Genetic Diversity of Stocked Lake Sturgeon in Oneida Lake, NY

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Abstract

Many lake sturgeon (*Acipenser fulvescens*) populations in the Great Lakes have not recovered from previous threats and stocking is a management strategy that can potentially help increase populations. However, there are genetic risks with stocking, including loss of genetic diversity. Stocked lake sturgeon in Oneida Lake, NY were analyzed at 12 microsatellite loci to compare their genetic diversity to their source populations. When stocking was conducted in a single year, there was a reduction in heterozygosity and number of alleles and an increase in relatedness of the offspring. Continued stocking over several years retained most of the genetic diversity of the source population. The number of parents used did not have an effect on heterozygosity or the number of alleles in the offspring, but there was a negative correlation with relatedness. Lake sturgeon stocking should occur over several years in order to obtain an adequate representation of genetic diversity in the stocked population.

Introduction

Many lake sturgeon (*Acipenser fulvescens*) populations throughout the Great Lakes have been substantially reduced in size relative to historic times (St. Pierre and Runstrom 2004). These declines can be attributed largely to overfishing and habitat alterations, including changes due to the presence and operation of dams. Although some of these problems have been addressed, many populations have yet to rebound, likely due to the life history traits of lake sturgeon. The lake sturgeon is a long-lived species with late sexual maturity and intermittent spawning (Bruch et al. 2001). Therefore, even when given sufficient habitat, impacted lake sturgeon populations will likely take several generations to fully recover. Stocking is a management strategy that can be used to reestablish populations in places where they have been extirpated or to supplement them to locations within their historic range where reduced population size makes natural recovery unlikely. However, there are genetic risks that should be considered prior to stocking.

Many genetic changes can occur as a result of the stocking process, including a loss of genetic diversity. This loss can first occur during broodstock sampling. Only a small subset of

the population is selected as the broodstock and, subsequently, only a small subset of the source population's genetic diversity is represented in the resulting offspring. An additional loss of genetic diversity can occur depending on how the crosses among males and females are conducted. If crosses result in a large proportion of full- or half-siblings, then relatedness among the offspring will be high and inbreeding will be a concern in the resulting population.

In Oneida Lake, New York, lake sturgeon have been stocked since 1995 using two different stocking scenarios (Jackson et al. 2002). In the first scenario, large numbers of offspring were released in a single year (1995). At that time, two females and four males were collected from the Des Prairies River, Quebec. Female lots were divided and each male was used only once. From the crosses, 5000 age-0 sturgeon were stocked into Oneida Lake. The second scenario collected broodstock over several years, releasing a smaller number of individuals each year. After 1995, broodstock were collected from the St. Lawrence River in Massena, NY (Table 1). The mating strategy remained the same and the number of parents used each year varied. Fewer numbers of sturgeon were released compared to the 1995 cohort.

The goal of our study was to assess the genetic diversity of the stocked sturgeon in Oneida Lake. The objectives were to i) determine if there were genetic differences between the stocked sturgeon and their respective source populations, ii) examine the effects of the number of parents used on the genetic diversity of the resulting offspring, and iii) identify the stocking scenario that maximizes the genetic diversity of the offspring.

Methods

Sample collection. Tissues for genetic analyses came from pectoral fin ray sections collected for aging and population analyses in Oneida Lake. Most sturgeon were collected by gill net. Younger sturgeon were occasionally collected in variable mesh gill nets (mesh sizes ranging from 38-102 mm stretch in 13 mm increments) sampled annually as part of the Oneida Lake walleye monitoring program. From 1999-2002, sturgeon were targeted with a 152-mm stretch mesh gill net. In 2002, more directed sampling was initiated using a variable mesh gill net with mesh sizes ranging from 152-305 mm in 51-mm increments. The later sampling approach

produced the majority of tissues used in this study. Most fish were aged using pectoral fin ray sections, which were prepared by taking thin sections and mounting on slides for examination under a dissecting microscope (Bruch et al. 2009), however in 1999 all stocked sturgeon were tagged prior to release with individually numbered carlin dangler tags and/or PIT tags, and ages of recaptured fish were determined from tagging records. For fish aged using fin rays, at least two independent readers were utilized. Cohort assignment was calculated by subtracting the age of the fish from the year of capture. Source population data from Welsh et al. (2008) were used, with the exception of 28 additional samples from the St. Lawrence River captured in 2006. Those additional samples were processed as part of this study.

Laboratory techniques. Genomic DNA was extracted using the Promega Wizard SV96 Genomic DNA Purification System from a total of 373 collected samples (1995: n=273; 1996: n=33; 1998: n=23; 1999: n=12; 2000: n=7; 2003: n=2; 2004: n=18). Extracted samples were then amplified at 12 standardized microsatellite loci (Welsh and May 2006) using the procedure described in Welsh and McLeod (2010). Amplified products were visualized on the Beckman Coulter CEQ8000 Genetic Analysis System.

Statistical analyses. A Bayesian approach was first used to determine the most likely number of populations represented in the dataset given the genetic data with no prior probabilities (using the software STRUCTURE; Pritchard et al. 2000).

The genetic diversity of stocked Oneida Lake sturgeon was compared to their respective source population. For the purpose of this analysis, the 1995 cohort represented stocked sturgeon from the Des Prairies River and all other cohorts were grouped together to represent the stocked sturgeon from the St. Lawrence River. To test for genetic differences between the stocked sturgeon and their respective source populations, pairwise F_{ST} values (using the software ARLEQUIN; Excoffier and Lischer 2010) and exact G test for genic differentiation (using the software GENEPOP; Raymond and Rousset 1995, Rousset 2008) were calculated. F_{ST} values can range between 0 (no genetic difference) to 1 (completely different). However, F_{ST} values between 0.05 and 0.15 are generally considered moderately differentiated and values ranging between 0.15 and 0.25 are considered highly differentiated (Wright 1978). To test for differences in levels of genetic diversity among stocked and wild sturgeon, heterozygosity (Nei

1987) and allelic richness (i.e., number of alleles corrected for differences in sample size; El Mousadik and Petit 1996) were calculated using the software FSTAT (Goudet 2001). High heterozygosity and a large number of alleles indicate high genetic diversity. Paired t-tests were conducted to identify significant differences between the source populations and their respective cohorts in Oneida Lake. Average relatedness (Queller and Goodnight 1989) was also calculated for all groups and significance was assessed based on 999 permutations (using the software GenAlEx; Peakall and Smouse 2006). In general, full-siblings and parents-offspring have relatedness values of 0.5, half-siblings have relatedness values of 0.25, and unrelated individuals have a relatedness value of 0.

The effect of the number of parents on genetic diversity of the offspring was tested. The effective population size (N_e) for each cohort was calculated using the equation $N_e = 4N_fN_m/(N_f+N_m)$, where N_f is the number of females used and N_m is the number of males used (Table 1). Only cohorts with an adequate sample size ($\geq$ 10 individuals) were used in this analysis (i.e., 1995, 1996, 1998, 1999, and 2004 cohorts). For each cohort, heterozygosity and allelic richness were calculated (using the software FSTAT). A regression analysis was performed to determine if there was a significant correlation between these measures of genetic diversity and the N_e of the cohort. Pairwise relatedness values were calculated for each cohort (using GenAlEx) and a Mantel test was performed to test the correlation between relatedness and N_e (using the software PopTools; Hood 2006).

Results

Based on the genetic data obtained from the stocked sturgeon in Oneida Lake, the most likely number of population clusters was four (Figure 1). Three distinct populations were identified in the 1995 cohort originating from the Des Prairies River. All the cohorts originating from the St. Lawrence River belonged to the fourth population cluster. The majority of individuals had a high proportion (>80%) of membership to a single cluster. A small number of individuals (n=14) in the 1996 cohort clustered with one of the Des Prairies population clusters. The age designation of the fin rays from those individuals was reexamined and, due to the

difficulty in accurately aging those particular individuals, it was determined that an aging error likely occurred. Those individuals were dropped from subsequent analyses.

Comparison to source populations. Both stocked groups had significant genetic differences from their respective source populations. The largest difference was between the Des Prairies River and its stocked cohort (F_{ST} = 0.06, p = 0.00). The St. Lawrence River cohorts were less differentiated from their source (F_{ST} = 0.02, p = 0.00). All stocked cohorts had significantly different allele frequencies from their source populations ($p < 0.00$).

Significant differences in genetic diversity were observed between the Des Prairies River and its stocked 1995 cohort in Oneida Lake. The 1995 cohort had significantly lower heterozygosity (0.60 vs. 0.45, p = 0.00; Figure 2), lower allelic richness (4.02 vs. 3.04, p = 0.00; Figure 3), and higher relatedness (-0.23 vs. 0.02, p = 0.00; Figure 4). No differences in genetic diversity were observed between the St. Lawrence River and its Oneida Lake cohorts (Figures 2-4).

Genetic effect of number of parents. No significant correlation was observed between the effective population size and heterozygosity (R^2 = 0.03, p = 0.21) or allelic richness (R^2 = 0.00, p = 0.98). However, relatedness decreased as the number of parents used increased (R = -0.02, p = 0.03).

Discussion

Stocking activities can potentially result in a loss of genetic diversity. One of the limitations of lake sturgeon stocking is the small number of individuals that are usually available for use as broodstock. This was demonstrated in Oneida Lake when a small number of parents from the Des Prairies River contributed a large number of offspring in a single year. The stocking event in 1995 resulted in a loss of heterozygosity, a loss of alleles, a shift in allele frequencies, and higher relatedness, relative to the source population. A significant loss of genetic diversity was observed in the stocked cohort, despite the sample size for the Des Prairies River source population being small (n=14). If a larger sample size was used, the differences between the stocked and wild fish may be even more pronounced.

Loss of genetic diversity was not observed when stocking continued over several years. Although a small number of parents was used each year, continual stocking using different parents resulted in a better representation of the source population's genetic diversity. There was still a significant shift in allele frequencies; however, the stocked population resulting from the St. Lawrence River cohorts had similar levels of heterozygosity, number of alleles, and relatedness compared to the wild source population. These results demonstrate the importance of stocking over several years in order to have levels of genetic diversity similar to those observed in natural wild lake sturgeon populations. Drauch and Rhodes (2007) also found that a single year-class generated from Lake Winnebago stock did not adequately capture the genetic diversity of the source population, while multi-year reintroductions in the Mississippi and Missouri Rivers had similar levels of genetic diversity and minimum genetic differentiation from their Lake Winnebago source.

The number of parents used in a given year should be maximized in order to reduce the relatedness among resulting offspring. As the number of parents increased, relatedness in the offspring decreased. When large numbers of related offspring are released, there is a greater chance that inbreeding will occur in the resulting self-sustaining population. The probability would be higher that an individual would mate with a full- or half-sibling. Inbreeding could result in inbreeding depression in the population (i.e., reduction in survival or reproductive success due to inbreeding).

One potential source of error in our study was the assignment of fish to specific stocking cohorts based on aging on fin ray sections. Fin sections have been shown to generally provide reliable age estimates for young fish, but the probability of underaging increases with fish 10 years old or greater (Bruch et al. 2009). Even with younger fish, the possibility of aging error cannot be completely discounted. While we cannot quantify the potential bias aging error might have introduced to our analyses, we were able to assess aging error from fish from the 1995 stocking due to their genetic differences from all subsequent stockings. Based on genetic results, 287 individuals from the 1995 stocking were represented in the tissue collections, however 14 of these fish were mis-aged and assigned to the 1996 stocking. These results indicate a 5% aging error rate for fish from the 1995 stocking, of which 57% were fish captured

at age-10 or 11. Given all tissues used were from fish captured in 2007 or earlier, age-10 or older fish were only represented in samples from the 1995 and 1996 cohorts, so presumably error rates were lower on fish from later stockings. Aging error should not be an issue with the 1999 and later stockings due to the use of unique tags applied to fish prior to stocking. However, incorrect aging of fish and resulting mis-assignment to a stocking cohort cannot be discounted as a potential source of error in our results. Another limitation of the study was that samples for genetic analysis were not available from the parents. Therefore, it is uncertain whether all parents contributed to the released offspring. Therefore, the effective population size may actually be lower than the values calculated for this study.

Genetic guidelines have been developed for lake sturgeon stocking in the Great Lakes (Welsh et al. 2010) and most of the recommendations in the guidelines are based on theoretical predictions. This study provides data supporting some of the recommendations. For example, the guidelines recommend that stocking continue for a lake sturgeon generation (i.e., 25 years) or until a targeted number of parents are acquired. The results from Oneida Lake support a multi-year stocking strategy in order to maximize the genetic diversity of the resulting population. The guidelines also recommend a minimum yearly target N_e of 8. Our results show that relatedness among offspring decrease with the greatest number of parents. However, with N_e=8, the relatedness of the offspring from a single cohort is still higher than relatedness values observed in wild populations. Therefore, that target should be a minimum and more parents should be used when possible. A multi-year stocking strategy helps to compensate for the higher relatedness because the combination of all cohorts results in a level of relatedness similar to the wild source population.

Oneida Lake has been stocked for many years, resulting in a healthy lake sturgeon population that may eventually be self-sustaining. As lake sturgeon stocking continues in New York State, there may be interest in using Oneida Lake as a source population. The cohorts from the St. Lawrence River have levels of genetic diversity similar to natural lake sturgeon populations and could provide donor individuals for stocking in locations in the Lake Ontario basin and St. Lawrence River system where lake sturgeon have been extirpated. However, the

1995 cohort from the Des Prairies River would not be a suitable source due to its low genetic diversity.

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Table 1. Summary of lake sturgeon stocking in Oneida Lake (year of stocking, source population, number of female and male parents used, N_e calculated based on unequal sex ratios of parents, and number of offspring released) and the samples analyzed in this study (sample size N).

Year	Source	# Females	# Males	N_e	# Released	N
1995	Des Prairies	2	4	5.3	5,000	273
1996	St. Lawrence	1	4	3.2	550	33
1998	St. Lawrence	1	3	3.0	387	28
1999	St. Lawrence	1	4	3.2	320	12
2000	St. Lawrence	1	4	3.2	300	7
2003	St. Lawrence	2	9	6.5	368	2
2004	St. Lawrence	3	6	8.0	1,200	18

Figure 1. Bayesian determination of most likely number of population clusters represented in the Oneida Lake samples based on the genetic data. Number of clusters with the highest likelihood and least variability among runs is 4.

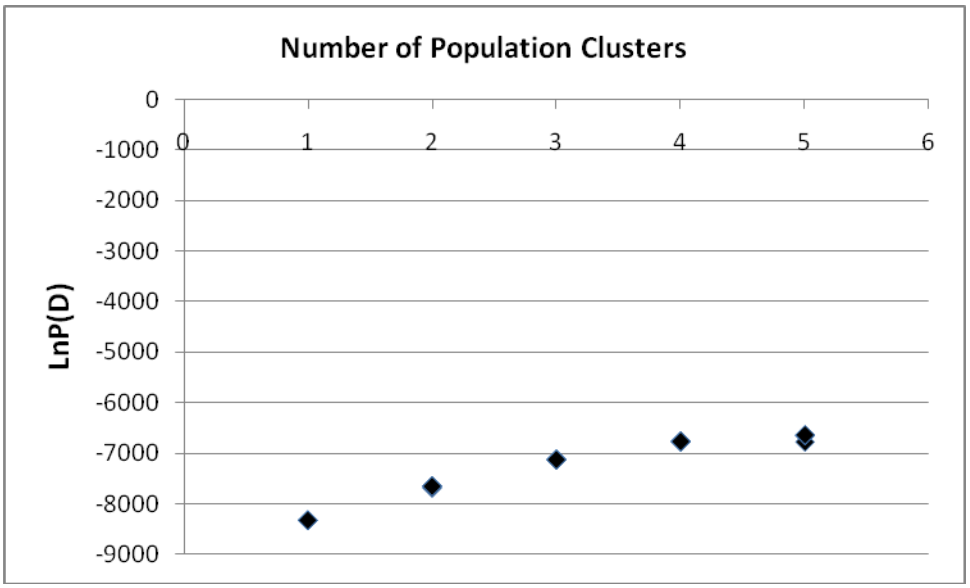


Figure 2. Heterozygosity observed in Oneida Lake sturgeon stocked in 1995 from the Des Prairies River (Des Prairies-Oneida) and in subsequent years from the St. Lawrence River (St. Lawrence-Oneida) compared to their respective source populations. * denotes significant comparisons (p=0.00).

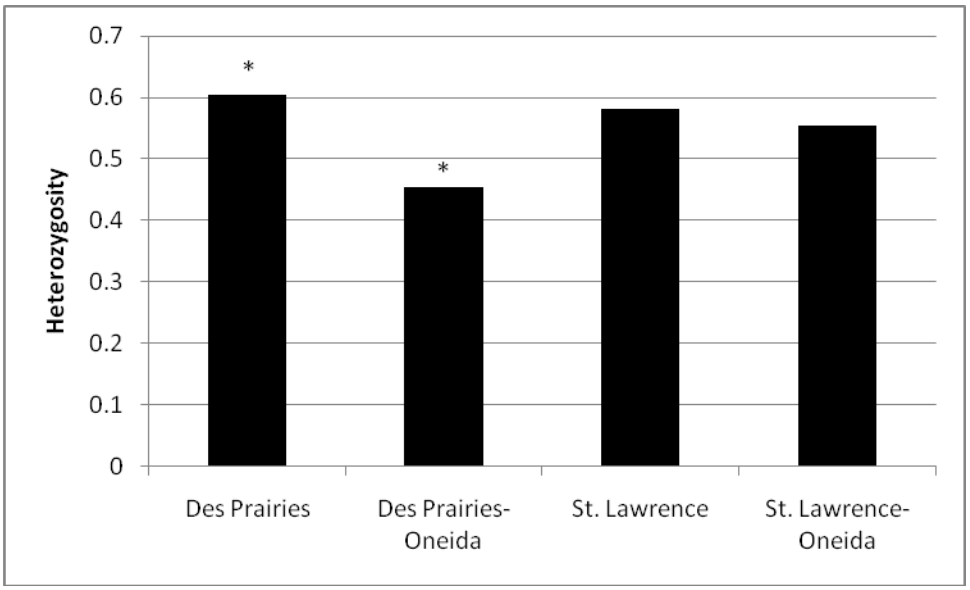


Figure 3. Allelic richness observed in Oneida Lake sturgeon stocked in 1995 from the Des Prairies River (Des Prairies-Oneida) and in subsequent years from the St. Lawrence River (St. Lawrence-Oneida) compared to their respective source populations. * denotes significant comparisons (p=0.00).

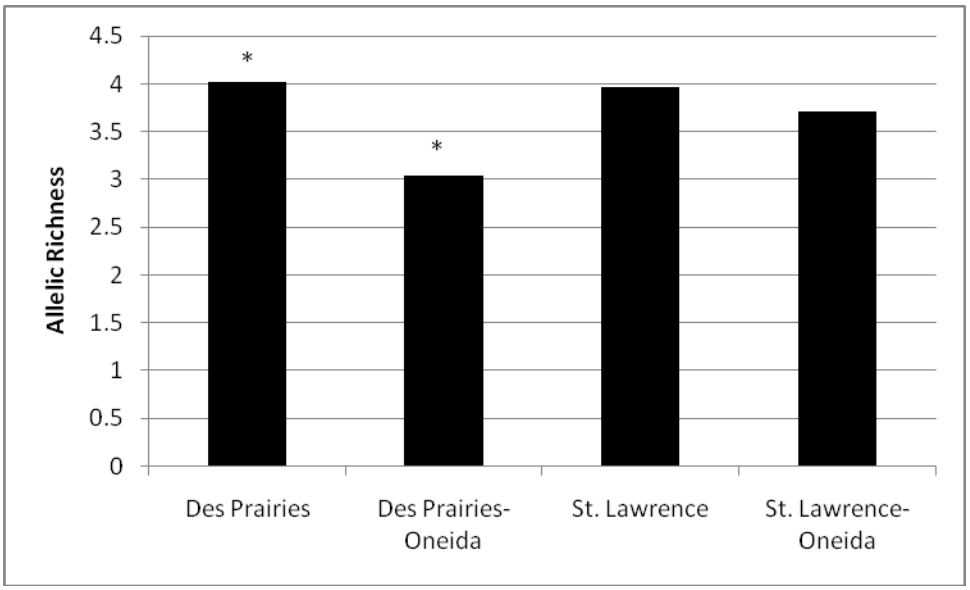
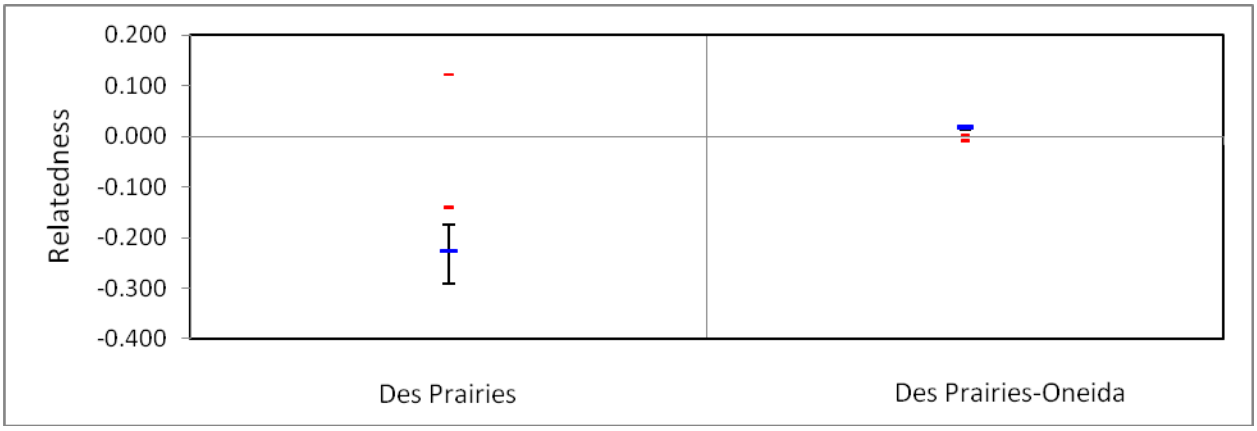
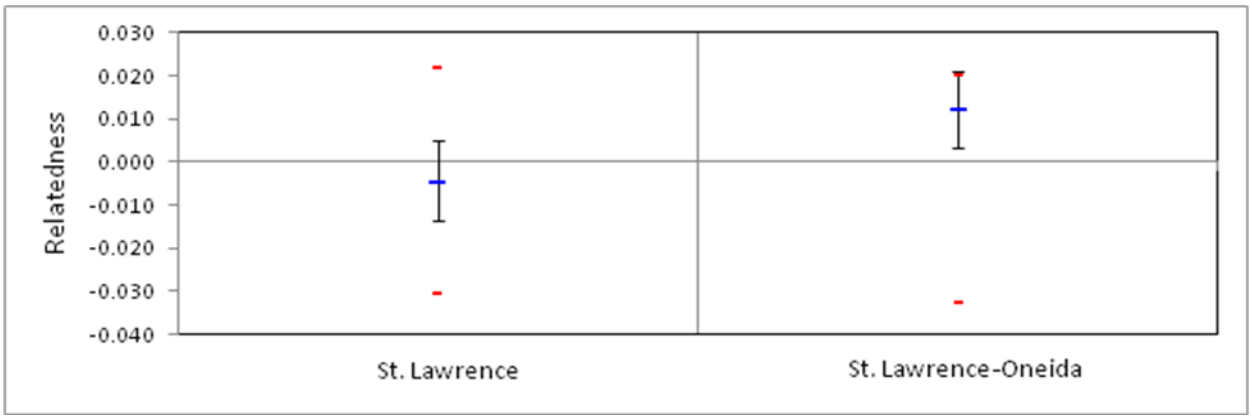


Figure 4. Relatedness observed in Oneida Lake sturgeon stocked A) in 1995 from the Des Prairies River (Des Prairies-Oneida) and B) in subsequent years from the St. Lawrence River (St. Lawrence-Oneida) compared to their respective source populations. Blue line and error bars represent the average relatedness and 95% confidence interval based on bootstrap resampling. Red bars represent the 95% confidence interval around the null hypothesis of "No difference" based on 999 permutations. Difference in relatedness between Des Prairies and Des Prairies-Oneida is significant (p=0.00).

A)



B)