Supplemental materials for:

Using transcriptomics to examine the physiological status of wild-caught walleye (*Sander vitreus*)

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Methods

Differential expression analysis

Salmon v0.13.3 (Patro et al. 2017) was used to estimate transcript abundances using a previously assembled reference transcriptome for walleye generated by Jeffrey et al. (2020) (Sequence Read Archive Accession SRP150633). The R/Bioconductor package "tximport" (Soneson et al. 2016) was used to estimate counts from abundances at the gene-level that were scaled using the average transcript length, averaged over samples, and library size (i.e., argument countsFromAbundance = lengthScaledTPM). Differential expression of genes was examined using the R/Bioconductor package "edgeR" (Robinson et al. 2010). Only genes with at least one count per million across eight samples were considered for further analysis, representing 34,185 genes, 12.9% of genes, and > 98.0 ± 1.9% (mean ± standard deviation) of reads per sample. An effective library size was calculated using "calcNormFactors" in "edgeR" to normalize library sizes across samples (Robinson and Oshlack 2010). A general linear model was run in "edgeR" with sampling site and year as factors, and quasi-likelihood *F*-tests were used for hypothesis testing (Lun et al. 2016). *A priori* contrasts were designed to compare sampling sites (i.e., Dauphin River *vs*. Red River, Dauphin River *vs*. Matheson Island, Matheson Island *vs*. Red River) within a year and genes were considered differentially expressed at a Benjamini-Hochberg corrected False Discovery Rate (FDR) < 0.05.

To identify differentially expressed genes that were specific to each sampling site, lists were generated for each sampling site and consisted of genes that were differentially expressed at that site compared to both other sites within a given year (e.g., Red River *vs.* both Matheson Island and Dauphin

River; Matheson Island *vs.* both Red River and Dauphin River; Dauphin River *vs.* both Red River and Matheson Island). The site-specific differentially expressed genes were then identified as being consistently different across years, different only within 2017, or different only within 2018. Annotation information for differentially expressed genes was retrieved from the previously annotated walleye reference transcriptome (Jeffrey et al. 2020). Differentially expressed genes that did not have available annotation information from the reference transcriptome or were identified as uncharacterized or non-vertebrate, were re-blasted using blastx and the non-redundant protein sequences database. Any non-vertebrate genes were removed prior to gene enrichment analysis.

Statistical analysis for selecting candidate genes for qPCR

Using the RNA-seq data, a set of 20 candidate genes were selected for qPCR using a strategy similar to that of Akbarzadeh et al. (2020) to develop qPCR assays for hypoxia biomarkers in salmonids. Annotated genes representing consistent differences among sampling sites, across years were subjected to a PCA using the scaled and normalized cpm values and the "FactoMinerR" package in R. The PC associated with the separation of sampling locations was identified and the genes most correlated to this PC axis were determined using the "dimdesc" function in "FactoMinerR". Candidate genes were selected based on their strong correlation with the PC that was associated with the separation of locations ($p < 0.05$) that were also not significantly correlated with the PC that separated years ($p > 0.1$). To verify separation of locations for the selected genes and to make comparisons to the qPCR data, an additional PCA using only the logCPM values for the 20 candidate genes was run. The PC representing separation by location was identified, and a two-way ANOVA was used to examine the fixed factors of location, year, and location × year for the PC scores of this axis. Residuals were examined for normality and equal variance and significant effects were further explored using a Tukey's HSD post-hoc test as above.

Comparison of RNA-seq data with qPCR

In addition to the Spearman's correlation analysis used to compare the RNAseq and qPCR $\log_2$ fold changes for the 20 candidate genes, additional analyses were carried out to establish whether the individuals examined in the RNA-seq study were representative of the larger sample size assessed using qPCR. Using only individuals from Red River, Matheson Island, and Dauphin River (i.e., analyzed across both platforms), a linear model was developed for each target gene with location, year, the interaction of location and year, the platform, as well as total length and mass as fixed factor. A stepwise Akaike Information Criterion (AIC) was used to compare models and determine the best fit for the data. The "best fit" model was run, and the residuals were examined for normality and equal variance using the "check model" function from the R package, "performance". If the data did not meet the assumptions of the linear model, the data were rank transformed (only for *actn4*). Significant effects of categorical factors were further explored with a Tukey's HSD post-hoc test using the "emmeans" package in R. There were no significant effects of total length or mass when these factors were included in the models.

Results

Enrichment analysis for Matheson Island differentially expressed genes

Fewer genes (180 total and 168 unique) were differentially regulated in walleye from Matheson Island compared to Red River and Dauphin River (Tables S3, S4), and significant enrichment of GO terms were only evident in 2018. In 2018, GO terms related to the immune response were enriched for genes up-regulated in Matheson Island fish compared to fish from the other two locations (Fig. S3a), and included genes such as *tyrosine-protein kinase Lck* (*lck*) and *ZAP-70* (*zap70*) and *lymphocyte cytosolic protein 2* (*lcp2*) (Table S5). For genes down-regulated in fish from Matheson Island in 2018, the 'endoplasmic reticulum lumen' GO term was significantly enriched (Fig. S3b), involving genes such as *cytoskeleton-associated protein 4* (*ckap4*) and *transport and Golgi organization protein 1 homolog*

(*mia3*) (Table S5). Significant enrichment of GO terms for genes of Matheson Island walleye that were down-regulated compared to Red River fish and up-regulated compared to Dauphin River fish in 2018 included GO terms related to the respiratory electron transport chain (Fig. S3c) and mitochondrial genes associated with complex I (*mt-nd3–6, mt-nd4l*) and complex III (*mt-cyp*) (Fig. 5; Table S6).

Table S1. Metadata for walleye (*Sander vitreus*) collected from Red River, Matheson Island, and Dauphin River in the Lake Winnipeg system included in the RNA-sequencing analysis.

Location	Year	Total *n*	Female *n*	Unknown sex *n*	Total length (mm)	Mass (kg)
Red River	2017	8	8	0	666 ± 42	3.29 ± 0.67
	2018	8	8	0	662 ± 47	3.68 ± 1.01
Matheson	2017	8	8	0	613 ± 57	2.30 ± 0.65
	2018	8	7	1	650 ± 65	2.80 ± 1.07
Dauphin River	2017	8	8	0	578 ± 61	1.97 ± 0.40
	2018	8	5	3	555 ± 57	1.83 ± 0.68

Data are presented as means ± sd.

Table S2. Oligonucleotide primers for qPCR in walleye (*Sander vitreus*).

Gene name	Protein name	Forward primer (5'–3')	Reverse primer (5'–3')	Product (bp)	Eff. (%)
Cytoskeleton organization					
actn4	Alpha-actinin-4	CTCTGCGAAAGAGGGTCTTCTC	GACCGTCCTTCCAGCTAATGTG	98	102
actr2	Actin-related protein 2	ACCCGATGGAGAACGGTATG	GGTCGGGACCAAAGGTGTAGT	75	107
arf6	ADP-ribosylation factor 6	ATCCTGATCTTCGCCAACAAA	GGCCTAGCTTCTCCTGGATCTC	73	99
arhgdia	Rho GDP-dissociation inhibitor 1	GCGGCACCTACACCATCAA	CACCAATCCCAGGAGAGATGA	70	104
cdc42	Cell division control protein 42 homolog	AGACAGCAACACGATCGAGAAG	TGAGCTCACGAGCCAGCTT	86	99
cotl1	Coactosin-like protein	CCTGGATCGGTGAGAACATCA	CTTTGACCAGCGCCTTGTC	69	103
fam49b (cyrib)	Protein FAM49B (CYFIP-reltated Rac1 interactor B)	GCACCTGGAGCAGAAACAGTCT	GCAGCGTGAAGTGCAGGAT	62	104
pfn2	Profilin-2	CAGCTACTGCATGCACCTGAA	GCCTTGCCGACACAGATGT	66	99
Stress response					
akr1a1b	Aldo-keto reductase family 1 member A1-B	AGCAGTTATTTGGGCATTGGA	CCAAATGTCTCATGCAGTGCTT	102	91
dnajc9	DnaJ homolog subfamily C member 9	TACGAGGTGCTCGGCATCA	TGGACTTTCAGCGACACTTTGT	80	94
gpx1	Glutathione peroxidase 1	CATGAGCGGTACACCAGCAA	TTCTCCTGGTGGCCGAACT	71	91
slc25a24	Calcium-binding mitochondrial carrier protein SCaMC-1	GGAGGGAGCATTTCCTGTTTAA	CACCAATATCCAGCACCGTAGA	87	101
ube2j1	Ubiquitin-conjugating enzyme E2 J1	TCATCCAGACAGGCAAGTGAGA	TGCAGACGTGGAGGTGTCTTC	81	107
DNA repair					
rpa2	Replication protein A 32 kDa subunit	TCCGGGCACGTATGTCAAA	GCCACGATAGATCGGTGGTT	66	97

Golgi protein transport					
ap1s1	AP-1 complex subunit sigma-1A	CAGGGAGCTGATGCAGATAGTG	GGTCCCTCCATTCGAGGAA	68	100
eipr1	EARP-interacting protein homolog	AAGTTCACCTCGGGCAAGTG	CTCGTATGGCCGTGTCATTG	76	104
Immunity					
tnfaip8l2	Tumor necrosis factor, alpha-induced protein 8-like protein 2	CACCAAAAGCTCTGTCCAAATG	GCGGTACAGTTCGTCCAGGAT	67	104
Ion transport					
mcu	Calcium uniporter protein, mitochondrial	TGCTTACACGCCAGGAGTATGT	CTTTTCACCCCCTTGTGGAA	79	95
cnnm4	Metal transporter CNNM4	GGTGTCATGGCACTCAATGC	GGTTCAACCCGCTGACGTTA	67	106
Signaling pathways					
dgka	Diacylglycerol kinase alpha	GCTGCACCTGTTGCAAGTACA	GCGACAACCAATCTTCGGTTT	104	107

Table S3. Number of differentially regulated genes with annotation information, the number of unique genes, and the number of enriched genes following enrichment analysis of gene ontology terms for walleye (*Sander vitreus*) sampled across the Lake Winnipeg system in 2017 and 2018.

Location	Contrast	Regulation	Annotated genes	Unique genes	Enriched genes
Red River	Both years	Up	43	40	6
		Down	104	90	50
	2017 only	Up	89	82	14
		Down	641	582	451
	2018 only	Up	248	234	0
		Down	224	206	126
Matheson Island	Both years	Up	0	0	0
		Down	4	3	0
		Up vs. R; Down vs. D	1	1	0
	2017 only	Up	27	27	0
		Down	22	22	0
		Up vs. R; Down vs. D	3	3	0
		Down vs. R; Up vs. D	2	2	0
	2018 only	Up	33	32	8
		Down	35	34	4
		Up vs. R; Down vs. D	33	26	0
		Down vs. R; Up vs. D	20	18	5
Dauphin River	Both years	Up	34	30	4
		Down	9	9	0
	2017 only	Up	64	62	0
		Down	25	25	0
	2018 only	Up	417	373	176
		Down	331	312	25

R, Red River; D, Dauphin River

Table S4. Differentially regulated genes in walleye (*Sander vitreus*) sampled from the Red River (R) Matheson Island (M), and Dauphin River (D) in 2017 (17) and 2018 (18). Log_2 fold-change (LogFC), false discovery rate (FDR), annotation source, and uniport ID, as well as E value, percent ID, and transcript sequence are provided in the .xlsx file.

Table S5. Differentially regulated genes associated with significantly enriched gene ontology (GO) terms (biological processes, BP; molecular functions, MF; cellular components, CC) for walleye (*Sander vitreus*) sampled from the Red River (R) Matheson Island (M), and Dauphin River (D) in 2017 (17) and 2018 (18). The GO category (GO cat.; biological process, BP; molecular function, MF; cellular component, CC), GO representative summary term (GO rep.), GO term description (GO desc.), as well as gene and protein names are presented in the .xlsx file.

Table S6. Differentially regulated genes associated with glycolysis and gluconeogenic pathways, metabolism, mTOR pathway, and hypoxic response in walleye (*Sander vitreus*) sampled from the Red River, Matheson Island, and Dauphin River in 2017 and 2018.

			D.17vsR.17		D.18vsR.18		M.17vsR.17		M.18vsR.18		D.17vsM.17		D.18vsM.18	
Gene id	**Gene**	**Contrast**	**logFC**	**FDR**	**logFC**	**FDR**	**logFC**	**FDR**	**logFC**	**FDR**	**logFC**	**FDR**	**logFC**	**FDR**
Glycolysis/gluconeogenesis														
walleye_DN102551_c0_g1	*pck1*	R.18 up	-0.92	0.033	-1.84	**0.000**	-0.17	0.816	-1.03	**0.031**	-0.75	0.223	-0.81	0.082
walleye_DN113477_c6_g3	*aldocb*	R.17 down, D.18 up	0.51	**0.010**	0.67	**0.000**	0.41	**0.048**	0.17	0.580	0.10	0.915	0.50	**0.019**
walleye_DN109278_c2_g1	*eno1*	R.17 down, D.18 up	0.56	**0.014**	0.95	**0.000**	0.54	**0.024**	0.25	0.447	0.03	0.990	0.70	**0.005**
walleye_DN113326_c7_g6	*gapdh*	R.17 down, D.18 up	0.38	**0.036**	0.36	**0.031**	0.46	**0.014**	-0.04	0.925	-0.07	0.944	0.40	**0.038**
walleye_DN113371_c0_g1	*ldha*	R.17 down, D.18 up	1.33	**0.001**	1.09	**0.002**	1.45	**0.000**	0.20	0.778	-0.12	0.963	0.89	**0.027**
walleye_DN113371_c4_g1	*ldha*	R.17 down, D.18 up	0.93	**0.002**	0.98	**0.000**	1.00	**0.001**	0.23	0.633	-0.07	0.978	0.75	**0.018**
walleye_DN108570_c4_g3	*tpi1b*	R.17 down, D.18 up	0.49	**0.015**	0.66	**0.000**	0.44	**0.037**	0.18	0.551	0.05	0.974	0.48	**0.026**
walleye_DN111971_c3_g8	*eno3*	D.18 up	0.53	0.040	0.86	**0.000**	0.49	0.072	0.19	0.663	0.05	0.986	0.67	**0.013**
walleye_DN105332_c1_g6	*fbp1*	D.18 up	0.34	0.224	0.57	**0.013**	0.41	0.132	0.00	0.996	-0.08	0.966	0.58	**0.033**
walleye_DN111191_c5_g1	*hk2*	D.18 up	0.33	0.040	0.36	**0.014**	0.29	0.086	0.01	0.986	0.04	0.975	0.35	**0.041**
walleye_DN104637_c6_g1	*pfkfb1*	D.18 up	0.21	0.561	0.85	**0.001**	0.14	0.767	0.13	0.809	0.08	0.971	0.72	**0.018**
walleye_DN109466_c13_g1	*pkm*	D.18 up	0.30	0.156	0.49	**0.006**	0.55	0.007	-0.12	0.733	-0.25	0.492	0.62	**0.004**
Metabolism														
walleye_DN104552_c6_g1	*mt-nd4*	R.both up, M.18 int., D.18 down	-0.43	**0.011**	-0.85	**<0.001**	-0.45	**0.011**	-0.40	**0.037**	0.02	0.992	-0.45	**0.012**
walleye_DN109940_c8_g1	*mt-nd5*	R.both up, M.18 int., D.18 down	-0.66	**0.000**	-1.17	**<0.001**	-0.48	**0.009**	-0.72	**0.000**	-0.19	0.623	-0.45	**0.016**
walleye_DN109940_c8_g3	*mt-nd5*	R.both up, M.18 int., D.18 down	-0.56	**0.002**	-1.00	**<0.001**	-0.41	**0.027**	-0.54	**0.008**	-0.15	0.769	-0.47	**0.016**
walleye_DN109940_c8_g7	*mt-nd5*	R.both up, M.18 int., D.18 down	-0.68	**0.000**	-1.13	**<0.001**	-0.50	**0.004**	-0.63	**0.001**	-0.19	0.584	-0.50	**0.005**
walleye_DN109940_c7_g12	*mt-nd6*	R.both up, M.18 int., D.18 down	-0.65	**0.000**	-1.10	**<0.001**	-0.48	**0.009**	-0.60	**0.003**	-0.17	0.690	-0.50	**0.009**
walleye_DN104552_c6_g4	*mt-nd3*	R.both up	-0.44	**0.008**	-0.92	**<0.001**	-0.36	**0.037**	-0.59	**0.002**	-0.08	0.922	-0.33	**0.066**
walleye_DN108955_c4_g10	*mt-cyb*	R.18 up,	-0.31	**0.098**	-0.81	**<0.001**	-0.36	**0.052**	-0.40	**0.047**	0.06	0.963	-0.41	**0.030**

		M.18 int. up, D.18 down												
walleye_DN112225_c12_g2	*mt-atp8*	R.18 up	-0.18	0.237	-0.46	**<0.001**	-0.26	0.082	-0.35	**0.028**	0.08	0.905	-0.11	0.557
walleye_DN108955_c4_g9	*mt-cyb*	R.18 up	-0.27	0.171	-0.77	**<0.001**	-0.35	0.071	-0.43	**0.036**	0.08	0.936	-0.34	0.094
walleye_DN107218_c0_g1	*mt-co1*	R.17 up, D.18 down	-0.49	**0.002**	-0.68	**<0.001**	-0.54	**0.001**	-0.31	0.090	0.05	0.960	-0.36	**0.036**
walleye_DN107218_c2_g1	*mt-co1*	R.17 up, D.18 down	-0.42	**0.011**	-0.64	**<0.001**	-0.51	**0.003**	-0.28	0.149	0.10	0.881	-0.36	**0.042**
walleye_DN107248_c63_g4	*mt-nd1*	R.17 up, D.18 down	-0.67	**0.001**	-1.09	**<0.001**	-0.49	**0.019**	-0.37	0.116	-0.18	0.720	-0.72	**0.002**
walleye_DN106456_c2_g1	*mt-co1*	R.17 up	-0.43	**0.004**	-0.61	<0.001	-0.54	**0.001**	-0.30	0.079	0.11	0.808	-0.31	0.055
walleye_DN110148_c1_g1	*mt-co1*	R.17 up	-0.60	**0.002**	-0.65	<0.001	-0.63	**0.002**	-0.31	0.182	0.03	0.986	-0.34	0.109
walleye_DN100513_c0_g1	*mterf3*	D.18 up	0.25	0.070	0.28	**0.021**	0.38	0.005	-0.11	0.577	-0.14	0.636	0.40	**0.005**
walleye_DN102460_c0_g1	*atp5h*	D.18 down	0.12	0.649	-0.62	**0.001**	0.07	0.842	-0.20	0.473	0.05	0.971	-0.42	**0.040**
walleye_DN112225_c12_g1	*mt-atp6*	D.18 down	-0.43	0.013	-0.84	**<0.001**	-0.29	0.127	-0.30	0.145	-0.14	0.761	-0.54	**0.004**
walleye_DN107218_c1_g1	*mt-co1*	D.18 down	-0.43	0.059	-0.61	**0.003**	-0.47	0.041	-0.12	0.771	0.04	0.984	-0.49	**0.041**
walleye_DN107248_c63_g5	*mt-co2*	D.18 down	-0.27	0.225	-0.82	**<0.001**	-0.17	0.518	-0.27	0.288	-0.09	0.926	-0.55	**0.010**
walleye_DN112462_c1_g1	*mt-co3*	D.18 down	-0.52	0.029	-0.84	**<0.001**	-0.33	0.213	-0.31	0.327	-0.19	0.780	-0.53	**0.039**
walleye_DN112462_c1_g3	*mt-co3*	D.18 down	-0.36	0.069	-0.86	**<0.001**	-0.24	0.274	-0.31	0.187	-0.11	0.880	-0.56	**0.006**
walleye_DN108955_c3_g4	*mt-cyb*	D.18 down	-0.27	0.202	-0.84	**<0.001**	-0.36	0.076	-0.41	0.061	0.09	0.921	-0.43	**0.038**
walleye_DN104552_c7_g6	*mt-nd4*	D.18 down	-0.45	0.008	-0.86	**<0.001**	-0.42	0.017	-0.36	0.063	-0.03	0.984	-0.50	**0.006**
Hypoxia														
walleye_DN110302_c3_g6	*nos1*	R.18 down	3.69	0.021	4.90	**0.001**	3.25	0.051	3.88	**0.022**	0.44	0.960	1.02	0.552
walleye_ DN110766_c1_g5	*egln3*	R.17 down	1.12	**0.047**	0.26	0.707	1.19	**0.038**	-0.11	0.939	-0.07	0.988	0.36	0.641
walleye_ DN113735_c3_g1	*slc2a1*	R.17 down	0.45	**0.002**	0.41	0.002	0.48	**0.002**	0.28	0.096	-0.03	0.984	0.13	0.477
walleye_ DN113735_c3_g8	*slc2a1*	R.17 down	0.94	**0.005**	0.72	0.021	0.76	**0.029**	0.18	0.784	0.17	0.913	0.54	0.144
walleye_ DN100261_c0_g1	*hmox*	D.18 up	0.24	0.614	0.74	**0.027**	0.16	0.784	-0.45	0.351	0.08	0.980	1.19	**0.003**
walleye_ DN109309_c3_g1	*hyou1*	D.18 up	0.48	0.063	0.61	**0.007**	0.90	0.001	-0.01	0.988	-0.42	0.247	0.63	**0.018**
walleye_ DN113024_c7_g1	*hbad*	D.18 down	-1.09	0.006	-1.45	**0.000**	-0.43	0.378	-0.09	0.930	-0.66	0.263	-1.36	**0.002**
mTOR pathway														
walleye_DN105880_c11_g2	*prkaa2*	R.18 up	-0.34	0.334	-1.27	**0.000**	0.13	0.791	-0.73	**0.034**	-0.48	0.316	-0.54	0.113
walleye_DN105603_c3_g10	*seh1l*	R.18 down	0.39	0.002	0.56	**0.000**	0.24	0.073	0.31	**0.029**	0.15	0.503	0.25	0.061
walleye_DN107366_c3_g3	*eif4a1*	R.17 down, D.18 up	0.51	**0.001**	0.65	**0.000**	0.40	**0.010**	0.30	0.079	0.11	0.813	0.35	**0.030**
walleye_DN111020_c1_g2	*eif4b*	R.17 down, D.18 up	0.31	**0.023**	0.42	**0.001**	0.38	**0.008**	0.10	0.656	-0.06	0.932	0.32	**0.029**
walleye_DN104291_c1_g1	*eif4g1*	R.17 down, D.18 up	0.77	**0.003**	0.91	**0.000**	0.77	**0.005**	0.21	0.624	0.00	0.999	0.70	**0.012**
walleye_DN113534_c6_g1	*hras*	R.17 down, D.18 up	0.46	**0.002**	0.53	**0.000**	0.35	**0.022**	0.14	0.543	0.11	0.822	0.39	**0.012**
walleye_DN111314_c0_g4	*atf4*	R.17 down	0.20	**0.037**	0.22	0.015	0.33	**0.001**	0.15	0.196	-0.13	0.444	0.06	0.643
walleye_DN106983_c0_g1	*eif4e*	R.17 down	0.42	**0.001**	0.14	0.275	0.35	**0.006**	-0.14	0.378	0.07	0.870	0.28	0.031
walleye_DN106499_c5_g4	*grb2*	R.17 down	0.47	**0.000**	0.09	0.377	0.32	**0.002**	0.10	0.460	0.14	0.368	-0.01	0.961

walleye_DN113534_c6_g4	*hras*	R.17 down	0.28	**0.006**	0.19	0.043	0.44	**0.000**	0.11	0.432	-0.16	0.283	0.08	0.568
walleye_DN108382_c6_g1	*kras*	R.17 down	0.43	**0.000**	0.25	0.020	0.50	**0.000**	0.09	0.656	-0.07	0.883	0.17	0.210
walleye_DN109684_c2_g3	*lamtor2*	R.17 down	0.31	**0.006**	0.24	0.020	0.25	**0.030**	0.14	0.364	0.06	0.912	0.10	0.468
walleye_DN105554_c3_g7	*mapk1*	R.17 down	0.33	**0.015**	0.31	0.013	0.29	**0.039**	0.09	0.731	0.04	0.969	0.23	0.131
walleye_DN113739_c6_g6	*mapk1*	R.17 down	0.51	**0.002**	0.17	0.319	0.37	**0.030**	-0.03	0.954	0.14	0.764	0.20	0.306
walleye_DN103901_c0_g1	*mtor*	R.17 down	0.33	**0.012**	0.30	0.011	0.32	**0.018**	0.18	0.290	0.01	0.994	0.13	0.435
walleye_DN105466_c8_g1	*pdpk1*	R.17 down	0.28	**0.006**	0.02	0.884	0.36	**0.001**	0.09	0.593	-0.07	0.824	-0.06	0.672
walleye_DN109353_c2_g8	*rragc*	R.17 down	0.23	**0.017**	0.21	0.023	0.26	**0.010**	0.15	0.234	-0.03	0.967	0.06	0.674
walleye_DN109353_c2_g5	*rragc*	D.18 up	0.55	0.001	**0.56**	**0.000**	0.14	0.526	0.10	0.757	0.40	0.060	**0.46**	**0.009**
walleye_DN112490_c3_g1	*nprl3*	D.18 down	-0.18	0.463	**-0.39**	**0.037**	-0.23	0.335	0.18	0.556	0.05	0.974	**-0.58**	**0.008**
PI3K/AKT/mTOR pathway														
walleye_DN112562_c7_g4	*foxo1a*	R.17 down	0.54	**0.016**	0.29	0.205	0.66	**0.005**	0.29	0.331	-0.11	0.917	0.00	0.995
walleye_DN103115_c0_g1	*acaca*	R.17 down, D.18 up	0.68	**0.003**	**1.10**	**0.000**	0.69	**0.004**	0.37	0.181	-0.01	0.999	**0.73**	**0.004**
walleye_DN108540_c0_g1	*acly*	R.17 down, D.18 up	0.65	**0.000**	**0.83**	**0.000**	0.68	**0.000**	0.31	0.100	-0.03	0.984	**0.52**	**0.003**
walleye_DN108540_c0_g2	*acly*	R.17 down, D.18 up	0.62	**0.000**	**0.81**	**0.000**	0.77	**0.000**	0.27	0.212	-0.16	0.717	**0.54**	**0.004**
Citric acid cycle														
walleye_DN107782_c2_g6	*idh1*	R.17 down	1.11	**0.001**	0.45	0.154	0.94	**0.006**	0.04	0.971	0.17	0.912	0.42	0.263
walleye_DN107754_c3_g2	*ogdh*	R.17 down	0.35	**0.012**	0.15	0.298	0.37	**0.011**	0.06	0.825	-0.02	0.988	0.09	0.668
walleye_DN107308_c5_g1	*sdha*	R.17 down	0.35	**0.008**	-0.06	0.742	0.30	**0.029**	-0.06	0.814	0.05	0.948	0.01	0.985

R.17, Red River 2017; R.18, Red River 2018; R.both, Red River both years; D.17, Dauphin River 2017; D.18, Dauphin River 2018; M.17, Matheson Island 2017; logFC, Log_2 fold-change; FDR, false discovery rate.

Significant differentially regulated genes are represented as bolded text. logFC values for significantly regulated genes are coloured to reflect genes differentially regulated in Red River (teal) and Dauphin River (blue) fish. In some cases, multiple contrasts may have been significant.

Table S7. Differentially regulated genes associated with ion regulation in walleye (*Sander vitreus*) sampled from the Red River, Matheson Island, and Dauphin River in 2017 and 2018.

			D.17vsR.17		D.18vsR.18		M.17vsR.17		M.18vsR.18		D.17vsM.17		D.18vsM.18	
Gene id	**Gene**	**Contrast**	**logFC**	**FDR**	**logFC**	**FDR**	**logFC**	**FDR**	**logFC**	**FDR**	**logFC**	**FDR**	**logFC**	**FDR**
walleye_DN108377_c4_g1	*aqp3*	M.17 up	0.04	0.987	1.56	0.288	**3.62**	**0.009**	1.32	0.540	**-3.58**	**0.035**	0.24	0.927
walleye_DN111678_c3_g2	*aqp8*	D.18 down	-1.48	0.098	**-2.90**	**0.001**	-0.57	0.641	-0.75	0.570	-0.92	0.618	**-2.15**	**0.031**
walleye_DN102608_c0_g1	*aqpa*	D.18 down	-1.14	0.013	**-1.99**	**0.000**	-0.59	0.263	-0.73	0.188	-0.55	0.535	**-1.26**	**0.010**
walleye_DN113771_c9_g2	*atp1a1*	R.18 up	-0.08	0.856	**-1.16**	**0.000**	0.17	0.692	**-0.76**	**0.016**	-0.25	0.725	-0.40	0.198
walleye_DN104577_c1_g1	*atp1a1*	R.17 up	**-0.59**	**0.009**	-0.84	0.000	**-0.52**	**0.027**	-0.44	0.094	-0.07	0.963	-0.41	0.101
walleye_DN104577_c1_g2	*atp1a1*	D.18 down	-0.22	0.651	**-1.66**	**0.000**	0.11	0.866	-0.47	0.310	-0.33	0.705	**-1.19**	**0.002**
walleye_DN113771_c14_g1	*atp1a1*	D.18 down	-0.11	0.843	**-1.50**	**0.000**	0.18	0.748	-0.49	0.292	-0.30	0.760	**-1.01**	**0.008**
walleye_DN113771_c14_g3	*atp1a1*	D.18 down	-0.09	0.890	**-1.52**	**0.000**	0.17	0.778	-0.43	0.394	-0.26	0.832	**-1.09**	**0.007**
walleye_DN106483_c6_g4	*atp1a3*	R.17 down	**0.80**	**0.002**	0.69	0.004	**0.58**	**0.031**	0.21	0.617	0.22	0.754	0.48	0.089
walleye_DN113771_c11_g1	*atp1a3*	D.18 up	0.23	0.140	**0.54**	**0.000**	0.23	0.141	0.08	0.787	-0.01	0.997	**0.47**	**0.003**
walleye_DN113771_c11_g5	*atp1a3*	D.18 up	0.26	0.292	**0.77**	**0.000**	0.36	0.133	0.23	0.459	-0.10	0.937	**0.54**	**0.022**
walleye_DN113859_c9_g1	*atp1a3*	D.18 up	0.20	0.190	**0.41**	**0.002**	0.24	0.109	-0.02	0.960	-0.04	0.965	**0.43**	**0.005**
walleye_DN113488_c5_g3	*atp2a2*	D.both down	**-0.42**	**0.016**	**-0.39**	**0.017**	0.03	0.921	0.00	0.996	**-0.45**	**0.041**	**-0.39**	**0.039**
walleye_DN106163_c0_g1	*atp2b4*	R.17 down	**0.72**	**0.000**	0.52	0.001	**0.58**	**0.001**	0.23	0.286	0.14	0.748	0.30	0.102
walleye_DN103456_c0_g1	*atp2b4*	M.18 up	2.63	0.267	-4.18	0.144	1.35	0.669	**5.29**	**0.030**	1.29	0.849	**-9.47**	**0.003**
walleye_DN111653_c1_g1	*atp6v0a2*	R.17 down	**0.25**	**0.044**	0.19	0.110	**0.37**	**0.004**	-0.03	0.933	-0.12	0.681	0.22	0.110
walleye_DN107940_c2_g1	*atp6v0e1*	R.17 down	**0.33**	**0.002**	0.10	0.386	**0.23**	**0.035**	0.01	0.970	0.10	0.704	0.09	0.542
walleye_DN106269_c5_g1	*atp6v1a*	R.17 down, D.18 up	**1.22**	**0.000**	**1.24**	**0.000**	**0.87**	**0.002**	0.43	0.184	0.36	0.436	**0.82**	**0.005**
walleye_DN102031_c0_g1	*atp6v1b2*	R.17 down, D.18 up	**0.43**	**0.002**	**0.49**	**0.000**	**0.34**	**0.020**	0.13	0.514	0.10	0.827	**0.36**	**0.016**
walleye_DN108788_c7_g1	*atp6v1c1a*	R.17 down, D.18 up	**0.47**	**0.001**	**0.51**	**0.000**	**0.39**	**0.005**	0.08	0.766	0.07	0.897	**0.43**	**0.004**
walleye_DN104855_c4_g3	*atp6v1c1a*	D.18 up	0.49	0.014	**0.56**	**0.003**	0.19	0.465	0.07	0.873	0.30	0.349	**0.49**	**0.024**
walleye_DN110109_c4_g3	*atp6v1e1*	R.17 down, D.18 up	**0.68**	**0.000**	**0.76**	**0.000**	**0.48**	**0.017**	0.16	0.588	0.21	0.618	**0.60**	**0.004**
walleye_DN106672_c2_g2	*atp6v1h*	D.17 down	**-6.37**	**0.008**	0.51	0.846	0.10	0.980	0.40	0.932	**-6.46**	**0.035**	0.10	0.982
walleye_DN104183_c4_g7	*camk1*	R.both down	**0.56**	**0.000**	**0.65**	**0.000**	**0.64**	**0.000**	**0.58**	**0.001**	-0.08	0.920	0.07	0.781
walleye_DN106317_c1_g1	*camk1d*	D.18 up	1.39	0.011	**2.38**	**0.000**	0.64	0.328	0.60	0.439	0.75	0.414	**1.79**	**0.003**
walleye_DN105627_c1_g1	*camk2g*	R.17 down	**0.38**	**0.004**	0.18	0.172	**0.31**	**0.020**	0.05	0.865	0.06	0.923	0.13	0.435
walleye_DN104069_c3_g1	*cdc42*	R.both down	**0.41**	**0.000**	**0.34**	**0.000**	**0.43**	**0.000**	**0.33**	**0.005**	-0.02	0.987	0.01	0.959
walleye_DN104069_c3_g2	*cdc42*	R.both down	**0.40**	**0.002**	**0.25**	**0.034**	**0.55**	**0.000**	**0.29**	**0.043**	-0.15	0.550	-0.04	0.861
walleye_DN107058_c0_g1	*clcn5*	R.17 down	**0.50**	**0.015**	0.17	0.436	**0.51**	**0.015**	0.07	0.876	-0.02	0.994	0.10	0.750
walleye_DN111682_c2_g1	*cldn1*	D.18 down	0.03	0.947	**-0.49**	**0.041**	0.52	0.054	0.26	0.476	-0.48	0.175	**-0.75**	**0.006**
walleye_DN111682_c2_g6	*cldn1*	D.18 down	0.30	0.432	**-0.60**	**0.039**	0.79	0.014	0.16	0.785	-0.49	0.301	**-0.76**	**0.023**
walleye_DN110492_c2_g9	*cldn22*	R.both up	**-1.14**	**0.013**	**-0.96**	**0.023**	**-1.43**	**0.003**	**-1.11**	**0.032**	0.28	0.869	0.15	0.855
walleye_DN100552_c0_g1	*cldn3*	R.17 down	**4.05**	**0.046**	1.05	0.633	**4.38**	**0.037**	4.64	0.044	-0.33	0.984	-3.58	0.091
walleye_DN106664_c5_g3	*cldn4*	D.18 down	-0.06	0.901	**-0.63**	**0.017**	0.39	0.221	0.09	0.894	-0.45	0.298	**-0.72**	**0.019**

walleye_DN83550_c0_g1	*cldn5*	R.both up	**-0.69**	**0.010**	**-1.07**	**0.000**	**-1.13**	**0.000**	**-1.05**	**0.001**	0.44	0.285	-0.02	0.977
walleye_DN110590_c3_g1	*cldn8*	D.18 down	-0.51	0.174	**-1.46**	**0.000**	0.05	0.939	-0.62	0.132	-0.56	0.291	**-0.85**	**0.023**
walleye_DN103849_c4_g3	*kcnj1*	R.18 up	-0.64	0.188	**-1.23**	**0.003**	-0.31	0.623	**-1.15**	**0.023**	-0.33	0.815	-0.08	0.934
walleye_DN104249_c1_g4	*kcnj16*	D.18 down	0.00	0.997	**-1.06**	**0.002**	0.26	0.616	-0.04	0.967	-0.26	0.827	**-1.02**	**0.009**
walleye_DN111684_c8_g2	*nr3c2*	R.17 down	**0.65**	**0.011**	0.74	0.002	**0.67**	**0.011**	0.51	0.083	-0.02	0.994	0.23	0.475
walleye_DN104639_c0_g1	*rhcg1*	R.18 up, M.18 Int, D.18 down	-0.54	0.123	**-2.07**	**0.000**	-0.09	0.882	**-0.77**	**0.041**	-0.45	0.436	**-1.31**	**0.001**
walleye_DN112974_c2_g2	*slc12a3*	D.18 up	0.35	0.688	**1.15**	**0.047**	-0.49	0.563	-0.45	0.674	0.84	0.437	**1.60**	**0.018**
walleye_DN106771_c6_g3	*slc12a8*	D.18 up	0.59	0.219	**1.74**	**0.000**	-0.19	0.798	0.20	0.825	0.78	0.220	**1.54**	**0.002**
walleye_DN105800_c0_g5	*slc4a1*	R.17 up	**-1.83**	**0.012**	-1.21	0.080	**-1.53**	**0.043**	-0.82	0.414	-0.30	0.941	-0.39	0.731
walleye_DN111084_c5_g1	*slc9a1*	R.18 up	-0.90	0.002	**-1.71**	**0.000**	-0.44	0.177	**-1.10**	**0.001**	-0.46	0.312	-0.61	0.057
walleye_DN105658_c3_g2	*slc9a3*	R.18 up, M18 Int, D.18 down	-0.16	0.764	**-1.92**	**0.000**	-0.07	0.918	**-0.81**	**0.037**	-0.09	0.974	**-1.11**	**0.004**
walleye_DN108637_c1_g1	*tjp3*	R.17 down	**0.38**	**0.012**	0.42	0.003	**0.45**	**0.004**	0.28	0.119	-0.07	0.929	0.14	0.485

R.17, Red River 2017; R.18, Red River 2018; R.both, Red River both years; D.17, Dauphin River 2017; D.18, Dauphin River 2018; M.17, Matheson Island 2017; logFC, Log_2 fold-change; FDR, false discovery rate

Significant differentially regulated genes are represented as bolded text. logFC values for significantly regulated genes are coloured to reflect genes differentially regulated in Red River (teal), Matheson Island (light blue), and Dauphin River (dark blue) fish. In some cases, multiple contrasts may have been significant.

Table S8. Results for linear models examining the consistency of candidate gene mRNA levels measured by qPCR of walleye sampled in the Lake Winnipeg system (Red River, Matheson Island, Dauphin River) in 2017 and 2018 that were or were not part of the RNA-seq study.

Gene	Factor[a]	Sum squares	df	*F*-value	*p*-value
actn4	Year	6136.00	1	8.46	**0.005**
	RNAseq	3954.00	1	5.45	**0.022**
	Total length	78.00	1	0.11	0.744
	Mass	41.00	1	0.06	0.814
actr2	Location	3.77	2	7.06	**0.001**
	Year	1.48	1	5.57	**0.020**
	Total length	0.10	1	0.37	0.546
akr1a1b	Year	6.00	1	19.46	**< 0.001**
	RNAseq	1.12	1	3.65	0.059
ap1s1	Location	1.20	2	2.52	0.086
	Year	2.08	1	8.75	**0.004**
	Total length	0.11	1	0.46	0.498
	Location × Year	0.95	2	2.01	0.141
arf6	Location	3.818	2	12.06	**< 0.001**
	Year	0.07	1	0.44	0.510
	Total length	0.02	1	0.10	0.755
	Location × Year	1.76	2	5.55	0.005
arhgdia	Year	0.79	1	2.63	0.108
	RNAseq	0.67	1	2.23	0.138
	Total length	0.06	1	0.21	0.652
	Mass	0.38	1	1.24	0.268
cdc42	Location	0.60	2	1.87	0.160
	Year	1.35	1	8.40	**0.005**
	RNAseq	0.17	1	1.05	0.309
cnnm4	Location	0.32	2	0.79	0.457
	Year	3.43	1	16.73	**< 0.001**
	RNAseq	0.50	1	2.45	0.121
	Total length	0.21	1	1.04	0.310
	Location × Year	1.14	2	2.78	0.067
cotl1	Location	16.90	2	42.46	**< 0.001**
	Year	0.34	1	1.72	0.193
	Total length	0.08	1	0.38	0.538
	Mass	0.00	1	0.02	0.879
dgka	Location	1.99	2	3.56	**0.033**
	Year	8.29	1	29.61	**< 0.001**
	RNAseq	0.87	1	3.12	0.081
	Total length	0.59	1	2.10	0.151
dnajc9	Location	1.39	2	3.59	**0.031**
	Year	3.56	1	18.36	**< 0.001**
	RNAseq	0.38	1	1.93	0.168

	Total length	0.00	1	0.01	0.931
eipr1	Location	0.99	2	1.98	0.144
	Year	3.38	1	13.57	**< 0.001**
	RNAseq	0.70	1	2.81	0.097
	Total length	0.25	1	1.02	0.316
	Location × Year	0.82	2	1.65	0.197
fam49b	Location	3.67	2	9.68	**< 0.001**
	Year	3.95	1	20.82	**< 0.001**
	RNAseq	0.23	1	1.21	0.275
	Mass	0.00	1	0.02	0.887
	Location × Year	0.91	2	2.40	0.097
gpx1	Location	7.21	2	12.69	**< 0.001**
	Year	7.59	1	26.72	**< 0.001**
	Mass	0.18	1	0.62	0.435
	Location × Year	1.41	2	2.48	0.089
mcu	Location	1.56	2	2.97	0.056
	Year	4.34	1	16.54	**< 0.001**
	RNAseq	1.01	1	3.84	0.053
	Total length	0.61	1	2.32	0.132
	Mass	0.34	1	1.29	0.259
pfn2	Location	2.63	2	5.69	**0.005**
	Mass	0.21	1	0.92	0.339
rpa2	Location	9.96	2	15.68	**< 0.001**
	RNAseq	0.05	1	0.17	0.679
slc25a24	Location	0.95	2	1.41	0.248
	Year	5.40	1	16.14	**< 0.001**
tnfaip8l2b	Year	3.28	1	16.02	**< 0.001**
	RNAseq	0.20	1	0.98	0.324
ube2j1	Location	3.78	2	7.81	**< 0.001**
	Year	0.29	1	1.19	0.279
	Mass	0.15	1	0.60	0.440
	Location × Year	4.33	2	8.94	**< 0.001**

[a] Akaike Information Criterion (AIC) was used to determine which fixed factors (location, year, RNAseq *vs*. non-RNAseq, total length, mass, location × year) were included in the linear model. Significant fixed factors are represented as bolded text. See Table S2 for gene abbreviations.

Table S9. Results for the linear models examining the relative mRNA levels measured by qPCR of 20 candidate genes for walleye (*Sander vitreus*) sampled from the Red River, Riverton, Matheson Island, and Dauphin River in 2017 and 2018.

Gene	Factor [a]	Sum squares	df	*F*-value	*p*-value
actn4	Location	1.32	3	1.45	0.232
	Year	5.42	1	17.87	**< 0.001**
actr2	Location	23909.00	3	5.18	**0.002**
	Year	8163.00	1	5.31	**0.023**
	Mass	320.00	1	0.21	0.649
akr1a1b	Location	1.95	3	2.31	0.079
	Year	1.57	1	5.58	**0.020**
	Mass	0.04	1	0.15	0.698
	Location × Year	2.27	3	2.69	**0.049**
ap1s1	Location	1.35	3	1.95	0.124
	Year	2.46	1	10.68	**0.001**
	Total length	0.12	1	0.53	0.468
arf6	Location	3.86	3	8.37	**< 0.001**
	Year	0.007	1	0.47	0.496
	Total length	0.02	1	0.14	0.710
	Location × Year	1.93	3	4.18	**0.007**
arhgdia	Year	1.41	1	4.85	**0.029**
	Mass	0.35	1	1.21	0.273
cdc42	Location	1.02	3	1.90	0.132
	Year	1.44	1	8.06	**0.005**
	Mass	0.04	1	0.23	0.630
cnnm4	Location	2.14	3	3.53	**0.017**
	Year	0.08	1	0.38	0.537
	Total length	0.30	1	1.50	0.222
	Location × Year	1.87	3	3.08	**0.030**
cotl1	Location	18.78	3	28.65	**< 0.001**
	Year	1.24	1	5.66	**0.019**
	Total length	0.12	1	0.54	0.464
	Mass	0.01	1	0.02	0.875
dgka	Location	2.15	3	2.73	**0.046**
	Year	9.10	1	34.77	**< 0.001**
	Total length	0.06	1	0.24	0.627
dnajc9	Location	1.98	3	2.80	**0.042**
	Year	3.79	1	16.11	**< 0.001**
	Mass	0.27	1	1.15	0.286
eipr1	Location	1.66	3	2.03	0.112
	Year	3.83	1	14.07	**< 0.001**
	Mass	0.172	1	0.63	0.427
fam49b	Location	5.46	3	9.87	**< 0.001**

	Year	4.65	1	25.24	**< 0.001**
	Mass	1.05	1	5.67	**0.018**
	Location × Year	0.86	3	1.55	0.205
gpx1	Location	9.23	3	9.86	**< 0.001**
	Year	10.28	1	32.91	**< 0.001**
	Location × Year	1.58	3	1.69	0.173
mcu	Location	1.58	3	2.05	0.110
	Year	5.22	1	20.32	**< 0.001**
	Total length	0.008	1	0.03	0.861
pfn2	Location	2.86	3	4.25	**0.007**
	Year	0.08	1	0.37	0.542
rpa2	Location	9.12	3	8.97	**< 0.001**
	Total length	0.02	1	0.05	0.817
slc25a24	Location	1.91	3	2.13	0.100
	Year	7.70	1	25.88	**< 0.001**
	Mass	0.34	1	1.13	0.290
tnfaip8l2b	Location	0.84	3	1.42	0.240
	Year	2.71	1	13.77	**< 0.001**
	Mass	0.32	1	1.60	0.207
ube2j1	Location	3.88	3	5.73	**0.001**
	Year	0.30	1	1.32	0.253
	Mass	0.33	1	1.47	0.227
	Location × Year	4.57	3	6.74	**< 0.001**

[a] Akaike Information Criterion (AIC) was used to determine which fixed factors (location, year, total length, mass, location × year) were included in the linear model.

Significant fixed factors are represented as bolded text. See Table S2 for gene abbreviations.

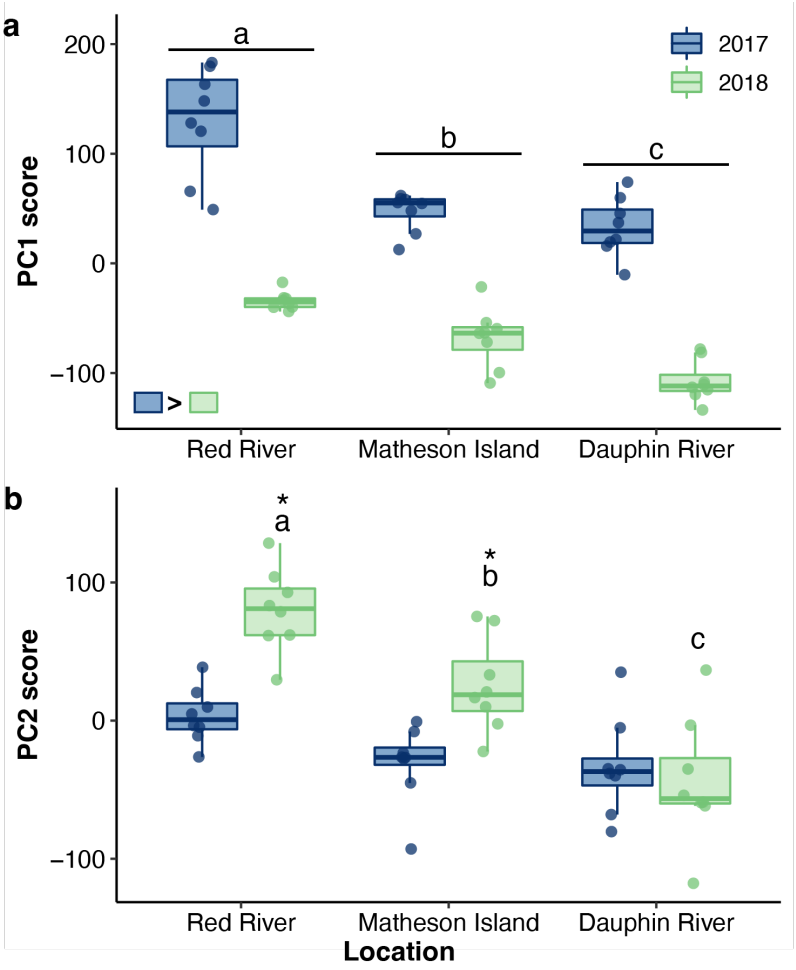

Figure S1. Principal component (PC) scores for PC1 (a) and PC2 (b) for whole-transcriptomic analysis of walleye (*Sander vitreus*) sampled in the Lake Winnipeg system in 2017 and 2018 (n = 8). Walleye were sampled from the Red River, Matheson Island, and Dauphin River, representing sites in the south basin, channel, and north basin, respectively. Locations that do not share a letter are significantly different from one another. An asterisk represents a significant effect of year within a location, while the inset represents an overall significant effect of year across locations (two-way ANOVA; see text for details). Horizontal bars in the boxplot represent the median response value and 75%, 50%, and 25% quartiles. Whiskers represent ± 1.5 times the interquartile range, and each dot represents an individual response value.

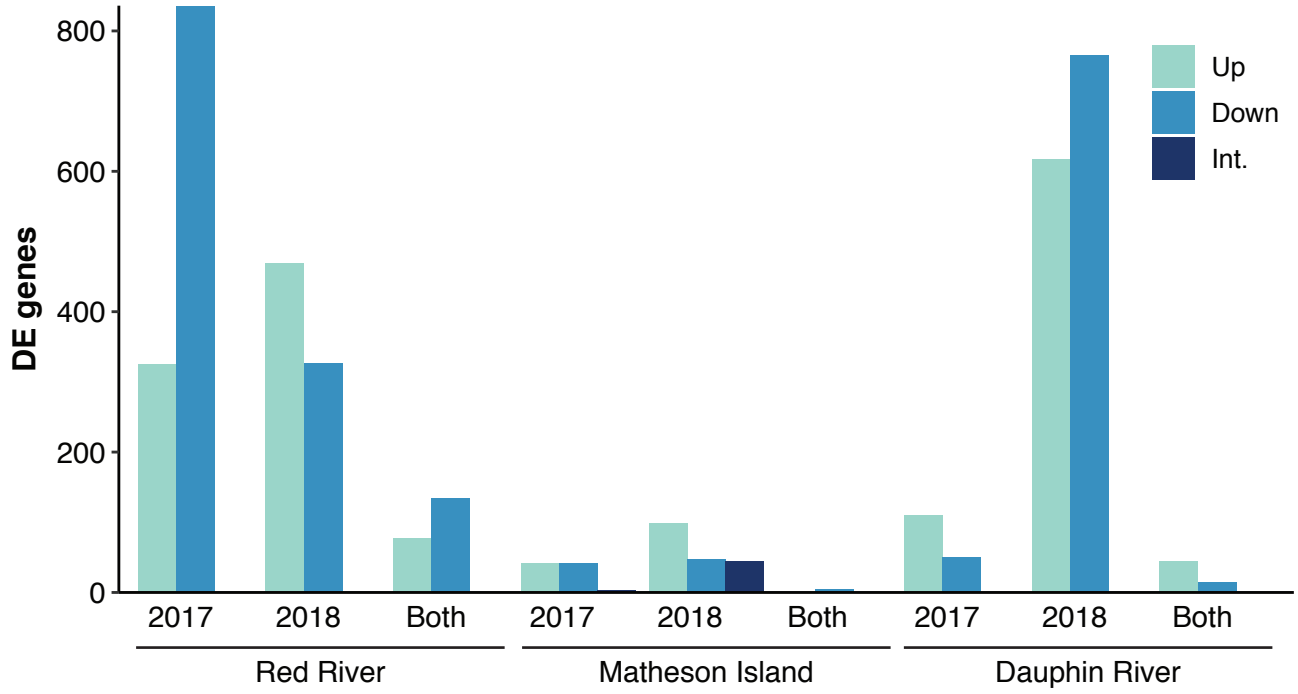

Figure S2. Number genes that were differentially expressed (DE) in walleye (*Sander vitreus*) from the Lake Winnipeg system in 2017 or 2018 only, or consistently across both years (Both). Walleye were sampled in the Red River, Matheson Island, and Dauphin River, representing sites in the south basin, channel, and north basin, respectively. Site comparisons represent differences of one site compared to the other two sites (i.e., Red River compared to both Matheson Island and Dauphin River; Matheson Island compared to both Red River and Dauphin River; Dauphin River compared to both Red River and Matheson Island). Genes are expressed in terms of their direction of regulation (up or down). Intermediate (int.) represents regulation in opposite directions relative to the two other sampling sites (e.g., for Matheson Island, up-regulated compared to Red River, down-regulated compared to Dauphin River).

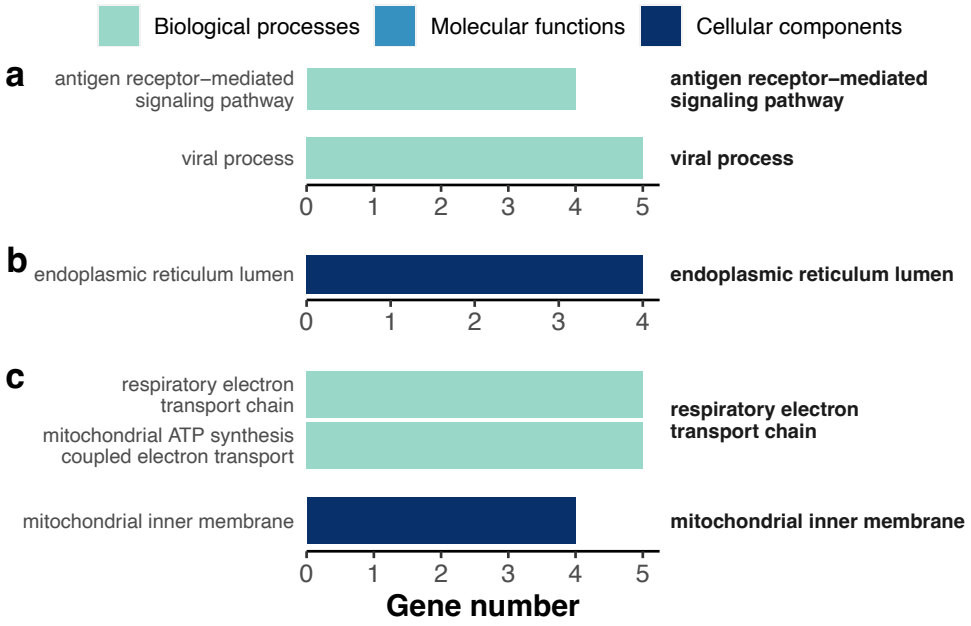

Figure S3. Summary of the enriched gene ontology (GO) terms of genes down-regulated in 2018 only for walleye (*Sander vitreus*; $n = 8$) sampled from Matheson Island compared to Red River and Dauphin River. Genes were considered differentially regulated at a false discovery rate < 0.05. Only GO terms from the functional analysis with an adjusted $p < 0.05$ with more than four transcripts were considered as significantly enriched. Significant GO terms were summarized using REVIGO to reduce redundancy and grouped according to similarity (right labels).

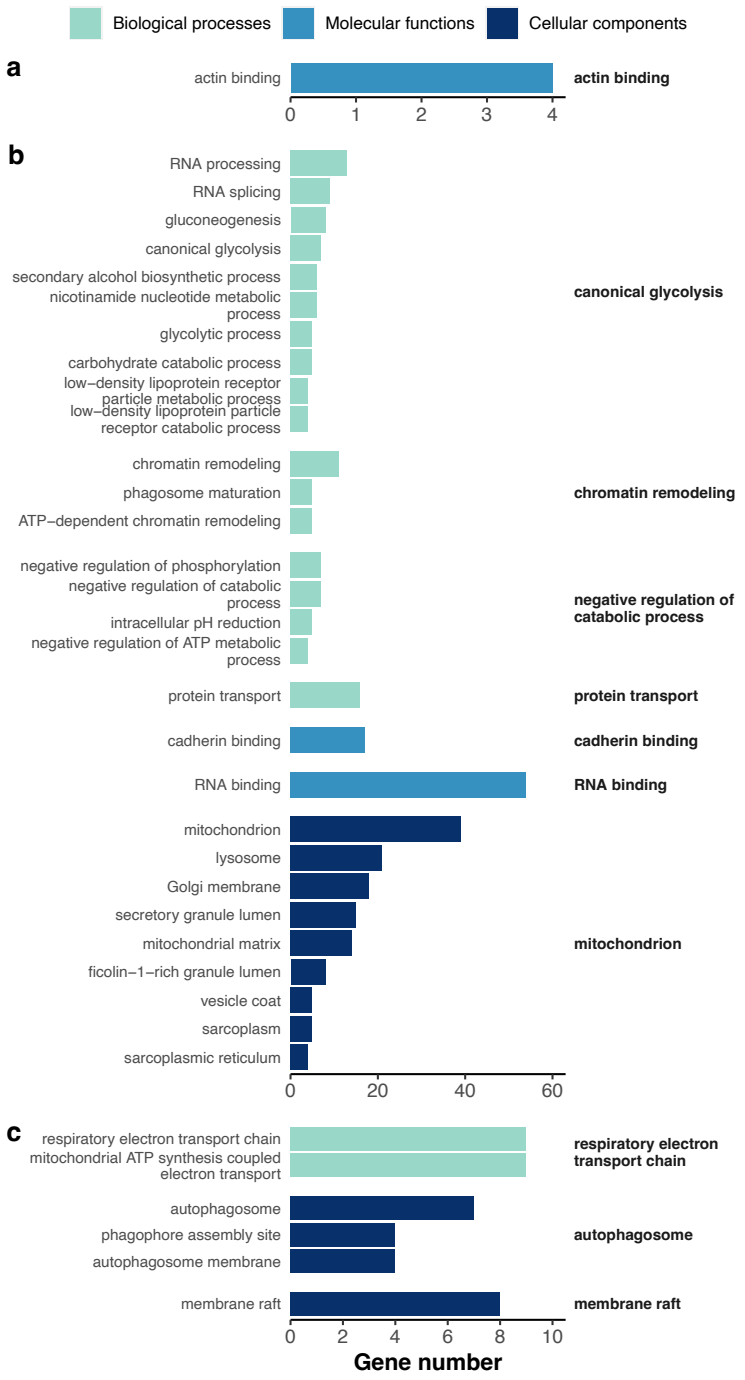

Figure S4. Summary of the enriched gene ontology (GO) terms of genes (a) up-regulated in both 2017 and 2018 as well as (b) up- and (c) down-regulated in 2018 only for walleye (*Sander vitreus*; $n = 8$) sampled from Dauphin River compared to Red River and Matheson Island. Genes were considered differentially regulated at a false discovery rate < 0.05. Only GO terms from the functional analysis with an adjusted $p < 0.05$ with more than four transcripts were considered as significantly enriched. Significant GO terms were summarized using REVIGO to reduce redundancy and grouped according to similarity (right labels).

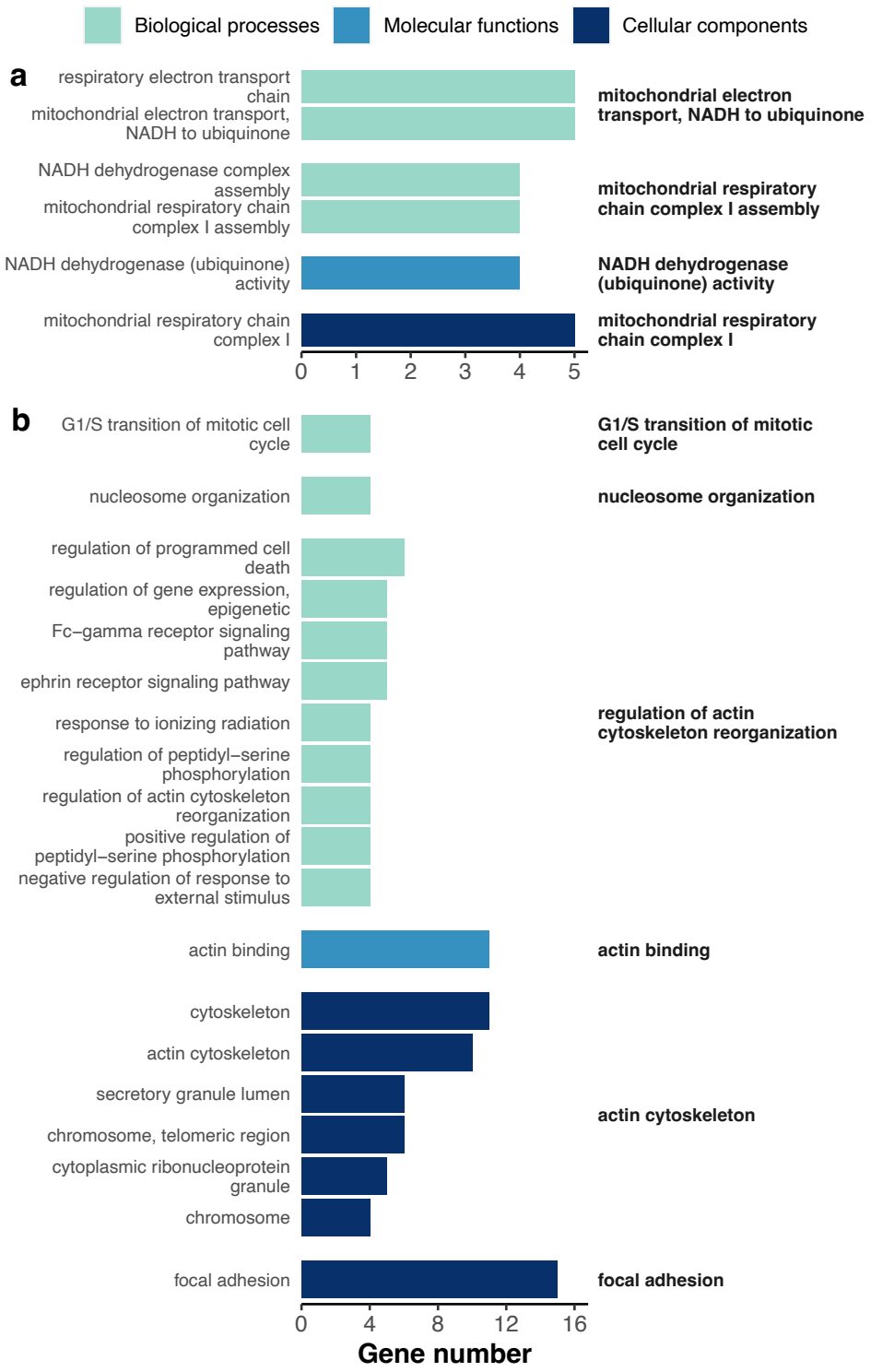

Figure S5. Summary of the enriched gene ontology (GO) terms of genes (a) up- and (b) down-regulated in both 2017 and 2018 for walleye (*Sander vitreus*; $n = 8$) sampled from the Red River compared to Matheson Island and Dauphin River. Genes were considered differentially regulated at a false discovery rate < 0.05. Only GO terms from the functional analysis with an adjusted $p < 0.05$ with at least four transcripts were considered as significantly enriched. Significant GO terms were summarized using REVIGO to reduce redundancy and grouped according to similarity (right labels).

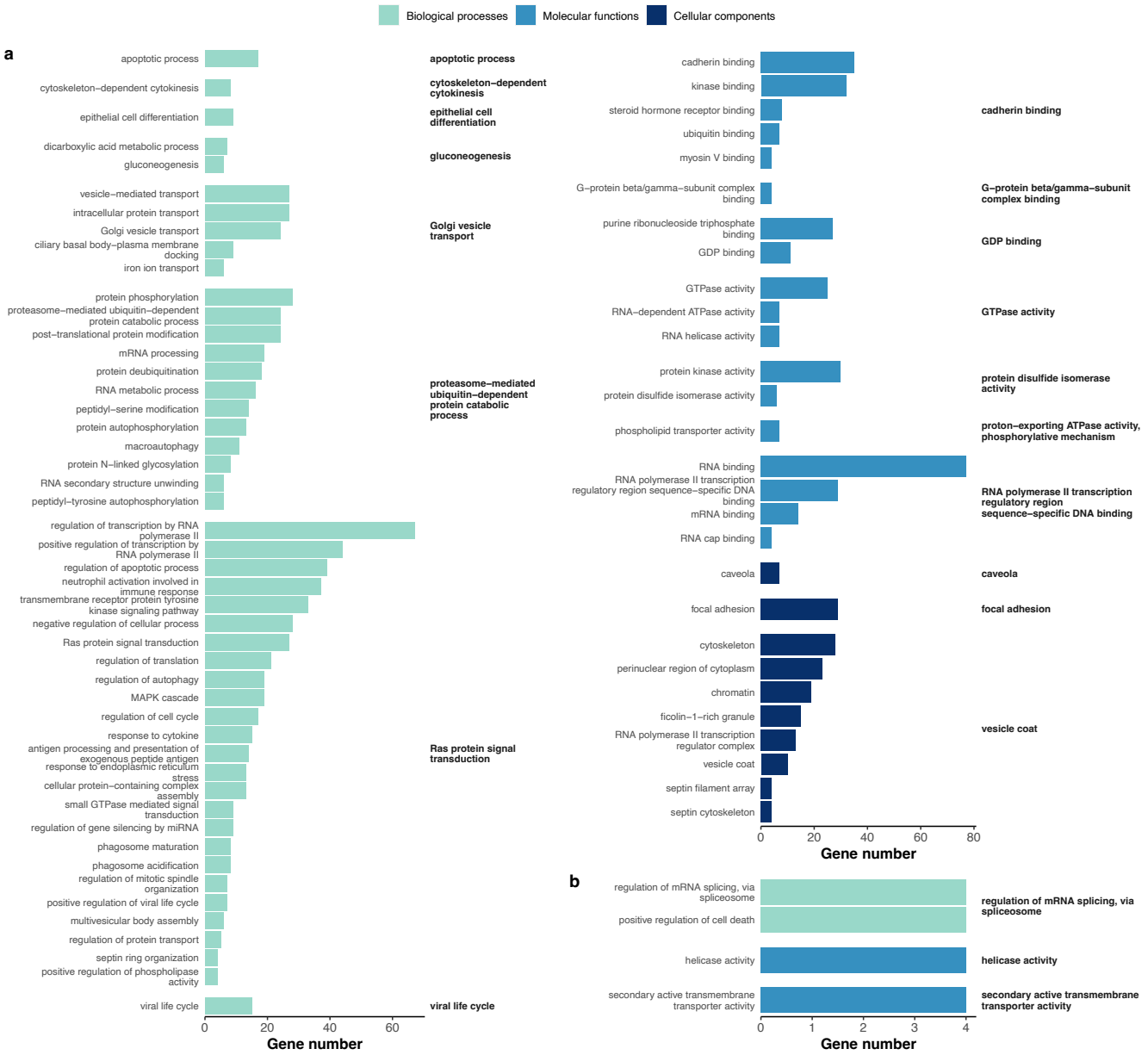

Figure S6. Summary of the enriched gene ontology (GO) terms of genes (a) down- and (b) up-regulated in 2017 only for walleye (*Sander vitreus*; $n = 8$) sampled from the Red River compared to Matheson Island and Dauphin River. Genes were considered differentially regulated at a false discovery rate < 0.05. Only GO terms from the functional analysis with an adjusted $p < 0.05$ with more than four transcripts were considered as significantly enriched. Significant GO terms were summarized using REVIGO to reduce redundancy and grouped according to similarity (right labels).

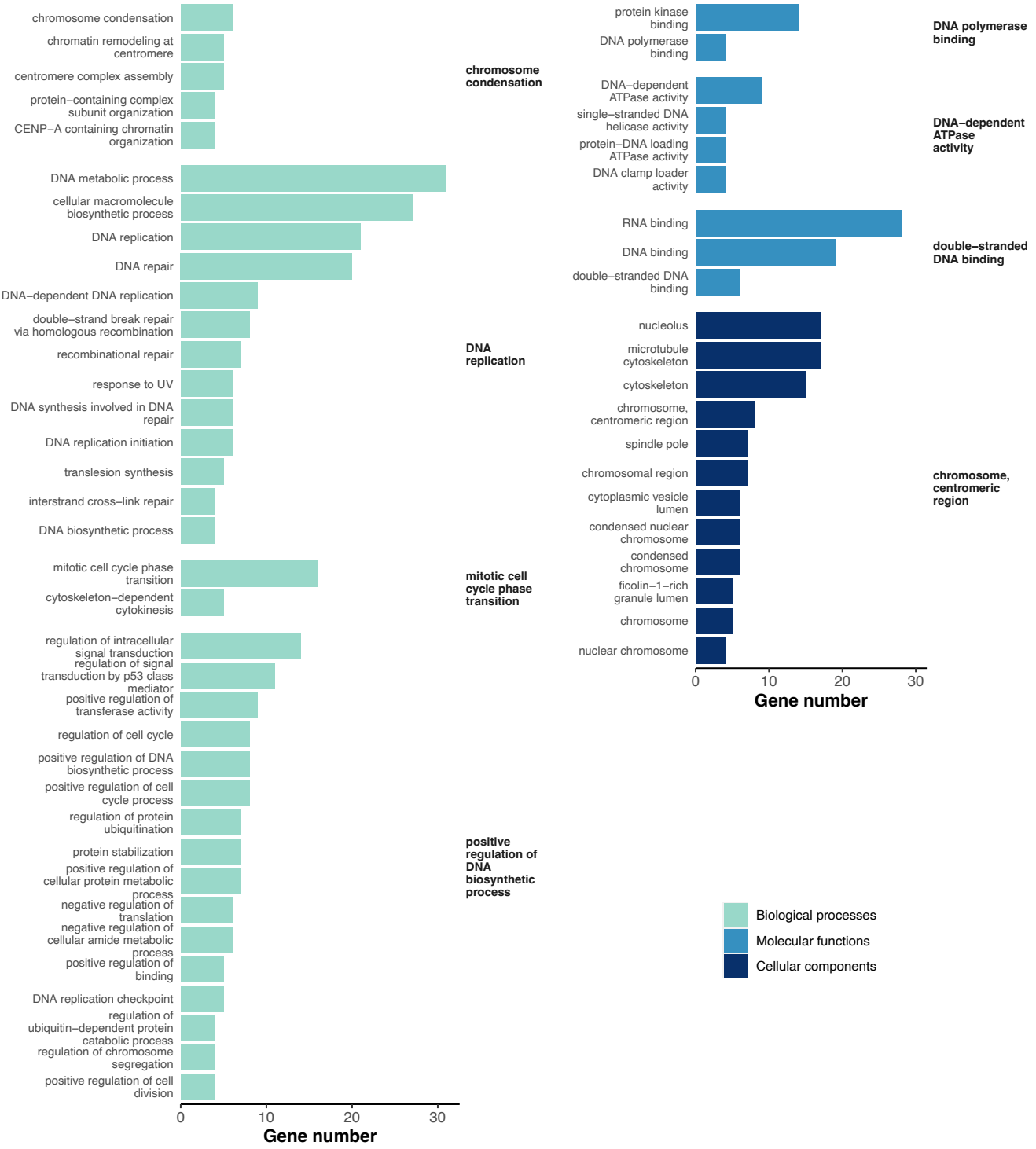

Figure S7. Summary of the enriched gene ontology (GO) terms of genes down-regulated in 2018 only for walleye (*Sander vitreus*; $n = 8$) sampled from the Red River compared to Matheson Island and Dauphin River. Genes were considered differentially regulated at a false discovery rate < 0.05. Only GO terms from the functional analysis with an adjusted $p < 0.05$ with more than four transcripts were considered as significantly enriched. Significant GO terms were summarized using REVIGO to reduce redundancy and grouped according to similarity (right labels).

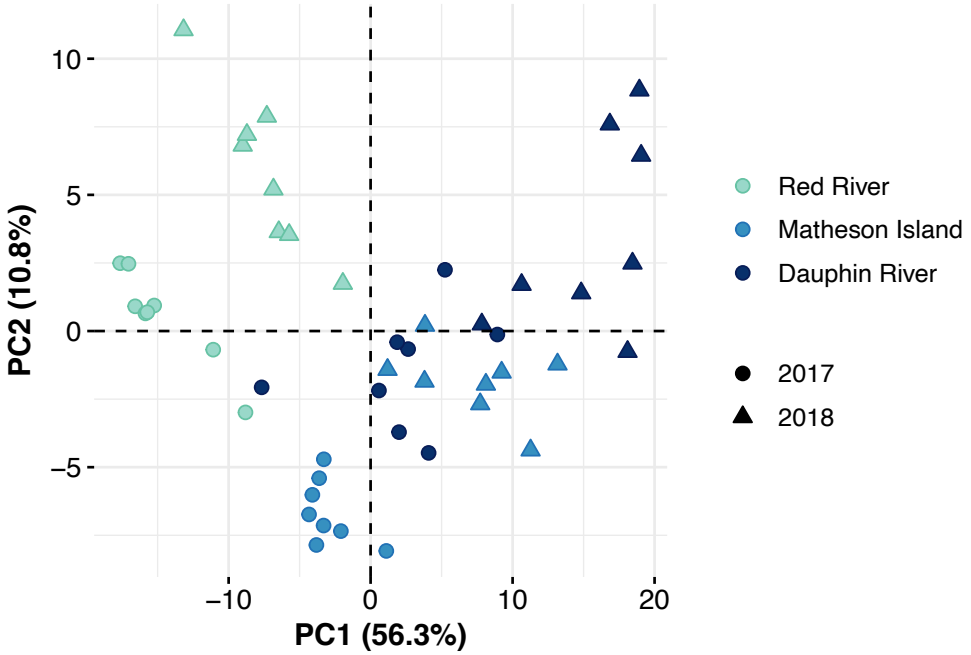

Figure S8. Principal components analysis (PCA) of the 195 annotated genes that were differentially regulated across both 2017 and 2018 in walleye (*Sander vitreus*) sampled in the Lake Winnipeg system. Walleye were sampled from the Red River, Matheson Island, and Dauphin River in 2017 and 2018 (n = 8 per site and year), and genes that were differentially regulated (FDR < 0.05) at one site compared to the other two sites were identified. The variance explained by each PC is indicated in brackets.

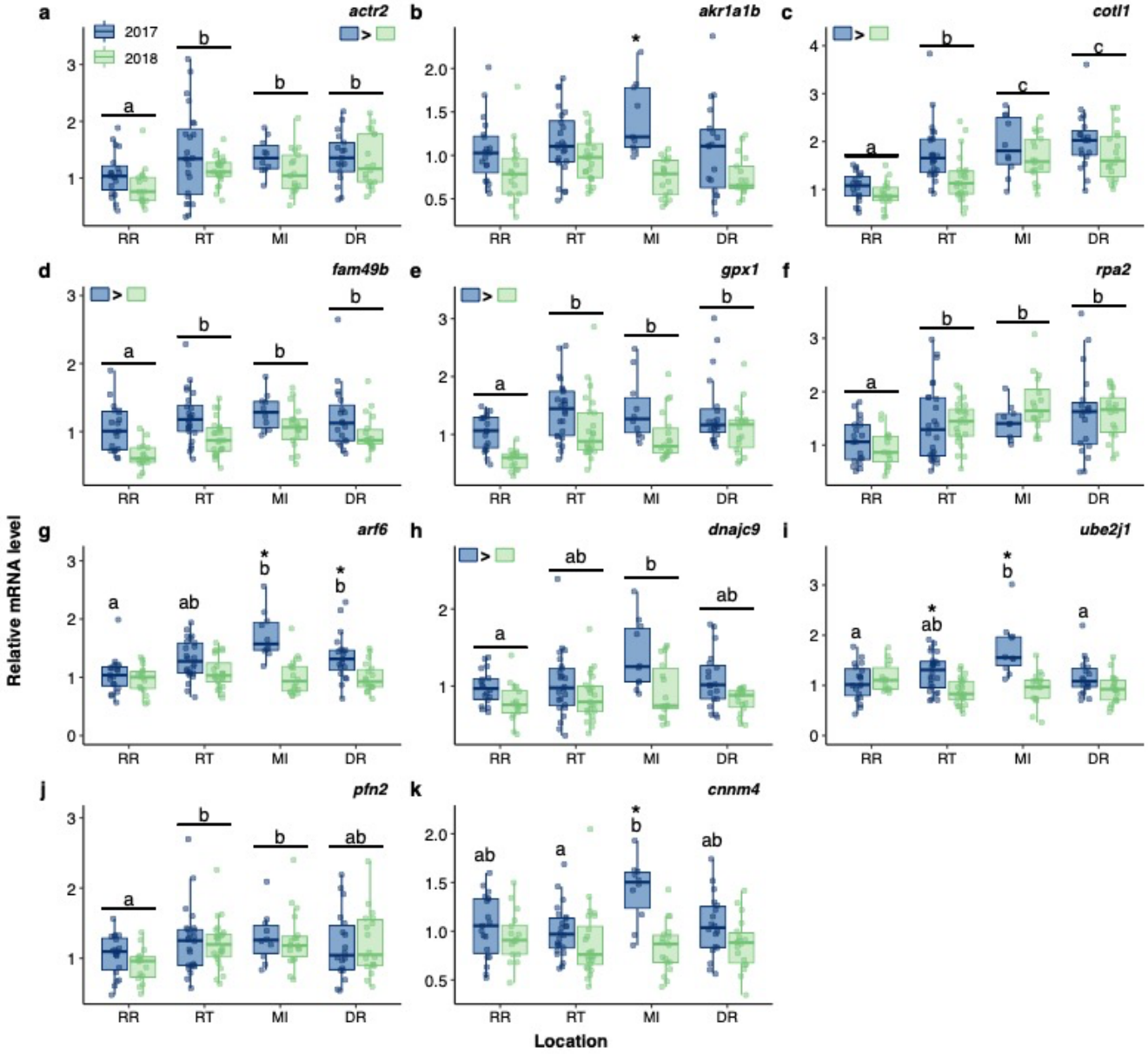

Figure S9. Relative mRNA levels for candidate genes of walleye (*Sander vitreus*) from the lake Winnipeg system. Walleye were sampled from the Red River (RR; n = 14–19), Riverton (RT; n = 23–24), Matheson Island (MI; n = 9–18), and Dauphin River (DR; n = 17–19) in 2017 and 2018. Locations that do not share a letter are significantly different from one another. An asterisk represents a significant effect of year within a location, while the inset represents an overall significant effect of year across locations (see Table S9). Horizontal bars in the boxplot represent the median response value and 75%, 50%, and 25% quartiles. Whiskers represent ± 1.5 times the interquartile range, and each dot represents an individual response value. See Table S2 for gene abbreviations.

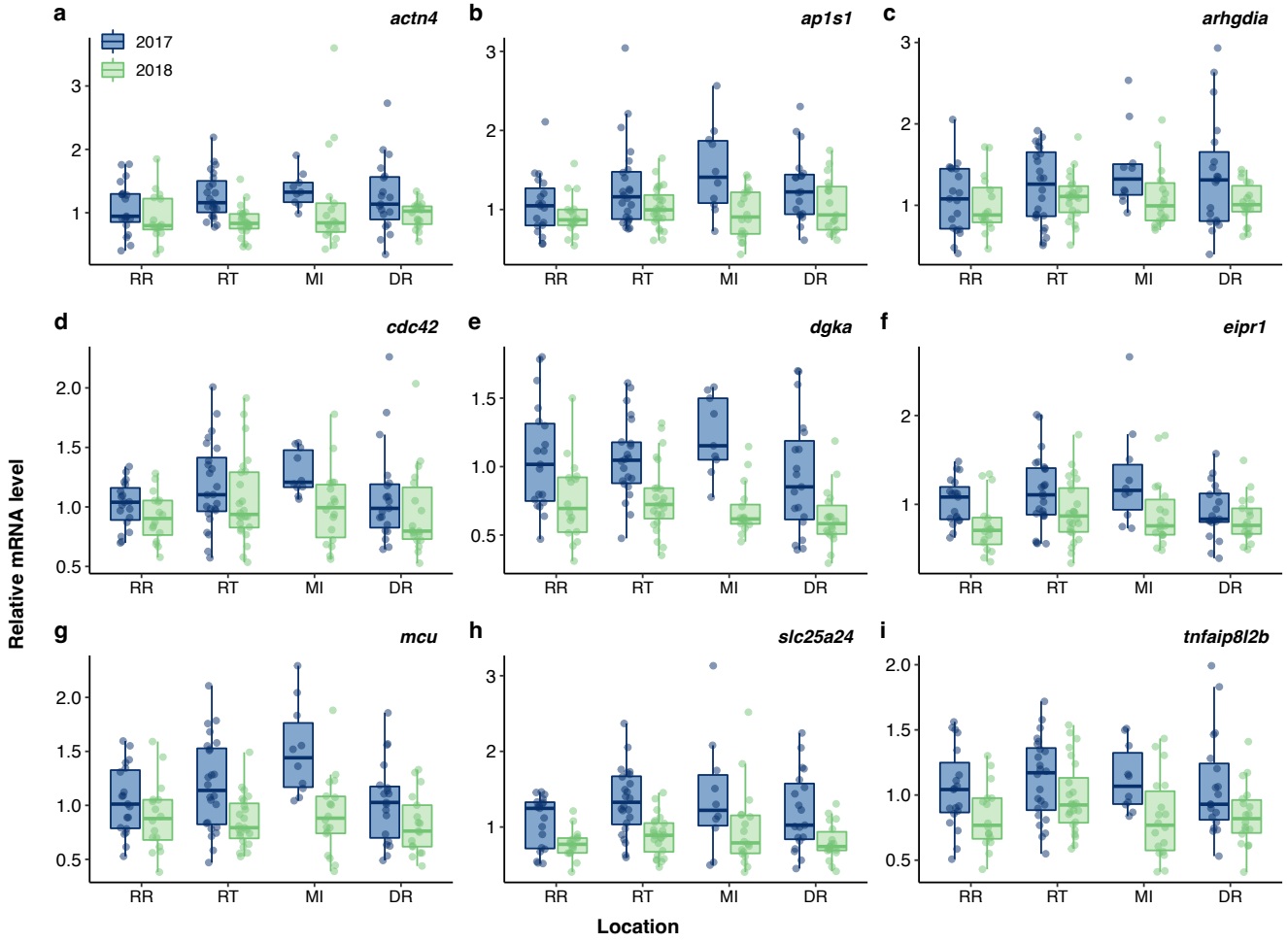

Figure S10. Relative mRNA levels for candidate genes of walleye (*Sander vitreus*) from the lake Winnipeg system. Walleye were sampled from the Red River (RR; n = 16–19), Riverton (RT; n = 22–24), Matheson Island (MI; n = 9–18), and Dauphin River (DR; n = 17–19) in 2017 and 2018. For each gene, there was a significant effect of year but not location (see Table S9). Horizontal bars in the boxplot represent the median response value and 75%, 50%, and 25% quartiles. Whiskers represent ± 1.5 times the interquartile range, and each dot represents an individual response value. See Table S2 for gene abbreviations.