SUPPORTING INFORMATION

Distinctive metabolite profiles in in-migrating Sockeye salmon suggest sex-linked endocrine perturbation

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Number of pages: 30 + excel file (4 pages)

Number of figures: 7

Number of tables: 12 + excel file (4 tables)

Method Validation

Results of spike/recovery experiments are provided in Tables S5-S8 of the supporting information. For amino acids and biogenic amines, average recoveries (*n*=5 replicates per spiking level) were 109, 111, and 116% at low, moderate, and high spiking levels, respectively. Average recoveries for all amino acids and biogenic amines were above 74% at all spiking levels and only lysine, sarcosine, and phenylalanine displayed recoveries exceeding 140%. Average percent relative standard deviation (% RSD) for all amino acids and biogenic amines was 9, 10, and 7 for low, moderate, and high spiking levels, respectively. Percent RSDs exceeding 20% were only observed for sarcosine at the lowest and highest fortification levels and spermine at the lowest fortification level. For hexose, recoveries were 103, 103, and 97 at low, moderate, and high spiking levels, with % RSDs of <6%, while fatty acid recoveries were 95, 105, and 96% for low, moderate, and high spiking levels, with % RSD of <22%. Due to the high number of lipids measured in the present work, it was not possible to validate accuracy for every target. Rather, we fortified salmon liver extracts with 10 authentic native lipid standards (6 carnitines, 3 phosphatidylcholines and a sphingomyelin) at 3 spiking levels and extrapolated the results to other lipid classes. Average recovery of carnitines was greater than 85% and less than 125% at all spiking levels with % RSDs of <17%. Lower recoveries were observed for phosphatidylcholines and sphingomyelins (average recovery 21-81% over all spiking levels), however % RSDs were reasonable with only lyso phosphatidylcholine (lysoPC) a C18:0 having a % RSD of >26% at the lowest fortification level.

Extraction efficiency experiments were conducted by primary and secondary extraction of salmon tissue with the refined method (extractions 1 and 2) followed by a third extraction with chloroform (extraction 3) with results presented in Table S9-S12. For all but 4 metabolite targets,

>90% of the total concentration was attributed to the first extraction. Exceptions were observed for putriscine, spermidine, and spermine, which were extracted primarily in the chloroform fraction. These biogenic amines are all structurally similar polyamines which are considerably less polar than other targets examined in the present work; therefore, their poor extraction efficiency using MeOH is perhaps not surprising. Results for these targets should be considered qualitative when using MeOH as an extraction solvent. For hexose, extraction efficiency experiments revealed that >97% was extracted using the first MeOH extraction procedure, with <3% observed in the second MeOH extraction, and none in the chloroform fraction. Similar results were obtained for fatty acids, with >90% observed in the first MeOH extraction. Lipid extraction efficiency experiments revealed that for all but one sphingomyelin (SM C18:0), over 90% of the concentration was observed in the first extraction.

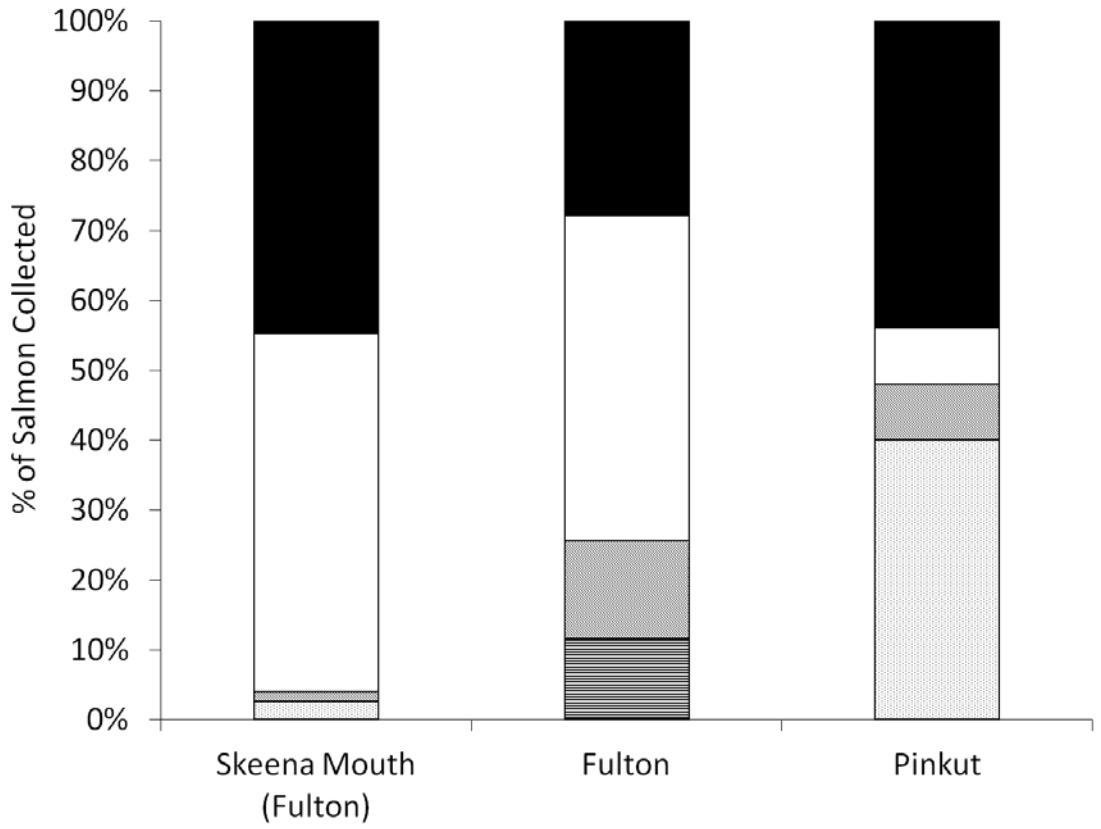

Figure S1. Distribution of salmon at each collection site based on the three letter genotype-phenotype sex classification: FFF (■), FFM (▧), MMM (□) and MMF (▩) animals. Animals not falling into the 4 classifications was grouped as "other" (▤).

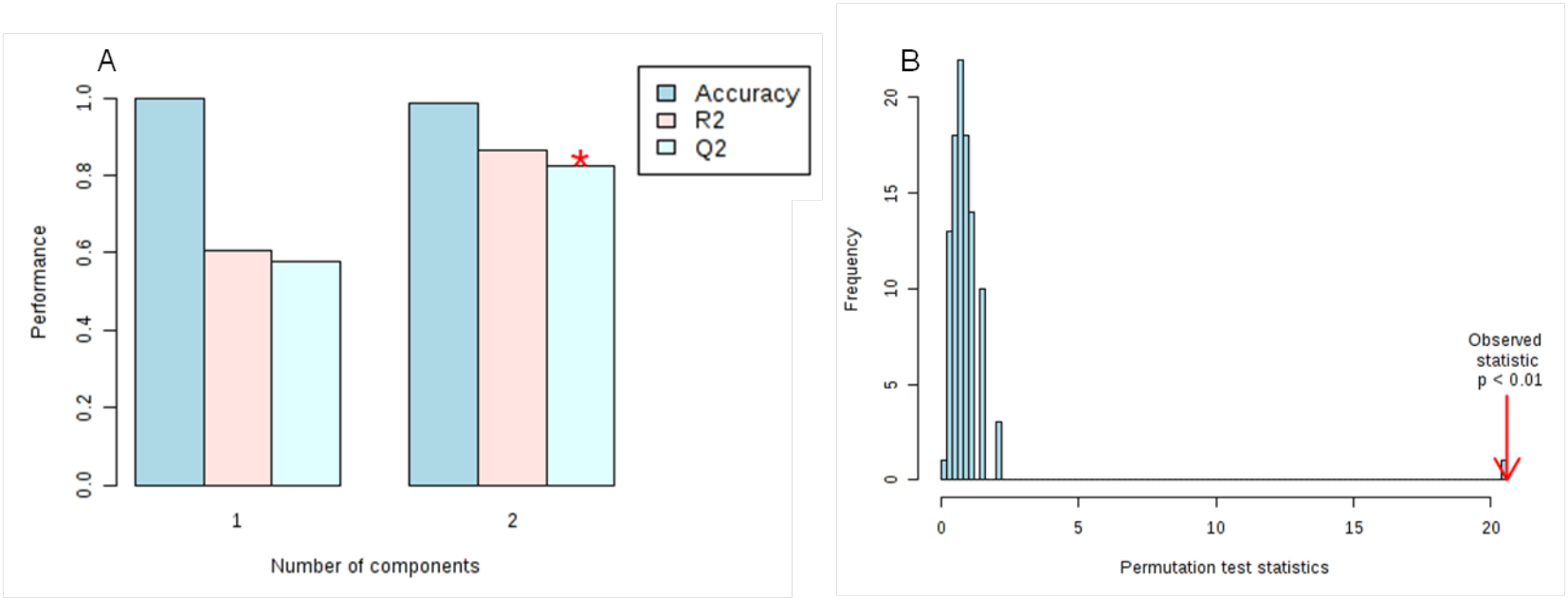

Figure S2. Validation of PLS-DA model (Figure 2 in main text) for migrating male Sockeye using A) 10-fold cross validation, and B) Permutation testing.

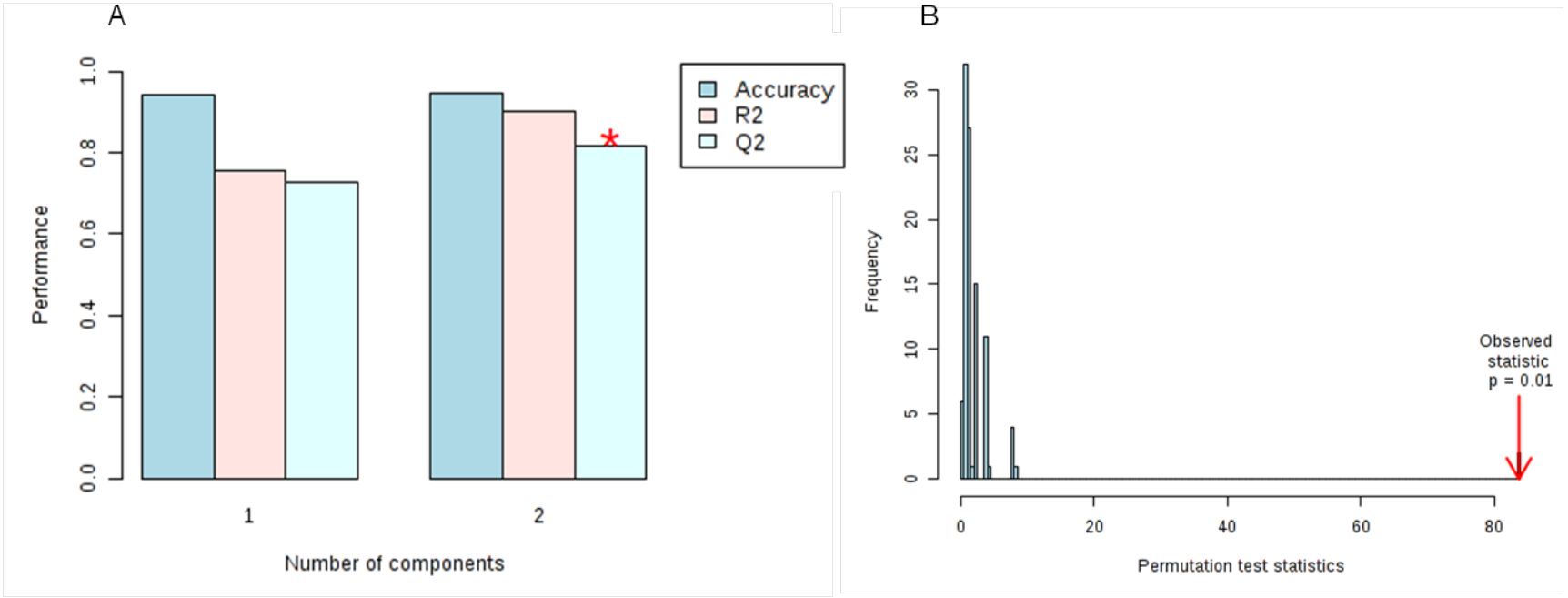

Figure S3. Validation of PLS-DA model (Figure 3 in main text) for migrating female Sockeye using A) 10-fold cross validation, and B) Permutation testing.

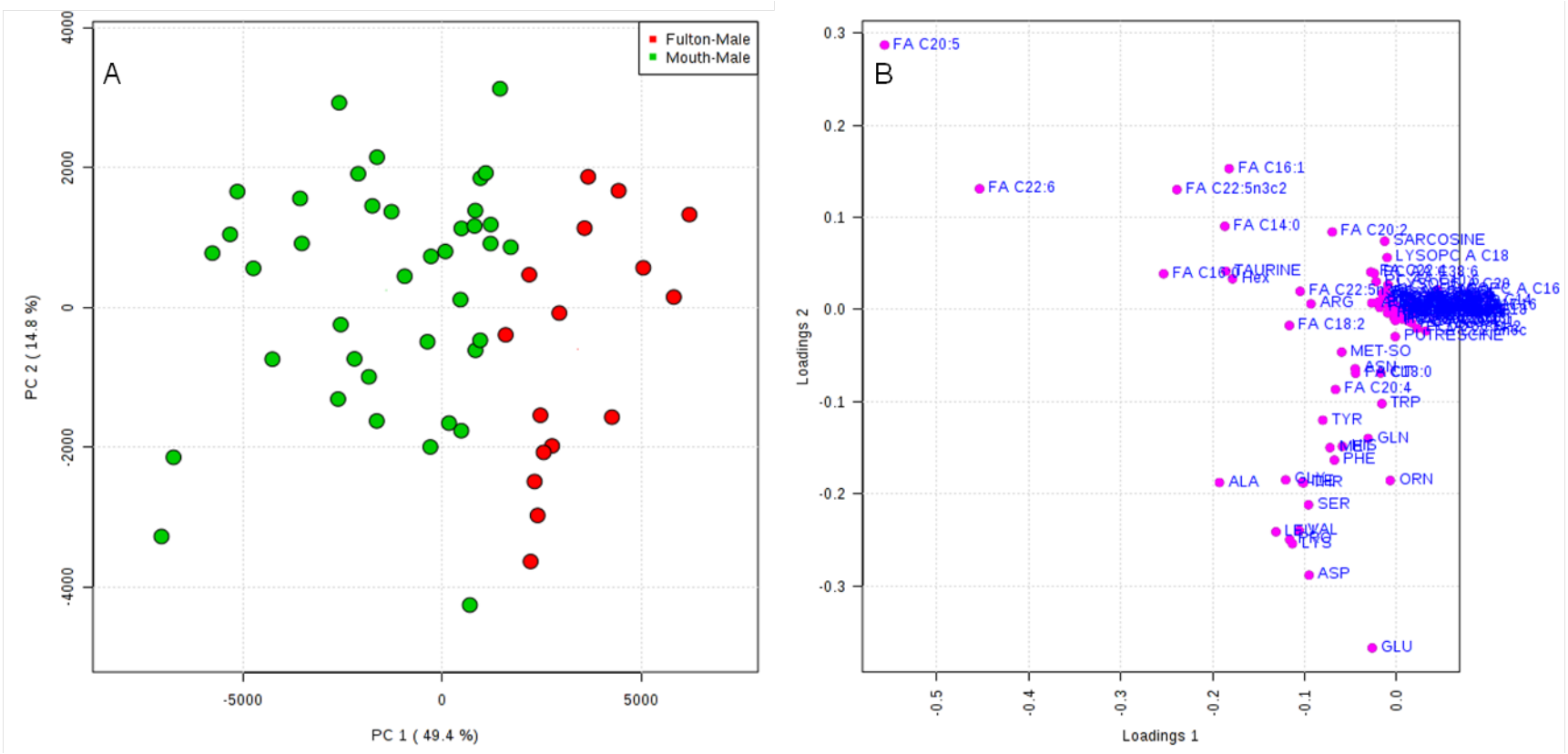

Figure S4. PCA scores plot (A) and loadings plot (B) of migrating male salmon (Fulton Stock) sampled at the mouth of the Skeena (green) and the Fulton River spawning grounds (red).

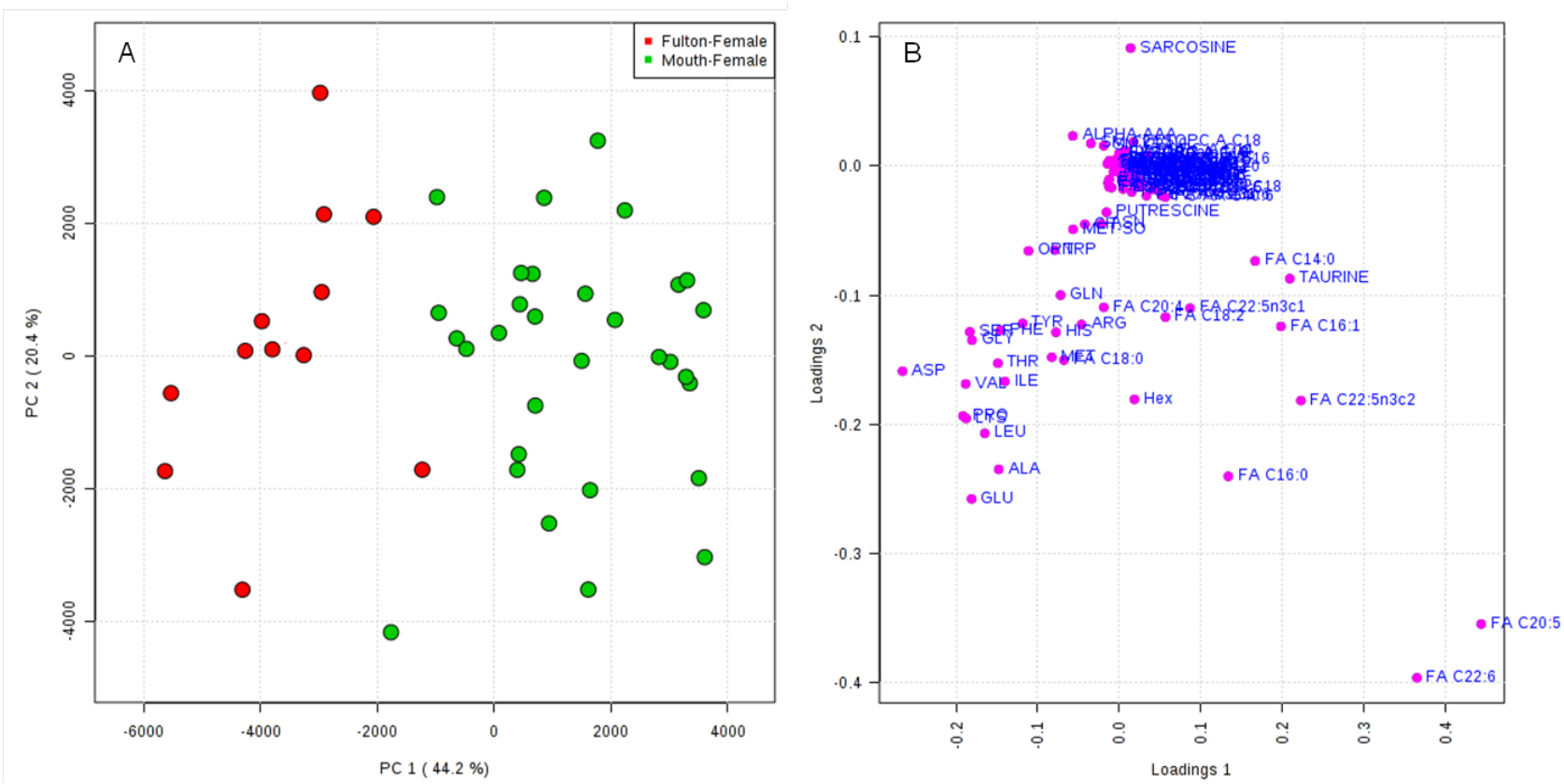

Figure S5. PCA scores plot (A) and loadings plot (B) of migrating female salmon (Fulton Stock) sampled at the mouth of the Skeena (green) and the Fulton River spawning grounds (red).

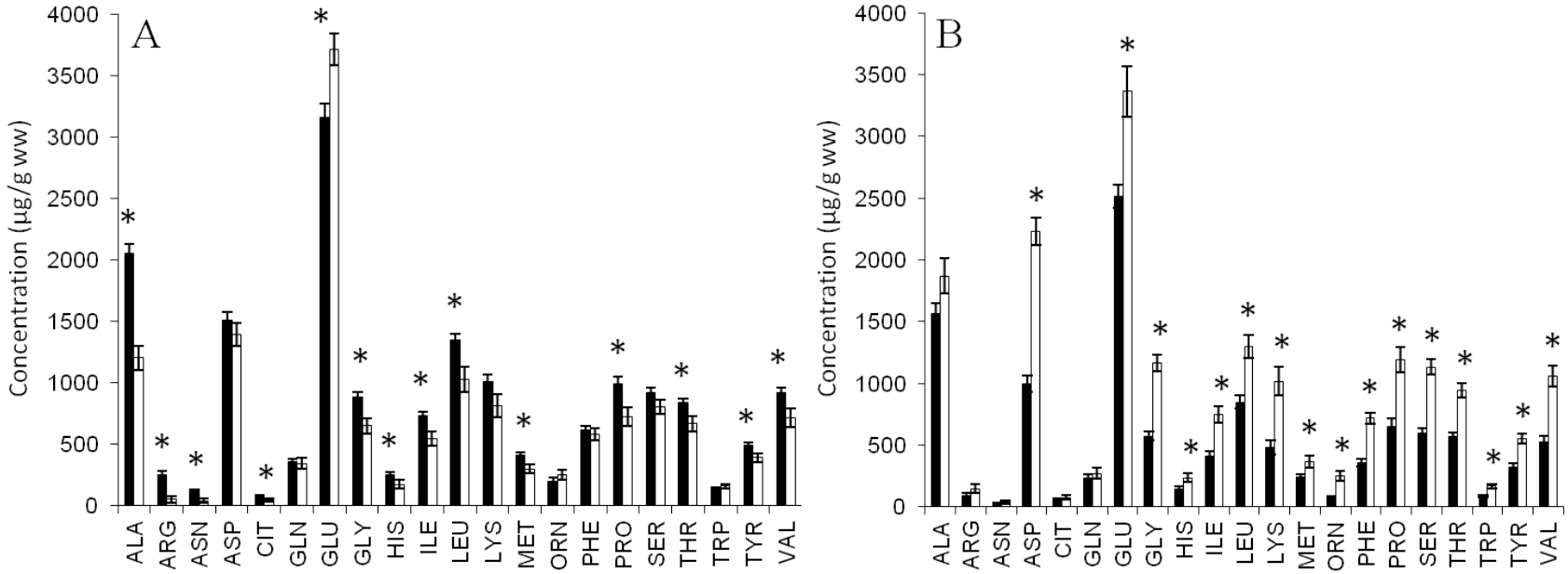

Figure S6. Amino acid concentrations for Fulton River male (A) and female (B) salmon collected from the mouth of the Skeena River (shaded bars) and spawning grounds (clear bars). An asterisk indicates a statistically (p<0.05) significant difference.

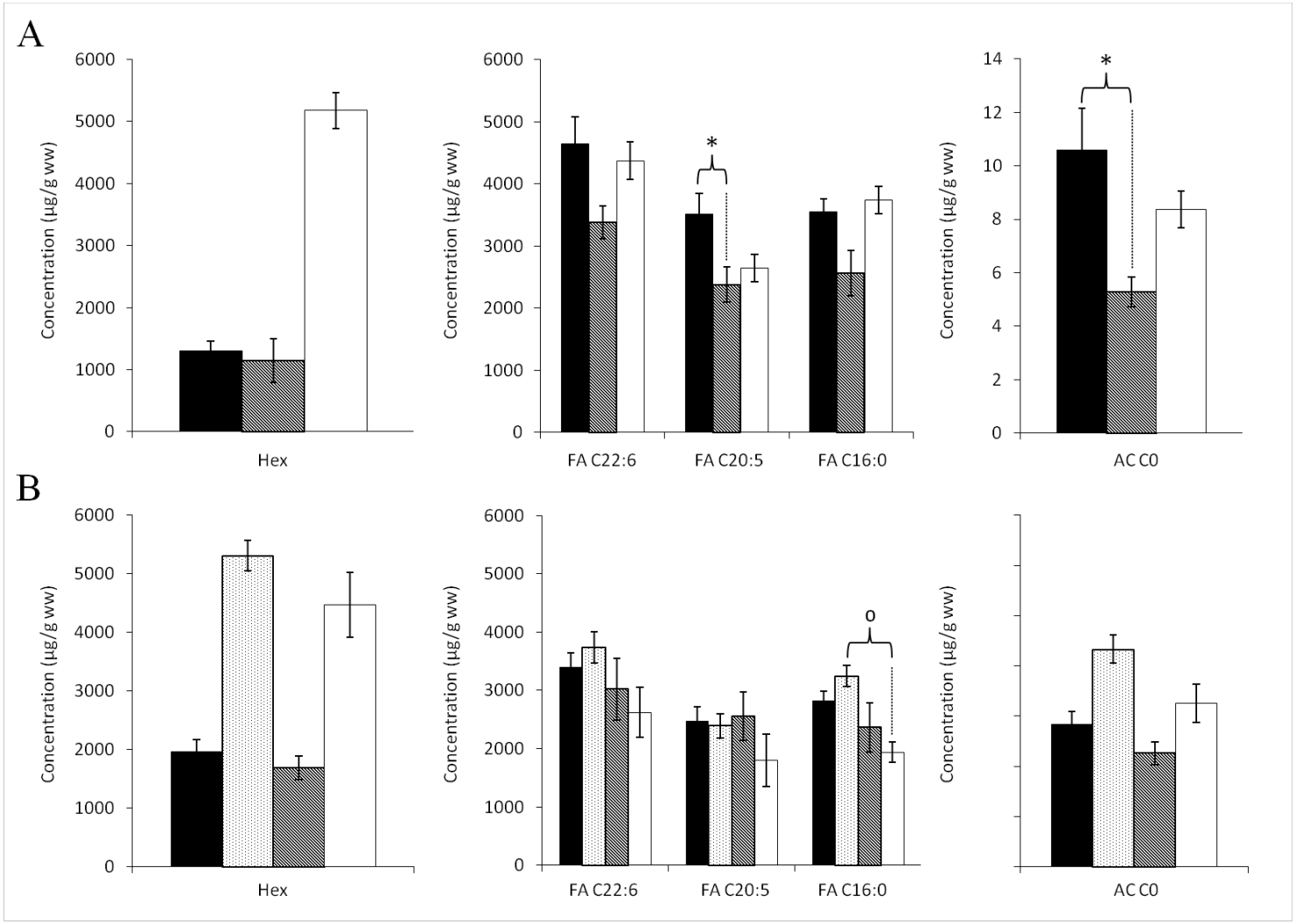

Figure S7. Concentrations of hexose (Hex), major hepatic fatty acids (DHA [FA C22:6]; EPA [FA C20:5]; and palmitic acid [FA C16:0]) and carnitine (AC C0) in Fulton River (A) and Pinkut Creek (B) Sockeye salmon. (*) indicates a statistically significant difference between FFF (■) and FFM (▨) animals while (°) indicates a statistically significant difference between MMM (□) and MMF (▩) animals.

Table S1. Amino acids and biogenic amines examined in the present study

Target ID	Class	Target	Abbreviation	Internal Standard
1	Amino Acid	Alanine	Ala	d4-Ala
2	Amino Acid	Arginine	Arg	15N2-Arg
3	Amino Acid	Asparagine	Asn	15N2-Asn
4	Amino Acid	Aspartate	Asp	d3-Asp
5	Amino Acid	Citrulline	Cit	13C-D4-Cit
6	Amino Acid	Glutamine	Gln	d5-gln
7	Amino Acid	Glutamate	Glu	d3-Glu
8	Amino Acid	Glycine	Gly	13C2-15N-Gly
9	Amino Acid	Histidine	His	13C6-His
10	Amino Acid	Isoleucine	Ile	13C6-Ile
11	Amino Acid	Leucine	Leu	13C6-Ile
12	Amino Acid	Lysine	Lys	d6-Orn
13	Amino Acid	Methionine	Met	d3-Met
14	Amino Acid	Ornithine	Orn	d6-Orn
15	Amino Acid	Phenylalanine	Phe	d5-Phe
16	Amino Acid	Proline	Pro	d7-Pro
17	Amino Acid	Serine	Ser	d3-Ser
18	Amino Acid	Threonine	Thr	13C4-Thr
19	Amino Acid	Tryptophan	Trp	15N2-Trp
20	Amino Acid	Tyrosine	Tyr	d4-Tyr
21	Amino Acid	Valine	Val	d8-Val
22	Biogenic Amine	Acetylornithine	Ac-Orn	d6-Orn
23	Biogenic Amine	Asymmetric dimethylarginine	ADMA	d7-ADMA
24	Biogenic Amine	Symmetric dimethylarginine	SDMA	d7-ADMA
25	Biogenic Amine	Alpha-Aminoadipic acid	Alpha-AAA	d6-Orn
26	Biogenic Amine	Carnosine	Carnosine	13C6-His
27	Biogenic Amine	Creatinine	Creatinine	d3-Creatinine
28	Biogenic Amine	Dopamine	DOPA	d3-DOPA
29	Biogenic Amine	3-hydroxytyrosine	Dopamine	d4-dopamine
30	Biogenic Amine	Histamine	Histamine	13C6-His
31	Biogenic Amine	Kynurenine	Kynurenine	d4-Tyr
32	Biogenic Amine	Methioninesulfoxide	Met-SO	d3-Met
33	Biogenic Amine	Nitrotyrosine	Nitro-Tyr	d4-Tyr
34	Biogenic Amine	Hydroxyproline	OH-Pro	d7-Pro
35	Biogenic Amine	Phenylethylamine	PEA	d4-Serotonin
36	Biogenic Amine	Putrescine	Putrescine	d4-Putrescine
37	Biogenic Amine	Sarcosine	Sarcosine	d3-Sarcosine
38	Biogenic Amine	Serotonin	Serotonin	d4-Serotonin
39	Biogenic Amine	Spermidine	Spermidine	d4-Putrescine
40	Biogenic Amine	Spermine	Spermine	d4-Putrescine
41	Biogenic Amine	Taurine	Taurine	13C2-Taurine

Table S2. Carbohydrates

Target ID	Class	Target	Abreviation	Internal Standard
42	Sugar	Hexose	Hex	$^{13}C_6$-glucose

Table S3. Carnitines, phosphatidylcholines, and sphingomyelins

Target ID	Class	Analyte	Abbreviation	Internal Standard
43	Acylcarnitine	Carnitine	AC C0	D9-AC C0
44	Acylcarnitine	Decanoylcarnitine	AC C10	D3-AC C8
45	Acylcarnitine	Decenoylcarnitine	AC C10:1	D3-AC C8
46	Acylcarnitine	Decadienylcarnitine	AC C10:2	D3-AC C8
47	Acylcarnitine	Dodecanoylcarnitine	AC C12	D9-AC C14
48	Acylcarnitine	Dodecenoylcarnitine	AC C12:1	D9-AC C14
49	Acylcarnitine	Dodecanedioylcarnitine	AC C12-DC	D9-AC C14
50	Acylcarnitine	Tetradecanoylcarnitine	AC C14	D9-AC C14
51	Acylcarnitine	Tetradecenoylcarnitine	AC C14:1	D9-AC C14
52	Acylcarnitine	Hydroxytetradecenoylcarnitine	AC C14:1-OH	D9-AC C14
53	Acylcarnitine	Tetradecadienylcarnitine	AC C14:2	D9-AC C14
54	Acylcarnitine	Hydroxytetradecadienylcarnitine	AC C14:2-OH	D9-AC C14
55	Acylcarnitine	Hexadecanoylcarnitine	AC C16	D3-AC C16
56	Acylcarnitine	Hexadecenoylcarnitine	AC C16:1	D3-AC C16
57	Acylcarnitine	Hydroxyhexadecenoylcarnitine	AC C16:1-OH	D3-AC C16
58	Acylcarnitine	Hexadecadienylcarnitine	AC C16:2	D3-AC C16
59	Acylcarnitine	Hydroxyhexadecadienylcarnitine	AC C16:2-OH	D3-AC C16
60	Acylcarnitine	Hydroxyhexadecanoylcarnitine	AC C16-OH	D3-AC C16
61	Acylcarnitine	Octadecanoylcarnitine	AC C18	D3-AC C16
62	Acylcarnitine	Octadecenoylcarnitine	AC C18:1	D3-AC C16
63	Acylcarnitine	Hydroxyoctadecenoylcarnitine	AC C18:1-OH	D3-AC C16
64	Acylcarnitine	Octadecadienylcarnitine	AC C18:2	D3-AC C16
65	Acylcarnitine	Acetylcarnitine	AC C2	D3-AC C2
66	Acylcarnitine	Propionylcarnitine	AC C3	D3-AC C3
67	Acylcarnitine	Propenoylcarnitine	AC C3:1	D3-AC C3
68	Acylcarnitine	Hydroxypropionylcarnitine	AC C3-OH	D3-AC C3
69	Acylcarnitine	Butyrylcarnitine	AC C4	D3-AC C4
70	Acylcarnitine	Butenylcarnitine	AC C4:1	D3-AC C4
71	Acylcarnitine	Hydroxylbutyrylcarnitine	AC C4-OH (C3-DC)	D3-AC C4
72	Acylcarnitine	Valerylcarnitine	AC C5	D9-AC C5
73	Acylcarnitine	Tiglylcarnitine	AC C5:1	D9-AC C5
74	Acylcarnitine	Glutaconylcarnitine	AC C5:1-DC	D9-AC C5

75	Acylcarnitine	Glutarylcarnitine (Hydroxyhexanoylcarnitine)	AC C5-DC (C6-OH)	D9-AC C5
76	Acylcarnitine	Methylglutarylcarnitine	AC C5-M-DC	D9-AC C5
77	Acylcarnitine	Hydroxyvalerylcarnitine (Methylmalonylcarnitine)	AC C5-OH (C3-DC-M)	D9-AC C5
78	Acylcarnitine	Hexanoylcarnitine (Fumarylcarnitine)	AC C6 (C4:1-DC)	D9-AC C5
79	Acylcarnitine	Hexenoylcarnitine	AC C6:1	D9-AC C5
80	Acylcarnitine	Pimelylcarnitine	AC C7-DC	D3-AC C8
81	Acylcarnitine	Octanoylcarnitine	AC C8	D3-AC C8
82	Acylcarnitine	Nonaylcarnitine	AC C9	D3-AC C8
83	Phosphatidylcholine	lysoPhosphatidylcholine acyl C14:0	lysoPC a C14:0	13:0 LPC
84	Phosphatidylcholine	lysoPhosphatidylcholine acyl C16:0	lysoPC a C16:0	13:0 LPC
85	Phosphatidylcholine	lysoPhosphatidylcholine acyl C16:1	lysoPC a C16:1	17:1 LPC
86	Phosphatidylcholine	lysoPhosphatidylcholine acyl C17:0	lysoPC a C17:0	13:0 LPC
87	Phosphatidylcholine	lysoPhosphatidylcholine acyl C18:0	lysoPC a C18:0	13:0 LPC
88	Phosphatidylcholine	lysoPhosphatidylcholine acyl C18:1	lysoPC a C18:1	17:1 LPC
89	Phosphatidylcholine	lysoPhosphatidylcholine acyl C18:2	lysoPC a C18:2	17:1 LPC
90	Phosphatidylcholine	lysoPhosphatidylcholine acyl C20:3	lysoPC a C20:3	17:1 LPC
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93	Phosphatidylcholine	lysoPhosphatidylcholine acyl C26:1	lysoPC a C26:1	17:1 LPC
94	Phosphatidylcholine	lysoPhosphatidylcholine acyl C28:0	lysoPC a C28:0	13:0 LPC
95	Phosphatidylcholine	lysoPhosphatidylcholine acyl C28:1	lysoPC a C28:1	17:1 LPC
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142	Phosphatidylcholine	Phosphatidylcholine acyl-alkyl C34:2	PC ae C34:2	37:4 PC
143	Phosphatidylcholine	Phosphatidylcholine acyl-alkyl C34:3	PC ae C34:3	37:4 PC

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170	Phosphatidylcholine	Phosphatidylcholine acyl-alkyl C44:5	PC ae C44:5	37:4 PC
171	Phosphatidylcholine	Phosphatidylcholine acyl-alkyl C44:6	PC ae C44:5	37:4 PC
172	Sphingolipid	Hydroxysphingomyeline C14:1	SM (OH) C14:1	SM 12:0
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174	Sphingolipid	Sphingomyeline C16:1	SM C16:1	SM 12:0
175	Sphingolipid	Hydroxysphingomyeline C16:1	SM (OH) C16:1	SM 12:0
176	Sphingolipid	Sphingomyeline C18:0	SM C18:0	SM 12:0
177	Sphingolipid	Sphingomyeline C18:1	SM C18:1	SM 12:0
178	Sphingolipid	Sphingomyeline C20:2	SM C20:2	SM 12:0

179	Sphingolipid	Sphingomyeline C22:3	SM C22:3	SM 12:0
180	Sphingolipid	Hydroxysphingomyeline C22:1	SM (OH) C22:1	SM 12:0
181	Sphingolipid	Hydroxysphingomyeline C22:2	SM (OH) C22:2	SM 12:0
182	Sphingolipid	Sphingomyeline C24:0	SM C24:0	SM 12:0
183	Sphingolipid	Sphingomyeline C24:1	SM C24:1	SM 12:0
184	Sphingolipid	Hydroxysphingomyeline C24:1	SM (OH) C24:1	SM 12:0
185	Sphingolipid	Sphingomyeline C26:0	SM C26:0	SM 12:0
186	Sphingolipid	Sphingomyeline C26:1	SM C26:1	SM 12:0

Table S4. Fatty Acids

Target ID	Analyte	Abbreviation	Internal Standard
187	decanoic acid (capric acid)	FA C10:0	d19-decanoic acid
188	tetradecanoic acid (myristic acid)	FA C14:0	d27-myristic acid
189	hexadecanoic acid (palmitic acid)	FA C16:0	d31-palmitic acid
190	hexadecenoic acid (palmitoleic acid)	FA C16:1	d31-palmitic acid
191	octadecanoic acid (stearic acid)	FA C18:0	d35-stearic acid
192	octadecadienoic acid (linoleic acid)	FA C18:2	d35-stearic acid
193	octadecatrienoic acid (γ-linolenic acid)	FA C18:3	d5-docosahexaenoic acid
194	11, 14-eicosadienoic acid	FA C20:2	d5-docosahexaenoic acid
195	11, 14, 17-eicosatrienoic acid (eicosatrienoic acid)	FA C20:3n3	d5-docosahexaenoic acid
196	eicosatrienoic acid (dihomo-γ-linolenic acid)	FA C20:3n6	d5-docosahexaenoic acid
197	Eicosatetraenoic acid (arachidonic acid)	FA C20:4	d5-docosahexaenoic acid
198	eicosapentaenoic acid (EPA)	FA C20:5	d5-eicosapentaenoic acid
199	docosatetraenoic acid (adrenic acid)	FA C22:4	d5-docosahexaenoic acid
200	C22:5 Isomer 1 (tentatively all-*cis*-4,8,12,15,19-docosapentaenoic acid)[1]	FA C22:5n3c1	d5-eicosapentaenoic acid
201	C22:5 Isomer 2 (all-*cis*-7,10,13,16,19-docosapentaenoic acid (DPA)	FA C22:5n3c2	d5-eicosapentaenoic acid
202	C22:5 Isomer 3 (tentatively all-*cis*-4,7,10,13,16-docosapentaenoic acid)[1]	FA C22:5n6c	d5-eicosapentaenoic acid
203	docosahexaenoic acid (DHA)	FA C22:6	d5-docosahexaenoic acid

Table S5. Results of spike/recovery experiments for amino acids and biogenic amines. See Table S1 for metabolite abbreviations.

	LOW SPIKING LEVEL (n=5)				MODERATE SPIKING LEVEL (n=5)				HIGH SPIKING LEVEL (n=5)			
Metabolite	**Spike Amt. (ng)**	**Mean % Rec.**	**Stdev**	**RSD**	**Spike Amt. (ng)**	**Mean % Rec.**	**Stdev**	**RSD**	**Spike Amt. (ng)**	**Mean % Rec.**	**Stdev**	**RSD**
Ala	712.5	104	5	5	1070	104	7	7	1430	110	4	4
Arg	348.5	106	11	10	523	107	8	8	697	116	8	7
Asn	264	102	10	10	397	104	9	8	529	102	5	5
Asp	342.5	107	6	6	514	106	10	9	685	113	6	5
Cit	350.5	107	10	10	526	107	10	9	701	111	2	2
Gln	1169	105	8	8	1750	115	12	10	2340	144	15	10
Glu	748.5	109	9	8	1120	109	14	13	1500	120	6	5
Gly	750.5	112	7	6	1130	117	12	10	1500	131	8	6
His	310.5	108	7	7	466	120	12	10	621	140	8	6
Ile	262.5	79	6	7	394	83	7	9	525	94	8	9
Leu	262.5	75	7	9	394	76	5	7	525	89	5	6
Lys	585	190	12	6	877	181	8	4	1170	203	14	7
Met	298.5	106	8	8	448	110	6	5	597	111	6	5
Orn	337	128	7	5	506	131	17	13	675	133	5	4
Phe	330.5	146	12	8	496	140	6	5	661	148	9	6
Pro	460.5	131	9	7	691	127	7	6	921	129	10	7
Ser	210	98	8	8	316	98	9	9	421	101	6	6
Thr	238	106	11	11	358	106	9	8	477	115	6	5
Trp	408.5	121	7	6	613	122	9	7	817	120	4	3
Tyr	362.5	112	7	6	544	117	13	11	725	119	6	5
Val	468.5	105	7	7	703	98	8	8	937	101	5	5
Ac-Orn	35	88	5	6	52.5	101	11	11	69.5	109	7	7
ADMA	27.5	106	10	10	41.5	108	19	18	55	103	3	3
SDMA	303.5	116	11	9	456	118	19	16	607	118	13	11
Alpha-AAA	64.5	96	4	4	96.5	100	8	8	129	104	4	4
Carnosine	45	82	7	8	68	82	5	6	90.5	86	2	2
Creatinine	452.5	105	7	7	679	106	5	5	905	108	5	5
DOPA	39.5	95	6	7	59	106	13	12	79	103	10	9
Dopamine	76	100	6	6	114	105	9	9	152	107	9	8
Histamine	73.5	91	10	11	111	93	10	11	148	113	11	10
Kynurenine	83.5	86	4	5	125	92	13	14	167	96	7	8
Met-SO	66	97	13	13	99	98	6	6	132	96	6	6
Nitro-Tyr	90.5	125	12	10	136	116	18	15	181	112	10	9
OH-Pro	52.5	93	8	9	78.5	97	4	4	105	98	8	8
PEA	6.5	121	8	7	9.5	126	22	17	12.5	137	11	8
Putrescine	6.5	116	1	1	9.5	136	23	17	13	155	17	11
Sarcosine	35.5	187	62	33	53.5	163	31	19	71.5	170	45	26
Serotonin	8.5	113	11	9	13	101	15	15	17	113	7	6
Spermidine	14.5	80	11	13	22	79	5	6	29	94	6	6
Spermine	35	90	25	28	52	74	6	7	69.5	88	5	6
Taurine	125	112	13	12	188	110	22	20	251	117	12	10

Table S6. Results of spike/recovery experiments for hexose.

	LOW SPIKING LEVEL (n=5)				MODERATE SPIKING LEVEL (n=5)				HIGH SPIKING LEVEL (n=5)			
Compound	**Spike Amt. (ng)**	**Mean % Rec.**	**Stdev**	**RSD**	**Spike Amt. (ng)**	**Mean % Rec.**	**Stdev**	**RSD**	**Spike Amt. (ng)**	**Mean % Rec.**	**Stdev**	**RSD**
Hex	195	103	6	6	927	115	27	24	2080	92	12	13

Table S7. Results of spike/recovery experiments for carnitines, phosphatidylcholines, and sphingomyelins. See Table S3 for metabolite abbreviations.

	LOW SPIKING LEVEL (n=5)				MODERATE SPIKING LEVEL (n=5)				HIGH SPIKING LEVEL (n=5)			
Compounds	**Spike Amt. (ng)**	**Mean % Rec.**	**Stdev**	**RSD**	**Spike Amt. (ng)**	**Mean % Rec.**	**Stdev**	**RSD**	**Spike Amt. (ng)**	**Mean % Rec.**	**Stdev**	**RSD**
AC C0	45	101	14	14	600	94	7	8	1200	90	3	3
AC C2	15	108	18	17	200	95	8	8	400	95	2	2
AC C4	15	125	16	13	200	107	8	7	400	99	2	2
AC C6 (C4:1-DC)	15	97	10	11	200	85	6	7	400	87	2	2
AC C10	15	113	12	10	200	96	8	8	400	92	3	3
AC C16	15	109	12	11	200	95	7	8	400	91	3	4
lysoPC a C18:0	38	63	26	42	400	83	7	9	1000	81	7	9
SM C18:0	38	41	9	22	400	41	4	9	1000	40	3	7
PC aa C36:2	38	26	7	26	400	32	4	12	1000	21	3	14
PC aa C48:0	38	35	6	16	400	46	5	11	1000	46	2	5

Table S8. Results of spike/recovery experiments for fatty acids. See Table S4 for metabolite abbreviations.

	LOW SPIKING LEVEL (n=5)				MODERATE SPIKING LEVEL (n=5)				HIGH SPIKING LEVEL (n=5)			
Compounds	**Spike Amt. (ng)**	**Mean % Rec.**	**Stdev**	**RSD**	**Spike Amt. (ng)**	**Mean % Rec.**	**Stdev**	**RSD**	**Spike Amt. (ng)**	**Mean % Rec.**	**Stdev**	**RSD**
FA C10:0	24000	109	5	5	50000	95	11	12	100000	67	5	7
FA C14:0	1200	86	4	5	2500	88	5	6	5000	80	3	4
FA C16:0	600	67	18	27	1250	77	13	17	2500	78	10	13
FA C16:1	1200	132	5	4	2500	110	3	3	5000	80	7	9
FA C18:0	1200	121	16	13	2500	92	4	4	5000	80	3	4
FA C18:2	1200	70	7	10	2500	212	8	4	5000	96	14	14
FA C18:3	1200	108	4	4	2500	104	3	3	5000	87	2	2
FA C20:2	1200	126	3	3	2500	129	5	4	5000	108	3	2
FA C20:3n3	1200	135	6	4	2500	146	15	10	5000	120	5	4
FA C20:3n6	1200	129	3	2	2500	144	12	9	5000	118	2	2
FA C20:4	600	122	8	6	1250	112	5	4	2500	98	2	2
FA C20:5	1200	87	8	10	2500	90	4	5	5000	79	3	4
FA C22:4	1200	139	5	4	2500	149	11	8	5000	135	4	3
FA C22:5n3c2	1200	125	1	1	2500	143	12	9	5000	111	2	2
FA C22:6	1200	85	1	1	2500	74	6	8	5000	58	4	8

Table S9. Results of extraction efficiency experiments for amino acids and biogenic amines. See Table S1 for metabolite abbreviations. ND=non-detect. NA=Not applicable due to lack of detection in any extract.

Targets	% extracted in Extraction 1	Extraction 1 (3× MeOH)			Extraction 2 (3× MeOH)			Extraction 3 (3× $CHCl_3$)		
		Mean ng/g	Stdev ng/g	RSD %	Mean ng/g	Stdev ng/g	RSD %	Mean ng/g	Stdev ng/g	RSD %
Ala	100	304000	10607	3	ND	ND	ND	ND	ND	ND
Arg	100	15086	10209	68	ND	ND	ND	ND	ND	ND
Asn	100	9080	10211	112	ND	ND	ND	ND	ND	ND
Asp	93	211000	9247	4	17060	3584	21	ND	ND	ND
Cit	100	10788	9195	85	ND	ND	ND	ND	ND	ND
Gln	100	32532	33695	104	ND	ND	ND	ND	ND	ND
Glu	97	695400	12341	2	21140	12688	60	ND	ND	ND
Gly	100	178600	15209	9	ND	ND	ND	ND	ND	ND
His	100	40920	7689	19	ND	ND	ND	ND	ND	ND
Ile	100	47140	4874	10	ND	ND	ND	ND	ND	ND
Leu	100	98820	11035	11	ND	ND	ND	ND	ND	ND
Lys	NA	ND	ND	ND	ND	ND	ND	ND	ND	ND
Met	100	50160	6252	12	ND	ND	ND	ND	ND	ND
Orn	NA	ND	ND	ND	ND	ND	ND	ND	ND	ND
Phe	100	26640	12815	48	ND	ND	ND	ND	ND	ND
Pro	100	70120	17031	24	ND	ND	ND	ND	ND	ND
Ser	98	119400	7301	6	2818	2441	87	ND	ND	ND
Thr	99	124200	11189	9	1496	2001	134	ND	ND	ND
Trp	100	15742	13418	85	ND	ND	ND	ND	ND	ND
Tyr	100	78500	6696	9	ND	ND	ND	ND	ND	ND
Val	100	39120	13405	34	ND	ND	ND	ND	ND	ND
Ac-Orn	NA	ND	ND	ND	ND	ND	ND	ND	ND	ND
ADMA	100	1123	627	56	ND	ND	ND	ND	ND	ND
SDMA	NA	ND	ND	ND	ND	ND	ND	ND	ND	ND
alpha-AAA	100	3082	1306	42	ND	ND	ND	ND	ND	ND
Carnosine	NA	ND	ND	ND	ND	ND	ND	ND	ND	ND
Creatinine	NA	ND	ND	ND	ND	ND	ND	ND	ND	ND
DOPA	NA	ND	ND	ND	ND	ND	ND	ND	ND	ND
Dopamine	NA	ND	ND	ND	ND	ND	ND	ND	ND	ND
Histamine	NA	ND	ND	ND	ND	ND	ND	ND	ND	ND
Kynurenine	NA	ND	ND	ND	ND	ND	ND	ND	ND	ND
Met-SO	100	16600	1185	7	ND	ND	ND	ND	ND	ND
Nitro-Tyr	NA	ND	ND	ND	ND	ND	ND	ND	ND	ND
OH-Pro	NA	ND	ND	ND	ND	ND	ND	ND	ND	ND
PEA	NA	ND	ND	ND	ND	ND	ND	ND	ND	ND
Putrescine	59	15980	1417	9	1160	402	35	10132	4155	41
Sarcosine	96	55040	31653	58	2525	1489	59	ND	ND	ND
Serotonin	NA	ND	ND	ND	ND	ND	ND	ND	ND	ND
Spermidine	NA	ND	ND	ND	ND	ND	ND	5306	2727	51
Spermine	NA	ND	ND	ND	ND	ND	ND	15316	8194	53
Taurine	95	464200	30483	7	24600	6669	27	ND	ND	ND

Table S10. Results of extraction efficiency experiments for hexose.

Targets	% extracted in Extraction 1	Extraction 1 (3× MeOH)			Extraction 2 (3× MeOH)			Extraction 3 (3× $CHCl_3$)		
		Mean ng/g	Stdev ng/g	RSD %	Mean ng/g	Stdev ng/g	RSD %	Mean ng/g	Stdev ng/g	RSD %
Hex	97	6552000	895835	14	169800	20933	12	ND	ND	ND

Table S11. Results of extraction efficiency experiments for carnitines, glycerophosopholipids, and sphingomyelins. See Table S3 for metabolite abbreviations. The final method used only the primary extraction procedure. ND=non-detect. NA=Not applicable due to lack of detection in any extract.

Targets	% extracted in Extraction 1	Primary Extraction (3× MeOH)			Secondary Extraction (3× MeOH)			Tertiary Extraction (3× $CHCl_3$)		
		Mean ng/g	Stdev ng/g	RSD %	Mean ng/g	Stdev ng/g	RSD %	Mean ng/g	Stdev ng/g	RSD %
AC C0	98	44	2	6	0.80	0	29	ND	ND	ND
AC C10	NA	ND	ND	ND	ND	ND	ND	ND	ND	ND
AC C10:1	NA	ND	ND	ND	ND	ND	ND	ND	ND	ND
AC C10:2	100	0.02	0	57	ND	ND	ND	ND	ND	ND
AC C12	NA	ND	ND	ND	ND	ND	ND	ND	ND	ND
AC C12:1	NA	ND	ND	ND	ND	ND	ND	ND	ND	ND
AC C12-DC	NA	ND	ND	ND	ND	ND	ND	ND	ND	ND
AC C14	NA	ND	ND	ND	ND	ND	ND	ND	ND	ND
AC C14:1	NA	ND	ND	ND	ND	ND	ND	ND	ND	ND
AC C14:1-OH	NA	ND	ND	ND	ND	ND	ND	ND	ND	ND
AC C14:2	NA	ND	ND	ND	ND	ND	ND	ND	ND	ND
AC C14:2-OH	NA	ND	ND	ND	ND	ND	ND	ND	ND	ND
AC C16	100	0.38	0	114	ND	ND	ND	ND	ND	ND
AC C16:1	NA	ND	ND	ND	ND	ND	ND	ND	ND	ND
AC C16:1-OH	NA	ND	ND	ND	ND	ND	ND	ND	ND	ND
AC C16:2	100	0.03	0	47	ND	ND	ND	ND	ND	ND
AC C16:2-OH	NA	ND	ND	ND	ND	ND	ND	ND	ND	ND
AC C16-OH	NA	ND	ND	ND	ND	ND	ND	ND	ND	ND
AC C18	NA	ND	ND	ND	ND	ND	ND	ND	ND	ND
AC C18:1	NA	ND	ND	ND	ND	ND	ND	ND	ND	ND
AC C18:1-OH	NA	ND	ND	ND	ND	ND	ND	ND	ND	ND
AC C18:2	NA	ND	ND	ND	ND	ND	ND	ND	ND	ND
AC C2	NA	ND	ND	ND	ND	ND	ND	ND	ND	ND
AC C3	NA	ND	ND	ND	ND	ND	ND	ND	ND	ND
AC C3:1	NA	ND	ND	ND	ND	ND	ND	ND	ND	ND
AC C3-OH	NA	ND	ND	ND	ND	ND	ND	ND	ND	ND
AC C4	NA	ND	ND	ND	ND	ND	ND	ND	ND	ND
AC C4:1	NA	ND	ND	ND	ND	ND	ND	ND	ND	ND
AC C4-OH (C3-DC)	NA	ND	ND	ND	ND	ND	ND	ND	ND	ND

AC C5	NA	ND	ND	ND	ND	ND	ND	ND	ND	ND
AC C5:1	NA	ND	ND	ND	ND	ND	ND	ND	ND	ND
AC C5:1-DC	NA	ND	ND	ND	ND	ND	ND	ND	ND	ND
AC C5-DC (C6-OH)	NA	ND	ND	ND	ND	ND	ND	ND	ND	ND
AC C5-M-DC	NA	ND	ND	ND	ND	ND	ND	ND	ND	ND
AC C5-OH (C3-DC-M)	NA	ND	ND	ND	ND	ND	ND	ND	ND	ND
AC C6 (C4:1-DC)	NA	ND	ND	ND	ND	ND	ND	ND	ND	ND
AC C6:1	NA	ND	ND	ND	ND	ND	ND	ND	ND	ND
AC C7-DC	NA	ND	ND	ND	ND	ND	ND	ND	ND	ND
AC C8	NA	ND	ND	ND	ND	ND	ND	ND	ND	ND
AC C9	NA	ND	ND	ND	ND	ND	ND	ND	ND	ND
lysoPC a C14:0	98	16	1	7	0.27	0	15	ND	ND	ND
lysoPC a C16:0	98	281	15	5	4.36	1	16	0.38	0.13	34
lysoPC a C16:1	98	15	1	7	0.29	0	23	0.01	0.02	209
lysoPC a C17:0	95	9	1	10	0.34	0	15	0.15	0.11	74
lysoPC a C18:0	99	58	5	8	0.82	0	19	ND	ND	ND
lysoPC a C18:1	98	136	12	9	2.45	1	29	0.10	0.10	103
lysoPC a C18:2	98	9	1	14	0.16	0	24	0.04	0.04	124
lysoPC a C20:3	NA	ND	ND	ND	ND	ND	ND	ND	ND	ND
lysoPC a C20:4	98	40	6	15	0.73	0	30	0.02	0.02	76
lysoPC a C24:0	NA	ND	ND	ND	ND	ND	ND	ND	ND	ND
lysoPC a C26:1	NA	ND	ND	ND	ND	ND	ND	ND	ND	ND
lysoPC a C28:0	NA	ND	ND	ND	ND	ND	ND	ND	ND	ND
lysoPC a C28:1	97	7.6	3.2	42	0.19	0	24	0.03	0.02	79
PC aa C24:0	NA	ND	ND	ND	ND	ND	ND	ND	ND	ND
PC aa C26:0	NA	ND	ND	ND	ND	ND	ND	ND	ND	ND
PC aa C28:1	NA	ND	ND	ND	ND	ND	ND	ND	ND	ND
PC aa C30:0	94	17	1	9	0.80	0	32	0.35	0.22	64
PC aa C30:2	NA	ND	ND	ND	ND	ND	ND	ND	ND	ND
PC aa C32:0	94	16	2	12	0.62	0	34	0.51	0.27	52
PC aa C32:1	100	18	5	29	ND	ND	ND	ND	ND	ND
PC aa C32:2	NA	ND	ND	ND	ND	ND	ND	ND	ND	ND
PC aa C32:3	NA	ND	ND	ND	ND	ND	ND	ND	ND	ND
PC aa C34:1	100	139	29	21	ND	ND	ND	ND	ND	ND
PC aa C34:2	100	18	4	22	ND	ND	ND	ND	ND	ND
PC aa C34:3	100	4	1	24	ND	ND	ND	ND	ND	ND

PC aa C34:4	NA	ND	ND	ND	ND	ND	ND	ND	ND	ND
PC aa C36:0	NA	ND	ND	ND	ND	ND	ND	ND	ND	ND
PC aa C36:1	100	49	11	23	ND	ND	ND	ND	ND	ND
PC aa C36:2	100	40	8	20	ND	ND	ND	ND	ND	ND
PC aa C36:3	100	8	2	19	ND	ND	ND	ND	ND	ND
PC aa C36:4	97	30	2	7	0.85	1	87	ND	ND	ND
PC aa C36:5	96	64	12	19	2.44	2	69	ND	ND	ND
PC aa C36:6	100	11	2	17	ND	ND	ND	ND	ND	ND
PC aa C38:0	98	19	6	31	0.34	0	25	ND	ND	ND
PC aa C38:1	96	7	2	21	0.33	0	151	ND	ND	ND
PC aa C38:3	96	9	3	33	0.36	0	120	ND	ND	ND
PC aa C38:4	91	19	1	7	1.29	1	42	0.61	0.49	80
PC aa C38:5	100	63	11	18	ND	ND	ND	ND	ND	ND
PC aa C38:6	98	136	35	26	3.26	3	88	ND	ND	ND
PC aa C40:1	NA	ND	ND	ND	ND	ND	ND	ND	ND	ND
PC aa C40:2	NA	ND	ND	ND	ND	ND	ND	ND	ND	ND
PC aa C40:3	NA	ND	ND	ND	ND	ND	ND	ND	ND	ND
PC aa C40:4	NA	ND	ND	ND	ND	ND	ND	ND	ND	ND
PC aa C40:5	99	20	2	10	0.23	0	136	ND	ND	ND
PC aa C40:6	98	75	20	27	1.69	1	86	ND	ND	ND
PC aa C42:0	NA	ND	ND	ND	ND	ND	ND	ND	ND	ND
PC aa C42:1	NA	ND	ND	ND	ND	ND	ND	ND	ND	ND
PC aa C42:2	NA	ND	ND	ND	ND	ND	ND	ND	ND	ND
PC aa C42:4	NA	ND	ND	ND	ND	ND	ND	ND	ND	ND
PC aa C42:5	NA	ND	ND	ND	ND	ND	ND	ND	ND	ND
PC aa C42:6	NA	ND	ND	ND	ND	ND	ND	ND	ND	ND
PC aa C48:0	NA	ND	ND	ND	ND	ND	ND	ND	ND	ND
PC ae C30:0	NA	ND	ND	ND	ND	ND	ND	ND	ND	ND
PC ae C30:1	NA	ND	ND	ND	ND	ND	ND	ND	ND	ND
PC ae C30:2	NA	ND	ND	ND	ND	ND	ND	ND	ND	ND
PC ae C32:1	97	5	3	59	0.18	0	256	ND	ND	ND
PC ae C32:2	NA	ND	ND	ND	ND	ND	ND	ND	ND	ND
PC ae C34:0	NA	ND	ND	ND	ND	ND	ND	ND	ND	ND
PC ae C34:1	100	9	3	30	ND	ND	ND	ND	ND	ND
PC ae C34:2	NA	ND	ND	ND	ND	ND	ND	ND	ND	ND
PC ae C34:3	NA	ND	ND	ND	ND	ND	ND	ND	ND	ND

PC ae C36:0	96	18	2	10	0.42	0	58	0.25	0.10	40
PC ae C36:1	100	13	4	31	ND	ND	ND	ND	ND	ND
PC ae C36:2	96	7	2	28	0.29	0	82	ND	ND	ND
PC ae C36:3	NA	ND	ND	ND	ND	ND	ND	ND	ND	ND
PC ae C36:4	NA	ND	ND	ND	ND	ND	ND	ND	ND	ND
PC ae C36:5	99	7	0	4	0.08	0	480	ND	ND	ND
PC ae C38:0	99	33	15	45	0.31	0	46	0.14	0.10	71
PC ae C38:1	98	12	2	20	0.25	0	152	ND	ND	ND
PC ae C38:2	NA	ND	ND	ND	ND	ND	ND	ND	ND	ND
PC ae C38:3	NA	ND	ND	ND	ND	ND	ND	ND	ND	ND
PC ae C38:5	96	10	1	10	0.47	0	83	ND	ND	ND
PC ae C38:6	98	14	2	11	0.29	0	114	ND	ND	ND
PC ae C40:1	NA	ND	ND	ND	ND	ND	ND	ND	ND	ND
PC ae C40:2	NA	ND	ND	ND	ND	ND	ND	ND	ND	ND
PC ae C40:3	NA	ND	ND	ND	ND	ND	ND	ND	ND	ND
PC ae C40:4	91	7	1	10	0.67	0	25	0.09	0.08	93
PC ae C40:5	NA	ND	ND	ND	ND	ND	ND	ND	ND	ND
PC ae C40:6	95	17	2	11	0.76	0	56	0.20	0.34	170
PC ae C42:0	NA	ND	ND	ND	ND	ND	ND	ND	ND	ND
PC ae C42:1	NA	ND	ND	ND	ND	ND	ND	ND	ND	ND
PC ae C42:2	NA	ND	ND	ND	ND	ND	ND	ND	ND	ND
PC ae C42:3	97	12	1	12	0.32	1	178	ND	ND	ND
PC ae C42:4	95	11	2	16	0.56	0	68	ND	ND	ND
PC ae C42:5	NA	ND	ND	ND	ND	ND	ND	ND	ND	ND
PC ae C44:3	NA	ND	ND	ND	ND	ND	ND	ND	ND	ND
PC ae C44:4	94	8	1	15	0.47	0	53	ND	ND	ND
PC ae C44:5	97	7	2	26	0.23	0	112	ND	ND	ND
PC ae C44:6	NA	ND	ND	ND	ND	ND	ND	ND	ND	ND
SM (OH) C14:1	NA	ND	ND	ND	ND	ND	ND	ND	ND	ND
SM (OH) C16:1	NA	ND	ND	ND	ND	ND	ND	ND	ND	ND
SM (OH) C22:1	NA	ND	ND	ND	ND	ND	ND	ND	ND	ND
SM (OH) C22:2	NA	ND	ND	ND	ND	ND	ND	ND	ND	ND
SM (OH) C24:1	NA	ND	ND	ND	ND	ND	ND	ND	ND	ND
SM C16:0	91	5	0	5	0.22	0	40	0.28	0.21	77
SM C16:1	NA	ND	ND	ND	ND	ND	ND	ND	ND	ND
SM C18:0	72	3	1	36	0.14	0	122	0.87	0.75	86

SM C18:1	NA	ND	ND	ND	ND	ND	ND	ND	ND	ND
SM C20:2	NA	ND	ND	ND	ND	ND	ND	ND	ND	ND
SM C22:3	NA	ND	ND	ND	ND	ND	ND	ND	ND	ND
SM C24:0	98	20	1	6	0.30	0	45	0.12	0.09	77
SM C24:1	98	45	3	6	0.65	0	39	0.37	0.19	52
SM C26:0	NA	ND	ND	ND	ND	ND	ND	ND	ND	ND
SM C26:1	100	1	0	45	ND	ND	ND	ND	ND	ND

Table S12. Results of extraction efficiency experiments for fatty acids. See Table S4 for metabolite abbreviations. ND=non-detect. NA=Not applicable due to lack of detection in any extract.

Targets	% extracted in Extraction 1	Extraction 1 (3× MeOH)			Extraction 2 (3× MeOH)			Extraction 3 (3× $CHCl_3$)		
		Mean ng/g	Stdev ng/g	RSD %	Mean ng/g	Stdev ng/g	RSD %	Mean ng/g	Stdev ng/g	RSD %
FA C10:0	NA	ND	ND	ND	ND	ND	ND	7980	ND	ND
FA C14:0	95	487800	17196	4	14240	586	4	10576	947	9
FA C16:0	94	2974000	129538	4	37000	ND	ND	149600	19087	13
FA C16:1	96	887600	31254	4	25240	991	4	15080	736	5
FA C18:0	90	1184000	82341	7	14208	19385	136	124240	33737	27
FA C18:2	100	1136600	110760	10	ND	ND	ND	ND	ND	ND
FA C18:3	100	24300	2299	9	ND	ND	ND	ND	ND	ND
FA C20:2	100	139200	12337	9	ND	ND	ND	ND	ND	ND
FA C20:3n3	100	13826	7184	52	ND	ND	ND	ND	ND	ND
FA C20:3n6	100	24400	2946	12	ND	ND	ND	ND	ND	ND
FA C20:4	100	452600	21927	5	940	724	77	ND	ND	ND
FA C20:5	99	3554000	98133	3	26360	3755	14	ND	ND	ND
FA C22:4	100	18600	2042	11	ND	ND	ND	ND	ND	ND
FA C22:5n3c1	99	4448000	179221	4	39100	8315	21	ND	ND	ND
FA C22:5n3c2	99	693000	40137	6	5722	163	3	ND	ND	ND
FA C22:5n6c	100	40160	3268	8	ND	ND	ND	ND	ND	ND
FA C22:6	99	4274000	230825	5	41880	3508	8	ND	ND	ND