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Barcoding a Lionfish's Last Meal: A Citizen Science Research Project for the Classroom

BY SHERRI ANDREWS, PH.D., AND NANCY KIM PHAM HO, MSC.

Lionfish may be found in various habitats such as reefs, sandy ocean bottoms, and estuaries. Their ability to adapt to this wide range of ecosystems are topics to discuss with students. Courtesy of Nancy Kim Pham Ho ©2017

ABSTRACT

Barcoding a Lionfish's Last Meal is a project that engages students in authentic research and uses molecular techniques to study an environmental issue—invasion of the lionfish. Red lionfish (*Pterois volitans*) are a voracious invasive species indigenous to Indo-Pacific waters that have the potential to greatly disrupt coral reef ecosystems. We report the initial data gathered from high school and university students as well as high school teachers attending professional development workshops. Data is shared on the iNaturalist website (see Resources) to allow widespread access for analysis. This project is now in its third year.

INTRODUCTION

As marine science educators, we need to build awareness of our fragile aquatic ecosystems. One area of concern is the introduction of an invasive species, *Pterois volitans* (red lionfish), to the marine ecosystem as well as temporarily living in some fresh water systems worldwide. Although beautiful, this species can reduce the native fish populations by an estimated 65 to 79%. (Bellaw et al. 2016; Green et al. 2012). Indigenous to Indo-Pacific waters, these voracious eaters have the potential to have profound effects on coral ecosystems in the Atlantic from North Carolina to Venezuela, the Caribbean Sea, and the Gulf of Mexico (Rocha et al. 2015; Albins et al. 2008). Alarmingly, there have also been sightings as far south as Brazil (Ferreira et al. 2015).

It is important to understand exactly what lionfish are eating in order to predict ecosystem changes (Valdez-Moreno et al. 2012), and over 100 studies have investigated the potential role lionfish may play in the Western Atlantic and the Caribbean (Cote and Smith 2018). In 2010, the National Oceanic and Atmospheric Administration (NOAA) initiated an "Eat Lionfish" campaign aimed at promoting consumption of lionfish as a viable seafood choice. Since then dozens of organizations have hosted lionfish hunting competitions in efforts to manage this invasive fish and educate local communities about this epidemic. A major area of focus has been on evaluating the prey items found in the guts of lionfish through visual identification, DNA barcoding, and metabarcoding (Dahl and Patterson 2014; Valdez-Moreno et al. 2012; Harmes-Tuochy CA et al. 2016).

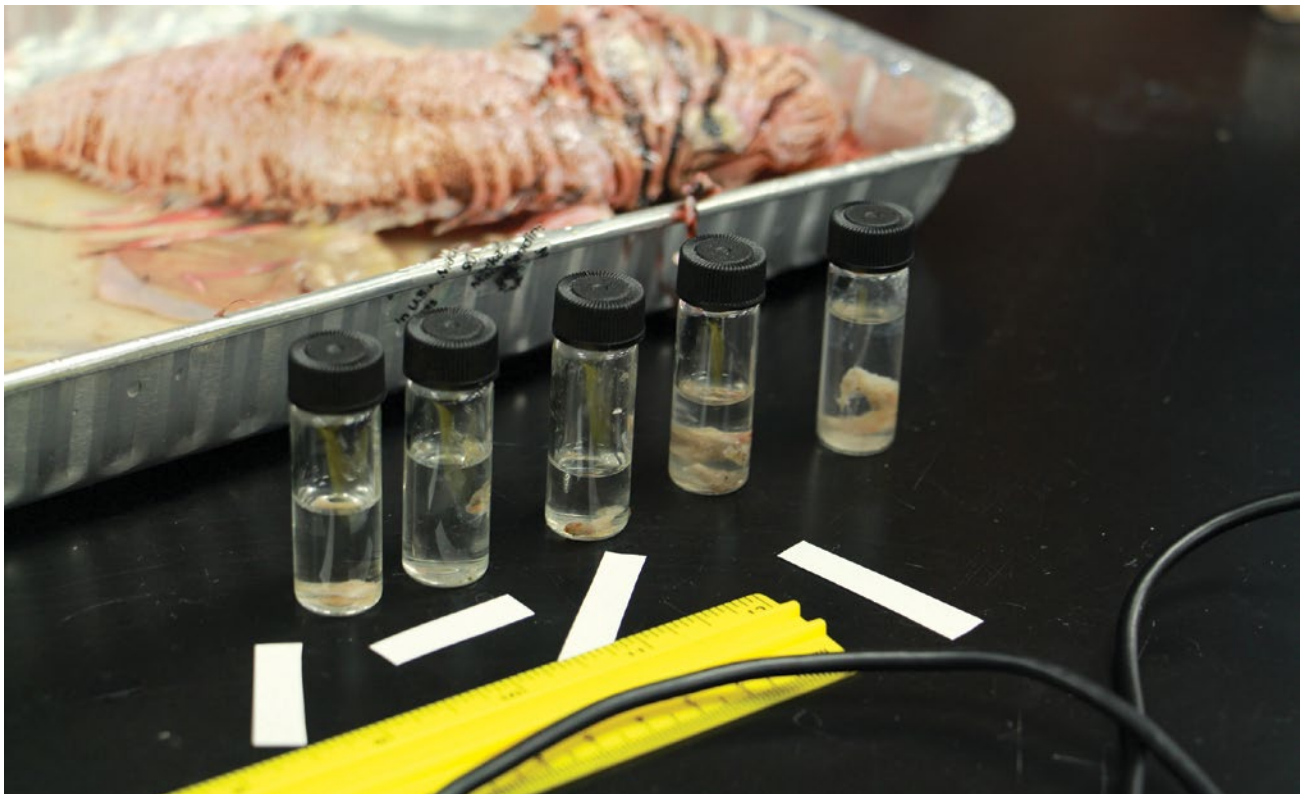
Carol Baldwin, Curator of Fishes at the Smithsonian Museum of Natural History, originally inspired the "Barcoding a Lionfish Last Meal: A Citizen Science Research Project for the Classroom." Her presentation to participants in the first-ever held workshop at Q?rious (a unique, interactive science education space) focused on the invasive lionfish and the need to use barcoding to identify what they were eating. The stomachs of lionfish, which are often discarded when gutted for fillets, gave rise to opportunities for educators to bring real-time ecology questions to the classroom.

Our lionfish project began in the summer of 2015, when a group of high school and middle school teachers participated in a workshop at the University of Western Florida. Participants spent two days learning about DNA barcoding and carrying out project procedures. Later that summer community college instructors and high school teachers attended a one-day workshop at Brunswick Community College in North Carolina. The following spring informal science educators, university students, high school teachers, and several high school students attended a one-day workshop at Vero Beach High School. These workshops resulted in several high school student research projects, intern projects, and classroom implementations at the middle school, high school, and university levels. Here we report the initial data collected by student researchers and teachers participating in these professional development workshops.

Participation in the project facilitates understanding of the scientific process related to DNA barcoding and it also can grow awareness of the magnitude of the problem this species is creating in our oceans. Participants dissect lionfish and obtain prey items for DNA sequencing or use previously processed samples. They learn how to extract DNA, amplify it, and determine if the amplification was successful. They can then prepare their samples for sequencing, receive the sequence, analyze the results, and add the data to the iNaturalist database. This data is then available to the public for analysis. (Please note that while there are many studies exploring this problem reported in the literature, many of these studies are using metabarcoding to identify lionfish diet.) This method is still quite expensive so it is for this reason the current study uses the Sanger Sequencing method.

The state of Florida hosts numerous lionfish events where a team of divers works together to help remove lionfish from local waters. Seven dive teams have donated their catch to this project to provide stomachs for students to analyze. Courtesy of Nancy Kim Pham Ho ©2017

Size matters: Removing lionfish is not the only goal of lionfish derbies. Biologists and other conservation organizations are able to collect valuable data on each fish removed from these public events. Courtesy of Nancy Kim Pham Ho ©2017



Once the lionfish stomach contents are processed, each of the contents may be preserved and cataloged for later analysis, allowing students to practice their data management skills. Courtesy of Adeljean Ho ©2017

Publishing data to the iNaturalist database allows access to teachers and students to ask questions such as: "Are there eating patterns among lionfish in different environments?"; "Does the size of the lionfish influence diet?"; "Can students identify species that are particularly vulnerable?"; and "If one species in particular is removed from the ecosystem, what might the long-term effects be on other populations?"

METHODS

The data presented here was collected from lionfish gut content obtained from several sources: fresh caught and dissected fish, fish obtained from teacher/divers in Pensacola in the summer of 2015, and the Sebastian River Lionfish Derby in May 2016. These fish were filleted by a team from Coastal Biology Inc. in exchange for stomach content. The fish were either weighed or measured, followed by dissection of the fish stomachs. Individual samples from stomach contents were placed in vials containing 95% ethanol. Alternatively, filleted fish were frozen. Each fish was assigned a unique ID, and numbers were assigned to each sample obtained from stomach contents.

If prey items were used in workshops, each participant got one sample. If whole lionfish were used, participants each dissected one fish. The number of prey items retrieved varied from sample to sample. Some fish had no prey items, most had two to four items, and the largest number of items found was fourteen.

Fish dissected at the professional development workshops followed NOAA Lionfish dissection guidelines. The fish were assigned a unique ID, measured, and weighed. The samples removed from the gut were assigned an ID number and washed in 10% Clorox™ solution followed by distilled water. This step was taken to minimize bacterial and cross contamination.

MATERIALS

DNA extraction was performed using Bio-Rad's Fish DNA Barcoding Kit (1665100EDU). Samples were amplified using PCR (also part of the Fish DNA Barcoding Kit) and amplicons were verified using gel electrophoresis.

The primer mix utilized was based on a literature search of universal primer cocktails for fish barcoding, and ultimately based on Ivanova et al. (2007). The mix contained degenerate primers that were tailed with M13 sequences in order to facilitate sequencing. Samples that had the appropriate sized 650bp PCR product (approximate 80% success rate) were sent for sequencing using Bio-Rad's DNA Barcoding Sequencing Module (1665115EDU). Upon receipt of data, it was entered into chromatograph reading software. The chromatograms were examined for quality, trimmed, and placed in a BLAST search. Samples that had an E-value of 0 or less, were at least 590 base pairs in length, and had 89% coverage were included in the database. Data was uploaded to iNaturalist (see Resources for websites) for easy access to any interested parties.

RESULTS

A total of 100 identified prey items found in lionfish stomach contents have been entered into the iNaturalist database (Figure 1). These were visually identified (shrimp and crab only) or met the quality criteria previously stated. We identified two genera and 23 different species. Percent of gut content was as follows:

- 13% red lionfish
- 12% shrimp
- 8% school bass
- 7% cigar minnow, tomtate, and cocoa damsel
- 6% decapoda (crustaceans)
- 5% twospot flounder
- 4% slippery dick, chameleon wrasse, and vermilion
- 3% bicolor damsel fish
- 2% flamefish, eyed flounder, sand perch, greenband wrasse, freckled cardinal fish, and green razor fish
- 1% pallid goby, wrasse (species nonspecific), crab, diamond lizardfish, and bluehead wrasse

It was interesting that 13% of total fish identified were lionfish, as metabarcoding studies did not report any lionfish found in stomach contents. It is likely that this data was removed from consideration in these studies due to the methods used in metabarcoding, which include next generation sequencing that would have also identified the dissected host fish tissue. It brings up the question of whether or not the observed 13% in the current study may be contamination from the dissected host fish itself. This was determined not to be the case as the samples were whole fish or large pieces of fish tissue, and the chromatographs did not indicate the presence of multiple sources of DNA due to mixed sample origin. Furthermore, additional studies using Sanger methods have reported that lionfish are cannibalistic (Valdez-Moreno et al. 2012), which corroborate the data from the current study.

Two of the fish identified are on The International Union for Conservation of Nature and Natural Resources (IUCN) Red List of Threatened Species™: the pallid goby and the vermillion snapper. This raises concerns that if lionfish continue to consume these species in great numbers, they could be moved to endangered status. If examining a larger data, the question arises, "Will there be more species added to the list of which we should be concerned?"

Two fish in the sample, redlizard fish and chameleon wrasse are not native to waters where the lionfish were speared. Redlizard fish are indigenous to the western Pacific. GenBank reported 89% sequence coverage and 99% sequence identity. Upon closer examination of the databases, an identification of *Synodus synodus* includes five common names of fish, one of which, the diamond lizard fish, is indigenous to the Eastern Atlantic. According to the IUNC redlists, there is only 5 to 6% difference in COI gene sequences in this species (see Resources for website). Perhaps the primer mix used in this study does not differentiate between these related species or data entered into GenBank is not as robust as expected.

The chameleon wrasse is indigenous to eastern pacific waters. GenBank reported 94% sequence coverage and 90% sequence identity. A close relative, the green banded wrasse is indigenous to Atlantic waters. This can be an area for further study.

Our findings are also supported by other publications. For example, Ballew et al. (2016) found that the tomtate populations were reduced by 45% during the study period. Tomtates represented 7% of our sample size. A total of 108 samples were submitted for sequencing. Eighty-seven of those samples were successfully sequenced (an 80.5% success rate). Bear in mind most of the participants in the project have very little experience in the technique which is the hallmark of Citizen Science projects. There are several contributing factors that limit barcoding success. These include:

- Samples may be too small and therefore not enough DNA is extracted.
- Exoskeletons and bones are more difficult for extraction.
- Proper technique for extraction is not followed (it is extremely important that a sterile mincing tool is used to retrieve the fish sample, and failure to do so can produce inconclusive chromatographs).
- Too much fish is used in the extraction procedure. Too much sample can also provide too many inhibitors of the PCR reaction and the reaction does not complete properly. Too much sample can also clog the DNA extraction columns.
- Improper micropipetting of solutions will inhibit extraction and PCR reactions.

FIGURE 1. Results of Prey Items Found in Lionfish Stomachs

Scientific Name	Common Name	Observations
Apogon maculatus	Flamefish	2
Bothus ocellatus	Eyed Flounder	2
Bothus robinsi	Twospot Flounder	5
Coryphopterus dicrus	Colon Goby	2
Coryphopterus eidolon	Pallid Goby	1
Decapoda	Decapods	6
Decapterus punctatus	Cigar Minnow	7
Dendrobranchiata	Shrimp and Prawns	12
Diplectrum formosum	Sand Perch	2
Haemulon aurolineatum	Tomtate	7
Halichoeres	Wrasse	1
Halichoeres bathyphilus	Greenband Wrasse	2
Halichoeres bivittatus	Slippery Dick	4
Halichoeres dispilus	Chameleon Wrasse	4
Phaeoptyx conklini	Freckled Cardinalfish	2
Pleocyemata	Crabs, Lobsters, and Allies	1
Pterois volitans	Red Lionfish	13
Rhomboplites aurorubens	Vermilion Snapper	4
Schultzea beta	School Bass	8
Scorpaenodes tredecimspinosus	Deepreef Scorpionfish	1
Stegastes partitus	Bicolor Damselfish	3
Stegastes variabilis	Cocoa Damselfish	7
Synodus synodus	Red Lizardfish	1
Thalassoma bifasciatum	Bluehead Wrasse	1
Xyrichtys splendens	Green Razorfish	2

Over spinning and allowing spin columns to dry out can inhibit binding DNA on the column until the proper step for DNA is released from the column.

Our most important finding is that this type of research can be accomplished at the high school and post-secondary levels. Our next step is to recruit and engage more teachers, university lab managers, and student independent researchers to participate in the project. This will allow the database to grow and will make more data accessible to teachers who do not have the bandwidth to participate in the data collection process. It will also allow teachers to use the data as a tool to teach data analysis as well as be of use for students to ask interesting research questions.

PARTICIPATION IN THE PROJECT

There are several considerations for participation in the project. At a minimum you will need a heat block or water bath, 2-20µl, 20-200µl, and 100-1000µl micropipets, ability to perform DNA electrophoresis, and a thermal cycler. If you are a high school teacher you may be able to borrow these items from another school, local community college, or university. For ease of prep you can order the kits or you can visit the literature to develop your own primer sets and purchase materials to extract, amplify, and verify PCR products. You will also need a source for sequencing services, which can be found at local core facilities, online resources, or through purchasing commercial kits.

You will need to secure a source of lionfish. If you use local divers, dive operations, or obtain samples from lionfish derbies, make sure to secure whole fish and clip the spines before handling, as they are venomous. Note that students can still be stung (although we have never experienced this) even though spines have been removed. For younger students, bellies or lionfish bits are suggested. We do have the ability to provide samples on a limited basis.

If you are interested in participating in this project, please contact the authors. We will try to provide lionfish stomachs, filleted lionfish, whole lionfish (first come first serve basis), or lionfish prey items in vials. You can also contact local dive shops to inquire about filleted lionfish and lionfish stomachs.

RESOURCES

BLAST Search:
https://blast.ncbi.nlm.nih.gov/Blast.cgi

iNaturalist:
https://www.inaturalist.org/projects/lion-fish-guts-barcoded

IUCN Red List of Threatened Species:
http://www.iucnredlist.org/details/13486169/0

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SHERRI ANDREWS has a Ph.D. in Curriculum and Instruction with a focus in the biological sciences, specifically biotechnology. Her area of expertise is developing and implementing curricula and teaching that brings cutting edge science to secondary and post-secondary classrooms. She has over 20 years of teaching experience at these levels.

NANCY KIM PHAM HO has a Master of Science in Biology with a focus on aquaculture, biology, and conservation and is the Director of Education for Coastal Biology Inc. She specializes in developing and teaching hands-on activities in the field of aquarium science for informal educators and mentoring those who are interested in pursuing a career in STEM fields.