Title
Biodiversity and Distribution of Marine Fishes in Indonesia inferred by Environmental DNA

Author
Marwayana, Onny Nurrahman

Publication Date
2018

Peer reviewed|Thesis/dissertation

UNIVERSITY OF CALIFORNIA

Los Angeles

Biodiversity and Distribution of Marine Fishes in Indonesia inferred by Environmental DNA

A thesis submitted in partial satisfaction

of the requirement for the degree Master of Science

in Biology

by

Onny Nurrahman Marwayana

2018

ABSTRACT OF THE THESIS

Biodiversity and Distribution of Marine Fishes in Indonesia inferred by Environmental DNA

by

Onny Nurrahman Marwayana

Master of Science in Biology

University of California, Los Angeles, 2018

Professor Paul Henry Barber, Chair

Indonesia is the heart of the Coral Triangle, the world's most diverse marine ecosystem. Preserving the biological and economic value of this marine biodiversity requires efficient and economical ecosystem monitoring. This study investigates the effectiveness of environmental DNA (eDNA) to capture fish biodiversity across a pronounced biodiversity gradient in Indonesia. A total of 15,219,431 sequence reads of 12S rRNA from 39 sites spanning 7 regions of Indonesia revealed 774 Amplified Sequence Variants (ASVs). Patterns of fish diversity based on eDNA partially conformed to expectations based on traditional biodiversity survey methods, with highest fish biodiversity in Raja Ampat, with generally lower diversity in Western Indonesia. However, eDNA performed relatively poorly compared to visual survey methods in

site by site comparisons, both in terms of total number of taxa recovered and ablity to assign species names to ASVs. This result stands in a stark contrast to eDNA studies temperate and tropical ecosystems with lower diversity. Analysis showed that while sequencing depth was sufficient to capture all fish diversity within individual samples, variation among samples from individual localities was high, and sampling effort was insufficient to capture all fish diversity at a given sampling site. Results of this study highlight two major challenge of eDNA in highly diverse ecosystems such as the Coral Triangle. First, reference databases are incomplete and insufficient to attach species names to ASVs. Second, sampling design based on studies from lower diversity temperate ecosystems may be inadequate to capture the diversity of high diversity ecosystems.

The thesis of Onny Nurrahman Marwayana is approved.

Thomas Bates Smith

Peggy Marie Fong

Paul Henry Barber, Committee Chair

University of California, Los Angeles

2018

Table of Contents

Acknowledgement viii

Introduction 1

Materials and Methods 5

Sampling Sites 5

eDNA Sampling 6

eDNA Extraction, Amplification, and Sequencing 7

Bioinformatics and Data Analysis 9

Comparison of eDNA Survey and Visual Census 11

Results 12

Raw eDNA Sequences 12

Regional Pattern and Identification of Amplicon Sequence Varian 13

Comparison eDNA diversity to that of visual census data in Raja Ampat 15

Discussions 21

Limitation of sample database 21

eDNA vs Visual Census 22

Importance of Sampling Intensity 24

Biodiversity patterns across Indonesia 24

Prospects for eDNA in high diversity ecosystems 27

Conclusion 29

References 30

List of Figures

Figure 1 5

Figure 2 14

Figure 3 16

Figure 4 17

Figure 5 19

Figure 6 20

Figure 7 20

List of Tables

Table 1.....6

Table 2.....8

Table 3.....12

Table 4.....14

Table 5.....15

Table 6.....26

Acknowledgements

This study was conducted as partial fulfillment of a Master of Science degree in the Department of Ecology and Evolutionary Biology (EEB) at the University of California, Los Angeles (UCLA). This work was funded by USAID PRESTASI Scholarship Program with additional funding from the National Science Foundation (OISE 1243541), and was a collaboration research between the Research Center for Oceanography, Indonesia Institute of Sciences (RCO – LIPI) and UCLA. Many thanks for the support and contributions of various colleagues, institutes, and research that supported this study, including Zack Gold for training me in eDNA methods, Gerald Allen and Mark Erdmann who shared data on fish diversity in Raja Ampat, and the Coral Reef Information and Training Center – Coral Reef Rehabilitation and Management Program – Coral Triangle Initiative – Indonesian Institute for Sciences (CRITIC Coremap CTI LIPI) that shared data on marine fish diversity in Batam-Bintan and other areas. Special thanks to my parents, advisor and committee members, lab partners, friends and many other colleagues who supported me and helped me keep my strength to conduct this research.

Introduction

Indonesia is the heart of the Coral Triangle, a region in Southeast Asia that includes the Philippines, Malaysia, Timor L'Este, Papua New Guinea and the Solomon Islands. Defined by the presence of more than 500 species of scleractinian coral (Veron *et al*., 2009), the Coral Triangle lies at the margins of the Pacific and Indian Oceans and is the world's most biologically diverse marine ecosystem (Allen, 2008; Roberts *et al*., 2002; Veron *et al*., 2009). This remarkable diversity has made the Coral Triangle the focus of numerous biogeographic studies addressing the origins of this biodiversity hotspot (Woodland 1983, Ladd 1960, Ekman 1953), as well as phylogeographic studies examining speciation processes in this region (Allen, 2008; Allen & Werner, 2002; Barber & Bellwood, 2005; Carpenter *et al*., 2010).

The biological importance of the Coral Triangle is matched only by its socio-economic importance. More than 370 million people depend on the ecosystem goods and services of the Coral Triangle, 120 million of which directly benefit from coastal and off shore fisheries production, and marine tourism (Coral Triangle Initiative, 2009; Foale *et al*., 2013). For example, the fishing industry in Indonesia accounted for 21% of Indonesia's agricultural economy and 3% of the national GDP in 2012 (FAO, 2018a). Similarly, the fishing industry in Philippines contributes an estimated 1.8% (valued at US$ 4 billion) to the country's Gross Domestic Product (GDP) in 2012 (FAO, 2018b). The biodiversity of the Coral Triangle also provides almost US$ 10 billion in nature-based marine tourism (NOAA, 2012).

Despite the biological and economic importance of the Coral Triangle, currently, 85% of coral reefs in the Coral Triangle are threatened or extremely threatened (Burke *et al*. 2012). Major threats include local population growth, increasing global demand for marine products,

coastal deforestation, marine pollution, and unsustainable fishing (Hoegh-Guldberg *et al*., 2009). Not only are reefs degrading, but even in "pristine" reef systems like Raja Ampat, larger members of the fish community, such as grouper and Napolean wrasse, have greatly reduced population sizes (Allen, 2003). Moreover, Indonesia wide, keystone species such as reef sharks are also depleted (Sembiring *et al*., 2015).

The losses of reef habitat and ecosystem function don't just impact marine biodiversity, they also impact the human communities that depend on the health reef ecosystems economically and socially (Burke *et al*., 2012). Unfortunately, the majority of the Coral Triangle is water, not land (e.g. Indonesia territory is 70% water). This lack of terrestrial habitat in this region means that it is difficult, if not impossible, to make up for lost marine productivity by increasing productivity on land. For example, even with Indonesia's current heavy reliance on marine resources for food, Indonesia's livestock production is insufficient to supply local demand (Permani *et al*., 2016). As such, it is critical to preserve the marine ecosystems within Indonesia and other Coral Triangle countries and the socio-economic benefits that these ecosystems provide.

One important aspect of achieving sustainability of marine ecosystems in the Coral Triangle is the ability to effectively monitor change over time. However, there are two major challenges to effective monitoring. First, research effort in the Coral Triangle is not proportional to its exceptional biodiversity (Fisher *et al.*, 2011; Barber *et al*., 2014; Keyse *et al*., 2014). As such we have an incomplete understanding of biodiversity in this biologically and economically critical ecosystem (e.g. Barber & Boyce, 2006) and limited funding and personnel to engage in the required biodiversity studies (Barber *et al*., 2014). A second challenge is that the most commonly used method to survey biodiversity, particularly coral and fish, is through visual

census. This method is expensive, time intensive, and may produce biased data as it depends on the skill of the observer and can overlook rare or cryptic taxa (Edgar *et al*., 2004). The latter is particularly problematic in Indonesia because areas like Raja Ampat have at least 1,704 species of marine fish (Allen, 2002), and few individuals have the taxonomic expertise to conduct such surveys. Furthermore, logistical issues and methodological biases hinder researchers' ability to do time-series visual surveys, limiting our ability to understand how these ecosystems are changing over time, precluding the collection of monitoring data that is critical to inform marine conservation efforts. As such, it is essential to develop novel methods that are 1) efficient, 2) inexpensive, 3) require no specific taxonomic expertise, and 4) are amenable to temporal sampling.

Environmental DNA (eDNA) is a revolutionary approach to the study of biodiversity. eDNA is freely associated DNA or cells that animals leave behind in their environment (Pilliod, 2012). Through sampling of soil or water, this DNA can be isolated, extracted, sequenced and then analyzed utilizing metabarcoding methods to document local biodiversity. While microbiologists pioneered initial eDNA efforts to understand soil microbial community diversity (Ogram *et al*., 1987; Taberlet, *et al*., 2012 for a review), eDNA is now widely applied to document the presence of endangered species (e.g. crested newt; Biggs *et al*., 2015; Rees *et al*., 2014, 2017), invasive species (e.g. the American bullfrog; Dejean *et al*., 2012; Rees *et al*., 2014; Takahara *et al*., 2013) and freshwater fish communities (Rees *et al*., 2014; Thomsen, *et al*., 2012), demonstrating the broad utility of this method.

More recently, eDNA is being applied to marine systems. Thomsen *et al*. (2012) recovered fifteen fish species using eDNA, including commercially valuable species as well as species rarely or never recorded by conventional monitoring methods. Similarly, Kelly *et al*.

(2014) assessed entire marine fish communities and showed that eDNA could differentiate between adjacent habitats within a coastal kelp forest ecosystem. Importantly, in some cases (e.g. Kelly *et al*, 2014; 2017), eDNA was more effective than traditional survey methods, recovering a greater diversity of taxa than traditional visual surveys.

While eDNA shows tremendous promise for revolutionizing the assessment and monitoring of marine ecosystems, eDNA methods have largely been employed in temperate marine, aquatic, and terrestrial environments where biodiversity is relatively low (Bohmann *et al*., 2014; Kelly *et al*., 2014; Miya *et al*., 2015; Rees *et al*., 2017; Takahara *et al*., 2013; Thomsen *et al*., 2012). It is unclear whether the methods, sampling design, and bioinformatics approaches employed in such studies will be as equally effective in megadiverse tropical regions like the Coral Triangle.

In this study, we employed eDNA methods to document the distribution and diversity of marine fish across the Indonesian archipelago and compare these results to conventional visual census methods. Specifically, this study seeks to determine (1) whether eDNA sampling methods are effective in regions of exceptional biodiversity, and (2) whether eDNA can capture more fish diversity than traditional survey methods, including rare and cryptic taxa, and (3) the potential for eDNA to provide a cost-effective method for assessing and monitoring fish communities in Indonesia in an effort to support marine conservation efforts across this region.

Materials and Methods

Sampling Sites

We collected eDNA samples across Indonesia, spanning a strong fish biodiversity gradient (Roberts *et al.* 2002; Bellwood & Meyer, 2009). Sampling focused on three regions, including: 1) outside the Coral Triangle in Western Indonesia (Aceh and Batam-Bintan), 2) lower diversity regions of the Coral Triangle in Central Indonesia (Derawan and Wakatobi), and 3) high diversity regions of the Coral Triangle (Eastern Indonesia: Lembeh Strait, Ternate, and Raja Ampat) that have the world's highest reef fish biodiversity (Allen & Werner, 2002; Roberts *et al.* 2002; Bellwood and Meyer 2009; Hoegh-Guldberg *et al.*, 2009).

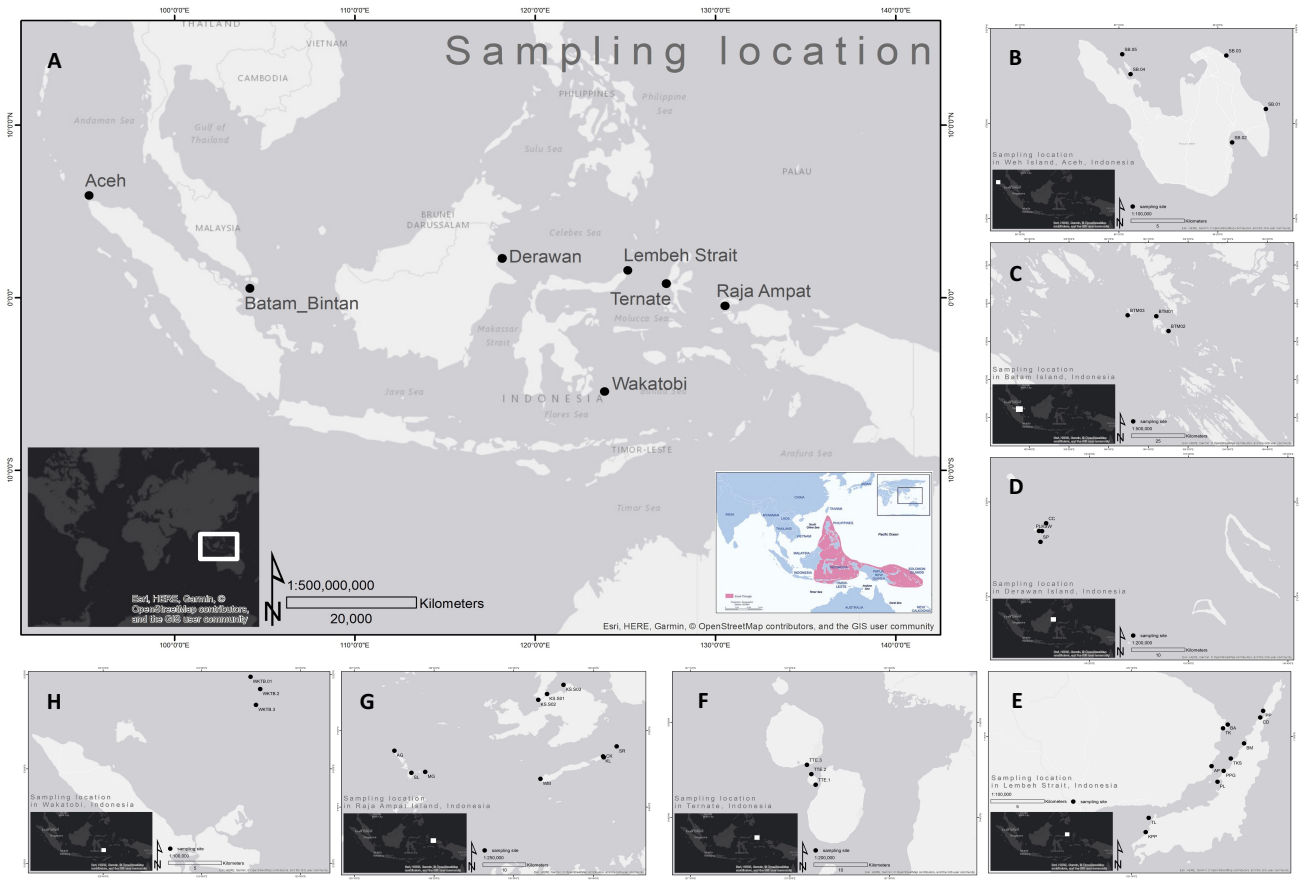

Figure 1. eDNA sampling locations in Indonesia (A) and the detailed sampling sites per location, which are Aceh (B), Batam-Bintan (C), Derawan (D), Lembeh Strait (E), Ternate (F), Raja Ampat (G), and Wakatobi (H). The colorful insert image is the coverage area of Coral Triangle.

eDNA Sampling

To assess marine fish diversity with eDNA, we employed a hierarchical sampling design across 7 regions (Table 1). Each sample consisted of one liter of seawater that was collected on SCUBA at depths between 11-15m to minimize variation in community composition associated with depth. Following standard sampling protocols used in temperate ecosystems (Miya *et al*., 2015), we collected 3 one liter water samples at each sampling site to maximize species diversity and to account for fine-scale heterogeneity in local eDNA signatures. To further maximize species diversity, we sampled multiple sites within each region, with sampling sites separated by at least 5 kilometers to capture spatial variability in habitat and eDNA signatures (Andruszkiewicz *et al*., 2017; Miya *et al*., 2015). For example, on the island of Derawan, we 3 one liter water samples from each of 4 sites, totaling 12 individual eDNA samples. Details of sampling are provided in Table 1.

Table 1. List of sample sites representing 7 different regions across Indonesia, ranging from west (Aceh) to east (Raja Ampat)

Region	Island	Site Codes	Site Names	Latitude	Longitude
Aceh	Pulau Sabang	SB01	Benteng	05º 50.774' N	095º 22.434' E
		SB02	Jaboi	05º 49.007' N	095º 20.731' E
		SB03	Sumur Tiga	05º 53.370' N	095º 20.683' E
		SB04	Rubiah Sea Garden	05º 52.608' N	095º 15.596' E
	Pulau Seulako	SB05	Seulako	05º 53.658' N	095º 15,176' E
Batam – Bintan	Pulau Abang Besar	BTM01	Abang Besar	00º 36.569' N	104º 12.023' E
	Pulau Abang Kecil	BTM02	Abang Kecil	00º 32.693' N	104º 15.016' E
	Pulau Petong	BTM03	Petong	00º 36.824' N	104º 04.814' E
Derawan	Pulau Derawan	CC	Coral Canyon	02º 17.754' N	118º 15.648' E
		PLK	Pelatak	02º 16.938' N	118º 14.928' E
		SP	Snapper Point	02º 15.792' N	118º 15.084' E
		SW	Ship Wreck	02º 16.926' N	118º 15.234' E
Wakatobi	Wakatobi	WKTB01	Wakatobi-1	05º 25.128' S	123º 52.480' E
		WKTB2	Wakatobi-2	05º 25.774 'S	123º 52.974' E
		WKTB3	Wakatobi-3	05º 26.616' S	123º 52.760' E
Lembeh Strait	Pulau Sulawesi	AP	Aer Perang	01º 28.441' N	125º 14.046' E
		BA	Batu Angus	01º 30.642' N	125º 14.860' E
		TK	Teluk Kembahu	01º 30.445' N	125º 14.623’ E
	Pulau Lembeh	BM	Batu Merah	01º 29.646' N	125º 15.690' E

		CD	California Dreaming	01º 30.958' N	125º 16.473' E
		KPP	Kampung Paudean	01º 24.945' N	125º 10.716' E
		PL	Pantai Labuhan	01º 27.614' N	125º 14.348' E
		PP	Pulau Putus	01º 31.368' N	125º 16.649' E
		PPG	Pantai Parigi	01º 28.174' N	125º 14.670' E
		TKS	Tanjung Kusu-kusu	01º 28.839' N	125º 15.016' E
		TL	Tanjung Lampu	01º 25.657' N	125º 11.031' E
Ternate	Pulau Ternate	TTE1	Ternate-1	00º 43.442' N	127º 22.602' E
		TTE2	Ternate-2	00º 44.522' N	127º 22.175' E
		TTE3	Ternate-3	00º 45.528' N	127º 21.730' E
Raja Ampat	Pulau Waigeo	KS_1	Kabui Strait_1	00º 25.164' S	130º 34,204' E
		KS_2	Kabui Strait_2	00º 25.930' S	130º 33.107' E
		KS_3	Kabui Strait_3	00º 23.972' S	130º 36.267' E
	Pulau Fam	AG	Anita's Garden	00º 32.608' S	130º 15.038' E
		MG	Melissa's Garden	00º 35.390' S	130º 18.909' E
		SL	Secret Lagoon	00º 35.514' S	130º 17.180' E
	Pulau Mansuar	CK	Cape Kri	00º 33.485' S	130º 41.334' E
		KL	Kri Lagoon	00º 33.354' S	130º 41.252' E
		SR	Sardine Reef	00º 32.044' S	130º 42.949' E
		WM	West Mansuar	00º 36.306' S	130º 33.387' E

To isolate eDNA from water samples, we filtered one liter of seawater through a 0.22 micron Sterivex™ filter (Millipore®, SIGMA MILLIPORE) following the methods of Miya *et al*. (2015) with one key modification; we collected individual water samples in sterile 1 liter Kangaroo™ Gravity Feeding Bags (similar to intravenous drip bags) that allow for gravity filtration through the Sterivex collumns, a method ideally suited to remote field locations. In addition to the eDNA water samples, we also filtered one blank at each locality as a negative control. Filters were stored in a -20 freezer until eDNA was extracted.

eDNA Extraction, Amplification, and Sequencing

In total, we extracted 119 eDNA samples and 4 blanks. We extracted eDNA using the DNeasy Blood & Tissue Kit (QIAGEN, Germany) following the manufacturer's protocol. We amplified extracted eDNA using the Multiplex PCR Kit (QIAGEN, Germany), targeting a region

of 12S rRNA mitochondrial DNA specifically designed for marine fishes (Miya *et al.*, 2015) using primer detailed in Table 2.

Table 2. The details of eDNA primer

Primer Name (DNA target)	Forward Primer		Reverse Primer		References
12S	MiFish-U F	GTCGGTAAA ACTCGTGCC AGC	MiFish-U R	CATAGTGGGGTA TCTAATCCCAGT TTG	Miya et al., 2015

Each individual eDNA sample was amplified via Polymerase Chain Reactions (PCR) in triplicate to account for potential PCR bias (Andruszkiewicz *et al*., 2017; Miya *et al*., 2015; Taberlet *et al.*, 2012). Each PCR reaction consisted of 12.5 µL Qiagen 2x Master Mix, 2.5 µL (2 mM) of the primer, 6.5 µL nuclease free water, and 1 µL the DNA extract. Thermocyling parameters utilized a touchdown protocol, beginning with a 15 minute pre-denaturation step at a 95 °C, followed by a touchdown thermocycling profile consisting of 30 seconds denaturing at 94 °C, 30 seconds annealing at 69.5 °C, and 30 seconds extension at 72 °C, with the annealing temperature dropping by 1.5 °C per cycle until 50 °C. Following this initial touchdown phase, the main cycle consisted of 25 cycles of 94 °C for 30 seconds for denaturation, 50 °C for 30 seconds for annealing and 72 °C for 45 seconds for extension, concluding with a 10 minute final extension at 72 °C.

To visualize successful PCR reactions, we electrophoresed 5µL of all PCR products for 30 minutes at 150 volts on 2% agarose gels prepared with 6x SYBR™ Green (Invitrogen™, ThermoFisher Scientific). We then pooled the triplicate PCR products, representing a single one-liter eDNA sample, into a single tube, and purified these pooled PCR products using Sera-Mag™ and Sera-Mag SpeedBeads Magnetic Particles (SIGMA-ALDRICH®) following manufacturer's

protocols. Next, we quantified the DNA concentration (ng/µL) of each pooled PCR sample using the Qubit™ 4 NGS Starter Kit (ThermoFisher) following the manufacturer protocol and then adjusted concentrations of pooled PCRs to be equal across all samples. The Nextera DNA Library Preparation Kit (illumine®) was then used to index each PCR amplified eDNA sample using a unique combination of Illumina Nextera i5 and i7 primers in a second PCR reaction, following the manufacturer protocol. The indexing PCR reaction consisted of 12.5µL Kapa High Fidelity Master Mix, 0.625 µL of 1µM i5 Illumina Nextera indices, 0.625 µL of 1µM i7 Illumina Nextera indices, and 11.25 µL of PCR product for a total of 10ng of DNA. To bioinformatically distinguish among samples, we also added index barcodes to each sample utilizing an indexing PCR protocol that began with an initial denaturation of 95 ˚C for 5 minutes, followed by 8 cycles of: 98˚C denaturation for 30 seconds, 56˚C annealing for 30 seconds, and 72˚C extension for 3 minutes, ending with a 72˚C extension for 5 minutes. To ensure the indexing PCR was successful, we electrophoresed indexed PCR products at 120V for 45 minutes on a 2% agarose gel prepared with 6x SYBR™ Green. Indexed PCR products were then cleaned and quantified, as above, to creating a final sequencing library that contained equal DNA concentrations (10 ng/µl) of each sample. The final libraries were sequenced at the UC Berkeley sequencing core on an Illumina MiSeq platform utilizing 300 base pair paired end sequencing.

Bioinformatics and Data Analysis

We conducted analyses of fish biodiversity in a hierarchical fashion. First, we examined diversity at the level of individual sampling sites, where fish diversity was represented by eDNA sequences amplified from each of the three one liter water samples at a given sampling locality. Second we examined diversity at the regional level, where fish diversity was represented by

eDNA sequences amplified from each of the three one liter water samples taken from multiple sampling localities within each of the 7 regions sampled (Table 1).

We analyzed all eDNA sequences using the *Anacapa* pipeline (Curd *et al*., 2018). Briefly, *Anacapa* begins by creating a *de novo* sequence reference library by combining records from publically accessible databases, such as European Molecular Biology Laboratory (EMBL) and National Center for Biotechnology Information (NCBI) using the CRUX package (Creating Reference libraries Using eXisting tools; https://github.com/limey-bean/Anacapa/tree/New-Master/Anacapa_db). This feature is particularly important as there are not pre-existing 12S reference libraries for Indonesian fishes. *Anacapa* then de-multiplexes the amplicon reads based on the primer sequences (Table 2) and primers are trimmed from the reads. Next, the DADA2 algorithm (Callahan *et al.* 2016) performs denoising and error correction on the raw sequence data, merges paired-end reads, and assigns high quality reads to Amplicon Sequence Variants (ASVs) through ASV Parsing. Finally, ASVs are assigned to taxa by Bowtie 2 and the Bayesian Least Common Ancestor algorithm using a 60% likelihood threshold (BLCA; Gao *et al.* 2017.)

Analyses in *Anacapa* began with an exploratory set of diversity summary statistics created using the *Ranacap*a package (Kandlikar *et al*., 2018). We then calculated standard alpha and beta biodiversity statistics in R by using the *Phyloseq* package (https://www.bioconductor.org/) with supporting analysis packages, including *devtools* and *vegan*. For most analyses, we excluded singletons to provide the most conservative measures of diversity. However, when comparing to fish visual census data, we include singletons to maximize diversity recovered from eDNA.

To examine patterns of fish diversity, ASV tables output from DADA2 were converted to the BIOM format and imported into QIIME 2 for diversity analyses, statistical testing, and data

visualization. The core-metrics pipeline from the QIIME 2 diversity plugin was used to compute alpha and beta diversity metrics using an even sampling (rarefaction) depth of 25,000 reads per sampling locality (i.e. individual reefs where 3 one liter water samples were sampled). Alpha diversity metrics examined included species richness (as measured by total number of ASVs), the Shannon's diversity index, Simpson's diversity index and number of observed ASVs; beta diversity was measured using DCA Jaccard index visualized in Detrended Correspondence Analysis (DCA).

Comparison of eDNA Survey and Visual Census

To determine the efficacy of eDNA as a biodiversity survey tool in high biodiversity ecosystems, we compared eDNA results to existing fish biodiversity data obtained from visual census surveys. We compared the results of fish eDNA metabarcoding survey results with high-quality visual census data developed by Gerry Allen (Allen, 2002), in Raja Ampat, a region representing extremely high fish diversity.

Because the performance of eDNA can be hindered by incomplete reference databases, we compared the effectiveness of eDNA and visual census in two different ways. First, we compared total species richness by comparing the total number of ASVs at each site identified through eDNA and the total number of species from species lists generated through visual surveys. Second, a list of taxa identified from eDNA that only included ASVs identified to species was compared to the species lists from visual surveys. A custom Python script was used to determine the overlap of species identified by the two survey methods and Venn diagrams were created to show the overlap of species identified between the two methods. In contrast to

initial diversity analyses that excluded singletons, we included singletons in this analysis because visual census protocol count taxa, even if only observed once.

Results

Raw eDNA Sequences

We collected 119 eDNA samples from June-August 2017, and from these samples we generated 15,219,431 12S raw sequence reads. After quality control filtering and removal of blanks, non-target taxa (i.e. not marine fishes), and the negative and positive controls, this total reduced to 9,496,999 high quality sequence reads. Total number of high quality sequences per region ranged from a high of 2,136,430 in Raja Ampat to 670,394 in Batam-Bintan (Table 3).

Following taxonomic assignment in the *Anacapa Classifier* module, we recovered 776 groupings of ASVs that are assumed to represent distinct species (hereafter referred to as ASVs). However, this number dropped to 650 after removing blanks, negative and positive controls, non-marine fishes, singletons, and after normalizing sequence reads across to 25,000 reads per sample. Number of ASVs ranged from a high of 388 in Raja Ampat to a low of 86 in Batam-Bintam. Numbers of ASVs that could be identified to species ranged from a high of 52% in Raja Ampat to a low of 13.23% in Batam-Bintan (Table 3).

Table 3. Number of sequence reads along data processing per region and location targeted by 12S rRNA mitochondrial DNA. R_0 is total sequence reads from MiSeq running. R_1 is sumary sequence reads produced by Anacapa pipeline. R_2 is total final sequence reads (after removing blanks, negative and positive controls, non-marine fishes, singletons, and after standardizing sequence number across all samples)

Regions	Data Processing Steps	Total number of reads	Number of ASVs	Percent of ASVs identified species (%)
Aceh	R_0	1,285,220		
	R_1	1,178,502	225	28.99

	R_2	774,934	194	29.84
Batam-Bintan	R_0	862,244		
	R_1	799,528	112	14.43
	R_2	670,394	86	13.23
Derawan	R_0	1,186,195		
	R_1	1,101,778	304	39.18
	R_2	684,072	269	41.38
Wakatobi	R_0	853,755		
	R_1	812,652	219	28.22
	R_2	689,666	193	29.69
Lembeh Strait	R_0	2,199,746		
	R_1	2,009,913	381	49.10
	R_2	1,494,582	326	50.15
Ternate	R_0	1,009,469		
	R_1	945,517	208	26.80
	R_2	783,089	180	27.69
Raja Ampat	R_0	2,952,814		
	R_1	2,649,109	388	50
	R_2	2,136,430	338	52

Regional Pattern and Identification of Amplicon Sequence Variants

Of 650 non-singleton ASVs, analyses in *Anacapa* identified 530 species, representing 107 genera and 47 families of marine fish. Diversity was highest in Raja Ampat, but otherwise did not follow the expected pattern of decreasing fish diversity from east to west (Edinger et al., 1998; Roberts 2002, Bellwood and Meyer 2009). After Raja Ampat, the next most diverse population was Lembeh Strait, followed in order by Derawan, Aceh, Wakatobi, Ternate and Batam-Bintam (Table 3). Similarly, even when including singletons, Simpson's, Shannon's. and Faith's Indices did not follow the expected east-west pattern of decreasing diversity (Table 4). While diversity was highest in Raja Ampat, Ternate, the next eastern most region had the second lowest diversity, followed by Batam-Bintan the second western most region. Results of DCA based on ASVs showed substantial overlap among all seven regions. However, Raja Ampat, Batam-Bintan, and Aceh had the most different distributions (Figure 2).

Table 4. Biodiversity across Indonesia measured by Shannon's, Simpson's, Faith's Phylogenetic diversity index and ASV richness based on regional ASV diversity rarefied to 60k sequences.

Regions	Shannon's Index	Simpson's Index	Faith's Phylogenetic Diversity	ASV Richness
Aceh	6.156	0.956	34.51	440
Batam-Bintan	2.987	0.774	13.80	86
Derawan	6.486	0.964	39.78	493
Wakatobi	5.358	0.929	34.19	323
Lembeh Strait	7.052	0.982	48.46	687
Ternate	6.393	0.974	29.63	328
Raja Ampat	7.076	0.980	50.82	721

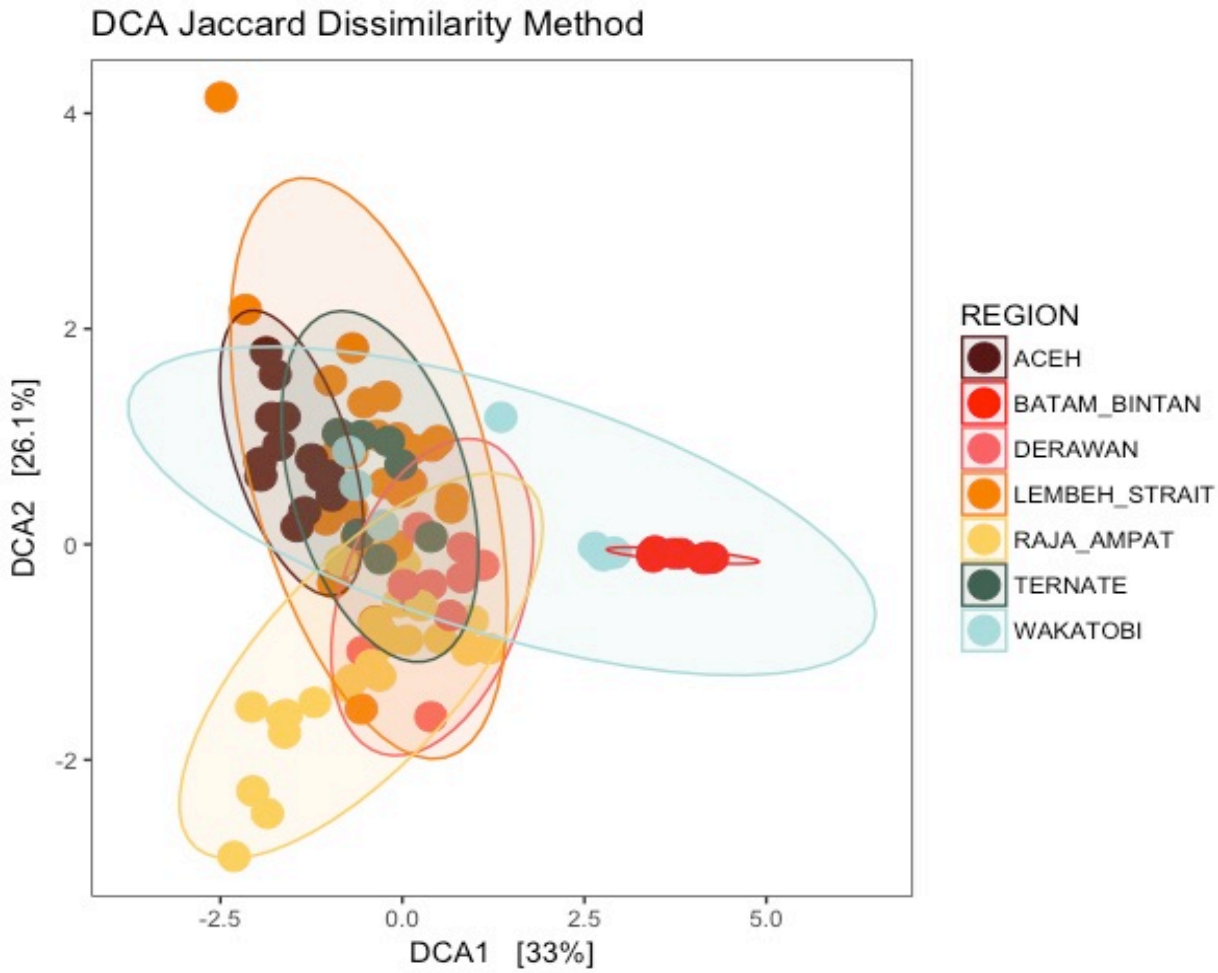

Figure 2. Beta biodiversity analyzed using Jaccard Dissimilarity Method and visualized in Detrended Correspondence Analysis (DCA)

Comparison eDNA diversity to that of visual census data in Raja Ampat

Comparing results from the eDNA method to visual census survey results from Allen and Erdmann (2002) showed very different patterns across Raja Ampat (Table 5). For all sampling sites with the exception of Kabui Strait, visual census surveys recovered more total fish diversity. This difference was even more pronounced when comparing ASVs identified to species rather than ASVs.

Table 5. Comparison of fish diversity in Raja Ampat based on 1) total ASVs, 2) ASVs identified to species, and 3) Species counts from visual census surveys

Region	Locality	Total ASVs	ASVs Identified to Species	Species in Visual Census
Raja Ampat	Anita Garden	166	57	218
	Cape Kri	146	29	272
	Kri Lagoon	167	71	302
	Kabui Strait	187	113	81
	Melissa Garden	131	75	214
	Secret Lagoon	127	52	89
	Sardine Reef	151	45	210
	West Mansuar	112	38	227

Directly comparing species identified through eDNA metabarcoding and the visual census conducted by Allen and Edrmann (Allen, 2002) showed minimal overlap between taxa identified through visual surveys and taxa identified by eDNA (Figure 3). The greatest percentage overlap in species was in Melissa's Garden where only 24 of 214 species were shared among eDNA and visual survey methods. The lowest percentage overlap was in Cape Kri with only 9 species shared among a total of 274 identified through both methods. Even though only a limited number of ASVs from eDNA analyses could be identified to species, many ASVs identified to species were not detected in visual surveys. Species identified only through eDNA spanned a wide diversity of taxa, spanning 47 different families (Figure 4).

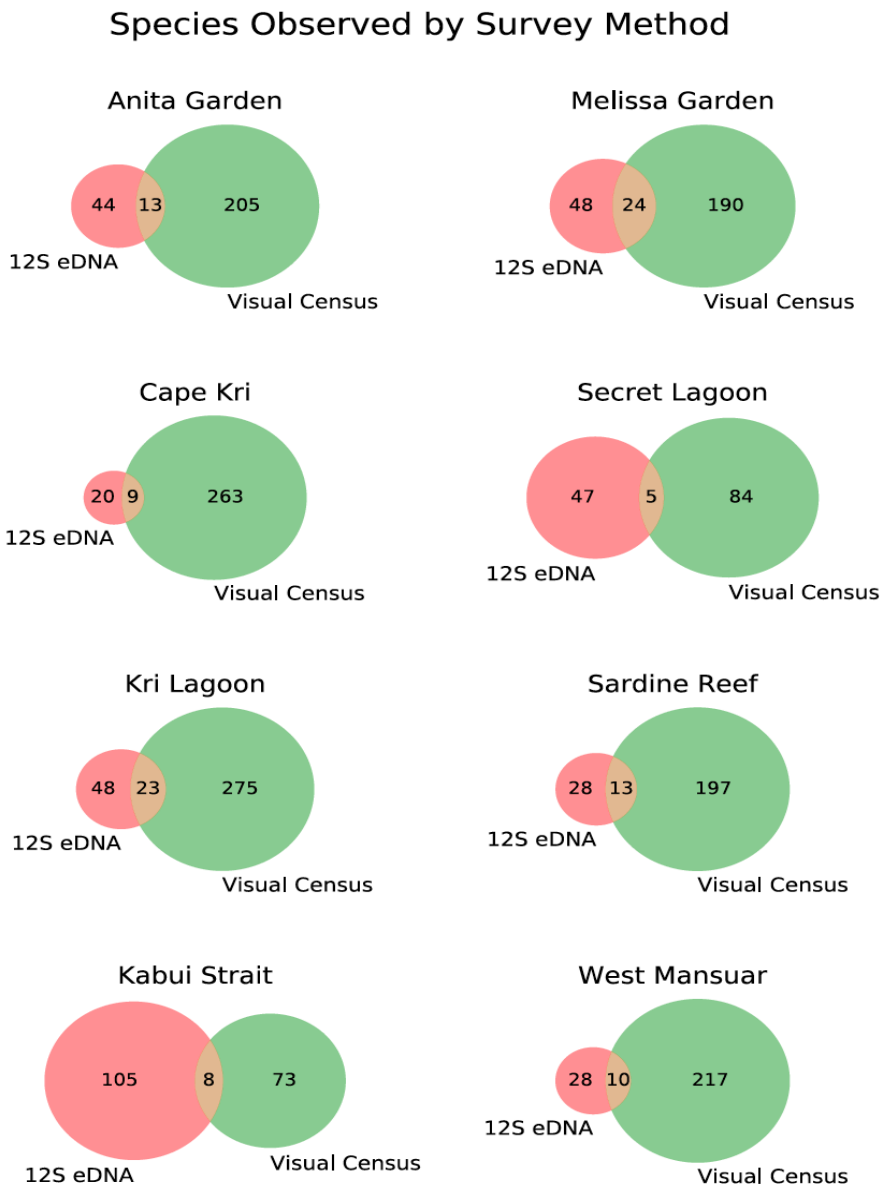

Figure 3. A comparison between the number of species identified through eDNA and visual census data in Raja Ampat. eDNA species in red represent ASVs that could be attributed to species based on reference databases, while visual census survey data in green comes from (Allen, 2002).

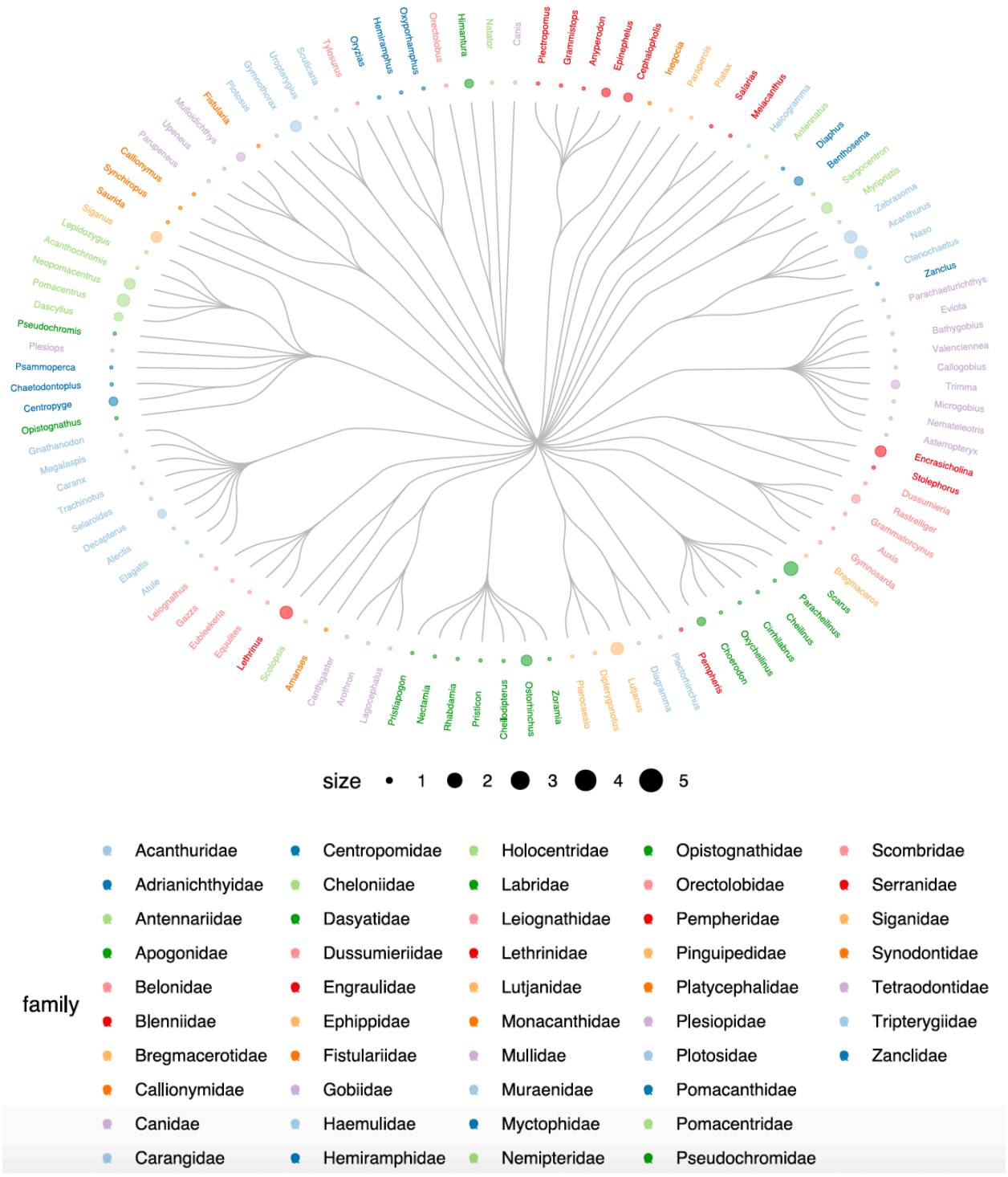

Figure 4. Phylogram showing the distribution of families and genera represented by samples recovered through eDNA analyses, but that were absent from visual census surveys by Allen (2002). Colors correspond to taxonomy and size of circle indicates numbers of species.

To determine whether these results were impacted by sequencing depth, we plotted rarefaction curves, comparing accumulation of ASVs versus the number of samples eDNA water samples taken in each region. ASV accumulation curves show that sequencing and sampling depth for any given sample and any given site was sufficient to capture all of the diversity present in eDNA (Figure 5). However, rarefaction curves showed that sampling depth was insufficient to capture the diversity in each of the 7 regions sampled, with the eastern most localities requiring the highest amount of additional sequencing and the western most sites requiring the least amount of additional sequencing to asymptote (Figure 6A).

To estimate the required number of samples needed to capture the full biodiversity of each region, we employed the iNEXT package in R to extrapolate the species accumulation curves to the number of samples required to saturated species accumulation relative to sampling depth. Results indicate that the lower-diversity regions (e.g. Batam-Bintan) needed 50-75 samples to capture all fish diversity present, while the higher-diversity regions, such as Raja Ampat required 100-150 or more samples to achieve saturation of fish diversity. Further evidence for the need of increased sampling is evidenced by limited overlap in species recovered in individual one liter samples obtained from the same sampling site (Figure 7).

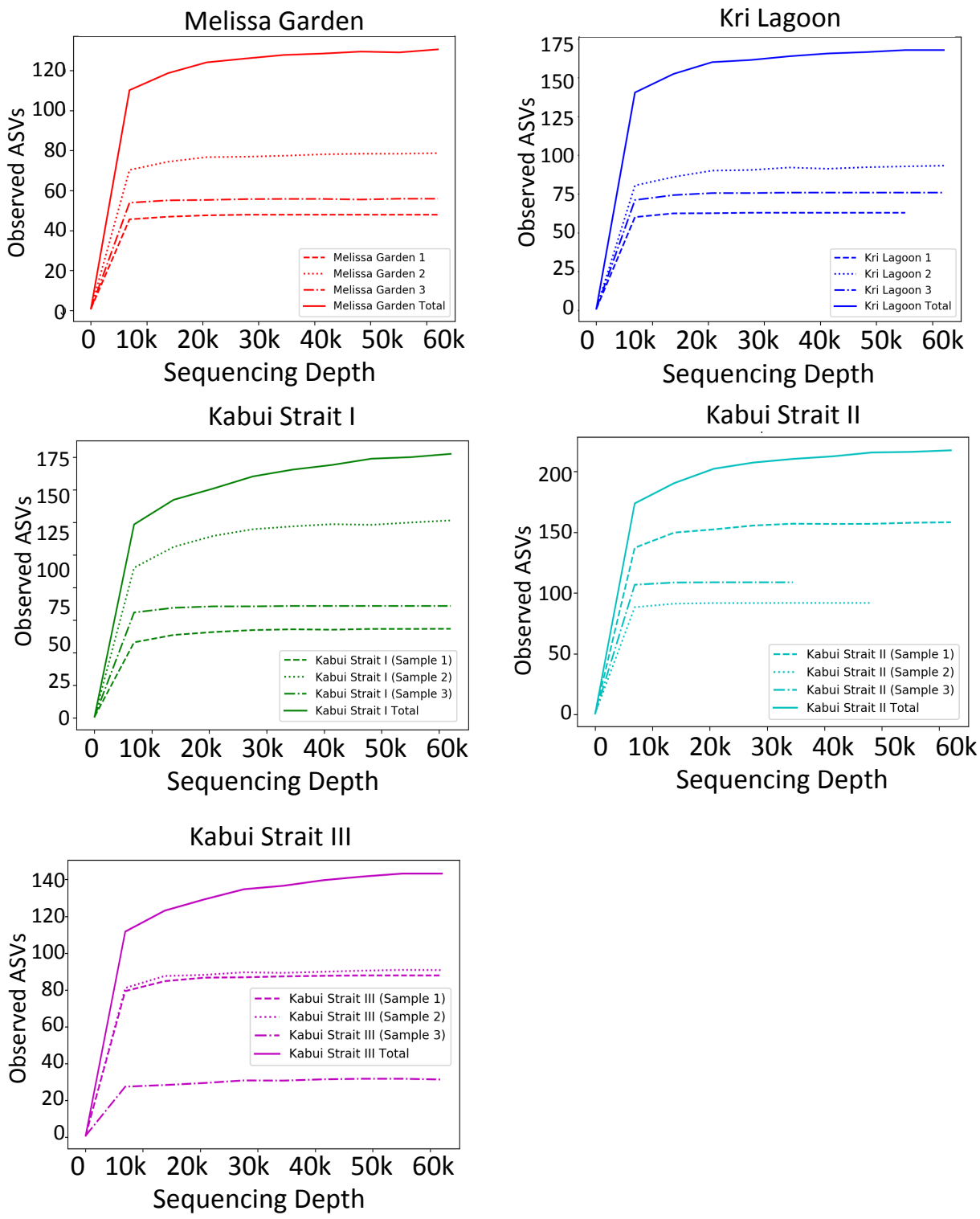

Figure 5. Species accumulation vs. sequencing depth for individual one liter eDNA samples across five sites in Raja Ampat showing that sequencing depth was sufficient to capture all species present in an individual eDNA sample.

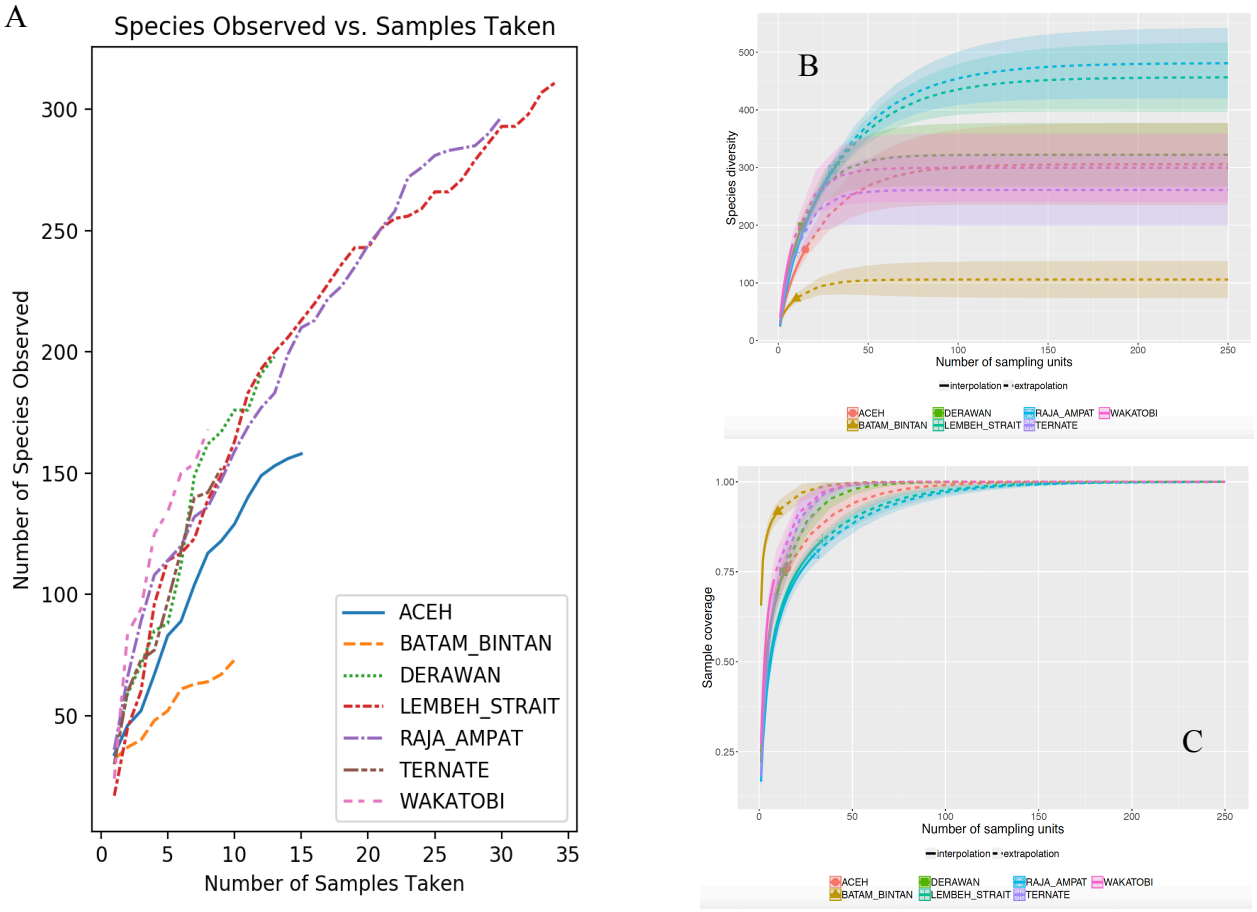

Figure 6. (A) Rarefaction curve comparing sample number to ASV diversity. (B) Species accumulation curves for all regions extrapolated to 250 sampling units, (C) Plot of sample coverage showing the percent of total biodiversity captured across total number of sampling units (e.g. 1 liter of water).

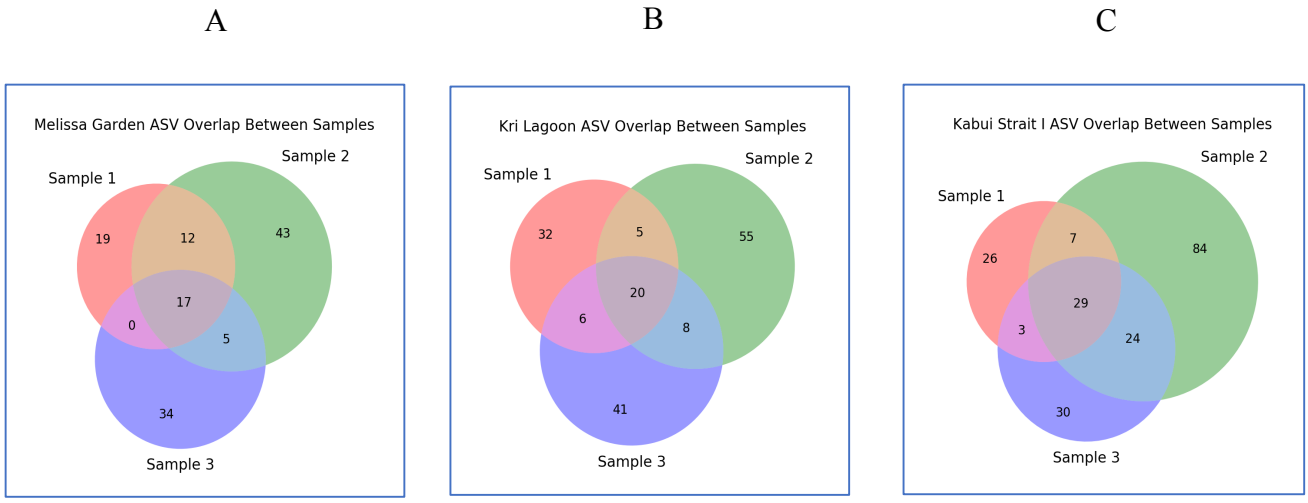

Figure 7. Ven diagrams showing minimal overlapping in ASVs captured in eDNA 3 individual eDNA samples collected from Melissa Garden (A), Kri Lagoon (B), and Kabui Strait I (C) in Raja Ampat.

Discussion

Environmental DNA (eDNA) can be a valuable tool for examining community diversity. However, while this technique has been effective in many lower diversity temperate marine ecosystems (Bohmann *et al*., 2014; Kelly *et al*., 2014; Miya *et al*., 2015; Rees *et al*., 2017; Takahara *et al*., 2013; Thomsen *et al*., 2012), the results of this study highlight significant challenges in regions of exceptional diversity like the Coral Triangle. While eDNA recovered a large amount of fish biodiversity in the form of Amplified Sequence Variants, only a fraction of this diversity could be identified to species, highlighting the limitations of existing reference databases.

A second major challenge observed in eDNA in high diversity ecosystems is the need for increased sampling depth. Even when ignoring the ability to identify ASVs to species, rarefaction curves plotting sample number vs. ASV showed that sampling protocols employed in less diverse aquatic ecosystems (Kelly *et al*., 2014; Miya *et al*., 2015; Thomsen *et al*., 2012) is insufficient to capture the high biodiversity of regions like the Coral Triangle, even as sequencing depth successfully captured all eDNA diversity within an individual water sample.

Limitations of sample databases

In contrast to previous studies, a large proportion of our ASVs could not be assigned to species. Interestingly, the highest percentage of ASV species assignment was in Raja Ampat with 52% of ASVs being assigned to species, despite supporting the most diverse reef fish communities in the ocean (Allen, 2002). In contrast, Batam-Bintam, the least diverse region sampled had nearly 87% of ASVs unidentified. This pattern highlights two key issues.

The first challenge for eDNA studies in Indonesia is that while DNA sequence databases like the National Center for Biotechnology Information (NCBI) and the European Molecular Biology Laboratory (EMBL) have a relatively large number of taxonomic coverage from temperate regions, these databases have a limited number of samples from high diversity regions like Indonesia. This disproportionate availability of sequencing resources in lower diversity regions of the world allows eDNA studies in these regions to be highly effective in capturing local diversity (e.g. Andruszkiewicz *et al*., 2017; Kelly *et al*., 2014; Miya *et al*., 2015; Thomsen *et al.*, 2012). As such, the limitations of eDNA in the Coral Triangle are likely a function of limited research focus in this region (Fisher *et al*. 2010, Barber *et al*., 2014; Keyse *et al*., 2014); there is no reason to believe that eDNA efforts in the Coral Triangle wouldn't be as effective, if research effort in this region was proportional to diversity

The second issue facing the Coral Triangle is that what limited research occurs in this region is often focused on the highest diversity regions like Raja Ampat, (Allen, 2008; Allen & Werner, 2002; Roberts *et al*., 2002), while largely ignoring less diverse regions like Batam-Bintam. Given the remarkable diversity of the Coral Triangle, it is highly likely that even low diversity regions harbor a substantial amount of unknown biodiversity, as highlighted by only 13% of eDNA sequences from Batam-Bintam being identified to species through matching to existing DNA sequence databases.

eDNA vs Visual Census

In all but one case eDNA recovered substantially less fish biodiversity than visual census data from the same locations. In side-by-side comparisons in Raja Ampat, not only did eDNA recover much less fish diversity than visual census methods, but it also recovered completely

different fish diversity. At most, only 10% of taxa overlapped in eDNA and visual census surveys (Figure 3).

While this result, in some ways, highlights the challenge of eDNA approaches in megadiverse ecosystems, it also highlights its utility. In total, eDNA recovered diversity from nearly 50 families of marine fishes that were missed by visual census surveys (Figure 4). While some of the diversity captured by eDNA included cryptic taxa like Blennidae that are obvious taxa to be missed in visual census surveys, it also captured a large number of taxa like Lutjanids, Pomacentrids, Siganids, and Epinephalids, among others, that are large, conspicuous fish that should be easily observed in visual surveys. While it is possible that eDNA at any given location could have been transported from a nearby reef or represent eDNA from larval stages, the comparison of taxa identified through eDNA and visual census was done at the regional, not local scale. Moreover, phylogeographic analyses show that Raja Ampat and Eastern Indonesia are phylo and biogeographically very unique (Barber *et al*., 2011; DeBoer *et al*., 2014a, 2014b) due to geology and physical oceanographic processes (Kool *et al*. 2011). As such, it is unlikely that this eDNA was transported from a different biogeographic region. However, because of the phylogeographic uniqueness of Raja Ampat, it is possible that sequences representing a particular species obtained from eDNA are genetically divergent from sequences in public reference databases, and that unique taxa assigned to eDNA 12s ASVs reflect deep phylogeographic structure, such as that observed in other marine taxa. Combined, these results highlight the complementary nature of eDNA to more traditional fish biodiversity survey methods.

Importance of Sampling Intensity.

The results of this study are likely a function of sampling intensity in two important ways. First, visual censuses of fish communities on coral reefs are done in multiple habitats over multiple days to maximize the amount of diversity recovered (Allen, 2002; Erdmann & Pet, 2002). While we sampled multiple liters of sea water from across individual reefs and from multiple reefs within a given region, this amount of sampling does not match the sampling intensity of visual census surveys. Second, while previous eDNA studies in temperate ecosystems recover high percentages of local biodiversity (Table 6), eDNA only captured a fraction of diversity within any region of Indonesia sampled in this study. Moreover, the ability of eDNA to capture all taxa within a single liter of water, and inability of eDNA to capture local fish diversity suggests that there may be a limit to how much diversity can be captured in a single liter of water through eDNA analysis. As such, eDNA studies in high diversity ecosystems will require greater sampling intensity. Given that our models indicate that high biodiversity regions like Raja Ampat may require in excess of 100 one-liter samples to capture all fish diversity present, eDNA surveys in this area will either need to increase the numbers of samples or volume of water per sample to be maximally informative. Further studies are necessary to determine the optimal sampling strategy.

Biodiversity patterns across Indonesia

One of the best-documented patterns of marine biodiversity is the extreme concentration of biodiversity in the Coral Triangle (Edinger *et al*., 1998; Veron, 1993; Wallace 1997; Roberts 2002, Bellwood & Meyer, 2009). Given that our study stretched from regions like Raja Ampat, known for the highest marine fish diversity in the world (Allen, 2002; 2008) to regions in

Western Indonesia that are outside of the Coral Triangle (Veron *et al*., 2009) and given that these patterns are based partially on fish biodiversity (Roberts 2002, Bellwood & Meyer, 2009), we expected to see clear gradients in diversity based on eDNA. However, while the Eastern Indonesian reefs of Raja Ampat had the highest fish biodiversity, and the Western Indonesian reefs of Batam-Bintan had the lowest, there were no other clear patterns of fish diversity based on eDNA.

There are two potential explanations for the failure of eDNA to capture this east-west biodiversity gradient in Indonesia. First, the rarefaction plots and models of sampling depth required to achieve species saturation, show that eDNA recovered a higher percentage of fish diversity present in Western Indonesia than in Eastern Indonesia. Thus, the inability to recover a well-known biodiversity pattern could be an artifact of undersampling the most biodiverse reefs in Indonesia, and that results from higher intensity eDNA sampling design could yield results that conform to predictions based on previous studies (Edinger *et al*., 1998; Veron, 1993; Wallace 1997).

A second, but not mutually exclusive explanation, is that visual surveys may not fully capture all of the diversity present. There are many taxa like blennies that live within the reef matrix (Böhm & Hoeksema, 2017; Kotrschal, 1988; Nursall, 1977; Wilson *et al.*, 2013). Moreover, visual surveys in Indonesia have been done on SCUBA, which can negatively impact fish counts (Lindfield *et al.*, 2014) As such, that the well-known biodiversity gradients in Indonesia may reflect visually conspicuous biodiversity, but not cryptic diversity or fish diversity sensitive to SCUBA.

A third potential explanation to the mismatch between eDNA and visual census data is that these studies were not conducted contemporaneously. Given that marine ecosystems are

dynamic, conducting eDNA and visual surveys at different times could result in capturing different communities. This possibility would be easily tested by including eDNA sampling as part of any fish visual census protocol, and doing so could add value to visual surveys as eDNA may provide insight into parts of the fish community (e.g. blennies) that are difficult to fully capture in visual surveys.

Finally, because eDNA and visual census survey data showed minimal taxonomic overlap it is possible that the more cryptic diversity captured by eDNA does not follow expected regional patterns in biodiversity, a result that could be confirmed through studies of cryptic invertebrate taxa or fish surveys on rebreathers, rather than open-circuit SCUBA. Given the above combined with the fact that 1) Aceh is located at the nexus between the Indian Ocean and Java Sea (Fadli *et al*., 2012; 2014), 2) this region is a suture zone between Pacific and Indian Ocean basins (Crandall *et al*. 2011), and 3) this region is receiving less scientific study that regions like Raja Ampat, it is possible that Western Indonesia isn't as biologically depurate as presently believed.

Table 6. Review of eDNA studies in temperate regions showing the ability of eDNA to effectively capture biodiversity in different habitats.

Sources	Reference	Environment	Region/Country	Target species	Result
Seawater	Andruszkiewicz *et al*., 2017	temperate east pacific	Monterey bay/ USA	marine vertebrates	72 species across 10 genera, 3 subfamilies, and 7 families
	Bakker *et al*., 2017	Temperate south pacific	New Caledonia and Carribia	sharks	22 elasmobranch molecular operational taxonomic units (MOTUs)
	Boussarie *et al*., 2018	temperate southwestern pacific	New Caledonian archipelago/ New Caledonia	sharks	13 shark species in 22 eDNA samples
	Guardiola *et al*., 2016	temperate ocean	Blanes Canyon/ Spain	deep-sea organisms	130 Metazoan, 30 Nematoda
	Kelly *et al*., 2014	temperate east pacific	California/ USA	marine fishes	230 OTUs (98% of

					variances)
	Miya *et al*., 2015	temperate ocean	Okinawa/ Japan	marine fishes	249 species across 64 families and 146 genera
	Thomsen *et al*., 2012	temperate ocean	The Sound of Elsinore/ Denmark	marine fishes	15 fish species of 9 orders and 11 families
	Yamamoto *et al*., 2017	temperate ocean	Maizuru bay/ Japan	marine fishes	2,323 fish species
Sediment	Ficetola *et al*., 2018	temperate lake	Lake La Poule/ Southern Pacific	freshwater invasive organisms	Various number of fungi spores, plants, mammals.
	Laroche *et al*., 2017	temperate south pacific	South Taranaki Bight/ New Zealand	marine benthic organisms	7,505 (78%) OTUs of bacteria, 2,317 (80%) OTUs of Eukaryotes
Freshwater	Rees *et al*., 2017	temperate freshwater ponds	Cambridgeshire/ United Kingdom	Great Crested newt (*Triturus cristatus*)	eDNA of species target detected in in all months in at least one of the ponds ponds
	Takahara *et al*., 2013	freshwater ponds	Hiroshima/ Japan	invasive bluegill sunfish (*Lepomis macrochirus*)	eDNA of species target detected in 19 of 70 ponds
	Thomsen *et al*., 2012	temperate freshwater ponds	Denmark, Sweden, Germany, Poland and Estonia	freshwater organisms	100% fish, 91–100% amphibians, 82% dragonfly, and 100% tadpole shrimp

Prospects for eDNA in high diversity ecosystems

Visual census studies in Raja Ampat recovered nearly 1000 species of marine fishes (Allen, 2002; Erdmann and Pet, 2002), a number that is significantly higher than the 721 ASVs including singletons or 650 excluding singletons obtained through eDNA. While the eDNA numbers are lower than the visual census surveys, it is important to put these numbers into context of sampling effort and cost. At it's worst, eDNA only recovered 49% as much fish diversity as visual census (Table 5), but this diversity was captured in a manner of minutes in only 3 liters of seawater, whereas the visual census data likely required dozens of man hours. Even when considering time and cost of lab work, eDNA is more time and cost efficient.

Moreover, while the eDNA required basic laboratory skills such as PCR, the visual census survey data required extensive taxonomic experience that took decades to develop. Obtaining almost half of the diversity from a small fraction of the effort speaks to the power of eDNA.

However, in two cases eDNA outperformed visual census data, recovering 143% of diversity in Secret Lagoon and 231% of the diversity in Kabui Strait. Even when eDNA underperformed visual census data, it still recovered an average of 61% of the diversity from visual surveys, ignoring the two cases where eDNA over performed. Moreover, it provided these results for approximately $50 per sample. As such, while there are clearly limitations to eDNA in high diversity ecosystems, the ability of eDNA to 1) provide substantial and insights into marine biodiversity with a minimum of taxonomic knowledge and, 2) provide complementary data on rare or cryptic taxa potentially overlooked by traditional visual surveys highlights the immense value and promise of eDNA data.

eDNA is clearly a valuable new tool for examining biodiversity across a variety of habitats. As the field of eDNA continues to mature, it is critical to do more research on the performance of eDNA in highly diverse ecosystems, such as Indonesia. Important next steps including working to developing reference databases in diverse tropical marine ecosystems like the Coral Triangle to increase the number of ASVs that can be assigned to species. In addition, performing eDNA and visual surveys at the same time in the same location, and increasing either the volume or number of eDNA water samples could provide important insights into the best sampling design for eDNA research, creating standard field protocols for eDNA and visual census surveys in the future.

Conclusions

The application of eDNA to assess biodiversity is still in its infancy. While the results of this study highlight the need for better sampling design for eDNA studies in exceptionally diverse regions like the the Coral Triangle, they also demonstrate that eDNA methods may be a powerful and economical valuable tool. In particular, eDNA is ideally suited for temporal monitoring of biodiversity and rapid biodiversity surveys spanning large geographic regions.

For eDNA to be maximally useful, it will be important to invest in the creation of more comprehensive reference barcode databases in biodiversity hotspots like the Coral Triangle to obtain the strongest results. As we refine sampling protocols and complete reference databases, there is no reason why eDNA can't equal or surpass the results of visual census surveys in even the most diverse marine ecosystems, providing a much needed tool to study and monitor marine ecosystems across the globe.

References

Aeby, G. S., Williams, G. J., Franklin, E. C., Kenyon, J., Cox, E. F., Coles, S., & Work, T. M. 2011. Patterns of Coral Disease across the Hawaiian Archipelago: Relating Disease to Environment. *PLoS ONE*, 6(5), e20370. https://doi.org/10.1371/journal.pone.0020370

Allen, G. R., & Werner, T. B. 2002. Coral Reef Fish Assessment in the 'Coral Triangle' of Southeastern Asia. *Environmental Biology of Fishes*, *65*(2), 209–214. https://doi.org/10.1023/A:1020093012502

Allen, G.R. 2002. Chapter 3. Reef Fishes of the Raja Ampat Islands, Papua Province, Indonesia. In: McKenna, S.A., Allen, G.R. and Suryadi, S. (eds.) A marine rapid assessment of the Raja Ampat Islands, Papua Province, Indonesia. *RAP Bulletin of Biological Assessment* 22. Conservation International, Washington, DC: 46-57 and 132-185.

Allen, G.R. 2003. Coral Reef Fishes of the Raja Ampat Islands in *Report on a rapid ecological assessment*. Donnelly, R., Neville, D., & Mous, P. Eds. The Nature Conservancy. Retrieved from http://epubs.aims.gov.au/handle/11068/8242, July 2018.

Allen, G.R. 2008. Conservation hotspots of biodiversity and endemism for Indo-Pacific coral reef fishes. Aquatic Conservation: *Marine and Freshwater Ecosystems*, 18(5), 541–556. https://doi.org/10.1002/aqc.880

Altizer, S., Ostfeld, R. S., Johnson, P. T. J., Kutz, S., & Harvell, C. D. 2013. Climate Change and Infectious Diseases: From Evidence to a Predictive Framework. *Science*, 341(6145), 514–519. https://doi.org/10.1126/science.1239401

Andruszkiewicz, E. A., Starks, H. A., Chavez, F. P., Sassoubre, L. M., Block, B. A., & Boehm, A. B. 2017. Biomonitoring of marine vertebrates in Monterey Bay using eDNA metabarcoding. *PLoS ONE*, 12(4), e0176343. https://doi.org/10.1371/journal.pone.0176343

Appeltans, W., Ahyong, S. T., Anderson, G., Angel, M. V., Artois, T., Bailly, N., … Costello, M. J. 2012. The Magnitude of Global Marine Species Diversity. *Current Biology*, 22(23), 2189–2202.

Bakker, J., Wangensteen, O. S., Chapman, D. D., Boussarie, G., Buddo, D., Guttridge, T. L., … Mariani, S. 2017. Environmental DNA reveals tropical shark diversity in contrasting levels of anthropogenic impact. *Sci. Rep.*, 7(1), 16886. https://doi.org/10.1038/s41598-017-17150-2

Barber, P. H., & Bellwood, D. R. 2005. Biodiversity hotspots: evolutionary origins of biodiversity in wrasses (Halichoeres: Labridae) in the Indo-Pacific and new world

tropics. *Molecular Phylogenetics and Evolution*, 35(1), 235–253. https://doi.org/10.1016/j.ympev.2004. 10.004

Barber, P. H., Ablan-Lagman, M. C. A., Ambariyanto, Berlinck, R. G. S., Cahyani, D., Crandall, E. D., … Willette, D. A. 2014. Advancing biodiversity research in developing countries: the need for changing paradigms. *Bulletin of Marine Science*, 90(1), 187–210. https://doi.org/10.5343/bms.2012.1108

Barber, P. H., Cheng, S. H., & Erdmann, M. V. 2016. Evolution and conservation of marine biodiversity in the Coral Triangle: insights from stomatopod Crustacea. *Phylogeography and Population Genetics in Crustacea*, 129.Barber, P.H., Cheng, S.H., Erdmann, M.V. Tengardjaja, K., Ambariyanto. 2011 Evolution and conservation of marine biodiversity in the Coral Triangle: insights from stomatopod Crustacea" In: Held, C., S. Koenemann & C.D. Schubart (eds), Crustacean Issues 19 *Phylogeography and Population Genetics in Crustacea*: pp. 129-156; CRC Press

Barber, P., & Boyce, S. L. 2006. Estimating diversity of Indo-Pacific coral reef stomatopods through DNA barcoding of stomatopod larvae. *Proc. Biol. Sci.*, 273(1597), 2053–2061. https://doi.org/10.1098/rspb.2006.3540

Bell, J. D., Kronen, M., Vunisea, A., Nash, W. J., Keeble, G., Demmke, A., … Andréfouët, S. 2009. Planning the use of fish for food security in the Pacific. *Marine Policy*, 33(1), 64–76. https://doi.org/10.1016/j.marpol.2008.04.002

Bellwood, D. R., & Meyer, C. P. 2009. Searching for heat in a marine biodiversity hotspot. J. *Biogeogr.*, 36(4), 569–576. https://doi.org/10.1111/j.1365-2699.2008.02029.x

Béné, C., Macfadyen, G., and Allison E.H. 2007. *Increasing the contribution of small-scale fisheries to poverty alleviation and food security*. FAO fisheries technical paper. No.481. Rome: FAO. p.125.

Biggs, J., Ewald, N., Valentini, A., Gaboriaud, C., Dejean, T., Griffiths, R. A., … Dunn, F. 2015. Using eDNA to develop a national citizen science-based monitoring programme for the great crested newt (Triturus cristatus). *Biol. Conserv.*, 183, 19–28. https://doi.org/10.1016/j.biocon. 2014.11.029

Böhm, T., & Hoeksema, B. W. 2017. Habitat selection of the coral-dwelling spinyhead blenny, Acanthemblemaria spinosa, at Curaçao, Dutch Caribbean. *Mar. Biodivers.*, 47(1), 17–25. https://doi.org/10.1007/s12526-016-0543-9

Bohmann, K., Evans, A., Gilbert, M. T. P., Carvalho, G. R., Creer, S., Knapp, M., … de Bruyn, M. 2014. Environmental DNA for wildlife biology and biodiversity monitoring. *Trends in Ecology and Evolution*. Elsevier Ltd.

Boussarie, G., Bakker, J., Wangensteen, O. S., Mariani, S., Bonnin, L., Juhel, J.-B., … Mouillot, D. 2018. Environmental DNA illuminates the dark diversity of sharks. *Sci. Adv*., 4(5), eaap9661. https://doi.org/10.1126/sciadv.aap9661

Bruno, J. F., Petes, L. E., Harvell, C. D., & Hettinger, A. 2003. Nutrient enrichment can increase the severity of coral diseases. *Ecology Letters*, 6(12), 1056–1061. https://doi.org/10.1046/j.1461-0248.2003.00544.x

Burke, L., Reytar, K., Spalding, M., & Perry, A. 2012. *Reefs at risk revisited in the Coral Triangle*. Washington, DC, USA: World Resources Institute.

Callahan, B. J., McMurdie, P. J., Rosen, M. J., Han, A. W., Johnson, A. J. A., & Holmes, S. P. 2016. DADA2: High-resolution sample inference from Illumina amplicon data. *Nat. Methods*, 13(7), 581. https://doi.org/10.1038/nmeth.3869

Carpenter, K. E., Barber, P. H., Crandall, E. D., Ablan-Lagman, M. C. A., Ambariyanto, Mahardika, G. N., … Toha, A. H. A. 2010. Comparative Phylogeography of the Coral Triangle and Implications for Marine Management. *Journal of Marine Biology*, 2011. https://doi.org/10.1155/2011/396982

Choi, H. C., & Turk, E. S. 2011. *Sustainability Indicators for Managing Community Tourism*. SpringerLink, 115–140. https://doi.org/10.1007/978-90-481-9861-0_7

Coral Triangle Initiative. 2009. *Regional Plan of Action, Coral Triangle Initiative on Coral Reefs, Fisheries and FoodSecurity (CTI-CFF)* .Manado. p.42.

Crandall, E. D., Sbrocco, E. J., DeBoer, T. S., Barber, P. H., & Carpenter, K. E. 2012. Expansion Dating: Calibrating Molecular Clocks in Marine Species from Expansions onto the Sunda Shelf Following the Last Glacial Maximum. *Mol. Biol. Evol*., 29(2), 707–719. https://doi.org/10.1093/molbev/msr227

Curd, E. E., Gold, Z., Kandlikar, G., Gomer, J., Ogden, M., O'Connell, T., … Meyer, R. S. 2018. Anacapa Toolkit: an environmental DNA toolkit for processing multilocus metabarcode datasets. BioRxiv, 488627. https://doi.org/10.1101/488627

Danovaro, R., Bongiorni, L., Corinaldesi, C., Giovannelli, D., Damiani, E., Astolfi, P., … Pusceddu, A. 2008. Sunscreens Cause Coral Bleaching by Promoting Viral Infections. *Environmental Health Perspectives*, 116(4), 441–447. https://doi.org/10.1289/ehp.10966

DeBoer, T. S., Naguit, M. R. A., Erdmann, M. V, Ablan-Lagman, M. C. A., Ambariyanto, Carpenter, K. E., … Barber, P. H. 2014a. Concordant phylogenetic patterns inferred from mitochondrial and microsatellite DNA in the giant clam Tridacna crocea. *Bull. Mar. Sci*., 90(1), 301–329. https://doi.org/10.5343/bms.2013.1002

DeBoer, T. S., Naguit, M. R. A., Erdmann, M. V, Ablan-Lagman, M. C. A., Ambariyanto, Carpenter, K. E., … Barber, P. H. 2014b. Concordance between phylogeographic and biogeographic boundaries in the Coral Triangle: conservation implications based on comparative analyses of multiple giant clam species. *Bull. Mar. Sci.*, 90(1), 277–300. https://doi.org/10.5343/bms.2013.1003

Dejean, T., Valentini, A., Miquel, C., Taberlet, P., Bellemain, E., & Miaud, C. 2012. Improved detection of an alien invasive species through environmental DNA barcoding: the example of the American bullfrog Lithobates catesbeianus. *J. Appl. Ecol.*, 49(4), 953–959. https://doi.org/10.1111/j.1365-2664.2012.02171.x

Delgado, C. L. 2003. *Fish to 2020: Supply and demand in changing global markets* (Vol. 62). WorldFish.

Donnelly, R., Neville, D., & Mous, P. 2003. *Report on a rapid ecological assessment*. The Nature Conservancy. Retrieved from http://epubs.aims.gov.au/handle/11068/8242, July 2018

Edgar, G. J., Barrett, N. S., & Morton, A. J. 2004. Biases associated with the use of underwater visual census techniques to quantify the density and size-structure of fish populations. *Journal of Experimental Marine Biology and Ecology*, 308(2), 269–290. https://doi.org/10.1016/j. jembe.2004.03.004

Edinger, E. N., Jompa, J., Limmon, G. V, Widjatmoko, W., & Risk, M. J. 1998. Reef degradation and coral biodiversity in Indonesia: effects of land-based pollution, destructive fishing practices and changes over time. *Marine Pollution Bulletin*, 36(8), 617–630.

Ekman, S. 1953. *Zoogeography of the sea*. Sidgwick and Jackson, London.

Erdmann, M.V. and & Pet, J., 2002. *A Rapid Marine Survey of the Northern Raja Ampat Islands*. Henry Foundation. The Nature Conservancy: NRM/EPIQ June 2002.

Fadli, N., Campbell, S. J., Ferguson, K., Keyse, J., Rudi, E., Riedel, A., & Baird, A. H. 2012. The role of habitat creation in coral reef conservation: a case study from Aceh, Indonesia. *Oryx*, 46(04), 501–507.

Fadli, N., Muchlisin, Z. A., Affan, M., & El Rahimi, S. A. 2014. The status of coral reefs in Aceh Besar district, Aceh Province, Indonesia. *Aquaculture, Aquarium, Conservation & Legislation-International Journal of the Bioflux Society* (AACL Bioflux), 7(5).

FAO Fisheries & Aquaculture - Fishery *and Aquaculture Country Profiles - The Republic of Indonesia*. 2018a. Retrieved from http://www.fao.org/fishery/facp/IDN/en# CountrySector-Statistics on September 2018

FAO Fisheries & Aquaculture - *Fishery and Aquaculture Country Profiles - The Republic of the Philippines*. 2018b. Retrieved from http://www.fao.org/fishery/facp/PHL/en on September 2018

FAO. 1996. *Report of the World Food Summit 13–17 November 1996*. Rome: Food and Agriculture Organization of the United Nations.

Ficetola, G. F., Miaud, C., Pompanon, F., & Taberlet, P. 2008. Species detection using environmental DNA from water samples. *Biology Letters*, 4(4), 423-425. https://doi.org/10.1098/rsbl.2008.0118

Ficetola, G. F., Poulenard, J., Sabatier, P., Messager, E., Gielly, L., Leloup, A., … Arnaud, F. 2018. DNA from lake sediments reveals long-term ecosystem changes after a biological invasion. *Sci. Adv*., 4(5), eaar4292. https://doi.org/10.1126/sciadv.aar4292

Fisher, R., Radford, B. T., Knowlton, N., Brainard, R. E., Michaelis, F. B., & Caley, M. J. 2011. Global mismatch between research effort and conservation needs of tropical coral reefs. *Conservation Letters*, 4(1), 64–72. https://doi.org/10.1111/j.1755-263X.2010.00146.x

Foale, S., Adhuri, D., Aliño, P., Allison, E. H., Andrew, N., Cohen, P., … Weeratunge, N. 2013. Food security and the Coral Triangle Initiative. *Marine Policy*, 38, 174–183. https://doi.org/10.1016/j.marpol.2012.05.033

Frey, M. A., & Vermeij, G. J. 2008. Molecular phylogenies and historical biogeography of a circumtropical group of gastropods (Genus: Nerita): Implications for regional diversity patterns in the marine tropics. *Molecular Phylogenetics and Evolution*, 48(3), 1067–1086. https://doi.org/10.1016/j.ympev.2008.05.009

Fukami, H., Budd, A. F., Paulay, G., Solé-Cava, A., Allen Chen, C., Iwao, K., & Knowlton, N. 2004. Conventional taxonomy obscures deep divergence between Pacific and Atlantic corals. *Nature*, 427(6977), 832. https://doi.org/10.1038/nature02339

Gao, X., Lin, H., Revanna, K., & Dong, Q. 2017. A Bayesian taxonomic classification method for 16S rRNA gene sequences with improved species-level accuracy. *BMC Bioinf*., 18(1), 247. https://doi.org/10.1186/s12859-017-1670-4

Garren, M., Raymundo, L., Guest, J., Harvell, C. D., & Azam, F. 2009. Resilience of Coral-Associated Bacterial Communities Exposed to Fish Farm Effluent. *PLoS ONE*, 4(10), e7319. https://doi.org/10.1371/journal.pone.0007319

Guardiola, M., Wangensteen, O. S., Taberlet, P., Coissac, E., Uriz, M. J., & Turon, X. 2016. Spatio-temporal monitoring of deep-sea communities using metabarcoding of sediment DNA and RNA. *PeerJ*, 4, e2807. https://doi.org/10.7717/peerj.2807

Haapkylä, J., Unsworth, R. K. F., Flavell, M., Bourne, D. G., Schaffelke, B., & Willis, B. L. 2011. Seasonal Rainfall and Runoff Promote Coral Disease on an Inshore Reef. *PLoS ONE*, 6(2), e16893. https://doi.org/10.1371/journal.pone.0016893

Hall, R. 2002. Cenozoic geological and plate tectonic evolution of SE Asia and the SW Pacific: computer-based reconstructions, model and animations. *Journal of Asian Earth Sciences*, 20(4), 353–431. https://doi.org/10.1016/S1367-9120(01)00069-4

Harzhauser, M., Kroh, A., Mandic, O., Piller, W. E., Göhlich, U., Reuter, M., & Berning, B. 2007. Biogeographic responses to geodynamics: A key study all around the Oligo–Miocene Tethyan Seaway. *Zoologischer Anzeiger - A Journal of Comparative Zoology*, 246(4), 241–256. https://doi.org/10.1016/j.jcz.2007.05.001

Hoegh-Guldberg, O., Hoegh-Guldberg, H., Veron, J. E. N., Green, A., Gomez, E. D., Ambariyanto, A., & Hansen, L. 2009. *The Coral Triangle And Climate Change: Ecosystems, People And Societies at Risk*. WWF Australia. Retrieved from http://eprints.undip.ac.id/1394

Huang, Y., & Coelho, V. R. 2017. Sustainability performance assessment focusing on coral reef protection by the tourism industry in the Coral Triangle region. *Tourism Management*, 59, 510–527. https://doi.org/10.1016/j.tourman.2016.09.008

Kandlikar, G. S., Gold, Z. J., Cowen, M. C., Meyer, R. S., Freise, A. C., Kraft, N. J. B., … Curd, E. E. 2018. ranacapa: An R package and Shiny web app to explore environmental DNA data with exploratory statistics and interactive visualizations. *F1000Research*, 7. https://doi.org/10.12688/f1000research.16680.1

Kawarazuka, N., & Béné, C. 2010. Linking small-scale fisheries and aquaculture to household nutritional security: an overview. *Food Security*, 2(4), 343–357. https://doi.org/10.1007/s12571-010-0079-y

Kelly, R. P., Closek, C. J., O'Donnell, J. L., Kralj, J. E., Shelton, A. O., & Samhouri, J. F. 2017. Genetic and Manual Survey Methods Yield Different and Complementary Views of an Ecosystem. *Frontiers in Marine Science*, 3, 283.

Kelly, R. P., Port, J. A., Yamahara, K. M., & Crowder, L. B. 2014. Using environmental DNA to census marine fishes in a large mesocosm. *PloS ONE*, 9(1), e86175. Retrieved from http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0086175

Keyse, J., Crandall, E. D., Toonen, R. J., Meyer, C. P., Treml, E. A., & Riginos, C. 2014. The scope of published population genetic data for Indo-Pacific marine fauna and future research opportunities in the region. *Bull. Mar. Sci.*, 90 (1), 47–78. https://doi.org/10.5343/bms.2012. 1107

Klanten, S. O., Herwerden, L. van, Choat, J. H., & Blair, D. 2004. Patterns of lineage diversification in the genus Naso (Acanthuridae). *Molecular Phylogenetics and Evolution*, 32(1), 221–235. https://doi.org/10.1016/j.ympev.2003.11.008

Kool, J.T., Paris, C.B., **Barber, P.H.,** Cowen, R.K. **(**2011) Connectivity and the development of population genetic structure in Indo-West Pacific coral reef communities. *Global Ecology and Biogeography*. **20:** 695-706.

Kotrschal, K. 1988. Blennies and Endolithic Bivalves: Differential Utilization of Shelter in Adriatic Blenniidae (Pisces: Teleostei). *Mar. Ecol.*, 9(3), 253–269. https://doi.org/10.1111/j.1439-0485.1988.tb00332.x

Ladd, H.S. 1960. Origin of the Pacific island molluscan fauna. *American Journal of Science*, 258, 137–150.

Laroche, O., Wood, S. A., Tremblay, L. A., Lear, G., Ellis, J. I., & Pochon, X. 2017. Metabarcoding monitoring analysis: the pros and cons of using co-extracted environmental DNA and RNA data to assess offshore oil production impacts on benthic communities. *PeerJ*, 5, e3347. https://doi.org/10.7717/peerj.3347

Lindfield, S. J., Harvey, E. S., McIlwain, J. L., & Halford, A. R. 2014. Silent fish surveys: bubble-free diving highlights inaccuracies associated with SCUBA-based surveys in heavily fished areas. *Methods Ecol. Evol.*, 5(10), 1061–1069. https://doi.org/10.1111/ 2041-210X.12262

Malaquias, M. A. E., & Reid, D. G. 2009. Tethyan vicariance, relictualism and speciation: evidence from a global molecular phylogeny of the opisthobranch genus Bulla. *Journal of Biogeography*, 36(9), 1760–1777. https://doi.org/10.1111/j.1365-2699.2009.02118.x

McKenna, M.C. 1973. *Sweepstakes, filters, corridors, Noah's arks, and beached Viking funeral ships in palaeogeography*. Implications of continental drift to the earth sciences, Vol. 1 (ed. by D.H. Tarling and S.K. Runcorn), pp. 295–308. Academic Press, London.

Miya, M., Sato, Y., Fukunaga, T., Sado, T., Poulsen, J. Y., Sato, K., … others. 2015. MiFish, a set of universal PCR primers for metabarcoding environmental DNA from fishes: detection of more than 230 subtropical marine species. *Open Science*, 2(7), 150088. Retrieved from http://rsos.royalsocietypublishing.org/content/2/7/150088.abstract

Moore, G. E. 1965. Cramming more components onto integrated circuits. *Electronics*, 38.

National Oceanic and Atmospheric Administration (NOAA). 2012. *Tourism and recreation*. Retrieved from: http://coralreef.noaa.gov/aboutcorals/values/tourismrecreation/ Accessed 10.03.18.

Nursall, J. R. 1977. Territoriality in Redlip blennies (Ophioblennius atlanticus-Pisces: Blenniidae). J. Zool., 182(2), 205–223. https://doi.org/10.1111/j.1469-7998.1977.tb04156.x

Ogram, A., Sayler, G. S., & Barkay, T. 1987. The extraction and purification of microbial DNA from sediments. *J. Microbiol. Methods*, 7(2), 57–66. https://doi.org/10.1016/0167-7012(87)90025-X

Outra, M. I. H., Sari, S. K., Sukandar, H., Malik, D. A., & Prabuning, D. 2016. *Engaging dive tourism in sustainable financing and coral reef data collection for better management of Karimunjawa National Park, Indonesia*. Abstract. In Thirteenth International Coral Reef Symposium. Honolulu, Hawaii.

Patterson, K. L., Porter, J. W., Ritchie, K. B., Polson, S. W., Mueller, E., Peters, E. C., … Smith, G. W. 2002. The etiology of white pox, a lethal disease of the Caribbean elkhorn coral, *Acropora palmata*. *Proceedings of the National Academy of Sciences*, 99(13), 8725–8730. https://doi.org/10.1073/pnas.092260099

Permani, R., Setyoko, N. R., Trewin, R., and David Vanzetti, D. 2016. Chapter 4 in *Crucial Agricultural Policy: Analysis of Key Threats to Food Security*. World Scientific Publishing Co. Pte. Ltd. pp. 81-114.

Pilliod, D.S., Goldberg, C.S., Laramie, M.B., & Waits, L.P. 2012. *Application of environmental DNA for inventory and monitoring of aquatic species.* US Geological Survey Fact Sheet, 3416, p. 4

Plaisance, L., Caley, M. J., Brainard, R. E., & Knowlton, N. 2011. The Diversity of Coral Reefs: What Are We Missing? *PLoS ONE*, 6(10), e25026. https://doi.org/10.1371/journal.pone.0025026

Randall, J.E., 2005. *Reef and shore fishes of the South Pacific. New Caledonia to Tahiti and the Pitcairn Islands.* University of Hawaii Press, Honolulu, Hawaii. 720 p.

Raymundo, L. J., Halford, A. R., Maypa, A. P., & Kerr, A. M. 2009. Functionally diverse reef-fish communities ameliorate coral disease. *Proceedings of the National Academy of Sciences*, ps.0900365106. https://doi.org/10.1073/pnas.0900365106

Read, C. I., Bellwood, D. R., & van Herwerden, L. 2006. Ancient origins of Indo-Pacific coral reef fish biodiversity: A case study of the leopard wrasses (Labridae: Macropharyngodon). *Molecular Phylogenetics and Evolution*, 38(3), 808–819. https://doi.org/10.1016/j.ympev.2005.08.001

Rees, H. C., Baker, C. A., Gardner, D. S., Maddison, B. C., & Gough, K. C. 2017. The detection of great crested newts year round via environmental DNA analysis. *BMC Research Notes*, 10(1), 327. https://doi.org/10.1186/s13104-017-2657-y

Rees, H. C., Bishop, K., Middleditch, D. J., Patmore, J. R. M., Maddison, B. C., & Gough, K. C. 2014. The application of eDNA for monitoring of the Great Crested Newt in the UK. *Ecol. Evol.*, 4(21), 4023–4032. https://doi.org/10.1002/ece3.1272

Rees, H. C., Maddison, B. C., Middleditch, D. J., Patmore, J. R. M., & Gough, K. C. 2014. REVIEW: The detection of aquatic animal species using environmental DNA: a review of eDNA as a survey tool in ecology. *J. Appl. Ecol.*, 51(5), 1450–1459. https://doi.org/10.1111/1365-2664.12306

Renema, W., Bellwood, D. R., Braga, J. C., Bromfield, K., Hall, R., Johnson, K. G., … Pandolfi, J. M. 2008. Hopping Hotspots: Global Shifts in Marine Biodiversity. *Science*, 321(5889), 654–657. https://doi.org/10.1126/science.1155674

Ricard, D., Minto, C., Jensen, O. P., & Baum, J. K. (2012). Examining the knowledge base and status of commercially exploited marine species with the RAM Legacy Stock Assessment Database. *Fish and Fisheries*, 13(4), 380–398. https://doi.org/10.1111/j.1467-2979.2011.00435.x

Roberts, C. M., McClean, C. J., Veron, J. E. N., Hawkins, J. P., Allen, G. R., McAllister, D. E., … Werner, T. B. 2002. Marine Biodiversity Hotspots and Conservation Priorities for Tropical Reefs. *Science*, 295(5558), 1280–1284. https://doi.org/10.1126/science.1067728

Sadovy, Y.J., 2002. The Humphead wrasse: a threatened reef fish. *SPC Live Reef Fish Information Bulletin* 10: 6.

Sembiring, A., Pertiwi, N. P. D., Mahardini, A., Wulandari, R., Kurniasih, E. M., Kuncoro, A. W., … Mahardika, G. N. (2015). DNA barcoding reveals targeted fisheries for endangered sharks in Indonesia. Fish. Res., 164, 130–134. https://doi.org/10.1016/j.fishres. 2014.11.003

Souhoka, J. and Turak E. 2003. Coral Diversity and the Status of Coral Reefs in the *Raja Ampat Islands in Report on a rapid ecological assessment*. Donnelly, R., Neville, D., & Mous, P. Eds. The Nature Conservancy. Retrieved from http://epubs.aims.gov.au/handle/11068/8242, July 2018.

Stein, L. D. 2010. The case for cloud computing in genome informatics. *Genome Biol.*, 11(5), 207. https://doi.org/10.1186/gb-2010-11-5-207

Taberlet, P., Coissac, E., Hajibabaei, M., & Rieseberg, L. H. 2012. Environmental DNA. *Molecular Ecology*, 21(8), 1789–1793. https://doi.org/10.1111/j.1365-294X.2012.05542.x

Takahara, T., Minamoto, T., & Doi, H. 2013. Using Environmental DNA to Estimate the Distribution of an Invasive Fish Species in Ponds. *PLoS ONE*, 8(2), e56584. https://doi.org/10.1371/journal.pone.0056584

Thomsen, P. F., Kielgast, J., Iversen, L. L., Møller, P. R., Rasmussen, M., & Willerslev, E. 2012. Detection of a Diverse Marine Fish Fauna Using Environmental DNA from Seawater Samples. *PLoS ONE*, 7(8), e41732. https://doi.org/10.1371/journal.pone.0041732

Thomsen, P. F., Kielgast, J., Iversen, L. L., Wiuf, C., Rasmussen, M., Gilbert, M. T. P., … Willerslev, E. 2012. Monitoring endangered freshwater biodiversity using environmental DNA. *Molecular Ecology*, 21(11), 2565–2573.

Vermeij, G. J. 2001. *Community assembly in the sea: geologic history of the living shore biota*. in Marine Community Ecology, M. Bertness, S. D. Gaines, and M. E. Hay, Eds., Sinauer Associates, Sunderland, Mass, USA.

Veron, J. E. N. 1993. A biogeographic database of hermatypic corals. *Aust. Inst. Mar. Sci.* Monograph Series 10, 433.

Veron, J. E. N., Devantier, L. M., Turak, E., Green, A. L., Kininmonth, S., Stafford-Smith, M., & Peterson, N. 2009. Delineating the coral triangle. *Galaxea, Journal of Coral Reef Studies*, 11(2), 91–100.

Wallace, C. C. 1997. Separate ocean basin origins as the explanation for high coral species diversity in the central. *Indo-Pacific 8th Int. Coral Reef Symp*. 1, 365-370.

Wallace, C. C., & Rosen, B. R. 2006. Diverse staghorn corals (Acropora) in high-latitude Eocene assemblages: implications for the evolution of modern diversity patterns of reef corals. *Proceedings of the Royal Society of London B: Biological Sciences*, 273(1589), 975–982. https://doi.org/10.1098/rspb.2005.3307

Wetterstrand, K. A. 2018. *DNA Sequencing Costs*: Data from the NHGRI Genome Sequencing Program (GSP). Retrieved November 8, 2018, from https://www.genome.gov/sequencingcostsdata

WFP. 2009. *Emergency Food Security Assessment Handbook*—Second Edition. Rome: World Food Programme.

Williams, S. T., & Reid, D. G. 2004. Speciation and Diversity on Tropical Rocky Shores: a global phylogeny of snails of the genus Echinolittorina. *Evolution*, 58(10), 2227–2251. https://doi.org/10.1111/j.0014-3820.2004.tb01600.x

Wilson, M.E.J and Rosen, B.R. 1998. *Implications of paucity of corals in the Paleogene of SE Asia: plate tectonics or Centre of Origin*. in Biogeography and Geological Evolution of SE Asia, R. Hall and J. D. Holloway, Eds., pp. 165–195, Backhuys Publishers, Leiden, The Netherlands, 1998.

Wilson, S. K., Fisher, R., & Pratchett, M. S. 2013. Differential use of shelter holes by sympatric species of blennies (Blennidae). *Mar. Biol.*, 160(9), 2405–2411. https://doi.org/10.1007/s00227-013-2235-3

Woodland, D. J. 1983. Zoogeography of the Siganidae (Pisces): An Interpretation of Distribution and Richness Patterns. *Bulletin of Marine Science*, 33(3), 713–717. Retrieved from https://www.ingentaconnect.com/content/umrsmas/bullmar/1983/00000033/00000003/art00017

Yamamoto, S., Masuda, R., Sato, Y., Sado, T., Araki, H., Kondoh, M., … Miya, M. 2017. Environmental DNA metabarcoding reveals local fish communities in a species-rich coastal sea. *Scientific Reports*, 7, 40368. https://doi.org/10.1038/srep40368

York, R., & Gossard, M. H. 2004. Cross-national meat and fish consumption: exploring the effects of modernization and ecological context. *Ecological Economics*, 48(3), 293–302. https://doi.org/10.1016/j.ecolecon.2003.10.009