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Groundwater fauna in an urban area: natural or affected?

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Abstract. In Germany 70 % of the drinking water demand is met by groundwater, whose quality is the product of manifold physical-chemical and biological cleaning processes. As healthy groundwater ecosystems help to provide clean drinking water, it is necessary to assess the ecological conditions of these ecosystems. This is particularly true for densely populated, urban areas, where faunistic groundwater investigations are still scare. The aim of this study is therefore to provide a first-tier assessment of the groundwater fauna in an urban area. Thus, we assess the ecological condition of an anthropogenically influenced aquifer by analysing the groundwater fauna in 39 groundwater monitoring wells in Karlsruhe (Germany) and a nearby forest land. For classification we apply the scheme from the Federal Environmental Agency (UBA), in which a threshold of more than 70 % of Crustaceans and of less than 20 % of Oligochaetes serves as an indication for good ecological conditions. In our study 35 % of the wells in the urban area fulfil these criteria, and even in the pristine forest land only 50 % of the wells indicate fine ecological conditions. While the assessment reveals that ecological conditions in the studied urban area are predominantly not in a good ecological state, there is no clear spatial pattern with respect to land use and anthropogenic impacts. However, there are noticeable differences in the spatial distribution of species and abiotic groundwater characteristics between wells in forest land and the urban area, which indicates that more comprehensive assessment methods are required to fully capture the different effects on groundwater fauna.



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1. Introduction

In Germany 70 % of the drinking water demand is met by groundwater, whose quality is the product of physical-chemical and biological cleaning processes (Avramov et al., 2010). Groundwater ecosystems are responsible for several services that help to provide clean drinking water, which is a vital resource for humanity (Griebler and Avramov, 2015). Bacteria and also fauna play an important role in the biological self-purification of groundwater by the retention of organic matter, natural attenuation of pollutants, storing and buffering of nutrients as well as the elimination of pathogens. Organic matter and pollutants can be degraded and converted to valuable biomass or tied by microbial activity. Protozoa and higher animals can graze resulting biofilms, loosen the substrate and therefore stimulate the biological self-purification (Avramov et al., 2010).

Yet, only healthy groundwater ecosystems can provide clean drinking water. Groundwater ecosystems are sensitive to external influences, such as chemical and thermal disturbances. The latter drives hydro-geochemical and biological processes in groundwater systems, which are relatively isothermal (Brielmann et al., 2009; 20011). Groundwater fauna mainly consists of stygobiote species, which spend their entire life in groundwater and are adjusted to this habitat (Hahn, 2006). Hence, they are assumed to be cold stenotherm, which means that they prefer cold temperatures and can hardly persist water temperatures over 16 °C (Brielmann et al., 2009) or rather 14 °C (Spengler, 2017) for a longer time period.

Nevertheless, groundwater is not yet recognized as a protected habitat in the German and European legislation, as in many countries globally, and there is no common ground on the best practice of assessing groundwater ecology (Hahn et al., 2018; Spengler and Hahn, 2018). The assessment of surface water is typically based on biological, physical-chemical and supported by hydro-morphological criteria (European Water Framework Directive and German legislation article 5 of the 'Regulation on the Protection of Surface Water'). While groundwater quality is mostly assessed by physical-chemical and quantitative criteria, very few quantifiable ecological criteria are available for the assessment of ecosystem health of groundwater.

Results from previous faunistic groundwater analyses are contained in a Germany-wide data record (data record by Hahn, 2005; Berkhoff, 2010; Stein et al., 2012; Gutjahr, 2013; Spengler, 2017; Spengler and Hahn, 2018). A closer look at the southwestern part of Germany, the German federal state Baden-Württemberg, is given by Hahn and Fuchs (2009). This large-scaled study focuses on defining stygoregions, which extend over several square kilometres, and are based on different hydrogeological units. They conclude that observed patterns of groundwater communities reflect a high spatial and temporal heterogeneity of groundwater, with respect to hydrogeological aquifer types (habitat structure, food, oxygen supply). Accordingly, stygobiotic biodiversity is likely to be underestimated at present.

Regional investigations on the spatial variation of groundwater fauna, i.e. stygobiont occurrences, and corresponding environmental parameters, such as geological site characteristics and altitude, are rare (Dole-Olivier et al., 2009; Gibert et al., 2009). An approach to elucidate groundwater biodiversity patterns in six European and seven North-American regions was conducted in the PASCALIS project (Protocol for the ASsessment and Conservation of Aquatic Life In the Subsurface) (Gibert et al., 2009), which aimed at mapping biodiversity and endemism patterns (Deharveng et al., 2009). The study shows that regional processes, such as hydrological connectivity, in a specific habitat (e.g. river floodplains as in Ward and Tockner,



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2001) have a much stronger influence on species composition than local habitat features such as permeability and saturation. Within a region, hydrogeology, altitude, palaeographical factors and human activities can interact in complex ways to produce dissimilar patterns of species compositions and diversity (Gibert et al., 2009). Unfortunately, the PASCALIS sampling protocol recommends selecting hydro-geographic basins that are not strongly affected by human activities such as groundwater pollutions (Malard et al., 2002), and does not biogeographically classify a groundwater system (Stein et al., 2012).

In urban areas anthropogenic impacts, such as a dense building development, underground car parks, open geothermal systems and injections of thermal wastewater from industry result in local thermal alteration of groundwater up to several degrees (e.g. Taylor and Stefan, 2009; Zhu et al., 2011; Menberg et al., 2013b; Tissen et al., 2019). According to Brielmann et al. (2011) annual temperature fluctuations in aquifers, caused by shallow geothermal energy systems, range between 4 °C in winter and 20 °C in summer. In 2000, the European Union (EU) (Water Framework Directive) defined the release of heat in the groundwater as a pollution, whereas the cooling of the groundwater is not particularly mentioned. Until now, there are no scientifically derived threshold values for groundwater temperature in the case of thermal (heat) pollution (Hähnlein et al., 2010; 2013). This results in a tension between conservation, exploitation and thermal use of groundwater. Yet, in an aquifer ecosystem downstream of an industrial facility in Freising (Germany), where groundwater is used for cooling resulting in a warm thermal plume, no relation between faunal abundance and groundwater temperature could be identified (Brielmann et al., 2009). Investigation of hydro-geochemical parameters, microbial activities, bacterial communities and groundwater faunal assemblages indicates that bacterial diversity clearly increases with temperature, while faunal diversity usually decreases with temperature (Brielmann et al., 2009). How exactly these groundwater communities react to changes in temperature and concentration of nutrients, dissolved organic carbon and oxygen, is not yet fully understood (Brielmann et al., 2009; 20011; Spengler, 2017).

Several approaches exist that allow a local assessment of the ecological state of groundwater based on different faunistic, hydro-chemical and physical parameters. The Federal Environmental Agency of Germany (Umweltbundesamt, UBA), for example, developed a concept for an ecologically based assessment scheme for groundwater ecosystems. This two-step scheme characterizes groundwater on two different levels by using the most important physico-chemical parameters, such as content of dissolved oxygen, as well as microbiological and faunistic characteristics such as amount of Oligochaetes and Crustaceans, and comparing these to reference values for natural, undisturbed and ecologically intact groundwater ecosystems (Griebler et al., 2014). Moreover, Korbel and Hose (2017) introduced the Groundwater Health Index (GHI), which is a tiered framework for assessing the health of groundwater ecosystems. The GHI uses biotic and abiotic attributes of groundwater ecosystem to set benchmarks, which provide an indication of ecosystem health. In fact, their study shows that ecosystem health benchmarks are probably more associate with aquifer typology, than being applicable for local areas. The common ground of both studies is the assessment of the ecological condition relative to a reference aquifer and the aim of classifying the locations (GHI: impacted or non-impacted groundwater).

Furthermore, the Groundwater-Fauna-Index (GFI), introduced by Hahn (2006), quantifies the ecological relevant conditions in the groundwater as a result of hydrological exchange between surface and groundwater. It incorporates ecologically



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important groundwater parameters such as relative amount of detritus, variation of groundwater temperature and concentration of dissolved oxygen (Hahn, 2006). Gutjahr et al. (2014) used the GFI as part of a proposal for a groundwater habitat classification at local scale, which introduce five types of faunistic habitats as a result of surface water influence, content of dissolved oxygen and amount of organic matter. Moreover, in the study of Berkhoff (2010) the GFI was used to examine the impact of the surface water influence on groundwater with the aim to develop a faunistic monitoring concept for hydrological exchange processes in the surrounding of waterside filtration plants. Spengler and Hahn (2018) argued for the definition of a regional and ecological temperature threshold and an ecology based assessment of thermal stress in groundwater.

The objective of this study is to investigate specifically the groundwater fauna under an urban area in comparison to a natural forest land. Hence, the groundwater fauna as well as thermal and chemical properties are sampled in 39 groundwater monitoring wells in Karlsruhe, Germany. In our study the classification scheme by the Federal Environmental Agency of Germany (UBA) is applied. The wells are characterized regarding the state of their ecosystem quality. Hence, we finally aim to distinguish areas with natural state of groundwater ecology from anthropogenically disturbed areas.

2. Material and methods

2.1 Study site

The study is performed in Karlsruhe, a city in the Upper Rhine Valley in south-western Germany. The urban region covers an area of 173 km² and has about 310,000 inhabitants (Amt für Stadtentwicklung - Statistikstelle, 2018). The Cenozoic continental rift valley is filled with Tertiary and Quaternary sediments, which are dominated by sands and gravels with minor contents of silt, clay and stones (Geyer et al., 2011). Sporadic layers with lower permeabilities lead to a separation of up to three aquifer levels (Wirsing and Luz, 2007). The upper aquifer is unconfined with a water table between 2 and 10 m below the ground. The flow direction is towards northwest to the Rhine River with groundwater flow velocities ranging between 0.5 and 1.5 m/d (Technologiezentrum Wasser, 2018).

Based on the land use plan of Karlsruhe, about 20 % of the study area is covered by buildings. The rest is characterised by vegetation (~ 56 %) and artificial surface covers (~ 24 %), showing the complexity and heterogeneity of the urban environment. According to Benz et al. (2016), the annual mean groundwater temperature (GWT) in Karlsruhe in the years 2011 and 2012 was 13.0 ± 1.0 °C. Distinct temperature hotspots occur mainly below the city centre, where building densities are highest. In the north-western part of Karlsruhe, the increase of GWT with about 3 K warmer than the land surface temperature (LST) is mainly caused by several groundwater reinjections of thermal wastewater (Benz et al., 2016).

In general, groundwater in the region of Karlsruhe is of good quality so that the local drinking water supplier (Stadtwerke Karlsruhe) only removes oxidised iron and manganese from the pumped groundwater. However, two main contaminations, which affect groundwater quality, are known in the urban area (Stadt Karlsruhe, 2006). A contaminant plume of 200 m length over the entire aquifer thickness is located in the east of Karlsruhe (Figure S1b). This plume contains a polycyclic aromatic hydrocarbons concentration of up to 500 µg/l caused by the former gas plant in the east of Karlsruhe (Kühlers et al., 2012).



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Moreover, three parallel contamination plumes, of 2,500 m length each, can be found in the southeast of Karlsruhe (Figure S1b), where highly volatile chlorinated hydrocarbons (7 μ g/l - 26 μ g/l) and their degradation products were detected (Wickert et al., 2006).

2.2 Material and sampling

From 2011 to 2014, samplings of groundwater parameters and fauna were performed in 39 groundwater monitoring wells in Karlsruhe. At the beginning of each sampling process, temperature and electrical conductivity were measured with an electric contact gauge (Type 120-LTC, Hydrotechnik) at a depth interval of 1 m. Using a bailer (Aqua Sampler, Cole-Parmer), water from the bottom of the groundwater monitoring wells was sampled and the pH value (Multiline Type 3430; WTW GmbH, Weilheim Germany) as well as the contents of dissolved oxygen (Multiline Type 3430; WTW GmbH, Weilheim Germany), iron, nitrate (NO₃-) and phosphate (PO₄-) (RQflex® plus 10 Reflectoquant®; Merck Millipore KGaG, Darmstadt Germany) were measured.

In accordance with suggestion made by Hahn and Gutjahr (2014), several integrative samplings (at least three) were conducted to achieve an ecologically representative sampling of groundwater fauna, which also reflects the occurring species at a community level. As the aim of this study is to provide a first-tier screening of the groundwater ecology, we sample the fauna in the monitoring wells in accordance with the sampling manual of the European PASCALIS Project (Malard et al., 2002) and the procedure described by Hahn and Fuchs (2009), using a modified Cvetkov net.

The organism communities of the groundwater consist of microorganisms and invertebrates (in particular Crustaceans) (Griebler et al., 2014). Crustaceans, especially Amphipods and Copepods, represent the majority of the groundwater fauna. The identification keys from the following studies were used to identify the different groups in the samples: Einsle (1993), Janetzka et al. (1996), Meisch (2000), Schellenberg (1942) and Schminke et al. (2007). The sampled fauna for this study can be assigned to the subphylum *Crustacea* and four other subordinate taxa (Table 1).





145 Table 1: Overview of the sampled fauna, divided into the subphylum *Crustacea* and other subordinate taxa.

Subphylum: Crustacea	Size [mm]	Habitats	Species number
Order: Cyclopoida	0.4 - 0.71	Fresh and marine water, groundwater ¹	22 species of the genus <i>Cyclops</i> worldwide ²
Genus: Parastenocaris	0.3 - 0.51	Tertiary relic living in moss, cavity rooms of streams and in groundwater ¹	16 (most stygophile and stygobiotic) species in Baden-Württemberg ¹
Order: Bathynellacea	0.5 - 5.4 ³	Cavity systems ³ and in groundwater ⁴ (foreign tropical origin) ⁵	Exclusively 160 real groundwater species (stygobiotic) ⁵
Order: Amphipoda	$0.5 - 30^{1}$	Sea, fresh water ¹ and in healthy groundwater ecosystems (important ecosystem service providers ⁶ & biodiversity indicators in Europe ⁷)	321 stygophile and stygobiotic species for Europe ⁸
Other subordinate taxa	Size [mm]	Habitats	Species number
Subclass: Oligochaeta	< 1 – 39	Colonise every habitat, groundwater ⁹	27 stygobiotic species in Europe ⁹ and 100 species worldwide ¹⁰
Phylum: Nematoda	$1 - 3^5$	Colonise every habitat ⁵ and can live under unfavourable conditions ¹¹	20,000 species worldwide ¹²
Class: Turbellaria	$0.4 - 5^{13}$	Sea, brackish and fresh water and groundwater ¹³	The class includes 3,400 species worldwide ¹³
Subclass: Acari	a few mm ⁵	Colonize every habitat, also groundwater, have high demands on water quality ⁵	Worldwide more than 5,000 water mite species ¹⁴
¹ Fuchs et al. (2006) ² Einsle (1996) ³ Sauermost and Freudig (199 ⁴ Camacho (2006) ⁵ Hunkeler et al. (2006)	⁷ Stoch e ⁸ Botosa ⁹ Sauerm	et al. (2009) 12 Ect neanu (1986) 13 Sau	hn et al. (2013) kert et al. (2008) uermost and Freudig (1999c) Sabatino et al. (2000)



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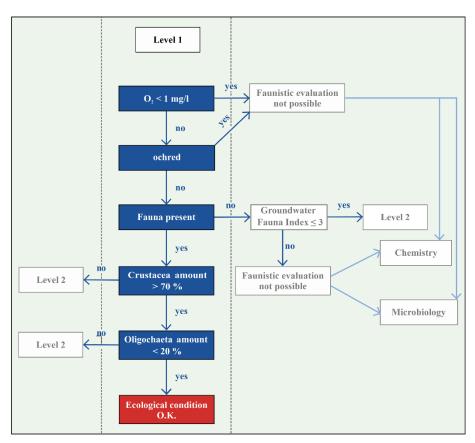
2.3 Classification scheme by the Federal Environmental Agency of Germany

Griebler et al. (2014) developed a two-step classification scheme for the Federal Environmental Agency of Germany (UBA) for characterization of groundwater ecology. They also defined spatially dependent reference values of ecologically intact groundwater ecosystems. In order to enable a statement about the exposure (organic, chemical, structural) of the groundwater at a specific site, biotic and abiotic parameters are used to distinguish locations with ecological conditions, which are O.K., i.e. very good or good ecological conditions, or fail these criteria, i.e. affected (Figure 1). Five criteria, of which at least three biological/ecological indicator parameters are to be selected, are taken as basis for a reliable assessment. The measured indicator parameters of any location have to be compared with the reference values provided by Griebler et al. (2014). Unstressed or natural groundwater habitats are defined as areas with a content of dissolved oxygen > 1.0 mg/l, that are not ochred and have an existing fauna, i.e. an amount of > 50 % of Stygobites, of > 70 % of Crustaceans and of < 20 % of Oligochaetes (Figure 1). This results in a qualitative interpretation of the ecological condition of the groundwater system. If the result indicates affected ecological condition, which means that one or more biological/ecological indicators are out of the reference range, an assessment according to the Level 2 scheme is necessary. This requires to obtain also a qualitative and quantitative interpretation of the ecological conditions. As our aim is a first-tier screening of an urban area, we only apply Level 1 in our study. In addition, Level 2 requires a determination of reference values at local reference locations, which are

well protected and have only a weak surface influence, and a subsequent comparison of these values with measured data.







5 Figure 1: UBA classification scheme according to Level 1 for groundwater ecosystems (modified after Griebler et al. (2014)).

3. Results and discussion

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3.1 Physical and chemical parameters

First, the groundwater conditions in the study site are evaluated by their physical-chemical characteristics. The following values are average values of the individual samplings of each monitoring well. In order to allow a spatially differentiated assessment, the study site is classified into two separate zones based on land use types: (1) Forest area (local name: Hardtwald), (2) Urban area containing industrial, commercial and residential areas (Figure 2a).



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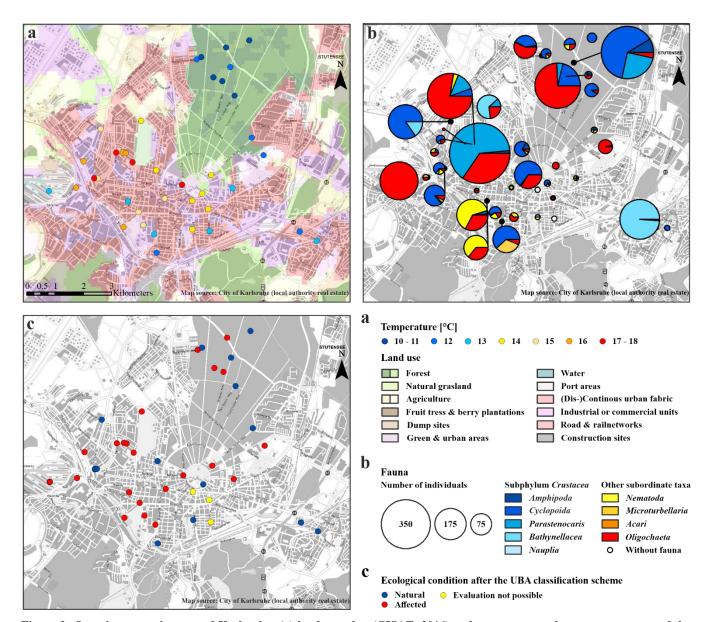


Figure 2: Overview map city area of Karlsruhe: (a) land use plan (GISAT, 2016) and average groundwater temperature of the multiple measurements [°C] at the bottom of the monitoring wells; (b) detailed groundwater fauna: colours of the circles shows the different taxa in the sample [%], the size indicates the number of individuals; (c) faunistic evaluation according to UBA classification scheme.

As expected, measured GWT at the bottom of the wells, in 8.5 to 39.0 m depth, are mainly constant over the repeated measurements. The lowest GWT ranging between 10.5 and 10.9 °C were measured in the eight wells of the forest area (Table S1). In contrast, the highest average GWT with 17.5 °C was measured in a well near the city hospital (T113) (Figure 2a). The mean value of all wells is 13.5 ± 2.1 °C, which is similar to the results from Benz et al. (2015) with 13.0 ± 1.0 °C. According to Benz et al. (2017), annual shallow GWT vary between 6 and 16 °C in the area of Karlsruhe, which is in line with the



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temperatures measured during fauna sampling (Figure 3a). For the urban area in the north-western part of the city, Figure 2a shows a clear warming trend, which was also observed by Menberg et al. (2013a,b). The increased GWT in this area can be traced back to effects of urban infrastructures and industries, which use groundwater for cooling purposes.

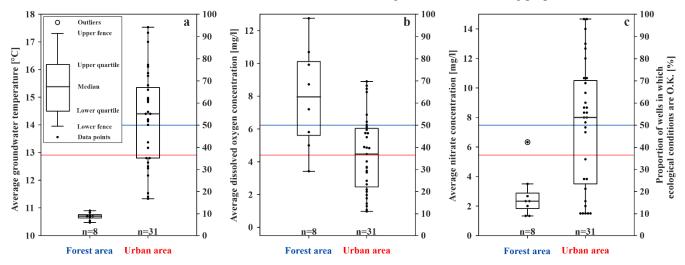


Figure 3: Boxplots of the physical and chemical parameters for the forest and urban area in the study site and the proportion of wells in which ecological conditions are O.K. [%], second axis; (a) average temperature of the repeated measurements [$^{\circ}$ C] at the bottom of the monitoring wells; (b) average content of dissolved oxygen [mg/l] of the monitoring wells; (c) average nitrate content [mg/l] of each monitoring well. (n = number of wells)

The content of dissolved oxygen acts as a limiting factor for groundwater fauna, since groundwater is usually under-saturated with a varying oxygen content between 0 and 8 mg/l (Griebler et al., 2014; Kunkel et al., 2004). In this study, the average content of dissolved oxygen in all wells is between 1.0 and 12.8 mg/l (Figure 3b and Figure S1a). As expected, the monitoring wells, located in the forest area (Hardtwald) show the highest content, while the lowest values are found in urban areas, which is likely linked to aquifer contamination and other anthropogenic effects. Urban water can be polluted in multiple ways, which affects the chemical and biological oxygen consumption in the groundwater. The higher the pollution and/or biological activity, the lower the dissolved oxygen (Griebler et al., 2014; Kunkel et al., 2004).

Nitrate is often named as an important pollutant in groundwater. The natural and geogenic concentrations of nitrate in groundwater is usually under 10 mg/l (Griebler et al., 2014). In our study area, the average nitrate content of all wells varies between 1.3 and 14.7 mg/l. In the urban area average nitrate concentrations are high and correlate inversely with the dissolved oxygen showing the link between pollution and oxygen consumption. The lowest nitrate concentrations are found in the forest area (Figure 3c and Figure S1c), where atmospheric nitrogen is hold back by forest soils. At the same time, anthropogenic impact is minimal as fertilization is forbidden due to the presence of water protection areas in the forest area (Aber et al., 1998; Schönthaler and von Adrian-Werburg, 2008).

Within the study, the average contents of iron and phosphate are low and in most cases below the detection limit of the test (Figure S1d, e) and also below the natural and geogenic concentrations (phosphate: 0.05 mg/l (Griebler et al., 2014) and iron: 3.3 mg/l (Kunkel et al., 2004)).



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Considering these findings, clear differences in the spatial distribution of abiotic groundwater characteristics are noticeable. The rural forest area shows a lower average GWT, lower nitrate concentrations and higher dissolved oxygen concentrations, which indicates a correlation between abiotic groundwater characteristics and land use in the study area.

210 **3.2** Groundwater fauna

In the entire samples 3,633 individuals were detected in 37 of 39 wells (Table S2). With 2,014 individuals, the group of *Crustacea* was found to be the most abundant group (55 %). 976 individuals (27 %) of the order of *Cyclopoida* dominated this group, followed by the genus *Parastenocaris* with 599 individuals (17 %), by the order of *Bathynellacea* (371), *Amphipoda* (66) and *Nauplia*. The communities of the monitoring wells also frequently contained Oligochaetes (1,343 individuals, 37 %). Furthermore, individuals of the phylum *Nematoda* (228 individuals) and *Microturbellaria* (46 individuals) were also often present.

Overall, there is a noticeable difference in the spatial distribution of species within the study area. Individuals of the subphylum *Crustacea* were found in larger numbers, with regard to the number of wells, in the monitoring wells in the forest area (660 individuals in eight wells) than in the urban area (1,354 individuals in 31 wells). Furthermore, no individuals of the order *Bathynellacea* and only 135 individuals of the genus *Parastenocaris* were found in the forest area. In contrast, larger numbers of the latter species as well as of Oligochaetes are characteristics of the wells in the urban area. However, in contrast to the abiotic characteristics, no clear pattern of faunal diversity and land use was observed, as Crustaceans and individuals of other subordinate taxa were found both in the rural forest and in the urban area.



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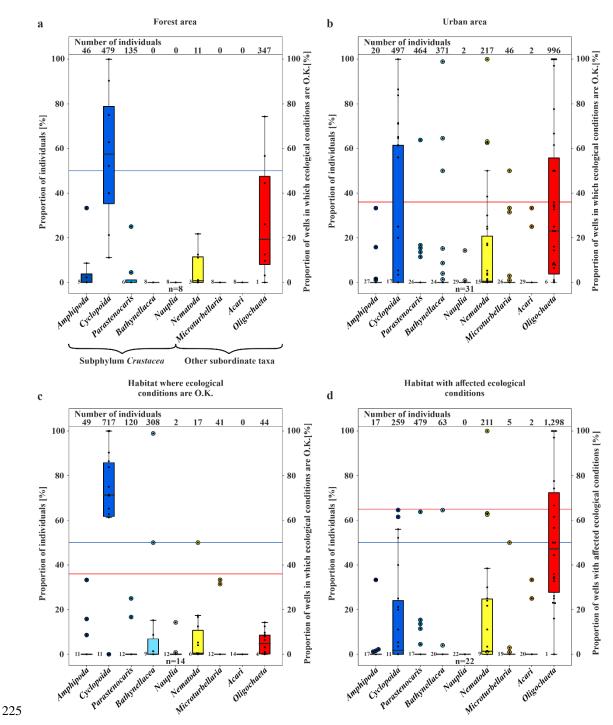


Figure 4: Boxplots of the amount of fauna [%]: (a) proportion of individuals and of wells in which ecological conditions are O.K. (second axis) [%] of the forest area; (b) proportion of individuals and of wells in which ecological conditions are O.K. [%] of the urban area; (c) proportion of individuals and of wells in which ecological conditions are O.K. [%] divided based on the results of the UBA classification scheme; (d) proportion of individuals and of wells with affected ecological conditions [%] divided based on the results of the UBA classification scheme. The colour of the boxes shows the different taxa in the samples. (n = number of wells)



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Stygobiotic Amphipods, large-bodied invertebrates that predominantly live within wells (Table 1) (e.g. Hahn and Matzke, 2005; Korbel et al., 2017), were found only in three wells (Figure 2c). 46 individuals of this order were detected in the forest and 20 individuals in the urban area (Figure 4a,b). As mentioned above, Amphipods indicate healthy groundwater ecosystems, as they react most sensitive to disturbances such as pollutants (Korbel and Hose, 2011) as well as groundwater temperature (11 ± 5 °C (Brielmann et al., 2011) up to 17 °C (Spengler, 2017; Issartel et al., 2005)). They are important ecosystem service providers in terms of bioturbation and organic decomposition (Boulton et al., 2008), as they show an active movement, with possible migration speeds between 1.7 and 3.5 x 10^4 m per year observed in laboratory experiments (Smith et al., 2016). If Amphipods were found, in most cases higher amounts of individuals of the order *Cyclopoida* were also identified. Individuals of the latter order were mostly be found in larger quantities in the majority of the wells (479 in the forest area and 497 in the urban area), as they are the largest group of Crustaceans in this environment (Fuchs et al., 2006) and can tolerate a wide temperature range (Spengler, 2017).

Lager numbers of *Parastenocaris* (464 individuals), which can tolerate GWT from 8 to > 20 °C (Fuchs et al., 2006) (e.g. *Parastenocaris phyllura* up to 22.5 °C in laboratory tests; Glatzel, 1990), were found in the urban area, especially in the northwest area (Figure 2b). This area is characterised by GWT between 16 and 18 °C, the highest at the study site. This observation is comparable with previous studies (Hahn, 2006; Hahn et al., 2013; Spengler, 2017), which showed that the genus *Parastenocaris* is particularly non-competitive and can often be found isolated in structurally burdened and physico-chemically altered areas. Accordingly, only 135 individuals were detected in the forest area.

In addition, quantities of *Bathynellacea* were found in five monitoring wells in a depth of 9 to 13.5 m and by medium GWT (12-15 °C), all located in the urban area (371 individuals), respectively (Figure 4b). This order typically inhabits the interstitial groundwater, which is characterised by a dominant exchange with the surface water and high variations in GWT, and can tolerate temperatures up to 18 °C (Stein et al., 2012). Interestingly, one location in the southern city area with 272 individuals is characterised by a high fluctuation in GWT (standard deviation of 3.4 °C) and a rather high nitrate content (8.3 mg/l), which are both indications for a disturbed and stressed habitat.

Besides the group of Crustaceans, Oligochaetes, which can tolerate a wide temperature range, were also found in large abundance in the study site. A significant amount of the subclass *Oligochaeta* (996 individuals) was found in the urban area (Figure 4b), compared to an overall number of 1,343 individuals. In general, the number of Oligochaetes is larger in locations with high GWT (12.6 -17.3 °C) and high nitrate concentrations (up to 14 mg/l).

Finally, Nematodes and Microturbellarians were found at locations with unfavourable living conditions, such as a low content of dissolved oxygen, or a high amount of fine substrates as also reported by Hahn et al. (2013). Both can tolerate high temperature ranges (Turbellaria: $2-20^{\circ}$ C (Herrmann, 1985), Acari: $9.1-18.5^{\circ}$ C (Więcek et al., 2013)). Here, both were found in larger quantities in the urban area of Karlsruhe (Figure 4b). This area has the lowest content of dissolved oxygen, high relative amount of detritus (> 2) and the highest nitrate concentrations (> 6 mg/l).

Eventually, correlation analysis between groundwater fauna and the chemical parameters showed that Stygobites are only slightly affected by groundwater quality. Only the Spearman's rank correlation coefficient ρ between the number of taxa and



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the content of dissolved oxygen is significant with a value of $\rho = 0.53$ (p-value = 0.0005, n = 39). The natural influence on porosity, groundwater flow and nutrient delivery were also discussed as primary influence on natural Stygobites distribution by previous studies (Hahn, 2006; Korbel and Hose, 2015).

Some limitations regarding the sampling method have to be considered when interpreting the faunistic results. In this study, a simple basic screening of well water was conducted, using net sampler and bailer, to assess conditions in the groundwater monitoring wells (39 wells with an average diameter of 132.5 mm, which corresponds to an area of 0.003 ‰ of the total urban area). According to the sampling manual of the PASCALIS Project 'the use of a phreatobiological net alone is considered as a satisfactory method for sampling groundwater fauna in large diameter wells' (Malard et al., 2002). Yet, several studies (e.g. Scheytt, 2014) report that scooped samples of wells are not representative, and therefore the water remaining in a well has to be purged and discarded before sampling. Other studies, on the other hand, found no significant differences in hydro-chemical values between the surrounding groundwater and the standing water in a well (Hahn and Matzke, 2005; Korbel et al., 2017). The sampled groundwater fauna of corresponding wells and aquifers were also shown to be similar with respect to types of faunal communities, in terms of total abundance however, as well as the numbers of individuals per litre, monitoring wells appear to exhibit larger numbers, caused by filtration effects (Hahn and Matzke, 2005; Hahn and Gutjahr, 2014; Korbel et al., 2017). In order to achieve a representative sampling of groundwater fauna in the aquifer and to reflect the occurring species at a community level a more comprehensive sampling method is required, e.g. the use of a defined standard sampling method using a pump to collect animals (Malard et al., 2002).

3.3 Classification scheme by the Federal Environmental Agency of Germany

In three wells evaluation with the UBA classification scheme was not possible due to ocherous conditions in two monitoring wells and low content of dissolved oxygen (<1 mg/l) in the third well. According to the UBA classification scheme, unstressed or natural groundwater habitats have an amount of more than 70 % of Crustaceans and less than 20 % of Oligochaetes. In 36 % of the sampled wells, i.e. 14 out of 39, these criteria are fulfilled, indicating O.K. ecological conditions or in other words a natural groundwater habitat (Figure 4c). These natural areas tend to contain more individuals of the orders *Amphipoda*, *Cyclopoida* and *Bathynellacea*. Monitoring wells, which do not fulfil these criteria and are accordingly defined as affected areas not having natural ecological conditions, contain more Oligochaetes and also Nematodes, which is partly explained by the used criteria of this classification scheme (Figure 4d).

Surprisingly, only 50 % of the wells in the rural forest, which is also the catchment area of the drinking water supply of Karlsruhe, are described as natural groundwater habitats. An identical number of wells yielded habitats with affected ecological conditions. The main difference between natural and affected wells in the forest area arises from the occurrence of specific species. Natural wells have an amount of Crustaceans of 86 to 100 %, in contrast to affected wells with only 33-67 % (Table S1 and Table S2). However, the abiotic parameters scarcely differ between natural and affected wells (average values for GWT: 10.8 and 10.6 °C, dissolved oxygen: 7.1 and 8.8 mg/l, nitrate: 2.5 and 3.0 mg/l), indicating that there are other processes or parameters that influence the groundwater fauna in these wells. One reason could be the varying local geology as fine sands





and silts are typical rather harsh environments resulting in an impoverishment of specific groundwater fauna such as *Crustacea* (Hahn, 1996).

In contrast to the forest land, the majority of wells (65 %) in the urban area are categorised as affected habitats. As expected, this indicates anthropogenically influenced groundwater ecosystems beneath the studied urban area. Once more no significant differences between the abiotic parameters of natural and affected wells are observed (e.g. median of dissolved oxygen: 4.7 and 5.8 mg/l, median of nitrate: 7.2 and 7.8 mg/l). On the other hand, the remaining 35 % of the wells in the urban area show natural ecological conditions, even though some of them are located in areas with anthropogenic impacts such as increased groundwater temperatures. Hence, no distinct spatial pattern of the ecological condition with respect to land use could be identified. This observed spatial heterogeneity in ecological conditions and heat anomalies in an urban area therefore also offer the potential to use groundwater for heating and cooling, and even to locally store energy in form of aquifer thermal energy storage (ATES) systems (e.g. Fleuchaus et al., 2018).

4. Conclusion

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The aim of this study is to provide a first-tier assessment of the ecological state of groundwater in an urban area and to distinguish areas with a natural state of the groundwater ecology from anthropogenically affected areas. To achieve this, we examine the groundwater fauna, as well as abiotic parameters in 39 groundwater monitoring wells in the urban area of Karlsruhe, Germany, and a nearby forest land using the simple UBA classification scheme to characterise the sampled monitoring wells.

We found a noticeable difference in the spatial distribution of abiotic groundwater characteristics and special species within the study area. The rural forest area shows lower GWT, lower nitrate concentrations and higher dissolved oxygen concentrations, which indicates a correlation between abiotic groundwater characteristics and land use. However, no clear spatial pattern regarding faunal diversity and land use was found, as both in the rural forest and in the urban area Crustaceans and individuals of other subordinate taxa were widely found. In terms of faunal quantity, Crustaceans were found in larger numbers, with respect to the number of wells, in the monitoring wells in the forest area than in the urban area. Larger amounts of the genus *Parastenocaris* as well as of Nematodes and Oligochaetes were found to be characteristics for wells in the urban area.

Furthermore, no clear spatial pattern of ecological groundwater conditions according to the UBA classification scheme could be observed. Surprisingly, only 50 % of the sampled wells in the rural forest were described as natural (undisturbed) groundwater habitats, while the other four were characterised as habitats with affected ecological conditions. Yet, the majority of wells (65 %) in the urban area were classified as affected locations, which suggest, that there are noticeable differences in the groundwater ecosystems between the surrounding rural areas and urban areas. Thus, further studies with larger-scale and repeated measurement campaigns are needed to verify our findings also in other cities, and to provide undisturbed local reference values which are required for a more reliable and also quantitative ecological assessment of urban aquifers. Finally,





city and also energy planning should seriously consider urban groundwater ecosystems as they provide valuable information for a sustainable use of the subsurface.

Data availability

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Author contributions

PB and HJH provided the topic and supervised the work, together with KM. SS and CS executed the field work and evaluated the samples. FK evaluated the collected data and interpreted as well as visualised the results and wrote the first draft of the paper. KM, CS, HJH and PB participating in editing the paper.

Competing interests

The authors declare that they have no conflict of interest.

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Review statement





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