UNIVERSITÉ CLAUDE BERNARD LYON 1

Parcours de Master en Génomique Environnementale

Environmental DNA for detection, quantification and monitoring of invasive alien species: a literature review

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Introduction

Since life first appeared on Earth, species have regularly moved outside their natural range. Ecological barriers change due to geographic and climatic events and this leads to natural migration and dispersion of species colonizing new favorable habitats. Nowadays, human activities at a global scale caused a significant disruption of the natural ecosystem equilibrium. Intentional or accidental introduction of species across geographic barriers has been known for decades to be detrimental to native species, communities, and ecosystems (Elton 2020; Simberloff and Stiling 1996). According to the BISE[1], around 10 000 alien species have been registered in Europe. Invasive species are today considered as one of the five major causes of biodiversity erosion in the IPBES[2] 2023 summary. These species establish a new range in which they persist, spread and proliferate to the detriment of the environment and other species. The fate of an alien/non-native species is most of the time decided by stochastic evolutionary forces. If a non-native species succeeds in its new environment, this is called naturalization. Naturalized ones can then potentially become invasive when they cause environmental damage (Mack, 2000). Such species not only negatively impact native biodiversity, but also ecosystem services and the human economy and well-being. The IUCN[3] added these notions to the definition of the term invasive alien species (IAS). Therefore the management and detection of these species is in considerable demand, both for biodiversity conservation and for human activities. To better manage invasive alien species, the most effective way is to detect newly established populations early in the invasion process (Larson et al 2020).

The environmental DNA approach is increasingly used for the early detection of IAS. It uses non-invasive methods for the detection of DNA traces left and/or released by organisms in the environment. The samples contain intracellular DNA, from living cells and unicellular organisms. They also include extracellular DNA resulting from cell death and physical, chemical or biological degradation (Levy-Booth et al. 2007; Pietramellara et al. 2009). Two main strategies can be adopted to study eDNA. Metabarcoding (Taberlet et al 2012) is based on DNA sequencing and results in an indicative list of the organisms present in the environment. It gives information on diversity (Burki, Sandin, and Jamy 2021; Elbrecht et al. 2018), community structure (Xie et al. 2021) and relative abundance (Fonseca 2018). This method can be used for many applications, especially invasive species detection (Ruppert, Kline, and Rahman 2019). On the other hand, quantitative PCR (qPCR) and droplet digital PCR (ddPCR) can be used for species-specific detection. They can also both permit absolute estimations of DNA concentrations in samples (Nathan et al 2014). Quantitative PCR is also supposedly cheaper, faster and more sensitive than metabarcoding (Bylemans et al. 2019). Although eDNA technologies are promising for the monitoring of invasive species, this topic is still rather low-key, all the more on terrestrial species.

This review presents highlights of recent eDNA studies answering biological questions about invasive alien species. The objective is to show where research currently stands on the subject and what avenues future studies should explore to move forward on this issue.

[1] Biodiversity Information System for Europe

[2] Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services

[3] International Union for Conservation of Nature

Detection

a. What are we able to detect today ?

Current studies on IAS using genomic tools focus mainly on aquatic and semi-aquatic habitats and communities. Since the 2010s, numerous qPCR assays have been designed to detect invasive aquatic taxa. There are many examples of successful detections of invasive fish (Jo et al. 2020 ; Loeza-Quintana et al. 2021 ; King et al 2022 ; Dubreuil et al. 2022 ; Riaz et al. 2023), crabs (Roux et al. 2020), bivalves (Smith et al. 2012; Xia et al. 2018), tunicates (Gargan et al. 2022; LeBlanc et al. 2020), amphibians (Lin, Zhang, et Yao 2019; Secondi et al. 2016), snakes (Piaggio et al. 2014) and many more (**cf Fig.1**). Like qPCR, eDNA metabarcoding studies from aqueous substrates still largely focus on invasive vertebrate (Bessey et al. 2020; Dufresnes et al. 2019; Ficetola, Manenti, et Taberlet 2019; Miya, Gotoh, et Sado 2020) and invertebrate communities (Klymus, Marshall, et Stepien 2017; Wu et al. 2023). While animals are well represented in the literature, little is known about aquatic flora (Espinosa Prieto et al. 2023), and even less about invasive flora (**cf Fig.1**). However, initial surveys have produced some interesting results. For instance, some scientists used metabarcoding to detect several taxa of aquatic vascular plants (Coghlan, Shafer, and Freeland 2021). They found invasive plants like *Stratiotes aloides* that were thought to have been eradicated five years previously. Fujiwara et al. (2016) designed a qPCR assay for the species *Egeria densa* and successfully detected it. It was therefore adopted by a few scientists in Japan for detection and abundance estimates of the invasive macrophyte in lotic habitats. (Doi et al. 2021 ; Miyazono et al. 2021). Others investigated the accumulation and degradation process of DNA signals for aquatic plants and applied this for field surveillance of the invasive *Hydrilla verticillata*. They have succeeded in detecting its DNA by qPCR in the laboratory and in the field in regions where *Hydrilla* distribution was known, and in identifying the mechanisms of its DNA degradation over time (Gantz et al. 2018). Results like these hold great promise for the future of aquatic plant monitoring. Overall, eDNA metabarcoding and qPCR early detection at low population densities are trustworthy and recommended in complement to conventional monitoring tools for aquatic IAS (Fonseca et al. 2023; Pilliod et al. 2013).

As water bodies are perfect vectors for eDNA over an entire watershed, they also proved effective for detecting terrestrial species. Research into invasive wild boar has demonstrated the effectiveness of qPCR for detecting specific terrestrial species from water samples (Hauger et al. 2020; Williams et al. 2018). More recently, Villacorta Rath et al. (2022) confirmed that terrestrial invertebrates can be successfully amplified from adjacent or downstreams waterbodies. Sales et al (2020) also highlighted the potential of metabarcoding to fully capture the signal from rare and invasive terrestrial or semi-aquatic mammal species, using eDNA from water in river systems. However, they suggest combining it with conventional methods to maximize monitoring efforts.

Although aquatic detection of terrestrial species has proved effective, these can be successfully detected from other substrates. For instance, DNA traces of invasive insect pests have been found on vegetation foliage and stems (Allen et al. 2021). Some mammals such as the large white-toothed shrew and the grey squirrel have been identified from native pine marten feces (O'Meara et al. 2014). However studies using soil are rare concerning IAS and as a reflection of the overall research on eDNA, surveys for terrestrial plants are still underrepresented. The ability to detect plants in the soil with eDNA is known and can, for example, be used to monitor vegetation responses on spatial and temporal scales. Actually, soil eDNA metabarcoding seems to give complex information describing past and present communities (Capo et al. 2021). This

information can, however, be a limitation for IAS monitoring, as the DNA present in the samples does not necessarily represent the current occupation by the targeted taxa. Moreover, this technique detects abundant taxa better than rare ones in soil (Ariza et al. 2023). Some qPCR studies have been carried out on rare terrestrial species too. For example, Hartvig et al. (2021) detected the DNA of rare orchids in samples located close to the nearest orchids (around 10 cm) but no DNA was found in soil where the plant had been found a few years before the study. These results show that soil is a delicate but informative substrate for the detection of rare terrestrial species and thus for the early detection of IAS.

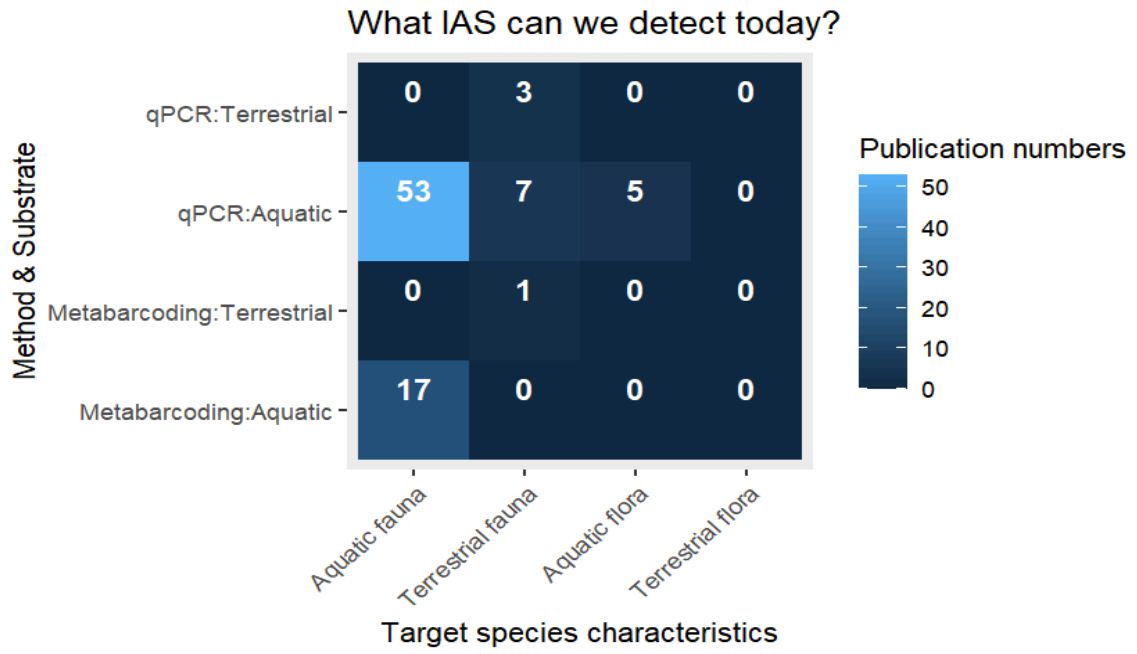

Figure 1: Plot of 86 articles grouped according to the type of organism(s) studied, the substrate chosen and the detection method used for the study. Search in Google Scholar on February 22, 2024 with the terms: *allintitle : environmental DNA (OR eDNA) detection invasive OR alien OR non-native -review* followed by manual filtering on a total of 106 publications.

b. Crucial considerations for a successful detection of IAS, especially on terrestrial species and soil substrates.

When attempting to understand eDNA ecology, researchers define a four-step framework highlighting the origin, state, transport and fate of genetic "extraorganismal" material (Barnes and Turner 2016) (**cf Fig. 2**). Many environmental and biotic factors have an impact on each of these four steps of the eDNA "life cycle", and ultimately on the collection and processing of eDNA samples. Methodological choices for the analysis of eDNA have therefore to be carefully pondered.

For example, substrate selection is one of the first points to consider. The organism and/or community studied has a significant impact on its DNA detectability. Studies have demonstrated that individual production rates, density and population size, residence times and even behavior influence DNA detectability and concentration (Pilliod et al. 2014; Smith et al. 2012; Villacorta-Rath et al. 2023). Physicochemical properties such as DNA fragment size, shape and interaction with other particles also play a role in detection capacity and may influence the choice of substrate to sample (Pietramellara et al. 2009). In fact, the distribution of eDNA in aquatic systems differs from that of terrestrial ones. In water bodies, the detection range is large due to the transport of DNA in water flows. However, the degradation process in this type of ecosystem is a matter of hours or days depending on environmental conditions and the origin and state of the particles studied. In contrast, the DNA shed by terrestrial organisms can remain very localized. In soil, DNA is adsorbed onto colloids and sand particles, protecting it from degradation by nucleases and allowing it to be stored for days or even years (Pietramellara et al. 2009).

As a result, designing a sampling plan for the early detection of IAS requires prior knowledge of the organism and environment studied. Choosing the right conditions and appropriate substrate to optimize detection can be very delicate. As seen above, the lack of terrestrial IAS studies suggests that soil sampling may not be the easiest and most efficient way to detect invasive species. In addition, the detection of terrestrial plants in soil is limited due to their sessility. This means that the sampling effort will be substantial, and even more so when it comes to detecting small quantities of DNA in the case of rare or invasive alien species. In this case, the number and volume of field samples and replicates is crucial in order to ensure a faithful representation of species occupancy (Macher et al. 2021; Schabacker et al. 2020). The detection probability can actually be maximized by increasing the number of PCR replicates, and the sequencing effort in the case of metabarcoding. Even though, sampling design for detection of terrestrial species, especially in soil, remains a key point to focus on for future research.

The lack of studies on terrestrial IAS implies a deficit of qPCR and metabarcoding markers limiting the choice of assays. For most IAS, robust qPCR assays remain to be designed (Hunter et al. 2017; Langlois et al. 2021), but this design step relies on availability of genetic sequences for the target species. (O'Meara et al. 2014). In general, IAS species are well-known and easily found in databases making this step quite affordable for anyone tackling the issue. On the other hand, eDNA metabarcoding is less stringent, and more generalist markers are most of the time sufficient to detect terrestrial IAS but the design of more specific markers could improve detection.

Depending on the assay used, laboratory protocols need to be adapted for successful amplification of target DNA. Extracellular or intracellular protocols target molecules present in different compartments, which can affect detection sensitivity and reveal different levels of information (Nagler et al. 2022). DNA extracts will be differing in terms of quantity and quality depending on the protocol chosen. Intracellular DNA (iDNA) is protected by cell walls from

enzymatic degradation so it is supposedly of higher quality. However, intracellular protocols can extract a mixture of non-target intracellular bacterial or fungal DNA hampering the amplification of target DNA, which is not the case for extracellular protocols. Therefore, choosing an extraction method not adapted to the scientific question, the life cycle of the targeted DNA and the type of sampling could limit IAS early detection. Furthermore, working with eDNA involves a high risk of contamination at every study level, which will generate false positives and negatives regardless of the protocol chosen (Sepulveda et al. 2020). Today, control procedures such as strict lab conditions, extraction and PCR negative controls and replication can make contamination less of a problem for data processing and results (Ficetola, Taberlet, and Coissac 2016).

In metabarcoding, artifacts like tag jumps are also a limitation for early IAS detection. They are chimeric sequences with false tag combinations (Schnell, Bohmann, and Gilbert 2015) generated during library preparation and identified through the bioinformatic process. These sequences can lead to false assignments of sequences to samples (Esling, Lejzerowicz, and Pawlowski 2015) and create a background noise in the data (Rodriguez-Martinez et al. 2023) that can hide the rare DNA signal (Taberlet et al. 2018). In eDNA metabarcoding, sequencing information undergoes many filtration steps and quality checks such as removing spurious sequences and comparing samples with controls. Rare sequences may not pass through these stages. Therefore, for IAS early detection, the difficulty is to sort out the background noise and differentiate it from a rare species.

Overall, the current literature shows that eDNA methods are applicable to IAS, but there are still technical considerations to keep in mind (**cf Fig. 3**). Detection limits can be improved in the future through a better understanding of the eDNA life cycle and the development of new sampling plans, new assay designs, machines and bioinformatic pipelines. This will enable progress to be made in the early detection and management of IAS.

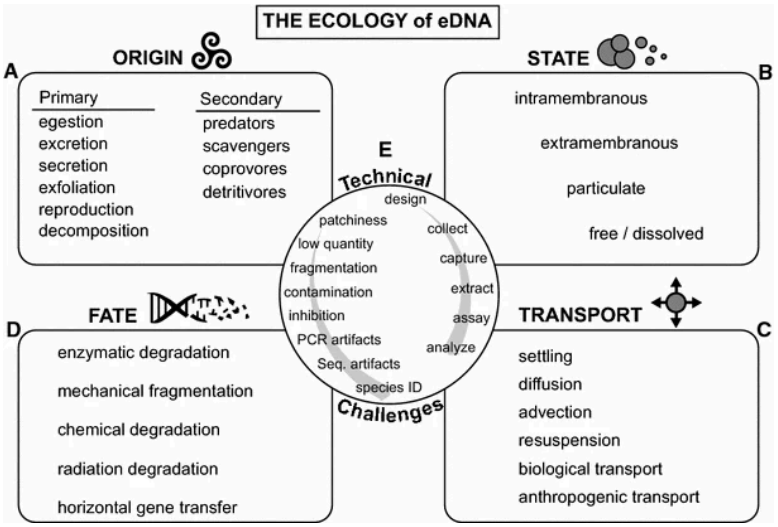

Figure 2: Processes and properties of eDNA through its "life cycle" and technical challenges associated, described in "The ecology of environmental DNA and implications for conservation genetics." by Barnes et al in 2016.

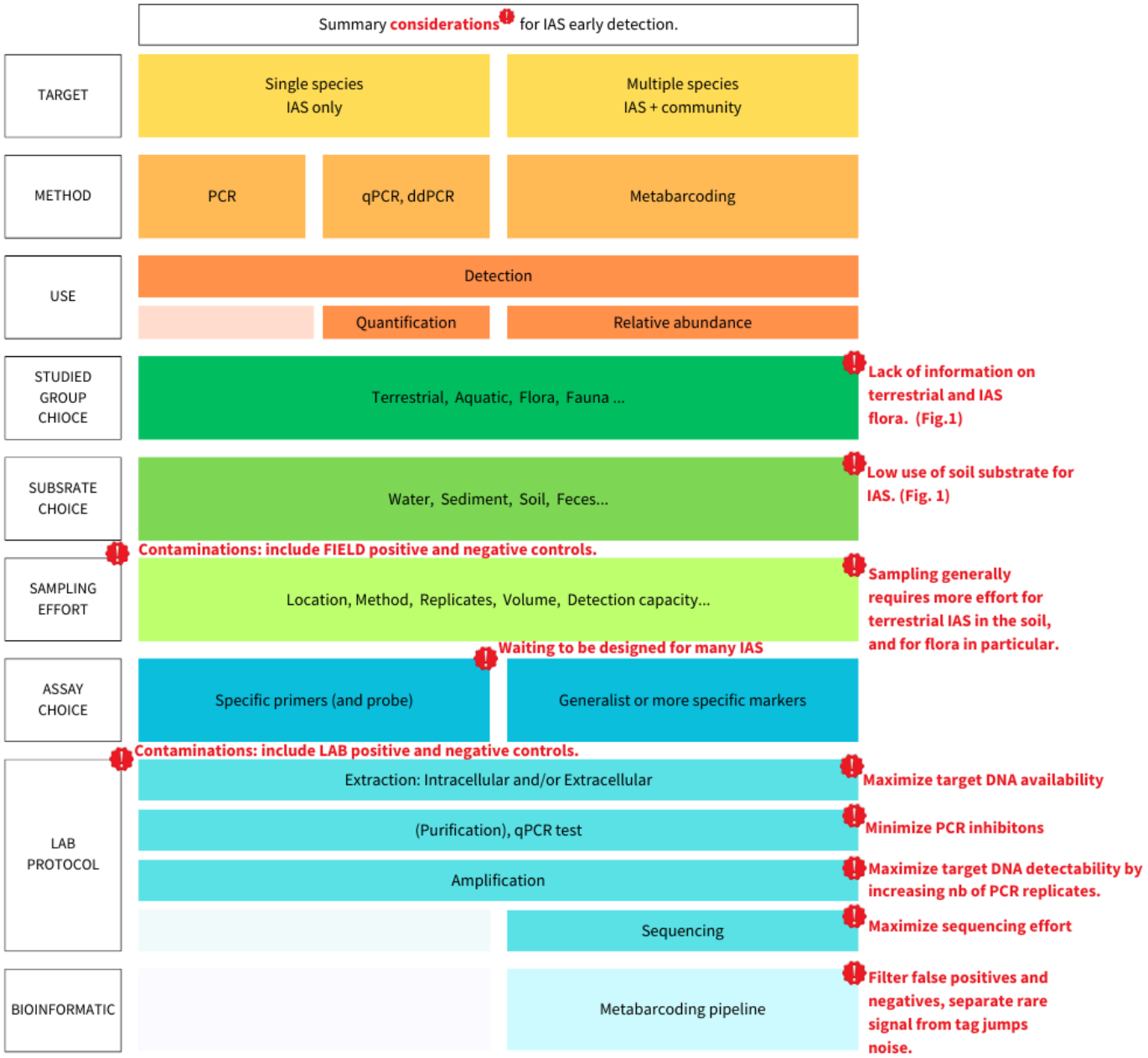

Figure 3: Summary of the main considerations for a successful IAS early detection.

Quantification: Is there a link among eDNA concentration, number of gene copies, biomass and number of individuals?

As mentioned in the previous section, eDNA analysis can be used for detection and provide qualitative information on organism presence or absence in the environment. However,

eDNA can also be used for quantitative purposes. With qPCR and ddPCR technologies, the initial number of eDNA copies in the studied sample can be estimated. Given these possibilities, some (water-based) studies have revealed an interesting correlation between a number of copies for a given volume and species biomass. For example, aquarium experiments on endangered salmonid species found significant correlations between eDNA concentrations, fish density and body size, suggesting that it could be an indicator of species biomass (Mizumoto et al. 2018). Other studies on invasive common carp demonstrated that biomass data estimated from eDNA concentrations can reflect the spatial distribution of this species in natural ecosystems (Eichmiller, Bajer, and Sorensen 2014; Takahara et al. 2012). Doi et al (2017) confirmed that qPCR is useful to estimate fish biomass. They also highlighted that a small number of qPCR replicates is sufficient for estimations when eDNA concentrations are high. Some scientists compared the absolute concentrations of fish eDNA obtained with ddPCR (highly accurate quantification) and number of individuals in a fish population (Capo et al. 2021). Their results were in line with previous studies, a positive correlation was observed but important unexplained variations remain. As seen above, they suggest improving sampling strategies and methods to obtain highly accurate estimates of fish population abundance. Recently, a study on the invasive American bullfrog in pond water found correlation in eDNA concentrations and total of individuals caught during a national eradication campaign (Everst et al. 2022). These types of results could be standardized in the future to relate estimated eDNA concentrations in samples to the number of individuals. Li et al (2016) also demonstrated a positive relationship between the relative number of reads obtained from eDNA metabarcoding and relative abundance or density of anuran species estimated with conventional methods. In this short-term study and after a robust sampling, metabarcoding data allowed more reliable measurements of large-scale anuran presence than the transect method. They also found no important effects of physicochemical factors on read counts. This shows that metabarcoding can also provide abundance estimates, which, although relative, can lead to interesting conclusions for biodiversity monitoring. These findings promise great possibilities in terms of IAS or rare species monitoring.

However, the studied group might influence this tight correlation (Kuehne et al. 2020). In addition, the correlation has been confirmed for a few species in the lab, but in natural environments, abundance estimations are most of the time complicated by many factors. Yates et al (2019) have collected data from experiments and quantified the strength of the correlation between eDNA and two metrics of abundance (density and biomass) across lab and *in situ* environments. According to the review, the observed variation in abundance was at 82% explained by eDNA concentrations in the lab, compared with 50% in natural environments. Given the ecological, biological and technological limitations of environmental DNA detection, a strong causal link between these metrics remains to be proven. A year later, Yates et al (2021) published an article eDNA production scales allometrically with organism mass. Therefore, future studies on this topic are encouraged to pay particular attention to individual variations in eDNA production, which will impact detected eDNA concentrations and are likely to bias abundances estimates. In addition, one can suggest expanding research on other individuals and taxonomic groups in order to strengthen the link between eDNA concentration/number of copies and biomass/abundances to provide a reproducible tool for monitoring.

Monitoring: eDNA as a biomonitoring tool for IAS, does it work ?

Monitoring IAS is of immediate concern to identify newly established populations, evaluate efficacy of current management measures but also to develop new indicators in order to improve these measures and minimize harmful consequences of a spreading population on biodiversity and human activities. Methods based on eDNA could help decision making for managers (Fonseca et al. 2023). This technique has already proved to be more sensitive and cost-efficient for some aquatic species IAS detection compared to more conventional ones (Dubreuil et al. 2022; Ota et al. 2020; Sard et al. 2019). As seen previously, early detection and relative or absolute quantification of species eDNA can be estimated and may bring supplementary information. In addition, eDNA can be used in unexplored or endangered environments thanks to its low-invasive sampling protocols. Managers are therefore encouraged to familiarize themselves with these tools. However, the use of eDNA is still in its infancy and managers still have reluctance on its use for IAS monitoring, because a positive detection may not indicate the current presence of a species and vice-versa. This is not only due to false and true positives, but also to the spatial and temporal large-scale detectability of eDNA analysis. Actually, eDNA reliability for aquatic IAS management is only part of the problem. The major limitation for its use might be the interface between eDNA results and management considerations (Sepulveda et al. 2020). Yet, there is a consensus on the fact that there is a need for guidance on the use of eDNA to support decision making for managers of rare or IAS (Morisette et al. 2021; Sepulveda et al. 2020). In addition, managers may still find it difficult to control the IAS population even if detection is efficient. These tools should not be used to prevent the establishment of IAS, but rather to limit their dispersal using appropriate monitoring methods, such as those used for Asian carp in the USA or Canada (LeBlanc et al. 2020). However, quantitative advances might help with this issue. The control of population size and dispersion can be even better if the number of individuals can be estimated with eDNA. In the future, detection might be even more affordable with the development of techniques recently applied for eDNA analysis such as LAMP (Loop-mediated isothermal AMPlification) (Zhou et al. 2021), RPA (Recombinase Polymerase Amplification) (Williams et al. 2023) or MinIon metabarcoding (Truelove, Andruszkiewicz, and Block 2019; van der Reis et al. 2023)). The minimization and speed of use of these technologies will permit direct analysis in the field and rapid results. As a result, field agents can work directly *in situ*, and detection can take a few hours instead of several days in the laboratory. Somes studies showed exciting results for IAS monitoring (Williams et al. 2017). However, this type of result has yet to be generalized in future studies.

Conclusion

Although traditional IAS monitoring tools are effective, eDNA has proved to provide complementary information. Early detection of IAS using eDNA is reliable, particularly in aquatic environments. However, little is known about the detection of invasive plants and terrestrial species and future studies on this issue are recommended. The correlation between eDNA particle concentration and population abundance still needs to be investigated for a larger number of taxa to consolidate the results obtained in preliminary studies. Therefore, eDNA monitoring for IAS will be enhanced by the development of new technologies and the contribution of future studies on methodological choices and technical considerations for early detection and quantification.

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