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Study on existence of the fisheries resources abundance by using environmental deoxyribonucleic acid (e-DNA) approach at fishing grounds in the Sulawesi Sea, Indonesia

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Abstract. Here, we report the results of our preliminary study on deep sea eDNA at fishing ground to approach the fisheries resources abundance at Sulawesi sea by using deep-sea water sampling collected from 10 sites ranging from 110m-200m in depth at front side of the International Coelacanth Research Center and Museum Base at Lolak Waters and Manado Bay North of Sulawesi using Nansen Bottle Sampler (1500 cc). The collected waters were filtered using Power Water Sterivex DNA Isolation Kits and preserved with the DNAiso Reagent then transported to Center for Strategy Research Project, University of the Ryukyus, Okinawa Japan where eDNA analyses were conducted. Our results revealed that the concentrations of eDNA has a good quality were measured with a NanoDrop Lite spectrophotometer, indicating eDNA was successfully extracted. Therefore, by using universal primers for eDNA, MiFish-U-F/R for the 1st-PCR (mt-12S amplification) and 2nd-PCR (tag-indexing) for library preparation to accommodate sequence variations and show that intense signal of MiFish eDNA amplification. Using a high-throughput Illumina MiSeq platform for sequencing analyses, we detected eDNA from 40 fish's species with dominantly by *Caranx sexfasciatus*, *Engrasicholina punctifer*.

1. Introduction

Indonesia is an Archipelago country are consisting of the 18.110 islands with a coastline length about 99,000 Km and consisting of about 9,900,000 tons of marine resources potency per year. This potency is distributing around the surface, middle and bottom waters layers. To monitor these marine resources existence abundance, the lots of the budget and times are needed.



A new technology approach can be overcoming of this problem were available can be implemented at around Indonesian marine waters by using the environmental DNA approach. Environmental DNA (eDNA) in aquatic environments refers to genetic material found in the water column. In the case of multicellular organisms, eDNA originates from various sources, such as metabolic waste, damaged tissue or sloughed skin cells [1]. Ficetola et al., was the first study demonstrating the use of eDNA for detecting an aquatic vertebrate species (invasive American bullfrog) from controlled environments and natural wetland, published in 2008 [2]. Here, we report the results of our preliminary study on deep sea eDNA at fishing ground to approach the fisheries resources abundance at Sulawesi sea.

2. Materials and Methods

Deep-sea water sampling was collected from 10 sites ranging from 110m-200m in depth at front side of the International Coelacanth Research Center and Museum Base at Lolak Waters and Manado Bay North of Sulawesi using Nansen Bottle Sampler (1500 cc) as shown in Figure 1. The positions were follows the discovered of coelacanth by Green Eye Project on 2007-2015 [3].

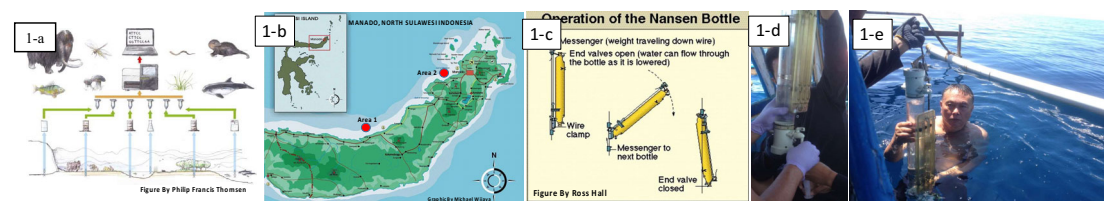


Figure 1. The concept of preliminary study of eDNA and the collections sites of deep seawaters Sampling.

The collected waters were filtered using Power Water Sterivex DNA Isolation Kits and preserved with the DNAiso Reagent and kept in a deep freezer -25°C at Faculty of Fisheries and Marine Science, Sam Ratulangi University, until they were transported to Center for Strategy Research Project, University of the Ryukyus, Okinawa, Japan (Figure 2.) where eDNA analyses were conducted following MiFish protocol [4]. Therefore, e-DNA extraction was conducted at Center for Strategy Research Project at University of the Ryukyus Okinawa Japan by using PowerWater Sterivex DNA Isolation Kit Samples by followed its protocol as follow [5].

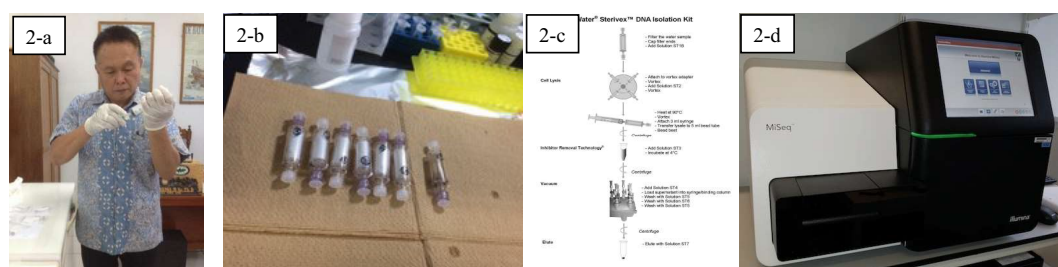


Figure 2. Laboratory Works on Deep-sea Environmental DNA Research at Faculty of Fisheries and Marine Science Sam Ratulangi University and University of the Ryukyus.

3. Results and Discussion

Our Results of eDNA Water sampler quality After Extraction and Amplification of Ten Collection sites at Manado Bay and Lolak Waters, Sulawesi Sea, Indonesia was used to analysis the fisheries

resources existence abundance after check the quality of DNA as shown in Table 1. Some number of the species have been analyses at The Center the Strategic research Projects of University of the Ryukyus. The eDNA water sampler was collected on 2018.06.17 and kept under -25°C , then was extracted on 2018.07.18. According to this table, the quality of DNA from 10 locations at the sea and one water sample site from the freshwater river as positive control shows that the DNA quality ranging from 1.8-12.6 ng/mL.

Table 1. Results of eDNA Water sampler quality After Extraction and Amplification of Ten Collection sites at Manado Bay and Lolak Waters, Sulawesi Sea, Indonesia

Sample ID	Date of Collection	Date of Extraction	Collection Sites	Quality of DNA	Nanodrop Concentration
				(ng/mL)	(ng/mL)
478	2018.06.17	2018.07.18	Tanjung Ompu 1	1.8	2.4
479	2018.06.17	2018.07.18	Tanjung Ompu 2	2.3	2.22
480	2018.06.17	2018.07.18	Lolak River	3.5	2.7
481	2018.06.17	2018.07.18	Molosing Island 1	3.1	2.28
482	2018.06.17	2018.07.19	Molosing Island 2	1.9	1.63
483	2018.07.03	2018.07.19	Big Fish Manado 1	2.9	2.12
484	2018.07.03	2018.07.19	Big Fish Manado 2	12.6	2
485	2018.07.03	2018.07.19	Minanga Manado 3	4.8	2.2
486	2018.07.03	2018.07.19	Recla. Manado 4	3.3	3.39
487	2018.07.03	2018.07.19	Trad. F.G. Manado 5	5	2.13
488	2018.04.20	2018.07.19	Sariouw River (*)	10.7	1.79

(*) Water sampler from Sariouw River as a positive control

Suggesting that the quality of the DNA is good enough to conduct for sequencing analysis of the next process. That argument are strong support by the results are showing at Figure 3a. The blue line are the quality DNA (ng/ml) of the seawater samples and the orange color indicated the standard of the the quality DNA as a control. From this figure shows, all of the seawater samples are laid at the upper sites of the orange colors. We conducted electrophoresis for each part 1st-PCR and 2nd-PCR

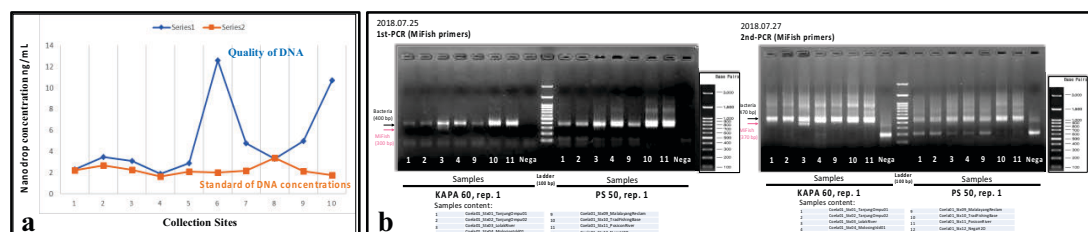


Figure 3. a. The comparison between Nanodrop concentration and Standard Concentration of water samples (ng/mL) of each collection site using in this study, **b.** The 1st-PCR and 2nd-PCR Shows Intense

Signal of MiFish eDNA by Using Universal Primers MiFish-U-F/R

amplification products and the results show as River samples as positive control exhibited intense signal of MiFish eDNA amplification and negative controls (DW) showed no clear bands. It's clearly showed (Figure 3b) that the water samples was collected from those two areas of Sulawesi waters are so cleared and feasible to be continued to next of the genome work. Then it can be explained (table 2) from all 10 collection sites we found that the number of fish species have been obtained based on fish base database. By using a high-throughput Illumina MiSeq platform for sequencing analyses, we detected eDNA from 40 fish's species and the dominantly are *Caranx sexfasciatus* and *Encrasicholina punctifer* fishes.

Table 2. Results of collections site, satellite positions, sea's depth and the number of fish's species was detected at Lolak Waters and Manado Bay North of Sulawesi.

Stations	Positions (Degrees)		Sea's Depth (Meter)	Species (Number)
	Latitude (N)	Longitude (E)		
1	0.9298	124.0351	115	8
2	0.9338	124.0066	150	6
3	0.9339	123.9961	196	14
4	0.9217	124.9889	170	2
5	0.9128	123.9869	110	11
6	1.4699	124.8199	95	3
7	1.4707	124.8179	120	2
8	1.4686	124.8153	135	8
9	1.4659	124.8112	120	6
10	1.4661	124.8156	90	4

According to the fisherman around the surveyed areas that about 2 weeks before those locations were plenty of the *Encrasicholina spp.* were caught there. In addition, to eDNA, this metabarcoding approach is applicable for species identification of the marine fisheries resources' existence abundance in Sulawesi sea. Then this bioinformatics data was compared to the fish's distributions and ecological as reported from The Green eye project 2007-2015 where the site also known as fishing ground of those target fishes. Therefore, the results of collections site, satellite positions, sea's depth and the number of fish's species was detected at Lolak Waters and Manado Bay North of Sulawesi, explained that the collection site at Lolak Waters relatively higher fisheries resources existence abundance compared to other collection site in Manado Bay (table 2).

The relationships between the collection sites number and sea's depth as shown at Figure 4-a by radar chart approach and the number of the fishes' species detected (Figure 4-b) was analyzed by using the balloons pattern. Therefore, Figure 4-c performed the relationships between two variables, seas' depth and the number of the fish species have been detected by environmental DNA tools approach and examined by simple regression analysis applied. Based of this results we found that the relationships between variable sea's depth and the number of the fish species are had any relation even relatively low indicated by value of $r = 0.257$ with the equation is Number Fish Spec. = $1.309 + 0.0401$ Sea's Depth (M). Comparing to these three graph, there are strong supported each other. On the other hand, in these figures explained that the surveyed locations have been taken the water sample at Manado bay are relatively shallower the sea depth than them at Lolak water of Bolaang Mongondow regency.

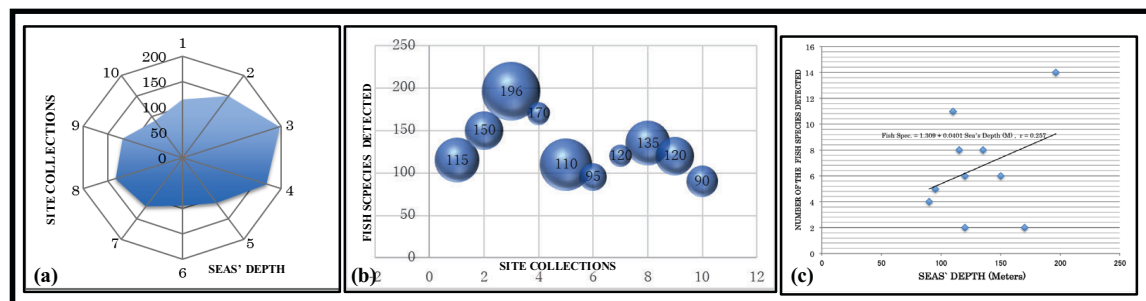


Figure 4. The Relationships between Collection site, Sea's Depth and the Number of Fish Species detected.

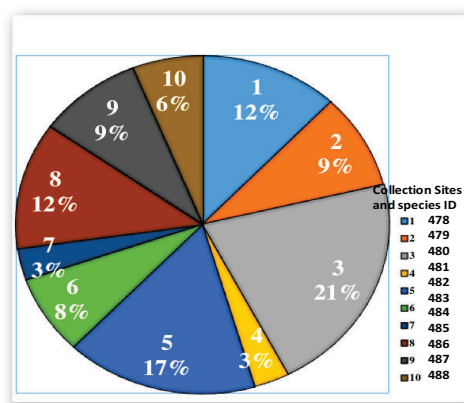


Figure 5. Comparison of the number of fish's species detected in percentage at each surveyed sample locations (from 1-10 sites), where each color represented each sample ID.

In Figure 5, explained that the number of the fish's species detected in percentage at each surveyed sample locations. From this figure shows that the location number 3 were the biggest fish's species in the number are located at Lolak waters as indicated at number 1-5 than them at Manado Bay of locations number of 6-10 in accumulations of 64 fish's species. From this information could be said that the fisheries abundance of Lolak waters were detected 41 species (64.06%) and are bigger than that at Manado bay of 23 fish's species (35.94%). This indicated the deep-sea marine protected area of The International Coelacanth Research Center and Marine Museum base site of Lolak waters are relatively higher of the fisheries resources existence abundance than at the Manado bay.

4. Concluding Remarks


Based on our results analysis to developed universal primer MiFish in a metabarcoding approach to fish eDNA we confirmed that the Lolak Waters are having relatively higher the fisheries resources existence abundance comparing of that on Manado bay. In implementation of the deep-sea environmental DNA research, the un-contamination aspect during fields work is absolutely necessary, therefore for effectiveness and efficiencies research of the marine fisheries resource's existence abundance point of view, the environmental DNA technology approach is suitable to be applied.

Finally, this marine environmental DNA technology could be got fruitful if can be implemented to Indonesian marine and freshwater due to Indonesia country have very wider territorial.

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