Journal of Great Lakes Research 46 (2020) 622–632

Contents lists available at ScienceDirect

Journal of Great Lakes Research

journal homepage: www.elsevier.com/locate/ijglr

Genetic versus demographic stock structure of rainbow smelt in a large fragmented lake

Peter T. Euclide [a,b,*], Bernie Pientka [c], J. Ellen Marsden [a,b]

[a] University of Vermont, Department of Biology, Marsh Life Science Building, 109 Carrigan Drive, Burlington, VT 05405, United States
[b] University of Vermont, Rubenstein Ecosystem Science Laboratory, 3 College St., Burlington, VT 05401, United States
[c] Agency of Natural Resources, Vermont Fish and Wildlife Department, 111 West St, Essex Junction, VT 05452, United States

ARTICLE INFO

Article history:
Received 25 September 2019
Accepted 10 February 2020
Available online 12 March 2020
Communicated by Wendylee Stott

Keywords:
Lake Champlain
Habitat fragmentation
Forage fish
Fisheries
Management
Osmerus mordax

ABSTRACT

Identification of fish stocks plays an important role in fisheries management, but stock identification often depends on the techniques used and the management goals as much as on actual population structure. Historically, stocks were identified by place of capture, population demography and morphology, but genetic stock identification has become a standard approach. Here, we evaluate the stock structure of rainbow smelt (*Osmerus mordax*) in three basins of Lake Champlain separated by causeways using genotype data from six microsatellite loci and 26 years of demographic data. No genetic differences among rainbow smelt from the different basins were evident, which suggests that gene flow occurs among basins. However, length, age, and catch-per-unit-effort of rainbow smelt suggests asynchronous population dynamics in the different basins, and thus each basin may hold populations that are at least partially isolated from one another. Consequently, we conclude that while rainbow smelt in Lake Champlain consist of at least three demographic stocks they may form only a single genetic stock. Our results concur with other studies that suggest care should be taken when only a single method of stock identification is used.

Introduction

Stock structure analysis is central to successful fisheries management (Cadrin et al., 2005). Fishing jurisdictions, harvest quotas, and stocking efforts based on stock-specific data from both harvested species and their forage help to limit the risk of overexploitation and ensure a more sustainable fishery (Bernatchez et al., 2017; Dahle et al., 2018). While many different techniques can be used to delineate stock structure, each technique has limits. Stock assessment usually falls into two categories, genetic and demographic, but the insight gained from each type of stock analysis differs (Lowe and Allendorf, 2010). While genetic assessment provides direct evidence of reproductive isolation among stocks, genetic methods often lack the sensitivity to detect ecologically meaningful differences in population connectivity (Waples and Gaggiotti, 2006). In contrast, demographic and behavioral analyses based on geometric morphometrics, demography, or tagging can provide evidence of prolonged post-larval isolation of stocks in the presence of gene flow (e.g. Begg and Waldman, 1999; Faust et al., 2019), but these types of studies are difficult or impractical to conduct in many systems. As genetic tools have become more accessible in the last few decades, molecular techniques have become a central part of stock assessment (Shaklee and Bentzen, 1998; Bernatchez et al., 2017) and now the definition of 'stock' almost always implies a discrete unit with genetic continuity among individuals (Ihssen et al., 1981; Cadrin et al., 2005). Focusing only on genetic connectivity, however, limits our understanding of demographic connectivity which can be equally as important for predicting population trends and informing year-to-year management decisions (Waples, 1998; Lowe and Allendorf, 2010).

The type of stock delineation technique used depends on the system and species in question, but integrating molecular and demographic data has been a challenge (Waples et al., 2008). Prior to development of molecular techniques, and still to a large degree, stock delineation is based primarily on where or when fish were captured and differences among stocks were identified using a combination of morphometrics, demographics, life history variation, mark-and-recapture, and, more recently, otolith microchemistry and acoustic telemetry (Begg et al., 1999; Pangle et al., 2010; Faust et al., 2018). Though molecular and phenotypic/behavioral methods are valid for stock analysis, the two methods can

* Corresponding author.
E-mail addresses: peter.euclide@uwsp.edu (P.T. Euclide), ellen.marsden@uvm.edu (J. Ellen Marsden).

https://doi.org/10.1016/j.jglr.2020.02.009
0380-1330/

contradict each other which makes their integration more difficult (e.g., Swain and Foote, 1999). In Lake Erie, otolith microchemistry and mark-and-recapture research indicate walleye (*Sander vitreus*) have a robust homing behavior; whereby upwards of 94% of individuals may return to their natal site to spawn (Zhao et al., 2011; Chen et al., 2017). Multiple genetic studies of these same Lake Erie populations, however, find almost no differences among sites, suggesting that these walleye populations are a single stock (Strange and Stepien, 2007; Stepien et al., 2009, 2012). The contradiction between methods is in part because in large populations, even a small number of migrants (less than 1% of the population) can be enough to eliminate strong signals of genetic differentiation between groups while demographic differences may be able to persist with up to 10% migration between stocks (Hastings, 1993). Therefore, taking a holistic approach that combines traditional fisheries and genetic techniques can help to evaluate how fish populations are structured.

In many systems, stocks are simply defined by geographic barriers or jurisdictional boundaries. This is the case for fishes in Lake Champlain which are physically isolated in three major basins, the Main Lake, Malletts Bay, and the Northeast Arm, that are separated by three large islands connected by six causeways built between 1850 and 1900. A fourth basin, Mississquoi Bay, was formerly separated from the Main Lake by a historic causeway. Lake Champlain is located between New York, Vermont and Quebec and has a surface area of 1127 km^2. The causeways are all located in the northern portion of the lake, are built on existing sandbars or in narrow openings between islands, and all have at least one small opening

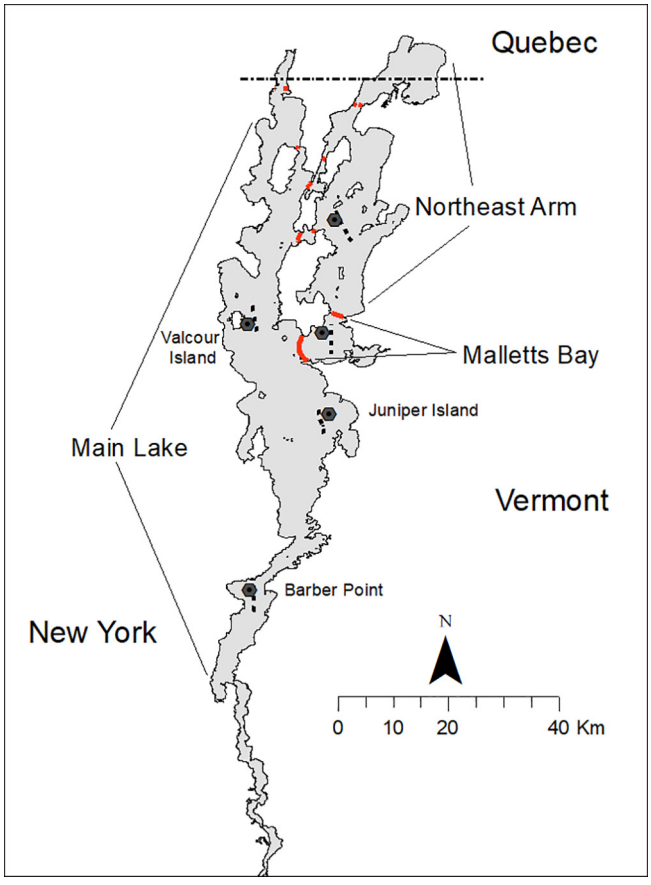

Fig. 1. Locations of genetic samples (gray dots) and forage fish survey trawling paths (dotted lines) in Lake Champlain. Red lines indicate the location of a causeway. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

for the movement of water, fish and boats between basins (Fig. 1; Marsden and Langdon, 2012). The physical fragmentation of Lake Champlain has led state agencies to focus assessment and management at the basin level, but very little research has been conducted to determine how interconnected fish populations are among basins.

There is reason to believe that causeways could isolate fish into different stocks. The three basins of Lake Champlain vary in size, trophic status, depth, and biological community (Table 1; Potash et al., 1969; LCBP, 2015). The Main Lake is oligotrohic and has a surface area of 682.5 km^2 and a maximum and mean depth of 122 m and 30.8 m, compared with the mesotrophic Northeast Arm (268.5 km^2, 49 m, 12.8 m) and oligotrophic Malletts Bay (54 km^2, 32.4 m, 13.3 m) (Myer and Gruendling, 1979). Further, while each causeway contains at least one opening, the openings are shallow (<10 m), warm (22–25 C during July and August), and narrow (19–85 m in width) forcing fish to cross through what is likely to be unfavorable habitat to pass between basins. Therefore, causeways may act as barriers, especially to coldwater fishes. Lake trout (*Salvelinus namaycush*) and walleye have been periodically observed passing through causeway openings from the Main Lake into the two smaller basins (Malletts Bay and the Northeast Arm) during the winter but appear to be absent from the openings during the summer (Pinheiro et al., 2017; Marsden unpublished data; Pientka, unpublished data). While causeways physically fragment the lake and may even limit fish movement, (Euclide et al., 2018) found that collections of slimy sculpin (*Cottus cognatus*), a deep cold-water species with low dispersal capabilities, were genetically panmictic among basins. Therefore, even if causeways restrict movement of fish in Lake Champlain, they do not necessarily restrict gene flow, or at least not enough to result in genetic divergence among basins.

Another species that is likely to be affected by lake causeways is rainbow smelt (*Osmerus mordax*). Unlike sculpin, rainbow smelt are an extremely active fish that can move long distances in large groups (Scott and Crossman, 1973). Such life history differences mean that rainbow smelt will consistently come into contact with lake causeways throughout their life, while slimy sculpin do not. Therefore, while causeways might not be a major concern for species like slimy sculpin, they may have significant influence on more mobile species, like rainbow smelt. An earlier study found that density, growth, and diet of age-0 and age-1 rainbow smelt appeared to differ among basins and suggested that the restriction of fish movements between basins by causeways has resulted in demographically distinct stocks (Stritzel Thomson et al., 2011), but this hypothesis has never formally been tested. Further, management of forage fish, including rainbow smelt, in Lake Champlain is largely based on the assumption that fish populations differ independently in each basin. Historically, annual surveys of forage fish were conducted separately in each basin by the Vermont Fish and Wildlife Department (VTFWD) under the presumption that causeways at least partially isolated these basins. Because rainbow smelt are a key forage fish species for walleye and salmonids, understanding the population dynamics of this species is important for future management actions (Marsden and Langdon, 2012). Rainbow smelt are also an important forage fish in many other lakes, but we still know very little about how their populations are structured, especially in smaller, fragmented systems outside of the Great Lakes or marine systems. A lack of stock structure makes predictions of future recruitment more difficult (Lorenzen et al., 2016) and could have lasting, system-wide consequences. Lake Champlain offers a study system that contains physically isolated populations to evaluate both genetic and demographic stock structure and improve our understanding of the life history of rainbow smelt as a species. Here we use thirty years of annual forage fish surveys conducted by the VTFWD in each basin and genetic

Table 1
Summary of basin characteristics of the three major basins in Lake Champlain evaluated in this study.

	surface area (km^2)	mean depth (m)	max depth (m)	mean phosphorous (μg/L)
Main Lake	683	19.5	122	15
Northeast Arm	269	13	48	19
Malletts Bay	54	21	30	10

data from six microsatellite loci to describe the stock structure and population dynamics of rainbow smelt in a closed lake system. We also aimed to test the hypothesis that the construction of lake causeways has led to detectable levels of genetic and demographic population structure of rainbow smelt in Lake Champlain. By combining genetic and demographic data in a single study, we are able to make inferences about the degree of isolation among basins and investigate the utility of the long-term forage fish monitoring program for identifying key differences among presumed rainbow smelt stocks.

Methods

Study species

Rainbow smelt are native to Lake Champlain and were the main pelagic planktivore until alewife (*Alosa pseudoharengus)* invaded the lake in 2004 (Marsden and Langdon, 2012). Unlike adfluvial rainbow smelt in the Great Lakes and elsewhere, rainbow smelt in Lake Champlain spawn in the open lake, though the exact location and substrate of spawning sites is unknown (Plosila, 1984; Marsden and Langdon, 2012). Rainbow smelt larvae are planktonic and can be found in the water column throughout the summer (Tin and Jude, 1983). Young-of-year (YOY) remain in warm water (10–20 °C) near or above the thermocline, while age-1 and older individuals are found in cool (<10–12 °C), deep water (Simonin et al., 2012).

Fish sampling (genetics)

Rainbow smelt for genetic analyses were sampled from Malletts Bay, the Northeast Arm, and two sites in the Main Lake (Barber Point and Valcour Island) of Lake Champlain by the VTFWD on the R/V *Doré* during the annual forage fish survey in 2012 using a 5 m × 5 m midwater-trawl with 12.7 mm mesh (Fig. 1). Additional samples for genetic analysis were collected from Juniper Island in the Main Lake during bottom trawls on the University of Vermont R/V *Melosira* during June 2015. Individuals were euthanized by cooling directly on ice, measured to the nearest mm (total length), and caudal fin clips were collected following protocols outlined in LaHood et al. (2008) or taken from whole frozen fish. Samples were collected during summer after rainbow smelt spawning finished in early spring. While spawning site fidelity has not been documented in Lake Champlain, this strategy ensured that fish sampled in different basins were representative of the whole basin and differed due to physical isolation, not spawning site fidelity.

Genetic analysis

DNA was extracted from 167 rainbow smelt fin clips using standard procedures from a DNeasy Blood and Tissue Kit (Qiagen). DNA concentration was measured on a NanoDrop and ranged from 6 to 100 ng/μl, though most samples contained between 30 and 50 ng/μl. Samples with more than 50 ng/μl of DNA were diluted with molecular Biology Grade Water (Mediatech, inc) to 50 ng/μl. Following extraction, polymerase chain reaction (PCR) amplification was conducted for eight previously identified microsatellite loci (Table 1). Markers were multiplexed when possible in 25 or 12.5 μl reactions. Loci *Osmo12*, *Osmo16*, *Osmo45*, and *Osmo157* (Saint-Laurent et al., 2003) were amplified using 2X Q5 High Fidelity DNA Polymerase Master Mix (New England BioLabs Inc.), 20 pmol of a fluorescently labeled forward primer and unlabeled reverse primer, and 5–50 ng of the DNA template. The general PCR program used for these loci was 98 °C for 2 min, 30 cycles at 98 °C for 30 s at marker-specific annealing temperature (Electronic Supplementary Material (ESM) Table S1), 72 °C for 45 s, followed by a final extension of 72 °C for 10 min. Loci *Omo1*, *Omo3*, *Omo5*, and *Omo11* (Coulson et al., 2006) were amplified using 2X *Taq* Master Mix (New England BioLabs Inc.), and 20 pmol of a fluorescently labeled forward primer and unlabeled reverse primer, and 5–50 ng of the DNA template. The general PCR program used for these loci was 95 °C for 2 min, 30 cycles at 95 °C for 30 s, 20 s at marker-specific annealing temperature, 68 °C for 30 s, followed by a final extension of 68 °C for 10 min. 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 ROX 500 size standard and scored using GENEMAPPER software (Applied Biosystems). A subset of individuals from each plate 96 sample plate was re-genotyped and scored at all markers to check for scoring errors. Additionally, any genotype that was difficult to score and could not be called identified with confidence (e.g. low peak height, high degree of stutter) was re-run; and, if the genotype could still not be scored confidently, it was left out of the dataset.

All loci were assessed for the presence of null alleles with MICRO-CHECKER version 2.2.3 (Van Oosterhout et al., 2004) and linkage disequilbrium using log likelihood ratio test and default setting GENEPOP version 4.7.3 (Raymond and Rousset, 1995). 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 (Keenan et al., 2013; R Core and Team, 2015; ESM Table S2). Any deviations from HWE following Benjamini-Hochberg false-detection rate corrections (Benjamini and Hochberg, 1995) for multiple comparisons were assessed for heterozygote excess or deficiency in the diveRsity package for R. To evaluate whether basins supported genetically distinguishable stocks of rainbow smelt, genetic distance among sample sites was measured using pairwise comparisons F_{ST}. Pairwise F_{ST} and log-likelihood tests for significance were calculated in GENODIVE (Meirmans and Van Tienderen, 2004) and corrected using Benjamini-Hochberg corrections for multiple comparisons. Because populations can be demographically independent when migration among stocks is relatively high and genetic differences are small (Waples and Gaggiotti, 2006), understanding the probability of type-2 error at small values of F_{ST} is important for the interpretation of null results. We tested for the statistical power to detect genetic differentiation for the sample sizes, number of loci and allele frequencies used in this study at five different expected levels of F_{ST} (0.001, 0.0025, 0.005, 0.01, and 0.05) using POWSIM (Ryman and Palm, 2006; Ryman et al., 2006). POWSIM simulates the sampling of genes from a specified number of populations that have diverged by drift for t number of generations. Samples from the simulated populations were then used to test for genetic homogeneity using Fisher's exact test and Chi-Square tests. Power was

then defined as the proportion of significant results obtained over multiple replicate simulations (2000 for this study). To estimate the number of genetically-distinguishable groups of rainbow smelt without *a priori* assumptions of number of populations, two different clustering models were used. First, clustering was assessed using discriminant analysis of principal components (DAPC) which is a multivariate analysis that summarizes genetic differentiation between groups while overlooking within-group variation (Jombart et al., 2010). All DAPCs were conducted in R version 3.3.0 using the ADEGENET version 2.0.1 (Jombart, 2008; R Core and Team, 2015) using sample site as the group variable. The degree of overlap was assessed visually by looking for distinct clustering in DAPC scatter plots as well as evaluating reassignment accuracy summarized by the DAPC analysis and visualized using the assignplot() function. Reassignment accuracy <50% was considered to be poor and indicative of sites having limited descriptive power. Second, the Bayesian genetic clustering algorithm, STRUCTURE, was implemented in the program ParallelStructure package in R (Pritchard et al., 2000; Besnier and Glover, 2013) for each value of K = 1–5 with settings of 900,000 replicates and an initial burn-in of 100,000 replicates. All STRUCTURE runs primarily used default settings which, in short, did not use location as a prior, used admixture models, assumed alleles were correlated within sites, and assumed no linkage disequilibrium. Replicate runs of each K were combined using CLUMPAK (Kopelman et al., 2015) and visualized in R. The optimal number of clusters was chosen using the ΔK method of Evanno et al., (2005).

Demographic analysis

Rainbow smelt were sampled annually from 1985 to 2015 in the three major main basins of Lake Champlain by the VTFWD. Sampling consisted of stepped oblique midwater trawling at night (Kirn and LaBar, 1991), between late July and early August. Each station was trawled four times with only one station sampled per night. Trawls were deployed from 35 to 10 m in 3-m steps of 5 min each. Due to variability in the early sampling protocol, only data from 1990 to 2015 were used. Because the objective of this study was to identify differences among basins that are physically isolated by causeways, and the Main Lake is much larger than either Malletts Bay or the Northeast Arm, rainbow smelt from multiple locations in the Main Lake (Barber Point, Juniper Island, Valcour Island) were combined and analyzed together generate a more representative sample of the population structure in the entire basin.

Catch-per-unit-effort (CPUE) was expressed as catch per 55 min of trawling. For each trawl replicate, all age-1 and older fish were counted and up to 200 fish were measured (total length, mm), weighed (g), and otoliths were extracted for age estimation. Whole otoliths were viewed at 30–70× magnification after clearing in 2:3 solution of glycerin and 70% ethyl alcohol (Kirn and LaBar, 1996). Age estimation of age 5+ rainbow smelt was found to be inconsistent among independent scorers in pilot studies conducted by the VTFWD, therefore, only age 1–4 smelt were used in our analysis.

Evaluation of demographic differences among basins focused on three principal metrics: age distribution, length-at-age, and CPUE. A complete time-series and catch-at-age analysis among the three basins and five sampling sites for the 30-year dataset is outside the purpose of this paper, and is being addressed separately. Spearman rank correlations were used to compare basins across years because Shapiro-Wilk Normality tests (Royston, 1995) generally showed that data were not normally distributed and because the large magnitude of differences among years could bias non-rank-based correlation methods such as a Pearson's correlation. All analyses and graphics were conducted in R version 3.3.3 and the ggplot2 package version 2.2.1 (Wickham, 2009; R Core and Team, 2015).

Variation in age distribution among basins was evaluated using Chi-Square analysis of the number in each age class summed across all years of data. Because age structure can be highly variable among years, depending on recruitment to age-1, the consistency in year class strength of age-1 fish among basins was evaluated using non-parametric Spearman rank correlations with annual mean number of age-1 fish as the response variable. We predicted that, if Lake Champlain consisted of a single demographic stock of rainbow smelt, a strong positive correlation in the proportion of age-1 fish between any two basins would be evident.

Preliminary von Bertalanffy growth analyses showed that rainbow smelt generally did not have asymptotic growth in Lake Champlain. Therefore, differences in growth among basins were evaluated using average length-at-age across all sampled years for age 1 to 4 fish and variation in length of age-1 individuals by year. To estimate differences in length-at-age for all age classes among basins, we first analyzed the entire dataset using two-way analysis of covariance with mean length of fish as the response variable, basin and year as the principal factors, and age as a covariate. Next, to evaluate trends across the time, we restricted the dataset to only age-1 fish. We compared mean length of age-1 fish among basins using a two-way analysis of variance (ANOVA) with length as the response variable, and basin and year as the principal factors. We then used post-hoc Tukey HSD tests to detect comparisons with significant differences and evaluate the consistency of length-at-age-1 differences among basins.

If rainbow smelt growth is basin-specific, we would expect no relationship in age-1 length among basins across years. However, if basins are interconnected, then yearly growth should be synchronous across years among basins. To test whether the length of age-1 fish was synchronous among basins across years, we used Spearman rank correlations to determine whether the mean length of age-1 fish for a given year could be predicted by the mean length of age-1 fish for the same year in a different basin. In addition to synchrony in growth, length could be simply related to population density. Therefore, we tested whether age-1 length was correlated with smelt density within each basin with a Spearman rank correlation.

Variation in CPUE among basins was evaluated using a 2-way ANOVA with CPUE for a given year as the response variable, and basin and year as the principal factors. To investigate which years and in how many years significant differences occurred among basins we tested for significant differences using post-hoc Tukey HSD tests. The consistency of CPUE among basins across years was evaluated using Spearman rank correlation. Because CPUE can easily be driven by one or two strong year classes, a second set of correlations using CPUE of only age-1 rainbow smelt was conducted to assess whether age-1 CPUE alone might drive differences among basins. Significance for all tests was determined using alpha = 0.05.

Results

Genetic stock structure

Prior to subsequent analyses, loci *Osmo45* and *Omo3* were removed from the data due to inconsistencies in allele scoring as a consequence of high stutter across all samples (*Osmo45*) and evidence of homozygosity excess indicating the presence of null alleles (*Omo3*) identified using MICRO-CHECKER and consultation with other experienced microsatellite scorers. Prior to their removal, a subset of samples with ambiguous genotype calls were re-run and re-scored by a second trained scorer which confirmed that high stutter was not an artefact of the original PCR and that

genotypes could not be confidently identified for either locus. The remaining six loci were generally in HWE following Benjamini-Hochberg correction (corrected p-value = 0.01). However, *Omo5* was significantly different from HWE expectations in the Northeast Arm samples but was not found to have significant heterozygote or homozygote excess and out of linkage disequilibrium with *Omo11* and *Omo1* ($p < 0.05$). Because *Omo5* was in HWE and linkage disequilibrium at all other sites, it was included in all analyses. Genetic diversity was similar across all sites and among all basins (Table 2). The remaining 6 loci were highly polymorphic containing 5 to 23 alleles (*Osom16* = 5, *Omo1* = 8, *Omo11* = 12, *Omo12* = 19, *Omo5* = 23, *Osmo157* = 23). Site-specific observed heterozygosity ranged from 0.65 in the Northeast Arm and Barber Point to 0.67 in Malletts Bay while allelic richness ranged from 8.52 in Valcour Island to 9.75 at Juniper Island.

Tests of statistical power indicated that with our current sample sizes and set of loci the probability of detecting a genetic distance between two samples of $F_{ST} = 0.005$ was 92% and the probability of detecting an F_{ST} of greater or equal to 0.01 was 100%. Estimates of pairwise F_{ST} did not indicate that sampled sites were significantly different from one another following correction for multiple comparisons, including those in different basins separated by at least one causeway (Table 3). Interpretation of both the genetic clustering algorithm, STRUCTURE, and DAPC indicated that a single, panmictic, lake-wide population of rainbow smelt was the most likely genetic stock structure in Lake Champlain (Fig. 2). STRUCTURE cannot directly estimate a single-population hypothesis; however, changes in ln likelihood for all values of K = 2–5 were small and posterior probabilities indicated that individual cluster membership was equally likely for all inferred clusters; therefore, these results provided indirect evidence that rainbow smelt formed a single, genetically homogenous population, and genetic variance could not be partitioned parsimoniously into more than a single cluster. DAPC also identified a single panmictic population as indicated by the high degree of overlap among sites when plotted and by low overall reassignment accuracy (49%; Fig. 2).

Demographic stock structure

From 1990 to 2015, age was estimated from otoliths for a total of 22,332 rainbow smelt and measured from 678 separate trawls. The age distribution of rainbow smelt was skewed heavily, such that age-1 to age-4 fish comprised 98% of all fish and the remaining 2% was composed of age 5 and older fish and some YOY which are not fully recruited to the gear. When data were combined across all available years, the age structure of rainbow smelt was dependent on sample basin ($X^2 = 169.41$; df = 6; p-value <2.2e-16). However, the effect size of the differences in age specific abundance was small (Pearson's residuals = 1–10; Fig. 3). Cohorts of age-1 rainbow smelt appeared to be in synchrony among basins since the start of the dataset in 1990. The proportion of age-1 fish was positively correlated between the Northeast Arm and the Main Lake and between the Northeast Arm and Malletts Bay ($p < 0.01$), but not between Malletts Bay and the Main Lake (Table 4).

Rainbow smelt length-at-age differed significantly among basins (Fig. 4A; Table 5). Rainbow smelt were smaller in Malletts Bay than the Northeast Arm or the Main Lake at all ages (Fig. 4A). Length-at-age of rainbow smelt in the Main Lake and Northeast Arm also differed from each other at all ages, but there was an interaction with age such that Northeast Arm rainbow smelt have a slower linear growth rate (11.2 mm/yr) compared to the Main Lake (16.4 mm/yr) or Malletts Bay (14.1 mm/yr) but a larger y-intercept (109.9 mm) than the Main Lake (99.3 mm) or Malletts Bay (90.5 mm). Differences among basins were fairly consistent for most of the 26-year dataset; however, a significant basin:year interaction was identified. Year-by-year comparisons of 9,305 age-1 rainbow smelt lengths suggested individuals from Malletts Bay were generally smaller than the other two basins in most years; length of age-1 rainbow smelt also varied significantly by year and a basin:year interaction was identified (Fig. 4B). Tukey HSD *post-hoc* comparisons indicated that age-1 rainbow smelt from Malletts Bay were significantly smaller than age-1 rainbow smelt in the Main Lake during 15 out of 26 years compared and only significantly larger in one out of 26 years. Overall, age-1 rainbow smelt in Malletts Bay were 12 mm smaller on average than Main Lake rainbow smelt. Malletts Bay rainbow smelt were significantly smaller than Northeast Arm rainbow smelt in 17 out of 26 years and larger only one of 26 years. Overall, Malletts Bay rainbow smelt were 16 mm smaller on average than Northeast Arm rainbow smelt. Age-1 rainbow smelt in the Main Lake were significantly smaller on average than Northeast Arm rainbow smelt in 8 out of 26 years and averaged 4 mm smaller than rainbow smelt in the Northeast Arm. No significant correlation in annual mean length at age-1 between basin pairs was identified (Table 4). Annual age-1 length and total CPUE in any basin was also not correlated between basin pairs ($p > 0.6$ for all).

Total CPUE (number of smelt per 55 min of trawling) differed significantly among basins (Fig. 5; Table 5) and when years were combined CPUE was significantly lower in the Main Lake (mean = 271, SD = 194) than the Northeast Arm (mean = 818, SD = 895) or Malletts Bay (mean = 815, SD = 1080). However, CPUE also varied across sample years (Fig. 5) and appeared to generally be driven by periodically high CPUE in the Northeast Arm and Malletts Bay associated with strong year classes, while CPUE in the Main Lake was much less variable. This interannual variability led to a significant interaction between year and CPUE. Tukey HSD *post-hoc* comparisons of models run with each trawl as a replicate indicated that CPUE in Malletts Bay was higher than the Main Lake in 4 of 26 years and higher than the Northeast Arm in 2 of 26 years, but smaller than the Northeast Arm in 3 of 26 years. CPUE was higher in the Northeast Arm than in the Main Lake in 6 of 26 years. Changes in CPUE across time were correlated between the Northeast Arm and Malletts Bay, but neither the CPUE in Northeast Arm or Malletts Bay were correlated with CPUE in the Main Lake (Table 4). The relationships in CPUE among basins were partially driven by strong age-1 cohorts in the Northeast Arm and Malletts Bay as indicated by the correlation between age-1 CPUE in the Northeast Arm and age-1 CPUE in Malletts Bay but lack of cor-

Table 2
Site-specific summary statistics of rainbow smelt genotypes of six microsatellite loci grouped by basin and site in Lake Champlain. N = number of individuals sampled for genotyping, *efN* = mean number individuals genotyped across loci, H_O = observed heterozygosity, H_E = expected heterozygosity, F_{IS} = inbreeding coefficient, and *AR* = mean allelic richness across all loci based on minimum sample size of 32 individuals.

	N	*efN*	*Ho*	*He*	F_{IS}	*AR*
Barber Point	33	30.83	0.65	0.64	−0.01	9.41
Juniper Island	32	29.67	0.66	0.65	0.01	9.75
Valcour	34	31.00	0.64	0.63	−0.01	8.52
Malletts Bay	32	31.17	0.67	0.66	−0.02	9.29
Northeast Arm	36	35.17	0.65	0.65	−0.01	9.14

Table 3
Pairwise F_{ST} (below diagonal) and uncorrected log-likelihood p-values (above diagonal) estimated for rainbow smelt sampled from five sites in Lake Champlain.

	Barber Point	Juniper Island	Valcour Island	Malletts Bay	Northeast Arm
Barber Point	–	0.80	0.96	0.53	0.48
Juniper Island	−0.006	–	0.90	0.62	0.29
Valcour Island	−0.007	−0.005	–	0.07	0.17
Malletts Bay	−0.001	−0.004	0.003	–	0.05
Northeast Arm	0.004	0.002	0.003	0.011	–

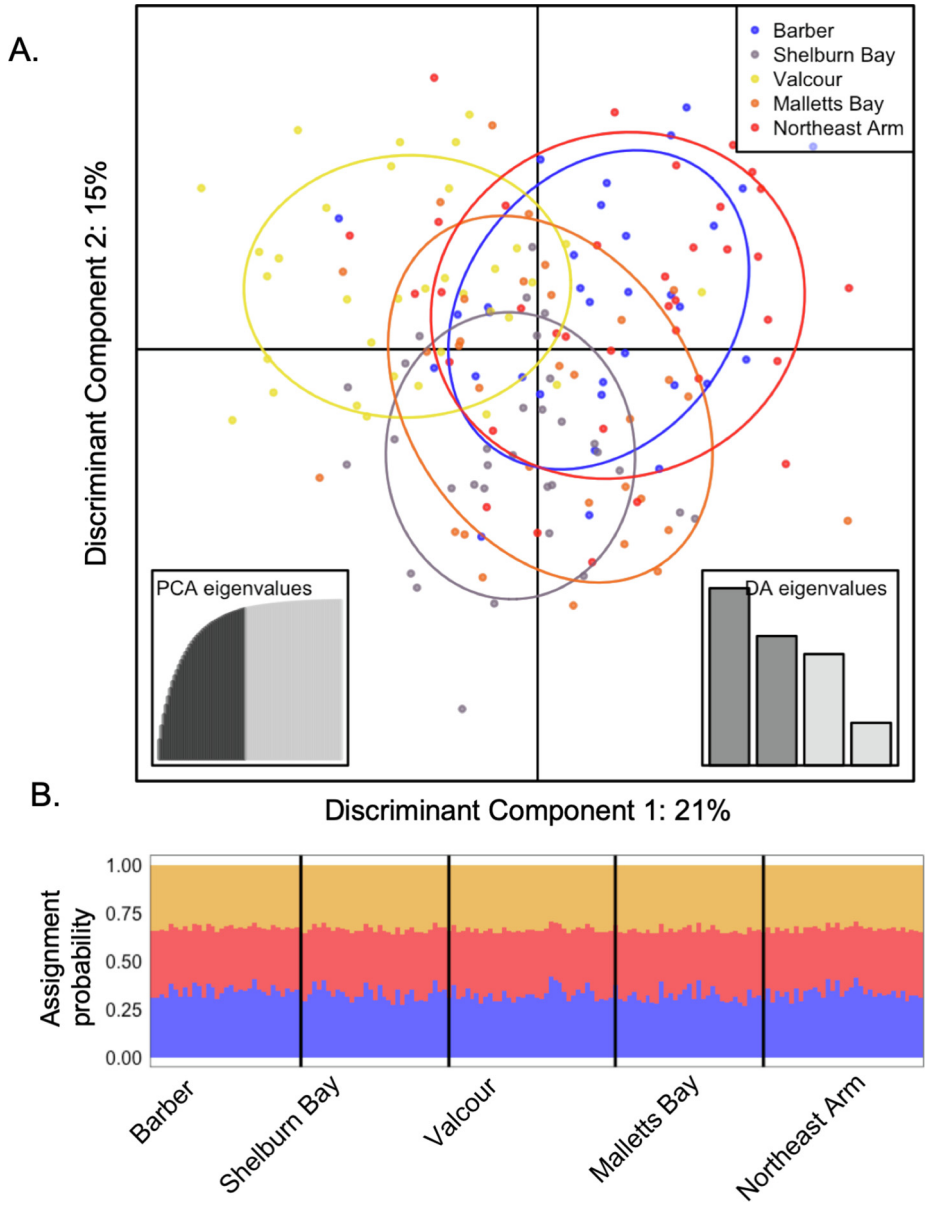

Fig. 2. Clustering model outputs from DAPC (top) and the genetic clustering algorithm STRUCTURE (K = 3; bottom). Each individual dot in the DAPC bi-plot represents a single genotyped individual and the color of the dot indicates the site the where the individual was sampled. The STRUCTURE barplot is a graphical representation of individual membership coefficient to each cluster (vertical bars). Colors represent estimated membership in each cluster for each individual. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

relation between age-1 CPUE in the Main Lake and age-1 CPUE in either the Northeast Arm or Malletts Bay (Table 4).

Discussion

Rainbow smelt in Lake Champlain appeared to form a single, genetically-connected stock and basin-specific demographic stocks. Therefore, causeways and sandbars that separate the basins likely limit enough of the dispersal among basins to result in basin-specific growth and mortality, but not enough to cause reproductive isolation. Our results suggest that conclusions based on only genetic or demographic data would be incomplete and misrepresent the true stock structure of the population. We discuss potential mechanisms for the observed lack of genetic structure in

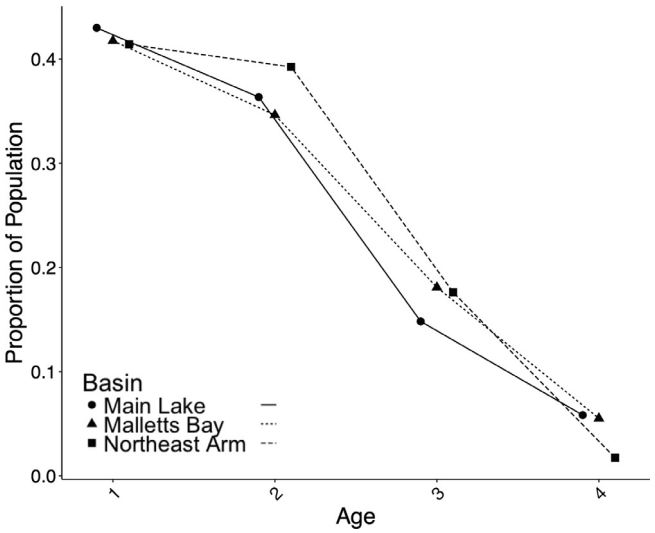

Fig. 3. The proportion of rainbow smelt age 1–4 captured during forage fish surveys between 1990 and 2015 in the three partially isolated basins of Lake Champlain.

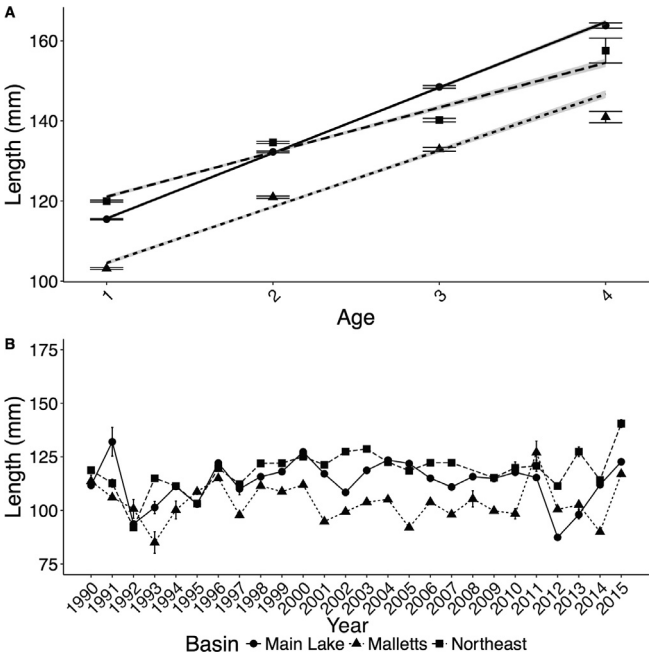

Fig. 4. A) length-at-age of rainbow smelt averaged across 26 years of forage fish surveys. Lines represent line of best fit, gray background indicate 95% confidence intervals around line of best fit. B) average length of age-1 rainbow smelt per year in each Lake Champlain basin.

conjunction with demographic differences among physically isolated basins.

Absence of genetic structure

We were unable to detect any genetic structure in rainbow smelt among basins; and based on genetics alone, we would therefore conclude that rainbow smelt consist of a single stock. However, we stress that our inability to detect any genetic structure does not mean that no structure exists. The level of genetic structure present in the rainbow smelt population may be small, and therefore undetectable using our current study design. Power estimates indicated that our sample size of individuals and loci genotyped at each site were sufficient to detect all but small levels of genetic distance (F_{ST} < 0.01). However, all except one pairwise comparison had F_{ST} < 0.01, suggesting that the use of larger sample sizes or additional loci may increase statistical power to the point that significant F_{ST} could be detected. Therefore, currently we can only conclude that rainbow smelt form a single genetic stock at a genetic distance threshold of F_{ST} = 0.01, but we cannot rule out the presence of shallow genetic structure left undetected. In fact, shallow genetic structure and low absolute values of F_{ST} is common for highly mobile species with large population sizes like rainbow smelt (e.g., McLean and Taylor, 2001) and not necessarily a predictor of demographic dependence (Waples and Gaggiotti, 2006).

Limited genetic structure among basins could be explained by either population size or gene flow. Based on forage fish surveys, we know that rainbow smelt are abundant in Lake Champlain. When effective population size is large, changes in allele frequencies due to genetic drift can take a long time to manifest. Therefore, even 100–150 years after causeways were constructed the populations isolated by causeways may be only starting to diverge (Hedrick, 2005). Alternatively, causeways may not sufficiently block gene flow to result in genetic divergence among basins. The amount of gene flow needed to maintain genetic connectivity could be easily reached by dispersal of early age classes, before large demographic differences begin to appear. Because causeway openings are less than 10 m deep and reach temperatures of 20–25 °C in the summer, YOY and older rainbow smelt likely avoid causeway openings, at least in the summer when water temperature in the openings are well above their preferred range (Marsden and Langdon, 2012; Simonin et al., 2012). Larval rainbow smelt, however, are pelagic and are the primary source of genetic connectivity in other systems, including the St. Lawrence River estuary and along the Atlantic coast (Baby et al., 1991; Bernatchez and Martin, 1996; Kovach et al., 2013). If rainbow smelt in Lake Champlain also follow this pattern, then all of Lake Champlain may be genetically connected by larval drift, while YOY and older fish are restricted by causeways.

In all likelihood, the genetic connectivity observed among basins is a combination of both gene flow and large effective population size. Even with periodic movement of larval and adult

Table 4
Sample size of number of years compared (N), rho test statistic, and significance for Spearman correlations testing the between-basin relationships of proportion of age-1 smelt, length at age-1, and catch-per-unit-effort (CPUE) across 26 years of trawling surveys in Lake Champlain.

		Main Lake : Northeast Arm	Main Lake : Malletts	Northeast Arm : Malletts
	N	26	26	26
Proportion age-1	rho	0.60	0.28	0.63
	p-value	<0.01	0.17	<0.01
Length at age-1	rho	0.38	0.33	0.29
	p-value	0.08	0.12	0.17
CPUE	rho	0.12	0.06	0.60
	p-value	0.56	0.79	<0.01
Age-1 CPUE	rho	0.25	0.30	0.81
	p-value	0.23	0.14	<0.01

Table 5
ANOVA results for analysis comparing length and CPUE of rainbow smelt among basins. "–" indicates that the effect was not calculated for the given response.

Effect		Response		
		length-at-age	length-at-age-1	CPUE
	N	21,945	9,305	678
Basin	degrees freedom	2	2	2
	f-value	2,597.1	1,941.2	141.5
	p-value	<0.001	<0.001	<0.001
Year	degrees freedom	25	25	25
	f-value	269.3	124.5	12.3
	p-value	<0.001	<0.001	<0.001
Basin:Year	degrees freedom	50	49	50
	f-value	99.1	48.33	17.4
	p-value	<0.001	<0.001	<0.001
Age	degrees freedom	3	–	–
	f-value	10,420.8	–	–
	p-value	<0.001	–	–

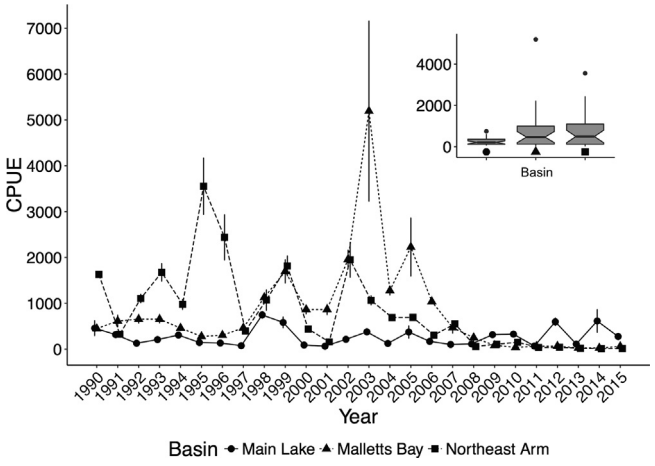

Fig. 5. Total catch-per-unit-effort (CPUE) of rainbow smelt in each Lake Champlain basin for each year. Error bars represent standard error. Inset plot indicates the across-year CPUE (y-axis) for each basin (shapes), lines indicate median values.

smelt though some of the causeway openings, it is likely that causeways still form a partial barrier that restricts gene flow. Incomplete barriers, often referred to as resistance barriers, are known to influence gene flow in many species (McRae, 2006) and are the basis of landscape genetics (Holderegger and Wagner, 2008). However, these types of barriers can be difficult to study in aquatic systems where species tend to have large effective population size and passive dispersal (i.e. larval drift) that make identifying differences among stocks difficult (e.g. Corander et al., 2013). Therefore, without additional information about how rainbow smelt interact with causeways it is difficult to determine exactly why we did not detect significant population structure in Lake Champlain.

Presence of demographic structure

The demographic structure of rainbow smelt varied among basins. The combination of variable age structure, length-at-age, and CPUE of rainbow smelt among all three basins and a lack of correlation in length-at-age and CPUE among basins across the 26 years of sampling all indicate that populations separated by causeways fluctuate independently. Rainbow smelt CPUE in Malletts Bay and the Northeast Arm was also more variable year-to-year than in the Main Lake, suggesting that populations may be more stable in the Main Lake. Therefore, basins may be genetically connected, but still experience source/sink dynamics that influence rainbow smelt stock dynamics (Waples, 1998).

Similar demographic differences among putative stocks of rainbow smelt have been observed in Lake Superior and Lake Erie (Luey and Adelman, 1984; Henderson and Nepszy, 1989). Demographic differences in Lake Superior were associated with genetic differences and attributed to adaptive isolation between stocks that experience high levels of predation and competition (Schreiner et al., 1984). However, in Lake Erie, demographic differences were attributed to limnological differences between sites; but genetic connectivity between stocks was not evaluated (Henderson and Nepszy, 1989). In both Great Lakes, stocks were not separated by geographic barriers other than distance; whereas the physically isolated basins we studied in Lake Champlain differ in productivity and community composition which could lead to differential growth and mortality among rainbow smelt stocks. The differences in productivity between the east and west basins of Lake Erie that are hypothesized to influence rainbow smelt growth (MacCrimmon et al., 1983) are similar to the differences in productivity among the three Lake Champlain basins. While the entire lake is small enough to experience similar annual weather conditions, basins are physically different from one another in trophic status, community composition, and bathymetry, all of which could contribute to the demographic differences we observed. Malletts Bay is more oligotrophic than the Northeast Arm or the Main Lake; mean chlorophyll of Malletts Bay is approximately 40% lower than the Northeast Arm and 20% lower than the Main Lake (LCBP, 2015). If most rainbow smelt are constrained to a single basin, the low productivity in Malletts Bay could result in the downward shift in average length at age we observed. Additionally, Malletts Bay lacks *Mysis diluviana* which are abundant and are fed upon extensively by age-1 and older rainbow smelt in the Main Lake (Johnson et al., 2004; Stritzel Thomson et al., 2011; Euclide, unpublished data). Therefore, differences in productivity and prey community could lead to the growth and mortality differences of rainbow smelt among basins as observed in this study.

Additional factors such as differential success among spawning sites or the distribution of predators may play a role in rainbow smelt recruitment and CPUE. Slight annual differences in climate effects, such as increased nutrients from run-off, could improve early survival of one basin over the others in a given year, similar to what has been noted for other species with isolated spawning stocks such as Pacific salmon or walleye (Schindler et al., 2010; DuFour et al., 2015). Variation in CPUE among basins could further be influenced by differences in predation rate. Rainbow smelt in Lake Champlain are consumed extensively by lake trout, Atlantic

salmon (*Salmo salar*), and walleye. Lake trout are known to utilize Malletts Bay and the Northeast Arm seasonally, avoiding the basins in the summer (Pinheiro et al., 2017; Pientka, unpublished data). Therefore, if predators do not or cannot actively redistribute among basins due to causeways, predation of rainbow smelt in a particular basin could vary annually.

Although rainbow smelt from different basins varied in length-at-age and CPUE, the basins do not appear to be completely disconnected. The proportion of age-1 rainbow smelt was correlated among basins which suggests that there may be some synchrony in new rainbow smelt cohorts. If cross-basin connections exist via larval drift, favorable environmental conditions could lead to periodic similarities among basins. This synchrony may then break down as cohorts age and cross-basin connections become more limited for older fish whose temperature and depth preferences are more restrictive (Simonin et al., 2012). This partial synchrony of rainbow smelt cohorts and lack of evidence of genetic divergence among basins indicates that rainbow smelt in Lake Champlain likely interact as connected sub-stocks, such that genetic diversity may be maintained by gene flow through causeway openings; but growth and recruitment are largely basin-specific from year to year (Morrissey and de Kerckhove, 2009).

The genetic connectivity between basins is intriguing but should be interpreted with caution. Because of the removal of loci due scoring issues, our study includes only six microsatellite loci which limits the power of genetic analyses. However, the loci used were highly polymorphic and our individual sample sizes were moderate, therefore we feel confident that our study should have been able to detect all but small genetic differences among basins. While not a justification for the present design, it is important to note that the sample sizes we used are similar to those reported in previous genetic studies (e.g., Simon et al., 1999; Hansen et al., 2010), many of which are still the basis of management decisions in use today. However, re-evaluation of stock structure using updated genomic techniques could help to result in different stock boundaries than previously identified. Additionally, the genetic patterns observed in rainbow smelt are identical to those observed previously in slimy sculpin in the same system suggesting that observed lack of genetic population structure may be associated with more than a lack of statistical power (Euclide et al., 2018). The consistency in panmixia observed between two species with such divergent life histories and behavior suggests that some factor other than adult movement, habitat, or spawning behavior influences observed population genetic structure of these, and perhaps other, Lake Champlain fishes. Slimy sculpin and rainbow smelt have large, unexploited populations in Lake Champlain; therefore it is quite possible that large effective population size, rather than dispersal, is responsible for the lack of observed genetic structure among basins. Because the causeways are only 100–150 years old, populations may also have simply not had sufficient enough time to diverge enough to detect differences with the current marker set.

Broader implications

Our study indicates that the populations of rainbow smelt in Lake Champlain may be genetically homogenous, but demographically independent. The demographic differences were small, but our results suggest that basin-specific populations may be vulnerable to environmental change and populations fluctuations. For example, if ecological/recruitment processes within the Northeast Arm and Malletts Bay are independent from the Main Lake, the smaller populations in these basins are likely less stable and more vulnerable to large changes in population abundance than they would be if the basins were completely interconnected (Lowe and Allendorf, 2010). Additionally, the difference in conclusions drawn from genetic and demographic techniques suggests that using either microsatellite or demographic data alone would have misidentified rainbow smelt stock structure and lacked the nuance gained from a dual-method strategy. Contradiction between demographic and genetic stock structure is not uncommon. While rainbow smelt demographic differences among regions in Lake Superior corresponded to genetic differences, similar differences were attributed to trophic state in Lake Erie where no genetic data were collected (MacCrimmon et al., 1983; Schreiner et al., 1984). Additionally, two different ecotypes of rainbow smelt in Lac Saint-Jean, Quebec showed only modest genetic differentiation despite large morphological differences between ecotypes (Saint-Laurent et al., 2003). Thus, demographic differences do not necessarily indicate genetically distinct fish stocks, and vice versa, emphasizing that caution should be used when using only a single method to identify new stocks or monitor existing stocks.

Our analysis suggests that although rainbow smelt abundance appears to have declined in the Northeast Arm and Malletts Bay in the last decade, the lake-wide rainbow smelt population genetic diversity remains high and genetic structure low. If smelt abundance continues to be suppressed in the smaller basins where gene flow from the Main Lake is less likely, over time these populations may begin to show signs of genetic isolation from the Main Lake because genetic drift has a stronger effect on small than large populations (Gillespie, 2010). Historically, high inter-annual variability in abundance in the two smaller basins may have been offset by dispersal from the Main Lake, where CPUE has remained comparatively stable since 1990 when sampling began. The recent declines emphasize the need for continued monitoring of all three basins, and further investigation of potential causes of the demographic differences among basins.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

This project was supported by funding from the Great Lakes Fishery Commission made available with the assistance of Senator Patrick Leahy. We thank Ben Block for his help with early data analysis, thoughtful discussion, and helpful comments. Undergraduate intern Edwin Gonzalez conducted DNA extraction and genotyping of early rainbow smelt samples and was funded by the National Science Foundation Research Experiences for Undergraduates (REU) Program (DBI-1358838). We also thank Matthew J. Wargo for the use of his laboratory and guidance developing basic PCR genotyping protocols, Captain Steve Cluett and deckhand Krista Hoffsis for help collecting the 2015 rainbow smelt samples, and Jason Stockwell for providing comments on the manuscript. Finally, we thank Nick Staats of US Fish and Wildlife for his time and initial analysis of the forage fish dataset.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jglr.2020.02.009.

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