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Large-scale eDNA monitoring of multiple aquatic pathogens as a tool to provide risk maps for wildlife diseases

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Abstract

Multiple parasites and pathogens cause disease in aquatic wildlife and in aquaculture species, generating a need for monitoring and management. Conventional disease monitoring methods involve laborious, costly, and invasive capture and examination of host species, and require specialized expertise for every host and pathogen of interest. Environmental DNA could provide simultaneous occurrence data for multiple pathogens across different host taxa, valuable for using parasite diversity as, for example, a bioindicator of ecosystem disturbance. Here, we tested the potential for simultaneous detection of four wildlife pathogens in water samples from 280, mainly riverine, sites across Switzerland. We targeted the crayfish pathogen *Aphanomyces astaci*, the amphibian pathogen *Batrachochytrium dendrobatidis*, and the fish pathogens *Saprolegnia parasitica* and *Tetracapsuloides bryosalmonae*. The eDNA detection showed a widespread distribution of *A. astaci*, *S. parasitica*, and *T. bryosalmonae*, although *A. astaci* and *T. bryosalmonae* were not detected in some alpine river catchments. *B. dendrobatidis* was detected only rarely, which was expected since the sampling did not target amphibian breeding sites. Co-detection rates were higher in rivers than in lakes, likely reflecting the habitat preferences and distributions of the host species. We discuss the advantages and limitations of eDNA-based pathogen monitoring and list a set of recommendations for managers. Our study illustrates how eDNA-based techniques can monitor several pathogen species concurrently, thus facilitating more comprehensive disease monitoring schemes. Combined with metabarcoding approaches in the future, eDNA-based sampling and detection can facilitate the incorporation of parasite and pathogen occurrence and diversity as an indicator for aquatic ecosystem health, and for revealing the hidden biodiversity and structure of parasite communities.

KEYWORDS
Aphanomyces astaci, *Batrachochytrium dendrobatidis*, disease surveillance, environmental DNA, pathogen monitoring, *Saprolegnia parasitica*, *Tetracapsuloides bryosalmonae*

1 | INTRODUCTION

The emergence of infectious diseases among many aquatic species (Daszak et al., 2000) continues with on-going climatic and environmental change (Marcogliese, 2008; Mitchell et al., 2005) and species introductions (Peeler et al., 2011). Mitigating the spread of wildlife diseases and reducing their consequent negative impacts is of both conservation and economic interest (Gupta et al., 2020). At the same time, pathogens are sensitive bioindicators of various anthropogenic stressors and may thus serve as bioindicators of environmental degradation (Shea et al., 2012; Taglioretti et al., 2018). Either goal requires detailed knowledge of the occurrence and abundance of pathogens to employ successful management strategies. This can be gained by implementing regular and comprehensive monitoring campaigns. However, most conventional disease monitoring methods involve capture of host species and subsequent examination for infection and are therefore detrimental to the host, laborious, and costly. For example, electrofishing is used to capture brown trout (*Salmo trutta)*, their kidneys are subsequently extracted and examined for *Tetracapsuloides bryosalmonae* infections which cause proliferative kidney disease (PKD; Wahli et al., 2007). Conventional pathogen and disease surveys are also often limited to specific host species, or species inhabiting specific habitats. Thus, conventional methods are not practical for surveys with the goal of providing a comprehensive picture of the distribution of multiple aquatic pathogens across taxonomic and habitat boundaries. Environmental DNA (eDNA) sampling is an increasingly applied monitoring method with the potential to detect multiple pathogens directly from an environmental matrix such as water (Thomsen & Willerslev, 2015). Non-invasive pathogen eDNA detection may be particularly important when the host species is rare, threatened or of fisheries and conservation importance. For example, several salmonid disease agents, particularly those with water-borne transmission stages, have been detected using eDNA techniques: *Ceratonova shasta* (Hallett & Bartholomew, 2006), *Gyrodactylus salaris* (Rusch et al., 2018) and *Myxobolus cerebralis* (Richey et al., 2018), the PKD agent, *T. bryosalmonae* (Fontes, Hartikainen, Holland, et al., 2017) and the shellfish pathogen, *Perkinsus marinus* (Audemard et al., 2004). The possibility of detecting the DNA of multiple pathogens from the same environmental matrix could facilitate comprehensive monitoring of parasite and pathogen communities, which is of importance for disease risk mapping, wildlife management and advancing the use of parasites as bioindicators.

The aim of this study was to map the occurrence of four aquatic pathogens (*A. astaci*, *B. dendrobatidis*, *S. parasitica* and *T. bryosalmonae*) using eDNA from water samples and to compare with previous pathogen occurrence data. All four target pathogens are transmitted through water-borne stages and are distributed across the study area in Switzerland. The hosts of the studied pathogens exhibit partially overlapping habitat requirements and seasonal development patterns, which were used to interpret the eDNA detection frequencies. A newly developed eDNA sampling method (Sieber et al., 2020) was used in 280 sites to collect triplicate 5 L water samples, and four species-specific quantitative real-time PCR (qPCR) assays were used to analyze each eDNA extract. The country-wide pathogen occurrence derived from eDNA data was compared to several geographical factors and earlier studies investigating disease occurrence in host species. The research demonstrates the applicability of pathogen eDNA detection for updating and extending existing pathogen surveillance and monitoring and highlights opportunities for its use in population and ecosystem health monitoring.

2 | METHODS

2.1 | Study species

All four pathogens we monitored are associated with disease outbreaks and are ecologically important in aquatic environments. *Aphanomyces astaci* causes the crayfish plague and is associated with population collapses of native European crayfish species (Holdich et al., 2009). While European crayfish species are highly susceptible to the disease, the introduced invasive North American crayfish species are asymptomatic carriers and act as pathogen reservoirs (Alderman et al., 1987; Nyhlén & Unestam, 1980). *A. astaci* zoospores are released from cadavers and during host molting (Oidtmann et al., 2002) and are transmissive to susceptible crayfish in water for at least several weeks (Svoboda et al., 2017).

The chytrid fungus *Batrachochytrium dendrobatidis* (Longcore et al., 1999) causes chytridiomycosis in amphibians (Kilpatrick et al., 2010), and is also spread by anthropogenic means, including the illegal trading of animals. Most amphibian species are susceptible (Daszak et al., 2004; Lips et al., 2006), and chytridiomycosis has been linked with the decline and extinction of many amphibian species worldwide (Cunningham et al., 2017), however no mass mortalities have been noted in Switzerland (Tobler et al., 2012). *B. dendrobatidis* releases free-swimming zoospores (Berger et al., 2005), which can survive several months in sterile lake water (Johnson & Speare, 2005).

Proliferative Kidney Disease is caused by *Tetracapsuloides bryosalmonae* (Phylum Cnidaria, Subphylum Myxozoa), severely impacting wild and farmed salmonids (Okamura et al., 2011). *T. bryosalmonae* has a complex life cycle and transmits between fish and freshwater bryozoans (Phylum Bryozoa, Class Phylactolaemata) via malacospores released into the water (reviewed in Hartikainen & Okamura, 2015). The bryozoans may act as long-lived parasite reservoirs through condition-dependent development of the parasite in asexually reproducing colonial hosts (Fontes, Hartikainen, Taylor, & Okamura, 2017). Once released, the malacospores are infective for 12–24 h (De Kinkelin et al., 2002; Feist et al., 2001), although duration of detection in eDNA is not known.

The “water mold” *Saprolegnia parasitica*, an oomycete pathogen, has a global freshwater distribution and causes significant economic losses in aquaculture (van West, 2006). Outbreaks of highly virulent strains of *S. parasitica* have also been observed in wild fish populations of brown trout (*S. trutta*) and grayling (*Thymallus thymallus*; Paul & Belbahri, 2012). Further infections of *S. parasitica* have been observed in arctic char (*Salvelinus alpinus*), common barbel (*Barbus barbus*), catfishes (*Silurus* sp) common minnow (*Phoxinus phoxinus*), European perch (*Perca fluviatilis*), marble trout (*Salmo marmoratus*), northern pike (*Esox lucius*), rainbow trout (*Oncorhynchus mykiss*), and whitefish (*Coregonus wartmanni*; Ravasi et al., 2018). The transmission of *S. parasitica* is via swimming primary and secondary zoospores, which are released into water in multiple cycles from infected fish (van West, 2006).

The use of eDNA to detect pathogens directly from water samples creates an opportunity to simultaneously discover all four discussed pathogens, even though they affect a range of different hosts. Detection of *B. dendrobatidis* (Kirshtein et al., 2007) and *A. astaci* (Strand et al., 2011) using water samples has been previously established (Barnes et al., 2020; Hyman & Collins, 2012; Kamoroff & Goldberg, 2017; Strand et al., 2014; Wittwer et al., 2018). Detection of *A. astaci* has been tested in large lakes (Strand et al., 2014) and streams (Wittwer et al., 2019); whereas, *B. dendrobatidis* was detected in ponds (Hyman & Collins, 2012; Walker et al., 2007), wetlands (Chestnut et al., 2014), and lakes (Kamoroff & Goldberg, 2017). Assays detecting *T. bryosalmonae* (Carraro et al., 2018; Fontes, Hartikainen, Holland, et al., 2017; Hutchins et al., 2018) and *S. parasitica* (Rocchi et al., 2017) have been tested in rivers, however, the eDNA collection methods and isolation procedures vary widely between the different pathogens.

2.2 | Study sites and eDNA sampling

Sampling sites were chosen to cover the major waterways throughout Switzerland. Sampling sites were located in eleven major watersheds: Aare, Adda, Adige, Alpenrhein, Doubs, Inn, Limmat, Reuss, Rhein, Rhône, and Ticino (Figure 1). The sites were chosen to reflect the distribution of host species and therefore most sites were situated in the Swiss midlands. Distribution maps of a selection of host species are depicted in Figure S1 in the supplement. Additional sites of interest were chosen by the cantonal authorities who provided funding to this project. Consequently, 280 sites, 212 running, and 68 standing waters were selected (Figure 1 and see Table S1 in the Supplement for site details). Sampling was conducted from May to September in 2017 and from May to October in 2018. Water samples were collected using an in situ filtration system described in Sieber et al. (2020). Briefly, three 5 L water samples were taken per site. In case the filter clogged before reaching 5 L, a maximum of six filters were collected. A negative control was performed using 5 L of clean MilliQ water pumped through the equipment before sampling at each site. The tubes containing the eDNA filters were stored on ice; however, for multi-day trips in canton Grisons and Ticino a dry shipper was used for transportation, which was cooled with liquid nitrogen before the trip. After returning from the field, collected samples were stored at −80°C until extraction.

2.3 | DNA extraction and real-time quantitative PCR

Extraction of eDNA samples was conducted in two laboratories which were only used for pre-PCR processes (Eawag Dübendorf and FHNW Muttenz). The DNEasy Power Water kit (Qiagen AG) with modifications described in Sieber et al. (2020; Text S1 in the Supplement) was used and extracted samples were stored at −20°C until further analysis.

All water sample extracts were analyzed for all four pathogen species in separate reactions using probe-based TaqMan real-time quantitative PCR (qPCR) performed on a LightCycler® 480 (Roche). Multiplexing was avoided to reduce potential interference between assays. Triplicate reactions were set up using a QIAgility pipetting robot (Qiagen AG) with negative controls included in each run. A standard dilution series of each pathogen's target DNA sequence was run on each detection plate (see below, based on GBlocks fragments). The samples were tested in separate runs for different pathogen species. An overview of the assays and their original references are listed in Table S2 in the Supplementary materials. Reaction setup and thermal cycling for *B. dendrobatidis* and *T. bryosalmonae* are described in Sieber et al. (2020). For *A. astaci* amplification, we targeted the ITS region according to Vrålstad et al. (2009), with a modified thermal cycling regime for increased specificity (Strand et al., 2011, 2014). The 10 μL reactions contained 5 μL of the LightCycler® 480 Probes Master buffer (Roche), forward primer (AphAstITS-39F) at a concentration of 50 nM, reverse primer (AphAstITS-97R) at 900 nM, the MGB probe (AphAstITS-60T) at 200 nM and 2.5 μL of the template DNA. Thermal cycling was initiated for 10 min at 95°C, followed by 50 cycles for 15 s at 95°C and 30 s at 62°C. At the end, a cooling step for 10 s at 40°C was implemented as suggested by the manufacturer of the thermal cycler. The assay for *S. parasitica* detection (Rocchi et al., 2017) targets the ITS1 and ITS2 regions. The reactions contained 5 μL of the LightCycler® 480 Probes Master buffer (Roche), the forward primer (Primer-F) and the reverse primer (Primer-R), each at a concentration of 900 nM, as well as containing the probe (Probe-R) at 200 nM and 2.5 μL of template DNA. Thermal cycling for *S. parasitica* included an initial 10 min at 95°C followed by 45 cycles for 15 s at 95°C and 60 s at 60°C, with a final 10 s cooling step at 40°C. In addition to the pathogen assays, and to test for inhibition, an internal positive control (IPC) was spiked into each eDNA sample in a separate qPCR run (see Sieber et al. (2020) for detailed procedures).

To quantify the DNA content of samples and to determine both the limit of detection (LOD) and quantification (LOQ) for each assay we used a double-stranded Gblocks fragment (Integrated DNA Technologies) containing target sequences of all pathogen assays (see Text S2). The definition of LOD and LOQ we discussed in previous publications (Sieber, 2020; Sieber et al., 2021). LOD and LOQ for *B. dendrobatidis* and *T. bryosalmonae* are reported in Sieber et al. (2020). For *A. astaci*, the LOD was determined to be at quantification cycle value (Cq-value) of 38.844 and the LOQ at a

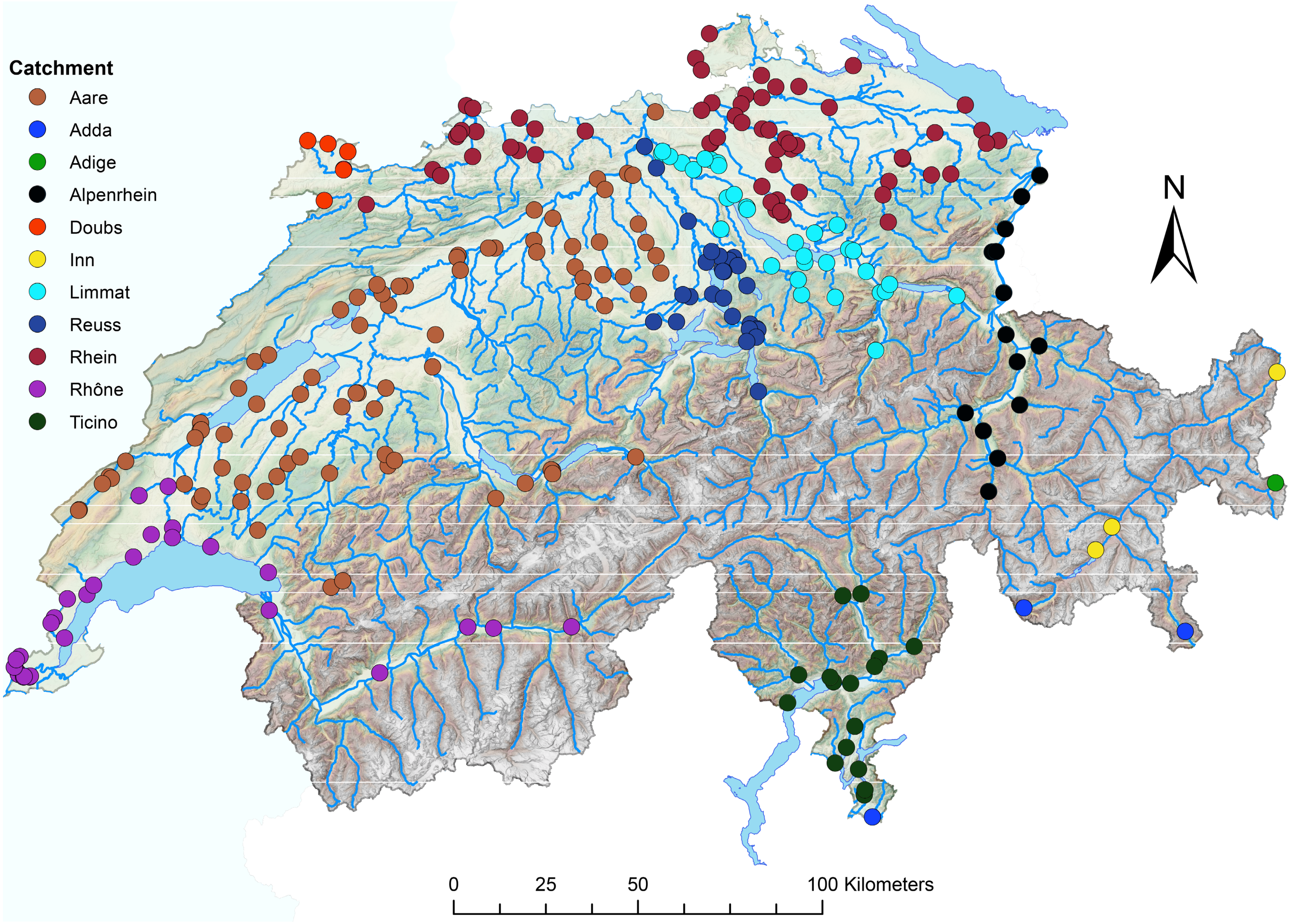


FIGURE 1 Map of Switzerland with sampling sites color-coded according to catchment. Background maps were drawn by swisstopo (Swiss Federal Office of Topography, Berne).

concentration of 11.09 copies μL^{-1} (Sieber et al., 2021). For *S. parasitica*, the LOD was determined to be at Cq-value of 35.752 and the LOQ at a concentration of 22.2 copies μL^{-1} using the same procedures as in Sieber et al. (2020). Dilution series results are listed in Table S3 in the Supplementary materials. The standard curves which were used to define to LOD and LOQ of all four pathogens are visualized in Figure S2 in the Supplementary materials.

2.4 | Data analysis

The qPCR raw output data were processed as described in Sieber et al. (2020). The pathogen DNA concentration from each water sample was defined as above LOD, and therefore as positive detection, if the DNA concentration in one or more of the qPCR replicates exceeded the LOD. A site was considered positive for the presence of a pathogen if at least one of the three replicate water samples from that site was positive for the tested pathogen DNA. Mean DNA concentrations per water sample were calculated with positive qPCR replicate values. Mean DNA concentrations per site were calculated from mean sample concentrations. Some sites were excluded from analysis for *A. astaci*, *S. parasitica* and *T. bryosalmonae* due to amplification in the sampling negative controls ($n = 18$ for *A. astaci*, $n = 2$ for *S. parasitica* and $n = 1$ for *T. bryosalmonae*). All analyses were conducted in R version 3.6.1. (R Core Team, 2019) and with following packages installed: car (Fox & Weisberg, 2019), dplyr (Wickham et al., 2023), lme4 (Bates et al., 2015), lmerTest (Kuznetsova et al., 2017), and nlme (Pinheiro et al., 2023). The gradient of sampled river stretches was assigned into flat, semi-steep, and steep slope categories according to Swiss river typology categories (Zeh Weissmann et al., 2009). The effect of the waterbody type (lake or river), sampling site elevation (meters above sea level, m.a.s.l.), lake surface area, river Strahler order, and gradient on pathogen detection was investigated using binomial generalized linear mixed effects models with watershed as the random factor.

The estimates of pathogen DNA concentration in the water samples were power-transformed for analysis to meet parametric assumptions, using Tukey's Ladder of Powers (Tukey, 1977). We then applied linear mixed effects models to test the effects of waterbody type and elevation, lake surface area, river Strahler order, and gradient on mean DNA concentrations per survey site. Since surface area and Strahler order are only defined for lakes and rivers, respectively, they were tested separately for each waterbody type. Waterbody type was not analyzed for *B. dendrobatidis* DNA concentrations because *B. dendrobatidis* was only found in rivers. The same analyses were also conducted on a reduced dataset which only included DNA concentrations above LOQ. Samples where *T. bryosalmonae* DNA concentrations were above the LOQ were only found in rivers, therefore, they were not analyzed with waterbody type and lake surface area. Association between DNA concentrations of two disease agent species in the same water sample were examined using linear mixed models with survey site as random factor for each pathogen pairing, except for the pairing of *B. dendrobatidis* and *T. bryosalmonae*. For that pairing, a linear model was implemented without random effect, because only single samples from sampling sites had concentrations for both agents. Effects of waterbody type, elevation, lake surface area, river Strahler order, and river gradient on the number of pathogens co-detected at each site (no detections to all four detected: 0–4) were analyzed using binomial generalized linear mixed effects models with watershed as the random factor. Co-detection between pathogen pairs was investigated with Pearson's Chi-squared tests with Yates' continuity correction for each agent pairing. The inhibition in water samples was measured as described in Sieber et al. (2020) using an internal positive control.

Hierarchical occupancy models were run according to Dorazio and Erickson (2018) to calculate occupancy probabilities at the level of sampling site, water sample and qPCR replicate for each pathogen except *B. dendrobatidis*, which was excluded from this analysis due to very low detection rates. All models were run in R with 11,000 Markow-Chain-Monte-Carlo (MCMC) iterations and the effects of elevation, waterbody type, river Strahler order and lake surface area were analyzed by model selection using Posterior Predictive Loss (PPLC, Gelfand & Ghosh, 1998) and Watanabe-Akaike Information criterion (WAIC, Watanabe, 2010) criterions. Furthermore, we used the equation $1-(1-\theta)^n \geq 0.95$ to determine the number of water samples (n) required per sampling site to reach detection success of 95% or above for estimated pathogen DNA detection probabilities per water sample (θ).

A. astaci and *T. bryosalmonae* eDNA occurrence data was compared with *A. astaci* and PKD prevalence data from crayfish and fish tissue, respectively, which was collected in previous studies (Jean-Richard, 2013; Wahli et al., 2008). From these surveys, 45 sites for *A. astaci* and 79 sites for *T. bryosalmonae* were surveyed in the same river stretch or the same lake and could therefore be compared to our survey data. The association between pathogen detection in the hosts and in water was analyzed with a Pearson's Chi-squared test with Yates' continuity correction and a McNemar's Chi-squared test with continuity correction.

We used binomial generalized linear mixed effects models to investigate the effect of waterbody type, elevation, lake surface area, river Strahler order, and gradient on qPCR inhibition in water. The effect of inhibition on detection rates in water samples was analyzed for each disease agent with Pearson's Chi-squared tests with Yates' continuity correction. Furthermore, influence of inhibition on disease agent concentrations in water samples were analyzed with linear mixed effects models with survey site as a random factor.

3 | RESULTS

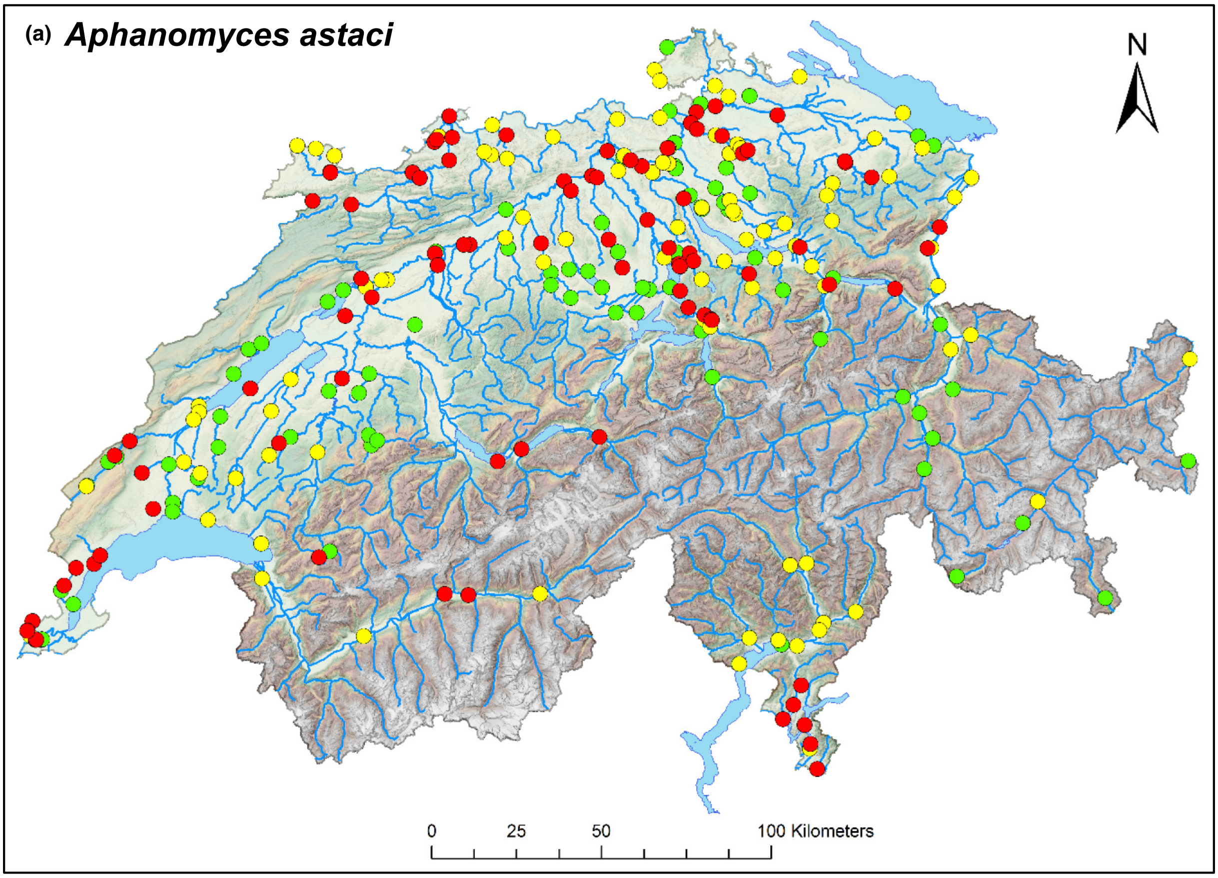
3.1 | Pathogen detection in water

Aphanomyces astaci was detected in 87 of 262 (33.21%) sites (Figure 2a), *B. dendrobatidis* in 5 out of 280 (0.02%) sites (Figure 2b), *S. parasitica* in 205 out of 278 (73.74%) sites (Figure 2c) and *T. bryosalmonae* in 59 out of 279 (21.15%) sites (Figure 2d). The corresponding site occupancy estimates for each species are shown in Table 1. Detailed results are listed in Table S1 in the Supplementary materials. *A. astaci* was found in all major watersheds except for the two alpine rivers Inn and Adige (Figure 2a). *B. dendrobatidis* was detected in single locations of the Adda, Alpenrhein, Limmat, Rhein, and Ticino catchments (Figure 2b). *S. parasitica* was present in all major catchments, while *T. bryosalmonae* was not found in the alpine Adda, Adige and Inn catchments (Figure 2d). *A. astaci* ($\chi^2=5.773$, df $=1$, $p=0.016$), *S. parasitica* ($\chi^2=23.650$, df $=1$, $p<0.001$) and *T. bryosalmonae* ($\chi^2=9.815$, df $=1$, $p=0.002$) were significantly more often detected in river than in lake sites. Due to low detection rate, *B. dendrobratidis* occurrence in waterbody type was not assessed ($n=5$ detections, all in rivers). *A. astaci*, *T. bryosalmonae* and *S. parasitica* detection frequency did not change significantly with elevation ($\chi^2=3.329$, df $=1$, $p=0.068$; $\chi^2=1.973$, df $=1$, $p=0.160$; $\chi^2=0.670$, df $=1$, $p=0.413$, respectively), despite a trend for more detections at lower elevations in the former two species. Elevational differences regarding detection were not assessed for *B. dendrobatidis* due to low detection rate. *S. parasitica* detection increased with lake surface area ($\chi^2=14.668$, df $=1$, $p<0.001$), which was not observed for *A. astaci* ($\chi^2=0.177$, df $=1$, $p=0.674$) nor for *T. bryosalmonae* ($\chi^2=0.200$, df $=1$, $p=0.655$). However, the sample sizes in lakes for the latter two species were lower. Neither river Strahler order nor river gradient categories had a significant effect on the detection of any pathogens (see Table S4 in the Supplementary material for analysis results).

Hierarchical occupancy models revealed a consistent (≥80%) qPCR replicate level detection for all analyzed pathogens (Table 1) and high site occupancy probability of *S. parasitica* ($\Psi(.)=0.808$). The occupancy probability among the three replicate water samples was similar for all three pathogens included. The inclusion of elevation, water type, river Strahler order, and lake surface area did not improve model fit (see Table S5 in the Supplementary material for detailed results).

3.2 | Pathogen DNA concentrations in water

The mean DNA concentration of *S. parasitica* decreased in water samples with increasing elevation ($r=-0.272$, $\chi^2=9.807$, df $=1$, $p=0.002$); however, this was not observed for the other pathogen



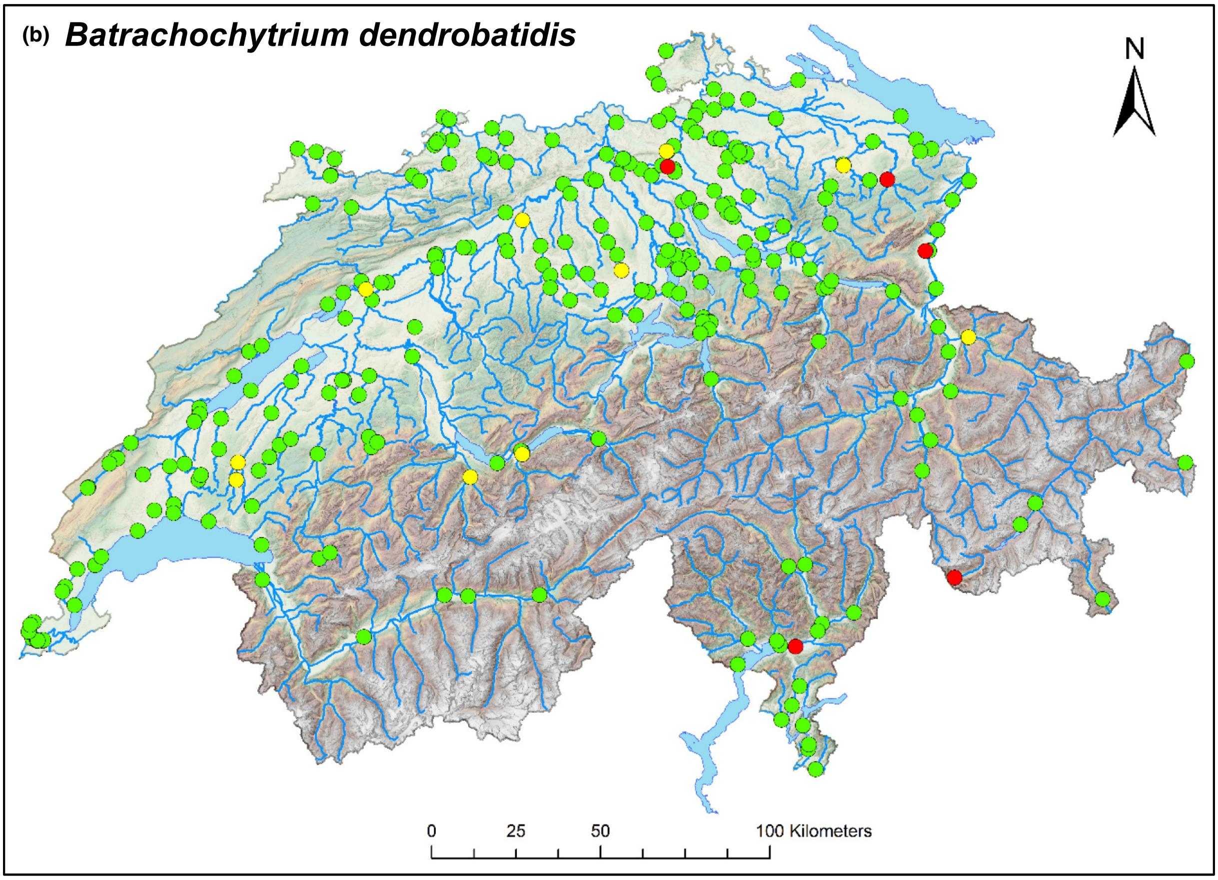


FIGURE 2 (Continued)

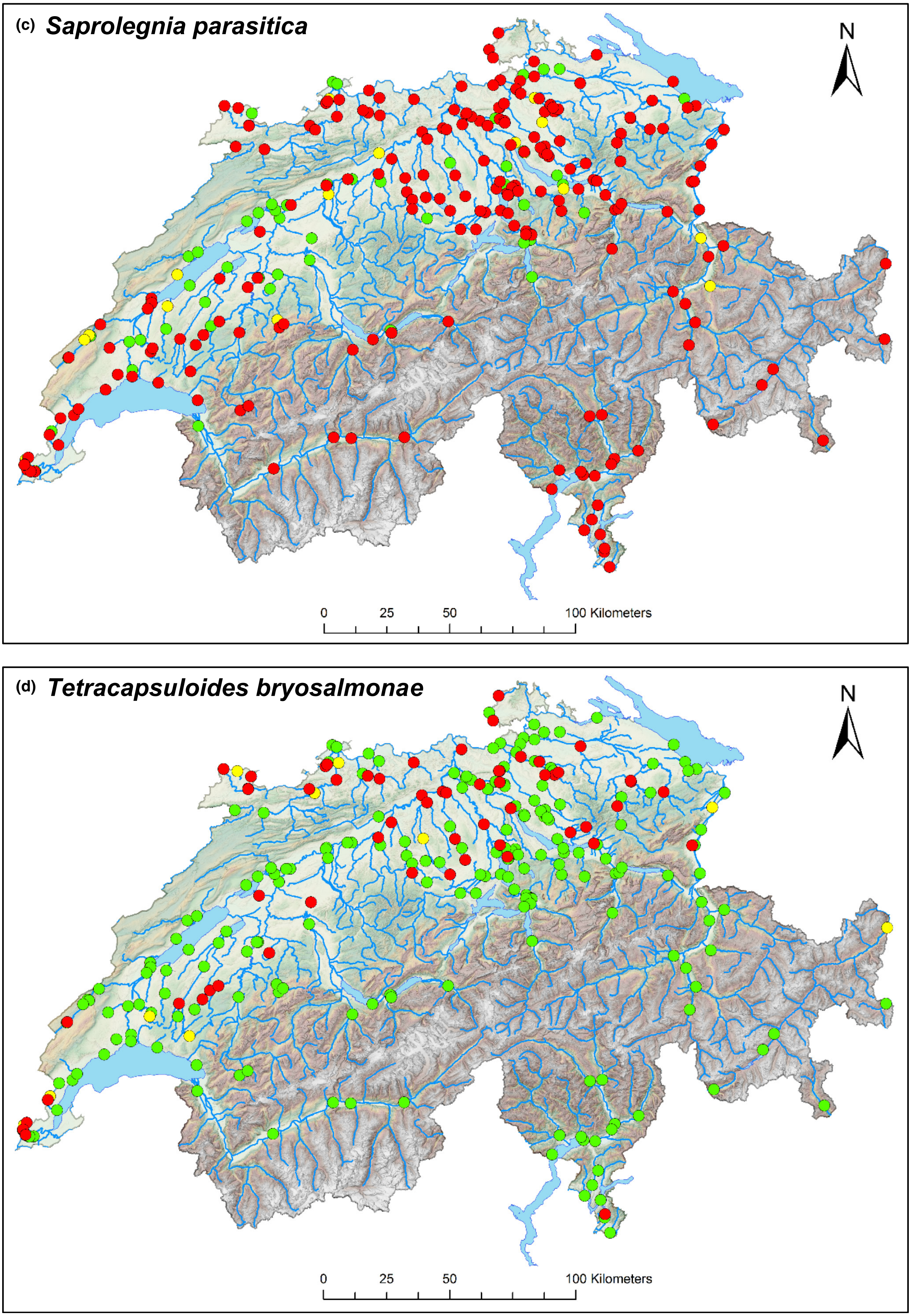


FIGURE 2 Distribution maps across Switzerland indicating the occurrence of (a) *Aphanomyces astaci*, (b) *Batrachochytrium dendrobatidis*, (c) *Saprolegnia parasitica* and (d) *Tetracapsuloides bryosalmonae*. Red = positive detection, yellow = uncertain detection (below the LOD), green = negative detection. Background maps were drawn by swisstopo (Swiss Federal Office of Topography, Berne).

TABLE 1 Occupancy probabilities of sampling site Ψ(.), water sample θ(.), qPCR replicate p(.), and number of water samples (n) required per sampling site to reach detection probabilities of ≥95% according to θ(.). Probability estimates are listed per pathogen species.

Species	Ψ(.)	θ(.)	p(.)	# water samples (n)
Aphanomyces astaci	0.371	0.544	0.804	4
Saprolegnia parasitica	0.808	0.605	0.908	4
Tetracapsuloides bryosalmonae	0.247	0.490	0.810	5

species. No other explanatory variables had a significant effect on pathogen DNA concentrations (see Table S6 in the Supplementary material for test results). When only above LOQ DNA concentrations were included, negative elevation-dependent DNA concentrations of both *T. bryosalmonae* ($r = -0.356$, $\chi^2 = 5.171$, df = 1, $p = 0.023$) and *S. parasitica* ($r = -0.186$, $\chi^2 = 4.048$, df = 1, $p = 0.044$) were observed; while this effect was not observed for *A. astaci* ($r = -0.296$, $\chi^2 = 3.195$, df = 1, $p = 0.074$). *S. parasitica* DNA concentrations above LOQ decreased with increasing river Strahler order ($r = -0.134$, $\chi^2 = 4.246$, df = 1, $p = 0.039$). Higher *A. astaci* DNA concentrations correlated with larger lake surface area ($r = 0.803$, $\chi^2 = 20.773$, df = 1, $p < 0.001$). River gradient was not found to influence the DNA concentration above LOQ for any of the pathogens (see Table S7 for test results).

3.3 | Patterns of co-occurrence

All four infectious agents were detected at one site (in the river Simmi, in the Alpenrhein catchment), all tested pathogens except *B. dendrobatidis* were present in 29 sites and all tested pathogens except *A. astaci* occurred in two sites. In 70 sites, two of the four pathogens were detected. Co-detection was higher in rivers compared to lakes ($\chi^2 = 33.860$, df = 1, $p < 0.001$). In rivers, most co-detections were observed in sites with semi-steep slopes ($\chi^2 = 15.707$, df = 2, $p < 0.001$). Elevation, lake surface area and river Strahler order did not show significant association with number of co-detected pathogens ($\chi^2 = 2.321$, df = 1, $p = 0.128$; $\chi^2 = 0.094$, df = 1, $p = 0.760$ and $\chi^2 = 0.276$, df = 1, $p = 0.599$, respectively). The detections of *A. astaci* and *S. parasitica*, *A. astaci* and *T. bryosalmonae*, and *S. parasitica* and *T. bryosalmonae* were positively correlated ($\chi^2 = 12.488$, df = 1, $p < 0.001$, $\chi^2 = 17.736$, df = 1, $p < 0.001$, and $\chi^2 = 14.643$, df = 1, $p < 0.001$, respectively). Pairwise co-detection of *A. astaci* with *S. parasitica* and of *A. astaci* with *T. bryosalmonae* did not show significant patterns with any sampling site properties (see Table S8 in the Supplementary materials for test results). As an exception, pairwise co-detection of *S. parasitica* with *T. bryosalmonae* decreased with elevation ($\chi^2 = 4.164$, df = 1, $p = 0.041$) and a similar, but insignificant trend was observed for pairwise detection of *A. astaci* with *T. bryosalmonae* ($\chi^2 = 2.912$, df = 1, $p = 0.088$).

DNA concentrations in water samples correlated positively between *S. parasitica* and *T. bryosalmonae* ($r = 0.355$, $\chi^2 = 19.209$, df = 1, $p < 0.001$), *S. parasitica* and *A. astaci* ($r = 0.103$, $\chi^2 = 22.591$, df = 1, $p < 0.001$), *S. parasitica* and *B. dendrobatidis* ($r = 0.729$, $\chi^2 = 22.294$, df = 1, $p < 0.001$), and to a lesser degree between *A. astaci* and *B. dendrobatidis* ($r = 0.025$, $\chi^2 = 4.053$, df = 1, $p = 0.044$). No other associations between disease agent DNA concentrations in water samples were found (*A. astaci* – *T. bryosalmonae*: $r = 0.132$, $\chi^2 = 1.238$, df = 1, $p = 0.266$; *B. dendrobatidis* – *T. bryosalmonae*: $r = 0.462$, $F_{1,4} = 1.181$, $p = 0.338$).

3.4 | Comparison of pathogen DNA detection in water with previous survey data

Among the 208 sites sampled here, 45 had previously been investigated for presence of *A. astaci* using tissue samples from invasive crayfish (Jean-Richard, 2013). Environmental DNA confirmed the presence of *A. astaci* in 9 of these sites and was absent in eDNA samples of 13 sites where no *A. astaci* had been detected in crayfish before. *A. astaci* DNA was found in eDNA samples in additional 12 sites where no infected crayfish had previously been recorded but failed to detect it in 11 sites with previous detection in crayfish. *T. bryosalmonae* was detected in both water samples and fish in 25 of the 79 river sites where both datasets were available (see Table S9 in the Supplementary material for site information and detection results). In 27 sites, neither of the sample types (water or tissue) detected the parasite. *T. bryosalmonae* DNA was present in eDNA samples of only one additional site where no infected fish had been previously recorded but failed to detect it in 26 sites with previous detection in fish. While the detection results of the two methods were correlated ($\chi^2 = 14.914$, df = 1, $p < 0.001$), in the 79 analyzed river sites, *T. bryosalmonae* was more commonly detected in fish (McNemar's $\chi^2 = 21.333$, df = 1, $p < 0.001$) compared with water samples.

3.5 | Inhibition of water samples

Only 2.37% ($n = 21/887$) of the eDNA samples from 13 sites (total sites analyzed $n = 266$) showed signs of inhibition, meaning they exhibited IPC Δ Cq-values ≥3. The elevation of a sampling site, lake surface area, river Strahler order, and river gradient (χ^2 tests, all $p > 0.195$) did not influence the probability of inhibited water samples. When present, inhibition affected detection rates of *S. parasitica* ($\chi^2 = 14.59$, df = 1, $p < 0.001$): the oomycete was detected in only one of the 21 samples with an IPC Δ Cq-value ≥3; whereas, *S. parasitica* detection rate in uninhibited samples (with IPC Δ Cq-values <3), was almost 50% ($n = 424/859$). Detection was not significantly influenced by inhibition in other pathogens (data not shown).

4 | DISCUSSION

Applying eDNA and molecular detection to parasites of diverse host groups that share the same habitat provides an efficient approach to characterizing parasite communities. Parasite communities can serve as indicators of ecosystem state, and eDNA methods may improve the incorporation of pathogens into ecosystem health assessment. Ultimately, understanding pathogen distribution and coexistence can lead to better disease management strategies. Here, we evaluate the potential of large-scale eDNA surveys to update and re-direct the surveillance and monitoring efforts of harmful amphibian, fish, and crayfish pathogens from the same water samples.

4.1 | Pathogen eDNA distribution, detection, and quantification

The 280 sites sampled were mainly rivers and streams in Switzerland. The most widespread pathogen was *S. parasitica* (Figure 2c), aligning with its endemic distribution throughout freshwater environments within the temperature range found in most Swiss lakes and rivers (Kitancharoen et al., 1996; van West, 2006). Although clinical saprolegniosis in fish is most often observed during stressful periods, particularly during the breeding season (Howe & Stehly, 1998; Neish, 1977) and during winter (Quiniou et al., 1998), the pathogen is detectable in many populations and environments year-round (Pickering & Willoughby, 1982; van West, 2006). The frequent *S. parasitica* detection across the entire study area, and the lack of association of detection probability with elevation in this study demonstrates the suitability of the sampling method for capturing pathogen stages from water samples with differing water chemistries and varied catchment features.

Conversely, *B. dendrobatidis* was detected very infrequently, with only 5 sites out of 280 with positive detection (Figure 2b). The amphibian host species mostly occur in smaller streams and ponds than targeted in this survey (e.g., midwife toad [*Alytes obstetricans obstetricans*], natterjack toad [*Bufo calamita*]), and presence in larger waterbodies is seasonal (Barnes et al., 2020; Kamoroff & Goldberg, 2017; Mosher et al., 2018). Furthermore, the sampling period from end of May to October did not closely consider species-specific temporal dynamics. *Ranavirus* and *B. dendrobatidis* concentrations in pond water were observed to be closely linked to host developmental stages, rising when anuran hosts reach later tadpole stages (Hall et al., 2018; Julian et al., 2019; Miaud et al., 2019). The strong developmental synchronicity of most amphibian species requires a more focused sampling period for maximized detection success of pathogens The contrasting detection patterns of *S. parasitica* (a generalist pathogen known to persist and occur in most types of aquatic environments) and *B. dendrobatidis* (a specialist pathogen whose amphibian host prefers a habitat not targeted in the study) thus indicate both sensitivity and specificity of the eDNA method for characterizing pathogen occurrence patterns across broad spatial scales.

A. astaci and *T. bryosalmonae* have in previous surveys been found across the study area, with distributions aligning largely with their invasive North American crayfish and brown trout/bryozoan host ranges, and with some evidence for decreasing occurrence with increasing elevation. Accordingly, *A. astaci* was detected in a third of all surveyed sites (87 of 262) and in all major Swiss waterways, consistent with the occurrence of plague-carrying invasive North American crayfish in all major lowland rivers and lakes across Switzerland (Stucki & Zaugg, 2011). *A. astaci* was not detected in the high alpine catchments of the Adige and the Inn rivers, where crayfish are absent due to low temperatures (Chucholl, 2017), supporting our findings of higher occurrence of *A. astaci* in lower elevations. *T. bryosalmonae* was found in 21% (59 of 279) of sites and appears mostly at low elevations in the northern part of Switzerland (Figure 2d). Wahli et al. (2002) observed a similar association with elevation in a survey of PKD among trout populations.

The eDNA survey showed that *T. bryosalmonae* DNA concentrations in water samples increased at lower elevations (when considering samples where the pathogen concentration was above LOQ). PKD affected trout populations are generally not found above 800 meters above sea level (m. a. s. l.) in Switzerland (Wahli et al., 2008) and this study independently confirms this pattern for *T. bryosalmonae* presence from water samples. Only one detection of *T. bryosalmonae* was found at an elevation of more than 800 m.a.s.l. This positive water sample was taken from the river Orbe, at 1014 m.a.s.l., where fish infected with *T. bryosalmonae* have been observed (Wahli et al., 2008). The eDNA evidence suggests that the absence of PKD in most sites above 800 m.a.s.l. could be attributed to infrequent occurrence of the parasite, rather than lack of PKD development at low temperatures associated with high elevations. The proliferation of *T. bryosalmonae* and the severity of PKD increases with temperature in laboratory studies in the bryozoan host (Tops et al., 2009) and fish host (Bailey et al., 2018), and therefore detection rates of *T. bryosalmonae* in water likely reflect the temperature dependence of parasite development in both hosts (Fontes, Hartikainen, Holland, et al., 2017). Further studies should investigate the elevation limits of bryozoan distribution, as the specific habitat requirements of this primary host may rarely be met at high elevation and thus limit the distribution *T. bryosalmonae* (trout commonly occur above 800 m.a.s.l.). The rare occurrence, or even absence, of *A. astaci* and *T. bryosalmonae* in alpine regions indicates the importance of high elevation refugia from disease agents, which may become threatened by the changing climate.

Despite collecting relatively large 5 L water samples, the limit of quantification for each pathogen was reached in relatively few of the samples with positive detection (proportion of samples exceeding LOQ; *A. astaci*: 21%, *B. dendrobatidis*: 0%, *S. parasitica*: 61%, *T. bryosalmonae*: 28%). Parasites and pathogens often show aggregated distribution among host populations, which could result in heterogenous release of pathogen eDNA into the water, making pathogen eDNA detection and quantification in larger water bodies challenging. Accordingly, the results for the widely distributed *S. parasitica* showed that pathogen eDNA concentration (when above the LOQ) decreased in rivers with higher Strahler order. Therefore, higher sampling effort, involving sampling at several locations of a lake or a large river is required to reach similar detection levels as in smaller waterbodies. Contrary to these results, we found that the detection frequency of *S. parasitica*, and the DNA concentration of *A. astaci*, increased with increasing lake surface area. This result could reflect different dilution factors and eDNA degradation dynamics in lakes and rivers, or the low number of lakes sampled in this study, or high variation in qPCR quantification of low DNA content samples (Mauvisseau et al., 2019).

Qualitative associations have previously been described between disease agent DNA concentrations and infection prevalence in host populations for the amphibian parasite, *Ribeiroia ondatrae* (Huver et al., 2015), *A. astaci* (Strand et al., 2014), *Ceratomyxa shasta* (Hallett et al., 2012) and various species of *Enterococcus* (Wade et al., 2010). However, to use pathogen eDNA detection to understand disease dynamics, further experimental data are needed to improve the understanding of quantitative or semi-quantitative relationships between pathogen DNA concentrations in water and disease prevalence and risk in host populations.

4.2 | Comparisons with previous surveys

To assess the variation in pathogen eDNA detection, we contrasted the eDNA survey results with previous surveys of parasite presence in host populations (*A. astaci* surveyed in 2012 and *T. bryosalmonae* in 2000–2006) (Jean-Richard, 2013; Wahli et al., 2008). It should be noted that these surveys were conducted >15 years ago in some cases, highlighting the need for methods that can be used to update such monitoring results more frequently.

The pathogen eDNA survey confirmed the results of the previous surveys in approximately half of the sampled sites for both pathogens. Notably, *T. bryosalmonae* eDNA revealed only one site where no infected fish were previously discovered (see Table S8 in the Supplement). Previous work shows a positive relationship between increasing water temperatures and the occurrence of PKD, and our results indirectly suggest that since 2006, when some of these sites were last tested, *T. bryosalmonae* distribution may not have changed significantly. This underlies the importance of temperature in provoking PKD outbreaks in areas where *T. bryosalmonae* is found to be present now and in the past.

A. astaci eDNA revealed pathogen presence in half of the sites where tissue samples from 2012 did not detect crayfish plague, however, *A. astaci* was not detected in eDNA from a similar number of sites where crayfish plague had previously been detected in crayfish. The timing and duration of the previous surveys, as well as disease dynamics and pathogen life-cycle differences may explain the variation in eDNA detection and survey results. *A. astaci* often appears in low prevalence in host populations of invasive crayfish species which are asymptomatic carriers (Sieber et al., 2021; Unestam & Weiss, 1970). Detection of the low zoospore release from asymptomatic hosts requires large sampling effort, yet is important when there is a danger of pathogen introduction to susceptible native crayfish populations (Schrimpf et al., 2013). Previous work has highlighted the importance of combined application of both conventional and DNA-based methods to create a more accurate indication of *A. astaci* distribution (Sieber et al., 2021).

4.3 | Patterns of co-occurrence of pathogen eDNA

The detections of *A. astaci* and *T. bryosalmonae* were correlated, with the two pathogens co-occurring more frequently than expected by chance. Although *A. astaci* and *T. bryosalmonae* parasitise different hosts (crayfish and bryozoans/fish, respectively), their co-occurrence may reflect similarity in the host habitat requirements. Further, *S. parasitica* occurrence was positively associated with both *T. bryosalmonae* and *A. astaci* occurrence, and eDNA concentrations were also observed to correlate between *A. astaci* and *S. parasitica* and *T. bryosalmonae* and *S. parasitica*. As *S. parasitica* can infect both fish and crayfish (Diéguez-Uribeondo et al., 1994), this result suggests some sites are particularly prone to harbor multiple pathogens. This could arise via opportunistic *S. parasitica* infection of fish and crayfish already weakened by either *A. astaci* or *T. bryosalmonae* (Edgerton et al., 2002; van den Berg et al., 2013). Furthermore, multiple pathogens might be more abundant in sites where their host populations are exposed to the same stressors, such as habitat degradation or temperatures near their thermal tolerance limits (Shea et al., 2012; Taglioretti et al., 2018). This suggests that the occurrence and concentration of generalist pathogens could be explored as an indicator for the severity of stressors driving disease in aquatic systems.

Inhibition was tested in this study using an internal positive control, showing minimal impacts on detection, and DNA was extracted using a standardized protocol. Despite this, we cannot fully exclude that frequent co-detection could have a technical reason, such as variation in eDNA extraction efficiency or inhibition among samples. Therefore, to confirm the co-occurrence patterns and ground-truth the eDNA-derived conclusions, complimentary sampling of hosts could be conducted in selected sites in future.

4.4 | Considerations for eDNA-based pathogen indicators

Pathogen detection in water samples is complicated by the host–parasite metapopulation dynamics, which can impact the distribution, magnitude, and timing of release of pathogen transmission stages into water (Miaud et al., 2019; Wittwer et al., 2018). In this study, each site was visited once and if the pathogens were not producing transmission stages at that time, their detection in water samples would have been unlikely. Thus, the timing of eDNA surveys to capture water-borne pathogens is a crucial consideration, and temporally repeated sample collection may be even more important than when assessing the occurrence of a free-living species.

Aquatic systems are complex and dynamic, leading to stochasticity in detection and limiting the accuracy of eDNA quantification due to heterogeneous distribution of pathogen DNA in the system (Sieber et al., 2020). In this study, sampling generally consisted of three 5 L water samples per site, exceeding the volume sampled in previous studies by a large margin (up to 5 water samples with volumes between 60 and 600 mL [Chestnut et al., 2014]). Despite the large sample volumes, occupancy modeling suggested similar number of samples ($n = 5$) should have been collected to exceed 95% probability of detection (Table 1). The results therefore suggest that for *B. dendrobatidis*, and possibly other pathogens with heterogenous zoospore distribution in water, pooled or integrated sampling from different positions of a site may be particularly beneficial (Goldberg et al., 2018). Occupancy models can be used to support sample collection decisions, particularly when large-scale surveillance and monitoring operations are planned. Our results suggest that managers should remain open to adapting collection methods to account for parasite aggregation and seasonal developmental cycles, in lieu of increasing water volumes and sample numbers.

5 | CONCLUSIONS

Pathogen surveys in wildlife populations currently require the collection of large numbers of hosts and are often limited in scope and restricted to a single species of host or pathogen. Yet, it is clear that host populations are threatened by multiple disease agents, which may have synergistic or cumulative effects on population and ecosystem health. eDNA sampling and qPCR detected multiple aquatic pathogen occurrence from the same water samples. We confirmed the widespread distribution of *A. astaci* and *T. bryosalmonae*, and the ubiquitous presence of *S. parasitica*, which has also been observed in previous surveys (Jean-Richard, 2013; Wahli et al., 2008). Factors that likely affect pathogen detection in water samples include the timing of surveys and the aggregated distribution of pathogens, which should be considered when planning sample collection. A major limiting factor in using eDNA measurements to understand disease risk are the mostly unknown relationships between disease agent DNA concentrations in water and disease prevalence in host populations. Experimental data on host–pathogen dynamics and field method comparisons are therefore required to better integrate pathogen eDNA detection to inform management decisions. We conclude that careful compilation of environmental factors and conventional disease monitoring methods, in addition to data gleaned from eDNA methods, could inform on host–parasite ecology and evolution, as well as parasite community ecology. This information will greatly help in enforcing appropriate and effective disease management plans, drive the development of new bioindicators for ecosystem and population health and provide a toolset for rapidly updating existing monitoring records.

AUTHOR CONTRIBUTIONS

All authors contributed to the study conception and design. All authors contributed to sample collection. Laboratory work was conducted by Natalie Sieber and Alex King. Data analysis and interpretation were performed by Natalie Sieber, Christoph Vorburger, and Hanna Hartikainen. The first draft of the manuscript was written by Natalie Sieber and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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CONFLICT OF INTEREST STATEMENT

All authors have no conflict of interest to declare.

DATA AVAILABILITY STATEMENT

The datasets generated and/or analyzed during the current study are available on dryad: https://datadryad.org/stash/share/QOUQA7WHcYOyhsc0G3oPISomPxaqw_txNMXwQwLOtJY.

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REFERENCES

Alderman, D. J., Polglase, J. L., & Frayling, M. (1987). *Aphanomyces astaci* pathogenicity under laboratory and field conditions. *Journal of Fish Diseases*, *10*, 385–393.

Audemard, C., Reece, K. S., & Burreson, E. M. (2004). Real-time PCR for detection and quantification of the protistan parasite *Perkinsus marinus* in environmental waters. *Applied and Environmental Microbiology*, *70*, 6611–6618.

Bailey, C., Schmidt-Posthaus, H., Segner, H., Wahli, T., & Strepparava, N. (2018). Are brown trout *Salmo trutta fario* and rainbow trout *Oncorhynchus mykiss* two of a kind? A comparative study of salmonids to temperature-influenced *Tetracapsuloides bryosalmonae* infection. *Journal of Fish Diseases*, *41*, 191–198.

Barnes, M. A., Brown, A. D., Daum, M. N., de la Garza, K. A., Driskill, J., Garrett, K., Goldstein, M. S., Luk, A., Maguire, J. I., Moke, R., Ostermaier, E. M., Sanders, Y. M., Sandhu, T., Stith, A., & Suresh, V. V. (2020). Detection of the amphibian pathogens chytrid fungus (*Batrachochytrium dendrobatidis*) and ranavirus in West Texas, USA, using environmental DNA. *Journal of Wildlife Diseases*, *56*, 702–706.

Bates, D., Mächler, M., Bolker, B. M., & Walker, S. C. (2015). Fitting linear mixed-effects models using lme4. *Journal of Statistical Software*, *67*, 1–48.

Berger, L., Hyatt, A. D., Speare, R., & Longcore, J. E. (2005). Life cycle stages of the amphibian chytrid *Batrachochytrium dendrobatidis*. *Diseases of Aquatic Organisms*, *68*, 51–63.

Carraro, L., Hartikainen, H., Jokela, J., Bertuzzo, E., & Rinaldo, A. (2018). Estimating species distribution and abundance in river networks using environmental DNA. *Proceedings of the National Academy of Sciences of the United States of America*, *115*, 11724–11729.

Chestnut, T., Anderson, C., Popa, R., Blaustein, A. R., Voytek, M., Olson, D. H., & Kirshtein, J. (2014). Heterogeneous occupancy and density estimates of the pathogenic fungus *Batrachochytrium dendrobatidis* in waters of North America. *PLoS One*, *9*, e106790.

Chucholl, C. (2017). Niche-based species distribution models and conservation planning for endangered freshwater crayfish in South-Western Germany. *Aquatic Conservation: Marine and Freshwater Ecosystems*, *27*, 698–705.

Cunningham, A. A., Daszak, P., Wood, J. L. N., & Cunningham, A. A. (2017). One health, emerging infectious diseases and wildlife: Two decades of progress? *Philosophical Transactions of the Royal Society B: Biological Sciences*, *372*, 20160167.

Daszak, P., Cunningham, A. A., & Hyatt, A. D. (2000). Emerging infectious diseases of wildlife—Threats to biodiversity and human health. *Science*, *287*, 443–449.

Daszak, P., Strieby, A., Cunningham, A. A., Longcore, J. E., Brown, C. C., & Porter, D. (2004). Experimental evidence that the bullfrog (*Rana catesbeiana*) is a potential carrier of chytridiomycosis, an emerging fungal disease of amphibians. *Herpetological Journal*, *14*, 201–207.

De Kinkelin, P., Gay, M., & Forman, S. (2002). The persistence of infectivity of *Tetracapsula bryosalmonae*, infected water for rainbow trout, *Oncorhynchus mykiss* (Walbaum). *Journal of Fish Diseases*, *25*, 477–482.

Diéguez-Uribeondo, J., Cerenius, L., & Söderhäll, K. (1994). *Saprolegnia parasitica* and its virulence on three different speciesof freshwater crayfish. *Aquaculture*, *120*, 219–228.

Dorazio, R. M., & Erickson, R. A. (2018). Ednaoccupancy: An r package for multiscale occupancy modelling of environmental DNA data. *Molecular Ecology Resources*, *18*, 368–380.

Edgerton, B. F., Evans, L. H., Stephens, F. J., & Overstreet, R. M. (2002). Review article: Synopsis of freshwater crayfish diseases and commensal organisms. *Aquaculture*, *206*, 57–135.

Feist, S. W., Longshaw, M., Canning, E. U., & Okamura, B. (2001). Induction of proliferative kidney disease (PKD) in rainbow trout *Oncorhynchus mykiss* via the bryozoan *Fredericella sultana* infected with *Tetracapsula bryosalmonae*. *Diseases of Aquatic Organisms*, *45*, 61–68.

Fontes, I., Hartikainen, H., Holland, J. W., Secombes, C. J., & Okamura, B. (2017). *Tetracapsuloides bryosalmonae* abundance in river water. *Diseases of Aquatic Organisms*, *124*, 145–157.

Fontes, I., Hartikainen, H., Taylor, N. G. H., & Okamura, B. (2017). Conditional persistence and tolerance characterize endoparasite-colonial host interactions. *Parasitology*, *144*, 1052–1063.

Fox, J., & Weisberg, S. (2019). *An R companion to applied regression, third edit*. Sage.

Gelfand, A. E., & Ghosh, S. K. (1998). Model choice: A minimum posterior predictive loss approach. *Biometrika*, *85*, 1–11.

Goldberg, C. S., Strickler, K. M., & Fremier, A. K. (2018). Degradation and dispersion limit environmental DNA detection of rare amphibians in wetlands: Increasing efficacy of sampling designs. *Science of the Total Environment*, *633*, 695–703.

Gupta, P., Robin, V. V., & Dharmarajan, G. (2020). Towards a more healthy conservation paradigm: Integrating disease and molecular ecology to aid biological conservation. *Journal of Genetics*, *99*, 1–26.

Hall, E. M., Goldberg, C. S., Brunner, J. L., & Crespi, E. J. (2018). Seasonal dynamics and potential drivers of ranavirus epidemics in wood frog populations. *Oecologia*, *188*, 1253–1262.

Hallett, S. L., & Bartholomew, J. L. (2006). Application of a real-time PCR assay to detect and quantify the myxozoan parasite Ceratomyxa shasta in river water samples. *Diseases of Aquatic Organisms*, *71*, 109–118.

Hallett, S. L., Ray, R. A., Hurst, C. N., Holt, R. A., Buckles, G. R., Atkinson, S. D., & Bartholomew, J. L. (2012). Density of the waterborne parasite *Ceratomyxa shasta* and its biological effects on salmon. *Applied and Environmental Microbiology*, *78*, 3724–3731.

Hartikainen, H., & Okamura, B. (2015). Ecology and evolution of malacosporean-bryozoan interaction. In B. Okamura, A. Gruhl, & J. L. Bartholomew (Eds.), *Myxozoan evolution, ecology and development* (pp. 201–216). Springer International Publishing.

Holdich, D. M., Reynolds, J. D., Souty-Grosset, C., & Sibley, P. J. (2009). A review of the ever increasing threat to European crayfish from non-indigenous crayfish species. *Knowledge and Management of Aquatic Ecosystems*, *2009*, 394–395.

Howe, G. E., & Stehly, G. R. (1998). Experimental infection of rainbow trout with *Saprolegnia parasitica*. *Journal of Aquatic Animal Health*, *10*, 397–404.

Hutchins, P. R., Sepulveda, A. J., Martin, R. M., & Hopper, L. R. (2018). A probe-based quantitative PCR assay for detecting *Tetracapsuloides bryosalmonae* in fish tissue and environmental DNA water samples. *Conservation Genetics Resources*, *10*, 317–319.

Huver, J. R., Koprivnikar, J., Johnson, P. T. J., & Whyard, S. (2015). Development and application of an eDNA method to detect and quantify a pathogenic parasite in aquatic ecosystems. *Ecological Applications*, *25*, 991–1002.

Hyman, O. J., & Collins, J. P. (2012). Evaluation of a filtration-based method for detecting *Batrachochytrium dendrobatidis* in natural bodies of water. *Diseases of Aquatic Organisms*, *97*, 185–195.

Jean-Richard, P. (2013). *Krebspesterhebung in der Schweiz Kampagne 2012* (p. 13). BAFU.

Johnson, M. L., & Speare, R. (2005). Possible modes of dissemination of the amphibian chytrid *Batrachochytrium dendrobatidis* in the environment. *Diseases of Aquatic Organisms*, *65*, 181–186.

Julian, J. T., Glenney, G. W., & Rees, C. (2019). Evaluating observer bias and seasonal detection rates in amphibian pathogen eDNA collections by citizen scientists. *Diseases of Aquatic Organisms*, *134*, 15–24.

Kamoroff, C., & Goldberg, C. S. (2017). Using environmental DNA for early detection of amphibian chytrid fungus *Batrachochytrium dendrobatidis* prior to a ranid die-off. *Diseases of Aquatic Organisms*, *127*, 75–79.

Kilpatrick, A. M., Briggs, C. J., & Daszak, P. (2010). The ecology and impact of chytridiomycosis: An emerging disease of amphibians. *Trends in Ecology & Evolution*, *25*, 109–118.

Kirshtein, J. D., Anderson, C. W., Wood, J. S., Longcore, J. E., & Voytek, M. A. (2007). Quantitative PCR detection of *Batrachochytrium dendrobatidis* DNA from sediments and water. *Diseases of Aquatic Organisms*, *77*, 11–15.

Kitancharoen, N., Yuasa, K., & Hatai, K. (1996). Effects of pH and temperature on growth of *Saprolegnia diclina* and *S. parasitica* isolated from various sources. *Mycoscience*, *37*, 385–390.

Kuznetsova, A., Brockhoff, P. B., & Christensen, R. H. B. (2017). LmerTest package: Tests in linear mixed effects models. *Journal of Statistical Software*, *82*, 1–26.

Lips, K. R., Brem, F., Brenes, R., Reeve, J. D., Alford, R. A., Voyles, J., Carey, C., Livo, L., Pessier, A. P., & Collins, J. P. (2006). Emerging infectious disease and the loss of biodiversity in a Neotropical amphibian community. *Proceedings of the National Academy of Sciences of the United States of America*, *103*, 3165–3170.

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.

Marcogliese, D. J. (2008). The impact of climate change on the parasites and infectious diseases of aquatic animals. *Revue Scientifique et Technique (International Office of Epizootics)*, *27*, 467–484.

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. *Scientific Reports*, *9*, 1–10.

Miaud, C., Arnal, V., Poulain, M., Valentini, A., & Dejean, T. (2019). EDNA increases the detectability of ranavirus infection in an alpine amphibian population. *Viruses*, *11*, 1–15.

Mitchell, S. E., Rogers, E. S., Little, T. J., & Read, A. F. (2005). Host–parasite and genotype-by-environment interactions: Temperature modifies potential for selection by a sterilizing pathogen. *Evolution*, *59*, 70–80.

Mosher, B. A., Huyvaert, K. P., & Bailey, L. L. (2018). Beyond the swab: Ecosystem sampling to understand the persistence of an amphibian pathogen. *Oecologia*, *188*, 319–330.

Neish, G. A. (1977). Observations on saprolegniasis of adult sockeye salmon, *Oncorhynchus nerka* (Walbaum). *Journal of Fish Biology*, *10*, 513–522.

Nyhlén, L., & Unestam, T. (1980). Wound reactions and *Aphanomyces astaci* growth in crayfish cuticle. *Journal of Invertebrate Pathology*, *36*, 187–197.

Oidtmann, B., Heitz, E., Rogers, D., & Hoffmann, R. W. (2002). Transmission of crayfish plague. *Diseases of Aquatic Organisms*, *52*, 159–167.

Okamura, B., Hartikainen, H., Schmidt-Posthaus, H., & Wahli, T. (2011). Life cycle complexity, environmental change and the emerging status of salmonid proliferative kidney disease. *Freshwater Biology*, *56*, 735–753.

Paul, C., & Belbahri, L. (2012). Clonalité de *Saprolegnia parasitica*, le parasite des poissons du Doubs.

Peeler, E. J., Oidtmann, B. C., Midtlyng, P. J., Miossec, L., & Gozlan, R. E. (2011). Non-native aquatic animals introductions have driven disease emergence in Europe. *Biological Invasions*, *13*, 1291–1303.

Pickering, A., & Willoughby, L. (1982). Saprolegnia infections of salmonid fish. In *Fiftieth annual report* (pp. 38–49). Freshwater Biological Association.

Pinheiro, J., Bates, D., & R Core Team. (2023). Nlme: linear and nonlinear mixed effects models.

Quiniou, S. M. A., Bigler, S., Clem, L. W., & Bly, J. E. (1998). Effects of water temperature on mucous cell distribution in channel catfish epidermis: A factor in winter saprolegniasis. *Fish & Shellfish Immunology*, *8*, 1–11.

R Core Team. (2019). *R: A language and environment for statistical computing*. R Foundation for Statistical Computing https://www.r-project.org/

Ravasi, D., De Respinis, S., & Wahli, T. (2018). Multilocus sequence typing reveals clonality in *Saprolegnia parasitica* outbreaks. *Journal of Fish Diseases*, *41*, 1653–1665.

Richey, C. A., Kenelty, K. V., Hopkins, K. V. S., Stevens, B. N., Mart, B., Barnum, S. M., Hallett, S. L., Atkinson, S. D., Bartholomew, J. L., & Soto, E. (2018). Distribution and prevalence of *Myxobolus cerebralis* in post fire areas of Plumas National Forest: Utility of environmental DNA sampling. *Journal of Aquatic Animal Health*, *30*, 130–143.

Rocchi, S., Tisserant, M., Valot, B., Laboissière, A., Frossard, V., & Reboux, G. (2017). Quantification of *Saprolegnia parasitica* in river water using real-time quantitative PCR: From massive fish mortality to tap drinking water. *International Journal of Environmental Health Research*, *27*, 1–10.

Rusch, J. C., Hansen, H., Strand, D. A., Markussen, T., Hytterød, S., & Vrålstad, T. (2018). Catching the fish with the worm: A case study on eDNA detection of the monogenean parasite *Gyrodactylus salaris* and two of its hosts, Atlantic salmon (*Salmo salar*) and rainbow trout (*Oncorhynchus mykiss*). *Parasites and Vectors*, *11*, 1–12.

Schrimpf, A., Maiwald, T., Vrålstad, T., Schulz, H. K., Śmietana, P., & Schulz, R. (2013). Absence of the crayfish plague pathogen (*Aphanomyces astaci*) facilitates coexistence of European and American crayfish in Central Europe. *Freshwater Biology*, *58*, 1116–1125.

Shea, J., Kersten, G. J., Puccia, C. M., Stanton, A. T., Stiso, S. N., Helgeson, E. S., & Back, E. J. (2012). The use of parasites as indicators of ecosystem health as compared to insects in freshwater lakes of the inland northwest. *Ecological Indicators*, *13*, 184–188.

Sieber, N. (2020). *Detection of aquatic wildlife pathogens from eDNA in water samples - validation and application in Switzerland*. ETH Zürich.

Sieber, N., Hartikainen, H., Krieg, R., Zenker, A., & Vorburger, C. (2021). Parasite DNA detection in water samples enhances crayfish plague monitoring in asymptomatic invasive populations. *Biological Invasions*, *24*, 281–297.

Sieber, N., Hartikainen, H., & Vorburger, C. (2020). Validation of an eDNA-based method for the detection of wildlife pathogens in water. *Diseases of Aquatic Organisms*, *141*, 171–184.

Strand, D. A., Holst-Jensen, A., Viljugrein, H., Edvardsen, B., Klaveness, D., Jussila, J., & Vrålstad, T. (2011). Detection and quantification of the crayfish plague agent in natural waters: Direct monitoring approach for aquatic environments. *Diseases of Aquatic Organisms*, *95*, 9–17.

Strand, D. A., Jussila, J., Johnsen, S. I., Viljamaa-Dirks, S., Edsman, L., Wiik-Nielsen, J., Viljugrein, H., Engdahl, F., & Vrålstad, T. (2014). Detection of crayfish plague spores in large freshwater systems. *Journal of Applied Ecology*, *51*, 544–553.

Stucki, P., & Zaugg, B. (2011). *Aktionsplan Flusskrebse Schweiz. Artenförderung von Edelkrebs, Dohlenkrebs und Steinkrebs*. Bundesamt für Umwelt.

Svoboda, J., Mrugała, A., Kozubíková-Balcarová, E., & Petrusek, A. (2017). Hosts and transmission of the crayfish plague pathogen *Aphanomyces astaci*: A review. *Journal of Fish Diseases*, *40*, 127–140.

Taglioretti, V., Rossin, M. A., & Timi, J. T. (2018). Fish-trematode systems as indicators of anthropogenic disturbance: Effects of urbanization on a small stream. *Ecological Indicators*, *93*, 759–770.

Thomsen, P. F., & Willerslev, E. (2015). Environmental DNA—An emerging tool in conservation for monitoring past and present biodiversity. *Biological Conservation*, *183*, 4–18.

Tobler, U., Borgula, A., & Schmidt, B. R. (2012). Populations of a susceptible amphibian species can grow despite the presence of a pathogenic chytrid fungus. *PLoS One*, *7*, 1–8.

Tops, S., Hartikainen, H. L., & Okamura, B. (2009). The effects of infection by *Tetracapsuloides bryosalmonae* (Myxozoa) and temperature on *Fredericella sultana* (Bryozoa). *International Journal for Parasitology*, *39*, 1003–1010.

Tukey, J. (1977). *Exploratory data analysis*. Addison-Wesley.

Unestam, T., & Weiss, D. W. (1970). The host–parasite relationship between freshwater crayfish and the crayfish disease fungus *Aphanomyces astaci*: Responses to infection by a susceptible and a resistant species. *Journal of General Microbiology*, *60*, 77–90.

van den Berg, A. H., McLaggan, D., Diéguez-Uribeondo, J., & van West, P. (2013). The impact of the water moulds *Saprolegnia diclina* and *Saprolegnia parasitica* on natural ecosystems and the aquaculture industry. *Fungal Biology Reviews*, *27*, 33–42.

van West, P. (2006). *Saprolegnia parasitica*, an oomycete pathogen with a fishy appetite: New challenges for an old problem. *Mycologist*, *20*, 99–104.

Vrålstad, T., Knutsen, A. K., Tengs, T., & Holst-Jensen, A. (2009). A quantitative TaqMan MGB real-time polymerase chain reaction based assay for detection of the causative agent of crayfish plague *Aphanomyces astaci*. *Veterinary Microbiology*, *137*(1–2), 146–155. https://doi.org/10.1016/j.vetmic.2008.12.022

Wade, T. J., Sams, E., Brenner, K. P., Haugland, R., Chern, E., Beach, M., Wymer, L., Rankin, C. C., Love, D., Li, Q., Noble, R., & Dufour, A. P. (2010). Rapidly measured indicators of recreational water quality and swimming-associated illness at marine beaches: A prospective cohort study. *Environmental Health: A Global Access Science Source*, *9*, 1–14.

Wahli, T., Bernet, D., Segner, H., & Schmidt-Posthaus, H. (2008). Role of altitude and water temperature as regulating factors for the geographical distribution of *Tetracapsuloides bryosalmonae* infected fishes in Switzerland. *Journal of Fish Biology*, *73*, 2184–2197.

Wahli, T., Bernet, D., Steiner, P. A., & Schmidt-Posthaus, H. (2007). Geographic distribution of Tetracapsuloides bryosalmonae infected fish in Swiss rivers: An update. *Aquatic Sciences*, *69*, 3–10.

Wahli, T., Knuesel, R., Bernet, D., Segner, H., Pugovkin, D., Burkhardt-Holm, P., Escher, M., & Schmidt-Posthaus, H. (2002). Proliferative kidney disease in Switzerland: Current state of knowledge. *Journal of Fish Diseases*, *25*, 491–500.

Walker, S. F., Salas, M. B., Jenkins, D., Garner, T. W. J., Cunningham, A. A., Hyatt, A. D., Bosch, J., & Fisher, M. C. (2007). Environmental detection of *Batrachochytrium dendrobatidis* in a temperate climate. *Diseases of Aquatic Organisms*, *77*, 105–112.

Watanabe, S. (2010). Asymptotic equivalence of Bayes cross validation and widely applicable information criterion in singular learning theory. *Journal of Machine Learning Research*, *11*, 3571–3594.

Wickham, H., François, R., Müller, K., & Vaughan, D. (2023). Dplyr: A grammar of data manipulation.

Wittwer, C., Stoll, S., Strand, D., Vrålstad, T., Nowak, C., & Thines, M. (2018). EDNA-based crayfish plague monitoring is superior to conventional trap-based assessments in year-round detection probability. *Hydrobiologia*, *807*, 87–97.

Wittwer, C., Stoll, S., Thines, M., & Nowak, C. (2019). EDNA-based crayfish plague detection as practical tool for biomonitoring and risk assessment of *A. astaci*-positive crayfish populations. *Biological Invasions*, *21*, 1075–1088.

Zeh Weissmann, H., Könitzer, C., & Bertiller, A. (2009). *Strukturen der Fliessgewässer in der Schweiz. Zustand von Sohle, Ufer und Umland (Ökomorphologie); Ergebnisse der ökomorphologischen Kartierung.* Stand: April 2009. Umwelt-Zustand Nr. 0926. Bundesamt für Umwelt.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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