*SAMPLE ROUGH DRAFT*

Population Genetics to Determine Life History Strategy of Habitat Selection of *Lagodon rhomboides* in the Indian River Lagoon Near Fort Pierce Inlet

XXXXXXX X. XXXXXXX

4-29-2012

Indian River State College

Biological Sciences Department

BACKGROUND

*Lagodon rhomboides,* commonly known as the pinfish, is an inshore fish which is a member of the family Sparidae. This fish is found along the east coast of the United States from New England to Florida as well as in the Gulf of Mexico, and is one of the most abundant fish that live in these areas (Hansen 1965). They prefer to live in shallow subtropical waters and around estuarine and vegetated areas like seagrass beds (Levin et al 1997). Pinfish are able to tolerate low temperatures and have been found in temperatures lower than 6˚C (Bennett and Judd 1992). These fishcannot live in fresh water but endure very low salinities down to 5ppt (Carrier and Evans 1976). For this reason, they are able to survive in a wide variety of niches including areas of high freshwater discharge found throughout the Indian River Lagoon.

Pinfish are known to not be affected by habitat isolation events since they tend to occupy several different shallow water habitats (Meyer and Posey 2009). A study was done involving traps with the purpose of finding whether pinfish tend to return to the same areas throughout their lifespan (Potthoff and Allen 2003). However, throughout the course of this experiment not one fish was ever proven to return back to the same area. This indicates that the fish are not staying in the same locations and their genomes should be widely dispersed over many locations or this could indicate that the study methods were not proper for accuracy. For instance, it is possible that the fish become trap-shy and do not re-enter the traps (Irlandi and Crawford 1996).

Previous studies that characterize pinfish populations as having high emigration and immigration rates with lack of habitat isolation events indicate that if pinfish are sampled from six different locations they should have very closely related genomes without clumps of genetically similar fish (Meyer 2009). Also, the pinfish at opposite sides of the sampling locations should be no more genetically different than the fish sampled from within the same location. On the other hand, if Irlandi and Crawford’s (1997) research is true, then the fish collected from different locations will be more genetically similar in each location, with much more diversity found between locations. Studies in population genetics can be used to determine which of these two ideas are more accurate.

JUSTIFICATION

The purpose of this research will be to determine genome differences in populations of L. rhomboides. After the DNA samples are sequenced, they will be comparatively analyzed for percentage of identical genetic sequences. PCR and the use of restriction enzymes and gel electrophoresis will be utilized during the procedures. This data can be used to help determine the life history of habitat selection of *L. rhomboides*. Knowing this information can help to establish greater detail in the behaviors of *L. rhomboides* and similar fish which are able to thrive in different coastal marine estuarine areas.

PROPOSED METHODS

Fish will be caught from six locations which will include three locations to the north of the Fort Pierce Inlet and three to the south. Each location will be set in a GPS so the distance from the inlet can be later estimated. Five fish from each location will be caught using cast nets when possible or a fishing pole when necessary. Fish will be selected based on size (10cm fork length) and immediately put on ice. After collection, the fish will be filleted and skinned with a stainless steel knife on a polyethylene cutting board previously cleaned with deionized water between the slicing of each fish. Utensils will be cleaned with deionized water between fish samples to eliminate DNA contamination. Before being placed on ice, samples will be rinsed with deionized water and placed in labeled bags. The samples will be frozen and stored at -20˚C within twelve hours of collection until they are analyzed.

Samples will be removed from freezer and thawed for approximately thirty minutes at room temperature. Approximately 0.2g sample of each fillet will be placed into a 1.5 mL microcentrifuge tube (Formosa et al 2010). Meanwhile, a 32 mL of Proteinase K Digestion Buffer will be pre-warmed to 65˚C for five minutes in a water bath. For each sample, a solution with 200 µL of the 65˚C Proteinase K Digestion Buffer and 20 µL of Proteinase K will be prepared and the solution added to the 1.5 mL tube containing the sample. The tubes will be incubated for ten minutes in a 65˚C water bath. The tubes will next be microcentrifuged at 14,000 rpm for three minutes. After centrifugation, 150 µL of supernatant should be transferred from each tube to new 1.5 mL microcentrifuge tubes, being careful not to disturb or transfer any of the pellet. In the new tube, 490ul of sulfolan/Binding buffer (11.20 mL of 90% sulfolane and 5.95mL of Nucleic Acid Binding buffer) will be added bringing the total volume to 640ul. Each sample will be transferred into a DNA Binding Spin Cup. The samples will be microcentrifuged for one minute at 14,000 rpm, loading the DNA into the spin cup matrix. The filtrates will be discarded from each tube. Next, 500 µL of 1X High Salt Wash Buffer will be added to the spin cup and microcentrifuged at 14,000 rpm for one minute. The filtrate will be discarded from each tube. Next, 500 µL of 80% ethanol added to each spin cup and microcentrifuged at 14,000 rpm for one minute and this step repeated two more times, making sure to the filtrates are discarded between each step. Each spin tube will be transferred to a new 1.5 mL microcentrifuge tube. Next, 100 µL of Elution Buffer added directly to the fiber matrix of each spin tube and incubated at room temperature for one minute. The samples will be microcentrifuged at 14,000 rpm for one minute. A nano drop spectrophotometer will be used to measure the concentration of each sample and the samples will be stored at -20˚C until ready to run PCR.

In a 1.5 µL microcentrifuge tube 350 µL of sterile H20, 437.5 µL of Green Go Taq PCR solution, and 87.5 µL of Primer Mix will be combined, vortexed for ten seconds on high, and microcentrifuged at 14,000 rpm for one minute. Next, 24 µL of the PCR mixture will be combined with 1 µL of each 50 ng/µL sample of pinfish DNA in separate PCR tubes. Fresh pipette tips will be used for each DNA sample to avoid cross contamination. One negative sample with 1 µL of sterile H20 being added instead of DNA will be included. Each of the tubes will be sealed, vortexed on high for ten seconds, and microcentrifuged at 14,000 rpm for one minute. PCR will be run for one cycle of 95˚C for five minutes; forty cycles of 95˚C for 30 seconds, 50˚C for 30 seconds, and 72˚C for 30 seconds; and finished with one cycle of 72˚C for seven minutes. The remainder of the 50 ng/µL DNA samples will be sent to the genomics lab to be sequenced.

For each PCR reaction three 0.5 mL tubes will be prepared, one for each restriction enzyme: DdeI, HaeIII, and NIaIII. For the DdeI reaction, 52.5 µL of sterile H20, 17.5 µL of 10X Ddel Buffer, and 17.5 µL of 10X Ddel enzyme combined, vortexed for ten seconds on high, and microcentrifuged at 14,000 rpm for one minute (Formosa et al 2010). Next, 2.5 µL of this solution will be added to each tube labeled Ddel. These steps will be repeated with the HaeIII Buffer and enzyme and the NIaII Buffer and enzyme. Next, 2.5 µL of each PCR reaction sample will be added to the appropriate labeled tubes. The digestion tubes will be incubated at 37˚C for two hours in a thermal cycler, which can be left over night at 37C. The reactions will be incubated at 65˚C for fifteen minutes in a thermal cycler. Next, 1 µL of 60 mM EDTA will be added to each tube and vortexed for thirty seconds on high. Three 1% agarose gel solutions (combine .5 g of agarose with 50 mL of TAE Buffer). The 1% agarose solution plus 2.5uL of ethidium bromide will be poured into the gel slot and two 20 well combs added to the gel. Once the gels are solidified the gels will be loaded with 10 µL of each pinfish DNA sample along with one DNA Marker in each row. Gels will next be run on 120 V for 30-45 minutes. There will be a comparative analysis of the sequenced genome of each of the fish sampled with the gel electrophoresis data being used as an alternate comparison.

BUDGET

|  |  |
| --- | --- |
| Gas | $50 |
| Cast net | $16.95 |
| Hooks | $4.23 |
| Shrimp | $3.17 |
| Ziplock bags | $3.18 |
| Ice | $5.30 |
| Stainless Steel knife | $21.19 |
| Polyethylene Cutting board | $11.65 |
| Deionized water | $21.44 |
| Microcentrifuge tubes | $66 |
| Proteinase K | $95.40 |
| 25 mL beaker | $18.02 |
| Nucleic Acid Binding Buffer | $33.92 |
| DNA binding spin cup | $10 |
| High Salt Wash Buffer | $10.60 |
| 80% ethanol | $42.24 |
| Elution buffer | $39.22 |
| DNase-free water | $58.30 |
| Sterile water | $25.44 |
| EDTA | $20.14 |
| Agarose | $89.04 |
| TAE buffer | $73.00 |
| Ethidium bromide | $35.62 |
| DNA marker | $66.80 |
| Total Budget | $770.85 |

Literature Cited

Bennett WA, Judd FW. 1992. Comparison of Methods for Determining Low Temperature Tolerance: Experiments with Pinfish, *Lagodon rhomboides*. Copeia. 1992(4) : 1059-1065.

Carrier JC, Evans DH. 1976. The Role of Environmental Calcium in Freshwater Survival of the Marine Teleost, *Lagodon rhomboides*. J. exp. Biol. 1976(65) : 529-538.

Formosa R, Ravi H, Happe S, Huffman D, Novoradovskaya N, Kincaid R, Garrett S. 2010. DNA-based Fish Species Identification Protocol. J. Vis. Exp. 2010(38) : 1871.

Hansen DJ. Food, Growth, Migration, Reproduction, and Abundance of Pinfish, *Lagodon rhomboides*, and Atlantic Croaker, *Micropogon undulates*. Near Pensacola, Florida, 1963-65. 1969. Fish. Bull. 68(1) : 135-146.

Irlandi EA, Crawford MK. 1997. Habitat linkages: the Effect of Intertidal Saltmarshes and Adjacent Subtidal Habitats on Abundance, Movement, and Growth of an Estuarine Fish. Oecologia. 1997(110) : 222-230.

Potthoff MT, Allen DM. 2003. Site Fidelity, Home Range, and Tidal Migrations of Juvenile pinfish, *Lagodon* rhomboides, in Salt Marsh Creeks. Env. Biol. Fish. 2003(67) : 231-240.

Levin P, Petrik R, Malone J. 1997. Interactive Effects of Habitat Selection, Food Supply and Predation on Recruitment of an Estuarine Fish. Oecologia. 1997(112): 55-65.

Meyer DL, Posey MH. 2009. Effects of Life History Strategy on Fish Distribution and Use of Estuarine Salt Marsh and Shallow-Water Flat Habitats. Estuar. Coast. 2009(32) : 797-812.