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MARINE ECOLOGY

Genetics from a drop in the ocean

Analysis of environmental DNA (eDNA) extracted from just 30 litres of seawater from the Arabian Gulf provides genetic insights into populations of the largest fish in the world.

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Understanding the relationship between population viability and genetic diversity is paramount for the effective conservation of endangered species[1]. However, determining the genetic diversity and structure of populations depends upon effective sampling, which may be costly, or require expert taxon-specific identification. Sampling can also be invasive for organisms and the environment, or suffer from inaccuracy if the target species is rare.

Building on seminal contributions in the field of 'macrobial' eDNA analysis[2], Sigsgaard and colleagues[3] demonstrate that high-throughput sequencing of seawater (eDNA) can provide useful estimates of genetic diversity of the elusive and endangered whale shark, *Rhincodon typus* (pictured). Until now, eDNA assessments have been restricted to species detection only, with Sigsgaard *et al.* now providing an important proof of principle that non-invasive eDNA techniques can be extended to provide useful population-level information.

Effective species conservation and management of natural resources is reliant upon our understanding of population dynamics[4]. This viewpoint is highly prevalent in fisheries management, where stocks are identified and managed based on their genetic identity[5]. Likewise, management strategies for large herbivores, such as caribou, are also based on herd characteristics, including detailed age structure and extensive mark recapture methods[6]. These same principles apply in the field of conservation genetics[7]. Therefore, whether the goal is species conservation or resource harvesting, effective population management is essential for maintaining healthy populations by ensuring that standing genetic variation is preserved. It is this standing genetic variation that provides the raw material to allow species to remain healthy and adapt via natural selection to changing environmental conditions[8].

Recent developments in the analysis of eDNA for macrobial species detection[9] has

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initiated a revolution in the fields of ecology, and natural resource and conservation management[10]. With eDNA analysis, DNA is isolated directly from an environmental sample without first isolating any type of organism (for example, soil, sediment, faeces, water or air)[2]. A good example is the original usage of macrobial eDNA detection, whereby traces of DNA or cells (for example, from shed skin or excrement) deposited into pond environments were used for species detection of invasive American bullfrogs (*Rana catesbeiana*) in France[9]. Compared with traditional methods, eDNA offers a rapid collection method that is cost effective, utilizes standardized molecular approaches, has minimal impact on the environment and effectively detects low-abundance species[2]. Several major methodological and technical advances in eDNA-based detection have enabled eDNA-based biodiversity assessments to be conducted across a wide range of species and ecosystems, potentially providing standardization for biodiversity assessment. The promise of eDNA as an all-encompassing assessment method has prompted extensive stakeholder interaction, private company start-ups and utilization by government agencies in developing biomonitoring programmes[11] and resource management strategies[2].

Whale sharks are known to form large feeding aggregations at several sites across the globe, and analysis of tissue samples has previously identified strong genetic differences between Atlantic and Indian–Pacific ocean populations[12]. In order to establish whether population genetic information could be gathered via this new technique, Sisgaard and colleagues fine-filtered a number of small volume seawater samples totalling 30 litres, and employed whale-shark-specific mitochondrial DNA markers[12] with high-throughput sequencing[13] to identify the genetic diversity of a well-characterized seasonal aggregation in the Arabian Gulf near Qatar.

Following extensive quality control of sequencing data, the team were able to demonstrate that eDNA analysis recovered more variants of the mitochondrial gene markers (haplotypes) than physical sampling, but that the haplotype frequencies were comparable between seawater samples and a companion analysis of actual whale shark tissue samples. By comparing the eDNA data with population genetic data from a whale shark reference database, the eDNA data was able to distinguish the closer relationship between the Arabian Gulf and the Indo-Pacific populations from the more distant relationship between the Atlantic and the Arabian or Indo-Pacific populations. Furthermore, population genetic estimates of global numbers of breeding females derived from the eDNA data, showed similarities, but not direct coincidence, with estimates derived from tissue analyses[12,14,15].

A notable limitation of estimating population genetic diversity from eDNA is that the number of sampled individuals (a vital metric in population genetic analysis) is unknown. However, here the team were able to rely on existing data from *in situ* observations and modelling to effectively

compare population measures. Analysis of maternally inherited mitochondrial DNA is also not capable of providing insights into genetic variability within individuals, which is often a useful metric for estimating potential fitness and levels of inbreeding. Future work will need to consider the challenging concept of age structure too, in order to assess effectively the genetic composition of populations comprising multiple cohorts.

Nevertheless, given that the remarkable insights were achieved by the genetic analysis of just 30 litres of seawater from a vast marine ecosystem demonstrates just how far the field of macrobial eDNA analysis has come in the past eight years. The general consensus emerging from the field is that the genetic information derived from aqueous eDNA samples is cellular material, freely dispersed and mixed particularly well in aquatic samples. Therefore, the team's findings suggest that if appropriate sampling is employed, further case studies will emerge. If achievable, combining insights into population genetic variability together with the species detection capability of eDNA[16] will provide a potent combination for assessing the genetic health of endangered species. ❐

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