**Integration of visual surveys and eDNA**

The integration of eDNA with traditional visual survey methods as shown in the study here can enhance the robustness of impact assessments. For instance, updating the stress impact categorization using the coral hard cover results of the PLITs resulted in Okinawa collection sites that were categorized as low impact using DiBattista et al. (2021) method being downgraded to the medium category. DiBattista et al. (2021) used numbers of hard coral cover reported in governmental monitoring and from published literature (see Japan Ministry of Environment <http://www.biodic.go.jp/trialSystem/top_en.html> and (Heery et al., 2018), which are crucial for understanding environmental trends and informing policy, but they might not directly overlap with the actual collection sites or may overlook localized variations or emerging hotspots of environmental change. Additionally, if monitoring efforts are not updated regularly, the data can quickly become outdated, reducing their relevance for addressing current conditions or guiding timely interventions. Gathering information about benthic coverage using PLITs while also collecting water samples for eDNA is done rather quickly and further visual observations about conditions on the reef as marine litter, disease emergence or bleaching patterns can be done. Therefore, combining eDNA data within situ visual surveys and photography can provide a more comprehensive picture of immediate coral reef health. Visual surveys can validate eDNA detections, while eDNA can uncover taxa that are overlooked due to visual or depth-related limitations (Everett & Park, 2018; Gösser et al., 2022; Stat et al., 2019). This complementary approach is particularly valuable for detecting subtle biodiversity changes that might precede visible reef degradation.

**Species richness between impact levels**

One of the key strengths of eDNA is its ability to non-invasively detect biodiversity across various taxa, including cryptic, rare, or invasive species, which are often challenging to identify through traditional survey methods (Dugal et al., 2022; Gaither et al., 2022; Stat et al., 2019; West, Adam, et al., 2021). For example, eDNA can detect shifts in communities, from relatively small scales up to indicating biogeographical breaks (DiBattista et al., 2022; Gert‐Jan Jeunen et al., 2019; Kelly et al., 2016; West, Michael J. Travers, et al., 2021).

The sensitivity of eDNA to anthropogenic impacts is particularly noteworthy. Studies have demonstrated its utility in detecting biodiversity gradients along pollution or nutrient enrichment gradients (Boulanger et al., 2021; DiBattista et al., 2022; Downie et al., 2024; Kelly et al., 2016; Manel et al., 2024). These shifts, often subtle and difficult to quantify visually, can be critical for identifying early signs of reef degradation. Okinawa, Japan as a mosaic of human influences, with highly developed urban areas, agriculture, touristic and recreational areas and National Parks on land and in the ocean is one of the prime regions to monitor anthropogenic impacts (Heery et al., 2018; Masucci et al., 2021).

Species richness based on unique ESVs assigned from COI exhibited noticeable differences at small spatial scales between our sampling sites. Our results showed a significant reduction in species richness with medium and high impact levels, although some sampling sites still maintained relatively high species richness. This result could reflect high species diversity associated with coral reefs and the loss of diversity that goes with the degradation of this system under anthropogenic impact (Heery et al., 2018). However, using species richness as the only proxy to infer ecosystem conditions might be misleading, as the taxa in focus, the source of eDNA (e.g. sediment versus seawater) as well as the ecosystem investigated could lead to different results. Another study also utilizing eDNA to investigate anthropogenic impact on coral reefs around Okinawa came to differing results using 18S (DiBattista et al., 2020). Here species richness based on assignments on the family level showed higher alpha diversity levels with medium and high impact levels for seawater samples while sediment samples did not show this (DiBattista et al., 2020). This could be due to the different taxonomic resolution of the primers in this study (COI) and the 18S primer, as COI more reliably reflects dominant sessile phyla, while 18S provides a more holistic representation of overall taxonomic diversity (Casey et al., 2021). For microbial diversity it was also reported that impacted sites had higher species richness on Okinawa than lower impact sites (Mars Brisbin et al., 2024) and could be quite diverse between different habitats (Hamamoto et al., 2024). In estuaries, those with anthropogenic influence showed either the highest eDNA derived taxonomic richness (Chariton et al., 2015) or at least for certain taxonomic groups as microbes and plants (Downie et al., 2024).

We also found an influence of depth in our species richness results. While there was no statistical difference in species richness between the two sampling depths in total and within impact levels, there was a clear pattern of increased variation (range between the lowest value and the highest value) in shallow water samples with high impact levels. While the deep high impact water samples were statistically different to both- deep and shallow- low impact samples, only the deep impact samples showed a significant difference to shallow water samples in high impacted sampling sites. The high variation of species richness in the shallow water samples could be due to the increased instability of the shallow habitat compared to deeper parts of the coral reef. Shallow parts of coral reefs are subjected to more impacts by waves, most likely higher impacts of marine heatwaves and runoff events. While this should be true for all impact levels, it could be that the ones already dealing with anthropogenic disturbances are not as resilient as those under lower anthropogenic impact (Gove et al., 2023).

However, relying only on alpha-diversity indices to infer coral reef impact status could be skewed as direct comparison between studies are difficult given variances in methodology, habitat and seasonality. Therefore, more evidence is needed if the findings of high levels of taxonomic richness may be really indicative of a healthy coral reef environment, at least in our study design. Also, not completely relying on taxon richness metrics, but examining community assemblages of the sampling sites should always be warranted.

**Community Assemblages under anthropogenic stress**

We found a clear distinction of community assemblages between our anthropogenic stress categories. This pattern was already visible for the nMDS plots using benthic cover inferred by the PLITs where low impact sites were clearly distinguished from medium and high impact sites and become more apparent for our eDNA results. There is a clear split between the sampling sites in the Keramashoto National Park and all other sites on Okinawa. Low-pressure sites showed an increase abundance of reads belonging to Cnidaria. These sites showed also high coverage of hard corals in the PLITS. However, the results of the eDNA are rather a reflection of other cnidarian taxa as most ESVs identified as Cnidaria belonged to Hydrozoans, but a family this group was also positively correlated with low anthropogenic impact in another study around Okinawa (DiBattista et al. 2020), as well as certain taxa of Porifera. A taxon also more abundant in low impact levels in this study. These shifts underscore the utility of eDNA in detecting subtle ecological transitions that may signal broader reef degradation around Okinawa. In high impact sites we found an increase of Chlorachniophytes and Amoebozoa. Maybe increased levels of microbial prey for those species or higher nutrient conditions in these sites led to the increase in both Chlorarachniophytes and Amoebozoa (Eckmann et al., 2023; Shiratori et al., 2017; Tekle et al., 2021; Yoo & Palenik, 2021). However, more work is needed in understanding the role of these taxa on coral reefs and if both groups could serve as bioindicators indicating organic pollution or eutrophication in marine ecosystems.

**Challenges and Considerations**

Despite its potential, eDNA methodologies face several challenges when applied to anthropogenic impact studies (Hinz et al., 2022). One significant issue is the influence of environmental factors on eDNA persistence and degradation. For example, high temperatures or UV exposure can degrade DNA molecules, potentially skewing results in heavily impacted reef areas (Maruyama et al., 2014; Tsuji et al., 2017). Additionally, the spatial resolution of eDNA data depends on the hydrodynamic properties of the sampling site; strong currents or wave action may transport eDNA away from its source, complicating the interpretation of results in relation to specific stressors (Gert‐Jan Jeunen et al., 2019; Jeunen et al., 2020).

Another challenge lies in the reliance on high-quality reference databases for taxonomic assignments (DiBattista et al., 2020; Dugal et al., 2022; Ip et al., 2021). Many marine species remain undescribed, and existing databases may be incomplete or contain misidentifications, especially challenging in sites with high diversity and endemism as the Ryukyu archipelago (Reimer et al., 2019). This limitation can result in unclassified or erroneously assigned sequences, reducing the utility of eDNA in precisely characterizing biodiversity changes due to anthropogenic impacts. Continued efforts to expand and curate reference databases, particularly for coral-associated taxa, are essential for maximizing the reliability of eDNA analyses.

**Conclusion**

In conclusion, eDNA represents a powerful and versatile tool for investigating anthropogenic impacts on coral reefs. Its sensitivity, scalability, and non-invasive nature make it uniquely suited to addressing the challenges of monitoring these dynamic and threatened ecosystems. Incorporating eDNA collection with traditional visual surveys enhances monitoring efforts by combining the breadth of eDNA's taxonomic detection with the contextual insights provided by direct observation. While challenges remain, particularly regarding data interpretation and taxonomic resolution, this integrated approach holds great promise for advancing coral reef conservation in the Anthropocene. Continued efforts to refine methodologies, expand reference databases, and effectively merge eDNA with visual surveys will be critical for unlocking its full potential in coral reef ecology and beyond.

Boulanger, E., Loiseau, N., Valentini, A., Arnal, V., Boissery, P., Dejean, T., Deter, J., Guellati, N., Holon, F., Juhel, J.-B., Lenfant, P., Manel, S., & Mouillot, D. (2021). Environmental DNA metabarcoding reveals and unpacks a biodiversity conservation paradox in Mediterranean marine reserves. *Proceedings of the Royal Society B: Biological Sciences*, *288*(1949), rspb.2021.0112, 20210112. https://doi.org/10.1098/rspb.2021.0112

Casey, J. M., Ransome, E., Collins, A. G., Mahardini, A., Kurniasih, E. M., Sembiring, A., Schiettekatte, N. M. D., Cahyani, N. K. D., Wahyu Anggoro, A., Moore, M., Uehling, A., Belcaid, M., Barber, P. H., Geller, J. B., & Meyer, C. P. (2021). DNA metabarcoding marker choice skews perception of marine eukaryotic biodiversity. *Environmental DNA*, *3*(6), 1229–1246. https://doi.org/10.1002/edn3.245

Chariton, A. A., Stephenson, S., Morgan, M. J., Steven, A. D. L., Colloff, M. J., Court, L. N., & Hardy, C. M. (2015). Metabarcoding of benthic eukaryote communities predicts the ecological condition of estuaries. *Environmental Pollution*, *203*, 165–174. https://doi.org/10.1016/j.envpol.2015.03.047

DiBattista, J. D., Berumen, M. L., Priest, M. A., de Brauwer, M., Coker, D. J., Sinclair–Taylor, T. H., Hay, A., Bruss, G., Mansour, S., Bunce, M., Goatley, C. H. R., Power, M., & Marshell, A. (2022). Environmental DNA reveals a multi–taxa biogeographic break across the Arabian Sea and Sea of Oman. *Environmental DNA*, *4*(1), 206–221. https://doi.org/10.1002/edn3.252

DiBattista, J. D., Reimer, J. D., Stat, M., Masucci, G. D., Biondi, P., de Brauwer, M., Wilkinson, S. P., Chariton, A. A., & Bunce, M. (2020). Environmental DNA can act as a biodiversity barometer of anthropogenic pressures in coastal ecosystems. *Scientific Reports*, *10*(1), 8365. https://doi.org/10.1038/s41598-020-64858-9

Downie, A. T., Bennett, W. W., Wilkinson, S., De Bruyn, M., & DiBattista, J. D. (2024). From land to sea: Environmental DNA is correlated with long-term water quality indicators in an urbanized estuary. *Marine Pollution Bulletin*, *207*, 116887. https://doi.org/10.1016/j.marpolbul.2024.116887

Dugal, L., Thomas, L., Wilkinson, S. P., Richards, Z. T., Alexander, J. B., Adam, A. A. S., Kennington, W. J., Jarman, S., Ryan, N. M., Bunce, M., & Gilmour, J. P. (2022). Coral monitoring in northwest Australia with environmental DNA metabarcoding using a curated reference database for optimized detection. *Environmental DNA*, *4*(1), 63–76. https://doi.org/10.1002/edn3.199

Eckmann, C. A., Eberle, J. S., Wittmers, F., Wilken, S., Bergauer, K., Poirier, C., Blum, M., Makareviciute-Fichtner, K., Jimenez, V., Bachy, C., Vermeij, M. J. A., & Worden, A. Z. (2023). Eukaryotic algal community composition in tropical environments from solar salterns to the open sea. *Frontiers in Marine Science*, *10*, 1131351. https://doi.org/10.3389/fmars.2023.1131351

Everett, M. V., & Park, L. K. (2018). Exploring deep-water coral communities using environmental DNA. *Deep Sea Research Part II: Topical Studies in Oceanography*, *150*, 229–241. https://doi.org/10.1016/j.dsr2.2017.09.008

Gaither, M. R., DiBattista, J. D., Leray, M., & Heyden, S. (2022). Metabarcoding the marine environment: From single species to biogeographic patterns. *Environmental DNA*, *4*(1), 3–8. https://doi.org/10.1002/edn3.270

Gert‐Jan Jeunen, Jeunen, G.-J., Michael Knapp, Knapp, M., Hamish G. Spencer, Spencer, H. G., Miles D. Lamare, Lamare, M. D., Helen R. Taylor, Taylor, H. R., Michael Stat, Stat, M., Michael Bunce, Bunce, M., Neil J. Gemmell, & Gemmell, N. J. (2019). Environmental DNA (eDNA) metabarcoding reveals strong discrimination among diverse marine habitats connected by water movement. *Molecular Ecology Resources*, *19*(2), 426–438. https://doi.org/10.1111/1755-0998.12982

Gösser, F., Schweinsberg, M., Mittelbach, P., Schoenig, E., & Tollrian, R. (2022). An environmental DNA metabarcoding approach versus a visual survey for reefs of Koh Pha–ngan in Thailand. *Environmental DNA*. https://doi.org/10.1002/edn3.378

Gove, J. M., Williams, G. J., Lecky, J., Brown, E., Conklin, E., Counsell, C., Davis, G., Donovan, M. K., Falinski, K., Kramer, L., Kozar, K., Li, N., Maynard, J. A., McCutcheon, A., McKenna, S. A., Neilson, B. J., Safaie, A., Teague, C., Whittier, R., & Asner, G. P. (2023). Coral reefs benefit from reduced land–sea impacts under ocean warming. *Nature*, *621*(7979), 536–542. https://doi.org/10.1038/s41586-023-06394-w

Hamamoto, K., Mizuyama, M., Nishijima, M., Maeda, A., Gibu, K., Poliseno, A., Iguchi, A., & Reimer, J. D. (2024). Diversity, composition and potential roles of sedimentary microbial communities in different coastal substrates around subtropical Okinawa Island, Japan. *Environmental Microbiome*, *19*(1), 54. https://doi.org/10.1186/s40793-024-00594-1

Heery, E. C., Hoeksema, B. W., Browne, N. K., Reimer, J. D., Ang, P. O., Huang, D., Friess, D. A., Chou, L. M., Loke, L. H. L., Saksena-Taylor, P., Alsagoff, N., Yeemin, T., Sutthacheep, M., Vo, S. T., Bos, A. R., Gumanao, G. S., Syed Hussein, M. A., Waheed, Z., Lane, D. J. W., … Todd, P. A. (2018). Urban coral reefs: Degradation and resilience of hard coral assemblages in coastal cities of East and Southeast Asia. *Marine Pollution Bulletin*, *135*, 654–681. https://doi.org/10.1016/j.marpolbul.2018.07.041

Hinz, S., Coston-Guarini, J., Marnane, M., & Guarini, J.-M. (2022). Evaluating eDNA for Use within Marine Environmental Impact Assessments. *Journal of Marine Science and Engineering*, *10*(3), 375. https://doi.org/10.3390/jmse10030375

Ip, Y. C. A., Tay, Y. C., Chang, J. J. M., Ang, H. P., Tun, K. P. P., Chou, L. M., Huang, D., & Meier, R. (2021). Seeking life in sedimented waters: Environmental DNA from diverse habitat types reveals ecologically significant species in a tropical marine environment. *Environmental DNA*, *3*(3), 654–668. https://doi.org/10.1002/edn3.162

Jeunen, G.-J., Lamare, M. D., Knapp, M., Spencer, H. G., Taylor, H. R., Stat, M., Bunce, M., & Gemmell, N. J. (2020). Water stratification in the marine biome restricts vertical environmental DNA (eDNA) signal dispersal. *Environmental DNA*, *2*(1), 99–111. https://doi.org/10.1002/edn3.49

Kelly, R. P., O’Donnell, J. L., Lowell, N. C., Shelton, A. O., Samhouri, J. F., Hennessey, S. M., Feist, B. E., & Williams, G. D. (2016). Genetic signatures of ecological diversity along an urbanization gradient. *PeerJ*, *4*, e2444. https://doi.org/10.7717/peerj.2444

Manel, S., Mathon, L., Mouillot, D., Bruno, M., Valentini, A., Lecaillon, G., Gudefin, A., Deter, J., Boissery, P., & Dalongeville, A. (2024). Benchmarking fish biodiversity of seaports with eDNA and nearby marine reserves. *Conservation Letters*, *17*(2), e13001. https://doi.org/10.1111/conl.13001

Mars Brisbin, M., Dudley, K. L., Yonashiro, Y., Mitarai, S., & Ares, A. (2024). Urbanization of a Subtropical Island (Okinawa, Japan) Alters Physicochemical Characteristics and Disrupts Microbial Community Dynamics in Nearshore Ecosystems. *Estuaries and Coasts*, *47*(5), 1266–1281. https://doi.org/10.1007/s12237-024-01366-3

Maruyama, A., Nakamura, K., Yamanaka, H., Kondoh, M., & Minamoto, T. (2014). The Release Rate of Environmental DNA from Juvenile and Adult Fish. *PLoS ONE*, *9*(12), e114639. https://doi.org/10.1371/journal.pone.0114639

Masucci, G. D., Biondi, P., & Reimer, J. D. (2021). Impacts of coastal armouring on rubble mobile cryptofauna at shallow coral reefs in Okinawa, Japan. *Plankton and Benthos Research*, *16*(4), 237–248. https://doi.org/10.3800/pbr.16.237

Reimer, J. D., Biondi, P., Lau, Y. W., Masucci, G. D., Nguyen, X. H., Santos, M. E. A., & Wee, H. B. (2019). Marine biodiversity research in the Ryukyu Islands, Japan: Current status and trends. *PeerJ*, *7*, e6532. https://doi.org/10.7717/peerj.6532

Shiratori, T., Fujita, S., Shimizu, T., Nakayama, T., & Ishida, K. (2017). Viridiuvalis adhaerens gen. Et sp. Nov., a novel colony-forming chlorarachniophyte. *Journal of Plant Research*, *130*(6), 999–1012. https://doi.org/10.1007/s10265-017-0961-1

Stat, M., John, J., DiBattista, J. D., Newman, S. J., Bunce, M., & Harvey, E. S. (2019). Combined use of eDNA metabarcoding and video surveillance for the assessment of fish biodiversity. *Conservation Biology*, *33*(1), 196–205. https://doi.org/10.1111/cobi.13183

Tekle, Y. I., Lyttle, J. M., Blasingame, M. G., & Wang, F. (2021). Comprehensive comparative genomics reveals over 50 phyla of free-living and pathogenic bacteria are associated with diverse members of the amoebozoa. *Scientific Reports*, *11*(1), 8043. https://doi.org/10.1038/s41598-021-87192-0

Tsuji, S., Ushio, M., Sakurai, S., Minamoto, T., & Yamanaka, H. (2017). Water temperature-dependent degradation of environmental DNA and its relation to bacterial abundance. *PLOS ONE*, *12*(4), e0176608. https://doi.org/10.1371/journal.pone.0176608

West, K. M., Adam, A. A. S., White, N., Robbins, W. D., Barrow, D., Lane, A., & Richards, Z. (2021). The applicability of eDNA metabarcoding approaches for sessile benthic surveying in the Kimberley region, north–western Australia. *Environmental DNA*. https://doi.org/10.1002/edn3.184

West, K. M., Michael J. Travers, Travers, M. J., Michael Stat, Stat, M., Euan S. Harvey, Harvey, E. S., Zoe T. Richards, Richards, Z. T., Joseph D. DiBattista, DiBattista, J. D., Stephen J. Newman, Newman, S. J., Alastair V. Harry, Harry, A., Harry, A. V., Alastair Harry, Craig L. Skepper, Skepper, C. L., … Bunce, M. (2021). Large-scale eDNA metabarcoding survey reveals marine biogeographic break and transitions over tropical north-western Australia. *Diversity and Distributions*, *27*(10), 1942–1957. https://doi.org/10.1111/ddi.13228

Yoo, Y. D., & Palenik, B. (2021). Growth and grazing of the chlorarachniophyte *Bigelowiella natans* (Chlorarachniophyceae) on the marine cyanobacterium *Synechococcus*. *Phycologia*, *60*(4), 375–383. https://doi.org/10.1080/00318884.2021.1941567