Genomic signals found using RNA sequencing support conservation of walleye (*Sander vitreus*) in a large freshwater ecosystem.

Matt J. Thorstensen[1], Jennifer D. Jeffrey[1], Jason R. Treberg[1], Douglas A. Watkinson[1], Eva C. Enders[2], & Ken M. Jeffries[1]

[1]Department of Biological Sciences, University of Manitoba, Winnipeg, Canada

[2]Freshwater Institute, Fisheries and Oceans Canada, Winnipeg, Canada

Abstract

RNA sequencing is an effective approach for studying an aquatic species with little prior molecular information available, yielding both physiological and genomic data, but its genetic applications are not well-characterized. We investigate this possible role for RNA sequencing for population genomics in Lake Winnipeg, Manitoba, Canada, walleye (*Sander vitreus*). Lake Winnipeg walleye represent the largest component of the second-largest freshwater fishery in Canada. In the present study, large female walleye were sampled via nonlethal gill biopsy over two years at three spawning sites representing a latitudinal gradient in the lake. Genetic variation from sequenced messenger RNA was analyzed for neutral and adaptive markers to investigate population structure and possible adaptive variation. We find low population divergence (F_{ST} = 0.0095), possible northward gene flow, and outlier loci that vary latitudinally in transcripts associated with cell membrane proteins and cytoskeletal function. These results indicate that Lake Winnipeg walleye may be effectively managed as a single demographically connected metapopulation with contributing subpopulations, and suggest genomic differences possibly underlying observed phenotypic differences. Because RNA sequencing data can yield

physiological in addition to genetic information discussed here, we argue that it is useful for addressing diverse molecular questions in the conservation of freshwater species.

Key Words: outlier loci, gene flow, population genomics, adaptive variation, transcriptomics

Introduction

Population abundances in aquatic systems are in decline globally, with a 36% decline in the marine Living Planet Index (LPI, http://livingplanetindex.org) between 1970 and 2012, and an 81% decline in the freshwater LPI during the same period (WWF 2016). These estimates are especially alarming for freshwater ecosystems, which cover 2.3% of the earth's global land surface area but are disproportionately high in species richness—for instance, one-third of all described vertebrate species live in freshwater (Reid et al. 2019; WWF 2018). It is therefore a significant concern that freshwater species are declining in abundance more rapidly than terrestrial or marine species (Reid et al. 2019). This decline underscores an urgent need for research supporting conservation efforts for these diverse freshwater species.

To take effective action, conservation practitioners require research on the environmental stressors a population faces, as well as population structure and evolutionary patterns to determine a species' adaptive potential (Connon et al. 2018; Russello et al. 2011; Waples & Gaggiotti 2006). Transcriptomics has been discussed in the context of differential gene expression, for identifying important physiological thresholds in species of conservation concern that can support risk assessments and setting management thresholds, thus, ultimately benefiting species conservation (Connon et al. 2018). An advantage of using RNA sequencing for conservation research is that it provides information about both genetics and molecular physiology by returning transcript abundances and single nucleotide polymorphisms (SNPs)

allowing researchers to gather a diverse array of information within one data set. These advantages make transcriptomics approaches useful for studying species of conservation concern, especially for species that do not have extensive molecular databases like those available for model species (e.g., zebrafish, *Danio rerio*).

Applications for transcriptomics to address population genomics questions in non-model species is relatively poorly characterized. A large topic of interest in conservation genomics is population structure, or genomic divergence between different groups of individuals, which can support decisions on whether those groups should be managed as a single or several units (Funk et al. 2012). Complementary to population structure analyses, outlier SNP detection may reveal adaptive variation useful for conservation (Funk et al. 2012; Russello et al. 2011). While many genomic methods rely on genes in linkage with outlier SNPs of interest to interpret the functional significance of data, the functional significance of SNPs in mRNA is more readily interpretable because those SNPs may represent *cis*-regulatory mechanisms within an annotated transcript (Verta & Jones 2019). Within a transcript, the effects of SNPs in open reading frames can be predicted, indicating how protein function may be modified by genetic variation (Cingolani et al 2012). Therefore, RNA sequencing may be an effective method for characterizing physiological patterns, population structure, and adaptive variation in species and systems with little prior information available.

Walleye (*Sander vitreus*) in Lake Winnipeg, Manitoba, are the largest component of the second largest freshwater fishery in Canada. Lake Winnipeg is characterized by a north and a south basin connected by a narrow channel (Johnston et al. 2012; Figure 1). While previous microsatellite research showed slight population differentiation between groups in each basin (Backhouse-James & Docker 2011), morphological, life history, dietary, and environmental

differences among Lake Winnipeg walleye suggest diverging genetic histories (Environment Canada 2011; Johnson et al. 2012; Moles et al. 2010; Sheppard et al. 2015, Sheppard et al. 2018; Watkinson & Gillis 2005). Within Lake Winnipeg, walleye have shown declining biomass and body condition, decreased catches, and commercial harvests above maximum sustainable yields for several years (Manitoba Government 2018; Manitoba Sustainable Development 2018). Observations of dwarf walleye suggest signs of selection against large, economically desirable fish (Johnston et al. 2012; Moles et al. 2010). These trends highlight the need to gain information on population structure and biological differences in Lake Winnipeg walleye to support future conservation efforts.

The current study aimed to show how mRNA sequencing can be an effective approach for developing critical pieces of information directly applicable to fisheries and conservation practitioners. We used RNA sequencing for genetic characterization of Lake Winnipeg walleye sampled from known spawning locations that potentially represent fish from the north and south basins. We also sampled fish collected at the channel that connects the north and south basins as an intermediate site. We hypothesized that walleye populations within Lake Winnipeg show evidence of distinct population differentiation identified using RNA sequencing data, despite the weak signatures from microsatellite data (Backhouse-James & Docker 2011). We predicted that the walleye population divergence may partially reflect the different environments and natural histories between the north and south basins of Lake Winnipeg.

Methods

RNA extraction and sequencing

Gill tissue was collected from large (≥ 1.2 kg) predominately female (44 female, 4 unidentified sex) (Supplementary Table 1) walleye over two years from three sites in the Lake Winnipeg system (Red River, Matheson Island, and Dauphin River, representing sites in the south basin, channel, and north basin, respectively; Figure 1; Supplementary Table 1; n = 8 per year and site, n = 48 total). These fish were sampled during the spawning season (approximately May through early June in 2017 and 2018). Walleye were collected by electrofishing, held in a live well for no longer than one hour, and anaesthetized using a Portable Electroanesthesia System (PES™, Smith Root, Vancouver, Washington, USA) in accordance with approved animal use protocols of Fisheries and Oceans Canada (FWI-ACC-2017-001, FWI-ACC-2018-001), the University of Manitoba (F2018-019) and the University of Nebraska-Lincoln (Project ID: 1208). Fish were sampled non-lethally for gill tissue, where 2–3 mm of the terminal ends of 3–4 filaments from the left side each fish were collected and placed in RNA*later* (Thermo Fisher Scientific, Waltham, Massachusetts, USA) that was kept at 4 °C for 24 h prior to storage at -80 °C. As part of a larger study on the physiological status and movement of Lake Winnipeg walleye, other samples collected were the first dorsal spine, scales, blood, a muscle biopsy, and fin clips. Fish were surgically implanted with VEMCO acoustic tags prior to release (VEMCO, Bedford, Nova Scotia, Canada). For the purposes of the present study, only gill tissue was analyzed. Total RNA extractions were performed on gill tissue using RNeasy Plus Mini Prep Kits (QIAGEN, Venlo, Netherlands) following manufacturer's protocols, with minor modifications (provided in the supplementary materials).

The quantity and quality of RNA was assessed with a Nanodrop One Spectrophotometer (Thermo Fisher) and electrophoresis on a 1% agarose gel, respectively. Total RNA was normalized to 80 ng μL^{-1} and sent to the McGill University and Génome Québec Innovation

Centre sequencing facility (http://gqinnovationcenter.com) for cDNA library preparation and sequencing. Total RNA was used to prepare 48 separate cDNA libraries to produce 100 base pair paired end reads using the NEBNext Ultra II Directional RNA Library Prep Kit for Illumina (New England Biolabs, Ipswich, Massachusetts, USA). Each library was individually barcoded with NEBNext dual adaptors (New England Biolabs) prior to sequencing. All 48 fish were sequenced on a single lane of a NovaSeq 6000 (Illumina, San Diego, California, USA). 2.17 billion reads total were sequenced, with an average of 45,225,548 reads per sample collected (5,071,090 s.d.) (Supplementary Table 1).

SNP calling

Raw read files were uploaded to the Graham and Cedar clusters on the Westgrid section of the Compute Canada partnership (https://www.westgrid.ca/). Read files were checked for quality using FastQC version 0.11.8 (Andrews 2010) and trimmed using Trimmomatic version 0.36 (Bolger et al. 2014). When using FastQC version 0.11.8 (Andrews 2010), the program was set to allow two seed mismatches, a palindrome clip threshold of 30 nucleotides, and simple clip threshold of ten nucleotides. A sliding window size of 4 base pairs was used to filter data for a minimum Phred 64 quality of five, with five nucleotides trimmed from both the leading and trailing ends of reads, and a minimum read length of 36. After trimming, FastQC was used again to verify data quality. Scripts used for the analyses in this manuscript are provided at https://github.com/BioMatt/Walleye_RNAseq.

The SuperTranscripts pipeline was used to align reads (Davidson et al. 2017) for SNP calling. First, Salmon version 0.11.3 (Patro et al. 2017) was used to quantify read counts, as compared to a previously assembled reference transcriptome for walleye (Sequence Read Archive Accession SRP150633; Jeffrey et al, in revision). In Salmon, validate mappings, range

134 factorization bins of size 4, sequencing bias, and GC bias options were all used, along with
135 dumping equivalence classes for subsequent steps. Using the count estimates from Salmon,
136 Corset version 1.07 (Davidson & Oshlack 2014) was used to cluster the data for assembly into
137 SuperTranscripts. A linear representation of the transcriptome was constructed with Lace version
138 1.00 (https://github.com/Oshlack/Lace) using information from Corset and the original
139 transcriptome, where 263,272 genes from the original transcriptome were gathered into 148,165
140 super clusters. Following Lace, STAR version 2.7.0a (Dobin et al. 2013) was used in 2-pass
141 mode to align trimmed reads to the reassembled transcriptome. Here, annotated junctions from
142 Lace were provided along with the new transcriptome, and sjdbOverhang of 99 was chosen
143 following recommended settings of 1 base pair below read length. A minimum of 79.6% reads
144 uniquely mapped to the Lace-clustered transcriptome (mean 81.5% ± 0.5% s.d.) (Supplementary
145 Table 1).

146 For calling SNPs, the STAR-aligned reads were processed with Picard version 2.18.9 by
147 adding read groups, splitting cigar ends, and merging bam files (Broad Institute 2018), then
148 SNPs were called using FreeBayes version 1.2.0 (Garrison & Marth 2012). Detailed methods for
149 calling SNPs are provided in the Supplementary Materials. We filtered the VCF file from
150 FreeBayes in two ways. This resulted in 2,458,947 SNPs and 586,556 indels, which were used as
151 unfiltered data for subsequent steps. To study SNPs as close to neutrality as possible, we used
152 vcftools version 0.1.14 (Danecek et al. 2011) to filter for biallelic SNPs of genotype and site
153 quality 30 with a minor allele frequency of 0.05 with no missing data, in Hardy-Weinberg
154 Equilibrium with a p-value < 0.005. SNPRelate version 1.16.0 (Zheng et al. 2012) was then used
155 to prune SNPs for linkage disequilibrium at a threshold of 0.20, where super clusters were coded
156 as chromosomes for the purposes of linkage disequilibrium pruning. These steps resulted in a

putatively neutral data set of 52,372 SNPs used for population structure analyses. For a broader subset of SNPs for which neutrality was not assumed, vcftools was used to filter for genotype and site with quality 30, minor allele frequency 0.05, and a maximum of two missing genotypes out of 48 possible. 222,634 SNPs were retained from these filtering steps, which were then used for outlier tests and functional analyses.

Population structure

To investigate population structure using the 52,372 putatively neutral SNPs, we used a combination of exploratory analyses, either with no prior information or with sampling location provided as priors, and population reassignment and differentiation tests to find genetic clusters despite possible signals of admixture or gene flow. Structure version 2.3.4 (Falush et al. 2003; Falush et al. 2007; Hubisz et al. 2009; Pritchard et al. 2000) was run with no prior location or population information, an initial value of alpha of 1.0, a maximum value of alpha of 10.0, prior mean F_{ST} of 0.01, lambda of 1.0, a burn in period of 10,000 repetitions, and 110,000 Markov Chain Monte Carlo repetitions after burn in. Structure plots were visualized with pophelper version 2.2.7 (http://royfrancis.github.io/pophelper/). Ten replicates of K = 2–5 were tested.

For analyses performed in R (R Core Team 2019), the package vcfR was used to format genotype data for use with other programs (Knaus & Grünwald 2017). Adegenet version 2.1.1 (Jombart et al. 2010) was used in two ways. First, in an exploratory capacity to perform Discriminant Analysis of Principal Components (DAPC), where sampling location was provided for the DAPC as prior population information. Second, population structure was investigated irrespective of sampling location by using cluster identification from successive K-means, as implemented in the find.clusters function in Adegenet. Here, different numbers of clusters were explored in the data (40 principal components were retained for exploratory steps) and evaluated

with a Bayesian Information Criterion (BIC), where the most well-supported number of clusters with lowest BIC was 2 (Supplementary Figure 1). In addition to exploring the two clusters, the population assignments from three clusters were used to explore genetic differentiation in the data because fish were sampled from three sites (Supplementary Table 1). With Hierfstat version 0.04-22 (Yang 1998; Weir & Cockerham 1984), the Weir & Cockerham's pairwise F_{ST} was calculated among the three sampling locations, then between the two reassigned clusters described by Adegenet (Supplementary Table 1). We generated 95% confidence intervals for these F_{ST} values in Hierfstat using a bootstrap approach over 1,000 iterations.

To visualize population differentiation, we used a PCA as implemented in Adegenet version 2.1.1 (Jombart et al. 2010) and t-SNE as implemented in Rtsne version 0.15 (van der Maaten & Hinton 2008). For the t-SNE, two final dimensions were used, with 100 initial dimensions, 15 perplexity, theta of 0.5, and 5,000 iterations. These approaches were used with the same settings applied to the putatively neutral SNPs, and visualizations were thus comparable between data sets.

Temporal stability & kinship

To test for temporal stability in the data, we created subsets of individuals caught in 2017 and 2018. As with the whole dataset, Weir & Cockerham's pairwise F_{ST} was calculated both among sampling locations and between the two reassigned clusters, and generated 95% confidence intervals over 1,000 bootstrapped iterations in hierfstat (Supplementary Table 1). Modest results that are consistent over time support confidence in a real genetic signal, as opposed to results driven by bias which are more likely to be inconsistent over time (Waples 1998).

To address the possibility that sample collection, extraction, sequencing, or another process introduced an erroneous year effect into the data, we identified SNPs that differed between fish sampled in 2017 and 2018 with an F_{ST} above 0.01 using hierfstat, then filtered out those SNPs from the data using VCFtools version 0.1.14. Following these steps, 13,640 SNPs (26.04% of 52,372 neutral SNPs total) were identified as having a large effect between years and were thus removed, leaving 38,732 SNPs. Analyses for population structure were then re-run with this smaller set of SNPs. F_{ST} was calculated both between sites and between two reassigned clusters described by Adegenet (Supplementary Table 1). Data was also visualized by using a PCA as implemented in Adegenet version 2.1.1, and t-SNE as implemented in Rtsne version 0.15.

To test if our estimates of population structure were not driven by family groups (Waples 1998), we used Colony version 2.0.6.4 to reconstruct pedigrees in our sample of 48 individuals, with consideration of possible full-siblings (Jones & Wang 2010; Wang 2004). The putatively neutral SNPs were converted to the Colony format using a script by D. deWaters (https://github.com/dandewaters/VCF-File-Converter). The Colony command-line input file was then generated to run the program with updated allele frequencies, dioecy, inbreeding possible, polygamy allowed, no clones, full sibship scaling, no sibship prior, unknown population allele frequencies, ten runs of medium length, full likelihood inference, and high precision. This Colony input file was generated using a script originally written by M. Ackerman (used in Ackerman et al. 2017), modified and posted with permission for the present study. Independent of Colony's maximum likelihood-based approach, we also used the method of moments as implemented in SNPRelate version 1.18.0 (Zheng et al. 2012) to estimate a kinship coefficient between individuals, also using the putatively neutral SNPs.

Outlier SNPs

Using the full list of SNPs filtered for genotype quality 30, minor allele frequency > 0.05 and two missing individuals allowed, but not filtered for Hardy-Weinberg Equilibrium or Linkage Disequilibrium, we tested for outlier SNPs using an unsupervised approach in pcadapt version 4.1.0 (Luu et al. 2017). The unsupervised approach was used because weak population differentiation and the likely presence of admixed individuals in the data would either lower our sample size by filtering admixed individuals out, or lead to false-positive outlier loci by their inclusion when using a supervised approach with population structure included (Liu et al. 2016). While this may lead to issues of false positives from multiple tests (Foll & Gaggiotti 2008), we addressed this issue by using a q-value of 0.05 and focusing our interpretation on transcripts that contain two or more outlier SNPs. Two PCs were chosen for this analysis by observing the scree plot visualizing K = 1–20 following Cattell's rule, where the point that a smooth decrease in eigenvalues levels off on a scree plot is the last important PC for explaining the data (Cattell 1966).

By relating the transcript ID of a significant outlier SNP (q-value < 0.05) to that transcript's putative function and gene ID from the annotated reference transcriptome, a database of transcripts which diverged by sampling location or year was created for the Lake Winnipeg walleye in the present study. From this database, a list of transcripts relevant to either sampling location or year was used for gene set enrichment analysis using EnrichR (Chen et al. 2013; Kuleshov et al. 2016), thereby summarizing genes by gene ontology (GO) terms. In addition, transcripts were filtered to find those with two or more significant outlier SNPs that diverged by either sampling location or year, and these transcripts were few enough that enrichment analysis

was not necessary. By only including genes with multiple outlier SNPs, we sought to reduce the presence of false positive signals in this outlier test.

Results

Population structure

Our data suggested weak but significant population structure between the north and south basins of Lake Winnipeg. The Red River and Matheson Island locations slightly diverged (F_{ST} = 0.0012), while the Dauphin River fish were the most genetically distinct group sampled (F_{ST} = 0.0068 and 0.0043 compared to the Red River and Matheson Island, respectively) (Figures 2, 3, Table 1, and Supplementary Figure 2). Moreover, Structure and the DAPC returned similar results with respect to which fish were admixed, although membership probabilities differed (Figure 2, 3). Between K = 2–5, Structure consistently separated the Dauphin River fish from the Matheson Island and Red River fish, while the Red River and Matheson Island fish did not separate from each other by site, but instead separated between years (Figure 2).

The PCA and t-SNE used with the putatively neutral SNPs show similar patterns of Matheson and Red River fish separated, but more similar to each other than either with the Dauphin River fish (Supplementary Figure 2). When comparing the PCA and t-SNE plots between the neutral linkage disequilibrium-pruned SNPs and the broader collection of SNPs used for outlier analyses, genetic differentiation between the Red River and Matheson Island fish disappears when using all of the SNPs with the t-SNE, whereas separation between the two sites persists when only using Hardy-Weinberg Equilibrium filtered and LD-pruned SNPs.

Population assignment

Using two clusters for reassignment from Adegenet, out of 48 fish, 36 clustered in one group (Cluster 1), and twelve in the other (Cluster 2; Supplementary Table 1). Cluster 1 was characterized by a combined Red River and Matheson Island group of fish with few Dauphin River fish (six were collected from the Dauphin River, 14 from Matheson Island, and 16 from the Red River), while Cluster 2 was characterized by Dauphin River fish and a small number of Matheson Island fish (ten fish from the Dauphin River and two from Matheson Island). Weir and Cockerham's pairwise F_{ST} between these two reassigned clusters was 0.0095 with a 95% confidence interval between 0.0090–0.010.

Using three clusters for reassignments from Adegenet, out of 48 fish, 19 were in one group (Cluster 1), ten fish were in another group (Cluster 2), and 19 fish in a final group (Cluster 3). Clusters 1 and 3 were characterized by a year effect, where every individual in Cluster 1 was captured in 2018 and every individual in Cluster 3 was captured in 2017. Both Clusters 1 and 3 had 16 out of 19 fish coming from the Red River or Matheson Island sites. Meanwhile, all ten fish in Cluster 2 were from the Dauphin River with five fish each collected in 2017 and 2018 (Supplementary Table 1).

Temporal stability and kinship

When partitioning individuals by sampling location and year collected, all confidence intervals for between-site pairwise F_{ST} estimates overlapped over both sampling years, indicating consistent patterns of between-site divergence in 2017 and 2018. However, values between the Dauphin River and Matheson Island varied the most, with an estimate of 0.0044 (0.0035–0.0052) in 2017, and 0.0060 (0.0051–0.0070) in 2018 (Table 2).

Using the 38,732 SNPs filtered for loci, which showed F_{ST} between years of > 0.01, F_{ST} between the two reassigned clusters found using Adegenet (Supplementary Table 1) was 0.010 (0.0094–0.011). With these same year effect-filtered SNPs, pairwise F_{ST} between sites did not significantly differ from values found using the neutral SNPs either overall or in a subset by year (Supplementary Table 2). The PCA and t-SNE on the SNPs filtered for a year effect showed patterns of spatial differentiation consistent with other analyses, with the Dauphin River fish being more separate from the Red River and Matheson Island group of fish (Supplementary Figure 3).

We found no evidence of kinship using either Colony or the method of moments. Over ten replicate runs in Colony, individuals belonged to separate families with inclusive and exclusive probabilities of 1.0000 each. Using the method of moments implemented in SNPRelate (Zheng et al. 2012), the highest kinship coefficient between two individuals was 0.096 (mean 0.053 ± 0.019 s.d.), where a kinship coefficient of approximately 0.5 would indicate full-siblings.

Outlier SNPs

There was site-specific differentiation across Principal Component 1 (PC1) in the pcadapt analysis (Figure 4). In total, 1,177 SNPs were outliers at q < 0.05, with 386 SNPs contributing to PC1 where fish separated by site, and 791 SNPs contributing to PC2 where fish separated by year (Figure 4). For the 386 SNPs associated with PC1 (Figure 4), 120 uniquely annotated transcripts were available for enrichment analysis using EnrichR. These transcripts corresponded to GO terms such as purine ribonucleoside triphosphate binding, ATP binding, and adenyl ribonucleotide binding, all significant at Benjamini-Hochberg adjusted *p*-values < 0.05 (Supplementary Table 3). By filtering for uniquely annotated transcripts with ≥ 2 outlier SNPs associated with PC1, 19 transcripts were identified (Table 3) that varied by sampling location.

Six of these genes were associated with ion channels and cell membrane transport, including claudin-10, ankyrin-3, sodium/hydrogen exchanger 6, sodium/potassium-transporting ATPase subunit alpha-3, perforin-1, and ATP-binding cassette sub-family A member 12. Additionally, four genes that varied spatially were associated with the cytoskeleton, such as myosin-9, beta/gamma crystallin domain-containing protein 1, tubulin beta-4B chain, and interferon-induced protein 44.

Using the 791 SNPs associated with Principal Component 2 (PC2), which varied by year (Figure 4), 130 uniquely annotated transcripts were available for enrichment analysis; however, no GO terms were significant at an adjusted p-value < 0.05. For transcripts with ≥ 2 PC2 outlier SNPs, 17 uniquely annotated genes were identified of which six were either transposons, transposable elements, or fragments of transposons (Table 4). Two genes that code for the proteins serine/threonine-protein phosphatase 6 catalytic subunit and protein BTG3, which regulate cell division in the G1 to S phase transition were also identified (Table 4).

Discussion

We observed weak population structure characterized by groups collected at the Red River and Matheson Island sampling locations, representing south basin and channel fish, contrasted with a group collected at the Dauphin River, representing north basin fish. As such, the north and south basin walleye in Lake Winnipeg may be separate groups with an F_{ST} of 0.0095, but with gene flow between them primarily at the channel connecting the two basins. Consistent with results in the present study, a study using microsatellites found a similar weak, but significant, differentiation between Lake Winnipeg walleye from sites in the north and south basins (e.g., F_{ST} = 0.022 between the Grand Rapids in the north and Red River in the south; see Backhouse-James & Docker 2012), suggesting genomic divergence between walleye from the two basins. One

335 important factor that may have contributed to weak population structure are historical stocking
336 programs, which may have introduced walleye from nearby Lake Manitoba to Lake Winnipeg
337 (Backhouse-James & Docker 2012). This unknown amount of gene flow from other systems,
338 including up to 26.5 million fish annually between 1970 and 1983 (Lysack 1986), may have
339 masked signatures of spatial population differentiation in Lake Winnipeg.

340 *Temporal differentiation*

341 Differentiation between years was strongest in the south basin, where the Red River and
342 Matheson Island fish separated by year to a greater extent than the Dauphin River walleye. Three
343 hypotheses may explain these patterns of stronger temporal differentiation in the south basin.
344 First, a cohort effect may underlie this pattern, where different year classes were more strongly
345 represented in the lake during a given sampling year. A cohort effect could be the result of
346 greater fishing pressure in the south basin than the north basin, as indicated by smaller allowed
347 net mesh sizes in the south basin (Manitoba Sustainable Development 2019). Fishing pressure
348 can change population dynamics and age structure in exploited species (Anderson et al. 2008;
349 Murphy et al. 2001), therefore large fisheries operating since at least 1890 may have affected age
350 structure in Lake Winnipeg fish (Department of Fisheries 1891). Cohort effects may alternatively
351 be influenced by environmental conditions including predation intensity, water temperature, and
352 time to hatch as observed in Lake Erie, Oneida Lake, and Lake Huron walleye (Busch et al.
353 1975; Fielder et al. 2007; Forney 1976). A second hypothesis is that some Lake Winnipeg
354 walleye may engage in unobserved skipped spawning or alternate year spawning, which have
355 been unexpectedly found in several species and may be present in walleye (Carlander et al. 1960;
356 Henderson et al. 1996; Moles et al. 2008; Rideout & Tomkiewicz 2011). Third, the observed
357 year effect may be an artifact of error introduced during sampling, extraction, sequencing, or

bioinformatics. While some error contributing to between-year differentiation is impossible to rule out, the possibility that a particular analysis or filtering method introduced the year effect is reasonably small given that distinct tests showed consistent year effects. Moreover, the data reveal a consistency in spatial patterns with and without the year effect, demonstrating that at least the spatial population differentiation in Lake Winnipeg walleye is likely real.

While pairwise site F_{ST} values were temporally consistent (i.e., no significant differences between years), the greatest pairwise F_{ST} confidence interval difference between years was between walleye collected at Matheson Island and the Dauphin River, where confidence intervals for F_{ST} estimates overlapped by only 0.0001 between 2017 and 2018. Following Amrhein et al. (2019), we interpret here the possibility that the entire range of these confidence intervals reflect meaningful patterns in the data, and that F_{ST} between the Dauphin River and Matheson Island was different between 2017 and 2018. Because Matheson Island represents a narrow channel connecting the north and south basins of Lake Winnipeg, fish which would normally spawn in the Dauphin River may have used the channel more often in 2017, thus, lowering F_{ST} when performing a site-wise comparison. This difference in habitat use may have arisen from an undetermined environmental variable, such as time of ice melt, which in the north basin was ten days later in 2018 than in 2017 (D. Watkinson, unpublished data found using https://zoom.earth/).

Notably, gene flow appears to be one way from the southern Red River, northward. Going by capture location, no fish caught in the Red River showed a genetic background consistent with the Dauphin River fish, while with the Adegenet-reassigned clusters, no fish assigned to the mostly Dauphin River group was found in the Red River. On the other hand, fish

380 which showed a genetic background consistent with the Red River group were found in the
381 Dauphin River, both based on capture location and population reassignment.

382 *Biological significance*

383 Several studies report morphological and life history differences between basins in Lake
384 Winnipeg walleye consistent with the two delineated groups found in this study. Furthermore,
385 environmental data show a north-south basin distinction with temperature, turbidity, mean depth,
386 suspended solid, sulphate, sodium, chloride, and nutrient differences between the two basins
387 (Brunskill et al. 1980; Environment Canada 2011). Walleye in the south basin show a bimodal
388 growth pattern, where fisheries-induced selection may have contributed to the observation of
389 dwarf walleye (Johnston et al. 2012; Mole et al. 2010; Sheppard et al. 2018). Harvest-induced
390 genetic changes have been linked to size reductions in other walleye within two generations
391 (Bowles et al. 2019). If walleye were panmictic throughout Lake Winnipeg, we might expect the
392 dwarf morphotype to occur with similar frequency in the north basin. However, out of 616 total
393 walleye caught in 2010 and 2011 (178 in the north basin, 438 in the south basin), only two out of
394 32 dwarf fish were caught in the north basin (Sheppard et al. 2018). Diet has also been shown to
395 differ between north and south basin walleye, possibly because of prey or turbidity differences
396 between the two basins (i.e., higher turbidity in the south basin) (Brunskill et al. 1980; Sheppard
397 et al. 2015). Between 1979 and 2003, population characteristics such as age and length at 50%
398 maturity were higher, while growth rate was slower in the north basin walleye, suggesting some
399 level of isolation among walleye between basins (Johnston et al. 2012). These population
400 characteristics may no longer be higher in the north basin following the collapse of the rainbow
401 smelt (*Osmerus mordax)*, after which walleye body condition has decreased since 2010
402 (Manitoba Government 2018). Scale morphometry further suggests differences among spawning

403 aggregations of walleye, especially between the north and south basins (Kritzer & Sale 2004;
404 Watkinson & Gillis 2005). Taken together, the results of our study and those of previous studies
405 suggest weak population structure among Lake Winnipeg walleye, with differentiation between
406 walleye in the north and south basins. This pattern of weak population structure, high
407 connectivity, but biologically significant differentiation is common in marine fishes such as the
408 Atlantic cod (*Gladus morhua*) or Atlantic salmon (*Salmo salar*) (Aykanat et al. 2015; Knutsen et
409 al. 2011), and of other walleye such as those observed in Lake Erie (Chen et al. 2019; Stepien et
410 al. 2018).

411 The results of the present study suggest that the genetic differences between Lake
412 Winnipeg walleye populations may have functional consequences. Out of 19 transcripts that had
413 multiple SNPs that varied by sampling location, eight were related to membrane function,
414 particularly ion channel activity. One of these proteins, Claudin-10 mRNA expression levels
415 have been related ammonia exposure (Connon et al. 2011), rearing density (Sveen et al. 2016),
416 and salinity (Bossus et al. 2015; Kolosov et al. 2013; Marshall et al. 2018) in fishes. Spatial
417 variation in cell membrane proteins is consistent with environmental differences between basins
418 in chemicals such as sodium, chloride, and phosphorous (Environment Canada 2011), although
419 the biological impacts of these spatial chemical differences is unknown. Four cytoskeletal
420 proteins were represented in the outlier SNPs that vary by sampling location as well.
421 Cytoskeletal function is connected to cell growth in plants (Hussey et al. 2006; Wasteneys &
422 Galway 2003), yeast (Li et al. 1995; Pruyne & Bretscher 2000), mouse cells (Kim et al. 2006;
423 Kim & Coulombe 2010), and zebrafish (Johnston et al. 2011). Spatial variation in genes related
424 to cell growth may thus be consistent with growth rate differences observed among walleye in

425 Lake Winnipeg, where north basin fish had higher growth rates in 2010 and 2011 (Sheppard et
426 al. 2018).

427 *Limitations*

428 Despite its advantages, there are some limitations to using mRNA sequencing in the
429 context of population genetics. The depth of sequencing required for differential gene expression
430 and differential exon usage leads to greater costs associated with mRNA sequencing studies
431 relative to reduced representation methods such as RAD-seq (Davey & Blaxter 2011). This often
432 translates to a lower sample size, as is the case in our study. Reduced sample sizes can bias
433 aspects of population genetic analyses, including identifying population structure (Waples &
434 Gaggiotti 2006) and outlier SNPs (Luu et al. 2016). Second, mutations in mRNA are widely
435 under selection (Chamary & Hurst 2005), therefore, caution must be exercised when interpreting
436 SNPs from mRNA in genetic tests assuming neutrality. Third, linkage disequilibrium is useful
437 for analyses of selective sweeps and demographic history (Catchen et al. 2017; Garrigan &
438 Hammer 2006; Hoffmann & Willi 2008), among other approaches, but mRNA data may not be
439 appropriate for these analyses because the extent of linkage between transcripts and marker
440 density across the genome may be unknown. Finally, one key element of how transcriptomics
441 was used in the present study is that it measured expressed mRNA in gill tissue. Messenger RNA
442 expression provides useful information for transcript quantification-based analyses, but likely
443 biases SNP discovery toward more highly expressed transcripts in the tissue collected. It is
444 unknown how this expression-specific bias may influence population genomics. Nevertheless,
445 mRNA sequencing has proven useful for recapitulating population structure discovered with
446 traditional genetic methods (Jeffries et al. 2019) and describing previously uncharacterized
447 population structure (Ellison et al. 2011; Yan et al. 2017).

448 *Conservation applications*

449 We used population genetics and outlier detection to characterize weak, but biologically
450 significant population structure, possible one-way gene flow, and genetic variation possibly
451 underlying biological differences among Lake Winnipeg walleye. These results are consistent
452 with observations of behavioural differences leading to fine-scale divergence in the walleye of
453 other systems (Stepien et al. 2009). The low levels of population differentiation and possible
454 gene flow from the south basin northward, indicate that this system may be effectively managed
455 as a demographically connected metapopulation with two contributing populations (Kritzer &
456 Sale 2004), consistent with conclusions from scale morphology presented in Watkinson & Gillis
457 (2005) and with observations of subtle stock structure in Lake Erie walleye (Chen et al. 2019;
458 Stepien et al. 2018).

459 The results from this study provide valuable information for walleye management,
460 especially because the status of Lake Winnipeg walleye is becoming a concern and conservation
461 action may be necessary to sustain the fishery. Signs of a declining fishery include a decrease in
462 biomass and body condition between 2010 and 2015 (Manitoba Government 2018), possible
463 unnatural selection against larger, economically desirable fish (Allendorf & Hard 2009; Bowles
464 et al. 2019; Moles et al. 2010), models showing walleye harvests have been above maximum
465 sustainable yields since the early 2000s, and a trend in harvest decline since 2010 (Manitoba
466 Sustainable Development 2017–2018). The data gathered here, particularly the spatial variation
467 in genes that may drive functional differences among Lake Winnipeg walleye, is useful for
468 generating hypotheses that test and explain organismal responses to environmental stressors,
469 thereby providing additional information for resource managers. For instance, life-history trait
470 differences can inform conservation in threatened fishes by identifying resilient populations in a

system (Hamidan & Britton 2015). Therefore, possible functional variation identified in this study may underlie heritable genetic differences among Lake Winnipeg walleye that change important traits such as tolerance to environmental conditions and growth rate differences. This information may be useful for integrating demographic connectivity and functional differences among walleye into a cohesive management framework.

We have shown how RNA sequencing data can be used for a population genomic scan in a non-model fish, even in a system where little molecular information is available. Filtering for Hardy-Weinberg equilibrium and linkage disequilibrium allows investigators to draw neutral markers from mRNA sequence data, making it useful for classical population genetic approaches. By contrast, the wide selective effects present in species' transcriptomes allow for hypothesis-generating outlier tests that may reveal variation underlying phenotypic differences among populations. Non-lethal sampling makes RNA sequencing useful for species with low population sizes and for follow-up studies, such as the potential to track tagged individuals from which tissue has been collected. Because RNA sequencing data can yield physiological in addition to genetic information discussed here, we argue that it is useful for addressing diverse molecular questions in the conservation of freshwater species.

Acknowledgements

We thank D. Watkinson, C. Charles, C. Kovachik, D. Leroux, N. Turner, M. Gaudry, S. Glowa, and E. Barker for helping to sample these fish in the field. D. Watkinson and C. Charles also assisted with ice on/ice off data and a map of Lake Winnipeg, respectively. Dr. C. Garroway provided valuable suggestions in data analysis, and Dr. G. Anderson engaged in useful conversations about Lake Winnipeg ecology. We thank the Lake Winnipeg Research Consortium Inc. for supporting the open access publication of this manuscript. Many analyses were enabled by our chance to use computing resources provided by WestGrid (www.westgrid.ca) and Compute Canada (www.computecanada.ca). Sequencing was performed at the McGill University and Génome Québec Innovation Centre sequencing facility. This work was supported by a Fisheries and Oceans Canada Ocean and Freshwater Science Contribution Program Partnership Fund grant awarded to J.R.T., K.M.J. and Darren Gillis, and Natural Sciences and Engineering Research Council of Canada Discovery Grants awarded to K.M.J. (#05479) and J.R.T. (#06052). Work by J.R.T. is also supported by the Canada Research Chairs program (#223744) and the Faculty of Science, University of Manitoba (#319254).

Data Archiving Statement

Raw sequence reads are available through the National Center for Biotechnology Information Sequence Read Archive (accession #PRJNA596986, https://www.ncbi.nlm.nih.gov/sra/PRJNA596986).

514 Works Cited

515 Ackerman, M. W., Hand, B. K., Waples, R. K., Luikart, G., Waples, R. S., Steele, C. A., Garner,
516 B.A., McCane, J., Campbell, M. R. (2017). Effective number of breeders from sibship
517 reconstruction: empirical evaluations using hatchery steelhead. *Evolutionary*
518 *Applications*, *10*(2), 146–160. https://doi.org/10.1111/eva.12433

519 Amrhein, V., Greenland, S., & McShane, B. (2019). Scientists rise up against statistical
520 significance. *Nature*, *567*(7748), 305–307. https://doi.org/10.1038/d41586-019-00857-9

521 Anderson, C. N. K., Hsieh, C. H., Sandin, S. A., Hewitt, R., Hollowed, A., Beddington, J., May,
522 R., Sugihara, G. (2008). Why fishing magnifies fluctuations in fish abundance. *Nature*,
523 *452*(7189), 835–839. https://doi.org/10.1038/nature06851

524 Andrews, S. (2010). *FastQC: a quality control tool for high throughput sequence data*.
525 https://www.bioinformatics.babraham.ac.uk/projects/fastqc/

526 Aykanat, T., Johnston, S. E., Orell, P., Niemelä, E., Erkinaro, J., & Primmer, C. R. (2015). Low
527 but significant genetic differentiation underlies biologically meaningful phenotypic
528 divergence in a large Atlantic salmon population. *Molecular Ecology*, *24*(20), 5158–5174.
529 https://doi.org/10.1111/mec.13383

530 Backhouse-James, S. M., & Docker, M. F. (2012). Microsatellite and mitochondrial DNA
531 markers show no evidence of population structure in walleye (*Sander vitreus*) in Lake
532 Winnipeg. *Journal of Great Lakes Research*, *38*, 47–57.
533 https://doi.org/10.1016/j.jglr.2011.05.005

534 Bolger, A. M., Lohse, M., & Usadel, B. (2014). Trimmomatic: A flexible trimmer for Illumina
535 sequence data. *Bioinformatics*, *30*(15), 2114–2120.
536 https://doi.org/10.1093/bioinformatics/btu170

537 Bowles, E., Marin, K., & Fraser, D. J. (2019). Size reductions and genomic changes associated
538 with harvesting within two generations in wild walleye populations. *BioRxiv Preprint*,
539 (September). https://doi.org/10.1101/787374
540 Braje, T. J., & Erlandson, J. M. (2013). Human acceleration of animal and plant extinctions: A
541 Late Pleistocene, Holocene, and Anthropocene continuum. *Anthropocene*, *4*, 14–23.
542 https://doi.org/10.1016/j.ancene.2013.08.003
543 Broad Institute. (2019). Picard Tools. *Broad Institute, GitHub Repository*. Retrieved from
544 http://broadinstitute.github.io/picard/
545 Brunskill, G.J., Elliot, S.E.M., & Campbell, P. (1980). Morphometry, hydrology, and watershed
546 data pertinent to the limnology of Lake Winnipeg. Canadian Manuscript Report of Fisheries
547 & Aquatic Sciences No. 1556. 1–32.
548 Busch, W.-D. N., Scholl, R. L., & Hartman, W. L. (1975). Environmental factors affecting the
549 strength of walleye (*Stizostedion vitreum vitreum*) year-classes in western Lake Erie, 1960–
550 70. *Journal of the Fisheries Research Board of Canada*, *32*(10), 1733–1743.
551 https://doi.org/10.1139/f75-207
552 Carlander, K. D., Whitney, R. R., Speaker, E. B., & Madden, K. (1960). Evaluation of Walleye
553 Fry Stocking in Clear Lake, Iowa, by Alternate-Year Planting. *Transactions of the*
554 *American Fisheries Society*, 89(3), 249–254. https://doi.org/https://doi.org/10.1577/1548-
555 8659(1960)89[249:EOWFSI]2.0.CO;2
556 Catchen, J. M., Hohenlohe, P. A., Bernatchez, L., Funk, W. C., Andrews, K. R., & Allendorf, F.
557 W. (2017). Unbroken: RADseq remains a powerful tool for understanding the genetics of
558 adaptation in natural populations. *Molecular Ecology Resources*, *17*(3), 362–365.
559 https://doi.org/10.1111/1755-0998.12669

Chamary, J., & Hurst, L. D. (2005). Evidence for selection on synonymous mutatations affecting stability of mRNA secondary structure in mammals. *Genome Biology*, *6*(9), R75. https://doi.org/10.1186/gb-2005-6-9-r75

Chen, K. Y., Euclide, P. T., Ludsin, S. A., Larson, W., Sovic, M. G., Gibbs, H. L., & Marschall, E. A. (2019). RAD-seq refines previous estimates of genetic structure in Lake Erie walleye (*Sander vitreus*). *Transactions of the American Fisheries Society*. https://doi.org/10.1002/tafs.10215

Chen, E. Y., Tan, C. M., Kou, Y., Duan, Q., Wang, Z., Meirelles, G., … Ma'ayan, A. (2013). Enrichr: Interactive and collaborative HTML5 gene list enrichment analysis tool. *BMC Bioinformatics*, *14*(1), 128. https://doi.org/10.1186/1471-2105-14-128

Cingolani, P., Platts, A., Wang, L. L., Coon, M., Nguyen, T., Wang, L., … Ruden, D. M. (2012). A program for annotating and predicting the effects of single nucleotide polymorphisms, SnpEff. *Fly*, *6*(2), 80–92. https://doi.org/10.4161/fly.19695

Connon, R. E., Jeffries, K. M., Komoroske, L. M., Todgham, A. E., & Fangue, N. A. (2018). The utility of transcriptomics in fish conservation. *The Journal of Experimental Biology*, *221*(2), jeb148833. https://doi.org/10.1242/jeb.148833

Danecek, P., Auton, A., Abecasis, G., Albers, C. A., Banks, E., DePristo, M. A., … 1000 Genomes Project Analysis Group. (2011). The variant call format and VCFtools. *Bioinformatics*, *27*(15), 2156–2158. https://doi.org/10.1093/bioinformatics/btr330

Davey, J. W., & Blaxter, M. L. (2010). RADSeq: Next-generation population genetics. *Briefings in Functional Genomics*, *9*(5–6), 416–423. https://doi.org/10.1093/bfgp/elq031

581 Davidson, N. M., Hawkins, A. D. K., & Oshlack, A. (2017). SuperTranscripts: A data driven
582 reference for analysis and visualisation of transcriptomes. *Genome Biology*, *18*(1), 148.
583 https://doi.org/10.1186/s13059-017-1284-1
584 Davidson, N. M., & Oshlack, A. (2014). Corset: Enabling differential gene expression analysis
585 for de novoassembled transcriptomes. *Genome Biology*, *15*(7), 410.
586 https://doi.org/10.1186/s13059-014-0410-6
587 Department of Fisheries. (1891). *Report of the Department of Fisheries for the Year 1890.*
588 Ottawa. Retrieved from https://waves-vagues.dfo-
589 mpo.gc.ca/Library/40758606_1890_pt.1.pdf.
590 Dirzo, R., Young, H. S., Galetti, M., Ceballos, G., Isaac, N. J. B., & Collen, B. (2014).
591 Defaunation in the Anthropocene. *Science*, *345*(6195), 401–406.
592 https://doi.org/10.1126/science.1251817
593 Dobin, A., Davis, C. A., Schlesinger, F., Drenkow, J., Zaleski, C., Jha, S., Batut, P., Chaisson,
594 M., Gingeras, T. R. (2013). STAR: Ultrafast universal RNA-seq aligner. *Bioinformatics*,
595 *29*(1), 15–21. https://doi.org/10.1093/bioinformatics/bts635
596 Ellison, C. E., Hall, C., Kowbel, D., Welch, J., Brem, R. B., Glass, N. L., & Taylor, J. W. (2011).
597 Population genomics and local adaptation in wild isolates of a model microbial eukaryote.
598 *Proceedings of the National Academy of Sciences*, *108*(7), 2831–2836.
599 https://doi.org/10.1073/pnas.1014971108
600 Falush, D., Stephens, M., & Pritchard, J. K. (2007). Inference of population structure using
601 multilocus genotype data: Dominant markers and null alleles: *Molecular Ecology Notes*,
602 *7*(4), 574–578. https://doi.org/10.1111/j.1471-8286.2007.01758.x

603 Falush, D., Stephens, M., & Pritchard, J. K. (2003). Inference of Population Structure Using
604 Multilocus Genotype Data: Linked Loci and Correlated Allele Frequencies. *Genetics*,
605 164(4), 1567 LP – 1587. Retrieved from
606 http://www.genetics.org/content/164/4/1567.abstract
607 Fielder, D. G., Schaeffer, J. S., & Thomas, M. V. (2007). Environmental and ecological
608 conditions surrounding the production of large year classes of walleye (*Sander vitreus*) in
609 Saginaw Bay, Lake Huron. Journal of Great Lakes Research, 33(SUPPL. 1), 118–132.
610 https://doi.org/10.3394/0380-1330(2007)33[118:EAECST]2.0.CO;2
611 Foll, M., & Gaggiotti, O. (2008). A genome-scan method to identify selected loci appropriate for
612 both dominant and codominant markers: A Bayesian perspective. *Genetics*, *180*(2), 977–
613 993. https://doi.org/10.1534/genetics.108.092221
614 Forney, J. L. (1976). Year-class formation in the walleye (*Stizostedion vitreum vitreum*)
615 population of Oneida Lake, New York, 1966–73. *Journal of the Fisheries Research Board*
616 *of Canada*, *33*(4), 783–792. https://doi.org/10.1139/f76-096
617 Funk, W. C., McKay, J. K., Hohenlohe, P. A., & Allendorf, F. W. (2012). Harnessing genomics
618 for delineating conservation units. *Trends in Ecology & Evolution*, *27*(9), 489–496.
619 https://doi.org/10.1016/j.tree.2012.05.012
620 Garrigan, D., & Hammer, M. F. (2006). Reconstructing human origins in the genomic era.
621 *Nature Reviews Genetics*, *7*(9), 669–680. https://doi.org/10.1038/nrg1941
622 Garrison, E., & Marth, G. (2012). Haplotype-based variant detection from short-read sequencing.
623 *ArXiv:1207.3907 [q-Bio]*. Retrieved from http://arxiv.org/abs/1207.3907

624 Grabherr, M. G., Haas, B. J., Yassour, M., Levin, J. Z., Thompson, D. A., Amit, I., … Regev, A.
625 (2011). Full-length transcriptome assembly from RNA-Seq data without a reference
626 genome. *Nature Biotechnology*, *29*(7), 644–652. https://doi.org/10.1038/nbt.1883
627 Hamidan, N., & Britton, J. R. (2015). Age and growth rates of the critically endangered fish
628 *Garra ghorensis* can inform their conservation management. *Aquatic Conservation: Marine*
629 *and Freshwater Ecosystems*, *25*(1), 61-70.
630 Healy, T. M., & Schulte, P. M. (2019). Patterns of alternative splicing in response to cold
631 acclimation in fish. *The Journal of Experimental Biology*, *222*(5), jeb193516.
632 https://doi.org/10.1242/jeb.193516
633 Henderson, B. A., Wong, J. L., & Nepszy, S. J. (1996). Reproduction of walleye in Lake Erie:
634 Allocation of energy. *Canadian Journal of Fisheries and Aquatic Sciences*, *53*(1), 127–133.
635 https://doi.org/10.1139/f95-162
636 Hoffmann, A. A., & Willi, Y. (2008). Detecting genetic responses to environmental change.
637 *Nature Reviews Genetics*, *9*(6), 421–432. https://doi.org/10.1038/nrg2339
638 Hubisz, M. J., Falush, D., Stephens, M., & Pritchard, J. K. (2009). Inferring weak population
639 structure with the assistance of sample group information. *Molecular Ecology Resources*,
640 *9*(5), 1322–1332. https://doi.org/10.1111/j.1755-0998.2009.02591.x
641 Hussey, P. J., Ketelaar, T., & Deeks, M. J. (2006). Control of the actin cytoskeleton in plant cell
642 growth. *Annual Review of Plant Biology*, *57*(1), 109–125.
643 https://doi.org/10.1146/annurev.arplant.57.032905.105206
644 Jeffries, K. M., Connon, R. E., Verhille, C. E., Dabruzzi, T. F., Britton, M. T., Durbin-Johnson,
645 B. P., & Fangue, N. A. (2019). Divergent transcriptomic signatures in response to salinity

exposure in two populations of an estuarine fish. *Evolutionary Applications*, eva.12799. https://doi.org/10.1111/eva.12799

Johnston, I. A., Bower, N. I., & Macqueen, D. J. (2011). Growth and the regulation of myotomal muscle mass in teleost fish. *Journal of Experimental Biology*, *214*(10), 1617-1628.

Johnston, T. A., Lysack, W., & Leggett, W. C. (2012). Abundance, growth, and life history characteristics of sympatric walleye (*Sander vitreus*) and sauger (*Sander canadensis*) in Lake Winnipeg, Manitoba. *Journal of Great Lakes Research*, *38*, 35–46. https://doi.org/10.1016/j.jglr.2010.06.009

Jombart, T., Devillard, S., & Balloux, F. (2010). Discriminant analysis of principal components: A new method for the analysis of genetically structured populations. *BMC Genetics*, *11*(1), 94. https://doi.org/10.1186/1471-2156-11-94

Jones, O. R., & Wang, J. (2010). COLONY: a program for parentage and sibship inference from multilocus genotype data. *Molecular Ecology Resources*, *10*(3), 551–555. https://doi.org/10.1111/j.1755-0998.2009.02787.x

Kim, S., & Coulombe, P. A. (2010). Emerging role for the cytoskeleton as an organizer and regulator of translation. *Nature Reviews Molecular Cell Biology*, *11*(1), 75–81. https://doi.org/10.1038/nrm2818

Kim, S., Wong, P., & Coulombe, P. A. (2006). A keratin cytoskeletal protein regulates protein synthesis and epithelial cell growth. *Nature*, *441*(7091), 362–365. https://doi.org/10.1038/nature04659

Knaus, B. J., & Grünwald, N. J. (2017). VCFR: a package to manipulate and visualize variant call format data in R. Molecular Ecology Resources, 17(1), 44–53. https://doi.org/10.1111/1755-0998.12549

669 Knutsen, H., Olsen, E. M., Jorde, P. E., Espeland, S. H., André, C., & Stenseth, N. C. (2011).
670 Are low but statistically significant levels of genetic differentiation in marine fishes
671 'biologically meaningful'? A case study of coastal Atlantic cod: Biologically relevant
672 genetic signals. *Molecular Ecology*, *20*(4), 768–783. https://doi.org/10.1111/j.1365-
673 294X.2010.04979.x
674 Kolosov, D., Bui, P., Chasiotis, H., & Kelly, S. P. (2013). Claudins in teleost fishes. *Tissue*
675 *Barriers*, *1*(3), e25391. https://doi.org/10.4161/tisb.25391
676 Kritzer, J. P., & Sale, P. F. (2004). Metapopulation ecology in the sea: From Levins' model to
677 marine ecology and fisheries science. *Fish and Fisheries*, *5*(2), 131–140.
678 https://doi.org/10.1111/j.1467-2979.2004.00131.x
679 Kuleshov, M. V., Jones, M. R., Rouillard, A. D., Fernandez, N. F., Duan, Q., Wang, Z., …
680 Ma'ayan, A. (2016). Enrichr: A comprehensive gene set enrichment analysis web server
681 2016 update. *Nucleic Acids Research*, *44*(W1), W90–W97.
682 https://doi.org/10.1093/nar/gkw377
683 Lake Winnipeg Basin Indicator Series: Fish Populations. (2018). *Manitoba Government*, 6.
684 Lawson, D. J., van Dorp, L., & Falush, D. (2018). A tutorial on how not to over-interpret
685 STRUCTURE and ADMIXTURE bar plots. *Nature Communications*, *9*(1), 3258.
686 https://doi.org/10.1038/s41467-018-05257-7
687 Li, H., Handsaker, B., Wysoker, A., Fennell, T., Ruan, J., Homer, N., … 1000 Genome Project
688 Data Processing Subgroup. (2009). The sequence alignment/map format and SAMtools.
689 *Bioinformatics*, *25*(16), 2078–2079. https://doi.org/10.1093/bioinformatics/btp352

690 Li, R. (1995). Regulation of cortical actin cytoskeleton assembly during polarized cell growth in
691 budding yeast. *The Journal of Cell Biology*, *128*(4), 599–615.
692 https://doi.org/10.1083/jcb.128.4.599
693 Liu, L., Ang, K. P., Elliott, J. A. K., Kent, M. P., Lien, S., MacDonald, D., & Boulding, E. G.
694 (2017). A genome scan for selection signatures comparing farmed Atlantic salmon with two
695 wild populations: Testing colocalization among outlier markers, candidate genes, and
696 quantitative trait loci for production traits. *Evolutionary Applications*, *10*(3), 276–296.
697 https://doi.org/10.1111/eva.12450
698 Luu, K., Bazin, E., & Blum, M. G. B. (2017). pcadapt: An R package to perform genome scans
699 for selection based on principal component analysis. *Molecular Ecology Resources*, *17*(1),
700 67–77. https://doi.org/10.1111/1755-0998.12592
701 Manitoba Fishery Regulations, 1987. (1987). SOR/87-509. Retrieved from https://laws-
702 lois.justice.gc.ca/eng/regulations/sor-87-509/index.html
703 Manitoba Sustainable Development. (2018). *Manitoba Sustainable Development Annual Report*
704 *2017-2018*(p. 206). Retrieved from http://www.gov.mb.ca/finance/publications/annual.html
705 Manitoba Sustainable Development. (2019). Lake Winnipeg Measures to Enhance Sustainability.
706 Retrieved from
707 https://www.gov.mb.ca/sd/pubs/fish_wildlife/fish/quota_buyback_proposed_reg.pdf
708 Marshall, W. S., Breves, J. P., Doohan, E. M., Tipsmark, C. K., Kelly, S. P., Robertson, G. N., &
709 Schulte, P. M. (2018). Clauding-10 isoform expression and cation selectivity change with
710 salinity in salt-secreting epithelia of *Fundulus heteroclitus*. *The Journal of Experimental*
711 *Biology*, *221*(1), jeb168906. https://doi.org/10.1242/jeb.168906

712 Moles, M. D., Johnston, T. A., Robinson, B. W., Leggett, W. C., & Casselman, J. M. (2008). Is
713 gonadal investment in walleye (*Sander vitreus*) dependent on body lipid reserves? A
714 multipopulation comparative analysis. *Canadian Journal of Fisheries and Aquatic Sciences*,
715 *65*(4), 600–614. https://doi.org/10.1139/F07-186
716 Moles, M. D., Robinson, B. W., Johnston, T. A., Cunjak, R. A., Jardine, T. D., Casselman, J. M.,
717 & Leggett, W. C. (2010). Morphological and trophic differentiation of growth morphotypes
718 of walleye (*Sander vitreus*) from Lake Winnipeg, Canada. *Canadian Journal of Zoology*,
719 *88*(10), 950–960. https://doi.org/10.1139/Z10-062
720 Murphy, M. D., & Crabtree, R. E. (2001). Changes in the age structure of nearshore adult red
721 drum off west-central Florida related to recruitment and fishing mortality. *North American*
722 *Journal of Fisheries Management*, *21*(3), 671–678. https://doi.org/10.1577/1548-
723 8675(2001)021<0671:CITASO>2.0.CO;2
724 Patro, R., Duggal, G., Love, M. I., Irizarry, R. A., & Kingsford, C. (2017). Salmon provides fast
725 and bias-aware quantification of transcript expression. *Nature Methods*, *14*(4), 417–419.
726 https://doi.org/10.1038/nmeth.4197
727 Pritchard, J. K., Stephens, M., & Donnelly, P. (2000). Inference of population structure using
728 multilocus genotype data. *Genetics*, *155*(2), 945–959.
729 Pruyne, D., & Bretscher, A. (2000). Polarization of cell growth in yeast. *Journal of Cell Science*,
730 113(4), 571 LP – 585. Retrieved from http://jcs.biologists.org/content/113/4/571.abstract
731 R Core Team (2019). R: A language and environment for statistical computing. R Foundation for
732 Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

733 Reid, A. J., Carlson, A. K., Creed, I. F., Eliason, E. J., Gell, P. A., Johnson, P. T. J., … Cooke, S.
734 J. (2019). Emerging threats and persistent conservation challenges for freshwater
735 biodiversity. *Biological Reviews*, *94*(3), 849–873. https://doi.org/10.1111/brv.12480
736 Rideout, R. M., & Tomkiewicz, J. (2011). Skipped spawning in fishes: More common than you
737 might think. *Marine and Coastal Fisheries*, *3*(1), 176–189.
738 https://doi.org/10.1080/19425120.2011.556943
739 Russello, M. A., Kirk, S. L., Frazer, K. K., & Askey, P. J. (2011). Detection of outlier loci and
740 their utility for fisheries management: Outlier loci for fisheries management. *Evolutionary*
741 *Applications*, *5*(1), 39–52. https://doi.org/10.1111/j.1752-4571.2011.00206.x
742 Sheppard, Katie T., Davoren, G. K., & Hann, B. J. (2015). Diet of walleye and sauger and
743 morphological characteristics of their prey in Lake Winnipeg. *Journal of Great Lakes*
744 *Research*, *41*(3), 907–915. https://doi.org/10.1016/j.jglr.2015.05.006
745 Sheppard, K.T., Hann, B. J., & Davoren, G. K. (2018). Growth rate and condition of walleye
746 (*Sander vitreus*), sauger (*Sander canadensis*), and dwarf walleye in a large Canadian lake.
747 *Canadian Journal of Zoology*, *96*(7), 739–747. https://doi.org/10.1139/cjz-2017-0276
748 State of Lake Winnipeg: 1999 to 2007. (2011). *Environment Canada*. Retrieved from
749 http://publications.gc.ca/pub?id=9.652328&sl=0
750 Stepien, C. A., Murphy, D. J., Lohner, R. N., Sepulveda-Villet, O. J., & Haponski, A. E. (2009).
751 Signatures of vicariance, postglacial dispersal and spawning philopatry: population genetics
752 of the walleye *Sander vitreus*. *Molecular Ecology*, *18*(16), 3411-3428.
753 Stepien, C. A., Snyder, M. R., & Knight, C. T. (2018). Genetic divergence of nearby walleye
754 spawning groups in central Lake Erie: implications for management. *North American*
755 *Journal of Fisheries Management*, *38*(4), 783-793.

Sveen, L. R., Timmerhaus, G., Torgersen, J. S., Ytteborg, E., Jørgensen, S. M., Handeland, S., … Takle, H. (2016). Impact of fish density and specific water flow on skin properties in Atlantic salmon (*Salmo salar L.*) post-smolts. *Aquaculture*, *464*, 629–637. https://doi.org/10.1016/j.aquaculture.2016.08.012

Vähä, J.-P., Erkinaro, J., Niemelä, E., & Primmer, C. R. (2007). Life-history and habitat features influence the within-river genetic structure of Atlantic salmon. *Molecular Ecology*, *16*(13), 2638–2654. https://doi.org/10.1111/j.1365-294X.2007.03329.x

Van Der Maaten, L. J. P., & Hinton, G. E. (2008). Visualizing high-dimensional data using t-sne. *Journal of Machine Learning Research*, *9*, 2579–2605. https://doi.org/10.1007/s10479-011-0841-3

Verta, J.-P., & Jones, F. C. (2019). Predominance of cis-regulatory changes in parallel expression divergence of sticklebacks. *ELife*, *8*, e43785. https://doi.org/10.7554/eLife.43785.001

Wang, J. (2004). Sibship Reconstruction From Genetic Data With Typing Errors. *Genetics*, *166*(4), 1963–1979. https://doi.org/10.1534/genetics.166.4.1963

Waples, R. S. (1998). Separating the wheat from the chaff: Patterns of genetic differentiation in high gene flow species. *Journal of Heredity*, *89*(5), 438–450. https://doi.org/10.1093/jhered/89.5.438

Waples, Robin S., & Gaggiotti, O. (2006). What is a population? An empirical evaluation of some genetic methods for identifying the number of gene pools and their degree of connectivity: What is a population? *Molecular Ecology*, *15*(6), 1419–1439. https://doi.org/10.1111/j.1365-294X.2006.02890.x

778 Wasteneys, G. O., & Galway, M. E. (2003). Remodeling the cytoskeleton for growth and form:
779 An overview with some new views. *Annual Review of Plant Biology*, *54*(1), 691–722.
780 https://doi.org/10.1146/annurev.arplant.54.031902.134818
781 Watkinson, D. A., & Gillis, D. M. (2005). Stock discrimination of Lake Winnipeg walleye based
782 on Fourier and wavelet description of scale outline signals. *Fisheries Research*, *72*(2–3),
783 193–203. https://doi.org/10.1016/j.fishres.2004.11.002
784 Weir, B. S., & Cockerham, C. C. (1984). Estimating *F* -statistics for the analysis of population
785 structure. *Evolution*, *38*(6), 1358–1370. https://doi.org/10.1111/j.1558-5646.1984.tb05657.x
786 Whitlock, M. C., & Lotterhos, K. E. (2015). Reliable detection of loci responsible for local
787 adaptation: Inference of a null model through trimming the distribution of F_{ST}. *The*
788 *American Naturalist*, *186*(S1), S24–S36. https://doi.org/10.1086/682949
789 WWF (2016). Living Planet Report 2016: Risk and resilience in a new era. WWF
790 International, Gland, Switzerland. Retrieved from
791 http://awsassets.panda.org/downloads/lpr_living_planet_report_2016.pdf
792 WWF (2018). Living Planet Report 2018: Aiming higher (Eds M. Grooten and R. E. A.
793 Almond). WWF International, Gland, Switzerland. Retrieved from
794 http://assets.wwf.ca/downloads/lpr2018_full_report_spreads.pdf
795 Yan, J., Song, Z., Xu, Q., Kang, L., Zhu, C., Xing, S., … Sang, T. (2017). Population
796 transcriptomic characterization of the genetic and expression variation of a candidate
797 progenitor of *Miscanthus* energy crops. *Molecular Ecology*, *26*(21), 5911–5922.
798 https://doi.org/10.1111/mec.14338
799 Yang, R.-C. (1998). Estimating hierarchical *F* -statistics. *Evolution*, *52*(4), 950–956.
800 https://doi.org/10.1111/j.1558-5646.1998.tb01824.x

Zheng, X., Levine, D., Shen, J., Gogarten, S. M., Laurie, C., & Weir, B. S. (2012). A high-performance computing toolset for relatedness and principal component analysis of SNP data. *Bioinformatics*, *28*(24), 3326–3328. https://doi.org/10.1093/bioinformatics/bts606

820 Table 1. Weir & Cockerham's pairwise F_{ST} calculated with hierfstat between the Red River in
821 the south basin, Matheson Island in the channel, and Dauphin River in the north basin for all 48
822 walleye (*Sander vitreus*) sampled in both 2017 and 2018. 95% confidence intervals are provided
823 in parentheses. Collection site locations are available in Figure 1. This analysis was performed
824 with 52,372 Hardy-Weinberg Equilibrium filtered and linkage disequilibrium pruned, putatively
825 neutral single nucleotide polymorphisms.

826

827

	Red River (south basin)	**Matheson Island (channel)**	**Dauphin River (north basin)**
Red River	-	0.0012 (0.0009–0.0016)	0.0068 (0.0064–0.0074)
Matheson Island		-	0.0043 (0.0039–0.0048)
Dauphin River			-

828

829

830

831

832

833

834

Table 2. Weir & Cockerham's Pairwise F_{ST} calculated with hierfstat between the Red River in the south basin, Matheson Island in the channel, and Dauphin River in the north basin. F_{ST} values above the diagonal represent the 24 walleye (*Sander vitreus*) collected in 2017, and values below the diagonal represent the 24 collected in 2018. 95% confidence intervals are provided in parentheses. Collection site locations are available in Figure 1. This analysis was performed with 52,372 Hardy-Weinberg Equilibrium filtered and linkage disequilibrium pruned, putatively neutral single nucleotide polymorphisms.

	Red River (south basin)	**Matheson Island (channel)**	**Dauphin River (north basin)**
Red River	-	0.0023 (0.0015–0.0031)	0.0073 (0.0064–0.0082)
Matheson Island	0.0019 (0.0011–0.0028)	-	0.0044 (0.0035–0.0052)
Dauphin River	0.0067 (0.0058–0.0077)	0.0060 (0.0051–0.0070)	-

850

851

852 Table 3. Genes that vary along a latitudinal gradient in Lake Winnipeg walleye (*Sander vitreus*)
853 with ≥ 2 outlier single nucleotide polymorphisms (SNPs) from pcadapt, each significant at a
854 Benjamini-Hochberg adjusted *p*-value < 0.05 (PC1 in Figure 4). SwissProt gene names and
855 corresponding proteins are provided, and general cellular location or function of these genes are
856 described in Summary Function. This analysis was performed using a set of 222,634 single
857 nucleotide polymorphisms (SNPs) not filtered for Hardy-Weinberg Equilibrium or pruned for
858 linkage disequilibrium, unlike the putatively neutral set of SNPs used for population structure
859 analyses.

SwissProt Gene Name	**Protein**	**Summary Function**
ABCA12	ATP-binding cassette sub-family A member 12	Cell Membrane
ANK3	Ankyrin-3	Cell Membrane
atp1a3	Sodium/potassium-transporting ATPase subunit alpha-3	Cell Membrane
CLDN10	Claudin-10	Cell Membrane
Prf1	Perforin-1	Cell Membrane

SLC9A6	Sodium/hydrogen exchanger 6	Cell Membrane
CRYBG1	Beta/gamma crystallin domain-containing protein 1	Cytoskeleton
IFI44	Interferon-induced protein 44	Cytoskeleton
MYH9	Myosin-9	Cytoskeleton
TUBB4B	Tubulin beta-4B chain	Cytoskeleton
DNASE1L1	Deoxyribonuclease-1-like 1	DNase
EIF4G1	Eukaryotic translation initiation factor 4 gamma 1	Expression regulation
srebf2	Sterol regulatory element-binding protein 2	Expression regulation
Znf879	Zinc finger protein 879	Expression regulation
CLPX	ATP-dependent Clp protease ATP-binding subunit clpX-like, mitochondrial	Protease
CXCR3	C-X-C chemokine receptor type 3	Signaling
MATK	Megakaryocyte-associated tyrosine-protein kinase	Signaling
Ralgds	Ral guanine nucleotide dissociation stimulator	Signaling
Pol	LINE-1 retrotransposable element ORF2 protein	Transposable Element

863

864 Table 4. Genes that vary between 2017 and 2018 in Lake Winnipeg walleye (*Sander vitreus*)
865 with transcripts containing ≥2 outlier single nucleotide polymorphisms (SNPs) from pcadapt,
866 each SNP significant at a Benjamini-Hochberg adjusted *p*-value < 0.05 (PC2 in Figure 4).
867 SwissProt gene names and corresponding proteins are provided, and general cellular location or
868 function of these genes are described in Summary Function. This analysis was performed using a
869 set of 222,634 single nucleotide polymorphisms (SNPs) not filtered for Hardy-Weinberg
870 Equilibrium or pruned for linkage disequilibrium, unlike the putatively neutral set of SNPs used
871 for population structure analyses.

SwissProt Gene Name	**Protein**	**Summary Function**
Gp1bb	Platelet glycoprotein Ib beta chain	Cell Adhesion
MFAP4	Microfibril-associated glycoprotein 4	Cell Adhesion
BTG3	Protein BTG3	Cell Division
Ppp6c	Serine/threonine-protein phosphatase 6 catalytic subunit	Cell Division
Slc12a3	Solute carrier family 12 member 3	Cell Membrane
MMD	Monocyte to macrophage differentiation factor	Expression regulation
Srsf10	Serine/arginine-rich splicing factor 10	Expression regulation
Ube2a	Ubiquitin-conjugating enzyme E2 A	Expression regulation
Znf18	Zinc finger protein 18	Expression regulation
St6galnac2	Alpha-N-acetylgalactosaminide alpha-2,6-sialyltransferase 2	Protein Modification

MYO9A	Unconventional myosin-IXa	Signaling/Cytoskeleton
Pol	LINE-1 retrotransposable element ORF2 protein	Transposable Element
RTase	Probable RNA-directed DNA polymerase from transposon BS	Transposable Element
TC1A	Transposable element Tc1 transposase	Transposable Element
TN6	Putative transposase in *Dicentrarchus labrax* (European seabass)	Transposable Element
TY3B-G	Transposon Ty3-G Gag-Pol polyprotein	Transposable Element
YTX2	Transposon TX1 uncharacterized protein (Fragment)	Transposable Element

872

873

874

875

876

877

878

879

880

881

882

883

884 Figure 1. Sampling locations within Lake Winnipeg. Eight walleye (*Sander vitreus)* per year and
885 per spawning site were collected, for *n* = 48 fish over 2017 and 2018. The Red River represents
886 the south basin, Matheson Island represents the channel connecting the two lake basins, and the
887 Dauphin River represents the north basin.

888

889 Figure 2. Representative Structure runs from ten replicates testing K = 2–5, organized by
890 collection site (Red River in the south basin, Matheson Island in the channel, and Dauphin River
891 in the north basin) and year collected (2017 and 2018) for all walleye (*Sander vitreus*) used in
892 the present study. Collection site locations are available in Figure 1. This analysis was performed
893 with 52,372 Hardy-Weinberg Equilibrium filtered and linkage disequilibrium pruned, putatively
894 neutral single nucleotide polymorphisms.

895

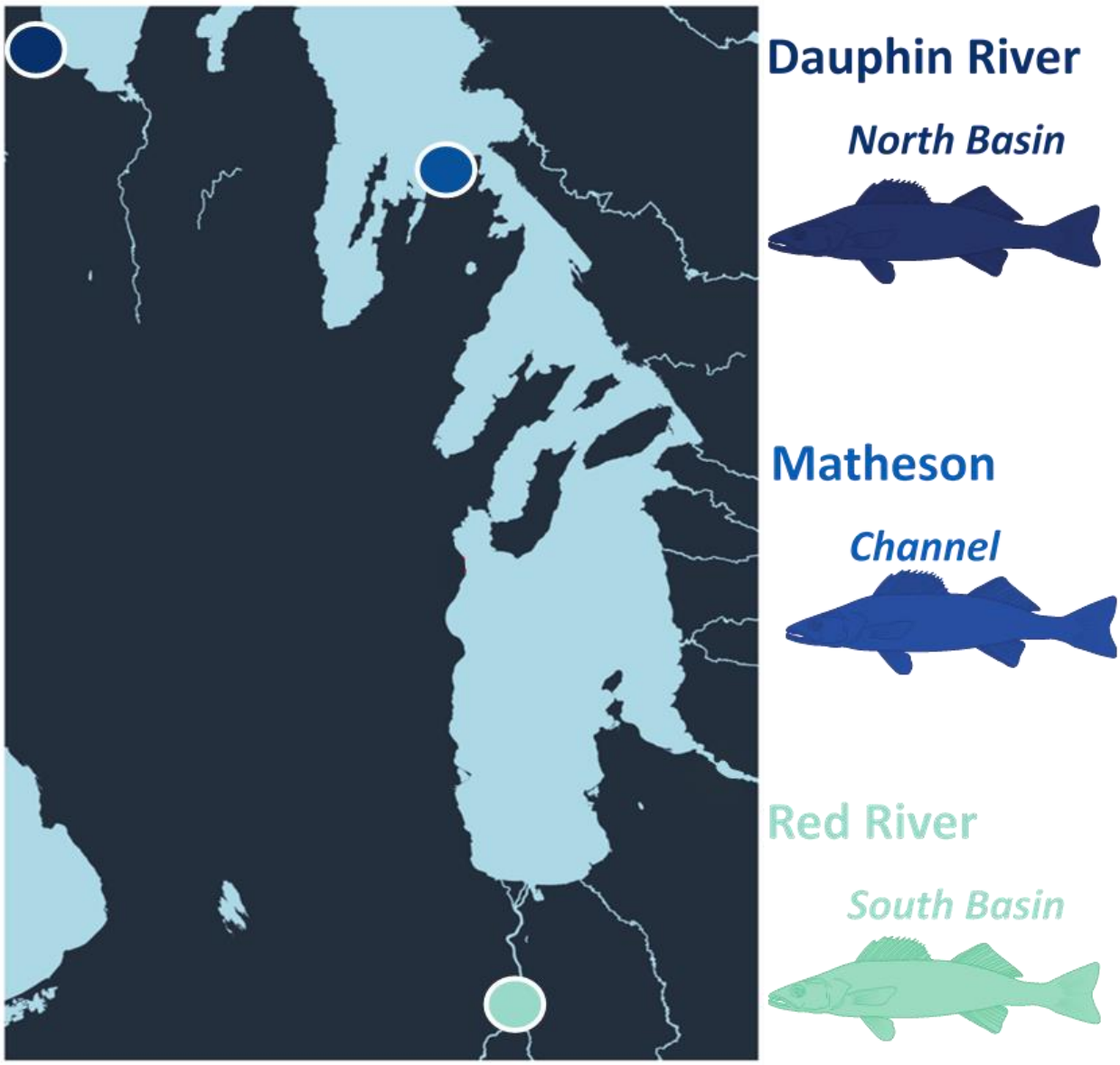
896 Figure 3. Membership probability plot of discriminant analysis of principal components using
897 prior collection site information (Red River in the south basin, Matheson Island in the channel,
898 and Dauphin River in the north basin) on walleye (*Sander vitreus*) collected over 2017 and 2018,
899 performed using Adegenet. Collection site locations are available in Figure 1. This analysis was
900 performed with 52,372 Hardy-Weinberg Equilibrium filtered and linkage disequilibrium pruned,
901 putatively neutral single nucleotide polymorphisms.

902

903 Figure 4. Principal Components Analysis implemented in pcadapt with color showing site
904 collected (red for Red River in the south basin, yellow Matheson Island in the channel, and blue
905 Dauphin River in the north basin), circles showing walleye (*Sander vitreus*) collected in 2017,

and triangles showing walleye collected in 2018. Collection site locations are available in Figure 1. This analysis was performed using a set of 222,634 single nucleotide polymorphisms (SNPs) filtered for quality, minor allele frequency > 0.05, and a maximum of two out of 48 missing individuals, but not filtered for Hardy-Weinberg Equilibrium or pruned for linkage disequilibrium. These SNPs are, thus, more likely to represent patterns of adaptive variation in the system, and outlier analyses were performed using this set of SNPs. Principal Component 1 (PC1) represents a latitudinal gradient, while Principal Component 2 (PC2) represents a genetic divergence between sampling years. Outlier SNPs that contribute to each of these axes were selected for functional analyses (Tables 3, 4, 5), with 386 SNPs contributing to PC1 and 791 SNPs contributing to PC2, significant at Benjamini-Hochberg adjusted *p*-values < 0.05.

917



918

919 .

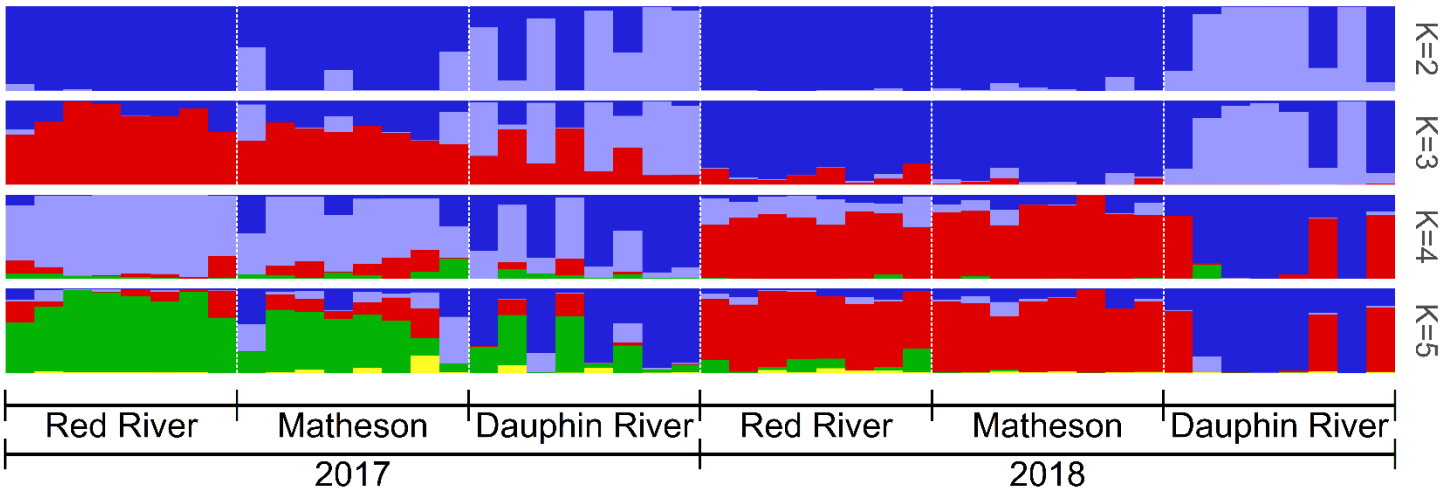
920

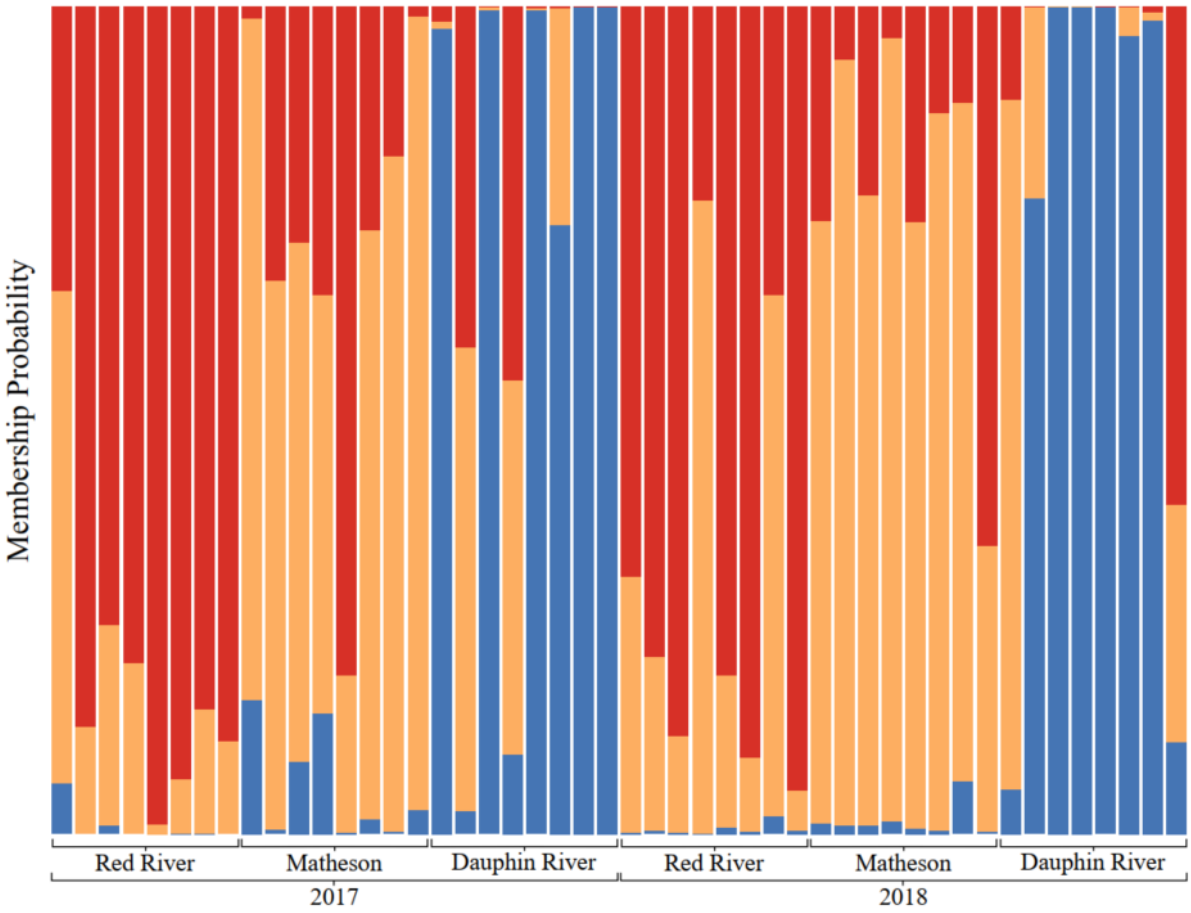
921

922

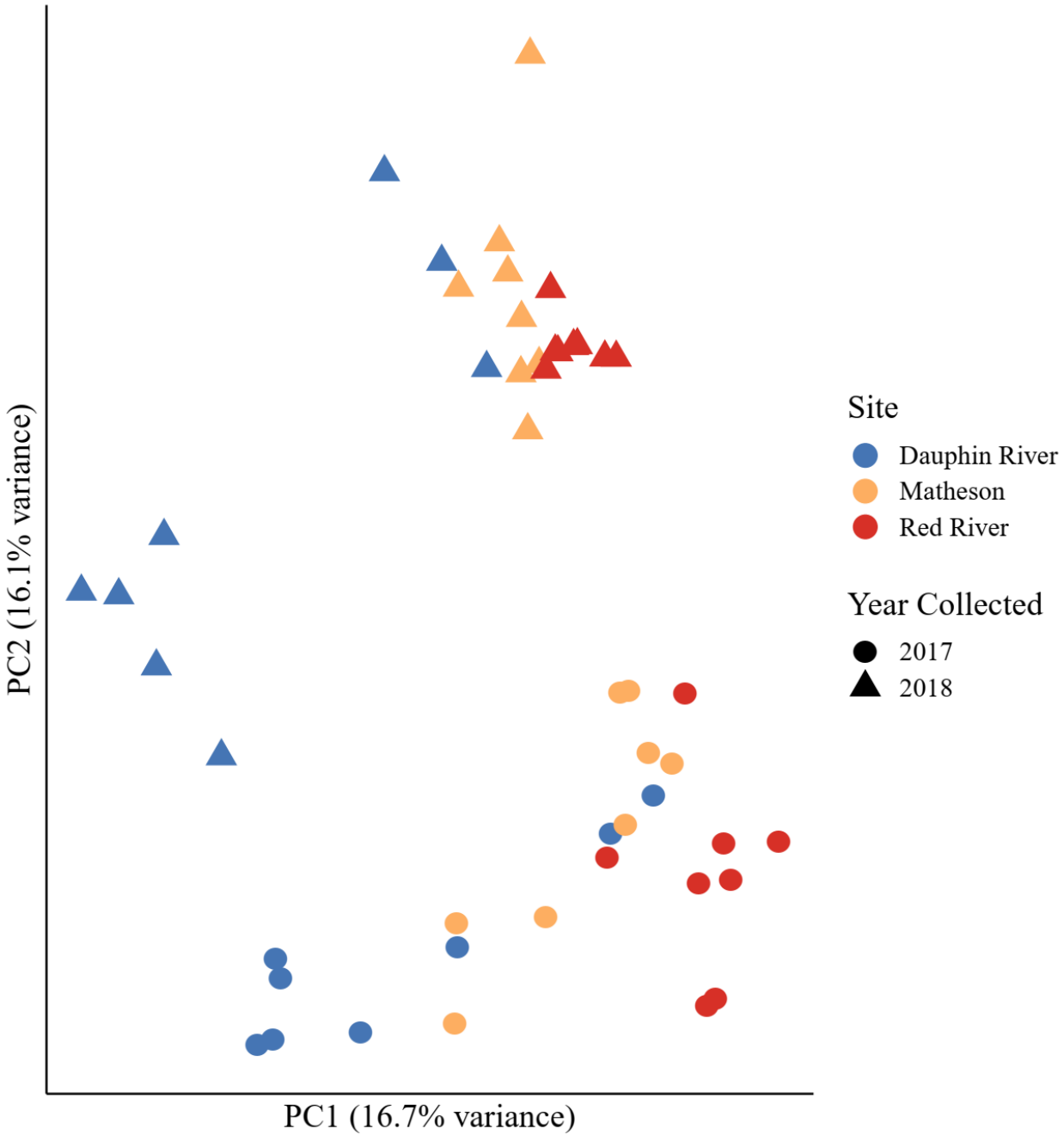
923

924





925



926