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REVIEW, CONCEPT, AND SYNTHESIS

A review of dietary DNA metabarcoding in marine vertebrates: a new frontier in sea turtle foraging ecology?

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Abstract

Diet characterisation is important for understanding trophic roles of animals across space and time, including in response to climate change. This has led to the development of a large range of dietary analysis techniques, from centuries-old morphological stomach analysis to recent molecular techniques. Given the difficulties and limitations of direct analysis in marine animals, here we review DNA-based methods of marine vertebrate diet analysis, examining the proliferation of studies over the last two decades. We identify a keystone taxon, sea turtles, where DNA-based approaches have had limited use, but offer great potential for characterising diet across species, life stages and regions. We show that contemporary molecular techniques can overcome some limitations of traditional methods based on morphological identification, such as the ability to identify rapidly digested food items. We report on the development of DNA metabarcoding protocols that enable simultaneous identification of many diet item sequences from heterogeneous samples. DNA metabarcoding can increase taxonomic resolution, improve the identification of certain items (e.g., gelatinous organisms), and increase the comprehensiveness of diet characterisation, particularly in combination with other techniques. However, careful methodological development and finer optimisation of metabarcoding protocols (e.g., appropriate primer selection, blocking of host DNA amplification) are necessary to improve results. Combination approaches to sea turtle dietary analysis and further experimentation with metabarcoding methodology will help to characterise variations and effectively monitor shifts in diet composition in response to environmental changes such as rising sea temperatures and displacement to alternative foraging grounds.

Keywords Diet analysis · Molecular ecology · Marine vertebrate · DNA barcoding · Marine turtles · Sea turtle diet

Introduction

The diet of an animal is a profoundly important component of their life history (Swanson et al. 2016), and characterising diet is key to understanding food webs and the ecological roles of different species (Duffy et al. 2007). Monitoring trophic changes is critical for understanding how marine species are responding to escalating environmental pressures and the significant implications on the stability and resilience of food webs (Myers et al. 2007; Rossoll et al. 2012; Hastings et al. 2020; Gomes et al. 2024). Although direct observation of feeding has long been used to gather information on the diet composition of terrestrial animals (Litvaitis 2000), opportunities for direct observation are limited for many marine animals due to the many obstacles associated with accessing underwater environments. Hence, the development of indirect dietary technologies has been key to improving knowledge of marine diets and ecosystems (Bowen and Iverson 2013; Nielsen et al. 2018).

Diet analysis methods have been in development for centuries: as early as the nineteenth century, postmortem stomach analysis on whaling boats contributed to our understanding of whale diet, e.g., the discovery that baleen whales consumed Crustacea (Scoresby 1820). Early studies

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mostly assessed marine diet via the analysis of stomach content or faeces and subsequent examination of semi-digested food items (e.g., McAtee 1912). In the last 35 years, stable isotopes have been used (and continue to be used) to give an indication of marine trophic ecology and diets integrated over time, following the concept that the stable isotopic composition of tissue from an organism higher up the food chain tissue reflects, to some extent, the isotopic composition of what it has eaten, albeit with some isotopic enrichment (Oelbermann and Scheu 2002; West et al. 2006; Eglite et al. 2023; Hobson 2023). Within the last decade, molecular approaches have become widely used across marine taxa (e.g., Berry et al. 2015; Berry et al. 2017; van Zinnicq Bergmann et al. 2021).

Given these recent developments in diet technology, it is timely to review DNA-based diet analysis methods in marine animals and adopt them to assess the effect of escalating environmental pressures on diet (e.g. climate change; Donaton et al. 2019; Hobson 2023). While there have been general reviews of DNA-based diet analysis in animals across the years (Pompanon et al. 2011; Alberdi et al. 2019; Sousa et al. 2019; Book chapter, Deagle et al. 2023), this review provides an up-to-date, targeted review of dietary metabarcoding studies in marine vertebrates for those entering the field. We review DNA-based dietary studies where samples were taken directly from marine vertebrates or their faeces to characterise diet (studies on prospective diet communities only were not assessed). We then identify a major marine taxon, sea turtles, where DNA-based studies are still in their infancy, presenting some of the key considerations for implementing studies on this taxon and providing a forward-looking framework for how dietary DNA metabarcoding may be used in future sea turtle research.

Dietary DNA metabarcoding in marine vertebrates

What is DNA metabarcoding?

Dietary DNA metabarcoding is the analysis of a sample from a host organism that could contain DNA from multiple food items (e.g., faeces, homogenate stomach slurry, a swab). DNA is extracted, amplified using polymerase chain reaction (PCR) and sequenced using high-throughput sequencing (HTS). Taxonomic identities are then assigned by comparison of the query sequences against DNA barcode reference libraries (Fig. 1).

DNA metabarcoding can be effective at providing short-term diet composition information, depending on the sample type, environmental conditions, digestion rate of the host and digestibility of food items. Results will typically represent diet items consumed between several hours to days pre-sampling (Thuo et al. 2019; van Zinnicq Bergmann et al. 2021). The validity, accuracy and reliability of dietary DNA metabarcoding results are determined by many factors, including but not limited to the barcoded region, primer selection and PCR conditions, sample preservation and DNA extraction, bioinformatic analysis, and the comprehensiveness and accuracy of reference databases. Other considerations include: the amplification of host DNA dominating PCR reactions; the inability to detect cannibalism, as prey DNA cannot be distinguished from host DNA (e.g., Martin et al. 2021); and difficulty separating intentionally eaten food items from secondary or incidental ingestion (e.g., de Bruyn et al. 2021). However, there are effective ways to assess and mitigate many of these potential limitations. For example, controlled DNA metabarcoding studies on captive animals have been utilised for bony fish, pinnipeds, penguins and cartilaginous fish (Deagle et al. 2010, 2013; Thomas et al. 2014; Corse et al. 2015; van Zinnicq Bergmann et al. 2021), providing great opportunities to test these biases. Despite these difficulties, many ecological studies have used dietary DNA metabarcoding and found increased taxonomic specificity and higher diversity in comparison with traditional dietary assessment techniques (e.g., Coker et al. 2023), particularly if used in combination with morphological or other biomarker-based techniques (Bonin et al. 2020; Martin et al. 2021). DNA metabarcoding has been recognised as a viable avenue for characterising diet, constructing ecological networks and monitoring trophic change (D'Alessandro and Mariani 2021; Cuff et al. 2022).

DNA-based dietary studies on marine vertebrates have been increasing, with a shift in the last couple of decades from simpler DNA-based techniques (e.g., targeted PCR to amplify select dietary items or DNA barcoding to identify distinct tissues from stomach contents) to metabarcoding protocols based on high-throughput sequencing (HTS) and bioinformatic analysis (Fig. 2, see Fig. 1 for depiction of workflows). This is likely reflective of the development, decreasing cost and increasing accessibility of HTS technologies. Diet studies on bony fish dominate marine vertebrate metabarcoding studies (36.5% of 85 metabarcoding studies reviewed), followed by seabirds (23.5%) and pinnipeds (17.6%, Fig. 3a). The dominance of faecal and gut sampling is likely due to faecal sampling of seabird and pinnipeds being relatively more accessible because of defaecation close to roosts or haulouts, and gut analysis being relatively more feasible in bony fish due to opportunities provided by fisheries (Fig. 3b). Meanwhile, swab-based studies are relatively scarce but have been used in marine vertebrates where faecal sampling is less accessible, such as cartilaginous fish and sea turtles (Díaz-Abad et al. 2022a; Clark et al. 2023; Olin et al. 2023). Overall, DNA metabarcoding

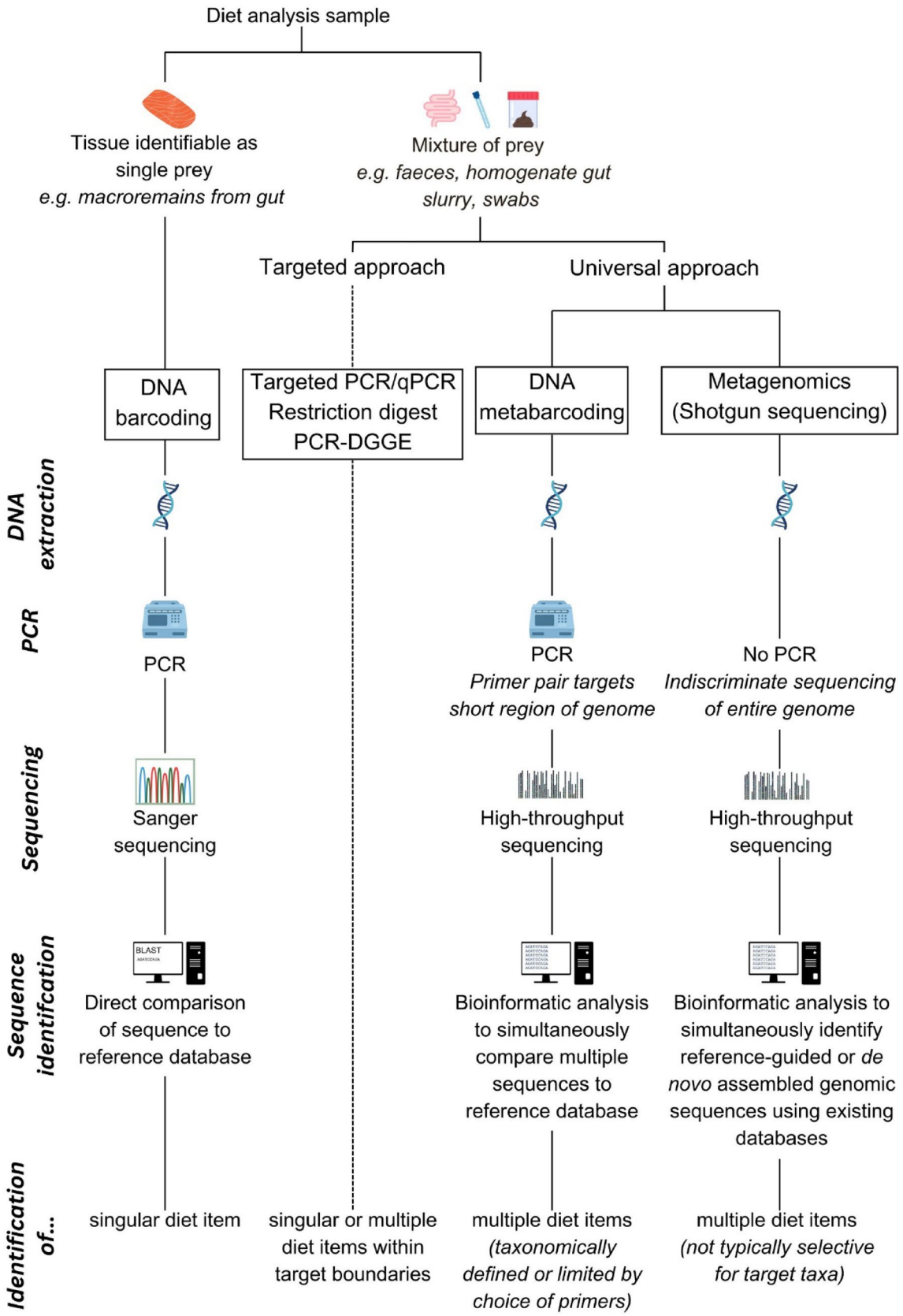

Fig. 1 A comparison of the main molecular dietary analysis workflows that are discussed in this review. Targeted workflows (dashed line) are not depicted in detail

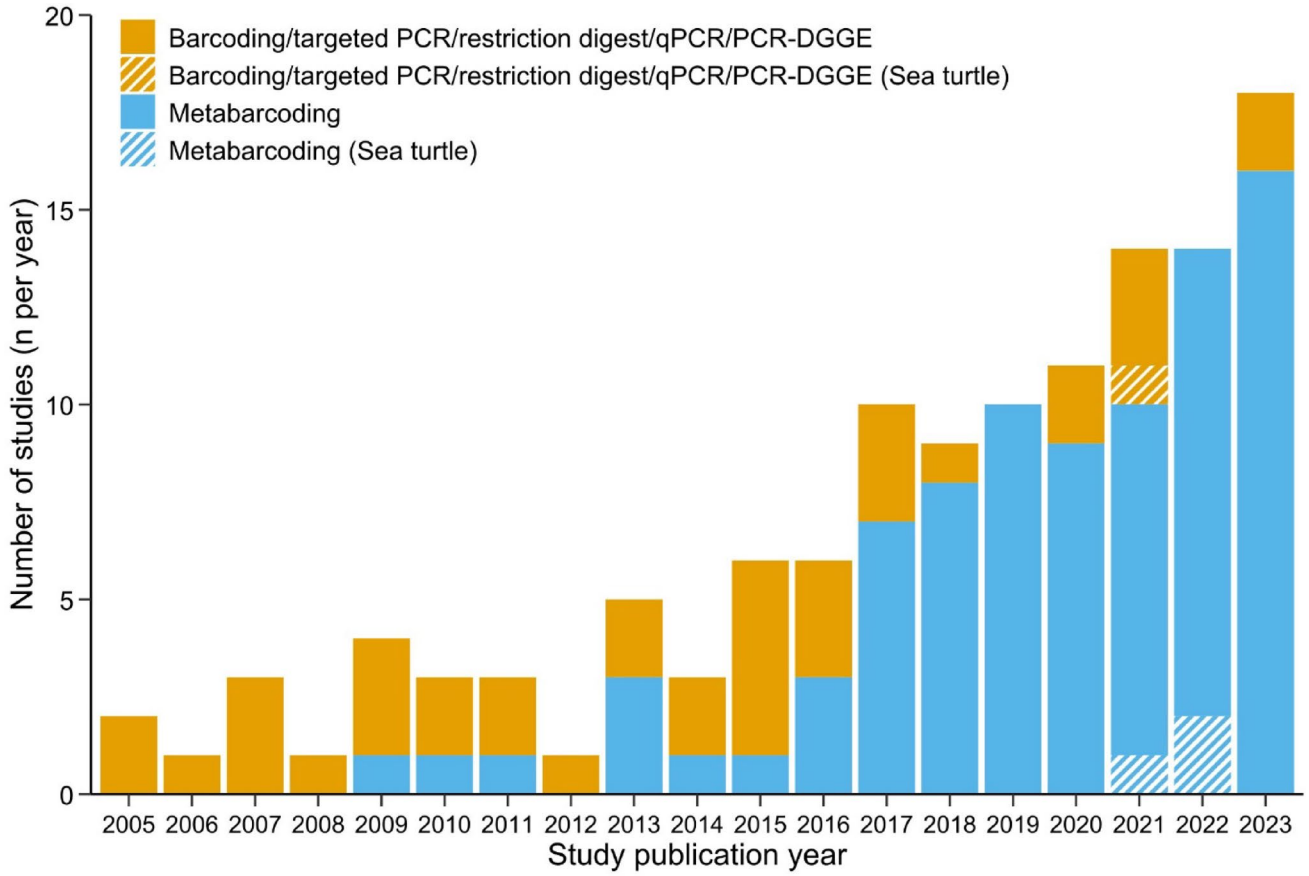

Fig. 2 A review of 124 DNA-based diet studies on marine vertebrates from 2005–2023 (Table S1). Four studies on sea turtles are separated (barcoding $n=1$; metabarcoding $n=3$). The metabarcoding category includes some studies that used metabarcoding combined with barcoding/targeted PCR/qPCR

has played an important role in expanding our understanding of marine vertebrate dietary composition by enabling increased sample sizes, enabling efficient sampling of some less accessible host species, improving the detection of fragile diet items, and increasing taxonomic resolution.

When is DNA-based analysis the best strategy?

DNA-based diet analysis can improve the identification of food items that may be routinely underrepresented or missed due to the limitations of traditional methods. Although numerical comparisons across metabarcoding studies can be difficult due to differences in methodology and reporting, some taxa are identified more or less frequently in metabarcoding studies compared to other techniques. DNA metabarcoding has identified prominent differences compared to previous diet studies, as well as previously undiscovered dietary components. This includes previously undocumented or understated fish species in pinniped diets (Boyi et al. 2022) and jellyfish in white shark (*Carcharodon carcharias*) diet (Clark et al. 2023). In pinniped studies, traditional hard part diet analysis on faecal samples often misses certain types of fish e.g., flatfish (Jeanniard-du-Dot et al. 2017; McCosker et al. 2023) and clupeids (Flanders et al. 2020; McCosker et al. 2023) as predators may avoid eating the head and therefore otoliths are not present in the digestive system or faeces. These fish are commonly identified if DNA metabarcoding is employed (Jeanniard-du-Dot et al. 2017; Flanders et al. 2020; Dufault et al. 2021). The same phenomenon has been found for species with particularly fragile otoliths, for example, increased identification of California sardines (*Sardinops sagax*) in harbour seal (*Phoca vitulina*) diet (Brassea-Pérez et al. 2019).

Across taxa, DNA metabarcoding improves the detection of gelatinous food items, increasing its identification when compared to morphological data (McInnes et al. 2016; Günther et al. 2021). Enhanced detection was noted for ctenophores and cnidaria (Martin et al. 2021; Clark et al. 2023) and cephalopods in some cases (Xavier et al. 2018; Brassea-Pérez et al. 2019), likely due to the easily digestible nature of these prey items, which makes them harder to pick up via morphological analysis.

DNA metabarcoding was also reported to be, in some cases, less sensitive than morphological identification-based

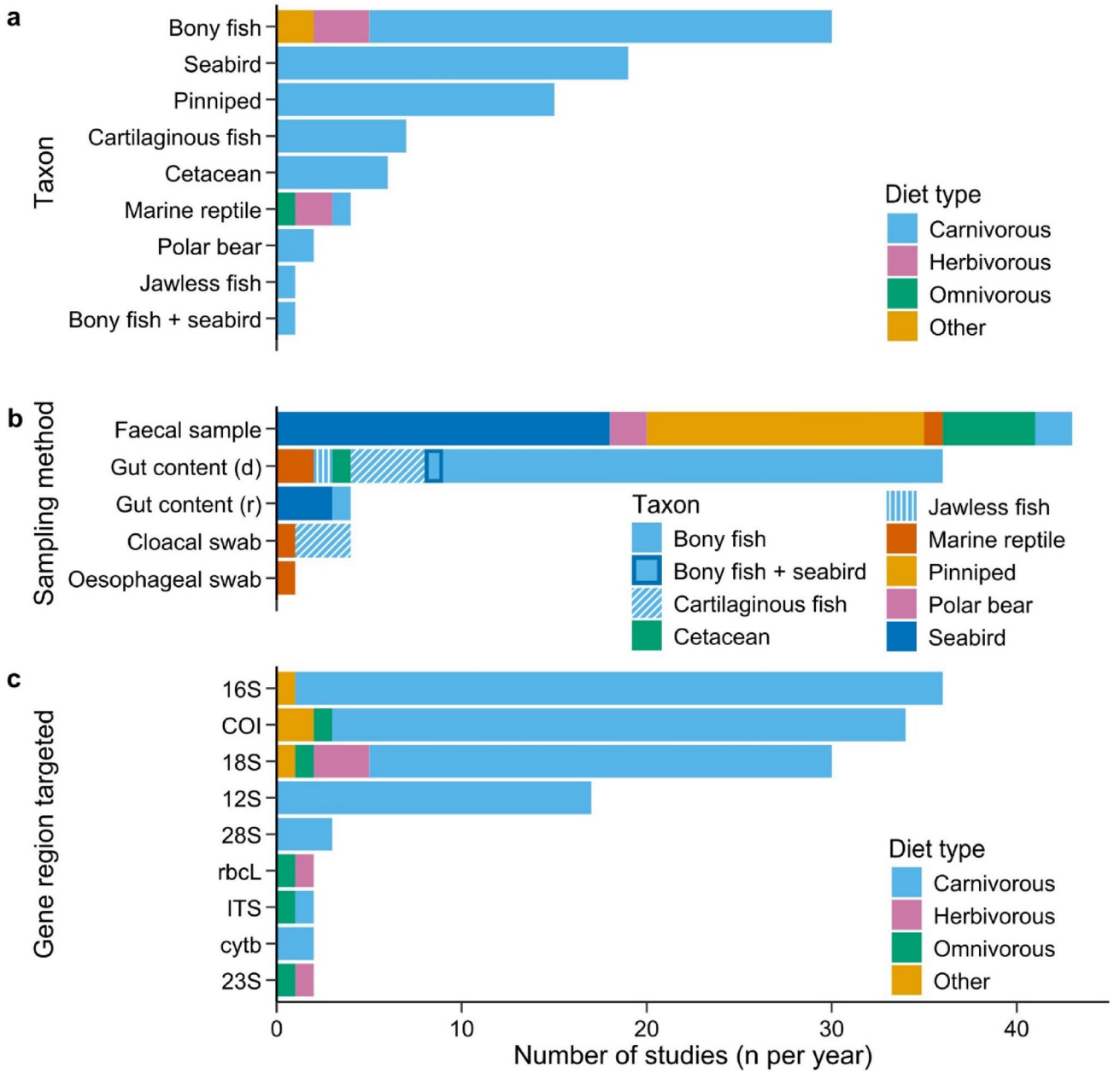

Fig. 3 The number of dietary DNA metabarcoding studies on marine vertebrates based on a review of 85 studies (Table S1). (**a**) Number of studies by broad taxonomic grouping and predominant diet type of host organism(s). (**b**) Number of studies by sampling method and broad taxonomic grouping of host organism(s). Includes some studies that utilised more than one sampling method. Gut content: (d) = gut content analysis from dead organisms; (r) = gut content analysis from regurgitate/lavage. (**c**) Number of studies by gene region targeted by primer set and predominant diet type of host organism(s). Diet type 'Other' indicates studies with multiple species for which it was inappropriate to group diet types

methods for certain diet items (e.g., crustaceans, cephalopods, echinoderms, annelids and insects; Jeanniard-du-Dot et al. 2017; Martin et al. 2021; Clark et al. 2023). This seems to be related to the detection accuracy of particular taxa, i.e., due to poor design of primers for certain target groups (McCosker et al. 2023), rather than DNA metabarcoding as a technique being unsuitable for whole groups of diet items. DNA degradation as well as tissue digestion may play a role, e.g., a hard cephalopod beak may be more likely to be found during scat analysis compared to DNA identified from heavily digested water-based diet tissue (Jeanniard-du-Dot et al. 2017). However, DNA from highly water-based species can still be found using sampling methods relying on DNA from nearer the end of the digestive process, despite the easier digestion (Clark et al. 2023). Studies assessing the differences in DNA degradation and factors like mtDNA

density among marine taxa are still limited and the field would benefit from further investigation into these factors, e.g., more studies on digestion/tissue correction factor analysis (Thomas et al. 2014). Studies of captive animals have assessed quantitative analysis and digestion bias, successfully using controlled mixes of prey tissues (a form of mock community analysis) to develop tissue correction factors, in turn improving quantitative diet estimates, and assessing prey-specific digestive biases to generate digestion correction factors (Thomas et al. 2014). Although the practical application of this technique for every possible food item in a species' diet will often be difficult, the value of mock community analysis in parallel to sampling is acknowledged as a valuable mitigation control for digestion and quantitative assessment bias.

Lack of an expected diet item may also be the result of an ecological bias. Crustacean DNA was detected in seabird regurgitate but not in chick faecal samples of the same species in a metabarcoding study (Alho et al. 2022). Whilst this could be due to methodology (e.g., lack of crustacean DNA amplification, lower DNA yield, increased degradation of DNA as a result of gut vs. faecal sampling) it could also be due to the different life stages assessed, with the authors hypothesising that adults may choose to feed chicks with higher quality food. Our review highlights the importance of comparisons across life stages, sampling types and parts of the digestive tracts (Alho et al. 2022), as well as the use of positive controls and testing of experimentally controlled conditions to avoid false negatives (e.g., multilocus primer sets can help reduce primer-based limitations; Zhang et al. 2023). Where an entire group of taxa is likely to have been missed, it is important to review primer selection and test whether the primers may have led to the exclusion of this taxa.

Importance of primer selection

Primers are responsible for which taxa are targeted to be amplified in preparation for sequencing. Primer selection is a complex balance between finding a gene region that is varied enough between taxa for sufficient taxonomic resolution and being adequately conserved across taxonomic ranks to increase the breadth of diet items that can be amplified and identified (Sousa et al. 2019; Sarkis et al. 2022). The target fragment of DNA also needs to be short, given that dietary DNA metabarcoding relies on the amplification of degraded DNA, making it harder to encompass enough variation for high taxonomic resolution. Degenerate primers (primers incorporating 'degenerate' bases that are not specific to one base and thus can enable more flexible taxonomic targeting) can enhance the breadth of diet items amplified, but amplification can be less reliable or stable as different annealing temperatures favour different targets. Furthermore, the development of reference databases has played a key role in the evolution of DNA metabarcoding studies, and the extent of these databases varies between gene regions. The most frequently used primer sets have an advantage in that they often correspond to regions with the best database coverage and allow direct comparison with existing studies. However, all primer sets naturally have some biases; therefore, primer selection should be carefully considered depending on the specific characteristics and aims of a study. Multilocus, even multi-region, coverage can improve resolution and reduce biases (e.g., Komura et al. 2018; Ravache et al. 2020). Even within gene regions, there can be considerable variation in the primer sets selected. We reiterate that dietary metabarcoding studies should detail at minimum, the name of the primers used the name of the primers used (consistent with previous use of the same primer), the sequence, the amplicon length and the original reference in either the main text or supplementary information to provide transparency and facilitate collaborative research in the field (see Table S2 for information on primers used in reviewed metabarcoding studies). While this best practice is generally followed, sometimes these details are incomplete, even in recent studies (e.g., de Bruyn et al. 2021; Novotny et al. 2022).

At present, marine taxa with primarily carnivorous diets have been most commonly assessed, with herbivorous or omnivorous diets lacking representation (Deagle et al. 2023; Fig. 3a). Predominantly piscivorous megavertebrate studies have frequently used primers targeting the 16S mitochondrial rRNA gene region (Fig. 3c), and taxon-specific primers targeting Chordata and Cephalopoda (e.g., Deagle et al. 2009; Brassea-Pérez et al. 2019), a reasonable approach when research aims are aligned with limiting diet discovery to chordates and cephalopods. Primer selection and the balance between taxonomic resolution and breadth of diet items is a less complicated feat for carnivorous/piscivorous and herbivorous diets, which are relatively constrained in comparison to omnivorous diets consisting of plant, protist and animal matter. DNA metabarcoding of omnivorous diets is recognised to be more difficult and lacks studies in marine vertebrates (Tercel et al. 2021; Deagle et al. 2023). These studies can benefit from a multi-locus/multi-region primer approach (Sarkis et al. 2022). Taxon-specific primers have had important roles in marine megavertebrate diet analysis, for example, in detecting a particular commercially important species in pinniped diets to assess fishery-pinniped interactions (Granquist et al. 2018). However, in wide-ranging, omnivorous diets, e.g., the diet of several sea turtle species, taxon-specific primers may be less applicable unless a similar specific outcome is required. When characterising a varied diet, the use of taxon-specific primers, perhaps based on previously reported food items, can prevent

the finding of rarer or novel dietary components. Further, it would not be cost- or time-effective to use taxon-specific primers across all possible taxa in a varied diet. However, as the use of universal primers comes with the caveat of reduced resolution, the use of universal primers could help to inform future, more taxon-specific studies.

Another factor that can affect primer selection is the corresponding reference databases, as they differ in comprehensiveness between amplified regions. For example, the COI region is relatively well represented for most vertebrate and invertebrate animals, particularly insects and fish, due to its longstanding use as a barcoding region for eukaryotes and its use in global barcoding initiatives. While the uptake of gap analysis and dedication to sequencing biodiversity has been considerable in the last five years (Weigand et al. 2019; Marques et al. 2021; Keck et al. 2023), libraries are also subject to significant geographical and taxonomic gaps and biases in the marine environment (Weigand et al. 2019; Vieira et al. 2020). Hence, a taxon present in a diet study sample may be missing from post-analysis results if there is no reference sequence for taxonomic assignment. For an in-depth overview of the challenges of taxonomic reference databases for metabarcoding protocols see Keck et al. (2023).

Blocking amplification of host DNA

In dietary DNA metabarcoding samples, host DNA is normally expected to be present alongside dietary DNA, often at higher concentrations. Host DNA can be amplified by universal primers, resulting in high proportions of sequence reads attributed to the host rather than dietary species. This can result in little useable data unless huge sequence coverage is applied, which increases costs. For example, in Díaz-Abad et al. (2022a), it was reported that host DNA made up a large proportion of total reads: 98.7% in cloacal swabs, 59.6% of oesophageal swabs and 99.8% of hatchling intestinal swabs. The need to block the amplification of host DNA depends on the amplification primers used and the nature of the sample. If taxon-specific primers are used (or primers that are not applicable to the host genome), host DNA amplification may not occur; however, using universal primers that cover more possible diet items means that host amplification is to be expected. Swab-based diet studies on sharks support this (van Zinnicq Bergmann et al. 2021; Clark et al. 2023; Olin et al. 2023). Where more taxon-specific primers targeting the 12S gene region were used, host DNA proportions were low (< 1% of reads, with and without blocking primers; Clark et al. 2023; van Zinnicq Bergmann et al. 2021). However, where universal COI or 18S-targeting primers were used with no blocking primer, host DNA proportion was high, for example, 90.3% of dietary-associated reads (Olin et al. 2023) and 99.2% of total reads (Clark et al. 2023). Methods of blocking host DNA include the design of primers to exclude predator DNA (Ford et al. 2016), restriction digestion (Dunshea 2009), blocking primers (Martin et al. 2021) and PNA clamps (Homma et al. 2022b; Box 1).

Box 1: Blocking primers vs. PNA clamps: how to block host DNA

Blocking primers are frequently used in dietary metabarcoding studies to block host DNA amplification (Martin et al. 2021; Alho et al. 2022; Clark et al. 2023). However, if inappropriately designed, blocking primers can prevent amplification of dietary DNA (Piñol et al. 2015). Whilst blocking primers are based on DNA, constituting a deoxyribose phosphate backbone, PNA clamps are an alternative method of PCR blocking based on peptide nucleic acids (PNAs) with a synthetic pseudopeptide backbone. An absence of charge in this backbone makes the PNA clamp more stable and more specific than blocking primers. The PNA clamp is resistant to nucleases/proteases and has a higher affinity, specificity and stringency when binding to DNA, meaning that binding can be determined by changes to a single base and mismatches are less likely than with a traditional blocking primer. Further, whereas successful blocking primers usually overlap PCR primer regions, competing with the PCR primers during the PCR annealing stage (Fig. 4, Vestheim and Jarman 2008), PNA clamps can be effective at any position between PCR primers. Blocking primers targeting the host taxon have been widely used in marine vertebrates, although several studies state not using this method due to concerns that dietary DNA amplification would be inhibited (e.g., McInnes et al. 2017). PNA clamps were originally applied to biomedicine (Orum et al. 1993) and more recently applied to ecology in endophytic fungal/bacterial metabarcoding studies (e.g., Lefèvre et al. 2020; Viotti et al. 2024). However, PNA clamps have not been widely adopted in dietary research (Fig. 5).

PNA clamps have been used in combination with universal 18S primers in bony fish with high success and in a direct comparison with blocking primers using mock communities, they were found to have higher suppression efficiency (59.28% more efficient; Homma et al. 2022a, b). Further, they were successfully used in combination with 18S primers to look at the diet of Bryde's whales (Carroll et al. 2019). PNA clamps are, at least up front, more expensive (around ten-fold depending on modifications, complexity and length) and optimisation can be challenging, requiring careful design and testing.

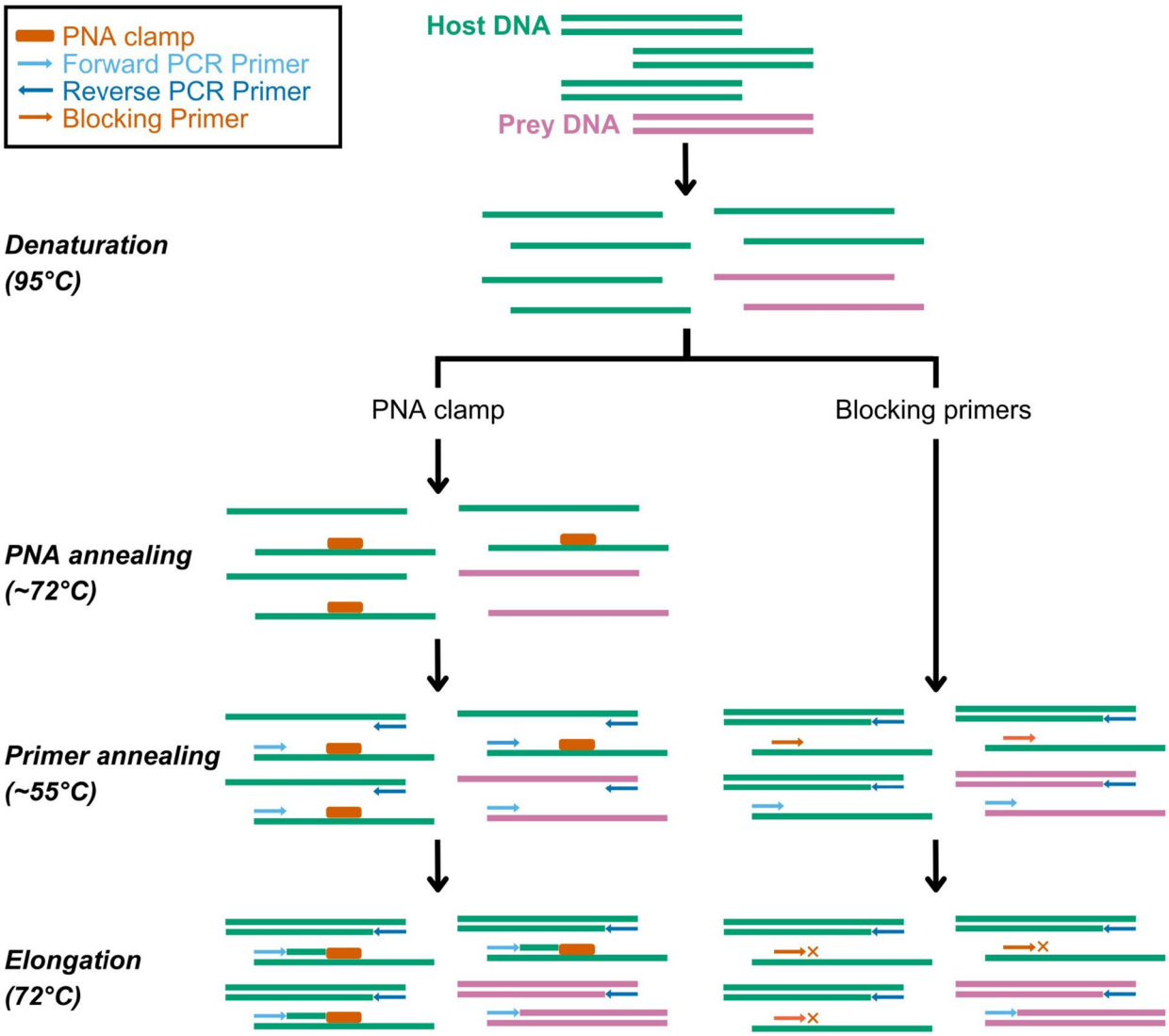

Fig. 4 Blocking primers (modified primers, usually incorporating a C3 spacer at the 3' end) and peptide nucleic acid (PNA clamps, synthetic nucleic acids made up of nucleobases on a peptide backbone) can prevent host DNA from dominating PCR reactions during metabarcoding. This diagram shows a common blocking primer mechanism where the primer overlaps with the PCR primers, competing with the primers to bind to the host DNA. PNA clamps bind at a higher annealing temperature before primer-binding. Blocking primers repress DNA amplification. PNA clamps prevent polymerase elongation and PCR amplification (diagram adapted from Kawasaki and Ryan 2021)

However, in studies where universal primers are used and host DNA is likely to dominate reactions, effective blocking may be more cost-effective by reducing the sequencing depth required to get the desired sequencing outputs. Furthermore, blocking primers may need to be used in higher concentrations, reducing cost-effectiveness. Homma et al. (2022b) found that blocking primer effectiveness depended on concentration, as their highest blocking primer concentration trial (2.0 µm, 10x the concentration of the normal PCR primers) was the most effective, whereas their PNA clamp worked with 100% efficiency even at a ten-fold lower concentration (0.2 µm).

The decision of whether to block host DNA and which method to use is dependent on the budget, aims and specifics of a study. Blocking mechanisms may be more valuable when universal primers are being used and when the sampling method is likely to incur high amounts of host DNA (e.g., with swabs).

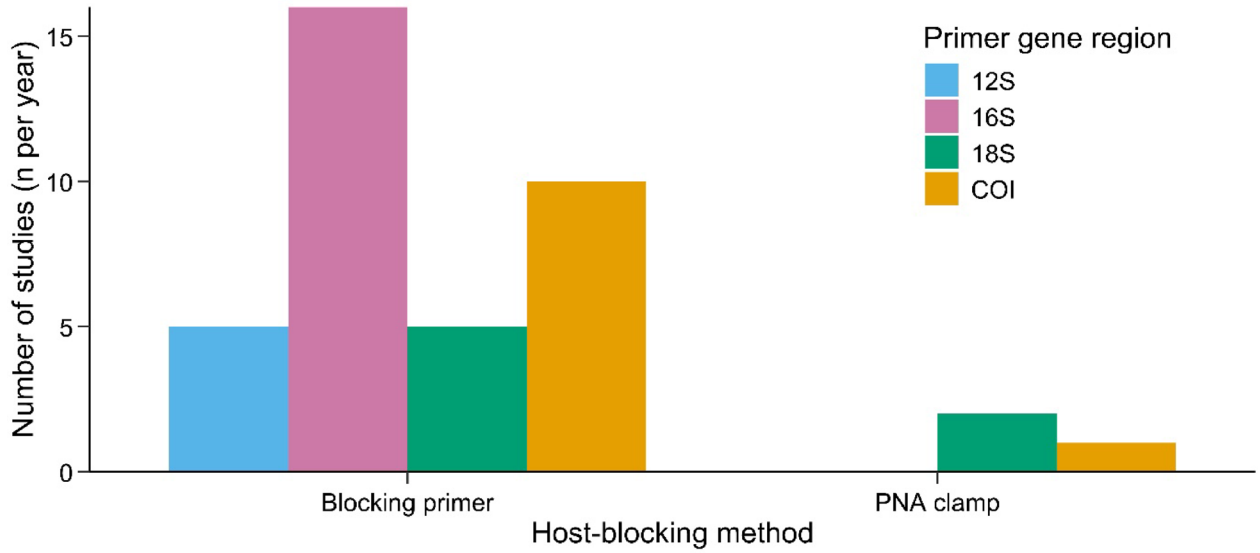

Fig. 5 Comparison of studies using blocking primer or PNA clamp host-blocking methods by primer gene region, based on the review of 85 metabarcoding studies ($n = 33$ studies, see Table S1)

Secondary/incidental predation and cannibalism

The capture of secondary and incidental predation is an important consideration in DNA metabarcoding, as it is difficult to distinguish between the organisms eaten intentionally, food items of those organisms, organisms accidentally ingested, and epibionts or epiphytes. For example, de Bruyn et al. (2021) showed extensive intermixing of taxonomic composition between shark stomachs and stomach contents of whole prey. However, there are ways of minimising obscurity caused by secondary diet items, for example, by comparing the co-occurrence of prey and potential food-of-prey (McInnes et al. 2017). Comparing different areas of the digestive tract may also help. Faecal analyses may be less affected by secondary food items than stomach analyses, as the double-digested DNA may be more heavily degraded by this point (Deagle et al. 2019; Zhang et al. 2023).

Epiphytes and epibionts may be incidentally ingested alongside intentionally eaten diet items. For example, Díaz-Abad et al. (2022a) attribute a high proportion of diatom DNA recovered from green turtle swabs to the epiphytic presence of diatoms on seagrasses like *Halodule sp.*, a common component of green turtle diet. However, it is relevant to consider that, even if not intentionally eaten, epiphytes such as diatoms may still have nutritional benefits, e.g., as a source of unsaturated fatty acids (Ackman et al. 1992; Yi et al. 2017).

Detection of cannibalism, however, is an issue that is yet to be effectively solved. Effective blocking primers or other host DNA-blocking methods will prevent amplification of same-species prey, and where same-species DNA is present, it cannot be distinguished between host and prey if cannibalism is a possibility (a phenomenon which has been reported, albeit infrequently, in sea turtles; Frick et al. 2009).

The difficulty of obtaining quantitative dietary DNA data

The difficulty of assessing the relative importance, quantity and size of food items is a key limitation of DNA metabarcoding. Percentage frequency of occurrence (%FOO), one of the key metrics that DNA metabarcoding can provide, only informs about the presence or absence of dietary components. FOO may be more reliable and conservative than relative read abundance (RRA) values due to being less affected by sequence recovery biases, but they can still present other biases, e.g., overestimation of the importance of small quantities of diet items (Deagle et al. 2019). RRA allows the comparison of relative dietary taxa abundance via frequencies of sequence reads, although there are conflicting results regarding how much relative abundance of sequence reads corresponds to relative quantities of diet items eaten, as this can be highly affected by sequence recovery biases (Deagle et al. 2019).

Deagle et al. (2010, 2013) studied captive penguins and seals, examining the proficiency of making quantitative prey conclusions based on metabarcoding data. The studies found that sequence proportions varied greatly when compared to diet proportions, suggesting that careful experimental design would be required to provide quantitative information. Alternatively, some studies have suggested that despite PCR bias, DNA metabarcoding can still provide useful relative quantification (Jarman et al. 2013) and semi-quantitative analysis in some cases (Günther et al. 2021).

The quantitative capabilities of dietary DNA metabarcoding analysis are expected to progress (Deagle et al. 2019).

Barriers to metabarcoding accessibility

Cost is a key factor when planning projects and considering whether a new technique could be broadly applicable to a particular field or organism. DNA metabarcoding, being a molecular technique, requires specialised laboratory equipment or outsourcing to a commercial provider. For researchers starting out in the field who do not have easy access to equipment, are unlikely to do repeated studies in the research area, or do not have suitable laboratory support or collaborations, commercial outsourcing may be the best approach. Considering a basic metabarcoding protocol in today's market, costs would likely come to around £35 per sample for DNA extraction, library preparation, and sequencing. Costs would be less for researchers with access to laboratory facilities and in-house sequencing platforms. For 200 samples, in-house extraction, preparation and sequencing on an Illumina MiSeq System would cost approximately £4500 per sample. Costs can further decrease for researchers with much larger sample sizes who can choose to utilise higher-throughput sequencing platforms. In comparison, outsourcing laboratory processing of 200 samples would cost around £7000. However, to put this into a wider perspective, a video camera diet study on a large marine vertebrate such as a sea turtle would cost between £1250–7000, depending on the specification of the equipment. Whilst equipment could be reused on another individual, this can involve expensive additional time in the field. Attachment and recovery of an animal-borne camera on a sea turtle would require around 3 days per turtle, whereas field-sampling of a sea turtle using swabs can be completed in around 30 minutes.

The affordability and accuracy of high-throughput sequencing continue to improve with technological development, but remain a key driver in the decision to conduct DNA metabarcoding studies. While Illumina sequencing remains the current standard for metabarcoding studies, Oxford Nanopore technology, which offers the additional options of portability and longer-read sequencing, has improved in terms of base calling accuracy and throughput options, and can be more cost-effective, meaning that it is likely to become much more commonly used in the near future. Recent studies confirm a similar performance (taxonomic assignments) for both Illumina and Nanopore technologies when used for dietary metabarcoding (van der Reis et al. 2022).

The bioinformatics analysis of DNA metabarcoding, wherein raw sequences are processed and assigned to identified taxa, is often considered a barrier to the accessibility of DNA metabarcoding. The choice of bioinformatic pipeline can influence results, requires careful consideration, and involves a significant amount of time, effort and coding proficiency (see Hakimzadeh et al. 2024 for a recent review of bioinformatic pipelines for DNA metabarcoding). Further, in-house bioinformatic analysis will require access to high-performance computing. Whilst bioinformatic analysis can be outsourced, increased availability of bioinformatics teaching resources and pre-made pipelines (such as the SimpleMetaPipeline, Williams et al. 2024) are making in-house analysis a more viable option for researchers new to the field.

Opportunities for sea turtle foraging ecology

The evolution of diet analysis in sea turtles

As keystone species, sea turtles are ecological engineers and hold important places within marine food webs. For example, green turtle (*Chelonia mydas*) consumption of seagrasses can have key impacts on seagrass productivity and resilience (Moran and Bjorndal 2005; Christianen et al. 2019) and hawksbill turtle (*Eretmochelys imbricata*) consumption of sponges can relieve corals from space competition (León and Bjorndal 2002). Further, over the last few decades, increased and improved diet analysis has proved sea turtle diet to be more variable than originally believed (Table 1). For instance, while green turtles were originally thought to be strict herbivores, diet is now understood to vary across age and location (e.g., Arthur et al. 2008; Esteban et al. 2020), with animal matter consumption (e.g., jellyfish, salps, fish and invertebrates; Holloway-Adkins and Dennis Hanisak 2017; Fukuoka et al. 2019; Piovano et al. 2020) now well-reported. Variations occur among species, life stages, geographies and environmental conditions, for example, interspecific niche separation (Martins et al. 2020), alternative food preferences in gravid individuals (Stokes et al. 2019), foraging dichotomies in geographically close habitats (Madeira et al. 2022), and intraspecific differences correlated with sea surface temperature (Esteban et al. 2020).

Traditionally, sea turtle diet analysis techniques have been based on morphological identification. However, several limitations mean that morphological identification alone may not give comprehensive results (see Table 1 for a comparison of techniques). By combining morphological-based techniques with more recently developed molecular and biochemical approaches, we can evaluate sea turtle diet more comprehensively (e.g., Williams et al. 2014; Bonin et al. 2020; Martin et al. 2021). Stable isotope analysis (SIA) has had extensive use over the last two decades and can

Table 1 A comparison of diet analysis methods that can be applied to sea turtles (includes adapted information from Bjorndal 1997; Wyneken et al. 2013; Díaz-Abad et al. 2022a; Deagle et al. 2023)

Method type		Method	Metrics	Advantages	Limitations	Recent sea turtle studies
Morphological identification	**Observational**	Visual observation (SCUBA/snorkel survey, observer-led photo/video)	• Presence/absence • FOO • Count	• Non-invasive • Direct assessment	• Only represents food consumption at a particular moment • Time-consuming • Difficult to standardise methods to obtain comparable information • Observer error	(Wood et al. 2017; Hanna et al. 2021)
		Video observation (animal-borne video, drone)	• Presence/absence • FOO • Count	• Can be non-invasive (excludes animal-borne video) • Ability to store and review footage for further analysis	• Retrieval of cameras can be difficult and costly • Sample size due to cost	(Patel et al. 2016; Weber et al. 2023)
	Gastrointestinal tract analysis	Oesophageal lavage	• Presence/absence • FOO • Count • Mass	• Safe for use without injury to animal • Inexpensive • Relatively easy and quick sampling • Can provide a good sample from anterior stomach/oesophagus	• Very short-term 'snapshot' of food consumption • Requires adequate training • Inappropriate for hard food samples • Diet items missed due to retention by the oesophageal papillae	(Holloway-Adkins and Dennis Hanisak 2017; Méndez-Salgado et al. 2020; Rezaie-Atagholipour et al. 2021; Quiñones et al. 2022)
		Gastric lavage (stomach flushing)	• Presence/absence • FOO • Count • Mass	• Can provide a clear view of what a turtle has been eating (composition, quantity and occurrence)	• Stomach could be empty at that time • Invasive and difficult • May underrepresent easily digestible food items, e.g., gelatinous organisms	(Burkholder et al. 2011)
		Gut content analysis (necropsy)	• Presence/absence • FOO • Count • Mass	• Can provide a clear view of what a turtle has been eating (composition, quantity and occurrence) • Inexpensive • Opportunity to obtain large sample sizes (depending on the scope and nature of the study)	• Stomach could be empty at that time • Potential for small sample sizes as requires dead animals (depending on region and activities of research program) • Often carried out on stranded individuals and may not be representative • Fragmentation of food items can make identification difficult • May underrepresent easily digestible food items e.g., gelatinous organisms	(Donaton et al. 2019; Stokes et al. 2019; Palmer et al. 2021; Kim et al. 2021; Martin et al. 2021; Baldi et al. 2023)
	Faecal examination		• Presence/absence • FOO • Count • Mass	• Can be relatively non-invasive • Generally inexpensive if access to live animals has been established	• Difficult to obtain large, representative sample sets in wild sea turtles • May underrepresent easily digestible and soft food items e.g., gelatinous organisms	(Baldi et al. 2023; Schmid and Tucker 2018)

Table 1 (continued)

Method type	Method	Metrics	Advantages	Limitations	Recent sea turtle studies
Biochemical	Stable isotope analysis (Bulk tissue and amino acid stable isotope analysis, CSIA-AA)	• Relative composition (Bayesian mixing models)	• Can offer a long-term view of food consumption (depending on tissue used)	• Relies on knowledge of isotopic composition of diet and dietary organisms having different stable isotopic values • Low taxonomic resolution – does not allow species-level identification • Requires reference knowledge • Can overestimate animal matter in the diet	(Méndez-Salgado et al. 2020; Wedemeyer-Strombel et al. 2021; Clyde-Brockway et al. 2022; Ramirez et al. 2023; Weber et al. 2023; Arends et al. 2024)
	Fatty acid analysis	• Relative composition (Bayesian mixing models)	• May provide information about health/nutrition	• Low taxonomic resolution • Limited knowledge of temporal scale • Requires reference knowledge	(Cardona et al. 2015; Koutsos et al. 2021)
	Trace element analysis	• Trophic transfer factor	• Broad spatial scales • May provide information about health	• Low taxonomic resolution (broad information about dietary taxa or foraging habits) • Limited knowledge of temporal scale • Requires reference knowledge	(Nicolau et al. 2017; Ramirez et al. 2019; Shaw et al. 2021)

Table 1 (continued)

Method type	Method	Metrics	Advantages	Limitations	Recent sea turtle studies
Molecular	Targeted PCR/qPCR (samples containing multiple unknown diet items)	• Presence/absence • FOO • Number of reads (qPCR)	• Low cost (PCR) or moderate cost (qPCR) per sample • Can be highly specific and sensitive • Potential to incorporate quantitative information	• Requires a priori dietary knowledge • Can become time-consuming and expensive for multiple targets	*No sea turtle diet studies found*
	DNA barcoding (individual, distinct diet items)	• Presence/absence • FOO • Count • Mass	• Can have less methodological development than metabarcoding	• Only suitable for individual, distinct diet items • Limited view of diet • Time-consuming for large sample sizes of mixed diet items • May underrepresent easily digestible good items e.g., gelatinous organisms • Likely to have contamination issues	(Kim et al. 2021)
	DNA metabarcoding (samples containing multiple unknown diet items)	• Presence/absence • FOO • Relative read abundance (subject to biases)	• Relatively low invasiveness (faecal, swabs) • Time-efficient and increasingly cost-efficient • High taxonomic resolution (if baseline of putative diet items available) • Easily reproducible, standardised sampling and molecular analysis • Can tie in with microbiome characterisation • Can work well for degraded DNA	• Dependent on a reference library • Taxonomic resolution can be limited • Hard to distinguish secondary/incidental consumption and detect cannibalism • PCR bias • Depending on sampling, it may underrepresent digestible items (faecal sampling, cloacal swabs) • Requires methodological development • Difficult to determine quantity/size of diet items • Variable digestion rates of diet items • Prone to non-target DNA amplification • Relatively high cost compared to visual gastrointestinal/fecal sample identification and bulk stable isotope analysis • Can be bioinformatically challenging	(Martin et al. 2021; Díaz-Abad et al. 2022a; Sarkis et al. 2022)
	Metagenomics	• Presence/absence • FOO • Relative read abundance (subject to biases)	• No PCR bias • High taxonomic resolution • No a priori knowledge required • Useful for additional analyses beyond diet (e.g., host population, microbiome characterisation)	• Expensive • Can be bioinformatically challenging • Difficult to deplete host DNA • Reference genome database is currently not rich enough to provide comprehensive results across taxa • Limitations with degraded DNA • Sensitive to degradation • May be inaccurate at high-resolution	*No sea turtle diet studies found*

FOO = Frequency of occurrence

Table 2 A comparison of the main methodological components of sea turtle dietary DNA metabarcoding studies ($n = 3$)

Study reference	Species	*N*	Sampling method	Gene region targeted by primers	Blocking primer	Host DNA	Methodological notes
Martin et al. (2021)	Loggerhead turtle	21	Homogenised gut content (necropsy, stranded)	18S (V7)	Yes	Present in 57% of samples despite blocking primer	Use of blocking primer
Díaz-Abad et al. (2022a)	Green turtle	15	Swabs: cloacal, oesophageal, intestinal (hatchling)*	18S (V7)	No	Cloaca: 98.7% of TR Oesophagus: 59.6% of TR Intestine: 99.8% of TR	Use of swabs and comparison of start/end of digestive tract
Sarkis et al. (2022)	Green turtle	39	Homogenised gut content (necropsy, stranded)	COI, 18S (V1-V3), 18S (V4), rbcL, UPA, ITS	No	0% - >90%** of TR	Comparison of six primer sets

TR = total DNA sequence reads

*Intestine of hatchling used as a control

**Host DNA depended on primer used

provide long-term, broad diet data, helping to make inferences about foraging areas and diet composition over time, depending on the tissue type that is sampled (Reich et al. 2008). For example, whilst blood serum can be used to look at recent consumption due to its short half-life, epidermal tissues inform about diet months prior due to a longer half-life (Prior et al. 2016; Haywood et al. 2019). Meanwhile bone and scute tissue can offer chronological foraging history information via analysis of tissue layers (Vander Zanden et al. 2010; Avens et al. 2013; Wedemeyer-Strombel et al. 2021).

Meanwhile, advances in molecular methods have established a new frontier in diet analysis. Openness to new technologies for investigating sea turtles' diet and careful development of methodologies based on experimental evidence may help to identify previously unknown dietary patterns and monitor future changes. This is particularly relevant in today's changing climate, as environmental shifts such as changing ocean temperature can lead to displacement to alternative foraging grounds (Chatzimentor et al. 2021) and shifts in diet composition (Donaton et al. 2019), exacerbating changes in foraging ecology. As diet composition has also been linked to variations in growth rates within species (Ramirez et al. 2020, 2023), diet characterisation may also provide information about the effect of climatic and distribution changes on growth dynamics and enable a greater understanding of resource partitioning among species and how this may change as a result of distributional or climatic changes.

The current state of molecular studies in sea turtles

DNA metabarcoding has been employed in several aspects of sea turtle research, including gut microbiome (Díaz-Abad et al. 2022b), epibiotic diatom assemblage (Rivera et al. 2018) and diet analysis (Díaz-Abad et al. 2022a). In the last decade, studies of sea turtle microbiomes have made substantial associations between bacterial communities and diet. As diet affects the gut microbiome, sea turtle microbiome may reflect whether a diet is primarily omnivorous or herbivorous. Microbial communities were shown to change in green turtles, shifting from omnivorous while free-living to predominantly herbivorous while in rehabilitation (Bloodgood et al. 2020). Further, distinct microbial communities were found between green and Kemp's ridley turtles (*Lepidochelys kempii*) from the same location, with higher levels of Clostridiales found in green turtles compared to Kemp's ridleys. Some Clostridiales bacteria have an important role in herbivorous digestion (Flint et al. 2008), and so this corroborates evidence that green turtles generally eat more plant matter than Kemp's ridley turtles (McNally et al. 2021). Microbiome characterisation has also been used to support dietary results derived from DNA metabarcoding (Díaz-Abad et al. 2022a).

DNA barcoding protocols, where individual food tissues are identified by DNA extraction, PCR and sequencing, are rarely undertaken or reported in sea turtles, despite being a useful complementary tool in gut content analysis studies (Kim et al. 2021). DNA barcoding was used to supplement morphological analysis of gut content tissue from necropsied sea turtles, increasing the resolution of food item identification (Kim et al. 2021). However, this method relies on obtaining DNA from gut tissue and so is subject to the limitations that come with sampling via necropsy (e.g., possible lack of representation of a wild population, low sample sizes) or oesophageal/gastric lavage (e.g., invasive procedures, may underrepresent easily digested food tissue).

To our knowledge, there are three published sea turtle dietary metabarcoding studies across two sea turtle species and two sampling methods at the time of writing (Table 2).

DNA metabarcoding, particularly studies utilising cloacal or oesophageal swabs, could enable increased sampling in a relatively less invasive manner than traditional gut sampling with relatively little training, particularly if field and laboratory researchers collaborate effectively. This offers a novel opportunity for obtaining sea turtle diet data.

Applying DNA metabarcoding to wide-scale sea turtle foraging ecology will require adaptations in methodology according to variations across sea turtle species, geography and life stage. For example, primer selection will vary. Optimal primer selection for adult green turtles that consume large proportions of plant-based material will not necessarily be the same as primer selection for adult leatherbacks (*Dermochelys coriacea*), for which gelatinous metazoan prey dominates diet composition. At present, sea turtle diet metabarcoding studies have used a combination of different universal primers, with Sarkis et al. (2022) carrying out a comparison and suggesting an optimal primer combination in a metazoan-targeting primer (targeting the COI gene region) and a eukaryote-targeting (18S region) primer to assess the omnivorous juvenile green turtle diet. They found this combination to be useful in characterising both animals, plants and algae in the juvenile green turtle diet. The 18S primers utilised by Díaz-Abad et al. (2022a) and Martin et al. (2021) targeted a different area of the gene and so direct comparisons are difficult to make. Further studies and experimentation with primers will help to validate these findings.

The use of swab-based sampling

A small proportion of dietary metabarcoding studies reviewed utilised oesophageal or cloacal swabs (one in sea turtles: Díaz-Abad et al. 2022a; three in sharks: Bergmann et al. 2021; Olin et al. 2023; Clark et al. 2023). The different swab sites present different outlooks on diet; cloacal swabs are likely to retrieve different results compared to oesophageal swabs due to some food items being easier to digest than others (Díaz-Abad et al. 2022a). Oesophageal samples may present a snapshot of more recent consumption (and could be dominated by the most recently ingested items), whereas cloacal samples may provide a more integrated view of diet over a longer timeframe. Van Zinnicq Bergmann et al. (2021) carried out a controlled feeding study on captured sharks and a concurrent study on a wild population of sharks to show that cloacal swab sampling can be used effectively and reliably. However, we suggest that swab sampling in combination with universal primers incurs high amounts of host DNA and may benefit from effective host-blocking mechanisms (Díaz-Abad et al. 2022a; Clark et al. 2023). Oesophageal and cloacal swabs offer a relatively less invasive method for assessing live animals compared to methods based on gut analysis (Campbell et al. 2023). Whilst utilising swabs is relatively understudied at the moment, the technique offers great opportunities for monitoring the diet of living marine vertebrates like sea turtles.

Understanding the contribution of gelatinous taxa to sea turtle diet

Gelatinous organisms that are easily digested can be difficult to identify in some traditional methods of diet analysis, such as morphological gut analysis. Improved identification of gelatinous organisms via DNA metabarcoding could be highly relevant for marine sea turtles. Jellyfish make up an important part of sea turtle diet, famously in the gelatinivorous leatherback (Heaslip et al. 2012), but also in other species (e.g., green turtles; Fukuoka et al. 2019). Given recent suggestions that gelatinous taxa (Cnidarian cubozoans, scyphozoans, hydrozoans; ctenophores, pelagic tunicates) may contribute to energy budgets of predators marine trophic systems more than previously believed (Hays et al. 2018; Chi et al. 2021) and that morphological analysis may underestimate gelatinous components of diet, it is plausible that we may be underestimating the contribution of gelatinous organisms to sea turtle diet. This hypothesis is supported by isotopic findings (González Carman et al. 2014; Fukuoka et al. 2019). Further, the presence of gelatinous components in sea turtle diet could have important implications in light of observed and predicted shifts in the abundance and distribution of gelatinous taxa driven by environmental change (Mills 2001; Attrill et al. 2007; Pantiukhin et al. 2024). Recent ecosystem modelling has indicated that the contribution of gelatinous taxa to food webs may be particularly susceptible to change following marine heatwaves (Gomes et al. 2024). Understanding gelatinous components of diet and how changes in populations of diet items may affect sea turtle food availability will be important in the coming years.

The importance of long-term diet studies in sea turtles

DNA metabarcoding has practical advantages when applied to diet studies, e.g., it is relatively efficient for obtaining large numbers of samples from minimal time in the field. Ontogenetic shifts are integral to most sea turtle lifespans, making long-term diet monitoring particularly critical for understanding their foraging ecology (Reich et al. 2007; Arthur et al. 2008; Witherington et al. 2012; Schmid and Tucker 2018). Whilst long-term diet studies have provided great insight into sea turtle diet (e.g., Seney and Musick 2007; Donaton et al. 2019), they are rare and logistically challenging, largely on account of the limitations

that come with traditional methods like gut content analysis (Table 1). DNA metabarcoding has the potential to facilitate long-term monitoring of sea turtle diet, providing large amounts of diet data that could enable large-scale comparisons across life stages and ontogenetic shifts (Sousa et al. 2016; Takahashi et al. 2020), seasonal variations (Hardy et al. 2017), and variable environments (Urquía et al. 2024). Further, DNA metabarcoding can help to predict the knock-on effects of range shifts on trophic systems (Ramos et al. 2023). Consequently, dietary DNA metabarcoding could be a valuable tool for identifying and monitoring key areas for the protection of sea turtles and the food they depend on, informing marine protected area planning and conservation policy (McInnes et al. 2017; Schwarz et al. 2018).

The future of molecular dietary analysis

As the costs of molecular techniques continue to decrease, it is important to consider how techniques will evolve in the coming years and the suitability of different methods to particular scenarios. Diagnostic PCR and DNA barcoding-based approaches can both have advantages depending on the study goal. The choice of whether metabarcoding is the best technique should be made based on the research questions being posed (for an in-depth comparison of molecular dietary techniques, see Deagle et al. 2023). For example, if the aim of a study is to identify whether a host organism is eating a particular species, targeted approaches may suffice (e.g., targeted PCR to assess pinniped-fishery interactions, Dufault et al. 2021). As another example, more targeted approaches may be appropriate where the focus is on a particular dietary item (e.g., DNA barcoding, Phillips et al. 2023). However, a targeted approach would not be appropriate in a study where prior knowledge of diet is poorly understood (e.g., Sousa et al. 2016) or the study aims to characterise all aspects of a broad diet (e.g., Sarkis et al. 2022).

Metabarcoding is susceptible to PCR bias, which can result in the failure to amplify particular dietary items or overestimation of the importance of certain taxa (Alberdi et al. 2019). Metagenomic protocols, wherein all DNA present is sequenced using shotgun sequencing and PCR amplification does not occur, avoid PCR bias. This removes the difficulty of selecting primers and thus primer bias. Whilst studies have started to explore metagenomics in dietary analysis with some success (Chua et al. 2021; Serite et al. 2023), whether it provides better information than metabarcoding is up for debate. For example, a similar performance was found in the number of taxa and resolution for metabarcoding and metagenomics techniques for herbivorous dietary analysis of a grouse (Chua et al. 2021). A controlled study on a herbivorous monkey found that each technique had advantages and disadvantages. For example, metabarcoding failed to identify several species to genus level, but metagenomics failed to pick up rare diet items (Srivathsan et al. 2015).

Metagenomic dietary analysis has been explored less than metabarcoding, particularly in the marine field. However, a study assessing diet competition in two estuarine pipefishes found that metagenomics identified a key dietary group omitted by metabarcoding analysis, likely as a result of amplification bias (Serite et al. 2023). Metagenomics may be an important avenue to explore in the dietary analysis of marine vertebrates, including sea turtles, but it does not come without limitations. As with metabarcoding, metagenomics relies on a known database of reference sequences (reference genomes), which are much more difficult to develop. The reference genome database is not yet rich enough to provide comprehensive results across taxa and the technology is currently very expensive, although these limitations will likely improve with time. Host DNA bias is another common issue between metabarcoding and metagenomics, but it is even harder to mitigate in metagenomics. Despite the appeal of alleviating PCR bias, PCR-based methods may remain the best molecular methods for dealing with degraded DNA, and hence, dietary DNA.

Concluding remarks and future directions

When conducting diet analysis, a combination of molecular techniques such as DNA metabarcoding and alternative technologies (e.g., morphological analysis, stable isotope analysis) can reveal the most information (Jeanniard-du-Dot et al. 2017). We suggest that further experimentation with DNA metabarcoding, particularly from swabs, could offer the means to carry out time-efficient, cost-effective, widespread diet sampling in sea turtles. Controlled feeding studies and experimentation with mock community analysis could improve confidence in results and provide a better understanding of biases, such as the temporal resolution of dietary DNA degradation (Nielsen et al. 2018).

We propose that the top five research priorities for facilitating sea turtle dietary DNA metabarcoding are: (1) Robust primer pair recommendations for different sea turtle species and life stages; (2) More efficient blockage of host DNA in PCRs; (3) Better understanding of how to adapt metabarcoding protocols to omnivorous diets; (4) Optimisation of swab-based protocols; and (5) Increased bioinformatic pipeline standardisation, providing increased accessibility for non-specialists.

DNA metabarcoding could be a key driver in answering some outstanding questions about sea turtle diet. By identifying more gelatinous taxa, it could help us to understand

how much gelatinous taxa are contributing to the diet of sea turtles other than leatherbacks (Fukuoka et al. 2019). By enabling us to identify broad ranges of dietary taxa, it may expose some of the rarer components of sea turtle diet and develop our understanding of where these animals deviate from herbivory/carnivory. By enabling wide-scale diet analysis, it could encourage subsequent global diet reviews across sea turtle species and help us to answer the question: how variable are sea turtle diets across different locations (Esteban et al. 2020)? Due to methodological compatibility with gut microbiota analysis (Díaz-Abad et al. 2022a, b), it could help answer the question: to what extent does diet influence gut microbiota in sea turtles (Kuschke 2022)?

Collaboration between sea turtle researchers across the world, from rehabilitation centres to field sites to laboratories, could enable quick, effective progression in this research area. Dietary DNA metabarcoding could facilitate long-term monitoring of sea turtle diet and their trophic interactions in response to environmental change-induced fluctuations such as migration to alternative foraging grounds (Chatzimentor et al. 2021), changes in diet composition (Donaton et al. 2019) and shifts in the abundance and distribution of food items (Hastings et al. 2020).

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Declarations

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