Mitochondrially encoded COX-1 DNA and eDNA in *Haliotis rufescens* is more similar to *Oryzias latipes* than *Danio rerio*.

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Summary

- Sequenced *COX-1* gene of japanese medaka, fruit fly, red abalone, roundworms, zebrafish, and human.
- Extracted eDNA and sequenced the *COX-1* gene.
- Created a phylogenetic tree based on sequencing data.
- Analyzed phylogenetic trees to compare the relation of each species.
- Analyzed gel electrophoresis with COX-1 and sequence alignments with fruit flies and japanese medaka

Abstract

The hypothesis we created (*COX-1* genes of the *Oryzias latipes* is closer related to the *Haliotis rufescens* than the *Drosophila melanogaster)* was incorrect. To come to this conclusion, first we extracted DNA from our samples. Next we purified the DNA, performed a PCR, and sequenced the samples. We analyzed our results and with phylogenetic trees, gel electrophoresis, and sequence alignments. These results described how the *COX-1* gene from *Haliotis rufescens* is closer related to the *COX-1* gene of the *Drosophila melanogaster* than the *Oryzias latipes.*

Introduction

Hypothesis 1: The *COX-1* gene extracted from the *Oryzias latipes* is more similar to the *Haliotis rufescens* than to that of a *Drosophila melanogaster* [1 , 2]

Hypothesis 2: eDNA will produce results more consistent with the NCBI database

The Black Abalone [3]:

- Endangered marine sea snails, scientifically known as *Haliotis cracherodii*
  - Uses a "muscular foot" for movement and suction
  - Susceptible to Withering Shell Disease and rising ocean temperatures/climate change
- Herbivores/Scavengers- feeds on algae, plankton, bacteria
- Habitat:
  - Rocky intertidal places (where the ocean meets the sand) up to 6-meters deep
  - Shallow waters on California's coastlines
- The question that arises: What can we do to help conserve *Haliotis rufescens?*

Image from the University of Atlas LA

COX-1 [4]:

- *COX-1* (mitochondrially encoded cytochrome C oxidase I) is a gene sequence that is used to identify species
  - It exists in the main subunits of complex IV (in the mitochondrial inner membrane)
  - It is the gene itself we use for barcoding - if DNA is similar the organisms are evolutionarily similar

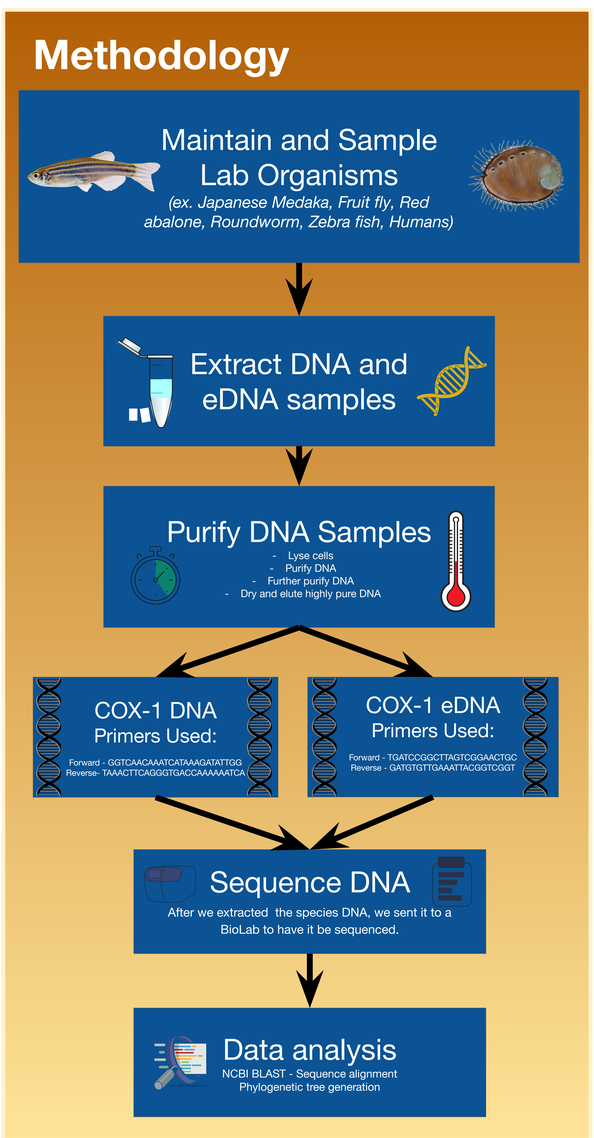
Investigation methods:

- Species identification through the observation of their respective DNA sequences
- Identified species from different locations in the environment using eDNA

Materials

- Micropipettes
- Buffer solutions
- Organism tissues
- Centrifuges
- PCR machine
- Electrophoresis Gel
- Agarose

Methodology



Results

DNA

Figure 1. Phylogenetic tree showing in-house organism's DNA sequences (excluding Danio rerio [zebrafish], which was a sequence given from the NCBI database.) The phylogenetic tree was created using Clustal Omega™ multiple sequence alignment program and customised with iTOL.

Figure 2. Phylogenetic tree showing organism's DNA sequences from the NCBI database. The phylogenetic tree was created using Clustal Omega™ multiple sequence alignment program and customised with iTOL.
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eDNA

Figure 3. Phylogenetic tree showing relations between in-house organism's eDNA. The phylogenetic tree was created using Clustal Omega™ multiple sequence alignment program and customised with iTOL.

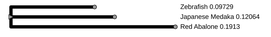

Figure 4. Phylogenetic tree showing relations between organism's DNA sequences from the NCBI database. The phylogenetic tree was created using Clustal Omega™ multiple sequence alignment program and customised with iTOL.

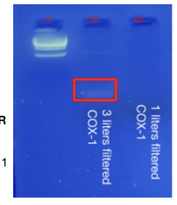

Figure 5. (right) Agarose gel electrophoresis (1.5% agarose) of PCR amplified products using species specific PCR primer sets. Lanes 2,3 are filtered COX-1 eDNA samples. Lane 1 shows a 500bp DNA ladder.

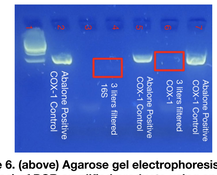

Figure 6. (above) Agarose gel electrophoresis (1.5% agarose) of PCR amplified products using species specific PCR primer sets. Lane 2 is the abalone positive COX-1 control. Lanes 4-7 are abalone tank eDNA.

MEDAKA			
Query	17	GCCAGGGGCACICTTGGGGGATGACCAACICTATAACGTAATTGTAACAGCCCACGCTTT	76
Subject	69	…T…T.T.A..A..T..C……A……T……….C..G..T..C..	128
Query	77	CGTAATAANCTTCTTCCTAGTTATCCCA	104
Subject	129	……..TT…..TA….A..A…	156

FRUIT FLY			
Query	33	GGGGATGACCAACTCTATAACGTAATTGTAACAGCCCACGCTTTCGTAATAANCTTCTTC	92
Subject	79	..A…..T…A.T…..T……….T..A..T…..TA.T….TT..T..T	138
Query	93	CTAGTTATCCC	103
Subject	139	A.G…..A..	149

Figure 7. Multiple sequence alignment of Japanese Medaka COX-1 sequence. The sequence was created using Clustal Omega™ multiple sequence alignment program.

Figure 8. Multiple sequence alignment of Fruit Fly COX-1 sequence. The sequence was created using Clustal Omega™ multiple sequence alignment program.

Description	Scientific name	Max score	Total score	Query cover	E value	Percent identity	Acc. len
Medaka	*Oryzias latipes*	75.2	75.2	82%	2e-18	78.41%	427
Fruit fly	*Drosophila melanogaster*	49.1	49.1	66%	3e-10	74.65%	653

Figure 9. Comparison of Japanese Medaka and Fruit Fly COX-1 sequence alignment. The alignment/comparison was created using Clustal Omega™ multiple sequence alignment program.

Discussions & Conclusions

- Figures 1 and 2 show that the phylogenetic tree from our lab differed from the phylogenetic tree created from NCBI database samples
- Our hypothesis is correct because figure 2 shows a closer evolutionary distance between the Japanese Medaka and Red Abalone than to fruit flies
- Using the eDNA that we collected, we created an accurate phylogenetic tree that compared NCBI database sequences to our own
- eDNA vs DNA
  - Figures 3 and 4 - eDNA samples revealed a similar phylogenetic tree to that of the NCBI database
  - Our DNA samples show a different phylogenetic tree with different evolutionary distances compared to the NCBI database.
    - We can conclude that the eDNA extraction will produce results more consistent with the NCBI database than extracted DNA
  - eDNA extracted from tank water must be filtered through 3 liters of water to acquire a sufficient amount of DNA.
- Figure 5. (Gel Electrophoresis) only shows a bar for 3 liters of filtered COX-1 as opposed to 1 liter filtered COX-1
- COX-1 Japanese Medaka and Fruit Fly
  - The japanese medaka sample shows a 78.41% percent identity (to NCBI) and 82% query cover
  - The fruit fly sample shows a 74.65% percent identity (to NCBI) and 66% query cover

Study Limitations/ Future Directions

Some setbacks we faced included-

- Universal COX-1 primer was not consistent throughout all species
- Poor/insufficient abalone DNA samples
- Limited amount of organism samples
  - Could not create a full phylogenetic tree

Future Directions:

- Maintain 3 microliters of eDNA water samples when performing gel electrophoresis
- Use organism specific COX-1 primers to run PCR for accurate results
- Gel electrophoresis: include more DNA samples as there are higher chances of seeing visual bands
- Use eDNA as apposed to DNA for results more consistent with the NCBI database
- Protect the red abalone from withering disease by improving eDNA extraction for further research

Sources