001	.042	.002	**-.252**	**-.074**
	$\delta^{13}C$ GLY	.031	.011	.082	.692	-.122	.238

a. Unless otherwise noted, bootstrap results are based on 2000 bootstrap samples

Table S6. Multivariate Analysis of variance (MANOVA) of various variables using the harvesting areas FL 1642 and FL 1662 as the fixed factor. Five amino acids exceeding 95% confidence are shown in bold letters with an asterisk symbol.

		Sum of Squares	df	Mean Square	F	Sig.
Pb concentration	Between Groups	.029	1	.029	1.754	.202
	Within Groups	.292	18	.016		
	Total	.321	19			
Cd concentration	Between Groups	.000	1	.000	.001	.979
	Within Groups	.395	18	.022		
	Total	.395	19			
δ^{13}C ASX	Between Groups	1.362	1	1.362	.070	.794
	Within Groups	349.352	18	19.408		
	Total	350.714	19			
δ^{13}C GLX	Between Groups	13.025	1	13.025	4.429	.050
	Within Groups	52.933	18	2.941		
	Total	65.958	19			
δ^{13}C SER	**Between Groups**	**88.915**	**1**	**88.915**	**12.279**	**.003***
	Within Groups	**130.345**	**18**	**7.241**		
	Total	**219.259**	**19**			
δ^{13}C GLY	**Between Groups**	**37.131**	**1**	**37.131**	**8.271**	**.010***
	Within Groups	**80.805**	**18**	**4.489**		
	Total	**117.935**	**19**			
δ^{13}C VAL	**Between Groups**	**21.221**	**1**	**21.221**	**7.639**	**.013***
	Within Groups	**50.001**	**18**	**2.778**		
	Total	**71.222**	**19**			
δ^{13}C XLE	Between Groups	13.286	1	13.286	4.016	.060
	Within Groups	59.546	18	3.308		

	Total	72.832	19			
δ^{13}C LYS	**Between Groups**	**31.914**	**1**	**31.914**	**7.947**	**.011***
	Within Groups	**72.286**	**18**	**4.016**		
	Total	**104.200**	**19**			
δ^{13}C PHE	**Between Groups**	**47.761**	**1**	**47.761**	**21.788**	**.000***
	Within Groups	**39.458**	**18**	**2.192**		
	Total	**87.220**	**19**			
δ^{13}C ARG	Between Groups	17.835	1	17.835	3.215	.090
	Within Groups	99.841	18	5.547		
	Total	117.677	19			