Background on lake sturgeon biology and management related to Lesson 10 on population variability in allele frequency and assignment testing

Introduction

Factors related to natural rates of straying among rivers - The tendency for individuals to return to spawn in natal streams is of ecological and managerial importance. For example, in Pacific salmon the tendency to stray (i.e., disperse to a stream other than an individual's stream of origin to spawn) is important for the colonization of new or previously extirpated streams. Straying does not necessarily result in gene flow to the destination population because immigrants may not breed or may be selected against based on pre-zygotic (population differences in mate preference) or post-zygotic (e.g., reduced offspring fitness). outbreeding depression) factors. Source-sink dynamics where population numbers are sustained by immigration of individuals from other populations can result in stable populations over generations. Conservation hatchery programs often adopt strict rearing and release policies to facilitate imprinting to natal waters to minimize straying. In locations where limited harvest of numerically depressed species is permissible, regulations often are established based on assessments of risk associated with potential harvest of individuals from numerically less abundant, non-target populations that may have strayed into the population subjected to legal harvested.

Many variables potentially affect the likelihood of individuals straying. Natal philopatry is observed in many fish species, and is believed to result from responses to site-specific olfactory signals detected during migration to spawning areas. Demographic parameters such as gender, age, and population size also may influence probabilities of straying. Individuals originating from populations that are temporally and spatially reproductively isolated have lower expectations of straying relative to geographically proximal populations that spawn at similar times. Habitat quality has the potential to affect the tendency of individuals to stray from a particular stream whether differences in quality are of anthropogenic or natural origin. Fish produced in hatcheries have been shown to stray more than their wild counterparts; however, juveniles that are properly imprinted to target streams are generally more likely to home.

Straying related to supplementation of populations – Increasingly, populations of lake sturgeon are being supplemented using juveniles reared in hatcheries. Fishery managers are increasingly aware of the importance of utilizing fish that were produced from their streams of origin or from near-by streams. Genetic policies recommended in the Great Lakes are described by in a publication by Walsh et al. (2010). A pdf copy can be down-loaded from a link in the Genetics Section of the Great Lakes sturgeon web site. In previous decades, stocking was routinely conducted without consideration of source and recipient population and lake sturgeon were transplanted widely between rivers, even between populations of different lake basins.

Methods used to quantify straying rates and movement patterns have traditionally included telemetry and capture-mark-recapture. Tag loss detracts from the usefulness of direct tagging methods when estimating population size and straying rates. Additionally, certain species have life history characteristics that make the implementation of direct straying estimators difficult. For instance, species with long generation times require long-term studies that extend until first reproduction occurs. Extended inter-spawning intervals and long distance

migrations between reproductive episodes also present challenges for collecting adequate numbers of recaptures from physical tags for accurate assessments of movements.

Molecular techniques can be used when opportunities to employ direct tagging studies are limited or impractical. Indirect genetic methods have been implemented successfully to quantify rates of straying. If sufficient levels of genetic variation exist between populations, samples obtained from a single capture can be used to determine an individual's population of origin based on individual assignment testing.

Applications of genetic techniques for species such as lake sturgeon are especially important to elucidate information on straying due to aspects of the species' ecology. Lake sturgeon are a long-lived potamodromous fish species that reach sexual maturity in 15-25 years, depending on sex. Through time, natal philopatry has resulted in genetically distinct lake sturgeon populations throughout the Great Lakes and have facilitated the use of genetic markers as a means of detecting individuals occupying non-natal habitats.

Scenario for Lesson 10

During the 1980's gametes were taken from the Wolf River drainage in Wisconsin and offspring were released into Burt and Mullett Lakes in Michigan. During the same period gametes were taken from adults in the Cheboygan River in Michigan and offspring were also released into Burt and Mullett Lakes. During gill net surveys, biologists collected a number of fish of a size that was consistent with stocking during the period when Wisconsin fish were released. Biologists were interested in determining evidence for differential suvival of Wisconsin and Michigan stocked fish. Since the appearance of Wisconsin and Michigan fish don't differ, they requested a genetic analysis of these individuals.

Students are provided an Excel spreadsheet with genetic data at 12 microsatellite loci for 32 individuals from the Wolf River population from Wisconsin and the Cheboygan River population in Michigan. These are real data from actual fish genotyped in the Scribner lab. We have made up data for Mullett Lake fish. There are several worksheets in the spreadsheet. One worksheet contains information on the exercise and poses several questions for the student to answer. Another worksheet contains the actual genotype data that the students will use for the exercise. Two other worksheets contain summaries of allele counts by locus for each population. There is an example loci where we show how allele frequencies can be estimated. The background pdf files contain other background information for estimation of other summary measures of diversity for the Fox and Cheboygan River populations.

Methods

Sample collection – Adult lake sturgeon (N=32) were sampled from Wisconsin tributaries to Lake Michigan including the lower Fox River (Fig. 1). Spawning adults (N-32) were also captured from the Cheboygan River drainage. Individuals were selected for analysis based on their physical presence at or near spawning areas in each river during the spawning season (April 15 through June 15). Upon capture, a 1 cm^2 portion of the dorsal or caudal fin was collected from each individual for genetic analysis and stored in uniquely marked scale envelopes. Sex and maturity status were not apparent for all fish at the time of sampling. Consequently, only individuals of at least the minimum expected size at sexual maturity for either sex (> 110 cm) were included in analyses.

Laboratory analysis

DNA was extracted from fin tissue using QIAGEN DNeasy kits (Qiagen, Inc., Valencia, CA) according to manufacturer's specifications. DNA was quantified using a NanoDrop spectrophotometer (NanoDrop Technologies, Thermo Scientific, Wilmington, DE) and diluted to a concentration of 20 ng/μL. All individuals were genotyped at twelve microsatellite loci: *AfuG68* (May et al. 1997), *Afu68b* (McQuown et al. 2002), *Spl120* (McQuown et al. 2000), *Aox27* (King et al. 2001), *AfuG9*, *AfuG160*, *AfuG63*, *AfuG74*, *AfuG204*, *AfuG195*, *AfuG56* and *AfuG112* (Welsh et al. 2003). Microsatellite polymerase chain reactions (PCR) were conducted in 25 μL volumes containing 100 ng of template DNA, 2.5 μL of 10X PCR buffer (1 Mtris-HCl, 1.5 MMgCl2, 1 MKCl, 10% gelatin, 10% NP-40, and 10% triton X), and 0.8 mM deoxy-nucleotide-triphosphates (dNTPs), 10 pm fluorescently labeled forward and unlabeled reverse primers, sterile water, and 0.5 U Taq polymerase. PCR was performed using Robocycler 96 thermocyclers (Stratagene, Inc., La Jolla, CA). PCR was performed under conditions detailed in Homola et al. (2010). Amplified PCR product were visualized on 6% denatured polyacrylamide gels using an FMBIO II scanner (Hitachi Software Engineering Co., Ltd., Yokohama, Japan). Allele size was determined by comparison to lake sturgeon samples of known genotype and based on molecular size standards. Genotype scores were confirmed by independent scoring by two experienced laboratory personnel.

Statistical analysis

Students will select 2 loci and

(a) The students will review all data for all 12 populations and select the loci that are most 'informative' or which exhibit the greatest difference in allele frequency. The students will estimate allele frequencies at two loci for the Fox and Cheboygan River populations. The students will preform a chi-square test to see if allele counts differ significantly
(b) The students will provide summaries of genetic diveristy (numbers of alleles and observed heterozygosity.
(c) For each of the 5 Mullett Lake fish, the students will estimate the 'expected' likelihood of observing each fish in each of the spawning populations. Using the population estimates of allele frequency the students will derived, the expected frequency of a heterozygous genotype is 2 times the frequency of each alleles. The expected frequency of a homozygous genotype is the square of the frequency of the allele. The students will estimate the likelihood of origin for each locus for each population. The students will estimate the 'confidence' in assignment decisions using 1 and 2 loci. The students will draw conclusions about the sampling requirements to achieve higher confidence in their decisions.
(d) The students will discuss the implications of their findings for fish managers.