The European Catfish *(Silurus glanis)* as an Invasive Species – eDNA Detection Methods

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Abstract
The European or Wels catfish *(Silurus glanis)* is an opportunistic apex predator which is the largest freshwater fish species in Europe, making it a popular choice as an angler fish or biological control agent. Moreover, in recent years in many places they have also become increasingly important in the food industry too. They mainly live in fishponds or rivers, and their natural area of distribution is Central-Eastern Europe and Western Asia. Over time they have been introduced into many aquatic systems in several countries, but they have become an invasive species due to their excellent adaptability. The European catfish is a highly successful, aggressive apex predator, with a broad diet and thus has a profound impact on the ecosystem. As a result, their monitoring of natural waters has become an important task at the national and international levels. Hence, as with other invasive species, for their detection, a variety of traditional methods are applied. However, with recent technological advances, non-invasive, sensitive, cost- and time-effective approaches have emerged that utilize environmental DNA (eDNA) as a basis. The aim of this review is to present these recent technologies and their application to European catfish species.

Keywords: environmental DNA, invasive species, review, *Silurus glanis*

1. Introduction

A key component of freshwater ecosystems are the fish communities, which provide important ecosystem services and are an excellent indicator of river and lake ecosystems due to their vulnerability to water pollution and habitat degradation [1, 2]. In his study in 2003, Jenkins reported that, globally, freshwater ecosystems are among the most threatened ecosystems, with species declining at a faster rate than those found in terrestrial and marine habitats since the 1970s and this trend is continuing today [3, 4]. To make matters worse, natural ecosystems are threatened by the introduction of various non-native fish species, because although not all species have an impact on the ecosystem, some may have not only ecological but also evolutionary and economic effects [5]. This is due to the fact that they can affect the organisms in their environment both directly and indirectly, through several levels of biological organisation (from genes to ecosystems) [1].

The European catfish, also known as Wels catfish or Sheatfish, has a natural distribution in Eastern Europe and Central-West Asia, but since the 1800s it has been introduced into several countries in Northern and Western Europe before making its way beyond the continent. In Portugal, for example, it was introduced in the Tagus River around 2006. Apart from this species, one other *Siluridae* is found in Europe, namely the *Silurus aristotelis*, which is native to Greece. The popularity of *S. glanis* is due to its economic importance in several aspects: as a sport fish (e.g. in France, Italy, UK) and as a food fish (e.g. in Hungary, Poland) [6]. It possesses excellent features, such as the ability to tolerate low oxygen

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levels, and high temperature [7], or its high growth rate [8]. These are make it suitable for aquaculture, but also facilitate its spread in natural waters especially during this undergoing global warming [7].

According to an article by Boulêtreau and Santoul in 2016 [6], although the catfish is the largest freshwater fish in Europe, and according to some legends (from the 18th century to the present day) can grow to 5 m in length and 306 kg in weight, evidence suggests that it is only around 2.7 m long and weighs around 130 kg. The *S. glanis* is a mainly dark-coloured fish with a large broad head, wide mouth and small eyes. It has two long barbs on its upper jaw and four short ones on its lower jaw, which are used to navigate and hunt on the lower part of the waterbodies [9]. The European catfish is a bottom-dwelling, opportunistic predator, with wide range of prey it eats birds in addition to fish [10-12]. Due to its size, adaptability and predatory nature, it is considered as an invasive apex predator species that can pose a threat to native species and ecosystems.

A particularly good example of the beneficial traits that have led to the spread of *S. glanis* is its ability to survive in brackish waters, which is part of its natural habitat. One study used stable isotope analysis (SIA) at a dam on the Garonne River (France) to show that although the dam did not affect the diet of *S. glanis*, the average amount of carbon from marine sources in the diet of European catfish was 53% [13]. Therefore, it is not surprising that the Sea lamprey *(Petromyzon marinus)*, which is listed as endangered on the European Atlantic coast and in North America, is potentially predated by the invasive *S. glanis* in that area. In France, Boulêtreau et al. [14] also demonstrated in a survey that catfishes cause increased damage to the population by hindering their spawning. In this experiment, 49 fish were tagged and released during their spawning period. As they swam upstream into freshwater, it is highly likely that the resident population of non-native European catfish consumed 50% of the fish within 8 days and 80% (39) in one month. This phenomenon is extremely dangerous, as *S. glanis* can establish in all areas where the endangered *P. marinus* can settle. Evidence of this can be seen from a previous report that examined the stomach contents of catfish in Portugal and found that their prey species ranged from small crabs to larger fish depending on the habitat (e.g. flow strength, water temperature) and size (i.e. larger mouth, larger prey) of *S. glanis*, and this range included sea lamprey along with other endangered native species such as the European eel *(Anguilla anguilla)*. Research carried out in Slovakia using data from the Danube River examined the population status of the last remaining sturgeon species in the river, the Sterlet *(Acipenser ruthenus)*, and found that as catches are getting heavier (1-2 kg in 2006; average 3.3 kg in 2021), the population there is presumably ageing. In turn, *S. glanis* is a potential predator of juvenile Sterlet specimens, as evidenced by the presence of a Sterlet marking "tag" in the gut contents of a catfish caught by an angler. However, this was not evidence that they were involved in the development of the stock ageing status [15].

Therefore, like in case of all other invasive species, monitoring the distribution and ecological impact of *Silurus glanis* has become an important task. To this day, researchers are developing and optimising new technologies to detect the species.

2. Literature review methods

The literature reviews used for this research were conducted in 2023 April, using the Google Scholar Web search engine, which such a Web search engine that is specifically searches the scientific literature and scholarly resources. Here, we used the advanced settings and searched for the following keywords: 'invasive' *'Silurus glanis*' 'environmental DNA'. We made a condition that it searches the whole literature and only shows those in which all keywords were present. While we also searched PubMed, CORE, BASE (Bielefeld Academic Search Engine), Semantic Scholar, RefSeek, and Science.gov with these settings, they turned out not to be the most efficient database search engines for the conducted study.

The Google Scholar search engine initially produced 637 results, the earliest of which was a book written in 1988, which ultimately proved to be irrelevant to the topic under study. Thus, the search results were then first narrowed down to articles published or available for early access from 2018 to April 2023 at the latest, as recently used technologies are the subject of this article. Hence, we reduced the search results to a total of 328 hits, of which based to the research engine 35 were review articles. In order to ensure the

reliability of the results of the present publication, we first selected these 328 literature papers in English, which totaled 273, whose content was personally screened. During the process of this, we searched scientific articles, books, book chapters, conference proceedings, abstracts, dissertations and scientific/official reports found in Google Scholar. Of these, we initially wanted to filter the literature used only to those published in journals with Q1 ranking in the SCIMAGO Journal and Country Rank (SJR). We selected them based on this, because the SJR is not judged on the quality of the article, but instead on the value of the journal in which the article is published. The list is ranked Q1 for the top 25% of journals, Q2 for those in the 25-50% group, Q3 for papers in the 50-75% group and Q4 for the 75-100% group. However, few Q1-ranked full-length articles containing all three search terms were published in the last 5 years. However, there were several instances where single literature appeared repeatedly in the results and several instances where all of the search terms were not met in the outcomes. Thus, the previously selected, relevant articles were supplemented with other Q2 articles, book, and non-Q ranked article. While we processed these literatures, we also included their older references into this review, but the methods discussed are all from the last 5 years. Thus, in total, the results were produced from 2 types of documents: 'article' (n=35) and 'book' (N=1), which were manually checked for their content (Figure 1). From this, we could see that methods based on environmental DNA are currently underused for the detection of *Silurus glanis*.

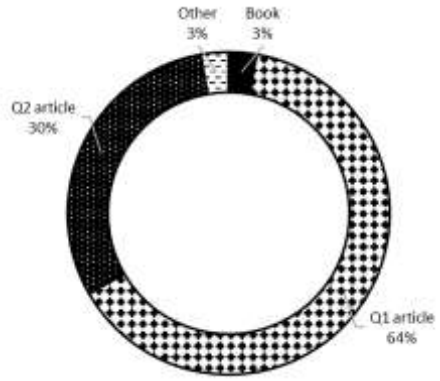

Figure 1. Types of the relevant literatures

3. Results and discussion

3.1. Traditional methods used to monitoring fish stocks

To begin with, it is worth mentioning the most common sources of information for catching and analyzing fish in different areas: sampling with fishing nets is a leading method currently used to study fish species in aquatic ecosystems. Examples of such methods include gillnets, drift nets, entangling nets and seine nets [16]. Frequently, these are accompanied by morphological and DNA barcoding through tissue samples from the specimens, to accurately identify the species of the captured fish. In the past, various data (e.g. weight, length) collected with gillnets have been used extensively in Europe to analyse the ecological state of the lakes, the potential of reservoirs, the size spectrum of lake fish communities and fish population diversity. This technique is simple, inexpensive and there is also available standard sampling protocol for its use. Furthermore, it can be applied to a wide range of habitat types and provides information on the species and age structure of populations. However, gillnets are only able to provide data on the relative abundance and biomass of fish, and their performance depends on species- and size-dependent fish activity. Moreover, the method is time- and energy-consuming as well as lethal to fish, thus its use for monitoring studies is being discouraged in a growing number of countries across the European Union (EU) [17].

By processing the information from similar studies, Kubala et al. [15] used a method to assess the status of the population by using catch (e.g. species, number of individuals, weight) information from different sources (e.g. from fishing associations) recorded during seine fishing within a given time period and area. Alternatively, a similar method was used, e.g. in a 2016 research using online and published data (blogs, angling forums, youtube) on European catfish for forensic analysis, as the species is considered invasive in the Tago watershed (Iberian Peninsula). This has allowed to assess the distribution range of the catfish species (increased by 700 km), the frequency of occurrence (mainly in reservoirs, high river sections) and the direction of its distribution (downstream), which has expanded since its first record in 1998 [18]. Another

approach could be Radio-telemetric and acoustic monitoring, where fish are tagged and their movements tracked when released. However, this would only track already known and tagged individuals, as was done in a study previously referred to. Hence, in the case of *S. glanis*, it may be possible to detect the predator by tagging the prey, but only by capturing the predator after it has consumed the tagged fish. This task is more challenging in medium to deep water, and is not appropriate for monitoring the distribution of predatory fish, although it is a good method for monitoring the behaviour and survival rate of the prey animal [14]. Koutsikos et al. [5] worked with information provided by traditional methods: those from literature surveys - scientific literature and grey sources - and information from surveys on electrofishing (EF) in water bodies (e.g. EU Water Framework Directive (WFD)). In the end, they created a matrix based on the data from their literature review, summarizing the ichthyofauna of 140 Greek river basins, including all species inhabiting the entire watershed of the system, regardless of whether the system consisted of only moving (lotic) or stationary (lentic). Samples taken by complementary electrofishing were entirely from the lotic systems. Unsurprisingly, for both methods used in this study, the invasive European catfish was among the non-native species. However, both in the data of the literature review (e. g. "cryptic" or "rare" species, thus difficult to observe) and in the results of the EF, errors could have occur (e.g. escape of the specimen from the net, like in case of catfish). Thus, the authors proposed the use of eDNA-based technologies to achieve even more accurate monitoring of the alien species, allowing early detection of invasive non-native species in the Greek waters.

Detection methods based on eDNA

As technology has advanced over the past decades, more and more attention has been paid to non-invasive, rapid, reliable, high-sensitivity environmental DNA (eDNA) technologies for species detection, which have revolutionised biodiversity monitoring [4,19]. Opportunities for this were opened up by the emergence of next-generation sequencing (NGS) technology in the mid-2000s, though the method took a long time to develop and spread. The concept of eDNA refers to the genetic material released by different organisms into their environment and which is present in environmental samples such as air, water, sediment or even faeces. However, environmental DNA can also come from any kind of secretions, gametes, blood or decomposition products. By total eDNA we mean both intracellular and extracellular DNA. The intracellular refers to DNA from living cells (organisms) that were found in the environmental sample. We refer to extracellular hereditary material when DNA is released from cells into the environment as they die. This type of sample is more degraded, as it can be damaged by mechanical, chemical and biological processes during cell death [20-24].

Methods based on eDNA start with sampling and continue with the processing of the isolated genetic material [19]. Two techniques based on the eDNA polymerase chain reaction (PCR) methods are widely used, and according to the objective we have in mind, we can choose the one that suits us best. The first method is DNA metabarcoding, which is a non-specific method that is used when we want to detect all the species present in a sample. This can be done without amplifying the metabarcodes, by the so-called shotgun sequencing of the eDNA. Therefore, metabarcoding can be used to generate large datasets of the biodiversity profile of a targeted taxonomic group from water samples, which can reveal the biodiversity structure of entire lake or river systems. Such datasets are processed in the form of sequence reads, subject to quality control, filtering, merging, error correction, cheme cleaning. The resulting data is zero radius operational taxonomic units (zOTUs) or clustered operational taxonomic units (OTUs) which are assignes taxonomic names based on reference sequences [19]. The second eDNA method is more specific and is based on qPCR (quantitative PCR) or ddPCR (Droplet Digital PCR) techniques and can be used to confirm the presence of a single species of our interest [23].

1. Benefits of eDNA methods:

- A cost-effective method for monitoring entire ecosystems.
- Can estimate the length of time that a species has been present in the ecosystem.
- Detection of evasive or invasive species with small numbers of individuals.

- Can overcome spatial heterogeneity (e.g. detection of organisms at different water depths).
- All water bodies can be easily sampled (e.g. temporary ponds, rivers, lakes, reservoirs, large heterogeneous waters, sea, etc.) [20, 21, 25, 26].

2. Sampling problems:

- The location of DNA (e.g. bound or free DNA).
- The environment of the sample (e.g. temperature, sunlight, pH, oxygen).
- The type of sample (e.g. water; soil).
- The movement of the sample (e.g. running water, wind).
- Age of sample (e.g. the older it is, the more degraded it is).
- Correct transport of the sample (e.g. in liquid nitrogen, in absolute ethanol) [20, 21, 25, 26].

3. Problems arising during processing:

- Selection of the right sampling time (e.g. animal migration).
- Possibility of contamination in the sample (e.g. DNA of a foreign species is introduced into the sample).
- The selection of an unsuitable primer (e.g. incorrect genetic identification).
- The longevity of already degraded DNA (e.g. bound on biofilms, intact for longer periods).
- Ability to detect DNA from already dead individuals (e.g. false result in population analysis).
- The spatial distribution of eDNA (e.g. species habitat preferences and activity, oxygen, trophic conditions, precipitation, etc.) [20, 21, 25, 26].

4. Problems arising from the handling of the sequencing data:

- The use of incorrect reference sequences > In 2017, 16.3% of the Channidae COI sequences that have been deposited in GenBank were based on incorrect identification.
- Sequence databases biased towards common species (e.g. detection of rare or poorly studied taxa may not be possible).
- Sequences from several databases are merged (e.g.: sequence duplications, different nomenclatures) [20, 21, 25, 26].

The aquatic ecosystem is particularly suitable for eDNA analysis, as water is a convenient medium for the deposition and transport of intracellular or extracellular environmental DNA from aquatic, semi-aquatic and terrestrial organisms [24]. Nevertheless, further research is still needed to better evaluate the spatial and temporal variability of eDNA and eventually to design an optimal sampling strategy for eDNA monitoring, in spite of the acknowledged ability of eDNA metabarcoding to describe fish populations.

One study sought to find a way to eliminate the time-consuming nature of filtered water samples used in fish eDNA metabarcoding by using passive DNA samplers, which have been used to extract DNA from freshwater macroinvertebrates. For this purpose, an aquatic biofilm was used, and the experiment was validated on a test community carried out in a large pond. In this way, the capability of fish eDNA metabarcoding from this matrix was verified, by comparison with conventional fish eDNA approach from filtered water samples. The two approaches yielded similar results for biofilms from environmentally protected habitats (waves degrade biofilms), but differed for rare taxa. Thus, the researchers have demonstrated that aquatic biofilms can serve as passive eDNA samplers of fish eDNA and thereby be used for efficient, rapid monitoring of fish communities. Additionally, they have opened up research opportunities to investigate the fish-fish relationships of simpler organisms on biofilms isolated from eDNA in an environmental matrix, while saving sampling effort, analysis time and costs [27].

We need to mention, that optimisation of the practical aspects of eDNA-based techniques is not the only thing that needs to be addressed, as over the years there are also more and more options for the methods of processing the data obtained from eDNA. Therefore, Blackman et al. [19], by using an eDNA sequence dataset of large freshwater fish (e.g. *Silurus glanis*), have compared the commonly used zero radius Operational Taxonomic Unit (zOTU) and the Operational Sequence Units (OSU) approach. As a result, they showed that the two methods produced similar results in the case of common species, while they could not be compared for rarer species. The reliability of zOTU depended on the size of the reference database, while the OSU was based on the reliability of the DNA sequences. So, its

efficiency decreased in the direct proportion to the phylogenetic age of the studied species. Possible solutions to these problems included the integration of target group coverage, outgroups and full taxonomic annotations into the reference databases. In this way, the misleading annotations that occur due to short amplicons are eliminated.

Surveys based on eDNA can be optimised in terms of sampling conditions to achieve the highest overall detection rate with the method. This influences the potential spread of the technique, as both management and policy related awareness can help to make it more popular among the users. This has been demonstrated by Blabolil et al. [28], who sampled eDNA from three fish ponds with high fish densities and broad species diversity in summer and autumn, using a large number of spatially evenly distributed replicates over the lake surface. With eDNA, most common species were detected, but there were exceptions that were caught in nets but were not detected during eDNA barcoding (e.g. *S. glanis*). Also, a higher species diversity was observed in autumn samples compared to summer samples and in rivers compared to stagnant waters. Thus, it could be said that the fish detected in the pooled samples may reflect the overall community structure and that the detectability of species increases with increased filtration rates.

In order to expand its potential, researchers have regularly sampled a large river (the Rhône) that runs from Lake Geneva to the Mediterranean Sea (524 km long). Here, they tested the ability of MOTU (Molecular Operational Taxonomic Units) readings to allow comparison of relative abundance of species between sites and the relationship with the detection rate of each MOTU. The results were also compared with conventional electrofishing (TEF) surveys to see the ability to estimate species richness and relative species abundance, as well as fish biodiversity along the entire length of the river. In addition, eDNA-metabarcoding detection distances were examined for the range of hydrological conditions. It was found that eDNA metabarcoding is able to qualitatively and quantitatively reveal the structure of fish assemblage (relative abundance of species) and that eDNA integrates a larger space than classical sampling sites. Moreover, it has been shown that eDNA behaves like a particle of organic matter in the water column, with detection distances ranging from a couple of km in a small stream to more than 100 km in a large river. This was the first proof that eDNA metabarcoding can describe the longitudinal fish assemblage pattern of a large river, and is therefore a reliable, cost-effective method for monitoring species diversity [29].

The parallel use of eDNA and traditional methods

Effective management of introduced invasive fish depends on the availability of up-to-date information on their ranges and spatial distribution [21]. In a study [20] the traditional electrofishing (EF) method, long-term electrofishing surveys (LTES) and eDNA barcoding. Researchers sampled eDNA from 30 L of water at nine sites on both banks of the river in the same week as traditional EF surveys for two consecutive years (2017-2018). Since this quantity was theoretically sufficient to detect species within 95% accuracy from the material stuck on the filter, they were able to compare their assessments of species richness using eDNA metabarcoding, EF and LTES. As a result, they concluded that the study confirms that the detection of different fish species using eDNA metabarcoding is more efficient than conventional EF. For 6 species, their presence in the sampled water was probably due to human consumption so, these were not taken into account. Combined, the two methods detected 4 new species in the region. Occasionally, the eDNA of some species was not detectable on both shores while they were caught by the EF method. This could have been due to, among other reasons, the high temperature of the water during summer. For LTES, the 3-6 year EF survey was less effective than eDNA, the 14-19 year was equivalent or slightly better (4 more species caught), while the 29 year survey allowed 6-7 more species to be detected than with the two year eDNA survey. In addition to these, the study demonstrated that the relative number of reads was positively correlated with the number of fish species detected with EF over the past two decades. Hence, it can be concluded that eDNA is a better detection method while it is a more cost effective solution compared to conventional methods. In 2019, another group in a similar study wanted to compare EF with eDNA metabarcoding method in 3 rivers (Ain, Rhône, and the Tier

River). Herein in this particular case, they were able to detect the presence of eDNA in the Rhône River using both method, in the Ain River they could only identify eDNA, whereas in the Tier River they could not. The water yield naturally influences the downstream transport of eDNA, and with it affected the taxonomic richness of all fish, while their spawning season affected only certain species. These results could assist to select the best sampling strategy in order of the research objectives [30].

One year after the previous research, a study was published by Pont et al. [21], offering an explanation and solution to the issue of using the methods described above. As we know, even today, the main methods used for monitoring and detecting native and invasive species are still mainly traditional methods or their improved versions. The reason for this is that, although it is now clear that technologies based on eDNA barcoding are advantageous in ecological areas, more than half of the member's states in the European Union methods include at least one aspect (e.g. age, size-coupled value) in their measurement systems that cannot be detected from eDNA, and their systems are therefore currently unsuited for this method. Therefore, they continue to use traditional electrofishing (TEF) data in accordance with the EU Water Framework Directive (WFD) across Europe. However, a review of the 25 current WFD-compliant methods for river fish shows that 81% of the measurements used in these methods are compatible with eDNA samples. Nevertheless, most trait-based enrichment measures still give different results when calculated from eDNA and when calculated from TEF samples. An existing predictive fish index was adjusted for compatibility, resulting in ecological scores comparable to the current validated method for 22 of the 25 study sites. Thus, they predict that an assessment methodology will become available within a few years, with high sensitivity, better reproducibility and lower uncertainty, aligned to fish eDNA.

A Czech study [25] involved the sampling of three reservoirs in two seasons with a spatially and temporally distributed pattern covering the main environmental gradients. This meant that 2 L water samples were collected at 1 km intervals from each other in the 5-5 sites at Klíčava and Žlutice reservoirs and 8 sites at Římov. Where possible, these were sampled from 3 depths: 5, 10 and 20 m. Thus, a total of 31 fish taxa were detected at all sampling sites during the eDNA metabarcoding. The reliability of the data was supported by the strong positive correlations between the scores of individual taxa, derived from the comparison of gill netting method described earlier in this paper, and the eDNA site occupancy. The analyses supported the expected results, such as the highest number of taxa compared to open water was in the largest water body, where rivers connected with other waterbody and in river shore regions [25].

In order to improve the precision of eDNA lentic water sampling, Hervé et al. [4] collected 4 types of samples every 6 weeks for 1 year from a dam reservoir (Aiguebelette), a shallow eutrophic lake (Etang des Aulnes) and a deep oligotrophic lake (Serre-Ponçon) to investigate the spatial variability of the eDNA signal in the lakes. Thus, a total of 92 eDNA samples were analysed using a metabarcoding method, with results compared to those of standardised gillnet sampling. In contrast to eDNA matearcoding, *Silurus glanis* was only detected by gillnet sampling in Etang des Aulnes lake. But beyond this, many of the species known from the lakes were only detectable using eDNA, confirming the greater sensitivity of this technique compared to gillnetting. As for the spatial variation in the concentration of the eDNA signal, it was found that the shore samples were the most species-rich, while in the regularly mixed ponds the detected eDNA was homogeneously distributed across depth. Furthermore, the presence of eDNA was shown to be related to the phenology of the species. Therefore, these results could contribute to the design of sampling protocols by determining where and when to collect eDNA in ponds for fish monitoring.

Apart from freshwater and coastal areas, there has been relatively little eDNA research in the open ocean. One example of this was when species-specific qPCR and metabarcoding with universal primers were compared for a total of 6 fish species in the Kuroshio Extension area. Eventually, the 2 methods were compared with the results of the conventional mesh method. Results showed that the qPCR method resulted in a higher detection rate than the metabarcoding method, but still produced similar results in estimating the spatial distribution and proportion of species. Therefore, the combined use of both methods was proposed for pelagic fish species [31].

Application of the eDNA method to detect invasive *Silurus glanis*

Complex and heterogeneous areas are highly challenging environments for biomonitoring. For example, fish in deep waters are present in a wide range of habitats, so traditionally a combination of survey methods is used [25]. A good example, when both the widely used EF and the eDNA barcoding have detected the presence of *S. glanis* in expected habitats [21]. In a single study, the presence of both EF and eDNA barcoding was detected at all 5 sampling sites. Furthermore, with the exception of one site (RS B river section), we could report that the combined results of the measurement types used for catfish (benthic feeding, phytophilic and tolerant to pollution species) showed a higher relative percentage abundance with the eDNA method. The study by Blabolil et al. [25] also showed that European catfish was detectable in eDNA barcoding, as it was among the species found in 3 reservoirs, which was confirmed by the conventional gillnet study.

Between November 2018 and December 2019, eDNA methods were applied at two sites in the Venetian Lagoon to monitor bony fish communities, detect non-native species and reveal seasonal variation in fauna. In the end, 62 fish taxa were identified to detect the majority of the species known to inhabit or invade the Venetian lagoon (e.g. the established *S. glanis*). Their DNA distribution reflected the differences in fish communities between the freshwater and marine parts of the lagoon. They also detected the oceanic pufferfish *(Lagocephalus lagocephalus)* for the first time, providing evidence of the species' northern distribution in the higher part of the Adriatic Sea. Furthermore, eDNA successfully profiled fish communities by season and habitat in the Venice lagoon. The study also demonstrated the potential use of eDNA to monitor the possible ecological consequences of MOSE (MOdulo Sperimentale Elettromeccanico) closures [32].

As in a previously discussed experiment too, a study on the Volga confirmed that qPCR techniques, due to their species specificity, can detect targeted species with higher yields than metabarcoding. This is how *S. glanis* was detected using a TaqMan qPCR method, yet since it did not appear in metabarcoding, the possibility of false negative results was highlighted for the used primers [33].

On this river, a prior study using eDNA metabarcoding was carried out in 2019 [34]. In this, the species richness and spatial distribution of fish were investigated using three mitochondrial DNA markers in the upper free-flowing river section and in the selected tributaries. In this way, 23 fish species and their distribution were identified in the upper Volga. However, several species present in the study were not detected by barcoding, such as *S. glanis*. This was due to the limitations of PCR primer sets or the quality of reference databases. In the case of European catfish, none of the markers used (Cytb; 12S; 16S) were identical to the reference sequence in this case, so amplification could not be performed correctly. However, as we have read several times before, this kind of problem can be solved by the eDNA qPCR method using species-specific primers, as described in this article, to exclude the possibility of technical error as a background for the lack of species. A recent study [35] in the Laurentian Great Lakes region on the US-Canada border conducted eDNA metabarcoding to assess the status of currently known species from the region, as well as non-native fish species identified as threats. They then evaluated 23 primer pairs commonly used in fish eDNA metabarcoding, and eventually determined the native and potentially invasive non-native fishes whose genetic sequencing should be prioritized to ensure reliable eDNA metabarcoding of the region. The 23 primer pairs used for species identification were located within either one of the four routinely targeted mitochondrial gene regions (Cytb, 12S, 16S - also used in previous experiments; COI) or a nuclear gene region (18S). Thus, a total of 75 potentially invasive species were identified, including *S. glanis*, which species was positive for 4 of the 7 risk factors. In this study, it was highlighted that the use of eDNA metabarcoding would be the most useful tool for conservation management of existing fish stocks, as it would allow early detection of invasive species and estimation of total biodiversity.

In one paper [36], the *S. glanis* catfish was used as a case study. During the research, online blogs, websites, videos, magazines and newspapers were searched for news about the presence and distribution of European catfish in the Iberian Peninsula. These were then cross-referenced with

official reports (e.g. national government reports) to produce a map showing the supposed invasion pattern of *S. glanis* across freshwater ecosystems. The status quo has highlighted the presence of the studied catfish in six of the seven main river basins of the region. To validate this, a molecular pilot study was also initiated, in which genus-specific primers were designed to detect this species in eDNA from reported sites. In the experimental set up, the primers were tested on eDNA samples taken from an experimental aquariums and on real environmental samples from different basins of Spain (Ebro, Douro and Tagus). *S. glanis* was detected in all basins, but beside these, 83% of the official *Silurus* reports were confirmed by two molecular markers, and in the 66% of the three unofficial reports, which originated from fishermen's websites and newspaper reports. The combination of citizen alerts and the eDNA detection method could be a useful tool for early detection of invasive species, allowing rapid and effective management actions against them. An article published in 2018, using eDNA metabarcoding on the Rhône River, found that it is possible to investigate the longitudinal species diversity of a river from several perspectives. For example, on this occasion, *S. glanis* was present in the lower reaches of the river, with more stagnant water-preferring and migratory species, while in the upper reaches of the river, mainly lotic species were detected [29].

Conclusion

This review has shown that environmental DNA-based technologies can be used for accurate and early detection of the presence and spread of invasive species, such as *S. glanis*, for monitoring the biodiversities of fresh, brackish and saline waters, and for monitoring the effects of artificial factors on the ecosystem, as well as for monitoring the diversity of species in standing and flowing water bodies. However, as these methods require further optimisation and are influenced by several factors, the best results are obtained when used in combination with other traditional techniques. This could be seen from the fact that among the eDNA methods, metabarcoding did not always detect European catfish, while other traditional methods were able to confirm its presence. This may be due to, amongst other things, the incompatibility between eDNA barcoding primers (as demonstrated in a study) and the genetic material of the catfish, but environmental influences may also play a role. To overcome this problem, the use of a species-specific qPCR approach or a combination of traditional EF and net techniques along with metabarcoding may be a solution. From the present review, it can be observed that although the application of eDNA-based methods is recognised as efficient, they are not yet well designed and widespread for the case of *Silurus glanis* monitoring.

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