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Application of molecular technologies to monitor the ecosystem of the WCPO

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Abstract

DNA metabarcoding combines DNA based identification with high-throughput DNA sequencing. It uses universal PCR primers to mass-amplify DNA Barcodes from an entire environmental sample to infer the species composition without the need to sort specimens. Metabarcoding can be applied to samples where DNA is highly degraded, such as in stomach contents to analyse diet composition or a water sample of the ocean. This method was trialled on 17 tuna stomachs from the Bismarck Sea to assess its utility as a method for monitoring ecosystem structure. The Bismarck Sea has been identified as an important sentinel area for detecting the effects of climate and fishing on ecosystem structure in the WCPO. The results from the trial are encouraging with more taxon identified by the metabarcoding approach than that obtained by traditional morphometric analyses by visual inspection of material in the stomach. This is particularly promising for the identification of species that are difficult to detect or identify and are typically missed and consequently their role in structuring pelagic ecosystems is unknown and are probably miss represented in the ecosystem model used to describe the WCPO (e.g. EB-WP-07). The method is relatively inexpensive with annual monitoring costs for the Bismarck Sea approximately AUD40000 to AUD50000 for 1000 samples.

Background

Environmental DNA and metabarcoding are two new molecular technologies that have the potential to provide state-of-the-art monitoring of the ecosystems in the western and central Pacific Ocean (WCPO). To date monitoring and trophic analyses of the pelagic ecosystems in the WCPO have primarily used the species identified by traditional survey methods such as the visual identification of predator dietary items or scientific cruises that focus of the capture of organisms. A shortcoming of these methods is that those species that are difficult to detect or identify are typically missed and consequently their role in structuring pelagic ecosystems is unknown. They are however thought to be a large proportion of the WCPO biodiversity and biomass (eg. squid and jelly fish). Environmental DNA and metabarcoding utilise advanced DNA detection technology within a robust analytical framework to enable identification of a wide range of taxa and are capable of filling this gap in our current knowledge.

Environmental DNA (eDNA) is defined as the use of genetic material obtained directly from environmental samples, without any obvious signs of biological source material. This is based on the fact that as species interact with the environment, they will continuously expel DNA into their surroundings, which may come from excreted cells or tissues such as skin, urine, intestinal lining in faeces, tissue separated from the body by injury or predation, cells released during reproduction, and decomposition of dead organisms. Contained in each of these cells is an individual's entire set of DNA, which eventually break down to release extra-cellular DNA that becomes embedded in sediment. The preservation of DNA in the environment can vary depending on a range of conditions but marine systems have proven to be rich in eDNA, with extracellular DNA in marine sediments considered as the largest reservoir of DNA in the oceans. As such, water or sediment samples from the ocean potentially contain genetic signatures of species assemblages and can enable comparisons in metazoan diversity at micro-geographical scales.

DNA metabarcoding combines DNA based identification with high-throughput DNA sequencing. It uses universal PCR primers to mass-amplify DNA Barcodes from an entire environmental sample to infer the species composition without the need to sort specimens. The combination of eDNA and metabarcoding can be applied to samples where DNA is highly degraded, such as in stomach contents to analyse diet composition or a water sample of the ocean. Through the analysis of this DNA, it is possible to show the presence of a species without actually catching or seeing the individuals. Due to its high specificity & sensitivity it is particularly useful in detecting low-density organisms.

In order to be able to fully apply these techniques to ecosystem monitoring in the WCPO, some a priori knowledge about the potential species present and their DNA sequences is required to allow identification in the stomachs contents or from water samples. Ongoing large scale DNA barcoding initiatives (www.barcodeoflife.org; www.ncbi.nlm.nih.gov/genbank) are establishing developing reference databases with the barcodes of many WCPO species. The number of barcodes available is increasing continuously and this can be further enhanced for ecosystem monitoring in the WCPO by accessing the reference collections used in the visual identification of stomach contents throughout the WCPO (e.g. SPC, CSIRO, NOAA, University of Tokyo).

Ecosystem Monitoring of the Bismarck Sea

Past research includes detailed analyses on the vulnerabilities of Pacific fisheries to climate, the study of population dynamics of equatorial tunas to support regional stock assessments, and investigations to describe the trophic ecology of the west Pacific. It is clear from this work that fishing mortality rates in the Bismarck Sea are among the highest in the region, and its tuna

habitats are predicted to degrade in association with future climate regimes. Analyses of tuna stomach contents was very effective for monitoring lower trophic levels which were identified as early warning indicators of environmental change due to their sensitivities to changes in water chemistry and ecosystem structure. Over the last decade, ~4,000 tuna stomachs have been collected and analysed from PNG which have been used to draft preliminary trophic models. This has provided a quantified baseline for monitoring the impacts of environmental change and harvest on tuna fisheries. However this analyses has been limited by some observed prey species being under represented in the diet analyses (e.g. pelagic anchovy). In the case of skipjack tuna it is hypothesised that this is due to their fast metabolism and digestion of prey. Gelatinous organisms and molluscs are also hypothesised to be under-represented in the diet of pelagic predators. The consequence of this under representation is that trophic models built to evaluate the effects of harvest of one or more species on the dynamics of other species and ecosystem structure are likely to be missing important pathways. The failure to account for these pathways could significantly impact upon the resilience of this ecosystem to disturbances and the longer term sustainability of the fisheries operating in this system that is estimated by the current trophic models.

The Pilot study

To pilot the application of metabarcoding for ecosystem monitoring in the WCPO, DNA was extracted from 17 tuna stomachs (11 skipjack, 2 bigeye and 4 yellowfin) collected from the Bismarck Sea. Independent stomach content analyses using morphological keys to visually identify whole and part diet items was carried out to verify that the molecular approaches retrieved all taxa that were identified by traditional methods. Generic PCR primers (short synthetic DNA tags) were used to target three DNA barcode regions each designed for fish, crustaceans, and cephalopods. A threshold of >95% match to the DNA was set to accept species identification from an individual sequence. The NIH genetic sequence database (Genbank) was accessed for matching stomach DNA with existing barcode sequences.

The metabarcoding proved successful for fish species (Figure 1). The species named in black text in Figure 1 represent those that were identified by the visual identification and the metabarcoding. The green text represents those only identified by the metabarcoding. The orange text represents those that were identified to family level in the visual analyses but to species level in the metabarcoding analyses. The DNA metabarcoding identified more species than the morphological analyses by visual inspection of the stomach contents (6 prey items in comparison to 2 by visual examination). Neither analysis was able to differentiate between the two species of Pomfret or Driftfish. Buccaneer anchovy was identified by the metabarcoding but only to the family level by the visual analysis. The study also examined the differences between DNA analyses of stomach solid particles and liquid. The solid particles analyses

identified one extra species. Differences were also detected between the species. Only buccaneer anchovy were detected in the stomach contents of bigeye and skipjack whereas all 6 prey items were detected in yellowfin.

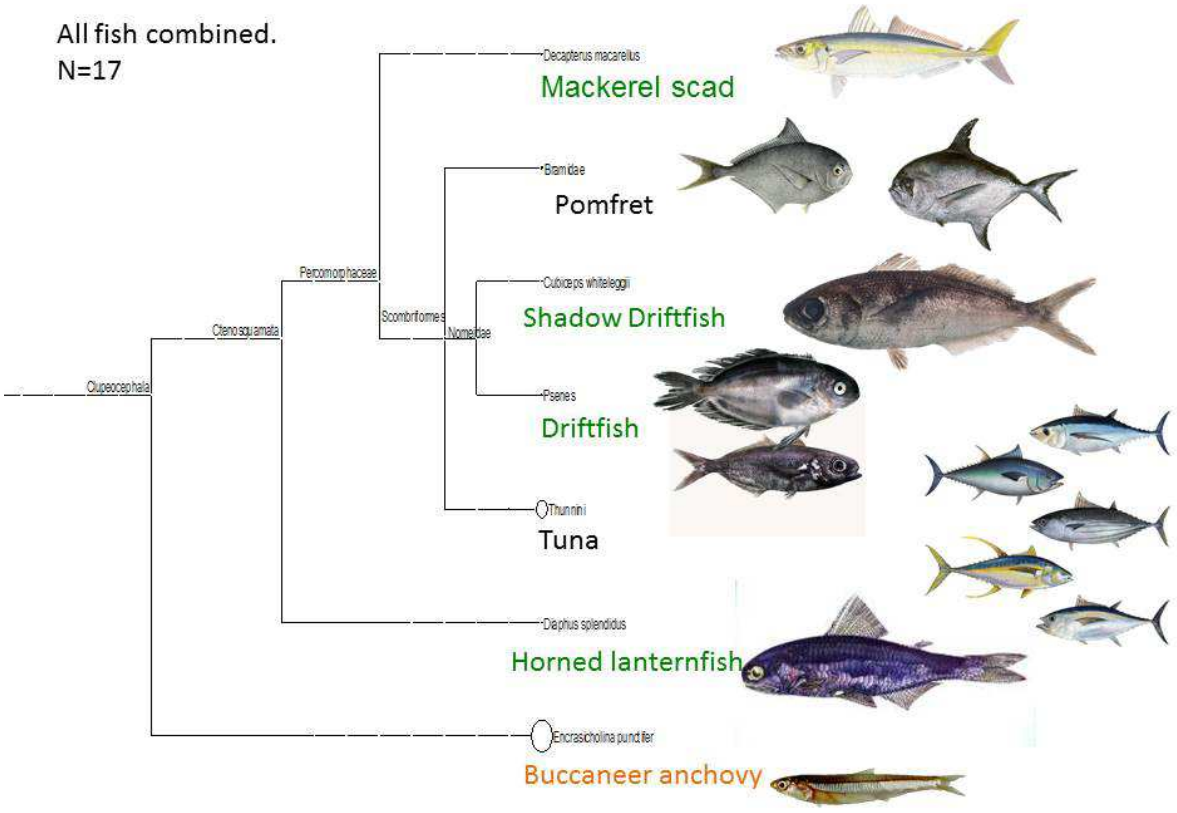

Figure 1. Identification of fish species from the DNA barcoding analyses of 17 tuna stomachs collected from the Bismarck Sea, Paua New Guinea.

The primers used for amplifying the DNA of crustacea and cephalopods were unable to generate sufficient quality amplicons for sequencing. These primers were relatively new and still require further optimisation so will be trialled again on these samples.

WCPO Applications

The results from this initial trial of DNA metabarcoding to identify prey items in tuna stomachs are encouraging and suggest that further application is warranted. There is currently some potential for misidentification of species due to the incomplete reference databases. When there is only one species for a genus in the database the analyses will identify to this species. For some genera where there are multiple species but only one barcoded this error may be prevalent. The databases are continually improving and adding to the databases by barcoding the reference libraries used for visual identification will help resolve this issue. Most of the

current applications for stomach contents analyses however do not require species level identification with genus or family sufficient. However cannibalism by skipjack and consumption of skipjack by larger yellowfin and bigeye has been reported and consideration may need to be given to improving the primers used to amplify the tuna DNA for species identification. The primers used in the pilot study are not able to differentiate between species.

Inferring abundance from metabarcoding remains in its infancy and consequently any estimate of proportion of stomach content attributed to one species over another would be highly uncertain. Similar uncertainties exist for morphomentric analyses by visual examination as the highly degraded or partially digested items are rarely identified.

Although the crustacean and cephalopod primers were unable to provide data on these species for this trial, these are currently undergoing further optimisation. As a consequence, these taxa should also be able to be identified using the metabarcoding approach.

The current cost per stomach for the DNA metabarcoding is approximately AUD40-50 depending on the number of samples and sequences required per sample to adequately represent the stomach contents. At this price the method would provide a cost effective addition to the ecosystem monitoring of the WCPO. Monitoring the Bismarck Sea ecosystem to measure climate and fisheries impacts for example could cost as little as AUD40000/yr for 1000 samples plus the cost of stomach collection and preparation. The price may further decrease for high quantity production.