Marine Genomics xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Marine Genomics

journal homepage: www.elsevier.com/locate/margen

Method paper

Metagenomic sequencing of environmental DNA reveals marine faunal assemblages from the West Antarctic Peninsula

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ARTICLE INFO

Keywords:
eDNA
Antarctica
Metagenomics
King crabs
Benthic invertebrates

ABSTRACT

The West Antarctic Peninsula (WAP) is the fastest warming region in Antarctica where climate impact on the cold-adapted marine ecosystem is already visible. To monitor faunal changes in remote vast bodies of Antarctic waters, efficient and informative tools are essential. High-throughput sequencing of environmental DNA (eDNA) has emerged as one such tool for monitoring biodiversity and ecosystems, as it increases detection sensitivity of taxa, and sampling is often simpler and less costly than traditional collection methods. We collected water samples from four WAP shallow (≤ 300 m) shelf regions, recovered the eDNA therein, and performed metagenomic shotgun sequencing and analyses to determine the effectiveness of this method to assess marine benthic faunal diversity; this includes the detection of deep-water predatory king crabs whose potential shoreward expansion to warming shelves has sparked much concern. Using a customized bioinformatics pipeline, we identified abundant signatures of common benthic invertebrate fauna, endemic notothenioid fishes, as well as lithodid king crabs. We also uncovered species richness and diversity comparable to biological inventories compiled by the use of traditional survey methods, supporting the efficacy of the eDNA shotgun sequencing approach. As the rate of eDNA degradation affects faunal detection sensitivity, we also quantified mitochondrial ND2 gene copies in eDNA derived from a WAP icefish and found *ND2* copies persisted to at least 20 days in the cold WAP water, much longer than values reported for temperate environments. We propose that eDNA metagenomic sequencing complements traditional sampling, and combining both will enable more inclusive biodiversity detection and faunal change monitoring in the vast Southern Ocean.

1. Introduction

The Southern Ocean hosts a unique biota as a result of Antarctica's distinctive climatic and geologic history (Clarke and Crame, 1989; Clarke and Johnston, 2003). The marine fauna that had evolved in the cold stable Antarctic environment over millions of years now face climate warming trends that have broad influence on community composition and overall ecosystem functioning (Clarke et al., 2007; Schofield et al., 2010). Organisms can respond and adapt to slow increases in environmental temperatures, while rapid changes are likely to cause physiological stress, driving migrations, extinctions and invasions by non-native taxa (Frenot et al., 2005; Cheung et al., 2009; Trivelpiece et al., 2011; Peck et al., 2014). The effects of increasing temperatures are most pronounced at the West Antarctic Peninsula (WAP) and its adjacent waters, which are experiencing the fastest rates of warming in Antarctica (Vaughan et al., 2003; Meredith and King, 2005; Mulvaney et al., 2012). To understand the full impact of rapidly shifting climate on the WAP ecosystem, efficient and effective tools are required to record spatial and temporal variations in biological communities.

1.1. Benthic marine animal communities of the Southern Ocean

Contemporary Antarctic marine macrofaunal communities have intermediate levels of benthic diversity that are comparable to temperate and tropical "non-reef" habitats (Clarke, 2008). Similar to these warmer waters, the West Antarctic continental shelf is populated by a variety of macro-invertebrates that include porifera (sponges), cnidarians (hydroids, stylasterids and anthozoans), bryozoans, annelids (polychaetes), echinoderms (asteroids, ophiuroids and echinoids), arthropods (amphipods, isopods and pycnogonids), mollusks (gastropods and bivalves) and tunicates (*see*De Broyer and Koubbi, 2014 and references therein). The Southern Ocean has also been a remarkable "evolutionary hotspot" that witnessed the adaptive radiation of the morphologically and ecologically diverse Antarctic notothenioid fishes (Eastman, 2005). Notothenioids constitute a major component of the

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https://doi.org/10.1016/j.margen.2017.11.003
Received 17 September 2017; Received in revised form 18 October 2017; Accepted 15 November 2017

benthos; in the coastal waters of the WAP, many species of the red-blooded family Nototheniidae and the white-blooded Channichthyidae are frequently found occupying shallow to deep-water habitats (600 to ≥ 1000 m) (DeWitt et al., 1990; Iwami and Kock, 1990; Kock, 1992).

In contrast, durophagous "bone-crushing" predators including sharks, rays, benthic reptant (walking) crabs and lobsters are absent or rare south of the Polar Front (Dayton et al., 1994; Aronson et al., 2007a; Aronson et al., 2015a). Durophagous crabs were previously thought to have disappeared with the late Eocene cooling and completely excluded from Antarctic waters for at least 14 my (Aronson et al., 2007b). Durophagous brachyurans (true crabs) are indeed absent in Antarctic waters (Hall and Thatje, 2011; Griffiths et al., 2013); their exclusion was attributed to their inability in hypo-regulating hemolymph Mg^{2+} levels at subzero temperatures, limiting crucial muscular functions including heartbeat, ventilation and locomotion (Frederich et al., 2001; Thatje and Arntz, 2004; Thatje et al., 2005). Anomuran crabs of the family Lithodidae (king crabs) however, proved not to be Mg^{2+} limited (Wittmann et al., 2012) and various lithodid populations were discovered south of the Polar Front in the recent decades, although still limited to deep, salty (> 34.6 psu), non-freezing (> 0.5 °C) habitats of the Upper Circumpolar Deep Water (UCDW) (Klinck et al., 2004; Dinniman et al., 2011; Griffiths et al., 2013; Griffiths et al., 2014). The most recent lithodid populations have been observed within the UCDW, at Palmer Deep basin (Smith et al., 2012) and Marguerite Bay (Aronson et al., 2015b). Although the non-freezing UCDW periodically intrudes onto surface waters (Smith et al., 1999; Prézelin et al., 2000; Bentley et al., 2011), no lithodid crabs have yet been observed on shallow shelves of the WAP, or of the Weddell and Ross Seas where temperatures remain consistently colder than 0 °C (Hall and Thatje, 2011). Regardless of the current debate on the historical origin of Antarctic lithodids (Griffiths et al., 2013), there is a common concern that rising shallow water temperatures could enable the predatory king crabs to expand from their current bathyal niche onto shelf ecosystems and alter benthic community structures (Turner et al., 2014a). Efficient and informative tools for tracking their movement over time and space are essential to allow evaluation of the risk of lithodid range expansion.

1.2. Environmental DNA as an ecosystem monitoring tool

Traditional biomonitoring methods typically rely on the observation and collection of whole organisms, combined with morphology-based identifications. Major challenges that traditional methods face include logistic costs of collection, as well as difficulties in reaching remote environments, which would bias sample inventories towards organisms and locations that are easier to access (Baird and Hajibabaei, 2012; Valentini et al., 2016). Additionally, rare or elusive organisms may go unobserved using traditional sampling (Jerde et al., 2011; Rees et al., 2014). The obvious difficulties of monitoring marine biodiversity within large swaths of oceanic provinces calls for more efficient approaches to survey aquatic communities. The sequencing of environmental DNA (eDNA) – genetic material extracted from cells and tissue fragments shed by organisms into the environment (Thomsen and Willerslev, 2015; Taberlet et al., 2012a) – has emerged as a useful tool for assessing biodiversity. Compared to traditional surveys, eDNA has increased detection sensitivity of organisms, as animal remains or early life stages too small to identify by eye can be detected at the DNA sequence level (Bohmann et al., 2014). eDNA has been widely employed in aquatic ecosystems to assess overall community composition (Thomsen et al., 2012a,b; Kelly et al., 2014; Leray and Knowlton, 2015) as well as to identify rare, endangered or invasive species (Jerde et al., 2011; Thomsen et al., 2012a; Goldberg et al., 2013; Dougherty et al., 2016; Larson et al., 2017).

A common approach for eDNA analyses is metabarcoding, which uses one or more barcoding genes to elucidate the taxonomic composition of complex eDNA samples (Hebert et al., 2003; Taberlet et al., 2012b; Valentini et al., 2016). Metabarcoding relies on PCR amplification of gene fragments with inherent uncertainties of whether all taxa within the complex sample could be evenly amplified using a given primer set or sets, which would lead to gene-specific taxonomic biases and skewed biodiversity assessments (Coissac et al., 2012; Zhou et al., 2013; Cowart et al., 2015; Pedersen et al., 2015). In contrast, shotgun metagenomic sequencing that involves the direct sequencing of total eDNA could bypass the PCR limitations associated with metabarcoding while still providing insights into community composition (Tringe and Rubin, 2005; Taberlet et al., 2012b). For Antarctic organisms, the metagenomic approach has been applied at the level of targeted sequencing of small subunit rRNA-enriched libraries derived from marine bacterial and archaeal metagenomes to investigate seasonal variations in bacterioplankton community composition and functional diversity (Brown et al., 2012; Grzymski et al., 2012; Cavicchioli, 2015). No study to-date has utilized metagenomic shotgun sequencing of eDNA to investigate Antarctic marine macrofaunal community composition.

In the present study, we applied high-throughput shotgun metagenomic sequencing and analyses of eDNA extracted from seawater samples collected from shallow (≤ 300 m) shelf locations at four WAP regions. We evaluated the efficacy of this approach for assessing overall marine invertebrate and vertebrate metazoan biodiversity as a method of recording spatial and temporal variations in communities facing climatic shifts. Antarctic notothenioid fish distributions in WAP waters are well known (Duhamel et al., 2014), and thus detection of their sequences in the eDNA samples serve as positive controls for this assessment. Furthermore, we determined the usefulness of this approach for detecting DNA signatures of lithodid king crabs in these shelf locations, and thus a potentially simpler and less expensive sampling method than the current logistically expensive remotely operated vehicles and mechanical trapping.

The use of eDNA to detect organisms is predicated upon the presence of their DNA at the sampling locations, which is directly influenced by the rate of eDNA degradation and water transport within aquatic environments (Lindahl, 1993; Deiner and Altermatt, 2014; Strickler et al., 2015). Therefore, we also estimated the rate of eDNA degradation in coastal WAP water via controlled aquarium experiments using eDNA derived from a common WAP icefish (*Chionodraco rastrospinosus*), to gain insight on the effect of sub-zero temperatures and the persistence of eDNA in polar marine ecosystems.

2. Materials and methods

2.1. Water sampling for environmental DNA

Eleven seawater samples were collected during the austral winter (July and August) of 2014 from four coastal regions near Anvers and Brabant Islands, West Antarctic Peninsula (Table 1). These sites include Dallmann Bay, Gerlache Strait, Bismarck Strait/Port Lockroy, and Palmer Station vicinity, approximating a North-South transect within 150 km of Palmer Station (USA) (Fig. 1, Table 1). Collections were made using sterilized, standard 5 L oceanographic water sampling Niskin bottles (Model 1010, General Oceanics, Miami), deployed using nylon rope from the R/V *Laurence M. Gould* at ocean sites and from a Zodiac rubber boat at sites near Palmer Station. The cap of the tubular bottle at each end was held open under tension with the elastic cord/holding pin assembly on the side of the bottle. On vertical descend, the chamber was continuously and freely flushed by ambient water as it transited the water column. On reaching the bottom, a stainless steel weight (Devil messenger 1000-MG, General Oceanics) was sent from surface sliding down the rope to dislodge the pin and trip the tension, shutting the caps and sealing the tube before retrieval. The water captured within the bottle therefore consisted of bottom water. Sampling depths varied from about 5 m (near surface) to 300 m depending on sampling sites. Two bottles were deployed together from the ship at each ocean sampling location, and single collections were made when