Aquatic Conservation: Marine and Freshwater Ecosystems

WILEY

ARTICLE OPEN ACCESS

An Update on the Conservation Status Assessment of two Endangered Freshwater Mussel Species in Bavaria, Germany

Andreas H. Dobler | Philipp Hoos | Juergen Geist

Aquatic Systems Biology Unit, Department of Life Science Systems, TUM School of Life Sciences, Technical University of Munich, Freising, Germany

Correspondence: Juergen Geist (geist@tum.de)

Received: 4 June 2024 | **Revised:** 14 August 2024 | **Accepted:** 19 September 2024

Funding: This study was funded by the Bavarian State Ministry of the Environment and the Bavarian Environment Agency (LfU).

Keywords: bivalves | conservation status | endangered species | European habitats directive | *M. margaritifera* | Natura 2000 | population trends | *U. crassus*

ABSTRACT

The two highly endangered European mussel species *Margaritifera margaritifera* and *Unio crassus* are target species of conservation. Based on a recently completed systematic state-wide monitoring of each 22 *M. margaritifera* and 22 *U. crassus* streams in Bavaria, Germany, we present an update on population trends, conservation status, habitat quality and threats for both species. Populations status and habitat quality varied strongly between *M. margaritifera* and *U. crassus* streams, but there was also great variability within each of those groups. The population decline of *M. margaritifera* has continued, albeit higher proportions of juveniles originating from artificial breeding programmes have been established in some streams. Habitat quality often did not match known requirements as evident from poor stream bed quality, lack of hosts and elevated nutrient levels. In contrast, *U. crassus* populations showed a better status, with an increase in population size over all sampled streams. Successful recruitment was indicated by high proportions of juveniles. However, no mussels older than 16 years were found, probably due to predation and structural stream maintenance measures. Climate change effects, such as extreme droughts, affected both species. This study demonstrates different needs in conservation management for both species. Although mitigation of drought effects is commonly needed for both species, tackling host fish management and direct threats such as predation should be prioritized in *U. crassus*, whereas restoration of prime habitat quality and intact catchments is key to enable natural recruitment and sustainable populations of *M. margaritifera*.

1 | Introduction

Freshwater mussels (Unionidae) are a crucial component of freshwater biodiversity but are particularly susceptible to many threats (Aldridge et al. 2023). Strong declines in global freshwater mussel diversity have been observed by several authors such as Lydeard et al. (2004), Lopes-Lima et al. (2018) and Lopes-Lima et al. (2017). These declines are often linked to the deleterious effects of pollution, unsustainable exploitation, alterations of natural habitats and the introduction of invasive species (Geist 2011; Lopes-Lima et al. 2017; Geist, Benedict, et al. 2023; Sousa et al. 2023). Additionally, the emerging threat of climate change on freshwater mussels, especially related to changes in temperature and hydrological cycles, is eminent (Lopes-Lima et al. 2018; Lopes-Lima et al. 2021). The analysis of mussel population trends over time is a crucial part of monitoring their well-being, and it is also an early warning system for detecting adverse effects, and of adapting suitable conservation strategies (Boon et al. 2019).

Andreas H. Dobler and Philipp Hoos are equal contribution authors.

Some species such as the highly endangered freshwater pearl mussel (*Margaritifera margaritifera*) and thick-shelled river mussel (*Unio crassus*), which both had a wide distribution across Europe, have experienced strong declines in the past decades (Araujo and Ramos 2001; Young, Cosgrove and Hastie 2001; Geist 2010; Lopes-Lima et al. 2017). Along with these declines, important ecosystem functions and services such as water purification that are provided by freshwater mussels are also decreasing (Zieritz et al. 2022; Lopes-Lima et al. 2018; Vaughn 2018).

Due to their complex life cycle, freshwater bivalves are considered to be indicator, umbrella and keystone species making them ideal targets for conservation (Geist 2010, 2011; Haag and Williams 2014; Lummer, Auerswald and Geist 2016; Vaughn 2018). However, this property must always be understood in the context of species-specific habitat requirements. Even if *M. margaritifera* and *U. crassus* overlap in their native range (Lopes-Lima et al. 2017), both species are known to have different habitat requirements, particularly in terms of substrate conditions (Denic et al. 2014; Inoue, Stoeckl and Geist 2017; Stoeckl and Geist 2016). Various life history traits of the species, such as the known host fish spectra or the time juvenile mussels spend in the substrate, help to illustrate and explain these differences (Denic et al. 2014; Lopes-Lima et al. 2017).

The effects of anthropogenic use and climate change on aquatic habitats and freshwater mussels are well understood and documented (Sousa et al. 2021; Höök et al. 2019; Heino, Virkkala and Toivonen 2009; Markovic et al. 2014). Without measures to stop or minimize these effects, generalist species are more likely to benefit from these developments, in contrast to highly specialised species, such as *M. margaritifera* (Markovic et al. 2014; Sousa et al. 2018; Santos et al. 2015; Hastie et al. 2003; Sousa et al. 2015).

To counteract these negative trends in the European Union, specific habitat types and species that are considered to be of European importance have been protected under the Habitats Directive (Bouchet, Falkner and Seddon 1999; Council of the European Communities 2013) since the year 1992. With these regulations, each member state is obliged to ensure a high level of protection and to implement conservation measures to maintain or to reach a favourable conservation status of all listed habitats and species. However, conservation areas often do not adequately cover the distribution of protected aquatic species such as freshwater mussels (Dobler et al. 2019).

In this study, the recent conservation status of the two highly endangered unionid species, *M. margaritifera* and *U. crassus*, in the federal state of Bavaria in Germany was assessed. Based on the regular monitoring for Natura 2000, the population status, habitat conditions and potential threats were evaluated using the German assessment schemes and monitoring guide (BfN and BLAK 2017). In addition, this study critically examines the methodology and assessment process used considering the data collected and existing scientific knowledge on the two target species. It also compares recent trends in mussel population status, host fish availability and general habitat quality with earlier reports for both species in the same area (Stoeckl, Denic and Geist 2020). Specifically, we hypothesised that (i) population declines have stopped or have been reversed due to ongoing conservation efforts for both species such as captive breeding and habitat restoration projects, at least in *U. crassus* which has a faster development time and lower habitat requirements than *M. margaritifera*, (ii) existing main threats continue to differ for both species with predation being more relevant for *U. crassus* and deficient juvenile habitat quality being more relevant for *M. margaritifera* and (iii) that conservation action therefore needs to set different priorities for future conservation and restoration.

2 | Materials and Methods

2.1 | Study Area

This study was conducted in all 22 *M. margaritifera* and in 22 *U. crassus* streams across the federal state of Bavaria in Germany between the years 2021 and 2023. As Bavaria is home to the largest number of recent *M. margaritifera* populations in Germany, this federal state and its conservation efforts have a special responsibility and importance in protecting these animals. Consequently, as part of the monitoring process, all recent populations of *M. margaritifera* were surveyed. For *U. crassus*, samples were randomly chosen by the Bavarian Environmental Agency (LfU) to ensure the full representativeness required for this monitoring. Although *U. crassus* is widely distributed across Bavaria, *M. margaritifera* naturally occurs only in siliceous headwater streams in the north-east (e.g., Dobler et al. 2019; Figure 1).

2.2 | Mussel Survey

The mussel survey was conducted in accordance with previous monitoring (Stoeckl, Denic and Geist 2020), considering the recommendations as defined in the CEN standard for freshwater pearl mussel (Boon et al. 2019; British Standards Institution 2017). The area surveyed in each stream covered the formerly documented colonised stretches. A complete census monitoring was performed for *M. margaritifera* streams with an expected population size of less than 1.000 individuals. For bigger populations, every 100 m, 10–20 m cross-channel transects were applied. Size classes were determined for *M. margaritifera* by measuring the total length (±0.5 mm) of at least 100 individuals per population with callipers. For populations below 100 individuals, all found mussels were measured. In more widespread populations with high densities, up to 300 individuals were measured to ensure representativeness. To distinguish juveniles from adult mussels, a threshold of 65 mm of total length was used (Hastie, Boon and Young 2000).

For *U. crassus*, population size estimation was based on sampling cross-channel transects using a systematic approach (Strayer and Smith 2003). The intensity of the survey (length of and distance between transects) depended on the procedure of the previous survey or, in the case of an initial monitoring, on the population density. Distance between transects was 80 m and the length of each transect was 20 m. As with *M. margaritifera*, the total shell length of individual mussels was also measured for *U. crassus*. In addition, the clearly visible annuli were counted for age determination. The threshold for juvenile mussels was set to 5 years and lower.

In all streams, visual and tactile searches were applied by wading upstream using aquascopes or by snorkelling, depending on the water depth and current of the streams. After the measurements, all living mussels were immediately returned to their original locations.

2.3 | Habitat Assessment

Habitat assessments for both species comprised aspects related to water chemistry, stream bed quality and fish community assessments since both species depend on suitable fish hosts for their development. To ensure representativeness, depending on the length of the stream stretches colonised by mussels, physicochemical habitat parameters were analysed at 5–10 sites per stream. Where possible, readings were taken at the exact same spots as previous monitoring (Stoeckl, Denic and Geist 2020). Temperature (°C), dissolved oxygen concentration (O_2, mg L^{-1}), pH value and electrical conductivity (EC, μS/cm relative to 25°C) were measured in the free-flowing water using a handheld Multi 3630 IDS (WTW GmbH, Weilheim, Germany). Turbidity (NTU) was measured using a handheld turbidity meter Turb 430 IR (WTW, Weilheim, Germany). Flow velocity (v, m s^{-1}) was measured with a handheld flow meter (Flowtherm NT; Höntzsch, Waiblingen, Germany) 5 cm below the water surface and 5 cm above ground. Redox potential (Eh), as an indicator for long-term oxygen supply and hydrological exchange between free-flowing water and the interstitial zone, was measured according to Geist and Auerswald (2007), in free-flowing water and at 10-cm depth in the substratum using a handheld pH-meter pH 3110 (WTW, Weilheim, Germany) with a platinum electrode and an $Ag/AgCl_2$ reference electrode.

For substratum texture analyses, sediment samples were taken at three sites per stream using a box sampler following Pander, Mueller and Geist (2015). Grain size was fractionated using a wet sieving tower (Fritsch, Idar-Oberstein, Germany) with different mesh sizes (63.0, 20.0, 6.3, 2.0 and 0.85 mm). Fractions were dried for 24 h at 100°C and weighed to the nearest gram. According to Geist and Auerswald (2007), grain sizes > 20 mm were generally excluded from the further analyses. At the same spots at which sediment was sampled, water samples with a volume of 50 mL were taken to analyse water chemistry. Samples were filtered through an ash-free filter paper (MN640d) with retention of particles > 2 μm (Macherey-Nagel, Düren, Germany) at site and stored at 4°C until further processing. Anions and cations of the filtered water samples were determined using an ion chromatograph ICS-1100 (Thermo Fisher Scientific, Dreieich, Germany) following the USEPA standard procedure (Method 300.1). A mixture of 1.8-mM disodium carbonate and 1.7-mM sodium hydrogen carbonate was used for eluting anions (AG-23 as guard column and AS-23 separation column), and a 30-mM methane sulfonic acid was used for eluting cations (CG-16 as guard column and CS-16 separation column). Additional samples for the analysis of total organic carbon (TOC, mg/L) were taken. Analyses of the TOC samples were conducted at the Bavarian Environment Agency (LfU) following a standard protocol (DIN EN 1484:1997-08 (H3)).

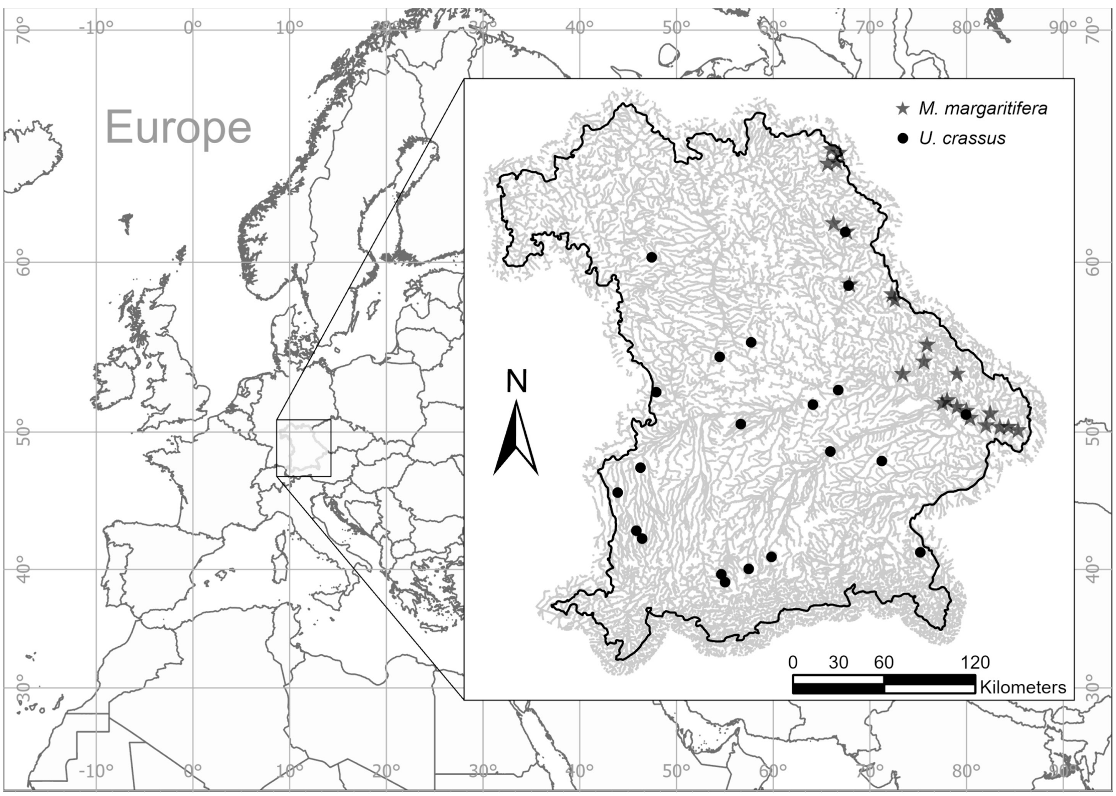

FIGURE 1 | Overview map of the distribution of the studied areas of *Margaritifera margaritifera* (grey stars) and *Unio crassus* (black circles) in the federal state of Bavaria, Germany.

Conditions of host fish stocks were determined by electro-fishing. Depending on the size of the stream and the on-site conditions (conductivity and water depth), electro-fishing was conducted using different kinds of units [1.7-kW portable electro-fishing backpack (EFGI 659, Fa. Brettschneider Spezialelektronik, Chemnitz, Germany), 1.7-kW portable electrofishing backpack (FEG 1700, Efko, Leutkirch, Germany), 3.0-kW portable electrofishing backpack (ELT 62 II, Fa. Hans Grassl GmbH, Schoenau, Germany) and 11-kW stationary electrofishing device (FEG 11000, Efko, Leutkirch, Germany)]. At each stream, between 5% and 10% of the surveyed river sections were examined by electro-fishing, wading in an upstream direction, and following the German standard (VDFF 2000). All fish caught were identified to the species level and their total length measured before being released back into the same stretch where they were caught. Species richness and host fish density were calculated based on the investigated surface area as described in Geist, Porkka and Kuehn (2006). In 16 cases, recent data on the fish community were available from the local fisheries administration ('Fachberatung für Fischerei') using the same methodology.

2.4 | Threat Assessment

According to the German assessment schemes and monitoring guide, threats were assessed qualitatively, based on a matrix that assigns the expressions of the individual factors to

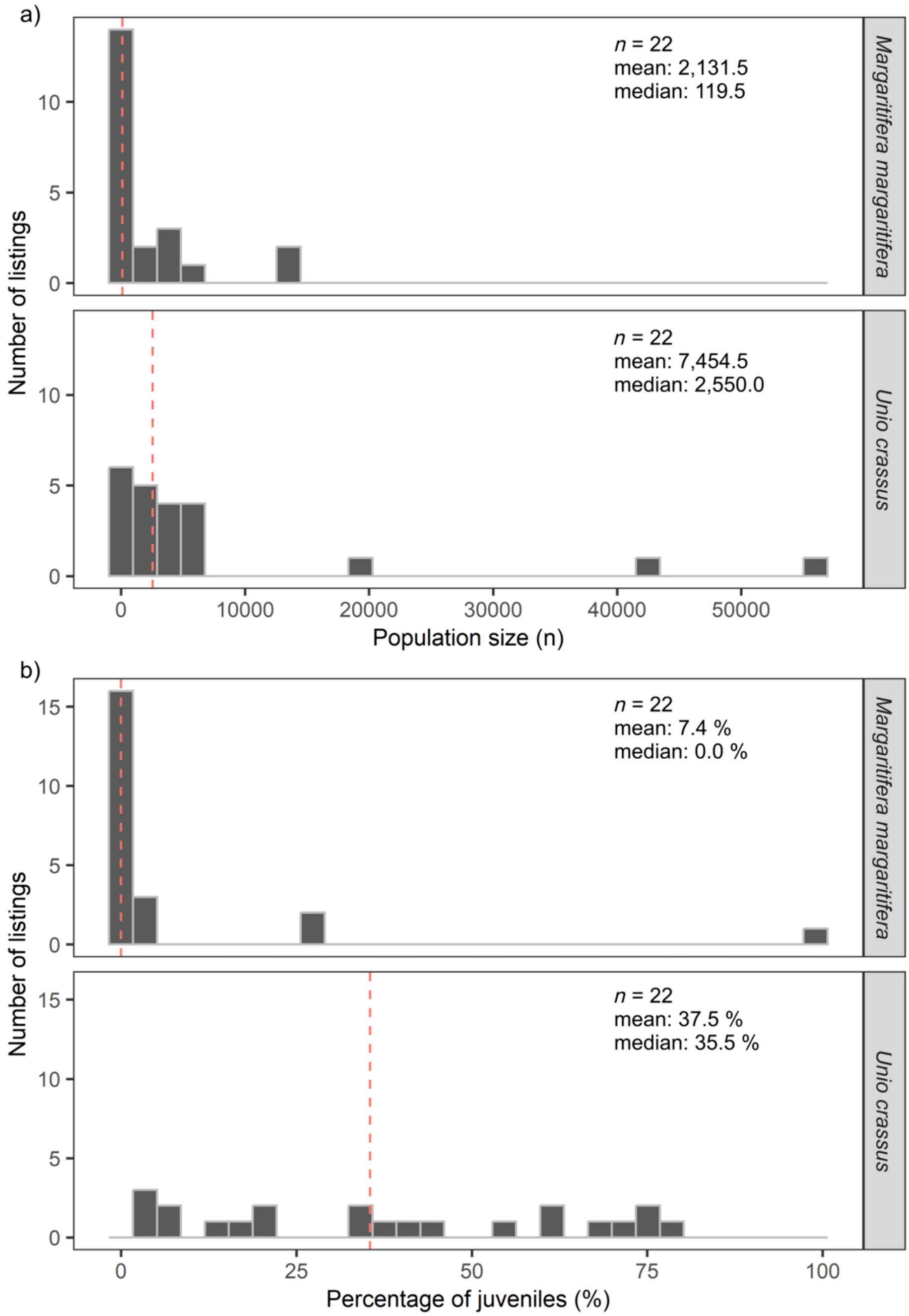

FIGURE 2 | Frequency distribution of (a) population size (*n*) and (b) percentage of juveniles (%) for 22 *Margaritifera margaritifera* and 22 *Unio crassus* populations in Bavaria, Germany. Red vertical dashed lines represent the median.

corresponding assessment levels (A, B and C), with Category A representing the best and Category C representing the worst assessment. Each section between two transects was examined. For the identification of the main threats to the mussel populations, eutrophication, substratum mobility and input, river maintenance, predation pressure, tourism effects and longitudinal connectivity were evaluated (see Table S1). Additional threats that are not listed but were identified during the monitoring were also addressed as 'other factors' (BfN and BLAK 2017).

2.5 | Data Analysis

For the qualitative assessments, monitoring results were used to rate the categories population status, habitat quality and existing threats following the German assessment schemes and monitoring guide (BfN and BLAK 2017). Each parameter was categorized either as A ('excellent'), B ('good') or C ('medium to poor') for population status and habitat quality and as A ('none to low'), B ('medium') or C ('strong') for existing threats. The worst score of each subcategory determined the rating of the superordinate category.

Statistical analyses were carried out using R (v. 4.3.2, Boston, MA). All data were tested for normality using the Shapiro–Wilk test and for homogeneity of variances using Levene's test. A Kruskal–Wallis test was used to examine differences in population sizes between both species because the normality of the data was not met. Principal Component Analyses (PCA) were performed using PRIMER (version 7) with PERMANOVA+ add-on (Plymouth Marine Laboratory, Plymouth, UK; Anderson, Gorley and Clarke 2008). Unless stated otherwise, values are given in mean ± standard deviation.

3 | Results

3.1 | Recent Status of *M. margaritifera* and *U. crassus* Populations

Across all studied streams, recent mussel populations were found in 42 out of the monitored 44 streams. The population sizes varied between 0 and 13,500 individuals for *M. margaritifera* with a mean of 2232 ± 3961 and between 5 and 56,000 for *U. crassus* with a mean of 5600 ± 12,265. Mean population sizes significantly differed between both species (Kruskal test; $p < 0.01$) (Figure 2a). For both species, two populations had an estimated size of more than 10,000 individuals. For *U. crassus*, the mean occupied area was 51.4% ± 19.2 *SD*, and *M. margaritifera* was found in 42.6% ± 34.7 *SD* of the surveyed stretches with a mean density of 0.41 individuals m^{-1} ± 0.71 *SD*.

Juveniles were found in seven out of 22 (31.8%) *M. margaritifera* and in 22 (100%) of *U. crassus* populations. Mean proportions of juveniles differed between both species with 7.4% ± 22.1 *SD* for *M. margaritifera* and 37.5% ± 26.6 *SD* for *U. crassus* (Figure 2b). However, in one *M. margaritifera* population,

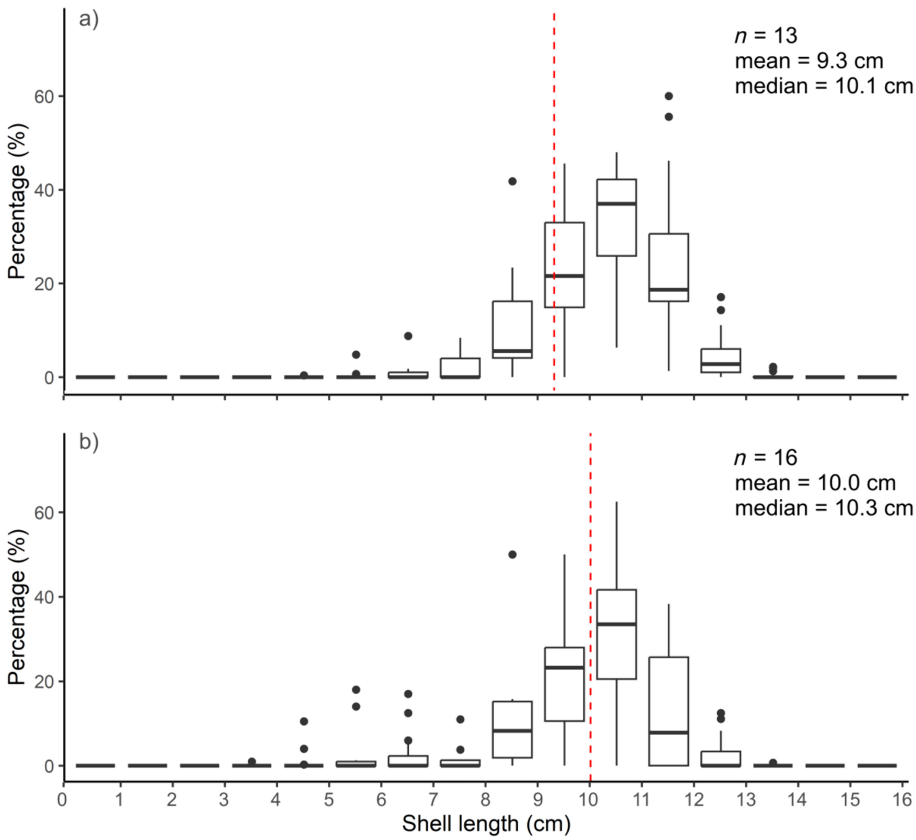

FIGURE 3 | Boxplots of the percentage of each size class of *Margaritifera margaritifera* for (a) 13 streams of the monitoring in 2012–2015 and (b) 16 streams of monitoring in 2021–2023. Boxplots represent the 25 and 75 percentiles with the median marked by a horizontal line. The dots represent outliers, and the red vertical dashed lines represent the mean size and age.

the proportion of juveniles exceeded 95%, which results from a past total extinction of this species in this stream followed by a reintroduction via infested fish since 2006 (C. Schmidt, pers. communication). A total of 1117 *M. margaritifera* individuals from 14 populations were measured and had a mean size of 10.0 cm ± 2.8 *SD* (Figure 3b). Compared to the results of the monitoring from 2015 (9.3 cm ± 3.7 *SD*), the mean size of the mussels increased by 7.5% (Figure 3). For *U. crassus*, the ages of a total of 4182 individuals were determined from 22 populations. The average age of the mussels was 6.3 years ± 2.6 *SD*. Comparing 18 *U. crassus* populations investigated in 2015 and the recent monitoring, a decrease from an average of 7.4 years ± 3.3 *SD* to 6.4 years ± 2.6 *SD* was evident (Figure 4). In both surveys, the age structures of *U. crassus* showed high proportions of juveniles (mean: 41.4% in 2015; mean: 37.5% in 2024). In 2024, 5-year-old mussels represented the highest percentage. Contrary to the monitoring in 2015, no mussels older than 16 years could be detected (Figure 4).

All investigated *M. margaritifera* populations were assessed with the lowest status ‘C’ according to the national classification system, which was mainly based on their low population density (Figure 5). Even in streams with a higher proportion of juveniles, the calculated population density was below the threshold of 5.0 individuals per meter of stream length and thus had to be assessed with ‘C’. Contrary to *M. margaritifera*, many *U. crassus* populations were classified with ‘A’ (13.6%) or ‘B’ (59.1%), since the population structure was often optimal (54.5%) or good (40.9%) as was the population size (13.6% ‘A’, 59.1% ‘B’, respectively) (Figure 6).

3.2 | Habitat Conditions in *M. margaritifera* and *U. crassus* Streams

3.2.1 | Host Fish Status

Host fish status was examined in 21 of 22 studied *M. margaritifera* streams and in 19 of the 22 studied *U. crassus* streams. In the host fish assessment, a total of 31 fish and lamprey species were recorded in the studied *U. crassus* (mean fish species: 8.3 ± 4.5 *SD*; Table S2) and 27 in *M. margaritifera* waters (mean fish species: 6.6 ± 5.3 *SD*; Table S3).

Brown trout (*Salmo trutta fario* L.), the only existing suitable host fish species in the Bavarian pearl mussel streams, was detected in each *M. margaritifera* stream with a total mean of 10.1 individuals 100 m^{-2} ± 10.3 *SD*. In line with previous reports across Europe (Geist, Porkka and Kuehn 2006), the mean density of *S. trutta f.* was also higher in non-recruiting populations (12.7 individuals 100 m^{-2} ± 11.0 *SD*) than in streams with observed natural recruitment (3.5 individuals 100 m^{-2} ± 3.4 *SD*; Table S3) in this dataset. Across all *U. crassus* streams, the mean density of the three primary hosts Eurasian minnow (*Phoxinus phoxinus* L.), European chub (*Squalius cephalus* L.) and three-spined stickleback (*Gasterosteus aculeatus* L.) was 26.8 individuals

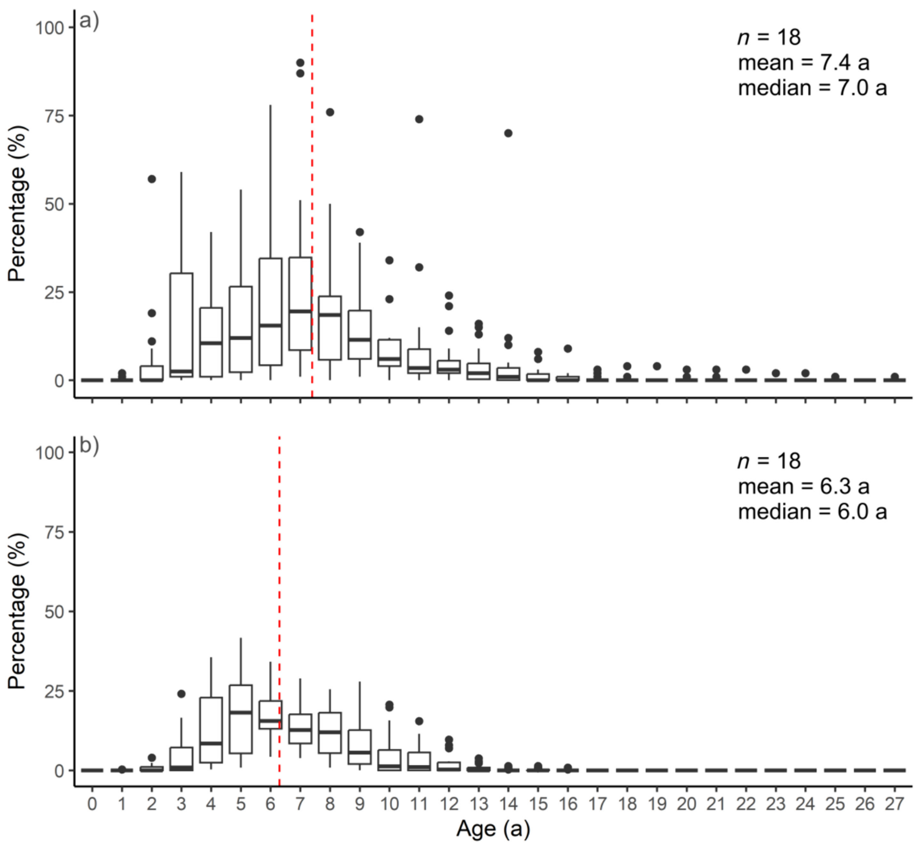

FIGURE 4 | Boxplots of the percentage of each age class of *Unio crassus* for (a) 18 streams of the monitoring in 2012–2015 and (b) 18 streams of the monitoring in 2021–2023. Boxplots represent the 25 and 75 percentiles with the median marked by a horizontal line. The dots represent outliers, and the red vertical dashed lines represent the mean size and age.

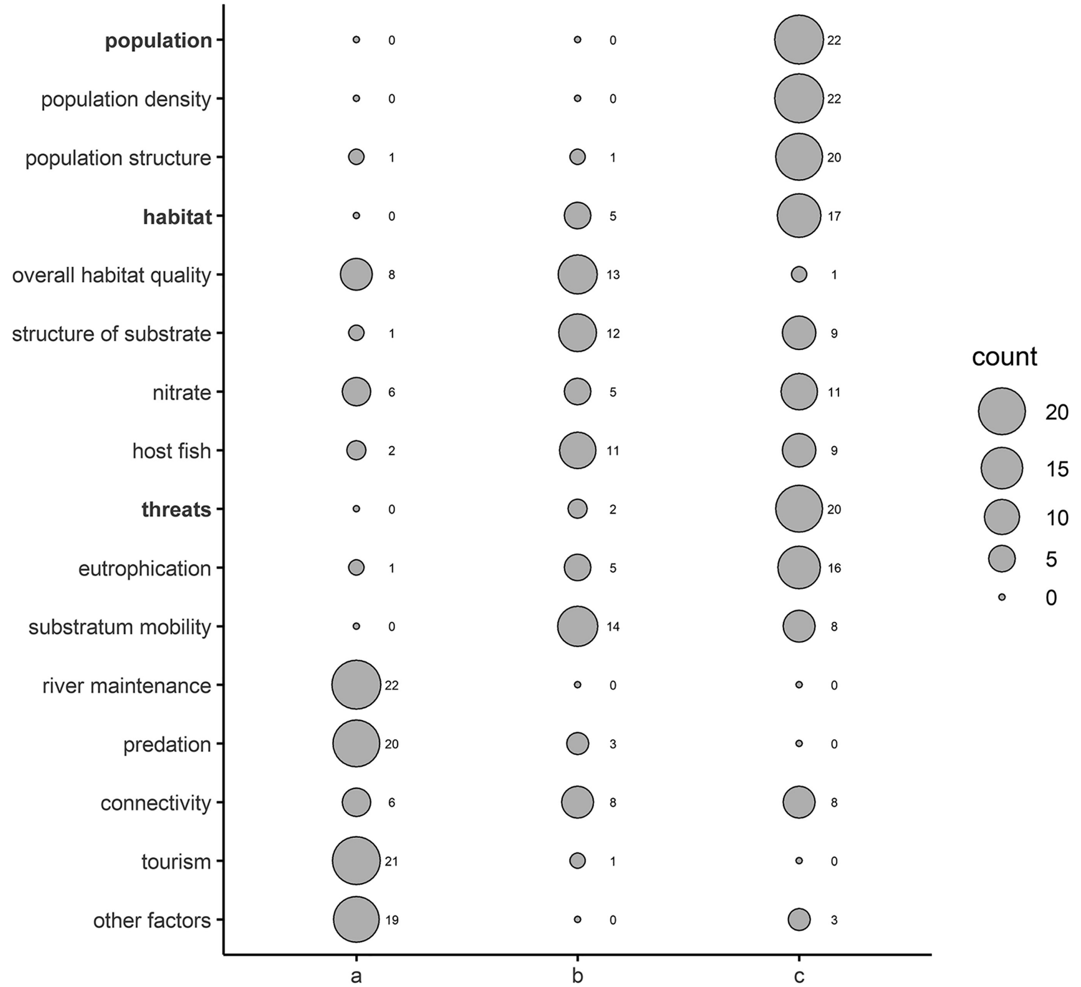

FIGURE 5 | Balloon plot of the assessment of 22 *Margaritifera margaritifera* populations. The three main categories (in bold) and their subcategories are based on the German assessment scheme and assessed by three indicator categories ('A', excellent; 'B', good; 'C', medium-poor). Ballon size indicates the frequency of each score.

100 m^{-2} ± 37.9 *SD* with *S. cephalus* being the most common species (in 16 of 19 streams) and *P. phoxinus* being the most abundant species (32.2 individuals 100 m^{-2} ± 49.3 *SD*). Only in one stream, no suitable host could be detected (Table S2).

The mean proportion of host fish was 46.0% ± 34.5 *SD* for *M. margaritifera* streams (Table S3) and 48.0% ± 28.2 *SD* for *U. crassus* streams (Table S2). The percentage of host fish for streams with *M. margaritifera* recruitment ranged from 0.2% to 100.0% with a lower mean (35.9% ± 40.6 *SD*) than in streams without recruitment (47.9% ± 33.3 *SD*; Table S3).

Besides the suitable host fishes, European bullhead (*Cottus gobio* L.) (recorded in five streams with recruitment and in 12 without recruitment) and *S. cephalus* (recorded in four streams with recruitment and six without recruitment) were the most common species in *M. margaritifera* streams (Table S3). For *U. crassus*, stone loach (*Barbatula barbatula* L.) and common gudgeon (*Gobio gobio* L.) (both recorded in 11 of the 19 studied streams) and common roach (*Rutilus rutilus* L.) (recorded in nine of the 19 studied streams) were the most common species. As a non-native fish species, the topmouth gudgeon, *Pseudorasbora parva* T. & S., was detected in one *M. margaritifera* stream with and in two without recruitment. In *U. crassus* streams, three non-native fish species were found. *P. parva* was detected in seven streams, one stream additionally contained Western tubenose goby (*Proterorhinus semilunaris* Heckel) and pumpkinseed (*Lepomis gibbosus* L.; Table S2).

Total fish density greatly varied among *U. crassus* streams with a mean of 70.5 individuals 100 m^{-2} ± 68.5 *SD* (max: 258.2 individuals 100 m^{-2}; min: 3.0 individuals 100 m^{-2}; Table S2) and *M. margaritifera* with a mean of 32.5 individuals 100 m^{-2} ± 29.5 *SD* (max: 137.5 individuals 100 m^{-2}; min: 1.0 individuals 100 m^{-2}). For *M. margaritifera*, mean number of total fish species (9.0 ± 7.9 *SD*) and the total fish density (39.7 individuals 100 m^{-2} ± 49.8 *SD*) were higher in streams with recruitment than in streams with no recruitment (5.6 species ± 3.8 *SD*; 29.7 individuals 100 m^{-2} ± 18.1 *SD*; Table S3).

3.2.2 | Abiotic Parameters

As expected, the physicochemical measurements in the 22 surveyed *M. margaritifera* and 21 *U. crassus* streams revealed very different habitat conditions. Measured pH values in *M.*

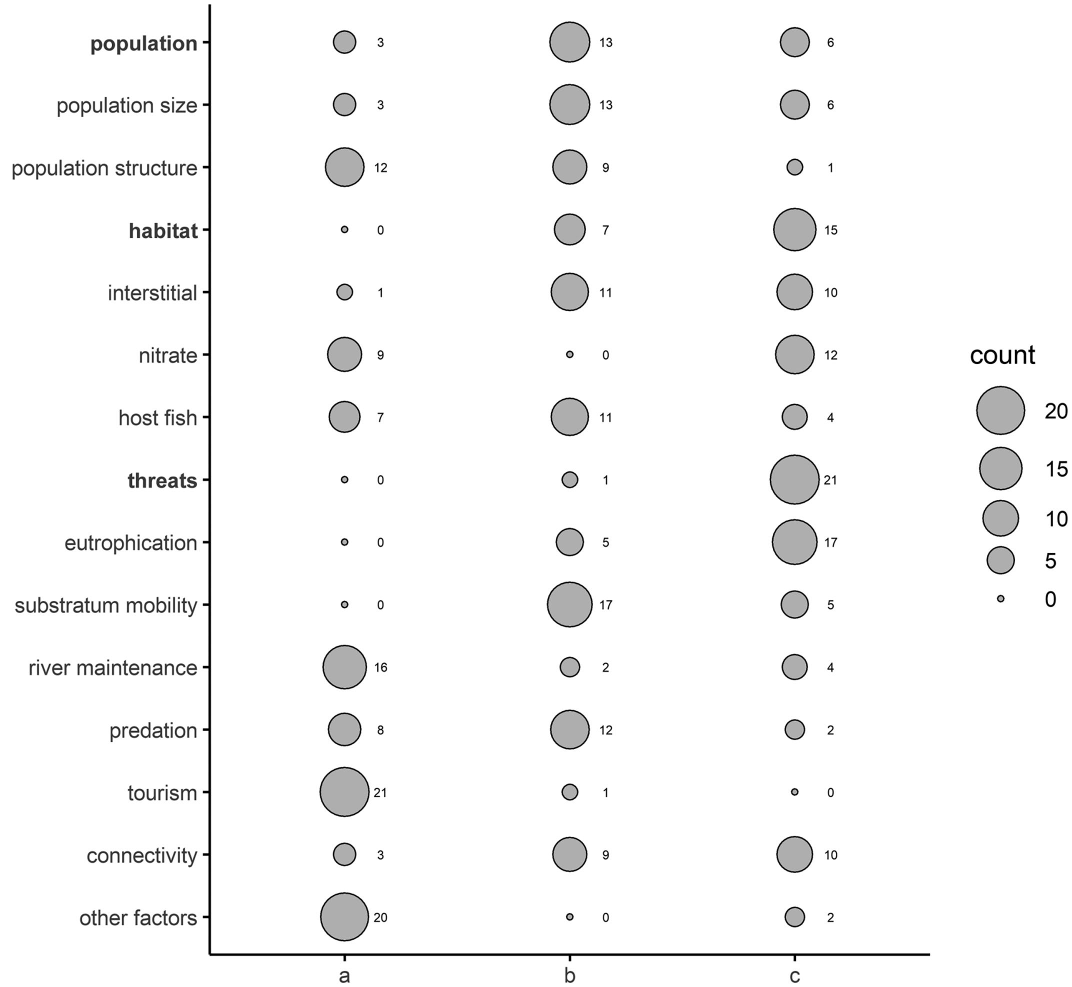

FIGURE 6 | Balloon plot of the assessment of 22 *Unio crassus* populations. The three main categories (in bold) and their subcategories are based on the German assessment scheme and assessed by three indicator categories ('A', excellent; 'B', good; 'C', medium-poor). Ballon size indicates the frequency of each score.

margaritifera streams ranged from 5.6 to 8.3 with a mean of 7.3 ± 0.55 *SD* (Figure 7a). In 15 measurements, values outside the suitable range (>6.1, <8.0) proposed by Sachteleben et al. (2004) were observed. Across all *U. crassus* streams, pH values were higher ranging from pH 7.0 to pH 8.5 with a mean of pH 8.0 ± 0.3 *SD* (Figure 8a). Redox values, as indicators for a long-term oxygen supply, always showed oxic conditions in the free-flowing water with a minimum of 380 mV and a mean of 521 ± 54 mV in *M. margaritifera* streams. In the interstitial zone, only 68.8% of the measurements showed oxic conditions (>300 mV; Schlesinger 1991). The mean delta redox (δEh) potential between free-flowing water and the interstitial zone, which indicates a potential obstruction of the exchange between the water column and the interstitial zone, was 154 ± 108 mV (29.6% ± 20.8 *SD*) in *M. margaritifera* streams (Figure 7b). In contrast, *U. crassus* streams showed a higher delta redox potential between free-flowing water and the interstitial (mean delta redox: 259.2 mV ± 95.1 *SD*, 60.7% ± 24.5 *SD*), indicating more anoxic conditions in the substratum (Figure 8b) and a greater tolerance of this species concerning this parameter. The measured oxygen values varied widely between 5.9 and 13.2 mg L^{-1} (mean: 9.6 mg L^{-1} ± 1.34 *SD*) in *M. margaritifera* streams and between 3.44 and 16.13 mg L^{-1} (mean: 8.4 mg L^{-1} ± 1.8 *SD*) in *U. crassus* streams (Figure 7c; Figure 8c). The given thresholds of 9.0 mg L^{-1} for *M. margaritifera* streams (Moorkens 2000) and of 7.0 mg L^{-1} for *U. crassus* streams were undercut in 59 (from nine *M. margaritifera* streams) of 170 measurements (34.7%) and in 35 (from 12 *U. crassus* streams) of the 179 recorded values (19.5%). With 140 µS cm^{-1} ± 56 *SD* in *M. margaritifera* streams (Figure 7d), the mean conductivity ranged below the threshold of 150 µS cm^{-1} (Sachteleben et al. 2004). In 50 of the 170 measurements (29.4%) this threshold was exceeded. In *U. crassus* streams, conductivity was higher and varied greatly between 138.4 and 1948.0 µS cm^{-2} (mean: 660.7 µS cm^{-2} ± 329.4 *SD*; Figure 8d). The turbidity threshold of 0.96 NTU (Österling, Arvidsson and Greenberg 2010) for *M. margaritifera* streams was only met in eight of the 172 measurements (4.7%), with a mean of 5.44 ± 4.74 NTU (Figure 7e). In *U. crassus* streams, turbidity was more than twice as high with a mean of 13.1 NTU ± 10.1 *SD* (Figure 8e). Flow conditions in *M. margaritifera* streams ranged widely from stagnant water to a maximum flow velocity of 1.89 m s^{-1} (mean: 0.20 m s^{-1} ± 0.25 *SD*). Similar flow conditions were found in *U. crassus* streams with flow velocity ranging from 0.0 to 1.35 m s^{-1} (mean: 0.32 m s^{-1} ± 0.26 *SD*).

Total organic carbon was lower in *M. margaritifera* than in *U. crassus* streams. In *M. margaritifera* streams, total organic

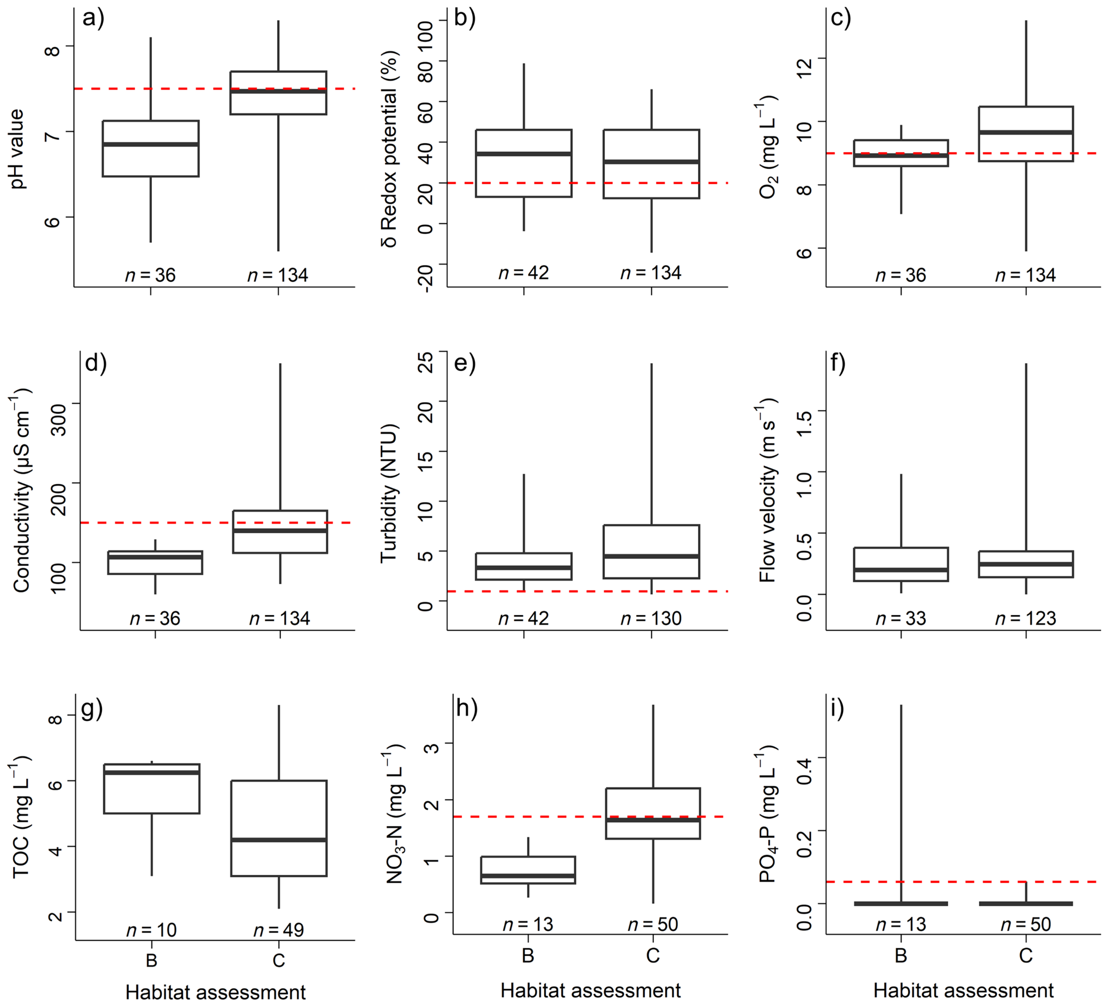

FIGURE 7 | Boxplot of physicochemical parameters in *Margaritifera margaritifera* streams. Boxplots represent the 25 and 75 percentile with the median marked by a horizontal line. The dots represent outliers and red vertical lines show threshold values according to the European Committee for Standardization (CEN) standard (DIN EN 16859) defined for the species.

carbon ranged from 2.1 to 8.3 mg L^{-1} (mean: 4.7 mg $L^{-1} \pm 1.7$ *SD*) and in *U. crassus* streams from 1.8 to 22.0 mg L^{-1} (mean: 6.8 mg $L^{-1} \pm 3.4$ *SD*).

As a eutrophication indicator, mean nitrate-nitrogen values were 1.57 ± 0.84 mg L^{-1} in *M. margaritifera* streams (Figure 7h). The threshold of 1.7 mg L^{-1} (Sachteleben et al. 2004) was exceeded in 23 of 63 measurements (36.5%). In *U. crassus* streams, nitrate-nitrogen ranged from 0.1 to 8.4 mg L^{-1} (mean: 2.1 mg $L^{-1} \pm 1.8$ *SD*) (Figure 8h). The guide value for *U. crassus* streams of 2.0 mg L^{-1} (LfU 2013) was exceeded in 40 of 69 recordings (58.0%). These recordings came from 11 different rivers. Denic et al. (2014) already showed that this could not be a valid threshold for successful recruitment of *U. crassus*, which is also supported by our dataset. Ortho-phosphate-phosphorous was above detection limits (<0.02 mg L^{-1}) in only three of 63 *M. margaritifera* samples and only in one of 69 *U. crassus* samples. The threshold of 0.06 mg L^{-1} for *M. margaritifera* streams (Moorkens 2000) was exceeded in two samples with a maximum amount of 0.54 mg L^{-1} (Figure 7i).

The percentage of fine sediments (<0.85 mm) within the 57 samples from *M. margaritifera* streams ranged from 3.4 to 100.0% (Figure 9a), with a mean value of 24.2 ± 18.9%. The mean percentage of fine sediment in streams with recruitment was $20.5\% \pm 22.5$ *SD* and $25.8\% \pm 17.1$ *SD* in streams without recruitment. In contrast, *U. crassus* streams showed a much higher proportion of fine sediment with a mean of $48.6\% \pm 30.2$ *SD*. For *U. crassus*, no clear trend could be observed between recent population status and the amount of fine sediment (Figure 9b).

3.2.3 | Habitat Evaluation

The habitat quality for *M. margaritifera* was assessed as 'C' in 17 (77%) of the 22 studied streams, which was mainly based on nitrate concentrations > 6.5 mg NO_3 L^{-1} in 11 streams (50.0%), unsuitable structure of substrate and host fish stocks (both 'C' in nine streams; 40.9%) (Figure 5).

For *U. crassus* streams, the habitat quality was rated as 'B' in seven (32%) and as 'C' in 15 (68%) streams (Figure 6). For *U. crassus* streams, the maximum nitrate concentration was >10 mg NO_3 L^{-1} leading to a total 'C' in 12 streams (54.5%) and in 10 streams (45.5%) the interstitial had to be rated as 'C'. However, in

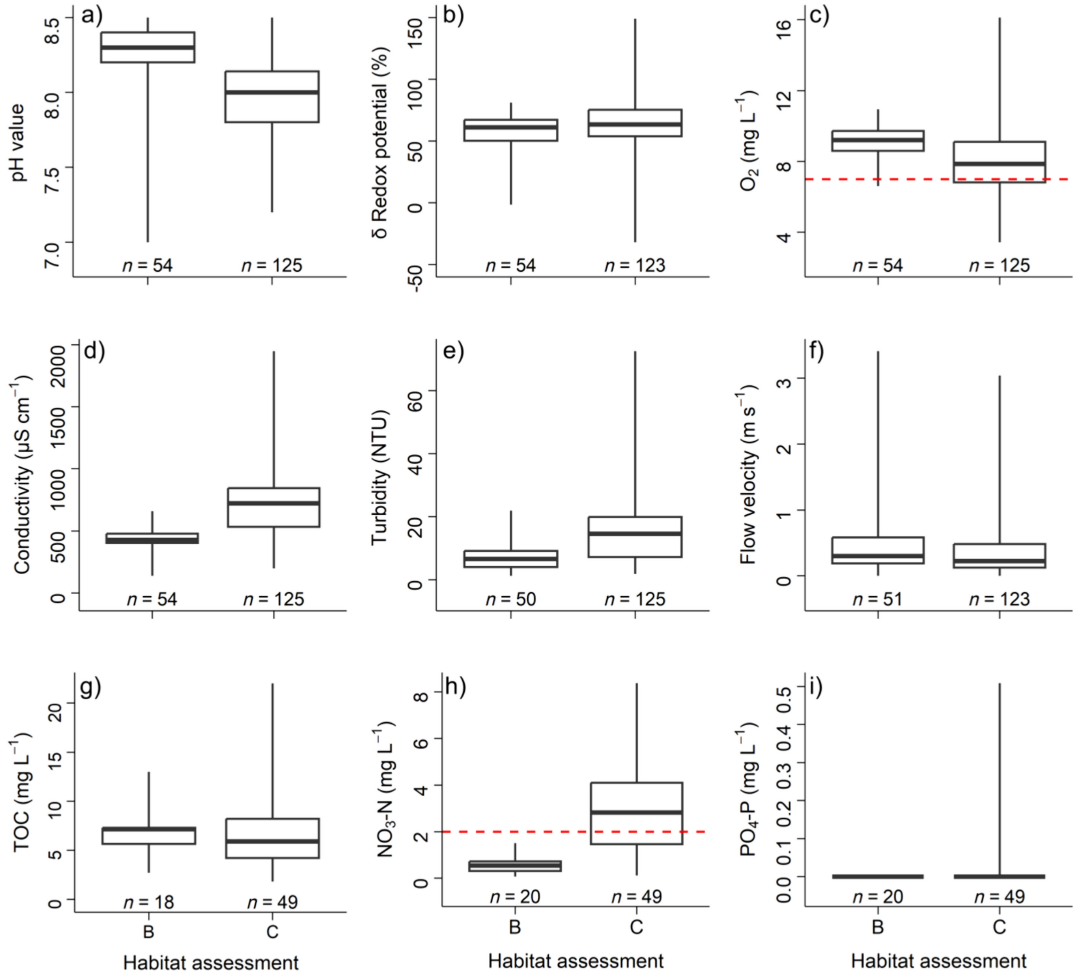

FIGURE 8 | Boxplot of physicochemical parameters in *Unio crassus* streams. Boxplots represent the 25 and 75 percentiles with the median marked by a horizontal line. The dots represent outliers and red vertical lines show threshold values according to the 'Leitfaden Bachmuschelschutz' (LfU 2013).

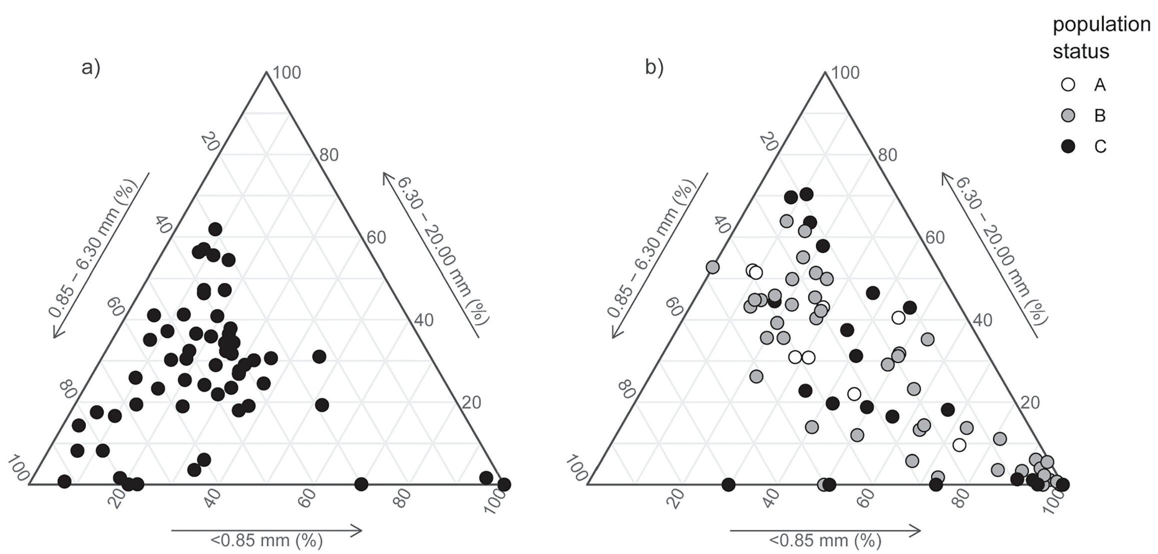

FIGURE 9 | Triangle plots of grain size distribution in a) *Margaritifera margaritifera* and b) *Unio crassus* streams. Different colours represent the population status 'A' (white), 'B' (grey) and 'C' (black).

only four streams (18.2%) a bad condition (rating 'C') of the host fish stock was assessed (Figure 6).

3.2.4 | Threat Assessment

For *M. margaritifera* streams, the threat assessment showed severe impacts (Category C) in 20 (90.9%) of the streams. Impacts on the other two streams (9.1%) were rated as medium (Category B). For *U. crassus* streams, severe impacts (Category C) from threats were observed in 21 of the streams (95.5%). For only one stream, disturbances were rated as weak (Category B). In none of the studied streams, threats were assessed as 'good' (Category A).

Discharge of nutrients and pollutants was found to be the main factor having a severe impact in 72.7% of the investigated *M. margaritifera* and in 77.3% of the investigated *U. crassus* streams. Only one *M. margaritifera* stream did not show indications of corresponding pollution.

Permanently impassable obstacles for host fish (Category C) and a highly elevated sediment shift and input (Category C) were determined in 36.4% of the investigated *M. margaritifera* streams. For *U. crassus*, permanently impassable obstacles for host fish were determined in 45.5%, and sediment shift and input and stream maintenance were rated as severe impacts in 22.7% and 18.18% of the studied streams. Maintenance measures were not found to have any impact on *M. margaritifera* habitats or the mussel populations.

Three *M. margaritifera* streams were given an exceptional rating due to the high risk of drying out. Such events were documented in these streams repeatedly in the years before, so the criterium was rated as a severe impact. This was not the case for any of the *U. crassus* streams.

For *U. crassus* populations, moderate to severe predation damage of more than 10% of the total population (Category C) was detected in 9.1% of the studied streams, and in 54.5%, moderate impact from predation (Category B) was observed. The most common cause was predation by the non-native muskrat (*Ondatra zibethicus* L.), which was identified by typical piles of empty shells on the stream banks. For *M. margaritifera*, predation only by invasive signal crayfish (*Pacifastacus leniusculus* Dana) was observed in three streams (13.6%). Crayfish predation was identified by typical marks on the edge of the shells (Dobler and Geist 2022).

Tourist use did not appear to have any evident and serious impact on the habitats or the mussel populations of both species.

3.2.5 | Habitat Parameters and Population Properties

The PCA revealed a clear, species-specific clustering of the sampling sites. Although *M. margaritifera* sites indicated higher oxygen saturation, lower delta redox and pH values, *U. crassus* streams generally showed a wider range across the variables, between the habitat of the biggest Bavarian population with high TOC values, and streams with high nitrate nitrogen values (Figure 10). The streams containing both, *M. margaritifera* and *U. crassus*, consequently showed indifferent characteristics between the two species-specific clusters (information on stream names is not shown for species protection reasons). Species-specific PCAs also revealed a clear pattern related to the specific overall habitat quality assessment.

3.2.5.1 | *M. margaritifera*. Concentration of nitrate nitrogen, as a criterion for the habitat quality assessment, plays a major role in the comparison between 'B'- and 'C'-rated habitats. In addition, lower pH and conductivity values seem to be distinctive features (Figure 11a).

No correlation was identified between the abiotic factors considered and the proportions of juvenile mussels (Figure 11a). However, in some cases, higher proportions of juvenile mussels resulted from a combination of low population size and high numbers of juveniles from supportive breeding actions (Geist, Thielen, et al. 2023). In the case of *M. margaritifera*, the PCA showed a negative correlation between high nutrient loads, in combination with low TOC values and population size (Figure 11a).

3.2.5.2 | *U. crassus*. In addition to the nitrate nitrogen values, conductivity, turbidity and delta redox values mostly contributed to the difference between the 'B'- and 'C'-rated habitats (Figure 11b). Neither the distribution of juvenile mussel proportions nor the population sizes show any correlations with abiotic parameters (Figure 11b).

4 | Discussion

This study shows a clear contrast between the population status and habitat assessments of *U. crassus* and *M. margaritifera* within the same geographic area. Although for *U. crassus*, stable population sizes and high proportions of juveniles across most populations indicate successful recruitment, populations of *M. margaritifera* are still decreasing and natural reproduction has serious deficiencies. However, the first effects of conservation efforts, such as the rearing and releasing of young mussels, were evident. Despite this situation, the conservation status of both species is still poor, which is mainly based on deficient habitat quality and increasing threats, for example, related to climate change.

4.1 | Population Status

4.1.1 | *M. margaritifera*

Hypothesis (i) was not confirmed for *M. margaritifera*, where an ongoing negative trend in population size was observed. Compared to the results in Stoeckl, Denic and Geist (2020) based on monitoring in 2015, the mean population size in the investigated streams further decreased by about 39% which is highly alarming. Contrary, the proportion of juvenile mussels of *M. margaritifera* increased by 238%. However, this success is mainly a result of the artificial breeding and stocking efforts in prioritized streams which has become a key conservation measure across Europe (Geist, Thielen, et al. 2023). Even though the number of streams presented in Stoeckl, Denic and Geist (2020) is lower ($n=9$) than in this study ($n=22$), juveniles are mainly found in the populations that were investigated in both studies. Nevertheless, a sufficient level of natural recruitment could not be observed in any of the streams. Consequently, no population can be identified as sustainably ‘functional’ (Geist 2010). This is also still the case for the one population that consists entirely of juvenile mussels derived from stocking with infested hosts, as these mussels have not reached maturity and the full reproduction cycle has thus not yet been completed. Since the critical stage of juveniles living buried within the sediment for several years was successfully completed by a high number of mussels and a sufficient amount of hosts being present, this population located in an extensively managed and well-protected area may reach the desirable status in the near future. For all other populations, an ongoing overageing can be seen by the proportions of size classes. Even with higher proportions of juveniles, this trend could not yet be compensated. An increased mortality due to mussels reaching their maximum lifespan will also be likely within the next years, as evidenced by the high proportion of mussels ≥10 cm representing the oldest and largest individuals recorded in this area. It is therefore urgently needed to further increase the proportion of juveniles either from natural reproduction or artificial breeding to stabilize and conserve these populations, along with increased efforts for catchment and habitat restoration (Geist, Thielen, et al. 2023).

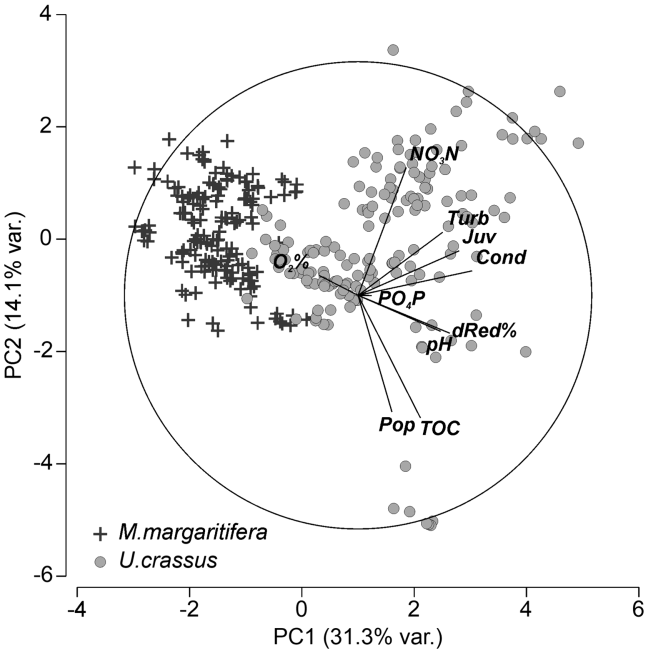

FIGURE 10 | Principal component analysis (PCA) based on the abiotic factors O_2% (oxygen saturation in %), Turb (turbidity in NTU), Cond (conductivity in μS cm^{-1}), pH (pH value), dRed% [percentage difference between the redox value (in mV) in the free water column and the interstitial water in 10 cm depth], water chemistry measurements of NO_3N (nitrate nitrogen in mg L^{-1}), TOC (total organic carbon in mg L^{-1}), PO_4P (ortho-phosphate-phosphorous in mg L^{-1}) recorded at the different monitoring points of the individual streams and the biotic factors Pop (estimated population size) and Juv (percentage of juveniles in %) for the respective stream.

4.1.2 | *U. crassus*

Contrary to the results of *M. margaritifera* and in line with Hypothesis (i), the population status results for *U. crassus* are mostly positive. Compared to Stoeckl, Denic and Geist (2020), the mean population size even increased by about 34%. In this study, population increase was reported for 10 of the studied streams, whereas five populations remained stable and seven declined. The proportion of juveniles slightly decreased by 9% over the studied populations compared with Stoeckl, Denic and Geist (2020). Compared to monitoring results from 2015 (Stoeckl, Denic and Geist 2020 and national monitoring database), the mean age of *U. crassus* in the dataset presented herein slightly decreased in 18 populations from which age structures were available for both periods (Figure 4). This decrease mainly results from mussels older than 16 years, which are missing in

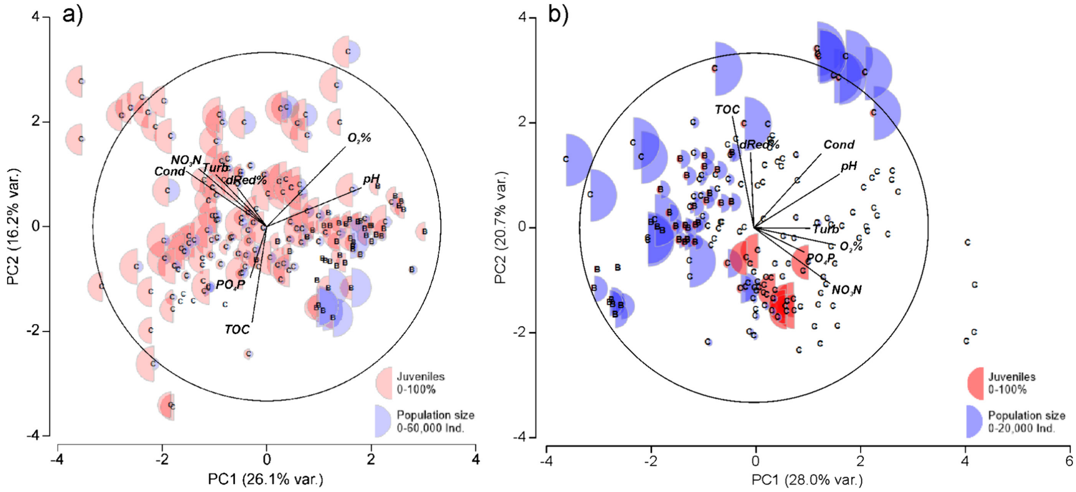

FIGURE 11 | Principal component analysis (PCA) based on the abiotic factors O_2% (oxygen saturation in %), Turb (turbidity in NTU), Cond (conductivity in μS cm^{-1}), pH (pH value), dRed% [percentage difference between the redox value (in mV) in the free water column and the interstitial water in 10 cm depth], water chemistry measurements of NO_3N (nitrate nitrogen in mg L^{-1}), TOC (total organic carbon in mg L^{-1}) and PO_4P (ortho-phosphate-phosphorous in mg L^{-1}) recorded at the different monitoring points of the individual streams. With an overlay indicating the estimated population size (blue) and percentage of juveniles (red) for the respective stream. Labels (B and C) indicate the assessment of the habitat quality of the respective streams. (a) *Unio crassus* and (b) *Margaritifera margaritifera*.

the most recent survey whereas older mussels were present before. In addition, the proportion of mussels older than ten years was observed to be lower than in the monitoring of 2015 (Stoeckl, Denic and Geist 2020). Due to the earlier maturation of *U. crassus* compared to *M. margaritifera* (Lopes-Lima et al. 2017) and the fact that the population sizes often increased (this study), reproduction does not seem to be the bottleneck for this species. The susceptibility of the age structure for *U. crassus*, in contrast to *M. margaritifera*, was found in the older age group (>10 years). Therefore, species-specific constraints need to be considered.

4.2 | Potential Species-Specific Constraints

In line with Hypothesis (ii), differences in threats were identified for both species. At first glance, it is surprising that the monitoring results show different population developments of the two investigated species, even though the ratings for the habitat and threat assessments were similar. This indicates that the habitat requirements and tolerances of the species are different than previously assumed at the time when the assessment schemes were conceptualised. However, previous work (e.g., Dobler et al. 2019; Inoue, Stoeckl and Geist 2017) also suggested a wider ecological niche and greater ecological tolerance of *U. crassus* compared to *M. margaritifera*.

Predation is known to be one of the major threats to European freshwater mussels (Geist, Benedict, et al. 2023; Aldridge et al. 2023; Meira, Byers and Sousa 2024), yet it greatly differs among species. Many species, including muskrat (Haag 2012), crayfish (Meira et al. 2019; Dobler and Geist 2022) and feral pigs (*Sus scofra* L.; van Ee, Nickerson and Atkinson 2020) are known to prey on freshwater mussels. However, predation has only been observed as a threat to *M. margaritifera* in a few individual cases and then only with a minor impact. In addition, only the signal crayfish was observed as a predator for *M. margaritifera* within the study region. Contrary to this, predation plays a major role for *U. crassus* across its distribution range in Bavaria. Not only were direct effects of predation by muskrat evident during our monitoring from typical piles of shells, but predation effects are also well reflected in the age structure. With higher age, the probability of being affected by predation increases for mussels. The missing individuals of the older fraction are likely a result of the generally high predation pressure. Especially in streams where maintenance measures were not found to be a high risk for the mussel population, predation seems to be the only plausible cause for this trend. However, the German assessment scheme (BfN and BLAK 2017) which only results in a classification of 'C' if already >10% of the total mussel population has documented predation effects, obviously underestimates the severity of this impact. The problem is exacerbated by the fact that muskrat predation often occurs mainly in winter (e.g., Zahner-Meike 2000; Stoeckl, Denic and Geist 2020). Since mapping of mussel populations can be carried out until autumn, traces of damage caused by predation can be erased by then, for example, by drifted shells.

For *M. margaritifera*, the lack of recruitment defines the main bottleneck in the life cycle. High proportions of fine sediment and the clogging of macropores were found to be the main driver here, which is in line with previous studies (Bauer 1988; Geist 2010; Österling, Arvidsson and Greenberg 2010; Geist and Auerswald 2007; Hastie, Boon and Young 2000; Stoeckl, Denic and Geist 2020; Denic and Geist 2014). This does not hold true for *U. crassus*, which is much more tolerant to high loads of fine sediment and which even seems to prefer such areas (Denic et al. 2014; Stoeckl and Geist 2016). Based on the dataset of the most recent monitoring presented here, reproduction of *U. crassus* was independent of fine sediment proportions. This contrast to *M. margaritifera* may be explained by the faster development, earlier maturation and the associated shorter time in the interstitial.

4.3 | Host Fish Dependency Considering Land Use and Climate Change Effects

Effects of climate change and land use in the catchment areas such as agriculture, pond management and forestry leading to habitat shifts, have likely resulted in the ongoing colonisation of former *M. margaritifera* habitats by *U. crassus*. In particular, lack of shading from absent riparian vegetation in combination with a high number of ponds draining into the stream can lead to a significant increase in water temperatures during the summer season (Hoess et al. 2022; Garner et al. 2014, 2017). With increasing water temperatures, especially in summer times, when *M. margaritifera* is releasing their glochidia, *S. trutta f.*, depending on low water temperatures (Elliott and Elliott 2010), might withdraw in upstream direction into smaller, colder brooks. With warming waters host fish accessibility, especially for *M. margaritifera* in larger streams decreases, since fish community compositions are changing. In addition to the increased oxygen deficiency within the stream bed at elevated fine sediment concentrations and higher temperatures (Wild, Nagel and Geist 2023, 2024), this effect might intensify the lack of recruitment in both the salmonid hosts and *M. margaritifera*.

Contrary, *U. crassus* reproduction and host fish community, consisting of much more tolerant species (e.g., Taeubert, Gum and Geist 2012; Taeubert et al. 2012), is favoured by these changes (Murdoch, Mantyka-Pringle and Sharma 2020), which might support the colonisation areas further upstream by *U. crassus*. This is in line with the observation of an ongoing shift of the front line of both species in an upstream direction (pers. observation). In addition to temperature changes, increased fine sediment and nutrient input, as effects of land use in the river basin, are known to have an impact on the development of fish communities which exacerbates this development (Murdoch, Mantyka-Pringle and Sharma 2020; Geist, Porkka and Kuehn 2006). As a logical ecological consequence, the different characteristics of the host fish spectra of the two mussel species, further support the different habitat requirements and tolerances of the two species, which consequently require different priority settings for conservation (Hypothesis III).

4.4 | Need for Adjusting Assessment Schemes

The approach to translating complex monitoring data into a harmonized system, such as the German assessment scheme (BfN and BLAK 2017) used in this study and in Stoeckl, Denic and Geist (2020) can be a useful tool to ensure the comparability of the results and to make data on the conservation status widely and easily accessible. This can be a necessary base for the documentation of population development, habitat quality and emerging threats, which are essential for targeted conservation action. However, as shown in this study, several weaknesses of the existing assessment scheme are evident and should be adjusted: First, to show a population development based on monitoring results, comparability over time needs to be ensured (Boon et al. 2019). This was not the case for *M. margaritifera*, for which three populations had to be downgraded from 'B' to 'C' compared to the results of the previous monitoring (Stoeckl, Denic and Geist 2020) due to an alteration in the assessment scheme. Instead of evaluating the population size as was done in 2015 (Stoeckl, Denic and Geist 2020), the population density had to be considered for the recent monitoring. For one of the affected populations, even an increase in population size could not avoid this downgrading. Consequently, this positive development, afforded by successful rearing, hatching and restoration efforts, could not even find recognition in the assessment.

Second, it is also essential that categories are well structured. For a clear and comprehensible assessment of the conditions of habitat, population and other circumstances, these should be structured accordingly. For example, substratum mobility and transport as well as fine sediment input are listed in the German assessment scheme as a threat. Although substratum mobility and transport rather describe an actual characteristic of the habitat, the fine sediment input, resulting, for example, from excessive erosion and unsustainable land use outside of the waterbody, clearly describes a threat, mostly caused by human activities in the tributary. As another example, the availability of suitable hosts is a key factor for the successful recruitment of unionid mussels, and information on this is needed for prioritization of conservation measures (Schwalb et al. 2011). However, the evaluation of the host fish aspect is very brief in the German assessment scheme, in the case of *M. margaritifera*. It is only questioned, whether the fish population is adapted to the specific stream type (BfN and BLAK 2017). In the CEN standard this very significant point receives more attention (see Boon et al. 2019). They propose spring and autumn fishing and the survey of the densities of 0+ or 1-year-old host fish to assess the host fish aspect. In addition, infestation control should be carried out and the number of infested fish should be recorded in the spring (Boon et al. 2019). This information provides an important basis for assessing the reproductive capacity and fertility of pearl mussel populations, which is particularly important for populations living in precarious conditions such as low mussel densities and overaged populations like those in Bavaria.

A third issue that is important for the usability of monitoring results is to adjust the guideline values to the actual, identified demands of the target species to ensure that these values meet their life history traits. As this and previous studies have shown, no connection can be established between the condition of a population and the prevailing substrate composition for *U. crassus* (Denic et al. 2014; Stoeckl and Geist 2016). The previously required guideline values for the fine sediment content of basic substrates for the assessment of a habitat should therefore be revised accordingly. The same applies to the guideline values for nitrate nitrogen, which should be adapted to recent scientific findings (e.g., this study; Douda 2010; Denic et al. 2014; Stoeckl, Denic and Geist 2020). Previous studies have postulated a significantly higher tolerance of *U. crassus* than previously expected (Denic et al. 2014), which is consistent with the results of this study.

5 | Conclusion

This study gives an update on the conservation status of *M. margaritifera* and *U. crassus* in Bavaria. A further decline and progressive ageing of the population was observed for *M. margaritifera*. Again and in line with previous studies, the main drivers for this development are identified and well established. Habitat and catchment restoration, especially in the prioritised streams, must be pushed forward quickly to prevent the extinction of remaining populations. Until the functionality of the habitats and catchments has been restored, it is necessary to maintain the captive breeding of juvenile mussels as an emergency measure to prevent extinction. This method has proven effectiveness and worth, even if this is not fully reflected by the monitoring results yet.

Contrary, *U. crassus* populations surveyed have, on average, shown an increase in numbers and rejuvenation in recent years. The main causes of past population declines do not seem to be fully understood. Because of their comparatively high tolerance to diverse habitat requirements including a wider spectrum of fish hosts, and their wide distribution in anthropogenically created waters, *U. crassus* is also increasingly affected by human influence through construction and maintenance measures (e.g., mowing and digging ditches). This proximity to humans and water bodies heavily used by them, including for transport and recreation, also highly exposes native freshwater mussels such as *U. crassus* to the impact of invasive species (e.g., *O. zibethicus* and *Dreissena ploymorpha*) (Geist, Benedict, et al. 2023; Hillebrand et al. 2024). These existing threats are also clearly identified in this study. In addition to the obvious issues of habitat degradation and predation, there is a much more specific aspect for the thick-shelled river mussel. According to recent genetic findings, this species complex consists of two different species in Bavaria, *U. crassus* and *U. nanus* stat. Rev. (Lopes-Lima et al. 2024). It is therefore necessary to obtain basic knowledge of the habitat requirements of both species, which should be the base for future management approaches.

Despite the differences in their ecological requirements, climate change and extended periods of drought were identified as a common threat for both species. Consequently, restoration of resilient catchments and preparation of emergency and mitigation measures are key.

This study has shown that regular monitoring of the same water bodies assessing population status, habitat quality and host fishes as well as potential threats as proposed by the CEN-standard for *M. margaritifera* can be advantageous to understanding long-term effects within each system, which is key to effective conservation. In this context, a harmonized and evidence-based approach adequately mirroring updated knowledge on the habitat requirements of both species is essential and should be implemented in national monitoring guidelines and across entire species distribution ranges.

Author Contributions

Andreas H. Dobler: conceptualization, funding acquisition, methodology, investigation, project administration, data curation, formal analysis, writing – original draft, writing – review and editing, validation, visualization. **Philipp Hoos**: conceptualization, funding acquisition, methodology, investigation, project administration, data curation, formal analysis, writing – original draft, writing – review and editing, validation, visualization. **Juergen Geist**: Conceptualization, methodology, investigation, project administration, funding acquisition, resources, supervision, validation, writing – review and editing.

Acknowledgements

We thank all of our colleagues and students, Ortwin Ansteeg, Dr. Helmut Bayerl, Alia Benedict, Kilian Boedrich, Hans Buxbaum, Dr. Marco Denic, Nico Geveke, Verena Hagengruber, Dr. Susanne Hochwald, Dr. Rebecca Hoess, Dr. Erwin Kalis, Helen Kehoe, Lea Kirch, Melina Klarl, Lukas Kohl, Merjem Musoska, Lydia Pech, Dr. Stefanie Rüegg, Dima Sarhan, Pauline Schmarsow, Victoria Schottländer, Rebecca Sessa, Dr. Ondrej Spisar, Nathalie Swoboda, Marie von Wesendonk and Amy Yang for their assistance with fieldwork and data preparation. We also thank Dr. Juergen Diemer and Gottfried Forster from the Bavarian Environmental Protection Agency for analysing TOC. This study was financed by the Bavarian Environmental Protection Agency. Open Access funding enabled and organized by Projekt DEAL.

Ethics and Integrity Statements

No ethics approval was required since this study only involved freshwater invertebrates (mussels). With respect to species protection and nature conservation legislation, all samplings were reviewed and approved by the District Governments of Upper Bavaria (reference number: ROB-55.1-8646.NAT_02-9-12-3), of Swabia (reference number: 55.3-8646-2/964), of Middle Franconia (no specific reference number provided), of Lower Bavaria (no specific reference number provided) and of Upper Palatinate (reference number: ROP-SG55.1-8646.4-1-160-7).

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

References

Aldridge, D. C., I. S. Ollard, Y. V. Bespalaya, et al. 2023. "Freshwater Mussel Conservation: A Global Horizon Scan of Emerging Threats and Opportunities." *Global Change Biology* 29: 575–589. https://doi.org/10.1111/gcb.16510.

Anderson, M., R. Gorley, and K. Clarke. 2008. *PERMANOVA+ for PRIMER: Guide to Software and Statistical Methods*. Plymouth, UK: Primer-e.

Araujo, R., and M. A. Ramos. 2001. Action Plan for *Margaritifera margaritifera* in Europe. In *Convention on the Conservation of European Wildlife and Natural Habitats (Bern Convention)*, (Ed.) *Nature and Environment*. Strasbourg: Council of Europe Publishing.

Bauer, G. 1988. "Threats to the Freshwater Pearl Mussel *Margaritifera margaritifera* L. in Central-Europe." *Biological Conservation* 45: 239–253. https://doi.org/10.1016/0006-3207(88)90056-0.

BfN, and BLAK. 2017. "Bewertungsschemata für die Bewertung des Erhaltungsgrades von Arten und Lebensraumtypen als Grundlage für ein Bundesweites FFH-Monitoring." In *BfN-Skripten 480*. Bonn, Germany: Bundesamt für Naturschutz (BfN) & Bund-Länder-Arbeitskreis (BLAK) FFH-Monitoring und Berichtspflicht.

Boon, P. J., S. L. Cooksley, J. Geist, I. J. Killeen, E. A. Moorkens, and I. Sime. 2019. "Developing a Standard Approach for Monitoring Freshwater Pearl Mussel (*Margaritifera margaritifera*) Populations in European Rivers." *Aquatic Conservation: Marine and Freshwater Ecosystems* 29: 1365–1379. https://doi.org/10.1002/aqc.3016.

Bouchet, P., G. Falkner, and M. B. Seddon. 1999. "Lists of Protected Land and Freshwater Molluscs in the Bern Convention and European Habitats Directive: Are They Relevant to Conservation?." *Biological Conservation* 90: 21–31. https://doi.org/10.1016/s0006-3207(99)00009-9.

British Standards Institution. 2017. *Water Quality—Guidance Standard on Monitoring Freshwater Pearl Mussel (Margaritifera margaritifera) Populations and Their Environment*. London, UK: BSI.

Council of the European Communities. 2013. "Consolidated Text: Council Directive 92/43/EEC of 21 May 1992 on the Conservation of Natural Habitats and of Wild Fauna and Flora." *Official Journal of the European Union*.

Denic, M., and J. Geist. 2014. "Linking Stream Sediment Deposition and Aquatic Habitat Quality in Pearl Mussel Streams: Implications for Conservation." *River Research and Applications* 31: 943–952. https://doi.org/10.1002/rra.2794.

Denic, M., K. Stoeckl, B. Gum, and J. Geist. 2014. "Physicochemical Assessment of *Unio crassus* Habitat Quality in a Small Upland Stream and Implications for Conservation." *Hydrobiologia* 735: 111–122. https://doi.org/10.1007/s10750-013-1467-z.

Dobler, A. H., and J. Geist. 2022. "Impacts of Native and Invasive Crayfish on Three Native and One Invasive Freshwater Mussel Species." *Freshwater Biology* 67: 389–403. https://doi.org/10.1111/fwb.13849.

Dobler, A. H., J. Geist, K. Stoeckl, and K. Inoue. 2019. "A Spatially Explicit Approach to Prioritize Protection Areas for Endangered Freshwater Mussels." *Aquatic Conservation: Marine and Freshwater Ecosystems* 29: 12–23. https://doi.org/10.1002/aqc.2993.

Douda, K. 2010. "Effects of Nitrate Nitrogen Pollution on Central European Unionid Bivalves Revealed by Distributional Data and Acute Toxicity Testing." *Aquatic Conservation: Marine and Freshwater Ecosystems* 20: 189–197. https://doi.org/10.1002/aqc.1076.

Elliott, J. M., and J. A. Elliott. 2010. "Temperature Requirements of Atlantic Salmon Salmo Salar, Brown Trout Salmo Trutta and Arctic Charr Salvelinus Alpinus: Predicting the Effects of Climate Change." *Journal of Fish Biology* 77: 1793–1817. https://doi.org/10.1111/j.1095-8649.2010.02762.x.

Garner, G., I. A. Malcolm, J. P. Sadler, and D. M. Hannah. 2017. "The Role of Riparian Vegetation Density, Channel Orientation and Water Velocity in Determining River Temperature Dynamics." *Journal of Hydrology* 553: 471–485. https://doi.org/10.1016/j.jhydrol.2017.03.024.

Garner, G., I. A. Malcolm, J. P. Sadler, C. P. Millar, and D. M. Hannah. 2014. "Inter-Annual Variability in the Effects of Riparian Woodland on Micro-Climate, Energy Exchanges and Water Temperature of an Upland Scottish Stream." *Hydrological Processes* 29: 1080–1095. https://doi.org/10.1002/hyp.10223.

Geist, J. 2010. "Strategies for the Conservation of Endangered Freshwater Pearl Mussels (*Margaritifera margaritifera* L.): A Synthesis of Conservation Genetics and Ecology." *Hydrobiologia* 644: 69–88. https://doi.org/10.1007/s10750-010-0190-2.

Geist, J. 2011. "Integrative Freshwater Ecology and Biodiversity Conservation." *Ecological Indicators* 11: 1507–1516. https://doi.org/10.1016/j.ecolind.2011.04.002.

Geist, J., and K. Auerswald. 2007. "Physicochemical Stream Bed Characteristics and Recruitment of the Freshwater Pearl Mussel (*Margaritifera margaritifera*)." *Freshwater Biology* 52: 2299–2316. https://doi.org/10.1111/j.1365-2427.2007.01812.x.

Geist, J., A. Benedict, A. H. Dobler, R. Hoess, and P. Hoos. 2023. "Functional Interactions of Non-native Aquatic Fauna With European Freshwater Bivalves: Implications for Management." *Hydrobiologia*. https://doi.org/10.1007/s10750-022-05121-2.

Geist, J., M. Porkka, and R. Kuehn. 2006. "The Status of Host Fish Populations and Fish Species Richness in European Freshwater Pearl Mussel (*Margaritifera margaritifera*) Streams." *Aquatic Conservation: Marine and Freshwater Ecosystems* 16: 251–266. https://doi.org/10.1002/aqc.721.

Geist, J., F. Thielen, L. Lavictoire, et al. 2023. "Captive Breeding of European Freshwater Mussels as a Conservation Tool: A Review." *Aquatic Conservation: Marine and Freshwater Ecosystems* 33: 1321–1359. https://doi.org/10.1002/aqc.4018.

Haag, W. R. 2012. *North American Freshwater Mussels: Natural History, Ecology, and Conservation*. Cambridge, UK: Cambridge University Press.

Haag, W. R., and J. D. Williams. 2014. "Biodiversity on the Brink: An Assessment of Conservation Strategies for North American Freshwater Mussels." *Hydrobiologia* 735: 45–60. https://doi.org/10.1007/s10750-013-1524-7.

Hastie, L. C., P. J. Boon, and M. R. Young. 2000. "Physical Microhabitat Requirements of Freshwater Pearl Mussels, *Margaritifera margaritifera* (L.)." *Hydrobiologia* 429: 59–71. https://doi.org/10.1023/a:1004068412666.

Hastie, L. C., P. J. Cosgrove, N. Ellis, and M. J. Gaywood. 2003. "The Threat of Climate Change to Freshwater Pearl Mussel Populations." *AMBIO: A Journal of the Human Environment* 32: 40–46. https://doi.org/10.1579/0044-7447-32.1.40.

Heino, J., R. Virkkala, and H. Toivonen. 2009. "Climate Change and Freshwater Biodiversity: Detected Patterns, Future Trends and Adaptations in Northern Regions." *Biological Reviews* 84: 39–54. https://doi.org/10.1111/j.1469-185X.2008.00060.x.

Hillebrand, V., A. H. Dobler, A. N. Schwalb, and J. Geist. 2024. "Physiological Effects of Interacting Native and Invasive Bivalves Under Thermal Stress." *Biological Invasions*. 26: 2281–2293. https://doi.org/10.1007/s10530-024-03315-4.

Hoess, R., K. A. Generali, J. Kuhn, and J. Geist. 2022. "Impact of Fish Ponds on Stream Hydrology and Temperature Regime in the Context of Freshwater Pearl Mussel Conservation." *Water* 14: 2490. https://doi.org/10.3390/w14162490.

Höök, T. O., C. J. Foley, P. Collingsworth, et al. 2019. "An Assessment of the Potential Impacts of Climate Change on Freshwater Habitats and Biota of Indiana, USA." *Climatic Change* 163: 1897–1916. https://doi.org/10.1007/s10584-019-02502-w.

Inoue, K., K. Stoeckl, and J. Geist. 2017. "Joint Species Models Reveal the Effects of Environment on Community Assemblage of Freshwater Mussels and Fishes in European Rivers." *Diversity and Distributions* 23: 284–296. https://doi.org/10.1111/ddi.12520.

LfU. 2013. *Leitfaden Bachmuschelschutz*. Augsburg: Bayerisches Landesamt für Umwelt (LfU).

Lopes-Lima, M., L. E. Burlakova, A. Y. Karatayev, K. Mehler, M. Seddon, and R. Sousa. 2018. "Conservation of Freshwater Bivalves at the Global Scale: Diversity, Threats and Research Needs." *Hydrobiologia* 810: 1–14. https://doi.org/10.1007/s10750-017-3486-7.

Lopes-Lima, M., J. Geist, S. Egg, et al. 2024. "Integrative Phylogenetic, Phylogeographic and Morphological Characterisation of the *Unio crassus* Species Complex Reveals Cryptic Diversity With Important Conservation Implications." *Molecular Phylogenetics and Evolution* 195: 108046. https://doi.org/10.1016/j.ympev.2024.108046.

Lopes-Lima, M., N. Riccardi, M. Urbanska, et al. 2021. "Major Shortfalls Impairing Knowledge and Conservation of Freshwater Molluscs." *Hydrobiologia* 848: 2831–2867. https://doi.org/10.1007/s10750-021-04622-w.

Lopes-Lima, M., R. Sousa, J. Geist, et al. 2017. "Conservation Status of Freshwater Mussels in Europe: State of the art and Future Challenges." *Biological Reviews* 92: 572–607. https://doi.org/10.1111/brv.12244.

Lummer, E. M., K. Auerswald, and J. Geist. 2016. "Fine Sediment as Environmental Stressor Affecting Freshwater Mussel Behavior and Ecosystem Services." *Science of the Total Environment* 571: 1340–1348. https://doi.org/10.1016/j.scitotenv.2016.07.027.

Lydeard, C., R. H. Cowie, W. F. Ponder, et al. 2004. "The Global Decline of Nonmarine Mollusks." *Bioscience* 54: 321–330. https://doi.org/10.1641/0006-3568(2004)054[0321:tgdonm]2.0.co;2.

Markovic, D., S. Carrizo, J. Freyhof, et al. 2014. "Europe's Freshwater Biodiversity Under Climate Change: Distribution Shifts and Conservation Needs." *Diversity and Distributions* 20: 1097–1107. https://doi.org/10.1111/ddi.12232.

Meira, A., J. E. Byers, and R. Sousa. 2024. "A Global Synthesis of Predation on Bivalves." *Biological Reviews of the Cambridge Philosophical Society* 99: 1015–1057. https://doi.org/10.1111/brv.13057.

Meira, A., M. Lopes-Lima, S. Varandas, A. Teixeira, F. Arenas, and R. Sousa. 2019. "Invasive Crayfishes as a Threat to Freshwater Bivalves: Interspecific Differences and Conservation Implications." *Science of the Total Environment* 649: 938–948. https://doi.org/10.1016/j.scitotenv.2018.08.341.

Moorkens, E. A. 2000. "Conservation Management of the Freshwater Pearl Mussel *Margaritifera margaritifera*—Part 2: Water Quality Requirements." *Irish Wildlife Manuals* 9: 1–44.

Murdoch, A., C. Mantyka-Pringle, and S. Sharma. 2020. "The Interactive Effects of Climate Change and Land Use on Boreal Stream Fish Communities." *Science of the Total Environment* 700: 134518. https://doi.org/10.1016/j.scitotenv.2019.134518.

Österling, M. E., B. L. Arvidsson, and L. A. Greenberg. 2010. "Habitat Degradation and the Decline of the Threatened Mussel *Margaritifera margaritifera*: Influence of Turbidity and Sedimentation on the Mussel and Its Host." *Journal of Applied Ecology* 47: 759–768. https://doi.org/10.1111/j.1365-2664.2010.01827.x.

Pander, J., M. Mueller, and J. Geist. 2015. "A Comparison of Four Stream Substratum Restoration Techniques Concerning Interstitial Conditions and Downstream Effects." *River Research and Applications* 31: 239–255. https://doi.org/10.1002/rra.2732.

Sachteleben, J., C. Schmidt, G. Wenz, and R. Vandré. 2004. *Leitfaden Flussperlmuschelschutz*. Augsburg: Bayerisches Landesamt für Umweltschutz.

Santos, R. M., L. F. Sanches Fernandes, S. G. Varandas, et al. 2015. "Impacts of Climate Change and Land-Use Scenarios on *Margaritifera margaritifera*, an Environmental Indicator and Endangered Species." *Science of the Total Environment* 511: 477–488. https://doi.org/10.1016/j.scitotenv.2014.12.090.

Schlesinger, W. H. 1991. *Biogeochemistry*. San Diego, CA: Academic Press, Inc.

Schwalb, A. N., K. Cottenie, M. S. Poos, and J. D. Ackerman. 2011. "Dispersal Limitation of Unionid Mussels and Implications for Their Conservation." *Freshwater Biology* 56: 1509–1518.

Sousa, R., Â. Amorim, E. Froufe, S. Varandas, A. Teixeira, and M. Lopes-Lima. 2015. "Conservation Status of the Freshwater Pearl Mussel *Margaritifera margaritifera* in Portugal." *Limnologica* 50: 4–10. https://doi.org/10.1016/j.limno.2014.07.004.

Sousa, R., A. Ferreira, F. Carvalho, M. Lopes-Lima, S. Varandas, and A. Teixeira. 2018. "Die-Offs of the Endangered Pearl Mussel *Margaritifera margaritifera* During an Extreme Drought." *Aquatic Conservation: Marine and Freshwater Ecosystems* 28: 1244–1248. https://doi.org/10.1002/aqc.2945.

Sousa, R., D. Halabowski, A. M. Labecka, et al. 2021. "The Role of Anthropogenic Habitats in Freshwater Mussel Conservation." *Global Change Biology* 27: 2298–2314. https://doi.org/10.1111/gcb.15549.

Sousa, R., T. Zajac, D. Halabowski, et al. 2023. "A Roadmap for the Conservation of Freshwater Mussels in Europe." *Conservation Biology* 37: e13994. https://doi.org/10.1111/cobi.13994.

Stoeckl, K., M. Denic, and J. Geist. 2020. "Conservation Status of two Endangered Freshwater Mussel Species in Bavaria, Germany: Habitat Quality, Threats, and Implications for Conservation Management." *Aquatic Conservation: Marine and Freshwater Ecosystems* 30: 647–661. https://doi.org/10.1002/aqc.3310.

Stoeckl, K., and J. Geist. 2016. "Hydrological and Substrate Requirements of the Thick-Shelled River Mussel *Unio crassus* (Philipsson 1788)." *Aquatic Conservation: Marine and Freshwater Ecosystems* 26: 456–469. https://doi.org/10.1002/aqc.2598.

Strayer, D. L., and D. R. Smith. 2003. *Guide to Sampling Freshwater Mussel Populations*. Bethesda, Maryland: American Fisheries Society.

Taeubert, J. E., B. Gum, and J. Geist. 2012. "Host-Specificity of the Endangered Thick-Shelled River Mussel (*Unio crassus*, Philipsson 1788) and Implications for Conservation." *Aquatic Conservation: Marine and Freshwater Ecosystems* 22: 36–46. https://doi.org/10.1002/aqc.1245.

Taeubert, J. E., A. M. P. Martinez, B. Gum, and J. Geist. 2012. "The Relationship Between Endangered Thick-Shelled River Mussel (*Unio crassus*) and Its Host Fishes." *Biological Conservation* 155: 94–103. https://doi.org/10.1016/j.biocon.2012.06.005.

van Ee, B. C., Z. L. Nickerson, and C. L. Atkinson. 2020. "Picky Pigs Prefer Pigtoes: Evidence for Species-Selective Feral pig Predation on Freshwater Mussels." *Freshwater Mollusk Biology and Conservation* 23: 92–98. https://doi.org/10.31931/fmbc.v23i2.2020.92-98.

Vaughn, C. C. 2018. "Ecosystem Services Provided by Freshwater Mussels." *Hydrobiologia* 810: 15–27. https://doi.org/10.1007/s10750-017-3139-x.

VDFF. 2000. *Fischereiliche Untersuchungsmethoden in Fließgewässern*. Offenbach: Verband Deutscher Fischereiverwaltungsbeamter und Fischereiwissenschaftler (VDFF).

Wild, R., C. Nagel, and J. Geist. 2023. "Climate Change Effects on Hatching Success and Embryonic Development of Fish: Assessing Multiple Stressor Responses in a Large-Scale Mesocosm Study." *Science of the Total Environment* 893: 164834. https://doi.org/10.1016/j.scitotenv.2023.164834.

Wild, R., C. Nagel, and J. Geist. 2024. "Multiple Climate Change Stressors Reduce the Emergence Success of Gravel-Spawning Fish Species and Alter Temporal Emergence Patterns." *Science of the Total Environment* 949: 175054. https://doi.org/10.1016/j.scitotenv.2024.175054.

Young, M. R., P. J. Cosgrove, and L. C. Hastie. 2001. "The Extent of, and Causes for, the Decline of a Highly Threatened Naiad: *Margaritifera margaritifera*." In *Ecological Studies. Ecology and Evolution of the Freshwater Mussels Unionoida*, edited by G. Bauer and K. Waechtler. Heidelberger Platz 3, D-14197, Berlin, Germany: Springer-Verlag GmbH and Co. KG Springer-Verlag New York Inc., 175 Fifth Avenue, New York, NY, 10010-7858, USA.

Zahner-Meike, E. 2000. "Die Gefährdung der großen Süßwassermuscheln durch den Bisam (*Ondatra zibethicus*)." *Mitteilungen des Badischen Landesvereins für Naturkunde und Naturschutz* 17: 525–540.

Zieritz, A., R. Sousa, D. C. Aldridge, et al. 2022. "A Global Synthesis of Ecosystem Services Provided and Disrupted by Freshwater Bivalve Molluscs." *Biological Reviews of the Cambridge Philosophical Society* 97: 1967–1998. https://doi.org/10.1111/brv.12878.

Supporting Information

Additional supporting information can be found online in the Supporting Information section.