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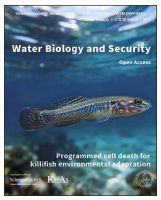

Research Article

How does the efficiency of Nanopore compare to Illumina sequencing in tracking the spread of aquatic invasive species?

Théo Deremarque [a], Rodolphe Elie Gozlan [a], Ravo Ravaozafindrasoa [a,b], Giuliano Mucci [a,c], Lucie Delalex [d], Jean-Michel Foissy [d], Michaël Cagnant [d], Mathieu Clair [d], Justina Givens [a], Fabienne Justy [a], Alice Valentini [e], Delphine Nicolas [f], Pascal Contournet [f], Claire Tetrel [g], Marc Thibault [f], Marine Combe [a,*]

[a] ISEM, Univ Montpellier, CNRS, IRD, Montpellier, France
[b] Laboratoire Eau, Environnement et Systèmes Urbains, Marne-La-Vallée, 77455, France
[c] Maladie Infectieuses et Vecteurs: Écologie, Génétique, Évolution et Contrôle (MIVEGEC), University of Montpellier, CNRS, IRD, 34090, Montpellier, France
[d] DIR OFB Paca Corse Domaine du Petit Arbois Pavillon Laënnec – Hall B, Avenue Louis Philibert, 13547, Aix-en-Provence, France
[e] SPYGEN, Le Bourget-du-Lac, France
[f] Tour du Valat, Research Institute for the Conservation of Mediterranean wetlands, Le Sambuc, 13200, Arles, France
[g] Direction Interrégionale PACA Corse, Service Appui aux Acteurs et Mobilisation des Territoires, Office français de la biodiversité, Domaine des Grandes Cabanes, France

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ABSTRACT

eDNA-based methods offer a non-invasive, easy-to-use approach for monitoring aquatic biodiversity, particularly for tracking invasive or cryptic species. While Illumina sequencing is commonly used, few studies have explored pairing eDNA sampling with Nanopore sequencing, a third-generation technology that allows for on-site sequencing but with a higher error rate. Here, we compared the performance of both technologies in detecting the invasive host-parasite complex *Pseudorasbora parva-Sphaerothecum destruens*. Water samples were collected from sites in Camargue and Corsica (Southern France), followed by DNA extraction, PCR amplification, and sequencing using either Illumina or Nanopore. Both technologies showed similar detection rates of *P. parva*, but only when Nanopore sequencing was performed under optimal conditions. However, for *S. destruens*, results varied: Illumina failed to detect the parasite, while Nanopore identified its DNA in multiple sites. We suggest that this discrepancy may be due to the different bioinformatic approaches used to process the reads and/or the higher error rate of Nanopore sequencing which could result in misassignments during species identification.

1. Introduction

Better understanding invasion biology is crucial given the severe ecological, economic and societal concerns due to the detrimental impact of non-native species on biodiversity (Bellard et al., 2016; Falaschi et al., 2020), food security (agriculture/aquaculture) (Paini et al., 2016) and human health (Medlock et al., 2012; Vilà and Hulme, 2017). In aquatic ecosystems, invasive species drive significant losses in fish, macrophytes, zooplankton (Gallardo et al., 2016), amphibians (Longcore et al., 1999) and crustaceans (Edgerton et al., 2004). Monitoring these environments is challenging due to water opacity and limited access to remote areas. However, efficient, non-invasive detection methods are vital for tracking invasive species and guiding conservation strategies (Reaser et al., 2020). Among these, environmental DNA (eDNA) surveys stand out as a sensitive, low-cost, and easy-to-use tool, and perfectly suited for detecting invasive species expansion (Larson et al., 2020; Thomas et al., 2020).

eDNA sampling (from soil, water, air, feces, etc.) can be followed by either species-specific approaches (Mauvisseau et al., 2019; LeBlanc et al., 2020), targeting a particular species, or metabarcoding (Ruppert et al., 2019), which analyzes the presence of multiple species in a clade (Combe and Gozlan, 2022). Metabarcoding is typically done using Illumina sequencing (Deiner et al., 2017; Nakao et al., 2021) a highly reliable technology. However, in recent years, Nanopore sequencing, a third-generation method, has gained attention due to its speed, portability, and suitability for fieldwork (Leggett and Clark, 2017). Although,

* Corresponding author.
E-mail address: marine.combe@ird.fr (M. Combe).
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few studies have used Nanopore for eDNA metabarcoding, results have shown consistent species detection compared to illumina, particularly for bivalves (Egeter et al., 2022) and fish (Doorenspleet et al., 2021). Yet, more validation is needed for other taxa, especially invasive or cryptic species in natural aquatic environments.

Pseudorasbora parva is a small cyprinid fish native to East Asia that thrives in lentic freshwater systems. As a highly invasive species, it spread across Europe in the 1960's (Gozlan et al., 2010; Combe and Gozlan, 2018). Owing to its reproductive traits and tolerance for a wide range of temperatures (Davies and Britton, 2015), *P. parva* has successfully colonized waterways in Asia, Europe, and Africa, posing a serious threat to local biodiversity. Notably, *P. parva* is also a healthy carrier host of the fungal parasite *Sphaerothecum destruens*, a generalist parasite capable of infecting various fish species and further endangering native ecosystems (Gozlan and Combe, 2023). However, the chronic nature of *S. destruens* infection and its low abundance in *P. parva* make detection difficult (Combe et al., 2022). This study compares the efficiency of Nanopore and Illumina sequencing technologies in detecting this invasive fish-parasite complex in freshwater ecosystems.

2. Material and methods

2.1. Study sites

The study sites in the Camargue (4 areas) and Corsica (Lake Calacuccia, Golo river) are detailed in Supplementary Table S1 S*destruens* detection was assessed only in the Camargue, while *P. parva* detection focused exclusively on Corsica. Additional details on eDNA sampling and DNA extraction protocols can be found in Supplementary Note 1.

2.2. PCR amplifications of host-parasite DNA

To dilute PCR inhibitors, each DNA extract was diluted fivefold in DNase/RNase-free water. For *P. parva* DNA amplifications, we targeted a 147 bp sequence of the mtDNA 16S rRNA genes using PparvaF-PparvaR (Robinson et al., 2019) primers and a 100 bp sequence of the mtDNA 12S rRNA genes using teleo_F-teleo_R (Valentini et al., 2016) primers. For amplification of *S. destruens* DNA, we targeted a 154 bp sequence of the 18S rRNA genes using the ChF2-ChR2 (Cherif et al., 2022) primers. PCR amplifications used for further Nanopore sequencing were performed in 0.2 mL PCR tubes using the following mix: 1X GoTaq G2 Green master mix (LifeTechnologies), 0.5 μM of each primer, 3 μL of DNA and made up to 25 μL with DNase/RNase-free water. PCR amplification cycles consisted of an initial denaturation at 95 °C during 5 min followed by 35 cycles of 30 s denaturation at 94 °C, 90 s hybridisation at 60 °C and 60 s elongation at 72 °C, ending with a final elongation at 72 °C for 5 min after the 35 cycles. PCR amplifications for Illumina sequencing were performed using teleo_F-teleo_R primers and following the protocol of Pont et al. (2018) with 12 replicates, whereas, due to budget constraints, only a single PCR replicate was carried out for Nanopore sequencing. For each sample, 3 μL of PCR product was loaded onto a 2 % agarose gel and migrated by electrophoresis at 100 V for 60 min. Migration profiles were checked under UV light and a band was scored of the expected size given the primers used. All PCR products, either positive or negative on the gel, were sequenced either using an Illumina MiSeq 1000 sequencer (Illumina, San Diego, CA, USA) or a MinION mk1C sequencer (Oxford Nanopore Technologies, Oxford, UK).

2.3. Nanopore sequencing

First, DNA quantification of PCR amplicons was performed using Qubit Fluorometric Quantification (ThermoFisher Scientific). The samples were then standardised to start the preparation of the sequencing libraries with the same amount of DNA for each sample (i.e., 110 fmol). Oxford Nanopore Technologies (ONT) libraries were prepared using native barcoding 24 v14 (SQK-NBD114.24, Oxford Nanopore Technologies) according to the manufacturer's instructions for barcoding of amplicon libraries. At the end of the library preparation, 20 fmol of DNA was loaded onto a flow cell (R10.4.1) and sequencing was performed on an Oxford Nanopore Technologies (Oxford, UK) Mk1c device (MinKNOW software v23.07.12) with a maximum run time of 48 h. Reads were basecalled and demultiplexed in real time using the guppy software, with default parameters, integrated into the MinION device resulting in fastq files. The bioinformatic analysis of the reads was performed using the pipeline developed by Egeter et al. (2022). The pipeline relied on minimizing the sequencing error rate by polishing the reads using different steps: (1) The raw reads were trimmed on both ends using CUTADAPT (Martin, 2011) to remove low quality bases and keep only reads ranging between 100 and 200 bp; (2) after filtration the reads were clustered using ISONCLUST (Sahlin and Medvedev, 2020) and a consensus sequence was created using RACON (Vaser et al., 2017); (3) the consensus sequences were clustered at 99 % sequence identity using CD-HIT (Fu et al., 2012) followed by the selection of a centroid for each cluster; (4) the primers were trimmed from the centroids, completing the production of polished reads. Those polished reads were then BLAST against a reference database restricted to either *P. parva* and some other species from the cyprinodei suborder (Supplementary Table S2) or all Mesomycetozoean species using the same settings as those used in the original pipeline. Stringent percentage identity was used to analyze the BLAST results: 99 % for species level, 97 % for genus level, 95 % for family level and 93 % for higher-than-family-level. In order to avoid taking into account spurious detections, sequences displaying less than 10 reads or with a frequency of occurrence <0.001 per sequence and per library were excluded.

2.4. Illumina sequencing

Prior to Illumina sequencing, amplified samples were quantified using capillary electrophoresis, purified using MinElute PCR purification kit and quantified again. PCR products were pooled in equal volumes to achieve a theoretical sequencing depth of 500,000 reads for fish primers and 50,000 for *S. destruens*. Libraries were prepared using the True Seq protocol (Illumina, San Diego, CA, USA) and paired-end sequencing was carried out on an Illumina NextSeq 1000 sequencer. Sequenced reads were analyzed using the programs implemented in the OBITools package (http://metabarcoding.org/obitools, Boyer et al., 2016). Forward and reverse reads were assembled using the illuminapairedend program and assigned to each sample using the ngsfilter program. Sequences shorter than 20 bp, occurring <10 times per sample or labeled as 'internal' by the obiclean program (likely due to PCR/sequencing errors) were discarded. The taxonomic assignment of ASVs (Amplicon Sequence Variant) was performed using the program ecotag with the local reference database (Valentini et al., 2016) and sequences (standard sequences) were extracted from GenBank® release 247.0. To account for the incorrect assignment of a small number of sequences to the sample due to tag jumps (Schnell et al., 2015), all sequences with a frequency of occurrence <0.001 per sequence and per library were excluded. Subsequently, the data were curated for index-hopping (MacConaill et al., 2018) with a threshold empirically determined using experimental blanks (i.e., combinations of tags not present in libraries), for a given sequencing batch between libraries.

3. Results

3.1. Detection of the invasive P. parva fish host from water samples

Illumina sequencing generated a mean number of 455,529 raw reads per site across 12 PCR replicates, while Nanopore generated a mean number of 145,438 reads per site with just 1 PCR replicate. After filtering, this difference became even more pronounced: Illumina averaged 274,400 filtered reads per site compared to just 5285 for Nanopore.

Illumina detected *P. parva* DNA in 7 out of 10 sites, including 4 in

Lake Calacuccia and 3 in the downstream Golo River. In contrast, the initial Nanopore run, using a flow cell with a low number of active pores, failed to detect *P. parva* DNA but did identify DNA from the Gobionidae family, to which *P. parva* belongs, but the resolution was insufficient to determine the taxonomic level beyond the family. Gobionidae DNA was detected at five sites, including 3 sites in the lake and 2 in the river—matching sites positive for *P. parva* in Illumina. The average number of mapped reads (to *P. parva* for Illumina sequencing and to the Gobionidae family for Nanopore sequencing) was consistently higher in Lake Calacuccia (92,704 for Illumina and 113 for Nanopore) compared with the Golo river (2533 for Illumina and 55 for Nanopore).

A second Nanopore run, using a new flow cell and focusing on two sites where *P. parva* was detected by Illumina but not Nanopore (Gol7 and Cal1), revealed *P. parva* DNA at the Gol7 site, with more reads than Illumina (43,618 vs. 1379 reads, respectively). The Cal1 site remained negative for *P. parva*, consistent with Illumina's results, which only detected it in 1 of 12 PCR replicates.

3.2. *Detection of the cryptic fungal parasite Sphaerothecum destruens from water samples*

Illumina sequencing failed to detect *S. destruens* DNA at any site, but other species from the same Mesomycetozoea class were found (Fig. 1). In the Camargue, species like *Sphaeroforma tapetis, Creolimax fragrantissima, Anurofeca richardsi,* and *Sphaerothecum caipira* were identified. Notably, *S. tapetis* had the highest read count (33,948 reads) and was consistently detected in the Fumemorte canal, while *S. caipira*, closely related to *S. destruens*, appeared at every sampling point of the study in the Grandes Cabanes domain.

Of 25 Mesomycetozoean occurrences identified by Illumina, 10 were also found by Nanopore. In all cases, Illumina produced significantly more mapped reads, with the ratio ranging from 1.6 to 400 times higher. Despite its lower sensitivity, Nanopore detected 14 occurrences of Mesomycetozoean missed by Illumina, including three species/genus found only by Nanopore: *Dermocystidium* spp., *Amphibiocystidium ranae*,

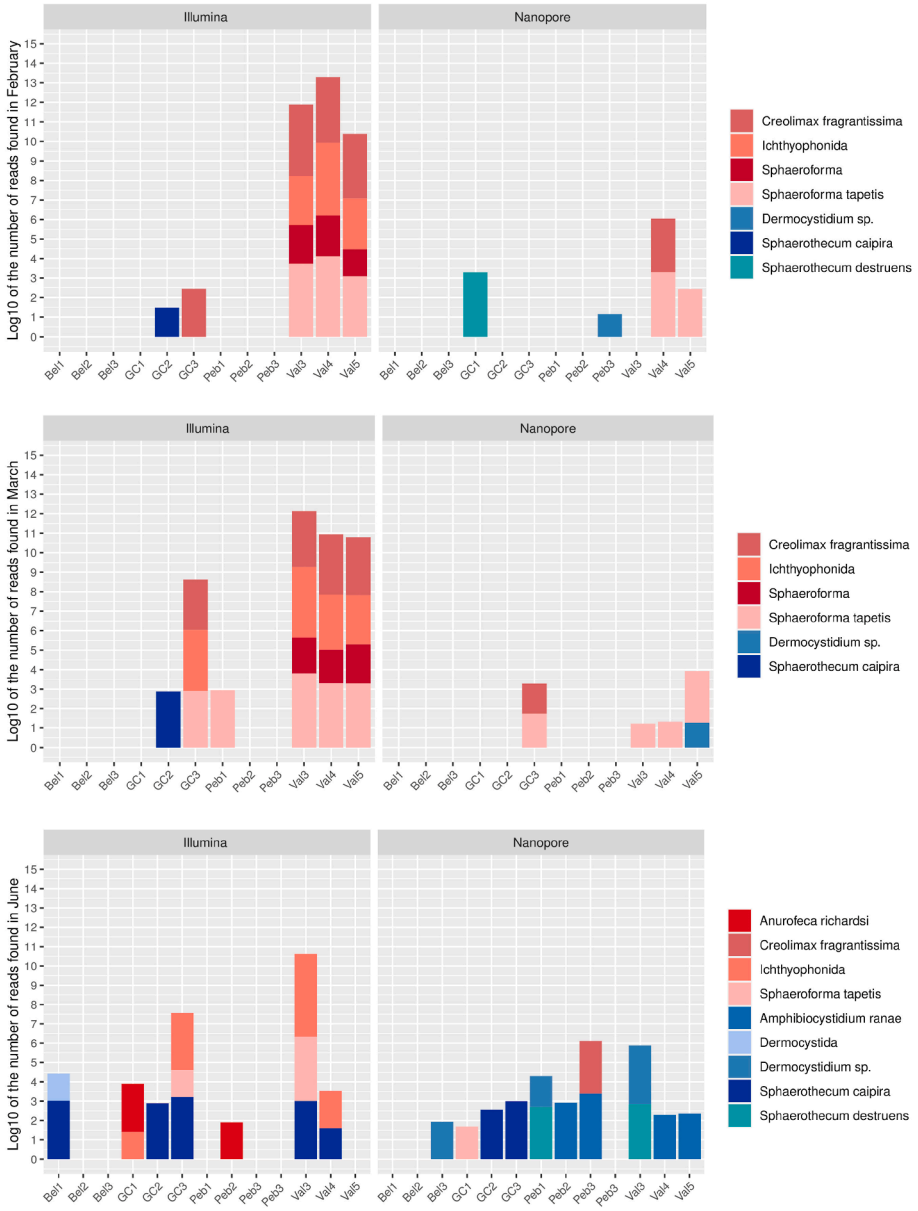

Fig. 1. Log 10 of the number of reads mapping clades from the class Mesomycetozoea resulting from an Illumina or Nanopore sequencing of eDNA samples collected in different sites of the Camargue in February, March, and June. The clades indicated in a shade of red in the figure belong to the order Ichthyophonida while the clades indicated in a shade of blue belong to the order Dermocystida.

and *S. destruens*. *S. destruens* was detected in February 2023 in the Grandes Cabanes domain, and in June 2023 in both the Pebre and Fumemorte canals, with more reads recovered in February (1943 reads) than in June (503 and 694 reads, respectively).

Interestingly, both technologies were more effective at detecting species from the Dermocystida and Ichthyophonida orders. Illumina attributed 18 % of occurrences to Dermocystida (8/45) and 82 % to Ichthyophonida (37/45), while Nanopore found 58 % from Dermocystida (14/24) and 42 % from Ichthyophonida (10/24).

4. Discussion

Our results highlight the importance of using closely matched protocols when comparing Nanopore and Illumina sequencing technologies for effective results. Notably, during the sequencing of *P. parva,* the initial flow cell's low pore count negatively impacted the outcomes. This was evident when we resequenced DNA from the Gol7 site with a new flow cell, which yielded significantly improved read quantity and quality. This could explain why Nanopore sequencing struggled to assign reads at the species level initially, yet successfully detected *P. parva* with a new flow cell. The only site where Nanopore failed to assign taxonomical reads while Illumina succeeded was Cal1, but even then, Illumina only detected it in one replicate. When we compared the average reads generated—145,438 for Nanopore versus 455,529 for Illumina—it was clear that Nanopore was at a disadvantage. These results underscore the necessity for Nanopore sequencing to be performed across multiple replicates and over a longer duration than the 40 h we used in this study, to enhance chances of detection. In addition to sequencing depth, the primers used also differed: Nanopore used PparvaF-PparvaR targeting the 16S rRNA gene, while Illumina used teleo_F-teleo_R targeting the 12S rRNA gene. Previous metabarcoding studies using eDNA samples have shown comparable efficiencies in detecting species or taxa with primers for either the 12S or 16S rRNA gene (Sard et al., 2019; Kumar et al., 2022). However, discrepancies between primer sets for certain species, like *Neogobius melanostomus*, were also noted (Sard et al., 2019). Furthermore, detection rate varied even among primers targeting the same gene (Li et al., 2024; Polanco Fernández et al., 2021) demonstrating that the effectiveness of a primer set depends not just on the targeted gene but also on its design, the size of the amplified fragment, and the targeted species.

Similar to our findings with *P. parva*, the comparison of Illumina and Nanopore sequencing revealed contrasting results for *S. destruens*. While both technologies detected Mesomycetozoean species, Illumina consistently showed a greater number of mapped reads than Nanopore. This underscores the necessity of sequencing multiple PCR replicates to enhance detection sensitivity for all species, particularly rare and cryptic ones (Wen et al., 2017; Shirazi et al., 2021). Despite similar outcomes at certain sites, the two technologies displayed distinct preferences, favoring the detection of either Ichthyophonida (Illumina) or Dermocystida (Nanopore). Notably, Nanopore successfully detected *S. destruens*, while Illumina did not. These discrepancies could stem from several factors: (i) The bioinformatic treatment of the reads differed depending on the sequencing technology. To address limitations specific to each technology: an ASV approach was adopted for the treatment of Illumina data while the reads generated by Nanopore were polished and clustered to obtain consensus sequences; (ii) Despite recent improvements, Nanopore sequencing still has a higher error rate than Illumina (Delahaye and Nicolas, 2021), making it more challenging to assign sequences accurately at the species level (Winand et al., 2020; Egeter et al., 2022). Our results contrast with previous studies on bivalves or on the gut content of lanternfish, which used multiple PCR replicates and found comparable detection levels for both technologies (Egeter et al., 2022; van der Reis et al., 2023). Ultimately, our study reveals ongoing challenges in efficiently detecting cryptic intracellular parasites in freshwater environments using eDNA techniques.

5. Conclusion

Illumina sequencing proved accurate enough to detect *P. parva* DNA whereas Nanopore sequencing failed to achieve species-level detection for most samples. However, using a flow cell with a higher number of pores improved the accuracy, enabling the detection of *P. parva*.

When it comes to detecting the intracellular *S. destruens*, this study highlights the challenges eDNA methods face in identifying cryptic species. Illumina sequencing was unable to detect the parasite, and Nanopore sequencing demonstrated limitations in accurately assigning reads at the species level.

Overall, this study emphasizes the necessity of following standardized protocols (e.g., PCR primers, number of PCR replicates, bioinformatic processing of the reads) to establish a meaningful comparison between Illumina and Nanopore sequencing.

CRediT authorship contribution statement

Théo Deremarque: Writing – review & editing, Writing – original draft, Investigation, Formal analysis. **Rodolphe Elie Gozlan:** Writing – review & editing, Writing – original draft, Investigation, Conceptualization. **Ravo Ravaozafindrasoa:** Formal analysis. **Giuliano Mucci:** Investigation. **Lucie Delalex:** Investigation. **Jean-Michel Foissy:** Investigation. **Michaël Cagnant:** Investigation. **Mathieu Clair:** Investigation. **Justina Givens:** Investigation. **Fabienne Justy:** Investigation. **Alice Valentini:** Investigation. **Delphine Nicolas:** Investigation. **Pascal Contournet:** Investigation. **Claire Tetrel:** Investigation. **Marc Thibault:** Investigation. **Marine Combe:** Writing – review & editing, Writing – original draft, Investigation, Funding acquisition, Conceptualization.

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Declaration of competing interest

Authors declare no conflict of interest. REG is an editorial board member for *Water Biology and Security* and was not involved in the editorial review or the decision to publish this article.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.watbs.2025.100423.

References

Bellard, C., Cassey, P., Blackburn, T.M., 2016. Alien species as a driver of recent extinctions. Biol. Lett. 12, 20150623. https://doi.org/10.1098/rsbl.2015.0623.

Boyer, F., Mercier, C., Bonin, A., Le Bras, Y., Taberlet, P., Coissac, E., 2016. obitools: a unix-inspired software package for DNA metabarcoding. Mol. Ecol. Resour 16, 176–182. https://doi.org/10.1111/1755-0998.12428.

Cherif, E., Deremarque, T., Justy, F., Gozlan, R.E., Combe, M., 2022. Assessing the specificity of the Rosette agent DNA amplification: An optimized protocol for the detection of standard DNA among studies. J Fish Dis 46, 177–180. https://doi.org/10.1111/jfd.13722.

Combe, M., Cherif, E., Charrier, A., Barbey, B., Chague, M., Carrel, G., Chasserieau, C., Foissy, J.-M., Gerard, B., Gozlan, Z., 2022. Towards unravelling the Rosette agent enigma: spread and emergence of the co-invasive host-pathogen complex, Pseudorasbora parva-Sphaerothecum destruens. Sci. Total Environ. 806, 150427.

Combe, M., Gozlan, R., 2022. Biodiversité, maladies émergentes, épidémies… Révéler l'invisible grâce à l'ADN présent dans l'environnement [WWW Document]. The Conversation. URL. http://theconversation.com/biodiversite-maladies-emergentes-epidemies-reveler-linvisible-grace-a-ladn-present-dans-lenvironnement-190389.

Combe, M., Gozlan, R.E., 2018. The rise of the rosette agent in Europe: an epidemiological enigma. Transbound. Emerg. Dis. 65, 1474–1481. https://doi.org/10.1111/tbed.13001.

Davies, G.D., Britton, J.R., 2015. Influences of population density, temperature and latitude on the growth of invasive topmouth gudgeon Pseudorasbora parva. Ecol. Freshw. Fish 24, 91–98. https://doi.org/10.1111/eff.12128.

Deiner, K., Bik, H.M., Mächler, E., Seymour, M., Lacoursière-Roussel, A., Altermatt, F., Creer, S., Bista, I., Lodge, D.M., de Vere, N., Pfrender, M.E., Bernatchez, L., 2017. Environmental DNA metabarcoding: Transforming how we survey animal and plant communities. Mol. Ecol. 26, 5872–5895. https://doi.org/10.1111/mec.14350.

Delahaye, C., Nicolas, J., 2021. Sequencing DNA with nanopores: troubles and biases. PLoS One 16, e0257521. https://doi.org/10.1371/journal.pone.0257521.

Doorenspleet, K., Jansen, L., Oosterbroek, S., Bos, O., Kamermans, P., Janse, M., Wurz, E., Murk, A., Nijland, R., 2021. High Resolution Species Detection: Accurate Long Read eDNA Metabarcoding of North Sea Fish Using Oxford Nanopore Sequencing. bioRxiv.

Edgerton, B.F., Henttonen, P., Jussila, J., Mannonen, A., Paasonen, P., Taugbøl, T., Edsman, L., Souty-Grosset, C., 2004. Understanding the causes of disease in European freshwater crayfish. Conserv. Biol. 18, 1466–1474. https://doi.org/10.1111/j.1523-1739.2004.00436.x.

Egeter, B., Veríssimo, J., Lopes-Lima, M., Chaves, C., Pinto, J., Riccardi, N., Beja, P., Fonseca, N.A., 2022. Speeding up the detection of invasive bivalve species using environmental DNA: a Nanopore and Illumina sequencing comparison. Mol. Ecol. Resour 22, 2232–2247. https://doi.org/10.1111/1755-0998.13610.

Falaschi, M., Melotto, A., Manenti, R., Ficetola, G.F., 2020. Invasive species and Amphibian conservation. Herpetologica 76, 216–227.

Fu, L., Niu, B., Zhu, Z., Wu, S., Li, W., 2012. CD-HIT: accelerated for clustering the next-generation sequencing data. Bioinformatics 28, 3150–3152. https://doi.org/10.1093/bioinformatics/bts565.

Gallardo, B., Clavero, M., Sánchez, M.I., Vilà, M., 2016. Global ecological impacts of invasive species in aquatic ecosystems. Glob. Change Biol. 22, 151–163. https://doi.org/10.1111/gcb.13004.

Gozlan, R.E., Andreou, D., Asaeda, T., Beyer, K., Bouhadad, R., Burnard, D., Caiola, N., Cakic, P., Djikanovic, V., Esmaeili, H.R., Falka, I., Golicher, D., Harka, A., Jeney, G., Kováč, V., Musil, J., Nocita, A., Povz, M., Poulet, N., Virbickas, T., Wolter, C., Serhan Tarkan, A., Tricarico, E., Trichkova, T., Verreycken, H., Witkowski, A., Guang Zhang, C., Zweimueller, I., Robert Britton, J., 2010. Pan-continental invasion of Pseudorasbora parva: towards a better understanding of freshwater fish invasions. Fish Fish. 11, 315–340. https://doi.org/10.1111/j.1467-2979.2010.00361.x.

Gozlan, R.E., Combe, M., 2023. Emergence of the fungal rosette agent in the world: Current risk to fish biodiversity and aquaculture. J. Fungi 9, 426. https://doi.org/10.3390/jof9040426.

Kumar, G., Reaume, A.M., Farrell, E., Gaither, M.R., 2022. Comparing eDNA metabarcoding primers for assessing fish communities in a biodiverse estuary. PLoS One 17, e0266720. https://doi.org/10.1371/journal.pone.0266720.

Larson, E.R., Graham, B.M., Achury, R., Coon, J.J., Daniels, M.K., Gambrell, D.K., Jonasen, K.L., King, G.D., LaRacuente, N., Perrin-Stowe, T.I., Reed, E.M., Rice, C.J., Ruzi, S.A., Thairu, M.W., Wilson, J.C., Suarez, A.V., 2020. From eDNA to citizen science: emerging tools for the early detection of invasive species. Front. Ecol. Environ. 18, 194–202. https://doi.org/10.1002/fee.2162.

LeBlanc, F., Belliveau, V., Watson, E., Coomber, C., Simard, N., DiBacco, C., Bernier, R., Gagné, N., 2020. Environmental DNA (eDNA) detection of marine aquatic invasive species (AIS) in Eastern Canada using a targeted species-specific qPCR approach. Manag. Biol. Invasions 11, 201–217. https://doi.org/10.3391/mbi.2020.11.2.03.

Leggett, R.M., Clark, M.D., 2017. A world of opportunities with nanopore sequencing. J. Exp. Bot. 68, 5419–5429. https://doi.org/10.1093/jxb/erx289.

Li, Y., Tang, M., Lu, S., Zhang, X., Fang, C., Tan, L., Xiong, F., Zeng, H., He, S., 2024. A comparative evaluation of eDNA metabarcoding primers in fish community monitoring in the East lake. Water 16, 631. https://doi.org/10.3390/w16050631.

Longcore, J.E., Pessier, A.P., Nichols, D.K., 1999. Batrachochytrium dendrobatidis gen. et sp. nov., a chytrid pathogenic to amphibians. Mycologia 91, 219–227. https://doi.org/10.1080/00275514.1999.12061011.

MacConaill, L.E., Burns, R.T., Nag, A., Coleman, H.A., Slevin, M.K., Giorda, K., Light, M., Lai, K., Jarosz, M., McNeill, M.S., Ducar, M.D., Meyerson, M., Thorner, A.R., 2018. Unique, dual-indexed sequencing adapters with UMIs effectively eliminate index cross-talk and significantly improve sensitivity of massively parallel sequencing. BMC Genom. 19, 30. https://doi.org/10.1186/s12864-017-4428-5.

Martin, M., 2011. Cutadapt removes adapter sequences from high-throughput sequencing reads. EMBnet.journal 17, 10–12. https://doi.org/10.14806/ej.17.1.200.

Mauvisseau, Q., Burian, A., Gibson, C., Brys, R., Ramsey, A., Sweet, M., 2019. Influence of accuracy, repeatability and detection probability in the reliability of species-specific eDNA based approaches. Sci. Rep. 9, 580. https://doi.org/10.1038/s41598-018-37001-y.

Medlock, J.M., Hansford, K.M., Schaffner, F., Versteirt, V., Hendrickx, G., Zeller, H., Bortel, W.V., 2012. A review of the invasive mosquitoes in Europe: Ecology, public health risks, and control options. Vector Borne Zoonotic Dis. 12, 435–447. https://doi.org/10.1089/vbz.2011.0814.

Nakao, R., Inui, R., Akamatsu, Y., Goto, M., Doi, H., Matsuoka, S., 2021. Illumina iSeq 100 and MiSeq exhibit similar performance in freshwater fish environmental DNA metabarcoding. Sci. Rep. 11, 15763. https://doi.org/10.1038/s41598-021-95360-5.

Paini, D.R., Sheppard, A.W., Cook, D.C., De Barro, P.J., Worner, S.P., Thomas, M.B., 2016. Global threat to agriculture from invasive species. Proceedings of the National Academy of Sciences 113, 7575–7579. https://doi.org/10.1073/pnas.1602205113.

Polanco Fernández, A., Marques, V., Fopp, F., Juhel, J.-B., Borrero-Pérez, G.H., Cheutin, M.-C., Dejean, T., González Corredor, J.D., Acosta-Chaparro, A., Hocdé, R., Eme, D., Maire, E., Spescha, M., Valentini, A., Manel, S., Mouillot, D., Albouy, C., Pellissier, L., 2021. Comparing environmental DNA metabarcoding and underwater visual census to monitor tropical reef fishes. Environ. DNA 3, 142–156. https://doi.org/10.1002/edn3.140.

Pont, D., Rocle, M., Valentini, A., Civade, R., Jean, P., Maire, A., Roset, N., Schabuss, M., Zornig, H., Dejean, T., 2018. Environmental DNA reveals quantitative patterns of fish biodiversity in large rivers despite its downstream transportation. Sci. Rep. 8, 10361. https://doi.org/10.1038/s41598-018-28424-8.

Reaser, J.K., Burgiel, S.W., Kirkey, J., Brantley, K.A., Veatch, S.D., Burgos-Rodríguez, J., 2020. The early detection of and rapid response (EDRR) to invasive species: a conceptual framework and federal capacities assessment. Biol. Invasions 22, 1–19. https://doi.org/10.1007/s10530-019-02156-w.

Robinson, C.V., Garcia de Leaniz, C., Rolla, M., Consuegra, S., 2019. Monitoring the eradication of the highly invasive topmouth gudgeon (Pseudorasbora parva) using a novel eDNA assay. Environ. DNA 1, 74–85. https://doi.org/10.1002/edn3.12.

Ruppert, K.M., Kline, R.J., Rahman, M.S., 2019. Past, present, and future perspectives of environmental DNA (eDNA) metabarcoding: a systematic review in methods, monitoring, and applications of global eDNA. Glob. Ecol. Conserv. 17, e00547. https://doi.org/10.1016/j.gecco.2019.e00547.

Sahlin, K., Medvedev, P., 2020. De novo clustering of long-read transcriptome data using a greedy, quality value-based algorithm. J. Comput. Biol. 27, 472–484. https://doi.org/10.1089/cmb.2019.0299.

Sard, N.M., Herbst, S.J., Nathan, L., Uhrig, G., Kanefsky, J., Robinson, J.D., Scribner, K. T., 2019. Comparison of fish detections, community diversity, and relative abundance using environmental DNA metabarcoding and traditional gears. Environ. DNA 1, 368–384. https://doi.org/10.1002/edn3.38.

Schnell, I.B., Bohmann, K., Gilbert, M.T.P., 2015. Tag jumps illuminated – reducing sequence-to-sample misidentifications in metabarcoding studies. Mol. Ecol. Resour 15, 1289–1303. https://doi.org/10.1111/1755-0998.12402.

Shirazi, S., Meyer, R.S., Shapiro, B., 2021. Revisiting the effect of PCR replication and sequencing depth on biodiversity metrics in environmental DNA metabarcoding. Ecol. Evol. 11, 15766–15779. https://doi.org/10.1002/ece3.8239.

Thomas, A.C., Tank, S., Nguyen, P.L., Ponce, J., Sinnesael, M., Goldberg, C.S., 2020. A system for rapid eDNA detection of aquatic invasive species. Environ. DNA 2, 261–270. https://doi.org/10.1002/edn3.25.

Valentini, A., Taberlet, P., Miaud, C., Civade, R., Herder, J., Thomsen, P.F., Bellemain, E., Besnard, A., Coissac, E., Boyer, F., Gaboriaud, C., Jean, P., Poulet, N., Roset, N., Copp, G.H., Geniez, P., Pont, D., Argillier, C., Baudoin, J.-M., Peroux, T., Crivelli, A. J., Olivier, A., Acqueberge, M., Le Brun, M., Møller, P.R., Willerslev, E., Dejean, T., 2016. Next-generation monitoring of aquatic biodiversity using environmental DNA metabarcoding. Mol. Ecol. 25, 929–942. https://doi.org/10.1111/mec.13428.

van der Reis, A.L., Beckley, L.E., Olivar, M.P., Jeffs, A.G., 2023. Nanopore short-read sequencing: a quick, cost-effective and accurate method for DNA metabarcoding. Environ. DNA 5, 282–296. https://doi.org/10.1002/edn3.374.

Vaser, R., Sović, I., Nagarajan, N., Šikić, M., 2017. Fast and accurate de novo genome assembly from long uncorrected reads. Genome Res. 27, 737–746. https://doi.org/10.1101/gr.214270.116.

Vilà, M., Hulme, P.E. (Eds.), 2017. Impact of Biological Invasions on Ecosystem Services. Springer International Publishing, Cham. https://doi.org/10.1007/978-3-319-45121-3.

Wen, C., Wu, L., Qin, Y., Nostrand, J.D.V., Ning, D., Sun, B., Xue, K., Liu, F., Deng, Y., Liang, Y., Zhou, J., 2017. Evaluation of the reproducibility of amplicon sequencing with Illumina MiSeq platform. PLoS One 12, e0176716. https://doi.org/10.1371/journal.pone.0176716.

Winand, R., Bogaerts, B., Hoffman, S., Lefevre, L., Delvoye, M., Van Braekel, J., Fu, Q., Roosens, N.H., De Keersmaecker, S.C., Vanneste, K., 2020. Targeting the 16S rRNA gene for bacterial identification in complex mixed samples: comparative evaluation of second (illumina) and third (Oxford nanopore technologies) generation sequencing technologies. Int. J. Mol. Sci. 21, 298. https://doi.org/10.3390/ijms21010298.