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Genetic diversity and structure of lake whitefish (*Coregonus clupeaformis*) 100 years after closure of the commercial fishery

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ABSTRACT

In small, inland fisheries even small perturbations to the ecosystem can quickly influence population abundance, size and age distribution, and genetic structure and diversity. In Lake Champlain, lake whitefish (*Coregonus clupeaformis*) experienced extensive commercial harvest from the mid-1800 s to1918 and habitat fragmentation due to the construction of multiple causeways between 1850 and 1899. We evaluated the influence these environmental perturbations had on lake whitefish population genetics 120 years later. We used historic catch records to determine whether fishing pressure could have been strong enough to reduce lake whitefish population abundance, and used genotype data from eight microsatellite loci to look for genetic signatures of population-sub structure and bottleneck. Catch records indicate lake whitefish were being harvested in Lake Champlain at a similar magnitude to the Great Lakes, and simulations suggest genetic diversity may have been lost as a result of harvest. However, we were unable to detect significant evidence of a genetic bottleneck, but we cannot conclusively suggest that harvest of lake whitefish did not result in a genetic bottleneck. Additionally, we found only slight evidence of population sub-structure among isolated basins, suggesting that either some gene flow among basins is possible, or that populations are just beginning to diverge and therefore differences in allele frequency were too small to detect. These data provide a perspective on effects of an inland lake commercial fishery that was closed prior to population collapse, and offer a comparison to the Laurentian Great Lakes where lake whitefish are still being harvested.

Introduction

Overfishing reduces population abundance, and frequently also results in loss of genetic diversity (Pinsky and Palumbi, 2014). After fishing pressure is released, populations can often recover demographically and return to size and age distributions similar to those present before the period of overharvest. Lost genetic diversity, however, takes much longer to recover (Hutchings and Reynolds, 2004) and can result in a loss of adaptive potential (e.g., Hoarau et al., 2005; Wright, 1931). Therefore, even if fish populations appear to have recovered from historic overharvest, they may be more vulnerable to environmental stressors.

In the face of increasing environmental change, assessing genetic diversity and managing fisheries for higher adaptive potential is important (Dudgeon et al., 2006). Commercial fishing for lake whitefish (*Coregonus clupeaformis*) in Lake Champlain was closed by 1912 in Vermont and by 1918 in Quebec (Marsden and Langdon, 2012). Since the fishery closure, there have been only two studies to evaluate the status of the lake whitefish population. Age and size structure, and estimates of growth and condition of adult fish from two commercially harvested locations were evaluated in the early 1930 s by Van Oosten and Deason (1939) who found that the length distribution of fish in Missisquoi Bay was characteristic of a disturbed population with a truncated length distribution skewed toward smaller individuals. A study that assessed age structure and growth from 2008 to 2010 found that lake whitefish exhibited characteristics of an unexploited population containing a diverse length and age distribution, indicative of a fully recovered population (Herbst et al., 2011).

Since the closure of the lake whitefish fishery by 1918, Lake Champlain has experienced significant changes which may have influenced lake whitefish populations and genetic structure. Deforestation, shoreline development, and agricultural runoff have led to

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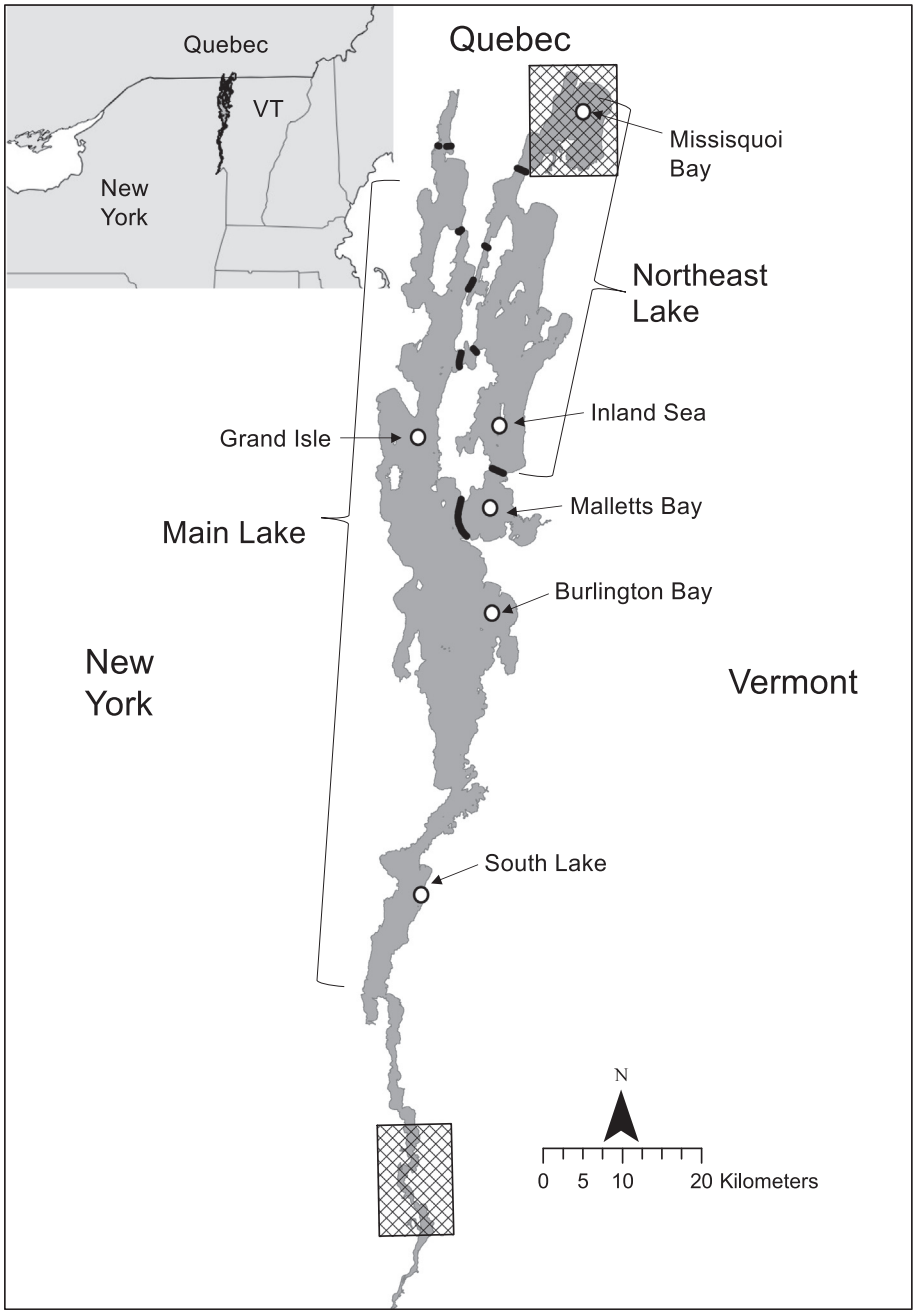

Fig. 1. Locations of lake whitefish samples (open circles), approximate locations of historic major fishing grounds (hashed boxes) and causeways (black lines). Major basins discussed in text are denoted using brackets.

high sedimentation and eutrophication of Missisquoi Bay, which is believed to have been one of the largest lake whitefish spawning areas in Lake Champlain (Fig. 1; Marsden & Langdon, 2012). As of 2017, 50 exotic species had colonized Lake Champlain, including alewife (*Alosa pseudoharengus*) which may prey upon larval lake whitefish (Marsden and Hauser, 2009). Additionally, when commercial fishing was at its highest in the late 1800 s and early 1900 s, nine causeways were built connecting the northern islands of Lake Champlain to each other and the mainland. While there is some unpublished evidence that at least a few fish are able to pass through the shallow openings in the causeways that allow boat traffic and water exchange between basins, the barriers have been hypothesized to restrict fish movement throughout the lake (Marsden and Langdon, 2012). Two of the major potential consequences of habitat fragmentation are loss of genetic diversity and population sub-structuring and both effects are amplified in a small or impaired population (Templeton et al., 1990). If commercial fishing and fragmentation by causeways reduced the population size and dispersal of lake whitefish in Lake Champlain, then the genetic diversity and population structuring of this species may have been altered. We hypothesized that the construction of causeways while lake whitefish populations were likely at their lowest may have had a long-term effect on the populations structure of lake whitefish that is detectable 165 years later.

At its peak in the early 1900 s, commercial harvest in Lake Champlain was removing 24,000–40,000 kg of lake whitefish annually from Missisquoi Bay, and unreported amounts from other parts of the lake (Marsden and Langdon, 2012). However, because

much of the fishery harvest in Lake Champlain was underreported, and the records that exist are mostly not digitized, estimation of lake-wide fishing pressure is difficult. The fishery occurred primarily in fall and used beach seines to harvest fish as they aggregated to spawn; harvested species were supported entirely by natural recruitment without any supplementation through stocking. Lake whitefish were being targeted during the same period in the Laurentian Great Lakes; however, commercial harvest there was not closed and stocks of lake whitefish began to decline severely by the early 1960 s (Allan et al., 2005; Mandrak et al., 2017). Therefore, if the magnitude of harvest in Lake Champlain was similar to the magnitude of harvest in the Great Lakes, then by the time commercial fisheries in Lake Champlain were completely closed in 1918 the lake whitefish populations may have already been substantially reduced. By combining historic records of lake whitefish harvest in the Great Lakes and Lake Champlain with population genetic data, we aimed to determine whether commercial harvest had a substantial genetic impact on lake whitefish populations in Lake Champlain. We hypothesized that if commercial fishing and causeways had a significant role in shaping the genetic structure of lake whitefish, then (1) the level of commercial harvest in Lake Champlain would need to have been similar to the level of harvest in the Great Lakes, (2) current genetic diversity of lake whitefish in Lake Champlain would be low, indicating the presence of a bottleneck and (3) lake whitefish populations would be genetically differentiated in basins isolated by causeways.

Methods

Lake Champlain

Lake Champlain extends 193 km from just north of the USA-Canada border in Quebec, Canada along the New York and Vermont, USA borders. The lake is narrow (20 km at the widest point), with an average depth of 19.5 m and a maximum depth of 122 m. Three large islands naturally divide the northern portion of Lake Champlain into eastern and western arms (Fig. 1). Six causeways built between 1850 and 1900 linked the islands to the mainland and fragmented the lake by reducing the width of open-water passages between the Main Lake, Malletts Bay, and the Inland Sea (Fig. 1; Marsden & Langdon, 2012). An additional causeway built in 1938 isolated Missisquoi Bay from the Inland Sea. The causeways, which are built out of stone, all have at least one opening (1–7 m deep) that allows some flow of water and passage of boats and fish, but the causeways have reduced the cumulative width of open-water passages among basins by 93%, from 11.9 km to 0.8 km. The width of the openings vary from 19 to 225 m, but most are<100 m wide (median = 69 m).

Commercial harvest data

Records of commercial fisheries harvest in Lake Champlain were compiled from Vermont Fish Commission reports. Attempts to obtain commercial harvest records for Quebec were unsuccessful. Because inconsistent catch units were used throughout the reports, catches recorded as "barrels of fish", "boxes of fish", and "number of fish" were converted to "lbs of fish", using estimated conversion factors (1 box = 100 lbs; 1 barrel = 200 lbs; 1 fish = 3.5 lbs). Conversion factors were initially estimated using Vermont Fish Commission reports that gave numbers of fish in barrels and boxes and corroborated with similar studies using historic data (Hall et al. 2012). Annual lakewide commercial harvest was then estimated as the total amount of whitefish harvested in each year in pounds, to compare with Great Lakes data that were also reported in imperial units.

Harvest was closed in Lake Champlain due to concerns about overfishing; however, it is unclear what evidence was used to make this decision, and it is difficult to determine the relative severity of harvest in Lake Champlain without some comparisons to other systems. To provide a context for fishing pressure, we used harvest data compiled by Baldwin et al. (2018) to estimate the amount of harvest in the Great Lakes during the same years that lake whitefish were harvested in Lake Champlain. In both Lake Champlain and the Great Lakes, lake whitefish are entirely demersal and have similar diets, habitat, and life history. Data were downloaded (http://www.glfc.org/databases/commercial/commerc.php) for all five Great Lakes and Lake St. Clair and filtered by species to include only lake whitefish. Data were also filtered by date to include only years between 1890 and 1963, spanning the period when data are available for Lake Champlain and all available data leading up to the population crash of lake whitefish throughout the Great Lakes (Ebener et al., 2008). Annual lake-wide harvest was estimated for each lake as the total weight of lake whitefish harvested in a year divided by the surface area of the lake. While surface area is not directly proportional to the available harvest area of a lake, this simplistic conversion allows for comparison with the Great Lakes where more harvest data are available. Harvest for the years where records were available in Lake Champlain (1895–1912) was then compared among lakes in R using a 1-way Analysis of Variance (ANOVA) with lake as the principal factor and standardized harvest as the response variable. Significant differences were identified using Tukey's honestly significant difference tests and plotted using ggplot2 (Tukey, 1949; Wickham, 2009). While our analysis of harvest data was crude, our objective was to estimate the approximate magnitude of harvest in Lake Champlain, not to conduct an exhaustive comparison of lake whitefish harvests.

Sample collection and microsatellite analysis

To evaluate contemporary genetic diversity and structure of lake whitefish in Lake Champlain, we obtained samples from the Northeast Lake (Missisquoi Bay and the Inland Sea), the Main Lake (Burlington Bay, Grand Isle) and the South Lake (Fig. 1). Adult lake whitefish were collected from the Inland Sea in 2008 using overnight sets of 1.8 m deep, 70.6–152.4 m long multi-panel gillnets with 7.6, 8.9, 10.2, 11.4, 12.7, 14, and 15.2-cm stretch mesh panels (Herbst et al., 2011). Adult lake whitefish from the Main Lake were collected as bycatch during bottom trawl surveys for lake trout during spring, 2016. Because lake whitefish in the Main Lake were captured eight years after samples in the Inland Sea, an additional 11 lake whitefish were collected in the Inland Sea during 2015 bottom trawls to compare to 2008 samples to evaluate temporal variation. Fish were frozen on board the boat, then later thawed in the laboratory for measurement; tissue samples or fin clips were either preserved in 95% ethanol or dried according to LaHood et al. (2008) for DNA extraction. Tissue samples of lake whitefish from Missisquoi Bay were provided by Dr. Louis Bernatchez, Laval University, Quebec (Lu et al., 2001).

Samples of muscle tissue (Inland Sea) were frozen in liquid nitrogen and reduced to a powder using a mortar and pestle before extraction; dried fin clips (Main Lake) were added directly to extraction tubes. DNA was extracted using Puregene Quiagen extraction kit guidelines. After extraction, DNA samples collected from the Inland Sea in 2008 were checked for degradation during storage using gel electrophoresis while samples collected in 2015 and 2016 were only checked for DNA concentration using a NanoDrop DNA analyzer. Samples were genotyped using polymerase chain reaction (PCR) at eight microsatellite loci previously identified for lake whitefish (BFW1, BFW2, Cocl-lav 28 (C28), Cocl-lav 45 (C45), Cocl-lav 68 (C68), Cocl-lav 6 (C6), Cocl-lav 4 (C4),

Table 1
Characteristics of the 8 microsatellite loci amplified in lake whitefish, with the marker name, forward and reverse primer sequence, fluorophore tail, amplified size range, annealing temperature (Ta) and citation for the source of the marker.

Marker	Primer (5′–3′)	Florophore	Size range	Ta	Source
BFW1	F: GATCAGAGAAATACACACAACGCATCAA R: CACGAGTCATTACCTTGGAGAC	FAM	198–226	60–55	Lu et al. (2001)
BFW2	F: GGGATACATCGGCAACCTCTG R: AAAAGAGTAACCCCTGACAGA	FAM	145–165	60–55	Lu et al. (2001)
CL23	F: GCTGTATGAGGATAGCATTC R: TGTGTTTTGCTGGATTACG	FAM	250–284	60–55	Lu et al. (2001)
C6	F: GCCATCATCCTCCCAGGAAAC R: CAGGGAATCTGCACTGGAGC	VIC	135–151	60–55	Rogers et al. (2004)
C28	F: ACAATAGCAGGCCATTCAGG R: CCAATCTTCAAAGCCATTTCA	VIC	171–185	62.5–59	Rogers et al. (2004)
C45	F: GAGTGACAGCAGGGAGCAG R: GGCTCGGTTGAAAGTTGAGA	VIC	237–255	62.5–59	Rogers et al. (2004)
C68	F: GTGTGTTACAAGTGGCTATG R: GTGATGGCTTTCAGAGGC	PET	173–179	62.5–59	Rogers et al. (2004)
C4	F: TGGTGTAATGGCTTTTCCTG R: GGGAGCAACATTGGACTCTC	VIC	133–152	62.5–59	Rogers et al. (2004)

Cocl-lav 23 (C23); Table 1) in 25 μl reactions containing primer-specific concentrations of forward and reverse primers (Lu et al., 2001; Patton et al., 1997; Rogers et al., 2004). Loci were amplified using a touchdown-based approach whereby the melting temperature (94 °C) and elongation temperature (72 °C) stayed the same for each cycle, but annealing temperature was lowered by 0.5 or 1.0 °C every 5 PCR cycles. All loci were amplified using one of two general programs: amplification of loci BFW1, BFW2, C23, and C6, PCR was initiated with a denaturing step of 94 °C for 3 min followed by 33 cycles of 30 s at 94 °C, 30 s at an annealing temperature which started at 60 °C and decreased by one degree every five cycles, and ended with 30 s at 72 °C (Table 1). The final annealing temperature (55 °C) was run for 8 cycles and followed by a final elongation at 72 °C for seven minutes. The process for loci C28, C45, C68, and C4, PCR was almost identical except the initial denaturing step was shortened to 30 s and annealing temperature was decreased by 0.5 °C every 5 cycles from 62.5 to 59.0 °C. Fragment analysis of PCR products was conducted in the University of Vermont Advanced Genome Technologies Core using an Applied Biosystems 3130 Genetic Analyzer and a LIZ 500 size standard and scored using GENEMAPPER software (Applied Biosystems).

Genetic diversity

All loci were assessed for the presence of null alleles with MICRO-CHECKER version 2.2.3 (Van Oosterhout et al., 2004). Conformance to Hardy-Weinberg equilibrium (HWE) expectations at each locus, observed (H_O) and expected (H_E) heterozygosity, F_{IS} and allelic richness were estimated using the basicStats function of the diveRsity package in R version 3.3.3 for each sampled site and then for all sites pooled to represent the total lake (Keenan et al., 2013; R Core Team, 2015). HWE was calculated using exact tests and allelic richness was calculated using rarefaction and scaled to the smallest sample size (21 individuals). Very few individuals (<6) were collected from the South Lake; and therefore, these individuals were excluded from allelic richness analysis. Any deviations from HWE following Bonferroni correction for multiple comparisons were assessed for heterozygote excess or deficiency using the diveRsity package for R. Private alleles were identified using GenAlEx (Peakall and Smouse, 2012, 2006). Linkage disequilibrium among loci in each population was tested using the log-likelihood ratio statistic conducted in GENEPOP using default settings (Raymond and Rousset, 1995; Rousset, 2008). The power of our current sample size and marker set to detect differentiation was evaluated using POWSIM (Ryman and Palm, 2006). Contemporary effective population size (*Ne*) was first calculated for each sampled location and then for the total lake using both linkage disequilibrium and heterozygote excess methods in N_eESTIMATOR (Do et al., 2014) with minimum acceptable allele frequency of 0.02.

Temporal stability of genetic diversity

Because samples were collected eight years apart, any genetic differentiation observed between lake whitefish from the Inland Sea and Main Lake could be the result of slight changes in population-wide allele frequency over the eight years between sampling. Lake whitefish reach maturity around age five and live more than twenty years; therefore, the eight-year gap in sampling is less than a single generation and so was not predicted to have an impact on observed genetic structure. However, to evaluate the amount of genetic differentiation than can be attributed to time between sampling events, three estimates of genetic differentiation were calculated. First, we conducted an analysis of molecular variance (AMOVA) to measure the amount of variation between samples of lake whitefish captured in the Inland Sea in 2008 and in 2015. The AMOVA was conducted using a permutation test GenAlEx with 999 permutations. We further accounted for temporal differences by calculating values of pairwise genetic distance (F_{ST} and G'_{ST}) between 2008 and 2015 samples from the Inland Sea calculated in the diversity R package. While G'_{ST} can bias genetic distance estimates making them appear higher than in reality, any values of G'_{ST} were always reported in conjunction with estimates of F_{ST} which can be less upwardly biased (Whitlock, 2011). If 95% confidence intervals around pairwise distance estimate included zero, the difference was considered to be negligible and non-significant.

Bottleneck analysis

Evidence of a bottleneck within the last 100 years was assessed using BOTTLENECK (Luikart and Cornuet, 1999) and m-ratio tests conducted using strataG and Critical_M (Archer et al. 2017; Garza and Williamson 2001) on the pooled dataset of 149 lake whitefish. BOTTLENECK evaluates the presence of recent reductions in effective population size by comparing observed heterozygosity to simulated theoretical expected heterozygosity at population equilibrium. Because low-frequency alleles are lost during bottlenecks faster than heterozygosity is reduced, excess heterozygosity indicates a recent loss of genetic diversity. Tests in BOTTLENECK were performed using both a stepwise mutation model (SMM) and the two-phase model of mutation (TPM) which has been shown to be more suitable for microsatellite loci. Significance of heterozygosity

excess following 1000 iterations of the model was determined using one-sided Wilcoxon's signed-rank tests. The variance of TPM was set to 30 and proportion of SMM in TPM was set to 70% (Cornuet and Luikart, 1997). The m-ratio test estimates evidence for a bottleneck by comparing the ratio of the number of microsatellite alleles to the range in allele size. Because exact effective population size and mutation rate in our system are unknown, we compared m-ratio tests across a range of critical values (Mc) to assess significance. M−ratios were estimated and averaged for microsatellite loci using strataG and compared to Mc values estimated in Critical_M using effective population sizes ranging from 500 to 10,000 and default additional settings.

To create a null model of diversity loss due to overfishing in Lake Champlain, we simulated the loss of genetic diversity associated with different overharvest scenarios and effective population sizes in the program BOTTLESIM (Kuo and Janzen, 2003). BOTTLESIM is designed to simulate genetic bottlenecks in populations with overlapping generations based on prior allele frequency data to estimate the expected reductions of genetic diversity following a bottleneck event. We based our simulations on historic knowledge of commercial harvest in Lake Champlain and current allele frequencies for the lake, and the assumption that harvest was on a single lake-wide population (i.e., no habitat fragmentation). Effective population size was set to either 10,000 or 2000 individuals. While the exact effective population size during commercial harvest is unknown, we chose values that were 1–2 orders of magnitude higher than we estimated (see results), and therefore should represent a conservative estimate of the effect harvest could have had on genetic diversity. The percent reduction of effective population size was set to 50, 75 or 90% to simulate various over-fishing scenarios. All simulations were run for 1000 iterations using random mating, with overlapping generations of 80% that assumes fish reproduce from the age of maturity at 5 to a maximum age of 25. To simulate the history of fishing in Lake Champlain as closely as possible, all simulations were run for 130 years, starting with 10 years of maximum Ne (10,000 or 2000) followed by 120 years of a 50, 75, or 90% reduction in effective population size representing the time-period of highest reported harvest in the late 1800 s and early 1900 s to the present day. While the census population size of lake whitefish likely returned to near pre-harvest levels after harvest was stopped, effective population size would not have returned to pre-harvest levels nearly as quickly without significant migration or mutation. BOTTLESIM assumes closed populations and no mutation; and therefore, we assumed that the reduction in effective population size would still be present today. Both assumptions are reasonable given the low likelihood of migration between other systems and Lake Champlain and the relatively short time period over which simulations were run.

Genetic structure

Possible genetic structure among sample sites was evaluated using pairwise comparisons of F_{ST} and G'_{ST}, and 95% bootstrapped confidence intervals calculated using the diveRsity R package. Significant difference from zero was determined using confidence intervals whereby any pairwise estimate that did not include zero was considered significant and by log-likelihood G-statistics calculated in GenoDive version 2.0b27 (Meirmans and Van Tienderen, 2004). To account for multiple comparisons, p-values were corrected with a Benjamini-Hochberg correction (Benjamini and Hochberg, 1995) calculated in R. To evaluate genetic structure without *a priori* assumptions of population structure we used the program STRUCTURE (Pritchard et al., 2000) deployed through the ParallelStructure package for R (Besnier and Glover, 2013). Each estimate of k 1–5 was run through five replicate runs of 100,000 replicates with a 10,000 cycles burn-in. The most likely value of K was determined using posterior probabilities and deltaK and ln′(K) calculated in Structure Harvester (Earl and vonHoldt, 2012; Evanno et al., 2005). Discriminate analysis of principal components (DAPC) was used as a second visualization of population clustering using *a priori* sites as groups. Population structure was visualized with bi-plots of the two most explanatory discriminant functions, and the fit of these clusters was evaluated based on the proportions of successful reassignment (Jombart, 2008; Jombart et al., 2010).

Results

Commercial harvest

Although commercial harvest of lake whitefish occurred throughout much of the 19th century, quantitative information on catches was only listed in Vermont Fish Commission reports between 1895 and 1912. During this period, over 340,000 lbs (154,221 kg) of lake whitefish were harvested from Lake Champlain. According to license sales, about 78% of harvest occurred in the northern portion of the lake, mostly in Missisquoi Bay and the northern portion of the Inland Sea. The harvest intensity reported for Lake Champlain was similar to the Great Lakes when standardized by lake surface area. While overall harvest differed among lakes ($F_{6,113} = 6.3$, $p < 0.001$), a Tukey HSD test revealed that harvest in Lake Champlain was similar to harvest in lakes Ontario, Huron, Superior, Michigan, and Erie but significantly lower than Lake St. Clair between 1895 and 1912 (Fig. 2; Electronic Supplementary Materials (ESM) Tables S1).

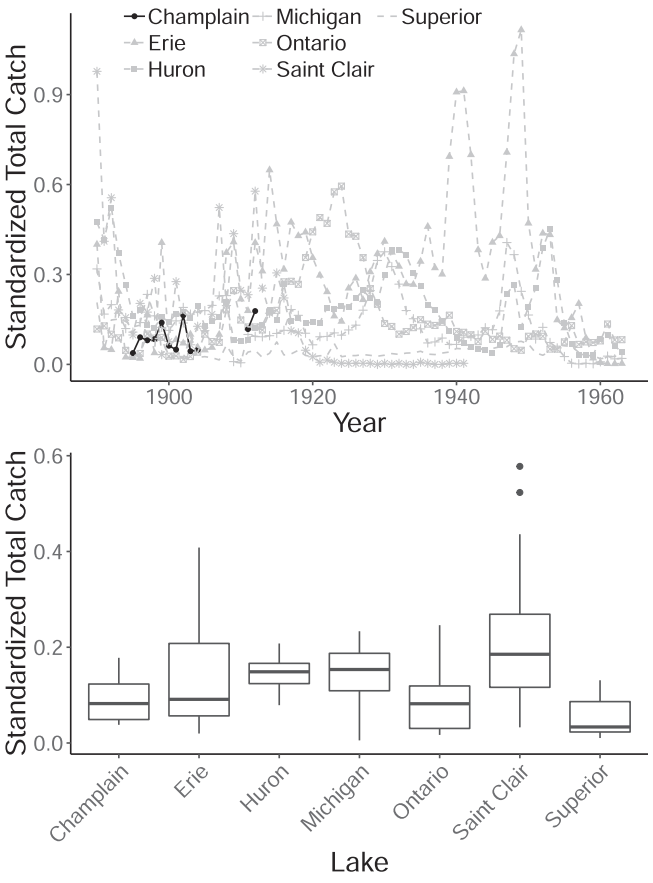

Fig. 2. Standardized total catch of lake whitefish between 1890 and a major decline in lake whitefish in the Great Lakes in 1963. Standardized total catch was calculated as the total lake-wide catch (in lbs) divided by lake surface area of the lake (in acres). Lake Champlain data were summarized from Vermont Fish Commission reports. Data from the remaining lakes were taken from Baldwin et al. (2018).

Table 2
Site-specific summary statistics of lake whitefish genotypes at eight microsatellite loci in Lake Champlain. AR = mean allelic richness across all loci based on minimum sample size of 21 individuals, efN = mean number individuals genotyped across loci, H_O = observed heterozygosity, H_E = expected heterozygosity, F_{IS} = inbreeding coefficient, HWE = P-value for Hardy-Weinberg equilibrium test, HWE_{hom} and HWE_{het} = P-values for heterozygosity deficit and excess, Ne = effective population size (lowest allele frequency used = 0.02). Grand Isle statistics are reported for all alleles, and with private alleles (PA) removed. NA = sample size too small to estimate.

Site	AR	N	efN	H_O	H_E	F_{IS}	HWE	HWE_{hom}	HWE_{het}	Ne
Burlington Bay	4.34	39	36.4	0.56	0.56	−0.032	0.02	0.93	0.15	173.6 (35.4–∞)
Grand Isle	5.09	36	27.8	0.52	0.59	0.100	0.19	0.73	0.00	47.3 (17.8–∞)
Inland Sea	4.18	43	34.1	0.58	0.58	−0.027	0.23	0.88	0.50	∞ (47.5–∞)
Miss. Bay	4.63	21	19.9	0.55	0.57	0.039	0.29	0.73	0.16	∞ (35.4–∞)
South Lake	NA	6	5.6	0.65	0.56	−0.166	1.00	0.92	0.99	NA
Whole lake combined	5.09	147	130.1	0.56	0.60	0.040	0.00	0.92	0.00	139.7 (67.7–643.9)

Population genetics

Locus C68 showed evidence of null alleles in Burlington Bay and Grand Isle, locus C6 showed evidence of a null allele at Grand Isle, and locus BFW2 showed evidence of a null allele in Missisquoi Bay. However, evidence was inconsistent across samples for both of the null alleles, and all populations other than Grand Isle were in HWE following Bonferroni corrections. Therefore, all loci were used in the following analyses. Grand Isle was the only sample site that deviated significantly from HWE (Table 2). The divergence from HWE at Grand Isle was due to heterozygosity excess resulting from a high number of private alleles (11 of the 38 genotyped individuals having private alleles at least one locus). The genotypes of all individuals with private alleles were re-analyzed and individuals GI_25 and GI_50 which had private alleles at four and five of the eight loci, respectively, were re-amplified and re-genotyped at each locus that showed private alleles. While all private alleles did not appear to be due to genotyping errors, all individuals GI_25 and GI_50 were considered outliers and removed from analysis. No loci were found to be in locus disequilibrium (log likelihood ratio statistic p-value greater than 0.05 for all comparisons). Power analysis indicated that the number of loci and sample sizes we used should be sufficient to correctly identify genetic distance (F_{ST}) greater than 0.01 more than 98% of the time and greater than 0.005 more than 78% of the time.

Inter-annual variation

Based on AMOVA results comparing 2008–2015 Inland Sea samples, 3% of variation was attributed to sampling date (p = 0.01). While the AMOVA results suggested that the amount of variation attributed to sampling was significantly greater than zero, confidence intervals of both G'_{ST} and F_{ST} included zero between 2015 and 2008; and therefore, these metrics were functionally zero, which indicates that genetic differentiation between years was negligible. Because very little variance was explained by sampling date and pairwise distance estimates were both zero, 2015 and 2008 Inland Sea samples were combined in all subsequent analyses.

Diversity and evidence of a bottleneck

Observed and expected heterozygosity ranged from 0.53 to 0.65 and 0.45 to 0.62 among sample sites and was 0.56 and 0.60 for the whole lake (Table 2). Mean allelic richness scaled to the lowest number of individuals sampled at any site (21) ranged from 4.18 to 5.95 among sample sites and was 5.09 for the whole lake. Effective population size ranged from 14.1 at Grand Isle to infinity for individual sample sites; however, jackknifed confidence intervals at all sites other than Grand Isle included infinity (Table 2). When samples were pooled, effective population size for the whole lake was estimated to be 139.7 (95% CI = 67.7–643.9). Inbreeding coefficient, F_{IS}, was negative for four of six sites, but positive in Grand Isle (0.13), Missisquoi Bay (0.04), and the pooled lake samples (0.04).

No evidence was found of a recent bottleneck in Lake Champlain lake whitefish populations as indicated by the lack of observed heterozygosity excess compared to simulated heterozygosity for either the SSM model or the TPM model ($SSM_{Wilcoxin}$ p = 1.00; $TPM_{Wilcoxin}$ p = 0.96). M−ratio tests also indicated a mean ratio of 0.76 which was higher than all values of Mc other than estimated for effective population size of 500 (ESM Table S3). Simulations indicated that starting effective population size had a large impact on the observed loss in genetic diversity following a bottleneck. At an Ne of 10,000 individuals, loss of genetic diversity over 120 years ranged from 0.9% loss of observed alleles (OA) and 0.2% loss of H_O when populations were reduced by 50%, to a loss of 14.2% in OA and a 0.9% loss of H_O when populations were reduced by 90%. Alternatively, for a population size five times smaller (2000 individuals), loss of genetic diversity ranged from a 14.3% loss in OA and 0.9% loss of H_O for a 50% reduction in population size, to 39.8% loss of OA and a 4.0% loss of H_O for 90% reduction in population size (Fig. 3).

Population sub-structuring

Only pairwise G'_{ST} and F_{ST} estimates between the Inland Sea and the Main Lake were significantly different from zero based on 95% bootstrapped confidence intervals and log-likelihood G-statistics (Table 3). Burlington Bay lake whitefish were also significantly different from Grand Isle lake whitefish based on G-statistics, but not 95% bootstrapped confidence intervals. Posterior probabilities from Bayesian STRUCTURE analysis indicated that there was very little support for any values of k greater than 1 evaluated, indicative of a lack of genetic structure. The program STRUCTURE does not directly estimate panmixia (k = 1). However, there were no large peaks present when using second-order statistics such delta K or lnK, suggesting no value of k was particularly explanatory. Additionally, the cluster assignment of all individuals became increasingly subdivided approximately proportional to the value of k, characteristic of a single genetic cluster (supplementary materials). Analysis using DAPC indicated similarly low levels of genetic structure. Bi-plots of DAPC of lake whitefish samples supported the lack of population clustering as indicated by a high degree of overlap in DAPC bi-plots and low reassignment accuracy to sample site of 43–69% (Fig. 4).

Discussion

Reduction in population size and habitat fragmentation often results in genetic changes in populations (Hedrick 2005). In Lake Champlain, lake whitefish populations experienced both types of impacts 120 years ago and our goal was to evaluate whether these environmental changes influenced the population genetic structure of the current lake whitefish population. We found that while

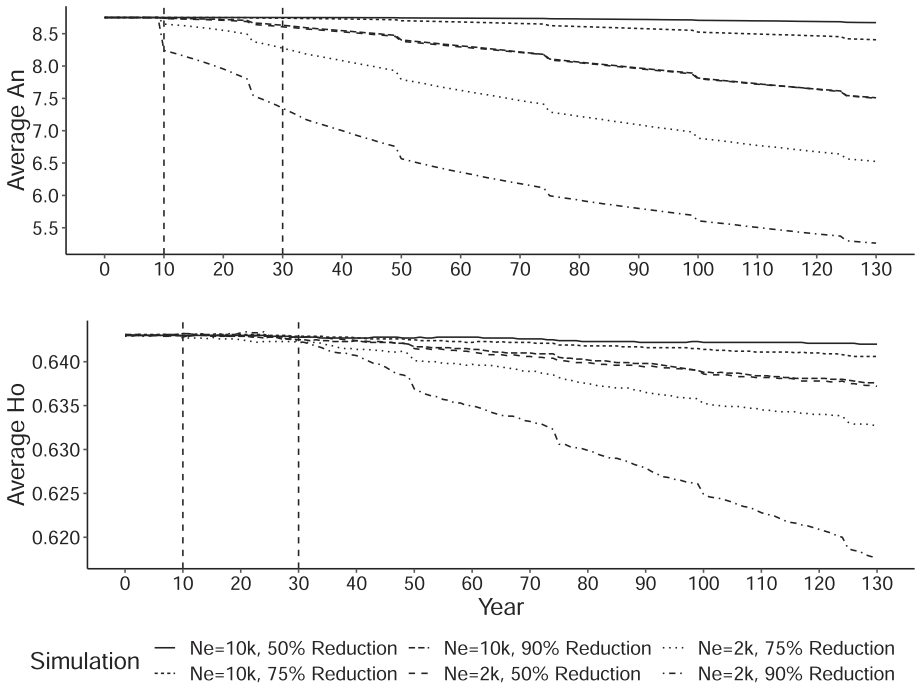

Fig. 3. Time series of simulated average number of alleles (An) and observed heterozygosity (H_O) following a reduction of effectve population size from either 10,000 or 2000 by 50%, 75% or 90% (line types). The simulated reduction in population size began after ten years (dotted line) and then population size was maintained at the reduced level for 120 years representing the time between peak lake whitefish harvest and present day.

Table 3
F_{ST} (above diagonal) and G'_{ST} (below diagonal) for all sites sampled for lake whitefish in Lake Champlain. Comparisons significantly greater than zero based on bootstrapped 95% confidence intervals are shown in bold and comparisons significantly greater than zero based on log-likelihood G-statistics are italicized.

	Burlington Bay	Grand Isle	Inland Sea	Missisquoi Bay	South Lake
Burlington Bay		*0.006*	***0.032***	0.011	0.021
Grand Isle	*0.004*		***0.020***	0.006	0.001
Inland Sea	***0.062***	***0.013***		−0.002	0.024
Missisquoi Bay	0.021	0.003	−0.003		0.017
South Lake	0.037	<0.001	0.046	0.035	

fishing pressure may have been sufficiently high to reduce the population size of lake whitefish in Lake Champlain (hypothesis 1), there was limited and contradictory evidence that lake whitefish genetic diversity has been substantially reduced (hypothesis 2). However, habitat fragmentation by causeways may have led to some genetic sub-structuring within the Lake Champlain lake whitefish population (hypothesis 3).

Based on our estimates of commercial harvest, fishing pressure in Lake Champlain was similar to the Great Lakes, where lake whitefish populations eventually crashed due to a combination of harvest and environmental changes (Ebener et al., 2008). While legal harvest of lake whitefish in Lake Champlain ended about 45 years prior to the collapse in the Great Lakes, the size structure of lake whitefish populations had already been truncated (Van Oosten and Deason (1939)). Therefore, commercial harvest in Lake Champlain was conducted at a rate now known to be unsustainable and led to demographic changes to the population. The combination of these findings suggests that fishing pressure in Lake Champlain was enough to influence the genetic diversity of lake whitefish populations.

Simulations of loss of genetic diversity following a bottleneck suggested that if harvest reduced effective population size by 50%, populations may have experienced a genetic bottleneck. Given the allele frequencies identified in our study, the observed heterozygosity in the starting population simulated by BottleSim was higher than what we observed in Lake Champlain, which could indicate that heterozygosity in whitefish is depressed in comparison to an idealized population. Further, the expected loss in allelic richness 120 years after a reduction in effective population size of 50% would be 10–20% for a starting effective population size of 2000 individuals. Therefore, the genetic diversity in the lake whitefish population observed today may be lower than it was 120 years ago as a result of harvest. However, without accurate historical data of effective population size it is difficult to infer just how much genetic diversity may have been lost. The lower confidence intervals of estimates of present day effective population size were low (17–67 individuals) so the effective population size of whitefish 100 years ago may not have been very large, but because the upper confidence intervals of all estimates included infinity, except when all samples were combined, the precision of estimates is low, and therefore should be interpreted with caution. To account for this lack of precision, we chose to run our simulations using an effective population size 3–15 times larger than we estimated for the whole lake population (∼100 to 700 individuals). Therefore our simulations should represent a conservative estimate of the potential reduction in genetic diversity associated with fishing in Lake Champlain so we could determine whether harvest could feasibly lead to a reduction in genetic diversity, especially in inland

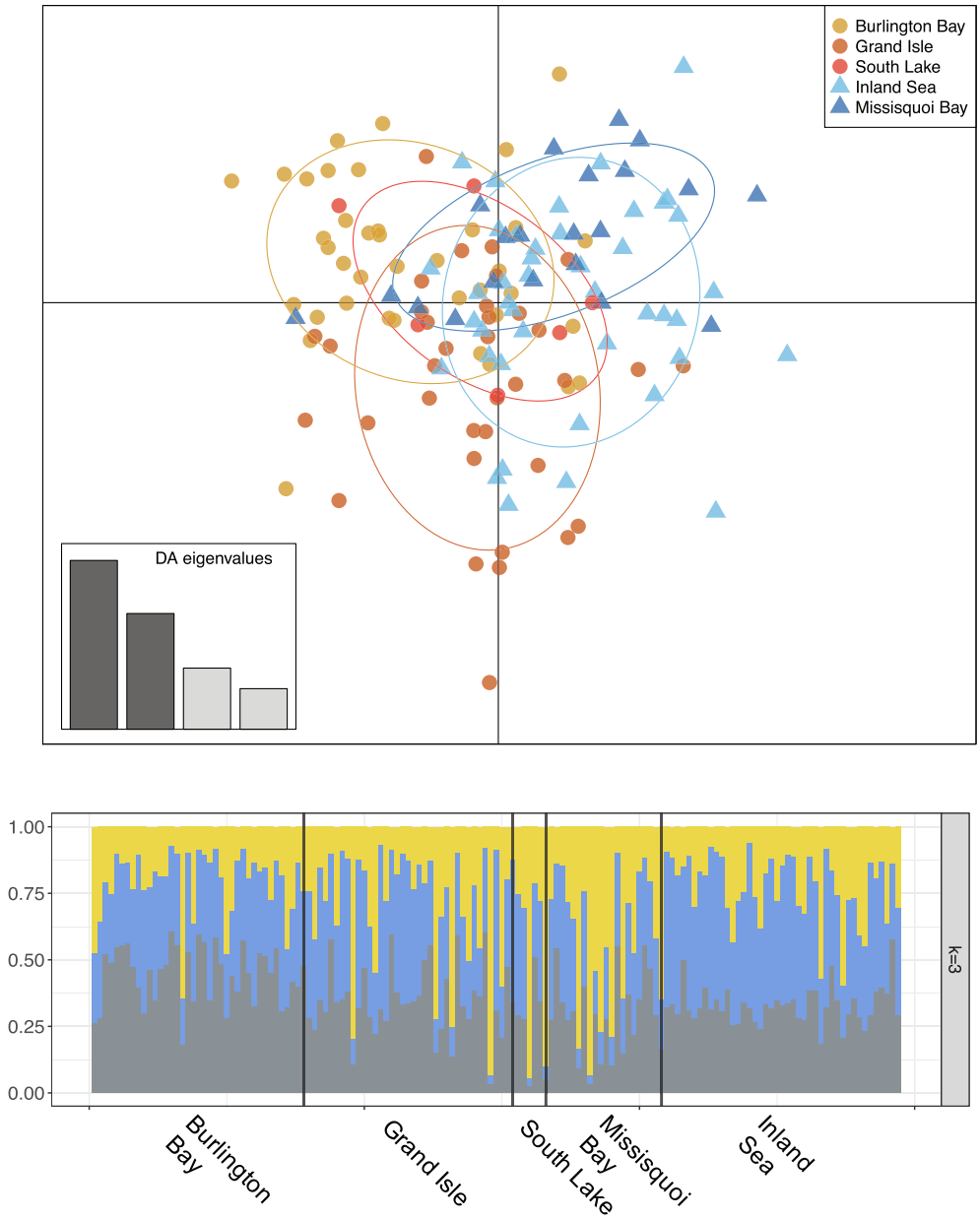

Fig. 4. Genetic clustering of all lake whitefish sampled in Lake Champlain using discriminant analysis of principal components (DAPC; top) and Bayesian STRUCTURE analysis with k = 3 (bottom). Each individual marker in the DAPC bi-plot represents a single genotyped individual, the color of the marker indicates the site the where the individual was sampled, and shape of the marker indicates the basin where the individual was collected (circle = Main Lake, triangle = Northeast Lake). The STRUCTURE barplot is a graphical representation of individual membership coefficient to each cluster (y-axis; vertical bars). Colors represent different estimated clusters of a single admixed individual. Vertical black bars indicate breaks between sampled populations (x-axis). Barplots for additional values of k can be found in supplemental materials. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

systems where effective population sizes are generally smaller and harvest can quickly reduce population abundance (Guinand et al., 2012).

While simulations suggest that harvest could have reduced genetic diversity, we found no evidence that populations experienced a genetic bottleneck with either heterozygosity excess methods or M−ratio tests. However, both heterozygosity excess methods or M−ratio tests often only detect severe bottlenecks of greater than 90% reduction in genetic diversity (Peery et al. 2012); therefore, it is possible that a bottleneck occurred but went undetected. Because the power of both heterozygosity excess and M−ratio tests is highest with larger sample sizes and higher numbers of loci than we were able to use here (Peery et al. 2012), so we only had statistical power to detect the presence of a very strong bottleneck. Therefore, we stress that the inability to detect a recent bottleneck does not mean genetic diversity of lake whitefish was unaffected by harvest. If lake whitefish experienced a bottleneck similar to those seen in our 50% removal simulations, the genetic impact would entail a loss of about 10–20% of diversity which would be below the detection threshold of bottleneck analyses used.

Comparisons of lake whitefish in Lake Champlain to other populations also suggest that any loss in genetic diversity due to overharvest may have been minimal. Observed heterozygosity and allelic richness of lake whitefish populations in lakes Michigan and Huron, which were also intensively fished, are similar to Lake Champlain ($H_O = 0.56$, $AR = 9.1$ Stott et al., 2010; $H_O = 0.63$, $AR = 14.5$ VanDeHey et al., 2009). While the average H_O of lake

whitefish at loci BFW1, BFW2, and C23 was higher in Lake Champlain (H_O = 0.67) than 21 out of 23 populations of lake whitefish evaluated by Lu et al. (2001). Comparisons of microsatellite genetic diverisity between studies should be done with caution (e.g., Moran et al. 2006), but the similar or higher genetic diversity of Lake Champlain compared to other exploited and unexploited populations do indicate that even if some genetic diversity was lost due to overfishing, much of the genetic diversity may remain intact.

Both G'_{ST} and F_{ST} indicated that there was modest but non-zero genetic differentiation among lake whitefish collected from the Main Lake and those collected in the Inland Sea, but not between the Main Lake and Missisquoi Bay. Additionally, the DAPC bi-plot did show some clustering by basin but reassignment accuracy to site was generally<70%. No genetic clustering was detected by STRUCTURE; however, differences among basins may be too small to reliably detect using this technique and the present number of genetic markers. DAPC generally performs as well as or better than STRUCTURE to identify clusters (Jombart et al., 2010), but STRUCTURE has difficulty identifying the correct number of clusters when F_{ST} is small (<0.02; Chen et al., 2007). Therefore, the low but positive genetic differentiation estimates between the Main Lake and the Inland Sea could indicate relatively recent geographic isolation between basins or genetic differentiation at the edge of our ability to detect with the current sample size and marker set.

The causeways that separate the Main Lake from the Inland Sea and Missisquoi Bay were not built until the 1900 s, but lake whitefish may have been partially reproductively isolated prior to causeway construction. Commercial harvest took place almost entirely in Missisquoi Bay in the Northeast Lake and southern portion of the Main Lake, presumably at large spawning aggregations. These areas are distant from one another (greater than100 km) and even prior to causeway construction would have been partially isolated due to the lake's bathymetry and islands (Fig. 1). Therefore the combination of physical isolation and modest spawning site fidelity of adult fish which are known to form genetically distinct spawning aggregation (VanDeHey et al., 2009) may have been enough to lead to genetic structure within the Lake Champlain population. The construction of causeways between spawning sites combined with stronger effects of genetic drift as a result of depressed lake whitefish spawning stock abundance due to commercial harvest would therefore likely have accelerated rates of genetic separation between Main Lake and Inland Sea fish.

Based on our results, lake whitefish in Lake Champlain appear to have similar genetic diversity as other populations of lake whitefish, including those in the Great Lakes (Lu et al., 2001; Stott et al., 2010; VanDeHey et al., 2009) and form a mostly unstructured lake-wide population indicative of either gene flow through causeway openings or insufficient time since isolation. Therefore, based on our analysis alone, we cannot conclusively suggest that harvest of lake whitefish did not result in a genetic bottleneck; but we did not find any strong evidence that a genetic bottleneck did occur. Given the wide confidence intervals around estimated effective population size, and limited number of loci used in the present study, we suggest that analysis with a more powerful dataset that includes more loci would help clarify the actual impact that harvest had on lake whitefish in Lake Champlain. Demographically (Herbst et al., 2011) and genetically (present study), the lake whitefish population appears to be in similar condition to an unexploited population with diverse age and length classes and equal or greater genetic diversity than other populations of lake whitefish.

Determining how historic events have impacted modern populations remains an important but difficult task for species conservation (Foster et al. 2006; Peery et al. 2012). Many of the major conservation issues facing populations today arose before detailed record-keeping and sample archives existed. Stressors such as fragmentation of tributaries by damming, introduction of invasive species, and overharvest or depletion of native fish populations (e.g. Parsons, 1973; Zimmerman and Krueger, 2009) are known to reduce population abundance, and result in loss of genetic diversity (Pinsky and Palumbi, 2014). However, without quality historic data much of the impact of humans on natural populations remains unquantified. Our study shows that incorporating historic demographic data into contemporary research can help contextualize results and improve our understanding of what populations looked like before major human interference.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jglr.2019.09.010.

References

Allan, J.D., Abell, R., Hogan, Z., Rrevenga, C., Taylor, B.W., Welcomme, R.L., Winemiller, K., 2005. Overfishing of inland waters. Bioscience 55, 1041. https://doi.org/10.1641/0006-3568(2005) 055[1041:OOIW]2.0.CO;2.

Archer, F.I., Adams, P.E., Schneiders, B.B., 2017. stratag: An r package for manipulating, summarizing and analyzing population genetic data. Mol. Ecol. Resour. 17, 5–11. https://doi.org/10.1111/1755-0998.12559.

Baldwin, N.A., Saalfeld, R.W., Dochoda, M.R., Buettner, H.J., Eshenroder, R.L., O'Gorman, R., 2018. Commercial Fish Production in the Great Lakes 1867-2015 [WWW Document]. Available online. http://www.glfc.org/databases/commercial/commerc.php.

Benjamini, Y., Hochberg, Y., 1995. Controlling the false discovery rate: a practical and powerful spproach to multiple hypothesis testing. J. R. Stat. Soc B. 57, 289–300.

Besnier, F., Glover, K.A., 2013. ParallelStructure: a R package to distribute parallel runs of the population genetics program STRUCTURE on multi-core computers. PLoS One 8. https://doi.org/10.1371/journal.pone.0070651.

Chen, C., Durand, E., Forbes, F., François, O., 2007. Bayesian clustering algorithms ascertaining spatial population structure: a new computer program and a comparison study. Mol. Ecol. Notes 7, 747–756. https://doi.org/10.1111/j.1471-8286.2007.01769.x.

Cornuet, J.-M., Luikart, G., 1997. Description and power analysis of two tests for detecting recent population bottlenecks from allele frequency data. Genetics 144, 2001–2014.

Do, C., Waples, R.S., Peel, D., Macbeth, G.M., Tillett, B.J., Ovenden, J.R., 2014. NeEstimator v2: re-implementation of software for the estimation of contemporary effective population size (Ne) from genetic data. Mol. Ecol. Resour. 14, 209–214. https://doi.org/10.1111/1755-0998.12157.

Dudgeon, D., Arthington, A.H., Gessner, M.O., Kawabata, Z.I., Knowler, D.J., Lévêque, C., Naiman, R.J., Prieur-Richard, A.H., Soto, D., Stiassny, M.L.J., Sullivan, C.A., 2006. Freshwater biodiversity: importance, threats, status and conservation challenges. Biol. Rev. Camb. Philos. Soc. 81, 163–182. https://doi.org/10.1017/S1464793105006950.

Earl, D.A., vonHoldt, B.M., 2012. STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. Conserv. Genet. Resour. 4, 359–361. https://doi.org/10.1007/s12686-011-9548-7.

Ebener, M.P., Kinnunen, R.E., Schneeberger, P.J., Mohr, L.C., Hoyle, J.A., Peeters, P., 2008. Management of commercial fisheries for lake whitefish in the Laurentian Great Lakes of North America. International Governance of Fisheries Ecosystems, American Fisheries Society, Bethesda, MD, pp. 99–143.

Evanno, G., Regnaut, S., Goudet, J., 2005. Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. Mol. Ecol. 14, 2611–2620. https://doi.org/10.1111/j.1365-294X.2005.02553.x.

Foster, D., Swanson, F., Aber, J., Burke, I., Brokaw, N., Tilman, D., Knapp, A., 2006. The Importance of land-use legacies to ecology and conservation. BioScience 53, 77–88. https://doi.org/10.1641/0006-3568(2003)053[0077:TIOLUL]2.0.CO;2.

Garza, J.C., Williamson, E.G., 2001. Detection of reduction in population size using data from microsatellite loci. Mol. Ecol. 10, 305–318.
Guinand, B., Page, K.S., Burnham-Curtis, M.K., Scribner, K.T., 2012. Genetic signatures of historical bottlenecks in sympatric lake trout (*Salvelinus namaycush*) morphotypes in Lake Superior. Environ. Biol. Fishes 95, 323–334. https://doi.org/10.1007/s10641-012-0005-6.
Hall, C.J., Jordaan, A., Frisk, M.G., 2012. Centuries of anadromous forage fish loss: Consequences for ecosystem connectivity and productivity. BioScience 62, 723–731.
Hedrick, P.W., 2005. Genetics of Populations. Jones and Bartlett Publishers Inc, Sudbury, MA.
Herbst, S.J., Marsden, J.E., Smith, S.J., 2011. Lake whitefish in Lake Champlain after commercial fishery closure and ecosystem changes. N. Am. J. Fish. Manage. 31, 1106–1115. https://doi.org/10.1080/02755947.2011.641068.
Hoarau, G., Boon, E., Jongma, D.N., Ferber, S., Palsson, J., Van der Veer, H.W., Rijnsdorp, A.D., Stam, W.T., Olsen, J.L., 2005. Low effective population size and evidence for inbreeding in an overexploited flatfish, plaice (*Pleuronectes platessa* L.). Proc. R. Soc. B Biol. Sci. 272, 497–503. https://doi.org/10.1098/rspb.2004.2963.
Hutchings, J.A., Reynolds, J.D., 2004. Marine fish population collapses: consequences for recovery and extinction risk. Bioscience 54, 297–309. https://doi.org/10.1641/0006-3568(2004) 054[0297:MFPCCF]2.0.CO;2.
Jombart, T., 2008. Adegenet: a R package for the multivariate analysis of genetic markers. Bioinformatics 24, 1403–1405. https://doi.org/10.1093/bioinformatics/btn129.
Jombart, T., Devillard, S., Balloux, F., 2010. Discriminant analysis of principal components: a new method for the analysis of genetically structured populations. BMC Genet. 11, 94. https://doi.org/10.1186/1471-2156-11-94.
Keenan, K., McGinnity, P., Cross, T.F., Crozier, W.W., Prodöhl, P.A., 2013. diveRsity: an R package for the estimation and exploration of population genetics parameters and their associated errors. Methods Ecol. Evol. 4, 782–788. https://doi.org/10.1111/2041-210X.12067.
Kuo, C.H., Janzen, F.J., 2003. BOTTLESIM: a bottleneck simulation program for long-lived species with overlapping generations. Mol. Ecol. Notes 3, 669–673. https://doi.org/10.1046/j.1471-8286.2003.00532.x.
LaHood, E.S., Miller, J.J., Apland, C., Ford, M.J., 2008. A rapid, ethanol-free fish tissue collection method for molecular genetic analyses. Trans. Am. Fish. Soc. 137, 1104–1107. https://doi.org/10.1577/t07-181.1.
Lu, G., Basley, D.J., Bernatchez, L., 2001. Contrasting patterns of mitochondrial DNA and microsatellite introgressive hybridization between lineages of lake whitefish (*Coregonus clupeaformis*); relevance for speciation. Mol. Ecol. 10, 965–985. https://doi.org/10.1046/j.1365-294X.2001.01252.x.
Luikart, P.S., Cornuet, J.M., 1999. BOTTLENECK: a computer program for detecting recent reduction in the effective population size using allele frequency data. J. Hered. 90, 502–503.
Mandrak, N., Bunnell, D., Muir, A., 2017. Ciscos (Coregonus subgenus Leucichthys) of the Laurentian Great Lakes and Lake Nipigon Canada [WWW Document]. Gt. Lakes Fish. Comm. http://www.glfc.org/pubs/misc/Ciscoes_of_the_Laurentian_Great_Lakes_and_Lake_Nipigon.pdf (accessed 3.29.18).
Marsden, J.E., Hauser, M., 2009. Exotic species in Lake Champlain. J. Great Lakes Res. 35, 250–265. https://doi.org/10.1016/j.jglr.2009.01.006.
Marsden, J.E., Langdon, R.W., 2012. The history and future of Lake Champlain's fishes and fisheries. J. Great Lakes Res. 38, 19–34. https://doi.org/10.1016/j.jglr.2011.09.007.
Meirmans, P.G., Van Tienderen, P.H., 2004. GENOTYPE and GENODIVE: two programs for the analysis of genetic diversity of asexual organisms. Mol. Ecol. Notes 4, 792–794. https://doi.org/10.1111/j.1471-8286.2004.00770.x.
Moran, P., Teel, D.J., Lahood, E.S., Drake, J., Kalinowski, S., 2006. Standardizing multi-laboratory microsatellite data in Pacific salmon: an historical view of the future. Ecol. Freshw. Fish 15, 597–605. https://doi.org/10.1111/j.1600-0633.2006.00201.x.
Parsons, J.W., 1973. History of salmon in the Great Lakes, 1850-1970, Technical Paper. U.S. Bureau of Sport Fisheries and Wildlife.
Patton, J.C., Gallaway, B.J., Fechhelm, R.G., Cronin, M.A., 1997. Genetic variation of microsatellite and mitochondrial DNA markers in broad whitefish (*Coregonus nasus*) in the Colville and Sagavanirktok rivers in northern Alaska. Can. J. Fish. Aquat. Sci. 54, 1548–1556. https://doi.org/10.1139/cjfas-54-7-1548.
Peakall, R., Smouse, P.E., 2012. GENALEX 6.5: genetic analysis in Excel. Population genetic software for teaching and research-an update. Bioinformatics 28, 2537–2539. https://doi.org/10.1093/bioinformatics/bts460.
Peakall, R., Smouse, P.E., 2006. GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. Mol. Ecol. Notes 6, 288–295. https://doi.org/10.1111/j.1471-8286.2005.01155.x.
Peery, M.Z., Kirby, R., Reid, B.N., Stoelting, R., Jonathan, N., Robinson, S., VáSquez-Carrillo, C., Pauli, J.N., Palsbøll, Per J., 2012. Reliability of genetic bottleneck tests for detecting recent population declines. Mol. Ecol. 21, 3403–3418. https://doi.org/10.1111/j.1365-294X.2012.05635.x.
Pinsky, M.L., Palumbi, S.R., 2014. Meta-analysis reveals lower genetic diversity in overfished populations. Mol. Ecol. 23, 29–39. https://doi.org/10.1111/mec.12509.
Pritchard, J.K., Stephens, M., Donnelly, P., 2000. Inference of population structure using multilocus genotype data. Genetics 155, 945–959. https://doi.org/10.1016/0379-0738(94)90222-4.
Raymond, M., Rousset, F., 1995. GENEPOP (Version 1.2): population genetics software for exact tests and ecumenicism. J. Hered. 86, 248–249.
Rogers, S.M., Marchand, M.-H., Bernatchez, L., 2004. Isolation, characterization and cross-salmonid amplification of 31 microsatellite loci in the lake whitefish (*Coregonus clupeaformis*, Mitchill). Mol. Ecol. Notes 4, 89–92. https://doi.org/10.1046/j.1471-8286.2003.00578.x.
Rousset, F., 2008. GENEPOP'007: a complete re-implementation of the GENEPOP software for Windows and Linux. Mol. Ecol. Resour. 8 (1), 103–106.
Ryman, N., Palm, S., 2006. POWSIM: a computer program for assessing statistical power when testing for genetic differentiation. Mol. Ecol. Notes 6, 600–602. https://doi.org/10.1111/j.1365-294X.2006.01378.x.
Stott, W., VanDeHey, J.A., Sloss, B.L., 2010. Genetic diversity of lake whitefish in lakes Michigan and Huron; sampling, standardization, and research priorities. J. Great Lakes Res. 36, 59–65. https://doi.org/10.1016/j.jglr.2010.01.004.
R Core Team, 2015. R: a language and environment for statistical computing.
Templeton, A.R., Shaw, K., Routman, E., Davis, S.K., 1990. The genetic consequences of habitat fragmentation. Ann. Missouri Bot. Gard. 77, 13. https://doi.org/10.2307/2399621.
Tukey, J.W., 1949. Comparing individual means in the analysis of variance. Biometrics 5, 99. https://doi.org/10.2307/3001913.
Van Oosten, J., Deason, H.J., 1939. The age, growth, and feeding habits of the whitefish, *Coregonus clupeaformis* (Mitchell), of Lake Champlain. Trans. Am. Fish. Soc. 68, 152–162. https://doi.org/10.1577/1548-8659(1938) 68[152:TAGAFH]2.0.CO;2.
Van Oosterhout, C., Hutchinson, W.F., Wills, D.P.M., Shipley, P., 2004. MICRO-CHECKER: software for identifying and correcting genotyping errors in microsatellite data. Mol. Ecol. Notes 4, 535–538. https://doi.org/10.1111/j.1471-8286.2004.00684.x.
VanDeHey, J.A., Sloss, B.L., Peeters, P.J., Sutton, T.M., 2009. Genetic structure of lake whitefish (*Coregonus clupeaformis*) in Lake Michigan. Can. J. Fish. Aquat. Sci. 66, 382–393. https://doi.org/10.1139/F08-213.
Whitlock, M.C., 2011. G'ST and D do not replace FST. Mol. Ecol. 20, 1083–1091. https://doi.org/10.1111/j.1365-294X.2010.04996.x.
Wickham, H., 2009. ggplot2: elegant graphics for data analysis.
Wright, S., 1931. Evolution in mendelian populations. Genetics 16, 97–159. https://doi.org/10.1007/BF02459575.
Zimmerman, M.S., Krueger, C.C., 2009. An ecosystem perspective on re-establishing Native deepwater fishes in the Laurentian Great Lakes. North Am. J. Fish. Manage. 29, 1352–1371. https://doi.org/10.1577/M08-194.1.