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Are long-term biomonitoring efforts overlooking crayfish in European rivers?

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Abstract

Background Long-term biomonitoring of macroinvertebrates is a popular and valuable approach for assessing the status of freshwater ecosystems, identifying the impact of stressors, and evaluating ecosystem health. Although macroinvertebrate-based biomonitoring can be effective in detecting changes in distribution patterns and community trends over time, crayfish often remain undetected or unreported by biomonitoring efforts despite their importance in maintaining the functioning of aquatic ecosystems.

Results By analyzing a comprehensive database of long-term macroinvertebrate time series, we found that most sampling methods and assessment schemes can detect both native and non-native crayfish in running waters if sites are continuously sampled. However, native crayfish were detected to a lesser extent and by fewer methods. Kick-net sampling and assessment techniques prevailed as the most efficient methods for capturing crayfish. However, the substantial number of time series lacking crayfish data calls into question whether these methods are sufficiently comprehensive to encapsulate crayfish populations accurately. The use of other targeted methods such as baited traps or hand catching may provide a more reliable estimate of their presence.

Conclusions Given the detrimental impacts of non-native crayfish and the decline in native crayfish populations, we strongly recommend that stakeholders and managers incorporate a combination of these approaches into their monitoring efforts. The use of different taxonomic levels (family vs. genus vs. species level) in estimating biological indices and biomonitoring tools can cause delays in identifying new non-native specie's occurrences, hindering effective water quality assessment and ecosystem management by governments and stakeholders. Therefore, whenever possible, we call for standardized taxonomic levels for biomonitoring studies and management strategies to accurately address these issues and make recommendations going forward.

Keywords Detection bias, Long-term data, Monitoring, Non-native species, Sampling, Water framework directive

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Introduction

Despite covering less than 1% of Earth's surface, freshwater ecosystems host remarkable biodiversity [96]. Human settlements have historically thrived near freshwater ecosystems due to the supply of fresh drinking water, crop irrigation, and various profitable economic activities as well as a source of food through fishing [16, 63, 103]. The multiple water uses interrelated with human activities have altered the structure and functioning of freshwater systems, involving losses in the availability, quality, and health of aquatic ecosystems [96]. Understanding, mitigating, and reverting these impacts requires a continuous monitoring of running waters and other freshwater systems that systematically assess the state of river ecosystems [89]. The introduction of non-native species has been recognized as one of the main drivers of biodiversity decline and species extinctions [5, 85]. Yet, aquatic invasions often proceed unnoticed, facilitating unhinged secondary spread [65] as well as multifaceted impacts on biodiversity [36, 40].

Crayfish are large and highly mobile freshwater crustaceans that play a vital role in freshwater systems [86]. They contribute to nutrient cycling via their omnivorous diet [6, 75] and serve as a food source for various predators [24], while contributing to the ecological balance of freshwater ecosystems [87]. Humans have had a long-lasting and intense relationship with crayfish, which often became important food items [81, 97]. People's interests in crayfish, as well as crayfish ability to be easily transported alive and establish self-sustaining populations have favored crayfish introductions since ancient times (e.g., [19]). The human-mediated spread of non-native crayfish has played an important role in their range expansion [36, 37, 76], with most water bodies in continental Europe having been invaded by non-native crayfish species [59, 106]. Meanwhile, the eradication of non-native crayfish is usually impossible [66, 94] and measures preventing their introduction and eliminating upstream spread are the main methods for their effective management [62, 68]. Today, non-native crayfish are known as a particularly pervasive and costly group of aquatic invaders [58, 69]. They are also considered among the most successful invaders globally, having led to the large-scale decline of biodiversity due to competition, predation, and habitat alteration, among others [36, 99, 105]. Monitoring crayfish populations, particularly non-native species, is therefore crucial, necessitating vigilant observation and management strategies to mitigate their significant detrimental effects on European freshwater ecosystems.

In this context, the European Union's Water Framework Directive [22] leveraged and expanded pre-existing national biomonitoring programs, enhancing Europe's approach to freshwater biodiversity conservation. Indeed, the WFD becomes crucial as a profound and fundamental basis for biodiversity assessments and subsequent conservation efforts. This network, which also includes the monitoring of phytoplankton, macrophytes and phytobenthos, and fish, was not specifically designed for studying non-native species or biodiversity in general, raising concerns whether biomonitoring under the umbrella of the WFD or prior biomonitoring efforts could effectively be utilized to monitor the distribution, abundance, and trends of crayfish species across European freshwater ecosystems [74]. Indeed, whereas local assessments of crayfish populations are commonly achieved with extensive one-time trapping efforts (of a varied array, either baited or not) [64], continuous long-term efforts are required to gather sufficiently long biodiversity monitoring data when aiming to tackle the challenges presented by demising native and spreading non-native crayfish [7, 38, 93]. Moreover, long-term biomonitoring data have recently proven useful to investigate the responses of community metrics to anthropogenic impacts across broad spatial scales [92], to detect novel non-native species introductions [71], and for investigating the temporal dynamics of long-term trends of non-native crayfish [93], simultaneously signaling the urgent need for applied management [9, 72]. Yet, despite the availability of a recently collated European long-term database [43], the adequacy of using long-term biomonitoring data for detecting native and non-native crayfish has not been tested and may not be without potential caveats, as widely applied approaches (i.e., kick net sampling) used to obtain macroinvertebrate long-term biomonitoring data may be inadequate.

The protocols used to collect data on aquatic diversity and monitor the ecological health of water ecosystems, as mandated by the Water Framework Directive, might offer unexplored opportunities for evaluating crayfish status. However, these methods may currently underestimate or overlook both native and non-native crayfish populations in biodiversity calculations. This could lead to overly optimistic assessments of ecological health and fail to attribute certain impacts on biodiversity correctly, especially those influenced by the varying abundance of non-native crayfish. Although we hypothesize that (*i*) the sampling approaches and protocols used for aquatic macroinvertebrates can detect both native and non-native crayfish, we anticipate that (*ii*) the efficacy of these protocols will vary, potentially leading to significant implications for the assessment of aquatic biodiversity. To this end, we used a recently collated database of macroinvertebrate time series, which were obtained using different sampling protocols, to investigate the presence of crayfish and their adequacy to monitor non-native crayfish.

Methods

We investigated the adequacy of long-term biomonitoring approaches for detecting native and non-native crayfish species in Europe (Additional file 1:Table S1) using the recently collated and to date most comprehensive European long-term database by Haase et al. [43]. This database contains 1816 macroinvertebrate community time series from 22 European countries. The data was collected for purposes such as research projects or regulatory biomonitoring that meet the following criteria: (*i*) each time series contained the abundance of macroinvertebrate taxa, (*ii*) sampled in a minimum of eight (not necessarily consecutive) years over a period of minimum 15 years and (*iii*) had consistent sampling effort per site (see Haase et al. 2024 for further details). Although macroinvertebrate community sampling protocols varied between time series, they were kept consistent over time within each time series (Additional file 1: Table S2). The nativeness of species in Haase et al. [43] was assessed at the country level by consulting three sources: the Global Alien Species First Record Database [90] and the Invasive Species Compendium (CABI, www.cabi.org). In case of a mismatch in the species' non-nativeness among countries assessment, we followed the Global Alien Species First Record Database [90] classification as the most reliable and updated database to date. For a comprehensive explanation of the data used, see Haase et al. [43].

The data collection methods employed to collect the time series constituting this database were diverse and lacked detailed information, hindering complex statistical analyses. Some methods were described as 'kick net sampling', 'hand netting', 'subsampling', or 'Ekman grabs', and were explicitly defined but lacked detailed clarity on the application (duration, area sampled, etc.). Others were vague and less specified, such as 'Multi Habitat Sampling' (MHS) in Germany, or they solely described the assessment schemes used to evaluate ecological health and water quality of rivers and streams via benthic macroinvertebrate community analysis. The more clearly defined information reported in [43] include national standard methods such as AFNOR XP T90-333 (for France [1]) or DIN 38410 (for Germany) [109], but also assessment methodologies or bioassessment tools, such as RIVPACS (River Invertebrate Prediction and Classification System) [108], IBGN (Indice Biologique Global Normalisé) [2], PERLA, AQEM (Aquatic Quality Evaluation in Mediterranean Rivers) [51], and 'STAR ICMi' (Standardisation of River Classifications) [12]. Less clear information included, among other 'artificial substrates', 'SUBS', or 'Multi-Habitat' (see Additional file 1: Table S2). Information provided by Haase et al. [43] did, however, not provide specific details.

To investigate detections of native and non-native crayfish in long-term biomonitoring over space and time and to infer the adequacy of long-term biomonitoring approaches for native and non-native crayfish detection, we first (*1*) identified time series containing native and non-native crayfish (considering European native and non-native species) [59], (*2*) investigated the continuity of native and non-native crayfish occurrences, i.e., if annual records were continuous over multiple years or isolated, and (*3*) compared the detections for both native and non-native crayfish across different sampling protocols. We compared differences in the occurrences of native and non-native species within long-term data across two levels. At the first level, we conducted a detailed spatial and temporal analysis at the species level by visually depicting occurrences of both native and non-native crayfish species in the database. We further inspected the occurrences of crayfish temporally across countries by identifying the records of crayfish split into native and non-native each time series identified at the species level. Subsequently, we computed several key metrics for each species, including the average duration of time series (in years), average number of samplings per time series, average number of records per time series, average abundance per occurrence, average period of records, and lag time between the first year of the time series and the first record of crayfish. At the second level, we compared the relative reporting rates of different methods and assessments that either reported occurrences of native or non-native crayfish, vs. those time series that did not report any crayfish occurrences at all. All analyses were performed in R version 4.3.1 (R core Team, 2023).

Results

From the 1816 macroinvertebrate community time series collected between 1968 and 2020, 1425 time series reported no crayfish. However, 391 time series reported one or multiple crayfish, of these, belonging to the families Astacidae (n = 210 time series; n = 634 annual occurrences) and Cambaridae (n = 237 time series; n = 641 occurrences) (Fig. 1).

From these, only 191 time series (10.5%) with a total of 542 crayfish occurrences were identified at the species level, including four native and three non-native species (Figs. 2, 3). Of them, 46 (2.5%) time series reported native crayfish. These were the native crayfish species *Astacus astacus* (n = 15 time series; n = 18 occurrences), *Pontastacus* (formerly *Astacus*) *leptodactylus* (n = 17 time series; n = 31 occurrences), *Austropotamobius pallipes* (n = 15 time series; n = 42 occurrences), and *Austropotamobius torrentium* (n = 2 time series; n = 2 occurrences). In contrast, we found 160 time series (8.8%) containing a total of 449 occurrences of non-native crayfish species. These were the *Pacifastacus leniusculus* ($\underline{n}$ = 85 time series; n = 252 occurrences), *Procambarus clarkii* (n = 18 time series; n = 56 occurrences), and *Faxonius* (formerly *Orconectes*) *limosus* (n = 66 time series; n = 141 occurrences). The remaining crayfish reports within these time series (n = 209) were not reported at the species level (Cambaridae, n = 153 time series; 444 occurrences; Astacidae, n = 93 time series, 289 occurrences). Note that different families and species can be reported within the same time series.

Identified time series that reported crayfish at the species level spanned on average 19.93 ± 8.21 (mean ± SD) years and contained 15.61 ± 6.39 sampling years, yet they

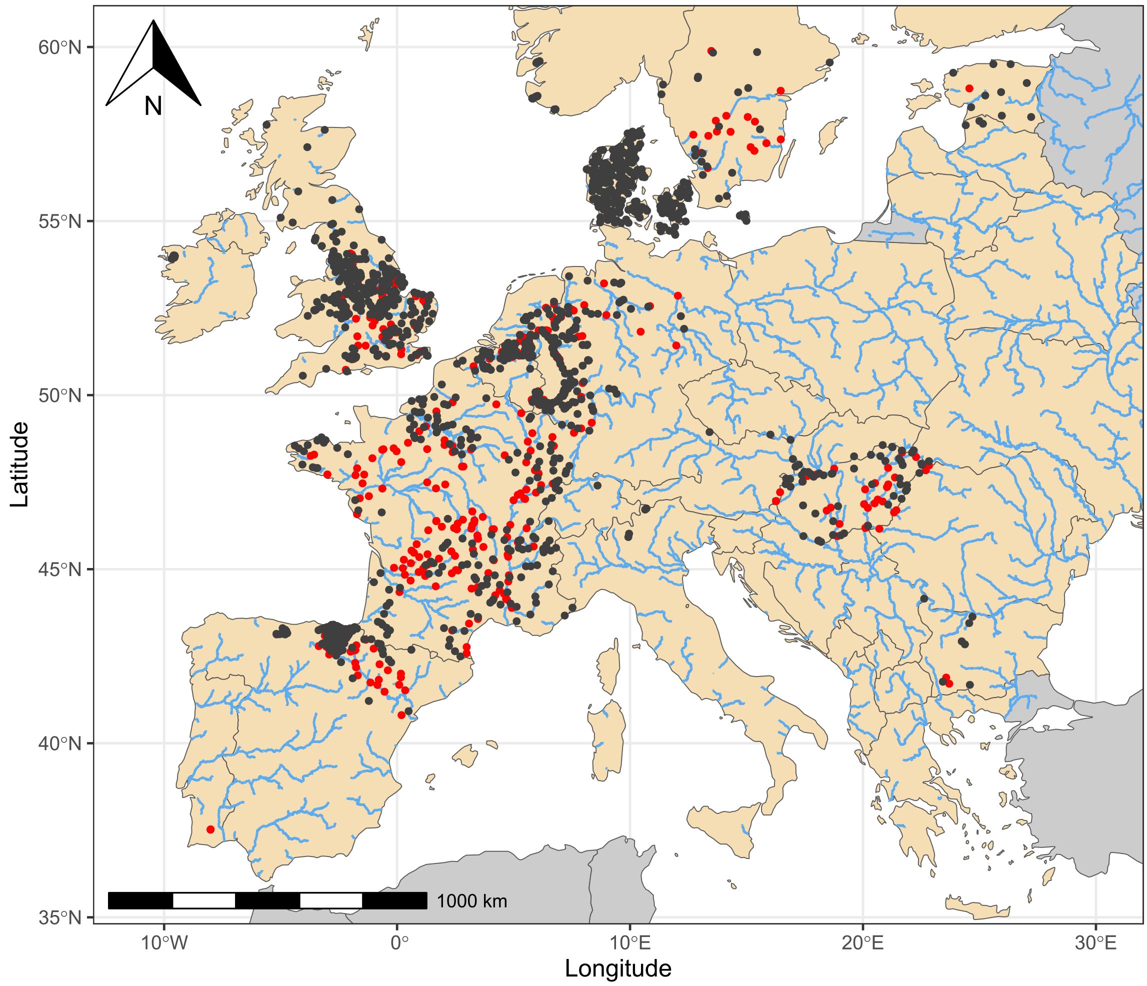

Fig. 1 Distribution of time series from Haase et al. [43] containing records of freshwater crayfish (red) vs. those reporting no crayfish (black). See Additional file 1: Fig. S1 for a more focused display on sites in the Rhine River

only recorded on average 3.88 ± 6.25 crayfish occurrences between 1980 and 2019. Time series also differed in duration and sampling frequency, with the average record of non-native crayfish per time series varying considerably (Table 1).

The first occurrence of a native crayfish (*A. astacus*) was detected in the 1980s in Bulgaria and the last occurrence of a native crayfish (*P. leptodactylus*) was in 2017 in Hungary. According to Haase et al., [43], the earliest records of non-native crayfish (*F. limosus*) were in 1983 from Germany. English, French, Portuguese, Spanish, and Swedish time series reported the first records of non-native crayfish in the early- to mid-1990s. Hungarian time series were the last to report non-native crayfish in the mid-2000s, whereas no records of non-native crayfish were available from Germany and Spain after the early 2000s (Fig. 2). Furthermore, we found that 16 (88.9%) observations of *A. astacus*, 20 (64.5%) of *P. leptodactylus*, 19 (45.2%) of *A. pallipes*, and 2 (100%) of *A. torrentium* were single occurrences. In the case of non-native crayfish, 226 (50.3%) observations were single occurrences, whereas 223 (49.67%) belonged to repetitive observations over multiple years (Fig. 4), with on average 2.28 (± 1.72 SD) years without having again identified a non-native crayfish.

A considerable share of time series ($n = 209$) contained species reported at a higher taxonomic level than the species level. These included members of the Cambaridae family (classified as Cambaridae spp.; $n = 153$ time series; 414 occurrences) that were reported in a time series listing various sampling and assessment schemes or tools (including AFNOR, IGBN, handnet, artificial substrate, standard invertebrate net). Crayfish belonging to the Astacidae family that were not reported at the species level ($n = 96$ time series, 289

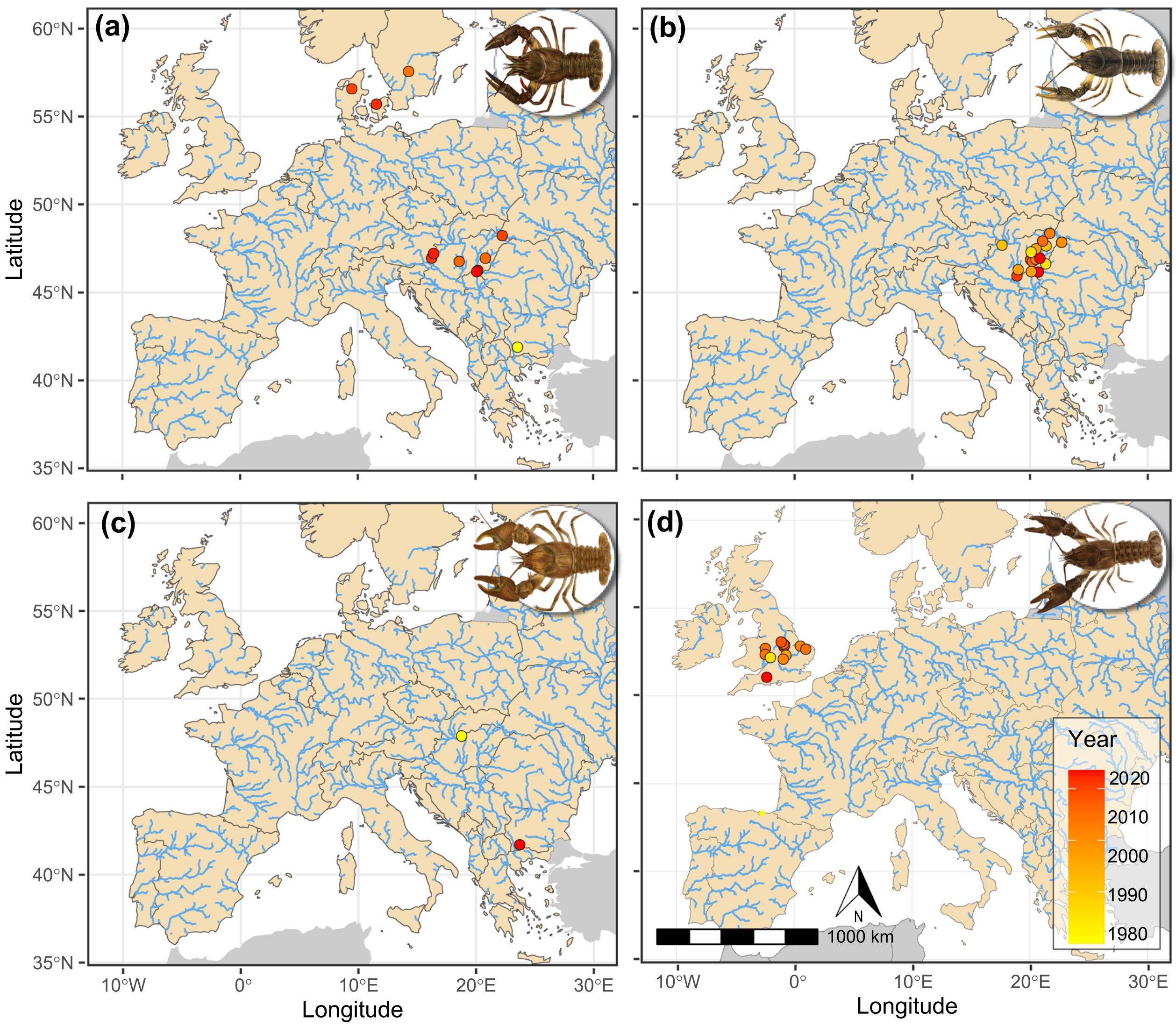

Fig. 2 Distribution of time series containing records of native crayfish **a** *Astacus astacus*, **b** *Pontastacus leptodactylus*, **c** *Austropotamobius torrentium*, and **d** *Austropotamobius pallipes* indicating the year the respective species was first recorded. Data were obtained from Haase et al. [43]. For the distribution of the different crayfish species please see Kouba et al. [59]. Crayfish drawings are the possession of the FFPW USB and drawn by the MgA. Radka Bošková

occurrences) were caught with methods described as AFNOR, IGBN, AQEM, kick net, and RIVPACS.

The 46 time series that contained native crayfish identified at the species level were all reported following predominantly AQEM and RIVPACS, followed by unspecific kick net sampling, Multi-habitat sampling, and Artificial substrate sampling (Fig. 5a). The 160 time series that identified non-native crayfish at the species level were mostly unspecific kick net sampling and RIVPACS, followed by AQEM, PERLA, Surber, and AFNOR XP (Fig. 5b). Time series that identified neither native nor non-native crayfish ($n = 1620$) were mostly collected following kick net sampling and RIVPACS, followed by IBGN and other assessment schemes, albeit to a lesser degree (Fig. 5c).

Discussion

Biodiversity monitoring in aquatic environments often serves the purpose of assessing the ecosystem's health and ecological condition. It achieves this by identifying various influential stressors, which in turn enables the evaluation of ecosystem health and functionality [35]. Although Target 5 (now Target 12) of the EU Biodiversity Strategy proclaims that native biodiversity needs to

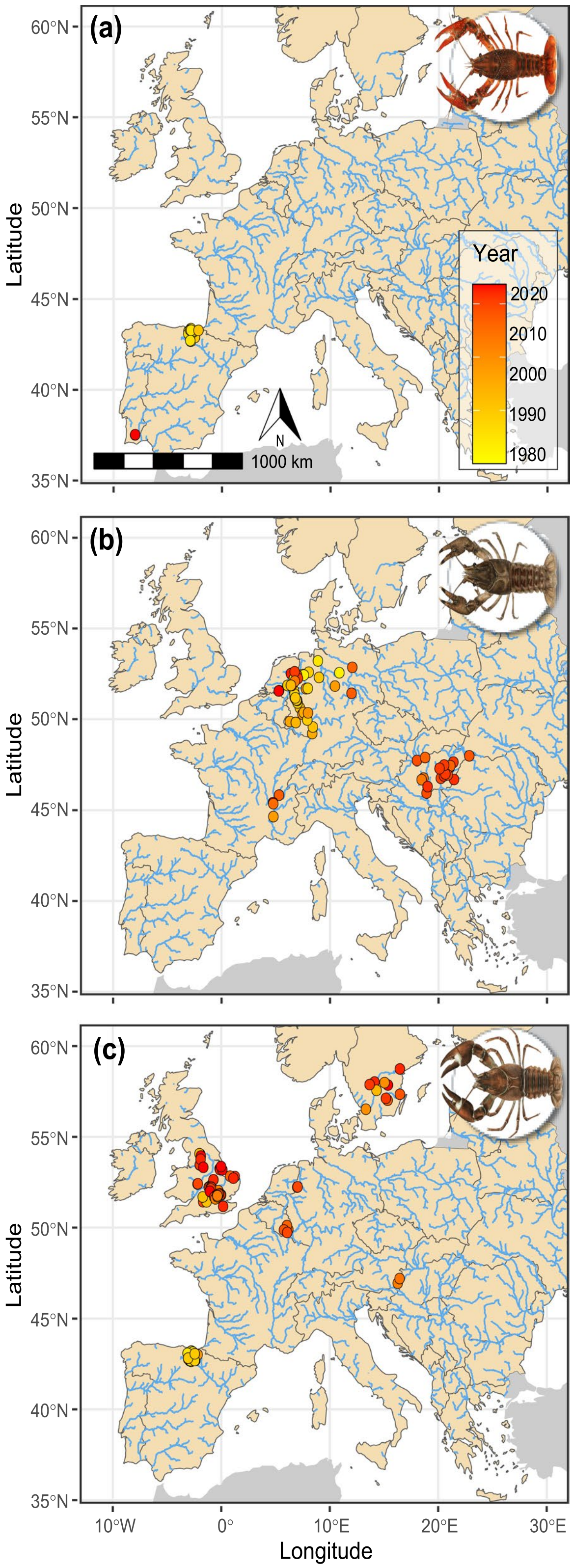

Fig. 3 Distribution of time series containing records of non-native crayfish **a** *Procambarus clarkii*, **b** *Faxonius limosus*, and **c** *Pacifastacus leniusculus*, indicating the year the respective species was recorded. Data were obtained from Haase et al. [43]. For the distribution of the different crayfish species please see Kouba et al. [59]. Crayfish drawings are the possession of the FFPW USB and drawn by the MgA. Radka Bošková

be protected by counteracting non-native species (European [27] and the updated EU Biodiversity Strategy 2030 [55] proclaims a "50% reduction in the number of Red List species threatened by invasive alien species", current monitoring practices (e.g., kick netting) relied on by the WFD may not be the most adequate way to monitor and differentiate between native and non-native species and thus, may undermine conservation efforts [11, 36].

Utilizing the most comprehensive European long-term data set that consisted of ~ 1.800 freshwater macroinvertebrate time series [43], we found numerous recent records of non-native crayfish and evidence of an increasing trend in the prevalence of non-native crayfish populations within long-term data. However, the overall reporting of crayfish in the studied long-term data set may not accurately reflect true distributions or abundances (see [93]). This is because the methodologies underlying these series varied significantly, including a mixture of national standards and frameworks, as well as ambiguous information about the used sampling approaches underlying the presented community data. This is a major shortcoming undermining the robustness of the collated data by Haase et al. [43], which should adhere to and clearly be identifiable by a European standard [56]. Moreover, varying taxonomic levels add another level of complexity. For instance, seven time series reported Decapoda, which could also include shrimps or crabs. We also found that the majority of time series reported species at the family level, which would indicate in the case of Cambaridae spp. a non-native species (e.g., *Procambarus clarkii*) as all members of this family are inherently non-native to Europe [20, 57]. In the case of an ambiguous entry Astacidae spp. could reflect both a native species belonging to the on the European continent native genus *Astacus* spp. (e.g., *Astacus astacus*), *Pontastacus* spp., or *Austropotamobius* spp. or the non-native species *Pacifastacus leniusculus*.

The ambiguous identification hinders precise ecological assessments and highlights the need for more species-specific reporting in biomonitoring studies. Interestingly, non-native species were first detected in the 1980s albeit some time series reaching back to as early as 1968. Reasons may include, among others possible explanations, that sites were either not invaded, or populations

Table 1 Averaged characteristics of time series and status (native vs. non-native to the European continent) of reported crayfish species occurrences (counts) and the lag time in reporting (i.e., the average years between the onset of time series and the first native or non-native crayfish being recorded)

Species	Status	Avg. time series duration (in years)	Avg. samplings per time series (in years)	Avg. number of records per time series	Avg. abundance per occurrence	Avg. period of records	Lag time
Overall		20.1 ± 8.4	15.5 ± 6.35	2.63	3.64	1980–2019	2.75
Astacus astacus	Native	10.4 ± 0.5	10 ± 0	0.46	8.80	1980–2015	8.60
Astropotamobius torrentium	Native	21.4 ± 9.9	11.5 ± 0.7	1	1.70	2008–2011	17.50
Astropotamobius pallipes	Native	21.4 ± 2.4	15.3 ± 3.90	2.8	1.78	1994–2019	3.8
Pontastacus leptodactylus	Native	11.2 ± 1.2	10.3 ± 1.2	0.88	2.80	2005–2017	5.70
Faxonius limosus	Non-native	24.2 ± 9.4	16.2 ± 7	2.13	2.79	1983–2019	0.63
Pacifastacus leniusculus	Non-native	25.1 ± 1.9	24.2 ± 5.4	3.00	7.14	1995–2011	8.62
Procambarus clarkii	Non-native	25 ± 1.8	24.4 ± 4.2	3.73	10.23	1994–2018	12.40

remained at low densities. Moreover, our time series revealed that after 2000, reports of non-native crayfish in Spain and Germany ceased, coinciding with the implementation of the Water Framework Directive [52]. This likely reflects a reporting issue rather than an actual disappearance, as non-native crayfish were not typically included in biomonitoring protocols. Despite this, the time series data continued to be collected, and evidence from other studies suggests that non-native crayfish populations remain highly abundant, particularly in the Basque Country of northern Spain [78, 93]. Furthermore, we found that the abundances of reported non-native crayfish were always low (mean ± SD, 4 ± 6.5 compared to numbers reported from the invaded range (see, e.g., Harper et al. [45]). Albeit being present in the respective regions (see, e.g., [50, 59, 77]), low crayfish abundances could be explained by crayfish being generally scarcer in deeper zones of larger rivers such as the Rhine or Rhône River or that the sampled sites were simply not occupied by native or invaded by non-native crayfish.

Comparability issues and sampling method biases in crayfish sampling methods

Although the majority of time series (78.58%) did not report any crayfish, most of the biomonitoring and assessment approaches used to collect long-term data reported the presence and status (i.e., native vs. non-native) of crayfish irrespective of the sampling protocols used, a point of contention previously raised by Gallagher et al. [31]. We, however, noted a considerable overlap in methods and assessment schemes for identifying both native and non-native crayfish and the relative proportion of crayfish detected varied, suggesting that unspecified kick net sampling was generally more effective at identifying non-native species. This conclusion is potentially misleading, considering that the *Aquatic Quality Evaluation in Macroinvertebrates* also employs kick net sampling [51], while *River InVertebrate Prediction And Classification System* and *Indice Biologique Global Normalisé* focus on macroinvertebrate data analysis and interpretation collected through various methods, including kick net or Surber sampling, D-frame nets, or hand picking [2, 18, 30]. Similarly, Multi Habitat Sampling incorporates techniques from both *Aquatic Quality Evaluation in Macroinvertebrates* and methodologies in the *Standardized Procedure for the Assessment of River Quality* [15], while techniques listed as Ekman grabs, handnets, or Surber samplers are tools that are reported in Haase et al. [43] without specific protocol details. Since Multi Habitat Sampling and Ekman grabs were used fewer than five times in the database, it remains challenging to determine if these techniques are adequate for sampling crayfish. Nevertheless, this heterogeneity in the database, underscored by the nested application of various sampling methods such as Ekman grabs within different assessment schemes, introduces a significant challenge in deriving conclusive insights. The complexity of these intertwined methodologies in such large data sets not only affects the comparability of data but also raises questions about the consistency and reliability of the findings, especially when applied to diverse ecological studies or species monitoring efforts.

There are several factors that might influence the probability of assessment schemes to detect crayfish [47]. These are, with the exception of site-specific hydromorphological conditions [102] or the flexibility in protocols

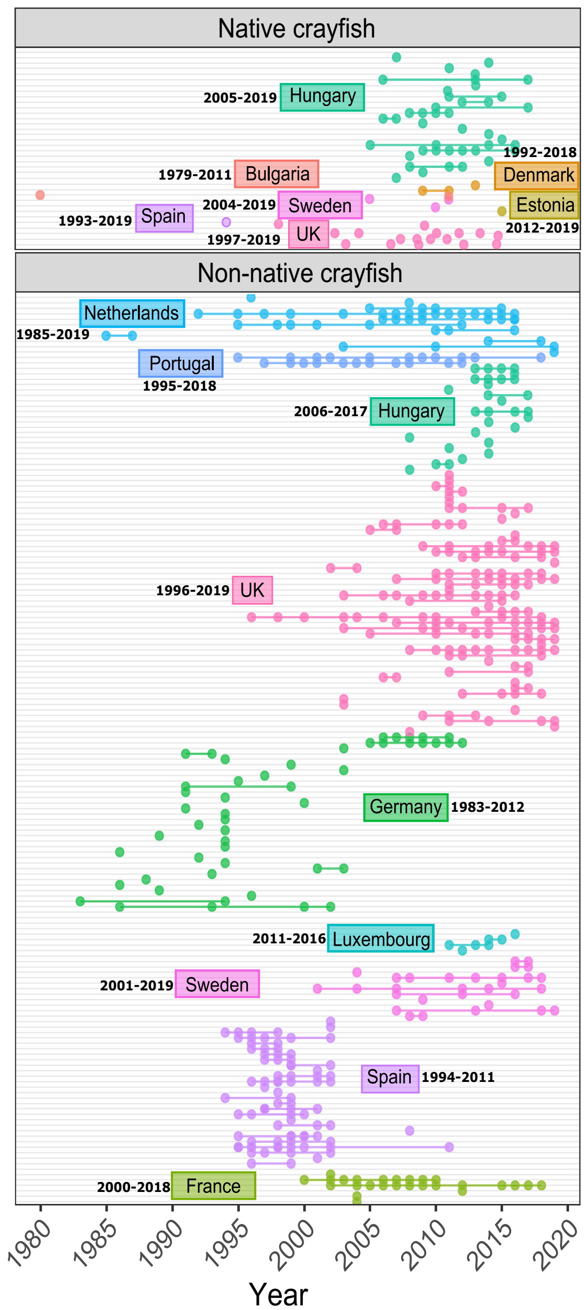

Fig. 4 Distribution of the occurrences of crayfish identified at the species level in each time series. The colors represent the geographic origin of each time series. Data were obtained from Haase et al., [43]. Dots represent annual occurrences while bars represent years without the detection of crayfish between occurrences

to sample rare habitats that might be occupied by crayfish [26], also the time the sampling was conducted. This is, because trapping (which was not underlying data reported by Haase et al. [43] should be conducted overlapping with crayfish activity patterns (i.e., between dask and dawn; [21] and information on different effectivities of kick net sampling between night and dark not being assessed. Moreover, the surface sampled by the respective method being small and thus inadequate to sample crayfish when occurring in lower densities, or if a method has more frequently been used in areas with remnant populations of native species. Indeed, while the scarce appearance of native crayfish species such as *A. astacus* likely relates to decade long decline in populations [70], two of the native species—*Austropotamobius pallipes* and *Austropotamobius torrentium*—have also declined substantially, being now listed in Annex II of the European Habitats Directive, meaning that EU Member States are required to designate Special Areas of Conservation for their protection [17]. The strikingly low number of these species' occurrences (0.82% and 0.10%) and simultaneously the low number of invaded time series (8.76% of species-level observations are considered, 17.46% if all crayfish records are considered) and generally low abundances raise concerns about the adequacy of the applied sampling methods (predominantly kick net sampling) in catching crayfish, and, respectively, the assessment scheme considering the presence of non-native crayfish.

Moreover, long-term biomonitoring efforts identified crayfish in every country that reported data in Haase et al., [43] except for Austria, Belgium, Cyprus, Czech Republic, Finland, Ireland, Italy, Latvia, Norway, Switzerland. The key issue, however, centers on the inconsistent and sporadic reporting of (especially non-native) crayfish populations over time. Given that efforts to manage non-native crayfish populations are often unsuccessful [37], the occurrence of crayfish sightings in isolated years—interspersed with periods where they are not observed—raises questions about the effectiveness of sampling methods in accurately capturing a representative sample of the benthic macroinvertebrate community, particularly for large-bodied crustaceans such as crayfish. It is conceivable that crayfish populations were present at low densities, resulting in their sporadic detection being merely coincidental. However, it is well-documented that non-native crayfish typically achieve high population densities [36], which underscores the necessity for more thorough research and investigation.

Limitations impact assessment outcomes

It is arguably true that all sampling methods for the WFD have certain weaknesses (see, e.g., [8]). In our case, however, an obvious weakness is the general lack of focus on crayfish, which is in part due to, e.g., kick netting-based biomonitoring protocols not taking into account the evading nature and burrowing ability of crayfish [95], their habitat use (i.e., sheltering under rocks, logs, and other debris [26], or the lack of a wider application

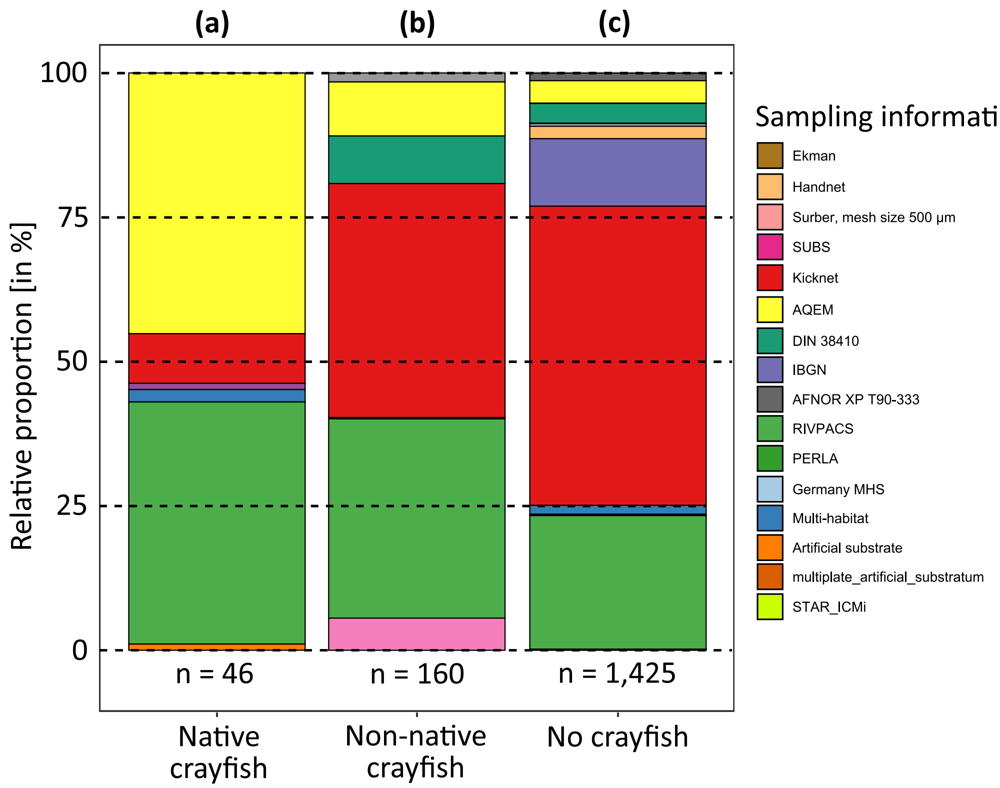

Fig. 5 Relative proportion of sampling methods and assessment schemes listed for time series collated by Haase et al. [43], reporting native (left), non-native (middle), or no crayfish species (right). See Additional file 1: Table S1 for the country of origin and sampling methods for each time series reporting crayfish and Additional file 1: Table S2 for details on acronyms. Note that some assessment schemes are ultimately based on methods that are also displayed

of baited traps or hand capturing [41] as these are not used in any long-term sampling protocols considered in this work. Furthermore, methods such as the IBGN (which utilizes a specific type of multi-habitat sampling) are seemingly biased against catching crayfish as electrofishing—another common way of capturing crayfish [33]—at the same site has previously resulted in more crayfish species and individuals being caught (pers. comm. Anthony Maire). However, compared to the on average 4 ± 6.5 non-native crayfish individuals and the observed maximum of 78 individuals recorded within one sample collected with kick net sampling in Spain in 1998 (although these might be early juveniles still associated with their mother) underlines that long-term biomonitoring is able to detect non-native crayfish [39]. Additional efforts should nevertheless assess the abilities of kick-netting to pragmatically and accurately assess crayfish abundances, as we were unable to identify the reasons why 23 samples between native and 152 samples between non-native occurrences recorded single crayfish occurrences. This is, as about half of the non-native crayfish observations were non-continuous occurrences (50.33%), whereas 83.20% of the data gaps were due to non-reporting or non-detections, and only 16.80% due to non-continuous sampling, ultimately suggesting that identified gaps most likely present false negatives.

Biomonitoring schemes may consequently underestimate non-native crayfish presences and shifts over time [93] due to the Water Framework Directive's focus on the ecological state rather than biodiversity (mirrored by the lack of [non-native] crustaceans in the methods used for assessing the ecological status in rivers based on invertebrates; [104]. Indeed, because non-native species are also often associated with water bodies of a lower ecological status and impacted biodiversity, non-native species were recognized as a Water Framework Directive pressure in 2002, but few Member States have explicitly included them in their assessments [104]. This is a significant shortcoming, as current Water Framework Directive assessments inadequately address the impacts of non-native crayfish on biological quality elements (BQEs). Arguments, however, exist for and against explicitly incorporating non-native species into the Water Framework Directive, with recommendations for their inclusion [10, 13, 28, 29, 100, 101]. Recent findings for instance demonstrated that non-native macroinvertebrates, such as *Dikerogammarus villosus*, have the potential to distort the assessment of degradation in the Water Framework Directive [3, 32]. They can influence the evaluation of zonation types and result in inaccurate assessments of the "general degradation" module or the degree of organic pollution, as they impact the estimated values of the German saprobic index based on the *Aquatic Quality Evaluation in Macroinvertebrates* taxa reference list [51]. Such a distortion can occur when non-native crustacean species dominate invaded ecosystems by attaining high local densities [3], developing the potential to cause the decline of native species and alter community compositions of both flora and fauna [23, 73]. Recent observations also indicate that fish invasions can influence the results of quality assessments by affecting the scoring system. Non-native fish, classified as 'intolerant,' often receive positive scores, suggesting a "good" ecological status [54]. While it may not be the case that benthic invertebrate protocols give non-native crayfish positive scores, misidentifications may have distorting outcomes.

The absence of crayfish in 75.6% of all time series (considering also entries at the genus and family level) and only 10.5% reporting crayfish at the species level raise the question if the sampling protocols consider non-native crayfish in the evaluation of samples and if specimens were simply avoided or willingly excluded—possibly being considered irrelevant for the respective estimation of the *Water Framework Directive*-compliant quality metrics evaluating the ecological status by local stakeholders. Indeed, the most frequently employed indices, such as the *Biological Monitoring Working Party* or its associated *Average Score Per Taxon*, assign scores to various taxa, including Astacidae, and consider them as indicator taxa or pressure. However, they do not consider species belonging to the genera *Procambarus* or *Faxonius*. This suggests the need to integrate non-native species into Water Framework Directive assessments more effectively, as their presence can significantly alter aquatic ecosystems. Furthermore, specific research groups seem to make decisions regarding the inclusion of Astacidae crayfish, which might be related to their native or endangered status or how frequently they are caught. This raises concerns that some non-native crayfish may be overlooked or willingly ignored when conducting community-based biomonitoring or that remaining populations are often of lower population density, protected, and subsequently kept secret [98, 107].

Recommendations

Long-term biomonitoring data present opportunities for studying native and non-native crayfish trends. The different sampling methods, sampling protocols, and Water Framework Directive compliant assessment systems used across European Union member states affect the detection of crayfish species, particularly when species are misidentified as seen by Krieg and Zenker [61]. The low detection rates of native crayfish further suggest the need for additional research into factors such as habitat changes, competition with non-native species, or the development of more effective, targeted sampling methods in standard biomonitoring protocols. Although kick net sampling has a large Catch Per Unit Effort and high detection probability [88], it is biased towards capturing smaller individuals of crayfish. These, however, constitute a significant share of crayfish populations [4], suggesting that kick net sampling should be an appropriate method for noticing the presence of crayfish. Trapping is also a relatively time-intensive approach that has produced limited large-scale and long-term information on non-native crayfish populations [25, 67, 80] and tend to overlook smaller individuals [14, 34, 64]. Factors such as weather conditions may also affect the effectiveness of baited traps [64, 82], resulting in non-native crayfish not being detected. Crayfish, however, inhabit areas that are not always effectively sampled using kick net sampling [31, 39, 79], such as under stones, logs, or in burrows and in greater depths (Larson and Olden, 2006 [60], or may evade capture through escape reactions [84]. The inability to accurately identify species (e.g., juvenile noble vs. signal crayfish), raises additional concerns that detected non-native crayfish species could falsely indicate good ecological quality in assessments (and vice versa), despite their significant negative impact on the invaded ecosystem. Considering that non-native crayfish pose a significant threat to native biodiversity, including native crayfish species which are of utmost relevance and conservation value [44, 99], their limited consideration and inclusion in long-term biodiversity monitoring through focused sampling further raises concerns about the accuracy of non-native species impact assessments using long-term data, as well as the assessment of stream quality following the WFD.

Biomonitoring as part of the WFD should also consider the effectiveness in using environmental DNA-based methods to detect non-native species [42, 53, 83] and continue to collate available information and make them openly accessible. This is of integral importance, because updateable biomonitoring data compiled in large data sets such as Haase et al. [43] can provide novel insights [48, 92, 93]. Moreover, a centralized European database could circumvent issues arising from questionable information on sampling procedures underlying community data. Despite these challenges, biomonitoring efforts remain crucial, particularly in evaluating non-native species and their impacts, as these are often abundance mediated [46]. Future efforts should be made to (a) unify how the WFD considers non-native species and (b) specifically target the capture of high-impact non-native species such as crayfish that may not be adequately sampled by existing protocols and methods (such as manual search assisted with a hand-held net at places of possible occurrences or baited traps at hardly accessible deep places). In the case of non-native crayfish, the establishment of such a 'best-practice' should be explored by future efforts to identify the most suitable (i.e., widely applicable and standardizable) approach. Concomitantly, (b) the timescale of non-native crayfish-focused monitoring should be assessed, delineating whereas (c) the implementation of native and non-native crayfish presences into schemes assessing the ecological state [104] should be explored, as their presence can have practical implications for management and conservation practices, including habitat restorations [91], the conservation of native [49], and the management of non-native populations [71].

Supplementary Information

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Additional file 1: Figure S1. Distribution of time series from Haase et al. [43] containing records of freshwater crayfish (red) vs. those not reporting no crayfish (black) in the Rhine river. **Table S1.** Country of origin and sampling methods for each time series reporting crayfish. **Table S2.** Detailing of Acronyms.

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Author contributions
PJH conceived the idea for this work. PJH and IS designed the methodology; IS prepared and analyzed the data; PJH led the writing of the manuscript. IS and IK contributed to the initial writing. AK contributed to a later version and supervised this project. All authors contributed critically to the drafts and gave final approval for publication.

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Data availability
The full data set underlying the analysis presented in this manuscript can be obtained from Haase et al. [43]. The filtered data set and associated R code can be obtained from the corresponding author upon reasonable request.

Declarations

Competing interests
The authors declare no competing interests.

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