

## Supplementary Materials

**Table S1.** Summary of 19 microsatellite loci used in preliminary genetic screenings of 40 individuals from four Walleye populations.

Locus	Size Range (bp)	Number of Alleles	Repeat	T <sub>m</sub> (°C)	Source
<i>Svi2</i>	190-208	9	(AC) <sub>18</sub>	54	[1]
<i>Svi4</i>	102-118	8	(AC) <sub>16</sub>	62	[2]
<i>Svi5</i>	178-191	NA	(AC) <sub>18</sub>	54	[1]
<i>Svi6</i>	135-169	18	(AC) <sub>19</sub>	60	[1]
<i>Svi7</i>	163-173	NA	(AC) <sub>14</sub>	53	[1]
<i>Svi10</i>	163-253	43	NA	54	[3]
<i>Svi11</i>	308-457	64	NA	5	[3]
<i>Svi16</i>	212-266	18	(AC) <sub>5</sub> n <sub>5</sub> (AC) <sub>30</sub>	55	[1]
<i>Svi17</i>	101-113	8	(AC) <sub>13</sub>	60	[2]
<i>Svi18</i>	118-126	5	(AC) <sub>18</sub>	58	[2]
<i>Svi20</i>	144-193	18	(AC) <sub>20</sub>	53	[1]
<i>Svi26</i>	156-189	16	(AC) <sub>13</sub> n <sub>2</sub> (AC) <sub>19</sub>	58	[1]
<i>Svi33</i>	90-102	7	(AC) <sub>14</sub>	60	[2]
<i>SviL1</i>	199-237	13	(GT) <sub>27</sub> GA(GT) <sub>3</sub> TT(GT) <sub>32</sub>	53	[4]
<i>SviL2</i>	175-203	11	(TAA) <sub>14</sub>	53	[4]
<i>SviL5</i>	188-224	11	(CA) <sub>18</sub>	55	[4]
<i>SviL6</i>	111-137	9	(AC) <sub>17</sub>	53	[4]
<i>SviL7</i>	195-235	11	(TG) <sub>22</sub>	53	[4]
<i>SviL8</i>	105-145	9	(TG) <sub>22</sub>	53	[4]

- [1] Eldridge, W.E.; Bacigalupi, M.D.; Adelman, I.R.; Miller, L.M.; Kapuscinski, A.R. Determination of relative survival of two stocked Walleye populations and resident natural-origin fish by microsatellite DNA parentage assignment. *Canad J Fish Aquat Sci* **2002**, *59*, 282-290.
- [2] Borer, S.O.; Miller, L.M.; Kapuscinski, A.R. Microsatellites in walleye *Stizostedion vitreum*. *Molec Ecol* **1999**, *8*(2), 336-338.
- [3] Cena, C.J.; Morgan, G.E.; Malette, M.D.; Heath, D.D. 2006. Inbreeding, outbreeding and environmental effects on genetic diversity in 46 Walleye (*Sander vitreus*) populations. *Molec Ecol* **2006**, *15*, 303-320.
- [4] Wirth, T.; Saint-Laurent, R; Bernatchez, L. Isolation and characterization of microsatellite loci in the Walleye (*Stizostedion vitreum*), and cross-species amplification within the family Percidae. *Molec Ecol* **1999**, *8*, 1960-1963.

**Table S2.** PCR protocols for amplification of 8 microsatellite loci used to assess population genetic structure of Walleye.

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Master mixes:

Master Mix 1: 22 $\mu$ l PCR solution contained 0.22 U/ $\mu$ l of 5X GoTaq Flexi Promega Buffer, 0.17 U/ $\mu$ l of 25 $\mu$ M of MgCl<sub>2</sub>, 2.5 $\mu$ M of dNTPs, 5 $\mu$ M concentration of both forward and reverse primer, with 0.01 U/ $\mu$ l of DNA Taq polymerase.

Master Mix 2: 17 $\mu$ l PCR solution contained 0.20 U/ $\mu$ l of 5X GoTaq Flexi Promega Buffer, 0.15 U/ $\mu$ l of 25 $\mu$ M of MgCl<sub>2</sub>, 2.5 $\mu$ M of dNTPs, 5 $\mu$ M concentration of both forward and reverse primer, with 0.012 U/ $\mu$ l of DNA Taq polymerase.

Master Mix 3: 22 $\mu$ l PCR solution contained 0.15 U/ $\mu$ l of 5X GoTaq Flexi Promega Buffer, 0.20 U/ $\mu$ l of 25 $\mu$ M of MgCl<sub>2</sub>, 2.5 $\mu$ M of dNTPs, 5 $\mu$ M concentration of both forward and reverse primer, with 0.016 U/ $\mu$ l of DNA Taq polymerase.

PCR protocols:

PCR Protocol 1: 95°C of initial denaturation for 5 minutes, followed by 10 cycles of 94°C for 1 minute, 50°C for 1 minute, and 72°C for 1 minute. This was followed by 29 cycles of 94°C for 1 minute, 59°C for 1 minute, and 72°C for 1 minute, with a final extension at 72°C for 10 minutes.

PCR Protocol 2: 94°C of initial denaturation for 3 minutes, followed by 30 cycles of 94°C for 1 minute, 57°C for 1 minute, and 72°C for 1 minute and 30 seconds, with a final extension at 72°C for 5 minutes.

PCR Protocol 3: 96°C of initial denaturation for 3 minutes, followed by 30 cycles of 96°C for 30 seconds, 53°C for 30 seconds, and 72°C for 1 minute, with a final extension at 72°C for 5 minutes.

PCR Protocol 4: 94°C of initial denaturation for 2 minutes, followed by 35 cycles of 94°C for 30 seconds, 48°C for 1 minute, and 72°C for 30 seconds, with a final extension at 72°C for 5 minutes.

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**Table S3.** Frequencies of null alleles in regional assemblages of populations estimated using Microchecker [5]. Because the Mobile Bay drainage and Great Lakes samples had high frequencies of missing data and a reduced number of polymorphic loci, estimation of null allele frequencies could not be completed.

<b>Population</b>	<i>Svi5</i>	<i>Svi6</i>	<i>Svi16</i>	<i>Svi17</i>	<i>Svi18</i>	<i>Svi33</i>	<i>SviL1</i>	<i>SviL7</i>
Mobile Bay drainage	-	-	-	-	-	-	-	-
Mississippi River	0.08	0.41	0.14	0.326	0.20	0.21	0.09	0.07
Tennessee River	0.06	0.14	0.11	0.25	0.32	0.26	0.26	0.29
New River	-	0.23	0.10	0.16	-	0.08	0.17	0.13
Ohio River	0.04	0.16	0.05	0.29	0.05	0.24	0.26	0.19
Great Lakes	-	-	-	-	-	-	-	-

[5] Van Oosterhout, C.; Hutchinson, W.F.; Willis, D.P.M.; Shipley, P. MICRO-CHECKER: software for identifying and correcting genotyping errors in microsatellite data. *Molec Ecol Notes* **2004**, *4*, 535-538.

**Table S4.** Full locus-by-locus genetic diversity indices for each population of Walleye.

<b>Population</b>	<b><i>N</i></b>	<b>Gene Copies</b>	<b>Alleles</b>	<b><math>H_o</math></b>	<b><math>H_e</math></b>	<b>Allele Range</b>	<b><math>M</math></b>
<b>Mobile Bay drainage</b>							
Coosa drainage, AL	<i>Svi17</i>	30	3	0.00	0.54	4	0.60
	<i>Svi33</i>	30	2	0.00	0.50	2	0.66
	<i>Svi18</i>	30	5	0.93	0.72	16	0.29
	<i>SviL1</i>	30	2	0.33	0.29	6	0.29
Tombigbee drainage, AL	<i>Svi6</i>	88	2	0.00	0.51	144	0.01
	<i>Svi5</i>	88	3	0.00	0.62	142	0.02
	<i>Svi17</i>	88	4	0.00	0.47	103	0.04
	<i>Svi33</i>	88	4	0.00	0.45	6	0.57
	<i>Svi18</i>	88	6	1.00	0.63	14	0.40
	<i>SviL1</i>	88	4	0.39	0.61	16	0.24
<b>Mississippi River drainage</b>							
Fellows Lake, MO	<i>Svi6</i>	18	5	0.78	0.80	8	0.56
	<i>Svi5</i>	18	10	0.89	0.93	42	0.23
	<i>SviL7</i>	18	6	0.44	0.81	10	0.55
	<i>Svi16</i>	18	8	0.67	0.89	20	0.38
	<i>Svi17</i>	18	4	0.22	0.76	103	0.04
	<i>Svi33</i>	18	3	0.89	0.63	83	0.04
	<i>SviL1</i>	18	9	0.67	0.91	179	0.05
Stockton Lake, MO	<i>Svi6</i>	42	7	0.43	0.79	146	0.05
	<i>Svi5</i>	42	13	0.81	0.92	186	0.07
	<i>SviL7</i>	42	9	0.24	0.84	239	0.04
	<i>Svi16</i>	42	13	0.57	0.86	205	0.06
	<i>Svi17</i>	42	7	0.71	0.81	109	0.06
	<i>Svi33</i>	42	5	0.48	0.89	85	0.06
	<i>Svi18</i>	42	4	0.00	0.52	120	0.03
	<i>SviL1</i>	42	9	0.29	0.61	181	0.05
Beaver/Table Rock Lake, AK/MO	<i>Svi6</i>	40	6	0.05	0.76	10	0.55
	<i>Svi5</i>	40	12	0.90	0.90	24	0.48
	<i>SviL7</i>	40	8	0.85	0.86	14	0.53
	<i>Svi16</i>	40	13	0.70	0.92	201	0.06
	<i>Svi17</i>	40	7	0.60	0.82	108	0.06
	<i>Svi33</i>	40	6	0.25	0.76	87	0.07
	<i>Svi18</i>	40	6	0.05	0.71	10	0.55
James/Table Rock Lake, MO	<i>Svi6</i>	40	6	0.10	0.59	12	0.46
	<i>Svi5</i>	40	8	0.50	0.82	18	0.42
	<i>SviL7</i>	40	9	0.85	0.83	229	0.04
	<i>Svi16</i>	40	10	0.40	0.85	199	0.05
	<i>Svi17</i>	40	6	0.35	0.81	107	0.06
	<i>Svi33</i>	40	7	0.15	0.79	95	0.07
	<i>Svi18</i>	40	7	0.40	0.79	122	0.06
Mozingo Lake, MO	<i>Svi6</i>	38	3	0.00	0.65	4	0.60

	<i>Svi5</i>	38	10	0.68	0.84	18	0.53
	<i>SviL7</i>	38	7	0.85	0.85	12	0.54
	<i>Svi16</i>	38	10	0.32	0.82	199	0.05
	<i>Svi17</i>	38	5	0.42	0.77	10	0.45
	<i>Svi33</i>	38	8	0.47	0.85	95	0.08
	<i>Svi18</i>	38	8	0.32	0.80	122	0.07
	<i>SviL1</i>	38	3	0.05	0.10	159	0.02
Smithville Lake, MO	<i>Svi6</i>	32	5	0.00	0.71	8	0.56
	<i>Svi5</i>	32	12	0.75	0.88	22	0.52
	<i>SviL7</i>	32	8	0.94	0.87	14	0.53
	<i>Svi16</i>	32	14	0.63	0.92	32	0.42
	<i>Svi17</i>	32	5	0.44	0.81	105	0.05
	<i>Svi33</i>	32	8	0.56	0.80	95	0.08
	<i>Svi18</i>	32	6	0.25	0.76	12	0.46
Current River, MO	<i>Svi6</i>	56	5	0.00	0.74	8	0.56
	<i>Svi5</i>	56	8	0.21	0.48	158	0.05
	<i>SviL7</i>	56	2	0.00	0.07	225	0.01
	<i>Svi17</i>	56	3	0.00	0.57	101	0.03
	<i>Svi33</i>	56	5	0.04	0.58	83	0.06
	<i>Svi18</i>	56	6	0.75	0.81	10	0.55
	<i>SviL1</i>	56	13	0.61	0.91	205	0.06
Black River, AK	<i>Svi6</i>	52	6	0.00	0.70	12	0.46
	<i>Svi5</i>	52	9	0.31	0.51	156	0.06
	<i>SviL7</i>	52	5	0.08	0.22	239	0.02
	<i>Svi17</i>	52	2	0.00	0.48	2	0.67
	<i>Svi33</i>	52	6	0.15	0.63	87	0.07
	<i>Svi18</i>	52	8	0.96	0.48	14	0.53
	<i>SviL1</i>	52	15	0.73	0.92	203	0.07
Tennessee River drainage							
Clinch and Powell Rivers, VA	<i>Svi6</i>	158	11	0.57	0.79	152	0.07
	<i>Svi5</i>	158	15	0.56	0.80	174	0.09
	<i>SviL7</i>	158	14	0.16	0.77	259	0.05
	<i>Svi16</i>	158	13	0.24	0.78	215	0.06
	<i>Svi17</i>	158	4	0.13	0.47	6	0.57
	<i>Svi33</i>	158	10	0.37	0.84	95	0.10
	<i>Svi18</i>	158	11	0.27	0.72	142	0.08
	<i>SviL1</i>	158	20	0.41	0.90	203	0.10
Lake Fontana, NC	<i>Svi6</i>	84	11	0.38	0.87	150	0.07
	<i>Svi5</i>	84	15	0.83	0.92	28	0.52
	<i>SviL7</i>	84	18	0.40	0.90	38	0.46
	<i>Svi16</i>	84	9	0.17	0.45	211	0.04
	<i>Svi17</i>	84	6	0.29	0.82	107	0.06
	<i>Svi33</i>	84	8	0.48	0.82	95	0.08
	<i>Svi18</i>	84	10	0.02	0.83	138	0.07
	<i>SviL1</i>	84	9	0.07	0.41	191	0.05

Nantahala Lake, NC	<i>Svi6</i>	54	8	0.41	0.82	14	0.53
	<i>Svi5</i>	54	12	0.78	0.89	26	0.44
	<i>SviL7</i>	54	13	0.41	0.83	36	0.35
	<i>Svi17</i>	54	5	0.15	0.80	105	0.05
	<i>Svi33</i>	54	5	0.07	0.46	87	0.06
	<i>Svi18</i>	54	7	0.00	0.76	134	0.05
Lake Santeetlah, NC	<i>Svi6</i>	36	9	0.56	0.89	18	0.47
	<i>Svi5</i>	36	11	0.50	0.91	172	0.06
	<i>SviL7</i>	36	14	0.61	0.93	261	0.05
	<i>Svi17</i>	36	5	0.56	0.73	105	0.05
	<i>Svi33</i>	36	5	0.28	0.63	95	0.05
	<i>Svi18</i>	36	6	0.06	0.82	128	0.05
Bear Lake, NC	<i>Svi6</i>	48	6	0.50	0.78	12	0.46
	<i>Svi5</i>	48	12	0.54	0.88	24	0.48
	<i>SviL7</i>	48	13	0.50	0.81	28	0.45
	<i>Svi17</i>	48	5	0.29	0.77	8	0.56
	<i>Svi33</i>	48	7	0.38	0.81	95	0.07
	<i>Svi18</i>	48	4	0.00	0.67	6	0.57
Wolf Lake, NC	<i>Svi6</i>	32	6	0.81	0.78	10	0.55
	<i>Svi5</i>	32	11	0.81	0.91	164	0.07
	<i>SviL7</i>	32	8	0.31	0.83	239	0.03
	<i>Svi16</i>	32	5	0.19	0.34	263	0.02
	<i>Svi17</i>	32	4	0.81	0.74	6	0.57
	<i>Svi33</i>	32	6	0.25	0.79	95	0.06
	<i>Svi18</i>	32	4	0.19	0.68	10	0.36
Lake James, NC	<i>Svi6</i>	100	8	0.54	0.83	14	0.53
	<i>Svi5</i>	100	15	0.86	0.91	30	0.48
	<i>SviL7</i>	100	15	0.36	0.88	34	0.43
	<i>Svi16</i>	100	15	0.98	0.88	277	0.05
	<i>Svi17</i>	100	5	0.48	0.68	105	0.05
	<i>Svi33</i>	100	6	0.26	0.78	87	0.07
	<i>Svi18</i>	100	10	0.36	0.84	18	0.53
Lake Hiwassee, NC	<i>Svi6</i>	32	7	0.50	0.81	12	0.54
	<i>Svi5</i>	32	9	0.81	0.86	18	0.47
	<i>SviL7</i>	32	7	0.19	0.79	24	0.28
	<i>Svi17</i>	32	4	0.31	0.72	103	0.04
	<i>Svi33</i>	32	4	0.19	0.55	85	0.05
	<i>Svi18</i>	32	7	0.94	0.85	128	0.05
Lake Glenville, NC	<i>Svi6</i>	48	8	0.53	0.86	14	0.53
	<i>Svi5</i>	48	11	0.75	0.91	20	0.52
	<i>SviL7</i>	48	11	0.25	0.88	255	0.04
	<i>Svi16</i>	48	4	0.13	0.23	269	0.01
	<i>Svi17</i>	48	5	0.42	0.75	8	0.56
	<i>Svi33</i>	48	6	0.38	0.76	89	0.07
	<i>Svi18</i>	48	10	1.00	0.90	18	0.53
New River, VA	<i>Svi6</i>	234	10	0.44	0.86	18	0.53

	<i>Svi5</i>	234	14	0.97	0.89	26	0.52
	<i>SviL7</i>	234	23	0.89	0.94	263	0.09
	<i>Svi16</i>	234	16	0.88	0.89	283	0.06
	<i>Svi17</i>	234	2	0.02	0.02	2	0.67
	<i>Svi33</i>	234	6	0.10	0.63	20	0.29
	<i>Svi18</i>	234	10	1.00	0.88	20	0.78
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Ohio River drainage							
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Barren River, KY	<i>Svi6</i>	140	11	0.24	0.82	148	0.07
	<i>Svi5</i>	140	19	0.83	0.92	40	0.46
	<i>SviL7</i>	140	17	0.67	0.91	36	0.46
	<i>Svi16</i>	140	13	0.69	0.79	273	0.05
	<i>Svi17</i>	140	5	0.07	0.62	105	0.05
	<i>Svi33</i>	140	7	0.41	0.79	95	0.07
	<i>Svi18</i>	140	11	0.67	0.83	136	0.08
	<i>SviL1</i>	140	11	0.04	0.53	175	0.06
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Goose Creek, KY	<i>Svi6</i>	54	6	0.15	0.67	10	0.55
	<i>Svi5</i>	54	12	0.93	0.88	32	0.36
	<i>SviL7</i>	54	13	0.70	0.86	257	0.05
	<i>Svi16</i>	54	9	0.56	0.77	263	0.03
	<i>Svi17</i>	54	4	0.00	0.59	103	0.04
	<i>Svi33</i>	54	5	0.19	0.52	83	0.06
	<i>Svi18</i>	54	7	1.00	0.82	12	0.54
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Rockcastle River, KY	<i>Svi6</i>	88	8	0.45	0.77	164	0.05
	<i>Svi5</i>	88	15	0.93	0.90	46	0.32
	<i>SviL7</i>	88	12	0.30	0.90	34	0.34
	<i>Svi16</i>	88	8	0.52	0.78	189	0.04
	<i>Svi17</i>	88	4	0.05	0.40	103	0.04
	<i>Svi33</i>	88	7	0.30	0.66	20	0.33
	<i>Svi18</i>	88	3	0.00	0.13	128	0.02
	<i>SviL1</i>	88	11	0.50	0.88	20	0.52
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Levisa and Russell Forks, KY	<i>Svi6</i>	30	6	0.53	0.74	142	0.04
	<i>Svi5</i>	30	12	0.93	0.90	44	0.27
	<i>SviL7</i>	30	7	0.13	0.83	12	0.54
	<i>Svi16</i>	30	10	0.53	0.77	203	0.05
	<i>Svi17</i>	30	4	0.53	0.73	6	0.57
	<i>Svi33</i>	30	7	0.67	0.79	20	0.33
	<i>SviL1</i>	30	12	0.53	0.92	24	0.48
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Allegheny River, PA	<i>Svi6</i>	42	6	0.90	0.79	144	0.04
	<i>Svi5</i>	42	10	0.57	0.88	18	0.53
	<i>SviL7</i>	42	12	0.95	0.90	30	0.49
	<i>Svi16</i>	42	6	0.29	0.82	10	0.55
	<i>Svi17</i>	42	3	0.33	0.36	4	0.60
	<i>Svi33</i>	42	9	0.33	0.85	20	0.43
	<i>Svi18</i>	42	4	0.05	0.70	6	0.57
	<i>SviL1</i>	42	11	0.52	0.90	24	0.44
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Great Lakes watersheds							
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Lake Erie	<i>Svi6</i>	52	5	0.19	0.65	8	0.56
	<i>Svi5</i>	52	7	0.65	0.80	16	0.41
	<i>SviL7</i>	52	8	0.50	0.84	26	0.30
	<i>Svi16</i>	52	6	1.00	0.79	10	0.55
	<i>Svi17</i>	52	2	0.04	0.04	2	0.67
	<i>Svi33</i>	52	5	0.35	0.78	14	0.33
	<i>Svi18</i>	52	6	1.00	0.79	10	0.55
Lake Ontario	<i>Svi6</i>	38	7	0.16	0.82	12	0.54
	<i>Svi5</i>	38	12	0.79	0.92	22	0.52
	<i>SviL7</i>	38	10	0.37	0.88	24	0.40
	<i>Svi16</i>	38	11	0.63	0.83	273	0.04
	<i>Svi17</i>	38	4	0.58	0.72	103	0.04
	<i>Svi33</i>	38	5	0.37	0.79	87	0.06
	<i>Svi18</i>	38	9	0.95	0.89	16	0.53
Mille Lacs, MN	<i>Svi6</i>	40	5	0.05	0.72	8	0.56
	<i>Svi5</i>	40	8	0.65	0.85	14	0.53
	<i>SviL7</i>	40	8	0.30	0.82	14	0.53
	<i>Svi17</i>	40	6	0.10	0.74	113	0.05
	<i>Svi33</i>	40	5	0.40	0.69	85	0.06
	<i>Svi18</i>	40	6	1.00	0.83	12	0.46
Lake Michigan	<i>Svi6</i>	40	5	0.10	0.64	10	0.45
	<i>Svi5</i>	40	9	0.80	0.77	16	0.53
	<i>SviL7</i>	40	7	0.20	0.71	12	0.54
	<i>Svi16</i>	40	5	0.20	0.36	261	0.20
	<i>Svi17</i>	40	4	0.40	0.73	103	0.04
	<i>Svi33</i>	40	6	0.35	0.77	87	0.07
	<i>Svi18</i>	40	7	1.00	0.78	12	0.54
Grand River, MI	<i>Svi6</i>	26	4	0.08	0.72	6	0.57
	<i>Svi5</i>	26	6	0.15	0.68	156	0.04
	<i>SviL7</i>	26	6	0.08	0.81	237	0.03
	<i>Svi16</i>	26	5	0.23	0.41	265	0.02
	<i>Svi17</i>	26	4	0.38	0.68	103	0.04
	<i>Svi33</i>	26	7	0.38	0.86	89	0.08
	<i>Svi18</i>	26	7	0.62	0.78	12	0.54
Flint River, MI	<i>Svi6</i>	40	7	0.80	0.85	12	0.54
	<i>Svi5</i>	40	11	0.75	0.88	22	0.48
	<i>SviL7</i>	40	10	0.45	0.87	22	0.43
	<i>Svi16</i>	40	8	0.95	0.81	273	0.03
	<i>Svi17</i>	40	5	0.15	0.77	105	0.05
	<i>Svi33</i>	40	5	0.30	0.80	858	0.06
	<i>Svi18</i>	40	8	0.95	0.84	14	0.53
St. Mary's River, MI	<i>Svi6</i>	38	9	0.32	0.81	16	0.53
	<i>Svi5</i>	38	12	0.84	0.91	24	0.48
	<i>SviL7</i>	38	6	0.37	0.83	14	0.40
	<i>Svi16</i>	38	8	0.37	0.59	273	0.03
	<i>Svi17</i>	38	5	0.11	0.72	105	0.05

	<i>Svi33</i>	38	7	0.26	0.81	89	0.08
	<i>Svi18</i>	38	9	1.00	0.86	16	0.53
Thunder Bay, Ontario	<i>Svi6</i>	12	5	0.17	0.80	10	0.45
	<i>Svi5</i>	12	7	1.00	0.89	18	0.37
	<i>SviL7</i>	12	5	0.67	0.79	233	0.02
	<i>Svi17</i>	12	3	0.00	0.55	101	0.03
	<i>Svi33</i>	12	3	0.17	0.62	83	0.04
	<i>Svi18</i>	12	7	1.00	0.91	12	0.54
Saginaw Bay, MI	<i>Svi6</i>	20	4	0.10	0.50	6	0.57
	<i>Svi5</i>	20	7	0.80	0.81	18	0.37
	<i>SviL7</i>	20	5	1.00	0.81	12	0.38
	<i>Svi16</i>	20	6	0.40	0.64	265	0.02
	<i>Svi17</i>	20	3	0.20	0.48	103	0.03
	<i>Svi33</i>	20	6	0.20	0.83	85	0.07
	<i>Svi18</i>	20	6	1.00	0.79	10	0.55
Oneida Lake, NY	<i>Svi6</i>	20	3	0.20	0.48	8	0.33
	<i>Svi5</i>	20	5	0.90	0.81	14	0.33
	<i>SviL7</i>	20	4	0.40	0.65	8	0.44
	<i>Svi17</i>	20	3	0.40	0.58	105	0.03
	<i>Svi33</i>	20	4	0.40	0.72	85	0.05
	<i>Svi18</i>	20	5	1.00	0.81	10	0.45
Lake Manitoba, Manitoba	<i>Svi6</i>	34	4	0.00	0.69	6	0.57
	<i>Svi5</i>	34	8	0.59	0.84	16	0.47
	<i>SviL7</i>	34	10	0.76	0.89	26	0.37
	<i>Svi16</i>	34	8	0.41	0.64	265	0.03
	<i>Svi17</i>	34	4	0.12	0.70	103	0.04
	<i>Svi33</i>	34	5	0.41	0.76	87	0.06
	<i>Svi18</i>	34	7	1.00	0.85	12	0.54
Lake Winnipeg, Manitoba	<i>Svi6</i>	38	8	0.16	0.83	16	0.47
	<i>Svi5</i>	38	16	1.00	0.93	36	0.43
	<i>SviL7</i>	38	10	0.63	0.86	20	0.48
	<i>Svi16</i>	38	8	0.42	0.65	267	0.03
	<i>Svi17</i>	38	4	0.11	0.59	103	0.04
	<i>Svi33</i>	38	5	0.26	0.64	85	0.06
	<i>Svi18</i>	38	10	1.00	0.89	18	0.53
Lac Mistassini, Quebec	<i>Svi6</i>	40	6	0.10	0.78	10	0.55
	<i>Svi5</i>	40	14	0.95	0.94	38	0.36
	<i>SviL7</i>	40	10	0.65	0.85	245	0.04
	<i>Svi16</i>	40	8	0.55	0.76	273	0.03
	<i>Svi17</i>	40	4	0.05	0.75	103	0.04
	<i>Svi33</i>	40	3	0.35	0.64	83	0.04
	<i>Svi18</i>	40	9	0.95	0.89	16	0.53

**Table S5.**  $F_{ST}$  values among populations of Walleye. Populations are (1) Hatchet Creek, Coosa Drainage, AL, (2) John Allen Fish Hatchery, Tombigbee drainage, AL, (3) Fellows Lake, MO, (4) Stockton Lake, MO, (5) Beaver/Table Rock Lake, AK/MO, (6) James/Table Rock Lake, MO, (7) Mazingo Lake, MO, (8) Smithville Lake, MO, (9) Current River, MO, (10) Black River, AK, (11) Clinch and Powell Rivers, VA, (12) Lake Fontana, NC, (13) Nantahala Lake, NC, (14) Lake Santeetlah, NC, (15) Bear Lake, NC, (16) Wolf Lake, NC, (17) Lake James, NC, (18) Lake Hiwassee, NC, (19) Lake Glenville, NC, (20) New River, VA, (21) Barren River, KY, (22) Goose Creek, KY, (23) Rockcastle River, KY (24) Levisa and Russell Forks, KY, (25) Allegheny River, PA, (26) Lake Erie, (27) Lake Ontario, (28) Mille Lacs, MN, (29) Lake Michigan, (30) Grand River, MI, (31) Flint River, MI, (32) St. Mary's River, MI, (33) Thunder Bay, ON, (34) Saginaw Bay, MI, (35) Oneida Lake, NY, (36) Lake Manitoba, MB, (37) Lake Winnipeg, MB, and (38) Lac Mistassini, QC.

	1	2	3	4	5	6	7	8	9	10	11	12
1	0.0											
2	0.191	0.0										
3	0.517	0.444	0.0									
4	0.400	0.355	0.062	0.0								
5	0.468	0.434	0.201	0.136	0.0							
6	0.463	0.409	0.225	0.148	0.069	0.0						
7	0.459	0.413	0.219	0.143	0.041	0.051	0.0					
8	0.493	0.447	0.202	0.143	0.026	0.056	0.022	0.0				
9	0.329	0.274	0.357	0.263	0.306	0.250	0.280	0.314	0.0			
10	0.292	0.222	0.351	0.260	0.311	0.280	0.279	0.328	0.049	0.0		
11	0.189	0.159	0.199	0.143	0.211	0.204	0.204	0.214	0.171	0.151	0.0	
12	0.346	0.306	0.154	0.080	0.120	0.110	0.100	0.127	0.218	0.221	0.128	0.0
13	0.482	0.421	0.259	0.160	0.216	0.204	0.192	0.203	0.300	0.321	0.208	0.064
14	0.458	0.405	0.264	0.147	0.192	0.172	0.160	0.191	0.260	0.273	0.196	0.079
15	0.474	0.418	0.272	0.161	0.157	0.162	0.143	0.175	0.264	0.271	0.224	0.087
16	0.472	0.426	0.252	0.148	0.131	0.144	0.126	0.144	0.258	0.273	0.212	0.089
17	0.418	0.397	0.176	0.100	0.102	0.127	0.126	0.110	0.288	0.299	0.191	0.097
18	0.492	0.430	0.256	0.132	0.173	0.169	0.165	0.197	0.265	0.299	0.211	0.066
19	0.423	0.365	0.227	0.108	0.152	0.137	0.130	0.149	0.255	0.365	0.168	0.035
20	0.435	0.359	0.242	0.146	0.190	0.178	0.195	0.167	0.310	0.306	0.170	0.142
21	0.323	0.273	0.111	0.053	0.134	0.123	0.126	0.125	0.219	0.214	0.104	0.049
22	0.444	0.386	0.218	0.131	0.157	0.184	0.188	0.180	0.316	0.307	0.202	0.119
23	0.378	0.327	0.094	0.104	0.264	0.255	0.251	0.256	0.304	0.282	0.141	0.167
24	0.415	0.366	0.084	0.077	0.244	0.243	0.224	0.242	0.311	0.290	0.143	0.145
25	0.413	0.350	0.172	0.147	0.219	0.226	0.222	0.208	0.301	0.280	0.077	0.166
26	0.526	0.451	0.271	0.170	0.202	0.207	0.230	0.188	0.363	0.362	0.210	0.190
27	0.422	0.370	0.171	0.080	0.113	0.123	0.120	0.118	0.277	0.272	0.150	0.059
28	0.456	0.374	0.245	0.116	0.185	0.182	0.180	0.196	0.278	0.277	0.181	0.083
29	0.471	0.406	0.233	0.114	0.172	0.179	0.178	0.180	0.294	0.305	0.200	0.098
30	0.389	0.334	0.243	0.117	0.179	0.170	0.170	0.185	0.204	0.212	0.123	0.085

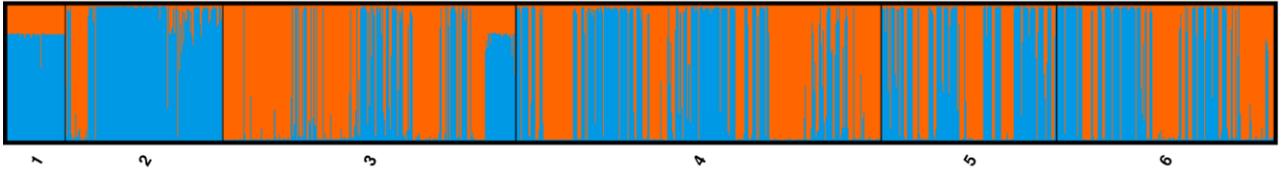
31	0.457	0.415	0.174	0.106	0.111	0.140	0.140	0.119	0.315	0.317	0.174	0.090
32	0.441	0.383	0.201	0.108	0.142	0.131	0.137	0.136	0.264	0.283	0.160	0.043
33	0.543	0.420	0.220	0.128	0.180	0.166	0.184	0.176	0.272	0.297	0.205	0.087
34	0.534	0.464	0.228	0.143	0.164	0.189	0.186	0.178	0.324	0.348	0.229	0.115
35	0.560	0.456	0.280	0.170	0.225	0.227	0.225	0.233	0.355	0.360	0.252	0.119
36	0.457	0.394	0.197	0.099	0.152	0.146	0.157	0.149	0.283	0.288	0.171	0.064
37	0.424	0.372	0.189	0.096	0.139	0.152	0.140	0.148	0.287	0.281	0.169	0.066
38	0.400	0.332	0.182	0.091	0.129	0.128	0.133	0.135	0.249	0.249	0.150	0.073

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14	0.130	0.0											
15	0.145	0.079	0.0										
16	0.154	0.073	0.014	0.0									
17	0.160	0.129	0.143	0.113	0.0								
18	0.112	0.100	0.088	0.094	0.113	0.0							
19	0.072	0.061	0.079	0.081	0.079	0.059	0.0						
20	0.189	0.200	0.209	0.214	0.128	0.214	0.131	0.0					
21	0.115	0.101	0.116	0.116	0.088	0.105	0.073	0.082	0.0				
22	0.191	0.166	0.169	0.169	0.127	0.139	0.138	0.164	0.093	0.0			
23	0.254	0.258	0.276	0.282	0.247	0.271	0.231	0.213	0.096	0.256	0.0		
24	0.256	0.244	0.269	0.261	0.227	0.258	0.217	0.237	0.093	0.262	0.020	0.0	
25	0.250	0.247	0.279	0.253	0.186	0.261	0.197	0.150	0.120	0.226	0.146	0.162	0.0
26	0.271	0.236	0.230	0.232	0.167	0.251	0.197	0.083	0.116	0.163	0.150	0.267	0.203
27	0.119	0.076	0.113	0.098	0.025	0.082	0.033	0.097	0.050	0.092	0.214	0.205	0.150
28	0.116	0.110	0.103	0.122	0.137	0.060	0.058	0.143	0.075	0.086	0.229	0.231	0.218
29	0.143	0.126	0.111	0.120	0.116	0.053	0.068	0.155	0.084	0.090	0.239	0.236	0.225
30	0.139	0.103	0.140	0.124	0.108	0.092	0.056	0.146	0.080	0.136	0.243	0.237	0.196
31	0.140	0.149	0.183	0.154	0.027	0.140	0.091	0.101	0.083	0.106	0.238	0.227	0.154
32	0.081	0.086	0.115	0.096	0.073	0.069	0.030	0.112	0.058	0.102	0.228	0.221	0.180
33	0.088	0.128	0.106	0.115	0.148	0.091	0.087	0.173	0.091	0.084	0.251	0.257	0.239
34	0.175	0.153	0.157	0.143	0.121	0.087	0.126	0.206	0.106	0.058	0.280	0.271	0.257
35	0.186	0.178	0.165	0.153	0.176	0.106	0.114	0.238	0.131	0.088	0.299	0.294	0.290
36	0.109	0.096	0.111	0.108	0.075	0.071	0.046	0.116	0.048	0.059	0.219	0.225	0.181

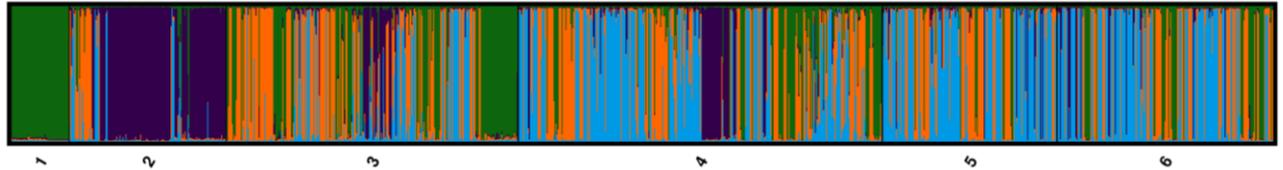
37	0.108	0.102	0.126	0.116	0.054	0.088	0.034	0.144	0.073	0.119	0.235	0.224	0.190
38	0.123	0.114	0.144	0.126	0.053	0.101	0.051	0.111	0.068	0.101	0.226	0.219	0.171
	26	27	28	29	30	31	32	33	34	35	36	37	38

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26	0.0												
27	0.152	0.0											
28	0.178	0.084	0.0										
29	0.158	0.081	0.013	0.0									
30	0.208	0.060	0.071	0.097	0.0								
31	0.170	0.021	0.136	0.132	0.112	0.0							
32	0.183	0.032	0.071	0.099	0.053	0.056	0.0						
33	0.210	0.099	0.048	0.076	0.112	0.125	0.051	0.0					
34	0.213	0.098	0.101	0.085	0.132	0.120	0.071	0.063	0.0				
35	0.262	0.141	0.061	0.062	0.151	0.178	0.112	0.081	0.050	0.0			
36	0.152	0.036	0.048	0.057	0.061	0.071	0.016	0.050	0.054	0.078	0.0		
37	0.215	0.008	0.045	0.101	0.065	0.065	0.049	0.103	0.131	0.158	0.066	0.0	
38	0.191	0.006	0.092	0.109	0.057	0.039	0.039	0.087	0.117	0.155	0.059	0.007	0.0

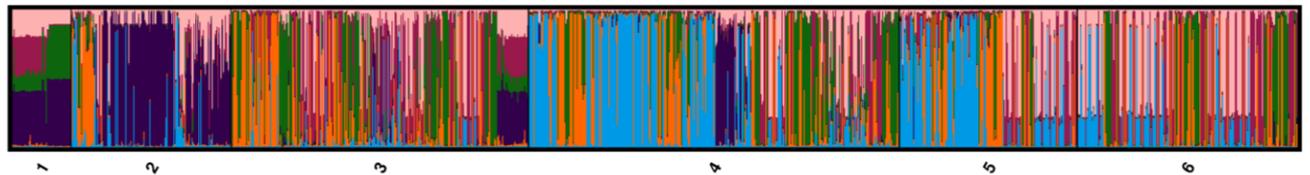
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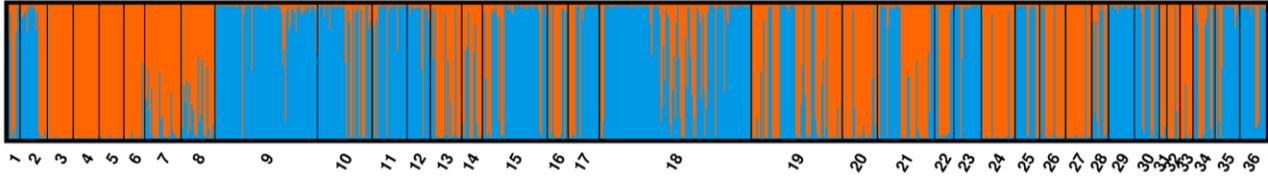


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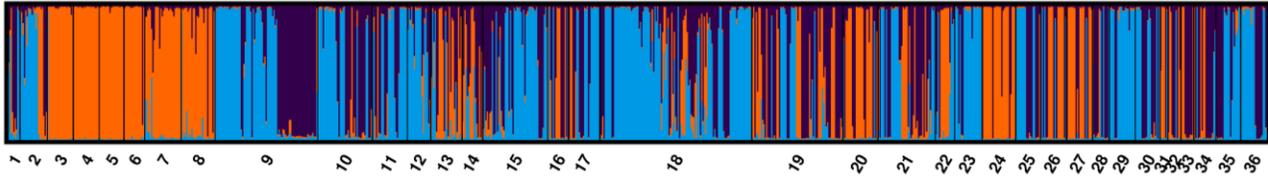


**Figure S1.** Structure plots representative of population genetic structuring of Walleye populations grouped within watersheds at different levels of  $K$ . The watersheds are designated: (1) Mobile Bay drainage, (2) Mississippi River, (3) Tennessee River, (4) New River, (5) Ohio River, and (6) Great Lakes.

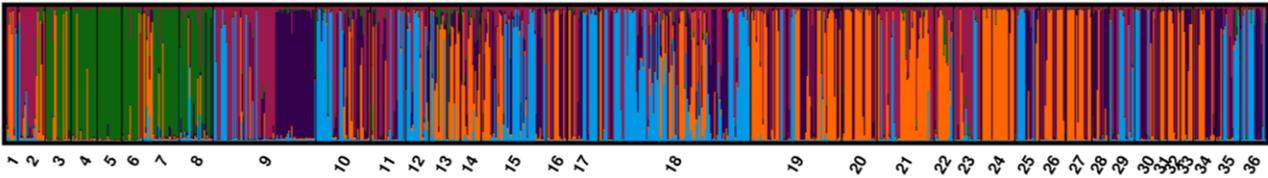
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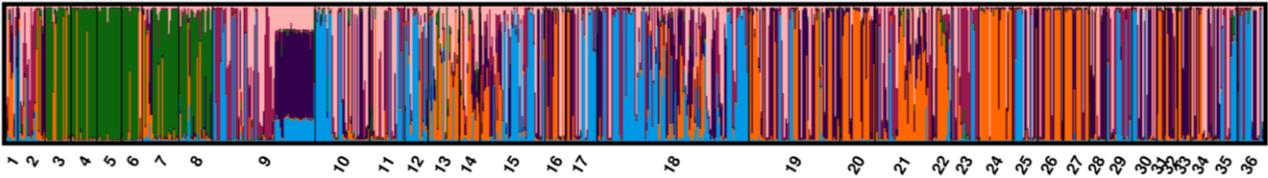
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$K = 5$

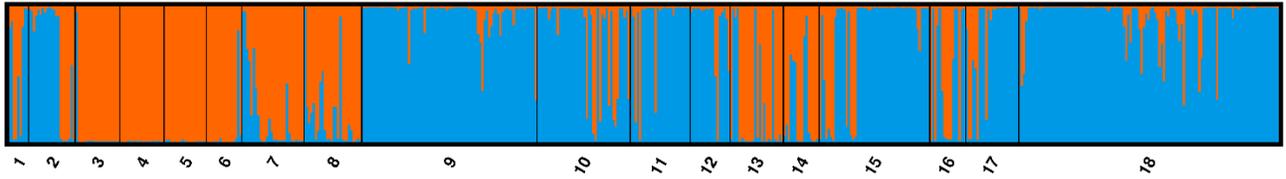


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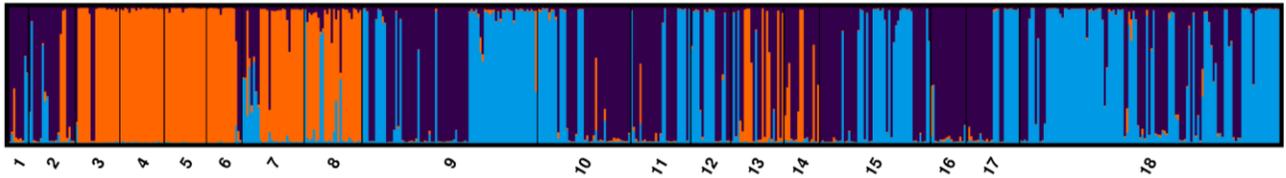


**Figure S2.** Structure plots representative of population genetic structuring of Walleye populations from the Mississippi, Tennessee, New, and Ohio River drainages and the Great Lakes at different levels of  $K$ . Populations are (1) Fellows Lake, MO, (2) Stockton Lake, MO, (3) Beaver/Table Rock Lake, AK/MO, (4) James/Table Rock Lake, MO, (5) Mozingo Lake, MO, (6) Smithville Lake, MO, (7) Current River, MO, (8) Black River, AK, (9) Clinch and Powell Rivers, VA, (10) Lake Fontana, NC, (11) Nantahala Lake, NC, (12) Lake Santeetlah, NC, (13) Bear Lake, NC, (14) Wolf Lake, NC, (15) Lake James, NC, (16) Lake Hiwassee, NC, (17) Lake Glenville, NC, (18) New River, VA, (19) Barren River, KY, (20) Goose Creek, KY, (21) Rockcastle River, KY (22) Levisa and Russell Forks, KY, (23) Allegheny River, PA, (24) Lake Erie, (25) Lake Ontario, (26) Mille Lacs, MN, (27) Lake Michigan, (28) Grand River, MI, (29) Flint River, MI, (30) St. Mary's River, MI, (31) Thunder Bay, Ontario, (32) Saginaw Bay, MI, (33) Oneida Lake, NY, (34) Lake Manitoba, Manitoba, (35) Lake Winnipeg, Manitoba, and (36) Lac Mistassini, Quebec.

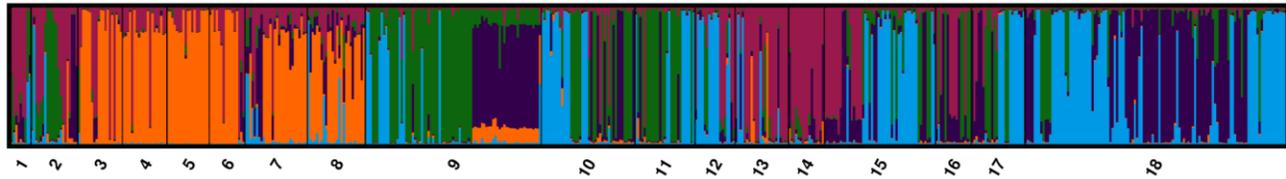
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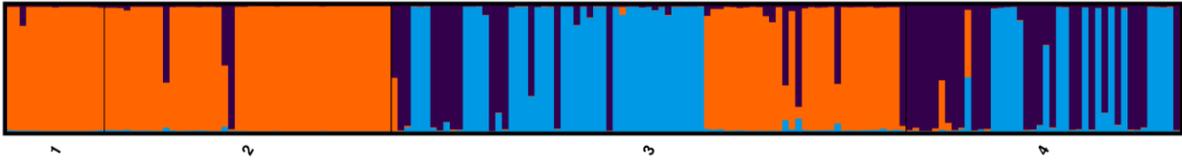


**Figure S3.** Structure plots representative of population genetic structuring of Walleye populations from the Mississippi, Tennessee, and New River drainages at different levels of  $K$ . Populations are (1) Fellows Lake, MO, (2) Stockton Lake, MO, (3) Beaver/Table Rock Lake, AK/MO, (4) James/Table Rock Lake, MO, (5) Mozingo Lake, MO, (6) Smithville Lake, MO, (7) Current River, MO, (8) Black River, AK, (9) Clinch and Powell Rivers, TN, (10) Lake Fontana, NC, (11) Nantahala Lake, NC, (12) Lake Santeetlah, NC, (13) Bear Lake, NC, (14) Wolf Lake, NC, (15) Lake James, NC, (16) Lake Hiwassee, NC, (17) Lake Glenville, NC, (18) New River, VA.

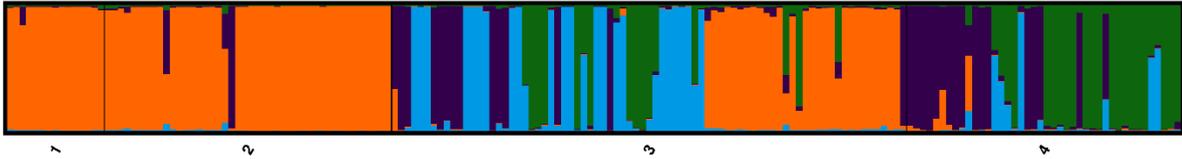
$K = 2$



$K = 3$



$K = 4$



**Figure S4.** STRUCTURE plots representative of population genetic structuring of Walleye populations across three southern drainages. Populations are (1) Hatchet Creek, Coosa Drainage, AL, (2) John Allen Fish Hatchery, Tombigbee drainage, AL, (3) Clinch and Powell Rivers, TN, and (4) Lake Fontana, NC.