様　式　Ｃ－１９、Ｆ－１９－１、Ｚ－１９　（共通）

科学研究費助成事業　　研究成果報告書

令和　６ 年　 ６ 月 ２１ 日現在

機関番号：１８００１
研究種目：国際共同研究加速基金（国際共同研究強化(B)）
研究期間：2020～2023
課題番号：２０ＫＫ０１６４

研究課題名（和文）Marine environmental DNA metabarcoding: understanding oceanic islands and mainland sites across anthropogenic gradients in two hemispheres

研究課題名（英文）Marine environmental DNA metabarcoding: understanding oceanic islands and mainland sites across anthropogenic gradients in two hemispheres

研究代表者
Ｒｅｉｍｅｒ　Ｊ・Ｄ（REIMER, JD）

琉球大学・理学部・教授

研究者番号：２０４５２９５６

交付決定額（研究期間全体）：（直接経費）　　14,000,000円

研究成果の概要（和文）：海洋島が本土のサイトと同じパターンの劣化を経験しているかどうかを調べるために、日本本土と小笠原のサイトの環境 DNA を調べた。オーストラリアの協力者と小笠原でフィールド調査を行い、日本南部周辺で eDNA サンプルを収集した。人間の影響を受けたサイトが必ずしも多様性が低いわけではなく、むしろ異なる生物多様性を持っている可能性があることを示している。初期分析では、影響を受けたサイトはさまざまな地域やサイトで共通点があることが示されており、おそらく人為的影響はすべて特定の方法で海洋生態系を変えていることを示しており、人為的影響による海洋生態系の劣化の一般理論が実現可能であることを示唆している。

研究成果の学術的意義や社会的意義
Our analyses indicate there are common denominators to impacted sites across different sites, showing that anthropogenic impacts all change marine ecosystems in particular ways, and there may be some general theory of marine ecosystem degradation under anthropogenic influences within reach.

研究成果の概要（英文）：To investigate whether oceanic islands experience the same patterns of degradation as mainland sites, we examined the environmental DNA of sites on the Japanese mainland and Ogasawara. We conducted field work in Ogasawara with Australian collaborators and collected eDNA samples around southern Japan. Our dataset shows that human-impacted sites are not necessarily less diverse, but rather may have different biodiversity. Initial analysis shows that the impacted sites have commonalities across different regions and sites, indicating that perhaps anthropogenic impacts are all altering marine ecosystems in specific ways, suggesting that a general theory of marine ecosystem degradation due to anthropogenic impacts may be within reach.

研究分野：海洋生態学

キーワード：eDNA　degradation　anthropogenic impacts　coral reefs　marine biodiversity　coral　fish

１．研究開始当初の背景
Introduction and background

UNESCO's 'a decade of ocean science' (https://en.unesco.org/ocean-decade) initiative (2017) states: "Nearly 3 billion people depend on marine and coastal biodiversity to meet their needs. It absorbs around a third of the CO2 produced by humans and reduces the impact of climate change. However, science has not yet managed to fully evaluate the cumulative effects of human activities on the ocean, including the impact of pollution, warming and acidification, which threaten this environment, which is vital for our survival". In addition, the United Nations Sustainable Development Goals (SDGs) includes SDG 14, to conserve and sustainably use oceans, seas, and marine resources. Approaches able to quantify how ocean biota are linked and are changing are vital to future management.

Marine ecosystems in general and coral reefs in particular are noted for their high levels of biodiversity, and also for the threat they face from local and global anthropogenic stressors. In Japan, government surveys have relied on the common method of assessing coral reef health through coral cover surveys and counts, but this method is time-consuming and costly. From this, two main problems have arisen: 1) surveys cannot cover more than a few sites in each target area, and data are lacking for most areas, and 2) even at sites where data exist, they are limited to coral cover and fish counts, and thus the large majority of marine biodiversity and taxa are ignored, and little understood by stakeholders and decision makers. Our knowledge of marine biodiversity is still fragmentary. Many marine taxa, and many marine regions, have only incomplete datasets available. For example, in coral reef ecosystems, data are present for fish and corals, but not for most other taxa (Hughes et al. 2002; Reimer et al. 2019). As well, data are often available for mainland or populated areas, but not oceanic regions (e.g. Chambers et al. 2017). This lack of data greatly inhibits our abilities to understand and conserve marine ecosystems (Hughes et al. 2002; Roberts et al. 2002; Reimer et al. 2019).

In Australia, marine scientists have recently (as of 2020) adopted a new rapid and more spatially and biologically complete methodology, namely environmental DNA (eDNA). The widespread application of eDNA is relatively new in the marine realm, and its application for assessing the health of ecosystems is in its infancy. The use of eDNA can potentially revolutionize our understanding of marine biodiversity, leading to better conservation and protection. The huge amount of biodiversity obtained from just a single seawater sample provides environmental snapshots, which constitute a powerful means to support baseline for monitoring efforts, including tracking of anthropogenic impacts (DiBattista et al. 2020). eDNA methodologies are developing rapidly, and analytical methods are becoming more and more powerful, with Australian scientists at the

forefront of eDNA innovation and application (Stat et al. 2017). At the same time, eDNA results consistently show the need for proper taxonomic understanding of marine taxa and knowledge of the sites being surveyed.

２．研究の目的

In this project, we collected environmental DNA (eDNA)* datasets over four years from pristine and anthropogenically impacted sites in Japan to 1) examine total coral community biodiversity, 2) examine how oceanic regions are linked with mainland sites, and 3) examine how human impacts affect biodiversity and linkages between these sites. Corresponding scientific questions to be answered in this study:

(1). What are the total biodiversity patterns of coral communities in Japan? What are the patterns of diversity of different marine taxa?

(2). How are biodiversity patterns of oceanic areas such as Ogasawara (currently data deficient) linked to their neighbouring mainland sites? Are there consistent (=biogeographical) patterns across different marine taxa?

(3). Regarding the patterns in question 2 above, how does these change with increasing anthropogenic impacts? Do linkages increase or decrease?

３．研究の方法

eDNA collection:

At each site for each year, we collected water and sediment eDNA; methods will follow DiBattista et al. (2020). Sediment and filtered water specimens were kept frozen until return to the laboratory, where they were will stored in a dedicated BioBank freezer until molecular analyses.

Data generation and analyses:

eDNA extraction and metabarcoding protocols utilized the methodologies and pipelines our team had already streamlined (DiBattista et al. 2019; 2020). We metabarcoded for all eukaryotes (=total biodiversity) using published 18S, COI, and ITS DNA marker primers, sequence on next-generation sequencers (NGS), and analyze generated sequence data using pipelines generally following Stat et al. (2017), with modification as needed. Acquired sequence data will be quality-filtered, checked for taxonomic accuracy, and analyzed based on molecular operational taxonomic units (MOTUs) (DiBattista et al. 2020). Comparisons of communities between years, taxa, and sites (ocean/mainland, pristine/anthropogenic) will be conducted via robust analytical methods such as PRIMER. PERMANOVA, and Principle Component Analyses (DiBattista et al. 2020). Support and knowledge from our Australian collaborators will aid in refinement of methods.

For Ogasawara fish and coral analyses, the first published data from this research, we utilized the following methods. Fish amplicons were generated with primers from Miya et al. (2020) (Table 2), with eight PCR replicates per sample (Minamoto et al., 2021). Each reaction replicate consisted of 2 µL of the eDNA sample and 10 µL of master

mix (6.0 µL KAPA HiFi, 1.2 µL Milli-Q Direct UltraPure Water, and 2.8 µL of the primer mixture with a 1:2:1 ratio of MiFish-E-F/R-v2, MiFish-U-F/R, and MiFish-U2-F/R, respectively). Thermocycler conditions also followed the suggestions of Minamoto et al. (2021), set as initial denaturation at 95°C for 3 min, 38 cycles of (1) denaturation at 98°C for 20 s, (2) annealing at 65°C for 15 s 35 cycles, and (3) extension at 72°C for 15 s, finally followed by final extension at 72°C for 5 min. The eight PCR replicates were pooled together and then purified and concentrated with the GeneRead Size Selection Kit to remove primer and adapter dimers. Products were then quantified with TapeStation 2200, and TruSeq DNA CD Indexes 96 Indexes were used to index the pooled sample PCR products with second PCR.

Metabarcoding for Scleractinia corals using the same eDNA samples was performed as described by Shinzato et al. (2021) and Nishitsuji et al. (2023) with their Scle-12S primers (Table 2). Scleractinian coral amplicons were generated with Tks GflexTM DNA Polymerase (Takara). Thermocycler conditions were set as 1 min at 94°C, followed by 35 cycles of 10 s at 94°C, 15 s at 60°C, and 30 s at 68°C, with an extension of 5 min at 68°C in the final cycle. PCR products were extracted and cleaned with a FastGene Gel/PCR Extraction Kit (NIPPON Genetics). Amplicon sequencing libraries of cleaned PCR products were prepared using a KAPA Hyper Prep Kit (NIPPON Genetics Co., Ltd.) without fragmentation. Sequencing of both fish and coral sample sets was performed on MiSeq with a MiSeq v3 600 cycle cartridge, at paired-end sequencing at 2 × 150 bp for MiFish products, and 2 × 300 bp for Scle-12S products on separate runs.

４．研究成果

Results and Discussion:

Our eDNA results of fish and corals from Ogasawara showed previously undetected records of corals and potentially fish from the islands (Acikbas-Oshima et al. 2024). We detected a total of 124 unique taxa of fish and 38 unique taxa of scleractinian corals. Overall, our eDNA results confirmed that the Ogasawara Islands host a rich variety of coral and fish fauna and underline the strength of eDNA surveys in rapidly obtaining targeted multi-taxa data using seawater samples, requiring comparatively little effort and a lack of requirement for in situ taxonomic expertise. We anticipate that continued biomonitoring using eDNA with high sampling effort will add to and complement the body of knowledge regarding species distributions, invasive species, and biodiversity hotspots within oceanic archipelagos. Our dataset also showed that human-impacted sites may not necessarily have lower diversity, but instead different biodiversity. Finally, the results also demonstrated the utility of eDNA, suggesting that even with 10 samples or so we can reach sampling saturation for corals, while for fishes this will take more time, perhaps 40 to 100 samples. Initial analyses indicate there are common denominators to impacted sites across different regions and sites, showing that perhaps anthropogenic impacts all change marine ecosystems in particular ways, hinting that there may be some general theory of marine ecosystem degradation under anthropogenic influences within reach.

While other portions of our data remain to be published, based on our recent publications (Stat et al. 2017; Huggett et al. 2018; Jones et al. 2018; DiBattista et al. 2019, 2020; Masucci et al. 2020) and other analyses (Glasl et al. 2020), we anticipate the following results to our core scientific questions.

1) Biodiversity patterns of Ogasawara will show unique features, with some endemism present, and differences in community structures from the nearest mainland sites. Overall diversity levels will be slightly lower than nearest mainland sites.

2) Despite being unique, there will be linkages between oceanic and mainland sites, particularly for taxa with comparatively longer pelagic larval dispersal, such as Cnidaria, and for generalist species with wide ranges.

3) Anthropogenic impacts will affect biodiversity at mainland and oceanic sites in similar ways, with alterations in biodiversity levels, but more importantly, a turnover in the fauna, and also a reduction and fragmentation in functionality (DiBattista et al. 2020). We may see an increased similarity in biodiversity among impacted sites.

Based on these results, we will be able to propose a general trend for how anthropogenic impacts change total marine biodiversity in marine communities in the Indo-Pacific Ocean, and provide researchers with several guidelines for eDNA detection of such changes. Results will also stress the need to strongly protect pristine and oceanic regions from further anthropogenic impacts given the already evident negative impacts combined with high rates of endemism.

References and related reading:

Açıkbaş-Oshima et al. Environmental DNA. 2024 6:e509; Chambers et al. Austral Ecology. 2017 42:20-30; De Goeij et al. Science. 2013 342:108-10; DiBattista et al. PeerJ. 2019 6;7:e6379; DiBattista et al. Scientific Reports. 2020 20;10:1-15; Elahi et al. Current Biology. 2015 20;25:1938-43; Glasl et al. ISME Journal. 2020 2:1-6; Holmlund & Hammer. Ecological Economics. 1999 1;29:253-68; Huggett et al. Environmental Microbiology. 2018;20:2769-82; Hughes et al. Ecology Letters. 2002;5:775-84; Jones et al. Frontiers in Microbiology. 2018 28;9:2000; Kelly et al. Frontiers in Marine Science 2017 3:283; Masucci et al. 2020;30:290-302; Minamoto et al. 2021. Environmental DNA. 3:8–13; Molnar et al. Frontiers in Ecology and the Environment. 2008;6:485-92; Nishitsuji et al. Proceedings of the Royal Society B. 2023, 290:20230026; Reimer et al. PeerJ. 2019 12;7:e6532; Roberts et al. Science 2002 295:1280-4; Shinzato et al. Frontiers in Marine Science. 2021 8:758207; Stat et al. Scientific Reports 2017 7:12240; Stat et al. Conservation Biology. 2019;33:196-205; Taberlet et al. Molecular Ecology. 2012;21:1789-93

５．主な発表論文等

〔雑誌論文〕　計3件（うち査読付論文　3件／うち国際共著　3件／うちオープンアクセス　2件）

１．著者名 Reimer JD, Gosser F	４．巻 290
２．論文標題 Can environmental DNA unlock the mysteries of biodiversity on coral reefs?	５．発行年 2023年
３．雑誌名 Proceedings of the Royal Society B	６．最初と最後の頁 20230605
掲載論文のDOI（デジタルオブジェクト識別子） 10.1098/rspb.2023.0605	査読の有無 有
オープンアクセス オープンアクセスではない、又はオープンアクセスが困難	国際共著 該当する

１．著者名 DiBattista Joseph D.、Reimer James D.、Stat Michael、Masucci Giovanni D.、Biondi Piera、De Brauwer Maarten、Wilkinson Shaun P.、Chariton Anthony A.、Bunce Michael	４．巻 10
２．論文標題 Environmental DNA can act as a biodiversity barometer of anthropogenic pressures in coastal ecosystems	５．発行年 2020年
３．雑誌名 Scientific Reports	６．最初と最後の頁 8365
掲載論文のDOI（デジタルオブジェクト識別子） 10.1038/s41598-020-64858-9	査読の有無 有
オープンアクセス オープンアクセスとしている（また、その予定である）	国際共著 該当する

１．著者名 Acikbas Ayse Haruka Oshima、Narisoko Haruhi、Huerlimann Roger、Nishitsuji Koki、Satoh Noriyuki、Reimer James Davis、Ravasi Timothy	４．巻 6
２．論文標題 Fish and coral assemblages of a highly isolated oceanic island: The first eDNA survey of the Ogasawara Islands	５．発行年 2024年
３．雑誌名 Environmental DNA	６．最初と最後の頁 -
掲載論文のDOI（デジタルオブジェクト識別子） 10.1002/edn3.509	査読の有無 有
オープンアクセス オープンアクセスとしている（また、その予定である）	国際共著 該当する

〔学会発表〕　計5件（うち招待講演　4件／うち国際学会　3件）

１．発表者名 AHO Acikbas et al. (incl. JD Reimer)
２．発表標題 Marine biodiversity patterns in an anthropogenically impacted oceanic archipelago: The first eDNA survey of the Ogasawara Islands
３．学会等名 1st Australian & New Zealand eDNA conference（国際学会）
４．発表年 2023年

１．発表者名
JD Reimer et al.

２．発表標題
Okinawa, Japan: A Model Location for Examining Anthropogenic Impacts on Coral Reefs

３．学会等名
Nha Trang University public lecture（招待講演）

４．発表年
2022年

１．発表者名
James Davis Reimer, Hirotaka Yamagiwa, Katie Midori Cook, Joseph D. DiBattista, Megan Huggett, Michael Stat, James Cant, Anthony A. Chariton, Maarten De Brauwer, Shaun P. Wilkinson, Giovanni Diego Masucci, Piera Biondi, Stuart Ross, Lee Hui Yian Theodora, Rick D. Stuart-Smith, Michael Bunce, Maria Beger

２．発表標題
Okinawa, Japan: A Model Location for Examining Anthropogenic Impacts on Coral Reefs

３．学会等名
The 2nd International Conference on Biodiversity, Ecology and Conservation of Marine Ecosystems（招待講演）（国際学会）

４．発表年
2022年

１．発表者名
James Davis Reimer, Piera Biondi, Giovanni Masucci

２．発表標題
サンゴ礁の礫の潜在的重要性と環境DNA技術

３．学会等名
さんご ｉｎ サンゴ礁ウィーク ２０２２ の海オンラインフエスタ（招待講演）

４．発表年
2022年

１．発表者名
James Davis Reimer & colleagues

２．発表標題
Using eDNA to understand the diversity of and anthropogenic impacts on coral reefs in southern Japan

３．学会等名
eDNA Society（招待講演）（国際学会）

４．発表年
2023年

〔図書〕　計0件

〔産業財産権〕

〔その他〕

―

6．研究組織

	氏名 （ローマ字氏名） （研究者番号）	所属研究機関・部局・職 （機関番号）	備考
研究分担者	成瀬　貫 (Naruse Tohru) (30398309)	琉球大学・熱帯生物圏研究センター・准教授 (18001)	
研究分担者	櫛田　優花 (Kushida Yuka) (90914341)	立正大学・地球環境科学部・助教 (32687)	
研究分担者	ＭＡＳＵＣＣＩ　ＧＩＯＶＡＮＮＩ・ＤＩＥＧＯ (Masucci Giovanni Diego) (80890053)	琉球大学・理工学研究科・博士研究員 (18001)	

	氏名 （ローマ字氏名） （研究者番号）	所属研究機関・部局・職 （機関番号）	備考
研究協力者	ラバシ　ティモシー (Ravasi Timothy)		
研究協力者	佐藤　矩行 (Satoh Noriyuki)		

7．科研費を使用して開催した国際研究集会

〔国際研究集会〕　計0件

8．本研究に関連して実施した国際共同研究の実施状況

共同研究相手国	相手方研究機関

オーストラリア	University of Newcastle	Griffith University		