



**HAL**  
open science

# Quantifying invasion dynamics: Quantitative polymerase chain reaction vs. droplet digital polymerase chain reaction in monitoring the alien invasive bloody-red shrimp *Hemimysis anomala* and its interaction with perch in Lake Geneva

Sirine Melliti, Marine Vautier, Cécile Chardon, Stéphan Jacquet

## ► To cite this version:

Sirine Melliti, Marine Vautier, Cécile Chardon, Stéphan Jacquet. Quantifying invasion dynamics: Quantitative polymerase chain reaction vs. droplet digital polymerase chain reaction in monitoring the alien invasive bloody-red shrimp *Hemimysis anomala* and its interaction with perch in Lake Geneva. *Limnology and Oceanography: Methods*, 2025, 10.1002/lom3.10666 . hal-04875710

**HAL Id: hal-04875710**

**<https://hal.science/hal-04875710v1>**

Submitted on 9 Jan 2025

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

NEW METHODS

# Quantifying invasion dynamics: Quantitative polymerase chain reaction vs. droplet digital polymerase chain reaction in monitoring the alien invasive bloody-red shrimp *Hemimysis anomala* and its interaction with perch in Lake Geneva

Sirine Melliti, Marine Vautier , Cécile Chardon, Stéphan Jacquet \*

Université Savoie Mont-Blanc, INRAE, CARRTEL, Thonon-les-Bains, France

## Abstract

Invasive alien species constitute a major threat to aquatic systems due to their potential impact on endemic biodiversity, ecosystem functioning, infrastructure, and possible sanitary issues. It is therefore crucial to obtain information on their presence, abundance, and distribution. The bloody-red shrimp *Hemimysis anomala*, which originated from the Ponto-Caspian area, has recently settled in Western European lakes, including Lake Geneva. Although divers have frequently reported the presence and development of this small crustacean over the last decade, no monitoring has yet been proposed. During a period of 2.5 yr, we tested and optimized an environmental deoxyribonucleic acid approach by comparing two polymerase chain reaction techniques, quantitative and digital droplet polymerase chain reaction, to assess the presence, abundance, and dynamics of the animal as well as that of a potential predator, the perch (*Perca fluviatilis*). We show and discuss the efficiency of the methods and reveal for the first time the seasonal dynamics of *Hemimysis anomala* at a selected site in Lake Geneva. We highlight, in agreement with diving observations, that the animal's abundance is high in winter and declines rapidly in early spring, concomitantly with temperature increase and perch presence.

Climate change and anthropization have facilitated the spread of invasive species by altering ecosystems and removing natural barriers (Barbet-Massin et al. 2013; IPBES 2023; Rahel and Olden 2008). However, most cases of alien species introduction are due to human activities, occurring accidentally through transport or intentionally for purposes like biological control, wildlife stock improvement, or aquaculture (Pysek et al. 2010; Sardain et al. 2019). In the mid-20<sup>th</sup> century, crustaceans were intentionally introduced into Eastern European lakes and reservoirs to enhance fish production. Increased driver and marine traffic has allowed many of these species,

particularly Ponto-Caspian mysids, to spread to Western Europe and the American Great Lakes (Brown et al. 2014).

*Hemimysis anomala*, also known as the bloody-red shrimp (Wittmann 2007; Audzijonyte et al. 2008; Daneliya et al. 2012), is a species native to the estuaries of the Black Sea, the Sea of Azov and the Caspian Sea (Chandra and Gerhardt 2008). Despite being critically endangered in its native region (Marty et al. 2010), it now occupies various ecosystems in Europe and North America as an Aquatic Invasive Species (AIS). *Hemimysis anomala* is a ubiquitous and opportunistic species with a euryhaline physiology and an omnivorous diet (Wittmann 2007; Frossard et al. 2023), adapting its diet to resource availability (Frossard et al. 2023). It exhibits diurnal vertical migration, hiding in dark areas during the day and migrating to feed in the pelagic zone at night (Borcherding et al. 2006; Rogissart et al. 2023).

The presence of *H. anomala* has significant impacts on food webs, due to its high consumption, reproductive rates, and migratory behavior. Thus, top-down but also bottom-up

\*Correspondence: [stephan.jacquet@inrae.fr](mailto:stephan.jacquet@inrae.fr)

**Associate editor:** Steven W. Wilhelm

**Data Availability Statement:** The data supporting the findings of this study are available in the open repository site ZENODO and referred to the DOI: <http://doi.org/10.5281/zenodo.14414711>.

controls have been highlighted in relation to the introduction of this species (Ellis et al. 2011; Marty 2008). While providing a lipid-rich food source for fish (Borcherding et al. 2007), it may also compete with planktivorous fish larvae and juveniles (Sinclair et al. 2016). This could reduce the abundance, growth, and productivity of pelagic fish, while benefiting to benthic fish (Marty et al. 2012; Ricciardi et al. 2012). The spread of *H. anomala* could result in complex interactions between species and trophic levels, restructuring food webs and energy flows (Marty 2008).

The impact of AIS is strongly related to their abundance (Sofaer et al. 2018), making it crucial to monitor their population abundance and dynamics for effective management. Regarding *H. anomala*, which colonized the large peri-alpine lakes for less than two decades (Frossard and Fontvieille 2018), divers have regularly observed swarms of several hundred thousand individuals in Lake Geneva in early winter since at least 2020 (Jacquet 2023; Rogissart et al. 2023). Over the last 4 yr, a survey conducted in Lake Geneva and using scientific and recreational diving allowed us to highlight the development of high-density swarms in the littoral zone and in surface waters in winter, when potential predators of the species, such as the European perch (*Perca fluviatilis*), leave this area to move into deeper waters (Craig 1977). At the beginning of spring, we observed that the abundance of swarms decreases simultaneously with the increase in water temperature and merely the return of the European perch to the littoral zone (Gillet and Dubois 2002; Guillard et al. 2006; Rogissart et al. 2022). Moreover, predation by this fish on *H. anomala* has been recorded using both acoustic and infrared/visible video cameras (Rogissart et al. unpublished). Hence, the decline in *H. anomala* observed by divers in early spring, and its concordance with the annual migration of the European perch has led to the hypothesis that predation by European perch may play a role in the regulation of *H. anomala* populations.

To answer this question, it is essential to acquire precise quantitative data that corroborate the information provided by scuba diving, which is limited to track small individuals at low abundance over a short time scale or assessing their density at high abundance. Environmental deoxyribonucleic acid (eDNA), increasingly used for biodiversity assessment and monitoring of various species, including rare or invasive species, appeared to be well-suited for this purpose (Ficetola et al. 2008; Jerde et al. 2011). Combined with quantitative analyses such as quantitative polymerase chain reaction (qPCR) or droplet digital polymerase chain reaction (ddPCR), eDNA can provide a highly sensitive approach allowing to detect the presence, absence, and abundance of one or more target species (Takahara et al. 2012; Doi et al. 2015). Additionally, eDNA sampling methods are less invasive than traditional sampling techniques and are unlikely to cause harm to species or ecosystems (Banerjee et al. 2022; Takahashi et al. 2023). This study aimed to investigate the

temporal dynamics of *H. anomala* in Lake Geneva using an eDNA approach. We targeted both *H. anomala* and the native fish *P. fluviatilis*, and quantified their eDNA concentrations to understand their interaction.

The study compared two widely used techniques for eDNA quantification: qPCR and ddPCR.

The first technique, qPCR, is used with SYBR Green, a fluorescent dye that detects all double-stranded deoxyribonucleic acid (DNA), including non-specific reaction products, and increases in fluorescence as the DNA is amplified. The second technique, ddPCR, is used with a TaqMan chemistry, which uses a fluorescent probe that binds specifically to the target DNA sequence, ensuring that only the target sequence is detected. Our choice to compare qPCR and ddPCR was driven by the fact that ddPCR technology is not yet accessible to all laboratories due to its high cost, while qPCR may still be a more affordable alternative. We therefore present a laboratory study designed to test the specificity and sensitivity of the two methods, using one primer set for SYBR Green qPCR and a different primer/probe set specifically developed in this study for TaqMan ddPCR. It was hypothesized that detection rates would be higher with ddPCR using a TaqMan approach than with qPCR using a SYBR Green approach, especially at low eDNA concentrations. Finally, we suggest ways to improve and optimize these tools for accurate use in biomonitoring and early detection of this AIS.

## Materials and procedures

### Study site

Lake Geneva, the largest natural perialpine lake in Western Europe, is located between France and Switzerland at an altitude of 372 m. It has a surface area of 582 km<sup>2</sup> and a maximum depth of 309 m. The sampling site was located in Saint-Disdille at Thonon-les-Bains (Lat = 46°24'05"N, Long = 6°30'07"E) at less than 0.1 km from the shore, due to large swarms of *H. anomala* reported by divers since 2020. Water samples were collected below the surface, at 0.5 m depth.

### Environmental deoxyribonucleic acid water sampling and filtration

Between January 2021 and June 2023, 54 water samples were collected from a lake using a 1-L glass jar. The jar was previously autoclaved and thoroughly rinsed with distilled water to reduce contamination risks. On site, the jar was rinsed again with lake water several times before being filled and sealed by a diver below the surface. To minimize eDNA degradation, the samples were stored on ice before being filtered in the laboratory on the same day using 0.45 µm Sterivex™ MILLIPORE filters as recommended by Vautier et al. (2021). Using a sterile 50 mL syringe attached to the Sterivex, 850 mL of water was filtered for each sample. Following filtration, the Sterivex was immediately stored at -20°C to minimize eDNA degradation prior to DNA extraction.

### Deoxyribonucleic acid extraction

Deoxyribonucleic acid extractions were performed in a laboratory dedicated to rare DNA and following the extraction protocol of the MACHEREY-NAGEL NucleoSpin® Soil kit adapted to Sterivex filters (Vautier et al. 2020). No-template controls (NTC) were included during extraction and used as negative controls in order to monitor for potential contamination or primer dimer formation. Additional DNA extractions from animal tissues were carried out using the MACHEREY-NAGEL NucleoSpin Tissue Kit to determine primers' specificity. Negative controls were performed on DNA from tissues of the crayfish *Pacifastacus leniusculus* and *Gammarus* sp., while positive controls were performed on DNA from *H. anomala* and *P. fluviatilis* tissues, all sampled by scuba diving and gillnetting.

A Nanodrop™ spectrophotometer was used to measure absorbance to assess extract quality and total DNA concentration. Deoxyribonucleic acid extracts were then stored at -20°C until further analysis. Pre-polymerase chain reaction (PCR) and post-PCR manipulations were physically separated and carried out in dedicated rooms.

### Primer selection

In this study, all PCR tests were carried out using primer sets that bind within the cytochrome oxidase subunit 1 (COI) gene. All details concerning the primers (sequences, amplification length, target, and references) are provided in Table 1.

### Quantitative polymerase chain reaction analysis

#### Primer specificity

To test the specificity of the SYBR Green *H. anomala* primer set (Table 1), we performed a qPCR run on two pools of eDNA samples. One pool, designated P1, contained eDNA from samples collected in January 2021 (with an expected high signal of *H. anomala* as observed by scuba diving), while the second pool (P2) contained eDNA extracted from the environmental samples collected in October/November 2021 (with an expected low signal of *H. anomala* as observed by scuba diving). The resulting PCR products were purified according to

the protocol provided in the QIAquick PCR Purification Kit to remove residual primers and reagents prior to cloning. The purified PCR products were cloned using a TOPO TA cloning kit according to the manufacturer's instructions (Invitrogen). Forty clones for the P1 pool and 39 clones for the P2 pool were collected and sequenced by Sanger technology by Eurofins Genomics (Germany) using the M13 forward primer. The sequences obtained were compared by BLAST (spring 2023) with the sequences contained in the NCBI databases (spring 2022) in order to determine the percentage of clones corresponding to *H. anomala*.

#### Quantitative polymerase chain reaction optimization and standard curve production

With the aim of optimizing qPCR assay conditions, we performed a series of qPCR assays using both SYBR Green and TaqMan techniques in simplex and multiplex. SYBR Green-based qPCR tests using the Ha Cox F and Ha COX R primer sets (targeting the cytochrome *c* oxidase subunit) revealed some issues such as non-specific amplification or dimerization of the primers. Numerous optimization tests were therefore carried out, which finally resulted in good reaction efficiency, specific amplifications without dimerization of the primers, and amplification curves with a clear plateau phase and without baseline deviation. Various parameters such as annealing time and temperature and primer concentration were adjusted to obtain better amplification results. The melting curves were carefully checked. Once the optimal qPCR conditions were determined, a standard curve was generated using DNA extracted from *H. anomala* tissues and amplified in SYBR Green-based qPCR. The PCR products were then purified, ligated into the pCR™2.1-TOPO® plasmid vector, and transformed into *E. coli* competent cells and cultured overnight. Colonies that successfully integrated the recombinant plasmid were selected for plasmid DNA extraction and linearization. After extraction, the Nanodrop™ spectrophotometer was used to measure the plasmid DNA concentration and its dilution, and to run standards ranging from 10<sup>8</sup> to 10<sup>1</sup>. Negative controls were run in parallel with the samples.

**Table 1.** Species-specific primers and probes used for the amplification of COX or COI gene region of *H. anomala* and *P. fluviatilis* in qPCR and ddPCR. F = Forward, R = Reverse, P = Probe.

Target species	Primer/probe	Sequence (5'-3')	Amplicon length (bp)	Reference
<i>H. anomala</i> (qPCR)	Ha Cox F	F: ACCTCCATCGTTAAGATTGCT	72	Brown et al. (2017)
	Ha COX R	R: TAGACTGTCCACCCCGTACC		
<i>H. anomala</i> (ddPCR)	H_anomala_COI-5P_F	F: AATTGGGTGAGCCCGGTAGG	128	Present study
	H_anomala_COI-5P_R	R: TAACCAGTTGCCGAAACCACC		
	H_anomala_COI-5P_P	Fam: ACAAACGCATGCGCTGTACAATCACC		
<i>P. fluviatilis</i> (ddPCR)	Perca_Rhone_COI-5P_F	F: CGCCGTTCTTCTCTTCTCT	116	Vautier et al. (2023)
	Perca_Rhone_COI-5P_R	R: GAATAGGGTCCGCTCCTCCT		
	Perca_Rhone_COI-5P_P	Hex: ACTTCCTGTTCTTGCCGCTGGCA		

### Environmental deoxyribonucleic acid sample analysis

The optimized PCR reaction mix conditions retained were a final reaction volume of 25  $\mu\text{L}$  containing 0.4  $\mu\text{M}$  of each primer in 1X Sybr Green Master Mix, along with 1  $\mu\text{L}$  of DNA solution. Two replicates of each PCR reaction were amplified in a thermocycler (15 min at 95°C, 40 cycles of three steps of 10 s at 95°C, 30 s at 58°C, and 30 s at 72°C) including no-template controls and standard samples. At the end of the qPCR, the amplification curve of each sample was analyzed using Rotor-Gene Q Series Software 2.3.1. The threshold cycle was determined and the initial concentration of the target gene in the environmental samples was obtained by plotting the Ct on the corresponding standard curve.

### Droplet digital polymerase chain reaction analysis

#### Primers and probe design for *H. anomala* detection

Cytochrome oxidase subunit 1 sequences for the target species, *H. anomala*, and the other macrocrustacean species found in Lake Geneva (gammarids and crayfish) were obtained from the Barcode of Life Database (BOLD) (<http://www.boldsystems.org/>). All available sequences were obtained regardless of geographical location in order to avoid intraspecific variation. Primer design and in silico validation were performed according to Vautier et al. (2023). The primer set selected for *H. anomala* were: H\_anomala\_COI-5P\_F, H\_anomala\_COI-5P\_R, and H\_anomala\_COI-5P\_P (Table 1).

#### Droplet digital polymerase chain reaction optimization

In the same way as for qPCR, the ddPCR assay was optimized by carrying out different tests targeting *H. anomala* and *P. fluviatilis*. Typically, adjusted parameters were DNA quantity, annealing temperature, the type of fluorescent dye (ddPCR EvaGreen Supermix or ddPCR Supermix for Probes), and the number of amplification cycles.

#### Primer specificity and sensitivity

To assess the sensitivity of the ddPCR primer/probe assay, the limits of detection (LOD) and limits of quantification (LOQ) were estimated. These parameters are critical when analyzing samples with low concentrations of target DNA and when inferring species abundance from eDNA data. To determine the LOD and LOQ values, a dilution series of DNA extracted from a tissue sample of *H. anomala* was prepared. A 1:5 serial dilution was conducted to obtain eight dilution points ranging from 1000 to 0.0124  $\text{pg } \mu\text{L}^{-1}$  as in Vautier et al. (2023). This series was then analyzed by ddPCR in 5 replicates, using 5  $\mu\text{L}$  of DNA template per ddPCR reaction. Five negative controls (reaction mix without DNA) were also included. The LOD was defined as the lowest concentration at which target DNA was detected in at least one replicate, and the LOQ was defined as the lowest concentration with a coefficient of variation value lower than 35% as proposed by Klymus et al. (2019).

### Environmental deoxyribonucleic acid sample analysis

Using the Bio-Rad QX600 ddPCR system, environmental DNA samples and DNA extraction controls were analyzed by duplex ddPCR, targeting both *H. anomala* and *P. fluviatilis*. Each ddPCR mix had a final volume of 22  $\mu\text{L}$  per sample and contained 1x Bio-Rad ddPCR Supermix for probes (no dUTP), 900 nM of each primer, 250 nM of each probe, 3  $\mu\text{L}$  of template DNA, 5 U of AflIII restriction enzyme, completed with DEPC-treated  $\text{H}_2\text{O}$ . For each sample, an emulsion was carried out with 20  $\mu\text{L}$  of ddPCR mix and 70  $\mu\text{L}$  of probe droplet generation oil using the QX600™ Droplet Generator. After droplet generation, 40  $\mu\text{L}$  of the product was carefully transferred into a 96-well ddPCR plate that was sealed with a pierceable foil using the PX1 PCR Plate Sealer and placed in a T100 Thermal Cycler. The PCR reaction was carried out with the following program: 10 min at 95°C, followed by 40 denaturation cycles of 30 s at 94°C and elongation of 60 s at 60°C, with a ramp rate of 2°C s<sup>-1</sup>, followed by 10 min at 98°C and a temperature hold at 4°C. Following amplification, the plate was transferred to a QX600 Droplet Digital PCR System droplet reader, which checked the fluorescence of all the droplets contained in each sample. QX Manager software was used to quantify copy number  $\mu\text{L}^{-1}$  of *H. anomala* and *P. fluviatilis*. The threshold separating positive and negative droplets was determined based on a positive control sample and according to the instructions in the QX Manager manual. The defined threshold value was the same for all samples. All droplets above the threshold were counted as positive events and those below as negative events. The number of positive and negative droplets (with an average number of droplets generated of 16,317  $\pm$  2471) was finally used by the QX Manager software to quantify the concentration (copy  $\mu\text{L}^{-1}$ ) of the target DNA sequences (Vautier et al. 2024).

### Statistical analysis

To measure the correlation between eDNA results (number of copies L<sup>-1</sup> of *H. anomala*'s target sequence) obtained by qPCR and ddPCR, we calculated Spearman's rank correlation coefficient using the R software (version 4.4.1).

## Assessment

### Quantitative polymerase chain reaction

#### Quantitative polymerase chain reaction primer specificity

To detect and quantify *H. anomala* DNA, we conducted qPCR tests using two different primer sets: the TaqMan-based primer-probe set designed during this study and the SYBR Green-based primer set. qPCR tests using the TaqMan primer-probe set showed specific amplification of *H. anomala*'s DNA and no amplification of the negative controls, namely DNA extracted from *Gammarus* and *Pacifastacus* tissues. However, despite its specificity for our target species, the primer-probe set did not allow reliable quantification of *H. anomala*'s DNA concentration due to baseline deviations

observed in the qPCR amplification curves. Therefore, we decided to optimize the SYBR Green-based qPCR assays and use this primer set to accurately quantify *H. anomala*'s DNA.

For the Ha Cox F and Ha Cox R primers used to amplify *H. anomala* DNA with the SYBR Green method, the results obtained after cloning and sequencing the PCR products from the two pools of environmental samples revealed a specificity of 91.9% for pool P1 and 72.7% for P2.

### Environmental sample analysis

A total of 51 environmental water samples were run in qPCR, of which 84.31% (43 samples) showed positive amplification. Estimation of DNA concentration by qPCR revealed significant variability and a marked seasonal dynamic of *H. anomala* (Fig. 1). Concentrations peaked at the beginning of each year (e.g., winter) and decreased significantly from the beginning of spring. Inter-annual variation was also observed. 2021 was the year with the highest DNA concentrations during the whole winter period and then the most heterogeneous. In contrast, 2022 showed only one concentration peak at the beginning of winter (January), followed by relatively moderate and slightly fluctuating concentrations during the rest of the year. In contrast to previous years, *H. anomala* DNA concentrations remained low in January and February 2023, with a peak occurring later, in March.

### Droplet digital polymerase chain reaction

#### Primer specificity and sensibility

Following the ddPCR analysis of the series of eight successive dilutions of the *H. anomala*'s target sequence, the LOD obtained (i.e., the lowest concentration at which the target DNA was detected in at least one replicate) was 0.07 copies  $\mu\text{L}^{-1}$  and the LOQ (i.e., the lowest concentration with a

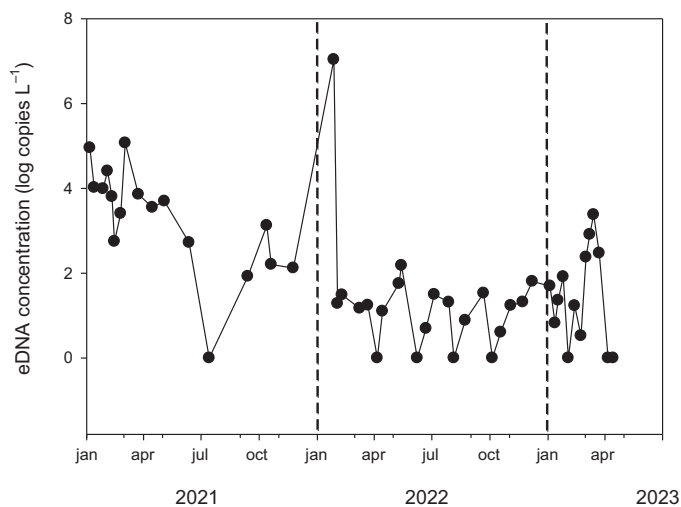
lower coefficient of variation value at 35%) was 0.54 copies  $\mu\text{L}^{-1}$ . Based on the work of Vautier et al. (2023), the LOD for *P. fluviatilis* DNA quantification assays was 0.11 copies  $\mu\text{L}^{-1}$  and the LOQ was 0.58 copies  $\mu\text{L}^{-1}$ . As expected, no amplification was observed for the negative (tissue) controls.

### Temporal dynamics of *H. anomala* and *P. fluviatilis* environmental deoxyribonucleic acid

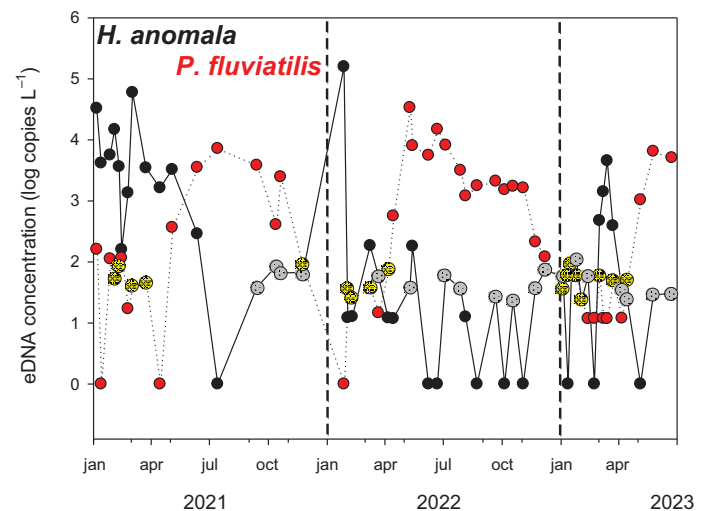
Fifty-one environmental samples were analyzed by ddPCR, of which 43 samples (84.31%) were positive for *H. anomala* compared to 48 samples for perch (94.12%). No sample had an eDNA concentration value below the LOD for *H. anomala*, while for perch 7 samples had values below the LOD. ddPCR analysis showed contrasting patterns in the eDNA concentration dynamics of *H. anomala* and *P. fluviatilis* during some periods of the year (Fig. 2). During the spring period, we observed a significant decrease in the signal of *H. anomala*'s signal, in parallel with an increase in the perch signal. Similar to qPCR, we observed a peak in *H. anomala* signal during winter, the latter being more pronounced in qPCR than in ddPCR.

### Quantitative polymerase chain reaction vs. droplet digital polymerase chain reaction

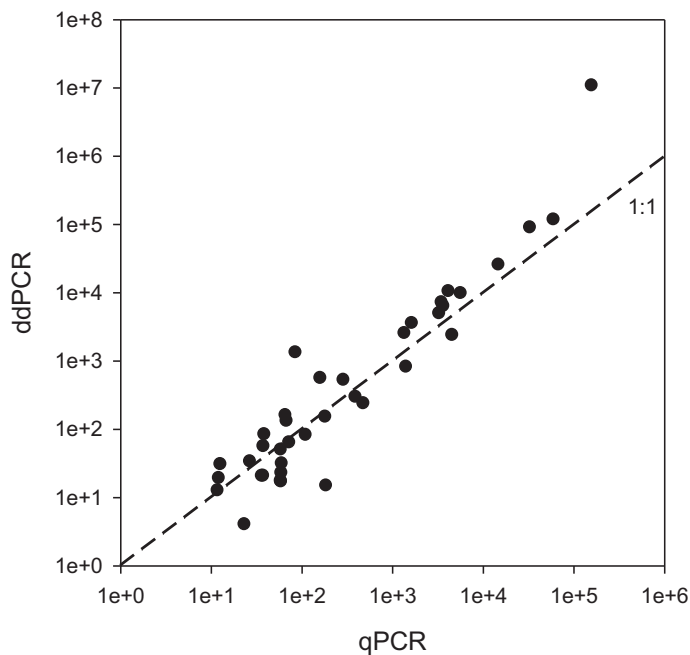
A strong correlation was found between the qPCR and ddPCR results ( $r = 0.92$ ,  $n = 51$ ,  $p < 0.01$ , Fig. 3). It is important to note that this comparison was based on the quantification of *H. anomala* DNA only. Indeed, the qPCR quantification assays for *P. fluviatilis* were unreliable, making it impossible to establish the necessary standard curve for the quantification of perch DNA by qPCR. For *H. anomala*, the qPCR assay using the SYBR Green-based primer set showed



**Fig. 1.** Temporal dynamics of eDNA concentration of *H. anomala* quantified by qPCR. Dashes lines symbolize the separation between each year investigated.



**Fig. 2.** Temporal dynamics of eDNA concentrations of *H. anomala* and *P. fluviatilis* quantified by ddPCR. Gray and yellow colored symbols correspond to values between LOD and LOQ. Dashes lines symbolize the separation between each year studied.



**Fig. 3.** Relationship between eDNA concentrations of *H. anomala* quantified by qPCR and ddPCR. The dashed line represents the 1 : 1 relationship.

a higher detection rate than the ddPCR assay using the dedicated primers/probe set, for 27 of the 51 samples. However, at low DNA concentrations ( $< 400$  copies  $L^{-1}$ ), a higher *H. anomala* signal was detected using the ddPCR primers/probe set. Furthermore, of the eight samples showing no amplification by qPCR, five samples showed a reliable *H. anomala* signal obtained by ddPCR.

## Discussion

### Methodological aspects

First, we discuss the limitations of this study. The results are based on data collected from a single site, at a single depth, and from a relatively small volume of water sampled. The sampling strategy could be improved by selecting a variety of sites, different depths or habitats, and by testing an optimal sampling volume. Although these choices were sufficient to test both PCR methods and to confirm the typical annual dynamics observed by diving (not shown), we are aware that such a sampling design can be considered a significant limitation. Indeed, the distribution of eDNA is likely to be influenced by various environmental factors and is rarely uniformly distributed in water, so such an approach may not fully capture the organism abundance and dynamics.

To obtain reliable results, qPCR required much more optimization than ddPCR, with key parameters like primer concentration, annealing time, and temperature. For ddPCR, the standardized protocol used with the Taq Man system did not require optimization for these three parameters. However, it is

worth noting that droplet generation, which is a crucial step of the ddPCR technique, requires attention and personal skills when performed manually. Indeed, the greater the number of droplets generated, the more reliable the quantification results.

Unlike qPCR, DNA quantification by ddPCR does not require the development of a standard curve, which, in our case required considerable optimization. The calculation of the number of target DNA copies in ddPCR is achieved by counting the number of positive droplets in relation to the total number of droplets. Therefore, there is no need to refer to a standard curve to determine the target DNA concentration. This can save time and resources in the experimental setup.

Another major advantage of using ddPCR is its greater tolerance to PCR inhibitors in DNA extracts, which are particularly problematic in environmental samples (Doi et al. 2015). The choice to use a large amount of DNA offers the advantage of increasing the likelihood of detecting the rare target DNA. However, the presence of DNA from organisms other than the target species is also likely to inhibit the amplification reaction (Harper et al. 2018). However, ddPCR offers several strategies to overcome the inhibition problem, such as the use of a restriction enzyme that allows access to the sequence in the presence of a significant amount of non-target DNA. Partitioning the reaction mixture into 15,000 to 20,000 nanodroplets also allows the inhibitory molecules to be partitioned into the generated droplets. PCR performed separately in each droplet reduces the effect of inhibitors and results in better sensitivity to low DNA concentrations (Goldberg et al. 2016; Cai et al. 2017). The ddPCR reaction is also an end-point reaction, unlike qPCR, so lower amplification efficiency caused by inhibitors will not affect the final results.

In terms of quantification, a strong and positive correlation was observed between the eDNA concentrations measured by both methods. However, we observed differences in DNA concentrations between the SYBR Green-based qPCR assay and the TaqMan-based ddPCR assay, which could be attributed to several factors. The specificity tests of SYBR Green primers from the sequencing of PCR products showed that the lower the amount of target DNA in environmental samples, the less specific the amplification. This may explain the higher levels of DNA detected by qPCR than by ddPCR observed in 53% of the samples analyzed, which may be due to non-specific amplifications. A higher concentration of DNA detected by qPCR does not necessarily indicate a higher sensitivity of the qPCR but rather a lower specificity. The SYBR Green system relies solely on the primers for amplification specificity, while TaqMan chemistry uses both primers and probes, resulting in a more specific assay. Conversely, the higher DNA concentrations estimated by ddPCR, observed in 41% of the analyzed samples may be attributed to its greater sensitivity. This is partly due to the sample partitioning in ddPCR, as it is based on precise counting of target molecules, allowing more

accurate detection of rare target DNA even in the presence of excess background.

In addition, SYBR Green binds to any double-stranded DNA, whereas TaqMan probes are specific to the target sequence. This difference in chemistry can affect the assay performance. Using different primer sets for qPCR and ddPCR can also lead to variations in target specificity and amplification efficiency. Primers are designed to anneal to specific regions of the target DNA sequence. If the primer sets used in qPCR and ddPCR are different, they may target slightly different regions or have variations in their binding sites. This can result in differences in target efficiency, affecting which DNA sequences are amplified and detected by each method.

The need to consider the unique requirements of qPCR and ddPCR primer design, as they may not be equally suited to the specific conditions of each method. While using SYBR Green for qPCR and TaqMan for ddPCR can provide a basis for comparison, the differences in chemistry and detection methods can introduce variability in results.

In this study, we found that when analyzing environmental samples containing a low quantity of the target DNA, the results obtained by qPCR using the SYBR Green chemistry tended to either overestimate *H. anomala*'s DNA concentration due to low specificity, or fail to detect it due to reduced sensitivity and the presence of inhibitory effects.

For an accurate detection and quantification of *H. anomala*'s DNA concentration, we would therefore recommend a TaqMan approach to limit the risk of non-specific amplification, and ddPCR for its ease of optimization and greater sensitivity and precision, which is particularly advantageous for detecting rare targets or low abundance nucleic acids.

### Environmental deoxyribonucleic acid approach for *H. anomala* detecting and monitoring

The study reveals that eDNA is an effective method for identifying organisms in aquatic ecosystems (Brys et al. 2021; Cardas et al. 2020; Doi et al. 2015). In addition to eDNA, diving and underwater cameras can also be used to detect *H. anomala*. The choice of method depends on the species' biology, study objectives, and available resources. Combining these methods is recommended for robust results, as each technique can provide insights into the animal's ecophysiology. The study emphasizes the importance of considering these factors when analyzing aquatic ecosystems.

eDNA analysis offers significant advantages in detecting rare species or AIS at the early stages of invasion, when population abundance is typically too low for visual detection. This method is particularly valuable when organisms are concealed in inaccessible habitats. The ability to detect species at low abundances is crucial, enabling managers to implement rapid response strategies to prevent, control, and mitigate AIS spread (Dejean et al. 2012; Takahara et al. 2013). While our eDNA approach demonstrated high species detection rates, it

had limitations in providing demographic information. For instance, adult to juvenile ratios and reproductive periods of *H. anomala* were better determined through diving observations. However, recent studies demonstrated the potential of using eDNA to assess demographic parameters in fish species, suggesting that similar approaches could be developed for *H. anomala* in the future (Maruyama et al. 2019).

The eDNA approach may also have some methodological limitations, and the target DNA may not be detected even when it is present. For example, false negatives can occur if inhibitors are present in the eDNA used as a PCR template (Jane et al. 2015) or if the PCR primers are not sensitive enough (Wilcox et al. 2013). Ecological and environmental factors such as seasonality, UV, pH, temperature, and currents are also likely to affect the concentration, dispersion, and degradation of DNA within aquatic ecosystems (Strickler et al. 2015). These factors must therefore be considered when planning sampling protocols. The concentration of target DNA may also depend on biological factors associated with the species itself. In contrast to fish or amphibians, crustaceans may be more difficult to detect due to their hard chitin exoskeletons, which prevent the release of DNA into the environment (Tréguier et al. 2014). It is therefore crucial to develop protocols to increase the sensitivity of the test.

Our study highlights the reliability of eDNA approach in detecting temporal dynamics in *H. anomala* populations, particularly during low-abundance periods. Similar conclusions were drawn by Cangelosi et al. (2024), who emphasized the method's potential to outperform conventional microscopic techniques in terms of sensitivity and cost-effectiveness. However, as they also noted, confirmatory traditional methods remain essential to address limitations such as the inability to distinguish between live and dead organisms. Interestingly, Sheehan et al. (in press, *Management of Biological Invasions*) provide complementary evidence, showing that eDNA signals for *H. anomala* decline rapidly in water, suggesting that positive eDNA detections are strong indicators of living organisms.

Finally, another promising approach that might be useful for such a survey is eRNA-based analysis, as such a method would be free of DNA signals left by organisms that are no longer at the site (either because they have moved elsewhere or deeper in the water column, or because they are dead). This method would therefore help to ensure that the signal refers to live and active individuals present in the immediate vicinity (Miyata et al. 2021).

### Population dynamics

Our eDNA monitoring revealed distinct seasonal variations in *H. anomala* signals, consistent with findings from visual detection and individual capture studies. However, the observed dynamics in our study differ from those reported in other ecosystems, highlighting the complexity of *H. anomala*'s population patterns. We detected a strong *H. anomala* signal during winter (January–March), followed by a significant



decline from late March–early April to low or zero concentrations until December. This pattern contrasts with observations in other ecosystems, suggesting that *H. anomala*'s population dynamics are highly context-dependent. (Table 2). It should be noted that the observations cannot be generalized to the ecosystem level, as only one site was studied. Indeed, we do not know whether the swarm dynamics might be different elsewhere in the lake or whether some of them might also migrate to the deeper zone for example. It is likely that the dynamics of *H. anomala* depend on a variety of factors, not only on the intrinsic properties of the species, but also on the context of the invasion, as well as the biotic and abiotic characteristics of the ecosystem in question, not to mention the sampling strategy used to detect and track this small crustacean. We are also aware that when an exotic species is introduced, the environmental conditions in the new ecosystem are likely to be different from those in the original habitat. An AIS must therefore be able to cope with new conditions, by adapting various aspects of its biology and ecology such as feeding and reproductive behavior and tolerance to a wide range of salinities and temperatures. Strong phenotypic plasticity may therefore explain the different dynamics that *H. anomala* exhibits in the different occupied ecosystems.

Considering the results obtained from the simultaneous monitoring of *H. anomala* and *P. fluviatilis*, two main factors could explain the decline of the blood-red shrimp, such as the increase in water temperature and the pressure exerted by the perch predation. Indeed, peaks in the *H. anomala* abundance were observed in winter (January–March), a period during which a total absence of perch in surface waters was reported by scuba-divers and confirmed by the eDNA approach. In addition, a shift in the concentrations of the two species was systematically observed each year from the month of April, a period during which the water temperature increases and the

perch return to the coastal zone (Jacquet 2023; Rogissart et al. 2023).

On the one hand, feeding, reproduction, and survival rates of *H. anomala* have been shown to be affected by water temperature (Sun et al. 2013). Jacquet (2023) also mentioned the effect of temperature as a key factor that could hinder the swarming of *H. anomala* in perialpine lakes. On the other hand, the predation of *H. anomala* by perch, demonstrated by both camera recordings and analysis of fish stomach contents, with more than 80 individuals found in just one perch (Rogissart et al. 2023; Rogissart et al. 2022), suggests that predation could exert a strong pressure that could significantly and relatively rapidly reduce the abundance of the blood-red shrimp. The shift observed here in the eDNA signal of the two species provides another element to confirm this interaction between *H. anomala* and *P. fluviatilis*. This is also consistent with the results of other authors, who have repeatedly observed the presence of perch near swarms of *H. anomala* as well as acts of predation (Ketelaars et al. 1999; Borcharding et al. 2006; Dumont and Muller 2010; Gallagher et al. 2015).

Our results do not exclude other possible explanations for the decline of *H. anomala* populations. Typically, interspecific competition could occur with the quagga mussel (*Dreissena rostriformis bugensis*), whose invasion in Lake Geneva dates back to 2015. This mussel is indeed a highly competitive AIS that has spread throughout the entire lake and established an important population. Its high filtration rate could therefore contribute to significantly reducing the plankton resources available to *H. anomala*, thereby contributing to the decline of its population (Beisel 2021; Jacquet 2023). The increase in water transparency since the mussel was installed in the lake (not shown) could be an indication of the extent of plankton consumption by this mollusk. At last, the natural mortality of individuals at the end of their lives could also contribute to the population decline.

**Table 2.** Temporal dynamics of *H. anomala*: present study and other localities.

Locality	Monitoring method	Temporal dynamics of <i>H. Anomala</i>	Reference
Saint-Disdille Lake Geneva (French side)	eDNA	<ul style="list-style-type: none"> <li>• High concentrations: January to March</li> <li>• Winter peak: January or March</li> <li>• Low concentrations/absence: April–December</li> </ul>	Present study
Boating marina connected to River Trent (UK)	Capturing	<ul style="list-style-type: none"> <li>• Increase in abundance from mid-May</li> <li>• Summer peak: End of June beginning of July</li> <li>• Total absence of capture: From July to October</li> </ul>	Nunn and Cowx (2012)
Regatta lake (UK)	Visual detection	<ul style="list-style-type: none"> <li>• Peak abundance: September</li> <li>• Gradual decrease in abundance until January</li> <li>• Absence of detection in February and March</li> <li>• Appearance of juveniles in April</li> </ul>	Stubbington et al. (2008)
Gravel-pit lake connected to the Lower Rhine (Germany)	Capturing	<ul style="list-style-type: none"> <li>• Increase in abundance from September</li> <li>• Winter peak in December</li> <li>• Decline in April</li> </ul>	Borcharding et al. (2006)

### Comments and recommendations

For an efficient detection and monitoring of the dynamics of the AIS *H. anomala* in lakes, it is proposed to use an eDNA approach coupled with ddPCR using the Taq Man-based primer set developed in this study. Although our sampling strategy could be improved by typically increasing the number of sites to be surveyed and possibly the volume of water to be sampled, the primers designed and the method appear to be sufficiently robust to be applied in other ecosystems.

### Author Contributions

Stéphan Jacquet conceived the research theme, coordinated the project, collected the samples, and participated in the writing and revision of the article; Sirine Melliti, Marine Vautier, and Cécile Chardon designed the methodology and made the analyses; Sirine Melliti and Marine Vautier led the analyses and participated to the writing and revision of the article. All authors contributed critically to the drafts and gave final approval before submitting the initial and revised form of the paper.

### Acknowledgments

We are grateful to Hervé Rogissart and Victor Frossard for their critical reading of the manuscript. Anonymous reviewers are also acknowledged for helping us to improve the initial submitted version of this article. This research was funded by the pole ECLA.

### Conflicts of Interest

None declared.

### References

- Audzijonyte, A., K. Wittmann, and R. Vainola. 2008. "Tracing Recent Invasions of the Ponto-Caspian Mysid Shrimp *Hemimysis anomala* Across Europe and to North America With Mitochondrial DNA." *Diversity and Distributions* 14, no. 2: 179–186. <https://doi.org/10.1111/j.1472-4642.2007.00434.x>.
- Banerjee, P., K. Stewart, G. Dey, et al. 2022. "Environmental DNA Analysis as an Emerging Nondestructive Method for Plant Biodiversity Monitoring: A Review." *AoB Plants* 14, no. 4: 1–14. <https://doi.org/10.1093/aobpla/plac031>.
- Barbet-Massin, M., Q. Rome, F. Muller, A. Perrard, C. Villemant, and F. Jiguet. 2013. "Climate Change Increases the Risk of Invasion by the Yellow-Legged Hornet." *Biological Conservation* 157: 4–10. <https://doi.org/10.1016/j.biocon.2012.09.015>.
- Beisel, J.-N. 2021. Biologie, écologie et impacts potentiels de *Dreissena rostriformis bugensis*, la moule quagga, espèce invasive au sein du Léman, 114. Rapport pour la CIPEL.
- Borcherding, J., B. Hermasch, and P. Murawski. 2007. "Field Observation and Laboratory Experiments on Growth and Lipid Content of Young-of-the-Year Perch." *Ecology of Freshwater Fish* 16, no. 2: 198–209. <https://doi.org/10.1111/j.1600-0633.2006.00212.x>.
- Borcherding, J., S. Murawski, and H. Arndt. 2006. "Population Ecology, Vertical Migration and Feeding of the Ponto-Caspian Invader *Hemimysis anomala* in a Gravel-Pit Lake Connected to the River Rhine." *Freshwater Biology* 51, no. 12: 2376–2387. <https://doi.org/10.1111/j.1365-2427.2006.01666.x>.
- Brown, M., B. Boscarino, S. Oyagi, M. Tibbetts, and C. Sanchez. 2017. Novel Methods for Early Detection of the Bloody red Shrimp, *Hemimysis anomala*. Great Lakes Research Consortium.
- Brown, M., B. Boscarino, J. Roellke, E. Stapyhton, and A. Driller-Colangelo. 2014. "Fifteen Miles on the Erie Canal: The Spread of *Hemimysis anomala* G.O. Sars, 1907 (Bloody Red Shrimp) in the New York State Canal System." *Bioinvasions Records* 3, no. 4: 261–267. <https://doi.org/10.3391/bir.2014.3.4.07>.
- Brys, R., D. Halfmaerten, S. Neyrinck, et al. 2021. "Reliable eDNA Detection and Quantification of the European Weather Loach (*Misgurnus fossilis*)." *Journal of Fish Biology* 98, no. 399: 414.
- Cai, Y., Y. He, R. Lv, et al. 2017. "Detection and Quantification of Beef and Pork Materials in Meat Products by Duplex Droplet Digital PCR." *PLoS One* 12, no. 8: e0181949. <https://doi.org/10.1371/journal.pone.0181949>.
- Cangelosi, A., M. Balcer, K. Prihoda, et al. 2024. "Evaluation of eDNA qPCR Monitoring as an Early Detection Tool for a Non-Native Mysid in Great Lakes Waters." *Journal of Great Lakes Research* 50, no. 102: 377. <https://doi.org/10.1016/j.jglr.2024.102377>.
- Cardas, B. J., D. Deconinck, I. Márquez, et al. 2020. "New eDNA Based Tool Applied to the Specific Detection and Monitoring of the Endangered European Eel." *Biological Conservation* 250: e108759. <https://doi.org/10.1016/j.biocon.2020.108750>.
- Chandra, S., and A. Gerhardt. 2008. "Recent Establishment of the Invasive Ponto-Caspian Mysid (*Hemimysis anomala* G.O. Sars, 1907) in the Hungarian Part of the Danube River." *Aquatic Invasions* 3, no. 1: 99–101. <https://doi.org/10.3391/ai.2008.3.1.17>.
- Craig, J. 1977. "Seasonal Changes in the Day and Night Activity of Adult Perch, *Perca fluviatilis*." *Journal of Fish Biology* 11, no. 2: 161–166. <https://doi.org/10.1111/j.1095-8649.1977.tb04109.x>.
- Daneliya, M. E., V. V. Petryashev, and R. Vainola. 2012. "Continental Mysid Crustaceans of Northern Eurasia." In Conference: Modern Problems of the Continental Crustacean Research.
- Dejean, T., A. Valentini, C. Miquel, P. Taberlet, E. Bellemain, and C. Miaud. 2012. "Improved Detection of an Alien Invasive Species Through Environmental DNA Barcoding: The Example of the American Bullfrog *Lithobates*

- catsebianus.” *Journal of Applied Ecology* 49, no. 4: 953–959. <https://doi.org/10.1111/j.1365-2664.2012.02171.x>.
- Doi, H., K. Uchii, T. Takahara, S. Matsushashi, H. Yamanaka, and T. Minamoto. 2015. “Use of Droplet Digital PCR for Estimation of Fish Abundance and Biomass in Environmental DNA Surveys.” *PLoS One* 10, no. 3: e0122763. <https://doi.org/10.1371/journal.pone.0122763>.
- Dumont, S., and C. Muller. 2010. “Distribution, Ecology and Impact of a Small Invasive Shellfish, *Hemimysis Anomala* in Alsatian Water.” *Biological Invasions* 12: 495–500. <https://doi.org/10.1007/s10530-009-9453-0>.
- Ellis, B., J. Stanforda, D. Goodmanc, et al. 2011. “Long-Term Effects of a Trophic Cascade in a Large Lake Ecosystem.” *Proceedings of the National Academy of Sciences* 108, no. 3: 1070–1075. <https://doi.org/10.1073/pnas.1013006108>.
- Ficetola, G. F., C. Miaud, F. Pompanon, and P. Taberlet. 2008. “Species Detection Using Environmental DNA From Water Samples.” *Biology Letters* 4: 423–425. <https://doi.org/10.1098/rsbl.2008.0118>.
- Frossard, V., and D. Fontvieille. 2018. “What is the Invasiveness of *Hemimysis anomala* (Crustacea, Mysidae) in the Large Deep Lake Bourget, France?” *Hydrobiologia* 814: 219–232. <https://doi.org/10.1007/s10750-018-3538-7>.
- Frossard, V., C. Vagnon, and S. Jacquet. 2023. “Seasonal Variations of the Trophic Niche of *Hemimysis anomala* Reveal that the Invasive Bloody-Red Mysid Crustacean is an Ecological Opportunist in Lake Geneva.” *Hydrobiologia* 851: 487–501. <https://doi.org/10.1007/s10750-023-05334-z>.
- Gallagher, K., R. Rosell, L. Vaughan, et al. 2015. “*Hemimysis anomala* G.O. Sars, 1907 Expands Its Invasive Range to Northern Ireland.” *BioInvasions Records* 4: 43–46. <https://doi.org/10.3391/bir.2015.4.1.07>.
- Gillet, C., and J. P. Dubois. 2002. La Reproduction de la Perche et du Gardon dans le Léman, hal-02833502. Genève, Suisse: Journée de la Société Française d’Ichtyologie.
- Goldberg, C. S., C. R. Turner, K. Deiner, et al. 2016. “Critical Considerations for the Application of Environmental DNA Methods to Detect Aquatic Species.” *Methods in Ecology and Evolution* 7: 1299–1307. <https://doi.org/10.1111/2041-210X.12595>.
- Guillard, J. M.-E., M. C. Perga, and N. Angeli. 2006. “Hydroacoustic Assessment of Young-of-Year Perch, *Perca fluviatilis*, Population Dynamics in an Oligotrophic Lake (Lake Annecy, France).” *Fisheries Management and Ecology* 13: 319–327. <https://doi.org/10.1111/j.1365-2400.2006.00508.x>.
- IPBES. 2023. Summary for Policymakers of the Thematic Assessment Report on Invasive Alien Species and Their Control of the Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services, edited by H. E. Roy, A. Pauchard, P. Stoett, T. Renard Truong, S. Bacher, B. S. Galil, P. E. Hulme, T. Ikeda, K. V. Sankaran, M. A. McGeoch, L. A. Meyerson, M. A. Nuñez, A. Ordóñez, S. J. Rahlao, E. Schwindt, H. Seebens, A. W. Sheppard, and V. Vandvik. Bonn, Germany: IPBES Secretariat. <https://doi.org/10.5281/zenodo.7430692>.
- Jacquet, S. 2023. “Are Warm Temperatures the Key Factor to Prevent *Hemimysis anomala* Winter Swarms in Peri-Alpine Lakes?” *Knowledge and Management of Aquatic Ecosystems* 424: 14. <https://doi.org/10.1051/kmae/2023008>.
- Jane, S., T. Wilcox, K. Mckelvey, et al. 2015. “Distance, Flow and PCR Inhibition: eDNA Dynamics in Two Headwater Streams.” *Molecular Ecology Resources* 15, no. 1: 216–227. <https://doi.org/10.1111/1755-0998.12285>.
- Jerde, C. L., A. R. Mahon, W. L. Chadderton, and D. M. Lodge. 2011. “‘Sight-Unseen’ Detection of Rare Aquatic Species Using Environmental DNA.” *Conservation Letters* 4: 150–157. <https://doi.org/10.1111/j.1755-263X.2010.00158.x>.
- Ketelaars, H. A. M., F. E. Lambregts-van De Clundert, C. J. Carpentier, A. J. Wagenvoort, and W. Hoogenboezem. 1999. “Ecological Effects of the Mass Occurrence of the Ponto-Caspian Invader, *Hemimysis anomala* G.O. Sars, 1907 (Crustacea: Mysidacea), in a Freshwater Storage Reservoir in the Netherlands, With Notes on Its Autecology and New Records.” *Hydrobiologia* 394: 233–248. <https://doi.org/10.1023/A:1003619631920>.
- Klymus, K. E., C. M. Merkes, M. J. Allison, et al. 2019. “Reporting the Limits of Detection and Quantification for Environmental DNA Assays.” *Environmental DNA* 2: 271–282. <https://doi.org/10.1002/edn3.29>.
- Marty, J. 2008. “Biological Synopsis of the Bloody Red Shrimp (*Hemimysis anomala*).” *Canadian Manuscript Report of Fisheries and Aquatic Sciences* 2842.
- Marty, J., K. Bowen, M. Koops, and M. Power. 2010. “Distribution and Ecology of *Hemimysis anomala*, the Latest Invader of the Great Lakes Basin.” *Hydrobiologia* 647: 71–80. <https://doi.org/10.1007/s10750-009-9990-7>.
- Marty, J., J. Ives, Y. De Lafontaine, S. P. Despatie, M. Koops, and M. Power. 2012. “Evaluation of Carbon Pathways Supporting the Diet of Invasive *Hemimysis anomala* in a Large River.” *Journal of Great Lakes Research* 38, no. 2: 45–51. <https://doi.org/10.1016/j.jglr.2012.01.003>.
- Maruyama, A., K. Nakamura, H. Yamanaka, M. Kondoh, and T. Minamoto. 2019. “Correction: The Release Rate of Environmental DNA From Juvenile and Adult Fish.” *PLoS One* 14, no. 2: e0212145. <https://doi.org/10.1371/journal.pone.0212145>.
- Miyata, K., Y. Inoue, Y. Amano, et al. 2021. “Fish Environmental RNA Enables Precise Ecological Surveys With High Positive Predictivity.” *Ecological Indicators* 128, no. 107: 796. <https://doi.org/10.1016/j.ecolind.2021.107796>.
- Nunn, A. D., and I. G. Cowx. 2012. “Diel and Seasonal Variations in the Population Dynamics of *Hemimysis anomala*, a Non-Indigenous Mysid: Implications for Surveillance and Management.” *Aquatic Invasions* 7, no. 3: 357–365. <https://doi.org/10.3391/ai.2012.7.3.007>.

- Pysek, P., V. Jarošík, P. E. Hulme, et al. 2010. "Disentangling the Role of Environmental and Human Pressures on Biological Invasions Across Europe." *Proceedings of the National Academy of Sciences. USA* 107, no. 27: 12157–12162. <https://doi.org/10.1073/pnas.1002314107>.
- Rahel, F. J., and J. D. Olden. 2008. "Assessing the Effects of Climate Change on Aquatic Invasive Species." *Conservation Biology* 22, no. 3: 521–533. <https://doi.org/10.1111/j.1523-1739.2008.00950.x>.
- Ricciardi, A., S. Avlijas, and J. Marty. 2012. "Forecasting the Ecological Impacts of the *Hemimysis anomala* Invasion in North America: Lessons From Other Freshwater Mysid Introductions." *Journal of Great Lakes Research* 38: 7–13. <https://doi.org/10.1016/j.jglr.2011.06.007>.
- Rogissart, H., V. Frossard, C. Goulon, et al. 2022. "A Multi-Faceted Approach to Determine the Importance of *Hemimysis anomala* in the Diet of Eurasian Perch (*Perca fluviatilis*) in Lake Geneva [Poster Presentation]." In 5<sup>th</sup> International Days of Limnology and Oceanography (JILO). Corte, France: INRAE CARRTEL & ECLA.
- Rogissart, H., V. Frossard, J. C. Guillard, C. Rautureau, and S. Jacquet. 2023. "Tracking the Real-Time Behaviour of *Hemimysis anomala*'s Winter Swarms Using Acoustic Camera." *Journal of Great Lakes Research* 2023: 102250. <https://doi.org/10.1016/j.jglr.2023.102250>.
- Sardain, A., E. Sardain, and B. Leung. 2019. "Global Forecasts of Shipping Traffic and Biological Invasions to 2050." *Nature Sustainability* 2: 274–282. <https://doi.org/10.1038/s41893-019-0245-y>.
- Sinclair, J. S., S. E. Arnott, and A. Cox. 2016. "The Quick and the Dead: Copepods Dominate as Cladocerans Decline Following Invasion by *Hemimysis anomala*." *Canadian Journal of Fisheries and Aquatic Sciences* 73: 793–803. <https://doi.org/10.1139/cjfas-2015-0111>.
- Sofaer, H. R., C. S. Jarnevich, and I. S. Pearse. 2018. "The Relationship between Invader Abundance and Impact." *Ecosphere* 9, no. 9: e02415. <https://doi.org/10.1002/ecs2.2415>.
- Strickler, K., A. Fremier, and C. Goldberg. 2015. "Quantifying Effects of UV-B, Temperature, and pH on eDNA Degradation in Aquatic Microcosms." *Biological Conservation* 183: 85–92. <https://doi.org/10.1016/j.biocon.2014.11.038>.
- Stubbington, R., C. Terrell-Nield, and P. Harding. 2008. "The First Occurrence of the Ponto-Caspian Invader, *Hemimysis Anomala* G. O. Sars, 1907 (Mysidacea) in the UK." *Crustaceana* 81: 43–55. <https://doi.org/10.1163/156854008783244816>.
- Takahara, T., T. Minamoto, and H. Doi. 2013. "Using Environmental DNA to Estimate the Distribution of an Invasive Fish Species in Ponds." *PLoS One* 8, no. 2: e56584. <https://doi.org/10.1371/journal.pone.0056584>.
- Takahara, T., T. Minamoto, H. Yamanaka, H. Doi, and Z. Kawabata. 2012. "Estimation of Fish Biomass Using Environmental DNA." *PLoS One* 7, no. 4: e35868. <https://doi.org/10.1371/journal.pone.0035868>.
- Takahashi, M., M. Saccò, J. H. Kestel, et al. 2023. "Aquatic Environmental DNA: A Review of the Macro-Organismal Biomonitoring Revolution." *Science of The Total Environment* 873: 162322. <https://doi.org/10.1016/j.scitotenv.2023.162322>.
- Tréguier, A., J.-M. Paillisson, T. Dejean, A. Valentini, M. A. Schlaepfer, and J.-M. Roussel. 2014. "Environmental DNA Surveillance for Invertebrate Species: Advantages and Technical Limitations to Detect Invasive Crayfish *Procambarus clarkii* in Freshwater Ponds." *Journal of Applied Ecology* 51, no. 4: 871–879. <https://doi.org/10.1111/1365-2664.12262>.
- Vautier, M., C. Chardon, and I. Domaizon. 2021. Fish eDNA: Water Sampling and Filtration Through Sterivex Filter Unit v1. ZappyLab.
- Vautier, M., C. Chardon, C. Galiegue, and I. Domaizon. 2024. ddPCR for Probes to Target eDNA Samples: From Sample Preparation to Droplet Reading v1. ZappyLab. <https://doi.org/10.17504/protocols.io.n92ldm47915b/v1>.
- Vautier, M., C. Chardon, C. Goulon, J. Guillard, and I. Domaizon. 2023. "A Quantitative eDNA-Based Approach to Monitor Fish Spawning in Lakes: Application to European Perch and Whitefish." *Fisheries Research* 264, no. 106: 708. <https://doi.org/10.1016/j.fishres.2023.106708>.
- Wilcox, T. M., K. S. McKelvey, M. K. Yong, et al. 2013. "Robust Detection of Rare Species Using Environmental DNA: The Importance of Primer Specificity." *PLoS One* 8, no. 3: e59520. <https://doi.org/10.1371/journal.pone.0059520>.
- Wittmann, K. 2007. "Continued Massive Invasion of Mysididae in the Rhine and Danube Systems, With First Records of the Order *Mysidacea* (Crustacea: Malacostraca: Peracarida) for Switzerland." *Revue Suisse de Zoologie* 114, no. 1: 65–86. <https://doi.org/10.5962/bhl.part.80389>.

Submitted 30 May 2024

Revised 12 December 2024

Accepted 16 December 2024