

Development and validation of non-lethal DNA sampling method for fish



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Introduction

Using genetic approaches to monitor fish population structure is widely used for analyzing marine connectivity in ecologically sensitive areas.

Field techniques used to collect fish tissue samples generally consist of capturing entire individual fish in order to obtain only a small fin clip needed for DNA extraction and analysis. This often results in unwanted injury or mortality, especially if the fish species under investigation are small to medium sized teleost reef fish species.

Effective non-lethal tissue sampling techniques are desirable as studies of genetic population structure become more common for small and medium sized teleost reef fish. This is particularly important in ecologically sensitive areas where a more robust understanding of population structure can lend insight into their preservation and management.

Objective

The intent of this study was to develop and test a method to collect DNA samples from small teleost reef fish (5-19cm) that significantly reduces or avoids mortality, and is reliable.

Methods

The fish:

- 24 small (5-7cm) sized mamo (*Abudefduf vaigiensis*.)
- 24 medium (15-19cm) sized pualu (*Acanthurus xanthopterus*)



Tagged *Abudefduf vaigiensis* in holding pen

Untagged *Acanthurus xanthopterus* just before release



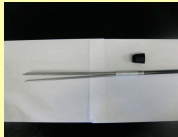
Methods

Fish were collected from the wild and placed in an open holding pen. Half the individuals in each species group were spaghetti tagged for recognition as experimental fish. The untagged other half were controls.

All fish were acclimated and monitored for overall health and survival two weeks following capture and tagging, and three following the experiment.

Biopsy collection equipment:

A black rubber stopper with an attached circular piece of black felt replaced the spear tips of Hawaiian three prong sling spears.



Sample collection and processing:

Divers entered the holding pen and used the modified spears to strike the sides of the tagged fishes, removing and replacing each tip after a successful strike.

DNA from the felt tip was transferred to Whatman FTA paper for storage. DNA purification, PCR amplification of a portion of the Cytochrome c Oxidase (COI) gene, and sequencing were performed on all samples.



Results

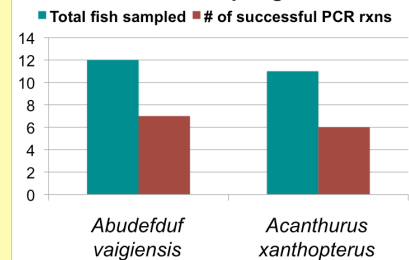
% Survival of fish three weeks following non-lethal tissue sampling		
Species	Control	Experimental
<i>Abudefduf vaigiensis</i>	100%	91.67%
<i>Acanthurus xanthopterus</i>	100%	100%

Literature Cited

Evans, R.D. 2008. Assessment of an underwater biopsy probe for collecting teleost fish tissue samples. *Marine Ecology Progress Series* 368:305-308.
 Zemlack, T.S., R.D Ward, A.D. Connell, B.H Holmes and P.D.N Hebert. 2009. DNA barcoding reveals overlooked marine fishes. *Molecular Ecology Resources* 9:237-242.

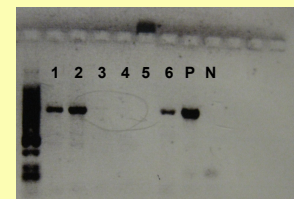
Results

Successful PCR reactions taken from fish tissues using a non-lethal sampling method



Fish species	# of DNA samples	# of successful PCR rxns	%
<i>Abudefduf vaigiensis</i>	12	7	58%
<i>Acanthurus xanthopterus</i>	11	6	55%

Gel image of COI gene fragment amplified from six *Abudefduf vaigiensis* experimental individuals with (+) and (-) controls



Conclusion

- 23 of 24 experimental fish survived the non-lethal tissue collection method
- On average, 56.4% of the DNA extracted yielded valid PCR products
- All sequenced PCR products were matched to expected species with high significance in BLAST

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