Are Craig Herring Reproductively Isolated from Sitka Sound Herring?

By Tyler Lantiegne and Kris Thorsteinson
Petersburg High School, Petersburg AK

Introduction

In this project, my partner and I are looking at different spawning clusters of herring and comparing them to determine if the clusters intermix reproductively. The pacific herring is a schooling fish, with schools numbering into the thousands; these schools stick together over long periods of time, so if there is any interbreeding there can relatively fast genetic change in a population. After comparing the sequences of DNA between Craig herring and Sitka Sound Herring, we believe that the herring populations will have reproductive intermixing and that the sequences of the DNA will show this.

Wildes et al. (2011) found that the allele frequency at various microsatellites varied slightly between spawning populations, with more of a difference noted between coastal and inner water populations. Between different regions, such as Alaska and British Columbia, had very slight genetic differentiation at various loci (Liu, et al, 2011). The Alaska Department of Fish and Game annual stock sampling found that the percentage of recruitment of age-3 herring was higher at lower latitudes, and lower and higher latitudes (Hebert, 2012). In our continuation of Sharon Wildes's study, we will be adding sequences at our two microsatellites for the spawning population of herring in Craig and offering values to help further these hypothesizes.

Life History

Pacific Herring, (*Clupea pallasii*), are a foragefish from the family Clupidae. This species is found throughout the Northern Pacific in both open and coastal waters. The herring aggregate in sheltered bays and sounds every year in early spring. Pacific Herring prefer to spawn on eelgrass, and release billions of gametes into the water column upon spawning (Funk, 2007). After an incubation period of 6-10 days in favorable conditions, the Pacific Herring once hatched quickly uses up its yolk sac and begins a period of high caloric intake until it reaches adulthood (Funk, 2007). The Pacific Herring has a homing instinct, which causes Pacific Herring to spawn again where they were born (Barnhart, 1988).

Purpose

In this project, my partner and I are looking at different spawning clusters of herring and comparing them to determine if the clusters intermix reproductively, a continuation of the study done by Wildes et al. (2011). We looked at 2 microsatellites, CPA 125 and CPA 134 from a population sample of Pacific Herring from the Craig area. Based on Wildes's and Herbert's findings, our hypothesis is that Craig and Sitka Sound would have adifference in allele frequencies at the two microsatellites because they both are on outer coastal areas and because Craig and Sitka are on either side of the latitude break described by Hebert (2012).

Procedure

Bait herring (C. pallasii) were obtained from the Craig, AK area, and we collected .1 to .2 gram of heart tissue from fifty samples (Figures 1 and 2). We extracted DNA with the following Promega Wizard DNA Purification Kit protocols (Promega). We deviated from protocols by straining out tissue in some samples because the tissue was not fully digested. PCR was conducted using CPA 125 and CPA 134 microsatellites (Miller, 2001) the CPA 125 forward sequence was 5' GCA AGA AAG AGC AGA 3' and the CPA 125 Reverse sequence was 5' GTT TCG ACT CAA CAG CTG GAA 3'. The CPA 134 forward sequence was 5' CAT TCT CTA CAA AGG GCA TAT and the CPA 134 reverse sequence was 5' GTT TCA TAC CAT TGA ATC CAG CTA (Miller, 1990).. For PCR, we used GE Healthcare PCR beads with 5 microliters of DNA template and a 0.5 uM concentration of forward and reverse primers. Microsatellites were amplified using a Bio-Rad thermocycler (Table 1).

PCR was size fractionated on a capillary sequencer at the UAF CORE Lab. Sharon Wildes provided the data for microsatellites for Sitka Sound Herring, and our final analysis of Craig Herring data was conducted manually viewing peaks with Peak Scanner software.

Table 1: Thermocycler program for Cpa125 and Cpa134.

	CPA 125	CPA 134
initial denature	10 minutes 95 degrees C	10 minutes 95 degrees C
denature	2 minutes 94 degrees C 2x	2 minutes 94 degrees C 2x
anneal	30 seconds 58 degrees C 2x	30 seconds 54 degrees C 2x
elongate	45 seconds 75 degrees C 2x	45 seconds 70 degrees C 2x
second denature	2 minutes 94 degrees C 38x	2 minutes 94 degrees C 38x
second anneal	30 seconds 58 degrees C 38x	30 seconds 54 degrees C 38x
Second elongate	45 seconds 75 degrees C 38x	45 seconds 70 degrees C 38x
final elongation	10 minutes 72 degrees	10 minutes 72 degrees C 38x

Figure 1: Kris Thorsteinson Extracting Heart Tissue

Figure 2: Tyler Lantiegne and Kris Thorsteinson preparing PCR product.

Results

Allele richness in the Craig herring at locus Cpa125 was 27 and at Cpa134, 18 alleles. Mean heterozygote diversity for Cpa125 was 72.4% (N=21) and for Cpa134 75% (N=18). After Chi Square analysis, we determined that random mating did account for close agreement between expected and observed heterozygosity values within population. Looking at the fragment lengths after doing a Fisher Exact test on our values generated a P value of .0001 for the CPA 125 microsattelite and .1010 for the CPA 134 microsattelite

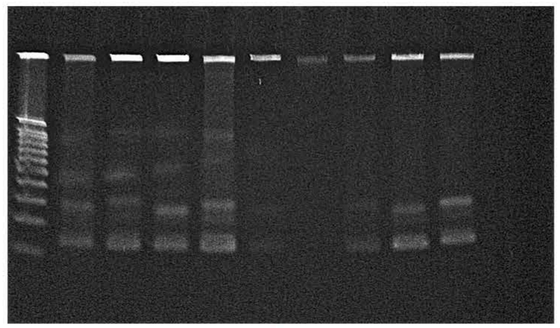
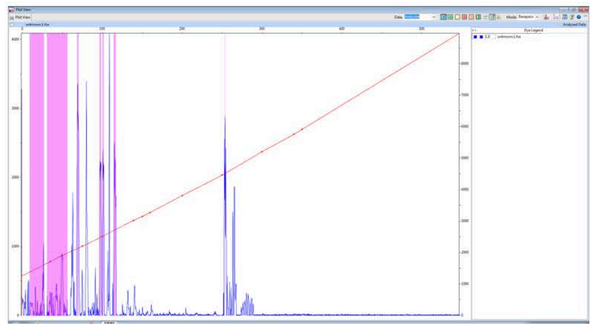


Figure 3: Polyacrimanide Gel of Cpa 125 PCR Product.



Conclusions

Our hypothesis was that the Craig Herring and Sitka Sound Herring spawning population would show a significant difference in the two microsattelites we tested due to the age structure difference between. After analysis with the Fisher Test we found that this was the case for one of our microsatellites, CPA 125. This means there was a significant difference between the Sitka and Craig herring alleles on one loci. This means that the CPA 125 locus was reproductively isolated between the two populations and the CPA 134 locus was then shared between the two. This could mean that the genes that code for the smaller herring size in Sitka Sound Herring could be on this allele, or that this difference in size is attributed to external environmental conditions.

In this study, our possible sources of error were PCR product found in the blank we ran, staining times, incomplete tissue digestion in some samples, and amount of PCR cycles. These sources could have changed the integrity of our extracted DNA and our PCR product, which then could have contributed to the amount of stutter and background noise found in our electropherogram readings.

From a management perspective, this information could elaborate on the size difference between Sitka Sound and Craig Herring, which then would give fisherman an answer to this size difference conundrum. Moreover, management decisions would be better informed. In order to be sure of this genetic origin we would have to undertake a larger study with more microsattelites and samples due to the polymorphism of Pacific Herring.

Literature Cited

Barnhart, R.A. 1988. Species profiles: life histories and environmental requirements of coastal fishes and invertebrates (Pacific Southwest) – Pacific herring. U.S. Fish Wildl. Serv. Biol. Rep. 82(11.79). U.S. Army Corps of Engineers, TR EL-82-4. 14 pp.

Funk, Fritz. "Pacific Herring". ADFG Wildlife Notebook Series. 2007.

Hebert, Kyle. 2012. Alaska 2010 herring stock assessment surveys. Alaska Department of Fish and Game, Fishery Data Series No. 12-46, Anchorage.

Miller, K.M., K. Laberee, A.D. Schulze and K.H. Kaukinen. 1990. Fisheries and Oceans, Canada, Science Branch/Aquaculture Division/Genetics Section, Pacific Biological Station, Nanaimo, B.C., Canada V9R 5K6, 2001. Web

Liu, Jin-Xian, Andrey Tatarenkov, Terry D. Beacham, Victor Gorbachev, Sharon Wildes, and John C. Avise. "Effects of Pleistocene Climatic Fluctuations on the Phylogeographic and Demographic Histories of Pacific Herring (Clupea Pallasii)." *Molecular Ecology* 20.18 (2011): 3879-893. Print.

U.S. Fish and Wildlife Service. 1983-19-. Species profiles: life historiesand environmental requirements of coastal fishes and invertebrates. U.S. FishWildl. Serv. Biol. Rep. 82(11). U.S. Army Corps of Engineers, TR EL-82-4.

Wildes, Sharon L., Johanna J. Vollenweider, Hanhvan T. Nguyen, and Jeffery R. Guyon. *Genetic Variation between Outer-coastal and Fjord Populations of Pacific Herring (Clupea Pallasii) in the Eastern Gulf of Alaska. Noaa.gov*. Noaa, 2011. Web. Oct. 2012

Wildes, Sharon. pers comm.