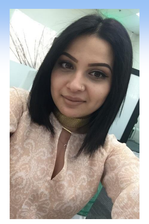

Is DNA Available for Analysis in Fry, Dead, or Preserved Convict Fish?

Yasamin Haider (STAR Fellow) and Dr. Ron Coleman (Research Mentor)

California State University, Sacramento *Department of Biology*

Introduction

Structure and relatedness of populations within the same species is crucial to understanding its evolutionary configuration. *Cryptoheros septemfasciatus* is a species of cichlid fish (family Cichlidae) that varies greatly from population to population where phenotypic differentiation is visible in many different rivers of Costa Rica. These variations are caused by the geography of the rivers, large-scale flooding, and tropical rainfall. The larger project being conducted aims to answer the questions of relatedness between the different populations of *C. septemfasciatus* in the many geographically close rivers of northern Costa Rica. To achieve this, fish samples must be taken from the different populations in the different rivers. My goal is to assist in creating and perfecting a protocol for DNA analysis using fish fry due to the fact that fry are much easier to capture and transport than adults. I aim to solve the following questions: do fry have enough DNA available for current methods of sequencing and analysis, and if so at what size is it possible to extract their DNA?

Figure 1: Male convict cichlid

Figure 2: Map of Costa Rica

Objectives

To answer the following questions:

1. Do cichlid fry have DNA that we are able to sequence for analysis?
2. If so, at what size fry is that DNA evident?
3. Can we obtain DNA from freshly dead, frozen, and preserved fishes?

Materials and Methods

Fry Collection and Preservation

Figure 3: Image of female convict with fry (indicated with arrows) in Tank 206

1. Set up my on tank of cichlids to spawn
2. Capture fry and collect fin clips
3. Preserve fry and fin clips in appropriately labeled 17x 60mm vial containing 95% EtOH
4. Store for DNA Extraction

Figure 4: Me cutting (a) and preserving (b) a fin clip for analysis

DNA Extraction

1. Conduct DNA Extraction on samples collected and stored
   a. Qiagen DNEasy Blood and Tissue Kit
2. Store properly for PCR

PCR

1. Conduct PCR
   a. Phusion High-Fidelity PCR Kit
   b. Primers used: Acit1F and Acit1R

Gel Electrophoresis

1. Create Gel using TAE buffer following appropriate gel protocol
   a. 2% gel (1g agarose in 50 ml of TAE)
2. Allow gel to harden with appropriate gel comb for samples
   a. 8, 12, and 15 combs
3. Load samples in wells and allow gels to run
   a. Around 110 Volts
4. Take UV image of gel using UV Transilluminator

Results

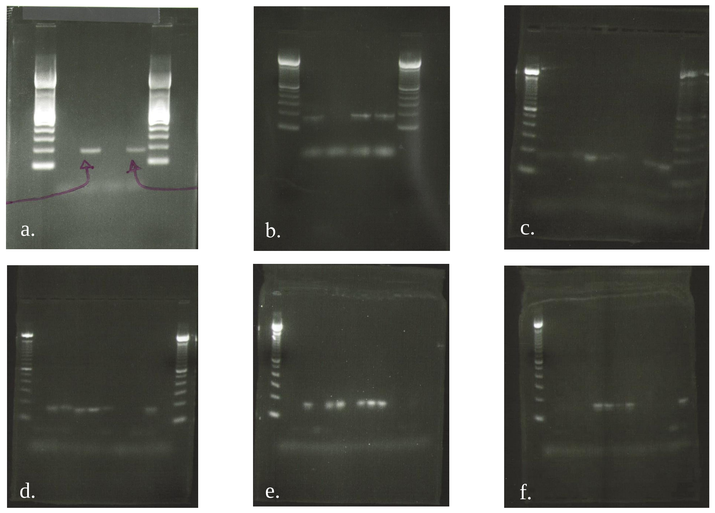

Figure 5: Gel images of all fry and fin clip samples. 5a is an image of samples P15, P21, 1.12, and 2.1 (left to right) where P21 and 1.12 were amplified. 5b is an image of the control samples (from left to right: CF2, CF1, CFC2, CFC1) where CFC1, CFC2, and CF2 were amplified. 5c is an image of fry samples 1.1-1.10 (left to right). 5d is an image of fry samples 1.11-2.7 excluding 1.12 and 2.1 (left to right). 5e is an image of fry samples 2.8-3.6 (left to right). 5f is an image of is an image of fry samples 3.7-3.15 and fin clips for *C. myrnae*, *C. sajica*, freshly dead, frozen, and preserved in formalin fishes.

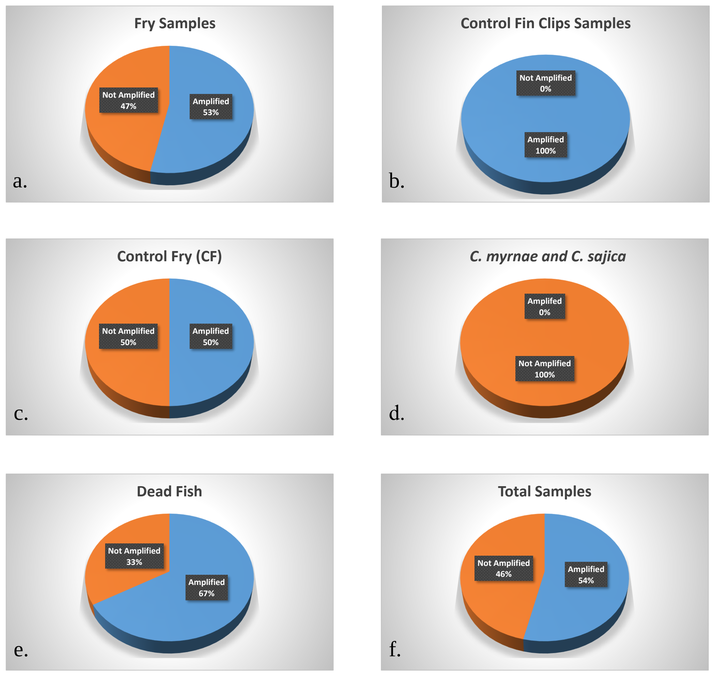

Figure 6: Pie charts to show the amplification rates. 6a is a pie chart for the fry samples (n=45). 6b is a pie chart for the control fin clips. 6c is a pie chart for the control fry. 6d is a pie chart to show *C. myrnae* & *C. sajica*. 6e is a pie chart to show the dead fish (freshly dead, frozen, and preserved in formalin). 6f is a pie chart of all the samples.

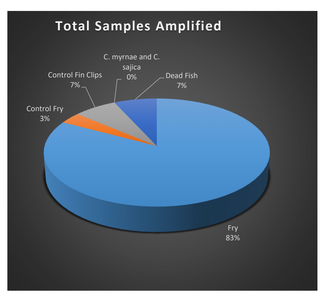

Figure 7: Pie chart of all samples where I was able to amplify DNA.

Conclusions

My research has shown that DNA is available for analysis in convict cichlid fry as small as having a Total Length (TL) of 5.2mm. The primers I used, Acit1F and Acit1R, were able to enhance both strands of DNA to be amplified which are shown in the gel images of Figure 5. I was also able to amplify DNA from a frozen convict fin clip and a fin clip from a *Pungu maclarani* that was preserved in formalin. In the end, knowing that we can extract and analyze DNA from convict fry will allow Sasha and Dr. Coleman's sample collecting process of different populations of *C. septemfasciatus* in the many different rivers of Northern Costa Rica to be more transparent and expeditious.

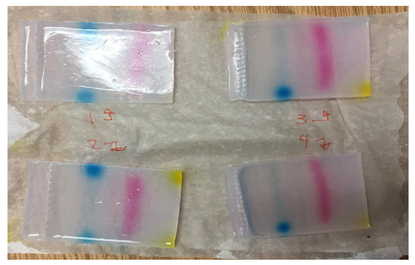

Figure 7: Image I took of the last four gels I created to show bands of samples as seen by the eye . Taking a UV image of the gels using a UV Trans illuminator allows us to see the bands much more clearly.

Discussion

Although I was successfully able to capture, preserve, extract, and amplify DNA in convict cichlid fry, I was not able to amplify DNA in all. In total, I had an outcome of amplifying 53% of the total 45 fry captured in my original sample sets. The smallest fry in which DNA was amplified measured at a TL of 5.2mm and the largest in which DNA was amplified measured at TL of 7.4mm. I also was able to amplify DNA in one out of the two control fry I experimented with, with this fry measuring at a TL of 11.9mm. Looking further into the reasons why this is the case, I can conclude that it could be a number of possibilities. The first, and possibly most explicable, reason could be the annealing temperatures. As a rule of thumb, I did set the annealing temperature to be 5°C lower than the Melting Temperature (Tm), however, that may still have been too high for some of the samples. If that was the case, then the primers were unable to bind to the template DNA which would not allow it to be amplified.

Overall, my work will allow Sasha and Dr. Coleman's research in Costa Rica to be more efficient. Now that a procedure has been created and we know that we can pull DNA from cichlid fry, then they can capture majority fry in Costa Rica. Fry are much easier to catch and store. Expediting the sample collection process will allow them to send their genetic material out for sequencing from Costa Rica this way they will not have to bring any samples/genetic material back which can become extremely difficult to do.

Bibliography

Bussing, W.A. (1998): Peces de las aguas continentales de Costa Rica (Freshwater fishes of Costa Rica). Revista de biologia tropical 46, 468 pp.

Chapman, L.J. and D.L. Kramer. 1991. The consequences of flooding for the dispersal of poeciliid fish in an intermittent tropical stream. *Oecologia* 87. 299-306.

Crispo, E. and L.J. Chapman.2010. Geographic variation in phenotypic plasticity in response to dissolved oxygen in an Afrian cichlid fish. *Journal of Evolutionary Biology*. 23:2091-2103

Streelman, J.T. and S.A. Karl. 1997. Reconstructing labroid evolution with single-copy nuclear DNA. *Proceedings of the Royal Society of London B: Biological Sciences.* 264. 1011-1020.

Zardoya, R., Vollmer, D.M., Craddock, C., Streelman, J.T., Karl, S., and A. Meyer. 1996. Evolutionary Conservation of Microsatellite Flanking Regions and their Use in Resolving the Phylogeny of Cichlid Fishes (Pisces: Perciformes). *Proceedings: Biological Sciences*. 263. 1589-1598.

Acknowledgements

This material is based upon work supported by the National Science Foundation through the Robert Noyce Teacher Scholarship Program under Grant #1136419. Any opinions, findings, and conclusions or recommendations expressed in this material are those of the author(s) and do not necessarily reflect the views of the National Science Foundation. The research was also made possible by the California State University STEM Teacher and Researcher Program, in partnership with Chevron (www.chevron.com), the National Marine Sanctuary Foundation (www.marinesanctuary.org), and California State University, Sacramento.

I would also like to thank Dr. Ronald Coleman for his guidance and mentorship through this research and Sasha Watkins for allowing me to be a small part of her research project.

www.PosterPresentations.com