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Metabarcoding of zooplankton to derive indicators of pelagic ecosystem status

Zooplankton play a key role in marine food webs and carbon cycling and are useful indicators of climate-related changes and ocean health in pelagic ecosystems. Zooplankton are traditionally identified to species through microscopy, but new molecular techniques have enabled the identification of individual specimens (DNA barcoding) or multiple species in the same sample (DNA metabarcoding). Metabarcoding has been tested and refined using zooplankton collected in South African waters for the first time. Challenges to the implementation of DNA-based methods to measure zooplankton biodiversity easily and routinely include an incomplete DNA barcode reference library, logistical complexity and uptake of the new technology by environmental management agencies. These challenges call for a national effort to intensify zooplankton barcoding initiatives and to effectively engage stakeholders in developing a roadmap towards application of DNA-based methods in marine environmental management.

Significance:

- Metabarcoding has been successfully applied to marine zooplankton for the first time in South Africa, demonstrating its potential as a tool to generate ecosystem indicators during routine ocean observations.
- National barcoding efforts must be intensified to provide a comprehensive reference library of zooplankton DNA.
- Effective engagement with stakeholders is required to overcome logistical and policy challenges, and to provide a roadmap towards application of DNA-based methods in marine environmental management.

The need for ocean indicators

Recent warming, acidification and deoxygenation associated with climate change have greatly affected physical and chemical conditions in oceans.[1] Altered marine habitats have led to shifts in the distribution and phenology of organisms, with major implications for biological productivity of marine ecosystems.[1] Yet, human reliance on the goods and services provided by oceans continues to grow.[2] Against a backdrop of climate change and increasing exploitation of marine resources, observations of key ocean indicators are critical to inform policy and support ocean governance and management.[2,3]

The importance of ocean observation systems is recognised by the United Nations Decade of Ocean Science for Sustainable Development (2021–2030), with one of the ten Ocean Decade Challenges being to expand the ocean observing system globally.[4] The Global Ocean Observing System (GOOS) programme has identified a suite of priority physical, biogeochemical and biological ecosystem variables, known as essential ocean variables (EOVs; Supplementary table 1), for routine and sustained observation to assess ocean changes globally, in support of ocean governance.[3] A complementary set of essential biodiversity variables (EBVs)[5], to monitor and reduce biodiversity loss, has been defined by the Group on Earth Observations – Biodiversity Observation Network (Supplementary table 1). Zooplankton biomass and diversity were included as biological EOVs that represent the base of marine food webs.[2] Here, we report on recent molecular methodology (DNA barcoding and metabarcoding) that allows for the rapid and accurate measurement of marine zooplankton biodiversity and relative abundance, and its potential application as a long-term indicator of pelagic ecosystem status in South African coastal and neritic waters. We comment on both methodological and logistical considerations.

Assessing zooplankton biodiversity

Zooplankton play a vital role in the functioning of marine ecosystems. As grazers in pelagic food webs, they provide the main energy pathway from primary producers to higher trophic levels such as fish, squid, and marine mammals. Their excretions fuel the microbial food web and contribute significantly to carbon sequestration via the biological pump.[6,7] Zooplankton are physiologically sensitive to temperature and have short life spans, thus thermal changes are rapidly reflected in their population dynamics.[8] Because they are not fished commercially, changes in zooplankton communities reflect actual environmental or ecosystem-mediated changes, largely unaffected by exploitation trends.[8,9] Zooplankton are therefore excellent 'sentinels' or indicators of change in pelagic ecosystems, with applications extending to climate, fisheries, invasive species, ecosystem health, definition of pelagic ecoregions, biodiversity and ecosystem assessments.[8,9]

Ongoing advancement of DNA barcoding reference data sets (e.g. BOLD, GenBank; Supplementary table 1) coupled with recent metabarcoding technologies now makes rapid and accurate processing of taxonomically complex zooplankton samples logistically feasible. This new technology prompted a shift from traditional microscopy methods to genes as a measure of marine diversity.[10] DNA barcodes can distinguish between visually similar or cryptic species, are independent of life stage, and reduce researcher bias through standardisation of reference systems.[10] DNA barcoding has been applied successfully to global biodiversity studies, including the Census of Marine Zooplankton (CMarZ).[11]

South Africa formally committed to the International Barcode of Life Project (iBOL) in 2011. Barcode reference databases with records of marine zooplankton from South African waters were reviewed recently.[12] Records were proportionally below global levels in nearly all taxa examined and were dominated by species from easily accessible habitats and those with commercially important life phases (i.e. drifting larvae of fish and benthic invertebrates) (Figure 1). Holoplanktonic species were grossly under-represented, despite making up the bulk of zooplankton biomass and with high importance as ecological indicators. The paucity of holoplanktonic barcodes stemmed from too few specialist taxonomists, the so-called 'taxonomic impediment'.[13]

Metabarcoding uses the same reference databases as barcoding but allows for identification of multiple taxa simultaneously from mixed samples by using high-throughput sequencing platforms.[14] Potential applications of metabarcoding are broad-ranging, including revolutionising biodiversity assessments in any environment for which DNA barcode reference databases are available[15], establishing time-series of diversity[16] and developing biotic indices for routine biomonitoring[17]. The progression from barcoding of individual specimens to metabarcoding of entire communities is now well underway in South Africa, with studies published on diatom communities in the St Lucia estuary[18], bacterial communities in waterholes in the Kruger Park[19] and biomonitoring of freshwater macro-invertebrates[20], among others.

Towards routine biodiversity monitoring

The adoption of molecular approaches such as metabarcoding in marine environmental management requires an iterative 'translational molecular ecology' approach (constant two-way communication between scientists and stakeholders).[17] Recent applications that demonstrate this process internationally include routine monitoring of ichthyoplankton, biosecurity monitoring for non-indigenous species, and ecological status assessments.[17] As part of the translational molecular ecology approach,

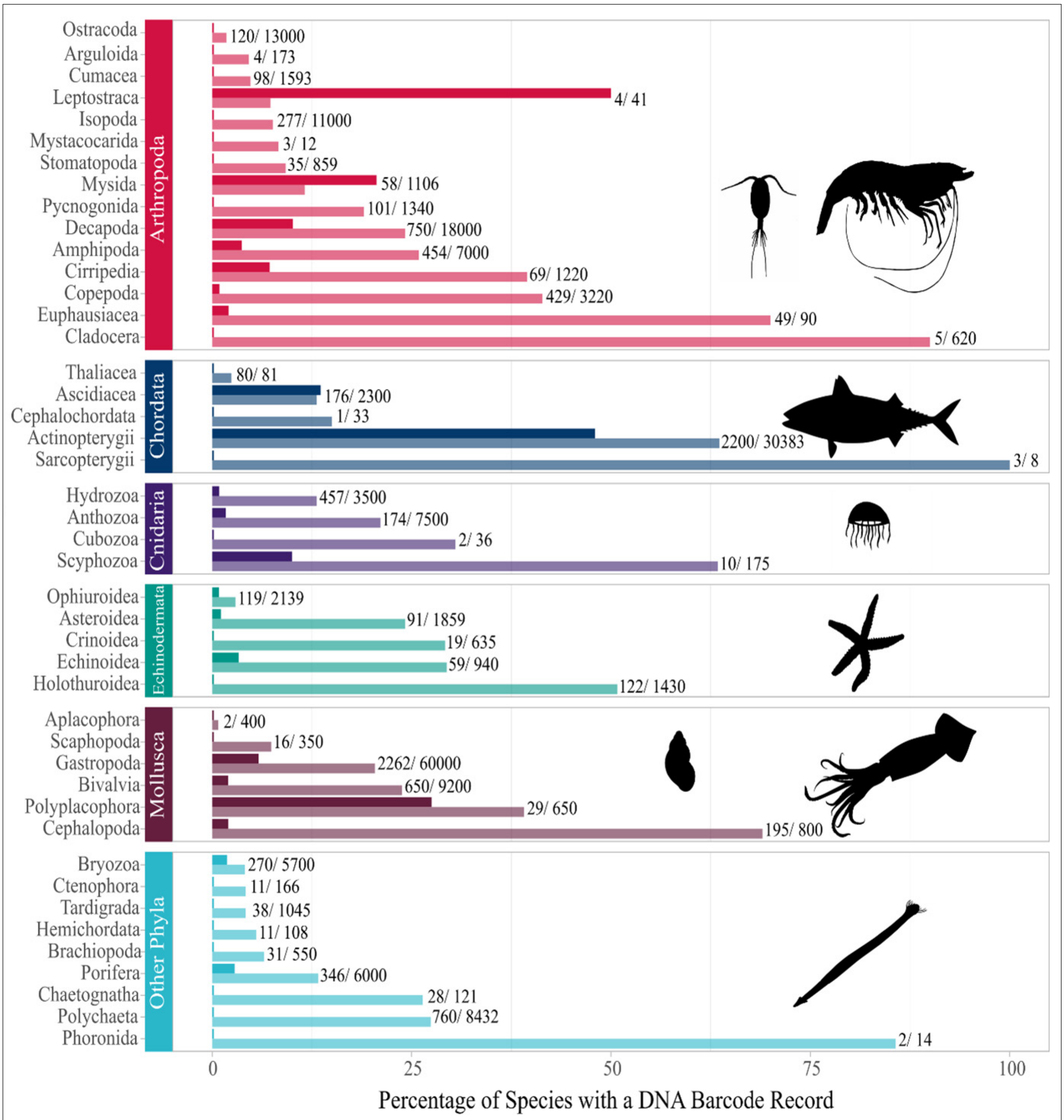

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Figure 1: The relative percentages of DNA barcode records available for marine zooplankton taxa, globally (pale bars) and for South Africa (dark bars). Numbers next to the bars are the numbers of species known locally/globally.

the development of and adherence to standardised protocols to guarantee data comparison across spatial and temporal scales are crucial.[17]

Metabarcoding of marine zooplankton in South Africa has been optimised to follow best-practice protocols set by the international Scientific Committee on Oceanic Research working group MetaZooGene (Supplementary table 1). Optimisation challenges were the design of taxon-specific mini-barcode primers to increase species detection rates[21], experimental validation of primer cocktails to test their efficiency in detecting rare species[22], and comparison of species identities obtained from metabarcoding and microscopy[12]. The strategic importance of an expanded DNA barcode reference database for the region is recognised by the Foundational Biodiversity Information Programme of the South African National Research Foundation. This Programme currently funds integrative molecular morphology projects to increase regional reference databases (including for zooplankton), with new records uploaded to open-access databases such as BOLD and GenBank.

Further incorporation of zooplankton biodiversity in ocean observation programmes as biological EOVs faces several logistical challenges in South Africa. Key among these are: access to sea-going vessels and sampling gear, laboratories for sample processing, technical and scientific expertise across various disciplines (genetics, taxonomy, bioinformatics, biodiversity, oceanography) and data management from the point of observation to implementation by users. To evaluate the underlying science, operational costs and benefits, a small-scale pilot project was initiated in 2018, along a single cross-shelf transect at the KwaZulu-Natal Bight Sentinel Site (Supplementary table 1; Supplementary figure 1). The pilot project is a collaboration between several academic institutions, which are in turn funded by the Department of Science and Innovation and the National Research Foundation.[12,21,22] Key findings were that costs, coordination and uptake will be the main challenges for a prospective long-term programme.

In addition to a successful 'proof of concept' exploratory project and an improved barcode reference database, progress towards the routine use of metabarcoding in monitoring of the marine environment in South Africa will require a 'translational molecular ecology' process to facilitate its uptake at environmental management and policy levels.[17] The role of national environmental observation agencies, primarily the South African Environmental Observation Network (SAEON) and the Department of Forestry, Fisheries and the Environment (DFFE), in providing operational budgets and coordination, and as long-term custodians of data and indices, will be critical for actionable progress beyond exploratory research. Our Research Letter describes the initial development of a DNA-based method for biodiversity assessments of South African pelagic ecoregions, including enabling conditions for its uptake in marine environmental management.

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Competing interests

We have no competing interests to declare.

Authors' contributions

J.A.H.: Conceptualisation, methodology, writing – the initial draft, writing – revisions, project leadership, project management. J.C.G.: Conceptualisation, methodology, writing – the initial draft, writing – revisions, project leadership, project management. S.P.S.: Methodology, data collection, data analysis, writing – the initial draft. S.W.-M.: Methodology, data analysis, writing – the initial draft, student supervision. A.G.: Methodology, data collection, data analysis, writing – the initial draft. R.C.: Methodology, data collection, data analysis, writing – the initial draft. S.H.P.D.: Methodology, data collection, data analysis, writing – the initial draft.

References

1. IPCC. The ocean and cryosphere in a changing climate: Special report of the Intergovernmental Panel on Climate Change. Cambridge: Cambridge University Press; 2019. Available from: https://www.ipcc.ch/srocc/

2. Muller-Karger FE, Miloslavich P, Bax NJ, Simmons S, Costello MJ, Sousa Pinto I, et al. Advancing marine biological observations and data requirements of the complementary essential ocean variables (EOVs) and essential biodiversity variables (EBVs) frameworks. Front Mar Sci. 2018;5:211. https://doi.org/10.3389/fmars.2018.00211

3. Miloslavich P, Bax NJ, Simmons SE, Klein E, Appeltans W, Aburto-Oropeza O, et al. Essential ocean variables for global sustained observations of biodiversity and ecosystem changes. Glob Change Biol. 2018;24:2416–2433. https://doi.org/10.1111/gcb.14108

4. United Nations Decade of Ocean Science for Sustainable Development. The ocean decade [webpage on the Internet]. c2021 [cited 2021 Dec 01]. Available from: https://www.oceandecade.org/

5. Pereira HM, Ferrier S, Walters M, Geller GN, Jongman RHG, Scholes RJ, et al. Essential biodiversity variables. Science. 2013;339:277–278. https://doi.org/10.1126/science.1229931

6. Passow U, Carlson CA. The biological pump in a high CO_2 world. Mar Ecol Prog Ser. 2012;470:249–271. https://doi.org/10.3354/meps09985

7. Steinberg DK, Carlson CA, Bates NR, Goldthwait SA, Madin LP, Michaels AF. Zooplankton vertical migration and the active transport of dissolved organic and inorganic carbon in the Sargasso Sea. Deep Sea Res I. 2000;47:137–158. https://doi.org/10.1016/S0967-0637(99)00052-7

8. Richardson AJ. In hot water: Zooplankton and climate change. ICES J Mar Sci. 2008;65:279–295. https://doi.org/10.1093/icesjms/fsn028

9. Batten SD, Abu-Alhaija R, Chiba S, Edwards M, Graham G, Jyothibabu R, et al. A global plankton diversity monitoring program. Front Mar Sci. 2019;6, Art. #321. https://doi.org/10.3389/fmars.2019.00321

10. Laakmann S, Blanco-Bercial L, Cornils A. The crossover from microscopy to genes in marine diversity: From species to assemblages in marine pelagic copepods. Phil Trans R Soc B. 2020;375(1814), Art. #20190446. https://doi.org/10.1098/rstb.2019.0446

11. Bucklin A, Peijnenburg KTCA, Kosobokova KN, O'Brien TD, Blanco-Bercial L, Cornils A, et al. Toward a global reference database of COI barcodes for marine zooplankton. Mar Biol. 2021;168, Art. #78. https://doi.org/10.1007/s00227-021-03887-y

12. Singh SP, Groeneveld JC, Huggett J, Naidoo D, Cedras R, Willows-Munro S. Metabarcoding of marine zooplankton in South Africa. Afr J Mar Sci. 2021;43(2):147–159. https://doi.org/10.2989/1814232X.2021.1919759

13. Engel MS, Ceríaco LMP, Daniel GM, Dellapé PM, Löbl I, Marinov M, et al. The taxonomic impediment: A shortage of taxonomists, not the lack of technical approaches. Zool J Linn Soc. 2021;193:381–387. https://doi.org/10.1093/zoolinnean/zlab072

14. Cristescu ME. From barcoding single individuals to metabarcoding biological communities: Towards an integrative approach to the study of global biodiversity. Trends Ecol Evol. 2014;29:566–571. https://doi.org/10.1016/j.tree.2014.08.001

15. Bucklin A, Lindeque PK, Rodriguez-Ezpeleta N, Albaina A, Lehtiniemi M. Metabarcoding of marine zooplankton: Prospects, progress and pitfalls. J Plankton Res. 2016;38(3):393–400. https://doi.org/10.1093/plankt/fbw023

16. Bucklin A, Yeh HD, Questel JM, Richardson DE, Reese B, Copley NJ, et al. Time-series metabarcoding analysis of zooplankton diversity of the NW Atlantic continental shelf. ICES J Mar Sci. 2019;76:1162–1176. https://doi.org/10.1093/icesjms/fsz021

17. Aylagas E, Borja A, Pochon X, Zaiko A, Keeley N, Bruce K, et al. Translational molecular ecology in practice: Linking DNA-based methods to actionable marine environmental management. Sci Total Environ. 2020;774, e140780. https://doi.org/10.1016/j.scitotenv.2020.140780

18. Nunes M, Adams JB, Van Aswegen S, Macher GF. A comparison between the morphological and molecular approach to identify the benthic diatom community in the St. Lucia estuary South Africa. Afr J Mar Sci. 2019;41:429–442. https://doi.org/10.2989/1814232X.2019.1689169

19. Farrell MJ, Govender D, Hajibabaei M, Van Der Bank M, Davies TJ. Bacterial diversity in the waterholes of the Kruger National Park: An eDNA metabarcoding approach. Genome. 2019;62(3):229–242. https://doi.org/10.1139/gen-2018-0064

20. Pereira-da-Conceicoa L, Elbrecht V, Hall A, Briscoe A, Barber-James H, Price B. Metabarcoding unsorted kick-samples facilitates macroinvertebrate-based biomonitoring with increased taxonomic resolution, while outperforming environmental DNA. Environ DNA. 2020;3:353–371. https://doi.org/10.1002/edn3.116

21. Govender A, Groeneveld J, Singh S, Willows-Munro S. The design and testing of mini-barcode markers in marine lobsters. PLoS ONE. 2019;14(1), e0210492. https://doi.org/10.1371/journal.pone.0210492

22. Govender A, Singh S, Groeneveld J, Pillay S, Willows-Munro, S. Experimental validation of taxon-specific mini-barcode primers for metabarcoding of zooplankton. Ecol Appl. 2022;32(1), e02469. https://doi.org/10.1002/eap.2469