Received: 31 August 2017 | Accepted: 22 February 2018

DOI: 10.1111/faf.12286

ORIGINAL ARTICLE

WILEY FISH and FISHERIES

The sceptical optimist: challenges and perspectives for the application of environmental DNA in marine fisheries

Brian Klitgaard Hansen[1] | Dorte Bekkevold[1] | Lotte Worsøe Clausen[1,2] | Einar Eg Nielsen[1]

[1]Section for Marine Living Resources, National Institute of Aquatic Resources, Technical University of Denmark, Silkeborg, Denmark

[2]International Council for the Exploration of the Sea (ICES), Copenhagen V, Denmark

Correspondence
Brian Klitgaard Hansen, Section for Marine Living Resources, National Institute of Aquatic Resources, Technical University of Denmark, Silkeborg, Denmark.
Email: bkha@aqua.dtu.dk

Funding information
Danish AgriFish Agency's Green Development and Demonstration Programme (GUDP-VIND); European Union; AtlantOS, Grant/Award Number: 633211; DiscardLess, Grant/Award Number: 633680

Abstract

Application of environmental DNA (eDNA) analysis has attracted the attention of researchers, advisors and managers of living marine resources and biodiversity. The apparent simplicity and cost-effectiveness of eDNA analysis make it highly attractive as species distributions can be revealed from water samples. Further, species-specific analyses indicate that eDNA concentrations correlate with biomass and abundance, suggesting the possibility for quantitative applications estimating abundance and biomass of specific organisms in marine ecosystems, such as for stock assessment. However, the path from detecting occurrence of an organism to quantitative estimates is long and indirect, not least as eDNA concentration depends on several physical, chemical and biological factors which influence its production, persistence and transport in marine ecosystems. Here, we provide an overview of basic principles in relation to eDNA analysis with potential for marine fisheries application. We describe fundamental processes governing eDNA generation, breakdown and transport and summarize current uncertainties about these processes. We describe five major challenges in relation to application in fisheries assessment, where there is immediate need for knowledge building in marine systems, and point to apparent weaknesses of eDNA compared to established marine fisheries monitoring methods. We provide an overview of emerging applications of interest to fisheries management and point to recent technological advances, which could improve analysis efficiency. We advise precaution against exaggerating the present scope for application of eDNA analysis in fisheries monitoring, but also argue that with informed insights into strengths and limitations, eDNA analysis can become an integrated tool in fisheries assessment and management.

KEYWORDS
commercial fisheries, environmental DNA, fisheries management, marine conservation, marine monitoring

1 | INTRODUCTION

Several hundred marine fish stocks are exploited by large-scale commercial fisheries, and collectively, these stocks provide a major food resource whose continued societal value is dependent on sustainable fisheries management (FAO, 2016). Stock assessment is commonly an integral part of fisheries management. However, robust assessment remains challenging because stock dynamics may be inherently unpredictable by nature but also because reliable estimates of

population sizes and stock structure are often difficult to obtain with current monitoring methods. For a range of fishes, stock assessment is tuned with continuous large-scale fisheries independent monitoring programmes, which are both time-consuming and involve expensive ship-borne surveys (Biber, 2011). Further, presently applied monitoring approaches are generally invasive, selective and rely on some degree of subjectivity related to the taxonomic expertise of the monitoring personnel. This is problematic due to a general decline in taxonomic expertise and related difficulties associated with correct species identification especially across egg and juvenile life stages (Daan, 2001; Fischer, 2013). Developing more reliable and cost-efficient methods for monitoring commercial fish stocks can hence improve stock assessment.

In recent years, the application of environmental DNA (eDNA) analysis has introduced a new paradigm in relation to how surveys of marine macro-organisms can be conducted, that is without observing the organism itself. eDNA is defined here as the genetic material obtained from a water sample containing no distinguishing signs of source macro-organisms. The method utilizes DNA which is continuously excreted by organisms into the surrounding environment, and captures, analyses and obtains the nucleotide sequence of this DNA based on an environmental sample, for example from water, air or soil (Pedersen et al., 2014; Rees, Maddison, Middleditch, Patmore, & Gough, 2014). As all organisms continuously shed DNA through their metabolic waste products (and gametes), the method has the potential to objectively identify either individual species using quantitative real-time polymerase chain reaction (qPCR); or entire biological communities across taxonomic groups using next generation sequencing platforms (NGS) (e.g., Kelly, Port, Yamahara, & Crowder, 2014; Miya et al., 2015; Port et al., 2015). Moreover, species-specific DNA concentrations have been shown to be positively correlated with biomass and abundance (Doi et al., 2015; Maruyama, Nakamura, Yamanaka, Kondoh, & Minamoto, 2014; Takahara, Minamoto, Yamanaka, Doi, & Kawabata, 2012), thus pointing to a large potential for many different quantitative monitoring applications. The collection and analyses of water samples for eDNA has in many cases been shown to be a cost-effective, sensitive and non-invasive method for presence/absence surveys of species, in contrast to established monitoring techniques relying on catching whole organisms (Davy, Kidd, & Wilson, 2015; Sigsgaard, Carl, Møller, & Thomsen, 2014; Turner, Miller, Coyne, & Corush, 2014). Fish is presently the most studied group of organisms with respect to eDNA, including surveys conducted in freshwater ponds and lakes (e.g., Sigsgaard et al., 2014; Takahara et al., 2012), running waters (e.g., Deiner & Altermatt, 2014; Jane et al., 2014) and the sea (Figure 1 and Table 1). Thus, eDNA analysis has rapidly developed into a promising monitoring and assessment tool not only for fish but across a large variety of organisms in both freshwater and marine ecosystems (Roussel, Paillisson, Tréguier, & Petit, 2015). The many potential applications, cost-effectiveness and apparent simplicity have in a very short time made eDNA analysis exceedingly popular in both the scientific community and among managers. The rapid uptake of eDNA analysis into many fields of aquatic monitoring has led to the suggestion that eDNA may also be a valuable substitute or supplement to established stock assessment of commercial fish (Mauvisseau et al., 2016; Thomsen et al., 2016). Accordingly, many institutions and managers responsible for commercial fish stock monitoring will soon be faced with deciding on whether to implement eDNA analysis in resource assessment or not. Despite the general attractiveness of eDNA analysis, the level of knowledge about the approach and its limitations is generally highly restricted beyond experienced practitioners within the research field. To provide a conceptual overview of the prospects and potential pitfalls of eDNA analysis in fisheries, we here outline how eDNA is generated, decays and is transported in aquatic environments. We contrast the information generated by application of eDNA with established fisheries monitoring approaches and highlight the five most important challenges related to interpreting quantitative analysis of commercial marine fish eDNA and outline

the factors that need to be considered when interpreting eDNA results. Finally, we provide an outlook on specific novel technical applications of interest and highlight perspectives for their future application in eDNA analyses for fisheries monitoring. This critical synthesis of the field of marine eDNA was put together using literature from both marine and freshwater eDNA research. Still we have kept the focus exclusively on marine applications, as there is a strong need to inform researchers, advisors, managers and other stakeholders about the many challenges (and opportunities) related to implementation of eDNA analyses in routine marine fisheries management. In addition, freshwater fish applications are generally inherently different from marine studies with respect to the natural setting and focus. Our search of literature has been conducted using Elsevier's Scopus and Thomson Reuters' Web of Science, scientific forums on eDNA and including references within identified papers of relevance. Specifically for the marine eDNA references we conducted a search 30th of November 2017 using the search terms "environmental DNA" OR "eDNA" OR "metabarcoding" AND "Marine" OR "brackish" on Scopus and Web of Science. Most published studies are recent and geographically biased towards Europe and North America with some studies from Japan and Australia (Figure 1 and Table 1). This pattern illustrates that marine eDNA research is still in its infancy, but that many new studies are expected to be published in the near future.

2 | WHEN A SIMPLE METHOD BECOMES COMPLEX

As organisms interact with the environment, they continuously shed DNA occurring either as extracellular molecules, free in solution or bound to particles, or as intracellular molecules residing inside cells (Turner, Barnes et al., 2014). For simplicity, the term "eDNA particles" will be used throughout to refer to all of the above states. The quantity of eDNA particles present in a given environmental sample is controlled by three processes: (1) organismal production rate, (2) degradation rate and (3) physical transport. The relative rate of each of these processes in a given environment determines how long DNA molecules from a particular organism will stay detectable in the local environment. Despite many examples that demonstrate efficacy of eDNA-based monitoring, few studies have taken rates of production, degradation and bulk transport into consideration and when they do, usually only focus on one or a few of these factors (Dejean et al., 2011; Pilliod, Goldberg, Arkle, & Waits, 2014; Sigsgaard et al., 2016). However, without considering environmental effects on the production, persistence and transport of eDNA, especially in marine ecosystems, it may be difficult to establish robust and reliable temporal and spatial relationships between recorded DNA and qualitative/quantitative monitoring data (Figure 2) (Sassoubre, Yamahara, Gardner, Block, & Boehm, 2016).

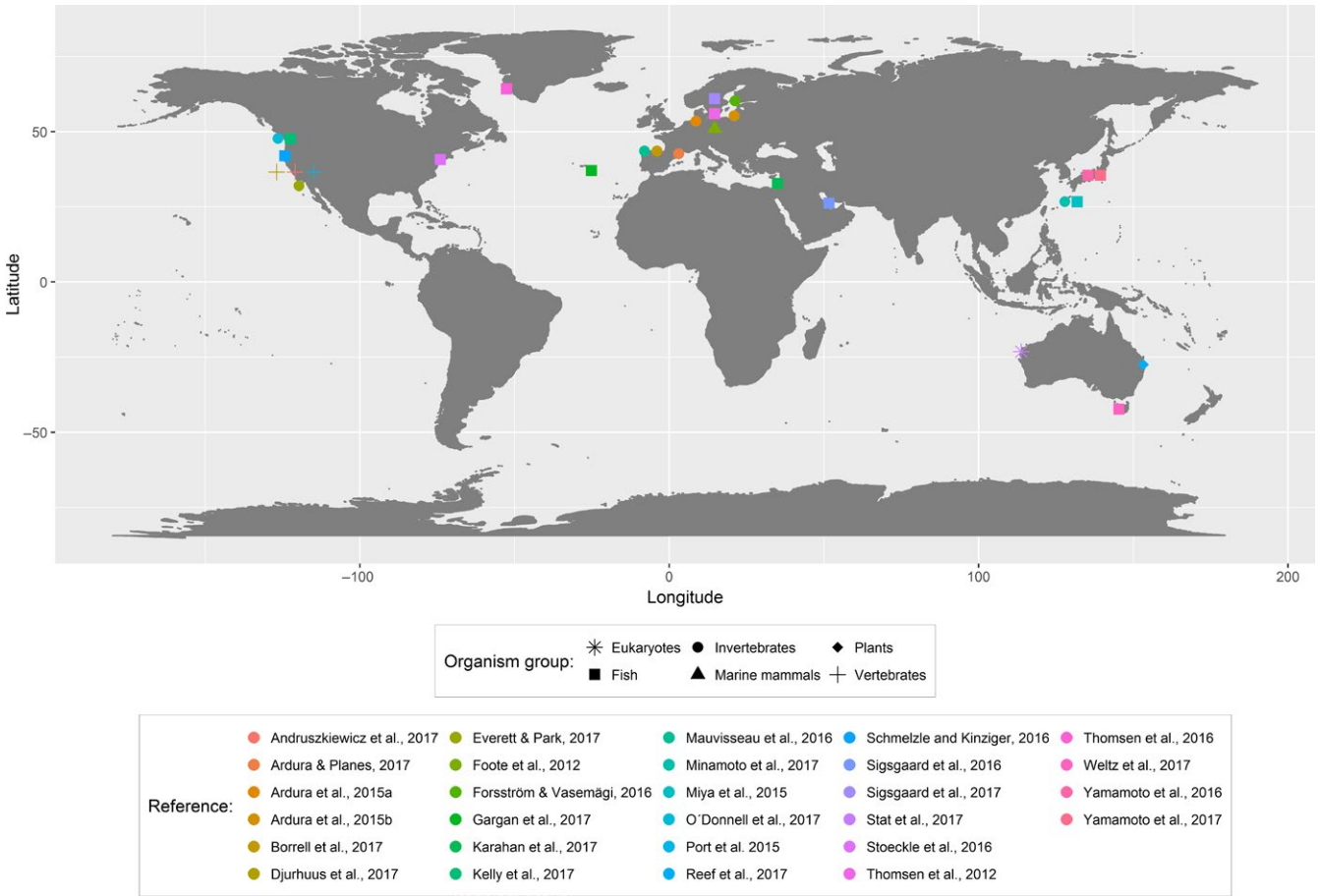

FIGURE 1 Geographical distribution of marine eDNA studies

TABLE 1 Marine eDNA studies

References	Title	Targeted organisms	Geographical location (country)
Andruszkiewicz et al. (2017)	Biomonitoring of marine vertebrates in Monterey Bay using eDNA metabarcoding	Vertebrates	USA (Pacific Ocean)
Ardura, Zaiko, Martinez, Samulioviene, Semenova, et al. (2015)	Environmental DNA evidence of transfer of North Sea molluscs across tropical waters through ballast water	Invertebrates	International
Ardura, Zaiko, Martinez, Samuiloviene, Borrell, et al. (2015)	eDNA and specific primers for early detection of invasive species—A case story on the bivalve *Rangia cuneata*, currently spreading in Europe	Invertebrates	Baltic Sea (Russia)
Ardura and Planes (2017)	Rapid assessment of non-indigenous species in the era of the eDNA barcoding: A Mediterranean case study	Invertebrates	Mediterranean Sea (France)
Borrell, Miralles, Do Huu, Mohammed-Geba, and Garcia-Vazquez (2017)	DNA in a bottle—Rapid metabarcoding survey for early alerts of invasive species in ports	Invertebrates	Atlantic Ocean (Spain)
Djurhuus et al. (2017)	Evaluation of filtration and DNA extraction methods for environmental DNA biodiversity assessments across multiple trophic levels	Vertebrates	USA (Pacific Ocean)
Everett and Park (2017)	Exploring deep-water coral communities using environmental DNA	Corals	USA (Pacific Ocean)
Foote et al. (2012)	Investigating the potential use of environmental DNA (eDNA) for genetic monitoring of marine mammals	Marine mammals	Oresund (Denmark)
Forsström and Vasemägi (2016)	Can environmental DNA (eDNA) be used for detection and monitoring of introduced crab species in the Baltic Sea?	Invertebrates	Baltic Sea (Finland)
Gargan et al. (2017)	Development of a sensitive detection method to survey pelagic biodiversity using eDNA and quantitative PCR: A case study of devil ray at seamounts	*Mobula tarapacana*, Mobulidae (Ray)	Atlantic Ocean (Azores)
Karahan et al. (2017)	Employing DNA barcoding as taxonomy and conservation tools for fish species censuses at the southeastern Mediterranean, a hot-spot area for biological invasion	Fish	Mediterranean Sea (Israel)
Kelly et al. (2017)	Genetic and manual survey methods yield different and complementary views of an ecosystem	Fish	Puget Sound (USA)
Mauvisseau et al. (2016)	On the way for detecting and quantifying elusive species in the sea: The *Octopus vulgaris* case study	*Octopus vulgaris*, Octopodidae (Octopus)	Cantabrian Sea (Spain)
Minamoto et al. (2017)	Environmental DNA reflects spatial and temporal jellyfish distribution	*Chrysaora pacifica*, Pelagiidae (Jellyfish)	Sea of Japan (Japan)
Miya et al. (2015)	MiFish, a set of universal PCR primers for metabarcoding environmental DNA from fishes: detection of more than 230 subtropical marine species	Fish	Sea of Japan (Japan)
O'Donnell et al. (2017)	Spatial distribution of environmental DNA in a nearshore marine habitat	Metazoans	Puget Sound (USA)
Port et al. (2015)	Assessing vertebrate biodiversity in a kelp forest ecosystem using environmental DNA	Vertebrates	Pacific Ocean (USA)
Reef et al. (2017)	Using eDNA to determine the source of organic carbon in seagrass meadows	Plants	Coral Sea (Australia)
Schmelzle and Kinziger (2016)	Using occupancy modelling to compare environmental DNA to traditional field methods for regional-scale monitoring of an endangered aquatic species	Fish	Pacific Ocean (USA)

(Continues)

TABLE 1 (Continued)

References	Title	Targeted organisms	Geographical location (country)
Sigsgaard et al. (2016)	Population characteristics of a large whale shark aggregation inferred from seawater environmental DNA	Fish	Arabian Gulf (Qatar)
Sigsgaard et al. (2017)	Seawater environmental DNA reflects seasonality of a coastal fish community	Fish	Oresund (Denmark)
Stat et al. (2017)	Ecosystem biomonitoring with eDNA: metabarcoding across the tree of life in a tropical marine environment	Eukaryotes	Australia (Indian Ocean)
Stoeckle et al. (2017)	Aquatic environmental DNA detects seasonal fish abundance and habitat preference in an urban estuary	Fish	Lower Hudson River estuary (USA)
Thomsen et al. (2012)	Detection of a diverse marine fish fauna using environmental DNA from seawater samples	Fish	Oresund (Denmark)
Thomsen et al. (2016)	Environmental DNA from seawater samples correlate with trawl catches of subarctic, deepwater fishes	Fish	Davis Strait (Greenland)
Weltz et al. (2017)	Application of environmental DNA to detect an endangered marine skate species in the wild	*Zearaja maugeana*, Rajidae (Skate)	Australia (Indian Ocean)
Yamamoto et al. (2016)	Environmental DNA as a "snapshot" of fish distribution: A case study of Japanese jack mackerel in Maizuru Bay, Sea of Japan	Fish	Sea of Japan (Japan)
Yamamoto et al. (2017)	Environmental DNA metabarcoding reveals local fish communities in a species-rich coastal sea	Fish	Sea of Japan (Japan)

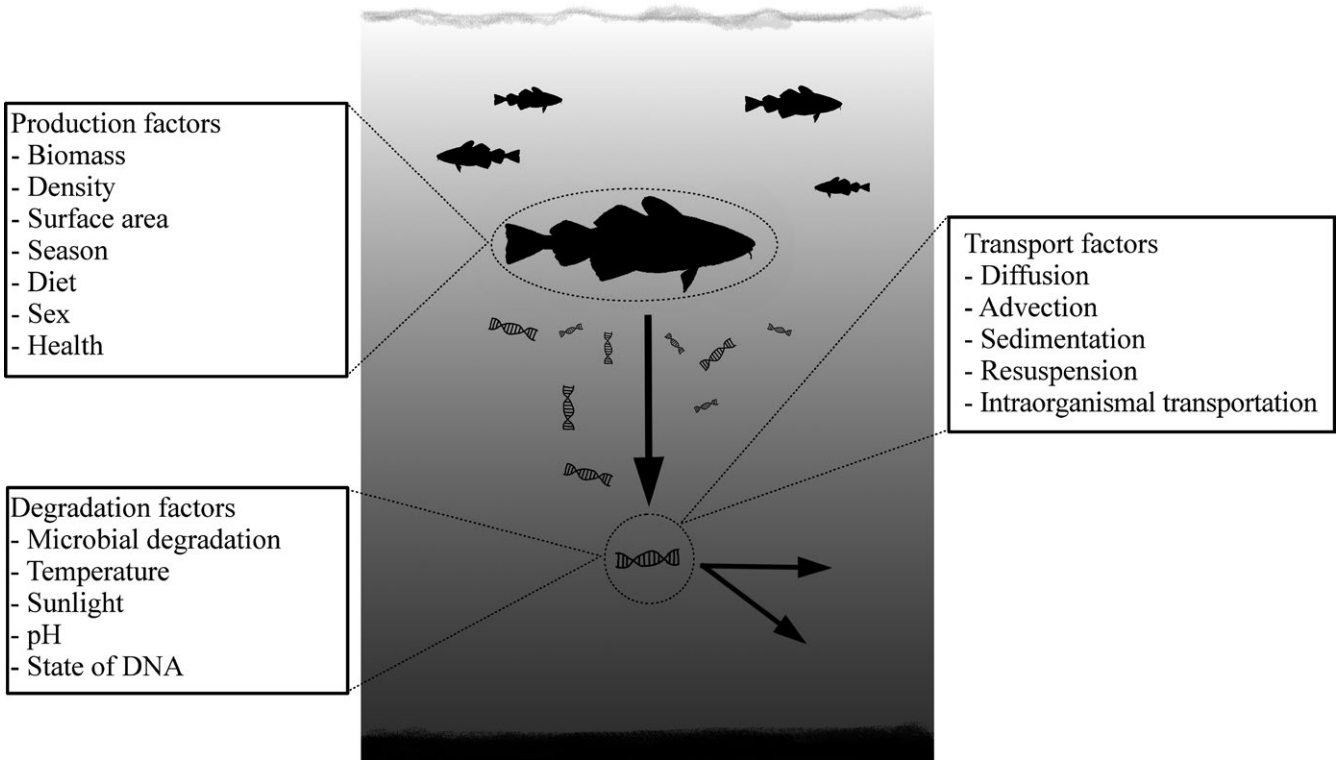

FIGURE 2 Conceptual diagram of factors likely to influence eDNA particle production and removal processes from a given water body

2.1 | Production

The exact origin and the relative amount of different metabolic sources of eDNA particles are largely unknown. There are multiple potential sources of eDNA particles from fish, including faeces, mucus, scales, tissue, gametes and other biological material (Alasaad et al., 2011; Livia, Antonella, Hovirag, Mauro, & Panara, 2006; Merkes, Mccalla, Jensen, Gaikowski, & Amberg, 2014; Pompanon et al., 2012). Klymus, Richter, Chapman, and Paukert (2014) found that fed, as opposed to non-fed, freshwater fish excrete more eDNA in two species of carp (*Hypophthalmichthys* spp., Cyprinidae), suggesting that large amounts of eDNA particles are shed from the gut lining. This finding coincides with the fact that the gastrointestinal tract is the largest external body surface facing the environment and its epithelial cells have the fastest renewal rate of all tissues in the vertebrate body (Crosnier, Stamataki, & Lewis, 2006; Helander & Fändriks, 2014). The eDNA particle production rate from individual macro-organisms are also affected by a variety of factors such as the size of the individual (Maruyama et al., 2014), biomass/density (Doi et al., 2015; Maruyama et al., 2014), diet (Klymus et al., 2014), health status (Pilliod, Goldberg, Arkle, Waits, & Richardson, 2013), species (Minamoto et al., 2017; Sassoubre et al., 2016; Tréguier et al., 2014), season (Spear, Groves, Williams, & Waits, 2014), and potentially sex. Further, changes in biotic and abiotic factors can potentially, directly or indirectly, through complex interactions, affect shedding rates due to changes in stress, metabolism, behaviour or health of the source organism. Environmental changes include variations in water oxygen content (Herbert & Steffensen, 2005) and temperature (Schurmann & Steffensen, 1997), which are known to cause physiological changes and could thus lead to altered particle production rates. Of such effects, only temperature variance has been assessed, with differing results. Takahara et al. (2012) and Klymus et al. (2014) found no significant relationship between temperature and eDNA shedding rates, where Lacoursière-Roussel, Rosabal, and Bernatchez (2016) found that a temperature of 14°C, as opposed to 7°C, increases eDNA shedding which in turn improved the ability to predict abundance and biomass of the freshwater species brook charr (*Salvelinus fontinalis*, Salmonidae). There is thus still limited understanding of how eDNA production co-varies with parameters such as temperature, and to which extent such variation needs to be accounted for in eDNA analyses.

2.1.1 | The geometric and metabolic challenge

Fish vary in size, age and life-stage and different individuals of the same species are therefore expected to generate highly variable outputs in terms of eDNA shedding, potentially influencing the interpretation of subsequent analytical results (Kelly, Port, Yamahara, & Crowder, 2014; Klymus et al., 2014; Maruyama et al., 2014). An important aspect is the total organismal surface area in direct contact with the external environment. As the total exposed body surface in direct contact with the environment is much larger for a group of small fish than for a single big fish of the same total biomass. The group of small fish will likely collectively shed more DNA than a single big fish (Kelly, Port, Yamahara, & Crowder, 2014), hampering inference about relationships between eDNA and biomass. The geometric aspect is also closely linked to the metabolic rate, which varies at specific size and life stages. A generalized principle states that the metabolic rate is proportional to body mass raised to the three-quarter power ($M^{0.75}$), which is assumed for a wide array of organisms, from single-celled to multicellular homoeothermic organisms (Clarke & Johnston, 1999; Gillooly, Brown, West, Savage, & Charnov, 2001). Consequently, small organisms have a relatively higher metabolic rate per unit bodyweight than larger organisms. Therefore, a number of small fish with the same total biomass as one large fish are expected to shed more eDNA particles (Maruyama et al., 2014). However, in some juvenile and larval stages of fish the general relationship between size and metabolic rate is mass-independent, which potentially further complicates attempts to relate quantities of metabolic waste, including eDNA, with numbers or biomass of fish (Post & Lee, 1996). Maruyama et al. (2014) hypothesized that eDNA shedding rate is likely to be a function of the developmental stage, and not just biomass. They compared eDNA shedding rates per body weight for juveniles and adults of the freshwater species bluegill sunfish (*Lepomis macrochirus*, Centrarchidae) and found that the eDNA shedding rate per unit body weight was almost four times higher in juveniles than in adults in this freshwater species. Using weight data from Maruyama et al. (2014) and the metabolic model described above, we estimated the theoretical relative metabolism and found that the juvenile group would have had a 2.0–3.5 times higher relative metabolism than the adult group. This is compatible with the 3–4 times difference in eDNA excretion rates found in their study. Hence, although our result is merely suggestive, it remains clear that neglecting relationships between eDNA shedding and size and metabolic parameters can lead to erroneous estimation of quantities of fish, especially in assemblages consisting of both juveniles and adults.

2.2 | Degradation

As soon as an eDNA particle is released into the environment it starts to degrade. The temporal persistence of an eDNA particle is dependent both on its molecular state, that is free or encapsulated in a cell or mitochondria, as well as on external abiotic and biotic environmental factors. Abiotic environmental factors, such as temperature, solar radiation and pH, cause large variation in the persistence of eDNA particles (Strickler, Fremier, & Goldberg, 2014). Temperature is generally one of the most influential factors, where a reduction in temperature can extend the preservation of eDNA particles from a couple of days in temperate aquatic ecosystems to hundreds of thousands of years under permafrost (Corinaldesi, Beolchini, & Dell'Anno, 2008; Minamoto et al., 2017). An illustrative study by Strickler et al. (2014) found evidence that a temperature of 5°C, compared with 25 and 35°C, increased the persistence time of eDNA particles from 10 days up to 53 days and decreased the decay rate more than fourfold. Several studies have assessed effects of

solar radiation on eDNA particles and some report that increased exposure can have negative effects on the persistence of eDNA particles, whereas others have found little or no effect (Andruszkiewicz et al., 2017; Barnes et al., 2014; Pilliod et al., 2014; Sigsgaard et al., 2016; Strickler et al., 2014). Finally, no clear relationship has been found between pH and the persistence of eDNA particles. However, Strickler et al. (2014) did find that the mean degradation rate was higher at pH 4 as opposed to pH of 7 and 10 (Strickler et al., 2014). In general, the environmental conditions where eDNA particles in aquatic environments seem to be best preserved are cold, alkaline and without exposure to solar radiation (Pilliod et al., 2014; Strickler et al., 2014). This also illustrates that the influence of, for example, depth, season and water chemistry should be taken into account for marine eDNA-based surveys as the persistence of eDNA particles may vary significantly across strata. Furthermore, abiotic factors can have complex interactions with biotic factors, for example by indirectly increasing degradation rate by providing favourable conditions for biota (Barnes et al., 2014; Strickler et al., 2014).

The biotic mediated decay conducted by microbial communities in marine ecosystems play a large part in the turnover of DNA, a process known as natural transformation (Lorenz & Wackernagel, 1994; Pietramellara et al., 2009). A broad variety of microorganisms in marine ecosystems have the physiological ability to take up DNA during normal growth, including both free extracellular DNA, DNA associated with particles, cellular debris, inactivated and even living cells (Paul, Jeffrey, & DeFlaun, 1987; Pietramellara et al., 2009). It is expected that biotic degradation will have some influence on the degradation of eDNA particles, but so far no clear correlation between microbial communities or abundance and eDNA degradation has been found (Baerwaldt et al., 2014; Barnes et al., 2014; Tsuji, Ushio, Sakurai, Minamoto, & Yamanaka, 2017). Barnes et al. (2014) found that the degradation rate of eDNA particles declined with increased physiochemical factors associated with high microbial activity (i.e., oxygen demand, chlorophyll and total eDNA). The degradative effect of microorganisms was suggested to be outweighed by a decrease in DNA degradation by solar radiation due to increased algal density. This illustrates that eDNA degradation processes are interconnected and complex in nature.

The persistence time of suspended eDNA particles can thus be highly variable and has been reported ranging from 1 to 58 days (Pilliod et al., 2014; Strickler et al., 2014). However, most suspended eDNA particles are not expected to last more than a couple of weeks, illustrating that in most cases eDNA analysis can be used as a fairly contemporary proxy of presence/absence on both spatial and temporal scales (Sigsgaard et al., 2016; Strickler et al., 2014; Thomsen et al., 2012). Molecular decay of eDNA has been investigated by measuring persistence time of eDNA after removal of the target organism or by modelling using an exponential decay model, allowing estimation of decay rates (e.g., Barnes et al., 2014; Thomsen et al., 2012). eDNA persistence time, as opposed to a decay rate, is dependent on the starting concentration and on the sensitivity of the detection methodology. Therefore, persistence time estimates cannot be compared among studies. Instead, we generally encourage reporting DNA concentration data from persistence studies and to refer estimates of decay rates rather than persistence times. In brackish and marine environments persistence time have been found to be relatively shorter than in freshwater, with eDNA falling below limit of detection after 0.9 days (*Platichthys flesus*, Pleuronectidae) (Thomsen et al., 2012), 6.9 days (*Gasterosteus aculeatus*, Gasterosteidae) (Thomsen et al., 2012), 4 and 7.8 days (*Rhincodon typus*, Rhincodontidae) (Sigsgaard et al., 2016), 3–4 days (*Engraulis mordax*, Engraulidae; *Sardinops sagax*, Clupeidae and *Scomber japonicas*, Scombridae) (Sassoubre et al., 2016) and <1–2.5 days (*Zearaja maugeana*, Rajidae) (Weltz et al., 2017). Decay rates show that there is a 1.5%–10.1% reduction in numbers of eDNA particles per hour in brackish and marine environments across several species of fish and one jellyfish (3% per hour) (Minamoto et al., 2017; Sassoubre et al., 2016; Thomsen et al., 2012). In comparison, freshwater studies have generally found longer persistence times for a range of organisms, for example 21–44 days (Goldberg, Sepulveda, Ray, Baumgardt, & Waits, 2013), 8–11 days (Pilliod et al., 2014), 14 days (Dejean et al., 2011) and 25 days (Dejean et al., 2011), but at a similar pace, at 5.1%–15% reduction of eDNA particles per hour (Eichmiller, Best, & Sorensen, 2016; Maruyama et al., 2014). It is, however, noteworthy that the current literature generally find higher persistence times in freshwater than in brackish or marine environments. We hypothesize that some of this difference potentially could be due to the difference in environmental factors or osmoregulation between fresh- and marine water species but more research is needed to support this hypothesis.

2.3 | Transport

Marine ecosystems constitute the most challenging aquatic environments for eDNA-based monitoring. This is not only due to high diversity in biotic and abiotic conditions affecting production and decay of eDNA, but also to the combined effects of current systems, tides and the immense dispersion through dilution which constantly removes particles from their source. In the sea, eDNA particles have been estimated to potentially travel more than 600 km in 1 week, based on an average current velocity and a measured maximum particle life time of 7 days (Thomsen et al., 2012). This combination of persistence time in the environment and ocean transport make the distribution and spread of eDNA particles highly variable and dependent on the specific biological and chemical composition of the water and pace of transport by the currents (Barnes et al., 2014; Pilliod et al., 2014; Strickler et al., 2014). eDNA particles in coastal regions are likely to be less affected by physical processes due to, for example, coastal morphology or retentive properties of seaweed which reduce advection and diffusion, but will likely be highly variable depending on the area specific oceanographic regime. Moreover, the majority of eDNA particles are too large (>1 μm) to remain suspended and will therefore be transported downwards by gravity, with a velocity depending on the density of the individual particle. Present studies of Common carps (*Cyprinus carpio*, Cyprinidae) in lakes and ponds have shown that the diameter of eDNA particles span from <0.2 μm to 180 μm< in diameter, with the highest

concentration (~40%–60%) in the size fraction 1–10 μm (Turner, Barnes et al., 2014; Turner, Uy, & Everhart, 2014).

3 | CHALLENGE I: CAN WE FIND WHAT WE ARE LOOKING FOR?

Studies of eDNA has shown overwhelming success in obtaining DNA from hundreds of species simultaneously and at the same time there is good concordance between eDNA surveys and established survey methods in terms of the species detected (e.g., O'Donnell, Kelly, & Lowell, 2017; Port et al., 2015; Thomsen et al., 2016). However, we encourage that more focus should be put on which species was not detected (false negatives) or more importantly, why they are not detected. This is especially important now as eDNA is slowly moving away from comparative studies where the eDNA approach is simultaneously compared to established methods. For example, Thomsen et al. (2012) found that eDNA outperform or perform equally well in terms of species richness estimates when compared to established survey methods. Surprisingly, their eDNA analysis did not detect any *Gobidae* spp. which are common in the study area and recorded using other survey methods. Such false-negative observations are general for eDNA analyses, and it is important to emphasize that detection is not only determined by the biology of the study organisms or environmental factors but also technical factors predominately related to metabarcoding approaches, see Textbox 1 (Everett & Park, 2017; Kelly, Port, Yamahara, & Crowder, 2014; Thomsen et al., 2016). These technical challenges illustrate that eDNA is not always a more objective approach than relying on "subjective" taxonomic expertise. Another general challenge related to eDNA species detection is the expectation that a water sample is a local contemporary snapshot of the presence of a single species or community of species. In other word, which spatial scale does a water sample represent? If eDNA is removed rapidly, due to transport or degradation, then organisms will be harder to detect increasing false negatives, but provide a more contemporary estimate. In contrast, slow removal will allow transport and detection of non-local and non-contemporary eDNA, that is false positives. This is just an example of why rates of degradation, production, transport and biology of the study organism needs to be considered for species detection. The issue of general detectability has been illustrated by Moyer, Díaz-Ferguson, Hill, and Shea (2014) who sampled four 189 m^3 mesocosms with different fish densities. Even with a relatively high fish density of 0.32 g fish/m^3, they estimated sample volume should be above 100 L to ensure more than 95% probability of detecting the fish present. This water volume-to-biomass ratio is considerably smaller for fish in marine environments, compared to fish densities in lentic and lotic environments (Moyer et al., 2014). Thus, all else being equal the probability of detecting a fish using eDNA in a marine ecosystem will likely be smaller than in freshwater systems. The effect of the low fish density in marine systems will not only challenge presence/absence assessments, but also, and in particular, estimation of quantitative relationships between eDNA and biomass or abundance due to quantitative limitations of the molecular techniques used at low eDNA concentrations (Kelly, Port, Yamahara, Martone, et al., 2014; Tréguier et al., 2014).

TEXTBOX 1 OVERVIEW OF MOLECULAR TOOLS USED IN EDNA

Molecular tools used to survey fish with eDNA

Currently, two techniques are predominantly used to detect eDNA from a target species: qPCR and metabarcoding using next-generation sequencing (NGS). Both approaches utilize PCR to enrich the concentration of target DNA templates in a given sample.

qPCR

Uses short DNA molecules (two primers and a fluorescent-labeled probe—combined known as an assay) that bind specifically to a complementary DNA strand of the target species. The probe consists of a fluorophore and quencher and binds between the two primers on the template strand. When the polymerase extends from the forward primer to the probe, it degrades the probe, separating the quencher and fluorophore, which cause emission of light. The fluorescence detected from the fluorophore is directly proportional to the amount of DNA template present in the sample. Quantification is conducted by comparing the unknown sample to standards of known DNA copy numbers. The same principal applies for digital droplet PCR (ddPCR) which is a technological development of qPCR utilizing another method for calculating quantity, but without the need for a standard curve.

NGS

Metabarcoding by NGS utilizes taxonomically broad universal primers which bind to (capture) DNA from all organisms whose DNA sequence match sufficiently with the primer sequences, and these primers often have broad taxonomic coverage, thus being vertebrate, invertebrate or plant specific. After binding, the DNA is amplified and the DNA sequences from all captured DNA molecules are obtained. These DNA sequences can then be compared with local or global DNA sequence databases and be assigned to lowest taxonomic unit of certainty; either to a specific species or some higher taxonomic level, depending on information content. There is also an increased interest in using PCR-free, also known as direct sequencing, NGS methods to sequence all DNA directly and bypass potential PCR amplification biases, which can obscure results (e.g., Miya et al., 2015; Thomsen et al., 2016). These methods are, however, hampered presently because the vast majority of the DNA in water originates from prokaryotes. Hence, finding the rare eukaryotic targets of interest is not yet cost-competitive as opposed to "traditional" amplicon-based metabarcoding (Stat et al., 2017).

4 | CHALLENGE II: WHAT IS THE SPATIAL ORIGIN OF EDNA?

One of the most fundamental questions, for eDNA-based species detection, is where the individual that shed the eDNA was located. Thus, is the DNA of local origin or has it been transported to the sampling location by currents? If physical transport, rather than local eDNA production, is the main factor responsible for eDNA distribution and abundance, particle transport would act to decouple inferences on presence/absence data from eDNA measurements and established observational surveys. However, both coastal (e.g., Andruszkiewicz et al., 2017; O'Donnell et al., 2017; Port et al., 2015; Thomsen et al., 2012; Yamamoto et al., 2017) and open ocean (Gargan et al., 2017; Sigsgaard et al., 2016; Thomsen et al., 2016) eDNA surveys report good concordance with established surveys in terms of local species detection. A study by Port et al. (2015) found concordance between eDNA and visual surveys of vertebrate communities, and also found that eDNA was able to distinguish vertebrate community assemblages in open marine habitats separated by <60 m. This and other studies suggest that local production and persistence of eDNA, in these specific cases, seem to be more important than overarching physical transport processes (O'Donnell et al., 2017; Port et al., 2015). Localized eDNA distribution in the ocean may be due to a general fast eDNA degradation, but could also be caused by fast dilution below detectable levels as DNA is transported away from the source. The dilution relates to the water volume-to-biomass ratio mentioned above. The effect of dilution is illustrated in a study by Foote et al. (2012) who found that the detection probability of marine eDNA particles from a small and relatively rare whale, the harbour porpoise (*Phocoena phocoena*, Phocoenidae) rapidly decreased with distance from the source. Three samples taken from inside an enclosure with the source organism all successfully detected its DNA, but beyond 10 m from the enclosure, only one of three samples was positive. These examples illustrate that local processes are important for eDNA detection in marine environments but also show that the large dilution factor and eDNA retention is likely to be a challenge in relation eDNA detection and quantification in marine ecosystems.

5 | CHALLENGE III: RELATIONSHIP BETWEEN EDNA AND BIOMASS/NUMBERS

Quantification of fish and other aquatic organisms is a central component of current fish stock assessment to facilitate timely and accurate fisheries management. Accordingly, there are strong incentives to move eDNA analyses beyond mere presence/absence detection to quantitative estimates that reflect the present status of the targeted stocks in terms of numbers and/or biomass. Generating quantitative stock estimates from eDNA analysis relies on the assumption that estimated eDNA particle concentration is correlated with living biomass. Although multiple studies have identified a strong linear relationship in experimental aquaria and ponds (e.g., Doi et al., 2015; Klymus et al., 2014), the relationship is either less pronounced (Schmelzle & Kinziger, 2016; Thomsen et al., 2016; Yamamoto et al., 2016) or lacking (Spear et al., 2014) in natural ecosystems. As discussed above, several factors can obscure relationships between eDNA and biomass or abundance when moving from experimental setups into natural aquatic systems. One important aspect is the size structure of the local population. For example, quantitative eDNA estimates are expected to vary between a school of 1,000 adult fish with a total biomass of 1 ton and a larval aggregation of the same total weight, consisting of a million individuals. Conservatively assuming that each larva sheds up to four times more DNA to the environment per unit body weight, this would lead to highly divergent estimates of biomass for adult versus larval aggregations (Maruyama et al., 2014). In natural situations, a given fish stock or population will typically consist of a mixture of small and large individuals in variable proportions, where indices for, for example, smaller juveniles and larger adults would constitute key stock assessment parameters, respectively, reflecting recruitment and spawning biomass indices. Therefore, generating an overarching stock estimate from eDNA, without knowing the proportions of small and large fish would in most cases not provide data of sufficient resolution for proper management under the current paradigm. Thus, obtaining knowledge of the size and age structure of the target species in the sampling area is still essential. Even without taking body geometrics or environmental factors into account, studies have found large interspecific variation in eDNA shedding among tank replicates of equally sized individuals (Minamoto et al., 2017; Pilliod et al., 2014). Klymus et al. (2014) found up to a 100-fold variation in day-to-day eDNA shedding from the same individual. Individual variation in eDNA shedding has fuelled a discussion of the overall predictability of quantitative eDNA-based analysis in aquatic environments and the often overly optimistic subsequent eDNA data processing and presentation (see discussions in Klymus et al. (2014) and Iversen, Kielgast, and Sand-Jensen (2015)). Iversen et al. (2015) argue that without the possibility to robustly measure shedding rates of individual fish, the conditional effects on eDNA content of, for example, temperature, food and biomass cannot be reliably estimated. Further, Sassoubre et al. (2016) published a conceptual mass-balance model taking both eDNA shredding and environmental effects into consideration to show how these processes interact to give widely disparate quantitative estimates on the number of fish present. Due to this natural complexity we believe that direct quantitative assessments will remain highly challenging in marine ecosystems under the existing analytical paradigm. However, an improved understanding of fundamental biological and environmental processes related to eDNA and development of a statistical modelling framework as for current stock assessment, quantification may become more feasible in future.

6 | CHALLENGE IV: APPLICATION TO FISHERIES MANAGEMENT

Analysis of eDNA has shown its primary strength in relation to presence/absence assessment of species, particularly for rare or elusive

species, which are otherwise hard to detect using visual methods alone (Mächler, Deiner, Steinmann, & Altermatt, 2014; Piaggio et al., 2014; Sigsgaard et al., 2014). A number of studies has compared eDNA analysis to established monitoring techniques, including angling, push net, fyke nets, gill net (Thomsen et al., 2012), visual surveys (e.g., Minamoto et al., 2017; Sigsgaard et al., 2017; Yamamoto et al., 2017), seines (Schmelzle & Kinziger, 2016; Thomsen et al., 2012), bottom trawl (e.g., Karahan et al., 2017; Stoeckle, Soboleva, & Charlop-powers, 2017; Thomsen et al., 2016), sonar (Yamamoto et al., 2016) and echo location (Foote et al., 2012). Direct comparisons of eDNA-based detection and established survey techniques demonstrated that most eDNA analyses either outperformed or did equally well as established methods in relation to the number of species detected (e.g., Schmelzle & Kinziger, 2016; Thomsen et al., 2012). However, in relation to fisheries applications, present eDNA analysis has several basic limitations compared to established monitoring methods, that is where the targeted species are captured whole and visually identified and inspected. First of all, as identified above, eDNA does not provide any direct information on the size and number of fish. Secondly, it does not provide any direct information on age, weight, life-stage or fecundity of the stock. Thus, as this information is crucial for established stock assessments to estimate productivity and reproductive capacity, eDNA analysis, as a standalone tool, cannot provide the information needed under the current stock assessment paradigm. Finally, as discussed above, mere eDNA species detection in an area does not necessarily only reflect local occurrence/abundance, but relies on spatial and temporal processes of eDNA production, persistence and transport at an often unknown scale (Jane et al., 2014; Roussel et al., 2015; Thomsen et al., 2012).

Further, it is also important to emphasize that neither eDNA nor established monitoring methods provide true species richness or abundance/biomass information. In fish stock assessment these issues are related to method selectivity and catchability. Where selectivity is the probability of catching specific individuals in a highly heterogeneous fish assemblage with individual traits (e.g., size, age, morphology and behaviour) with a particular gear type, while catchability is the relationship between how much is caught per unit effort and true abundance. It is vital to emphasize that both selectivity and catchability are of equal importance and applicability in eDNA-based analyses. Here, selectivity and catchability relate to catching eDNA rather than a whole fish, which can be challenging due to, for example, eDNA production differences in individual fishes, high decay rate or due to technical issues relating to sensitivity of DNA-based species detection technologies.

7 | CHALLENGE V: OTHER SOURCES OF EDNA IN FISHERIES APPLICATIONS

DNA analysis can reduce many of the drawbacks of established monitoring techniques, but also presents a unique set of challenges that must be recognized and addressed for implementation in marine monitoring (Merkes et al., 2014). As the performance of eDNA analysis is already being directly compared with established monitoring surveys, it is important to identify vectors of false positives originating from contamination by the use of fishing gear in close proximity or prior to water sampling (Baerwaldt et al., 2014; Merkes et al., 2014; Thomsen et al., 2016). The effect of widespread trawling activities within a fished area, where many tons of fish are dragged through the water for an extended period of time, can dramatically increase eDNA in the water, thus likely biasing quantitative estimates upwards and potentially decoupling presence/absence inference (Yamamoto et al., 2016). On the same note, even empty fishing gear is likely to contain DNA originating from slime, scales and other residues from previous catches. Furthermore, sediments have been shown to be massive reservoirs for eDNA particles, as the substrate adsorbs settled eDNA particles and delays the degradative processes (Eichmiller, Bajer, & Sorensen, 2014; Turner, Uy, et al., 2014). Turner, Uy, et al. (2014) found that adsorbed eDNA particles can persist up to five times longer and be up to 1,800 times more concentrated in the sediments than in suspension. This suggests that events such as bottom trawling or harsh weather conditions that disturb bottom sediments, can re-suspend eDNA particles and potentially bias biomass and density estimations or even reintroduce eDNA from species no longer present in the area (Jones, 1992). The largest effects are expected in relatively shallow coastal locations where the seafloor and water sampling sites are in close proximity. Another aspect is fish discards which presently still constitutes a large fraction of the total catches worldwide (Heath, Cook, Cameron, Morris, & Speirs, 2014). The presence of fish carcasses is a potential source of contamination as the method cannot differentiate between DNA from live or dead organisms. Merkes et al. (2014) assessed if carcasses from Silver carps (*Hypophthalmichthys molitrix*, Cyprinidae) could be a source of false positive detection and found that carcasses remained detectable in freshwater 28 days after introduction. The concentration and persistence of eDNA from these carcasses were also found to be enhanced by increased biomass and density. Intensively fished areas may contain large amounts of such decomposing discarded fish; however, not only whole dead fish constitute a source of contaminant eDNA. Yamamoto et al. (2016) suggested that fish markets and fish processing plants located in harbours are likely sources of eDNA to surrounding water bodies. Exactly how this issue should be resolved in practice for areas with high discards or runoffs from fish processing plants will depend on the study site, organism and sampling location. The contribution from carcasses is likely negligible in some cases, but more studies are needed to determine the magnitude of the problem.

8 | IMPROVING EDNA ANALYSES

Marine ecosystems are remarkably diverse, and biological, physical and chemical features vary significantly across space and time,

which will cause eDNA production, persistence and transport to vary extensively. However, many aspects of eDNA production, persistence and transport are only sparsely explored in general and especially in relation to marine environments and their organisms. The production of eDNA has in several studies been tied with metabolism (e.g., Goldberg, Pilliod, Arkle, & Waits, 2011; Lacoursière-Roussel et al., 2016; Takahara et al., 2012). However, presently, little research has been conducted to determine the direct relationship between metabolic rate and eDNA production. Insights into such relationships and associated variability can improve our understanding of the rate of eDNA production at specific body geometrics and life stages (Maruyama et al., 2014), which would be especially valuable for assessing timing and magnitude of, for example, larval production in spawning or retention areas. Another important factor that deserves additional research is the influence of the surrounding environmental conditions on the eDNA production by the source organism. Highly variable physical, chemical and biological factors will potentially influence eDNA shedding to a very large degree, as seen with food availability (Klymus et al., 2014) and temperature (Lacoursière-Roussel et al., 2016), potentially complicating broad temporal and spatial quantitative comparisons. Gaining knowledge of such processes can help to establish estimates of general relationships that can be applied to improve quantitative aspects of eDNA-based monitoring. The environmental diversity and dynamics in marine environments will also significantly vary the eDNA persistence time (Strickler et al., 2014). Therefore, more knowledge on which external drivers are primarily responsible for how long eDNA particles persist across diverse marine ecosystems will improve both qualitative and quantitative inferences from eDNA studies. A promising means to improve our understanding of the complex interactions and dynamics of eDNA in marine systems is to incorporate oceanographic modelling on eDNA transport and detection. Combining oceanographic modelling with eDNA persistence rates can allow researchers to backtrack the most likely spatial origin of eDNA and hereby improve the ability to interpret data (Díaz-Ferguson & Moyer, 2014; Kelly, Port, Yamahara, Martone, et al., 2014). A good outset can be to use rare sessile marine organisms (that constitute eDNA sources in known locations) as models, before trying to assess the dispersion patterns of eDNA from free-moving organisms, such as fish. However, such modelling would also require estimates of eDNA persistence and production rates. Presently, the best estimates of how environmental factors affect eDNA particles originate from experiments conducted in freshwater with freshwater organisms. To which extent these estimates are transferrable to specific marine ecosystems and organisms are not known (Minamoto et al., 2017; Strickler et al., 2014; Thomsen et al., 2012). Moreover, estimation of eDNA persistence time in aquatic environments may benefit from insights from parallel research relating to microbial pathogen and GMO detection in the environment (Barnes & Turner, 2015). Accordingly, research should focus more on understanding the basic processes of eDNA in marine environments, rather than the present focus on direct application.

9 | NOVEL EDNA FISHERIES AND MONITORING APPLICATIONS

Despite current uncertainties about the biology and ecology of eDNA in the marine environment, the prospects of the approach for monitoring marine environments are huge. Ten years after the pioneering study by Ficetola, Miaud, Pompanon, and Taberlet (2008) we are likely still only seeing the tip of the iceberg in relation to potential applications. Thus, there are many new and unexplored areas of interest to fisheries management and monitoring applications, including (1) ecosystem monitoring, (2) assessment of migration patterns and life-history events, (3) stock structure, (4) diet and processed fish product analysis.

9.1 | Ecosystem monitoring

Environmental DNA analysis can simultaneously generate richness and to some extent, with the caveats mentioned above, diversity estimates for a broad taxonomic range of organisms, which makes it particularly amendable and valuable for large-scale ecosystem monitoring and management approaches (Miya et al., 2015; Port et al., 2015; Yamamoto et al., 2017). Ecosystem-based management entails multispecies approaches, requiring managers to consider interactions of fished stocks with predators, competitors and prey, necessitating knowledge of the occurrence and abundance of several species. Presently, most of the distributional information available on marine species is from fish or shellfish of commercial value. In contrast, relatively little is known for many species in a given marine environment, even though they might be of fundamental importance for, for example, the productivity of fisheries targeting other species (Beaugrand, Brander, Alistair Lindley, Souissi, & Reid, 2003; Frank, Petrie, Shackell, & Choi, 2006; Thresher, Harris, Gunn, & Clementson, 1989). The application of eDNA-based analyses spanning multiple trophic levels, including fish, zoo- and phytoplankton, can potentially be an important means to link and understand trophic ecosystem interactions. A study by Sigsgaard et al. (2016) found a significant correlation (R^2 = .84) in eDNA concentration between whale shark (*R. typus*, Rhincodontidae) and its prey, mackerel tuna spawn (*Euthynnus affinis*, Scombridae), illustrating a potential for using eDNA as a tool to study predator-prey interactions. With a very broad taxonomic approach eDNA analysis can also inform about the general state of the ecosystem and possibly even provide proxies for ecosystem health based on biodiversity indicators (Bienert et al., 2012; Huver, Koprivnikar, Johnson, & Whyard, 2015). Further, a number of European Union member states have taken the first actions to implement eDNA in monitoring of indicator and invasive species in concordance with the Marine Strategy Framework Directive (Andersen et al., 2016; Bourlat et al., 2013; Walsh & Rhodes, 2016). Shipping is perhaps the most important artificial pathway in spreading of invasive species, as these provide a temporary suitable habitat in the ballast tanks or on the exterior of the ships (Ardura, Zaiko, Martinez, Samulioviene, Semenova, et al., 2015). Established approaches require numerous highly specialized

taxonomists to assess a survey site, for example ports, for occurrences of invasive species as these organisms span from phyto- and zooplankton to fish (Andersen et al., 2016). Accordingly, eDNA is especially valuable due to its sensitivity, broad taxonomic scope and non-specialized sampling technique. Further, in the coming years the time from sampling to result will drastically decrease, from months and days to hours (see Section below on "10.1") which is essential for some immediate mitigation measures.

9.2 | Assessment of migration and life-history events

The ability to quantify biomass precisely from eDNA particles in marine environments is currently challenging (see above). However, monitoring concentrations of eDNA may be applicable as an indirect indicator of population and life-history dynamics. Temporal and spatial shifts in species-specific eDNA concentrations has potential to be used for assessing migration patterns and timing of life-history events such as arrival at feeding/nursing grounds or spawning. Several studies have illustrated that temporal and spatial changes in marine environments can be detected with eDNA (e.g., Minamoto et al., 2017; Sigsgaard et al., 2017; Stoeckle et al., 2017). In particular, spawning events are expected to give rise to large fluctuations in eDNA concentration due to communal release of gametes, increased activity and congregation of often large numbers of individuals (Buxton, Groombridge, Zakaria, & Griffiths, 2017; Spear et al., 2014). Also, analysis of eDNA from sediments can potentially yield information about long-term migration and distribution patterns, as eDNA particles are more concentrated and preserved for longer periods in sediments than in water (Eichmiller et al., 2014; Pedersen et al., 2014; Turner, Uy, et al., 2014). Therefore, it is also likely that sediment cores can be used as eDNA banks for inferring both short-term and long-term distributional patterns, although sediment mixing and environmental conditions for DNA preservation may be a complicating factor (Anderson-Carpenter et al., 2011; Eichmiller et al., 2016; Matisoo-Smith et al., 2008; Turner, Uy, et al., 2014).

9.3 | Diet and processed fish products analysis

Although eDNA analysis by definition only applies to analysis of samples taken directly from a natural environment, the basic principle can be applied to other types of samples. One extension of the eDNA analysis concept for ecosystem-based management is the assessment of trophic interactions through diet analysis (Taberlet, Coissac, Hajibabaei, & Rieseberg, 2012). Diet analyses performed by taxonomic identification using macro- and microscopic examination of gut content provide input to ecosystem models and are used increasingly in fisheries management (Bourlat et al., 2013; Ovenden, Berry, Welch, Buckworth, & Dichmont, 2015). However, those approaches are often limited in their ability to visually identify prey items, especially at late digestion stages (Carreon-Martinez, Johnson, Ludsin, & Heath, 2011; Taylor, Fox, Rico, & Rico, 2002). DNA analyses of gut contents and faeces essentially use the same approaches as for eDNA, that is in analysing a mixture of degraded DNA from single or multiple prey species. The approach can potentially provide both qualitative and quantitative estimates, yielding detailed insights into diet preferences, predator-prey interactions and likely provide superior estimates of energy budgets compared to conventional approaches (Carreon-Martinez et al., 2011; Pompanon et al., 2012).

Another application worth pointing to, which uses the same methodology but is not strictly an eDNA approach, is fisheries forensics. Fisheries and food regulations rely on effective enforcement requiring the ability for high confidence in species identification of whole fish and fish products. Presently, DNA barcoding is a widely used approach to identify species in seafood products (Ivanova, Zemlak, Hanner, & Hebert, 2007; Smith, McVeagh, & Steinke, 2008). However, the methodological constraints of traditional DNA barcoding (Sanger sequencing) hinders analysis of complex products such as surimi, fish cakes or fish roe, where more species can contribute with DNA. Also, traditional DNA barcoding is not able to inform on the relative quantity of different species in a mixed-species product. Fish silage, used for animal feed or fish-oil production, is also problematic for effective fisheries control due to onboard processing (Ferraz De Arruda, Borghesi, & Oetterer, 2007; Larsen et al., 2013). Fish silage consists of minced fish dissolved in acid upon capture at sea, meaning there are no morphological characteristics useable for species identification or even estimation of biomass contribution from individual species when the product is landed, which is a necessity and a requirement by European law for proper fisheries control (European Commission, 2013). Utilizing the same approach for complex fish products as for eDNA analysis will likely allow better establishment of the relationship between DNA and biomass/proportion, as many of the traditional causes of uncertainty related to eDNA production, persistence and transport are almost eliminated. However, challenges such as likely variation in DNA content between different tissue types, which are differentially represented in different sized fish, and the presence of non-target (e.g., protected species) through contamination by stomach content, need to be addressed before DNA-based quantification in relation to fisheries forensic questions can be considered robust.

9.4 | Fish stock structure

Knowledge of genetic stock structure is increasingly applied in fisheries management and conservation of aquatic resources (Carvalho, Hauser, Martinsohn, & Naish, 2016; Ovenden et al., 2015). Here, fisheries genetic methods have played a crucial role in delineating the fundamental biological units (genetic populations) and their spatiotemporal distribution and mixing (Bekkevold et al., 2015; Bonanomi et al., 2015) to allow informed and sustainable exploitation of populations. These methods can also be applied in a forensic context to unambiguously assign individual fish back to population/area of origin, thus providing means to fight illegal, unreported and unregulated (IUU) fishing (Nielsen et al., 2012). The collection of sufficient samples for population genetic inferences, in particular for

species of low abundance can be a challenge and associated with high cost. Thus, eDNA has been suggested as a potential fast and cost-effective method to obtain non-invasive population samples. In a recent pioneering study of whale sharks from the Arabian Gulf, Sigsgaard et al. (2016) demonstrated that genetic marker (mitochondrial DNA haplotypes) frequencies obtained by eDNA analyses from water samples resembled marker frequencies obtained by tissue from population samples of individual sharks. The eDNA derived population data could be used to infer the genetic relationships between the Gulf and other whale shark populations as well as providing estimates of effective population size. More data are needed to evaluate the potential of eDNA for population genetics; however, there are inherent challenges. First of all population genetics is frequency based, relying on differences in distribution of genetic marker variants (e.g., mtDNA haplotypes, SNPs and microsatellites) among the sampled units to infer population structure. In general, marker frequency variation among natural populations of marine fish tends to be very small, commonly with less than two per cent of the total genetic variance distributed among populations (Ward, Woodwark, & Skibinski, 1994). Thus, the assumption that a sample is representative for the population as a whole is essential (Waples, 1998). Given the nature of an eDNA sample, non-random contributions from different individuals are likely to occur due to differences in production rates and distance from sampling site. In turn, this likely results in biased estimates of marker frequencies and potentially erroneous inferences on population structure. In addition, bulk samples of eDNA from multiple individuals do not allow more advanced fisheries genetic analyses, such as population assignment tests (Nielsen, 2016), as these methods are individual-based, that is rely on the combined information from multiple genetic markers genotyped in the same individual.

10 | NEW BETTER, FASTER AND CHEAPER TECHNOLOGIES

Quantitative real-time polymerase chain reaction (e.g., Klymus et al., 2014; Pilliod et al., 2014; Takahara et al., 2012) and more recently digital droplet PCR (hereafter ddPCR) (Doi et al., 2015; Nathan, Simmons, Wegleitner, Jerde, & Mahon, 2014) have been the gold standards for eDNA analysis. This is due to dual interests in increasing sensitivity (level of detection) and ability to quantify eDNA concentrations in attempts to estimate biomass or abundance. However, qPCR and ddPCR instruments are usually limited to analysing a restricted number of species in each eDNA sample (Herder et al., 2014). This limitation is mainly due to the standard analytical setup, which becomes cost- and time inefficient with multiple species, as each species require individual tubes and 3–12 replicates per sample to verify results (Rees et al., 2014). However, a number of high-throughput platforms are available that potentially enable cost-efficient and large-scale multispecies qPCR analysis. These include the Open Array from Life Technologies, the Light Cycler 1536 from Roche, the Smartchip from Wafergen and the Biomark from Fluidigm (Svec, Rusnakova, Korenkova, & Kubista, 2013). An example of the capacity of such systems is the Biomark HD, where sample reactions (assays and samples) mixes automatically on a chip in a mesh-like fashion, into individual reaction chambers. A number of different chips are available for this instrument (i.e. FLEXsix, 48.48 Dynamic Array and 96.96 Dynamic Array), which allow 12, 48 and 96 different assays and samples to be cross-analysed simultaneously, respectively, enabling up to 9.216 qPCR reactions in a single run (Korenková et al., 2015; Spurgeon, Jones, & Ramakrishnan, 2015; Svec et al., 2013). Illustrated in another way, the 96.96 Dynamic Array plate, can potentially screen up to 96 different species in 96 samples simultaneously (excluding various non-template controls and standards). However, the platform uses small reaction volumes (<10 nl) requiring high concentrations of eDNA and the time required to optimize numerous assays to work under identical reaction conditions remain a major challenge for utilizing these platforms for eDNA analysis (Korenková et al., 2015; Spurgeon et al., 2015). It should also be noted that the assay design process is time-consuming and can be complex due to the potential for cross-reactivity to DNA from non-target species and presently few species-specific qPCR assays are designed for marine species.

Metabarcoding by NGS is the most widely used technique in marine eDNA studies (e.g., Thomsen et al., 2016; Yamamoto et al., 2017). Metabarcoding allows for presence/absence monitoring of entire ecological communities through sequencing DNA fragments from a predetermined targeted group of species (see Textbox 1); however, up till now metabarcoding has been lacking the strong quantitative properties of ddPCR and qPCR (Evans et al., 2015; Kelly, Port, Yamahara, & Crowder, 2014; Port et al., 2015). Yet an interesting aspect within this field is the miniaturization, portability and read length offered by new range of sequencing systems, also known as third-generation sequencing (Goodwin, McPherson, & McCombie, 2016; Schadt, Turner, & Kasarskis, 2010). In 2014, the first prototype of the MinION sequencer from Oxford Nanopore Technologies was released, and more recently, a smartphone-powered sequencer the "SmidgION" was announced (Goodwin et al., 2016; Harley, 2016). These new sequencing systems have raised the standards with regard to size and portability, being smaller than an average smartphone. Although these systems presently are still limited in terms of throughput and direct field usage, the perspectives in having portable instruments will have a huge impact on how environmental monitoring in the field is carried out, as it with time can enable "on-site" data collection, almost in real time (Zaaijer et al., 2017). Such direct monitoring will also minimize many of the classical logistical and practical challenges of handling, storing and transporting environmental samples.

10.1 | Ecogenomic sensors

Remote real-time ocean observation systems and genomic technologies have developed substantially in the past decade, and the future

scenario of automated real-time eDNA measurements is now closer than ever (Ottesen, 2016; Ussler et al., 2013). The so-called ecogenomic sensors are instruments for DNA analysis that can be deployed outside the laboratory, providing the entire sample-to-result process without human interaction. They autonomously collect and store water samples and perform (near) real-time molecular analysis using qPCR and other molecular detection technologies (Ottesen, 2016; Scholin, 2009). The "environmental sample processor" (ESP) platform has already shown practical application and significant progress for monitoring a wide array of microorganisms (Ottesen et al., 2014; Robidart et al., 2014; Saito, Bulygin, Moran, Taylor, & Scholin, 2011) and zooplankton (Harvey et al., 2012), from diverse environments ranging from coastal to deep sea applications (Harvey et al., 2012; Ussler et al., 2013). The ESP can be set to monitor either a specified geographical location, for example moored to a dockside or buoy, or be mobile, for example free drifting or carried onboard vessels to perform autonomous sampling and analysis (Ottesen, 2016). Even automated self-propelling ecogenomic sensors are currently under development (Pargett et al., 2015). These sensors are combined relatively easily with already existing data collection instrumentation, for example CTD with water sampler. Such strongly integrated collections of physical, chemical and biological data could be of high value for understanding production, persistence and transport of eDNA (Barnes & Turner, 2015; Pedersen et al., 2014; Strickler et al., 2014). Ecogenomic sensors are costly, but in comparison with the cost of extensive ship time for visual monitoring or for continuous collection of water samples, they are already cost-competitive.

11 | CONCLUSIONS

Environmental DNA analysis is a rapidly emerging tool in marine monitoring and is increasingly applied not only a as research tool but also for direct management purposes. A diverse array of environmental and biological factors influence eDNA production, degradation and transport but the methodological challenges associated with analysing eDNA as a means to monitor commercial species are complex and still remain sparsely explored. A more thorough investigation of the key biological and environmental parameters and their interaction is essential for making the best use of eDNA's potential for both future research and management applications. We believe that with the current eDNA analytical paradigm, direct quantitative assessment will remain challenging, and we recommend focusing on the traditional strengths of eDNA analyses for presence/absence detection and on detection of relative changes in concentration which can be used to detect migration and life-history events. Implementation of eDNA in fisheries science can also enable a more broad ecological understanding, supplementing established tools used for diet analysis or fish product analysis and can further yield a much broader taxonomic coverage than current monitoring methods, providing basis for improved ecosystem-based management. Moreover, the pace of technical advancement in molecular biology will make eDNA analysis even more profitable, providing both instruments with higher throughput allowing cheaper and broader species coverage as well as mobile instruments which can be either handheld or submersible and fully automated providing on-site near real-time results. Although we stress that methodological challenges and shortcomings need careful consideration for proper application and use in management and policymaking, eDNA-based monitoring will continue to develop to have profound and valuable impact on how we will carry out fisheries research and management in the future.

ACKNOWLEDGEMENTS

We thank the Danish AgriFish Agency's Green Development and Demonstration Programme (GUDP-VIND) and European Union's Horizon 2020 research and innovation programmes, AtlantOS under grant no. 633211 and DiscardLess under grant no. 633680, for funding this work. We also want to extend our gratitude to three anonymous reviewers whose comments substantially improved the manuscript.

ORCID

Brian Klitgaard Hansen http://orcid.org/0000-0002-3257-8419

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How to cite this article: Hansen BK, Bekkevold D, Clausen LW, Nielsen EE. The sceptical optimist: challenges and perspectives for the application of environmental DNA in marine fisheries. *Fish Fish*. 2018;19:751–768. https://doi.org/10.1111/faf.12286