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Monitoring invasive alien macroinvertebrate species with environmental DNA

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Abstract

Regular monitoring of ecosystems can be used for the early detection of invasive alien species (IAS), and provide information for management and preventing them from becoming established or advancing into new areas. Current methods of monitoring freshwater systems for IAS can be both financially costly and time-consuming, with routine monitoring often carried out at low intensity and at only a small number of sites. In this study, we evaluate how environmental DNA (eDNA) metabarcoding for monitoring freshwater macroinvertebrate IAS compares to traditional kick-net sampling as part of a national (Switzerland) and a catchment monitoring programme. Kick-net sampling was more fruitful for the detection of several well-known target macroinvertebrate IAS. However, eDNA samples proved complementary for the detection of IAS that belong to species often being unnoticed by traditional sampling due to methodological or taxonomic reasons. Specifically, the invasive jellyfish Craspedacusta sowerbii, hardly detectable using classic kick-net sampling, was found to be widespread in both the national and the catchment-scale monitoring with the eDNA method only. Our study shows that IAS detection using eDNA is easily implemented in both national- and catchment-scale monitoring campaigns. However, successful detection of target IAS is still highly dependent on primer choice, species' biology, and availability of adequate markers. Specifically, multiple markers should be considered for detecting IAS from several different taxonomic groups, such as those under the 'freshwater macroinvertebrate' umbrella term. While eDNA is still developing in terms of these fundamental methodological requirements, surveillance for both target and non-target IAS using eDNA is likely to increase efficiency in early detection of IAS in freshwater systems.

KEYWORDS

biomonitoring, biosecurity, early detection, eDNA, IAS, management

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1 | INTRODUCTION

There has been a steep increase in the number of invasive alien species (hereafter IAS) spreading to new areas throughout all ecosystems in recent years due to the increase in trade, tourism, and travel (Hulme, 2006, 2009; IPBES, 2019; Sutherland et al., 2013). This particularly concerns freshwater ecosystems, which, as highly diverse habitats, are simultaneously facing increasing threats from other anthropogenic pressures (Dudgeon, 2019; Dudgeon et al., 2006) and are prone to be invaded by species from many taxonomic groups, including fish and invertebrates (Baltazar-Soares et al., 2019; De Ventura, Kopp, Seppälä, & Jokela, 2017). To prevent IAS from successfully invading a new habitat and for subsequent management, early detection is paramount, also to reduce the financial costs associated with any possible control attempts (Hulme, 2006). However, to do this, regular and accurate monitoring must be carried out (Dudgeon, 2019).

Current freshwater monitoring methods rely on sight or capture of specimens (e.g., electro-fishing, kick-net, or Surber sampling) with subsequent morphological identification either in the field (i.e., fish) or via microscope (i.e., macroinvertebrates). However, these methods may not be the most suitable tools as species in low abundance (e.g., IAS at early phase of establishment) are often missed. Freshwater macroinvertebrates are typically collected by kick-net sample, which aims to sample a representation of the community from subhabitats within a designated sample site (Barbour, Gerritsen, Snyder, & Stribling, 1999). This method is highly standardised but was developed to allow ecological assessment of rivers by using prior knowledge of specific macroinvertebrate community preferences and pollution tolerances, rather than early detection of macroinvertebrate IAS. Furthermore, cryptic, or closely related taxa, juvenile or damaged specimens may not be identified correctly, or only identified to a coarse taxonomic level, which may lead to incorrect or unsuccessful detection of an IAS via morphological methods (Haase et al., 2006; Mandelik et al., 2010; Blackman et al., 2017). Finally, the surveyed 'macroinvertebrates' are purely defined by their size and lifestyle (generally meaning benthic invertebrates that can be seen by the naked eye), and thus are biased and likely overseeing small species.

Developments of molecular tools for the identification of taxa via DNA, either from tissue or environmental samples (bulk and environmental DNA, respectively), offer potential solutions to the limitations of current approaches. Using environmental DNA (hereafter eDNA) to identify hidden biodiversity, including IAS from simple water sample collection, is a growing trend and 'game-changer' regarding biomonitoring (Taberlet, Coissac, Hajibabaei, & Reiseberg, 2012; Lawson-Handley, 2015; Deiner et al., 2017). In 2008, Ficetola and colleagues used DNA extracted from pond water to detect the American bull frog, a prominent IAS in Europe. Since this study, research application of eDNA has ballooned to include different taxonomic groups by either a species-specific method (conventional PCR, qPCR and ddPCR), or whole communities such as fish (Hänfling et al., 2016; Pont et al., 2018), macroinvertebrates (Fernández, Rodríguez-Martínez, Martínez, Garcia-Vazquez, & Ardura, 2019; Brantschen et al., 2021), and zooplankton (Brown, Chain, Zhan, MacIsaac, & Cristescu, 2016; Djurhuus et al., 2018) using a metabarcoding approach. A key advantage of eDNA sampling, which makes it particularly well suited for IAS monitoring, is its scalability. Water eDNA sample collection can be quick and simple to collect (dependant on water body type, water volume, and equipment). These samples can be processed in large numbers, therefore have an associated reduction in cost per sample compared to traditional sampling methods (Lacoursière-Roussel et al., 2018). Furthermore, monitoring programmes can cover large areas from catchment campaigns to national monitoring schemes, a scalability generally lacking for traditional methods (Altermatt et al., 2020).

Recently, several private companies have begun to offer eDNA services, such as protected species monitoring (e.g., great crested newt), and eDNA-based macroinvertebrate IAS monitoring has a large potential to transition from a still mostly academic to a more applied use. This is for example highlighted by the continued development of eDNA approaches by North American agencies and researchers for the detection of invasive Dreissenid mussels, primarily by a speciesspecific approach (Gingera, Bajno, Docker, & Reist, 2017; Sepulveda et al., 2020). The focus has remained on the species-specific approach. as it is thought to be more sensitive than a general metabarcoding approach (Blackman et al., 2020; Harper et al., 2018; Simmons, Tucker, Chadderton, Jerde, & Mahon, 2015). However, the metabarcoding approach can lead to the detection of unknown biodiversity previously not recorded, including 'unexpected' invasive or non-native species (Blackman et al., 2017; Simmons et al., 2015), and while a species-specific approach is an effective tool for monitoring known IAS, it does not take full advantage of the potential to detect these 'unexpected' or non-target IAS. Although several studies have compared eDNA metabarcoding and traditional kick-net sampling for freshwater macroinvertebrate (e.g., Fernández et al., 2019; Laini et al., 2020; Mächler et al., 2019), none have focused specifically on IAS within this group. Therefore, to assess the complementarity and potential advantages of eDNA to traditional kick-net methods for macroinvertebrate IAS detection, there first needs to be standardised testing of these tools at a large scale (Blackman et al., 2020).

Most national freshwater macroinvertebrate sampling programmes include a list of established IAS, which incorporates potential IAS that have been determined using a 'horizon scanning' approach (Altermatt, 2012; Roy et al., 2014). In Switzerland, 50 freshwater macroinvertebrate IAS are currently known to be present, and this list is supplemented with a further 32 taxa listed as 'likely to occur' due to documented invasions in neighbouring countries (See Table S1 and Wittenberg, 2005; Altermatt, 2012, Altermatt, Alther, Fišer, & Švara, 2019). While this is a suitable strategy for predicting IAS, it does not consider those species that may arrive 'unexpectedly'. Within the list of 82 taxa (present or likely to become present), several taxa are also not recorded as part of the standard kick-net survey methodology (Table S1). Furthermore, the standard morphological identification analysis of this taxonomic group (apart from Ephemeroptera, Plecoptera, and Trichoptera) is to family level only,

rather than species, which may in some instances facilitate the spread of some closely related IAS, such as the morphologically similar Dreissena polymorpha and Dreissena rostriformis bugensis (Peyer et al. 2011), or fail to separate native from non-native species (e.g., Crangonyx subterraneus from Crangonyx pseudogracilis; Altermatt et al., 2014, Altermatt et al., 2019). In combination with relatively low frequency in which kick-net monitoring campaigns are carried out, eDNA metabarcoding may negate some of the issues associated with specimen collection and morphological identification while also allowing monitoring at a finer spatial scale. However, to determine the practical application of eDNA monitoring for routine surveillance of macroinvertebrate IAS, comparisons and calibration experiments are needed with current methods (Blackman et al., 2020). Here, we carried out simultaneous eDNA collection and traditional kick-net sampling at two scales, namely at the national scale of Switzerland $(\sim 40,000 \text{ km}^2)$ and the catchment scale of the river Thur in Northeast Switzerland (\sim 700 km²), to compare the sample methods for the detection of invasive alien macroinvertebrates species in Swiss surface waters.

2 | METHODS

2.1 | National-scale monitoring

2.1.1 | eDNA sampling

As part of the Swiss Federal Office for the Environment (FOEN) routine monitoring for freshwater quality in Switzerland, eDNA and benthic macroinvertebrates were collected at 92 sites throughout Switzerland in 2019 (See Table S2). Environmental DNA samples were collected prior to kick-net sampling. For each of the sites, a total of 2 L of water filtered per site was collected using four Sterivex filters (500 ml per filter) with a 0.22 μ m pore size (Merck Millipore, Merck KgaA, Darmstadt, Germany). Filters were sealed with Luer fitting and placed in a cool box for transport to the lab where samples were stored at -20° C until further processing. Negative controls consisting of 500 ml of ddH₂O were filtered in the field and treated in the same way as samples (n = 8).

2.1.2 | Macroinvertebrate collection and determination

At each site, kick-net samples were collected by sampling eight microhabitats following Stucki (2010). Coarse organic particles, debris, amphibians, and fish were removed from the sample, and the remaining material was pooled and stored in 85% molecular grade ethanol. Identification was carried out by experienced taxonomists in the laboratory following the IBCH Labor-Protokoll (Stucki, 2010) except for the genera of Ephemeroptera, Plecoptera, and Trichoptera where individuals were further identified to species- or complex-level where taxonomically necessary.

2.2 | Catchment-scale monitoring

2.2.1 | eDNA sampling

Environmental DNA samples were collected at 20 sites in 2018 as a continued monitoring project of the Thur catchment (See Table S2 and Mächler et al., 2019; Carraro, Mächler, Wüthrich, & Altermatt, 2020; Blackman, Ho, Walser, & Altermatt, 2021). Environmental DNA samples consisting of 2 × 500 ml of water was collected directly from the river with single-use sterile syringes and filtered through 0.22 μ m Sterivex filters and sealed with Luer fittings and stored in a cool box for transporting to the lab where samples were stored at -20° C until further processing (n = 40). All samples were collected using sterile gloves from the bank of the river without entering the watercourse (to avoid contamination). Negative controls consisting of 500 ml of ddH₂O were filtered on each day of sampling (n = 7) and were treated in the same way as samples.

2.2.2 | Macroinvertebrate sampling

Kick-net samples were collected from 20 sites in the Thur catchment in Summer 2016 (Carraro, Stauffer, & Altermatt, 2021; Mächler et al., 2019). The method of collection differed from the national monitoring method in terms of sampling effort and seasonality: the sampling protocol was simplified to a total of three samples covering the prevailing substrates and fieldwork was done in July instead of March/April as scheduled by Stucki (2010). Identification was carried out by experienced taxonomists in the laboratory following the protocol introduced above.

2.2.3 | eDNA extraction and library preparation

DNA extractions from filters were performed in a clean room environment at Eawag, Switzerland (Deiner, Walser, Mächler, & Altermatt, 2015). The DNA was extracted using the QiAgen PowerWater Sterivex Extraction Kit (Qiagen, Germany). Filters from different sites were extracted in random batches including field and filter control that were treated equally to the samples. Extractions were performed as described by the manufacturer protocol. DNA was eluted into 100 μ l of elution buffer and stored until further processing at -20°C. Both sets of samples (national and catchment) from this study used the same library preparation. A two-step library preparation method was used targeting a 313 bp fragment of the COI barcode region with the degenerative primer pair: mICOlintF and jgHCO2198 (Table S3, Geller et al. 2013; Leray et al. 2013). These primers were modified to include the Nextera transposase sequences. A synthetic DNA strand, which amplified with the primer sequences, was used as PCR positive control (Table S4). Samples and controls were randomized over all 96-well PCR plates (four plates for the national sampling and three plates for the catchment sampling).

Each PCR reaction consisted of SigmaFree water, the provided 1x Buffer I (Thermo Fisher Scientific, MD), BSA (0.1 mg/µl), dNTP (0.2 mM), MgCl₂ (1 mM), mICOlintF, and jgHCO2198 primers (0.5 μ M each) and the polymerase AmpliTaq Gold 360° (1.25 U/µl) in a total volume of 25 µl. Exactly 2 µl of DNA template was used in each reaction. A touchdown protocol was used as follows: 95°C for 10 min, denaturation of DNA at 95°C for 15 s, annealing at 62°C for 30 s, followed by extension at 72°C for 30 s. For the first 16 cycles the annealing temperature was reduced by one degree per cycle, for the next 25 cycles, the annealing temperature remained at 46°C, followed by a final extension at 72°C for 5 min before the plates were cooled down to 10°C. All PCR were carried out in triplicate and PCR products were checked for amplification with the AM320 method on the QiAxcel Screening Cartridge (Qiagen, Germany); we did not experience any issues of inhibition. First round PCR products were cleaned using Zymo PCR 96 Plate clean-up Kit (Zymo Research, UK) according to the manufacturer's protocol. The clean amplicons were indexed using the Illumina Nextera XT Index Kits following the manufacturers' protocol (Illumina, Inc., San Diego, CA). The second-round PCR product was cleaned up with Thermo MG Magiet bead clean-up kit and a customized program for the KingFisher to remove excessive Nextera XT adaptors. The cleaned product of 50 µl was eluted in a new plate and stored at 4°C.

Samples were then quantified using the Qubit BR DNA Assay Kit (Life Technologies, Carlsbad, CA) in duplicates with the calibration standards on a Spark Multimode Microplate Reader (Tecan, US Inc., USA) following the manufacturer's protocol. According to these concentrations, samples were pooled in equimolar pools using a BRAND Liquid Handling Station (BRAND GMBH + CO KG, Wertheim, GE). All controls were added according to their concentration: if there was no measurable DNA, they were added to the lowest pool with a standardized volume (10 µl). The pools were combined in equimolar aliquots, considering the number of samples to reach a normalisation of DNA per sample. The final library was then pooled and cleaned with AMPure XP beads (Beckman Coulter, USA) and guantified using the Qubit fluorometer and the HS Assay Kit. The library was then prepared with a Nextera XT library prep Kit (Illumina, Inc. San Diego, CA) for loading on the flow cell. Both libraries were loaded at 16 pM and 15 pM with 10% PhiX for the national and catchment samples, respectively.

2.3 | Bioinformatics

After completion of each Illumina MiSeq PE300 (600 cycles) run, the data were demultiplexed (MiSeq Reporter V2.4) and reads were quality checked using usearch v11.0.667 (Edgar, 2010), FastQC (Andrews, 2015), and MultiQC (Ewels, Magnusson, Lundin, & Kaller, 2016). Raw reads were first 3'-end-trimmed, merged, and full-length primer sites were removed using usearch v11.0.667 (Edgar, 2010). The merged and primer trimmed reads were quality filtered using prinseq-lite (0.20.4). The UNOISE3 (usearch v11.0.667) workflow with an additional clustering identity was applied to obtain error corrected and

chimera-filtered sequence variants ZOTUs. Taxonomic assignment was performed with a 0.85 confidence threshold using SINTAX in the usearch software v11.0.667 with a custom reference database (Including MIDORI un-trimmed [V20180221] Leray, Ho, Lin, & Machida, 2018), see Appendix S1 Methods section for detailed parameters used for each library.

2.4 | Data analysis

After bioinformatic quality assurance, each dataset was subject to further quality assurance steps for tag-jumping or minor contamination in library preparation. For the national samples, only ZOTUs, which appeared in at least two out of the four replicates, were kept (See Brantschen, Blackman, Walser, & Altermatt, 2021 for full details). For the catchment sampling campaign, 0.1% of reads in each sample were removed from each taxon found in that sample, in line with other studies (Hänfling et al., 2016, see Blackman et al., 2021 for full details). All data were then transformed to presence/absence. ZOTUs were merged to species and genus level and then filtered for taxa on the BAFU IAS list (See Table S1). We used a Chi-square test on IAS that were detected by both methods to determine if there was a statistical difference in eDNA and kick-net sampling methods. We also tested whether the number of IAS detected by the two methods increased with upstream drainage area (km²), using a generalised linear model (GLM) with Poisson regression. All analysis was carried out in R version 3.6.3 (R Core Team, 2021). Map projections were made in SwissRiverPlot (Alther & Altermatt, 2021) for the national sampling campaign and tmap (Tennekes, 2018) for the catchment sampling campaign.

To confirm the detection of *Craspedacusta sowerbii*, we constructed a phylogenetic tree based on the 13 hydrozoan sequences from our data set (including 5 *C. sowerbii* sequences) and all NCBI records of tissue extracted samples from published papers. All analysis was conducted in MEGA version X (Kumar, Stecher, Li, Knyaz, & Tamura, 2018; Stecher, Tamura, & Kumar, 2020). All available published tissue derived sequences registered in NCBI (https://www.ncbi. nlm.nih.gov/) using the *COI* barcode region were downloaded (n = 13). We trimmed and aligned the 26 sequences using MUSCLE (Edgar, 2004). Sequences were mapped using a neighbour-joining (Saitou & Nei, 1987) and maximum composite likelihood method (Tamura, Nei, & Kumar, 2004) with 1,000 bootstrap replicates (See Figure S10 and Table S5 for NCBI accession numbers and source).

3 | RESULTS

The MiSeq runs generated 26.4 and 17.56 million raw reads from the national- and catchment-scale campaigns, respectively (the full description of both library outputs can be found in Brantschen et al. 2021 and Blackman et al. (2021) for the national- and catchment-scale campaigns, respectively). Of the 50 known macroinvertebrate IAS taxa listed as being in or at risk of arriving in Switzerland, 13 IAS taxa were detected over the national- and catchment-scale monitoring

campaigns using eDNA and kick-net sampling (Table 1 and Table S6). Of the 13 taxa at the national scale, one macroinvertebrate IAS was only detected with eDNA, nine taxa were detected with kick-net only, and three taxa were detected with both methods (Figure 1, Table 1). At the catchment level, three IAS taxa were detected in total, one taxon was detected with eDNA only, one taxon was detected with kick-net only, and one taxon was detected with both methods (Figure 1, Table 2).

3.1 | IAS detection with both methods

Only three taxa were detected by both sampling methods: Potamopyrgus antipodarum, Corbicula fluminea/fluvialis, and Dikerogammarus villosus. The taxon detected by both methods (eDNA and kick-net) were either detected at the same sites or were detected by both methods but at different sites, see Figures 2-4. Potamopyrgus antipodarum, the New Zealand mud snail, was the most common species found with both methods at the national and the catchment scale. We did not find any difference in the sampling methods at either sampling scale (Chi-Square test for homogeneity: national: $x^2 = 2.993$, df = 1, p = .833, catchment: $x^2 = 0.008, df = 1, p = .930$). At the national scale, P. antipodarum was detected at 12 sites with the eDNA method only, and 19 sites kick-net method only. At 10 sites, both sampling methods confirmed the presence of P. antipodarum (Figure 2a). At the catchment scale, P. antipodarum was detected at two sites with the eDNA method only and two sites with kick-net method only. At one site, P. antipodarum was detected with both sampling methods together (Figure 2b). The remaining taxa detected with both sampling methods were only found at the national sampling scale campaign. Firstly, we found Corbicula fluminea/fluvialis (called 'the Asian Clams'), with both methods but to different taxonomic resolution, as species-level identification of Corbicula fluminea/fluvialis was not determined as part of the standard morphological analysis.

Corbicula fluminea was recorded at two sites with eDNA at a national scale, and Corbicula fluminea/fluvialis at four sites with kick-net samples and one site with both methods (Figure 3a). We also detected no significant difference in the detection of Corbicula fluminea/fluvialis by either method or the combined methods (Chi-Square test for homogeneity: $x^2 = 0.761$, df = 1, p = .383). Secondly, Dikerogammarus villosus, known as the killer shrimp, and is widespread in Switzerland (Altermatt et al., 2014; Altermatt et al., 2019) was recorded at one site with eDNA at a national scale, three sites with kick-net samples, and a further site with both methods together (Figure 3b). We detected no significant difference in the detection by either method (Chi-Square test for homogeneity: $x^2 = 2.096$, df = 1, p = .148). Although we are unable to relate metabarcoding read number to the abundance of macroinvertebrates, *P. antipodarum*, *C. fluminea/fluvialis*, and *D. villosus*



FIGURE 1 Comparison of kick-net and eDNA method. Overlap in the IAS detected with each method at national (a) and catchment (b) scale monitoring. Kick-net in yellow, eDNA in blue, and both methods in green [Color figure can be viewed at wileyonlinelibrary.com]

Таха	Common name	Kick-net	eDNA	Both
Cambaridae	Crayfish (family)	1	0	0
Corbicula fluminea/fluvialis	Asian clam	4	2	1
Crangonyctidae	Shrimp (family)	6	0	0
Craspedacusta sowerbii	Peach blossom jellyfish	0	48	0
Dikerogrammarus villosus	Killer shrimp	3	1	1
Dreissenidae	Mussel (family)	5	0	0
Dugesia tigrina	Flatworm	11	0	0
Echinogammarus ischnus	Scud	1	0	0
Janiridae	Isopod (family)	1	0	0
Pacifastacus leniusculus	Signal crayfish	1	0	0
Physella acuta	Bladder snail	7	0	0
Physella heterostropha	Bladder snail	1	0	0
Potamopyrgus antipodarum	New Zealand Mudsnail	12	19	10

Note: The number of sites where freshwater IAS were detected with either kick-net, eDNA, or detected with both methods from the 92 study sites sampled in 2019.

TABLE 1Invasive alien taxa found aspart of the Swiss national-scale samplingcampaign

TABLE 2Invasive alien taxadetection from the catchment-scalesampling campaign of the river Thur

Таха	Common name	Kick-net	eDNA	Both
Craspedacusta sowerbii	Peach blossom jellyfish	0	12	0
Dugesiidae	Flatworm (family)	1	0	0
Potamopyrgus antipodarum	New Zealand Mudsnail	2	2	1

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Note: The number of sites where freshwater IAS were detected with either kick-net, eDNA, or detected with both methods from the 92 sites sampled in 2019. Kick-net samples collected in 2016 and with eDNA 2018.



FIGURE 2 Detection of *Potamopyrgus antipodarum* with kick-net and eDNA sampling. Sampling campaigns were carried out at a national scale (a) and catchment scale (b). Positive detection via kick-net sampling (yellow filled circle), eDNA sample (blue), and both (green). Absence of detection is represented with a grey filled circle. The Thur catchment is highlighted in orange on the map of Switzerland (a) and the blue arrow shows the direction of flow in the Thur catchment (b) [Color figure can be viewed at wileyonlinelibrary.com]

were detected at the most sites and with the highest read numbers and densities using eDNA and kick-net sampling, respectively (See Table S7 for further information).

Of those taxa not detected with both methods, kick-net sampling was more successful for the detection of macroinvertebrate IAS (n = 9). At the national-scale sampling campaign, this included a range of taxa that were identified morphologically to different taxonomic levels, that is, four family- and five species-level identifications (See Figures S1-S9 and Table S7). The taxa came from a variety of class/ order: 2 Decapoda, 2 Amphipoda, 1 Bivalvia, 1 Isopoda, 1 Tricladida, and 2 Gastropoda. The number of detections of each taxon also varied with Dugesia tigrana found at the most sites (11 sites), Physella acuta at seven sites, Crangonyctidae at six sites, and Dreissenidae at five sites. Although there are two species of Crangonyctidae found within Switzerland (the native, yet subterranean species Crangonyx subterraneus and the non-native Crangonyx pseudogracilis; Altermatt et al., 2014), species-level identification was not determined as part of the standard morphological analysis, yet all samples very likely only refer to the latter for biological reasons. The number of specimens found at each site also varied greatly (see Table S6). The remaining taxa were found at only one site each (Janiridae, Cambaridae, Echinogammarus ischnus, Physella heterostropha, and Pacifastacus leniusculus). In the catchment-scale sampling campaign, only one IAS

was found with kick-net and not with eDNA, namely a flatworm of the Dugesiidae family, which was found morphologically at two sites but not identified to species level.

Although eDNA sampling only detected one species that was not found with kick-net sampling, this finding is particularly interesting. *Craspedacusta sowerbii*, also known as the Peach blossom jellyfish, is widespread in Europe (Jankowski, Collins, & Campbell, 2008) and has been recorded in Switzerland since 1962 (Balvay, 1990). However, it is hardly ever (if at all) detected by kick-net sampling due to its size and form (polyp and medusa). However, in both national- (48 or 52% of sites) and catchment-scale (12 or 60% of sites) campaigns, we detected this IAS using eDNA at a high rate (Figure 4), indicating it being relatively widespread. Detection using eDNA often requires further verification to confirm the taxonomic assignment. This confirmation for *Craspedacusta sowerbii* can be found in Figure S10.

3.2 | Sample method detection over catchment size

To test the success of both methods to detect IAS at different scales, we plotted the upstream drainage area against the total number of IAS taxa found by either method (Figure 5). There is no change in the





relationship between the number of taxa detected and the drainage area when using eDNA (p = .5403), with a maximum of three IAS detected at a site (Figure 5a). However, using kick-net sampling, there is a positive and significant effect of drainage area on the total number of IAS detected (p < .001), with a maximum of five IAS detected at sites with the largest drainage area (Figure 5b, See Table S8 for full GLM output).

DISCUSSION 4

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Freshwater ecosystems face unprecedented impacts from anthropogenic pressures. The introduction of IAS into a new ecosystem threatens not only native flora and fauna but also ecosystem functioning via competition, predation, spread of disease, and parasitism (Simberloff, 2011) and represents one of the main threats facing freshwater ecosystems (Dudgeon et al., 2006). This study shows that eDNA and kick-net methods are valuable tools for the detection of macroinvertebrate IAS within freshwater rivers. However, our results demonstrate that the two methods gave different and nuanced views on the occurrence of macroinvertebrates IAS in Switzerland. The most macroinvertebrate IAS were detected using kick-net sampling across a national-scale sampling campaign (12), with only three of those taxa also detected using the eDNA methodology. However, at both national- and catchment-scale sampling, we detected a species with eDNA, which was not reported with kick-net. This species, Craspedacusta sowerbii, may be already widespread yet hitherto often overseen by classic survey methods. Our study demonstrates the benefit of using eDNA metabarcoding for the detection of taxa currently not recorded by traditional means or where kick-net sampling may not be appropriate. However, we also show the limitations of using a single marker eDNA metabarcoding approach to target such a broad group like freshwater macroinvertebrates, and a single marker is not able to cover all the breath of macroinvertebrates. Thus, while eDNA



FIGURE 4 Detection of *Craspedacusta sowerbii* with eDNA sampling. Sampling campaigns were carried out at a national scale in Summer (a) and catchment scale (b). Positive detection via kick-net eDNA sample (blue) and absence of detection is represented with a grey filled circle. The Thur catchment is highlighted in orange on the map of Switzerland (a) and the blue arrow shows the direction of flow in the Thur catchment (b). Detection via kick-net sampling was not recorded for this species at either national or catchment scales [Color figure can be viewed at wileyonlinelibrary.com]

metabarcoding offers new opportunities for early detection of macroinvertebrate IAS and can complement current traditional kicknet sampling campaigns, there are new methodological constraints to consider. Specifically, calibration of these two methods as well as expansion to more markers is needed for future applied IAS surveillance monitoring.

Although Craspedacusta sowerbii has previously been recorded in Switzerland (Balvay, 1990), due to its size and morphology, it is unlikely to be found in routine kick-net sampling, whereas eDNA metabarcoding is better suited for its detection. The finding of eDNA signals of this species at such a large extent in both sampling campaigns within Swiss rivers was surprising and alarming, as it indicates the possibility of major knowledge gaps. We therefore carried out further stringent quality controls, to prevent false positives from the high-throughput sequencing data (Darling, Pochon, Abbott, Inglis, & Zaiko, 2020). By constructing a phylogenetic tree of the C. sowerbii sequences produced in this study and reference sequences from NCBI of DNA tissue extracts, we find further evidence our identification is correct. However, like similar studies seeking to confirm 'unexpected' IAS, we would recommend physical collection of this species as a next step (Blackman et al., 2017). We are not able to indicate which form of the freshwater jellyfish (e.g., polyp or medusa) was detected in this study (and picked up in the eDNA samples). However, it is highly likely that the signal we detect is from the polyp form, as they can persist on river substrates, whereas flow velocity is a limiting factor in suitable habitat conditions for the free-floating medusa stage (Gasith, Gafny, Hershkovitz, Goldstein, & Galil, 2011). Both forms of C. sowerbii are zooplankton consumers, and although some work suggests it has minimal effect on a freshwater ecosystem (Dodson & Cooper, 1983), it is likely to influence algae grazer populations and

therefore have cascading effects on food webs due to algal accumulation, especially during jellyfish 'bloom' events where large numbers appear in a relatively short amount of time (Gasith et al., 2011; Jankowski, Strauss, & Ratte, 2005). This dataset and other eDNA metabarcoding sources are valuable resources, complementing also classical surveys, to map the extent of *C. sowerbii*'s occurrence in Switzerland and Europe wide. However, these data should be used in conjunction with eDNA models (e.g., Carraro et al., 2020, 2021) to reflect both the spatial extent to which the eDNA signal represents and sources of the signals both in the rivers where it was detected, and any lentic body outflows, which could also contribute to the signal.

Encouragingly, the most common taxa found in both data sets, P. antipordarum, was found at similar scales with no significant difference in the detection methods at both national- and catchment-scale campaigns, and approximately a third of positive detections made with both detection methods. Having both a strong overlap in the sites where detections was successful with both methods and several areas in the national-scale campaign where detection was by one method only reflects the different scales which the sampling methods represent and supports the use of eDNA as a complimentary tool to kick-net sampling for the detection of IAS. Similar patterns, albeit fewer, are true for both D. villosus and C. fluminea/fluvialis. While the kick-net sample is a point source collection of specimens from a single site, eDNA is an integrated signal of DNA shed by organisms upstream of the collection point (Deiner, Fronhofer, Mächler, Walser, & Altermatt, 2016) and therefore detection by these two methods is unlikely to synchronise at all sites. This is particularly useful when surveying for IAS. Where there is a positive eDNA detection, but no detection using kick-net, this may reflect the suitability of the site



FIGURE 5 Invasive alien species detected at site level by eDNA (a) and kick-net sampling (b). Plot shows the number of detections by either method as a function of upstream drainage area at the national (triangle) and catchment (circles) based sampling campaigns. Line indicates the GLM with shaded areas showing 95% confidence as calculated using model predictions and standard error [Color figure can be viewed at wileyonlinelibrary.com]

sampled. Traditional sampling campaigns are often carried out at a relatively low number of sites and are constrained to areas that are physically accessible for a sampler to wade into. Therefore, if a site does not have appropriate habitat for an IAS and so it is not present, it will not be detected by kick-net; however, it may still be present within the river reach if the habitat is suitable upstream. By collecting eDNA we increase the area, which is screened for IAS to a river stretch, rather than a single site. Combining this added benefit with the overall decreasing sample cost with increasing sample number (Altermatt et al., 2020; Lacoursière-Roussel et al., 2018), eDNA has the potential to be a highly beneficial monitoring method for IAS detection in particular.

In our dataset, we have several positive kick-net detections where IAS have not been detected with eDNA, which may be criticised as 'false negative'. We note this being a common pattern for eDNA metabarcoding studies in riverine systems for multiple reasons. Firstly, understanding low DNA quantity available (either from shedding rates or low biomass; Barnes & Turner, 2015) or optimisation of

the eDNA sampling protocol (Mächler, Deiner, Fabienne, & Altermatt, 2016; Muha, Robinson, Garcia de Leaniz, & Consuegra, 2019). Rather than sight or capture of specimen, for a positive detection via eDNA, sufficient DNA must be available in the water column. However, certain taxa due to their morphology (e.g., body armour or shell) may produce very low quantities of DNA (Martins et al., 2020). For example, Blackman et al. (2020) successfully detected Dreissenidae in a river in the United Kingdom using the same primers and sampling method as this study. However, here we do not detect Dreissenidae with eDNA metabarcoding, only with kick-net sampling. Blackman et al. (2020) noted a correlation between decrease in the metabarcoding read number and number of Dreissena rostriformis bugensis mussels found at sampling sites in their study. In this study, at sites where Dreissenidae were detected by kick-net between 1 and 11 specimens were collected. We therefore assume that this was not sufficient biomass or density, in relation to the size of the river, for the successful detection via eDNA metabarcoding with COI in our study, and further developments in our methodological understanding are still needed.

Traditional macroinvertebrate monitoring via kick-net sampling targets taxa based on their size, function, and resilience to different environmental pressures, thus including a varied and diverse number of taxonomic groups. However, as eDNA utilises the genetic material shed by a species, there are different considerations to be made. Successful detection using an eDNA metabarcoding approach is underpinned by the primers used to amplify target species. However, due to the traditional methods, which previously established the 'freshwater macroinvertebrate' group, this refers to detecting a polyphyletic group (Brantschen et al., 2021; Carew, Miller, & Hoffmann, 2011; Leese et al., 2021), which makes finding a conserved primer region for multiple groups difficult. Here, we used one general metazoan primer pair for the CO1 marker (Leray et al., 2013; Geller et al., 2013), which may not be ideal for the successful detection of some of the target IAS. When using a metabarcoding approach for other groups such as vertebrates, a conserved region of a single marker can be used, such as 12S. The COI marker, however, is a protein-coding gene that has codon degeneracy, which makes primer specificity difficult (Deagle, Jarman, Coissac, Pompanon, & Taberlet, 2014; Leese et al., 2021) and can lead to unspecific amplification (e.g., bacteria, fungi) and, as a result, lack of amplification for your target group. It is therefore harder to amplify all those taxa that are considered under the 'macroinvertebrate' umbrella term and consequently, when using a single COI marker for macroinvertebrate eDNA metabarcoding, the same coverage across the group cannot be expected.

Of the IAS that were detected with kick-net sampling in this study but not by eDNA, the taxa fall into three groups: Crustacea, Mollusca, and Turbellaria. Previous studies using the *COI* marker have already highlighted these groups as potential 'problems' often failing to be detected when using a single *CO1* primer approach (Klymus, Marshall, & Stepien, 2017; Komai et al., 2019; Martins et al., 2020). It can therefore be postulated that by increasing the number of markers we could detect these missing groups. However, our primary reason for using a *COI* marker is due to the taxonomic coverage of macroinvertebrates and the availability of reference sequences for

subsequent taxonomic assignment. Over 60% of freshwater invertebrates species have publicly available sequences for such taxonomic assignment (Weigand et al., 2019), and although some groups are more studied than others, IAS tend to be well documented and sequenced. Reference databases are a vital source that underpins the taxonomic steps of species identification from metabarcoding studies and all studies are reliant on the completeness and correctness of these reference database. Non-Arthropoda taxa detection may increase with the inclusion of an 18S marker; however, taxonomic resolution and coverage may not be to species level (Taberlet, Bonin, Zinger, & Coissac, 2018; Martins et al., 2020). Decisions when applying an eDNA metabarcoding approach to the detection of macroinvertebrates need to be weighed up between taxonomic resolution and potential taxon detection. However, by collecting eDNA samples in the first place, we uncover the potential to apply multiple primers (both universal and species specific) to the same samples (while sufficient sample remains) and repeatedly test for the presence of different taxa, which is another advantage of using this approach (Blackman et al., 2021).

The kick-net sampling strategy showed a positive increase in the number of IAS taxa detected with increased drainage area. Finding a higher number of IAS in larger water bodies is consistent with the increased opportunity for introductions in major water bodies, which have been reengineered and connected to new areas due to trade and transport across Europe (Leuven et al., 2009). The river Rhine in particular is a major source of invasive macroinvertebrate species due to its connection to areas such as the Ponto Caspian regions (Leuven et al., 2009). Therefore, these larger rivers (the Rhine, Rhone, and Aare) are where IAS become established and then spread upstream into other tributaries either by human intervention or natural means. Contradictorily, we do not find a correlation between the number of IAS detected and drainage area with eDNA sample collection. This likely reflects not only the comparable lower volumes of water collected in larger rivers to those collected at sites with smaller drainage area at the top of the catchment but could also the heterogeneously dispersed DNA across a river width (Macher & Leese, 2017). Increased detection may be achieved by sampling multiple locations within a site to capture eDNA (Macher & Leese, 2017), in a similar method to the multihabitat approach of a kick-net sample.

Although the taxa detected in this study will likely be impossible to be eradicated once established, it is important to use appropriate and timely methods to ensure the ecological integrity and possible management of the systems in which they occur. For example: while the impacts of *P. antipodarum* are limited in low numbers, it has high fecundity and has been recorded to impact primary production and nutrient in large densities (Goldberg, Sepulveda, Ray, Baumgardt, & Waits, 2013; Hall Jr, Dybdahl, & VanderLoop, 2006; Hall Jr, Tank, & Dybdahl, 2003), similarly *C. fluminea/fluvialis* in large numbers out-competes native unionid species and other filter-filters for space and food (Schmidlin & Baur, 2007). Accurate monitoring is therefore important to detect potential changes in freshwater ecosystems and ensure better understanding of the advance of IAS in Switzerland. As mentioned previously, a potential benefit of using eDNA metabarcoding for IAS detection would be the ability to upscale our monitoring campaigns to monitor at finer resolutions. This would enable us to include a higher numbers of sites and key IAS pathways, which are either not currently monitored or unsuitable to check with established methods.

5 | CONCLUSION

Our findings support the complementary use of both eDNA and kick-net sampling for macroinvertebrate IAS detection in freshwater river systems. We demonstrated the added benefit of eDNA as a complimentary tool to kick-net sampling. While not all IAS within the macroinvertebrate group can be detected by using a single primer, traditional and molecular methods do overlap for several common macroinvertebrate IAS. We especially see two major benefits of eDNA metabarcoding. Firstly, the ability to detect unexpected or overlooked IAS where traditional kick-net sampling and morphological identification may not be suitable. Secondly, by using an eDNA metabarcoding approach, monitoring can be upscaled both in terms of number of samples collected and the area in which they represent. These benefits justify the integration of eDNA metabarcoding as a complementary tool also for routine biomonitoring programmes.

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DATA AVAILABILITY STATEMENT

All IAS sequences generated during this study will be made available in a public repository upon publication.

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