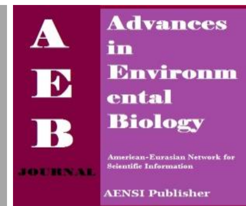
**Advances in Environmental Biology**, 8(7) May 2014, Pages: 3151-3157

AENSI Journals
**Advances in Environmental Biology**
ISSN-1995-0756 EISSN-1998-1066
Journal home page: http://www.aensiweb.com/aeb.html




# Genetic Diversity of Pike (*Esoxlucius*) In the South Caspian Sea Using Mtdna Sequences

[1]**Farzaneh Mehrabi and** [2]**Kaivan Hazaie**

[1]*Department of Fisheries, Qaemshahr Branch, Islamic Azad University, Qaemshahr, Iran.*
[2]*Assistant Professor, Department of Fisheries, Qaemshahr Branch, Islamic Azad University, Qaemshahr, Iran.*

ARTICLE INFO

***Article history:***
*Received 15 April 2014*
*Received in revised form 22 May 2014*
*Accepted 25 May 2014*
*Available online 15 June 2014*

***Key words:***
*Esox Lucius, genetic diversity, mitochondrial DNA (mtDNA), heterozygosity*

ABSTRACT

In study of genetic variation of Pike(Esoxlucius), the 30 sample fish were collated in 2 station (Mazandaran, Gilan). A piece of soft fin (3-5 g) of pectoral were separated and fixed in alcohol of 96 percent. DNA was extracted by phenol-chloroform method. The quality and quantity of DNA were determinate by spectro photometer and 1 percent gets Agaroz of electro phoresis. Poly merase chain reaction (PCR) by ther mocycler using primers equencing, CDL-D and PIDL showed polymorphs. The results indicate 9 Haplotypes in Gilan samples and 5 Haplotypes in Mazandaran samples.in the sampling location, the average of Haplotypes diversity was calculated 1 and the average of nucleotides diversity was 0.013. The number of alleles was studied in 14 variable positions. According to the findings, the maximum number of observed alleles was 2 alleles in Gilan and that's minimum was 1 in mazandaran. The average of observed alleles was 1.5± 0.7. The maximum and the minimum of expected heterozygosity was calculated 0.035±0.04 - 0.071 ±0.0009 and also the most and the least genetic distance was found 0.0004 - 0.027. The most genetic differences (Fst=0.27) was gotten between samples of Mazandaran station and Gilan station. The results have shown that, in the different stations, sampling of Esox Lucius genetically had different genetic group but this genetic differentiation hadn't any significant difference.

**© 2014 AENSI Publisher All rights reserved.**

**To Cite This Article:** Farzaneh Mehrabi and Kaivan Hazaie., Genetic Diversity of Pike(Esoxlucius) In The South Caspian Sea Using Mtdna Sequences. ***Adv. Environ. Biol., 8(7), 3151-3157, 2014***

## INTRODUCTION

All finite populations undergo random genetic change, known as genetic drift. One of the most important consequences of this random change is that populations continuously lose genetic variation A. n inverse relationship between population size and the rate of loss of genetic variation has long been established in population genetics theory. Loss of variation is not determined by the commonly measured census size; however, various demographic factors also play a role. Effective population size (Ne), a concept first developed by WRIGHT (1931, 1938), corrects for the influence of different demographic factors on genetic variation with in a population. In an ideal population Ne is equal to however, it is less than N in most real populations. Unequal sex ratio and nonrandom distribution of family size are the two factors that commonly reduce Ne below N. In addition, if Ne fluctuates over time, the appropriate value for all generations under consideration is the harmonic mean of Ne for each generation, the harmonic mean is then skewed toward the lowest values. The result of all these demographic factors is that individuals of one generation do not contribute evenly to future generations and therefore only a limited amount of a population's genetic material is maintained. Effective size can be estimated if the above demographic information is known, but this is rarely the case, especially for natural populations. The difficulty in estimating Ne directly from demographic data has led to the development of numerous methods for estimating it indirectly from molecular genetic data. One such method, called the temporal method, is based on the logic that if Ne determines rates of change in genetic variation, hen a measure of genetic change over time should allow Net be estimated. Despite the importance of Ne in determining genetic change, there has been little application of indirect genetic methods natural populations. Most of the estimates using the temporal method in natural populations have been for species with short generation times, such as insects [4] based on data from and plants use of the temporal method to estimate Ne for fish populations has often focused on hatchery populations. The degree to which genetic diversity is important for sustainability of populations is a topic of considerable debate. A fundamental assumption of conservation geneticists is that inbreeding and loss of genetic variability increase the risk of extinction argued that there is ample evidence to support this assumption and that the genetic contribution to population declines has been underestimated.

**Corresponding Author:** Farzaneh Mehrabi, Department of Fisheries, Qaemshahr Branch, Islamic Azad University, Qaemshahr, Iran
E-mail: fish.fm1367@yahoo.com

**Advances in Environmental Biology**, 8(7) May 2014, Pages: 3151-3157

In contrast, Caughley suggested that even though this assumption has theoretical support, genetic impoverishment has rarely been shown to be a factor in the extinction of natural populations. A related issue for managers of exploited wild populations is the degree to which genetic diversity influences the long-term productivity of populations that are large enough that extinction is not an immediate threat. The debate over the role of genetic diversity remain sun resolved, in large p art, due to the difficulty in distinguishing genetic from non genetic causes of population change. Making such distinction requires some means of monitoring genetic characteristics of populations over time. For example, FRANKHAM counted for non genetic factors and determined that the risk of extinction increased at a threshold level of inbreeding in laboratory population of Drosophila and mice. More studies are needed to clarify the relationship between genetic diversity and the dynamics of natural populations. This article present results of a study of historical genetic changes in a natural population of northern pike, Esoxlucius. We examined genetic variatioant three times over a period of 32 years by sampling fish scales from a historical collection. Variability was assessed from DNA extracted from the epithelial cells adhered to these unpreserved scales us in PgC R to amplify microsate llite DNA loci. Based on temporal changes in genetic]variation, we estimated Ne for three time intervals. Our results show the potential for using genetic methods based on microsatellite data to compare trends in Ne, an indicator of genetic diversity, with population dynamic parameters in natural populations. Such comparisons will help to evaluate the importance of genetic diversity to the long-term persistence and productivity of natural populations and determine whether or not conclusions reached in studies of captive populations can be extended to populations in natural environments.

## MATERIALS AND METHODS

Development of microsatellite markers: MILLER and KAPUSCINSKI reported the development of microsatellite markers from partial genomic librarie so f northern pike DNA. We have since screened a second library using two modifications of our initial methods. First, we isolated 250-1000-bpDNA fragments from northern pike genomic DNA before inserting them into a vector. This step made it possible to avoid large inserts, which are difficult to sequence completely. Second, we screened clones with an AC - GT alternating copolymer (Pharmacia) rather than with an oligonucleotide. We labeled this copolymer with 32P using a nick translation kit(Promega) and then proceeded with our original protocol. Use of the copolymer enhanced the signal given by positive clones because more than one radio nucleotide could be in corporate into a single hybridizing strand.

*Population study site: This study focused on the northern:*

Pike population of Lake Escanaba, WisconsinL.ake Escanaba is a 119-hectare lake located within the Northern Highlands Fishery Research Area of northern Wisconsin. Northern pike were introduced into Lake Escanaba in the late 1930’s and early 1940’s. The source population of the stocked fish is uncertain and more than one population may have been used[S. NEWMAN, Wisconsin Department of Natural Resources(WDNR) , Woodruff, WI, personal communication]. No stocking has taken place since that time. It has an inlet and outlet at high water but fish migration is thought to be unlikely. The WDNR has operated a check station since 1946 to monitor compulsory permit system for fishing on the lake. The WDNR has also monitored the status of the population by netting spawning fish each spring. Scales were removed from all fish taken in nets or registered by anglers, and ages were determined by WDNR personnel.

Collection of samples: We obtained samples from the historical scale collection maintained at the WDNR check station Unpreserved scales from each individual had been stored separately in envelopes that recorded information about the individual (e.g., date sampled, length, age). Samples from 3 years were chosen based on two criteria: ( 1 ) a

Large number of individuals were available, and (2) many years (several generations) separated the samples. To meet these criteria, we took most of the spring spawning samples from 1961 (86 individuals) and 1993 (’72), and a random subset (110) from the anglers’ harvest in 1977.

Analysis of genetic variation at microsatellite lock We determined sample allele frequencies at all loci following the procedures of MILLER and KAPUSCINSKI (1996). Briefly, microsatellite loci were amplified using PCR with epithelial cells adhered to fish scales as the source of DNA. A single scale was boiled in 200 p1 of a chelating resin (5% w/v Chelex, Sigma Chemical) and 10 p1 of the solution were added to aPCR reaction mix containing 25 pmol of each PCR primer, 0.2 mMdNTPs, 1 unit Taq DNA polymerase and 1X PCR buffer (Promega). After amplification in a thermal cycler, 4-8 p1 of the PCR product were electro phoresed on an 8%nondenaturing polyacrylamide gel and visualized with Wlight after staining with ethidium bromide. We excluded loci from further use if they were monomorphic after an initialscreening of 20 individuals. All polymorphic loci were scored for all sampled individuals. We tested for agreement with Hardy-Weinberg (H-W) expectations using chi-square statistics. Observed heterozygosities were calculataesdt he number of heterozygotes observed divided by the total number in a sample. Expected heterozygosities were determined from sample allele frequencies by assuming H-W frequencies for the genotypes.

Advances in Environmental Biology, 8(7) May 2014, Pages: 3151-3157

Estimation of effective population size: To estimate Ne, we used the following equation,
&= t / [ 2 ( P - 1 / s + ( l / N ) ) I , (1)
or the following,
Ne= t / [ Z ( P - l / S ) I , 121
Depending on the type of sampling that was done. For intervals beginning with 1961, Equation 1 was appropriate because

Sampling was nonlethal and therefore individuals had the opportunity to contribute to future generations (plan I,WAPLES 1989). The appropriate equation for the interval1977-1993 was not so evident. The sample from 1977 was collected lethally so these fish no longer contributed offspring( ie., Equation 2 following plan 11, WAPLES 1989). Many of

These fish were of reproductive age, however, and would have spawned in earlier years. For the interval 1977-1993, we estimated Ne using both equations and compared results. In addition to estimating the standardized variance of allele frequency change, (F), w e needed to estimate census population size ( N ) at time 0 and the number of generations between samples (t). Sample sizes (S) were the harmonic means of sample sizes at time 0 and t.

Estimation of F: Several methods of computing P have been proposed. WAPLES found that the measures 1', (NEIand TAJIMA 198) 1a ndFk led to similar results. We also found that the two methods led to similar results We therefore present results based on Fk only. POLLAKm'Seasure for one locus is where L is the number of alleles and the X's are the frequencies of allele i ( i = 1, 2, . . . L) at sampling times 0 and t. For multiple loci? we computed weighted means of single locus P values ash=Z(I+- l)Fkj/Z(L,- l),where the j's index the different loci ( NEI and TAJIMA19 84).Estimation of N: Estimates of the adult census size (N) were based on data collected by fisheries managers at Lake Escanaba. Unfortunately, the needed population estimates were not made in all years. Kempincer *et al*. and Kempinger and Carline reported population estimates for fish56 cm (hereafter designated W ) and for fish of all sizes susceptible to the sampling gear (N) based on mark-recapture methods for the years 1958-1972. The WDNR made population estimates of N* for all years since 1980. They also recorded harvests (N') of fish in all years including 1964-1972, when a 56 cm minimum size limit was in effect (S.NEW, personal communication). We indirectly estimated N for all years by two methods. First, we applied the average ratio of NN* from the years 1958-1972 to records of W from 1980-1993. We then took the average N and assigned this value to the years during which no population estimates were made, 1973-1979. Next, we applied the average ratio of NN' from the years 1958-1963 to records of N' from1973-1993. Average N was then assigned to the years the size limit was in effect, 1964-1972. We used the averages from these two methods as population estimates of N for the years Estimation of t h number of generations in an interual: Generation length for populations with overlapping generations is equal to the mean age of parents. We approximated this through a weighted mean age of spawners. The number of females in each age class was weighted by a value to account for their relative fecundity. Because fecundity increases regularly with growth in northern pike (SPmovSKAYA and SOLONINOV1A98 4), the youngest spawners were assigned a value of one and each older age class was given a weighting value equal to its proportional increase in size, as determined by mean weight. Male contribution was assumed equal across age classes; thus all males were weighted equally. We then took the average of these weighted values from the three sampling years. Finally, we divided the number of years separating samples by the estimate of generation length to determine the number of generations in an interval. Determination of confidence intervals: The 1 - a confidence limits for Fare.

(1 - a) Confidence interval for F1973- 1993.where n is the number of degrees of freedom associated with P[n=):(number of all elesper locus- l) andx2a/2[n] is the critical a / 2 chi-square value for n degrees of freedom( WAPLES1 989). The confidence limits obtained from Equation4 were used in place of Pin Equation l or 2 to determine the confidence interval for Ne.

*Results:*

Microsatellite isolation: In a first round of genomic library screening, we isolated a total of 14 positive clones from -4000 colonies. From these clones we developed nine pairs of primers for PCR amplification experiments. In screening a second library, we isolated nine positive clones from 990 colonies and developed six new pairs of PCR primers. Because this second library was size-selected we were able to estimate the number of ACn microsatellites in the northern pike genome. The average size of an insert was 625 bp. Thus, we screened -990 X 625 = 619 kb,or 0.024% of the northern pike's estimated 2600 Mbp genome. By assuming that the partial library was representative of the entire genome, we estimated that the northern pike genome contains38,000 A Cnmicrosatelliteso, r one approximately every69 kbp if they are evenly distributed throughout the genome. The rate of occurrence of ACn microsatellites was threefold higher in brown trout (Salmotmtta) and over ninefoldhigher in Atlantic cod (Gudhusmmhuu) (every 7 kbp.

Seven (47%) of the 15 microsatellite loci that we studied were polymorphic in the northern pike population of Lake Escanaba (designated as Elu, for E. lucius, followed by a locus identification number: 19, 37,

**Advances in Environmental Biology**, 8(7) May 2014, Pages: 3151-3157

51,76,78,87, and 276)C. ore sequences and sequences of the PCR primers for these polymorphic loci are reported.

In Table 1 with three of these repeated from. Comparisons of polymorphic and monomorphic loci showed that their cores equences had similar characteristics (Table 2). Each group had similar numbers in all repeat classes, as categorized by WEBER. Polymorphic loci had greater average numbers of repeats than monomorphicloci (28 us. 25 for the total number of repeats and 26vs. 20 for the longest continuous run of repeats), but their ranges overlapped substantially. We tested segregation at polymorphic loci using apedigreed population maintained at the University of Minnesota. All tests that we could conduct indicated that the loci were inherited in a Mendelian fashion (data not shown). Using chi-square tests, we found agreement between observed and expected genotypicratios of 20 offspring of parents who were variable at the Elu37, Elu76, and Elu276 loci (for each, P >0.05). Although these results were encouraging, it should be noted that our small population limited our ability to conduct powerful tests of or deviations from Hardy-Weinberg equilibrium. We previously reported that low microsatellitevariability in the pedigreed population prevented us from conducting segregation analysis at lociElu78 and Elu87. TheElu19 locus from the second library screening was also monomorphic in the pedigreed population. Analyses of additional pedigreed northern pike;' when possible, will be useful for confirming the Mendelian inheritance of these loci. Genetic variation: We determined allele frequencies.

**Table 1:** Characterietics of seven microsatellite loci found to be polymorphic in the Lake Escanaba northern pike population.

| Locus | Core sequence | PCR primer sequences (5'–3') | Alleles (bp) |
|---|---|---|---|
| Elu19[a] | $(AC)_{23}Ag(AC)_3$ $AAT(AC)_3$ | CATCATgAACATTCAgACgC gAgATgCTAATTCATCCACTg | 155, 149, 147 |
| Elu37[b] | $(AC)_{32}(AT)_5$ | ggCTACTCCAgAACCTTCCC CAAATTTTATgACCAgCACC | 150, 138 |
| Elu51[c] | $(AC)_{16}$ | gtgggCATTCAgCCgATATAgC CTgTCTCATTACTgCCTggCTC | 125, 123 |
| Elu76[a] | $(AC)_{17}$ | ACCACATTCCACATCTgATgg AATCCCTTATTCTgACCCTgC | 167, 165 |
| Elu78[a] | $(AC)_{13}$ | CTAgAgggggAAAACAAACC CACTgTCCATCATCACCCCTCTC | 136, 132 |
| Elu87[c] | $(AC)_{20}(N)_{14}$ $(AC)_4TT(AC)_5$ | AgCACTgCCACACATgACgTg CCAgCTgCCTCAgATTgCTCCCC | 161, 157, 153 |
| Elu276[a] | $[(CT)_2(gTCT)_3]_4$ $(CT)_2(gTCT)_2$ $(CT)_2(gTCT)_4$ | CTgTCACAgTTCAAAgATggC TCTTTAAACTgggggggAggAAAg | 165, 149 |

[a] PCR conditions: annealing temperature = 58°, 30 cycles.
[b] Annealing temperature = 60°, 27 cycles.
[c] Annealing temperature = 63°, 25 cycles.

At the seven polymorphic loci for the three sampling dates (Table 3) . Five of the seven loci had only two alleles each. The other two (Elu19 and Elu87) each had three alleles. We observed all of the alleles at ach sampling date, i.e., there were no new or lost alleles throughout the time period. The frequency of the most common allele at a locurasnged from 0.45 to 0.99, with most values (14 of 20) > 0.70. Alleles at the Elu51locus could not be resolved in the samples from 1961because of the poor quality of the PCR products. The bands on the acxylamide gels were often blurred, which made the similarly sized products (2bp difference). Difficult to distinguish. After attempting to score -40samples, of which the majority were blurred, we decided to discontinue use of Elu51 for this year's samples. Alleles that are difficult to resolve are not uncommon with microsatellites. Our findings suggest that sample age, or presumably DNA quality, can affect the ability.

To resolve alleles in a locus-specific manner. Genotypes at all loci were in agreement with Hardy-Weinberg (H-W) expectations except for Elu276 in1961. The Elu 276 locus included a homozygous genotype with zero observations and an expected value of6.4 in the 1961 sample; for such a situation, the chisquare test may be inappropriate. Therefore we used bootstrap resampling (EFRON and GONG 1983), which yielded P = 0.001. This locus was in agreement with HW expectations on the other two sampling dates. When averaged across all sampling years, observed heterozygote frequencies ranged from 0.11 to 0.62 per locus. When averaged across all polymorphic loci with in a sampling year (excluding Elu51 because the 1961data were missing), there were changes among years. Average observed heterozygosity decreased between1961 (0.41) and 1977 (0.32), but it increased slightly from 1977 to 1993 (0.33). Comparable expected heterozygosities, based on allele frequencies, were 0.35,0.30, and 0.32.Sample allele frequencies changed between years at all loci. We determined changes for three time intervals:1961-1977, 1977-1993, and the entire interval, 1961-1993. Magnitudes of change varied greatly among alleles and often among time intervafolsr the same allele. In the intervals 1961-1977 and 1977-1993, we found.

**Table 2:** Comparisons between polymorphic and monomorphic loci.

| Locus type | No. in repeat class | | | No. of repeats | |
|---|---|---|---|---|---|
| | Perfect | Imperfect | Compound | Total | Continuous |
| Poly | 3 | 2 | 2 | 28 (13–56) | 26 (13–56) |
| Mono | 2 | 4 | 2 | 25 (15–44) | 20 (6–44) |

Repeat classes correspond to those described by WEBER (1990). Number of repeats is reported as the total number in the entire core sequence (total) and as the longest stretch of uninterrupted repeats (continuous), with range in parentheses.

**Table 3:** Observed allele frequencies and heterozygosities at seven microsatellite loci m the northern pike population of W e Escanaba, W wonsin.

| Locus | Allele (bp) | 1961 | 1977 | 1993 |
|---|---|---|---|---|
| *n* | | 86 | 110 | 72 |
| Elu19 | 155 | 0.27 | 0.20 | 0.13 |
| | 149 | 0.24 | 0.25 | 0.24 |
| | 147 | 0.49 | 0.55 | 0.63 |
| H. obs. | | 0.69 | 0.64 | 0.53 |
| Elu37 | 150 | 0.85 | 0.87 | 0.86 |
| | 138 | 0.15 | 0.13 | 0.14 |
| H. obs. | | 0.30 | 0.23 | 0.25 |
| Elu51[a] | 125 | | 0.65 | 0.60 |
| | 123 | | 0.35 | 0.40 |
| H obs. | | | 0.43 | 0.44 |
| Elu76 | 167 | 0.85 | 0.99 | 0.96 |
| | 165 | 0.15 | 0.01 | 0.04 |
| H obs. | | 0.24 | 0.03 | 0.08 |
| Elu78 | 136 | 0.90 | 0.81 | 0.86 |
| | 132 | 0.10 | 0.19 | 0.14 |
| H obs. | | 0.20 | 0.33 | 0.19 |
| Elu87 | 161 | 0.10 | 0.08 | 0.06 |
| | 157 | 0.77 | 0.72 | 0.76 |
| | 153 | 0.13 | 0.20 | 0.18 |
| H. obs. | | 0.45 | 0.45 | 0.40 |
| Elu276 | 165 | 0.73 | 0.87 | 0.67 |
| | 149 | 0.27 | 0.13 | 0.33 |
| H obs. | | 0.55 | 0.25 | 0.5 |
| Average H[b] | | 0.41 | 0.33 | 0.34 |
| Without Elu51 | | 0.41 | 0.32 | 0.33 |

H obs., heterozygosities.
[a] We could not determine allele frequencies at locus Elu51 in 1961 because of difficulties in interpreting gels.
[b] Average heterozygosity does not include the nine loci that were monomorphic.

The greatest changes in the frequencies at the Elu276locus (0.14 and 0.20, respectively), but an allele of theElu19 locus had the greatest change from 1961-1993(0.14). The smallest changes occurred in the interval1961-1977 at a different allele of the Elu19 locus (0.01) and at this allele and those of Elu37 in the intervals1977-1993 and 1961-1993 (0.01 for all). Many alleles changed frequency in one direction in the first time period but shifted back toward the earlier frequency during the second period. In fact, the average magnitude of change was greatest from 1961 to 1977, rather than over the longer interval of 1961-1993.

Effective population size: We made three estimates of effective population size based on temporal changes in allele frequencies observed in the first (1961-1977) and second (1977-1993) time intervals, and over the entire interval (1961-1993). We also estimated Ne for the entire interval based on the sum of P s for the first two intervals, weighted by the number of independent alleles contributing to each estimate. For this case, the sampling correction in the denominator includes the terms 1 / S and 1 / N (where appropriate) for both sampling periods. When the number of alleles is equal, this estimate is the harmonic mean of the two component interval estimates of Ne.

*Discussion:*

The use of fish scales has given us the rare opportunity to examine historical trends in genetic diversity within a natural population. We estimated effective population size, a predictor of a population's rate of loss of neutral genetic variation, from temporal changes in allele frequencies at micro sateilite loci. Use of a historical tissue collection provided two benefits: (1) it increased the precision of our estimates by allowing us to increase our sampling interval and (2)it let us apply an equation developed for discrete generations without correcting for demographic parameters(JORDE and RYMAN 1995). Our estimates indicated that the study population of

northern pike has persisted at an Net hat was possibly as low as 48 over 3 0 year period, which implies that it lost as much as 8% of its heterozygosityint hat time. The relatively high levels of heterozygosity that we detected may be the remnants of highheterozygosity in the source population (s) used for

Stocking Lake Escanaba. This heterozygosity will, of course, continue to decline if the population remains closed. The importance of this loss to the sustainability of the population is uncertain. We suggest that the analysis of historical effective population size, made possible using procedures like ours, provides a means to compare population changes with rates of loss of genetic variation. Certain fish species, especially freshwater tiynpheas biting lakes, may comprise a large part of the group of species for which this type of study is possible because of the ability to identify closed populations and the presence of historical tissue collections. Fisheries. management agencies and research institutions have routinely collected fish scales as a means to age fish and

Examine growth characteristics. There may be no other taxa for which tissue from large numbers of individuals has been saved on a population basis. With the advent of PCR, however, tissue collection is often much easier and wise sampling now ( eg., bird feathers, hair samples;ELLECREN1 992) may make long-term, PCR-based genetic studies possible in other species.

Advantages of historical sampling: For estimating effective population size by the temporal method, the ability to examine samples taken several generations a part provided us two important advantages. First, because of the larger number of generations between samples we were able to make estimates with reasonable precision (WAPLES 199) 1. Second, we could apply disCrete generation formulas (Equations 1 and 2) without making demographic corrections as in JORDE and RYMAN( 1995). With overlapping generations, JORDE and RYMAN showed that changes in allele frequencies between cohorts are a function of Ne and age-specific survival and death rates. Based on simulation results, however, they showed that Ne could be estimated directly from allele frequency changes with little bias when intervals one generation (Table 1, JORDE and RYMAN 1995).

Possible bias: An important source of potential bias is the estimate of generation length. Because generation length, t, appears alone in the numerator of Equations4 and 5, bias in an estimate of Ne is directly proportional to the error in estimating t. For estimates of t, we weighted age frequencies of spawners by a measure of relative fecundity and then used the average from the three sampling dates. The weights certainly had some error associated with them. As shown in RESULTS, estimates of t would have been 4.3 if we did not weight ages, and 3.8 if we weighted all fish rather that just females. It is also likely that the average age of parents fluctuated among generations. Values for the three years we sampled ranged from 3.8 to 4.4. It is unlikely, however, that bias arising from estimates of t would significantly alter our conclusion that both Ne and Ne: N were low in this population. The greatest change resulting from the comparison values above would be a10% increase in N e .

Differing estimates for the intervai 1961-1993: Estimates of Ne for the interval 1961-1993 differed by a factor of 2.5, depending on whether the estimate was based on allele frequency changes from years 1961 to1993 or on the combined changes observed from 1961to 1977 and 1977 to 1993. Althougthh e estimates of Nediffered, their confidence intervals overlapped considerably, with the lower 95% limit of the higher estimate( 29) being less than the lower estimate (48) .Use of the discrete generations model on a population with overlapping generations may have contributed to the difference in estimates. In simulations of populations with overlapping generations, JORDE and RYMAN ( 1995) found that the discrete generations model overestimated true Ne by -50% when samples were taken one generation apart but by only 10% when the samples were three generations apart. Samples taken at longer intervals should produce estimates that more closely approach true Ne. Therefore, estimates based on an interval of eight generations should be lesssensitive to population structure than estimates based on four generations. Although the above argument favors the estimate based on the entire interval, 1961-1993, there is also support for the mean estimate of the shorter intervals. If the changes we observed at relatively few loci were by chance not typical of changes at all loci, then ignoring the intermediate sample resulted in loss of important information on the dynamics of allele frequency change. We may have been observing the realconsequences of the lower estimated N, over the short intervals and the stochastic drift of allele frequencies back toward their original values ovetrhe long interval. The above considerations do not answer the question of which estimate to use for the entire time interval, but they do illustrate an important point. Even though the use of historical collections allows increased precision by increasing generation interval, the importanceof examining a reasonable number of loci should not be overlooked. For some fish species the development of primers for many. microsatellite loci does not make this alimiting factor. The expected magnitude of Ne is an important guide in determining the best study design.WAPLE(S 1 989) has noted that the temporal method is best suited for the study of small populations. As Neincreases, the signal from sampling error quickly overwhelms that from drift. Therefore, if Ne is expected to be moderately large (severahlundred), the number of loci, the number of generations between samples, and sample size should all be as large as possible. Researchers need to use caution in accepting lower numbers ofloci and sample sizes in trade for easily increasing the generation interval by historical sampling. kssumpti~of~ t he model: Assumptions of the temporal method are that the loci are selectively neutral, a closed population, no mutations during the sampling period, and random sampling of the population(WAPLE1S9 89). All assumptions were likely met in this study.

**Advances in Environmental Biology**, 8(7) May 2014, Pages: 3151-3157

## REFERENCES

[1] Brighitte, J., M. Hansen, V. Loeschcker, 2005. Microsatellite DNA analysis of northern pike (Esoxlucius) populations: insights into the genetic structure and demographic history of a genetically depauperatee specious. Biology Journal of Linnaean Society, 84: 1-11.
[2] Bruzan, P., M. Luczynski, P.A. Kuznlar, 1998, Mitochondrial DNA in two samples of northern pike, Esoxlucius, AqualtherReasearch, 29: 521-526.
[3] Lin, Y.S., Y.P. Poh, S.M. Lin and C.S. Tzeng, 2002. Molecular techniques to identify freshwater eels. Zoological Studies, 41(4): 421-430.
[4] Nei, M., 1972. Genetic distance between populations.American Naturalist, 106: 283-92.
[5] Nei, M., 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics 89: 583-590.
[6] Sambrok, J., E.F. Fritsch and T. Maniatis, 1989. Electrophoresis of RNA throughgelscontaining formaldehyde: Molecular Cloning, 2nd edn. Cold.