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METHODS AND RESOURCES

An amplicon genotyping panel suitable for species identification and population genetics in sauger (*Sander canadensis*) and walleye (*Sander vitreus*)

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Abstract
Sauger (*Sander canadensis*) and walleye (*Sander vitreus*) are closely related North American fish species that are often managed by fishery agencies throughout their ranges. However, genotyping resources for sauger are presently limited to a small set of microsatellite loci. We evaluated whether primers in an existing walleye genotyping-in-thousands panel could amplify single nucleotide polymorphism loci (SNPs) in sauger. We identified 71 primer pairs that amplify 118 SNPs in both species. Allele frequency differences were large enough to confidently distinguish the species and identify hybrids. Additionally, we identified 41 loci with observed heterozygosity > 0.1 in sauger; these markers may be useful for simple population genetic analyses and parentage analysis when few contributors are present and for differentiating highly structured populations.

Keywords Amplicon genotyping · GT-seq · Sauger · Walleye · Stocking assessment

Sauger (*Sander canadensis*) and walleye (*Sander vitreus*) are closely related North American freshwater fish species. While both species have supported substantial recreational and commercial fisheries in parts of their ranges (Pegg et al. 1996; Radomski 2003; Schmalz et al. 2011), they are increasingly targets of restoration due to abundance declines associated with habitat fragmentation, overharvest, and climate change (Hoff 2001; Jaeger et al. 2005; Gillenwater et al. 2006; Hansen et al. 2017; Hartman et al. 2019). Given the species' dual roles as targets of both rehabilitation and harvest, they are often closely managed by natural resource agencies (e.g., Loukmas 2013; Gelwicks et al. 2014).

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Amplicon genotyping panels provide a cost-efficient means of collecting genetic data at hundreds of loci that can inform research of conservation interest (Meek and Larson 2019). A genotyping-in-thousands (GT-seq; Campbell et al. 2015) panel was recently developed for walleye with loci selected to both quantify population structure and inform parentage analyses (Bootsma et al. 2020). This panel consists of 436 primer pairs that generate ~ 140 bp amplicons, permitting genotyping on single-end 150 bp sequencing platforms. Given their taxonomically close relationship and capacity to hybridize (saugeye; Graeb et al. 2010; Quist et al. 2010), we expected a subset of loci from the existing walleye panel to amplify and be polymorphic in sauger. A genotyping panel applicable to both species could have conservation and management applications, including species identification, identification of saugeye (*Sander canadensis* X *vitreus*), and assessment of stocking success via parentage-based tagging.

To identify loci with utility for both sauger and walleye, we first genotyped 351 sauger from Wisconsin's Lake Winnebago using a 400-locus subset of the Bootsma et al. (2020) walleye GT-seq panel with demonstrated consistent amplification. Genomic DNA was extracted from ethanol-preserved fin clips using a chelating resin-based procedure

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(Campbell et al. 2015; Bootsma et al. 2020). PCR amplifications used 1.5 µl of the primer pool at 0.5 µM per primer pair and involved initial denaturing at 95 °C for five min, five touchdown amplification cycles (95 °C for 30 s, 5% ramp down, 57 °C for 2 min, 72 °C for 30 s), 10 additional amplification cycles (95 °C for 30 s, 65 °C for 30 s, 72 °C for 30 s), a final extension at 72 °C for 5 min, and a 4 °C hold. A barcoding PCR was then performed by combining 5 µl 2x Qiagen Multiplex PCR Plus master mix with 1 µl of 10 µM Illumina TruSeq i7 barcoding primer, 2 µl of 5 µM Illumina TruSeq i5 barcoding primer, and 2 µl 3:40 diluted PCR product. Barcoding amplifications involved initial denaturing at 95 °C for 5 min, 10 amplification cycles (95 °C for 10 s, 65 °C for 30 s, 72 °C for 30 s), a final extension at 72 °C for 5 min, and a 4 °C hold. PCR product was then normalized with SequalPrep normalization kits with an elution volume of 20 µl per well, followed by pooling 10 µl per well. The plate-specific 960 µl pools were concentrated to 100 µl with a QIAquick PCR purification and gel extraction kit. Eluates were purified with 0.9x AMPure XP beads and eluted in 40 µl TLE. We then performed a final purification via gel extraction. Libraries were quantified with Qubit high sensitivity assays and sequenced using MiSeq 2×150 runs. Sequence reads were then genotyped using GTscore v1.3 (McKinney et al. 2020).

Genotypes were filtered to remove SNPs with $>10\%$ missing data and individuals with $>50\%$ missing data. We used two walleye datasets to make comparisons to the sauger data; each genotyped according to Bootsma et al. (2020). First, we calculated locus-specific observed and expected heterozygosity and minor allele frequency using data from 254 walleye from a single population in Fox Lake, Wisconsin. Second, we used a random subsample of 351 walleye (i.e., the same number of sauger analyzed) drawn from a larger dataset of 45 walleye populations across Minnesota and Wisconsin to demonstrate differences in allele frequencies between the two species using a principal component analysis (PCA). Using multiple walleye populations allowed inter-specific variation to be assessed relative to intra-specific variation. Additionally, we used the hybridize function of the Adegenet (Jombart 2008) R package to simulate sauger x walleye genotypes to evaluate the panel's capacity to detect saugeye. PCA was performed on species-specific allele frequency data using the dudi.pca function of the R package ade4 (Dray and Dufour 2007; Thioulouse et al. 2018). Individuals with $>5\%$ missing data were removed from the PCA to limit bias caused by the necessary substitution of missing data with the average values of observed data.

Seventy-two primer pairs amplified 118 SNPs that were shared among sauger and walleye (1–7 SNPs per amplicon; Table 1). Two overamplifying loci (83,046 and 85,954) were identified, which could be removed from the genotyping panel to improve evenness of sequencing depth across loci (Table 1). Locus-specific measures of genetic diversity often differed substantially between the species (Table 1). Inter-specific allele frequency differences resulted in adequate power to distinguish the species and their hybrid along the first principal component axis (Fig. 1), with additional axes summarizing intraspecific variation (maximum 2.2% variation explained). These 118 SNPs may help inform future conservation genetic analyses of sauger, with the added benefit of enabling direct comparison to walleye.

Table 1 Locus IDs, SNP IDs, alleles, and primer sequences for 118 sauger (SAU; Sander canadensis) and walleye (WAE; Sander vitreus) primer pairs, along with proportion of reads attributable to each proper pair (Prop. reads), measures of genetic diversity, deviation from Hardy-Weinberg proportions for each SNP amplified by the listed primers

Locus	Pos	Forward primer	Reverse primer	T_MFor	T_MRev	Prop. reads	H_{obs}		H_{exp}		MAF		HW *p*-value	
							SAU	WAE	SAU	WAE	SAU	WAE	SAU	WAE
407	34	GCTTTSCCCCACCAACTACA	TGGGGACAAAACAAGACATTTGAG	57.8	55.9	0.002	0.03	0.50	0.03	0.48	0.01	0.40	1.00	0.52
2838	112	CAGTCATGGTGAAGTGGTACA	ATCGTCAGGTGTTCCCAACA	54.5	56.5	0.001	0.03	0.46	0.03	0.50	0.01	0.49	1.00	0.24
3807	82	GCCAAAGCCCGATCATACAA	GGGAGAGTACAAACTAACTGATGG	55.7	54.8	0.003	0.21	0.37	0.21	0.33	0.12	0.21	1.00	0.08
3816	131	CACCTGAACCGGGCTCTGAT	ACGGTCAGTRGGTTCAGTATCTC	59.5	56.8	0.004	0.98	1.00	0.50	0.50	0.49	0.50	0.00	0.00
5414	88	GTCTGCCTGGTGTACTCTCG	ATCACGATGCCAGGTCCAC	57.2	57.5	0.013	0.15	0.33	0.14	0.31	0.07	0.19	0.39	0.55
5649	119	GCCGAGGGTAAGAGACTTGT	GCGGCCTCCAACTTGAGTTT	56.5	58.3	0.001	0.04	0.45	0.04	0.46	0.02	0.35	1.00	1.00
8215	21	TGCAGGCTACAGGGAAGAC	TGTCTTGCAGGAAATCGCAC	56.9	56	0.02	0.04	0.47	0.04	0.36	0.02	0.23	1.00	0.00
8215	24						0.08	0.55	0.07	0.40	0.04	0.28	1.00	0.00
8215	41						0.01	0.34	0.01	0.32	< 0.01	0.20	1.00	0.43
8215	65						0.25	0.55	0.24	0.49	0.14	0.43	1.00	0.06
8672	69	CTGTCAAGATCAGCATAGAGGGA	CACACACACAAGTTCGAGCC	55.9	56.6	0.007	0.01	0.17	0.01	0.18	0.01	0.10	1.00	0.29
8672	75						0.03	0.50	0.03	0.45	0.01	0.34	1.00	0.11
8799	123	AAGGATAAATGTGACTCTAGCCTGA	CGGTGTAACGGAAGCAGGAT	55.4	57.3	0.002	0.00	0.48	0.00	0.47	< 0.01	0.38	1.00	0.91
9012	134	AAAGCAGCTGTCAACTGGA	GGTCAGTACAGAAACATTTCAGGT	54.5	55	0.001	0.47	0.16	0.47	0.15	0.38	0.08	1.00	1.00
10,127	69	TCAACACTGCATTACCGACT	CAAGACAGGCCTATAAGATCAGG	54.1	54.6	0.001	0.03	0.37	0.03	0.49	0.02	0.42	1.00	0.00
10,310	42	AGGTGCAGCAGCTCGGWAAA	AAGGCTCTAGTTACTYCTACTGTCT	59.6	55.4	0.001	0.18	0.31	0.21	0.38	0.12	0.25	0.01	0.00
10,310	59						0.01	0.49	0.01	0.47	< 0.01	0.37	1.00	0.38
10,310	104						0.35	0.16	0.38	0.17	0.26	0.09	0.17	0.48
12,951	40	AGAGCCGGGTGTCAGATCT	CCACGATGTCTGAGGTATAACT	58	53.7	0.001	0.38	0.19	0.36	0.25	0.23	0.14	0.30	0.00
12,951	52						0.01	0.34	0.01	0.36	< 0.01	0.24	1.00	0.26
12,951	102						0.01	0.36	0.01	0.40	0.01	0.28	1.00	0.16
14,727	58	CTGTCAGCATTAACGCRTTAA	CCACAAGGCTTTAATCCAGCT	52.4	55.4	0.002	0.90	0.91	0.50	0.50	0.45	0.45	0.00	0.00
14,727	65						0.21	0.63	0.19	0.45	0.11	0.33	0.19	0.00
15,072	25	GCTTCTACAGCGAGGAGGA	ACAATCTGTTTGCTTGCACTGG	56.3	56.5	0.004	0.17	0.03	0.16	0.03	0.09	0.01	0.47	1.00
15,430	124	GCAGGAAACATCAGGACACA	TTTCAGCTGCCGGACTCAG	55.2	57.6	0.002	0.02	0.07	0.02	0.07	0.01	0.03	1.00	1.00
18,971	39	GACCCGTACGGAACCAAGTC	CCCTTTCGGACCTTGGATAAA	57.7	54.6	0.002	0.05	0.46	0.05	0.42	0.03	0.30	1.00	0.22
27,091	101	CCTGTTCTCTTTGGTCTTCAAC	TCCTTTCCACCGGACTGTTT	53.6	56.5	0.002	0.01	0.26	0.01	0.25	< 0.01	0.14	1.00	0.81
35,129	36	AGAGCAGGAAAGGGTTAATCAT	TCAACTCTCTAGCACCTCCC	53.9	55.8	0.008	0.09	0.41	0.10	0.44	0.05	0.33	0.58	0.19
43,602	28	TGCAGGGACATACAGTACTCC	GCAGTTAAGCTATCTCTTGTGTGT	56	55	0.006	0.24	0.50	0.24	0.42	0.14	0.30	1.00	0.00
49,163	27	TCCGCCGCAACTCAGTTCT	AGAATAACAGTTCCAGTGCAGT	59.4	54.1	0.003	0.15	0.43	0.17	0.43	0.09	0.31	0.06	1.00
49,163	87						0.00	0.46	0.00	0.45	< 0.01	0.34	1.00	1.00
49,163	88						0.01	0.46	0.01	0.45	< 0.01	0.34	1.00	1.00
49,163	99						0.01	0.56	0.01	0.50	< 0.01	0.49	1.00	0.09
49,163	104						0.01	0.55	0.01	0.50	< 0.01	0.49	1.00	0.08
49,163	108						0.01	0.44	0.01	0.45	< 0.01	0.34	1.00	0.89
49,163	113						0.03	0.00	0.03	0.00	0.01	1.00	1.00	1.00
49,616	24	TGCAGGCAAAAGAAAGGAGA	GAGTTGTGTGGACGCGA	54.7	55.2	0.003	0.27	0.50	0.33	0.48	0.21	0.41	0.00	0.79
50,827	18	TGCAGGAGGTAGATATGYCCAG	GTTTAATCTTTGAGTTAGGGAAGGC	57	54	0.008	0.99	1.00	0.50	0.50	0.49	0.50	0.00	0.00

Table 1 (continued)

Locus	Pos	Forward primer	Reverse primer	T_MFor	T_MRev	Prop. reads	H_{obs}		H_{exp}		MAF		HW *p*-value	
							SAU	WAE	SAU	WAE	SAU	WAE	SAU	WAE
51,390	111	GAGGCCGCYGTTGTTTGATA	TCCTCCTCTGCCCTGTCTGA	56.8	59.8	0.001	0.08	0.48	0.09	0.46	0.04	0.37	0.51	0.78
51,689	31	GGTGAGAGACAACACATATCCAC	GCTTCAATATTTGTGGCACCT	55.1	53.7	0.001	0.02	0.46	0.02	0.49	0.01	0.44	1.00	0.21
51,969	47	TGCAGGCCAGGGACACAAA	CAATGATCACCRAGTGGCATCC	60	57.3	0.006	0.00	0.39	0.00	0.41	1.00	0.29	1.00	0.65
51,969	129						1.00	1.00	0.50	0.50	0.50	0.50	0.00	0.00
52,719	21	GGGTTCACCACAGGTTSATT	TCAGCATGCTCAATACCAGGG	55.3	57.2	0.003	0.52	0.40	0.38	0.32	0.26	0.20	0.00	0.00
52,719	70						0.00	0.51	0.00	0.49	< 0.01	0.42	1.00	0.43
53,047	88	TGCAGGGAGAAGACACCTC	GTGTYTGACCTGGTGTTGT	56.6	54	0.032	0.91	0.86	0.50	0.50	0.45	0.48	0.00	0.00
53,047	113						0.06	0.08	0.05	0.08	0.03	0.04	1.00	1.00
53,757	54	GGATGATCCTGGTCAGTCGG	TGTTAAACTGCGTTTCGTTGTC	57.2	54.2	0.014	0.00	0.52	0.00	0.50	1.00	0.48	1.00	0.61
53,757	71						0.49	0.35	0.48	0.36	0.40	0.24	0.84	0.72
57,228	64	GAGGCGACGGAGGTGGATTT	ATGTTTGCCAGTGTGACATCG	59.7	56	0.008	0.40	0.05	0.40	0.05	0.28	0.02	0.90	1.00
62,093	87	CGTGTTCTCCTGCTGTGTTC	CAGCTGCAGGACTGAGTCTG	56.1	57.8	0.012	0.00	0.45	0.00	0.45	< 0.01	0.34	1.00	1.00
63,600	65	TGCATATTCCCCTCCCTCCC	AATGGACATGCATGCCAGGC	58.8	58.9	0.008	0.08	0.53	0.08	0.47	0.04	0.38	1.00	0.06
63,600	105						0.10	0.40	0.09	0.39	0.05	0.27	1.00	0.86
64,864	127	GCTCAGCACTGTTCAGAGAGT	AGGTGGTTTAGCAGCTCATTGT	56.9	56.8	0.007	0.02	0.02	0.02	0.02	0.01	0.01	0.04	1.00
66,199	62	GTACAGTCAGAGGAGTTTGCT	CCTCTTGCAGCAGGAACCT	54.2	57.5	0.005	0.02	0.42	0.02	0.39	0.01	0.26	1.00	0.35
74,161	56	TCAGACATGTTAGGGCATCTG	GGCCTGCTGTTTAATGCAAGT	54.4	56.4	0.007	0.00	0.23	0.00	0.25	1.00	0.14	1.00	0.18
76,206	23	TGCAGGCCTAATGTGCTGAA	GTTAAGCAAAGTCCTGTAAACTCTC	57.1	53.8	0.001	0.02	0.03	0.02	0.03	0.01	0.02	1.00	1.00
76,358	17	TGCAGGCTGATATTAGYGTCCT	GGATCCATGTATTTACCAAATCTGC	57	54.1	0.004	0.91	0.86	0.50	0.49	0.45	0.43	0.00	0.00
77,004	72	CGATTGTTGGAGGTTTCTGGG	ATGCTGCATTTAATCCTGTCCA	56.1	54.8	0.013	0.49	0.53	0.48	0.49	0.40	0.44	0.65	0.32
78,262	38	GCTCAGGTAGGAGGACATGTC	GCCATCTAAGCTTACAAAGGTACA	56.8	54.8	0.002	0.38	0.09	0.35	0.09	0.23	0.04	0.23	1.00
78,262	98						0.02	0.47	0.02	0.50	0.01	0.48	1.00	0.33
78,784	55	GTCACTCAGTATACTCGTATGCT	CAGTCAGAGAACAGCTACAGG	53.5	54.7	0.009	0.26	0.45	0.25	0.45	0.15	0.34	0.40	1.00
78,784	129						0.00	0.48	0.00	0.50	1.00	0.46	1.00	0.69
78,833	124	GGCTTGGCTGGCTCTGATTA	ACGTTATATTGCTAGARGTGGGA	57.5	54.3	0.013	0.99	0.98	0.50	0.50	0.49	0.49	0.00	0.00
79,206	96	AGCAATGCTTCAAGGTGACTC	CCTCTGCCTGTACTGTTGAAAC	55.5	55.7	0.005	0.00	0.54	0.00	0.50	1.00	0.47	1.00	0.19
80,879	63	GAAGTGACCTCAGACTTGACGT	ATGCCAATAAACCAGAGCAGG	56.5	55.5	0.009	0.23	0.49	0.27	0.50	0.16	0.48	0.02	0.79
80,879	71						0.01	0.26	0.01	0.28	0.01	0.17	1.00	0.24
81,600	51	ACCAATGACAGCCTAGTAAGC	CCAAGCCACTTTGATCATCCA	54.6	55.4	0.002	0.04	0.00	0.04	0.00	0.02	0.00	1.00	1.00
81,600	91						0.00	0.54	0.00	0.50	1.00	0.46	1.00	0.22
82,477	53	TGTGAGGGTCCTTCCGTGGT	TCTGTCTCCGCGAAGTCCAG	60.7	58.9	0.006	0.02	0.45	0.02	0.41	0.01	0.29	1.00	0.15
82,477	88						0.10	0.51	0.10	0.47	0.05	0.38	0.25	0.19
82,518	69	CACAAACCTACTCAAACTCAACAGT	GGAACGGTTGTATCTTGGCA	55.4	55.2	0.002	0.03	0.03	0.09	0.03	0.05	0.02	0.00	1.00
82,518	88						0.02	0.47	0.02	0.47	0.01	0.37	1.00	1.00
82,518	114						0.20	0.31	0.41	0.30	0.28	0.18	0.00	0.57
83,046	44	AGGTTGTCAACTGCGTGGTT	GTTGGGTTGAGAGTCGGAGC	57.4	58	0.178	0.02	0.77	0.02	0.48	0.01	0.39	1.00	0.00
83,046	118						0.01	0.47	0.01	0.42	< 0.01	0.30	1.00	0.05
83,250	84	TGCAGGATATTCAACATCAGTAACG	TGGAAACAGCRCCATGGAAA	55.1	56.4	0.005	0.18	0.24	0.16	0.24	0.09	0.14	0.09	1.00
83,250	97						0.42	0.32	0.43	0.29	0.32	0.17	0.46	0.05

Table 1 (continued)

Locus	Pos	Forward primer	Reverse primer	T_MFor	T_MRev	Prop. reads	H_{obs}		H_{exp}		MAF		HW *p*-value	
							SAU	WAE	SAU	WAE	SAU	WAE	SAU	WAE
83,990	92	GGGGTTGGCGTTCATTTCT	GAGGGCAGATGGAGAGAGAGA	55.9	57.6	0.064	0.00	0.48	0.00	0.49	1.00	0.41	1.00	1.00
85,173	25	TTGAAAACACACAGGGCCRA	ACGGGTTTTATTGAGTTCGTTC	56.4	53.4	0.002	0.98	0.90	0.50	0.49	0.49	0.45	0.00	0.00
85,173	83						0.01	0.47	0.01	0.47	0.01	0.38	1.00	1.00
85,853	38	TGCAGGTTTTAAAGGAACAYGC	TTCCCAGTATGTATACAGAGTTACG	55.3	53.6	0.002	0.03	0.45	0.03	0.42	0.01	0.30	1.00	0.45
85,853	88						0.01	0.48	0.01	0.46	< 0.01	0.35	1.00	0.48
85,853	91						0.01	0.48	0.01	0.47	< 0.01	0.37	1.00	0.69
85,853	101						0.01	0.50	0.01	0.50	< 0.01	0.49	1.00	1.00
85,954	50	TGCAGGTTGTCAACTGTRGT	TTGAGAGTTGGAGCAGGGTT	55.6	56.3	0.168	0.89	0.13	0.49	0.12	0.44	0.07	0.00	0.61
89,495	69	GCAGGTATCAGTATCAGCAGGT	GCAAGAGAAACAAATTGCGCT	56.4	54.8	0.011	0.01	0.14	0.01	0.14	0.01	0.08	0.01	0.65
90,624	122	GCAGCTGGGCATTGGATCTT	GAGCTGCCTCATTACCGTCT	58.4	56.8	0.01	0.29	0.43	0.30	0.43	0.19	0.31	0.47	0.88
91,228	33	CAGGGAGGCATGCACATCTC	TTTGTCACAGCCCACTATACAT	58.3	54	0.005	0.01	0.10	0.01	0.10	< 0.01	0.05	1.00	0.53
91,228	71						0.02	0.33	0.02	0.33	0.01	0.21	1.00	1.00
92,099	54	TGCAGGAAACGCGTGATGG	GGGCTAAAGTCACGTACTGCT	58.7	56.9	0.013	0.04	0.44	0.04	0.44	0.02	0.33	1.00	1.00
92,649	92	GTAGATCTAAACTACTGCTGCTGC	TGGAACTCTGGTGGAACTGC	55.2	57.3	0.035	0.01	0.55	0.01	0.50	< 0.01	0.50	1.00	0.19
94,066	85	TGTGTACAAGTACCTCTGAGCTG	GGAGACAGGAACAAAGTCACC	56.1	55.5	0.008	0.02	0.51	0.02	0.50	0.01	0.46	1.00	0.81
95,180	12	TGCAGGGACTTYTGGGGTTT	TCACTGAGGTTTAGCAATGTGT	58.2	54.3	0.011	0.79	0.23	0.48	0.20	0.40	0.11	0.00	0.05
95,563	105	GTCTCCGCCAACACTCAAAC	CCACGTCAGGCTACATATCCC	56.4	57.1	0.011	0.06	0.08	0.06	0.08	0.03	0.04	1.00	1.00
97,392	84	ATGACTTCCTCTCGCCTTTCC	ACCACCCTCTGTCAGTACATG	56.8	56.3	0.093	0.02	0.31	0.02	0.28	0.01	0.17	1.00	0.18
98,061	32	GCAGGTATSTCAGTGGGATTGA	GGTCTCTGAGGCTATAAATAAACAG	56.6	53	8E-04	0.20	0.52	0.44	0.50	0.33	0.45	0.00	0.43
98,061	57						0.16	0.50	0.35	0.48	0.23	0.40	0.00	0.59
98,061	132						0.00	0.28	0.00	0.27	1.00	0.16	1.00	0.81
98,400	76	TCTGCGTTTCTTTCTCTGCG	TAACAGATGCTAACCGCCCG	55.6	57.3	0.017	0.02	0.39	0.02	0.42	0.01	0.29	0.04	0.36
98,787	33	TGCAGGAGAGGAGAGACACG	TGAACTGACCGTCTGTGATGA	58.5	55.8	0.013	0.09	0.52	0.08	0.50	0.04	0.48	1.00	0.70
98,787	64						0.02	0.03	0.02	0.03	0.01	0.02	1.00	1.00
99,095	59	CTGATCACACTGCGCTGGT	TGTTTGTGTTTCCGTCACCT	57.9	54.8	0.005	0.03	0.47	0.03	0.46	0.02	0.36	1.00	0.78
99,988	24	AGGCWCTGGGTGTTCTTGC	ACAGATTGAACCCTAATCACCC	58	54.5	0.005	0.02	0.52	0.02	0.46	0.01	0.37	1.00	0.07
99,988	48						0.49	0.19	0.50	0.19	0.46	0.11	0.74	0.74
99,988	93						0.01	0.52	0.01	0.46	0.01	0.36	1.00	0.05
99,988	136						0.02	0.55	0.02	0.48	0.01	0.41	1.00	0.05
101,686	91	TCCTCCGTCTCTTGCCTCCA	CATATTGTGTGATCTGAAAGGGCC	60.2	56	0.043	0.00	0.59	0.00	0.50	< 0.01	0.46	1.00	0.00
101,686	133						0.56	0.45	0.50	0.41	0.47	0.29	0.02	0.08
101,686	134						0.56	0.45	0.50	0.41	0.47	0.29	0.01	0.22
106,412	126	AGGAATCAAAACCTACCAGACACT	CACTGGCAACTTTCTTCAGCA	55.9	55.9	0.004	0.04	0.42	0.04	0.42	0.02	0.30	1.00	1.00
108,053	67	AGGGTGGAGAAGTGTAGCAT	TCTCTCTAATGACCAGGCTACA	55.6	54.6	0.004	0.01	0.37	0.01	0.33	0.00	0.21	1.00	0.09
108,053	114						0.03	0.26	0.03	0.25	0.01	0.15	1.00	0.64
113,244	68	GGTATGTTCACTGCTATCGCC	AGTCTTTGAGTGTCTTTCGCA	55.6	54.3	0.008	0.13	0.44	0.13	0.50	0.07	0.45	0.39	0.09
113,244	125						0.13	0.32	0.13	0.31	0.07	0.19	0.71	1.00
113,556	38	GGAGTGAAGCTGCTGTCTG	CAAGATCTGAGCCAGTGCCT	55.7	57.2	0.006	0.01	0.00	0.01	0.00	< 0.01	1.00	1.00	1.00
113,556	127						0.01	0.49	0.01	0.49	< 0.01	0.44	1.00	1.00

Table 1 (continued)

Locus	Pos	Forward primer	Reverse primer	T_MFor	T_MRev	Prop. reads	H_{obs}		H_{exp}		MAF		HW *p*-value	
							SAU	WAE	SAU	WAE	SAU	WAE	SAU	WAE
185,622	57	AGGATTCAGTAAAGGAAGGAGATGG	GGGCACTTCTCATAACACATACG	56	55.6	0.008	0.00	0.48	0.00	0.50	1.00	0.48	1.00	0.53
185,622	61						0.15	0.48	0.14	0.50	0.07	0.48	0.23	0.54

Pos: position within locus; T_MFor: forward primer melting temperature; T_MRev: reverse primer melting temperature; H_{obs}: observed heterozygosity; H_{exp}: expected heterozygosity; MAF: Minor allele frequency; HW p-value: exact test for Hardy-Weinberg proportion deviation p-value

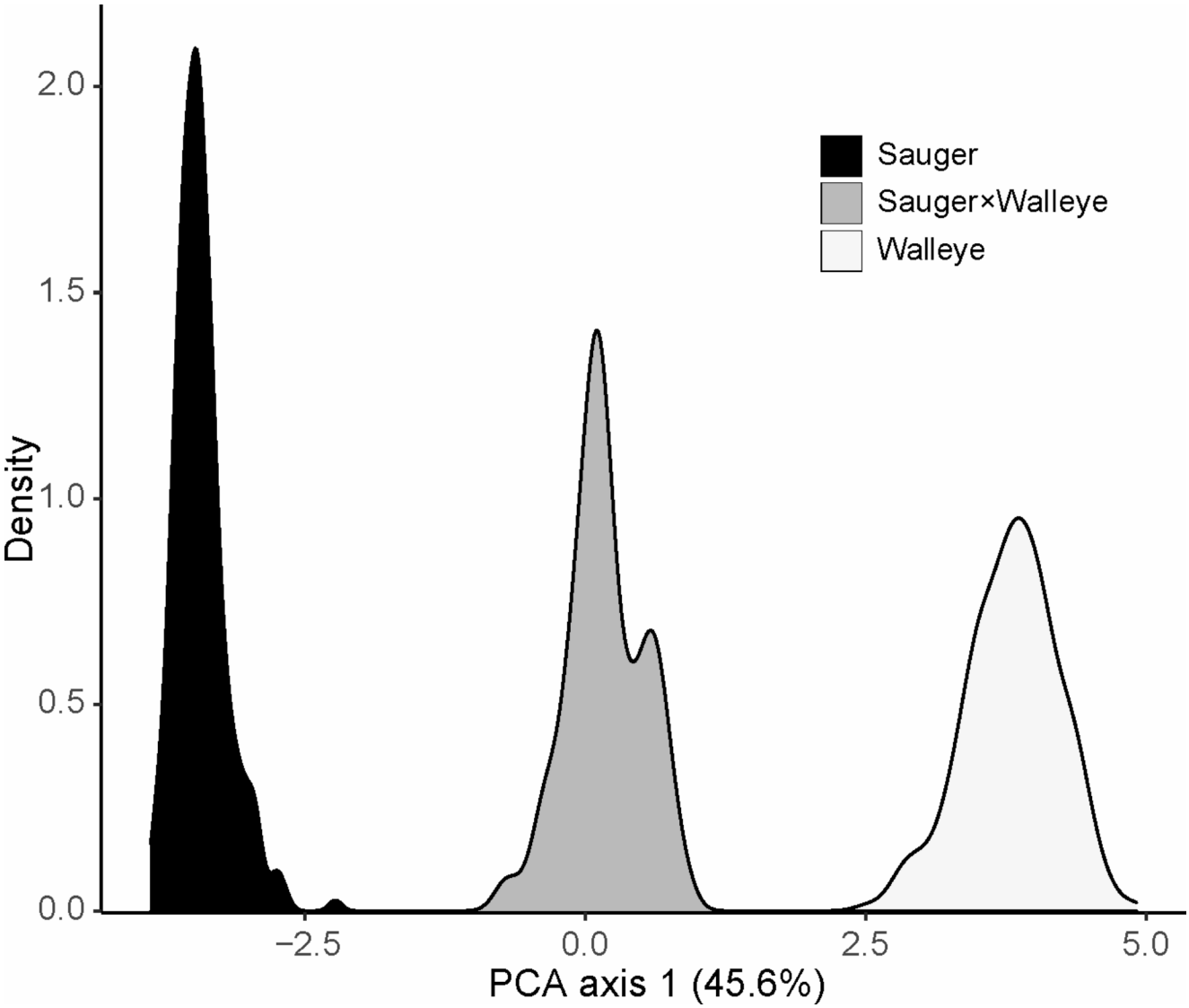

Fig. 1 Density plot of principal component analysis (PCA) axis 1 indicating divergence in allele frequencies between sauger (*Sander canadensis*), walleye (*Sander vitreus*), and simulated sauger×walleye at 118 SNP loci

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Data Availability Coauthor and data author, Paul Albosta, has made the GTscore primer-probe file and genotypes for walleye, sauger, and simulated saugeye available in an archived GitHub repository at https://github.com/palbosta/Winnebago_sauger_parentage (DOI https://doi.org/10.5281/zenodo.7683147). Questions about the data can be directed to the data author, Paul Albosta at palbosta@uwsp.edu.

Declarations

Competing interests The authors have no relevant financial or non-financial interests to disclose.

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