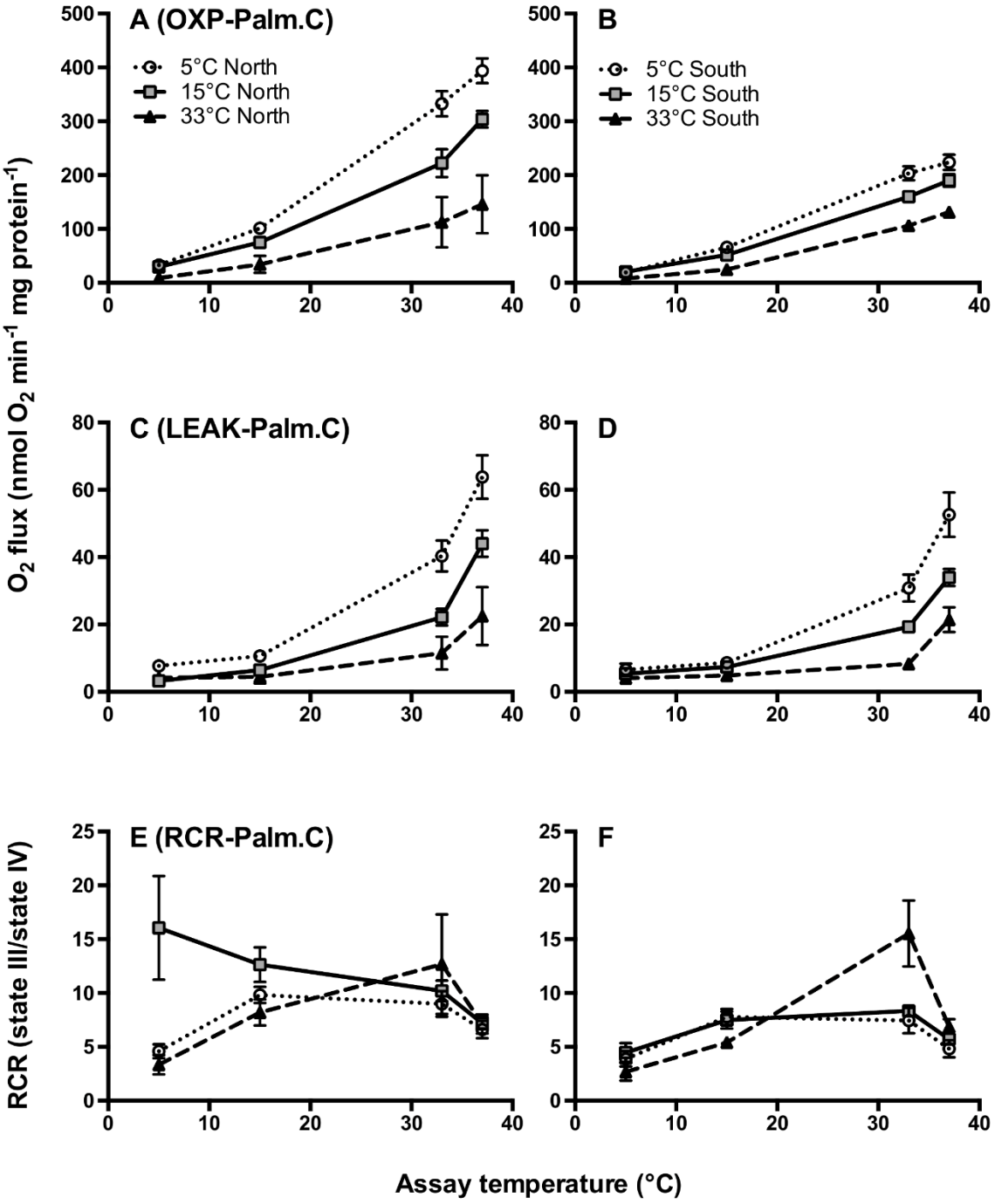

Figure S1. *Fundulus heteroclitus* coupled liver mitochondrial respiration and respiratory control ratio fuelled with fatty-acid substrates. Northern (A, C, E) and southern (B, D, F) *F. heteroclitus* were acclimated to 5, 15 or 33°C for four weeks. Oxidative phosphorylation (OXP, state 3; A, B) and LEAK (state 2; C, D) respiration rate were measured with fatty-acid substrates (palmitoyl carnitine and malate). Respiratory control ratio (E, F) was calculated as the ratio of OXP/LEAK. Data are mean ± SEM; see Table 1 for associated statistics (n = 7-8).

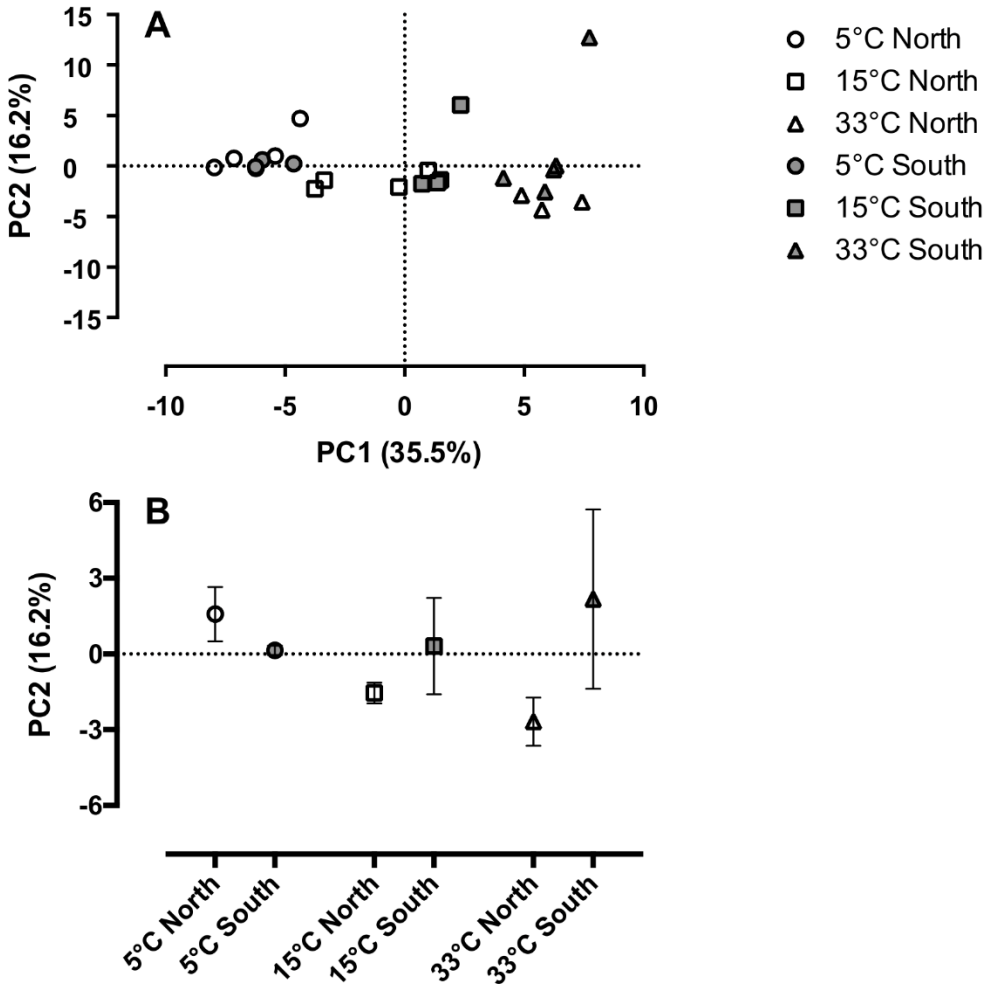

Figure S2. Second principal component score for liver mitochondrial membrane composition of thermally acclimated *Fundulus heteroclitus* subspecies. Northern (white symbols) and southern (grey symbols) were acclimated to 5, 15, or 33°C for four weeks. (A) the plot of individuals along PC1 and PC2 axes. PC2 did not significantly separate subspecies of thermal acclimation treatment (B). See the Results section for associated statistics, $n = 4$.

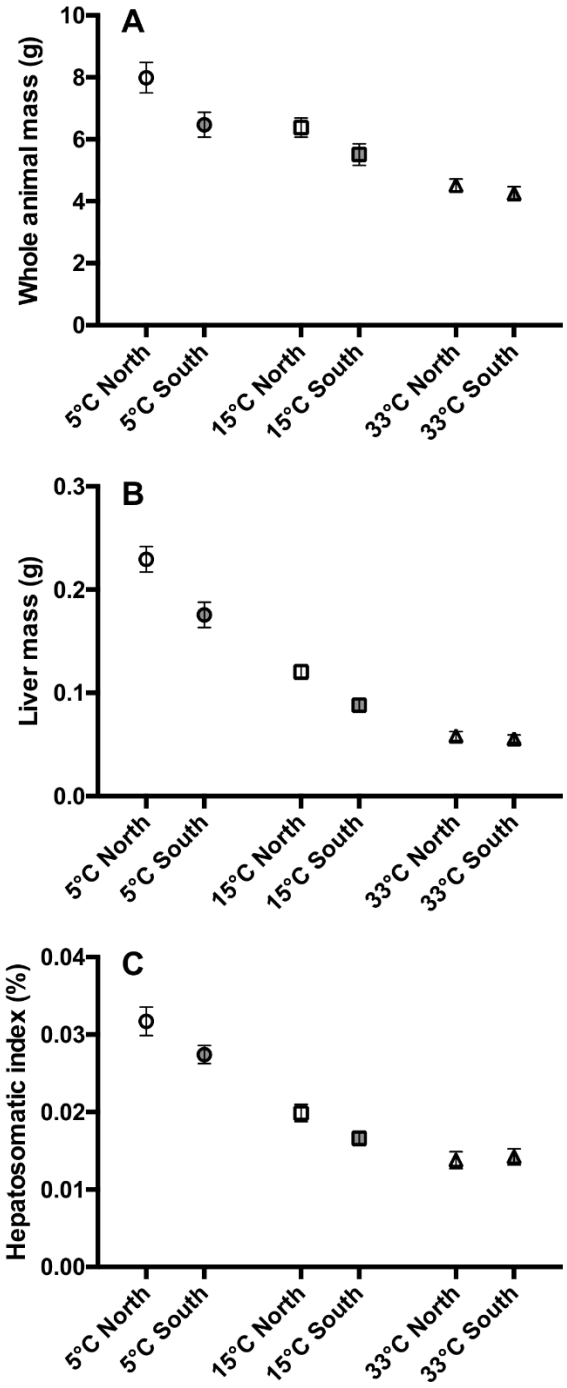

Figure S3. *Fundulus heteroclitus* whole-animal, liver wet mass, and somatic index. Northern and southern *F. heteroclitus* were acclimated to 5, 15, or 33°C for four weeks. Hepatosomatic index (C) was calculated as the ratio of wet liver mass to whole-animal mass. Data are mean ± SEM; see Results for associated statistics (n = 49-56).

Supplemental Table 1. Principal component analysis factor scores, percent contribution (Ctr) of individual variables to the components, and the squared cosines of the variables on principal components 1 and 3.

	F_1	F_3	Ctr_1	Ctr_3	Cos_1^2	Cos_3^2
CL	0.42	-0.24	0.67	0.80	17.6	5.6
Monolyso-CL	0.83	-0.16	**2.63**	0.39	69.5	2.7
PE	0.59	-0.56	**1.33**	**4.49**	35.1	31.7
PC	-0.35	0.75	0.45	**7.88**	12.0	55.6
CL_1402	0.74	0.29	**2.09**	1.20	55.1	8.5
CL_1404	0.91	0.10	**3.11**	0.15	82.0	1.0
CL_1424	-0.76	0.26	**2.17**	0.98	57.2	6.9
CL_1428	0.85	-0.04	**2.76**	0.02	73.0	0.2
CL_1448	-0.77	0.20	**2.26**	0.54	59.8	3.8
CL_1450	0.59	-0.04	**1.33**	0.02	35.1	0.2
CL_1452	0.75	-0.16	**2.14**	0.38	56.5	2.7
CL_1472	-0.75	0.37	**2.13**	**1.94**	56.2	13.7
CL_1476	0.81	0.36	**2.49**	**1.83**	65.6	12.9
CL_1496	-0.89	0.03	**2.98**	0.01	78.5	0.1
CL_1498	0.06	-0.85	0.02	**10.13**	0.4	71.4
CL_1500	0.88	0.19	**2.92**	0.49	77.2	3.4
CL_1520	0.66	-0.05	**1.66**	0.04	43.8	0.3
CL_1522	0.88	0.29	**2.96**	1.19	78.2	8.4
CL_1544	-0.72	-0.26	**1.95**	0.94	51.5	6.7
CL_1546	0.72	0.00	**1.94**	0.00	51.1	0.0
CL_1566	-0.74	0.08	**2.10**	0.10	55.3	0.7
CL_1568	-0.04	0.32	0.01	**1.44**	0.2	10.2
MLCL_1164	-0.22	0.59	0.19	**4.87**	5.0	34.4
MLCL_1166	-0.36	0.34	0.49	**1.64**	13.1	11.5
MLCL_1186	-0.81	0.07	**2.50**	0.07	66.0	0.5
MLCL_1188	-0.01	-0.15	0.00	0.30	0.0	2.1
MLCL_1190	0.05	0.19	0.01	0.49	0.3	3.5
MLCL_1210	0.29	0.42	0.32	**2.51**	8.4	17.7
MLCL_1212	0.47	0.49	0.85	**3.38**	22.4	23.8
MLCL_1234	-0.50	-0.52	0.95	**3.78**	25.1	26.7
MLCL_1236	0.90	-0.18	**3.04**	0.44	80.2	3.1
MLCL_1256	-0.23	0.10	0.19	0.15	5.1	1.0
MLCL_1258	0.71	0.29	**1.89**	1.23	49.9	8.7
MLCL_1282	-0.10	-0.42	0.04	**2.53**	0.9	17.8
PC_14:0	-0.08	0.21	0.02	0.65	0.7	4.6
PC_16:0	0.46	0.28	0.80	1.12	21.2	7.9
PC_16:1	0.80	-0.19	**2.40**	0.49	63.4	3.5
PC_18:0	-0.70	0.36	**1.85**	**1.86**	48.8	13.1
PC_18:1n9	0.25	0.04	0.24	0.03	6.3	0.2
PC_18:1n7	0.83	0.15	**2.63**	0.33	69.4	2.3

Supplemental Table 1. Principal component analysis factor scores, percent contribution (Ctr) of individual variables to the components, and the squared cosines of the variables on principal components 1 and 3.

	F_1	F_3	Ctr_1	Ctr_3	Cos_1^2	Cos_3^2
PC_18:2n6	0.09	-0.58	0.03	**4.76**	0.8	33.6
PC_18:3n6	-0.24	-0.08	0.21	0.08	5.6	0.6
PC_18:3n3	-0.26	-0.10	0.25	0.15	6.5	1.1
PC_20:0	-0.37	-0.11	0.51	0.16	13.5	1.1
PC_20:1	-0.14	-0.09	0.08	0.11	2.0	0.8
PC_20:2	-0.31	-0.16	0.36	0.35	9.5	2.5
PC_20:4n6	0.25	-0.16	0.25	0.37	6.5	2.6
PC_20:3n6	-0.51	0.00	1.00	0.00	26.4	0.0
PC_20:3n3	-0.48	-0.25	0.89	0.86	23.5	6.1
PC_20:5n3	0.22	-0.34	0.19	1.67	4.9	11.8
PC_22:0	-0.60	0.08	**1.36**	0.10	35.9	0.7
PC_22:1	-0.46	-0.07	0.82	0.07	21.5	0.5
PC_22:4	-0.31	-0.40	0.36	**2.31**	9.6	16.3
PC_22:5n3	-0.40	0.05	0.60	0.03	15.8	0.2
PC_22:6n3	0.72	0.07	**1.96**	0.08	51.7	0.5
PE_14:0	-0.81	0.06	**2.47**	0.05	65.2	0.3
PE_16:0	-0.75	-0.03	**2.13**	0.02	56.2	0.1
PE_16:1	-0.25	0.56	0.23	**4.47**	6.1	31.5
PE_18:0	-0.53	0.12	1.07	0.21	28.2	1.5
PE_18:1n9	0.73	0.34	**2.04**	**1.68**	53.8	11.9
PE_18:1n7	0.18	-0.42	0.12	**2.46**	3.1	17.4
PE_18:2n6	0.60	-0.49	**1.34**	**3.43**	35.5	24.2
PE_18:3n6	-0.35	0.31	0.46	**1.38**	12.2	9.7
PE_18:3n3	0.58	0.18	1.29	0.44	34.1	3.1
PE_20:0	0.19	-0.25	0.13	0.88	3.5	6.2
PE_20:1	0.73	0.16	**2.00**	0.35	52.8	2.5
PE_20:2	0.67	0.45	**1.71**	**2.82**	45.2	19.9
PE_20:4n6	-0.70	-0.26	**1.84**	0.99	48.5	7.0
PE_20:3n6	0.84	0.36	**2.69**	**1.84**	70.9	12.9
PE_20:3n3	-0.39	0.47	0.57	**3.16**	15.2	22.3
PE_20:5n3	0.69	0.11	**1.81**	0.17	47.8	1.2
PE_22:0	-0.82	0.21	**2.53**	0.65	66.7	4.6
PE_22:1	-0.42	-0.29	0.68	**1.16**	18.0	8.2
PE_22:4	0.72	-0.04	**1.98**	0.02	52.1	0.2
PE_22:5n3	-0.71	-0.10	**1.91**	0.14	50.3	1.0
PE_22:6n3	0.65	-0.35	**1.61**	**1.75**	42.5	12.4

The important contributions (Ctr) are in bold, indicating a greater than average variable contribution to the respective principal component (i.e., Ctr > $\frac{1}{76}$ or 1.31%). CL: cardiolipin; MLCL: monolysocardiolipin; PC: phosphatidylcholine; PE: phosphatidylethanolamine. Squared cosines have been multiplied by 100 for ease of interpretation. $n = 4$

Supplemental Table 2. Hepatic mitochondrial phospholipid class distribution and cardiolipin molecular species from thermally acclimated *Fundulus heteroclitus* subspecies.

Mitochondrial PL classes	5°C		15°C		33°C	
	North	South	North	South	North	South
CL	27.8±1.15	24.3±2.02	24.7±.93	21.3±.60	22.0±.77	22.4±2.11
MLCL (*†‡)	3.2±.28	1.7±.26	1.6±.12	9.7±.05	7.4±.09	7.2±.02
PE (†‡)	31.0±.83	24.6±1.21	30.0±1.47	30.6±.33	33.2±.99	28.2±1.39
PC (*‡)	41.1±.89	51.0±.90	45.1±1.92	48.0±.75	44.7±.68	49.3±1.08
PC/PE (*‡)	1.3±.04	2.1±.07	1.5±.14	1.6±.04	1.4±.05	1.8±.08
CL Molecular species						
CL_1402 (*)	0.9±.11	1.1±.11	0.5±.06	0.9±.19	0.1±.05	0.05±.01
CL_1404 (*)	$1.6±1.1x10^{-3}$	$1.4±6.7x10^{-4}$	$0.7±9.2x10^{-4}$	$0.9±2.2x10^{-3}$	$0.01±2.3x10^{-5}$	$0.03±1.3x10^{-4}$
CL_1424 (*‡)	0.6±.05	0.9±.04	0.9±.12	1.6±.16	1.7±.13	1.4±.20
CL_1428 (*)	3.3±.17	2.2±.19	1.7±.22	2.2±.39	0.2±.05	0.2±.03
CL_1448 (*)	2.4±.04	4.0±.11	4.0±.46	6.5±.35	9.6±1.08	7.7±1.93
CL_1450 (*)	5.6±.31	6.3±.33	6.3±.34	6.2±.62	3.7±.58	3.7±.82
CL_1452 (*)	4.4±.24	3.6±.19	4.2±.55	4.2±.26	1.7±.33	1.7±.24
CL_1472 (*‡)	5.7±.38	6.6±.55	6.0±.38	9.3±1.05	9.9±.24	8.7±.27
CL_1476 (*†)	6.6±.76	8.9±.25	4.0±.24	5.3±.21	2.3±.28	2.6±.32
CL_1496 (*)	6.7±.50	7.7±.36	11.8±1.47	13.9±1.12	23.2±.94	18.3±2.37
CL_1498 (*‡)	10.9±.23	7.9±.34	14.4±.56	9.6±.97	8.2±.95	11.7±.79
CL_1500 (*)	7.8±.33	9.1±.52	6.4±.27	6.7±.53	3.2±.35	3.8±.65
CL_1520 (*)	8.2±.28	7.0±.25	6.9±.21	6.7±.71	5.4±.58	5.4±.69
CL_1522 (*†)	4.8±.21	6.4±.08	2.5±.21	2.7±.24	1.0±.41	1.5±.32
CL_1544 (*)	11.8±.72	6.8±.58	15.9±.96	9.5±.84	21.9±2.06	24.4±3.97
CL_1546 (*)	6.6±.49	6.4±.25	5.3±.35	3.8±.96	3.5±.47	4.3±1.05
CL_1566 (*)	1.0±.14	0.68±.11	1.0±.14	1.0±.13	2.6±.37	2.2±.44
CL_1568	0.7±.11	1.1±.13	0.6±.10	0.7±.12	0.8±.32	1.0±.16
MLCL_1164 (*)	4.4±.53	6.1±.52	3.4±.27	8.3±.57	5.6±.95	5.8±.95
MLCL_1166	2.8±.39	4.1±.33	2.3±.69	3.5±.45	4.2±.29	4.0±1.20
MLCL_1186 (*)	5.4±.37	8.9±.30	9.6±1.54	13.4±.14	17.4±2.78	16.0±.17
MLCL_1188 (*)	7.9±.46	8.4±.88	11.9±.97	10.7±.27	9.5±1.21	6.8±1.95
MLCL_1190	6.0±.42	7.0±.47	5.4±.71	6.4±1.51	5.8±.56	6.1±1.86
MLCL_1210	10.5±1.05	11.4±.62	8.2±.49	10.2±.53	8.8±1.36	9.7±.85
MLCL_1212 (*)	7.6±.77	9.3±.84	3.8±.66	5.7±1.15	5.4±.81	5.5±.90
MLCL_1234 (*)	14.2±.78	12.2±1.54	20.9±.99	13.5±1.17	19.8±2.83	20.8±2.79
MLCL_1236 (*†)	17.0±1.06	13.7±1.38	14.7±2.08	9.5±.77	5.7±.69	5.2±.73
MLCL_1256	4.8±1.19	3.9±.33	4.9±.85	6.2±1.33	5.7±1.00	5.2±1.45
MLCL_1258 (*)	8.0±.48	6.9±.65	3.3±1.15	5.1±1.12	2.8±1.73	1.4±.20
MLCL_1282	10.8±1.65	7.5±.75	10.8±1.33	7.0±.83	8.6±1.12	13.0±2.94

Data are mean % of total head group or fatty acid ± SEM (n = 4). Symbols indicate significant effects from a two-way ANOVA *: acclimation; †: subspecies; ‡: acclimation x subspecies. The number following CL or MLCL corresponds to the MW of the PL species and corresponds to the FA chains as indicated below. CL: cardiolipin; MLCL: monolysocardiolipin; PC: phosphatidylcholine; PE: phosphatidylethanolamine. PC\PE: the ratio of phosphatidylcholine to phosphatidylethanolamine.

Supplemental Table 3. Fatty acid composition of cardiolipin and monolysocardiolipin molecular species determined by HPLC-ESI-MS/MS

CL Molecular Species (m/z)	16:0	16:1	18:1	18:2	18:3	20:4	22:6
CL_1402	1	1	2				
CL_1404	2		2				
CL_1424		1	1	2			
CL_1428		1	3				
CL_1448				4			
CL_1450			1	3			
CL_1452			2	2			
CL_1472				3		1	
CL_1476			2	1		1	
CL_1496				3			1
CL_1498			1	2			1
CL_1500			2	1			1
CL_1520				2		1	1
CL_1522			1	1		1	1
CL_1544				2			2
CL_1546			1	1			2
CL_1566					1	1	2
CL_1568				1		1	2
MLCL_1164		1	2				
MLCL_1166	1		2				
MLCL_1186				3			
MLCL_1188			1	2			
MLCL_1190			2	1			
MLCL_1210				2		1	
MLCL_1212			1	1		1	
MLCL_1234				1		2	
MLCL_1236			1			2	
MLCL_1256					1	1	1
MLCL_1258				1		1	1
MLCL_1282				1			2

CL: Cardiolipin, MLCL: monolysocardiolipin, m/z: mass to charge ratio (equivalent to molecular weight). The number following CL or MLCL corresponds to the MW of the PL species and corresponds to the FA chains.

Supplemental Table 4. Total hepatic mitochondrial phospholipid FA composition from thermally acclimated *Fundulus heteroclitus* subspecies.

	5°C acclimated		15°C acclimated		33°C acclimated	
Fatty acids	North	South	North	South	North	South
14:0 (*)	0.9±.06	1.0±.05	1.0±.06	1.0±.03	1.2±.06	1.0±.06
16:0 (*)	16.7±.32	17.2±.17	17.4±.31	17.2±.34	18.3±.37	17.5±.36
16:1 (*†)	2.1±.09	1.9±.13	1.8±.09	1.4±.14	1.1±.001	0.9±.02
18:0 (*†)	9.3±.24	10.7±.09	9.8±.11	12.5±.26	13.1±.44	13.6±.58
18:1n9	12.7±.37	13.8±.19	12.8±.43	11.5±.35	12.6±.61	13.4±.75
18:1n7 (*†)	4.4±.18	3.7±.07	3.4±.11	2.4±.06	1.5±.05	1.1±.14
18:2n6 (*†)	6.8±.23	5.6±.09	7.6±.34	6.4±.19	6.6±.30	6.3±.40
18:3n6 (*)	0.21±.031	0.27±.027	0.34±.015	0.19±.037	0.18±.036	0.17±.052
18:3n3 (*)	$0.19\pm1.4\times10^{-4}$	$0.15\pm1.0\times10^{-4}$	$0.18\pm7.1\times10^{-5}$	$0.17\pm 9.1\times10^{-5}$	$0.20\pm4.5\times10^{-5}$	$0.21\pm1.5\times10^{-4}$
20:0 (*†)	0.48±.068	0.29±.014	0.39±.036	0.28±.028	0.31±.025	0.24±.041
20:1 (*)	1.3±.07	1.2±.06	1.0±.04	0.8±.04	0.6±.06	0.7±.08
20:2 (*)	1.0±.11	1.0±.07	0.9±.06	0.7±.03	0.6±.03	0.8±.12
20:4n6 (*)	6.8±.23	7.2±.24	5.8±.27	6.2±.13	5.3±.20	5.7±.28
20:3n6 (*)	0.40±.053	0.50±.040	0.35±.062	0.34±.022	0.47±.037	0.57±.105
20:3n3 (*)	0.69±.086	0.59±.041	0.72±.065	0.68±.039	0.92±.055	0.97±.149
20:5n3 (‡)	2.6±.16	1.8±.14	1.7±.12	2.0±.11	1.3±.08	1.9±.23
22:0 (*)	0.9±.07	0.8±.06	1.1±.07	1.1±.06	1.4±.12	1.4±.16
22:1 (*)	0.61±.088	0.43±.021	0.62±.046	0.57±.025	0.68±.059	0.78±.097
22:4	1.8±.09	1.6±.09	1.2±.24	1.4±.05	1.4±.20	2.1±.26
22:5n3 (*†)	9.2±.21	8.1±.38	10.3±.23	10.4±.50	12.7±.89	10.7±.64
22:6n3 (*)	20.0±.37	21.6±.46	20.2±.60	21.2±.49	18.2±.68	17.5±.82

Fatty acid data are mean % of total ± SEM (n = 7-8). Symbols indicate significant effects from a two-way ANOVA *: acclimation; †: subspecies; ‡: acclimation x subspecies.

Supplemental Table 5. Hepatic mitochondrial phosphatidylcholine (PC) fatty composition from thermally acclimated *Fundulus heteroclitus* subspecies.

	5°C		15°C		33°C	
Phosphatidylcholine (PC) fatty acids	North	South	North	South	North	South
PC_14:0	0.7±.14	0.6±.03	0.5±.16	0.7±.04	0.5±.12	0.6±.10
PC_16:0	19.7±1.99	20.4±.61	18.0±.84	17.9±1.23	18.6±.73	15.9±2.18
PC_16:1 (*)	1.8±.09	1.5±.07	1.5±.18	1.3±.14	0.7±.04	1.0±.16
PC_18:0 (†)	7.6±.77	7.5±.41	7.3±.86	10.1±.27	11.3±.64	10.1±1.26
PC_18:1n9	12.2±.41	13.2±.34	12.8±.86	11.1±.73	13.0±.74	11.3±2.51
PC_18:1n7 (*)	2.7±.20	2.9±.06	1.9±.27	1.5±.15	1.0±.06	1.5±.48
PC_18:2n6	5.7±.52	4.7±.07	6.2±.82	5.3±.31	4.9±.38	5.5±.43
PC_18:3n6	0.8±.66	0.2±.03	0.1±.04	0.8±.55	0.1±.05	1.4±.69
PC_18:3n3	0.9±.83	0.1±.003	0.1±.05	0.9±.75	0.1±.04	1.8±.93
PC_20:0	0.8±.44	0.2±.01	0.3±.15	1.3±1.12	0.3±.03	2.8±1.45
PC_20:1	1.2±.49	1.0±.07	0.7±.08	1.0±.52	0.4±.09	1.9±.63
PC_20:2	1.2±.67	0.6±.08	0.8±.24	1.1±.66	0.5±.03	2.5±1.02
PC_20:4n6	3.8±.20	3.6±.25	3.3±.32	3.6±.21	2.2±.64	3.8±.66
PC_20:3n6	0.9±.35	0.5±.02	0.7±.27	0.9±.38	1.1±.29	1.7±.58
PC_20:3n3	0.5±.20	0.2±.01	0.3±.14	0.5±.17	0.5±.22	1.2±.42
PC_20:5n3	2.1±.19	1.6±.11	2.0±.26	1.9±.28	0.9±.11	2.0±.51
PC_22:0 (*)	1.2±.19	0.8±.08	1.1±.39	1.3±.17	1.7±.30	2.1±.65
PC_22:1	0.7±.11	0.4±.05	0.6±.24	0.5±.10	0.8±.19	1.7±.83
PC_22:4	0.3±.06	0.4±.21	1.3±.51	0.8±.12	1.1±.32	0.6±.27
PC_22:5n3	11.2±1.64	9.8±0.91	12.2±3.01	11.1±1.50	18.0±1.98	11.0±0.81
PC_22:6n3 (*)	18.3±2.24	25.0±1.00	20.6±3.31	18.0±2.13	14.8±0.96	11.6±2.07

Data are mean % of total head group or fatty acid ± SEM (n = 4). Symbols indicate significant effects from a two-way ANOVA *: acclimation; †: subspecies; ‡: acclimation x subspecies.

Supplemental Table 6. Hepatic mitochondrial phosphatidylethanolamine (PE) fatty composition from thermally acclimated *Fundulus heteroclitus* subspecies.

	5°C		15°C		33°C	
Phosphatidylethanolamine (PE) fatty acids	North	South	North	South	North	South
PE_14:0 (*)	0.9± .08	0.8±.11	1.1±.11	1.6±.16	1.5±.13	1.5±.03
PE_16:0 (*)	12.7±.43	11.6±.68	13.1±.75	14.5±.35	14.9±.31	14.5±.19
PE_16:1	0.7±.10	0.6±.05	0.3±.13	0.8±.18	1.2±.41	0.6±.16
PE_18:0 (*†)	13.1±.46	15.3±0.39	13.7±1.23	16.0±0.91	15.4±0.48	17.6±0.65
PE_18:1n9 (*)	6.9±.15	6.6±.36	3.1±1.08	2.7±.14	3.3±.18	3.1±.30
PE_18:1n7	4.4±.41	3.9±.13	4.3±.63	4.3±.18	3.7±.22	4.2±.25
PE_18:2n6 (*)	2.0±.21	1.6±.11	2.2±.45	1.2±.27	1.0±.13	0.8±.05
PE_18:3n6	0.2±.04	0.1±.05	0.1±.05	0.3±.05	0.3±.02	0.3±.11
PE_18:3n3 (*)	0.4±.04	0.3±.04	0.2±.08	0.3±.06	0.2±.02	0.1±.06
PE_20:0	0.4±.01	0.3±.04	0.5±.29	0.3±.06	0.3±.07	0.1±.09
PE_20:1 (*)	1.3±.11	1.3±.10	0.7±.27	1.1±.45	0.2±.14	0.1±.10
PE_20:2 (*)	0.8±.06	1.0±.07	0.4±.16	0.4±.16	0.5±.19	0.06±.06
PE_20:4n6 (*)	0.4±.03	0.3±.03	3.4±1.08	4.7±.68	3.9±.41	3.6±.06
PE_20:3n6 (*)	6.4±.24	7.7±.44	2.1±1.21	1.4±.15	1.4±.30	0.6±.38
PE_20:3n3 (*)	1.1±.08	1.1±.06	0.4±.29	0.7±.33	1.6±.33	1.7±.27
PE_20:5n3 (*)	2.3±.26	2.3±.10	1.8±.24	1.5±.25	1.4±.37	1.2±.22
PE_22:0 (*)	1.6±.09	1.6±.16	2.0±.10	2.5±.27	2.9±.31	2.6±.19
PE_22:1 (*)	0.6±.02	0.6±.02	1.4±.41	1.0±.12	1.1±.14	0.9±.11
PE_22:4 (*)	3.2±.74	3.1±.73	2.2±1.53	1.2±.91	1.1±.46	1.6±.39
PE_22:5n3 (*)	15.2±.40	16.1±0.72	21.7±1.96	22.3±2.47	22.4±1.23	21.2±0.65
PE_22:6n3 (*)	16.7±1.54	15.8±2.51	16.2±4.38	9.2±5.43	9.9±4.08	11.5±1.35

Data are mean % of total head group or fatty acid ± SEM (n = 4). Symbols indicate significant effects from a two-way ANOVA *: acclimation; †: subspecies; ‡: acclimation x subspecies.

Supplemental Table 7. Characteristics of hepatic mitochondrial phospholipid fatty acid composition from thermally acclimated *Fundulus heteroclitus* subspecies.

	5°C acclimated		15°C acclimated		33°C acclimated	
Total Phospholipids	North	South	North	South	North	South
MUFA/PUFA (*‡)	0.43±.013	0.43±.010	0.42±.019	0.36±.011	0.37±.021	0.41±.062
DBI (*)	2.5±.028	2.5±.019	2.5±.037	2.5±.019	2.4±.056	2.3±.062
n3/n6 (*‡)	2.3±.048	2.4±.069	2.4±.035	2.6±.047	2.7±.119	2.5±.117
Total n3 PUFA	0.328±.0043	0.323±.0043	0.332±.0064	0.347±.0037	0.334±.0105	0.314±.0111
Total n6 PUFA (*†‡)	0.143±.0020	0.135±.0028	0.141±.0017	0.132±.0011	0.126±.0021	0.127±.0017
Total n7 PUFA (*†‡)	0.078±.0018	0.068±.0015	0.062±.0017	0.045±.0017	0.031±.0008	0.027±.0008
Chain length	19.18±.037	19.15±.022	19.19±.035	19.27±.035	19.19±.063	19.24±.079
Phosphatidylcholine	North	South	North	South	North	South
PC-MUFA/PUFA	0.4±.02	0.4±.01	0.3±.01	0.4±.09	0.3±.02	0.4±.04
PC-DBI	2.4±.09	2.6±.05	2.5±.07	2.2±.25	2.4±.10	2.1±.04
PC-n3/n6 (‡)	3.1±.48	4.0±.19	3.4±.29	2.7±.49	4.1±.38	2.3±.33
PC-Total n3 PUFA	0.3±.02	0.3±.01	0.3±.01	0.3±.04	0.3±.01	0.2±.01
PC-Total n6 PUFA (‡)	0.11±.015	0.09±.003	0.10±.006	0.11±.014	0.09±.005	0.13±.017
PC-Total n7 PUFA (*‡)	0.06±.007	0.05±.001	0.04±.004	0.04±.007	0.02±.001	0.04±.005
PC-Chain length	19.0±.05	19.2±.05	19.3±.06	19.3±.14	19.2±.10	19.1±.11
Phosphatidylethanolamine	North	South	North	South	North	South
PE-MUFA/PUFA (*)	0.31±.009	0.27±.008	0.20±.012	0.24±.032	0.22±.019	0.23±.004
PE-DBI(*‡)	2.7±.03	2.7±.07	2.8±.14	2.4±.06	2.4±.08	2.4±.01
PE-n3/n6 (*‡)	3.9±.19	3.6±.19	5.1±.55	4.4±.45	5.5±.64	6.6±.44
PE-Total n3 PUFA (‡)	0.38±.003	0.38±.011	0.43±.021	0.38±.002	0.39±.013	0.40±.003
PE-Total n6 PUFA (*)	0.09±.004	0.10±.004	0.08±.004	0.08±.007	0.07±.007	0.06±.003
PE-Total n7 PUFA	0.06±.005	0.06±.002	0.05±.009	0.07±.008	0.05±.002	0.05±.002
PE-Chain length (‡)	19.6±.06	19.6±.06	19.7±.12	19.4±.04	19.4±.04	19.5±.03

Symbols indicate significant effects from a two-way ANOVA *: acclimation; †: subspecies; ‡: acclimation x subspecies. MUFA/PUFA: the ratio of monounsaturated fatty-acid to polyunsaturated fatty-acid. DBI: fatty acid double-bound (unsaturation) index. n3/n6: the ratio of n3 PUFA to n6 PUFA. n = 7-8 (total PL) or 4 (PE and PC).

Supplemental Table 8. Hepatic mitochondrial phospholipid fatty acid product/precursor ratios and their associated desaturase and elongase enzymes.

			5°C		15°C		33°C	
Product	**Precursor**	**Enzyme**	North	South	North	South	North	South
18:0	16:0	Elovl6 (*†‡)	0.55±.020	0.62±.009	0.56±.011	0.72±.021	0.71±.028	0.78±.033
18:1n7	16:1n7	Elovl6 (*)	2.1±.12	2.0±.12	1.8±.05	1.7±.16	1.4±.05	1.2±.14
20:0	18:0	Elovl6 (*†)	0.05±.018	0.03±.003	0.04±.009	0.02±.005	0.02±.004	0.02±.008
20:1n9	18:1n9	Elovl6 (*)	0.10±.008	0.08±.005	0.07±.005	0.06±.002	0.04±.002	0.05±.004
22:0	20:0	Elovl6	0.02±.014	0.04±.020	0.01±.009	0.03±.015	0.01±.008	0.01±.009
22:1n9	20:1n9	Elovl6 (*)	0.45±.051	0.36±.025	0.62±.030	0.76±.071	1.28±.191	1.22±.204
20:2n6	18:2n6	Elovl5 (*)	0.15±.020	0.18±.013	0.11±.011	0.11±.005	0.10±.004	0.13±.023
20:3n3	18:3n3	Elovl5	3.72±.650	3.86±.232	3.86±.398	4.01±.471	4.56±.290	4.60±.526
20:3n6	18:3n6	Elovl5 (*)	2.19±.534	1.89±.167	1.03±.196	2.07±.315	3.31±.793	2.98±.632
22:4n6	20:4n6	Elovl4/5 (*)	0.26±.007	0.23±.014	0.22±.041	0.23±.010	0.26±.028	0.36±.037
22:5n3	20:5n3	Elovl4/5 (*†‡)	3.54±.260	4.74±.588	5.94±.388	5.18±.451	9.54±.555	6.13±.758
18:1n9	18:0	SCD (*†‡)	1.38±.044	1.29±.023	1.30±.042	0.92±.034	0.96±.024	0.97±.031
16:1n7	16:0	SCD (*†)	0.12±.003	0.10±.006	0.10±.005	0.08±.008	0.05±.002	0.05±.001
18:3n6	18:2n6	Δ6D (‡)	0.03±.003	0.04±.004	0.04±.002	0.03±.005	0.02±.004	0.02±.010
22:6n3	22:5n3	Δ6D[#] (*†)	2.17±.074	2.69±.150	1.97±.090	2.07±.141	1.47±.133	1.66±.137
20:4n6	20:3n6	Δ5D (*)	18.9±2.24	14.7±1.08	19.1±2.16	18.5±1.72	11.6±1.11	11.5±1.70
20:4n6	18:2n6	Δ6D/Elovl5/Δ5D (*†)	1.02±.072	1.28±.055	0.79±.071	0.97±.053	0.81±.068	0.94±.109
20:3n6	20:2n6	Δ8D (*†)	0.38±.035	0.49±.021	0.40±.049	0.48±.027	0.71±.032	0.68±.041

Data are mean ± SEM (n = 7-8). Symbols indicate significant effects from a two-way ANOVA. *: acclimation; †: subspecies; ‡: acclimation x subspecies. [#]: Reaction also requires Elovl2/4/5 and peroxisomal β-oxidation; or can be catalyzed by Δ4D. Elovl, elongase; Δ6D, Δ-6 desaturase; Δ5D, Δ-5 desaturase; Δ8D, Δ-8 desaturase; SCD, stearoyl-CoA desaturase.