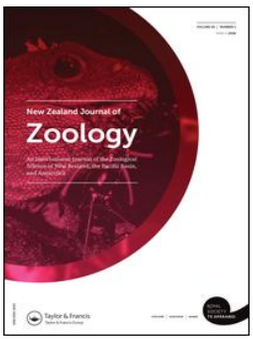

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REVIEW ARTICLE

Environmental DNA as a tool for monitoring Antarctic vertebrates

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ABSTRACT

Antarctica is home to numerous species that are vulnerable to environmental change, and assessing species responses requires long-term monitoring. However, Antarctica's extreme nature presents limitations to conducting the type of long-term or broad-scale studies necessary for understanding changes in community composition. In this paper, we evaluate the potential for the use of environmental DNA (eDNA) methods in expanding scientific research efforts for biodiversity monitoring and conservation genetics in Antarctica. Through a systematic literature review, we identify that most Antarctic eDNA studies have focused on microbial metabarcoding using samples from soil, sediment, snow, and water. Few eDNA studies in Antarctica have focused on vertebrate biodiversity or population genetics, but we highlight several examples that have effectively and creatively used eDNA to study vertebrates. We highlight the potential for the use of portable sequencing technologies in the future of Antarctic eDNA research. We conclude that eDNA could be a valuable tool for researchers in their efforts to assess, monitor, and conserve biodiversity in the Antarctic.

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Introduction

The extreme nature of the Antarctic environment requires a range of techniques to address the challenges of biodiversity assessment and targeted species detection (Chown et al. 2015; Czechowski et al. 2017; Gutt et al. 2018). Our regional knowledge of biodiversity is directly impacted by the degree of inaccessibility at different locations (Griffiths 2010), and research limitations are not only spatial but temporal, given that Antarctica is largely inaccessible over the dark winter months. A baseline understanding of species' distribution and community composition across the continent and throughout the year is vital for monitoring the effects of increasing human activity and for planning subsequent mitigation strategies (Chown et al. 2012). Assessing this disturbance requires long term data that cover a broad temporal and spatial range, but long-term studies are particularly susceptible to

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disruptions to planned data collection. Disruptions in fieldwork can result in low sample sizes, which can impede the incorporation of biologically important variables such as age and sex in data analyses (e.g. Nachtsheim et al. 2017). One way to increase scientific flexibility is to use multiple approaches to answer research questions, such as combining traditional visual surveys with molecular tools (Ammon et al. 2018). In this review, we will focus on one such tool, environmental DNA, and the opportunities this field offers for current biological survey efforts in the Antarctic region (continental Antarctica, the Southern Ocean, and the Sub-Antarctic Islands).

The term environmental DNA (eDNA) was first used by Ogram et al. (1987) for microbial DNA collected from marine sediments, and generally refers to any genetic material extracted from an environmental sample (Ogram et al. 1987; Taberlet et al. 2012). As an organism interacts with its environment, it sheds genetic material, such as through hair, faeces, or gametes. DNA 'traces' can be detected in a variety of environmental samples (e.g. water, soil, and sediments). Since the early aquatic application of eDNA to detect the presence of amphibians (Ficetola et al. 2008), the field has expanded, leading to a variety of novel methods, with samples extracted from salt licks (Ishige et al. 2017), honey (Bovo et al. 2018), and leaves (Valentin et al. 2020). Whilst it is possible to detect enough DNA for genome assembly in microbes (Taberlet et al. 2018), most eDNA from macrofauna is no longer protected by intact cells (Levy-Booth et al. 2007; Pietramellara et al. 2009; Taberlet et al. 2018), and such extracellular DNA decays relatively quickly (Collins et al. 2018; Wei et al. 2018). A complex interaction of factors causes rapid decay of DNA (Corinaldesi et al. 2008), such as exposure to UV radiation, lower pH, increased temperature, consumption by microbes, hydrolysis, and enzyme action (Finkel and Kolter 2001; Hebsgaard et al. 2005; Strickler et al. 2015; Barnes and Turner 2016). The short-lived nature of extracellular DNA in the environment may be useful for spatiotemporal inferences of species on detection (Barnes and Turner 2016), however, DNA may also be adsorbed onto organic material and preserved; for example Haile et al. (2009) used ancient DNA techniques to detect horse (*Equus* spp.) and mammoth (*Mammuthus* spp.) DNA in Alaskan permafrost sediments.

Within different environmental sources, the method of DNA amplification and sequencing can be approached in one of two ways depending on the research question (e.g. targeting single or multiple species; Taberlet et al. 2018). For single-species questions, such as the detection of an invasive, endangered, or cryptic species (Ficetola et al. 2008; Dejean et al. 2012; Goldberg et al. 2013; Uchii et al. 2016), primers specific to that species' DNA are required (Ficetola et al. 2008). Using these species-specific primers, genotypes from PCR or signals from quantitative PCR (qPCR) act as a fingerprint or 'barcode' to identify target species (Bohmann et al. 2014). Alternatively, a multi-species approach (called metabarcoding) allows for the identification of all taxa present in a sample (Brown et al. 2016; Valentini et al. 2016). Metabarcoding provides a snapshot of biodiversity at a given point in time. Generally, this process requires the use of high-throughput, next generation sequencing, which allows large amounts of data processing in a single sequencing run (Shokralla et al. 2012; Bohmann et al. 2014). Such a broad, community-level approach is preferable for studies on biodiversity (Bohmann et al. 2014), predator-prey

interactions (Deagle et al. 2005), and investigating potential invasions where species-specific information may be unknown, for example in ballast water (Bohmann et al. 2014; Gerhard and Gunsch 2019). Metabarcoding requires universal primers that bind to target DNA regions across taxonomic groups. With the development and commercialisation of high-throughput sequencing technology (Schuster 2008) and data systems such as the Barcode of Life Database (BOLD; Ratnasingham and Hebert 2007), the use of eDNA to monitor ecosystem diversity, particularly the diversity of vertebrate species, has become increasingly feasible (Valentini et al. 2009; Bálint et al. 2018; Cristescu and Hebert 2018). A discussion of the specific methods for metabarcoding is beyond the scope of this review. However, a technical review of high throughput sequencing metabarcoding in Antarctica is provided by Czechowski et al. (2017).

Antarctica is unique in that it is the only continent without an Indigenous human population, and therefore its vertebrates may be susceptible to human disturbance by researchers (Martín et al. 2004; Van Polanen Petel et al. 2007; Coetzee and Chown 2016). Environmental DNA surveys inherently do not require direct contact with target taxa and are considered both non-invasive and 'non-disruptive' to wildlife (Lefort et al. 2019). Whilst many traditional survey methods, such as visual or aerial surveys, do not require invasive samples, they may still lead to a stress response in the target organism. For example, approaching chinstrap penguins (*Pygoscelis antarcticus*) can affect fleeing behaviour (Martín et al. 2004), while prolonged exposure to helicopters inhibits foraging Adélie penguins (*Pygoscelis adeliae*) from returning to their nests (Wilson et al. 1991). An environmental sample, however, can be taken without the need to approach, or even sight, the target species. With this in mind, eDNA surveys present an ideal opportunity to continue to expand our knowledge on Antarctic biodiversity with minimal risk of harm.

In this paper, we review the literature on eDNA studies in Antarctica, the Southern Ocean, and the Sub-Antarctic islands. We focus on vertebrate eDNA studies and discuss how methods from Antarctic microbial studies could be translated into vertebrate research. Our review compares current and future applications of eDNA in Antarctica for a number of sample sources, drawing connections to eDNA surveys within the Arctic. The future is bright for the use of eDNA in improving our understanding of Antarctica's unique vertebrate species.

Literature search

We reviewed the literature in Scopus, using the term 'Antarctic' in combination with 'environmental DNA' or 'e*DNA'. Alternative abbreviations may be used such as sediment DNA (sedDNA; Ficetola et al. 2018), and further, the term eDNA may also be used to refer to 'extracellular DNA' (Fröls et al. 2012; Ricciardelli et al. 2019). Thus, we also ran multiple searches pairing 'Antarctic' with the terms '*barcoding', 'high throughput sequencing', 'next generation sequencing' or 'metagenomics'. We then went through the results to determine the source and to identify any papers that discussed environmental vertebrate DNA. We excluded literature reviews, book chapters, notes and errata. The review covers literature from 2000–2020, as of June 29, 2020 (Table S1, supplementary information).

Summary of review results

We found a total of 172 papers for all search terms. Of these papers, only 22 included search terms 'environmental DNA' and 7 'eDNA' (Table S1, supplementary information). These results included studies that directly sampled the environment as well as those that analysed data from previously collected metagenomes; we excluded studies that used other sources of genetic material (e.g. seal buccal swabs; Crane et al. 2018). The most common environmental source for DNA was soil ($n = 47$ studies) followed by water, which includes both marine samples ($n = 37$) and water collected from terrestrial water bodies ($n = 27$). Sediment samples returned 22 studies, and studies involving ice or snow samples returned the fewest, with 14 (Table S1, supplementary information). Samples classed as 'other' included faeces, rock and hypoliths, biofilms, microbial mats, and filtered air (Table S1, supplementary information).

Only 4 of the 172 studies in our search recovered vertebrate DNA from the environment (Table S1, supplementary information). These 4 studies used samples from seawater (Cowart et al. 2018; Mariani et al. 2019), sediment (Ficetola et al. 2018), and faeces (McInnes et al. 2016) as environmental sources (Figure 1). The vertebrates detected in these four studies ranged from Antarctic specialists such

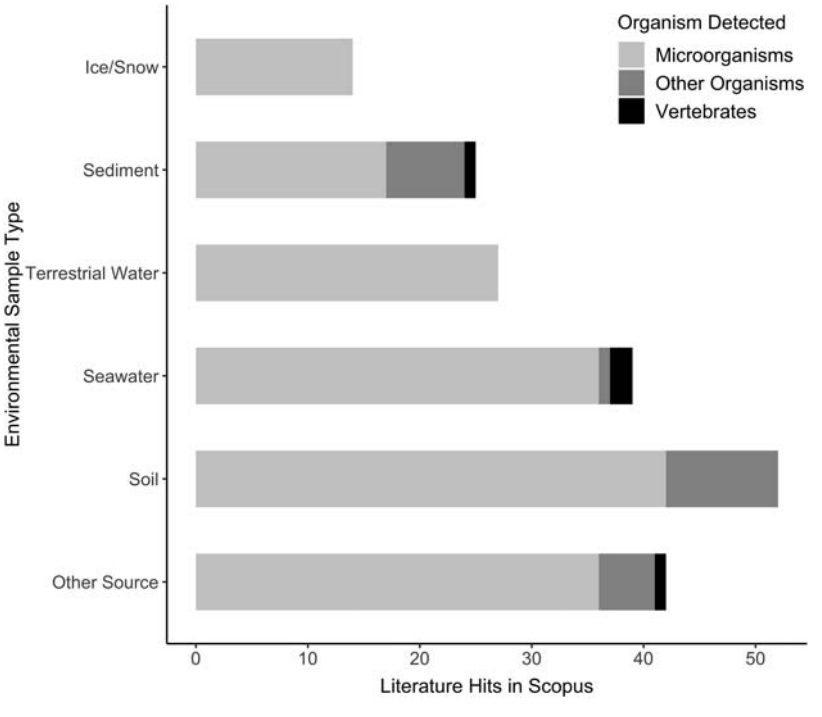

Figure 1. Number of papers found by in our systematic review of Antarctic environmental DNA. Categorised by 'Environmental Sample Type' and 'Organism detected', highlighting the discrepancy between microbial and vertebrate eDNA studies in Antarctica. Papers that overlapped between categories and they were counted as a hit for both. Papers in the 'Other Organisms' category included those that targeted one or more of the groups: meiofauna, benthic communities, invertebrates, moss, vascular plants, lichen, as well as macro algae and fungi.

as fishes (McInnes et al. 2016; Cowart et al. 2018; Mariani et al. 2019), Weddell seals, and chinstrap penguins (Mariani et al. 2019), to invasive rabbits (Ficetola et al. 2018).

Polar eDNA methods

Soil samples

Despite being the most common sample type in our review, none of the 43 soil studies detected vertebrate DNA (Table S1, supplementary information). However, Pansu et al. (2015) made links between the introduction of rabbits to the Sub-Antarctic Kerguelen Islands and soil community compositions, finding a reduction in fungal diversity in locations where rabbits were historically introduced using multi-species barcoding. Furthermore, Pansu et al. (2015) showed that the herbivores' preference for introduced plant species alters the characteristics of soil communities, demonstrating the applications of metagenomic analyses of soil samples for monitoring ecosystem shifts.

Water samples

We found 38 papers that reported extracting DNA from seawater samples. The vast majority report on microbial studies (89%), although two detected vertebrate eDNA from marine samples (Cowart et al. 2018; Mariani et al. 2019). One of these explored a novel method for collecting DNA using sponges (*Porifera* sp.) as 'natural samplers', capturing the DNA of a number of fishes, as well as both chinstrap penguin and Weddell seal DNA (Mariani et al. 2019). With further validation, sponges could be advantageous for eDNA sampling, filtering up to a thousand-fold more water than traditional filtering methods (Kahn et al. 2015; Mariani et al. 2019). Additionally, their ability to regenerate makes them easy to sample without long-term damage (Mariani et al. 2019).

A survey of invertebrate and vertebrate metazoan diversity in the West Antarctic Peninsula detected the eDNA of fishes using metagenomic analysis of shallow shelf water samples (Cowart et al. 2018). Results were comparable to inventories collated by the Scientific Committee on Antarctic Research-Marine Biodiversity Network (SCAR-MarBIN, www.scarmarbin.be, www.biodiversity.aq, Griffiths et al. 2011; De Broyer et al. 2014) from peer-reviewed literature, museum specimens and the Census of Antarctic Marine Life (CAML, www.caml.aq, De Broyer and Danis 2011). Cowart et al. (2018) suggest that a combination of the two approaches (eDNA and traditional surveys) will lead to improved detection of faunal assemblages, in turn improving biodiversity assessments and monitoring community change in the Southern Ocean. In-field eDNA detection was supported with laboratory-based assessments of DNA degradation in Antarctic conditions, using Antarctic icefish (*Chionodraco rastrospinosus*) held in tanks (Cowart et al. 2018). Mitochondrial DNA persisted for longer under Antarctic conditions than would be expected in temperate waters, potentially leading to a greater breadth of species detected in future Antarctic eDNA studies.

Fewer studies focused on terrestrial water samples, and all of those were microbial metagenomic studies. This microbial focus is unsurprising; few vertebrates have been associated with Antarctic lakes, with only two instances of fish being sighted in the brackish waters of Beaver Lake (*Trematomus scotti*; Cromer et al. 2005) and Ablation Lake (*Trematomus hemacchii*; Heywood and Light 1975). Nonetheless, eDNA from Antarctic lakes could have potential for genotyping birds that have been associated with a number of lakes on the continent, such as the South Polar skua (*Stercorarius maccormicki*; Laybourn-Parry and Pearce 2007) and Antarctic petrels (*Thalassoica antarctica*) which have been known to breed hundreds of kilometres inland (Hiller et al. 1988; Brooke et al. 1999).

Sediment samples

Of the papers we reviewed, 22 discussed the use of sediment samples to detect eDNA, with only one detecting vertebrate DNA (Ficetola et al. 2018). One of the benefits of sediment samples is that they provide ideal conditions for preserving DNA. By carbon dating the layers within a sediment core and extracting environmental DNA from those layers, we can collect historical data on species diversity and extinctions. Ficetola et al. (2018) extracted rabbit and vascular plant DNA from sediment cores collected in the sub-Antarctic Kerguelen Islands to assess the recovery of plant diversity from a herbivore invader. Plant communities were assessed before and after the first detection of rabbit DNA to draw conclusions on the dynamics of the ecosystem and the trajectories of change. Not only were they able to show that changes in plant communities were consistent with the first detection of rabbit DNA, but they accounted for climate change as another potential mechanism driving change. Although climate change explained some variation in plant communities, rabbit abundance explained a significantly higher percentage of variation. Defining the mechanisms affecting an ecosystem aids in differentiating between a system in 'crisis' versus one that has stabilised to a new equilibrium (Ficetola et al. 2018), a clearer understanding of ecosystem dynamics could impact what is considered the best response in a management situation.

Snow and ice samples

The advantage of snow samples as a source for eDNA is the snow acts as a natural freezer, helping to preserve the DNA within the samples (Hinlo et al. 2017). All of our hits for both snow and ice were microorganism studies; only 5 studies used snow samples and 11 focused on ice samples, with most of these from cryoconite holes (Webster-Brown et al. 2015; Sommers et al. 2018; Lutz et al. 2019; Weisleitner et al. 2019; Sommers, Fontenele, et al. 2019; Sommers, Porazinska, et al. 2019). To our knowledge, no studies of Antarctic vertebrate eDNA from snow samples have been published to date. This gap in the literature presents a potential for future eDNA studies on Antarctic wildlife (Box 1); importantly, eDNA could aid in monitoring of ice-dependent species that are likely to be negatively impacted by climate change in the near future (Siniff et al. 2008; Ainley et al. 2010; Jenouvrier et al. 2014; Trathan et al. 2020).

Box 1. Weddell seals case study.

Snow can limit the degradation of eDNA within a sample (Dalén et al. 2007; Kinoshita et al. 2019), and likewise eDNA is not dispersed as it would be in other systems, such as lotic water, making it a promising sample source for studies targeting nuclear eDNA. Preliminary work in the Arctic has shown promising potential for detecting nuclear eDNA in the footprints of polar bears (*Ursus maritimus;* Von Duyke et al. 2019) and captive lynx (*Lynx lynx*; Hellström et al. 2019). Due to the quality and quantity of DNA required for such studies, accessing freshly deposited DNA left in the environment is best practice. Whilst this may be a challenge in long ranging terrestrial mammals in the Arctic, Antarctica's megafauna are marine organisms, travelling limited distances across the ice and providing ample opportunities to collect fresh samples.

A potential candidate for snow-based eDNA surveys are Weddell seals, which are fast-ice (sea ice literally fastened to the Antarctic coastline) obligates (Stirling 1969), found only sporadically around Antarctica (LaRue et al. 2019). Weddell seals require time out of the water to rest between foraging and to raise their young in the austral spring (Stirling 1969). The proximity of a Weddell seal population to Scott Base and McMurdo Station, combined with their limited movement and breeding season behaviour, makes them the ideal target for an annual survey effort (Stirling 1969). Whilst much is known about this southernmost breeding marine mammal on the planet due to a mark-recapture study that has been ongoing since the mid-1960s (Hastings and Testa 1998; Cameron and Siniff 2004), nuclear eDNA surveys could provide additional information, such as monitoring genetic diversity.

Genetic studies of Weddell seals primarily use flipper skin punches (Gelatt et al. 2001; Harcourt et al. 2007). These skin samples provide valuable genetic information, but require a high level of training and permitting, and can be hazardous for the researchers involved. Environmental DNA surveys are not a replacement for traditional genetic survey methods, but the ease of collecting non-invasive environmental sampling is beneficial for building more extensive datasets, whilst limiting disturbance to Antarctic wildlife. Furthermore, using miniaturised technology (e.g. Johnson et al. 2017; Millán-Aguiñaga et al. 2019) would reduce the need to transport tissue samples out of Antarctica. One eDNA survey has successfully detected Weddell seals in the West Antarctic Peninsula (Mariani et al. 2019), but the potential for supplementing the traditional genetic sampling methods using eDNA has not been evaluated. We suggest future species-specific studies investigating the possibility of collecting nuclear eDNA from snow recently vacated by Weddell seals in the breeding colonies of Erebus Bay. As methodologies are developed, they could be applied to other, less accessible pinnipeds, such as the pack-ice inhabiting crabeater seals (*Lobodon carcinophaga*). Success in this field would allow us to draw population-level inferences for Antarctic megafauna from non-invasive eDNA samples (for a review of population genetic inference from eDNA, see Adams et al. (2019)).

Future development of eDNA surveys for Antarctic snow samples can look to recent studies in the Northern Hemisphere (Dalén et al. 2007; Franklin et al. 2019; Kinoshita et al. 2019). The varying success of these studies is attributed in part to sample size, ranging from single footprints (Dalén et al. 2007), five footprints (Kinoshita et al. 2019), to 2 L samples (Franklin et al. 2019). Detection rates can also be impacted by storage length (Kinoshita et al. 2019), exposure to UV (e.g. targeting shaded versus sunlit sites [Kinoshita et al. 2019]) and laboratory methods (e.g. the use of qPCR over PCR [Franklin et al. 2019]). Whilst the simplified environment of snow samples increases the likelihood of eDNA collection (Franklin et al. 2019), and tracks provide areas where eDNA is potentially condensed (Dalén et al. 2007), it can also increase the risk of contamination. These risks can be minimised through the use of gloves, sterilised equipment, and field blanks (Dalén et al. 2007; Franklin et al. 2019; Kinoshita et al. 2019). Care should be taken to ensure sampling teams have not recently been in contact with target species DNA (Franklin et al. 2019), similarly, it is important laboratories have not been previously contaminated with target species DNA (Dalén et al. 2007; Franklin et al. 2019). An additional benefit of snow samples is the preservation of DNA in compacted layers over time, which can be analysed through the collection of snow-columns (Franklin et al. 2019). By collecting columns from sites where camera traps had recorded lynx (*Lynx lynx*), Franklin et al. (2019) demonstrated the persistence of eDNA in snow and expanded the timeframe for species detection. Whilst there are no terrestrial mammals on continental Antarctica, applying these methodologies to Antarctic snow samples would provide further opportunity to study vertebrates that venture onto the ice (Box 1).

Challenges and limitations

A primary problem in working with genetic material is that DNA is vulnerable to degradation, which is exacerbated by exposure to UV, high temperatures, and deviations in pH (Strickler et al. 2015). These factors pose contrasting issues for studying eDNA under Antarctic conditions. For example, Strickler et al. (2015) found that cold, low-UV conditions resulted in less DNA degradation; thus, temperatures in Antarctica would be ideal, but 24 h sunlight over summer would increase UV exposure. These factors are important to consider in the design of eDNA surveys on the continent. In their review of the applications of DNA, Beng and Corlett (2020) suggested research using eDNA has expanded without a concordant expansion in the understanding of its limitations. In their review covering misuses of DNA, Cristescu and Hebert (2018) suggested the biggest issue with the adoption of widespread eDNA applications was uncertainty introduced by the rates of false positives and false negatives. Whilst false positives can indicate contamination at any stage during collection and analysis, and can be assessed using negative controls, false negatives can be caused by a number of factors, e.g. eDNA dispersal, sampling efforts and insufficient databases, making them difficult to evaluate (Cristescu and Hebert 2018). Misidentification of DNA (Furlan et al. 2020) can lead to incorrect interpretations, with inaccurate data informing management decisions. Drawing robust comparisons between eDNA datasets requires critical study design and methodological consistency (as reviewed in Goldberg et al. 2016; Dickie et al. 2018; Lear et al. 2018; Mathieu et al. 2020). Subjective sampling methods and inadequate methodological information leads to a low level of reproducibility between eDNA studies (Dickie et al. 2018), as does the lack of standardisation between laboratory protocols (Lear et al. 2018; Zinger et al. 2019). Through investigating potential bias, and introducing greater standardisation across eDNA workflows, we will be able to draw sounder conclusions and be better placed to advise on research questions.

Biodiversity and invasive species monitoring

Recently, international programmes have prioritised mapping Antarctic diversity (e.g. the Census of Antarctic Marine Life (CAML); Grant and Linse 2009), resulting in the creation of databases such as the Registry of Antarctic Marine Species (RAMS; Griffiths et al. 2011), which is a compilation of known taxa and distribution data in the Antarctic marine environment (De Broyer and Danis 2011). The goal was to assess the current understanding of biodiversity in the region and compile existing gaps that can be identified and targeted, including under-sampled taxonomic groups or specific locations with few existing observations (De Broyer and Danis 2011). RAMS also provides a baseline from which changes in species distributions over time can be identified (Griffiths 2010), and De Broyer and Danis (2011) highlighted the need to incorporate more efficient taxonomic tools to expand the database. One such tool is eDNA; by confirming species presence and mapping distributions we could utilise eDNA to improve the confidence in current databases, identifying the taxa and sites that should be targeted for future biodiversity studies.

The limitations of Antarctic biodiversity assessments in a number of regions arise from their inaccessibility (Griffiths 2010). For example, the Western Weddell and Eastern Ross Seas are particularly poorly sampled due to extensive ice cover, and lack of year-round field stations in the vicinity. Likewise, knowledge of the deep sea is heavily restricted to transit routes (Griffiths 2010). Sampling biases are also temporal, as many winter behaviours and distributions are poorly studied given most research is conducted during the relative safety of austral summer (Griffiths 2010). Environmental DNA offers a tool that addresses some of these existing biases in Antarctic biodiversity databases, enabling us to catalogue taxa in situations where traditional methods are not safe or cost appropriate (e.g. deep sea underwater surveys; Pawlowski et al. 2011). Furthermore, optimisation of population genetics using eDNA would allow researchers to non-invasively assess the genetic diversity of Antarctic species (Adams et al. 2019). For example, a population genetic eDNA study on orcas (*Orcinus orca*) detected haplotypic variation between orca communities (Baker et al. 2018, see case study in Box 2).

Box 2. Orca case study.

Whilst eDNA has been primarily utilised to establish the presence or absence of taxa, there is growing interest in population genetics applications (Adams et al. 2019). In marine systems outside of Antarctica, mitochondrial DNA haplotypes have been identified using eDNA for a number of marine vertebrates (Sigsgaard et al. 2016; Baker et al. 2018; Parsons et al. 2018; Tsuji et al. 2020). In one such example, resident and transient communities of Northern hemisphere Orcas (*Orcinus orca*) were distinguished using known mitochondrial haplotypes (Parsons et al. 2013) genotyped from eDNA samples (Baker et al. 2018). Multiple orca ecotypes are known to inhabit the Southern Ocean, with two ecotypes observed in McMurdo Sound (Andrews et al. 2008; Ainley and Ballard 2012). The success of distinguishing orca ecotypes from water samples in the Northern Hemisphere suggests that eDNA could provide a non-invasive method for differentiating between populations throughout Antarctica.

The specific methodologies used might determine the success of eDNA as a tool for population genetics. In their study on North Pacific orcas in Puget Sound, Baker et al. (2018) successfully identified orca DNA for up to two hours after encountering orcas using droplet digital PCR (Baker et al. 2018), a method based on fractioning samples into droplets which are individually amplified by PCR, aiding with absolute quantification (Hindson et al. 2011). Whilst fragmentation of eDNA limited barcoding, Baker et al. (2018) were able to identify the resident population ecotype in two of their samples, supporting audio and visual data collected during the encounter. In contrast, Pinfield et al. (2019) were unable to make population level inferences in their qPCR-based study of Northeast Atlantic orca, finding a high rate of false negatives. Detection may have been limited by weather conditions, animal behaviour and temperature-affected skin sloughing. Colder conditions may lead to a reduction in DNA deposition, as blood flow is restricted to outer skin layers and less skin is shed (Pinfield et al. 2019). Study design is likely also a contributor to the high rate of false negatives, with Pinfield et al. (2019) suggesting sampling behind whales and increasing PCR replicates may lead to more positive results. Future studies on Southern Hemisphere orcas should consider the relationship between detection rates and methodological choices. For eDNA surveys of whales to be viable, more research is needed on optimising workflows, particularly in Antarctica. Nonetheless, population genetic applications of eDNA could be an excellent approach for exploring regional differences across the continent.

The increasing development and availability of sampling technologies (e.g. remotely operated vehicles (ROVs) and icebreaker vessels) is expanding our capability to monitor Antarctic biodiversity (Griffiths 2010). Cowart et al. (2018) found lithodid king crabs (*Neolothodes yaldwyni*) in waters off the West Antarctic Peninsula, which is noteworthy as this area has been suggested as a possible location for such invasions (Thatje et al. 2005; Smith et al. 2012). Along coastal marine systems community eDNA signals remain distinct with minimal signal dispersal (Jeunen et al. 2019; 2020), thus eDNA could play a role in monitoring potential invasion fronts in the Antarctic. Likewise, eDNA sampling can assist biosecurity efforts, through monitoring both

present (e.g. ballast water; Rey et al. (2019)) and historic threats (Pansu et al. 2015; Ficetola et al. 2018).

Both Pansu et al. (2015) and Ficetola et al. (2018) used eDNA to show a lag in ecosystem recovery after the eradication of invasive rabbits in the Kerguelen Islands. Understanding how an ecosystem reacted during the initial introduction of a species, and analysing subsequent recovery after eradication, can help inform management on other Sub-Antarctic islands. Compared to eDNA surveys, which are often described as a 'snapshot' of a time and location in the current environment, ancient DNA allows us to look at historic 'snapshots' and construct a much larger picture of an ecosystem through time, informing future conservation efforts. Nonetheless, Pansu et al. (2015) and Ficetola et al. (2018) demonstrate that eDNA can be used as more than just a 'snapshot'.

Community sampling efforts

Community scientists have contributed to successful eDNA surveys in temperate regions, both in freshwater (Biggs et al. 2015) and marine environments (Miralles et al. 2016). Sampling techniques can be easily taught, and the risk of contamination can be minimised by using enclosed filters (Spens et al. 2017). Additionally, standard quality assurance procedures, such as field negatives or controls, are of even greater value when surveyors are inexperienced (Biggs et al. 2015). Though there are limits to non-specialists carrying out eDNA surveys (Biggs et al. 2015), the ability to generate large amounts of data across a greater spatiotemporal range is advantageous for increasing understanding of species distribution and composition. Community-based projects also educate the public about eDNA and raise awareness of species within the target ecosystem, acting as an immersive outreach tool that can enhance science communication (Biggs et al. 2015; Taylor et al. 2019).

Although Antarctica does not have an indigenous human population, communities at research stations and even tourists could contribute to data collection. Over the 2018–2019 summer season the International Association of Antarctic Tour Operators (IAATO) recorded 55,489 visitors (IAATO 2009), not including scientists, commercial fishers, or base staff. However, few peer-reviewed studies implementing community-engaged projects in Antarctica exist (Casanovas et al. 2015; Mascioni et al. 2019; Taylor et al. 2019; Cusick et al. 2020) and to our knowledge there are no published community science eDNA surveys. Antarctic tourism is predominantly cruise based, so staff can monitor data collection and implement a greater level of standardisation. Further, IAATO is responsive to involvement in research as it corresponds to Resolution 7 (2009) of the Antarctic Treaty system (Taylor et al. 2019). These principles encourage the education of tourists to foster connections with Antarctica, resulting in growing interest in tourist-based data collection and education opportunities e.g. the Polar Citizen Science Collective (http://www.polarcollective.org; Taylor et al. 2019). Collaborating with tourist companies across the summer season would provide a unique opportunity to build a large-scale baseline of biodiversity across the Antarctic Peninsula, generating a large amount of genetic data with a relatively small individual sampling effort. In addition to tourists, base staff and or researchers heading into the deep-field or overwintering could be provided with eDNA sampling kits, allowing for multiple snapshots of Antarctic biodiversity across a range of temporal and spatial scales.

Miniaturised technology and in situ eDNA analysis

The prospect of highly portable sequencing technology is promising for remote field research. Emerging technology such as the MinION sequencing device (Oxford Nanopore Technologies) could potentially accelerate the process of eDNA surveys, increasing the agility and adaptability of research in the field (Edwards et al. 2016; Johnson et al. 2017; Edwards et al. 2019; Gowers et al. 2019). For example, Truelove et al. (2019) successfully carried out ship based, rapid, in-field analysis of white shark (*Carcharodon carcharias*) DNA, extracted from seawater and sequenced using the MinION device. Results were ground-truthed on-shore with Illumina sequencing and found to be comparable, despite the higher error rate in the MinION device. A decreased turnaround time allows for further, informed sampling a quicker response on time-sensitive issues.

While application of the MinION to vertebrate DNA has been limited (Truelove et al. 2019), the MinION has been applied to microbial studies in both the Arctic (Edwards et al. 2016; Goordial et al. 2017; Ducluzeau et al. 2018; Edwards et al. 2019; Gowers et al. 2019; Millán-Aguiñaga et al. 2019) and Antarctica (Johnson et al. 2017; Millán-Aguiñaga et al. 2019). In one extreme example, Gowers et al. (2019) packed their entire laboratory onto a sled, travelled 135 km from the nearest field station onto the Vatnajökull ice cap in Iceland, and successfully sampled and sequenced eDNA from the ice for metagenomics analysis. These previous studies in similar environments to Antarctica have highlighted the primary challenge – extreme conditions. In particular, the cold temperatures affect battery life, reagents, and the function of the MinION device and laptops, and contribute to the brittling of resin-based equipment (Gowers et al. 2019). Suggestions for tackling these challenges include the design of an insulating containment system (Johnson et al. 2017) and the use of sheltered field laboratories, utilising either pre-existing structures (Edwards et al. 2016) or tents (Gowers et al. 2019).

Conclusions

We found 172 papers addressing eDNA studies across 20 years, the vast majority of which focused on microbial diversity. We found eDNA technologies have been used in Antarctica since the beginning of the millennium (Gordon et al. 2000), but few have used eDNA to sequence the DNA of Antarctic vertebrates (McInnes et al. 2016; Cowart et al. 2018; Ficetola et al. 2018; Mariani et al. 2019). This gap highlights an important opportunity for future Antarctic research, particularly for biodiversity analysis, invasive species monitoring, and community science programmes.

As genetic technologies develop, the scope for eDNA surveys will only increase. The MinION sequencer is an example of the opportunity presented by technological advances for in-field, real time analysis of eDNA, increasing the adaptability and the agility of genetic research on the ice (Edwards et al. 2016; Johnson et al. 2017; Edwards et al. 2019; Gowers et al. 2019). There is also the potential to look into differences within species using nuclear eDNA, both for comparisons between individuals (Box 1) or ecotypes in species such as orca (*Orcinus orca*) (Box 2). Though the development of eDNA workflows in fields such as population genetics is still in the early days, with much of the research currently in the preliminary phase, Antarctica and the Southern Ocean presents a potential region to explore these novel applications.

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