

Spatial and temporal analysis of groundwater ecosystems

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“Die Welt ist im Wandel. Ich spüre es im Wasser. Ich spüre es in der Erde. Ich rieche es in der Luft.“

- *Galadriel (Der Herr der Ringe - Die Gefährten von J. R. R. Tolkien)* -

Dedicated to all my loved ones who have accompanied me on this journey.

ABSTRACT

Groundwater is an important source of freshwater, drinking water, service water for irrigation, industrial and geothermal uses. Moreover, groundwater is the largest terrestrial freshwater biome of the world, with ecosystems inhabited mainly by invertebrates (stygo fauna) and microbes, that undertake important services, including water purification, as well as nutrient and carbon cycling. This habitat is naturally and anthropogenically threatened in many areas, yet only healthy groundwater ecosystems help to provide these important services. In many parts of the world, even the most basic knowledge of these ecosystems is lacking. Thus, this thesis aims to improve the understanding of ecological processes and conditions in groundwater, which is essential for sustainable resource and environmental management in times of competing groundwater uses. To achieve this groundwater fauna is analysed and assessed in detail on different spatial and temporal scales.

The first study of this cumulative thesis provides an overview on groundwater fauna (stygo fauna) research, including the historical evolution of research topics and the development of sampling methods. To investigate the global distribution of groundwater fauna research and identify resulting data gaps, data from 859 studies is reviewed. From this, it is apparent that there has been an exponential increase in the number of groundwater fauna studies over the last ten decades, together with changing paradigms in the research focus. Furthermore, sampling methods have developed from using simple nets, substrate samples and hand-pumps in the beginning to recent advances in molecular methods. Finally, studies on groundwater fauna are spatially unevenly distributed and are dominated by research in Australia and Europe, with few studies in Africa, Asia, and the Americas. This biased view on groundwater fauna hinders the identification of biodiversity patterns and ecosystem functions on a global scale.

The second study uses long-term groundwater data from South-West Germany to identify shifts in groundwater fauna due to natural and/or anthropogenic impacts. Thus, a comprehensive spatial and temporal analysis of metazoan groundwater fauna and abiotic parameters from 16 monitoring wells over two decades is conducted. No overall temporal trends for fauna abundance

and biodiversity and no significant large-scale trends in abiotic parameters are observed. Nine wells out of 16 show stable ecological and hydro-chemical conditions at a local level. The remaining wells exhibit shifting or fluctuating faunal parameters between individual years, indicating more complex temporal behaviours on a local scale. On the one hand, these temporal changes can be linked to natural causes, such as decreasing dissolved oxygen contents or fluctuating temperatures. Moreover, by examining aerial images of the surroundings of three individual wells, it is revealed that anthropogenic impacts, such as construction sites, can cause significant shifts in groundwater fauna and changes in the ecological status.

Changes in hydrology and surface conditions are increasingly occurring in densely populated urban areas, which is why investigating urban aquifers becomes increasingly important. Thus, the ecological status of an anthropogenically influenced aquifer is examined in the third study by analysing fauna and hydrogeological, physico-chemical parameters in 39 groundwater monitoring wells in an urban area and compared to a forested area outside the built-up area of the city of Karlsruhe (Germany). Statistical analyses confirm noticeable differences in the spatial distribution of abiotic groundwater characteristics, such as lower groundwater temperature and higher dissolved oxygen content in the forested area, and thus indicate a correlation between abiotic characteristics and land use. Moreover, spatial differences in the species distribution are visible. However, no clear spatial pattern is found regarding faunal diversity and land use. The groundwater ecosystem status index is applied for classification of the ecological status of groundwater, but shows no clear spatial patterns concerning land use and other anthropogenic impacts. Thus, it is therefore not possible to obtain a clear result on the ecological status with existing assessment approaches in urban areas.

This thesis demonstrates that groundwater is a complex ecosystem affected by multiple stressors. Groundwater shows heterogeneous conditions as a habitat, even at a local scale. Moreover, changes in hydro(geo)logy and surface conditions, such as land use, influence groundwater fauna. Thus, this thesis reveals that the understanding of this ecosystem is important to obtain clear information on its ecological status.

KURZFASSUNG

Grundwasser ist eine wichtige Quelle für die Versorgung mit Süßwasser, Trinkwasser sowie mit Brauchwasser für die Bewässerung, ebenso wie für industrielle und geothermische Zwecke. Darüber hinaus ist das Grundwasser das größte terrestrische Süßwasserbiom der Welt mit einem Ökosystem, das hauptsächlich mit wirbellosen Tieren (Stygofauna) und Mikroben besiedelt ist, die wichtigen Aufgaben wie die Wasserreinigung sowie die Erhaltung des Nährstoff- und Kohlenstoffkreislauf übernehmen. In vielen Regionen der Welt ist dieser Lebensraum jedoch durch natürliche und anthropogene Einflüsse bedroht. Dies ist kritisch, da nur gesunde Grundwasserökosysteme zu wichtigen Ökosystemleistungen beitragen. Zudem fehlt in vielen Teilen der Welt selbst das grundlegendste Wissen über diese Ökosysteme. Ziel dieser Arbeit ist es daher, die ökologischen Bedingungen und Prozesse im Grundwasser besser zu verstehen, was in Zeiten konkurrierender Grundwassernutzungen für ein nachhaltiges Ressourcen- und Umweltmanagement unerlässlich ist. Im Einzelnen wird die Grundwasserfauna dafür auf verschiedenen räumlichen und zeitlichen Skalen erfasst und bewertet.

Die erste Studie dieser kumulativen Dissertation liefert einen globalen Überblick über die Grundwasserfaunaforschung, einschließlich der historischen Entwicklung der Forschungsinhalte und der Probenahmeverfahren. Um die globale Verbreitung der Grundwasserfaunaforschung und die bestehenden Datenlücken zu ermitteln, werden Daten von 859 Studien analysiert. Es zeigt sich, dass in den letzten zehn Jahrzehnten die Zahl der Studien zur Grundwasserfauna exponentiell zugenommen hat, was mit einem Paradigmenwechsel bei den Forschungsschwerpunkten einherging. Außerdem haben sich die Probenahmemethoden von der anfänglichen Verwendung einfacher Netze, Substratproben und Handpumpen zu neueren molekularen Analysen weiterentwickelt. Zuletzt zeigt sich, dass Studien zur Grundwasserfauna global ungleichmäßig verteilt sind und von der Forschung in Australien und Europa dominiert werden, während in Afrika, Asien und Amerika nur wenige Studien vorhanden sind. Diese einseitige Sichtweise auf die Grundwasserfauna erschwert die Bestimmung von Biodiversitätsmustern und Ökosystemfunktionen in einem größeren Maßstab.

In der zweiten Studie dieser Arbeit werden Langzeitdaten für Grundwasser aus Südwestdeutschland verwendet, um durch natürliche und/oder anthropogene Einflüsse ausgelöste Veränderungen der Grundwasserfauna zu identifizieren. Dazu wird eine umfassende räumliche und zeitliche Analyse der metazoischen Grundwasserfauna und abiotischer Parameter an 16 Messstellen über zwei Jahrzehnte durchgeführt. Dabei wurden keine großskaligen zeitlichen Trends für die Abundanz und die biologische Vielfalt der Fauna sowie keine signifikanten großräumigen Trends bei den abiotischen Parametern festgestellt. Neun der 16 Brunnen zeigen stabile ökologische und hydrochemische Bedingungen auf lokaler Ebene. Die übrigen Brunnen weisen zwischen einzelnen Jahren wechselnde oder schwankende Faunenparameter auf, was auf komplexere zeitliche Zusammenhänge auf lokaler Ebene hinweist. Diese zeitlichen Veränderungen sind zum einen auf natürliche Ursachen zurückzuführen, wie z. B. abnehmende Gehalte an gelöstem Sauerstoff oder schwankende Temperaturen. Anhand von Luftbilderanalysen der Umgebung von drei einzelnen Brunnen wird zudem festgestellt, dass anthropogene Einflüsse, wie z. B. Baustellen, zu erheblichen Verschiebungen der Grundwasserfaunagemeinschaften und Veränderungen des ökologischen Zustands führen können.

Besonders in dicht besiedelten städtischen Gebieten kommt es zunehmend zu Veränderungen der Hydrologie und der Oberflächenbedingungen, weshalb die Untersuchung städtischer Grundwasserleiter immer wichtiger wird. Daher wird in der dritten Studie der ökologische Zustand eines anthropogen beeinflussten Grundwasserleiters durch die Analyse der Fauna und hydrogeologischer, physikalisch-chemischer Parameter in 39 Grundwassermessstellen in einem städtischen Gebiet im Vergleich zu einem Waldgebiet außerhalb des bebauten Gebietes der Stadt Karlsruhe (Deutschland) untersucht. Die Analysen bestätigen erkennbare Unterschiede in der räumlichen Verteilung der abiotischen Grundwassereigenschaften, wie z. B. niedrigere Grundwassertemperaturen und höhere Konzentrationen an gelöstem Sauerstoff im Waldgebiet, und weisen somit auf einen Zusammenhang zwischen abiotischen Eigenschaften und der Landnutzung hin. Darüber hinaus sind räumliche Unterschiede in der Artenverteilung zu erkennen, während bei der Faunendiversität und der Landnutzung kein klares räumliches Muster erkennbar ist. Zur Klassifizierung des ökologischen Zustands des Grundwassers wird der "Grundwasserökosystem Status Index" herangezogen, der keine klaren räumlichen Muster in Bezug auf die Landnutzung und andere anthropogene Einflüsse erkennen lässt. Eine eindeutige Bewertung des ökologischen Zustands mit den bestehenden Bewertungsansätzen im urbanen Raum ist daher nicht möglich.

Zusammenfassend zeigt diese Arbeit, dass Grundwasser ein komplexes Ökosystem ist, welches von zahlreichen Stressfaktoren beeinflusst wird. Selbst auf lokaler Ebene weist das Grundwasser als Lebensraum heterogene Bedingungen auf. Darüber hinaus beeinflussen Veränderungen der Hydro(geo)logie und der Oberflächenbedingungen, wie etwa durch Landnutzung, die Grundwasserfauna. Schlussendlich zeigt diese Arbeit, wie wichtig das Verständnis dieses Ökosystems ist, um klare Informationen über seinen ökologischen Zustand zu erhalten.

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ACRONYMS AND SYMBOLS

Acronyms

BW	Baden-Württemberg
CLC	CORINE Land Cover
DNA	Deoxyribonucleic Acid
EC-WFD	European Union Water Framework Directive
eDNA	environmental Deoxyribonucleic Acid
EH	Shannon Equitability/Evenness Index
eRNA	environmental Ribonucleic Acid
EPI	Ecophysiological Index
EU	European Union
GDE	Groundwater-Dependent Ecosystems
GFI	Groundwater Fauna Index
GHI	Groundwater Health Index
GWT	Groundwater temperature
H	Shannon Index
HS	Shannon Diversity Index
GESI	Groundwater Ecosystem Status Index
LST	Land Surface Temperature
LUBW	Landesanstalt für Umwelt Messungen und Naturschutz Baden-Württemberg

MDS	MultiDimensional Scaling
NIWA	National Institute of Water and Atmospheric Research
NSW	New South Wales
NZ	New Zealand
PASCALIS	Protocols for the ASsessment and Conservation of Aquatic Life In the Subsurface
PCR	Polymerase Chain Reaction
PHATE	Potential of Heat-diffusion for Affinity-based Trajectory Embedding
PPCS	Postojna–Planina Cave System
QLD	Queensland
RNA	Ribonucleic Acid
UBA	Federal Environmental Agency of Germany - Umweltbundesamt
UK	United Kingdom
VIC	Victoria
WA	Western Australia
wGHI^N	weighted Groundwater Health Index Nitrates

Constants

Latin symbols and variables

e.g. exempli gratia

etc. et cetera

i.e. id est

Greek symbols and variables

ρ Spearman rank correlation coefficient

Operators and math symbols

h hours

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1

INTRODUCTION

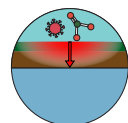
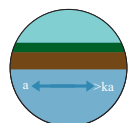
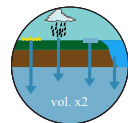
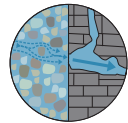
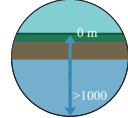
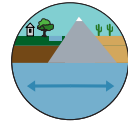
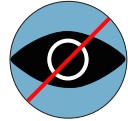
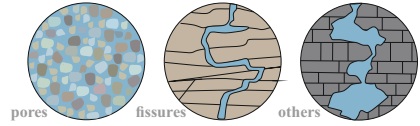
1.1 General motivation

Nearly 71 % of the earth's surface is covered by water (Shiklomanov and Rodda, 2003), which is the basic prerequisite for life. Nevertheless, only three per cent of the total water resources on Earth are freshwater (Meissner and Mampane, 2009), with groundwater accounting for approximately 99 % of all liquid freshwater (Shiklomanov and Rodda, 2003) or 15.9 million km³ (only the fresh groundwater component) (Ferguson et al., 2021).

Groundwater is part of the water cycle and, therefore, an open system in contact with terrestrial and aquatic systems. It is recharged via precipitation and infiltration of surface water or artificial recharge and discharged on the surface by springs, wetlands, entering surface water (exempli gratia (e.g.) lakes, rivers, et cetera (etc.)) and exploration, e.g. by wells. Per definition groundwater includes all water below the water table in the subsurface, the so-called saturated zone, where all cavities are entirely filled with water and whose movement is determined exclusively by gravity (Verein Deutscher Ingenieure e.V. (VDI), 1994).

Thus, groundwater

- is present in pores, fissures and other voids within geological formations,
- is invisible, hidden to the naked eye and often poorly known and understood by society,
- is a spatially distributed resource, which is virtually ubiquitous and extends laterally under most of the land surface,
- has a large lateral extent, but also a significant vertical dimension (3D geometry), from very close to the land surface to great depths, down to thousands of metres,
- is generally moving very slowly, except for karst formations, mainly because subsurface lithological matrix offers a hydraulic resistance to flow many orders of magnitude higher than the hydraulic resistance experienced in open channel flow,
- is stored in large volumes in the subsurface, exceeding annual groundwater replenishment by two orders of magnitude, on average,
- ages commonly vary widely from recent to tens of millennia, while groundwater quality (salinity and other quality parameters) may also be subject to significant variation due to long residence times and contact with the lithological matrix and subsurface biosphere and
- due to the overburden's resistance to flow, groundwater systems are usually better protected against pollution, but once polluted they are much more difficult to remediate, with shallow groundwater domains more vulnerable to pollution than deeper ones (United Nations, 2022).



In addition, groundwater is a vital resource for humanity, as it provides a variety of services. One existing scheme to classify services of whole ecosystems is the Millennium Ecosystem Assessment classification scheme, which divides groundwater ecosystem services into four categories: supporting, provisioning, regulating, and cultural services (Figure 1.1) (Millennium Ecosystem Assessment, 2005). Provisioning services are services that allow humans to use water for specific purposes. One-third of the world population uses groundwater as a source of drinking water (Sampat, 2000). Moreover, groundwater provides 49 % of the water volume for domestic use by the global population (Food and Agriculture Organization of the United Nations (FAO), 2023; Margat and Gun, 2013) and around 25 % of all water for irrigation, serving 38 % of the world's irrigated land. Alongside the use of groundwater for irrigation or other agricultural purposes (69 %) and drinking water/domestic use (22 %), 9 % of the total global amount of groundwater is used as cooling and process water for industrial purposes, mineral water and energy supply (geothermal use: heat and cold storage¹)(Avramov et al., 2010; Job, 2022; Siebert et al., 2010; Stauffer et al., 2013). According to the United Nations (2022), the demand of groundwater is projected to grow by 1 % per year over the next 30 years.

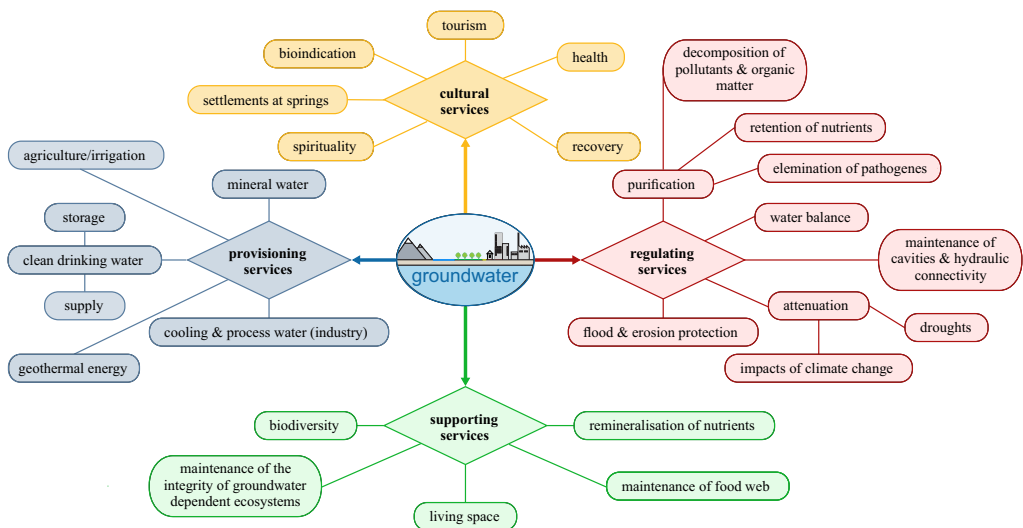


Figure 1.1: Services provided by groundwater and its ecosystem classified according to the Millennium Ecosystem Assessment classification scheme (Millennium Ecosystem Assessment, 2005).

Besides, there are some more important services provided by groundwater. Cultural services include more indirect services linked to leisure activities, local recreation, tourism, traditions, religion or spiritual values associated with a specific site (caves, springs, etc.) (Avramov et al.,

¹ numbers varied between the continents and is estimated by the United Nations, 2022

2010). In addition, there are regulating services, which are in-situ services regulating groundwater systems' quantity and quality regimes. For instance, these services reflect the buffer capacity of aquifers, as groundwater with surface connection is a retention body for higher amounts of surface water, which helps to reduce droughts, as well as floods and protects against erosion (United Nations, 2022).

On the contrary, in-situ services on which groundwater-dependent ecosystems and other groundwater-related environmental features rely are supporting services (United Nations, 2022). Groundwater is also the largest terrestrial freshwater living space of the world (Gibert et al., 1994; Griebler et al., 2014b; Hose et al., 2022) and its ecosystems are essential for energy and material flow (Boulton et al., 2008). For instance, they enable water transmission by bioturbation (movement, burrowing) as higher organisms maintain cavities and thus the hydraulic connectivity (Hose and Stumpp, 2019; Mermillod-Blondin et al., 2023; Mermillod-Blondin and Rosenberg, 2006; Nogaro et al., 2006; Stumpp and Hose, 2017). This contribution is also called ecosystem engineering activity, since organisms (ecosystem engineers) 'directly or indirectly control the availability of resources to other organisms by causing physical state changes in biotic or abiotic materials' (Jones et al., 1997). Moreover, water is purified by microorganisms. During the so-called self-purification of groundwater, microorganisms contribute to the biogeochemical cycling, as well as to the elimination of pathogens, organic matter and anthropogenic contaminants. In addition, they retain nutrients. If the distribution of substances in the underground is prevented solely by microbial degradation or sedimentation (in the case of metals), this is referred to as natural attenuation. Furthermore, groundwater fauna promote microbial growth and contribute to the food web as they process organic matter by grazing biofilms of bacteria (Avramov et al., 2010; Griebler and Avramov, 2015; Mermillod-Blondin et al., 2023). Last but not least, microorganisms and fauna in groundwater can be used as bioindicators. Knowing the natural boundary conditions, changes occurring in the ecological patterns (abundance, activity, species range and community composition) can be used to assess the ecological status of groundwater (Avramov et al., 2010).

Nevertheless, the ecosystem services concept is not yet commonly implemented in the routine of water-regulation practice (Carpenter et al., 2006). However, a 'framework of ecosystem services is a powerful tool to raise awareness in human society of the various benefits we receive and use from ecosystems each day' (Griebler and Avramov, 2015).

1.2 Groundwater ecosystems

Although groundwater is the largest terrestrial freshwater biome of the world (Gibert et al., 1994; Griebler et al., 2014b; Hose et al., 2022), groundwater ecology is a relatively young research discipline with research and knowledge lagging behind that of surface ecosystems such as lakes and streams (Griebler et al., 2014b). However, groundwater ecosystems have been investigated for over a hundred years, with the first description of eyeless and depigmented animals in a cave in 1537 (Myroie, 2004). Groundwater is a species-rich habitat, with over 25,000 species (up to 100,000 species in Culver and Holsinger (1992) and Martinez et al. (2018)) and communities consisting of a highly diverse biota (Malard et al., 2023; Marmonier et al., 2023).

The basis of groundwater ecosystems and their food web is built by bacteria, archaea and fungi (Griebler and Lueders, 2009; Humphreys, 2006). These microorganisms are ubiquitous in groundwater and build small colonies or occur as single cells, and are mainly attached to sediments as biofilms. Due to the low nutrient and oxygen supply, microbial diversity and activity in aquifers are very low compared to surface water (Griebler and Lueders, 2009). The community structure of groundwater fauna is mainly influenced by the intensity of surface influence, such as the input of carbon and oxygen, and by structure (id est (i.e.) available cavities) of the aquifer (Avramov et al., 2010).

Depending on the pore space in aquifers, groundwater can be colonised by micro-fauna (*Protozoa*, *Rotifera*, *Turbellaria* and *Nematoda*) (see Figure 1.2) and/or by large-bodied meio-fauna (Humphreys, 2006). Thus, pore space and the structure of the aquifer matrix are important determinants of the presence of organisms. Alluvial aquifers with sandy and silty sediments, and therefore small pore spaces, typically inhabit only a few small invertebrates. In contrast, aquifers with larger fractures, karstic voids or coarse sand and gravel deposits show more invertebrates (Hose et al., 2015). Due to their size, vertebrates such as salamanders and fishes occur only in karst and pseudokarst (Humphreys, 2006, 2009). Moreover, the meio- and macro-fauna in groundwater are mainly invertebrates dominated by arthropods. As can be seen in Figure 1.2, Crustaceans (*Copepoda*, *Syncarida*, *Amphipoda*, *Ostracoda* and *Isopoda* (Hose et al., 2014; Humphreys, 2006)) usually make up to 50 % of the species abundance and richness (Korbel and Hose, 2011). Still, also a wide range of other phyla like molluscs (snails), other arthropods (mites, insects (beetles)) and various worms (*Platyhelminthes*, *Annelida*) (Hose et al., 2015; Humphreys, 2009) can be found in groundwater. The group of *Copepoda* (red colour in Figure 1.2) occurs nearly ubiquitously in subterranean habitats, with over 1,000 known groundwater species in six orders (Galassi et al., 2009). Still, species richness in groundwater is largely unknown, and its distribution is only sketchily understood (Galassi et al., 2009; Gibert and Culver, 2009; Gibert and Deharveng, 2002; Stoch and Galassi, 2010).

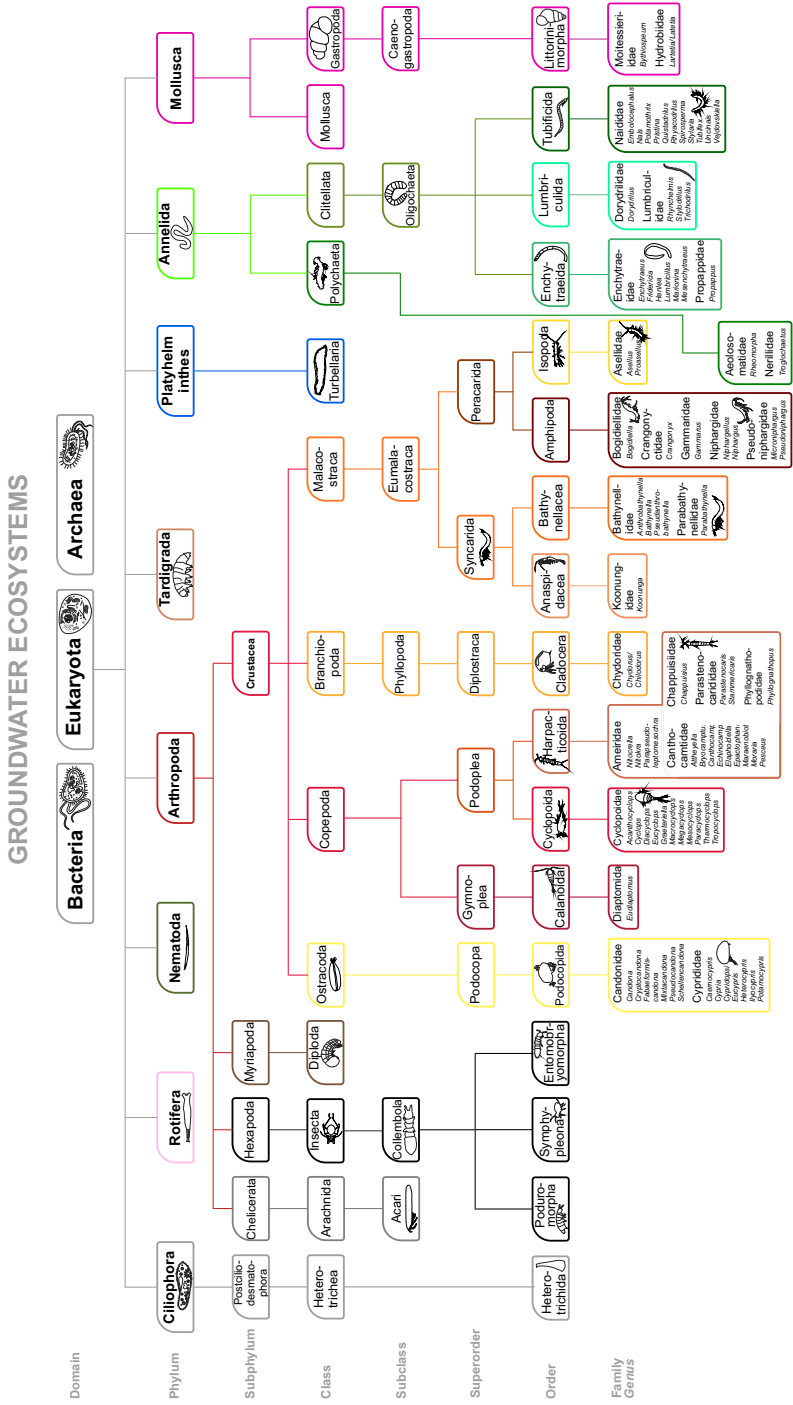


Figure 1.2: Taxonomy of groundwater ecosystems based on the underlying literature of this thesis.

1.2.1 Stygofauna

Fauna populating groundwater is known as stygofauna. In this context, the prefix ‘stygo’ derives from the Greek language and means ‘hateful’. This is in connection with the river Styx from ancient Greek mythology, which flowed underground and carried the souls of the dead to Hades. Thus, in the mythological context, stygobionts were the creatures living in the watery regions of the underworld (Botosaneanu, 1986). First, Lazare Botosaneanu used the term ‘stygofauna’ in the scientific literature in his study *Stygofauna Mundi* (Botosaneanu, 1986), describing the subterranean aquatic fauna of the world (Goater, 2009).

Groundwater is typified by extremes, like total darkness, lack of space, low oxygen and nutrient content, and no primary production of energy. These extreme conditions hinder the colonisation of this habitat. Thus, stygofauna comprises stygobionts, stygophiles, and stygoxenes species depending on their ecological preferences (Gibert et al., 1994; Hahn, 2006). Stygoxenic fauna inhabits surface water or soil and is transported to groundwater accidentally or occasionally, and is not able to reproduce there (Dumnicka et al., 2017; Mösslacher, 1998; Stein et al., 2010). Stygophilic species are affiliated with ground and surface waters and can survive considerable times in groundwater and reproduce there (Mösslacher, 1998; Stein et al., 2010). They use alluvial sediments or caves as a refuge from predators or changing environmental conditions at the surface (floods, droughts, etc.) (Knight and Penk, 2010). Stygobiontic species are obligate hypogean and exclusively inhabit groundwater. They are specialised to a subterranean life and spend their entire life cycle in this habitat. Thus, stygobiontic species show morphological, physiological and behavioural adaptations, such as a long, thin body shape and small size, ocular regression and hypertrophy of sensory organs, a lack of pigmentation, a relatively long life span and a reduced metabolic and reproduction rate, which are linked to environmental limitations (Galassi, 2001; Gibert and Culver, 2009; Gibert et al., 1994; Knight and Penk, 2010; Rétaux and Jeffery, 2023). Due to the dependence on food and oxygen supply from the surface, the abundance of stygofauna decreases with depth and distance from input pathways. Thus, stygofauna is rarely found below 100 m ground level or in locations with a dissolved oxygen concentration in groundwater below 0.3 mg/L. Moreover, stygofauna, which is stranded in unsaturated sediments, can hardly survive for more than 48 hours (h) and has limited capacity to recover from disturbances and changes in water quality from natural background (Hose et al., 2015).

Moreover, the number of stygobionts in a groundwater site is generally low compared to the surface diversity (Culver and Sket, 2000; Stoch and Galassi, 2010). The absence of higher amounts of energy in groundwater systems results in low biomass and biodiversity, which is why the food web in aquifers relies on inputs of nutrients and carbon from the surface (Hose et al., 2015). Thus, the colonisation of groundwater differs from that of surface water. Due to isolation and fragmentation stygobionts are characterised by a high degree of short-range endemism (Harvey, 2002), resulting

in a low local diversity compared to regional diversity, as much larger differences in species composition among sampling sites are found for surface-dwelling freshwater fauna in comparable spatial units (Gibert and Culver, 2009; Gibert and Deharveng, 2002). In addition, the occurrence of only a few lineages results in an over-representation of a few major taxa and under-representation of others. Moreover, there are many relict species ('living fossils') and truncated food webs with very few predators conceivably caused by a scarce food supply (Gibert and Deharveng, 2002). Pressure for food and resources has resulted in the diversification of niches in some fauna (e.g. Ercoli et al., 2019; Fišer et al., 2019), which is why similar morphological, physiological and behavioural traits are creating low inter-species variability across many biological attributes (Hose et al., 2022). Another characteristic of groundwater is the rarity of stygobiontic species, which is why typically only half of all stygobiontic species in any given region are found at less than five per cent of sites (Castellarini et al., 2004; Hahn, 2015; Martin et al., 2009). There are typically few species in one location, but many across different locations (Dumnicka et al., 2020).

1.2.2 Natural and anthropogenic impacts on groundwater ecosystems

In groundwater, with the exception of karst aquifers, environmental conditions are mostly stable, resulting in a low water flow, a small thermal range and low temperatures, with a variation of 1 – 2 °C over a year and little or no seasonality (Avramov et al., 2010; Gibert et al., 1994; Notenboom, 1991; Taylor and Stefan, 2009). Globally, multiple natural and human stressors threaten groundwater and its specialised ecosystems and have the potential to alter groundwater community structure (e.g. Korbel et al., 2022a) and compromise ecosystem functions, resulting in the deterioration of groundwater health (Hancock, 2002; Hancock et al., 2005). A distinction between quantitative and qualitative impacts can be made in this context. Quantitative impacts affect the volume or structure of a groundwater body (Danielopol et al., 2003). A common impact of this category is groundwater abstraction for drinking water, irrigation and mining activities (see Figure 1.3) (Danielopol et al., 2003; Hancock et al., 2005). The abstraction of groundwater alters groundwater levels and surface water groundwater connectivity, causing desiccation and death for particular stygobiontic biota (Korbel et al., 2019; Patel et al., 2020). These pressures are associated with rising global exploitation of groundwater relating to a general demographic increase. They are particularly prevalent in less developed countries in Asia, Africa and South America (Vörösmarty et al., 2000; Wada et al., 2010). Additionally, mining activities can result in dewatering and removal of surrounding material from the groundwater body (Danielopol et al., 2003; Hancock et al., 2005).

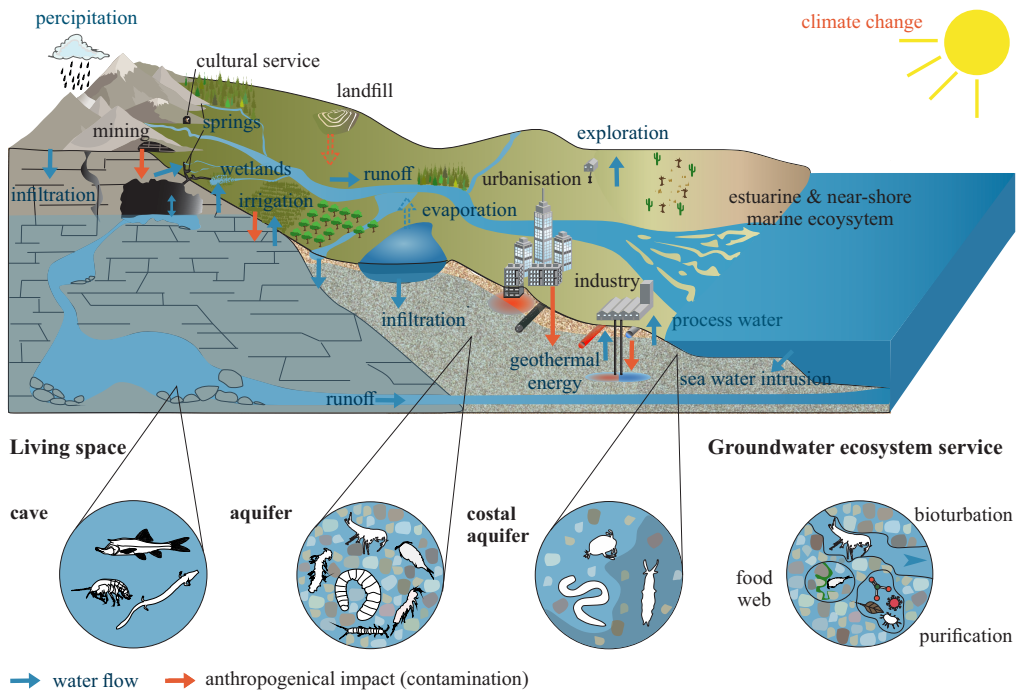


Figure 1.3: Block diagram showing the water cycle, including the water flow (blue arrows) and various anthropogenic impacts (red arrows) on groundwater. Different groundwater fauna habitats and ecosystem services are also illustrated.

An example of a qualitative impact is groundwater contamination (see Figure 1.3 red arrows), which is another frequent threat to stygofauna. Common pollutants derive from heavy industry (Hose et al., 2014), agriculture (Di Lorenzo et al., 2020b; Di Lorenzo and Galassi, 2013; Korbel et al., 2022a), urbanisation (Hallam et al., 2008), surface waters (Danielopol et al., 2003; Kristensen et al., 2018; Uhl et al., 2022) and thermal pollution (Griebler et al., 2016; Menberg et al., 2013a; Taylor and Stefan, 2009; Tissen et al., 2019; Zhu et al., 2010). These various sources of pollution differ in terms of the depth of contamination and its spread. Most of the named pollution sources are located near the land surface (agriculture, urbanisation, surface water), but there are also sources at greater depths (mining, gas and oil exploration). Concerning the spread of contamination, a distinction can be made between point sources (e.g. industry, households) and non-point sources, with diffuse sources and widespread pollution (e.g. agriculture). Latter often includes larger quantities of pollutants (United Nations, 2022). Generally, high sensitivity to organic contaminants and ammonium is observed for stygobiotic invertebrates in field and laboratory studies (Becher et al., 2022; Di Lorenzo et al., 2015; Romano and Zeng, 2013). Previous studies showed that ammonium impacts the organisms' growth and various physiological mechanisms, particularly respiratory metabolism (Romano and Zeng, 2013).

Examples of strong impacts are urban areas, which are characterised by multiple anthropogenic impacts such as dense building development, open geothermal systems, underground car parks and injections of thermal wastewater from industry resulting in local thermal alteration of groundwater by up to several degrees (Menberg et al., 2013a; Taylor and Stefan, 2009; Tissen et al., 2019; Zhu et al., 2010). Temperature changes can affect physico-chemical groundwater characteristics (Griebler et al., 2016) but also microbial processes, community composition and biodiversity, as physiological activity, especially metabolic rates, of stygobiontic species are temperature-dependant (Colson-Proch et al., 2009; Di Lorenzo and Galassi, 2017). Additionally, anthropogenic climate change significantly threatens biogeochemical processes and groundwater ecosystems (Griebler et al., 2016) due to altered recharge events, increased evapotranspiration, temperatures (Figura et al., 2011; Menberg et al., 2014; Tissen et al., 2019) and changing river-groundwater interactions. Threats due to climate change are most severe in areas with semi-arid to arid climates, humid regions of the Northern Hemisphere and (sub-)tropical areas (humid monsoonal countries) (Danielopol et al., 2003).

Finally, all those stressors can impact biodiversity, leading to species extinction and a shift in the community composition as ubiquitous surface-water species outcompete and replace groundwater species (Danielopol et al., 2003). In addition, there are implications for freshwater ecosystems (Hancock et al., 2005; Korbel et al., 2022a), terrestrial vegetation and fauna (Eamus and Friend, 2006) and estuarine and near-shore marine ecosystems (Moore, 1999) due to the ubiquity of groundwater dependence in terrestrial ecosystems (Hancock et al., 2005) (Figure 1.3 blue arrows). However, investigations on groundwater fauna and the impacts of humans on these ecosystems are still rare (M.-J. Dole-Olivier et al., 2009; Gibert and Culver, 2009; Martinez et al., 2018). Thus, there is a need for increased knowledge on subterranean ecosystems, their assessment and protection (Griebler et al., 2014b; Malard et al., 2023).

1.2.3 Assessment and protection approaches

During the last century, the subject of groundwater fauna research shifted from describing newly discovered species towards an ecosystemic and holistic view with research incorporating the whole ecosystem functioning (Danielopol and Marmonier, 1992; Malard et al., 2023). In the same context, social and political recognition of the importance of groundwater increased and topics of conservation and groundwater management began to emerge (Boulton et al., 2003a; Danielopol et al., 2003). Particularly noteworthy is the importance of the Swiss Water Protection Ordinance from 1998 for groundwater research. This was one of the first international authorities to include monitoring of both water quality and ecological criteria for groundwater systems (Danielopol and Griebler, 2008; Griebler et al., 2023; Schweizerischer Bundesrat, 1998). Another milestone and driver for groundwater ecosystem management and research in Europe was the European

Groundwater Directive 2006. This directive attempted to incorporate ecological knowledge into schemes for environmental planning and policies (Griebler et al., 2023; Steube et al., 2009). At the same time, groundwater initiatives in Australia began to gain momentum with the emergence of national policies to monitor groundwater ecosystems' stress and health (Griebler et al., 2023; NGC, 2004). Until now, global groundwater policy has primarily focused on the utilisation of groundwater after extraction and not on aquifer management, which aims to control groundwater abstraction and quality and to preserve groundwater system functions and services (United Nations, 2022).

Early approaches for monitoring groundwater ecosystems in Germany began with Hahn (2006) introducing the Groundwater Fauna Index (GFI), which quantifies relevant ecological conditions in the groundwater as a result of hydrological exchanges between the surface and groundwater. Shortly afterwards and triggered by Australian water management policies and industry, the first attempt to assess, measure and monitor ecosystem health was developed in 2011 in Australia. The Groundwater Health Index (GHI) uses a two-tiered framework consisting of a multi-metric suite of biotic and abiotic indicators (Korbel and Hose, 2011). Commissioned by the Federal Environmental Agency of Germany - Umweltbundesamt (UBA), Griebler et al. (2014b) developed a two-step, ecologically based classification scheme for characterising groundwater ecosystems. This assessment scheme is based on determining biotic and abiotic parameters, which are compared with reference values and are used to distinguish locations with very good or good ecological conditions, or locations that fail these criteria. A different approach is offered by the more recent assessment scheme of Fillinger et al. (2019). The microbial Density-Activity-Carbon index can be used to detect disturbances of groundwater ecosystems and is based on three microbial indicators: prokaryotic cell density, microbial activity and bioavailable carbon.

Despite all the mentioned efforts, there is still a lack of detailed information on groundwater ecosystems in most parts of the world. Even the most basic knowledge, for example, on essential properties, such as structural heterogeneity (e.g. ecosystem size, connectivity to neighbouring systems), inflow and out-flow of matter (e.g. sediment, detritus) and the spatial and temporal distribution of substrates (e.g. dissolved oxygen, pollutants and nutrients) is still missing (Larned, 2012). Furthermore, an almost untouched topic are groundwater foodweb interactions, especially between micro- and macroorganisms, as well as their link to processes of the carbon and nutrient cycle and services, such as attenuation of pollutants (Griebler and Avramov, 2015). Furthermore, international water law should pay more attention to transboundary aquifers, i.e. aquifers with groundwater flow crossing international boundaries, to avoid conflicts between countries. Moreover, there is a notable absence of an overall analysis of spatial and temporal distribution and research into groundwater fauna on a global scale. In some cases, this is certainly due to the fact that 'sharing data and information is often deficient, especially in low-income countries' (United Nations, 2022).

1.3 Objectives and approaches

This thesis aims to improve the understanding of ecological processes and conditions in groundwater, which are essential for sustainable resource and environmental management in times of competing groundwater uses. More specifically, this thesis aims to build a bridge between a basic understanding of biological issues and multidisciplinary approaches to understanding underlying processes. To do so, groundwater fauna is analysed and assessed on different spatial and temporal scales in this study.

In detail, this thesis seeks to

- build a common knowledge basis for an improved understanding, assessment and conservation of groundwater biodiversity on a larger scale. By obtaining a global overview of the historical evolution of stygofauna research, stygofauna sampling methodologies and an analysis of groundwater fauna research's spatial and temporal distribution, the thesis aims to summarise the current knowledge and thus point out knowledge gaps.
- identify shifts in groundwater fauna due to natural or anthropogenic impacts in recent decades and parameters that have a major influence on groundwater ecosystems. Comprehensive analysis of metazoan groundwater fauna and abiotic parameters over two decades, as well as statistical analyses are conducted to distinguish sites with stable and unstable faunal conditions. Also, the impact of specific hydrological and hydro-chemical parameters on this characterisation is assessed.
- assess the ecological status of urban aquifers and to identify parameters required for a more reliable, quantitative, ecological assessment. Groundwater ecosystems beneath an urban area are compared to a natural area, considering the status of their ecosystem and the impact of land use on groundwater faunal communities. They are investigated by analysing local hydrogeological, physico-chemical and faunal data and with the goal to detect causes for faunal changes.
- determine implications for environmental policies for sustainable groundwater management and monitoring requirements for groundwater fauna to ensure these ecosystems are maintained and preserved in the future. The final aim of this thesis is to verify the applicability of biomonitoring of groundwater on different spatial scales.

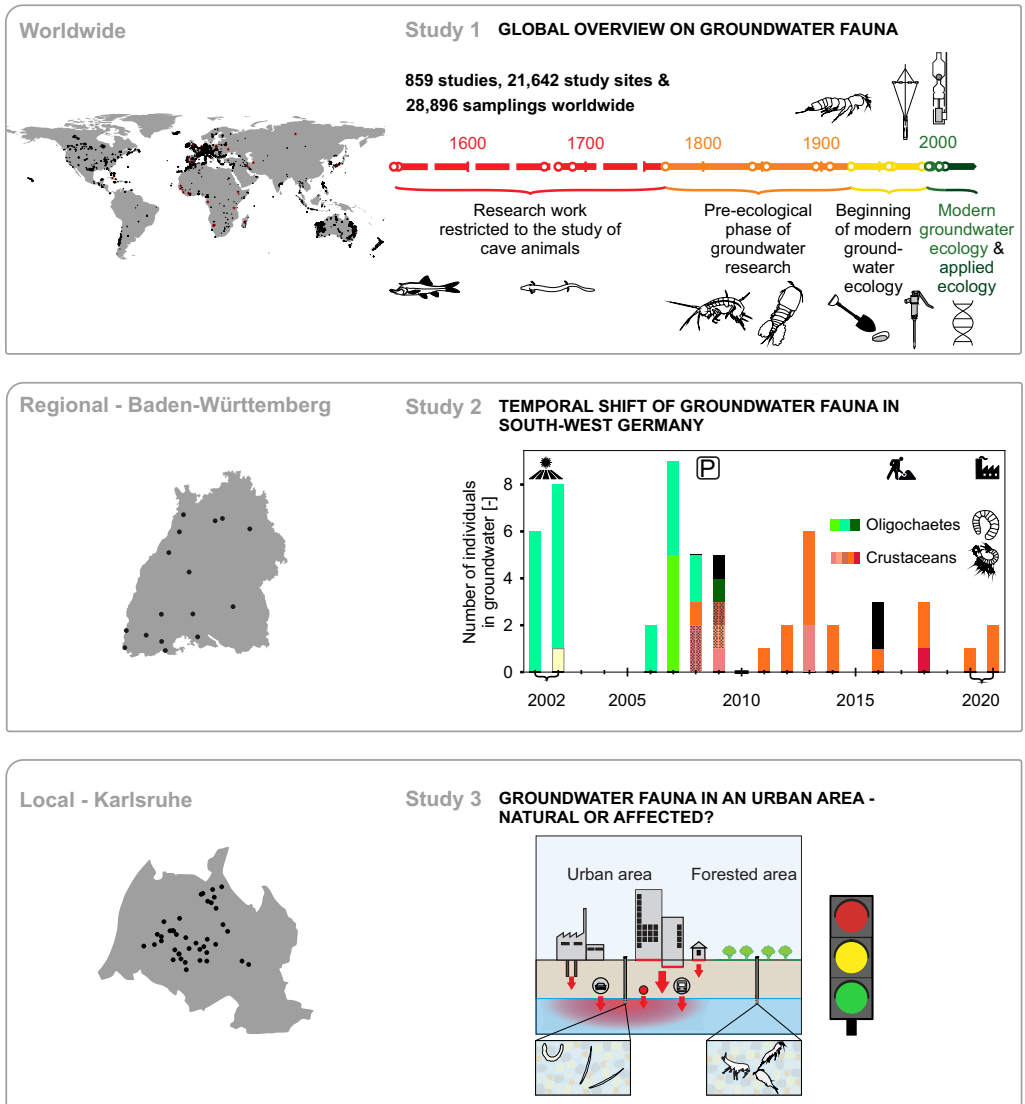


Figure 1.4: Graphical overview of the studies of this thesis on groundwater fauna. The investigations focus on different temporal and spatial scales and aim to better understand ecological processes and groundwater conditions.

1.4 Structure of the thesis

This cumulative thesis combines three individual studies, which are enclosed in Chapters 2, 3 and 4. All studies were submitted to peer-reviewed (ISI-listed) international journals, with Study 1 and Study 3 in Chapters 2 and 4 already published and Study 2 in Chapter 3 being under review.

The thesis is organised as follows:

- Chapter 2: GLOBAL OVERVIEW ON GROUNDWATER FAUNA

In this review a global overview on groundwater fauna (i.e. stygofauna) research is presented. To achieve this, an extensive review of accessible groundwater fauna data is conducted by analysing data from national and international publications in scientific journals, national reports, doctoral theses, historical writings, books and consisting online databases in various languages. On this basis, an overview of (i) the historical evolution of stygofauna research, (ii) stygofauna sampling methodologies, and (iii) an analysis of the global spatial and temporal distribution of groundwater fauna research and knowledge gaps is provided. So, a common knowledge basis for an improved understanding, assessment and conservation of groundwater biodiversity on a larger scale is built.

- Chapter 3: TEMPORAL SHIFT OF GROUNDWATER FAUNA IN SOUTH-WEST GERMANY

In this study long-term groundwater data from 16 monitoring wells in Baden-Württemberg (BW) South-West Germany is used to identify shifts in groundwater fauna due to natural or anthropogenic impacts. Therefore, the available groundwater data of the study site is reviewed, and observation wells for additional sampling in 2020 are selected. Metazoan groundwater fauna and abiotic parameters are temporally analysed on different spatial scales. To distinguish wells with stable and unstable faunal conditions and to assess the impact of specific hydrological and hydro-chemical parameters on this characterisation, a multivariate PHATE-analysis is conducted. Moreover, time series of multiple parameters and aerial images of three individual wells are analysed in detail concerning changes in land use, surface condition, and abiotic parameters.

- Chapter 4: GROUNDWATER FAUNA IN AN URBAN AREA - NATURAL OR AFFECTED?

In this last study, a first assessment of the groundwater fauna in an urban area is provided. Therefore, groundwater fauna beneath residential, commercial and industrial, i.e. urban areas in comparison to a forested area outside the built-up area of Karlsruhe (Germany)

is investigated to determine whether land use impacts groundwater faunal communities. Hence, the groundwater fauna is sampled in 39 groundwater monitoring wells, groundwater temperatures are measured, and chemical properties are analysed. For classification, the Groundwater Ecosystem Status Index (GESI) is applied, which characterises sites regarding the state of their ecosystem. To better understand large-scale relationships and the fine structures of high-dimensional biological data, a PHATE-analysis is conducted.

- Chapter 5: SYNTHESIS

The major results of the study are summarised and their contribution to improving the understanding of groundwater ecology is described. Finally, perspectives for future research are provided.

2

GLOBAL OVERVIEW ON GROUNDWATER FAUNA

Reproduced from Koch, F., Blum, P., Korbelt, K., Menberg, K., (2023) Global overview on groundwater fauna. *Ecohydrology* 17 (1). 28. <https://doi.org/10.1002/eco.2607>.

2.1 Introduction

Groundwater is an important source of freshwater, drinking water and service water for irrigation, industrial and geothermal uses (Job, 2022; Siebert et al., 2010; Stauffer et al., 2013). Moreover, groundwater is the largest terrestrial freshwater biome of the world (Griebler et al., 2014a) and is considered as a species-rich habitat (> 100,000 species) with many taxa displaying high endemism (Culver and Holsinger, 1992; Martinez et al., 2018). Groundwater communities consist of a highly diverse biota, including bacteria and archaea, viruses, protozoans, fungi, invertebrates, salamanders and fish (Marmonier et al., 2023). Fauna populating groundwater, known as stygofauna, comprise stygobiontic species (exclusively inhabiting groundwaters), stygophilic species (affiliated with both ground and surface waters) and stygoxenic fauna (accidentally or occasional groundwater inhabitants) (Gibert et al., 1994; Hahn, 2006).

Within groundwater communities, microorganisms play essential roles in water purification through biogeochemical cycling (Griebler and Avramov, 2015) and form biofilms, on which stygofauna feed. Stygofauna have roles in promoting microbial growth (Edler and Dodds, 1996; Mermillod-Blondin et al., 2002), enhancing aquifer water transmission (Hose and Stumpp, 2019; Stumpp and Hose, 2017), organic matter processing (Kinsey et al., 2007; Simon and Benfield, 2001) and contribute to the subterranean food web (Saccò et al., 2022b). Combined, these species provide several functions sustaining groundwater ecosystems, aiding groundwater health and water quality (Mermillod-Blondin et al., 2023).

Globally, groundwater and groundwater fauna are facing common threats, including abstraction (Wada et al., 2014), contamination (Burri et al., 2019) and climate change (Amanambu et al., 2020), all of which place multiple stresses on groundwater ecosystems. Groundwater abstraction for irrigation, potable water and mining activities (Danielopol et al., 2003; Hancock et al., 2005) alter groundwater levels, with duration and rate of abstraction known to strand particular stygobiotic biota, causing desiccation and death (Korbel et al., 2019; Patel et al., 2020). These pressures are particularly prevalent in Africa, Asia and South America (Wada et al., 2010) and are associated with demographic increases (Vörösmarty et al., 2000). Groundwater contamination is another frequent threat to stygofauna, with common pollutants derived from agriculture (Di Lorenzo et al., 2020b; Di Lorenzo and Galassi, 2013; Korbel et al., 2022a), heavy industry (Hose et al., 2014), urbanisation (Hallam et al., 2008), surface waters (Danielopol et al., 2003; Kristensen et al., 2018) and thermal pollution (Menberg et al., 2013a; Taylor and Stefan, 2009; Tissen et al., 2019; Zhu et al., 2010). Additionally, anthropogenic climate change poses a significant threat to groundwater ecosystems and biogeochemical processes (Griebler et al., 2016) due to altered recharge events, increased evapotranspiration, increased temperatures (Figura et al., 2011; Menberg et al., 2014; Tissen et al., 2019) and increased groundwater extraction caused by drying rivers. Climate change threats are most severe in areas with semi-arid to arid climate, humid areas of the Northern Hemisphere and (sub-)tropical areas (humid monsoonal countries) (Danielopol et al., 2003).

The multiple stressors that humans have placed on groundwaters globally (Becher et al., 2022) have the potential to alter groundwater community structure (e.g. Korbel et al., 2022a) and compromise ecosystem functions, resulting in the deterioration of groundwater health (Hancock, 2002; Hancock et al., 2005). Furthermore, these stressors can impact biodiversity and alter surface water groundwater connectivity, leading to species extinction and shift in the community composition as ubiquitous surface water species outcompete and replace groundwater species (Danielopol et al., 2003). Such changes to groundwater regimes, connectivity and biota can have implications for terrestrial vegetation and fauna (Eamus and Froend, 2006), freshwater ecosystems (Hancock et al., 2005; Korbel et al., 2022c) and estuarine and near-shore marine ecosystems (Moore, 1999) due to the ubiquity of groundwater dependence in terrestrial ecosystems

(Hancock et al., 2005). These impacts highlight the need for increased knowledge on subterranean ecosystems, their assessment and protection (Griebler et al., 2014a).

However, investigations on groundwater fauna and the impacts of humans on these ecosystems are still rare (M.-J. Dole-Olivier et al., 2009; Gibert and Culver, 2009; Martinez et al., 2018). A pioneering study of the global diversity of subterranean fauna, 'Stygofauna mundi', highlighted the biodiversity values of these ecosystems identifying 6,634 species of aquatic subterranean dwellers, from a variety of groundwater habitats (Botosaneanu, 1986; Malard et al., 2009). Later studies indicated over 7,800 subterranean species (Juberthie, 2000), with the most recent knowledge of global biodiversity synthesised in the revised edition of *Groundwater Ecology and Evolution* (Marmonier et al., 2023). As groundwater ecosystem diversity, functions and processes differ across landscapes (Korbel et al., 2013a; Zagnajster et al., 2023), knowledge of these ecosystems must be drawn globally from a variety of bioregions and climatic zones in order to implement effective management. Attempts have been made to improve knowledge of stygofauna diversity patterns in various regions of the world (e.g. Gibert et al., 2009: European Protocols for the Assessment and Conservation of Aquatic Life In the Subsurface (PASCALIS)-project) with broad-scale studies synthesising current knowledge of groundwater biological and habitat diversity in Europe (Cornu et al., 2013), Thailand and Vietnam in South-East Asia (Brancelj et al., 2013) and Australia (Hose et al., 2015). Despite these studies, there is a notable absence of an overall analysis of spatial and temporal distribution and research into groundwater fauna on a global scale.

The aim of this study is to provide a global overview on groundwater fauna (stygofauna) research. Hence, we conduct an extensive review of accessible groundwater fauna data by analysing data from national and international publications in journals, national reports, doctoral theses, historical writings, books, consisting online databases and others in various languages. In the following, we provide an overview of (i) the historical evolution of stygofauna research, (ii) stygofauna sampling methodologies and (iii) an analysis of the global spatial and temporal distribution of groundwater fauna research and knowledge gaps, with regional summaries. It is envisaged that by encapsulating such data, we can start to build a common knowledge basis for increased understanding, assessment and conservation of groundwater biodiversity on a larger scale. Moreover, we can identify where data is lacking, which has important implications for the implementation of environmental policies for sustainable groundwater management (Danielopol et al., 2003; Tomlinson et al., 2007).

2.2 Historical evolution of groundwater fauna research

According to Griebler et al. (2014a), groundwater ecology is a relatively young discipline with research and knowledge lagging behind that of surface ecosystems such as streams and lakes. Historically, data were collected both opportunistically and sporadically; however, more recent awareness of the importance of groundwater ecosystem processes and services (Mermillod-Blondin et al., 2023) has seen the increase in well-designed ecological research and monitoring programs. Much of this research has broadened the knowledge on the biological distribution of stygofauna (Marmonier et al., 2023) as well as efforts to conserve this biota (see Boulton et al., 2023). This review analyses over 800 publications published prior to 2022 (Table A1.4), providing an overview of the evolution of this research as well as temporal and spatial analysis of research sites, with a summary provided in Figure 2.1.

2.2.1 Early research phase

The earliest written observation in groundwater ecology dates back to 1537 with a sporadic observation in a cave (Figure 2.1) (Myloie, 2004). The 17th century saw increased research on

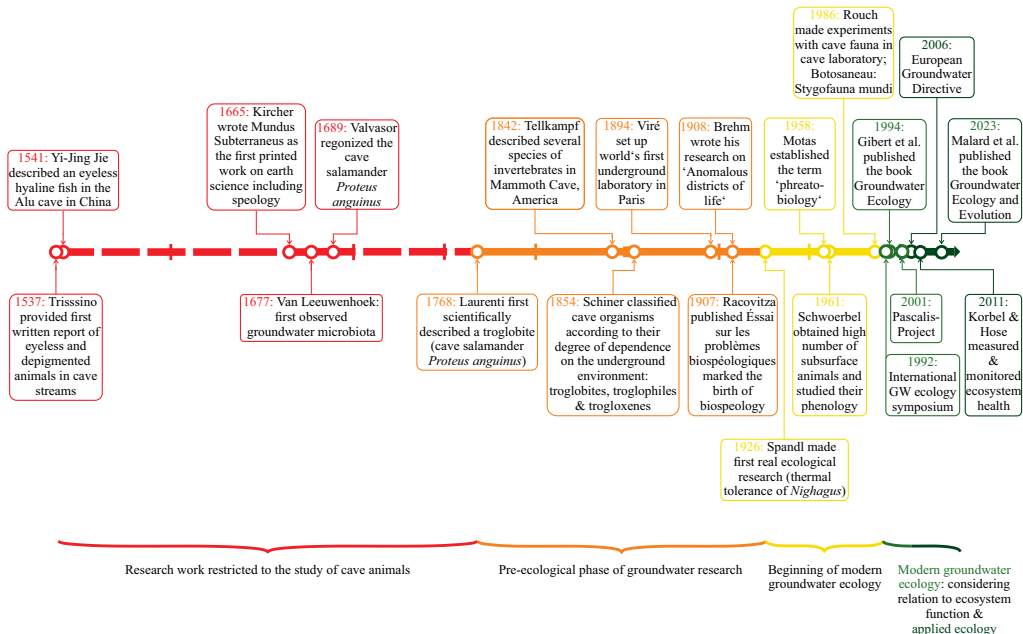


Figure 2.1: Chronology and milestones of groundwater fauna research and chronological overview over the change of research topics. The colouring points out the four phases of groundwater research.

groundwater fauna and microbiology, with the first stygobiontic species identified in scientific writing, namely a cave salamander in Slovenia (Culver and Pipan, 2013; Freiherr von Valvasor, 1689). Early research focused on groundwater fauna, while groundwater microbiology research became established in the second half of the 19th century (Griebler et al., 2014a). Likewise, early research concentrated on cave ecosystems (Figure 2.1, red phase), due to the accessibility of this habitat. During the second half of the 19th century, other subsurface habitats (e.g. aquifers) became more accessible, leading to the discovery of organisms previously unknown to science (Danielopol and Griebler, 2008) and the emergence of groundwater ecology as a research field.

2.2.2 Pre-ecological research phase

In the 18th and 19th centuries, during the so-called ‘Pre-ecological phase’ of groundwater research (Danielopol and Griebler, 2008), the emphasis of research was on cataloguing new species, their habitats and their biogeographical origin (Danielopol and Griebler, 2008) (Figure 2.2, orange phase). The term ‘biospeology’ was proposed by Racovitza (1907) and characterised the research activities at this time (Hancock et al., 2005).

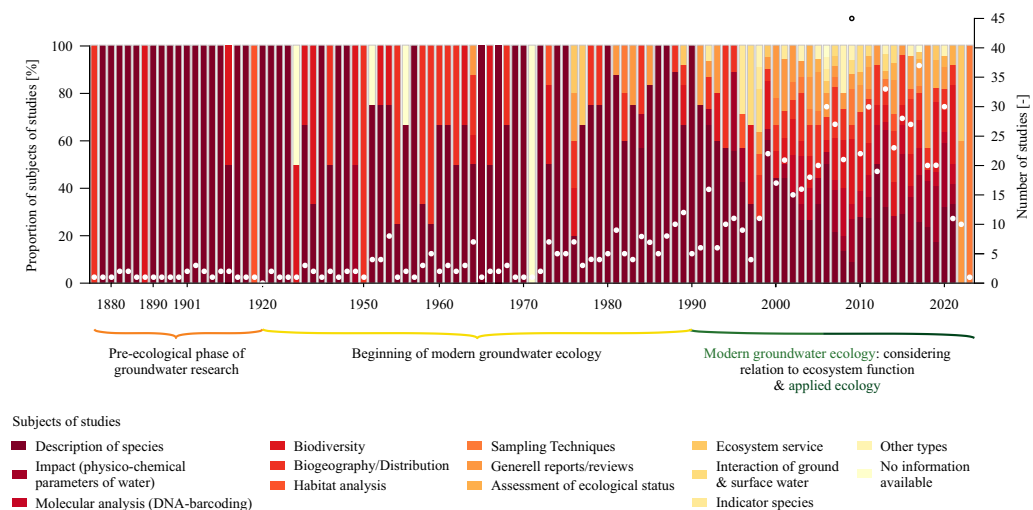


Figure 2.2: Proportion of the different subjects of all considered studies over time (first y-axis) and number of studies over time as white dots (second y-axis).

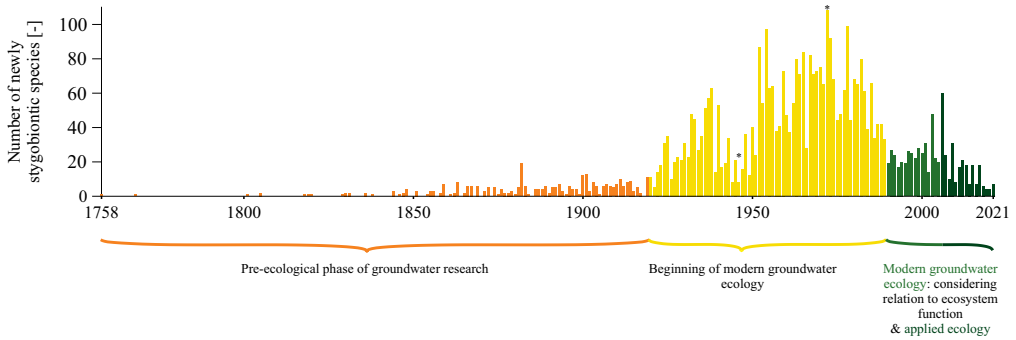


Figure 2.3: Number of newly discovered stygobiontic species mentioned in all considered studies. The colouring points out the four phases of groundwater research (Figure 2.1) and the * marked important years. A list with all 859 used studies is provided in Table A1.4.

2.2.3 Modern ecology research phase

The beginning of the ‘modern groundwater ecology phase’ (Figure 2.3, yellow phase) began in the 1920s, with a significant increase in the number of studies, sampling events (Figure A1.2) and, consequently, a sharp increase in the reporting of newly discovered stygobiontic species. In this context, the year 1971 is striking. No information on published studies was found for this year; however, many new stygobiontic species were found in this and the following years. One reason for this could be a time delay between the conduction of studies and the actual publication of results. The number of reported new stygobiontic species saw a dip around the early to mid-1940s (coinciding with global unrest), then peaked in 1972 and has shown a general declining trend since this time (Figure 2.3). During this period, the awareness of groundwater fauna increased and paved the way for modern groundwater research with the development of new integrated concepts, sampling methods and knowledge of ecology.

The ‘modern groundwater ecology phase’ of research saw the emergence of studies on the ecology of unconsolidated sediments (Hancock et al., 2005), sampling of wells and tap water (Brehm, 1930) as well as the ecology of alluvial aquifers and karst systems. Arguably, this began with Spandl (1926) (Hancock et al., 2005), who conducted the first true ecological research of groundwater fauna in his study of the thermal tolerance of the genus *Niphargus* in 1926 (see Figure 2.1). The term ‘phreatobiology’ was introduced by Motas (1958) to describe the research on the biology of groundwater organisms living in porous, unconsolidated sediments (Danielopol and Marmonier, 1992; Hancock et al., 2005; Motas, 1958). Other important innovations in this period included the description of groundwater and hyporheic fauna in relation to subsurface water chemistry (Danielopol and Marmonier, 1992) and the beginning of groundwater phenology studies (Danielopol and Griebler, 2008) in the 1960s (Schwoerbel, 1961). Moreover, the first

experimental studies under conditions in the cave laboratory ‘Laboratoire Souterrain du C.N.R.S’ in Moulis were conducted (Rouch, 1986). Besides the influx of new species discovered in this era, for the first time, studies began to concentrate on the ecosystem functions, e.g. studies on whole subsurface karstic drainage system (Danielopol and Marmonier, 1992).

The early 1990s saw the development of a novel view on biodiversity of subterranean aquatic organisms in relation to ecosystem functioning and services (Danielopol and Griebler, 2008) (Figure 2.3, green phase). The subject of studies shifted from the description of newly discovered species (i.e. descriptive typological approach) towards an ecosystemic and holistic view with research incorporating the whole of ecosystem functioning (Danielopol and Marmonier, 1992)(Figure 3.2). The growing interest on groundwater fauna research is reflected in the exponential increase in the number of studies over time since the 1920s, which peaked in 2009 (Figure 2.2). The importance of ecosystem function and functional traits of groundwater fauna continues to be recognised (Griebler and Avramov, 2015; Hose et al., 2022; Saccò et al., 2021).

Alongside the increased interest in groundwater fauna and ecology developing in the late 1990s, technological advances allowed the usage of Deoxyribonucleic Acid (DNA) methods for the identification of biota. Initially, the use of DNA centred on groundwater microbial studies (Barton et al., 2004; Hebert et al., 2003), with a focus on contamination and remediation (Alfreider et al., 2002; Geets et al., 2001; Ross et al., 2001). The emergence of molecular methods for stygofauna first saw DNA used to study stygofauna phylogeny, which added to our understanding of groundwater species and their evolutionary pathways (Cooper et al., 2002; Lefébure et al., 2006; Leys et al., 2003). Molecular methods were then pioneered for groundwater fauna studies in groundwater karst systems, utilising environmental DNA (environmental Deoxyribonucleic Acid (eDNA)) for the targeted detection of threatened subterranean species which was followed by the use of eDNA for studies in stygofauna biodiversity, ecosystems and routine ecosystem monitoring (Korbel and Hose, 2017). The continued shift in focus towards ecosystem functioning and whole-of-ecosystem analysis (occurring in the late 1990s and early 2000s) followed the path taken in surface ecology research and is likely aided by the emergence of molecular analysis and eDNA allowing the identification of unprecedented numbers of potentially new species.

2.2.4 Applied ecology research phase

During the late 1990s, the responses of fauna to external influences, such as chemical and thermal alterations, anthropogenic and natural and changes in habitat structure on groundwater ecosystems received more attention. By the late 20th century, scientists began to investigate the use of stygofauna as bioindicators, describing the sensitivities of these species to both natural and anthropogenic factors with research on human impacts on groundwater fauna rising in prominence

(Mösslacher and Notenboom, 1999). As scientists continued to discover the functions and roles of stygofauna, and the important biodiversity that groundwater hosts, a new research topic began to emerge and a new sub-phase of groundwater research began—the ‘applied ecology phase’ (Figure 2.3, dark green).

With increased social and political recognition of the importance of groundwater, the topics of conservation and groundwater management began to emerge as a focus of ecological research (Boulton et al., 2003a; Danielopol et al., 2003). However, due to limited knowledge of stygofauna biodiversity and spatial range, these early studies were largely unrecognised by governments worldwide. Accordingly, the development of new assessment schemes for the monitoring of ecological status or health of groundwater ecosystem received more attention in the 21st century. By 2010, the increased interest in bioindicator species and studies investigating natural and anthropogenic influences on groundwater fauna distributions (Danielopol et al., 2000; Goldscheider et al., 2006; Griebler, 2001; Griebler et al., 2002; Humphreys, 2006; Malard et al., 1994; Marmonier et al., 2000; Mösslacher et al., 2001; Mösslacher and Notenboom, 1999; Notenboom et al., 1995; Sinclair et al., 1993) had paved the way for the first studies attempting to monitor groundwater health and diversity using bioindicator. Groundwater scientists began to apply the notion of ‘health’ (which had recently been applied globally in environmental policies) to groundwater ecosystems; with connotations to human health, it is deemed an easy way for non-scientists and water managers to understand environmental conditions.

A milestone and driver for groundwater ecosystem management and research in Europe was the European Groundwater Directive 2006, which attempted to incorporate ecological knowledge into schemes for environmental planning and policies (Griebler et al., 2023; Steube et al., 2009). This directive saw the emergence of studies to assess groundwater ecosystems: Hahn (2006) utilising abiotic indicators and detritus, predictive methods (Stoch et al., 2009) to assess groundwater diversity, and Steube et al. (2009) suggesting the combination of both abiotic and biotic factors. Griebler et al. (2010) provided alternate methods for groundwater ecosystem assessments, suggesting the use of natural reference conditions for aquifers of differing typology. At this same time, other countries were also developing policies to conserve and protect groundwater quality and Groundwater-Dependent Ecosystems (GDE). In Australia, groundwater initiatives began to gain momentum in the mid-2000s with the emergence of national policies to monitor groundwater ecosystems’ health and stress (Griebler et al., 2023; NGC, 2004); however, the lack of scientific methods to implement such policies was recognised (Boulton et al., 2003b; Hatton and Evans, 1998). The first attempt to assess, measure and monitor ecosystem health, using a two-tiered framework consisting of a multi-metric suite of biotic and abiotic indicators, was in Australia in 2011 (Korbel and Hose, 2011). The development of this ‘Groundwater Health Index’ (GHI) framework was triggered by Australian water management policies and the heavy reliance on groundwater from irrigation-based industries. Methods to measure groundwater health

and monitor groundwater ecosystems continue to be refined and developed (Di Lorenzo et al., 2020a; Fillinger et al., 2019; Griebler et al., 2014b; Koch et al., 2021; Korbel and Hose, 2017), with these management tools being used by governments to monitor groundwater health (e.g. report Korbel et al., 2022a). Integral to these management tools is flexibility with emerging technologies, such as eDNA (Korbel et al., 2022c; Saccò et al., 2022c) able to be integrated into frameworks which allow for the integration of most recent science to inform management decisions.

Other important research areas emerging in the ‘applied ecology’ phase include attempts to better understand the links between groundwaters and adjoining ecosystems (Boulton et al., 1998; Danielopol and Marmonier, 1992; Hahn, 2006; Hancock et al., 2005; Korbel et al., 2022b,c). Such studies holistically investigated interconnectivity between ecosystem and the dynamic of exchange between the surface, hyporheic and subsurface zones. Again, these studies have been mainly prompted by management decisions and directives, for example understanding the mechanisms behind the loss of river baseflow due to over-extraction of groundwater is required for water allocations and management. As a last milestone, Malard et al. (2023) published the second edition of the book ‘Groundwater Ecology and Evolution’. This updated edition synthesises the current state of knowledge on groundwater ecology and evolution and highlights the opportunities and challenges for conserving and managing groundwater ecosystems.

Furthermore, groundwater research has begun to include multidisciplinary approaches combining experts from various fields to refine subterranean ecological patterns (Saccò et al., 2021). It is becoming more common for studies to combine skills of hydrogeology, geomorphology, hydrochemistry, molecular science, ecology and biology to answer complex questions (Burrows et al., 2017; Korbel et al., 2022c). Some other approaches have combined techniques, using isotopes, radiocarbon analysis (^{14}C) and DNA methods for the analyses of environmental samples (Hartland et al., 2011; Saccò et al., 2019). Such multidisciplinary techniques allow for more sophisticated ecological studies including the identification of energy flows and food web structure and biological and water exchanges between connected ecosystems (Hartland et al., 2011).

2.3 Sampling groundwater fauna

Traditionally, environmental sampling involves accessing numerous sites within a limited time frame, ideally with a representative spatial distribution of samples for the area under investigation (Hahn and Matzke, 2005). In the case of groundwater fauna sampling, this represents a great challenge because the living space, i.e. the aquifer, is hard to access (Korbel et al., 2017; Steube et al., 2009). Access points to springs, caves and sediments from the hyporheic zone of rivers are rare and selective (Maurice, 2009), and groundwater wells accessing aquifers are expensive to establish. Moreover, different technical requirements for sampling are required for differing aquifer

types (Hahn, 2002; Thulin and Hahn, 2008). Besides access, the patchy distribution and high endemism of groundwater fauna require a larger number of sampling points to obtain representative results (Gibert and Deharveng, 2002; Hahn and Matzke, 2005; Mösslacher, 1998; Thulin and Hahn, 2008). Additionally, the small and sensitive anatomy of stygobiontic species complicates the intact extraction of samples for morphological identification and representative aquifer sampling (Hahn, 2002). Over time several novel methods, described in detail in the Supporting Information (Chapter 5.2), were developed to account for these challenges (Figure 2.4).

2.3.1 Temporal development of sampling methods

As expected, the number of different sampling methods has increased with the number of studies and with the emergence of more advanced methods from the 1960s onwards (Figure 2.5). Hancock et al. (2005) and Danielopol and Griebler (2008) provide a short summary of the historical and technical background of sampling and analysis methods for groundwater ecology including microbiology and fauna. Our study highlights the different types of stygofauna methods used in each period of time, as a proportion of the studies completed (Figure 2.5).

Before and during the ‘pre-ecological phase’ of groundwater research simple nets, substrate samples and hand-pumps were used to sample fauna of wells, caves and springs. More complex methods were developed from 1934 onwards, in particular the rapid and qualitative Karaman–Chappuis method (Karaman, 1933) (Figure 2.4) and the Bou–Rouch method (Bou and Rouch, 1967), which allows pumping of animals living in sandy and gravel sediments and consequently led to discovering more diverse meio- and macro-organismal assemblages (Danielopol and Griebler, 2008). Nevertheless, this method is not strictly representative of *in situ* conditions as faunal diversity and density are not expressed per volume of sediment and larger species such as amphipods and isopods can be damaged (Malard et al., 2002; Pospisil, 1992).

Sampling methods were further developed between the mid- 1960s and 1990 in the beginning of ‘modern groundwater ecology phase’, with specific traps and pumps offering increased quantitative data (Danielopol and Griebler, 2008). However, issues still exist with such sampling methods, for example the commonly used balance and inverted bottle traps (Boutin and Boulanouar, 1983; Ginot and Decou, 1977) are species-selective, such that representative sampling requires a combination with other methods such as nets. Table 2.1 summarised the most important dis- and advantages and fields of applications of the sampling methods for groundwater fauna.

Recent sampling of stygofauna (‘modern groundwater ecology’ era) is still dominated by sampling well waters (Figure 2.5), with pumping and filtering animals the most common and well-established method for stygofauna studies (Hahn, 2002; Thulin and Hahn, 2008). Additionally, pumping well water enables a simultaneous sampling of fauna, sediment and water (Hahn, 2002) and is

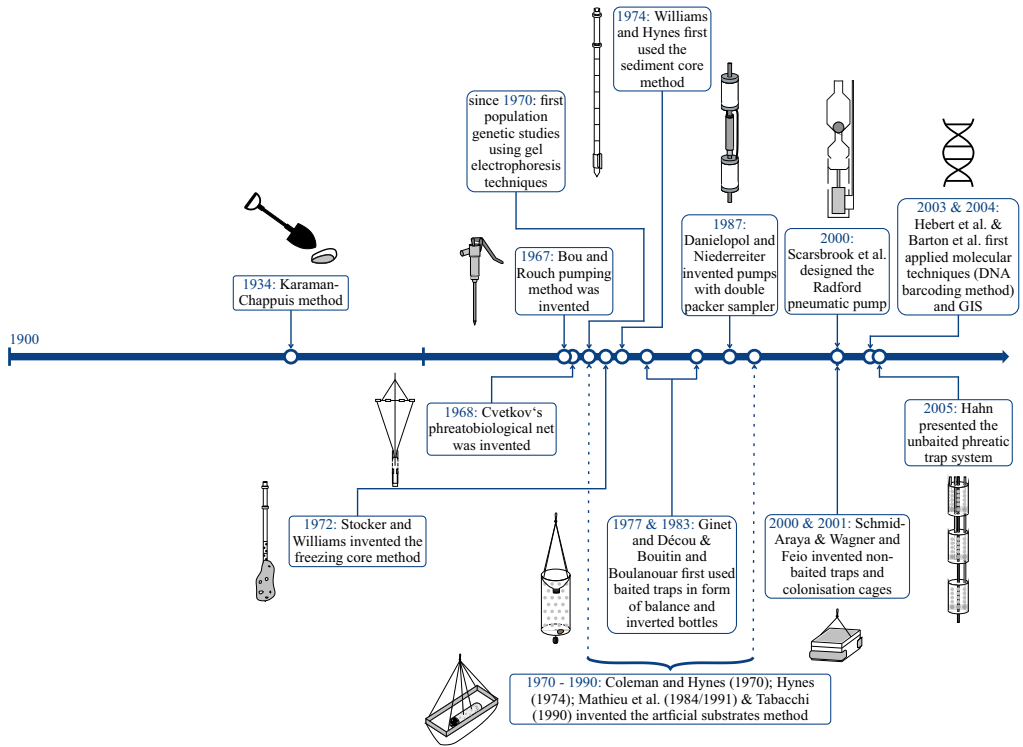


Figure 2.4: Temporal development of sampling methods with sketches of the invention (for more information, see Figure A1.1).

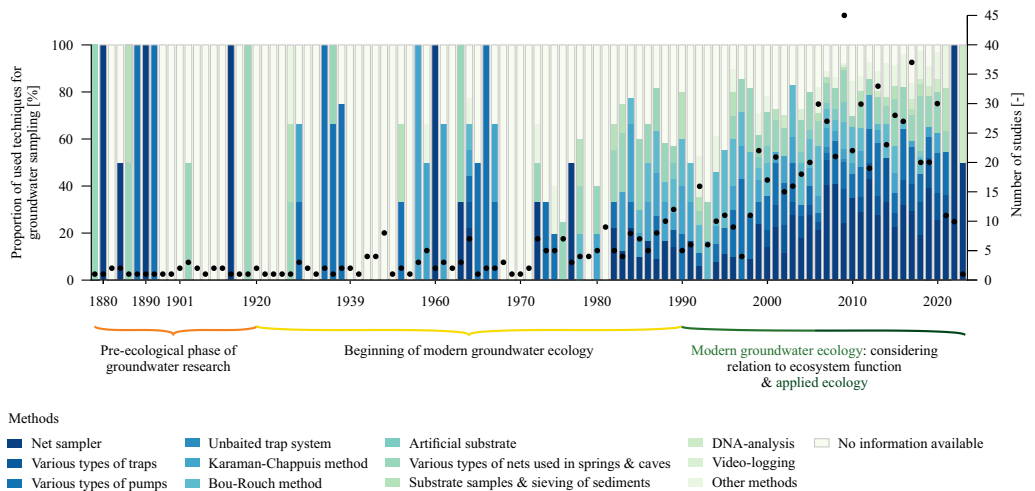


Figure 2.5: Proportion of applied methods for groundwater sampling and number of considered studies over time as black dots (secondary y-axis).

easy to standardise by extracting a defined volume of water (Hahn, 2002). However, pumping is considered as a selective sampling method, because of filtering effects and a resistance against the suction of pump. Hence, large, sessile or more active species can be underrepresented in the samples (Allford et al., 2008). The net sampler or phreatobiological net is a valuable alternative to pumping devices with respect to obtained numbers of taxa and community composition (Hahn and Matzke, 2005; Malard et al., 2002; Thulin and Hahn, 2008) and can be used for largescale faunal surveys (Allford et al., 2008) and wells up to 100-m depth (Hahn, 2002); however, like well sampling from pump, there are issues with these sampling methods collecting representative samples of stygofauna communities.

Table 2.1: Comparison of several methods for groundwater fauna sampling (++ = very good; + = good; o = moderate; - = negative; -- = very negative; NA = information not available) in order of their temporal development (Figure 2.3).

Sampling method	Expenditure of time			Condition of fauna sample	Efficiency	Potential animal findings	Habitat
	Feasibility	Sampling duration	Sample preparation				
Karaman-Chappuis method	++	+	++ ¹	++	+	Free swimming fauna of interstitial water ¹	Riverbed sediments (subterranean rivers, lakes) ¹ & groundwater
Bou and Rouch technique	++ ^{1,2}	+ ¹²	++	+ ¹²	+	Diverse meio- and macro-organisms ³ ; swimming organisms & species linked to sand particles ¹	Sandy sediments and gravel of rivers, lakes & shallow groundwater ¹ , max. depth 6 m ²
Phreatobiological net	++ ¹³	++ ^{7,13}	++ ¹³	++ ¹³	+ ^{13,14}	Fauna of well water & in the bottom sediments of the well ¹	Large diameter wells ¹ in alluvial sediments
Traps Baited Traps	++	- ¹	++	++	+ ^{8,14}	Limited to isopods, amphipods, planarians ¹	Wells, lakes, caves (siphons, pools) ¹
Non-baited Traps	++	-	++	++	+ ^{8,14}	NA	Mainly used for the hyporheos ¹ , upper part of groundwater body ¹⁵
Phreatic trap system	o	- ⁷	++	++	+	NA	Wells
Freezing core method	- ⁴	+	-	++	++ ²	Interstitial fauna ¹⁴	Stony streambeds ¹¹ ; restricted to superficial sediments ¹⁴
Artificial substrates	+	- ^{1,10}	++	++	+ ¹	Meiofauna ¹	Caves, along subterranean rivers & streams ¹
Sediment core	++	+	++	+	+	NA	In uniform & mixed gravels up to 10 mm in diameter ⁵ (coarse substrates of stony streams ⁴)

Sampling method	Feasibility	Expenditure of time			Condition of fauna sample	Efficiency	Potential animal findings	Habitat
		Sampling duration	Sample preparation	Expenditure of costs				
Pump Centrifugal pump	+	0 ⁸	++	0	- ⁹	NA	Small animals (mites & copepods), larger animals as fragments ⁹	Wells, aquifers, hyporheic zones; up to 50 m depth
Double packer pump	+	0 ⁸	++	- ⁷	NA	+	Small, less tenacious animals & larger animals ^{1,3}	
Pneumatic pump	+	0 ⁸	++	- ^{9,14}	++ ^{8,9}	+ ¹⁴	Small animals & larger amphipods, isopods ⁹ and syncarids	
Molecular techniques	o/+	++	-/o	+/o ⁸	NA	o ⁸	All, including cryptic species ^{16,17}	Water & sediment ¹⁶
Video-logging	+	NA	NA	NA	-	NA	Very low abundance taxa	Wells in sandy & silty environments ⁶
¹ Malard et al. (2002)			⁷ Hahn (2005)				¹³ Allford et al. (2008)	
² Pospisil (1992)			⁸ Hose and Lategan (2012)				¹⁴ Hahn (2002)	
³ Danielopol and Griebler (2008)			⁹ Scarsbrook et al. (2000)				¹⁵ Thulin and Hahn (2008)	
⁴ Boxshall et al. (2016)			¹⁰ Sket (2018)				¹⁶ Saccò et al. (2022b)	
⁵ Williams and Hynes (1974)			¹¹ Stocker and Williams (1972)				¹⁷ Trontelj et al. (2009)	
⁶ Datry et al. (2003)			¹² Stubbington et al. (2016)					

2.3.2 Discussion of efficiency and representativity

The issue of representative sampling is complicated for stygofauna, with sampling regimes needing to consider the inclusion or exclusion of purged well water in sample design, the volume of water sampled and the extraction rate of pumped waters (see Korbel et al., 2022b). Groundwater wells typically provide an artificial environment, in which there is a large column of water that may be atypical of the surrounding aquifer (particularly in alluvial aquifers) and may be enriched in oxygen organic matter (Hahn and Matzke, 2005). Due to these factors, wells often contain a larger abundance of stygofauna than the surrounding aquifer (Hahn and Matzke, 2005; Roudnew et al., 2014; Sorensen et al., 2013) and may favour taxa that prefer the open water column provided by well casings, thus purging wells prior to sampling becomes important for any study looking at richness and abundance measures (Korbel et al., 2017). However, studies have indicated that there are compositional differences in pre-purged and purged samples (Korbel et al., 2017, 2022b). To date, only 29 studies sample fauna exclusively by pumping aquifer water through purged wells, with Australia playing a leading role (13 studies: Castaño-Sánchez et al., 2020b; Cook et al., 2012; Hartland et al., 2011; Korbel and Hose, 2011, 2015, 2017; Korbel et al., 2019; Sorensen et al., 2013; Terramin, 2018). The issues of purging wells and sample volumes need to be considered in the aims and objectives of monitoring programs, in order to ensure that stygofauna communities are accurately represented (Korbel et al., 2022b).

2.3.3 The evolution of molecular methods for groundwater sampling

A methodological milestone that altered the view on the diversity of groundwater fauna was the application of molecular tools on individual specimens and water samples (see Boulton et al., 2023; Danielopol and Griebler, 2008). Initially, molecular methods for stygofauna were primarily used to identify new species and identify evolutionary processes (e.g. Cooper et al., 2002), which was followed by the adoption of environmental DNA (eDNA) techniques to detect threatened subterranean species (Gorički et al., 2017; Niemiller et al., 2018). However, eDNA promises more than just the identification of single species and their lineages; it offers a powerful tool for the rapid, non-invasive assessment of stygofauna within groundwaters, providing information on biodiversity, ecosystem functioning, phylogenetics and trophic interactions from a single sample (see Boulton et al., 2023). This can be seen in the most recent uses of eDNA metabarcoding to identify multiple species within the same environmental sample to characterise stygofauna communities, functions and biodiversity (Korbel et al., 2022b) and their trophic interactions (Saccò et al., 2021, 2022b).

Environmental DNA/Ribonucleic Acid (RNA) methods are based on the concept that organisms shed DNA/RNA in groundwater either while they are alive (e.g. exoskeleton shedding) or leaving DNA when they die. As DNA lasts much longer than RNA (which degrades very quickly), eDNA indicates animals that have either lived, been transient or died in groundwater, with eDNA indicating the animals that are functionally present at the time of sampling. Recent studies have indicated that, due to the low presence of RNA within groundwater, presumably due to low biotic abundances, eDNA is a more viable method for biodiversity studies than environmental Ribonucleic Acid (eRNA) (Korbel et al., 2022c). Both eDNA and eRNA analyses in groundwater follow a conventional workflow (see Boulton et al., 2023) whereby groundwater and/or sediment samples (e.g. from springs, caves and wells) are filtered and membranes frozen (Korbel et al., 2022c). DNA is extracted from the membrane and Polymerase Chain Reaction (PCR) is conducted, samples are then sequenced and results are interpreted using bioinformatics (see Saccò et al., 2022c).

Environmental DNA analysis has several benefits over traditional sampling techniques (see Boulton et al., 2023). This molecular method has increased our knowledge of the breadth of taxa within groundwater ecosystems, as it is able to detect very small protozoans as well as cryptic species (Sbordoni et al., 2000), clarifying genetic difference between morphologically similar specimens and allowing the study of entire phylogenetic lineages (Zakšek et al., 2007). In addition, this method does not require the removal of animals from their habitat, and as such provides a non-intrusive method for monitoring rare and endangered species (Niemiller et al., 2018). Furthermore, eDNA methods allow for the characterisation of entire communities (prokaryotic and eukaryotic) and their functional roles and can elude to potential interactions between taxa (Deiner et al., 2017). Molecular methods are still rare for groundwater stygofauna studies (Fenwick et al., 2021; Korbel et al., 2017, 2022c; Lennon, 2019; Saccò et al., 2022c; Vörös et al., 2017; West et al., 2020), although they show great potential for their ability to identify new species and metabolic functions of groundwater biota (Boulton et al., 2023; Korbel et al., 2017) with improvement in these methods for the detection of stygofauna promising (e.g. Heyde et al., 2023).

However, eDNA methods do not come without limitations. A lack of reference sequence databases (Korbel et al., 2022c) often results in large numbers of unidentified taxa in the bioinformatic processing of sequences (Lennon, 2019; Saccò et al., 2022c), which is compounded by a lack of taxonomic keys for stygofauna in many parts of the world. There are also several knowledge gaps surrounding the use of eDNA within groundwaters, many of which involve the detection of *Crustacea*, a dominant stygofauna taxa. Additional research on primers, the fate and transportation of DNA within aquifers and research on stygofauna DNA shedding capacity (Korbel et al., 2022c; Trimbois et al., 2021) is required before this method can replace traditional sampling (Korbel et al., 2022b; Saccò et al., 2022c).

In summary, net sampling and pumping well water are the dominating sampling methods worldwide. Nevertheless, each of the described methods has its limitations. Hence, a combination of methods, such as net sampling together with pumping and/or DNA-analysis, is recommended (Korbel et al., 2017; Saccò et al., 2022a).

2.4 Global groundwater fauna research

The investigation of the global distribution of groundwater fauna sampling sites, both temporally and spatially, is one of the main aims of this paper. Our analysis revealed the scale of the uneven spatial distribution of sampling events and the number of studies over the world (Table A1.4 and Figure 2.6). Most research in the northern hemisphere is concentrated in Europe, Northern America and Northern Africa. In the southern hemisphere, groundwater fauna research is focused on Australia, with New Zealand researchers increasingly contributing to the knowledge of stygofauna. A lack of temporal sampling in many parts of the world was noted, with repeated sampling over many years mainly occurring in regions of Europe, Australia and America. This lack of replicated sampling has limited the understanding of basic biology and ecology of many species until now. Below a more detailed analysis on stygofauna studies is presented, by geographic region, on the spatial and temporal distribution of studies (Figure 2.6), topic of studies (Figure 2.7a) and sample methods employed (Figure 2.7b).

2.4.1 Africa

Africa, as a whole continent, is one of the least-studied regions of the world in terms of stygobiontic organisms (Tuékam Kayo et al., 2012). We identified a total of 155 studies, 749 sampling sites and 1,505 individual samplings (i.e. individual sampling events at one site, including repeated measurements). Studies concentrated in the Maghreb (Northwest Africa), particularly in Algeria (24 studies, 223 sampling sites, 816 samplings) and Morocco (44; 266; 347), with limited studies in South Africa (9; 20; 20) and Madagascar (4; 9; 11). For the remaining continent, only a few studies and very limited sampling sites exist (Table A1.3). Some apparently un-sampled regions of Africa, especially the Sahara, can be explained by the absence, or at least the low occurrence, of shallow groundwater, as can be seen by the light colours indicating a low groundwater recharge in Figure A1.3. Also, worth mentioning is the number of studies (13) with no information about the exact location of the sampling sites (star symbol in Figure 2.6), which is due to the age of these studies (> 30 years old), which were incompletely available in secondary literature. Similar observations regarding the lack of specific locations of species findings in Africa have been made previously (Tuékam Kayo et al., 2012).

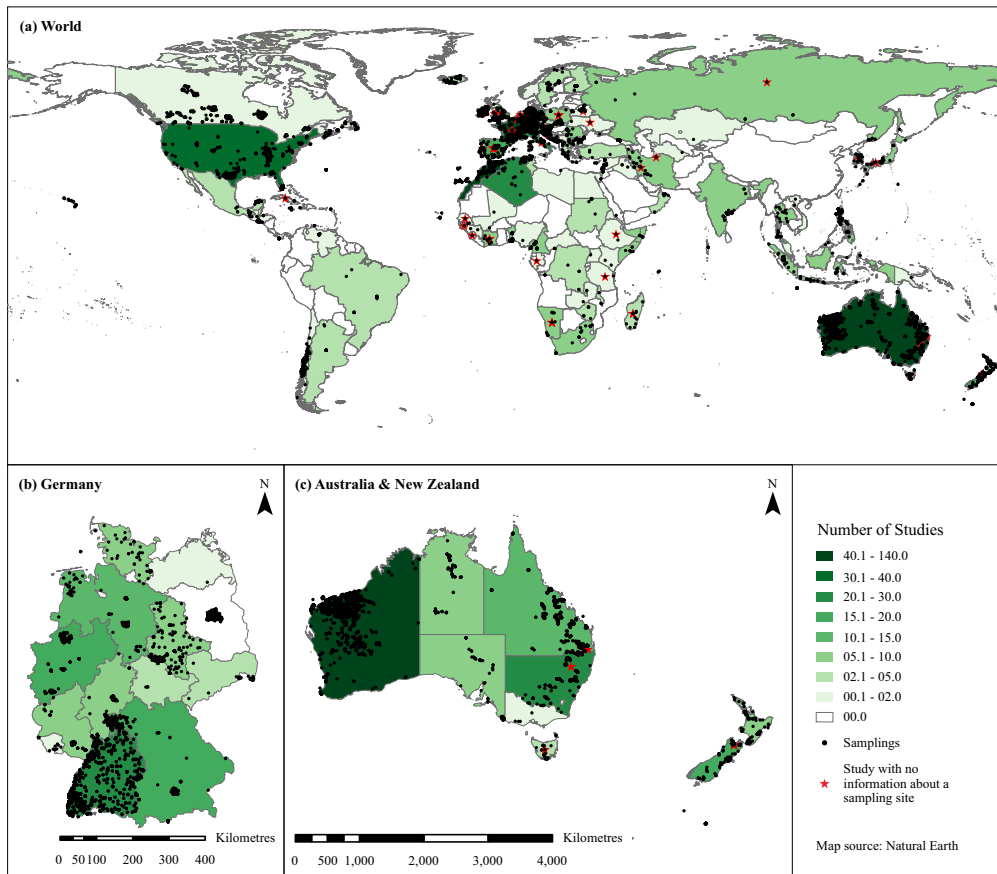


Figure 2.6: Overview of groundwater fauna samplings and number of studies (a) worldwide, (b) within Germany and (c) within Australia. Data sourced from 859 studies (Table A1.4).

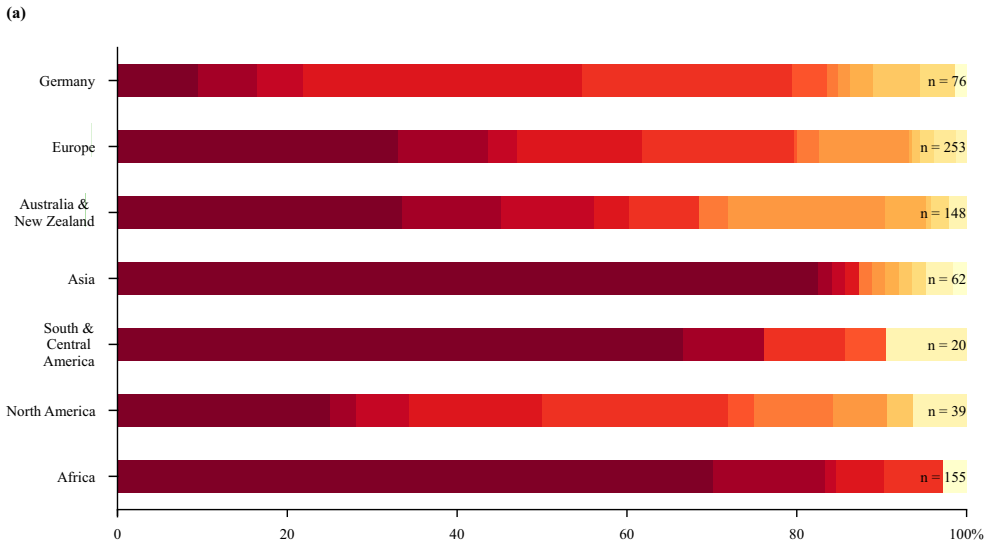
More detailed evaluation of the reviewed studies revealed that 70 % of studies in Africa concentrated on the description of newly discovered groundwater fauna (Figure 2.7a). Nevertheless, more than a dozen of studies in Algeria and Morocco conducted eco-toxicological investigations, revealing that faunal richness in some urban and mining areas is linked with groundwater quality and stygofauna abundance decreases with pollution (Aidaoui, 2019; Boughrou, 2007; Boughrou et al., 2007; Boulaassafer et al., 2021; Boulal et al., 2017; Boutin et al., 1995; Boutin and Idbennacer, 1989; El Adnani et al., 2007, 2006; El Moustaine et al., 2013, 2014; Hallam et al., 2008; Hichem et al., 2019; Laid and Zouheir, 2018; Merzoug et al., 2011, 2014; Ramzi et al., 2020) with authors proposing the use of stygofauna as bioindicators of water quality (Merzoug et al., 2011, 2014). Boulaassafer et al. (2021) study on evolutionary processes considerably expanded the knowledge of diversity and geographic range of a freshwater snail genus, and information on endemism and

biogeographical distribution of stygobiontic crustacean species of Africa and Madagascar has been recorded (Tuékam Kayo et al., 2012). Additional findings of a new Nematode species in deep (3.6 km) fractured aquifers of South Africa expanded the global knowledge of the understanding of life under extreme conditions such as high temperatures (41 °C), high pressures (1.3 to 6.8 kPa) or low dissolved oxygen concentrations (13 to 72 μM) and food shortages (Borgonie et al., 2011). Other research topics on the African continent such as biodiversity, biogeography and ecosystem service and ecological status are missing so far, as is any concerted effort from governments to monitor ecosystem health and biodiversity. Moreover, groundwater fauna of Africa is mainly sampled by using net samplers (in 25 % of the considered studies) or traps (14 %). In only 3 % of the considered studies pumps are used (Figure 2.7b).

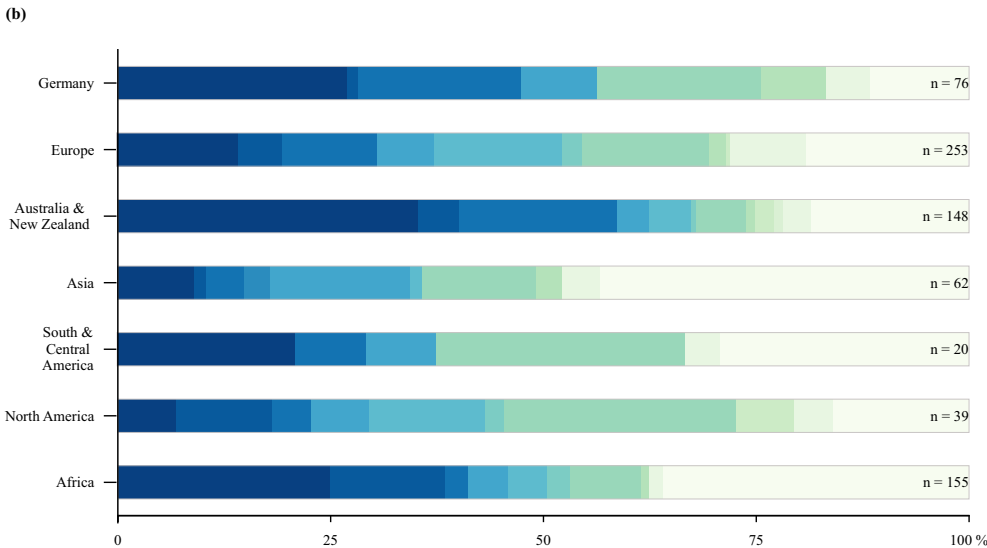
2.4.2 Americas

Combined, the Americas have a total of 57 studies focusing on groundwater fauna, showing a lack of research in this field, given the size of the continents. The distribution of studies is unevenly distributed across the Americas (Figure 2.6). In the United States, the first groundwater fauna research was conducted in 1842 in the Mammoth Cave by Tellkampf (Romero, 2001). Broader investigation started in the 1960s and 1970s, for example conducted by Culver and Holsinger (Culver and Holsinger, 1992; Holsinger and Longley, 1980). As can be seen in Figure 2.6, research is patchy and focussed on the federal state Texas (7 studies, 248 samplings) and the east coast, with the federal states New York (5; 23), Florida (4; 384), West Virginia (3; 443), the District of Columbia (2; 87) and Alabama (1; 1,529), contributing 29 groundwater fauna studies with a comparably large number of 3,368 individual samplings. In contrast to other continents, groundwater fauna is mostly sampled in caves, springs and the interstitial of rivers and is also linked to a limited use of net samplers (only 7 % of all studies; Figure 2.7b). The topic of most studies here is the description of newly discovered species including their traits (e.g. Wilhelm et al., 2006), followed by biogeographical analyses of groundwater fauna. In this context, studies on the influence of the last glaciation event on the present biogeographical distribution of stygobionts have to be emphasised. It is assumed that stygobionts are infrequent north of the glacial border and that more specialised species are unable to migrate into previously glaciated regions. Only less specialised species have invaded groundwater from surface after glacial retreat (Lewis and Reid, 2007; Strayer et al., 1995). Discussions on ecosystem service and biomonitoring on this continent are lacking.

As in North America, the main research focus in Central and South America are the description of newly discovered species (67 % of studies) and biogeographical analyses of groundwater fauna (10 %). In Central America, 14 studies were carried out at 128 sites, with the most studies conducted in Mexico (3 studies, 48 sampling sites). A similar number of studies (10) and samplings



Subjects of studies



Methods

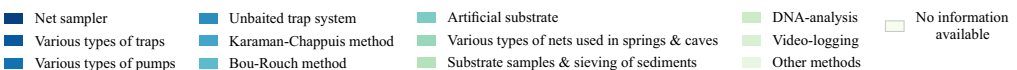


Figure 2.7: (a) Proportion of the different subjects of studies and (b) proportion of main techniques for sampling groundwater fauna for each continent and Germany (n = number of studies).

(235) were identified in South America, with a distinct lack of spatial distribution of studies across the continent (Figure 2.6). Groundwater research was conducted in both Argentina and Brazil, with studies concentrating on the impacts of land use and hydrogeological characteristics on groundwater invertebrate (Davaló Centurião et al., 2020; Tione et al., 2016).

In summary, North America groundwater fauna studies predominantly provide insight into newly discovered species and biogeographical analyses including factors influencing biogeographical distribution of stygobionts (e.g. glaciation), whereas Central and South America studies typically also concentrated on the discovery of new species and anthropogenic influences on these taxa. There were limited studies in porous aquifers, with groundwater fauna being also mostly sampled in caves, springs and the interstitial of rivers. Studies on the influence of aquifer types, climates, aquifer types and human disturbances on stygofauna are in their infancy, with very limited discussions on ecosystem service up to now.

2.4.3 Asia and the Middle East

Although it boasts one of the oldest records of cave fauna (a cave fish identified in China in 1537), studies on groundwater fauna in Asia are limited and unevenly distributed. Overall, 63 studies and 1,286 samplings were recorded in Asia on 745 sampling sites (excluding Russia, Turkey and Cyprus, which are counted as European). The main focus of groundwater fauna studies was the description of newly discovered species (83 %), with 119 new species and seven new genera described in the available studies. Similar to North America, groundwater fauna is mostly sampled in caves, springs and the interstitial of rivers, with the Karaman–Chappuis method and the usage of various types of nets being the dominating sampling methods.

In Eastern Asia, Japan and South Korea dominated the sampling effort (9; 237), with seven and nine groundwater fauna studies, respectively. In 1916, the first groundwater animal of Japan was described, while the first comprehensive and large-scaled study on faunal distribution was published in 1976 (Matsumoto, 1976), with the presence (or absence) of groundwater fauna revealing their potential as bioindicators (Matsumoto, 1976). Recent studies in Japan and South Korea have focused on the understanding of the subclass *Copepoda*, especially on the origin, relationship and distribution patterns of different species by analysing morphological micro-characters and phylogenetic relationships using modern techniques like scanning electron microscopy and molecular techniques (Karanovic, 2020; Karanovic et al., 2013, 2015; Karanovic and Lee, 2012; Karanovic et al., 2012). Berkhoff et al. (2003) was the first groundwater fauna research in South Korea in 2003 and related the distribution of fauna to land use (Berkhoff et al., 2003).

In the eastern part of India, seven studies with 43 samplings have been identified. The description of newly discovered species is the subject of all studies, while two recent studies have applied phylogenetic analyses to examine evolutionary relationships (Bandari et al., 2017; Karanovic and Ready, 2004). The research project 'Biodiversity of subterranean groundwater fauna of India, with special reference to *Copepoda* and *Bathynellacea*' from 2008 to 2013 increased the number of stygobiotic cyclopoid species of India from three to 11 (Totakura and Reddy, 2015). In all, one genus and 17 species have been described.

Within South-east Asia, there have been a total of 25 studies in the following three countries: Indonesia (9 studies; 51 samplings), Philippines (10; 139) and Thailand (6; 637). Descriptions and temporal analyses of species are the subject matter of all these studies, with research on caves dominating (e.g. Culver et al., 2006; Watiroyram et al., 2017). However, Husana and Yamamuro, 2013 have attempted to identify several factors impacting stygofauna distributions. According to Brancelj et al. (2013), 122 stygofauna species have been described within South-East Asia, with 24 species described after this review. Additionally, an extensive study on ecosystem health and monitoring in the Philippines is currently underway (Magbanua, 2022).

A larger number of groundwater fauna studies (27 studies with 129 samplings) can be found in the Middle and Near East. However, 37 % of the studies and 33 % of the samplings were conducted in Iran. Research on groundwater fauna, focused on the amphipod genus *Niphargus*, has been conducted in Iran (Esmaeili-Rineh et al., 2015). Morphological characters and phylogenetic analyses resulted in the identification of 17 new stygofauna species in 2018, along with studies comparing Iranian amphipods with European nipargids (Bargrizaneh et al., 2021; Esmaeili-Rineh et al., 2016, 2017a, 2018, 2015, 2017b; Hekmatara et al., 2013; Mamaghani-Shishvan and Esmaeili-Rineh, 2019). Other studies in Iran used molecular techniques to investigate the distributional ranges of amphipods, revealing that 'there is no evidence to consider that groundwater species are geographically more restricted than surface species' (Esmaeili-Rineh et al., 2020), thus adding to the global understanding stygofauna distribution.

Our findings reveal that in Asia and the Middle East, most studies are still focused on the description of newly discovered species, with limited studies investigating the origin, functioning and distribution of stygofauna or groundwater ecology. Spatially, studies are concentrated in Japan, South Korea, India and Iran. In relation to the large area of this continent, Asia is poorly investigated and more information is required to effectively describe the biodiversity of stygofauna in this region.

2.4.4 Australia and New Zealand

With 133 studies, 4,014 sampling sites and 5,826 samplings in total, extensive research on stygofauna and groundwater ecology is still conducted in different regions across Australia. Studies by Charles Chilton between 1882 and 1925 placed Australia at the forefront of groundwater fauna research in the late 19th and early 20th centuries. Much of the early focus of research on groundwater fauna was on discovery, species descriptions and biogeographic and evolutionary processes (Goater, 2009), with early studies on the origin and evolutions of groundwater biota occurring in western and central Australia (Bradford et al., 2010; Cooper et al., 2002; Leys et al., 2003). In the mid-late 1990s, groundwater fauna research saw a resurgence, as ecological analyses became a requirement for some environmental impact assessment (e.g. in Western Australia in 1998), with specific species gaining legislative protection (e.g. the crustacean *Lasionectes exleyi*). Government policies developed throughout the late 1990s to the early 2000s, aimed at protecting groundwater and their related ecosystems (Goater, 2009; Humphreys, 2006; Playford, 2001).

The close links between groundwater fauna research, water management policies and extractive industries (Hose et al., 2015) have seen sampling efforts unevenly distributed throughout the continent. Studies are focused in areas of intensive mining activities (Hose et al., 2015) and in the Murray Darling Basin, an area heavily reliant on groundwater for agriculture and potable water (Figure 2.6). As a result, the majority of groundwater fauna studies have been conducted in Western Australia (WA), New South Wales (NSW) and Queensland (QLD) (78, 23 and 15 studies, respectively), with notable descriptions of the stygofauna inhabiting Tasmanian cave systems (Eberhard, 1992, 2001) and research in South Australia that have contributed to knowledge stygofauna distribution and ecosystem functioning (e.g. Smith et al., 2016; Zeidler, 1985). Due to legislative requirements for sampling, groundwater fauna research has mainly focused on wells, using net and pump collection methods, with recent government initiatives investigating the effectiveness of sampling methods and eDNA for stygofauna monitoring (Korbel et al., 2022b).

In WA, research has focused on the description of new species (28) and phylogenetics (12), with more recent studies looking at stygofauna diversity (5 studies). Most sampling events in WA have been conducted in the iron-ore-rich areas of the Pilbara region (2,020 of 3,742 samplings). Early studies from the Pilbara unveiled one of the richest stygofauna diversities in the world (Eberhard et al., 2004, 2005; Humphreys, 2001), with descriptions of Amphipods (Bradbury and Eberhard, 2000), Isopods, Ostracods (Karanovic and Marmonier, 2003; Karanovic, 2006), Spelaeogriphaceans (Poore and Humphreys, 1998) and Copepods (De Laurentiis et al., 1999). The biodiversity of the areas was further uncovered in 2004 with extensive surveying detecting stygofauna in 71 % of sampled wells, with an average of 3.8 taxa and 23.3 individuals per sample (Eberhard et al., 2004). The Yilgarn region of WA has also seen a concentration of stygofauna genetics and evolution research, with 979 samplings in its numerous isolated calcrete aquifers,

leading to suggestions of evolution within individual calcretes following independent colonisation by their epigeal ancestors ('subterranean island hypothesis') (Allford et al., 2008; Cooper et al., 2007). West of the Pilbara region, the first discoveries of groundwater fauna occurred in the Cape Range and Barrow Island, with research here continuing (e.g. Saccò et al., 2022d). The Cape Range Province in the Gascoyne region is globally recognised for its subterranean fauna and karst systems (Goater, 2009). Research in calcrete aquifers has added to the global understanding of stygofauna distribution patterns (Humphreys, 2001; Saccò et al., 2020). In the Perth region, with 30 samplings, studies focused on Copepods from basins and craton aquifers (De Laurentiis et al., 2001). Other surveys were the result of legislative requirement on coal and iron ore projects, e.g. surveys in the Enneaba region which resulted in the discovery of an undescribed Bathynellidid (see Hose et al., 2015). More recently, functional ecology (Bradford et al., 2013, 2010; Saccò et al., 2019) and investigations into the use of eDNA techniques for stygofauna (e.g. Heyde et al., 2023) have become the object of stygofauna studies in WA. Such studies are contributing greatly to the worldwide understanding of ecosystem functioning, processes and stygofauna distribution.

Sampling in the Eastern states of Australia (QLD, NSW, Victoria (VIC)) is again linked with mining and agricultural groundwater dependencies. NSW is represented by 23 studies, 255 sampling sites and 794 samplings. The Hunter Valley contains over 20 of the world's largest coal mines, which resulted in numerous ecological surveys investigating stygofauna (including microbiota). Early work in the Hunter region improved the ecological knowledge of stygofauna, identifying the importance of organic matter supply for stygofauna richness (Hancock and Boulton, 2008), and leading to the discovery of the first stygobiontic beetle in eastern Australia (Watts et al., 2007). Other early work focused on biodiversity within karst ecosystems (e.g. Eberhard and Spate, 1995). However, most sampling sites in NSW (225) are located in the arid to semi-arid regions of the Murray Darling Basin, where industries extracting groundwater dominate (e.g. mining, agriculture). Here, groundwater studies have focused on the ecology of the alluvial deposits of the Namoi and Gwydir River catchments, improving knowledge of the environmental and human influences on stygofauna distribution (Eberhard et al., 2017; Menció et al., 2014), their connectivity with surface waters (e.g. Korbelt et al., 2022c), then using this information to develop frameworks for the assessment of groundwater ecosystem health (Korbelt et al., 2017, 2022a, 2013a; Korbelt and Hose, 2011, 2015, 2017; Korbelt et al., 2013b, 2019). Several of these Australian studies have been amongst the first to investigate the use of eDNA as a method for assessing biodiversity (e.g. Asmyhr and Cooper, 2012) and ecosystem functional processes in groundwaters. Others have been conducted in the alluvial aquifers of the Murray, Murrumbidgee, Lachlan and Macquarie catchments (Lennon, 2019; MacDonald, 2017; Nelson, 2020). Moreover, a review by Saccò et al. (2022a) on coastal groundwater ecosystems in Australia points out the importance of stygofaunal communities in coastal aquifers and the threats to them caused by size-reduction of the aquifer,

salinization from seawater intrusion, land clearing, anthropogenic contamination and impacts of mining and industry.

In 2011, Korbelt and Hose suggested a tiered multi-metric framework for assessing ecosystem health in groundwater, resulting in the Groundwater Health Index (see Section 2.2.4). This framework was first applied in the Gwydir River catchment, demonstrating differences in groundwater fauna and water quality under different land uses and allowing a numerical health ranking (Korbelt and Hose, 2011, 2017). The GHI was improved in 2017 (Korbelt and Hose, 2017) where its use was expanded into the Namoi and Macquarie catchment (Korbelt and Hose, 2017) and is currently being utilised by the NSW government to monitor groundwater health in several of the Murray Darling subcatchments (NSW Department of Planning and Environment, 2022) and has been adapted for Europe (e.g. Di Lorenzo et al., 2020a) and the Philippines (Magbanua, 2022).

In Queensland, sampling of stygofauna is geographically patchy and sparse (15 studies, 1,077 samplings), with many areas of the north and west un-sampled (Glanville et al., 2016). The spatial distribution is clustered around locations with extractive industry and intensive groundwater use (Glanville et al., 2016), for example in the Bowen (188 samplings) and Surat Basins (373 samplings), where Australia's largest known proven coal seam gas reserves are located (Hose et al., 2015). In the Surat Basin, consultant reports (Subterranean Ecology, 2012) fauna diversity in the Horse Creek alluvium and Walloon coal measures near Wandoan were described (Hose et al., 2015). The knowledge of stygofauna biogeography and biodiversity in Queensland has been contributed to by several studies (e.g. Little et al., 2016; Schulz et al., 2013) and is described in Glanville et al. (2016). A special feature of the state is the Queensland Subterranean Aquatic Fauna Database, which contains data from 755 samples of 582 sites provided by the Queensland Government and industry. In recent times, work describing groundwater species has occurred in the Northern Territory (Oberprieler et al., 2021; Rees et al., 2020).

Also, of note in this global region is the stygofauna research conducted in New Zealand (NZ), with 23 studies covering 305 sites. The first research on subterranean fauna in New Zealand was conducted by Charles Chilton (1882) who described the first amphipods. This study focused on range extensions and intraspecific variations, from the southern hemisphere in the alluvial groundwaters of the Canterbury Plains. An extensive sampling effort in the 1970s was mounted by Kuschel in the Waimea Plains, producing a collection of insects, crustaceans and molluscs (Fenwick, 2001). These early studies were followed by assessments of stygofauna distribution patterns (Fenwick and Scarsbrook, 2004; Scarsbrook and Fenwick, 2003; Wilson and Fenwick, 1999), hyporheic fauna (Boulton et al., 1997) and potential human impacts (Sinton, 1984). Such studies were succeeded by investigations into groundwater fauna ecology (Fenwick et al., 2021), interconnected hyporheic zones (Larned et al., 2007), human impacts on stygofauna (Hartland et al., 2011) and ecosystem functioning, including microbial studies (Close et al., 2008; Weaver et al.,

2016). Alongside the ecological studies, there have been significant collections of stygofauna in the BioHeritage Project, funded by the National Institute of Water and Atmospheric Research (NIWA), where reference databases from 65 wells were collected with the aim to develop invertebrate indicators of groundwater health (Greenwood and Fenwick, 2019).

Overall research within the Australasian region has aided the global knowledge of stygofauna and their functions. It has been stated that ‘Australia is considered world leading in its recognition of the need to protect groundwater resource and their dependent ecosystem through water resource policy’ (Goater, 2009). Australia is regarded as a pioneer in the field of stygofauna monitoring programs but has also contributed greatly to the global understanding of stygofauna evolution, distribution, sampling methods, ecosystem functions and processes as well as anthropogenic impacts on these ecosystems (e.g. Bradford et al., 2010; Hose and Stumpp, 2019; Humphreys, 2001; Korbel et al., 2022a, 2019; Leys et al., 2010; Murphy et al., 2009; Saccò et al., 2021). Due to the emergence of policies and legislation based on GDE in this region, researchers have been at the forefront in incorporating global knowledge on groundwater species, ecology and responses to disturbance to lead in the development of applied ecological research. This research is being used by governments to monitor, evaluate and report on groundwater health.

2.4.5 Europe

Groundwater fauna research in Europe dates back to the 17th century, with pioneering work in France (Hertzog, 1933; Moniez, 1889), Italy (Pesce, 1980), Austria (Spandl, 1926), Germany (Kiefer, 1957; Noll, 1939), Slovenia (Freiherr von Valvasor, 1689; Sket, 1999), Switzerland (Graeter and Chappuis, 1913; Schnitter and Chappuis, 1914) and Spain (Camacho, 1989; Notenboom and Meijers, 1985). In total, there are 358 studies in Europe, covering 12,524 sites. As in all continents, early groundwater fauna research in Europe began with descriptions of species and taxonomy as well as the development of more complex sampling methods (e.g. the Karaman–Chappuis method 1933 and the Bou and Rouch, 1967). The application of diverse sampling methods (net sampler, various types of pumps and nets and Bou-Rouch method), particularly in Germany, has led to groundwater fauna research over numerous stygofauna habitats, including interstitial and hyporheic zone of rivers, cave and springs (Figure 2.7b).

Spatial analysis of fauna sampling sites in Europe is concentrated near the latitude of 45 °N, along the Pyrenees in the west to the Dinaric Karst of Slovenia, Serbia, Montenegro and Croatia in the east. At this latitude, richness of aquatic and terrestrial species is high, resulting in the preferential examination of these fauna hotspots in many groundwater studies (Rapoport’s rule (Rapoport, 1982)) (Culver et al., 2006; Pipan et al., 2020; Zagmajster et al., 2014). Additionally, as many of these ‘hotspots’ are located in Europe’s vast cave system, some of which have special legislative

protection (e.g. Vjetrenica in Bosnia Herzegovina), there has been a concentration of studies in these regions. For example, the Postojna–Planina Cave System (PPCS) in Slovenia is one of the most-studied caves globally, with more known stygobiontic species than any other cave or subterranean location in the world (Culver and Sket, 2000). In addition to research on new species, ecological and species distribution studies have been conducted in these karst environments, highlighting the potential use of copepods as natural tracers of complex water movements in epikarst (Pipan and Culver, 2007). Contrastingly, northern Europe has had a low frequency of sampling, with studies here indicating stygofauna consist mainly of a few old stygobiontic species and ubiquists (Särkkä and Mäkelä, 1998; Thulin and Hahn, 2008).

European studies on groundwater fauna have produced much of the global knowledge on the impact of natural events (e.g. glaciation, earthquakes) on stygofauna distribution and patterns of endemism (Särkkä et al., 1998; Thulin and Hahn, 2008). Due to its status as an island and its glaciation during the last ice age, the United Kingdom (UK) is also interesting for groundwater fauna research; however, there is a distinct lack of research into groundwater ecology in this region with only 10 stygobiontic species, three of them endemic to Ireland or England, having been identified. Moreover, most groundwater taxa in England have been collected in cave systems (Maurice, 2009), with the known distribution of most stygobiontic taxa restricted to an area south of the maximum limit of the Devensian glaciation (Proudlove et al., 2003). Additional knowledge of the impacts of natural events on stygofauna endemism and distribution were uncovered after the 2009 earthquake in L'Aquila, Italy, with authors who observed a decrease in subterranean copepod species abundance as a result of the earthquake-induced aquifer strain and a consequential flushing of fauna (Galassi et al., 2014).

As in Australia, European groundwater ecology research has been advanced through policies and legislative requirements in the 1990s. Particularly worthy of mention is the importance of the Swiss Water Protection Ordinance in groundwater research, which was one of the first international authorities to include monitoring of both water quality (physical–chemical water standards) and ecological criteria for groundwater systems (Danielopol and Griebler, 2008). In 2006, the European Groundwater Directive also triggered groundwater ecological research by stating the importance of protecting groundwater ecosystems, noting ‘research should be conducted in order to provide better criteria for ensuring groundwater ecosystem quality’ (European Union, 2006).

These government initiatives precipitated the PASCALIS project, which was the first project to investigate groundwater biodiversity and endemism patterns across several countries (Gibert and Culver, 2009). PASCALIS not only introduced a standardised sampling technique but also uncovered the spatial distribution of stygofauna (locating 214 species new to six European regions) and 112 species new to science (Gibert and Culver, 2009). This project was important for a global understanding of the importance of hydrological connectivity on biotic distribution within

groundwaters. Additional research in Europe during this time also contributed to the global understanding of stygofauna with suggestions that altitude, hydrogeology, palaeographical factors and human activities in a region can interact in complex ways to influence species diversity and compositions (M. J. Dole-Olivier et al., 2009; Gibert and Culver, 2009).

Other research around the 1990s focused on the human impacts, ecotoxicology as well as functional roles of groundwater fauna (Avramov et al., 2013; Becher et al., 2022; Castaño-Sánchez et al., 2020a; Di Lorenzo et al., 2019; Reboleira et al., 2013) leading to the development of bioindicators, which were utilised in more recent applications of ecology into groundwater monitoring frameworks. These studies along with studies indicating stygofauna habitat tolerances and distribution patterns (e.g. M. J. Dole-Olivier et al., 2009) and human impacts on stygofauna (e.g. Di Lorenzo et al., 2015, 2020b; Di Lorenzo and Galassi, 2013) have indicated that groundwater organisms can be used as tools of landscape changes with the absence or presence of communities reflecting the impact of changes in regional groundwater quality (Marmonier et al., 1993). Adding to this research, studies investigated the agricultural impact in alluvial aquifers on groundwater communities, producing threshold values for nitrate, and produced faunal indicators of human impacts and thus groundwater health. Additional studies in Italy building on the Australian groundwater health index (Korbel and Hose, 2017) developed a European-based monitoring framework specific to nitrate (Di Lorenzo et al., 2020a). Castaño-Sánchez et al. (2020b) reviewed existing ecotoxicological studies and presented a database containing experimentally derived species' tolerance data for 28 contaminants and temperature for 46 terrestrial and groundwater species.

Due to the breadth of stygofauna studies in Europe, this region has been at the forefront of developing bioindicators of groundwater condition (e.g. Malard et al., 1996; Marmonier et al., 2018; Mösslacher and Notenboom, 1999). Early attempts to use stygofauna as indicators for monitoring formed in Europe (e.g. Hahn, 2006; Steube et al., 2009; Stoch et al., 2009). Other more recent studies in Germany (see below section) have resulted in the development of ecological assessment frameworks (Fillinger et al., 2019; Griebler et al., 2014b). Marmonier et al. (2018) used two combined methodological approaches to assess the ecological status of groundwater ecosystems in two alluvial plains in France. Composition analysis showed that the species richness, abundance and assemblage composition significantly changed with agricultural land use or urbanisation around the wells, and in wells with low oxygen and high nitrate concentrations, the Ecophysiological Index (EPI) decreased.

The understanding of subterranean biodiversity and human impacts on this ecosystem is a necessary step for incorporating current biological concepts within the framework of groundwater management (Danielopol et al., 2004). Along with Australia, the majority of research on groundwater ecology and applications for management and monitoring frameworks was conducted in Europe (Fillinger et al., 2019; Griebler et al., 2014b, 2010; Hahn, 2006; Koch et al., 2021; Stoch et al., 2009).

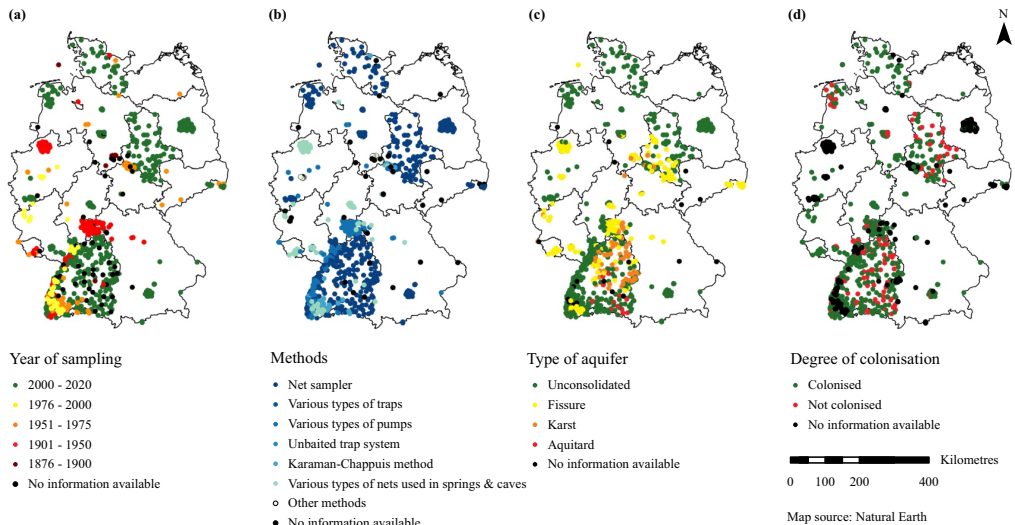


Figure 2.8: Overview over the (a) year of sampling; (b) used main techniques for groundwater sampling; (c) type of the aquifer and (d) the degree of colonisation of every sampling in Germany.

As can be seen, much of the current research on European groundwater health frameworks has been conducted in Germany and Italy (Di Lorenzo et al., 2020a; Stoch et al., 2009) with collaborations between German, Italian and Australian researchers a noted development (Castaño-Sánchez et al., 2020a,b; Danielopol et al., 2003; Di Lorenzo et al., 2019; Galassi et al., 2009; Korbel et al., 2022c; Stumpp and Hose, 2013).

2.4.6 A focus on Germany

Our study indicates that research on groundwater fauna began in Germany in 1876. Since then, there are records of 76 studies, 2,378 sampling sites and 4,232 samplings (sampling density: 1.18×10^{-2} [samplings/km²]) (Figure 2.8a). Although comparable high sampling densities can be found in Slovenia (4.59×10^{-2} [samplings/km²]), Luxemburg (2.48×10^{-2}), Austria (1.78×10^{-2}) and Belgium (1.46×10^{-2}), these countries are spatially smaller and have less than half the number of studies and samplings than Germany. The high density and frequency of sampling in Germany have placed Germany at the forefront of stygofauna research.

Much of the sampling effort in Germany has occurred since 2000, with sampling concentrated in the state of Baden-Württemberg (Figure 2.8a). Fauna samplings are dominated by net and pump sampling (wells) and nets (caves and springs) (Figure 2.8b). Sampling methods vary due to aquifer type, for example netting method dominates studies in fractured chalk rock springs

of the Baumberge area (Beyer, 1932). Nevertheless, most German research has occurred in unconsolidated aquifers (Figure 2.8c).

In the state of Baden-Württemberg, there have been 28 studies, at 950 sample sites with 2,026 samplings, at a density of 5.6×10^{-2} samplings per square kilometre (area: 35,751 km²), one of the highest worldwide (Table A1.3). By the early 2000s, 105 taxa, 60 of them stygobionts, had been found in Baden-Württemberg, with studies covering diverse hydrogeology, including karst, quaternary sediments, crystalline and sedimentary rocks (see Figure A1.3). As such, this region has contributed to the global understanding of stygofauna distribution, including the identification of relationships between subterranean fauna distribution and hydrogeological aquifer type (Hahn and Fuchs, 2009). The temporal resolution of data in Baden-Württemberg is also remarkable, with 44 sites sampled annually or bi-annually between 2002 and 2020. Such frequent sampling has enabled the temporal analysis of groundwater fauna assemblages, with results indicating that abiotic, microbiological and faunal parameters displayed limited changes between 2002 and 2014 (Stein et al., 2015).

Colonisation of groundwater by fauna has been a main topic of study within Europe. Stygofauna have surface water origins, with numerous theories surrounding their colonisation of groundwaters (Cooper et al., 2023), resulting in a high degree of endemism, many relict species ('living fossils') and a truncated food web consisting of few predators (Gibert and Deharveng, 2002). Another characteristic of groundwater is the scarcity of stygobiontic species so that often only half of all stygobiontic species in any given region are found at less than 5 % of sites (Castellarini et al., 2004; Hahn, 2015; Martin et al., 2009).

Distribution patterns within Europe are particularly impacted by glaciation (Stein et al., 2012; Stoch and Galassi, 2010). Our survey revealed that stygofauna were found in 63 % (2,662 samples) of samples in Germany. However, in 27 % of these samples, no information on the colonisation status was available, potentially due to the clustered results of older studies (early–mid-1900s), where results of individual measurements were not resolved on a site-specific level but interpreted as an overall result for a wider research area (Figure 2.8d).

The volume of data collected in Germany has resulted in the ability to investigate stygofauna distribution. Studies within Germany have indicated differences in distribution patterns of stygofauna spatially, with investigations of 'stygoregions' (Gibert et al., 2009) used to explain differences in distribution patterns explained by geological events (Hahn and Fuchs, 2009; Stein et al., 2012), such studies have been completed elsewhere in Europe (e.g. Stoch and Galassi, 2010). Studies have indicated that the degree of colonisation varies spatially within Germany, e.g. groundwater fauna is reported to be nearly absent in the Northern Lowlands, because of fine sediments and low oxygen concentrations (Stein et al., 2012). Yet, our analysis shows that 37 % (161 samples) of the samples in this region contained fauna. Nevertheless, the number of samples

with no available information is still high (226 samples; 52 %). Additionally, there has been important research conducted on the impacts of geology and water chemistry on stygofauna, with studies investigating the impacts of oxygen concentrations (Stein et al., 2012) and physical habitat characteristics (e.g. aquifer and pore sizes; Hahn, 2006; Hahn and Fuchs, 2009; Hahn and Matzke, 2005; Stein et al., 2012) on stygofauna distribution.

Interestingly, there is a clear difference in the main subjects studied between Germany and the rest of Europe. Within Germany, stygofauna biodiversity (33 %) and biogeography (25 %) are a distinct focus of research, with only 10 % of the studies in our literature review focused on the description of species. This differs from the European context, where 33 % of studies focused on taxonomy and species descriptions and 18 % on biogeographic distribution and evolutionary processes.

Besides the focus on biodiversity and ecology issues, Germany has seen itself at the forefront of applying ecological research to address groundwater management and monitoring requirements. Early approaches for monitoring groundwater ecosystems began in Germany (see Section 2.2.4). Hahn (2006) introduced early frameworks, and Stoch et al. (2009) developed a predictive model for assessing groundwater ecosystems. Further, Steube et al. (2009), supported by the German Federal Environment Agency, suggested the use of biotic and abiotic indicators in groundwater ecosystem assessment and acknowledged difficulties in establishing reference conditions and the need to test proposed methods. Additionally, Griebler et al. (2010) proposed groundwater assessment methods utilising aquifer typology. These significant early developments paved the way for the development of the groundwater health frameworks (Korbel and Hose, 2011, 2017) and, for Griebler et al. (2014b), ecologically-based assessment scheme for groundwater ecosystems. Much of this work has been aimed to support the European Union Water Framework Directive (EC-WFD) 2000. An assessment scheme using microbial indicators has since been developed the microbial Density-Activity-Carbon index (Fillinger et al., 2019), and Germany-based assessment schemes have been applied in numerous studies (Berkhoff, 2010; Gutjahr et al., 2014; Hahn et al., 2020; Koch et al., 2021; Spengler, 2017).

2.5 Conclusion

This study has provided a global perspective on groundwater fauna research including its historical and technical development and spatial distribution.

The main findings of the current study are as follows:

- a continuing, exponential increase in the number of studies on groundwater fauna over the last 10 decades,
- changing research paradigms from the description of newly discovered species and their evolution towards ecosystemic and more holistic analyses of fauna and their functions to the application of ecological management and monitoring programs,
- a change in sampling methods from simple nets and hand pumps to more complex methods,
- the recent emergence of molecular technologies, such as eDNA, which offer the potential to ease sampling and enable vast data collection,
- large gaps in the spatial and temporal distribution of groundwater fauna remain, particularly in Africa, Asia and the Americas,
- due to spatial biases, the knowledge on groundwater biota and their potential functions may be biased towards the intensively sampled aquifers studied in Europe, Australia and New Zealand.

As such, a comprehensive and broad overview of the global geographical distribution of groundwater fauna in diverse climatic zones, aquifer types and associated trends over time is still required. In the future, a shift from local studies to a global perspective is essential in order to provide a common knowledge basis for understanding, assessment, monitoring and conservation of groundwater biodiversity. A worldwide effort to collect information on groundwater ecosystems, functional roles and human impacts on them is required to implement stronger policies and monitoring requirements for groundwater fauna so as to ensure these ecosystems are maintained and preserved into the future.

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3

TEMPORAL SHIFT OF GROUNDWATER FAUNA IN SOUTH-WEST GERMANY

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3.1 Introduction

Groundwater is an important source of freshwater, drinking water and service water for irrigation, geothermal and industrial uses (Job, 2022; Siebert et al., 2010; Stauffer et al., 2013). Furthermore, groundwater ecosystems build the largest terrestrial freshwater biome of the world (Griebler et al., 2014a), which is considered a species-rich habitat (> 100,000 species) with many endemic taxa (Culver and Holsinger, 1992).

However, human activities and natural changes threaten this habitat in many areas (Becher et al., 2022), put pressure on its ecosystem and groundwater communities, and alter general biogeochemical processes (Griebler et al., 2016). Changes in water quality and water volume are driven by natural processes (Goater, 2009), yet groundwater abstraction for irrigation, drinking

water and mining activities changes groundwater volume and, therefore, groundwater levels (Danielopol et al., 2003; Hancock et al., 2005). Changes in groundwater quality can also be caused by pollutants originating from agriculture (Di Lorenzo et al., 2020b; Di Lorenzo and Galassi, 2013; Korbelt et al., 2022a), urbanisation (Hallam et al., 2008), surface waters (Danielopol et al., 2003; Kristensen et al., 2018), heavy industry (Hose et al., 2014) and thermal pollution (Menberg et al., 2013a; Taylor and Stefan, 2009; Tissen et al., 2019; Zhu et al., 2010). Moreover, climate change, which increases groundwater temperatures (Figura et al., 2011; Menberg et al., 2014; Tissen et al., 2019), puts pressure on groundwater and its ecosystems. Those environmental pressures can alter groundwater fauna community structures (Korbelt et al., 2022a) and, therefore, induce changes in groundwater biodiversity and species dominance (Goater, 2009). Typical implications of this are changes in the natural variation of population cycles, shifts in the composition of the community as ubiquitous surface-water species can outcompete and replace groundwater species, and, finally, species extinction (Danielopol et al., 2003). The potential decline or even loss of groundwater communities compromises the functioning of an aquifer and its ecosystem, resulting in deterioration of groundwater health (Hancock, 2002; Hancock et al., 2005).

To assess groundwater's health or ecological status, schemes for monitoring this ecosystem, i.e., biomonitoring, become increasingly important, as already implemented for surface water (Haase et al., 2023). Biomonitoring is defined as the 'use of biological systems (organisms and organism communities) to monitor environmental change over space and/or time [DIN EN 16413]' (Verein Deutscher Ingenieure e.V. (VDI), 2018). More specifically, by repeatedly or permanently monitoring organisms or organism communities, the quality of a habitat environment is recorded and assessed, and the state of an ecosystem and its dynamics in time and space are evaluated (Mösslacher and Notenboom, 1999; Underwood, 1997). For application in groundwater, two strategies are currently available (Friberg et al., 2011; Mösslacher et al., 2001; Mösslacher and Notenboom, 1999). In 'active' biomonitoring, standardised organisms with a known origin from the laboratory are inserted in groundwater, and their behaviour is monitored in this habitat (see the biological early warning system of the GroundCare project Spengler et al. (2017)). In contrast, in 'passive' biomonitoring, bioindicators (wildlife population) are sampled from their natural habitat and their behaviour is examined in the laboratory (Brielmann et al., 2011) or as in most studies, the faunal communities found are analysed to conclude the prevailing conditions (Fuchs et al., 2006; Hahn and Fuchs, 2009; Korbelt and Hose, 2015; Stein et al., 2010; Verein Deutscher Ingenieure e.V. (VDI), 2018).

The advantage of biomonitoring compared to monitoring abiotic parameters is that organisms and communities act as remote sensors integrating environmental conditions and stress over their lifetime, undergo quantitative and qualitative alterations and thus can provide information on medium- to long-term environmental conditions and changes (Conti, 2008). Hence, for most existing biomonitoring programs, faunal community composition, different species and taxa,

and an abundance of other taxonomic groups are considered (Conti, 2008; Friberg et al., 2011). Macroinvertebrates are often used for biomonitoring as their taxonomy is well known, they are sensitive to many stressors and can be sampled easily and repeatedly over specific time periods, which results in more accurate indications of diversity, richness and composition among all groundwater biota (Conti, 2008; Friberg et al., 2011; Lennon, 2019).

However, until now, a lack of repeated and long-term samplings of groundwater fauna has limited the understanding of the basic biology and ecology of many species, and there is a notable absence of analysis of temporal evolution and variation of groundwater fauna (Koch et al., 2022). Repeated samplings over multiple years can be found in very few locations, mainly in Central Europe and Australia (Goater, 2009; Korbelt and Hose, 2017; Marmonier et al., 2000; Menció et al., 2014). Goater (2009) analysed groundwater fauna data from an 8-year monitoring program (1999 - 2007) at 21 wells in Australia, observing changes in stygofauna species presence, population numbers and community assemblages, as well as a shift from an amphipod-dominated to a copepod-dominated system. A second study in Australia conducted four sampling campaigns between 2007 and 2010 at 20 wells to investigate stream–aquifer relationship (Menció et al., 2014). Further ecological research focused on examining environmental and human influences on the distribution of biota and groundwater ecosystem health. A study in New South Wales at 15 sites with six samplings revealed only minor variation in ecological conditions between 2007 and 2015 (Korbelt and Hose, 2017). Moreover, ecosystem health benchmarks are associated with aquifer typology rather than applied only to local areas. In Europe, Marmonier et al. (2000) also revealed limited temporal variations in the biodiversity of the interstitial fauna of artificial aquatic systems for three successive years (1995 - 1997). Long-term data on groundwater ecology and chemistry is available across the state of Baden-Württemberg (BW), Germany, from a continuous monitoring program of 44 sites sampled annually or bi-annually between 2002 and 2022 (Fuchs, 2007; Fuchs et al., 2006; Stein et al., 2015), which makes the state of BW one of the most densely investigated areas worldwide concerning groundwater fauna with 2,026 samplings at 950 sites (Koch et al., 2022). The present study uses this dataset to comprehensively analyse the temporal distribution of groundwater fauna and abiotic parameters, while specifically considering changes in natural or anthropogenically impacts, such as temperature, land use and nitrate concentration. Hence, the main objective of this study is to identify changes in groundwater fauna due to natural or anthropogenic stress in recent decades. Furthermore, changes in groundwater ecosystems on different spatial scales and the implications of the observed changes for biomonitoring are assessed. Finally, we identify ecological and physico-chemical parameters most suitable for robust biomonitoring.

3.2 Material and method

The following workflow was developed to address our objective (Figure 3.1). The available groundwater data of the study site (i.e. the state of Baden-Württemberg) was reviewed, and observation wells for additional sampling in 2020 were selected (Figure 3.1, step 1, site selection). Afterwards, the biotic and abiotic data was temporally and statistically analysed on different spatial scales (local to state-wide, Figure 3.1, steps 2 and 3, temporal and statistical analyses). Finally, three individual wells were analysed in detail concerning changes in land use and abiotic parameters (Figure 3.1, step 4, local scale analysis).

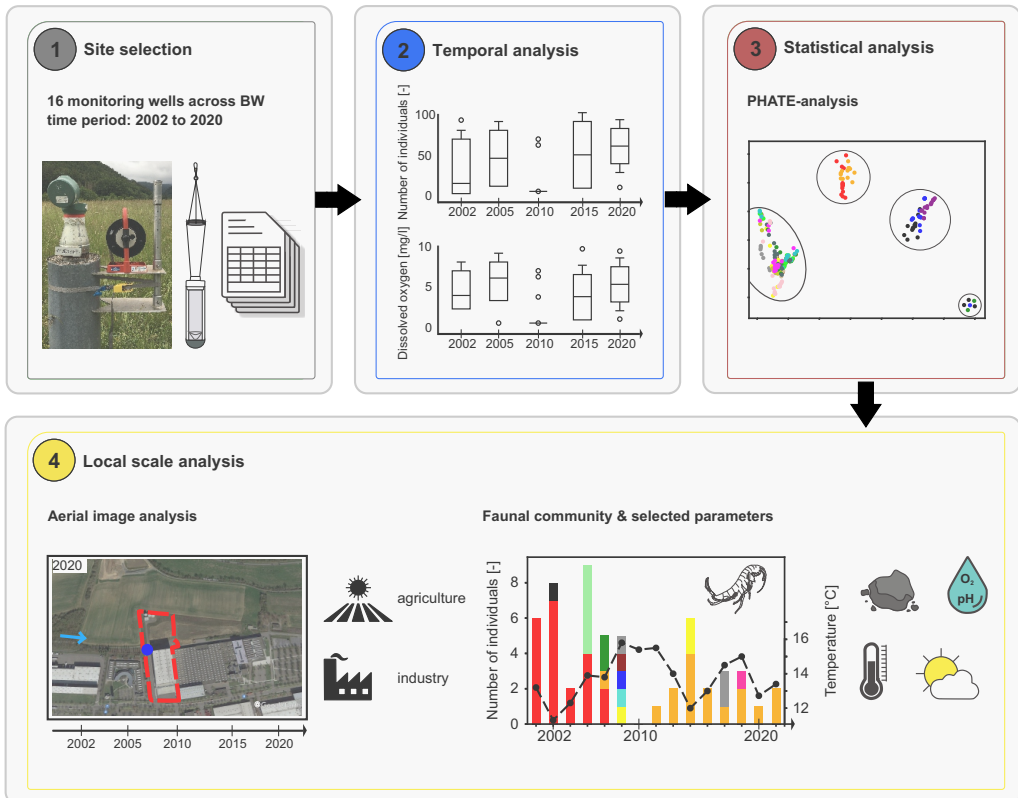


Figure 3.1: Developed workflow including four steps: (1) site selection and database, (2) temporal analysis, (3) statistical analysis and (4) local scale analysis (aerial images by Google LLC. (2022)).

3.2.1 Site selection

The State Office of Environment, Measurements and Nature Conservation (LUBW) maintains an extensive network of up to 2,600 groundwater observation wells (Landesanstalt für Umwelt Messungen und Naturschutz Baden-Württemberg, 2013). In addition, the locations of the groundwater level monitoring network and the database of all monitoring sites in Baden-Württemberg are also considered, which results in 50,000 initial sites. Of these, 304 wells were analysed faunalally at least twice in the past (Fuchs, 2007) (Figure 3.2). The 304 sites were selected based on a representative distribution within the different natural areas and aquifer types in the state of Baden-Württemberg, as well as accessibility and absence of installed measurement devices, pumps, etc. within the wells (Fuchs, 2007). Based on the initial measurement results in 2001/2002, out of the 304 sites, 44 were selected for annually or bi-annually sampling between 2006 and 2022 (Stein et al., 2015). Faunal colonisation, a good area- and aquifer-type coverage and availability of physico-chemical measurements were considered for this selection. Out of these 44, 16 wells were selected for the current study based on spatial coverage of the study area, aquifer type, land use type, well depth, faunal colonisation during the past two decades, availability of time series of physico-chemical parameters, and an average content of dissolved oxygen higher than 1 mg/l, which is a limiting factor for faunal colonisation (Griebler et al., 2014b).

3.2.2 Groundwater sampling

Faunal groundwater sampling in the 16 selected observation wells took place twice in 2001/2002 (for analysis in this study, only data from June - September was used), annually from 2006 to 2014, and then bi-annually in August/September until 2020. At the beginning of each sampling, the depth of the groundwater table and of the well were measured using an electrical contact gauge. Afterwards, water standing in the well (i.e. well water) was taken with a bailor (750 ml Aquasampler of the Bürkle GmbH, Lörrach) from the bottom of the well. In these samples, oxygen concentrations (Intellical LD0101, luminescence-based optical probe; accuracy: ± 0.1 mg/l), carbonate hardness, pH (PHC101; accuracy: ± 0.002) and electrical conductivity (TDS-measuring instrument Meditech/TenYua) were measured using an HQ40D portable 2-channel multimeter (Hach Lang GmbH, 2022). Additional samples were taken to determine the amount of sediment and further chemical analyses (such as the content of dissolved carbon, organic nitrogen, phosphate, etc.).

The faunal sampling was conducted using a modified Cvetkov net as described by Hahn and Fuchs (2009). More specifically, a plankton net consisting of a gauze funnel with a mesh size of $73 \mu\text{m}$ attached to a collection vessel (50 ml centrifuge tube) with a weight was used (Figure 3.1, step 1). With the help of a fishing rod or a winch for larger depths, the net sampler was lowered into the well

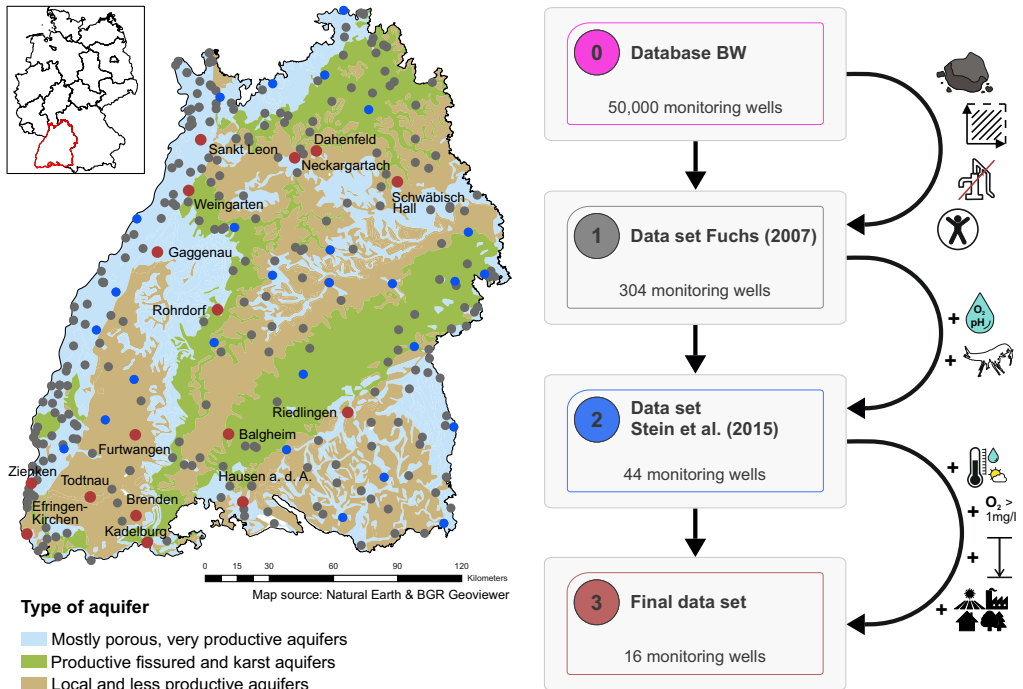


Figure 3.2: Study area, the state of Baden-Württemberg in South-West Germany, including the aquifer types according to the classification of the Federal Institute for Geosciences and Natural Resources (left corner) and selected monitoring wells according to the selection process on the right (Bundesanstalt für Geowissenschaften und Rohstoffe (BGR), 2019).

down to the bottom. To sample as much fauna as possible, the net sampler was quickly raised about 1.5 m and lowered again ten times. The collected samples were stored in a cooling box at about 8 °C until fixation with 96 % ethanol on the same day. The dye Rose Bengal ($C_{20}H_2Cl_4I_4Na_2O_5$ by Thermo Scientific Chemicals), which colours organic matter in a pink hue, is added for easier determination of groundwater fauna. Faunal samples were sorted on order level and determined on species level. Based on this determination, further faunal parameters were calculated, such as the total abundance, number of taxa or species, classification into stygofaunal classes (stygoxenic, stygophil, stygobiotic) and ecological status according to Griebler et al. (2014b).

Hydro-chemical groundwater sampling from pumped aquifer water was performed by the LUBW, with measurement results being provided in an annual catalogue, which is publicly accessible online (Landesanstalt für Umwelt Messungen und Naturschutz Baden-Württemberg, 2022). Of all 144 available parameters, 42 are used in this study, in particular standard cations and anions, heavy metals and inorganic trace substances, pesticides and aromatic hydrocarbons (Table A2.1). Parameters with a lack of temporal resolution are excluded. Annual air temperature data was taken from the German Meteorological Service (DWD Climate Data Center (CDC), 2022).

3.2.3 Statistical analysis

To better understand large-scale spatial relationships and the structure of the high-dimensional groundwater data, a PHATE (Potential of Heat-diffusion for Affinity-based Trajectory Embedding)-analysis is conducted (Moon et al., 2019). This method has shown to provide meaningful results for handling financial data (Grzybowska and Karwański, 2022), prediction of disease outbreaks (Kuchroo et al., 2022), learning of brain activation manifolds (Busch et al., 2022), RNA sequencing (Moon et al., 2019) and investigation of groundwater ecosystems (Koch et al., 2021).

PHATE is a dimensionality reduction method that generates a low-dimensional embedding specific for visualisation. Thus, an accurate, denoised representation of a data set's global and local structures is provided without imposing strong assumptions on the design of the data. The PHATE algorithm computes the pairwise distances from the data matrix. It transforms the distances to affinities to encode local information by applying a kernel function developed to Euclidian distances. Using diffusion processes, global relationships are learnt and encoded using the potential distance. Finally, metric MultiDimensional Scaling (MDS) embeds the likely distance information into low dimensions for visualisation (Moon et al., 2019). Thus, objects with similar characteristics are close to each other in the final graph. In this study, the analysis is conducted using 15 physical, biotical and (hydro-)geological input parameters (Table A2.2).

3.3 Results and discussion

3.3.1 Faunistic overview

Overall, there is no regional pattern in the spatial distribution of faunal abundance and biodiversity (Figure 3.3). Potential reasons for this are a high hydrogeological and hydro-chemical heterogeneity in combination with a small number of monitoring wells, superimposing effects of local influences and site-specific parameters linked to variations in topography and geology. Considering the entire investigation period (2002 - 2020), the faunal colonisation differs across the study area, with between 52 and 1,800 individuals and up to 42 different species per well, respectively (Figure 3.3). There is a visual relationship between abundance and biodiversity, as monitoring wells with many individuals also show more species (and vice versa).

The highest abundances in the state of Baden-Württemberg (BW) were found in the porous aquifers in the northeastern part of BW and in the southern Upper Rhine Valley, while the lowest abundances were found in fractured and karst aquifers, as well as in less productive aquifers (Figure 3.2). The spatial distribution of the faunal community according to taxonomic groups is shown in

Figure 3.3b. In the southeast of the state, Crustaceans (mainly Cyclopoids) dominate. In karst aquifers of the Swabian Alb and the local and less productive fissured aquifers of the southern Black Forest, Amphipods are more frequent (e.g., Balgheim, Todtnau, Brenden). Wells in the northern Black Forest and along the Rhine in more productive aquifers are additionally colonised by Isopods, Harpacticoids, Nematods, Ostracodes and/or Annelids. Synacrids were found only in wells in the northern part of BW (except for Kadelburg), in the north of Upper Rhine Valley and the catchment of the Neckar River in this study (see also Fuchs et al., 2006). This aligns with findings of distribution patterns of groundwater fauna from previous studies (Fuchs, 2007; Fuchs et al., 2006; Hahn and Fuchs, 2009; Stein et al., 2015, 2012).

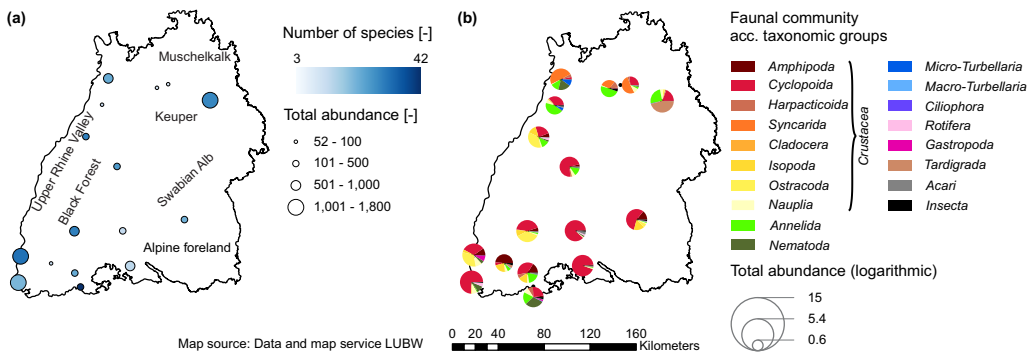


Figure 3.3: Maps of (a) the number of species and the total abundance and (b) the faunal community according to taxonomic groups as a sum of all measurements of all years (2002 – 2020) for each of the 16 monitoring wells in the study area.

3.3.2 Temporal analysis

From 2002 to 2020, the number of individuals of all 16 monitoring wells ranged between 10 and 50 on average per year, with no observable temporal change in the average total abundance or its variation (Figure 3.4a). The same applies to the number of species (Figure 3.4b), which indicates that the large-scale biodiversity was stable over the past two decades. Also, no significant changes are revealed over time for the abiotic parameters, suggesting stable hydro-chemical conditions over time (Figure A2.1). The year 2007 stands out with a high number of individuals and species (Figure 3.4), as well as a low share of stygobiontic species. This year also shows a higher content of dissolved oxygen and electric conductivity (Figure A2.1), hinting at a more pronounced surface influence and disturbed conditions, particularly in Efringen, Rohrdorf, Zienken, Schwäbisch Hall, and Gaggenau.

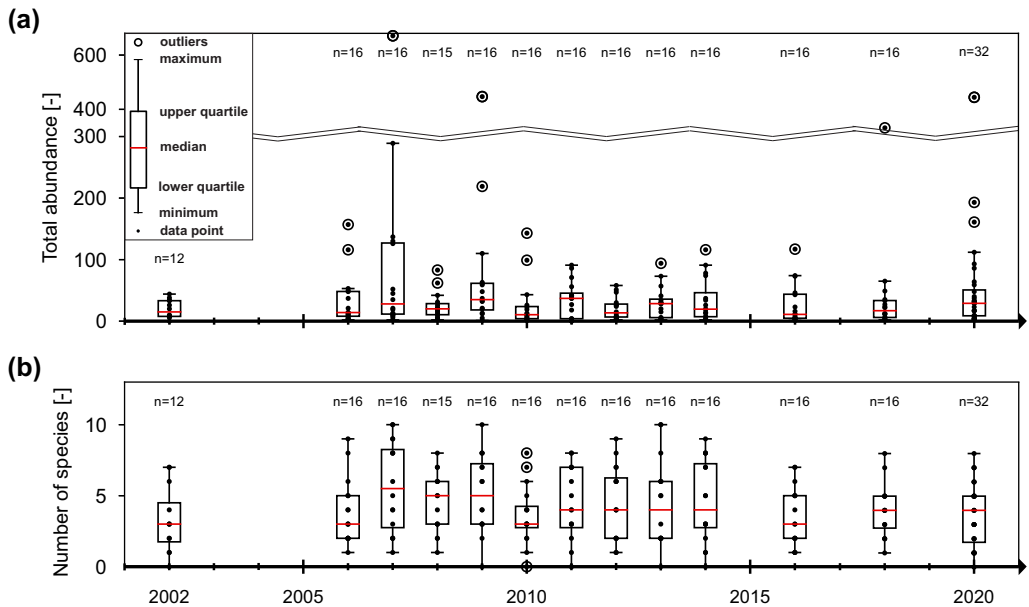


Figure 3.4: Boxplots of important biotic parameters between 2002 and 2020 of all 16 wells combined: (a) total abundance and (b) number of sampled species. "n" indicates the number of measuring points. No sampling was conducted in years with no boxplot.

While there is no apparent trend on the regional scale, there are significant variations between individual years for some individual wells, indicating more complex temporal behaviours on local scale (Figure 3.5). Regarding the faunal parameters, no single well exhibits a significant positive development concerning faunal abundance or biodiversity. Only the well in Schwäbisch Hall shows a slightly increasing trend in abundance over the last five years. This well is characterised by the presence of Tardigrads, which are typical for small surface water bodies, and wells with organic material from the surface (leaves, moss, etc.) (Schminke et al., 2007). Moreover, this well shows a gradual change in the faunal community from one single stygobiontic species to multiple stygobiontic and stygophile species, potentially linked to surface water influence (Schminke et al., 2007). On the other hand, there is a clear decrease in the abundance and faunal diversity in Todtnau und Zienken (Figure 3.5a & b), which in the case of Zienken is linked to decreasing dissolved oxygen contents with < 1 mg/l in 2014 (Figure 3.5e). This could be related to microbial oxygen degradation due to a high organic matter input (Stein et al., 2015). A strong surface influence in Zienken is linked to periods with higher abundances from 2007 to 2009 and 2013 to 2014, which coincided with higher numbers of ubiquitous species from the surface and a higher bacterial count (Stein et al., 2015).

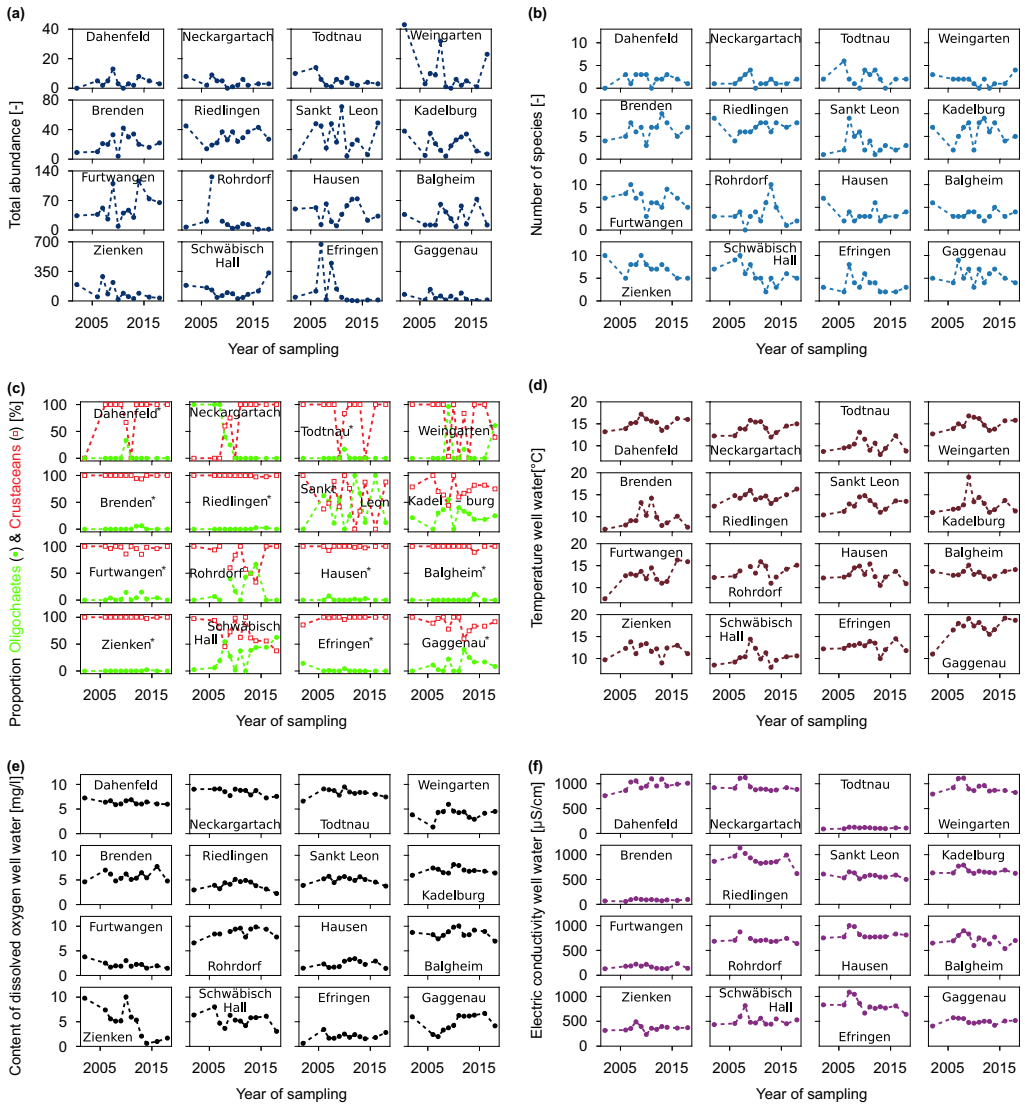


Figure 3.5: Time series of individual monitoring wells for (a) total abundance (please note the different scales of the y-axis); (b) number of species; (c) proportion of Crustaceans and Oligochaetes (* wells with a very good or good ecological status according to the assessment scheme of Griebler et al. (2014b)); (d) temperature of the well water; (e) content of dissolved oxygen of the well water and (f) electric conductivity of the well water.

Fluctuations of abundance and number of species are present in several wells, indicating unstable conditions likely caused by varying surface influence, e.g. in Gaggenau and Efringen (Figure 3.5a and 3.5b). Besides four stygobiontic indicator species in Gaggenau, all other species only appear sporadically, indicating an intermittent surface water input into this well (Figure 3.5b). This could also explain the increase in oxygen concentration after 2006, as well as the higher number of Oligochaetes and Nematodes (Figure 3.5c). These results are consistent with previous studies, which showed that groundwater communities strongly depend on the hydrologic exchange with surface water (Fuchs et al., 2006; Gutjahr et al., 2014; Hose et al., 2015).

An important parameter for the ecosystem status is the ratio of Crustaceans and Oligochaetes, which serves as a basis of the ecological assessment scheme by Griebler et al. (2014b). According to this scheme, monitoring wells with more than 70 % Crustaceans and less than 20 % Oligochaetes have a 'natural' status (indicating very good or good ecological conditions). Considering the entire study period, most monitoring wells in this study fall within this category (Figure 3.5c). Only six wells have a proportion of Oligochaetes higher than 20 % in one or multiple years, indicating disturbed conditions: Kadelburg, Schwäbisch Hall, Rohrdorf, Sankt Leon, Weingarten and Neckargartach (Figure 3.5c). While the first four wells show a fluctuating ecological status over time, probably related to varying surface water influence, the latter well shows a distinct change in the ecological status in 2008, which does not seem to be related to other biotic or abiotic parameters in Figure 3.5.

Abiotic parameters show mostly constant conditions in the individual wells (Figure 3.5d, e, f). One notable exception concerning electric conductivity is Efringen, where electric conductivity values decrease over time (Figure 3.5f). This may be caused by the reconstruction of the well from an underfloor to a surface observation well. In terms of well water temperature (Figure 3.5d), several sites show an increasing trend, e.g. Weingarten, Riedlingen and Furtwangen, which is in the same range as observed changes in groundwater temperature in previous studies (Figura et al., 2011; Menberg et al., 2014). More pronounced temperature changes, such as Kadelburg with temperature variations of > 5 °C between individual measurements and Furtwangen with an increase of 4.6 K between 2002 and 2020, are more likely related to varying surface influence or environmental conditions during measurements.

Overall, faunal and abiotic parameters are mostly constant over time in the individual wells of the study area. Nine out of 16 wells (Dahenfeld, Balgheim, Hausen, Riedlingen, Brenden, Kadelburg, Rohrdorf, Furtwangen, Todtnau) show stable conditions over time concerning the variance of the faunal and abiotic parameters (Table A2.3). In contrast, seven wells (Weingarten, Schwäbisch Hall, Efringen-Kirchen, Zienken, Gaggenau, Neckargartach, Sankt Leon) show higher standard deviations and, therefore, unstable conditions. These are often linked to a varying influence of

surface water, which aligns with previous studies (Dole-Olivier, 1998; Foulquier et al., 2011; Stein et al., 2012).

3.3.3 Statistical analysis

Overall, the PHATE analysis shows that biodiversity, illustrated by the number of taxa and individuals, and geological conditions, such as the type of aquifer, have the largest impact on the clustering of the monitoring wells into three distinct groups (Figure 3.6). Group I contains monitoring wells in the karst aquifer of the Muschelkalk and Lettenkeuper formations in the northeast of BW and a fissured aquifer in the southern Black Forest, which generally have a low abundance. In contrast, Group II includes samples in all other wells, with the remaining wells of the southern Black Forest (Brenden, Furtwangen) located at the edge of this group. Group III consists of samples that had no groundwater fauna. Previous studies also showed that hydrogeological parameters strongly influence the occurrence and composition of groundwater fauna (Koch et al., 2021; Stein et al., 2012). Geology and hydrological connectivity significantly influence water chemistry and habitat availability and, therefore, biotic distribution (Fuchs, 2007; Fuchs et al., 2006; Hahn, 2006; Korbel et al., 2018; Korbel and Hose, 2015; Tione et al., 2016). For instance, the abundance and species richness of crustacean fauna in the alluvial aquifers are most related to hydrological conditions, oxygen concentrations and geologic structures (Mösslacher, 1998), which is consistent with our findings. Moreover, this is consistent with the results from Korbel et al. (2018), who state that ‘sediment size, and thus the size of interstitial voids, is a key limiting factor’ for stygofauna.

Temporal variations at the individual sites are also noticeable in the PHATE results by observing the spread of samples of individual locations. Samplings of very stable locations (e.g. Todtnau, Dahlenfeld, Balgheim, and Riedlingen) with small parameter fluctuations over time are spatially more concentrated in the PHATE graph than wells with unstable conditions (e.g. Schwäbisch Hall, Weingarten). Generally, sites with stable conditions for both faunal and hydro-chemical parameters (as identified in Table A2.3) are concentrated in the lower right area of Group II as Sub-Group II.a (except for Dahlenfeld and Rohrdorf), and the upper-left area as Sub-Group II.b (Figure 3.6). Thus, this result here confirms the categorisation of the wells using the variance of their faunal and hydro-chemical parameters.

While the affiliation of the wells with a specific group is constant over time (except for the mentioned samplings with no fauna), the location of different samplings within the group can be associated with concrete changes in specific parameters, specifically abundance, sediment content and temperature. Despite stable overall conditions, the well in Rohrdorf shows one outlier further down in the graph (orange triangle) representing the measurement in 2007 with significantly more

individuals (126) than in other years. The amount of sediment is responsible for a clustering of samplings from different wells (Hausen, Gaggenau, Weingarten, among other) in the upper left corner of Group II, which are from 2009, 2011 and 2012 and lack information about the amount of sediment. The samplings Gaggenau (dark pink dots) show a further outlier in 2002, located more to the right, which is related to a relatively low sediment content < 1 ml. The same applies to Schwäbisch Hall with an outlier (red dots) to the right side of the graph, containing also a low sediment content, little detritus and low abundance (8 individuals). The samplings in Furtwangen (yellow triangles) are also spread over a wide area, with the ones at the bottom of Sub-Group II.a from 2002 and 2020 exhibiting significantly lower temperatures (4.3 °C and 8.7 °C), which are close to the measured temperatures in Brenden (light pink).

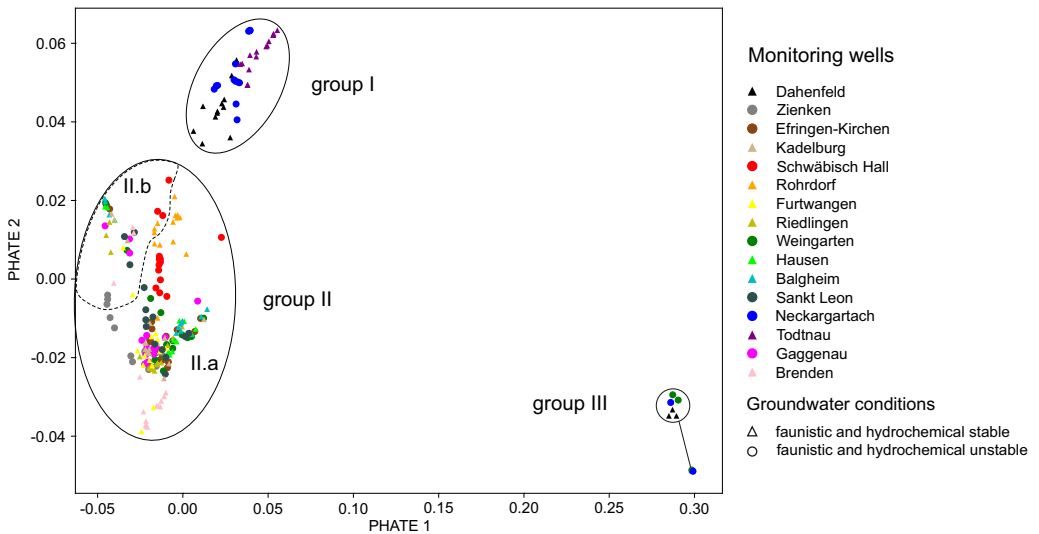


Figure 3.6: Graphical result of the PHATE-analysis for all 16 monitoring wells from 2001/2002 to 2020, presented by the affiliation of the monitoring wells of each sampling.

These findings show that the amount of sediment can be used as an indicator for the pore structure, which determines hydraulic conductivity and living space (Mösslacher, 1998), and can thus be used as a proxy for living conditions. Temperature, on the other hand, can be used as an indicator for surface influence, as it is an indirect marker of the degree of hydrological exchange with the surface (Hahn, 2006; Schönborn, 2003). This was also observed by Koch et al. (2021) on the city scale, where local geology influenced the occurrence of groundwater fauna, the number of individuals and the food supply. Other studies also observed a significant effect of groundwater temperature on fauna, as diversity decreased with increasing temperatures in laboratory and small-scale field studies (see Brielmann et al., 2009; Spengler, 2017).

3.3.4 Local scale analysis

As described above, certain changes in faunal parameters in individual wells can be related to changes in abiotic parameters, for example, to a decreasing dissolved oxygen content as in Zienken, while other changes, such as the fluctuating ecological status in Neckargartach and Sankt Leon, cannot directly be related to varying abiotic parameters (Figure 3.5). Hence, we assess these two unstable wells in more detail with respect to changes at the surface surrounding of the wells and compare them to the well in Todtnau, which shows very stable conditions.

3.3.4.1 Neckargartach

The well in Neckargartach is 35 m deep with filter screens between 27 and 34 m in a fractured and karst aquifer formed by a clay-containing limestone (Figure 3.2). The overall number of individuals is very low (maximum of nine individuals per sampling) and decreases during the observation period (Figure 3.7). Faunal analysis on species level shows a distinct change in the faunal community and dominating species between 2002 and 2020.

Between 2002 and 2007, Oligochaetes dominate the faunal community (Figure 3.7). Most of these individuals belong to the species *Dorydrillus michaelsoni*, which is only occasionally found in groundwater and is an indicator of slightly contaminated groundwater (Moog, 2002). In 2008 and 2009, the dominating species was *Chappuisius inopinus* (Crustacea: Harpacticoida), while from 2011 onwards, mainly Crustaceans were found, with the rare species *Parabathynella badenwuerttembergensis* being the dominating species. Overall, these faunal changes represent a change from stygophil species to domination of stygobiontic species, as well as a simultaneous change from dominating Oligochaetes to Crustaceans and thus to the aforementioned change in the ecological status (Figure 3.5c).

The observed faunal changes coincide with alterations in land use of the surrounding area (Figure 3.8). After the first period with dominating Oligochaetes, a previously unsealed area was first converted into a gravel-covered car park in 2008 and later into an industrial warehouse in 2018. During that time, different crustacean species began to dominate the faunal community (Figure 3.8). Surprisingly, this surface change is not reflected in the well water temperatures, even though groundwater under covered surfaces typically shows higher temperatures than under unsealed surfaces (Tissen et al., 2019 ERL). In Neckargartach, this is likely linked to groundwater inflow from the adjacent agricultural area into the monitoring well (Lang et al., 2004). The nitrate content decreases from 51.4 to 25.9 mg/l during the study period (Figure 3.7), most likely due to the absence or reduction of fertilisation. Also, dissolved oxygen content decreases slightly from 10.9 to 8.0 mg/l (Figure 3.7 & Figure A2.2), which is consistent with the shielding from the surface as

main source of oxygen and the change from stygophil to stygobiontic species (Bork et al., 2009; Hahn, 2006; Malard et al., 1996; Mösslacher, 1998; Pospisil, 1999; Sket, 1999).

Neckargartach

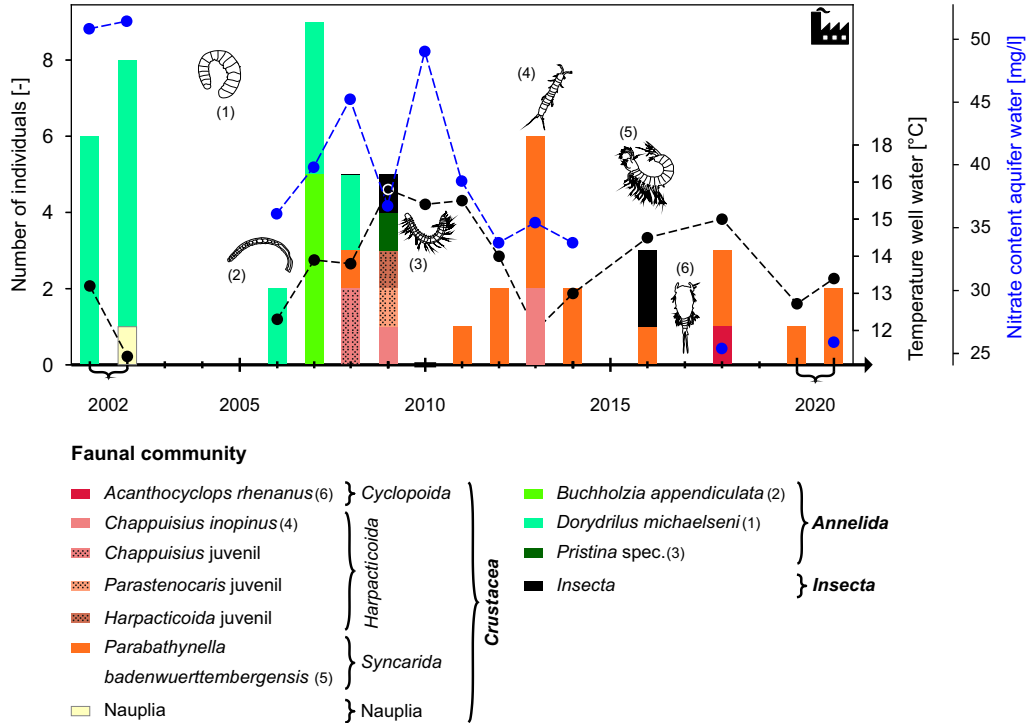


Figure 3.7: Temporal development of the faunal community (abundance and composition of the faunal community; higher taxa in bold letters) and the well water temperature (secondary y-axis) at the bottom of the monitoring well in Neckargartach during the period of investigation (2002 – 2020). No sampling was conducted in years with no bar.

Thus, there is a clear link in Neckargartach between decreasing surface influence, caused by increasing surface sealing, and dissolved oxygen content as well as the composition of groundwater ecosystems, as also observed by Korbel et al. (2013a). In this study, agriculture in areas with different land use affects groundwater ecosystems (composition of stygofauna and microbial assemblages) due to changes in groundwater quality (nitrate and phosphorus contents). Hence, a higher abundance of Cyclopoids, Harpacticoids and Oligochaetes was found under irrigated areas. However, it has to be mentioned that the improvement of the ecological status according to Griebler et al. (2014b) through surface sealing is a site-specific observation for Neckargartach. Generally, surface sealing and the related decrease in dissolved oxygen will more likely lead to the deterioration of groundwater ecosystems (Hervant and Malard, 1999; Korbel et al., 2022a; Mösslacher, 1998).

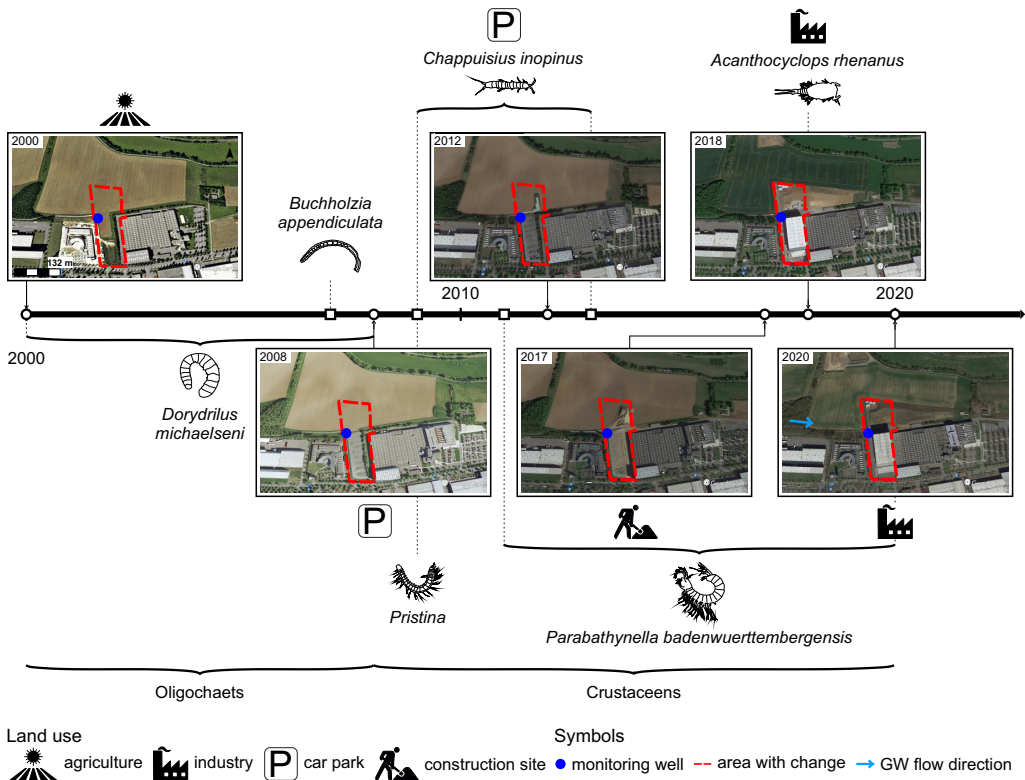


Figure 3.8: Temporal changes of land use using aerial image interpretation and faunal community structure of the monitoring well Neckargartach in the industrial park Böllinger Höfe between 2000 and 2020 (image source: Google Earth Pro (Google LLC., 2022)).

3.3.4.2 Sankt Leon

The well Sankt Leon is located next to a field path between a forest and an agricultural area near the village of Sankt Leon (Figure A2.3a), with filter screens between 4 and the well bottom in 10 m, in the quaternary glacial sand and gravels of the Upper Rhine Valley (Figure 3.2). The well shows unstable abiotic and faunal conditions (between 0 and > 400 individuals per sampling, Figure 3.5) and significant variations in the faunal compositions (Figure 3.9).

Common taxa in this well are Nematodes, as well as different Cyclopoids (*Crustacea*). In the first decade, rather ubiquitous species colonise the well in large numbers, with *Graeteriella unisetigera* and *Diacyclops languidoides* (*Crustacea: Cyclopoida*) being the dominating species. The latter is one of Germany's most common and widespread groundwater species (Matzke et al., 2009; Schminke et al., 2007). From 2010 onwards, fewer individuals and different taxa can be found, with *Bathynella freiburgensis* (*Crustacea: Syncarida*) being the dominating species in recent years.

Overall, the development of the faunal communities over time points to a change from ubiquitous to more stygobiontic species.

Sankt Leon

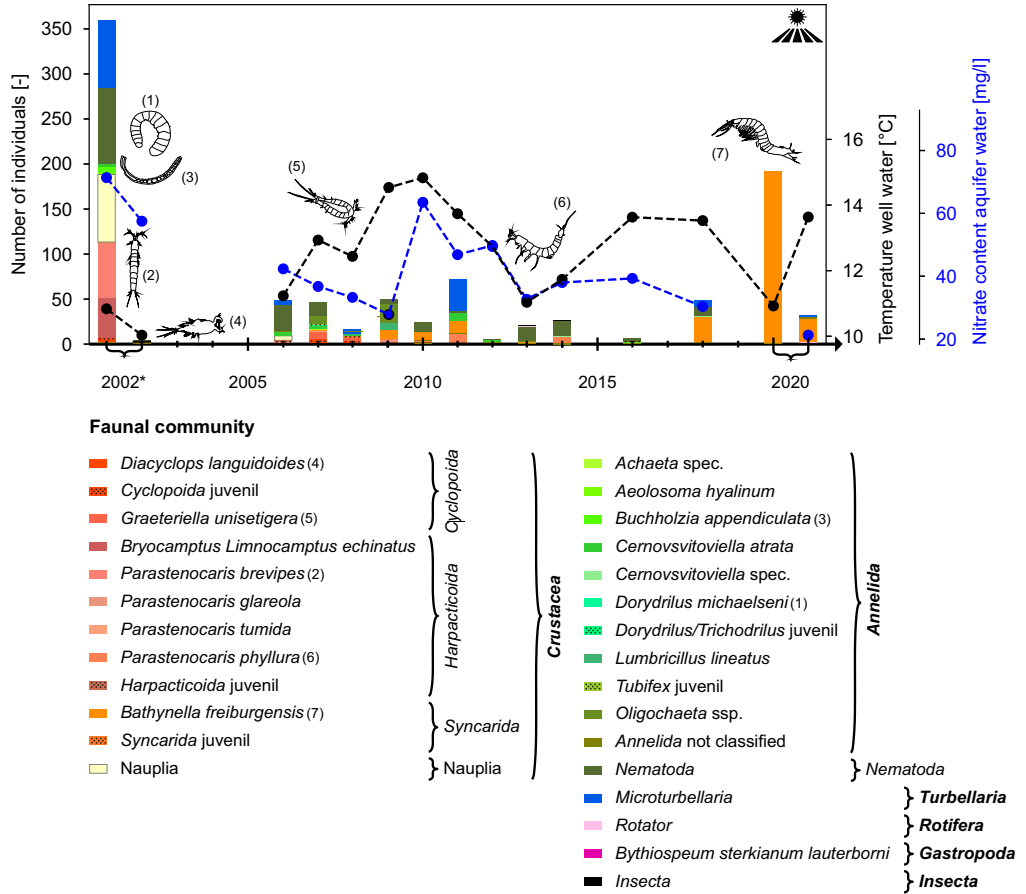


Figure 3.9: Temporal development of the faunal community (abundance and composition of the faunal community; higher taxa in bold letters), of the content of nitrate (data of the LUBW, blue y-axis on the right) and the well water temperature (black y-axis on the right) on the bottom of the well in Sankt Leon between 2002 (* fauna sampling in May and December) and 2020. No sampling was conducted in years with no bar.

Although these faunal changes indicate a weakening surface influence over time, no changes in land use or surface conditions were observed (Figure A2.3a). Well water temperature shows significant fluctuations between 11 °C and 14 °C, yet without visible trend. As *Bathynella freiburgensis* tolerates a large range of temperatures and is typical for the Upper Rhine Valley (Fuchs, 2007; Spengler, 2017), individuals of that species might have a competitive advantage over other species. The nitrate content decreased from 70 mg/l to below 20 mg/l (Figure 3.9). High nitrate contents in the first years are most likely linked to intensive agriculture, in particular asparagus cultivation,

which is typical for this region. However, these fluctuations do not seem to correlate with the observed faunal shifts. Furthermore, previous studies showed that a nitrate concentration below 50 mg/l has no direct impact on groundwater fauna (Di Lorenzo et al., 2020b; Di Lorenzo and Galassi, 2013; Fakher el Abiari et al., 1998; Mösslacher and Notenboom, 1999). Accordingly, more detailed and site-specific investigations (e.g. more accurate, in-situ groundwater temperature and dissolved oxygen measurements over depth) would be needed to clarify the drivers of the unstable conditions in Sankt Leon.

3.3.4.3 Todtnau

The well in Todtnau is also located in an agricultural area near a small stream (Prägbach) and the village of Todtnau-Geschwend (Figure A2.3b), with a depth of 39 m and well screens between 5 and 36 m in a fractured crystalline aquifer in the southern Black Forest. The number of individuals and species is generally low (Figure 3.10).

Todtnau

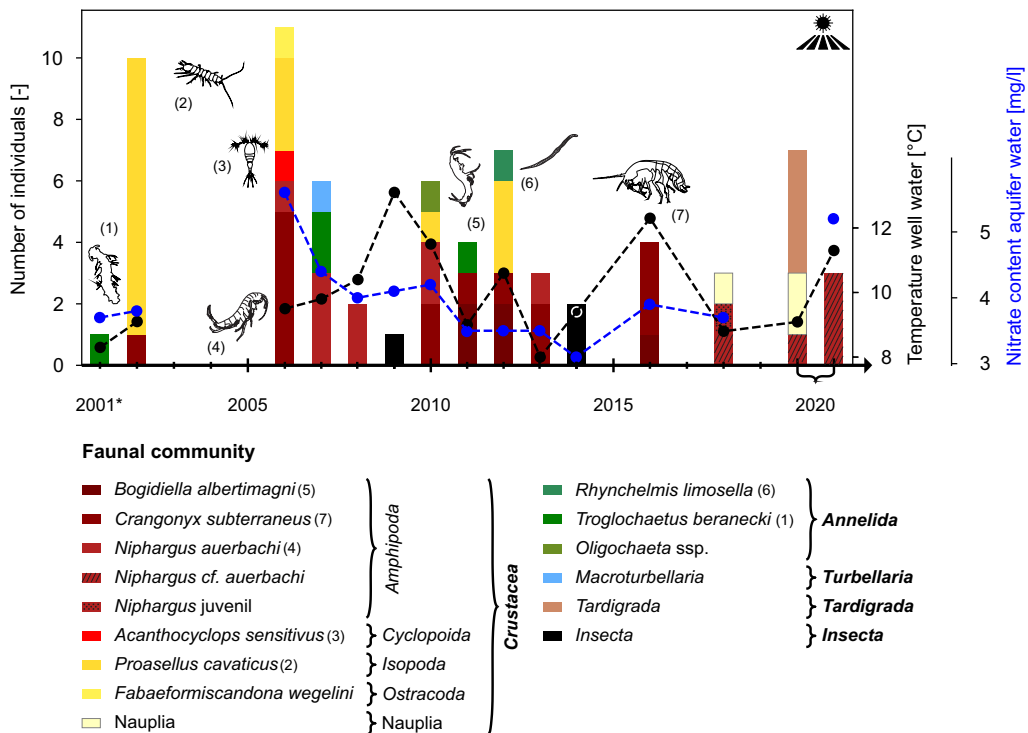


Figure 3.10: Temporal development of the faunal community (abundance and composition of the faunal community; higher taxa in bold letters), of the content of nitrate (data of the LUBW, blue y-axis on the right) and the well water temperature (black y-axis on the right) on the bottom of the well in Todtnau-Geschwend between 2001 (* fauna sampling in November) and 2020. No sampling was conducted in years with no bar.

In contrast to the two wells discussed previously, the well in Todtnau shows stable conditions (Table A2.3, Figure 3.5). Well water temperatures are generally low due to higher altitude in the Black Forest. The same applies to electric conductivity related to filter screens in crystalline rocks and organic matter content due to the depth of the well. Despite its location in an agricultural area, the nitrate content is below the geogenic background (Figure 3.10). However, despite stable physico-chemical conditions and no change in land use or surface conditions, certain changes in the faunal community can be observed as different species occur in different years, yet without any visible trends.

The well in Todtnau is colonised by stygobiontic species only, except for *Collembola* (*Insecta*) in two years, which live in the soil and on the water's surface. These are also the samplings that show a non-natural status of the ecosystem according to Griebler et al. (2014b) due to the absence of *Crustacea* (Figure 3.5c). The most common species during the past 20 years is *Crangonyx subterraneus* (*Crustacea: Amphipoda*), which is widespread, ecologically very flexible and inhabits all kinds of underground habitats, but prefers fractures and gravels (Schminke et al., 2007). The species *Proasellus cavaticus* (*Crustacea: Isopoda*) is also commonly present at this location. Individuals of this species can be up to 1 cm long and, therefore, prefer larger cavities, e.g. caves and fractured aquifers, such as the one in Todtnau. Another common species is *Niphargus auerbachii* (*Crustacea: Amphipoda*), a comparably big, stygobiontic groundwater species. The groundwater species *Troglochaetus beranecki* (*Annelida: Polychaeta*) found in 2001, 2007 and 2011 is cold-stenotherm, has low water chemistry requirements (Schminke et al., 2007) and also inhabits deep groundwater wells (Matzke et al., 2009). It is also noted that juvenile crustaceans, especially niphargids, are predominant in the last two years of sampling. This may indicate a recovery of the population after a lack of colonisation in 2014. Years without colonisation may point to bad living conditions with a lack of nutrients and, therefore, challenging living conditions. In general, the faunal composition in Todtnau reflects the hydrogeological conditions of the well, with many species typical of large cavities and higher altitudes.

3.4 Conclusion

This study analyses the faunal composition and abiotic parameters of 16 groundwater measurement wells in the German state of Baden-Württemberg over the past two decades. Statistical analyses are used to distinguish wells with stable and unstable faunal conditions and to assess the impact of specific hydrological and hydro-chemical parameters on this characterisation. Time series of individual wells are also discussed in combination with past aerial images to analyse the impact of changes in surface conditions.

Considering the entire study area, we observed no long-term changes or trends in abiotic or faunal parameters, indicating generally stable and ecological good conditions. However, temporal fluctuations in faunal parameters, such as total abundance and number of species, and thus unstable conditions are observed for seven out of 16 wells. In some cases, these changes are directly related to gradual changes in abiotic parameters, such as decreasing abundances due to reduced dissolved oxygen contents. Yet more often, there are no clear patterns in individual abiotic parameters; instead, superimposing effects of multiple parameters linked to increasing or weakening surface influence lead to changes in groundwater fauna. Results from a multivariate PHATE analysis confirm these findings and highlight the hydrogeological setting, the content of sediment and detritus in the well and the temperature as influential factors.

Examining faunal changes on species level for selected wells reveals that unstable conditions can be linked to changes in surface sealing by anthropogenic construction measures, which even changed the ecological status at one specific site. However, variable faunal composition and fluctuating abundances were also observed for sites with no visual changes in land use and surface influence and also (although less prominent) for a deep, well-shielded site with very stable abiotic groundwater conditions. Thus, more long-term studies of groundwater ecology with higher spatial and temporal resolution are necessary to further improve our understanding of faunal shifts over time.

These findings have direct implications for large-scale biomonitoring in groundwater, which is becoming increasingly important. Transferability of local observations to a larger scale is very limited due to small-scale heterogeneities in hydrogeological conditions and superimposing, site-specific effects. Noticeable environmental changes for wells in the state of Baden-Württemberg were often linked to changes in dissolved oxygen content, well water temperature and sediment content. Accordingly, these parameters should be accurately and representatively measured in the water column of the well (if possible depth-resolved) and assessed in combination with hydrogeological and surface conditions to obtain more reliable biomonitoring results. Furthermore, the observed faunal fluctuations in wells in natural, unaffected areas with stable abiotic conditions

stress that reference locations for ecological groundwater assessments and biomonitoring have to be carefully selected and ideally be based on multi-annual data.

Acknowledgments

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4

GROUNDWATER FAUNA IN AN URBAN AREA - NATURAL OR AFFECTED?

Reproduced from Koch, F., Menberg, K., Schweikert, S., Spengler, C., Hahn, H.J., Blum, P. (2021) Groundwater fauna in an urban area - natural or affected?. *Hydrology and Earth System Sciences* 25. 3053-3070. <https://doi.org/10.5194/hess-25-3053-2021>.

4.1 Introduction

In Germany, 70 % of the drinking water demand is met by groundwater, for which the quality is the product of multiple physical–chemical and biological processes (German Environment Agency, 2018). 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 fauna also 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 biomass or bound by microbial activity. Protozoa and higher organisms can graze resulting biofilms, loosen the substrate and, therefore, stimulate biological self-purification (Boulton et al., 2008; Griebler and Avramov, 2015; Hancock et al., 2005). Healthy groundwater ecosystems can provide clean

drinking water; however, they are sensitive to external influences such as chemical and thermal disturbances. The latter drives hydro-geochemical and biological processes in groundwater systems which are typically isothermal (Briemann et al., 2009, 2011). Groundwater fauna mainly consist of stygobiotic species which spend their entire life in groundwater and are adjusted to this habitat (Hahn, 2006). Hence, in central Europe, they are assumed to be cold stenotherm, which means that they prefer cold temperatures. A variability in temperature tolerance among groundwater faunal groups and species is reported in various studies, which explains why the use of individual temperature thresholds is more useful for capturing different preferences. According to Spengler (2017), faunal diversity is generally declining at a temperature above 14 °C. Various authors reported species-specific temperature preferences between 8 and 16 °C (for individuals of the species *Niphargus inopinatus* and *Proasselus cavaticus*; Briemann et al., 2009, 2011) and a specific temperature threshold of up to 19 °C (for *Parastenocaris phyllura*; Glatzel, 1990). Above these thresholds, the mortality of individuals raises until groundwater fauna is almost absent, for example at 22 °C in the study of Foulquier et al., 2011. However, temperature sensitivity is not only an issue at species level but also for the communities as a whole. Spengler (2017) reported 12 °C to be a temperature threshold value indicated by a shift in community structure for faunal communities of groundwater of the Upper Rhine valley.

Nevertheless, in German and European legislation, as in many countries globally, groundwater is not yet recognised as a habitat worthy of protection, and there is no common understanding of the best practice for assessing the ecological status of groundwater (Hahn et al., 2018; Spengler and Hahn, 2018). The assessment of surface water is typically based on biological and physical–chemical criteria and is also supported by hydro-morphological criteria (European Water Framework Directive and German legislation; article 5 – “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 the health of groundwater ecosystems. The availability of ecological criteria can only be increased by conducting a large number of studies dealing with the analyses of groundwater ecosystem health by investigating groundwater fauna. Results from previous faunal groundwater analyses are contained in a Germany-wide data record (Berkhoff, 2010; Gutjahr, 2013; Hahn, 2005; Spengler, 2017; Spengler and Hahn, 2018; Stein et al., 2012). The study by Hahn and Fuchs (2009) focuses on defining stygoregions based on different hydrogeological units located in Baden-Württemberg, Germany. They conclude that the observed patterns of groundwater communities reflect a high spatial and temporal heterogeneity in aquifer types with respect to habitat structure, food and oxygen supply. Although there are various studies on this topic (e.g. Deharveng et al., 2009; M.-J. Dole-Olivier et al., 2009; Gibert and Deharveng, 2002; Malard et al., 2002), stygobiotic biodiversity is still likely to be underestimated.

Regional investigations on the spatial variation in groundwater fauna, i.e. stygobiont occurrences, and corresponding environmental parameters, such as geological site characteristics and altitude, are rare (M. J. Dole-Olivier et al., 2009; Gibert and Culver, 2009). An approach for elucidating groundwater biodiversity patterns in six European regions was conducted in the PASCALIS (Protocol for the Assessment and Conservation of Aquatic Life In the Subsurface) project (Gibert and Culver, 2009), which aimed at mapping biodiversity and endemism patterns (Deharveng et al., 2009) and shows that regional processes, such as hydrological connectivity, in a specific habitat (e.g. river floodplains as in Ward and Tockner (2001)) have a much stronger influence on species composition than local habitat features, such as permeability and saturation. Within a region, hydrogeology, altitude, palaeogeographical factors and human activities can interact in complex ways to produce dissimilar patterns of species compositions and diversity (Gibert and Culver, 2009). The PASCALIS sampling protocol recommends selecting hydro-geographic basins that are not strongly affected by human activities, such as groundwater pollution (Malard et al., 2002), and do 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 by up to several degrees (e.g. Menberg et al., 2013a; Taylor and Stefan, 2009; Tissen et al., 2019; Zhu et al., 2010). 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 European Union (EU) Water Framework Directive defined the release of heat in the groundwater as pollution, whereas the cooling of the groundwater is not mentioned. Until now, there are scientifically derived threshold values for groundwater temperature in the case of thermal (heat) pollution published, but none of these have been implemented in official regulations or water law (Blum et al., 2021; Hähnlein et al., 2010, 2013). This results in a tension between conservation, exploitation and thermal use of groundwater. However, as seen in an aquifer ecosystem downstream from 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 increased with temperature, while faunal diversity decreased with temperature (Brielmann et al., 2009). Similar results are provided by Griebler et al. (2016), where potential impacts of geothermal energy use and storage of heat on groundwater are investigated. Temperature changes in groundwater correspond to changes in groundwater chemistry, biodiversity, community composition, microbial processes and function of the ecosystem. How exactly groundwater communities react to changes in the temperature and concentration of nutrients, dissolved organic carbon and oxygen is not yet fully understood (Brielmann et al., 2009, 2011; Castaño-Sánchez et al., 2020a; Spengler, 2017).

Several approaches exist that allow a local assessment of the ecological state of groundwater based on different faunal, hydro-chemical and physical parameters. Korbelt and Hose (2011, 2017) introduced the Groundwater Health Index GHI, which is a tiered framework for assessing the health of groundwater ecosystems. Here, both biotic and abiotic attributes of groundwater ecosystems are used as benchmarks for ecosystem health. Their study shows that ecosystem health benchmarks are probably more associated with aquifer typology than being applicable for local areas. This index is applied and tested by Di Lorenzo et al. (2020a) in unconsolidated aquifers in Italy located in nitrate vulnerable zones. They refined the index (weighted Groundwater Health Index Nitrates ($wGHI^N$)) and demonstrated its applicability on shallow and deep aquifers and also revealed that this new index is limited due to low correlations between the indicators. Commissioned by the UBA, Griebler et al. (2014b) developed a concept for an ecologically based assessment scheme for groundwater ecosystems, which builds on the assessment of Korbelt and Hose (2011, 2017). This two-step scheme characterises groundwater on two different levels by using the most important physical-chemical parameters, such as content of dissolved oxygen and microbiological and faunal characteristics, i.e. the number of oligochaetes and crustaceans, and comparing these to reference values for natural, undisturbed and ecologically intact groundwater ecosystems (Griebler et al., 2014b).

Furthermore, the GFI, introduced by Hahn (2006), quantifies the relevant ecological conditions in the groundwater as a result of hydrological exchanges between surface and groundwater. It incorporates ecologically important groundwater parameters, such as the relative amount of detritus, variation in 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 on a local scale, which introduced five types of faunal habitats as a result of surface water influence, the 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 of developing a faunal monitoring concept for hydrological exchange processes in the surrounding river bank 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 beneath residential, commercial and industrial, i.e. urban, areas in comparison to a forested area outside the built-up area of Karlsruhe to determine whether land use has an impact on groundwater faunal communities. Hence, in 39 groundwater monitoring wells in Karlsruhe, Germany, the groundwater fauna are sampled, the groundwater temperatures are measured and chemical properties are analysed. In our study, the classification scheme developed by Griebler et al. (2014b) is applied. The wells are characterised regarding the state of their ecosystem. Finally, we aim to distinguish areas with natural groundwater ecology from anthropogenically disturbed areas.

4.2 Material and methods

4.2.1 Study site

The study is performed in Karlsruhe, a city in the Upper Rhine valley in southwestern 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 northwest of the Rhine, 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 area (i.e. urban area, city centre, neighbouring districts, and parts of the Hardtwald forest and several outskirts) is covered by buildings. The rest is 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 hot spots occur mainly below the city centre, where building densities are highest. In the northwestern part of Karlsruhe, the increase in GWT was about 3 K warmer than the annual mean Land Surface Temperature (LST), which 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, and the local drinking water supplier (Stadtwerke Karlsruhe) only needs to remove 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, which contains a polycyclic aromatic hydrocarbons concentration of up to 500 µg/L, of 200 m length over the entire aquifer thickness is located at a former gas plant in the east of Karlsruhe (Figure A3.2b; Kühlers et al., 2012). Moreover, three parallel contamination plumes, of 2.5 km length each, can be found in the southeast of Karlsruhe (Figure A3.2b), where highly volatile chlorinated hydrocarbons (7 – 26 µg/L) and their degradation products were detected (Wickert et al., 2006).

4.2.2 Material and sampling

From 2011 to 2014, samplings of groundwater parameters and fauna were performed in 39 groundwater monitoring wells in the city area of Karlsruhe, of which eight wells are in the forested area and 31 in the residential, commercial and industrial areas (urban area). 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; Xylem Analytics, Weilheim, Germany) and the contents of dissolved oxygen (MultiLine type 3430; Xylem Analytics, Weilheim, Germany), iron, nitrate (NO_3^-) and phosphate (PO_4^{3-} ; (RQflex® plus 10 Reflectoquant®; Merck KGaA, Darmstadt, Germany) were measured.

In accordance with the suggestion made by Hahn and Gutjahr (2014), several integrative samplings (i.e. repeated samples taken over a period of time) were conducted to capture an ecological representation of groundwater fauna which reflects the occurring species at a community level. Every well was sampled at least three times. From 2011 – 2012, 22 measurement wells (mainly in the Hardtwald and the northwest of Karlsruhe) were sampled six times at a minimum interval of 2 months. In 2014, 17 measurement wells, mainly located in the south or in the inner city, were sampled three times (see Table A3.2). As the aim of this study is to provide a first-tier screening of the groundwater ecological status, we sampled 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.









Furthermore, the relative amount of sediment as an indication of the nutrient availability and the cavity system was measured. Before the fauna sample from the net sampler was passed over a sieve with a mesh size of $74 \mu\text{m}$, the sediment was separated and classified into different categories (sand, fine sand, ochre, detritus, and silt). It should be noted that the detritus content was not recorded quantitatively but on the basis of estimated frequency classes. The estimation of the relative amounts of sediment per sample is based on Table A3.1 in the Supplement. Mann–Whitney tests (U tests) were applied to detect the potential impacts of groundwater characteristics (physical–chemical parameters), geology and well design on the groundwater quality as well as on groundwater fauna. Samples were regarded as significantly different if the p value was $< 5.0 \times 10^{-2}$.

To better understand large-scale relationships and the fine structures of high-dimensional biological data, the PHATE analysis introduced by Moon et al. (2019) (<https://github.com/Krishna-swamyLab/PHATE>, last access: 15 April 2021) was used. This dimensionality reduction method generates a low-dimensional embedding specific for visualisation, which provides an accurate, denoised representation of both local and global structures of a data set without imposing strong

assumptions on the structure of the data. The PHATE algorithm computes the pairwise distances from the data matrix and transforms the distances to affinities to encode local information by applying a kernel function, which is developed to Euclidian distances. By using diffusion processes, global relationships are learnt and encoded using the potential distance. Finally, the potential distance information is embedded into low dimensions for visualisation with metric MDS (Moon et al., 2019). Objects that are close to each other in the final graph, therefore, have similar characteristics.

Crustaceans, especially amphipods and copepods represent the majority of 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 4.1).

Table 4.1: Overview of the sampled fauna, divided into the subphylum *Crustacea* and other subordinate taxa.

Subphylum: <i>Crustacea</i>	Size [mm]	Habitats	Species number
Order: <i>Cyclopoida</i> 	0.4 - 0.7 ¹	Fresh and marine water, groundwater ¹	298 species and subspecies worldwide ² , 8 stygobiotic species in Germany ³
Order: <i>Harpacticoida</i> 	< 0.5 ⁴	Marine, freshwater, semi-terrestrial environments and groundwater ⁵	599 (sub-)species worldwide ² , 20 stygobiotic species in Germany ³ , 17 stygophile* & stygobiotic species in Baden-Württemberg ⁶
Genus: <i>Parastenocaris</i>	0.3 - 0.5 ¹	Tertiary relict living in cavity rooms of streams, in groundwater and moss ¹	206 (sub-)species worldwide ² (16 stygophile & stygobiotic species in Baden-Württemberg ⁶)
Order: <i>Bathynellacea</i> 	0.5 - 5.4 ⁷	Cavity systems ⁷ and in groundwater ⁸ (foreign tropical origin) ⁹	Exclusively 160 stygobiotic species worldwide ⁹ , 8 species in Germany ³
Order: <i>Amphipoda</i> 	0.5 – 30 ¹	Sea, fresh water ¹ and in healthy groundwater ecosystems (important ecosystem service providers ¹⁰ & biodiversity indicators in Europe ¹¹)	321 stygophile & stygobiotic species in Europe ¹² , 24 stygobiotic species in Germany ³
Other subordinate taxa	Size [mm]	Habitats	Species number
Subclass: <i>Oligochaeta</i> 	< 1 – 3 ¹³	Colonise every habitat, groundwater ¹³	100 species worldwide ¹⁴ and 27 stygobiotic species in Europe ¹³
Phylum: <i>Nematoda</i> 	1 – 3 ⁹	Colonise every habitat ⁹ , can live under unfavourable conditions ¹⁵	20,000 species worldwide ¹⁶ , 60 stygobiotic species in Europe, 6 species in Germany ³
Class: <i>Turbellaria</i> 	0.4 – 5 ¹⁷	Sea, brackish and fresh water and groundwater ¹⁷	3,400 species worldwide ¹⁷ , 7 stygobiotic species in Germany ³
Subclass: <i>Acari</i> 	a few mm ⁹	Colonize every habitat, also groundwater, have high demands on water quality ⁹	< 5,000 water mite species worldwide ¹⁸ , 10 stygobiotic species in Germany ³

¹Fuchs et al. (2006)⁷Sauermost and Freudig (1999b)¹³Sauermost and Freudig (1999c)²Galassi (2001)⁸Camacho (2006)¹⁴Batzer and Boix (2016)³Zaenker et al. (2020)⁹Hunkeler et al. (2006)¹⁵Hahn et al. (2013)⁴Hahn (1996)¹⁰Boulton et al. (2008)¹⁶Eckert et al. (2008)⁵Galassi et al. (2009)¹¹Stoch et al. (2009)¹⁷Sauermost and Freudig (1999a)⁶Fuchs (2007)¹²Botosaneanu (1986)¹⁸Di Sabatino et al. (2000)

*Stygophile organisms are found primarily in surface water, but they can survive in shallow groundwater for a while (Preuß and Schminke, 2004).

4.2.3 Classification scheme by Griebler et al. (2014)

Commissioned by the Federal Environmental Agency of Germany (UBA), Griebler et al. (2014b) developed a two-step, ecologically based classification scheme for the characterisation of groundwater ecosystems and also defined spatially dependent reference values of ecologically intact groundwater ecosystems. In order to enable a statement about the exposure of the groundwater at a specific site, biotic and abiotic parameters, which are determined and compared with reference values, are used to distinguish locations with very good or good ecological conditions or locations which fail these criteria, i.e. affected areas (Figure 4.1). If an ecological assessment of groundwater ecosystems, which is based on the groundwater fauna analysis, takes place, some faunal criteria must be considered. Invertebrates avoid habitats that are ochred or have a low dissolved oxygen content. Thus, unstressed or natural habitats are defined as areas, with a dissolved oxygen content > 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 4.1). This allows a qualitative interpretation of the ecological condition of the groundwater system. If the results indicate affected ecological conditions, i.e. one or more biological and/or ecological indicators are out of the reference range, then an assessment according to the level 2 scheme is necessary. This requires a determination of the reference values at local reference locations, which are protected and have a weak surface influence, and a subsequent comparison of these values with measured data. As our aim is a first-tier screening of an urban area, we only apply level 1 in our study.

4.3 Results and discussion

4.3.1 Physical and chemical parameters

First, the groundwater conditions in the study area are evaluated by their physical–chemical characteristics. The following values are average values of the individual samplings from each monitoring well. In order to allow for a spatially differentiated assessment, the study site (city area of Karlsruhe) is classified into different zones based on land use types provided by the European seamless vector data of the CORINE Land Cover (CLC) inventory (European Environment Agency, 2016). Based on this data, the city area is subdivided into (1) forested area (forest; local name – Hardtwald) and (2) industrial, commercial and residential areas (urban area; Figure 4.2a). For simplification, the phrases forest and urban area are used in the following. A more detailed subdivision in the urban area did not appear reasonable due to the heterogeneous structure.

As expected, measured GWTs at the bottom of the wells with 8.5 to 39.0 m depth, are mainly constant over the repeated measurements. The lowest GWTs, ranging between 10.5 and 10.9 °C,

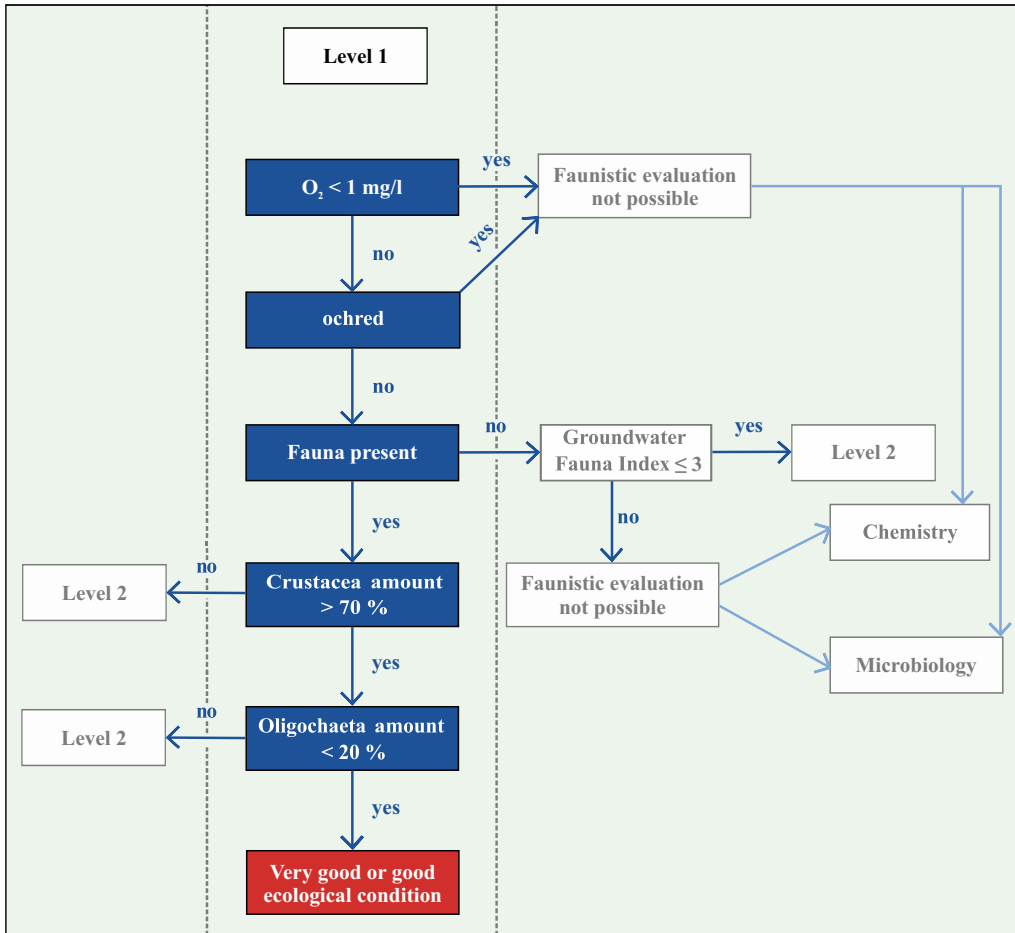


Figure 4.1: Classification scheme by Griebler et al. (2014b), according to level 1 for groundwater ecosystems, on the basis of groundwater fauna (modified after Griebler et al. (2014b)).

were measured in the eight wells of the forested area (Table A3.2). In contrast, the highest average GWT, at 17.5 °C, was measured in a well near the city hospital (T113; Figure 4.2a). The mean value of all wells is 13.5 ± 2.1 °C, which is similar to the results from Benz et al. (2014), with 13.0 ± 1.0 °C. According to Benz et al. (2017), annual shallow GWTs vary between 6 and 16 °C in the area of Karlsruhe, which is in line with the temperatures measured during fauna sampling (Figure 4.3a). For the urban area in the northwestern part of the city, Figure 4.2a 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.

The dissolved oxygen content acts as a limiting factor for groundwater fauna, since groundwater is usually undersaturated, with a varying oxygen content between 0 and 8 mg/l (Griebler et al., 2014b; 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 4.3b and Figure A3.2a). As expected, the monitoring wells located in the forested area (Hardtwald) show the highest content, while the lowest values are found in urban areas and are likely linked to aquifer contamination and other anthropogenic effects (dissolved oxygen content of forested versus urban area; U test – p value = 5.3×10^{-3} ; $n = 8$; 31). 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., 2014b; Kunkel et al., 2004). Moreover, it seems that, with a greater depth of the measurement wells, the dissolved oxygen content increases (U test – p value = $< 10^{-13}$; $n = 39$). This can be explained by the fact that shallow wells can have a low water column in which oxygen can rapidly be consumed by groundwater microorganisms, chemical reactions and/or groundwater fauna. In the upper, unscreened part of deeper wells, dissolved oxygen can be consumed, while in the lower, screened part, oxygen is continuously being refilled by oxic groundwater from the surroundings (Malard et al., 2002). Furthermore, reducing conditions in the overlaying soil can result in a low content of dissolved oxygen in groundwater.

Nitrate is often named as an important pollutant in groundwater. The natural and geogenic concentration of nitrate in groundwater is usually under 10 mg/l (Griebler et al., 2014b). In our study area, the average nitrate content of all wells varies between 1.3 and 14.7 mg/l. In the urban area, the average nitrate concentrations are generally higher and correlate with the content of dissolved oxygen (U test – p value = 4.0×10^{-3} ; $n = 39$), showing the link between nitrate content and oxygen consumption. Wells with a dissolved oxygen content below 1.5 mg/l have an average nitrate content of 1.5 mg/l, most likely caused by nitrate reduction under anoxic conditions. Groundwater with reducing conditions (< 5 mg/l dissolved oxygen) has an average nitrate content of about 7 mg/l, which is in contrast to groundwater with oxidising conditions that has 9 mg/l, which promotes the oxidation of ammonium to nitrate. The lowest nitrate concentrations are found in the forested area (Figures 4.3c and A3.2c), where atmospheric nitrogen is held back by forest soils (U test – p value = 1.7×10^{-3} ; $n = 8$), and fertilisation is prohibited due to water protection regulations in the forested area (Aber et al., 1998; Schönthaler and Adrian-Werburg, 2008). Moreover, the average concentrations of iron and phosphate are low and, in most cases, below the detection limit of the test (Figure A3.2d, e) and also below the natural and geogenic concentrations within the study site (phosphate – 0.05 mg/l; Griebler et al., 2014b; iron – 3.3 mg/l; Kunkel et al., 2004).

Considering these findings, clear differences in the spatial distribution patterns of abiotic groundwater characteristics are noticeable. The forested area shows lower average GWT than the urban area (U test – p value = 3.3×10^{-5} ; $n = 8$; 31), lower nitrate concentrations (U test – p value = 4.1×10^{-3} ;

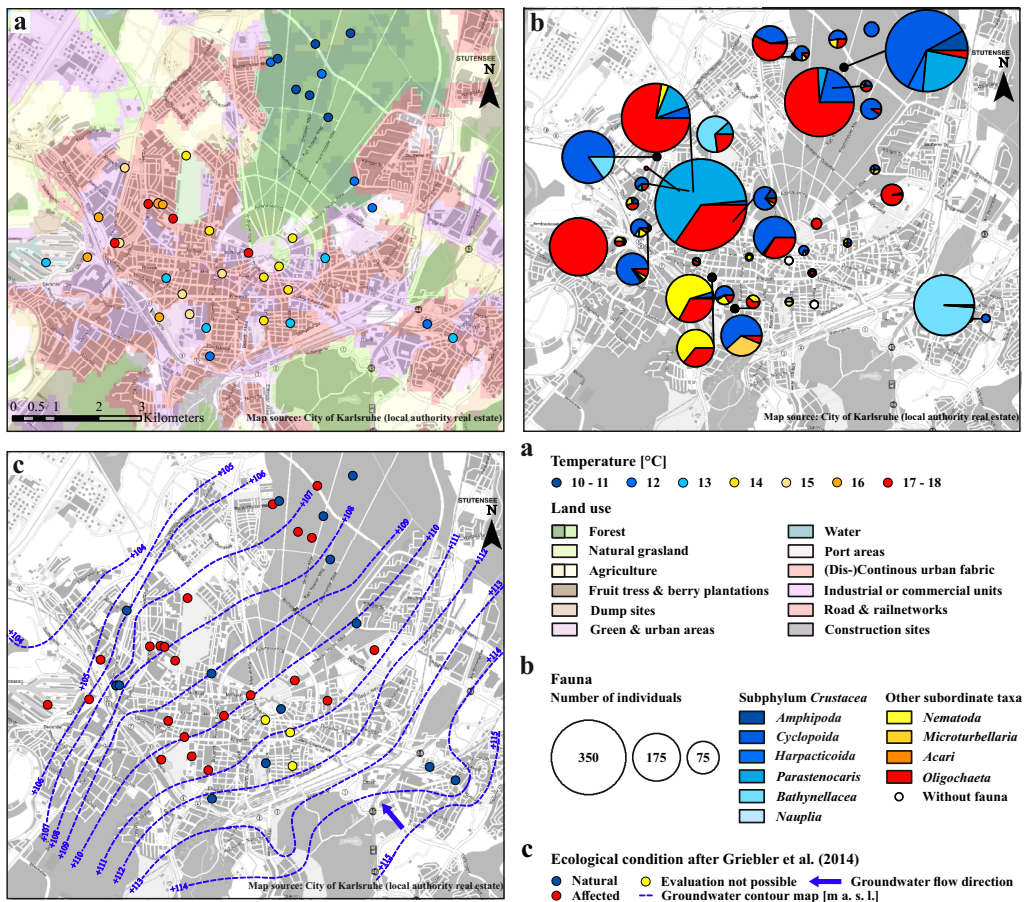


Figure 4.2: Overview map of the city area of Karlsruhe. (a) Land use plan (European Environment Agency, 2016) and average groundwater temperature of the multiple measurements (degrees Celsius) at the bottom of the monitoring wells. (b) Detailed groundwater fauna – colours of the circles show the different taxa in the sample (percent), and the size indicates the number of individuals. (c) faunal evaluation, after Griebler et al. (2014b), and groundwater contour map in metres above sea level (modified after the local authority real estate of Karlsruhe).

$n = 8; 31$) and higher dissolved oxygen concentrations (U test – p value = 5.3×10^{-3} ; $n = 8; 31$), which indicates a correlation between abiotic groundwater characteristics and land use in the study area. Moreover, no impact of groundwater originating from the urban area is observed on the wells in the forested area, as the groundwater flow direction in Karlsruhe is northwest (see Section 4.2.1 and Figure 4.2c). Further investigations demonstrated that, besides one larger and two smaller contamination sites (still with concentrations below the threshold values, however; Figure A3.2b), only minor groundwater pollution is documented in Karlsruhe (see the Chapter 5.2). The chemical and physical parameters considered in the long-term monitoring system are within the range of local background and below threshold values of the drinking water ordinance of Germany (see the

Supplement for more information). Thus, the main documented impacts on groundwater quality in the study area are related to temperature and oxygen.

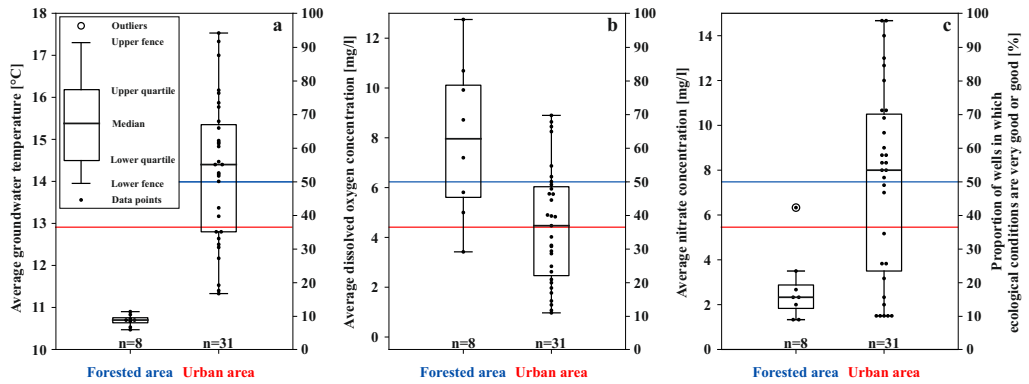


Figure 4.3: Box plots of the physical and chemical parameters for the forested and urban area in the study site and the proportion of wells in which ecological conditions are very good or good (in percentage), indicated by the blue (forested area) and red (urban area) lines (secondary axis). (a) Average temperature of the repeated measurements (degrees Celsius) at the bottom of the monitoring wells. (b) Average content of dissolved oxygen (milligrams per litre) of the monitoring wells. (c) Average nitrate content (milligrams per litre) of each monitoring well (n is the number of wells).

4.3.2 Groundwater fauna

The biotic communities of the groundwater consist of microorganisms and invertebrates (in particular crustaceans; Griebler et al., 2014b). In the pool of samples, 3,666 individuals were detected in 37 of 39 wells, which means that 95 % of the wells are colonised (Table A3.3). With 2,047 individuals, the group of *Crustacea* was found to be the most abundant (56 %). A total of 976 individuals (27 %) of the order of *Cyclopoida* dominated this group, followed by the genus *Parastenocaris*, with 599 individuals (16 %), the order of *Bathynellacea* (371), *Amphipoda* (66), *Harpacticoida* (33) 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 respect to the number of wells, in the monitoring wells in the forested area (690 individuals in eight wells) compared to those in the urban area (1,357 individuals in 31 wells). Furthermore, no individuals of the order *Bathynellacea* and only 135 individuals of the genus *Parastenocaris* were found in the forested area. In contrast, larger numbers of the latter species as well as of oligochaetes are characteristically found in 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 forested and in the urban area.

Stygobiontic amphipods, i.e. large-bodied invertebrates, which, due to their size, have a habitat preference for open spaces such as wells (Table 4.1; e.g. Hahn and Matzke, 2005; Korbel et al., 2017), were found in only three wells (Figure 4.2b). A total of 46 individuals of this order were detected in the forest and 20 individuals in the urban area (Figure 4.4a, b). Although statistical analysis showed no clear differences between the abundance of amphipods and land use (U test – p value = 1.5×10^{-1} ; $n = 8$; 31), the higher number of individuals in the forest area could support the hypothesis that amphipods indicate healthy groundwater ecosystems as they react most sensitively to disturbances such as pollutants (Korbel and Hose, 2011) and groundwater temperature. In laboratory experiments with a thermal tank, Brielmann et al. (2011) found that 77 % of the individuals of the studied amphipods (*Niphargus inopinatus*) preferred areas with a temperature between 8 and 16 °C. In addition, Spengler (2017) and Issartel et al. (2005) observed maximum temperatures of up to 17 °C. The lack of a statistically significant correlation might also be related to the low number of wells ($n = 8$ in the forested area) and individuals ($n = 46$). Amphipods are important ecosystem service providers in terms of bioturbation and organic decomposition (Boulton et al., 2008). As observed in laboratory experiments (Smith et al., 2016), they actively move, with migration speeds between 1.7 and 3.5×10^4 m per year. In most cases when amphipods were found, higher concentrations of individuals of the order *Cyclopoida* were also identified (abundance of *Amphipoda* versus *Cyclopoida*; U test – p value = 9.6×10^{-5} ; $n = 39$). Individuals of the latter order were generally found in larger quantities in the majority of the wells (479 in the forested 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 (e.g. upper thermal limit of 26.9 ± 0.2 °C in laboratory tests by Castaño-Sánchez et al., 2020a; Spengler, 2017).

The order *Harpacticoida*, which includes the genus *Parastenocaris*, have an elongated body shape and a stem-chiselling movement, which is why they are predestined for living in cavities and groundwater (Fuchs, 2007; Hahn, 1996) and prefer sand and gravel as a substrate (Galassi et al., 2009). Larger numbers of *Parastenocaris* (464 individuals), which can tolerate GWT from 8 to > 20 °C (Fuchs et al., 2006), e.g. *Parastenocaris phyllura* withstood up to 22.5 °C in laboratory tests (Glatzel, 1990), were found in the urban area, especially in the northwestern area (Figure 4.2b). This area is characterised by GWTs 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 physically and chemically altered areas. Accordingly, only 135 individuals were detected in the forested area.

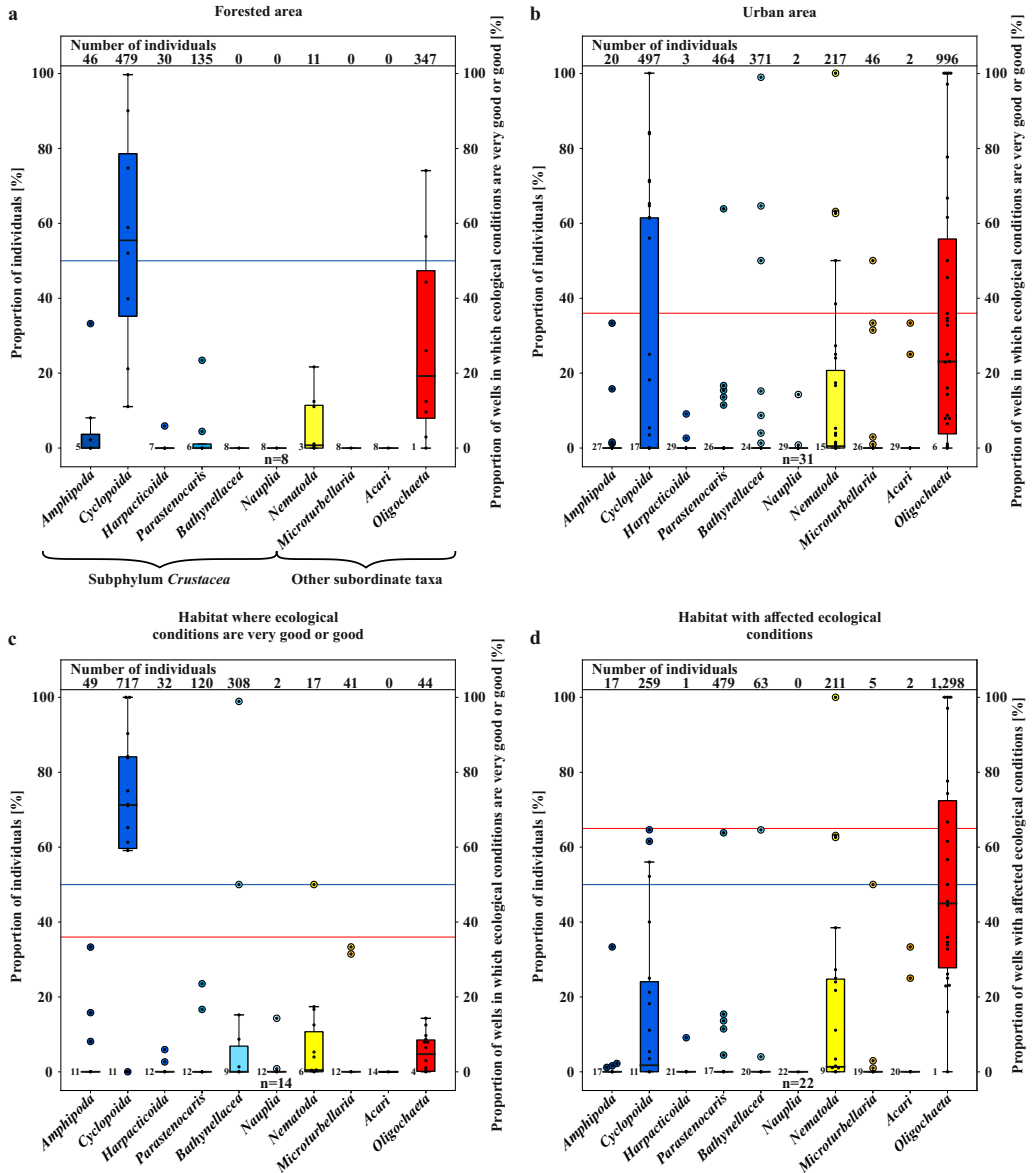


Figure 4.4: Box plots of the amount of fauna (percent). (a) Proportion of individuals and wells in which ecological conditions are very good or good (secondary axis; percent) in the forested area. (b) Proportion of individuals and wells in which ecological conditions are very good or good (percent) in the urban area. (c) Proportion of individuals and wells in which ecological conditions are very good or good (percent), divided based on the results of the classification scheme by Griebler et al. (2014b). (d) Proportion of individuals and wells with affected ecological conditions (percent), divided based on the results of the classification scheme by Griebler et al. (2014b). The colour of the boxes shows the different taxa in the samples (n is the number of wells).

In addition, quantities of *Bathynellacea* (371 individuals) were found in five monitoring wells all located in the urban area at a depth of 9.0 to 13.5 m with a GWT of 12 – 15 °C (Figure 4.4b). 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) compared to wells in the forested area, 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 4.4b), 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 nitrate concentrations up to 14 mg/l, which is above the geogenic concentration of 10 mg/l and higher compared to wells in the forested area.

Finally, nematodes and microturbellarians were found at locations with unfavourable living conditions, such as a low dissolved oxygen content or a high amount of fine substrates, as also reported by Hahn et al. (2013), and both can tolerate high temperature ranges (*Turbellaria* – 2 – 20 °C (Herrmann, 1985); *Acari* – 9.1 – 18.5 °C (Wiecek et al., 2013)). Here, both were found in larger quantities in the urban area of Karlsruhe (Figure 4.4b). This area has the lowest content of dissolved oxygen and a relatively higher amount of detritus (> 2).

Eventually, correlation analysis between groundwater fauna and the chemical parameters showed that stygobites are only slightly affected by groundwater chemistry (Hahn, 2006; Schmidt et al., 2007; Stein et al., 2010). Only the Spearman rank correlation coefficient (ρ) between the number of taxa and the dissolved oxygen content is significant, with a value of $\rho = 0.55$ (p value = 3.0×10^{-4} ; $n = 39$). Moreover, it is assumed that groundwater fauna can usually cope well with short-term changes in physical–chemical parameters (Griebler et al., 2016). Previous studies showed that some species can even benefit from pollutants (Matzke, 2006; Zuurbier et al., 2013). In case of nitrate, numerous studies emphasise that nitrate at concentrations below 50 mg/l does not directly affect groundwater fauna (Di Lorenzo et al., 2020b; Di Lorenzo and Galassi, 2013; Fakher el Abiari et al., 1998; Mösslacher and Notenboom, 2000). As the highest average nitrate content per well is below 15 mg/l in this study, a direct negative effect of the nitrate concentration on the groundwater fauna is unlikely. Thus, nitrate is only mentioned as one measured parameter and is not discussed as a potential anthropogenic impact in this study.

The natural influences on porosity, groundwater flow and nutrient delivery were also discussed as being a primary influence on natural stygobite distribution in previous studies (Hahn, 2006; Korbel and Hose, 2015). An important natural influence is the local geology, as fine sands and silts are typically rather harsh environments, resulting in an impoverishment of specific groundwater fauna

such as *Crustacea* (Hahn, 1996). The city of Karlsruhe is located on carbonate (Würm) gravel and river terrace sands, pervaded by bands of drifting sand and inland dune sands. These sediments are highly water permeable and show vertical seepage of water movement almost exclusively. Flood sediments (on top of river gravel) and bog formations, are located in the east and west of Karlsruhe (Regierungspräsidium Freiburg, 2019). This local geology limits the cavity size and, therefore, has impacts on the habitat of the groundwater fauna (Wirsing and Luz, 2007). For example, individuals of the genus *Parastenocaris* typically inhabit small-scale cavity systems (Spengler, 2017). Individuals of this genus can be found both in the wells drilled in gravel (four wells) and in drifting sand sediments (three wells; abundance of *Parastenocaris* versus geological units; U test – p value = 1.4×10^{-9} ; $n = 39$). Amphipods are predominantly found in measurement wells located in the Würm gravel (in five of seven wells; abundance of *Amphipoda* versus geological units; U test – p value = 9.0×10^{-11} ; $n = 39$). Moreover, it seems that differences in the geological units have an influence on the total amount of individuals (U test – p value = 1.7×10^{-9} ; $n = 39$) and the relative amount of detritus (U test – p value = 3.0×10^{-3} ; $n = 39$). As these results show, regional geology seems to have an influence on the occurrence of specific groundwater taxa and on the number of individuals and on food supply, in terms of available organic matter. However, it is not possible to give a reliable estimate of the strength of the anthropogenic impacts, e.g. if they are strong enough to overrule the regional selective forces. Hence, this should be investigated in more detail in future studies.

Limitations regarding the sampling method must be considered when interpreting the faunal results. In this study, a simple basic screening of well water was conducted using a net sampler and bailer to examine 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 [being] 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. Nevertheless, pumping can result in the selection of the taxa, especially in the presence of very fine sediments, and can result in changes in the sediment composition in the surrounding of the wells and, therefore, in habitat conditions. Other studies, on the other hand, found no significant differences in hydro-chemical values (temperature, pH, dissolved oxygen, etc.) 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 the types of faunal communities. However, in terms of total abundance, and the numbers of individuals per litre, monitoring wells appear to exhibit larger numbers caused by filtration effects (Hahn and Gutjahr, 2014; Hahn and Matzke, 2005; Korbel et al., 2017). As the aim of this study is to provide an overview of the groundwater fauna

community and to receive a first impression of groundwater ecology, sampling the fauna by using a net sampler is sufficient. 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). Care should also be taken when interpreting faunal results of sites that are sampled in different years. To improve comparisons of the biotic communities, a consistent sampling period of every well is necessary in the future.

4.3.3 Classification scheme by Griebler et al. (2014)

In three wells, evaluation with the classification scheme by Griebler et al. (2014b) was not possible due to ocherous conditions in two monitoring wells and low dissolved oxygen content (< 1 mg/l) in the third well. According to the classification scheme by Griebler et al. (2014b), unstressed (meaning no natural or anthropogenic stressors) or natural groundwater habitats have 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 were fulfilled, indicating very good or good ecological conditions or, in other words, a natural groundwater habitat (Figure 4.4c). These natural areas tend to contain more individuals of the orders of *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 criteria of this classification scheme (Figure 4.4d).

Surprisingly, only 50 % of the wells in the forest, which is also the catchment area of the drinking water supply of Karlsruhe, are described as being natural groundwater habitats. An identical number of wells yielded habitats with affected ecological conditions. The main difference between natural and affected wells in the forested area arises from the occurrence of specific species. A total of 86 % – 100 % of species found in natural wells are crustaceans, in contrast to affected wells with only 33 % – 67 % (Table A3.2 and A3.3). 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. A reason could be the varying local geology, as mentioned above. Moreover, food supply is one of the most limiting parameters for the survival of groundwater fauna (Datry et al., 2005; Hahn, 2006). If the organic carbon supply varies on a small scale, this can influence the microbiology and, therefore, the groundwater fauna as well, although short-term changes in nutrient supply can be compensated by groundwater fauna.

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.

In future, a further subdivision of a study area in more land use categories could be beneficial for specifically looking at typical anthropogenic impacts. Furthermore, the integration of more biological criteria is useful to improve the results of the assessment, according to Griebler et al. (2014b). Because of heterogeneous groundwater ecosystems in Germany, it is likely that the reference values provided by Griebler et al. (2014b) do not reflect the situation in Karlsruhe correctly. Considering site-specific characteristics and reference values would lead to a more robust assessment. Other assessments, like the similarly structured GHI or wGHI^N (Di Lorenzo et al., 2020a; Korbel and Hose, 2017) can, additionally, be used. Moreover, there are a few newly developed indexes, like the D–A–C (prokaryotic cell density – D; activity – A; bioavailable carbon – C) Index, which is based on microbiological indicators and shows whether groundwater reserves deviate from natural references (Fillinger et al., 2019), which can be used in the future. As mentioned in the introduction, another way of quantifying the relevant ecological conditions in the groundwater is the GFI. During the preparation of this study, the GFI was tested on the data (see Chapter 5.2); however, it did not provide any additional information or valuable insights and was therefore excluded. The influence of multiple stressors, such as the pollution of the groundwater through industrial plants, etc., and their effects on the governing parameters can bias the GFI. In general, the GFI seems to be suitable only for unpolluted and anthropogenically undisturbed groundwater with sufficient oxygen concentrations (> 1 mg/l). Moreover, under urban areas, changes in GWT are caused by anthropogenic heat inputs (Benz et al., 2014; Menberg et al., 2013b; Tissen et al., 2018) rather than being related to surface water influences. Hence, the GFI appears to be unsuitable for the assessment of the groundwater fauna in an urban setting. The same outcome emerges for the Shannon diversity index, which was also tested during the preparation of the study and showed no clear distribution pattern according to faunal diversity and was therefore not considered further.

4.3.4 PHATE analysis

A PHATE analysis is conducted using the following input parameters: depth, GWT, nitrate and phosphate content, the relative amount of detritus, geological unit, number of taxa, number of individuals, Shannon diversity, number of crustaceans and oligochaetes (according to Griebler et al. (2014b)) and the abundance of amphipods as well as of individuals of the orders *Cyclopoida* and

Bathynellacea and the genus *Parastenocaris*. The content of dissolved oxygen is not considered in this analysis since it was always above the limit of 1 mg/l, except in one case. Thus, dissolved oxygen is not expected to have an influence on the groundwater fauna in our study area.

There are four groups, which can be assigned predominant characteristics, that can be distinguished in the PHATE visualisation (Figures 4.5 and A3.3 - A3.4). A total of three measurement wells (group IV) contain neither oligochaetes nor crustaceans, indicating unfavourable living conditions. In contrast, the nine wells of group III contain high amounts of oligochaetes (100 % of oligochaetes according to the scheme of Griebler et al. (2014b), and an average GWT of 14.3 °C; Table A3.4). However, diversity and abundance was found to be low in group III.

An even higher average GWT of 15.0 °C was found for group II, which mostly consists of wells drilled in drifting sand sediments. Surprisingly, these wells also show the highest diversity (\geq three taxa per well), the highest Shannon diversity (see Chapter 5.2), and the highest number of individuals in total and of individuals of the genus *Parastenocaris*. Individuals of this genus are often found isolated in altered areas (Spengler, 2017). Moreover, in five wells of group II, individuals of the order *Bathynellacea*, which can tolerate temperatures up to 18 °C and typically inhabit interstitial groundwater (Stein et al., 2012), were found. The presence of individuals of the genus *Parastenocaris* and the order *Bathynellacea* in group II suggests that they may act as type species for urban situations. The observation that group II shows the highest GWT and the highest Shannon diversity is in contrast to findings of previous studies that noticed decreased diversity at elevated temperatures (Briellmann et al., 2009). These diverging observations suggest that faunal quantities, such as diversity or abundance, are not always suitable indicators for changes within organism communities. For example, if species disappear due to increased temperatures and are substituted by more tolerant species, the difference in diversity may be marginal and the change in the community may not be noticeable.

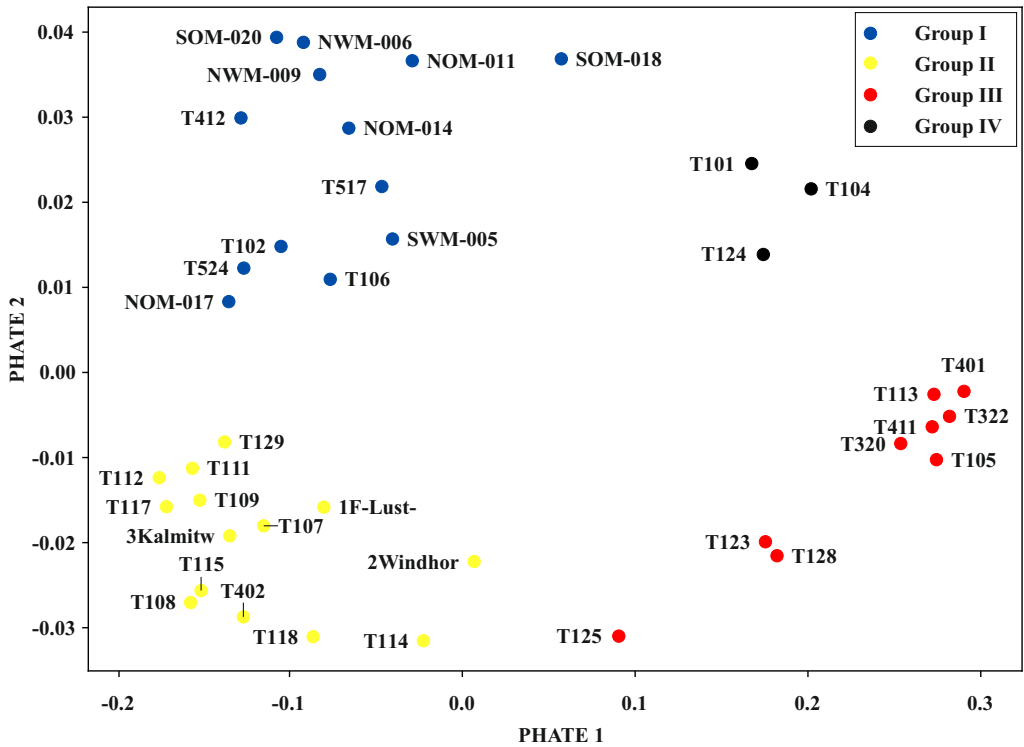


Figure 4.5: PHATE visualisation showing similarities between measurement wells. Different colours indicate the four clearly separable groups.

Wells of group I (blue) are drilled predominantly in Würm gravel (geological unit of group I versus group II; U test – p value = 8.2×10^{-3} ; $n = 13; 14$), while having the lowest GWT (GWT of group I versus group II; U test – p value = 2.0×10^{-5} ; $n = 13; 14$). These wells show a moderate diversity and number of individuals, yet have the highest average number of crustaceans and the highest number of amphipods and individuals of the order *Cyclopoida*. Considering these findings and the U test results (see Table A3.5), the grouping of the measurement wells seems to be influenced by the composition of the groundwater organism communities, the faunal diversity (numbers of taxa and individuals) and the geological unit and the GWT (Figures A3.3 - A3.4).

Considering the spatial distribution of the grouped wells in the study area, it becomes apparent that all wells in the forested area fall within group I (Figure 4.5). Those wells which are located outside the forested area are in locations with nearby green areas (parks, recreational areas, etc.). In contrast, the wells of the other three groups are heterogeneously distributed within the urban area. Many of the measurement wells of groups III and IV are associated with suspected or known contaminated sites (Figure A3.2b). Overall, a spatial pattern of abiotic groundwater characteristics

(GWT and nitrate content) and the occurrence of particular species (*Parastenocaris*) within the study area is apparent in the PHATE analysis, which confirms the classification according to land use. Yet again, no clear spatial pattern regarding faunal diversity in the study area could be identified, although a tendency of clustering of wells from group III with higher diversity and number of individuals can be seen in the northwestern area of the city.

4.4 Conclusion

The aim of this study is to provide a first assessment of the ecological state of groundwater in an urban area and to distinguish areas with a natural state of groundwater ecology from anthropogenically affected areas. To achieve this, we examine the groundwater fauna and abiotic parameters in 39 groundwater monitoring wells in residential, commercial and industrial areas (31 wells) and a forested area (eight wells) outside the built-up area of Karlsruhe, Germany, using the simple classification scheme by Griebler et al. (2014b) 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 forested area shows lower GWT, lower nitrate concentrations and higher dissolved oxygen concentrations, which indicates a correlation between abiotic groundwater characteristics and land use. Moreover, amphipods are more abundant in wells in the forested than in urban area. However, in both the rural forested and in the urban area, crustaceans and individuals of other subordinate taxa were widely found, and therefore, no clear spatial pattern regarding faunal diversity and land use was 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 forested area compared to those in the urban area. Larger numbers of the genus *Parastenocaris* and 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 classification scheme by Griebler et al. (2014b), could be observed. Surprisingly, only 50 % of the sampled wells in the forested area 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, suggesting that there are noticeable differences in the groundwater ecosystems between the surrounding forested and urban areas. The level 2 assessment from Griebler et al. (2014b) can help to achieve a more reliable and quantitative ecological assessment of urban aquifers as it divides groundwater ecosystems into ecological grades according to the intensity of anthropogenic disturbance. It is based on the use of local reference values and the collaboration with experts; however, is challenging to apply.

Therefore, further studies with large-scale and repeated measurement campaigns are needed to verify our findings. This should also include other cities and the determination of undisturbed local reference values which are required for a more reliable, but also quantitative, ecological assessment of urban aquifers. Moreover, a wider range of indicators should be considered in a classification scheme, such as temperature, porosity of the aquifer, groundwater flow, pollutants and nutrient supply, especially when investigating urban areas. In addition, an important adaptation for an improved evaluation method is the determination of fauna at species level, which will provide more information (i.e. about stygobionts, stygophiles and stygoxenes) and also consider the endemism of stygobiontic species. In this context, classification schemes should pay more attention to the different groundwater species and their potential use as indicator species. Finally, city and energy planning should seriously consider urban groundwater ecosystems as they provide valuable information for a sustainable use of the subsurface.

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5

SYNTHESIS

5.1 Summary and conclusion

Groundwater is an important global resource that harbours a complex ecosystem, which provides important services, including water purification, as well as nutrient and carbon cycling. Nevertheless, this ecosystem has hardly been investigated and even the most basic knowledge is still lacking in many parts of the world. This thesis aims to improve the understanding of ecological processes and conditions in groundwater, which are essential for sustainable resource and environmental management in times of competing groundwater uses. Therefore, a common knowledge basis on groundwater fauna is built, changes in faunal and abiotic parameters due to natural or anthropogenic influence are investigated, indicator parameters are identified, and implications for biomonitoring on different scales are discussed. Thus, groundwater fauna is described and assessed on different spatial and temporal scales (see Figure 5.1).

Since information on groundwater ecosystems is still lacking in many parts of the world, it is necessary to gain a **global overview on groundwater fauna** and to build up inventory and carry out analyses of available data. Thus, this thesis provides an overview on groundwater fauna (stygofauna) research, including the historical evolution of research topics and the development of sampling methods, and secondly, it identifies the global distribution of groundwater fauna research and existing data gaps. Data from 859 studies, such as from national and international publications

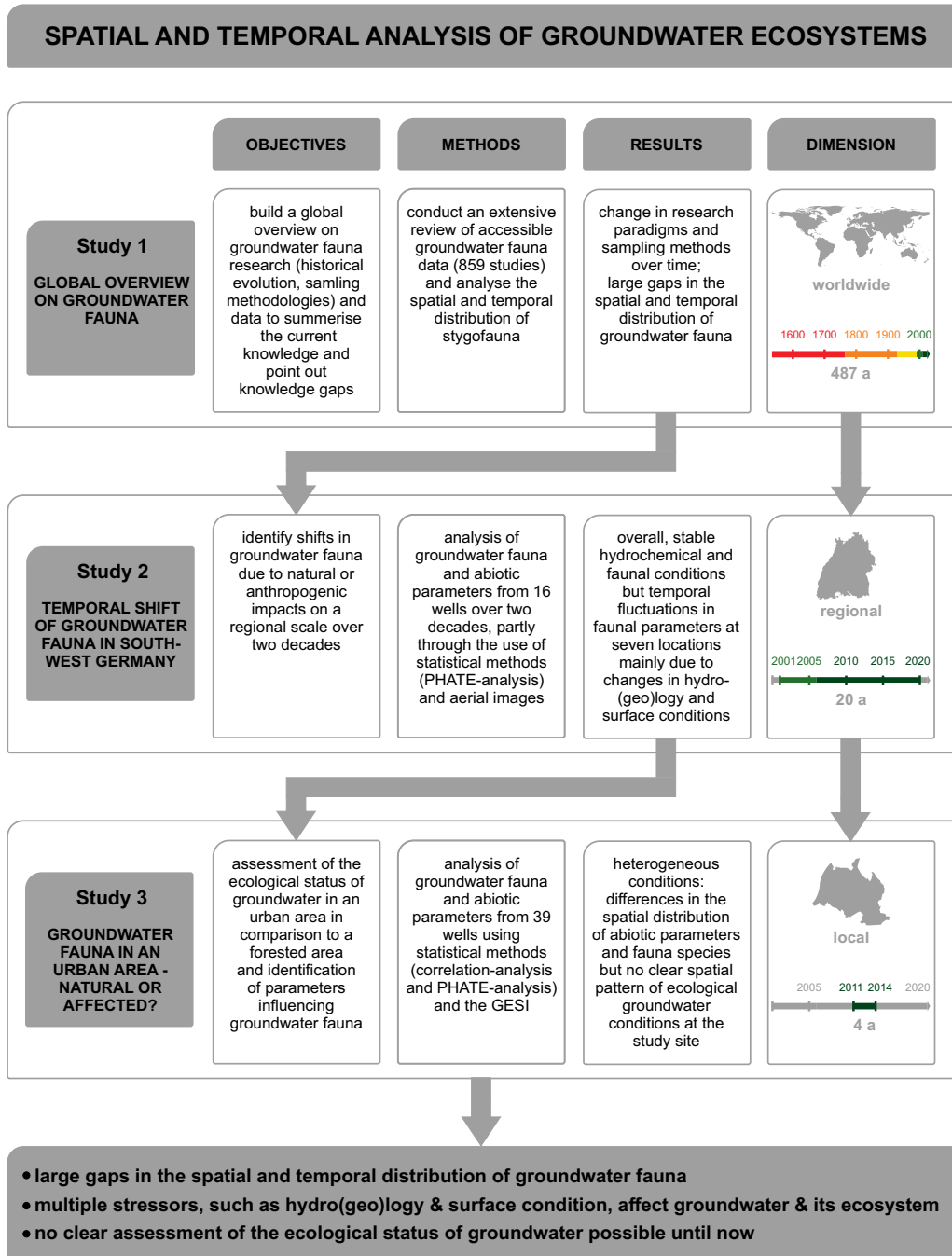


Figure 5.1: Objectives, methods, results and dimension of the three collected studies in this thesis.

in scientific journals, national reports, doctoral theses, historical writings, books, online databases and others, is analysed and an extensive review of accessible groundwater fauna data is conducted.

In the early research phase, sporadic observations of fauna date back to 1537, but it was not until the 17th century that groundwater fauna research increased. During the ‘pre-ecological research phase’, the emphasis of research was still on cataloguing new species, their habitats and their biogeographical origin. It was in the 1920s that the research focus changed from the description of newly discovered species and their evolution towards ecosystemic and more holistic analyses of fauna and their functions to the application of ecological management and monitoring programs. The growing interest in groundwater fauna research is reflected in the exponential increase in the number of studies over time since the 1920s, which peaked in 2009. Moreover, sampling methods have developed from using simple nets, substrate samples and hand-pumps in the beginning to recent molecular analyses (e.g. eDNA), which offer the potential to ease sampling and enable vast data collection. Until now, net sampling and pumping well water have been the dominating sampling methods worldwide. In the early 2000s, during the ‘applied ecological phase’, the first policies to conserve and protect groundwater quality and GDE are developed in Switzerland, Germany and Australia.

Studies on groundwater fauna are spatially uneven and are dominated by research in Europe and Australia, with few studies in Africa, Asia and the Americas. In Africa, very limited information about stygofauna distribution (i.e. the geographical spread of groundwater fauna), ecosystem services and ecological status is available, with information on associated trends over time also missing. American groundwater fauna studies predominantly provide insight into newly discovered species and biogeographical analyses, including factors influencing biogeographical distribution of stygobionts. In relation to its large area, Asia is poorly investigated, and studies are concentrated in Japan, South Korea, India and Iran. Moreover, most studies in Asia and the Middle East still focus on describing newly discovered species, with limited studies investigating the origin, functioning and distribution of stygofauna or groundwater ecology. These gaps in global research bias the view on groundwater biota, hindering the identification of biodiversity patterns and ecosystem functions on a wider geographic and climatic scale. Matters are quite different in Australia and large parts of Europe. Australia is regarded as a pioneer in the field of stygofauna monitoring programs. Australian studies also contributed greatly to the global understanding of stygofauna evolution, distribution, sampling methods, ecosystem functions and processes, anthropogenic impacts on these ecosystems and the recognition of the need to protect groundwater resources and their ecosystems. In Europe, research began with descriptions of species and taxonomy, as well as the development of more complex sampling methods. Moreover, European studies on groundwater fauna have contributed much to the global knowledge on the impact of natural events and resulted in the development of ecological assessment frameworks (Fillinger et al., 2019; Griebler et al., 2014b; Hahn, 2006). The high density and frequency of sampling and the research focus on

biodiversity and ecology issues have placed Germany at the forefront of stygofauna research and the application of ecological research to address groundwater management and monitoring requirements. This first global overview on groundwater fauna research is important to understand biodiversity and to determine future research fields. Thus, a worldwide effort to collect information on groundwater ecosystems, their functional roles and anthropogenic impacts on them is required to implement stronger policies and monitoring requirements for groundwater fauna.

One approach to improve the understanding of groundwater biodiversity and thus enhance a comprehensive assessment and protection of the ecosystem and the development of potential measures is biomonitoring. As already implemented for surface water (Haase et al., 2023), ecosystem monitoring can help to assess its health or ecological status. Thus, organisms and communities act as remote sensors integrating environmental conditions and stress over their lifetime, and provide information on medium- to long-term environmental conditions and changes (Conti, 2008). In this thesis, long-term groundwater data from **South-West Germany** is used to identify **shifts in groundwater fauna** due to natural or anthropogenic impacts. Available groundwater data of the study area (i.e. the state of Baden-Württemberg) is reviewed, and observation wells for additional sampling in 2020 are selected. In total, 16 observation wells are selected based on spatial coverage of the study area, aquifer type, land use type, well depth, faunal colonisation during the past two decades, availability of time series of physico-chemical parameters, and an average content of dissolved oxygen higher than 1 mg/l. Afterwards, the biotic and abiotic data is temporally and statistically analysed on different spatial scales (local to state-wide). Comprehensive analysis of metazoan groundwater fauna and abiotic parameters from 16 monitoring wells over two decades reveals no overall temporal trends for fauna abundance, biodiversity in terms of number of species, as well as no significant large-scale trends in abiotic parameters. This indicates that large-scale biodiversity and hydro-chemical conditions were stable over the past two decades on a regional scale. Nevertheless, there are significant variations between individual years for some specific wells, indicating a more complex temporal behaviour on a local scale. While nine wells out of 16 show stable ecological and hydro-chemical conditions, the remaining seven wells exhibit shifting or fluctuating faunal parameters. At some locations, these temporal changes are linked to gradual natural changes in abiotic parameters, such as decreasing dissolved oxygen contents or fluctuating temperatures. More often, however, there are no clear patterns in the abiotic parameters of individual wells. Instead, changes in groundwater fauna are caused by superimposing effects of multiple parameters linked to increasing or weakening surface influence (Schwäbisch Hall, Gaggenau and Efringen). Moreover, most monitoring wells in this study have a 'natural' status according to the assessment scheme of Griebler et al. (2014b). Only six wells (Kadelburg, Schwäbisch Hall, Rohrdorf, Sankt Leon, Weingarten and Neckargartach) have a proportion of Oligochaetes higher than 20 % in one or multiple years, indicating disturbed conditions. Abiotic parameters show mostly constant conditions in the individual wells.

A multivariate PHATE-analysis suggests that, besides the hydrogeological setting, varying contents of sediment and detritus impact faunal abundance. Finally, three individual wells are analysed in detail, as certain changes in faunal parameters in those individual wells could not be directly related to varying abiotic parameters. Thus, two unstable wells are analysed with respect to changes at the surface surrounding and compared to a deeper and rural well, which shows very stable conditions. By examining aerial images of the surroundings of individual wells over time, anthropogenic impacts, such as construction sites in Neckargartach were identified, and seem to cause significant shifts in groundwater fauna from dominating Oligochaetes to Crustaceans and thus to the aforementioned change in the ecological status. In Sankt Leon, faunal changes indicate a weakening surface influence over time, without visible changes in land use or surface conditions and thus no drivers of the unstable conditions. However, variable faunal composition and abundances are also observed for sites with very stable abiotic conditions in anthropogenically less affected areas, such as in Todtnau. These findings indicate that hydro(geo)logical changes and surface conditions, such as land use, should be assessed in line with hydro-chemical parameters to understand changes in groundwater fauna better. Accordingly, reference sites for natural conditions in ecological assessment and biomonitoring schemes for groundwater protection should be selected very carefully.

Changes in hydrology and surface conditions are increasingly occurring in densely populated urban areas, making it more challenging to find uninfluenced reference sites in such regions. Moreover, there are increasing conflicts over the use of groundwater, which is why the investigation of urban aquifers is becoming increasingly important for sustainable resource and environmental management. As groundwater quality is the product of multiple physical–chemical and biological processes and healthy groundwater ecosystems help to provide clean drinking water, it is necessary to assess their ecological conditions. Previous analyses within the framework of the global review have shown that faunal groundwater investigations are still scarce in urban areas. Therefore, this thesis assesses the **groundwater fauna in an urban area**. For this purpose, the ecological status of an anthropogenically influenced aquifer is examined by analysing fauna and hydrogeological, physico-chemical parameters in 39 groundwater monitoring wells in the residential, commercial and industrial, i.e. urban areas (31 wells) in comparison to a forested area (eight wells) outside the built-up area of the city of Karlsruhe (Germany). Analyses confirm noticeable differences in the spatial distribution of abiotic groundwater characteristics, such as lower GWT, lower nitrate concentrations and higher dissolved oxygen concentrations in the forested area. This indicates a correlation between abiotic groundwater characteristics and land use. Moreover, spatial differences in species distribution are observed, as amphipods are more abundant in wells in the forested than in the urban area, and larger numbers of the genus *Parastenocaris* and of nematodes and oligochaetes are typical for wells in the urban area. However, no clear spatial pattern is found for faunal diversity and land use as crustaceans and individuals of other subordinate taxa are

widely found in both the rural forested and the urban area. Moreover, correlation analyses between groundwater fauna and the abiotic parameters show that local geology influences the occurrence of specific groundwater taxa, the number of individuals and food supply. A PHATE-analysis reveals that similarities between wells can best be explained by the composition of the groundwater organism communities, the faunal diversity, the geological unit and GWT. Again, no clear spatial pattern regarding faunal diversity in the study area could be identified. For classification, the groundwater ecosystem status index GESI is applied, in which a threshold of more than 70 % of crustaceans and less than 20 % of oligochaetes serves as an indication for very good and good ecological conditions. Only 35 % of the wells in the residential, commercial and industrial areas and 50 % of wells in the forested area fulfil these criteria. However, no clear spatial patterns concerning land use and other anthropogenic impacts, particularly with respect to GWT are found. Thus, this study reveals heterogeneous faunal conditions in urban and also in 'natural' groundwater as a habitat, which do not allow a clear assessment of the ecological status with existing assessment approaches.

Summing up, groundwater is a complex system affected by multiple stressors, thus showing heterogeneous conditions as a habitat even at a local scale. Changes in hydro(geo)logy and surface conditions, such as land use, influence groundwater fauna. Thus, this thesis reveals the need to improve our understanding of this ecosystem further and to obtain clear information on its ecological status.

5.2 Perspective and outlook

Despite a continuing, exponential increase in the number of studies on groundwater fauna over the last ten decades, there is more inter- and transdisciplinary work required for a comprehensive assessment and protection of groundwater ecosystems worldwide. Studies 1 to 3 (Chapters 2 - 4) of the present thesis highlight the need for further investigation of this complex ecosystem. Based on the findings of this thesis, further research on the following specific subjects is suggested:

(I) Application of biomonitoring

Findings from this study can help to design and refine robust ecological monitoring and management tools for agencies and local authorities. To ensure representative results of future groundwater fauna sampling campaigns, it is advisable to employ shorter sampling intervals, e.g. on a monthly basis, to also address the effects of seasonality. Additionally, future research should focus on identifying and analysing reference sites for different settings, including forests, green areas, cities, industrial areas, and surface waters, to account for small-scale heterogeneities in hydrogeological conditions and land use. These measures are crucial for the transferability of findings from local

biomonitoring to larger scales and in the long run, as well as to identify sites at high risk. The principle is that we can only protect what we know.

(II) Development and use of a standardised assessment scheme

Investigations on groundwater fauna and the impacts of humans on the corresponding ecosystems are still rare. Until now, there is no common understanding of the best practice for assessing the ecological status of groundwater on a larger scale, although there are some approaches available, such as the GFI, GHI and the scheme according to Griebler et al. (2014b). Each of these approaches has advantages and disadvantages, as well as limited applicability due to the amount of data required and the complexity of the area to be analysed. Therefore, the aim must be to develop a more reliable, but also quantitative, ecological assessment. As a basis for a new assessment scheme, approaches from the surface water assessment could be considered, adapted and further developed for groundwater specific aspects. The present thesis offers findings for developing a new and standardised assessment scheme for groundwater health. It has been shown that various parameters, such as surface conditions (e.g. built-up areas, underground infrastructure, sealing, etc.), hydrogeological (e.g. type of aquifer, geological unit, size of pore cavities, groundwater flow), and physico-chemical (e.g. electric conductivity, temperature, content of dissolved oxygen, pollutants and nutrient supply) influence the health of the ecosystem. Thus, a more comprehensive range of indicators should be considered in an assessment scheme in the future. This is particularly true when investigating urban areas.

(III) Development of a standardised sampling method

To record groundwater biodiversity, comprehensive data on fauna is needed. Therefore, fauna sampling in groundwater should become standard practice alongside groundwater quality measurements and physico-chemical analyses. Likewise, it has to be ensured that representative samples of the entire faunal community are available. This is complicated for stygofauna as different sampling regimes need to be considered. Over time, several novel methods, described in detail in Chapter 2, were developed to account for these challenges. In summary, net sampling and pumping well water are the dominating sampling methods worldwide. Nevertheless, each of the described methods has its limitations. Hence, a combination of methods, such as net sampling together with pumping and/or DNA-analysis, is recommended (Korbelt et al., 2017; Saccò et al., 2022c).

(IV) Development of a common global database

Huge data sets are required to obtain extensive knowledge of stygofauna biogeography and biodiversity. Fauna databases are a useful tool for managing and sharing large amounts of data. An example of a national database is the Queensland Subterranean Aquatic Fauna Database in Australia, which contains data from 755 samples of 582 sites provided by the Queensland Government and industry. Moreover, a smaller reference database, with 65 wells in New Zealand

was developed during the BioHeritage Project, funded by the National Institute of Water and Atmospheric Research (NIWA) (Greenwood and Fenwick, 2019). In addition to national databases, there are transnational databases and databases that only cover individual groups of organisms, such as the European groundwater crustacean data set (EGCD). This database comprises a total of 21,700 occurrence data collectively representing 12 orders, 46 families, 165 genera and 1,570 species and subspecies of obligate groundwater crustaceans in Europe (European Union, 2020). To better record and protect biodiversity in the future and to be able to quantify a loss of biodiversity, a larger-scale overview is also required. The global data set from Chapter 2 of this thesis provides an initial basis for this.

(V) Map optimisation and expansion

One of the main aims of this thesis is to provide a global overview on stygofauna research, including an investigation on the global spatial and temporal distribution of groundwater fauna sampling sites. Based on data from 859 studies, Study 1 in Chapter 2 provides a world map giving an overview of groundwater fauna samplings and the number of studies. It would be desirable to provide this map interactively and online with regular updates to have a common knowledge basis for understanding, assessment, monitoring and conservation of groundwater biodiversity.

(VI) Extended studies on groundwater fauna in urban aquifers

Urban aquifers are complex and harbour a mostly unexplored ecosystem with a huge biodiversity of invertebrates and microorganisms. However, this thesis and various studies show that multiple stressors cause changes in groundwater quality and living conditions (Becher et al., 2022). Further large-scale studies in other cities with repeated measurement campaigns are needed to verify and extend the current knowledge. Moreover, sustainable management of the subsurface is essential in this special environment, which is characterised by different interests and conflicts of use. Thus, urban groundwater ecosystems should seriously be considered in city and energy planning.

(VII) Adaption and revision of national and international law

In many countries, legislation does not yet recognise groundwater as a habitat worthy of protection. In the mid-2000s, national policies to monitor groundwater ecosystems' health and stress emerged in Australia. Governments are using stygofauna to monitor, evaluate and report on groundwater health. A favourable example of groundwater ecosystem management and research in Europe is the European Groundwater Directive 2006, which attempted to incorporate ecological knowledge into schemes for environmental planning and policies. In September 2023, proposals by the European Parliament Environment Committee to amend the EU Commission's draft regarding the revision of the Water Framework Directive, Groundwater and Environmental Quality Standard Directives were voted for in plenary. The Environment Committee advocated for better research and more effective groundwater protection and its biocoenoses. Findings on the effects of heat input should

also be considered throughout the EU when making provisions for groundwater protection. Thus, a new article (6aa) is inserted in the directive to improve protection of groundwater ecosystems:

‘The Commission shall [...] publish an assessment of the impacts of physico-chemical elements, like pH, oxygenation, and temperature, on health of groundwater ecosystems, accompanied, where appropriate, by a legislative proposal to revise this Directive accordingly, to set the corresponding parameters, provide for harmonised monitoring methods, and define what would constitute a "good ecological status" for groundwater’ (European Parliament, 2023).

This is an important step, yet to implement stronger policies and monitoring requirements for protecting groundwater ecosystems, a worldwide effort to collect information on groundwater ecosystems, functional roles and human impacts is required.

(VIII) Impacts of climate change

Climate change significantly threatens biogeochemical processes and groundwater ecosystems (Griebler et al., 2016). These impacts are on-going, and surface temperature may not have reached subsurface areas yet, as can be seen in Study 2 (Chapter 3). According to Schmidt et al. (2023) it seems that the aquifers are still developing towards a new equilibrium. How climate change affects ecosystems and their services, such as drinking water supply, should be the subject of future research. Therefore, more data on groundwater quantity and quality is needed, which will increase the demand for ecological monitoring in the future.

APPENDICIES

Appendix Study 1

This Appendix refers to Study 1 (Chapter 2). The content was published in the journal Ecohydrology as a supporting information and is available online at: <https://doi.org/10.1002/eco.2607>.

S1 Groundwater fauna sampling methods

S1.1. Pumping of well water

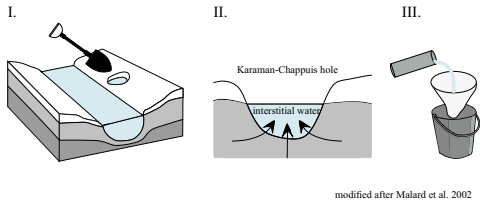
Pumping well water and filtering animals from it is one of the most common and well-established method for sampling groundwater fauna (Hahn, 2002; Thulin and Hahn, 2008). Furthermore, this method enables a simultaneous sampling of fauna, sediment and water (Hahn, 2002), and is easy to standardize by extracting a defined volume of water (Hahn, 2002). As reported in literature, the volume of pumped water varies between 20 to 1,000 L (Malard et al., 2002; Thulin and Hahn, 2008). An additional benefit of this method is that species can be extracted from several microhabitats (Allford et al., 2008; Hahn, 2002). However, existing studies also show the disadvantages of this method. Pumping is considered as a selective sampling method because of filtering effects and a resistance against the suction of pump. Hence, large, sessile or more active species can be underrepresented in probes (Allford et al., 2008). According to Hahn (2005) pumping might also alter the sediment structure in fine sediments and is quite expensive (up to 10,000 €) (Hahn, 2005). There are also issues with collecting representative stygofauna samples from wells that have not been purged (see Korbel et al., 2017).

Commonly used pumps for sampling groundwater fauna are centrifugal pumps, such as pressure pumps (e.g. Grundfos MP1) (Malard et al., 2002) as well as pumps with double packer samples and pneumatic pumps. Pumps with double packer sampler were first used by Danielopol and Nierreiter in 1987 and allow a selective sampling at defined depths (Danielopol and Niederreiter, 1987). Packers (i.e. expanding plugs) temporarily isolate an interval of a borehole, with a possible diameter between 2.5 and 7.5 cm (Pospisil, 1992), from which water is pumped (Price and Williams, 1993). The best method of obtaining undamaged invertebrates from deep groundwater wells (> 8 m below ground) is the use of a pneumatic pump, like the Radford pneumatic pump (Scarsbrook et al., 2000). This type of pump is built of a submersible pump placed around a pneumatic cylinder supplied with compressed air and water is pumped to the surface with a pumping rate of 0.25 m³ per hour (Scarsbrook et al., 2000).

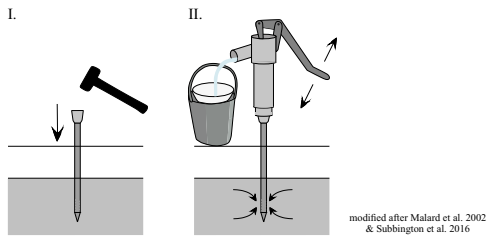
S1.2. Netsampling

A second commonly used method for sampling groundwater fauna is the net sampler or phreato-biological net. It is a valuable alternative to pumping devices with respect to obtained numbers of taxa and community composition (Hahn and Matzke, 2005; Malard et al., 2002; Thulin and Hahn, 2008) and can be used for large-scale faunal surveys (Allford et al., 2008) up to a well depth of 100 m (Hahn, 2002). Particularly suited for large wells dug in alluvial plains, organisms can be collected using a net sampler. Different methods for net sampling have been adopted around the world (e.g. Environmental Protection Authority (EPA), 2016; Hancock and Boulton, 2008), with a variety of mesh sizes (e.g. 50, 63, 74 or 150 μm) and the number of repeat hauls of nets at each site (e.g. Hancock and Boulton, 2008). The basic concept is the net is lowered and sediment agitated to capture stygofauna within the well. More information about the design and application of the net sampler can be found in the Supporting Information (Figure A1.1). Down- and upward movements (at least ten times) of the net in the well capture animals swimming in well water and whirled up from the bottom sediments (Boulton et al., 2008; Eberhard et al., 2009; Malard et al., 2002). However, net sampling does not provide information about the aquifer and is selective due to filtering effects of the well design (Allford et al., 2008), with some species of stygobiotic fish known to evade capture by nets. Nets can also become clogged with sediment reducing their efficiency, and this method can only sample organisms living within the well environment thus may not be truly representative of the wider aquifer community structure (Korbel et al., 2017).

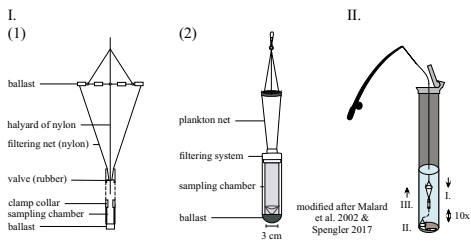
(a) Karaman-Chappuis method



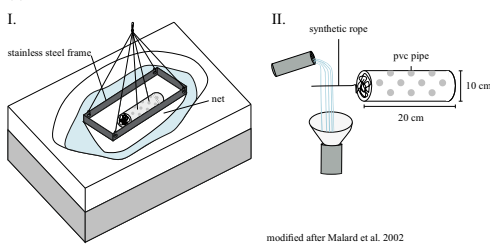
(b) Bou and Rouch pumping method



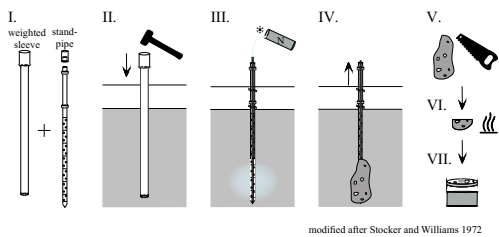
(c) Phrebotical net by Cvetkov (1) & modern (2)



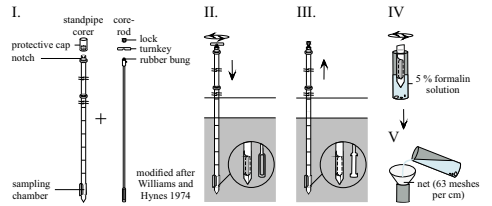
(d) Artificial substrates



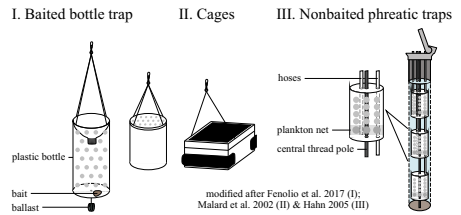
(e) Freezing core method



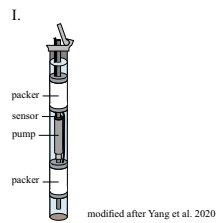
(f) Sediment core method



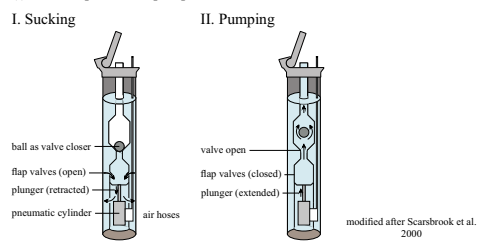
(g) (Non-)Baited traps



(h) Double packer pumping



(i) Radford pneumatic pump



(j) eDNA analysis

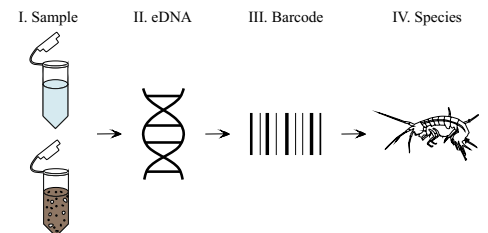


Figure A1.1: Detailed schemes of the different sampling techniques.

S1.3. Traps

Another method to sample fauna in wells, caves and the hyporheic zone is based on the principle that animals move actively or passively into traps (Hahn, 2002). In contrast to the methods presented above, traps exhibit a diverse design and function and can be either baited or non-baited. Non-baited traps and colonisation cages are mainly used for sampling the hyporheos (Malard et al., 2002) and therefore not in the focus of this study. Baited traps, where a bait is placed in a net or container that is left for at least 12 hours in a well, are commonly used for sampling stygofauna. The most popular design is the balance and inverted bottle trap (see also Figure A1.1). However, it must be mentioned that this method is species-selective, so that a combination with other methods (e.g. a net sampler) is highly recommended. Depending on the type of bait, the method can also influence physico-chemical parameters of well water (Malard et al., 2002). In 2005 Hahn presented the unbaited phreatic trap system, which allows sampling water from the well and the surrounding aquifer at defined depths. Here, three unbaited traps are fixed to a central pole in a well and the content of the traps is monthly pumped at the surface. As a result, this method provides newly colonized, artificial biotopes with a higher abundance in the traps than in the surrounding aquifer (Hahn, 2005).

S1.4. Further methods

Another rapid and qualitative method for groundwater fauna sampling is the Karaman-Chappuis method. In this method small holes are dug in riverbed sediments (Danielopol and Marmonier, 1992) and animals which flow into with the interstitial water are removed with the water and filtered afterwards (Malard et al., 2002) (Figure A1.1). As mentioned above, the sampling method of Bou and Rouch (1967) allows pumping animals living in sandy sediments and gravel of defined sections. This method uses steel pipes hammered into sediments to at least 2 m depth and extraction of groundwater from the pipe with a hand pump (also called Bou-Rouch pump). Afterwards, the water is filtered (Malard et al., 2002). However, this sampling is not strictly representative of in-situ conditions as faunal diversity and density are not expressed per volume of sediment. Also, amphipods as well as isopods can be damaged (Malard et al., 2002; Pospisil, 1992).

A rarely applied method is the freezing core method, during which a standpipe with a protective cap, two insulated copper rods and a weighted sleeve are drilled into a streambed. Then, fauna is paralysed by creating an electrical field for 10 minutes (Boxshall et al., 2016) and the cap is removed and liquid nitrogen is inserted for another 15 minutes. Afterwards, the core is removed with a vertical pull (Stocker and Williams, 1972) and prepared for analysis. This technique is the only method to obtain quantitative data (Hahn, 2002; Pospisil, 1992). However, it has the distinct disadvantage of destroying the habitat (Stocker and Williams, 1972) and is thus rarely applicable to groundwater (Hahn, 2002).

Similar to traps, the method of artificial substrates is often used in cave studies, and to study the distribution of groundwater fauna along subterranean rivers and streams. These substrates consist of a 20 cm long PVC pipe filled with a synthetic rope of 25 m length and with a diameter of 0.5 cm and placed in a net. After one month the pipe is pulled out of the lake or stream (Malard et al., 2002).

Developed by Williams and Hynes (1974) the sediment core method uses a standpipe corer to sample the hyporheos. For this purpose, a pipe with a diameter of 2.5 cm is drilled into the sediment up to 1 m depth. Afterwards a core-rod is placed inside the pipe and the pipe is rotated in order to fill the chamber of the core-rod. After rotating the core rod again to close the open wall of the pipe, the pipe is pulled out of the sediment. The disadvantages of this method are the selective sampling of species in case of a large average grain size (Williams and Hynes, 1974), the perturbation of the sediment caused by the mechanical stress, and a possible flight reaction of animals (Pospisil, 1992).

S1.5. New technologies

State-of-the-art methods for detection of groundwater fauna have been found in more recent studies. Video logging is an in-situ method to observe animals and aquifer conditions by inserting a video-camera in wells with transparent Perspex tubes (Pospisil, 1992). Using this method, biogenic structures, like worm galleries, < 0.05 mm are detectable in shallow depths up to 10 m (Datry et al., 2003).

S2 Additional Figures

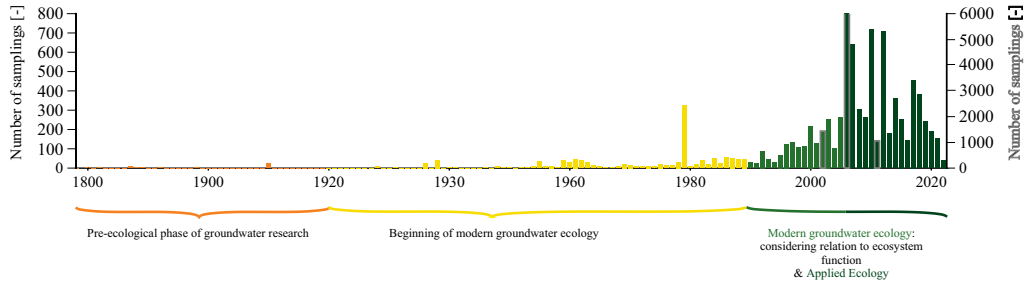


Figure A1.2: Number of samplings [-] over time.

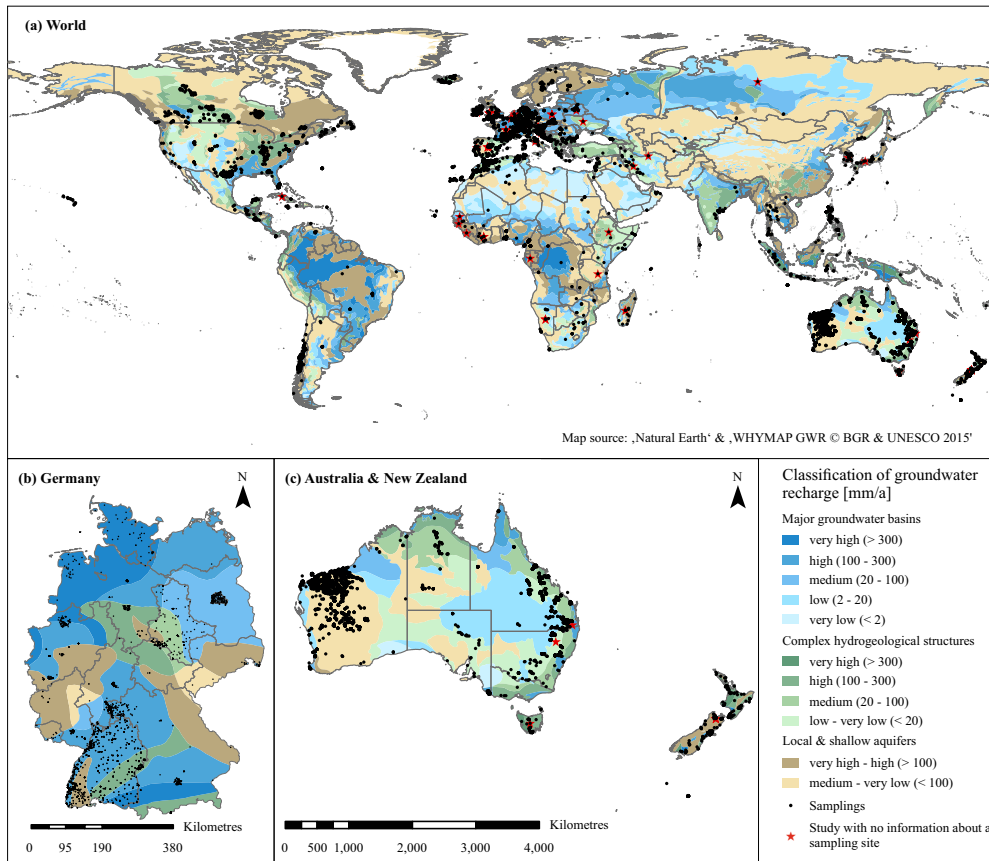


Figure A1.3: Overview over groundwater fauna samplings and the classification of groundwater recharge [mm/a] worldwide (a), of Germany (b) and of Australia (c), including data from about 800 studies worldwide (see Table A1.4 in the Supporting Information).

S3 Additional Table

Table A1.3: Number of studies [-], of sampling sites [-] and of samplings [-], country area [km²] and sampling density [sampling site/km²] or [samplings/km²] per country and continent.

Continent	Country	Number of studies [-]	Number of sampling sites [-]	Number of samplings [-]	Area [km ²]	Sampling density [sampling sites/km ²]	Sampling density [samplings/km ²]
Africa	Algeria	24	223	816	2,308,858	9.66E-05	3.53E-04
	Benin	6	55	119	116,113	4.74E-04	1.02E-03
	Cameroon	6	79	79	464,319	1.70E-04	1.70E-04
	Central African Republic	1	3	3	617,984	4.85E-06	4.85E-06
	Côte d'Ivoire	6	5	9	320,677	1.56E-05	2.81E-05
	Democratic Republic of the Congo	5	5	8	2,325,240	2.15E-06	3.44E-06
	Egypt	2	2	2	1,001,078	2.00E-06	2.00E-06
	Eritrea	1	1	1	122,538	8.16E-06	8.16E-06
	Ethiopia	2	2	2	1,127,375	1.77E-06	1.77E-06
	Gabon	1	1	1	259,968	3.85E-06	3.85E-06
	Guinea	1	2	2	244,302	8.19E-06	8.19E-06
	Guinea-Bissau	1	1	1	32,829	3.05E-05	3.05E-05
	Kenya	2	2	2	585,702	3.41E-06	3.41E-06
	Libya	1	1	1	1,623,759	6.16E-07	6.16E-07
Madagascar	4	9	11	592,982	1.52E-05	1.86E-05	
Malawi	3	3	3	119,398	2.51E-05	2.51E-05	

Continent	Country	Number of studies [-]	Number of sampling sites [-]	Number of samplings [-]	Area [km ²]	Sampling density [sampling sites/km ²]	Sampling density [samplings/km ²]
	Mali	2	2	2	1,252,723	1.60E-06	1.60E-06
	Morocco	44	266	347	591,744	4.50E-04	5.86E-04
	Namibia	6	11	11	822,714	1.34E-05	1.34E-05
	Nigeria	1	1	1	907,499	1.10E-06	1.10E-06
	Republic of Cabo Verde	2	6	6	3,883	1.55E-03	1.55E-03
	Senegal	2	2	2	196,225	1.02E-05	1.02E-05
	Sierra Leone	1	1	1	71,612	1.40E-05	1.40E-05
	Somalia	8	12	14	639,222	1.88E-05	2.19E-05
	South Africa	9	20	20	1,219,505	1.64E-05	1.64E-05
	Sudan	3	8	8	1,857,639	4.31E-06	4.31E-06
	Tanzania	2	1	1	939,006	1.06E-06	1.06E-06
	Tunisia	5	15	17	156,613	9.58E-05	1.09E-04
	Uganda	1	1	1	241,854	4.13E-06	4.13E-06
	Zambia	1	1	3	751,914	1.33E-06	3.99E-06
	Zanzibar	1	1	1	2,499	4.00E-04	4.00E-04
	Zimbabwe	3	7	10	389,338	1.80E-05	2.57E-05
	Sum:	155	749	1505	Average:	1.08E-04	1.39E-04
Asia	Georgia	1	5	48	66,615	7.51E-05	7.21E-04
	India	7	32	43	3,144,310	1.02E-05	1.37E-05
	Indonesia	9	48	51	1,879,826	2.55E-05	2.71E-05
	Iran	10	43	43	1,622,509	2.65E-05	2.65E-05
	Iraq	2	3	3	399,119	7.52E-06	7.52E-06
	Israel	5	10	13	21,901	4.57E-04	5.94E-04

Continent	Country	Number of studies [-]	Number of sampling sites [-]	Number of samplings [-]	Area [km ²]	Sampling density [sampling sites/km ²]	Sampling density [samplings/km ²]
	Japan	7	45	46	6,876	6.54E-03	6.69E-03
	Kazakhstan	1	1	1	2,714,265	3.68E-07	3.68E-07
	Lebanon	2	4	4	10,000	4.00E-04	4.00E-04
	Malaysia (Borneo)	2	2	2	327,885	6.10E-06	6.10E-06
	Oman	3	12	14	311,213	3.86E-05	4.50E-05
	Philippines	10	129	139	293,237	4.40E-04	4.74E-04
	Republic of Korea	9	227	237	92,620	2.45E-03	2.56E-03
	Syrian Arab Republic	2	2	2	185,674	1.08E-05	1.08E-05
	Thailand	6	179	637	514,454	3.48E-04	1.24E-03
	Turkmenistan	1	1	1	470,850	2.12E-06	2.12E-06
	Vietnam	1	2	2	328,892	6.08E-06	6.08E-06
	Sum:	63	745	1286	Average:	6.78E-04	8.01E-04

Continent	Country	Number of studies [-]	Number of sampling sites [-]	Number of samplings [-]	Area [km ²]	Sampling density [sampling sites/km ²]	Sampling density [samplings/km ²]
Europe	Austria	33	1418	1495	83,993	1.69E-02	1.78E-02
	Belarus	1	25	25	207,499	1.20E-04	1.20E-04
	Belgium	11	412	447	30,670	1.34E-02	1.46E-02
	Bulgaria	2	49	52	112,760	4.35E-04	4.61E-04
	Croatia	7	75	74	55,078	1.36E-03	1.34E-03
	Cyprus	1	2	2	5,395	3.71E-04	3.71E-04
	Czech Republic	5	223	249	78,759	2.83E-03	3.16E-03
	United Kingdom	16	595	684	243,783	2.44E-03	2.81E-03
	Federation of Bosnia and Herzegovina	2	30	39	28,814	1.04E-03	1.35E-03
	Finland	3	21	26	333,059	6.31E-05	7.81E-05
	France	47	2654	2813	539,175	4.92E-03	5.22E-03
	Corsica	1	3	3	8,666	3.46E-04	3.46E-04
	Germany	76	2378	4232	357,674	6.64E-03	1.18E-02
	Baden-Württemberg	28	950	2026	36,123	2.63E-02	5.61E-02
	Bavaria	17	429	697	70,024	6.13E-03	9.95E-03
	Berlin	1	181	181	889	2.04E-01	2.04E-01
	Brandenburg	0	0	0	30,038	0	0
	Bremen	0	0	0	386	0	0
	Hamburg	0	0	0	774	0	0
	Hesse	9	141	156	21,012	6.71E-03	7.42E-03
	Lower Saxony	13	141	179	47,595	2.96E-03	3.76E-03
	Mecklenburg-Western Pomerania	1	1	1	23,208	4.31E-05	4.31E-05

Continent	Country	Number of studies [-]	Number of sampling sites [-]	Number of samplings [-]	Area [km ²]	Sampling density [sampling sites/km ²]	Sampling density [samplings/km ²]
	North Rhine-Westphalia	16	199	383	34,537	5.76E-03	1.11E-02
	Rhineland-Palatinate	8	71	194	20,096	3.53E-03	9.65E-03
	Saarland	1	12	12	2,654	4.52E-03	4.52E-03
	Saxony	3	24	24	18,167	1.32E-03	1.32E-03
	Saxony-Anhalt	8	131	280	20,665	6.34E-03	1.35E-02
	Schleswig-Holstein	7	51	51	15,436	3.30E-03	3.30E-03
	Thuringia	5	47	48	16,068	2.93E-03	2.99E-03
	Greece	5	18	20	131,353	1.37E-04	1.52E-04
	Iceland	4	69	92	102,390	6.74E-04	8.99E-04
	Ireland	10	140	154	69,445	2.02E-03	2.22E-03
	Italy	25	2143	2279	251,203	8.53E-03	9.07E-03
	Sardinia	4	8	8	24,114	3.32E-04	3.32E-04
	Sicily	1	1	1	25,763	3.88E-05	3.88E-05
	Kosovo	1	1	1	10,913	9.16E-05	9.16E-05
	Luxembourg	1	72	74	2,608	2.76E-02	2.84E-02
	Macedonia	4	8	8	25,385	3.15E-04	3.15E-04
	Montenegro	4	20	20	13,727	1.46E-03	1.46E-03
	Netherlands	5	10	10	37,102	2.70E-04	2.70E-04
	Norway	1	1	1	319,477	3.13E-06	3.13E-06
	Poland	10	54	81	313,428	1.72E-04	2.58E-04
	Portugal	5	64	64	87,971	7.28E-04	7.28E-04
	Madeira	1	7	7	811	8.63E-03	8.63E-03
	Romania	7	45	71	236,377	1.90E-04	3.00E-04
	Russia	6	18	74	16,980,192	1.06E-06	4.36E-06

Continent	Country	Number of studies [-]	Number of sampling sites [-]	Number of samplings [-]	Area [km ²]	Sampling density [sampling sites/km ²]	Sampling density [samplings/km ²]
	Serbia	1	1	1	56,483	1.77E-05	1.77E-05
	Slovakia	1	44	133	48,458	9.08E-04	2.74E-03
	Slovenia	17	766	933	20,327	3.77E-02	4.59E-02
	Spain	19	871	972	494,272	1.76E-03	1.97E-03
	Balearic Islands	1	2	2	5,117	3.91E-04	3.91E-04
	Sweden	3	26	187	446,174	5.83E-05	4.19E-04
	Switzerland	13	231	480	41,436	5.57E-03	1.16E-02
	Turkey	3	17	19	780,080	2.18E-05	2.44E-05
	Ukraine	1	2	2	571,999	3.50E-06	3.50E-06
	Sum:	358	12522	15833	Average:	3.81E-03	4.50E-03
Central America	Bermuda	1	21	22	62	3.37E-01	3.53E-01
	Cuba	2	4	5	109,929	3.64E-05	4.55E-05
	Curacao	1	2	2	463	4.32E-03	4.32E-03
	El Salvador	1	27	27	20,539	1.31E-03	1.31E-03
	Guatemala	2	3	3	108,811	2.76E-05	2.76E-05
	Haiti	1	2	1	26,892	7.44E-05	3.72E-05
	Honduras	1	3	4	112,237	2.67E-05	3.56E-05
	Jamaica	1	8	8	11,033	7.25E-04	7.25E-04
	Mexico	3	46	48	1,957,845	2.35E-05	2.45E-05
	Panama	1	10	10	74,530	1.34E-04	1.34E-04
	Saint-Martin	1	2	3	68	2.93E-02	4.39E-02
	Sum:	10	128	133	Average:	3.39E-02	3.67E-02

Continent	Country	Number of studies [-]	Number of sampling sites [-]	Number of samplings [-]	Area [km ²]	Sampling density [sampling sites/km ²]	Sampling density [samplings/km ²]
North-America	Canada	2	299	299	9,945,528	3.01E-05	3.01E-05
	United States of America	34	2815	3456	7,942,143	3.54E-04	4.35E-04
	Alabama	1	1529	1529	133,822	1.14E-02	1.14E-02
	Arizona	2	2	10	295,070	6.78E-06	3.39E-05
	Arkansas	1	5	5	137,540	3.64E-05	3.64E-05
	California	1	14	14	409,737	3.42E-05	3.42E-05
	Colorado	1	8	27	269,202	2.97E-05	1.00E-04
	Connecticut	1	1	1	12,708	7.87E-05	7.87E-05
	District of Columbia	2	87	87	162	5.36E-01	5.36E-01
	Florida	4	384	384	146,732	2.62E-03	2.62E-03
	Georgia	2	19	19	152,164	1.25E-04	1.25E-04
	Hawaii	1	15	15	16,848	8.90E-04	8.90E-04
	Idaho	1	1	1	216,254	4.62E-06	4.62E-06
	Illinois	2	10	10	150,153	6.66E-05	6.66E-05
	Indiana	1	251	256	94,332	2.66E-03	2.71E-03
	Kansas	1	1	84	212,826	4.70E-06	3.95E-04
	Kentucky	2	181	181	104,578	1.73E-03	1.73E-03
	Maryland	1	4	4	25,467	1.57E-04	1.57E-04
	Massachusetts	1	2	2	21,162	9.45E-05	9.45E-05
	Michigan	2	2	3	250,100	8.00E-06	1.20E-05
	Montana	1	1	1	379,492	2.64E-06	2.64E-06
	New Hampshire	2	2	5	24,266	8.24E-05	2.06E-04
	New York	5	23	23	136,933	1.68E-04	1.68E-04

Continent	Country	Number of studies [-]	Number of sampling sites [-]	Number of samplings [-]	Area [km ²]	Sampling density [sampling sites/km ²]	Sampling density [samplings/km ²]
	Ohio	1	2	2	116,044	1.72E-05	1.72E-05
	Oklahoma	1	47	47	180,637	2.60E-04	2.60E-04
	Pennsylvania	1	1	1	119,357	8.38E-06	8.38E-06
	South Dakota	1	2	2	199,304	1.00E-05	1.00E-05
	Tennessee	2	18	18	109,298	1.65E-04	1.65E-04
	Texas	7	148	248	685,339	2.16E-04	3.62E-04
	Utah	1	27	30	219,762	1.23E-04	1.37E-04
	Virginia	2	3	3	102,236	2.93E-05	2.93E-05
	West Virginia	3	24	443	62,735	3.83E-04	7.06E-03
	Wisconsin	1	1	1	169,361	5.90E-06	5.90E-06
	Sum:	36	3114	3755	Average:	1.92E-04	2.33E-04
South America	Argentina	3	10	22	2,784,305	3.59E-06	7.90E-06
	Brazil	4	25	25	8,472,664	2.95E-06	2.95E-06
	Chile	2	130	180	736,274	1.77E-04	2.44E-04
	Venezuela	1	8	8	912,684	8.77E-06	8.77E-06
	Sum:	10	173	235	Average:	4.80E-05	6.60E-05
Oceania	Papua New Guinea	1	2	2	455,743	4.39E-06	4.39E-06
	Fiji	1	1	1	18,930	5.28E-05	5.28E-05
	Seychelles	1	3	3	436	6.89E-03	6.89E-03
	Maldives	1	4	4	109	3.68E-02	3.68E-02
	Sum:	4	10	10	Average:	1.09E-02	1.09E-02

Continent	Country	Number of studies [-]	Number of sampling sites [-]	Number of samplings [-]	Area [km ²]	Sampling density [sampling sites/km ²]	Sampling density [samplings/km ²]
Australia		133	4014	5826	7,622,937	5.27E-04	7.82E-04
	Australian Capital Territory	0	0	0	2,349	0	0
	Jervis Bay Territory	0	0	0	76	0	0
	Lord Howe Island	0	0	0	14	0	0
	Macquarie Island	0	0	0	119	0	0
	New South Wales	23	255	794	801,935	3.18E-04	9.90E-04
	Northern Territory	6	45	74	1,348,468	3.34E-05	5.49E-05
	Queensland	15	732	1077	1,730,136	4.23E-04	6.22E-04
	South Australia	10	70	90	983,875	7.11E-05	9.15E-05
	Tasmania	4	46	46	68,115	6.75E-04	6.75E-04
	Victoria	1	1	3	227,945	4.39E-06	1.32E-05
	Western Australia	78	2865	372	2,528,139	1.13E-03	1.53E-03
	Esperance			2	55,661		3.59E-05
	Gascoyne			92	135,065		6.81E-04
	Goldfields			779	714,525		1.09E-03
	Kimberley			21	419,245		5.01E-05
	Mid-West			720	466,795		1.54E-03
	Perth			30	6,417		4.68E-03
	Pilbara			2020	506,778		3.48E-03
	South West			17	24,802		6.85E-04
	Wheat Belt			38	159,457		2.38E-04
	Christmas Island			23	136		1.69E-01
	New Zealand	23	305	436	266,886	1.14E-03	1.63E-03
	Sum:	146	4327	6404	Average:	8.35E-04	1.21E-03

S4 Literature search

An extensive review of accessible groundwater fauna data was conducted by analysing information from national and international publications in peer-reviewed journals, national reports, doctoral theses, historical writings, books and existing online databases. Literature was searched by using web platforms such as Scopus and Google Scholar, as well as specific ecological databases, like ZOBODAT. Moreover, detailed searches were conducted on the homepages of universities or research institutes of known researchers in the field, social networks (ResearchGate), libraries from research institutes and museums (including their virtual services), conference proceedings and by direct request addressed to authorities and experts in the field (including companies).

The research terms, used for the literature research, include the following keywords:

- groundwater fauna/biology/biodiversity/ecology/habitat,
- stygo/aquifer/subterranean fauna,
- stygobiont/stygobiontic species names,
- authors names and
- fauna sampling methods (pumps, corer, nets, etc.).

To increase the breadth of searches, a combination of keywords, together with region/country names, proved useful. Keywords were also searched using English, German and less often French, Spanish and Italian.

From the set of results only studies including stygobiontic groundwater fauna and from groundwater-specific sample sites such as wells, boreholes, springs, interstitial/hyporheic zones of rivers and caves with sole groundwater sources (trogllobiont/stygobiontic colonisation) were used for this review.

Notwithstanding the limitations to the completeness of the database (access to studies prior to 1990, as well as theses and national reports may be limited), the study compiled 859 studies. The meta-analysis included studies dating from 1880 to 2022, with more recent literature used within the continent and method related sections of the paper but not in the data analysis.

Table A1.4: Overview over the used literature for the world map divided by continents.

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Appendix Study 2

This Appendix refers to Study 2 (Chapter 3). The content was published in the journal Hydrology and Earth System Sciences as a supplement and is available online.

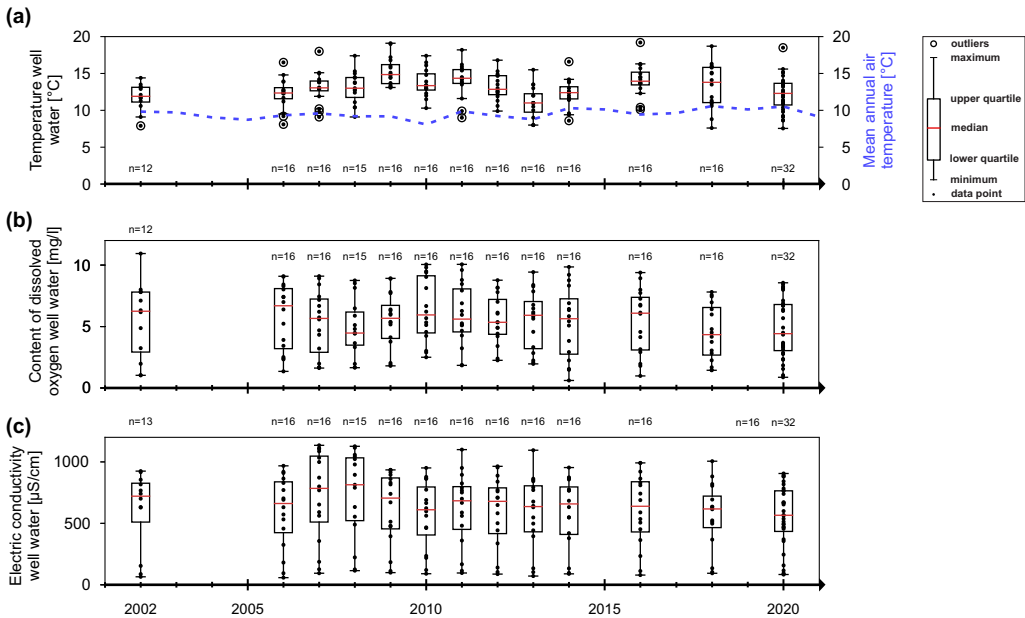


Figure A2.1: Boxplots of important abiotic parameters between 2002 and 2020: (a) temperature of the well water; (b) content of dissolved oxygen of the well water and (c) electric conductivity of the well water. For comparability of results, only data from June to September were used for 2002 and 2020, and the same monitoring sites as in subsequent years. "n" indicates the number of measuring points. No sampling was conducted in years with no boxplot.

Table A2.1: List of all parameters of the LUBW annual catalogue used in this study (Landesanstalt für Umwelt Messungen und Naturschutz Baden-Württemberg, 2022)

Parameter	Unit	Parameter	Unit	Parameter	Unit	Parameter	Unit
Physical-chemical complete analysis							
acid capacity up to pH 4.3	[mmol/l]	Heavy metals		Pesticides		Hydrocarbons	
calcium	[mg/l]	arsenic	[mg/l]	atrazine	[µg/l]	benzene	[µg/l]
chloride	[mg/l]	barium	[mg/l]	bromacil	[µg/l]		
dissolved oxygen concentration	[-]	beryllium	[mg/l]				
dissolved oxygen saturation	[mg/l]	boron	[mg/l]				
DOC (dissolved organic carbon)	[mg/l]	cadmium	[mg/l]				
electric conductivity	[%]	cobalt	[mg/l]				
fluoride	[mg/l]	cooper	[mg/l]				
iron	[mg/l]	lead	[mg/l]				
magnesium	[mg/l]	lithium	[mg/l]				
manganese	[mg/l]	molybdenum	[mg/l]				
nitrate	[mg/l]	nickel	[mg/l]				
ortho-phosphate	[mg/l]	mercury	[mg/l]				
pH-value	[-]	selenium	[mg/l]				
phosphorous	[mg/l]	silicate	[mg/l]				
potassium	[mg/l]	strontium	[mg/l]				
sodium	[mg/l]	thallium	[mg/l]				
spectral absorption coefficient at 436nm	[l/m]	uranium	[mg/l]				
sulphate	[mg/l]	zinc	[mg/l]				
sum alkali metals	[mmol/l]						
temperature	[°C]						

Table A2.2: Parameters of the PHATE-analysis.

Parameters	Unit
Physical	
temperature well water	[°C]
detritus content (classes with estimated values)	[-]
amount of sediment	[ml]
Biotical	
number of taxa	[-]
total abundance	[-]
proportion of Crustaceans (acc. to Griebler et al., 2014)	[%]
proportion of Oligochaetes (acc. to Griebler et al., 2014)	[%]
proportion of stygobiont to non-stygobiont individuals	[-]
abundance Amphipods	[-]
abundance Cyclopoids	[-]
abundance Harpacticoids	[-]
abundance Nematodes	[-]
(Hydro-)geological	
geological unit	[-]
well depth	[m]
Assessment scheme	
Groundwater-Fauna-Index (GFI)	[-]

Table A2.3: Standard deviation of different faunistic and hydro-chemical parameters over time of each well. Wells with an asterisk * show stable hydro-chemical and faunistic conditions and a variance of less than 13.

Location of the well	Standard deviation of the:									
	Temperature well water [°C]	Content of dissolved oxygen well water [mg/l]	Electric conductivity well water [µS/cm]	Total abundance [-]	Number of species [-]	Proportion of Crustaceans [%]	Proportion of Oligochaetes [%]	Proportion of stygobionts to no-stygobionts [-]		
Dahenfeld*	1.08	0.51	90.41	3.33	1.06	35.73	14.92	1.98		
Zienken	1.41	2.61	53.47	77.32	1.49	0.62	0.62	1.12		
Efringen-Kirchen	1.11	0.74	124.08	192.46	1.67	5.39	5.39	0.37		
Kadelburg*	2	0.55	57.88	11.4	2.17	16.25	16.25	0.29		
Schwäbisch Hall	1.56	1.45	97.23	115.85	2.12	22.26	22.26	4.01		
Rohrdorf*	1.41	0.93	54.38	31.53	2.19	23.38	23.38	1.16		
Furtwangen*	2.07	0.5	37.81	30.66	1.79	4.86	4.86	0.89		
Riedlingen*	1.14	0.86	114.06	8.96	1.33	1.48	1.48	1.16		
Weingarten	1.27	1.13	98.01	9.34	1.08	44.71	29	1.55		
Hausen*	1.53	0.7	81.65	19.61	1.4	2.05	2.05	0.92		
Balgheim*	0.87	1.16	107.63	21.25	1.02	2.77	2.77	0.75		
Sankt Leon	1.25	0.78	47.08	46.23	2.25	35.38	35.38	3.55		
Neckargartach	1.3	1.02	86.41	2.6	0.96	45.42	43.06	0.59		
Todtnau*	1.41	0.54	12.2	3.42	1.6	34.77	4.29	2.64		
Gaggenau	1.51	1.6	41.63	37.73	1.6	13.01	13.01	3.51		
Brenden*	1.93	0.93	16.6	10.29	1.76	2.11	2.11	3.12		

Neckargartach

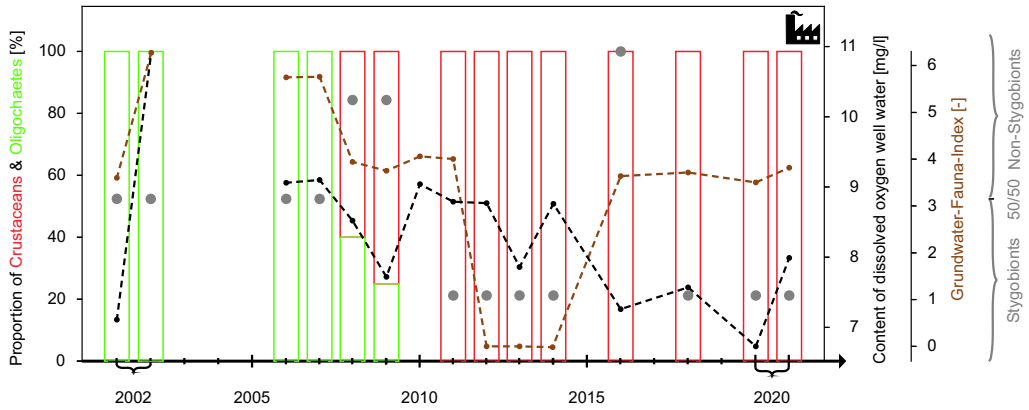


Figure A2.2: Temporal change of abiotic and faunal parameters over time in Neckargartach. The Groundwater-Faun-Index (GFI) changes from 6.3 in 2002 to 5.8 in 2007 and finally to 3.4 in 2020, which can be due to a decrease in the surface influence. This shows a clear connection between changes in GFI and land development. No sampling was conducted in years with no bar.

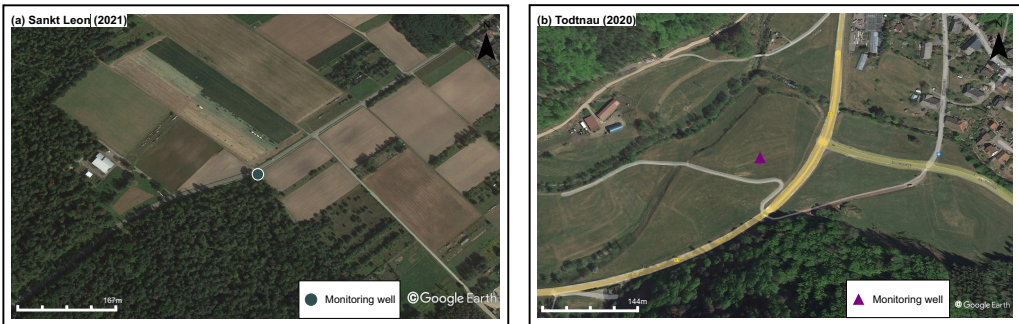


Figure A2.3: Aerial image of the location of the monitoring well in Sankt Leon (a) and Todtnau (b) (Source: Google Earth Pro (Google LLC., 2022)). The surrounding of the wells has not changed over the investigation period (2002 - 2020).

Appendix Study 3

This Appendix refers to Study 3 (Chapter 4). The content was published in the journal Hydrology and Earth System Sciences as a supplement and is available online at: <https://doi.org/10.5194/hess-25-3053-2021-supplement>.

Groundwater Fauna Index (GFI)

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 important groundwater parameters such as relative amount of detritus, variation of groundwater temperature and concentration of dissolved oxygen (Hahn, 2006) and is calculated by using this equation:

$$GWFaunaIndex = \sqrt{\text{Dissolved Oxygen} \left(\frac{mg}{l} \right) \times \text{Relative Amount of Detritus}} \times \text{Standard deviation of Temperature} \quad (A3.1)$$

The determined average GFI of all sampled wells is 6.0 ± 2.8 with a total variation between 0 and 14 and a heterogeneous distribution of the GFI-values. High GFI values (> 10 , Type III), indicating hydrological exchange with the surface (Hahn, 2006), were only found in three wells which share a high standard deviation of GWT (2.6 to 3.5 °C), higher dissolved oxygen (5.5 to 5.8 mg/l) as well as nitrate concentrations (7.7 up to 12 mg/l). These specific well locations have mainly no or minor sealed surfaces. Overall, 82 % of the measurement wells showed meso-alimonic conditions (GFI $> 2 - 10$, Type II) and therefore indicate a medium level of surface influence, at diverse urban and forested locations. Only four wells in this study were well insulated from surface influences (GFI < 2), with three wells located in densely built-up surroundings with sealed surfaces. Moreover, the average GFI in the forested area is 4.5 ± 1.9 and in the urban area 6.2 ± 2.7 .

Shannon diversity index

The Shannon-Index, introduced by Shannon and Weaver (1949) is an established standard method to quantify the ecological diversity of e.g. bacterial or faunal communities. The index describes the diversity by including the number of species and the relative frequency of individuals. The sampled wells in the forested area show the highest balance (median Shannon Equitability/Evenness Index (EH) = 0.47) and Shannon diversity index (median Shannon Diversity Index (HS) = 0.74).

The maximum diversity (median Shannon Index $(H)_{max} = 1.58$) is the same in both the forested and the urban area. The balance (median $EH = 0.42$) and Shannon diversity index (median $HS = 0.52$) are only a little bit lower in the urban area. These results are comparable with the study of Brielmann et al. (2009), where the Shannon diversity index of an anthropogenically influenced groundwater of an aquifer downstream of an industrial facility varies between 0.20 and 1.45. Nevertheless, no clear distribution pattern according to faunal diversity is recognizable. Thus, the Shannon diversity index was not considered further.

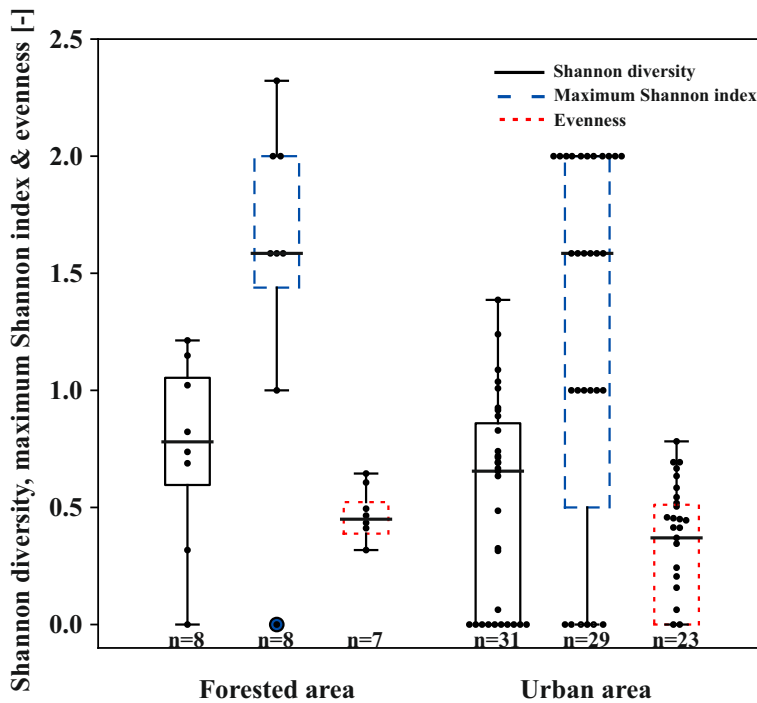


Figure A3.1: Boxplots of the Shannon diversity index, maximum Shannon index and evenness, divided in forested and urban area (n = number of wells, or number of wells at which the evaluation is applicable).

Urban impacts on groundwater quality

Urban impacts on groundwater systems can be manifold, such as increasing temperatures (urban heat islands (Menberg et al., 2013b)), contaminants (Kuroda and Fukushi, 2008), changes in the precipitation discharge due to sealing, falling water levels due to groundwater withdrawal (Foster, 1990). In our study, we intend to provide a first impression of the situation in Karlsruhe and therefore focus on the standard parameters. A first overview is given by the LUBW continuous monitoring program of groundwater wells (Landesanstalt für Umwelt Messungen und Naturschutz Baden-Württemberg, 2020), which provides profound groundwater analysis in Karlsruhe. Some of the considered measurement wells are close to the measurement wells of this study. Assessing the evaluation period of this study (2011 - 2014), most of the wells of the monitoring program show values within the range of the local background or below the thresholds of the drinking water ordinance of Germany and therefore no contamination.

One exception is a measurement well in the Kapellen-Street (next to T105), which shows higher ammonium (average: 0.55 mg/l, threshold drinking water ordinance: 0.5 mg/l), iron (5.1 mg/l, threshold value of the German drinking water ordinance: 0.2 mg/l) and manganese concentrations (0.55 mg/l, threshold drinking water ordinance: 0.05 mg/l). Moreover, this well has a noticeable concentration of arsenic (8.7 $\mu\text{g/l}$, threshold drinking water ordinance: 10 $\mu\text{g/l}$) and of the herbicide CGA 369873 (0.1 $\mu\text{g/l}$, threshold: 0.1 $\mu\text{g/l}$). This well is at the margin of one of the largest contaminated sites in Karlsruhe, the former gas plant.

Three other wells, which contain contaminants are in the Kaiserallee, Mathy-Street (next to T124) and near the municipal hospital. They showed noticeable concentrations of volatile hydrocarbons of up to 13 $\mu\text{g/l}$ during the evaluation period (in detail at the hospital: 3 - 6 $\mu\text{g/l}$; Kaiserallee: 5 - 8 $\mu\text{g/l}$; Mathy Street: about 3 $\mu\text{g/l}$). In comparison, the German threshold value of the drinking water ordinance is 20 $\mu\text{g/l}$.

The groundwater of one measurement well in the Hardtwald (next to SWM-005/SOM- 020) has a different chemical composition than the wells in the urban area. It shows lower concentrations of boron (30 - 45 $\mu\text{g/l}$, compared to the other wells: 50 - 98 $\mu\text{g/l}$), calcium (100 - 110 mg/l, compared to the other wells of up to 150 mg/l), chloride (25.5 mg/l in 2014 compared to the other wells: > 50 mg/l), potassium (3.2 mg/l) and sodium (11.3 mg/l). Furthermore, the content of dissolved oxygen is higher than in the wells of the urban area (average with 4.8 mg/l).

This overview indicates that beside one larger and two smaller contaminations, the groundwater beneath Karlsruhe contains only minor pollution. Groundwater fauna can usually cope well with short-term changes of chemical-physical parameters (Griebler et al., 2016). Previous studies showed that some species can even benefit from pollutants (Matzke, 2006; Zuurbier et al., 2013). Thus, the main documented impacts on groundwater quality in the study area are related to GWT, oxygen and nitrate concentration.

Table A3.1: Estimation of the relative amounts of sediment per sample (modified after Hahn (2006)).

Scale	Description	Characterisation
0	Absent	No sediments in the sampling vessel
1	Little	Bottom of the sampling vessel ($\varnothing \frac{1}{4}$ 7.6 cm) slightly covered by sediment
2	Much	Bottom of the sampling vessel covered by several millimetres of sediment
3	Very much	Bottom of the sampling vessel covered by one or more centimetres of sediment

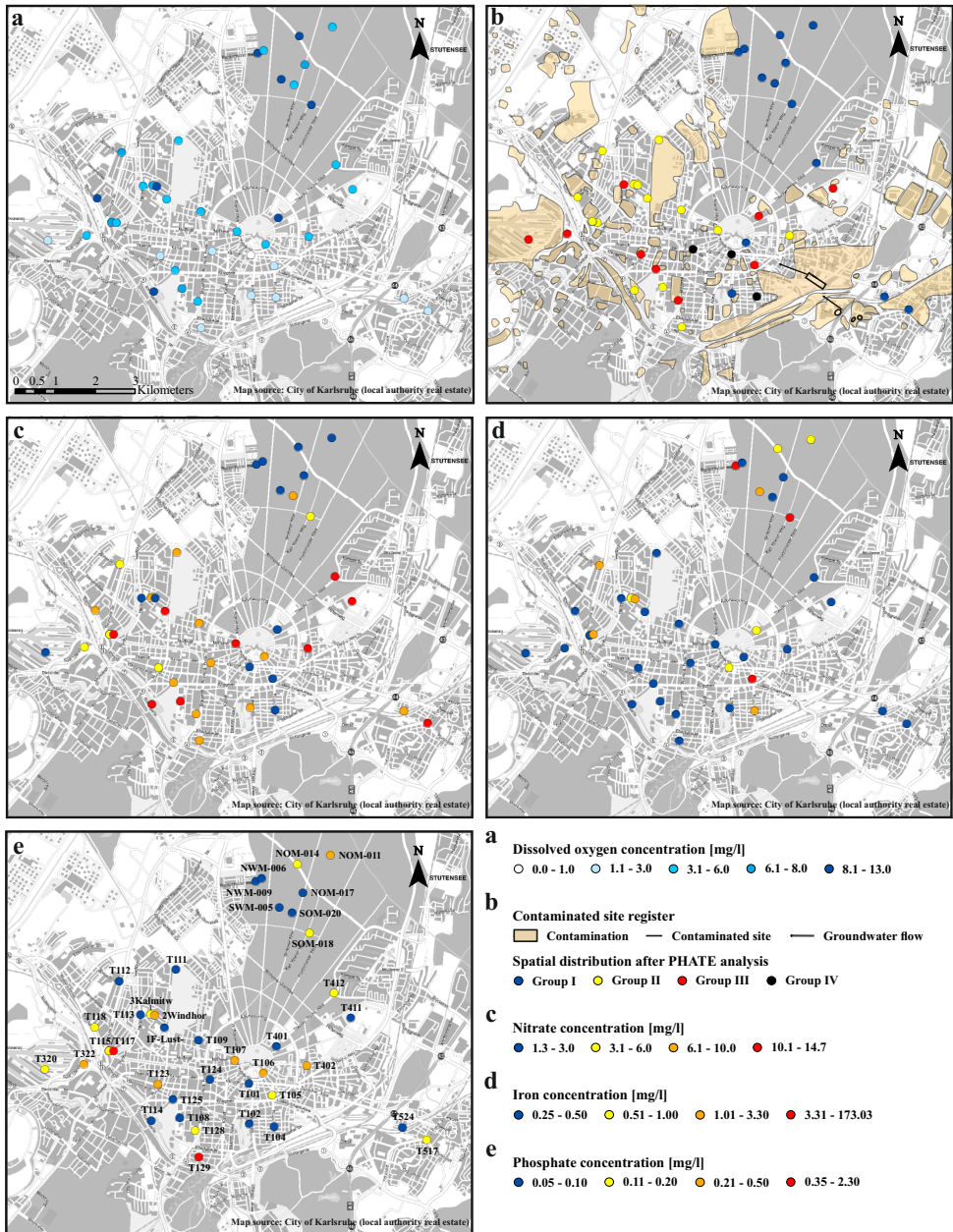


Figure A3.2: Overview map of Karlsruhe: (a) the average content of dissolved oxygen of the multiple measurements [mg/l]; (b) contaminated sites of the soil protection and contaminated site register (Bodenschutz- und Altlastenkataster) of Karlsruhe (modified after Kühlers et al. (2012), Stadt Karlsruhe (2006), and Wickert et al. (2006)) and the spatial distribution after the PHATE analysis; (c) average nitrate concentration [mg/l] of the repeated measurements; (d) iron concentration [mg/l] of the repeated 5 measurements and (e) the phosphate concentration [mg/l] of the repeated measurements at the bottom of the measurement wells.

Table A3.2: Well locations, information, sampled properties and result of the evaluation (GWT=groundwater temperature, *sampling 2011-2012; † sampling 2014; ‡ sampling 2014; 3 times).

Measuring point	Location	Area classification	Depth [m]	Average GWT [°C]	SD GWT [-]	Relative amount of detritus [-]	Average dissolved oxygen [mg/l]	Average GFI [-]	Amount crustaceans (acc. to Griebler et al. (2014)) [%]	Amount oligochaetes (acc. to Griebler et al. (2014)) [%]	Total amount of individuals [-]	Numbers of taxa [-]	Ecological condition (acc. to Griebler et al. (2014))
T101	Lammstr. No.7	* Urban area	39	14.4	0.05	3	0.97	0	0	0	0	0	Faunistic evaluation not possible
T102	Tulla Bad	* Urban area	10	14	2.99	2	1.45	5	100	0	4	2	Natural
T104	Arbeitsamt - Rankenstr.	* Urban area	15.8	12.5	1.28	3	1.07	2	0	0	0	0	Faunistic evaluation not possible
T105	Fritz-Erler-Str. No.21	* Urban area	9.3	14.4	3.29	3	1.29	6	0	100	1	1	Faunistic evaluation not possible
T106	Schloßplatz / Schloßbezirk	* Urban area	11	14.2	2.6	3	4.02	9	86	14	7	3	Natural
T123	Sophienstr. - Grillparzerstr.	* Urban area	14	12.8	1.65	1	2.18	2	0	100	3	2	Affected
T124	Kaiserplatz	* Urban area	13	14.9	1.95	3	1.97	5	0	0	2	1	Affected
T125	Kriegsstr. No.141	* Urban area	11.8	15	2.32	1	3.64	4	0	100	103	3	Affected
T128	Söldenstr. - Brauerstr.	* Urban area	9.5	13.2	3.21	2	5.5	11	0	100	13	2	Affected

Measuring point	Location	Area classification	Depth [m]	Average GWT [°C]	SD GWT [-]	Relative amount of detritus [-]	Average dissolved oxygen [mg/l]	Average GFI [-]	Amount crustaceans (acc. to Griebler et al. (2014)) [%]	Amount oligochaetes (acc. to Griebler et al. (2014)) [%]	Total amount of individuals [-]	Numbers of taxa [-]	Ecological condition (acc. to Griebler et al. (2014))
T129	Schule Beiertheim	Urban area *	8.5	12.2	3.67	1	2.31	6	91	9	124	4	Natural
T320	Städteckenstr. No.16	Urban area *	9	12.6	3.43	1	2.62	6	0	100	252	1	Affected
T322	Rheinhafenbad	Urban area *	10	15.9	2.99	2	3.45	8	0	100	6	2	Affected
T402	Am Fasanengarten - Parksstraße	Urban area *	9	12.4	3.43	2	3.68	9	50	50	4	4	Affected
T411	Gewann Blüsse	Urban area *	10.9	11.3	2.64	3	5.74	11	0	100	34	2	Affected
T412	Theodor-Heuss-Allee	Urban area *	10	11.4	2.99	1	4.47	6	100	0	6	4	Natural
T517	Auer Str. - Reichenbachstr.	Urban area *	9	12.8	3.43	2	2.84	8	100	0	5	1	Natural
T524	Dornwaldstr.	Urban area *	9	11.5	3.43	2	1.77	6	99	1	275	2	Natural
T401	Area next to the Wildpark-Stadion	Urban area †	11	14.2	2.6	2	8.9	10	0	100	8	1	Affected
T109	Erzbergerstr.	Urban area †	13.7	14.1	1.76	1	4.86	4	92	8	38	4	Natural
T108	Edgar-von-Gierke/Siegfried-Kühn-Straße	Urban area †	12	14.9	2.26	2	6.12	8	79	21	25	4	Affected
T114	Allotment garden at the Alb	Urban area †	12.8	15.4	2.01	1	8.25	6	13	88	171	4	Affected

Measuring point	Location	Area classification	Depth [m]	Average GWT [°C]	SD GWT [-]	Relative amount of detritus [-]	Average dissolved oxygen [mg/l]	Average GFI [-]	Amount		Total amount of individuals [-]	Numbers of taxa [-]	Ecological condition (acc. to Griebler et al. (2014))
									(acc. to Griebler et al. (2014)) [%]	oligochaetes (acc. to Griebler et al. (2014)) [%]			
T115	Sonnenstr. – Zietenstr.	Urban area	13.5	14.8	1.81	1	4.83	4	89	11	23	4	Natural
T117	Sonnenstr. – Zietenstr.	Urban area	13	17	1.95	3	6.24	8	92	8	76	5	Natural
T118	Schoemperlenstr.	Urban area	13.7	16.1	1.76	1	8.45	6	38	63	11	4	Affected
T112	Wattstr. – Annweilerstr.	Urban area	12	14.5	2.26	2	6.87	8	100	0	204	4	Natural
T111	Field near Kaiserslauterner-Straße	Urban area	8.9	13.4	3.48	3	5.75	14	77	23	96	4	Affected
T113	Hertzstr. – St. Barbara-Weg	Urban area	11	17.5	2.6	1	3.35	5	0	100	1	1	Affected
3Kalmittweg	Kalmitweg No.3	Urban area	15.5	15.3	1.34	1	6.44	3	77	23	13	3	Affected
2Windhor	Wilhelm-Windhorst-Straße	Urban area	15.2	15.8	1.34	2	8.64	6	20	80	353	4	Affected
1F-Lust	Schänzle Franz-Lust-Str. – Kußmaulstr.	Urban area	15.2	17.3	1.34	2	5.95	5	65	35	630	3	Affected
T107	Molkestr. – Willy-Brandt-Allee	Urban area	10.1	16.2	2.95	1	4.9	7	66	34	130	3	Affected

Measuring point	Location	Area classification	Depth [m]	Average GWT [°C]	SD GWT [-]	Relative amount of detritus [-]	Average dissolved oxygen [mg/l]	Average GFI [-]	Amount		Total amount of individuals [-]	Numbers of taxa [-]	Ecological condition (acc. to Griebler et al. (2014))
									crustaceans (acc. to Griebler et al. (2014)) [%]	oligochaetes (acc. to Griebler et al. (2014)) [%]			
NOM-011	†	Forested area	14.9	10.7	1.47	1	3.42	3	100	0	15	1	Natural
NOM-017	†	Forested area	15	10.9	1.45	3	7.2	7	97	3	506	6	Natural
SOM-020	†	Forested area	15	10.7	1.45	1	5.81	3	50	50	9	4	Affected
SOM-018	†	Forested area	27	10.3	0.26	3	12.75	2	90	10	31	2	Natural
SWM-005	†	Forested area	15.5	10.5	1.34	2	8.72	6	26	74	358	3	Affected
NWM-009	†	Forested area	15	10.8	1.45	2	10.69	7	43	57	90	4	Affected
NWM-006	†	Forested area	14.8	10.7	1.49	1	5	3	86	14	16	3	Natural
NOM-014	†	Forested area	15	10.5	1.45	1	9.92	5	67	33	23	3	Affected

Table A.3.3: Taxa-site matrix of the invertebrate fauna of each water gauge.

Official designation of the water gauges	Number of individuals																Percentage	
	T101	T102	T104	T105	T106	T123	T124	T125	T128	T129	T320	T322	T402	T411	T412	T517		T524
Amphipoda	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	2
Cyclopoida	0	0	0	0	5	0	0	0	0	76	0	0	1	0	0	5	0	87
Harpacticoida	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Parastenocaris	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1
Bathynelleacea	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	272	274
Nauplia	0	0	0	0	1	0	0	0	0	1	0	0	0	0	0	0	0	2
Amount Crustacea	0	2	0	0	6	0	0	0	0	77	0	0	1	0	3	5	272	366
Amount Crustacea %	0	50	0	0	86	0	0	0	0	62	0	0	25	0	50	100	99	44
Amount Amphipoda %	0	0	0	0	0	0	0	0	0	0	0	0	0	0	33.3	0	0	0
Amount Cyclopoida %	0	0	0	0	71.4	0	0	0	0	61.3	0	0	25	0	0	100	0	0
Amount Harpacticoida %	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Amount Parastenocaris %	0	0	0	0	0	0	0	0	0	0	0	0	0	0	16.7	0	0	0
Amount Bathynelleacea %	0	50	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	98.9
Amount Nauplia %	0	0	0	0	14.3	0	0	0	0	0.8	0	0	0	0	0	0	0	0
Nematoda	0	2	0	0	0	0	2	65	5	0	0	0	1	0	1	0	0	76
Oligochaeta	0	0	0	1	1	2	0	37	8	8	252	3	1	33	0	0	3	349
Acari	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	2
Mikturellaria	0	0	0	0	0	0	0	1	0	39	0	3	0	1	2	0	0	46
Amount others	0	2	0	1	1	3	2	103	13	47	252	6	3	34	3	0	3	473
Total amount	0	4	0	1	7	3	2	103	13	124	252	6	4	34	6	5	275	839
Amount Oligochaeta %	0	0	0	100	14.3	66.7	0	35.9	61.5	6.5	100	50	25	97.1	0	0	1.1	0
Amount Nematoda %	0	50	0	0	0	0	100	63.1	38.5	0	0	0	25	0	16.7	0	0	0
Amount Acari %	0	0	0	0	0	0	33.3	0	0	0	0	0	25	0	0	0	0	0
Amount	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Microturbellaria %	0	0	0	0	0	0	0	1	0	31.5	0	50	0	2.9	33.3	0	0	0

Official designation of the water gauges	SOM-018	SWM-005	NWM-009	NWM-006	NOM-014	Number of indi- viduals	Percen- tage	Number of all indi- viduals	Peren- tage of all
Amphipoda	0	0	2	0	0	64	2.3	66	1.8
Cyclopoida	28	76	36	12	12	889	31.4	976	26.6
Harpacticoida	0	0	0	0	0	33	1.2	33	0.9
Parastenocaris	0	16	0	0	0	598	21.2	599	16.3
Bathynellacea	0	0	0	0	0	97	3.4	371	10.1
Nauplia	0	0	0	0	0	0	0	2	0.1
Amount Crustacea	28	92	38	12	12	1681	59.5	2047	55.8
Amount Crustacea %	90	26	42	75	52				
Amount Amphipoda %	0	0	2.2	0	0				
Amount Cyclopoida %	90.3	21.2	40	75	52.2				
Amount Harpacticoida %	0	0	0	0	0				
Amount Parastenocaris %	0	4.5	0	0	0				
Amount Bathynellacea %	0	0	0	0	0				
Amount Nauplia %	0	0	0	0	0				
Nematoda	0	0	1	2	5	152	5.4	228	6.2
Oligochaeta	3	266	51	2	6	994	35.2	1343	36.6
Acari	0	0	0	0	0	0	0	2	0.1
Mikrurbellaria	0	0	0	0	0	0	0	46	1.3
Amount others	3	266	52	4	11	1146	40.5	1619	44.2
Total amount	31	358	90	16	23	2827	100	3666	100
Amount Oligochaeta %	9.7	74.3	56.7	12.5	26.1				
Amount Nematoda %	0	0	1.1	12.5	21.7				
Amount Acari %	0	0	0	0	0				
Amount Microturbellaria %	0	0	0	0	0				

Table A3.4: Average and standard deviation of faunistic, chemical and physical parameters with regard to the four groups (result of the PIATE analysis).

	Average amount crustaceans (acc. to Griebler et al. (2014)) [%]	Average amount oligochaetes (acc. to Griebler et al. (2014)) [%]	Average numbers of taxa	Average amount of individuals	Average Shannon diversity	Average abundance Amphipoda	Average abundance Cyclopoida	Average abundance Parastenocaris	Average abundance Bathynellacea
	[-]	[-]	[-]	[-]	[-]	[-]	[-]	[-]	[-]
Group I (n = 13)	80.3 (± 24.5)	19.7 (± 24.5)	2.9 (± 1.3)	103.5 (± 159.6)	0.6 (± 0.4)	3.7 (± 10.8)	37.6 (± 78.2)	10.5 (± 31.6)	21.1 (± 72.4)
Group II (n = 14)	67.8 (± 26.7)	32.4 (± 26.9)	3.9 (± 0.5)	135.6 (± 165.9)	0.9 (± 0.3)	1.3 (± 2.9)	34.8 (± 46.9)	33.1 (± 103.1)	6.9 (± 17.2)
Group III (n = 9)	0.0 (± 0.0)	100.0 (± 0.0)	1.7 (± 0.7)	46.8 (± 78.8)	0.2 (± 0.3)	0.0 (± 0.0)	0.0 (± 0.0)	0.0 (± 0.0)	0.0 (± 0.0)
Group IV (n = 3)	0.0 (± 0.0)	0.0 (± 0.0)	0.3 (± 0.5)	0.7 (± 0.9)	0.0 (± 0.0)	0.0 (± 0.0)	0.0 (± 0.0)	0.0 (± 0.0)	0.0 (± 0.0)

	Average geological unit	Average GWT [°C]	Average phosphate concentration [mg/l]	Average nitrate concentration [mg/l]	Average relative amount of detritus	Average depth [m]
	[-]	[°C]	[mg/l]	[mg/l]	[-]	[m]
Group I (n = 13)	2 (± 1)	11.5 (± 1.3)	0.1 (± 0.1)	5.4 (± 3.9)	1.8 (± 0.8)	13.9 (± 4.5)
Group II (n = 14)	3 (± 1)	15.0 (± 1.5)	0.3 (± 0.6)	9.1 (± 3.7)	1.7 (± 0.7)	12.4 (± 2.3)
Group III (n = 9)	3 (± 1)	14.1 (± 1.8)	0.1 (± 0.1)	4.9 (± 3.9)	1.8 (± 0.8)	10.7 (± 1.5)
Group IV (n = 3)	2 (± 1)	13.9 (± 1.0)	0.1 (± 0.0)	3.9 (± 3.4)	3.0 (± 0.0)	22.6 (± 11.7)

Table A.3.5: Results of the Mann-Whitney-Tests from the four groups of the PHATE analysis.

	Amount crustaceans (acc. to Griebler et al. (2014)) [%]	Amount oligochaetes (acc. to Griebler et al. (2014)) [%]	Numbers of taxa [-]	Total amount of individuals [-]	Shannon diversity [-]	Abundance Amphipoda [-]	Abundance Cyclopoida [-]	Abundance Parastenocaris [-]	Abundance Bathynellacea [-]
Group I vs. II (n = 13;14)	1.3×10 ⁻¹	1.3×10 ⁻¹	1.5×10 ⁻²	2.0×10 ⁻¹	3.2×10 ⁻¹	7.4×10 ⁻¹	5.6×10 ⁻¹	7.2×10 ⁻¹	4.0×10 ⁻¹
Group I vs. III (n = 13;9)	4.0×10 ⁻⁶	4.0×10 ⁻⁶	2.7×10 ⁻²	2.4×10 ⁻¹	3.9×10 ⁻¹	2.0×10 ⁻¹	8.9×10 ⁻⁴	3.7×10 ⁻¹	6.8×10 ⁻¹
Group IV vs. I (n = 3;13)	3.6×10 ⁻³	1.3×10 ⁻¹	1.1×10 ⁻²	3.6×10 ⁻³	2.0×10 ⁻¹	7.9×10 ⁻¹	7.1×10 ⁻²	1	1
Group II vs. III (n = 14;9)	2.5×10 ⁻⁶	2.5×10 ⁻⁶	9.8×10 ⁻⁷	3.4×10 ⁻²	3.3×10 ⁻²	4.1×10 ⁻¹	1.4×10 ⁻⁴	2.3×10 ⁻¹	1.2×10 ⁻¹
Group IV vs. II (n = 3;14)	2.9×10 ⁻³	1.2×10 ⁻²	2.9×10 ⁻³	2.9×10 ⁻³	2.9×10 ⁻²	1	2.9×10 ⁻²	8.4×10 ⁻¹	6.5×10 ⁻¹
Group IV vs. III (n = 3;9)	1	9.1×10 ⁻³	4.6×10 ⁻²	2.7×10 ⁻²	7.6×10 ⁻¹	1	1	1	1

	Geological unit [-]	GWt [°C]	Phosphate concentration [mg/l]	Nitrate concentration [mg/l]	Relative amount of detritus [-]	Depth [m]
Group I vs. II (n = 13;14)	8.2×10 ⁻³	2.0×10 ⁻⁵	5.2×10 ⁻¹	1.2×10 ⁻²	6.1×10 ⁻¹	2.8×10 ⁻¹
Group I vs. III (n = 13;9)	1.5×10 ⁻¹	3.8×10 ⁻³	1	9.9×10 ⁻¹	9.7×10 ⁻¹	7.4×10 ⁻²
Group IV vs. I (n = 3;13)	4.8×10 ⁻¹	2.1×10 ⁻²	3.6×10 ⁻³	6.1×10 ⁻¹	7.1×10 ⁻¹	2.2×10 ⁻¹
Group II vs. III (n = 14;9)	4.4×10 ⁻¹	3.2×10 ⁻¹	7.2×10 ⁻¹	4.5×10 ⁻²	8.5×10 ⁻¹	9.4×10 ⁻²
Group IV vs. II (n = 3;14)	3.5×10 ⁻¹	2.8×10 ⁻¹	2.9×10 ⁻²	9.1×10 ⁻²	2.9×10 ⁻²	1.1×10 ⁻¹
Group IV vs. III (n = 3;9)	8.4×10 ⁻¹	9.6×10 ⁻¹	9.1×10 ⁻³	1.7×10 ⁻¹	9.1×10 ⁻²	1.8×10 ⁻²

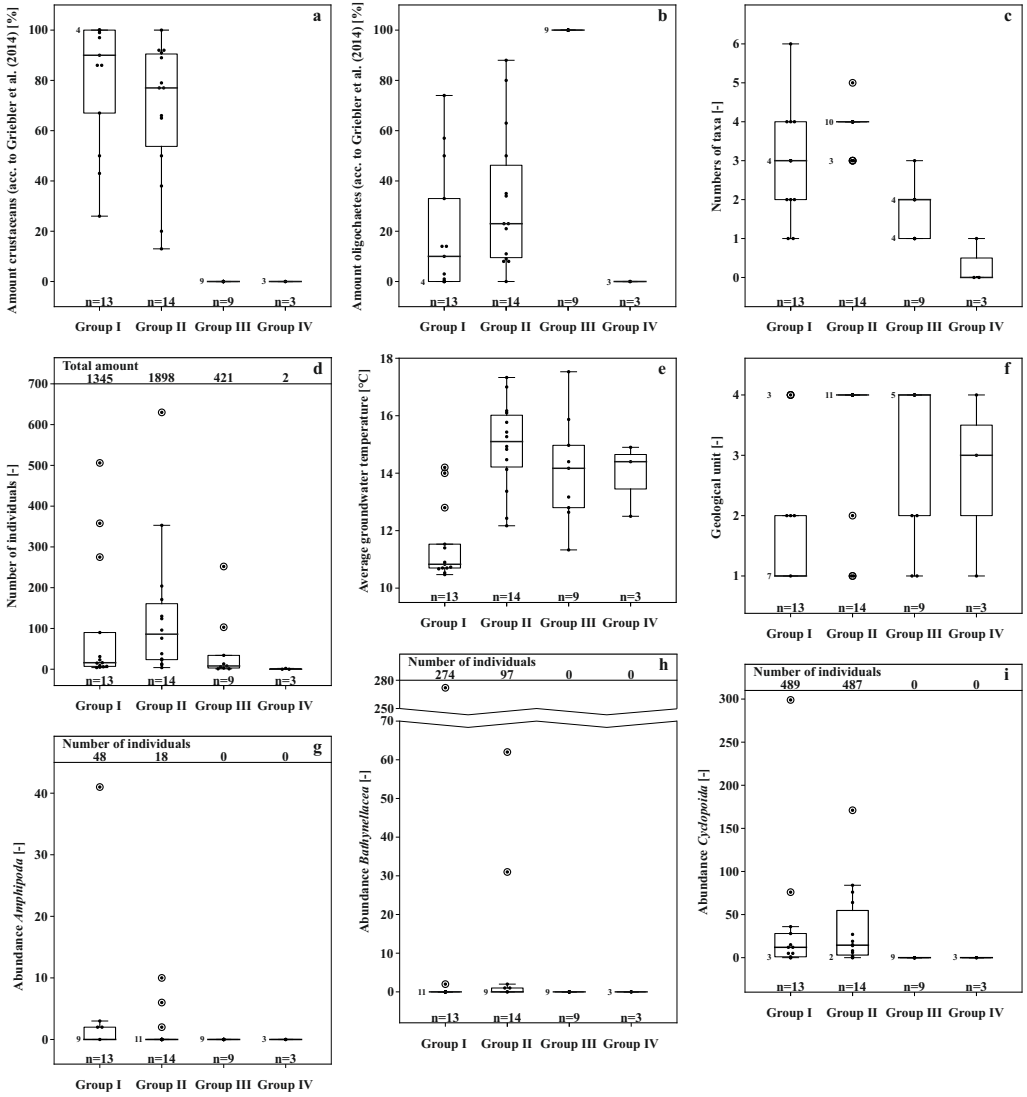


Figure A3.3: Boxplots of: (a) Amount of crustaceans [%] and (b) oligochaetes [%] according to the scheme of Griebler et al. (2014b); (c) numbers of Taxa [-]; (d) number of individuals [-]; (e) average GWT of the repeated measurements at the bottom of the measurement wells [°C] and (f) geological unit [-]; (g) Abundance of the order *Amphipoda* [-]; (h) of the order *Bathynellacea* [-] and (i) of the order *Cyclopoida* [-], divided into four groups according to the PHATE visualization (n = number of wells).

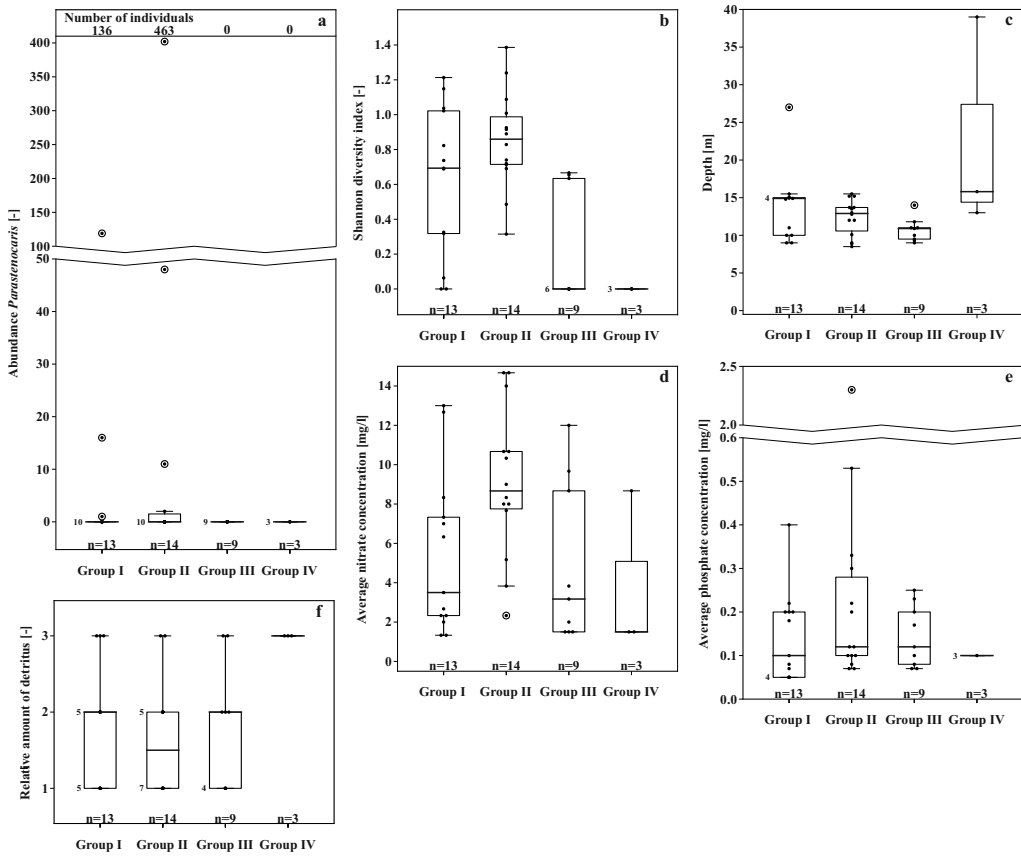


Figure A3.4: Boxplots of: (a) Abundance of the genus *Parastenocaris* [-]; (b) Shannon diversity index [-]; (c) depth of the measurement wells [m]; (d) of the average nitrate concentration [mg/l]; (e) average phosphate concentration [mg/l] of the repeated measurements at the bottom of the measurement wells and (f) of the relative amount of detritus [-], divided into four groups according to the PHATE visualization (n = number of wells).

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Declaration of authorship

Study 1 (Chapter 2)

Koch, F., Blum, P., Korbel, K., Menberg, K., (2023) Global overview on groundwater fauna. Ecohydrology 17 (1). 28. <https://doi.org/10.1002/eco.2607>

Fabien Koch (FK) did all the literature search and data analysis. Kathryn Korbel (KK) provided critical feedback and contributed to the interpretation of the results. Kathrin Menberg (KM) and Philipp Blum supervised the work. FK wrote the initial draft and all authors (FK, PB, KK, KM) discussed and interpreted the results and substantially contributed to editing and reviewing the manuscript.

Study 2 (Chapter 3)

Koch, F., Blum, P., Stein, H., Fuchs, A., Hahn, H. J., Menberg, K., (2024) Temporal shift of groundwater fauna in South-West Germany. Hydrology and Earth System Sciences (submitted)

Andreas Fuchs (AF) and one time also Fabien Koch (FK) executed the fieldwork and AF evaluated the fauna samples. FK evaluated the collected data, interpreted and visualised the results. Philipp Blum (PB) and Hans Jürgen Hahn (HJH) provided the topic and supervised the work, together with Kathrin Menberg (KM). FK and wrote the initial draft of the paper. All authors (KM, AF, HS, HJH and PB) discussed and interpreted the results and substantially contributed to editing and reviewing the manuscript.

Study 3 (Chapter 4)

Koch, F., Menberg, K., Schweikert, S., Spengler, C., Hahn, H.J., Blum, P. (2021) Groundwater fauna in an urban area - natural or affected?. Hydrology and Earth System Sciences 25. 3053-3070. <https://doi.org/10.5194/hess-25-3053-2021>

Svenja Schweikert (SS) and Cornelia Spengler (CS) executed the fieldwork. Fabien Koch (FK) evaluated the collected data, interpreted and visualised the results with support from CS. Philipp Blum (PB) and Hans Jürgen Hahn (HJH) provided the topic and scientifically supervised the work, together with Kathrin Menberg (KM). FK wrote the initial draft and all authors (FK, CS, HJH, KM and PB) discussed the results and substantially contributed to editing and reviewing the manuscript.

Publications and contributions

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Koch, F., Blum, P., Korbel, K., Menberg, K., (2023) Global overview on groundwater fauna. *Ecohydrology* 17 (1). 28. <https://doi.org/10.1002/eco.2607>.

Koch, F., Menberg, K., Schweikert, S., Spengler, C., Hahn, H.J., Blum, P. (2021) Groundwater fauna in an urban area - natural or affected?. *Hydrology and Earth System Sciences* 25. 3053-3070. <https://doi.org/10.5194/hess-25-3053-2021>.

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Conference contributions (as first author)

Koch, F., Menberg, K., Spengler, C., Stein, H., Fuchs, A., Hahn, H. J., Blum, P. (2023) Groundwater biomonitoring as a tool for identifying environmental trends. Conference: Transforming towards a sustainable society – challenges and solutions. 12. October 2023. Karlsruhe. https://indico.scc.kit.edu/event/3604/attachments/6805/10699/231009__BookofAbstracts_A5_09102023.pdf (presentation).

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Koch, F., Menberg, K., Schweikert, S., Hengel, J., Spengler, C., Hahn, H.J., Blum, P. (2023) Urban groundwater fauna – natural or anthropogenically influenced?. EGU23. The 25th EGU General Assembly. 24-28 April. 2023 in Vienna and online. id. EGU23-5773. 10.5194/egusphere-egu23-5773 (presentation).

Koch, F., Menberg, K., Schweikert, S., Spengler, C., Hahn, H.J., Blum, P. (2022) Urbane Grundwasserfauna: natürlich oder anthropogen beeinflusst?. FH-DGGV Jahrestagung. 23. March 2022. online (presentation).

Eidesstattliche Versicherung

Eidesstattliche Versicherung gemäß § 13 Absatz 2 Satz 1 Ziffer 4 der Promotionsordnung des Karlsruher Instituts für Technologie (KIT) für die KIT-Fakultät für Bauingenieur-, Geo- und Umweltwissenschaften

1. Bei der eingereichten Dissertation zu dem Thema „Spatial and temporal analysis on groundwater ecosystems“ handelt es sich um meine eigenständig erbrachte Leistung.
2. Ich habe nur die angegebenen Quellen und Hilfsmittel benutzt und mich keiner unzulässigen Hilfe Dritter bedient. Insbesondere habe ich wörtlich oder sinngemäß aus anderen Werken übernommene Inhalte als solche kenntlich gemacht.
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4. Die Richtigkeit der vorstehenden Erklärungen bestätige ich.
5. Die Bedeutung der eidesstattlichen Versicherung und die strafrechtlichen Folgen einer unrichtigen oder unvollständigen eidesstattlichen Versicherung sind mir bekannt.

Ich versichere an Eides statt, dass ich nach bestem Wissen die reine Wahrheit erklärt und nichts verschwiegen habe.

Karlsruhe, 08.02.2024

M.Sc. Fabien Koch