

Walleye in the Light on the Water

*A genomic and phenotypic evaluation
of walleye along the Waswanipi river*

For the Cree Nation of Waswanipi

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Introduction

In the southern part of the traditional Cree territory of Eeyou Istchee (Figure 1), the community of Waswanipi (the name in English translates to Light on the Water) have a long cultural history of harvesting fish populations for their subsistence and well-being. The fish community in the Waswanipi Lake region is composed mainly of cisco, lake whitefish, lake sturgeon, walleye, pike, burbot and long nosed and common sucker (Brooke & Penn, 1996). One of the important socio-economic and cultural fish species in this region is walleye (*Sander vitreus*), targeted by both Cree fishers, and by sport fishers who all compete at the famous Waswanipi Old Post Fishing Derby each summer.

With such demand for this species and the expansion of the sportfishing tourism industry in many regions in Canada an increased fishing pressure may be imposed. For example, in nearby Mistassini Lake, Cree elders and fishers have consistently reported reductions in body size and catch rates in walleye populations particularly in the southern parts of the lake, adjacent to human development (Bowles, Jeon, Marin, MacLeod, & Fraser, 2022). Genomic data can be used to evaluate the health and status of fish populations, as well as to identify distinct stocks and their contribution to the different fisheries (commercial, recreational, and subsistence). This knowledge will help to facilitate the Cree people of Eeyou Istchee to develop and enhance community-based sustainable fisheries management.

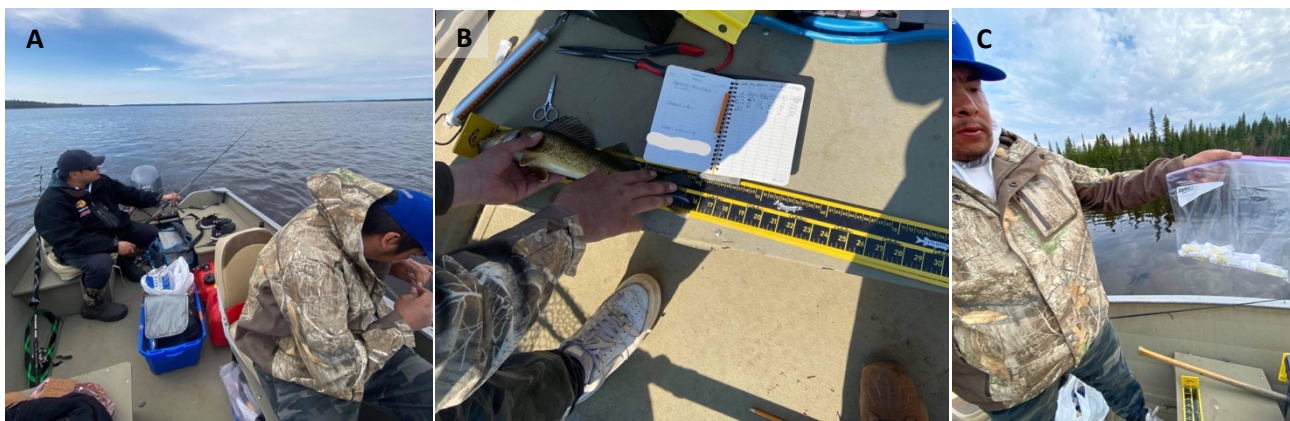
To date, no genetic analyses were conducted in the Waswanipi region to assess and define walleye stock(s). As part of the large-scale project Fostering Indigenous Small-scale fisheries for Health, Economy, and food Security (FISHES) and to address some concerns raised by the local community, we aimed to:

- characterize the genomic structure and phenotypic (body size) variation of walleye in Waswanipi, and
- compare with allopatric populations in Mistassini Lake and Mistasiniishish Lake tributaries.

Methods

Fish Sampling

Sampling along the Waswanipi River (Figure 1) was conducted during the spawning period (mid to end May) in two subsequent years (2021 and 2022) by our FISHES partner, the Cree Nation of Waswanipi. Walleye were captured via angling (Photo A) and were immediately placed in freshwater baths with aerators. From each walleye the fork length (FL, ± 1 mm), body weight (± 50 g) and sex were recorded (Photo B). Fin clips were also collected for genetic analyses (Photo C). Walleye were then either returned to the water near the location of capture or kept for human consumption. Despite considerable effort during the 2021 field season only a single sample was captured from Broadback River, and hence this sample is not considered further in the report.



Photos from the field: A) Field crew from Cree Nation of Waswanipi during the 2021 sampling, B) Standard fish measurements, C) Samples collected. Photo credit: Ian Saganash

Morphological analyses

Because walleye exhibit sexual dimorphism (i.e., females tend to be larger in body size compared to males) we looked at the different sexes separately within a river. For all samples from the Waswanipi River, we investigated variation in fork length and mass across and within each sampling year. However, due to male-biased sampling in 2022 (see results), we combined data from both years to test for differences between sexes using non-parametric tests. We also compared body size differences between Waswanipi River and tributaries from Mistassini and Mistasiniishish (Albanel) Lakes. All analyses were done in R v4.2.2 (R Core Team, 2020) and plots were generated using *ggplot2* (Wickham, 2009).

Genetic analyses

A subset of 40 samples, collected in 2021 along the Waswanipi River, were randomly selected (at equal sex ratio; 20F:20M) and combined with samples from Mistassini and Mistasiniishish tributaries for genetic analysis. We extracted DNA from fin (caudal) samples stored in 95% ethanol using the DNeasy Blood and Tissue Kit (Qiagen) according to the manufacturer's protocol with an overnight lysis step. DNA quality was evaluated on an 1.5% agarose gel electrophoresis and quantified using Qubit (Invitrogen). Whole-genome (low-coverage) libraries were prepared for each fish sample according to the protocol described in Therkildsen and Palumbi (2017) and adapted by Merot et al. (2021). Equimolar amounts of individual libraries were pooled and sequenced using paired-end 150 bp reads on an Illumina NovaSeq 6000 S4 at Genome Quebec.

Sequencing reads were cleaned, filtered, and aligned to the walleye reference assembly (Heiner et al., unpublished). We used *ANGSD* v0.933 (Korneliussen, Albrechtsen, & Nielsen, 2014), a software specifically designed to take genotype uncertainty into account instead of called genotypes, which is appropriated for low coverage data. In general, we followed the methodological guidelines by Lou et al. (2021) and the pipeline developed by Merot et al. (2021), available at https://github.com/claimeerot/angsd_pipeline. We investigated genomic structure and population differentiation across all walleye tributaries using three approaches. First, we ran PCAngsd (Meisner & Albrechtsen, 2018) to perform a principal components analysis (PCA). Then we ran NGSadmixture (Skotte, Korneliussen, & Albrechtsen, 2013) for several K-clusters (K2-K10) and 10 replicates to assess the optimal number of K that best explains our data and identify fine-scale structure. Finally, genetic differentiation between sampling sites was estimated by calculating pairwise genome-wide F_{ST} .

Results

Between 2021 and 2022, a total of 164 walleye samples were captured along the Waswanipi river (Figure 1, Table 1) with a sex-bias towards males (84% of the sampled fish). Such sampling bias from spawning areas is not uncommon for walleye, an observation also reported in Mistassini Lake (Marin & Fraser, 2022). However, this might be a result of specific sampling techniques (e.g., gillnet vs. angling) which target different sexes and body sizes.

Table 1. Summaries from fish sampling along the Waswanipi River.

Sampling Year	Total sample size (N)	Females			Males		
		N	Mean fork length (mm)	Mean weight (grams)	N	Mean fork length (mm)	Mean weight (grams)
2021	99	22	410	693	77	376	525
2022	65	5	414	709	60	379	447
2021-2022	164	27	411	696	137	378	491

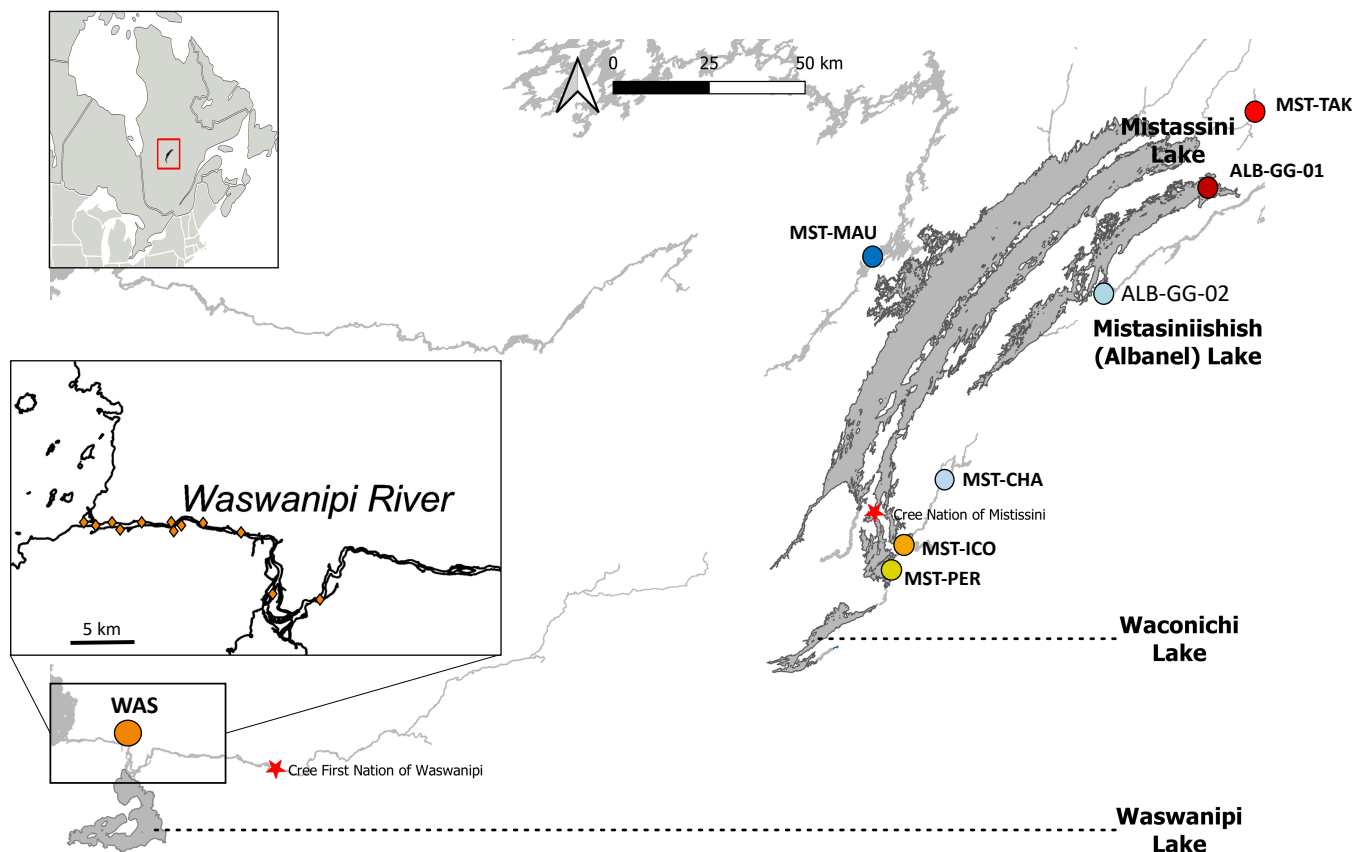


Figure 1. A map showing the location of each sampled tributary in the three lakes-regions (Waswanipi (WAS), Mistassini (MST) and Mistasiniishish (ALB)). The inset shows multiple sites along the Waswanipi river where walleye were caught.

Analyses of body size between the sexes, across the two sampling years in Waswanipi, showed that females were significantly larger (Wilcoxon test) than males (Figure 2, Table 1). Between years, there was an indication that the males were lighter in 2022 vs. 2021 (Table 1, Figure A in the appendix). On the other hand, albeit with a low sample size in 2022, female body size was more consistent between years (Table 1, Figure A in the appendix).

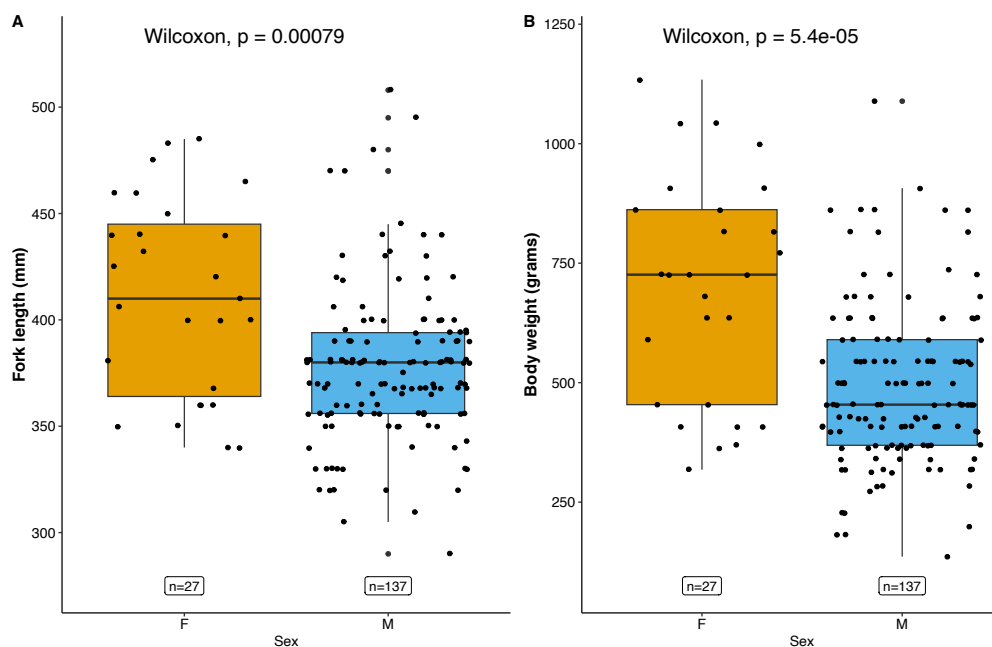


Figure 2. Body size variation among samples from Waswanipi river collected in 2021 and 2022 (combined). Females (orange box plots) are significantly larger than males (blue box plots) in A) fork length and B) body weight. The sample size (n) for each group is given below each box plot.

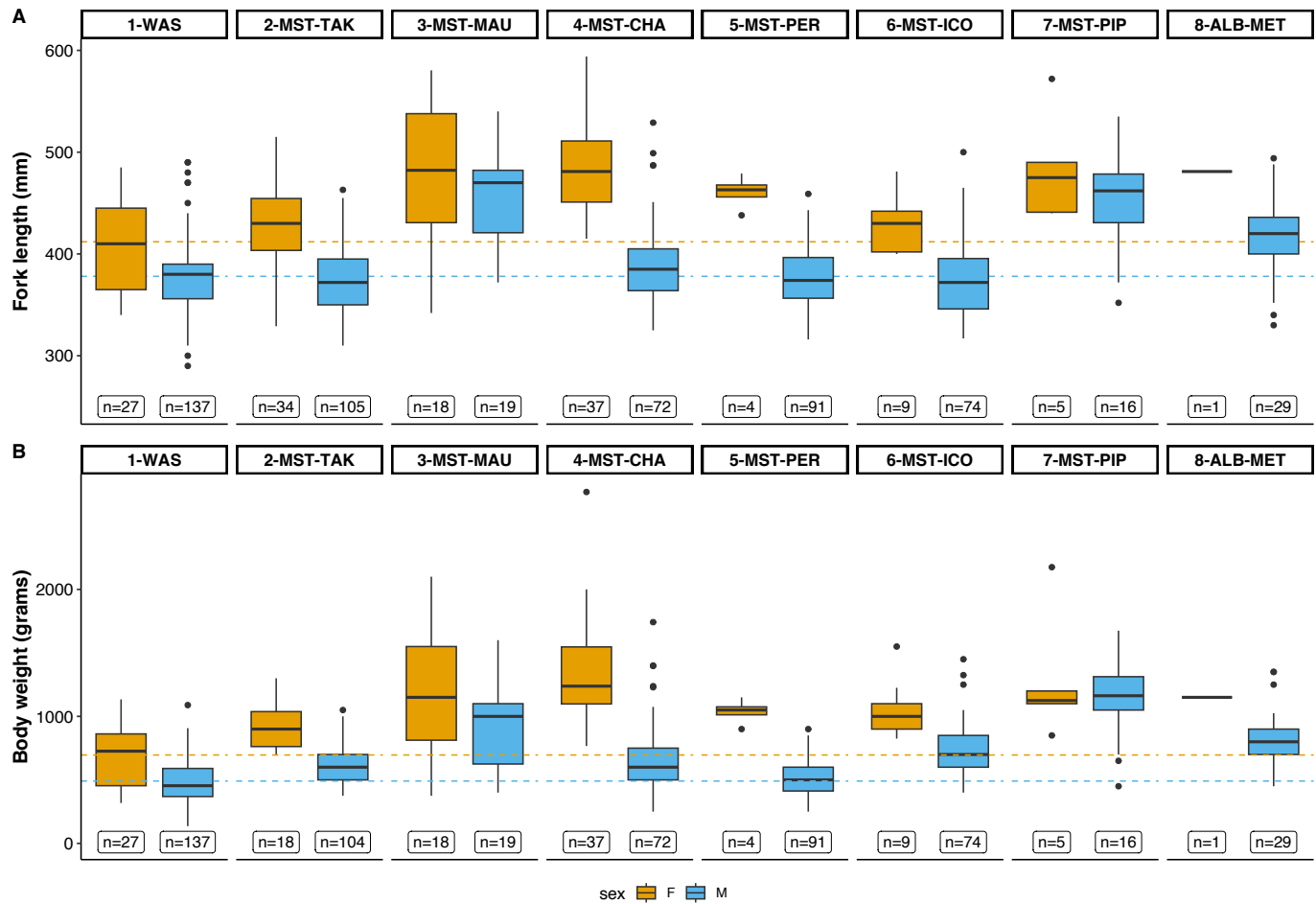


Figure 3. Body size variation among samples from tributaries in Waswanipi (WAS), Mistassini (MST) and Mistasiiniishish (ALB). The two dashed lines indicate the mean values of Waswanipi samples for fork length (A) and body weight (B) for each sex (orange for females and blue for males). The sample size (n) for each group is given below each box plot. Samples from MST-PIP and ALB-MET were not used in the genomic analyses.

Comparing Waswanipi samples with walleye from other tributaries in Mistassini (MST) and Mistasiiniishish (ALB) lakes, females appear to be smaller in fork length (mean = 411 mm) and in body weight (mean = 696 grams), whereas the males were more similar in fork length (mean = 378 mm) than in body weight (mean = 491 grams) to walleye from other rivers (Figure 3, Table A in the appendix).

For a subset of Waswanipi samples collected in 2021, we generated low-coverage whole-genome sequencing (lcWGS) data and analysed these along with samples from Mistassini (MST) and Mistasiiniishish (ALB). The principal component analysis (PCA) and the clustering analysis indicated that Waswanipi samples form one cluster with no further population subdivision (Figure 4A and 4C). These results preclude the occurrence of a second genetic stock in the region. The Waswanipi stock differs from all other populations as shown by the moderately-high F_{ST} estimates (Figure 4B). The closest population appears to be de Maures (MST-MAU), a tributary in Mistassini Lake, connected to the Rupert River (Figure 1).

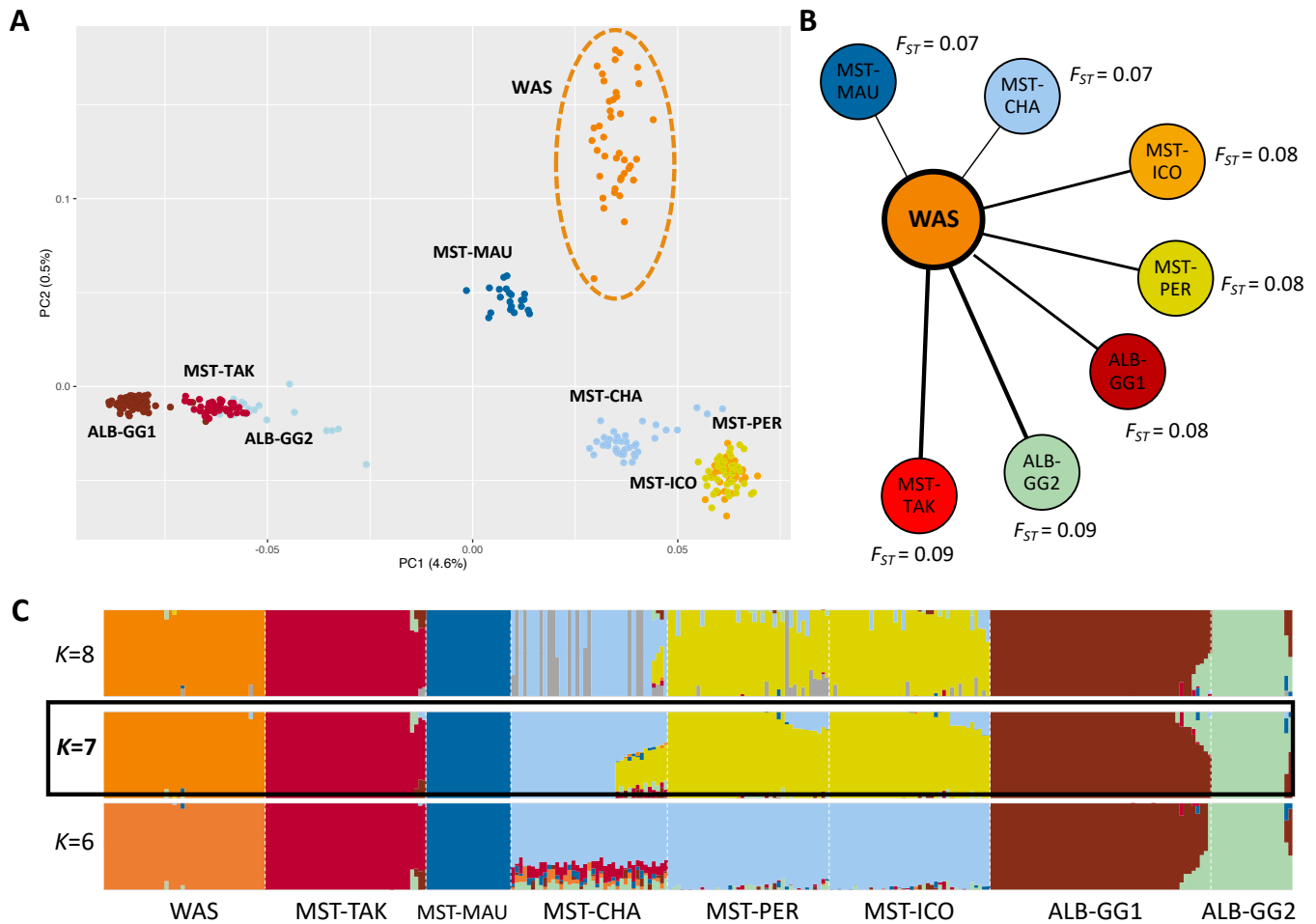


Figure 4. Population genomic structure and differentiation among walleye tributaries. (A) Principal component analysis (PCA) of walleye from sampling sites across the three lake-regions (Mistassini; MST, Mistassiniishish; ALB and Waswanipi; WAS). Each dot represents a fish sample color-coded by sampling site. Walleye from Waswanipi River are denoted by the dashed orange ellipse. (B) A schematic representation of pairwise F_{ST} estimates between Waswanipi (WAS) and other tributaries. The stronger the differentiation (higher F_{ST}) the thicker the line connecting two tributaries. (C) Visualization of NGSadmix clustering results across three consecutive K-cluster runs (K6-K8). Each sample is represented by a vertical line, denoting the proportion of membership to a defined K-cluster. All samples from Waswanipi (WAS) consistently belong to a single cluster across multiple runs of K. At K=7, we pick up the ‘optimal’ fine-scale structure for our dataset. The two sites in Mistassini (MST-PER and MST-ICO) are considered a meta-population.

Conclusions and future considerations/recommendations

In this report we evaluated the genomic and phenotypic (body size) variation of walleye in Waswanipi River and in relation to tributaries in two other lakes (Mistassini and Mistassiniishish). Despite a male-biased sampling, female walleye appear to be significantly larger than males. Both sexes, but especially females, tended to be smaller than their counterparts in the tributaries of the other lakes. The genomic analyses support the presence of one stock in the Waswanipi River, which differs from tributaries of the other lakes sampled in adjacent areas, a result of absence of contemporary migration/gene flow.

The observed decrease in body weight of Waswanipi males in 2022 relative to 2021 is slightly concerning however this result should be taken with caution. Implementing a monitoring program like the one adopted in Mistassini and re-evaluated recently (Marin & Fraser, 2016, 2022), to gather baseline data about

population characteristics (i.e. population size, age structure and genetic diversity) will enable a better understanding of the trends allowing for a sustainable management. For example:

- Consider yearly surveys in known spawning grounds with a traditional capture-mark-recapture study to obtain estimates of population sizes.
- Validate field records and standardize measuring units to preclude any erroneous inferences on body size trends. Total length and preferably size-at-age (via otolith extraction and aging) are also useful for comparing across multiple regions.
- If there are local concerns about increased fishing pressure, consider refraining from using techniques that size-select for larger walleye (i.e., gillnetting, scooping, snaring and night fishing) and consider setting a daily catch quota.

All samples from Waswanipi were caught along an approximately 20km stretch in the Waswanipi River and our genetic analysis indicates that there is only one stock in this region. However, other unsampled rivers in the area (e.g., Broadback river) might host different genetic stocks. Genetic data can add valuable information, delineating distinct stocks and assessing their contribution to mixed-stock harvests. Currently a panel of ~350 single nucleotide polymorphisms (SNPs), developed for the FISHES project is being used in genetic stock identification in Mistassini and Mistasiniishish Lakes. Given adequate sampling of all known spawning grounds around Waswanipi Lake, this panel can be applied to this region.

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Appendix

Table A. Summary results of walleye morphological variation across all tributaries

Lake/River	Year sampled	Location ID	Females			Males		
			Sample Size	Fork length (mm)	Weight (grams)	Sample Size	Fork length (mm)	Weight (grams)
Waswanipi river	2021-2022	WAS	27	411	696	137	378	491
Mistassini – Takwa river	2022	MST-TAK	34	430	925	105	374	600
Mistassini – de Maures river	2020-2022	MST-MAU	18	482	1171	19	457	916
Mistassini – Chalifour river	2022	MST-CHA	37	484	1323	72	391	679
Mistassini – Perch river	2022	MST-PER	4	461	1038	91	375	523
Mistassini – Icon river	2022	MST-ICO	9	432	1050	74	375	741
Mistassini - Pipichouane river	2022	MST-PIP*	5	484	1290	16	454	1142
Albanel – Metawashiish river	2022	ALB-MET*	1	481	1150	29	418	833

*Samples from MST-PIP and ALB-MET were not used in the genomic analyses

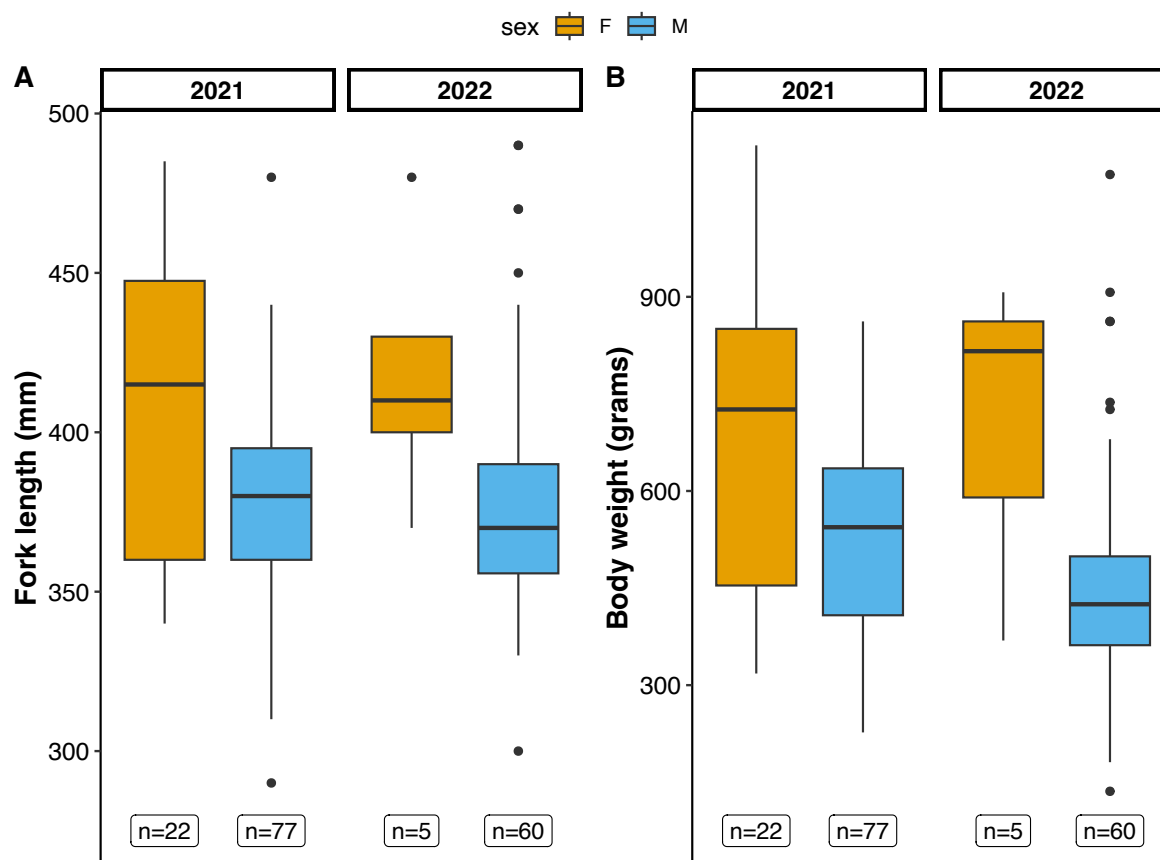


Figure A: Within-year body size variation of walleye sampled along the Waswanipi River. A) Fork length and B) body weight. The sample size (n) for each group is given below each box plot.