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Review Article

Groundwater fauna downtown – Drivers, impacts and implications for subsurface ecosystems in urban areas

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ABSTRACT

Groundwater fauna (stygofauna) comprises organisms that have adapted to the dark subterranean environment over a course of thousands and millions of years, typically having slow metabolisms and long life cycles. They are crucial players in the groundwater of oxygenic aquifers, and contribute to various ecosystem services. Today's knowledge of their sensitivity to anthropogenic impacts is incomplete and a critical analysis of the general relevance of local findings is lacking. In this review, we focus on those areas with the highest interference between humans and stygofauna: cities. Here is where local pollution by various contaminants and heat strongly stresses the unique groundwater ecosystems. It is demonstrated that it is difficult to discern the influence of individual factors from the findings reported in field studies, and to extrapolate laboratory results to field conditions. The effects of temperature increase and chemical pollution vary strongly between tested species and test conditions. In general, previous findings indicate that heating, especially in the long-term, will increase mortality, and less adapted species are at risk of vanishing from their habitats. The same may be true for salinity caused by road de-icing in cold urban areas. Furthermore, high sensitivities were shown for ammonium, which will probably be even more pronounced with rising temperatures resulting in altered biodiversity patterns. Toxicity of heavy metals, for a variety of invertebrates, increases with time and chronic exposure. Our current knowledge reveals diverse potential impacts on groundwater fauna by urban pollution, but our insights gained so far can only be validated by standardized and long-term test concepts.

1. Introduction

Groundwater is the most extracted raw material. It supplies half of the world's population with drinking water and is used extensively for irrigation and industry (Griebler and Avramov, 2015; Siebert et al., 2010). The perception of groundwater as an essential resource however ignores that aquifers host vulnerable ecosystems. To date, the protection of aquifers is mainly motivated by economic considerations for securing freshwater production. There is little attention to the biodiversity and functionality of hidden subterranean ecosystems that are much less understood than those of surface waters. In fact, aquifers are densely colonized and harbour highly diverse microbial (Ghiorse and Wilson, 1988; Griebler and Lueders, 2009; Hirsch et al., 1992; Karwautz and Griebler, 2022; Malard, 2022) and invertebrate (stygofaunal) communities including many relictual and endemic species (Gibert et al., 2009; Humphreys, 2009).

True groundwater fauna (stygobites) spend their whole life cycle in the subsurface and rarely establishes stable populations in surface waters (Gibert et al., 1994). One reason is their long-term adaptation to dark, temperature-stable, and generally energy-poor environments, resulting in a slow metabolism and long lifespans. The regression and lack of eyes and pigmentation, along with the slow metabolism, put groundwater invertebrates at a disadvantage when competing in surface water (Giere, 2008). Their high specialization augments sensitivity and vulnerability to anthropogenic impacts like temperature changes, groundwater pollution, altered hydrological conditions, and habitat loss due to water table drawdown (Danielopol et al., 2003; Korbel and Hose, 2015; Marmonier et al., 2013; Stein et al., 2010).

Aquifers deviate the most from their natural state in urbanized regions (Schirmer et al., 2013). This is where multiple direct anthropogenic stressors occur simultaneously, causing abrupt spatial and temporal changes in water quantity and quality. Quantitative impacts stem from local, well-based extraction and injection of water as a part of urban water management, from modifications of the hydraulic regime

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related to the installation of subsurface infrastructure, as well as from substantial changes in groundwater recharge due to urban land use and surface sealing (Benz et al., 2016; Wong et al., 2012). Changes in the hydraulic conditions and subsurface water circulation have consequences for groundwater quality. Groundwater quality beneath cities is influenced by local pollution from industrial and communal waste waters, underground pipe leakage, atmospheric deposition and traffic, as well as by a typically altered thermal regime (Fig. 1). As a consequence, aquifers located in rural regions are often favoured as primary freshwater sources to supply cities. This limits the attention to urban aquifers as safeguard environments and habitats which also need be protected.

Our current knowledge about subterranean ecosystems in urban areas is incomplete and disperse, and the role of multiple interacting factors in particular has hardly been tackled. Moreover, different urban realms are interconnected and can impact each other. Especially, pressures occurring localized in a terrestrial realm can have an impact on a larger spatial scale in the groundwater ecosystem causing changes in ecological and chemical characteristics (Bugnot et al., 2019). In the present review, the focus lies on groundwater fauna in urban environments and the knowledge available from diverse case-studies. In the following, first, fundamental properties of groundwater fauna are introduced. Second, key factors of anthropogenic disturbance in urban groundwater ecosystems such as warming, oxygen depletion, and chemical pollution including nutrients, organic pollutants, heavy metals and salt, are elaborated upon. Based on this, the major urban factors for survival, diversity and metabolic mechanisms of groundwater fauna are summarized along with conclusions and future research needs.

2. Characteristics of groundwater fauna

Aquifers contain unique subterranean ecosystems with a vast diversity of invertebrates including micro-, meio- and macrofauna. Stygofauna is adapted to its extraordinary environment – characterized by the absence of light and low inputs of nutrients and organic matter – through the reduction or even total loss of eyes and pigmentation, the elongation of the body shape and the development of sensory features enabling it to move and forage in this habitat (Gibert et al., 1994). Moreover, stygobionts are thought to be long-lived (Gibert et al., 1994; Voituron et al., 2011). They undergo slower growth and have fewer offspring than their surface relatives (Gibert et al., 1994; Humphreys, 2009). Groundwater fauna can also typically tolerate low oxygen concentrations (Malard and Hervant, 1999).

Stygofauna is typically dominated by arthropods, in particular crustaceans from the classes Ostracoda, Copepoda, Malacostraca (Amphipoda, Isopoda, Syncarida) are highly represented. Further arthropods resemble mites (Acari), beetles, and other insects. Additionally, abundant groups of organisms in groundwater are worms from the phyla Plathelminthes (Turbellaria), Annelida (Oligochaeta and Polychaeta), and Nematoda. Less frequently found members of stygofauna include molluscs (Gastropoda) (Fig. 2) (Humphreys, 2009). The stygobiont community is characterized by high endemism and relict species, whereby the latter successfully survived periods of geological and climatic change (aquifers are "living museums") (Humphreys, 2009). Groundwater fauna inhabits all kinds of aquifer types and geographic regions, usually with diversity hotspots in aquifers that provide sufficient space (e.g., karst, alluvial gravel deposits) (Culver and Sket, 2000; Humphreys, 2009; Iannella et al., 2020; Korbel and Hose, 2015).

In addition to invertebrates, microorganisms such as bacteria, archaea, protozoa, and fungi are an important part of groundwater communities (Griebler and Lueders, 2009). The microbial communities do not only constitute the main fraction of biomass and biodiversity in the subsurface (Whitman et al., 1998), but they also hold the key responsibility for biogeochemical processes and water purification (Feichtmayer et al., 2017; Griebler and Avramov, 2015; Herrmann and Taubert, 2022). Microbes mediate all important geochemical cycles, as they form colonies and biofilms on sediments, detritus, and rock surfaces which are grazed on by invertebrates, and therefore serve as the food base for the meso- and macrofauna (Barlocher and Murdoch, 1989; Saccò et al., 2022). The grazing of these biofilms by stygofauna in turn may stimulate microbial activity and productivity, and it may reduce clogging of pores and thereby modify local micro-scale conditions (Griebler and Avramov, 2015; Schmidt et al., 2017). Stygofauna is involved in the carbon cycle via retardation and decomposition of particulate organic matter (Griebler and Avramov, 2015). Movement and burrowing (bioturbation) of stygofauna influence the hydraulic properties of aquifers: voids are maintained open, as well as created in materials with low conductivity (e.g., clay and fine sand) (Danielopol, 1989; Stumpp and Hose, 2017).

Ecosystem services and goods are processes and products provided by natural ecosystems and their inhabitants, contributing to human well-being. Groundwater ecosystem services are manifold, including (1) purification, storage and provision of clean water via biodegradation, immobilization of contaminants and elimination of pathogens, and (2) supply of geothermal energy, to name two prominent examples

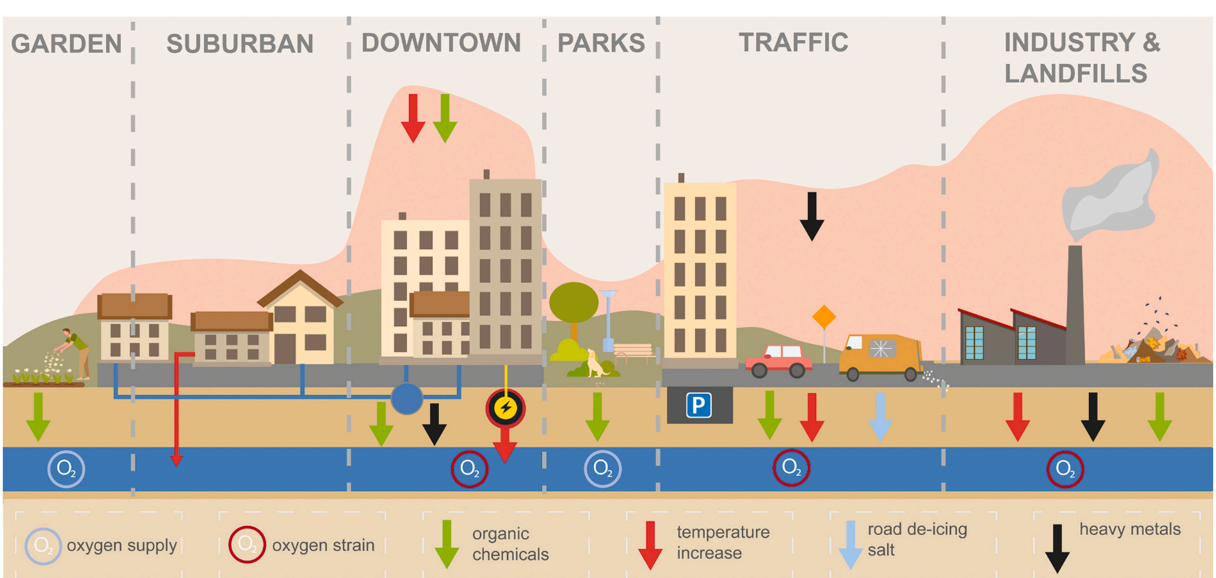

Fig. 1. Schematic overview of sources of anthropogenic pollution in an urban aquifer.

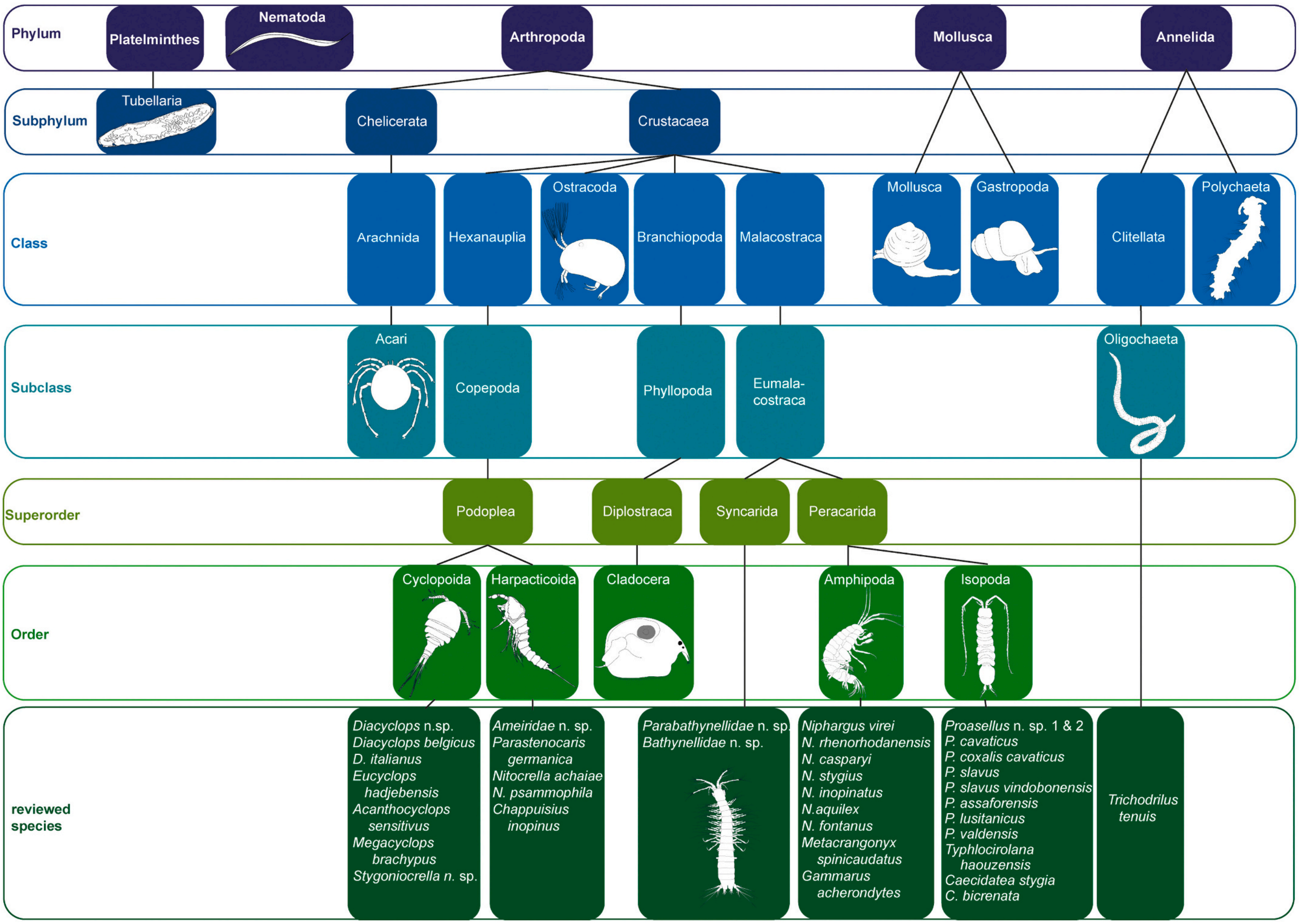

Fig. 2. Taxonomic classification of the species reported in this review.

(Griebler and Avramov, 2015). Until now, however, a fundamental understanding of the relationship between stygofauna and groundwater ecosystem services is lacking. Without doubt, due to the constantly increasing number and accumulation of pollutants in aquifers, as well as their overuse and exploitation particularly in urban areas, the risk of negative effects on biodiversity and ecosystem services is increasing (Di Lorenzo et al., 2021a; Di Lorenzo et al., 2018; Griebler et al., 2019).

3. Pressures in urban aquifers and groundwater fauna response

3.1. Rising temperature

3.1.1. Temperature in urban aquifers

In the following, we refer to absolute temperatures in degree Celsius (°C) and for clarity use the Kelvin (K) scale to indicate relative thermal changes. A prominent feature of urban aquifers is the altered thermal regime with elevated and highly spatially variable groundwater temperatures when compared to more natural and rural areas (Benz et al., 2016). Temperature is of particular importance in determining physiological activity and phenology of animals due to temperature dependence of enzymatic reactions (Colinet et al., 2015). Also, temperature effects on the groundwater physico-chemical characteristics are well known (see a summary in Griebler et al. (2016)). Reduced oxygen concentrations and pH values, for example, have frequently been observed with groundwater warming (Riedel, 2019). Groundwater is typically thermally stable with temperatures in shallow aquifers close to the annual mean of the air temperature. However, in case of infiltrating conditions from surface water and in highly dynamic aquifers, seasonal temperature variability may be more locally pronounced. For instance, in central Europe, natural groundwater temperatures are typically between 10 and 12 °C (Bannick et al., 2008) whereas they can be below 8 °C in high mountain areas and Scandinavian regions and above 18 °C in Mediterranean countries (Benz et al., 2017; Tissen et al., 2019).

Seasonally uniformity and long term stability of the thermal regime is considerably changed by anthropogenic drivers. Due to climate change, shallow quaternary aquifer temperatures are expected to rise by up to 5 K at a latitude of 45° within the next century (Menberg et al., 2014; Taylor and Stefan, 2009). In urban areas, these long-term background changes are superimposed by the direct effects of modified land use, heat release of basements and subsurface infrastructure, as well as geothermal use (Fig. 1). Subsurface urban heat islands (SUHI) have been observed in cities worldwide, which are measured as elevated shallow groundwater and soil temperatures in urbanized regions. The aquifers below more densely populated and older urban areas (e.g., city centres) often have higher temperatures than those below rural, undisturbed regions where the temperatures can be more than 5 K colder. Most relevant anthropogenic heat sources were observed to be sealing of ground surface, built-up-areas, and underground structures (e.g., basements, tunnels) (Benz et al., 2015; Böttcher and Zosseder, 2022; Previati et al., 2022). Locally, hot spots of more than 10 K elevation in temperature have been reported (Blum et al., 2021; Ferguson and Woodbury, 2007; Hemmerle et al., 2019; Huang et al., 2009). Nevertheless, groundwater warming is spatially heterogeneous and also dependent on the hydrogeological conditions such as flow velocity, saturated thickness, and depth-to-water and influence of anthropogenic heat sources differs strongly among cities (Bayer et al., 2019; Böttcher and Zosseder, 2022; Zhu et al., 2015).

3.1.2. Effects of temperature changes on groundwater organisms

Stygobionts are ectotherms with narrow thermal tolerance ranges and skewed temperature curves, describing the relationship between temperature and physiological functions. Groundwater invertebrate species that have been exposed to thermal conditions deviating from their habitat temperatures, exhibited thermal stress and a strong physiological response in incubation experiments. Of specific interest are upper thermal tolerance or/and lethal temperature values. Survival rates are frequently expressed as LT50 values, which describes the combination of temperature and time at which 50% of the observed population has died (LT = lethal temperature).

Issartel et al. (2005) compared the thermal stress response of the stygobiont amphipods *Niphargus rhenorhodanensis* and *Niphargus virei* with their surface water relative *Gammarus fossarum* under laboratory conditions. Survival rates of *N. rhenorhodanensis* were higher than those of *N. virei* when acclimated to 12 °C (natural habitat temperature) and

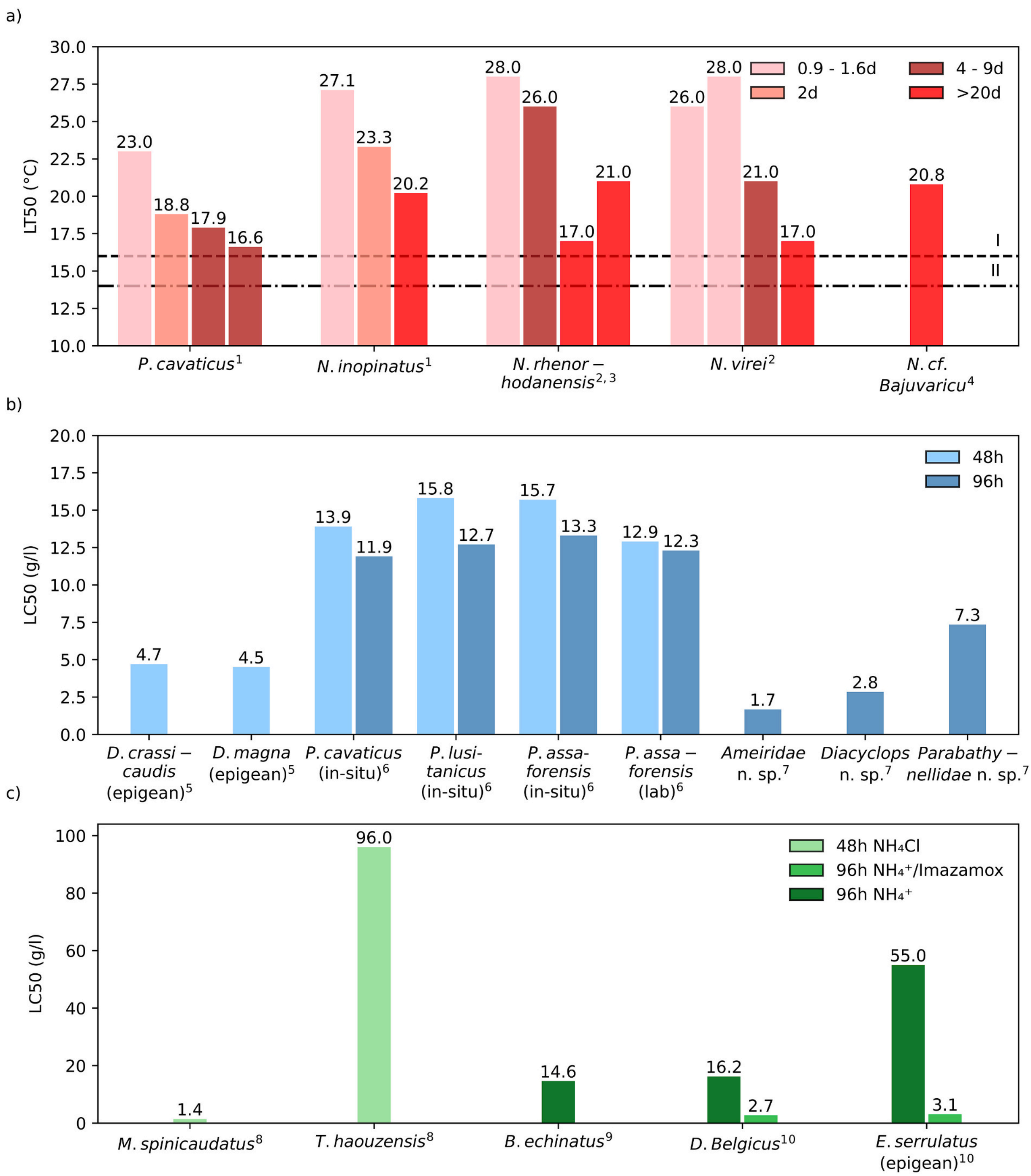

Fig. 3. Results from dose-response tests for a) temperature sensitivity of stygobiontic species, I – threshold estimated by Brielmann et al. (2011), II – threshold estimated by Spengler (2017); b) lethal concentration of 50% of the tested individuals (LC50) for NaCl; c) lethal concentrations of 50% of the tested individuals (LC50) for ammonium species and mixtures.

1: Brielmann et al., 2011, 2: Issartel et al., 2005, 3: unpublished data reported in Colson-Proch et al., 2010, 4: (Weber, 2008), 5: Castaño-Sánchez et al., 2021, 6: Castano-Sanchez et al., 2021, 7: Castano-Sanchez et al., 2020a, 2020b, 8: Boutin et al., 1995, 9: Di Marzio et al., 2009, 10: Di Marzio et al., 2018 were similar to the rates of the surface crustacean *G. fossarum*, which showed the highest thermal plasticity and longer duration of survival over the entire tested temperature range from −2 to 28 °C (Fig. 3a). The high survival rates of *N. rhenorhodanensis* were unexpected, since they colonize similar habitats as *N. virei* (Issartel et al., 2005). Brielmann et al. (2011) found *Niphargus inopinatus* to be more tolerant to temperature elevations than isopod *Proasellus cavaticus* (Fig. 3a). LT50 values of both species decreased the longer the test lasted (Brielmann et al., 2011). These results are in agreement with the observed narrow thermal tolerance breadths that apply to two *P. cavaticus* morphospecies (*Proasellus* n. sp. 1 and *Proasellus* n. sp. 2) reported in Mermillod-Blondin et al. (2013). In the same study, survival rates found for *Proasellus valdensis* were high (95% individual survivals at 11 °C) and comparable to those of *N. rhenorhodanensis* which did not substantiate a specific adaptation to their thermally stable environment (Mermillod-Blondin et al., 2013).

Brielmann et al. (2011) performed temperature tolerance tests in a temperature gradient chamber with the two groundwater species *N. inopinatus* (Amphipoda) and *P. cavaticus* (Isopoda). In doing so, a temperature gradient of 2 to 35 °C was established in an acrylic glass chamber and individuals of both species were positioned at a temperature of 12 °C within the gradient. The temperature with the mean residence time of *N. inopintatus* and *P. cavaticus* was 11.7 ± 3.4 °C and 11.4 ± 5 °C, respectively, with most observed individuals found at temperatures between 8 and 16 °C. They set a threshold of 16 °C above which no individuals showed survival or residence. According to the lower thermal tolerance of *P. cavaticus* detected during the temperature response tests, the individuals in the temperature gradient chamber demonstrated rigidity at 22.9, 23.5 and 25 °C which was not identified for *N. inopinatus* (Brielmann et al., 2011). Castano-Sanchez et al. (2020b) determined the upper thermal limits (UTL), whereby the UTL50 is the upper lethal temperature affecting 50% of the population and was estimated from the LT50s taken every 24 h within the warming period. Highest sensitivity was observed for members of the fauna group Harpacticoida (*Ameiridae* n. sp.) with UTL50 values of 24.8 ± 0.2 °C, followed by Cyclopoida (*Diacyclops* n. sp., UTL50: 26.9 ± 0.2 °C) and Syncarida (*Parabathynellidae* n. sp., UTL50 < 30 °C).

In other experiments, the influence of temperature changes on physiological features was investigated. Oxygen consumption or standard respiration rates (SRR) revealed no significant change for different stygobiontic species of different phylogenetic groups (e.g., *Diacyclops belgicus* (copepod), *N. virei, N. rhenorhodanensis* (Amphipoda)) with elevated temperatures (14–21 °C) (Di Lorenzo and Galassi, 2017; Issartel et al., 2005; Mermillod-Blondin et al., 2013). The unaffected physiological activities of *D. belgicus* may be due to adaptation to groundwater temperatures of 14–15.9 °C in a Mediterranean shallow aquifer and the wide geographical range of the species (Di Lorenzo and Galassi, 2017).

Furthermore, Q_{10} values, describing temperature dependence of a physiological process like respiration rate, indicate temperature sensitivity of organisms. Thereby, if the Q_{10} value is estimated to be 1, the respiration rate is independent of temperature. If $Q_{10} > 1$ or $Q_{10} < 1$, the process is considered to be temperature dependent and respiration is increasing or decreasing with temperature. For example, a Q_{10} value of 2 indicates a doubling of the respiration rate when temperature increases by 10 °C. Respiration rates and the Q_{10} value were estimated by Issartel et al. (2005). Very high or low Q_{10} values indicate temperatures that cause irreparable protein damage and therefore inhibition of the physiological system (Hochachka and Somero, 2002). Q_{10} values were always higher in *N. virei*, signifying a lower capacity to maintain enzymatic activities with elevated temperatures in comparison to *N. rhenorhodanensis* (Issartel et al., 2005). Q_{10} value for *D. belgicus* indicated a possible beginning of irreversible denaturation of enzymes at increased temperatures (Di Lorenzo and Galassi, 2017). Increasing temperatures stimulate metabolic processes, which may be even more pronounced in thermally sensitive stygobionts compared to epigean relatives, as presented by Simčič and Sket (2021) for the hypogean species *N. stygius* and the epigean species *N. zagrebensis*. With higher temperatures, the epigean *Eucyclops serrulatus* exhibited accelerated metabolic activities as well as shortened development times (nauplius to adult: 20 d at 15 °C, 14 days at 20 °C (Maier, 1990); 35 days at 18 °C, 21 days at 21 °C (Cifoni et al., 2017)) and life spans (42 d at 20 °C, 96.5 d at 15 °C) (Cifoni et al., 2017; Maier, 1990).

Another way to determine temperature sensitivity and tolerance of groundwater organisms is the qualitative and quantitative evaluation of stress responses, for example via catecholamine levels (Avramov et al., 2013), heat shock proteins (Colson-Proch et al., 2010) or immune response (detection of free amino acids and sugars) (Mermillod-Blondin et al., 2013). Catecholamines (noradrenaline, adrenaline, dopamine) are involved in the physiological response to stress. Groundwater species already exhibiting very high initial catecholamine levels showed an increased stress response with a sudden temperature increase of 6 and 12 K. Due to their storage of catecholamines, stygobionts are able to react faster to stress than their surface relatives, which may be an adaptation to food scarcity and their energy limited environment. However, if this storage is emptied, recovery may be critical (Avramov et al., 2013). Therefore, in the long-term, groundwater organisms may be less tolerant to thermal changes with respect to an inhibited catecholamine synthesis. This would be in agreement with the results of Colson-Proch et al. (2010) who observed a significant stress reaction *N. rhenorhodanensis* exposed to a heat shock of +6 °C after 1 and 2 months.

In a field study, Brielmann et al. (2009) reported a decreased faunal diversity with elevated temperatures in observation wells distributed within and outside a heat plume originating from an industrial facility. No effect was found with respect to the faunal abundance. In another field survey by Spengler (2017), the biodiversity of groundwater crustaceans in the Upper Rhine valley (Germany) was observed to change significantly above 14 °C. In this study, temperature increase was correlated with a decrease in stygobiont species abundance.

3.2. Salt pollution from road de-icing

3.2.1. Salt in urban aquifers

Salt in urban groundwater can originate from landfill leachate, septic tank effluents, animal feeds, industrial effluents, sewage effluents, water-conditioning salt, fertilizers (KCl), and seasonally from road de-icing (Fig. 1) (Aghazadeh et al., 2010; Kelly, 2008; Kelly et al., 2012). Since the late 1940s, the most commonly used de-icing salt is NaCl due to its low cost, easy use and storage, and its high efficiency (Jandová et al., 2020). Thirty five million metric tons of salt are used annually worldwide and the trend is increasing in response to the expansion of urban areas and impervious surfaces (Johnsson and Adl-Zarrabi, 2019; Kelly, 2008). Approximately 60% of the applied salt is subjected to surface runoff each year and transported to surface waters during the spring snow melt. The remainder accumulates in groundwater (Perera et al., 2013).

Chloride as the primary environmental contaminant in salt is not toxic to human health in concentrations usually found in drinking water (WHO, 2011) and highly soluble and mobile in water (Foos, 2003). Concentrations vary greatly depending on background concentrations, run-off behaviour, geology and hydrogeology, as well as salt application amounts in the study areas. A rapid degradation of water quality due to road salt in urban areas has been reported multiple times (Aghazadeh et al., 2010; Cooper et al., 2014; Daley et al., 2009; Kelly et al., 2012; Williams et al., 2000). Average concentrations of chloride in cities with intense road de-icing salt applications ranged between 415 mg L^{-1} and 1100 mg L^{-1} (Aghazadeh et al., 2010; Williams et al., 2000). Particularly high chloride concentrations were observed along heavily treated roads in urban regions (Bester et al., 2006; Jamshidi et al., 2020; Jandová et al., 2020).

Chloride contamination is a long-lasting problem for groundwater, as well as surface water. It might take decades to wash out the chloride even after salt application would immediately be stopped (Bester et al., 2006; Jamshidi et al., 2020; Ludwikowski and Peterson, 2018). Model scenarios by Bester et al. (2006) for southwest Ontario, Canada or Ludwikowski and Peterson (2018) for Illinois, USA showed that even with a reduction of salt application by 100%, it will take decades until groundwater reaches a safe drinking water quality again.

3.2.2. Salt effects on groundwater organisms

Studies which discuss the influence of (road) salt on groundwater organisms are rare. Research projects mainly focused on community-level effects and the characterization of aquifers which were not necessarily anthropogenically used or polluted. In Australian aquifers, most taxa were found in groundwater with electrical conductivity (EC) values below 1500 μS/cm (Hancock and Boulton, 2008), whereby no significant correlation between faunal abundance and EC was identified. In Portuguese and Algerian aquifers, wells with highly saline groundwater had the lowest stygofaunal abundances compared to the total of collected fauna (Mahi et al., 2019; Mencio et al., 2014). This was also the case for a coastal aquifer in Portugal affected by seawater intrusion. Gastropods as well as Ostracods seemed to be more abundant in wells with a lower salinity. Furthermore, the absence of the stygobiont species *Eucyclops hadjebensis* and *Acanthocyclops sensitivus* in high salinity bores was observed. However, the presence of *Megacyclops brachypus*, for example, was more related to high salinity bores (Shapouri et al., 2016). The authors found EC to be one of the major parameters affecting the faunal assemblage, which is in agreement with results of some other studies (Castano-Sanchez et al., 2020b; Dole-Olivier et al., 2009; Shapouri et al., 2016). In contrast to these findings, Schulz et al. (2013) detected high stygofaunal abundances of the three taxa Cyclopoida, Harpacticoida and Bathynellacaea in highly saline waters exceeding marine conductivities. Also, Tione et al. (2016) discovered exclusive copepod species (*Stygonitocrella* n. sp.) in a brackish well with high salinity (average 2737 mg L^{-1} chloride). The authors concluded that groundwater species are able to adapt to special hydrochemical conditions and that salinity may be one main influencing factor for species to occur.

In three papers, Castano-Sanchez et al. (2020b, Castaño-Sánchez et al., 2021, Castano-Sanchez et al., 2021) observed mortality rates of stygobiont species under laboratory conditions or in situ (Fig. 3b). First, the authors investigated the salinity tolerance of *Diacyclops* n. sp., *Ameiridae* n. sp. and *Parabathynellidae* n. sp. over a time period of 96 h. Syncarida were the most tolerant taxa, and Cyclopoida species were more tolerant to salinity than harpacticoid species (Fig. 3b). The more tolerant taxa thereby were sampled from an aquifer with higher salinity, and the authors assumed a pre-adaptation to local conditions or a broader tolerance in these taxa (Castano-Sanchez et al., 2020b). In a second study, adults and juvenile epigean *Diacyclops crassicaudis crassicaudis* were shown to be slightly more tolerant than *Diacyclops* n. sp. and twice as tolerant as the harpacticoid *Ameiridae* n. sp. (Castano-Sanchez et al., 2021). Thereby, copepodites were more sensitive than adult animals. In a very recent study, different isopods were tested in situ and under varying laboratory conditions. All three species – *Proasellus cavaticus, Proasellus lusitanicus* and *Proasellus assaforensis* – were more tolerant to NaCl under in situ conditions than the taxa tested in the previous studies (Fig. 3b). *P. assaforensis* was more tolerant under lab conditions than the other tested taxa, but had a lower mortality in situ, which may be explained by induced transportation stress or acclimation to the laboratory conditions (Castaño-Sánchez et al., 2021).

3.3. Organic chemicals

3.3.1. VOCs in urban aquifers

Groundwater pollution with organic chemicals is a major issue in urban and industrial landscapes and include a variety of contaminant groups such as aliphatic, aromatic or chlorinated organic chemicals, many being quite soluble in water, mobile and volatile (VOCs). VOCs are commonly represented by halogenated solvents (e.g., PCE, TCE, DCE, VC, DCM), BTEX (benzene, toluene, ethylbenzene, xylenes) and PAHs (e.g., naphthalene, phenanthrene, fluoranthene, pyrene and fluorene) (Bulatović et al., 2021; Gesels et al., 2021; Liu et al., 2020; Park et al., 2005; Squillace et al., 2004). These organic chemicals originate from diverse sources including former gasification plants, infrastructures like oil storage and gas stations, urban traffic and transport of oil and gas including accidental spills, textile and car industry with dry cleaning processes, manufactured products used in buildings (paints, cleaning agents, adhesives, furnishings, floor and wall coverings, combustion products) and leaking landfills (Fig. 1) (Han et al., 2013; Kuroda and Fukushi, 2008; Miller et al., 2020). In particular, big cities suffer from the legacy of gasification plants and the subsurface dumping of waste products such as tar oil (Meckenstock et al., 2010). Concentrations of organic chemicals in urban groundwater can span several orders of magnitude. While at former gasification plants and specific sites of accidental spill and leakage, BTEX and PAH concentrations in contaminated groundwater can reach values of >100 mg L^{-1} and > 10 mg L^{-1} (e.g. Anneser et al., 2008; Anneser et al., 2010; Bulatović et al., 2021; Griebler et al., 2004; Meckenstock et al., 2010), urban groundwater concentrations are generally more in the μgL^{-1} range (e.g., Han et al., 2013; Liu et al., 2020; Ilić et al., 2021). Similarly, chlorinated solvents at industrial megasites may exhibit concentrations up to the lower mg L^{-1} range (e.g. Wycisk et al., 2003). Frequently, contamination levels in urban groundwater exceed WHO limits (Gesels et al., 2021).

3.3.2. CECs in urban aquifers

Other groups of organic chemicals are summarized as contaminants of emerging concern (CECs), summarizing chemicals which were detected only recently and are not yet regulated but may be of future concern (Bunting et al., 2021). CECs include pesticides, pharmaceuticals, personal care products, fragrances, water treatment by-products, drugs, artificial sweeteners, among others (Richardson and Ternes, 2011), and mainly originate from effluents of waste water treatment plants, hospital effluents, private and public gardens/parks, landfills and livestock farming (Li et al., 2021; Mojiri et al., 2020; Stuart et al., 2012). CECs are not effectively removed by water treatment processes and are therefore released to the aquatic environment and infiltrate into shallow urban aquifers (Richards et al., 2021; Stuart et al., 2012). The top ten CECs in European groundwater were recently compiled by Bunting et al. (2021), i.e. carbamazepine, caffeine, sulfamethoxazole, bisphenol-A (BPA), ibuprofen, acetaminophen, N,N-diethyl-m-toluamide, diclofenac, chlorobenzene. A similar set of compounds is found in urban groundwater all over the world with local and regional particularities (e.g., higher fraction of pesticides and or drugs) (Koroša et al., 2016; Pinasseau et al., 2019; Sharma et al., 2019).

Pesticides in this context are of particular importance. Based on their mode of effect, they are classified into four main groups, namely fungicides, herbicides, insecticides and others (miticides, algaecides) (Mojiri et al., 2020). Especially in the vicinity of agricultural areas and due to their application in urban gardens and parks, pesticides can reach high concentrations in urban groundwater, as it is shown for Patna City, India (Richards et al., 2021), Maribor, Slovenia (Koroša et al., 2016), Lyon, France (Pinasseau et al., 2019) or Doncaster, U.K. (White et al., 2016). Even pesticides that are not authorized (anymore), like atrazine, propazine and simazine, are regularly found at elevated concentrations (2–230 ng L^{-1} (Koroša et al., 2016)).

3.3.3. Nitrate in urban aquifers

High nitrate concentrations are one of the primary causes of groundwater pollution. Nitrate is characterized by a high solubility, mobility in soils and sediments, and stability at oxic conditions (Zhang et al., 2015). It becomes toxic for humans at high concentrations and the drinking water threshold is set at 50 mg L^{-1} (WHO, 2011). Urban sources of nitrate are irrigation runoff from fertilized urban green areas/public gardens, animal waste, leaching of landfills, domestic waste, industrial wastewater, leaking sewage pipelines, and atmospheric depositions (Gu et al., 2013; Guimarães et al., 2019; Hosono et al., 2011; Ren and Zhang, 2020; Wakida and Lerner, 2005). Especially in areas with poor sanitation where pit latrines and septic tanks are common, elevated nitrate levels can be observed (Walraevens et al., 2015). In oxygenated aquifers, nitrate may originate from nitrification of ammonia. When conditions shift to hypoxic and anoxic, nitrate is biologically reduced to N_2O and N_2 (denitrification) or ammonia (dissimilar nitrate reduction to ammonia, DNRA).

Groundwater can be affected very seriously by human activity, with gradually increasing nitrate levels shown to accompany urbanization and economic development, even if concentrations were within limits set by the World health Organization (WHO) or only single wells exceeded these limits (e. g. Chicago, Lisbon, Milan, and Karlsruhe) (De Caro et al., 2017; Gu et al., 2013; Hwang et al., 2015; Koch et al., 2021; Martin Del Campo et al., 2014; Ren and Zhang, 2020; Teixeira et al., 2018). With concentrations of 89.52 (Shijiazhuang), 93.2 (Barcelona), 145 (Manila) or 60 (Porto) mg L^{-1} on average, groundwater in these cities is in an alarming state and certainly not suitable for drinking purposes (Guimarães et al., 2019; Hosono et al., 2011; Jurado et al., 2013; Zhang et al., 2019).

3.3.4. Ammonium in urban aquifers

Natural levels of ammonium are typically below 0.2 mg L^{-1} (WHO, 2011) and increased concentrations are usually caused by faecal pollution from sewage leakages, pit latrines, industrial and domestic wastewaters, fertilization, livestock-farming, and landfill leachates (Bruce and McMahon, 1996; Ikem et al., 2002; Kurilic et al., 2015; Zhang et al., 2015). Thresholds for drinking water quality are set at 0.5 mg/l (EU (European Union), 2020). Moreover, contrary to nitrate, ammonium is mainly present in reducing conditions in aquifers, since nitrification may lead to a transformation to nitrate under oxic conditions. High ammonium levels correlating with low levels of dissolved oxygen were observed in urban and industrial areas (Bruce and McMahon, 1996; Jurado et al., 2013).

Ammonium pollution in urban aquifers is of importance, as the following values indicate: some sites in Lisbon (0–2.04 mg L^{-1} (Teixeira et al., 2018)), Milan (0.1–43 mg L^{-1} (De Caro et al., 2017)), Krakow (1.957–2.618 mg L^{-1} (Binkowski et al., 2017)) or Temerin (0.07–4.2 mg L^{-1} (Kurilic et al., 2015)) exceeded national limits or natural background values. Especially urban and suburban areas where adequate sanitary conditions, infrastructure for local water supply and waste water treatment is lacking, high ammonium levels can frequently be detected, as observed in Serbia (Petkovic et al., 2011), Zimbabwe (Gumindoga et al., 2019), or Zambia (De Waele et al., 2004).

3.3.5. Effects on groundwater fauna

Data available on the ecotoxicological effects of organic pollutants to stygobiontic fauna are extremely scarce and interpretation is difficult. Most toxicity studies with organic chemicals and stygobionts were carried out with pesticides including α-endosulfan, 3,4-dichlorophenol, aldicarb, Ariane™, chlorpyrifos, desethylatrazine, Imazamox, penthaclorophenol, S-metolachlor and thiram. Sensitivity of groundwater organisms was highly variable and highest sensitivities were observed with the substances compiled in Table 1. For example, *N. rhenorhodanensis* showed high survival with the highest concentration of S-metolachlor and desethylatrazine tested, which exceeded concentrations typically found in the environment (Maazouzi et al., 2016). The authors therefore concluded that there is no short-term threat of those two pesticides to groundwater fauna. In comparison, *Parastenocaris germanica* was much more sensitive to pentachlorophenol or aldicarb (Table 1). Moreover, juveniles tended to be more sensitive and differences between higher and lower groundwater crustaceans, with respect to their organismic complexity, were found with the latter one seeming to be less tolerant (Notenboom and Boessenkool, 1992; Schäfers et al., 2001). Groundwater crustaceans reacted with a delay or were less sensitive to individual pesticides, when compared to related epigean species on the basis of metabolic activity measurements (Schäfers et al., 2001). Taking the slow metabolism of groundwater fauna into account, it is a specific deficit that data from chronic ecotoxicity tests are missing completely.

Volatile organic compounds (VOCs), such as toluene (acute LC50 64–68 mg L^{-1}) or BPA are obviously highly tolerated (LC50 - 24 h = 6 mg L^{-1}, respectively) by selected groundwater invertebrates in acute tests (Gerhardt, 2019). However, the few compounds tested are not at all representative for the vast diversity of VOCs present in urban groundwater, and again, information from chronic tests is extremely scarce (e. g., toluene chronic LC50 36–50 mg L^{-1}; Avramov et al., 2013).

Also pharmaceuticals like propranolol and diclofenac were tolerated at high concentrations by respectively one stgyobiont species *(Diacyclops belgicus* (Di Lorenzo et al., 2019a), *Nitocrella achaia*Di Lorenzo

Table 1
Acute and chronic LC50 (μg L^{-1}) for tested organic chemicals of stygobiontic species, values for normoxic/anoxic habitat conditions.

Name	Class	Tested stygobiont	LC50 acute	LC50 chronic
S-Metolachlor[1]	Pesticide-herbicide	*Niphargus rhenorhodanensis*	36,900 ($n = 2$)	
Desethylatrazine[1]	Pesticide	*Niphargus rhenorhodanensis*	112,300	
Chlorpyrifos[2]	Pesticide-insecticide	*Parastenocaris germanica*	57 ($n = 1$)	
Pentachlorophenol[2]	Pesticide-insecticide	*Parastenocaris germanica* copepodites adults	11 ($n = 4$) 90–160/90–120	
Aldicarb[2]	Pesticide	*Parastenocaris germanica* copepodites adults	360 2999/<1000	
PCP[3]	Pesticide	*Parastenocaris germanica*	36	
3,4-Dichlorophenol[3]	Pesticide	*Parastenocaris germanica*	4600	
Cyprodinil[4]	Pesticide-fungicide	*Niphargus fontanus*	2430	940
		Chappuisius inopinus	1320	170
Lambda-Cyhalothrin[4]	Pesticide-insecticide	Higher crustaceans e.g. *Niphargus fontanus*	300–595	42–124
		Lower crustaceans e.g. *Chappuisius inopinus*	277–4245	15–548
Bromoxynil[4]	Pesticide-herbicide	*Niphargus fontanus*	93 – >160	
		Chappuisius inopinus	113	
Toluene[5]	VOCs	*Niphargus inopinatus*	63,500–67,900 ($n = 2$)	35,500–55,300
Propranolol[6]	Pharmaceuticals	*Diacyclops belgicus*	4900 ($n = 1$)	
Diclofenac[7]	Pharmaceuticals	*Nitocrella achaiae*	12,000 ($n = 1$)	
BPA8	Diphenylmethane deriv.	*Proasellus slavus*	6300 ($n = 5$)	100
		Niphargus casparyi	12,300 ($n = 5$)	1000
NH_4^+/Imazamox9	Mixture	*Diacyclops belgicus*	2710	

1: Maazouzi et al. (2016), 2: Notenboom and Boessenkool (1992), 3: Notenboom et al. (1992) 4: Schäfers et al. (2001), 5: Avramov et al. (2013), 6: Di Lorenzo et al. (2019a), 7: Di Lorenzo et al. (2021a), 8: Gerhardt (2019), 9: Di Marzio et al. (2018)

et al., 2021a)) and a groundwater population of *Diacyclops crassicaudis crassicaudis* (Castano-Sanchez et al., 2021), especially by adult animals, and the substances may pose no or a moderate risk to European groundwater bodies (Di Lorenzo et al., 2019a)(Di Lorenzo et al., 2021a). Observed surface and groundwater concentrations of diclofenac, on the other hand, are between 0.0005 and 0.5 mg L^{-1} and already cause sublethal effects to crustaceans, which imply a risk of this substance to stygobionts(Castano-Sanchez et al., 2021).

Reviewing field studies at sites with intense agricultural use, ammonium contamination seems to affect the whole stygobiontic copepod assemblage, with the most affected species being *Diacyclops italianus* and *Nitocrella psammophila* in bores with higher ammonium concentrations (>0.032 mg L^{-1}) (Di Lorenzo et al., 2015a; Di Lorenzo et al., 2014). The value of 0.032 mg L^{-1} NH_4^+ was estimated by Di Lorenzo et al. (2014) to be the chronic lethal concentration for *Diacyclops belgicus*. In the same study, *D. belgicus* was also more sensitive than the epigean *Eucyclops serrulatus* to acute and chronic ammonium exposure, but showed similar sensitivity as the epigean *B. echinatus* (Fig. 3c) (Di Marzio et al., 2009). Due to synergistic effects, ammonium was even more toxic to stygobionts when combined with the herbicide Imazamox (Di Marzio et al., 2018). In another study, *Metacrangonyx spinicaudatus* had a higher sensitivity to ammonium chloride than *Typhlocirolana haouzensis* (Fig. 3c) (Boutin et al., 1995).

Di Lorenzo et al. (2015b) noticed that temperature affects the toxicity of ammonia. The stygoxene cyclopoid *E. serrulatus* showed chronic lethality at 1 mg L^{-1} NH_4^+ (Di Lorenzo et al., 2014), with increasing sensitivity under warmer conditions (LC50-96 h at 15 °C: 52 mg L^{-1} NH_4^+, at 18 °C: 24.76 mg L^{-1} NH_4^+) (Di Lorenzo et al., 2015b; Di Lorenzo et al., 2014). Ammonium has an impact on the growth and survival via the disruption of various physiological mechanisms (osmoregulation, immunology, acid/base balance, gas exchange) and induction of oxidative stress, pathogenic susceptibility and histopathological damage (Romano and Zeng, 2013).

Only few ecotoxicological studies with nitrate and stygobionts are available. Gerhardt (2020) examined a slightly increased mortality (from 50 to 100 mg L^{-1} nitrate) with chronic (5 weeks) nitrate exposure for the stygobiont amphipod *Niphargopsis casparyi*. *Proasellus slavus* indicated no sensitivity to nitrate. Nevertheless, mortality never reached 50%. Also, ventilatory activity was significantly reduced, whereas locomotor activity was independent upon nitrate levels. Furthermore, effects of potassium nitrate on survival rates of three stygobiont species *P. slavus vindobonensis*, *Metacrangonyx* n. sp. and *Typhlocirotana* n. sp. were merely moderate (El Abiari et al., 1998; Mösslacher and Notenboom, 1999), with LC50-96 h values above 50 mg L^{-1}. These results suggest that groundwater crustaceans may not be as sensitive to nitrate as expected.

Similar observations were made in field studies in Italy and the French Pyrénées, which revealed that even at sites heavily contaminated by nitrate, copepod or crustacean assemblages as a whole were highly diversified, and nitrate concentrations up to 150 mg L^{-1} had low effect on the stygobiontic community (Di Lorenzo et al., 2021b; Di Lorenzo and Galassi, 2013; Dumas et al., 2001; Dumas and Lescher-Moutoué, 2001; Galassi et al., 2009). However, Di Lorenzo et al. (2021b) also described low juvenile-to-adult and male-to-female ratios and altered copepod assemblages. Reasons may be, that the long-term nitrate pollution has induced the disappearance of sensitive species and the following exploitation of stygophile species. Again, juveniles may be more sensitive than adult animals (Di Lorenzo et al., 2021b).

Often, abundance and biomass did not correlate with physicochemical parameters or organic variables, concluding that nutrients only play a minor role as drivers for groundwater fauna (Di Lorenzo et al., 2015a; Dole-Olivier et al., 2009; Dumas et al., 2001; Galassi et al., 2009; Marmonier et al., 2018). Consistent with these findings, Stein et al. (2010) concluded that the effect of nitrate on groundwater fauna is indirect, displaying the correlation with groundwater/surface water interactions and land-use impacts.

3.4. Metal pollution

Metals and metalloids are present in soils and water in colloidal, particulate or dissolved form. Some of the most common metals in groundwater include iron (Fe), lead (Pb), mercury (Hg), cadmium (Cd), copper (Cu), chromium (Cr), manganese (Mn), zinc (Zn), nickel (Ni), and the metalloid arsenic (As). Groundwater naturally contains metals and although individual metals (including some heavy metals) are essentially required at low concentrations by (micro)organisms, at elevated concentrations these can have (neuro-)toxic, carcinogenic, mutagenic or teratogenic effects on organisms including microbes, fauna and humans (Momodu and Anyakora, 2010). Therefore, the WHO (2011) has evaluated maximum contaminant levels (mg L^{-1}) for critical metals in drinking water (Fig. 4).

Main causes for metal contamination and accumulation in groundwater today are urbanization, industrialisation, agriculture, and consequently acidification, which are factors that often appear side by side and can therefore interact with each other (Kumar et al., 2012). In urban areas, metals enter groundwater bodies mainly through road run-off, corrosion of building materials, spills (industrial/domestic waste) and surface water, with areas near to pollution sources as well as older and inner parts of cities being the most impacted (Kumar et al., 2012; Santos et al., 2002; Wieczorek et al., 2020). Analyses of urban soils have shown that the level of metal contamination can be connected to traffic volume and distance to roads (Wang et al., 2016). Urban agricultural areas (e.g., gardens, parks) impact groundwater through the application of pesticides/fertilizers, since these agents contain metals and alter groundwater properties like the pH, leading to higher mobility of metals (Khan et al., 2018).

Depending on the hydrogeology, the composition and concentrations of metals in groundwater is very heterogeneous in space and time. Some metals (e.g., Fe, Mn) can bind and release trace metals by changing from solid (under oxic conditions) to soluble state (under reducing conditions or low pH), making the redox conditions and pH important controlling factors (Vesper, 2019; Vranković et al., 2017). However, other influencing factors are CO_2, the partial pressure of O_2, the impact of microbial activity and the presence of organic compounds (e.g., humics), and other pollutants including nutrients (Hosono et al., 2011; Khan et al., 2018; Vesper, 2019).

Although a generalised ranking of the presence of metals in groundwater cannot be achieved due to the different geological settings and pollution situations worldwide, there are differences in the solubility and association of metals with each other. Santos et al. (2002) observed a high mobility for Zn (most soluble tested metal in water) and Cd, intermediate mobility for Cu and low mobility for Pb. Furthermore, Kumar et al. (2012) revealed positive correlations between the appearances of Cd, Cr and Cu as well as Pb and Zn in urban groundwater. The composition, mobility, toxicity and bioavailability of metals in groundwater is associated with the speciation, origin and age of the metals and other compounds (e.g., phosphates) and is, especially in urban areas, dependent on the type of industrial influence (Santos et al., 2002).

3.4.1. Effects on groundwater fauna

Since several metals can have highly toxic effects on organisms at low concentrations, they are the most tested contaminants in subterranean environments (Castano-Sanchez et al., 2020a; Momodu and Anyakora, 2010). The assimilation of essential metals, including some heavy metals (e.g., Fe, Mn, Cu and Zn) is obligatory at low levels, since they are needed for growth and energy metabolism; e.g., metals are frequent components of proteins (Momodu and Anyakora, 2010; Vranković et al., 2017). Stygofauna can reduce the availability of metals in solution in the groundwater ecosystem to a small extent due to their ability of bioaccumulation (Canivet and Gibert, 2002). It has been observed that cave-dwelling arthropods exhibit higher accumulation rates for nonessential metals than for essential metals, which makes them potential

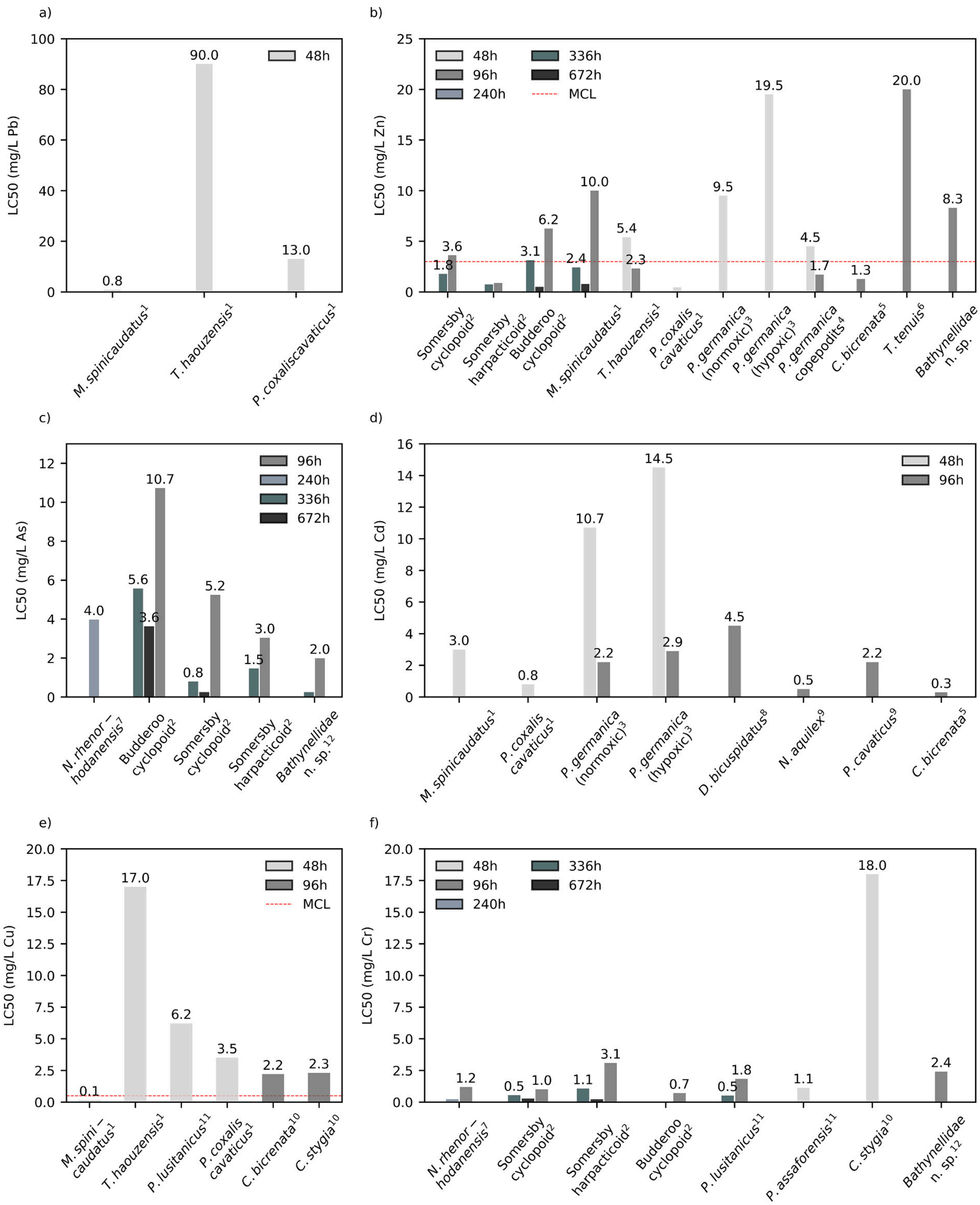

Fig. 4. Lethal concentrations at which 50% of the tested individuals died (LC50) for the six metals: a) lead (Pb), b) zinc (Zn), c) arsenic (As), d) cadmium (Cd), e) copper (Cu), and f) chromium (Cr); MCL – maximum concentration level defined in WHO (2011) for drinking water standards.
1: Boutin et al., 1995, 2: Hose et al., 2016, 3: Notenboom et al., 1992, 4: Notenboom et al., 1992, 5: Bosnak and Morgan, 1981a, 1981b, 6: Meinel and Krause, 1988, 7: Canivet et al., 2001, 8: Mösslacher and Notenboom, 1999, 9: Meinel et al., 1989, 10: Bosnak and Morgan, 1981a, 1981b, 11: Reboleira et al., 2013, 12: Hose et al., 2019

bioindicators for groundwater quality (Vranković et al., 2017).

In several laboratory studies, hypogean species were found to be less sensitive and to have a greater tolerance to metals than epigean species. Reasons for this could be that groundwater species have lower metabolisms, lower assimilation rates, lower growth and reproduction rates, and increased longevity (Canivet et al., 2001; Canivet and Gibert, 2002; Krupa and Guidolin, 2003; Plénet, 1999). Toxicity in groundwater species also increases with exposure time, and chronic stress is assumed to impact the durability of groundwater populations (Hose et al., 2016). Furthermore, the harsh conditions in underground habitats may be reasons for groundwater species being potentially more prone to devastating effects of metal contamination in the long run (Hose, 2005; Mösslacher and Notenboom, 1999).

Metal toxicity data are available for five groups of groundwater invertebrates, namely Amphipoda, Isopoda, Copepoda, Syncarida, and Oligochaeta; the metals that have been tested are As, Cd, Cu, Cr, Pb, and Zn (Fig. 4). Toxicity values of stygofauna within and between taxonomic groups are generally variable as the sensitivity of the individual species originating from different habitats is diverse and there are mostly no correlations found between water quality, taxon, and sensitivity to metals (Hose et al., 2016). However, some general patterns have been observed. In groundwater Copepoda, Cr was the most toxic metal in comparison to As and Zn (Hose et al., 2016). Canivet et al. (2001) reported that the crustacean *N. rhenorhodanensis* was more sensitive to Cr (LC50-96 h: 0.23 mg L^{-1} Cr) than to As (LC50-96 h: 3.97 mg L^{-1} As). In syncarids (*Bathynellidae* n. sp.), Hose et al. (2019) found As (LC50-336 h: 0.25 mg L^{-1} As) to be the most toxic metal followed by Cr (LC50-336 h: 0.51 mg L^{-1} Cr) and Zn (LC50-336 h: 1.77 mg L^{-1} Zn). The syncarids furthermore regulated Zn accumulation at exposure concentrations below 1 mg Zn/L with increased body concentrations and mortality above that level, while accumulation of As was not regulated. Additionally, Krupa and Guidolin (2003) found that groundwater amphipods (*Niphargus montellianus*) are more tolerant to Zn than to Cd, and they are most sensitive to Cu. Cr was the only metal that hypogean fauna was more sensitive to than epigean fauna in laboratory tests (Fig. 4).

The LC50/LC100 values did not differ greatly between three amphipod species *M. spinicaudatus*, *N. rhenorhodanensis* and *N. aquilex*, aside from one value which was Zn for *N. aquilex* (180 mg L^{-1}). The other two amphipods showed the least sensitivity to Cd (3–4.5 mg L^{-1} Cd) and As (3.97 mg L^{-1} As) in comparison to Cr (0.23–1.18 mg L^{-1} Cr), Pb (0.8 mg L^{-1} Pb), and especially Cu (0.15 mg L^{-1} Cu) and Zn (0.45 mg L^{-1} Zn), since the LC values for these were below the maximum contaminant level that is suggested for drinking water by the WHO (Fig. 4) (Boutin et al., 1995; Canivet et al., 2001; Meinel and Krause, 1988; Meinel et al., 1989; Plénet, 1999).

The genus that was tested most extensively in Isopoda was *Proasellus* including five species (*P. lusitanicus*, *P. assaforensis*, *P. cavaticus*, *P. coxalis cavaticus* and *P. slavus vindobonensis*), followed by *Caecidatea* including two species (*C. bicrenata* and *C. stygia*) and *Typhlocirolana haouzensis*. *T. haouzensis*, thereby, was observed as the most tolerant species over all with comparatively high LC50-48 h values for Cd (150 mg L^{-1} Cd), Cu (17 mg L^{-1} Cu) and Pb (90 mg L^{-1} Pb) and equally high values for Zn (19.5 mg L^{-1} Zn) compared to the other isopod species (Fig. 4). Furthermore, Isopoda generally seem to be less sensitive to Zn (9.5–127 mg L^{-1} Zn) than Amphipoda (0.45–180 mg L^{-1} Zn) and Copepoda (0.5–10 mg L^{-1} Zn). For Cd, Cr, Cu and Pb the values were highly variable between isopod species as well as Isopoda and other groups (Fig. 4) (Bosnak and Morgan, 1981a, 1981b; Boutin et al., 1995; Meinel and Krause, 1988; Meinel et al., 1989; Mösslacher and Notenboom, 1999; Reboleira et al., 2013).

With respect to Copepoda, which are generally the most abundant group in groundwater, many species were tested for metal toxicity, including the stygobite *Parastenocaris germanica* (incl. LC values for copepodites) (Notenboom and Boessenkool, 1992; Notenboom et al., 1992), two undetermined groundwater species of Cyclopoida and one species of Harpacticoida (Hose et al., 2016), stygophile populations of *Acanthocyclops vernalis, Diacyclops bicuspidatus, Megacyclops viridis* (Mösslacher and Notenboom, 1999), and *Bryocamptus echinatus* (Di Marzio et al., 2009), as well as stygoxene species including *Attheyella crassa* (Di Marzio et al., 2009), *Bryocamptus zschokkei* (Burton et al., 2002), *B. pygmaeus*, and *B. minutus*, all belonging to the genus *Bryocamptus* (Di Marzio et al., 2009). In comparison to other stygofauna groups, the LC values for Copepoda were in close range of the different species tested, indicating comparable metal tolerance. Toxicity for all tested species increased with test duration (Fig. 4). Furthermore, the tolerance of *B. zschokkei* for Cu (0.29 mg L^{-1} Cu) and of a harpacticoid species for Cr in 14- and 28- day experiments (0.03 and 0.02 mg L^{-1} Cr) as well as the EC50/LC50 values for Zn in several groundwater crustaceans including amphipods, copepods and syncarids were below the maximum contaminant levels for drinking water recommended by the WHO (2 mg L^{-1} Cu, 0.05 mg L^{-1} Cr and ~ 3 mg L^{-1} Zn) (Fig. 4) (Burton et al., 2002; Di Marzio et al., 2009; Hose et al., 2019; Hose et al., 2016; Notenboom and Boessenkool, 1992; Notenboom et al., 1992).

For the group Oligochaeta, only one groundwater species, *Trichodrilus tenuis*, has been tested. Tested metals (LC50/96 h) were Cd, of which the tolerance in *T. tenuis* was relatively low (1.05 mg L^{-1} Cd) compared to the other groundwater invertebrates, and Zn (8.3 mg L^{-1} Zn) of which the value was within the highly variable range between the groups (Fig. 4) (Meinel and Krause, 1988; Meinel et al., 1989).

3.5. Oxygen depletion

3.5.1. Dissolved oxygen in urban aquifers

In natural aquifers, dissolved oxygen (DO) levels are spatially heterogeneous at macro-, meso- and microscales. They also reflect sediment composition and structure, groundwater flow velocity, mineralogy and organic matter content, as well as abundance and activity of organisms (Malard and Hervant, 1999). Usually, groundwater is undersaturated with dissolved oxygen, and DO with concentrations below 0.1 mg/l is suspected to be a limiting factor for groundwater fauna (Strayer, 1994). For confined aquifers, Malard and Hervant (1999) indicated gradients between 9×10^{-5} and 1.5×10^{-2} mg L^{-1} O_2 m^{-2}, whereby no replenishment of oxygen from the surface is possible. In parafluvial aquifers, oxygen levels range from 2×10^{-2} to 1 mg L^{-1} O_2 m^{-2}, and in unconfined aquifers oxygen consumption can be compensated by DO replenishment from the atmosphere by groundwater recharge. Oxygen is more rapidly consumed if the groundwater table is near the surface, due to the degradation of soil dissolved organic carbon. If the groundwater table is far from the surface, DO is gradually consumed along the direction of flow (Malard and Hervant, 1999).

It is assumed that DO levels near industrial facilities, agricultural areas and urban areas are lower than in natural regions due to pollution and anthropogenic effects, which enhance chemical and biological oxygen consumption (Griebler et al., 2014; Kunkel et al., 2004). In the city of Karlsruhe, Germany, for example, high average DO levels of 7.96 ± 2.95 mg L^{-1} were measured in groundwater below forest areas ($n = 8$) and significant lower levels of 4.47 ± 2.31 mg L^{-1} in wells in the city centre ($n = 31$) (Koch et al., 2021).

3.5.2. Reaction of groundwater fauna

Low DO concentrations and oxidative stress are habitual to groundwater organisms (Malard and Hervant, 1999). Under anaerobiosis, the production of reactive oxygen species (ROS) is enhanced and results in cell damage (Lawniczak et al., 2013). To prevent damage, organisms have developed non-enzymatic and enzymatic antioxidant mechanisms (Simčič and Sket, 2021). Low metabolic rates of subterranean organisms may be favourable in such oxygen limited systems.

Stygobiont species show high survival rates under low DO levels (0.01–0.5 mg L^{-1}) (Danielopol, 1989; Malard and Hervant, 1999; Notenboom et al., 1992) and are able to cope with hypoxic to normoxic conditions (Mösslacher, 2000). For example, the copepod *Acanthocyclops* n. sp., sampled from a hypoxic habitat, had only small metabolic differences compared to the copepod *Diacyclops bicuspidatus* from a normoxic condition habitat. The stygobiont *Niphargus stygius* had a lower oxygen consumption in recovery after anoxia than the stygophile *Niphargus zagrebensis*. Also, oxygen consumption after hypoxia at 20 °C was greater in the stygobiont species than at 10 °C (Simčič and Sket, 2021).

Hypogean species tend to be less sensitive to hypoxic conditions and show lower metabolic rates than closely related surface organisms. This was shown for example for the hypogean species *Niphargus* n. sp. compared to the epigean species *Gammarus* n. sp. (Danielopol, 1989), or the hypogean copepod *D. belgicus* compared to the epigean *Eucyclops serrulatus* inhabiting the same aquifer (Di Lorenzo et al., 2015c). These results are in agreement with the findings of Wilhelm et al. (2006) who detected 2–4.5 times lower oxygen consumption rates in the stygobite *Gammarus acherondytes* than in the stygophile species *Gammarus troglophilus*. Hypogean and epigean populations of *Gammarus minus* on the other hand showed similar survival times and metabolic responses under hypoxic conditions (Hervant et al., 1999), both not being very tolerant to DO depletion.

The assumption may be, that hypogean species are able to regulate their respiration, which is highly efficient in environments with fluctuating oxygen levels. Lawniczak et al. (2013) perceived a very fast and effective system to cope with anoxic conditions in *N. rhenorhodanensis*. The animals were able to restore their cells' oxidative balance very quickly after hypoxia and therefore avoid oxidative cell damage due to free radicals. However, studies indicated that this is not universally the case for subterranean organisms and also demonstrated that metabolic rates are related to oxygen availability and/or the energetic state of the ecosystem (Malard and Hervant, 1999; Mösslacher and Creuzé Des Châtelliers, 1996). .

4. Synopsis

Urban aquifers are huge, complex and mostly unexplored ecosystems harbouring a vast biodiversity of microorganisms and invertebrates. These organisms are responsible for providing a diverse collection of essential ecosystem services and may be endangered by the various stressors associated with the concentrated human activity in urban areas. Here, not only single pollutants pose a risk to the groundwater organisms, but multiple stressors and contaminant mixtures can cause dramatic changes in groundwater quality and environmental conditions, turning the habitat hostile to groundwater communities. Especially, metals, a range of organic chemicals and elevated temperatures are stressors present in every city's subsurface and therefore impose a serious risk to the vulnerable groundwater ecosystems. Due to their persistence, organic chemicals introduced to groundwater may remain for decades and are toxic to non-targeted organisms even at low concentrations (Li et al., 2021). Some organic chemical (e.g., BTEX) show a high mobility with groundwater (Bulatović et al., 2021) and an increase in temperature will further accelerate the solubility and mobility of many organics (Niculae et al., 2018). As a consequence of increased temperatures and organic pollution, low levels of dissolved oxygen are very common to occur in urban groundwater with conditions frequently switching from oxic to hypoxic and anoxic (Brielmann et al., 2011). This is resulting in a highly stressful environment for groundwater invertebrates. In urban areas, point source pollution, such as sewage leakage, may play an important role at a very local scale with altered faunal communities (Notenboom et al., 1995; Scarsbrook and Fenwick, 2003; Tione et al., 2016). For example, higher numbers of Oligochaeta as well as Microturbellaria and Nematoda – which are known to cope with less favourable conditions, low DO contents and higher temperatures (Hahn et al., 2013) – were spotted in urban wells compared to wells in forest areas in the city of Karlsruhe, Germany (Koch et al., 2021).

Stygobionts have shown to react sensitively to some of these stressors, with increased mortality and changes in community diversity. Yet the few data available are often inconclusive and especially testing of chemical mixtures or long-term chronic tests are missing. By this, the conclusions that can be drawn so far are affected by a high uncertainty.

In field and laboratory studies, stygobiontic invertebrates showed high sensitivity to ammonium and organic contaminants including a wide range of pesticides. Due to accumulation of these pollutants in groundwater organisms (invertebrates and microbes) and interactions with other substances, the whole ecosystem functioning may be affected. For example, Michel et al. (2021) observed an inhibition of denitrification due to ESA-metolachlor (10 μg L^{-1}) and propiconazole, as well as 1,2,4-triazole (2 and 10 μg L^{-1}), which are metabolites of the pesticide S-metolachlor. Thereby, the reduction of nitrate to nitrite was affected on the protein level in microorganisms.

Also, the toxicity of several metals (e.g., LC50 values below the WHO drinking water limits for Cr, Cu, and Zn observed in some Copepoda, Amphipoda, and Synacrida) and the sensitivity to mid- to long-term groundwater temperature increases or repeated heat stress may be of high risk for urban stygobionts. Groundwater organisms with narrow tolerance breadths are likely to be at risk at increasing habitat pollution and will be replaced by competitive stronger (surface) relatives, which may result in altered structures of trophic chains and related ecosystem processes (Mermillod-Blondin et al., 2013). Little is known about these complex interactions involving microorganisms and invertebrates and potentially affecting groundwater ecosystem services. Several studies targeting surface ecosystems demonstrated that a reduction in biodiversity has negative effects on ecosystem functions (Boulton et al., 2008; Loreau et al., 2001), and same may be expected for groundwater habitats. Moreover, in the subsurface, extinction of species will not be easily reversible, as groundwater ecosystems are highly fragmented, harbouring extremely rare and often endemic species.

Physiological and biogeochemical responses in organisms which are temperature dependent are also linked to increasing temperature in urban groundwater (Hochachka and Somero, 2002). Increasing temperatures stimulate metabolic rates (Lushchak, 2011; Simčič and Sket, 2021). Consequently, an elevated temperature in combination with organic pollution increases the pollution uptake as observed for juveniles of *E. serrulatus* by Di Lorenzo et al. (2015b). Also, juveniles seem to be more sensitive than adults. This may be due to a thinner exoskeleton (pollutant uptake mainly through the body surface) and higher metabolic rates compared to adults (elevated influx rate).

No or just a low risk seems to come from low oxygen levels, for which stygofaunal animals were shown to be very tolerant to. Studies have shown that oxygen levels below 0.01 mg L^{-1} are lethal to stygobionts in the long run, even though stygobiont species are much more tolerant to low oxygen levels than epigean species and have evolved strategies to cope effectively with hypoxic conditions. Nevertheless, due to fast oxygen depletion occurring with high amounts of organic matter and stimulated microbial metabolisms (due to higher temperatures for example), anoxic zones in urban aquifers may arise. With these, invertebrates will be eliminated and anaerobic microbial metabolisms will prevail. Toxic by-products (e.g., hydrogen sulphide) of such processes will additionally impair groundwater quality. Those zones typically occur only locally in urban aquifers but can easily spread to larger areas due to import of organic chemicals and heat. Fauna in groundwater is reported within a wide range of DO levels. Several studies indicated DO levels as well as land-use to be drivers of faunal assemblage patterns and shifts in niche occupation (Dole-Olivier et al., 2009; Dumas and Lescher-Moutoué, 2001; Hahn, 2002; Marmonier et al., 2018; Tione et al., 2016). For example, Koch et al. (2021) found a significant correlation between the number of taxa and the DO levels in the city of Karlsruhe, Germany.

For nitrate, altered juvenile-to-adult and female-to-male ratios were detected, indicating that long-term nitrate pollution may have altered the stygofaunal assemblage as a whole and less tolerant species may have vanished over time, which is also likely to occur in cities existing and developing for decades. Many field studies also observed the vanishing of stygobiont species in aquifers with high salinity, indicating the high sensitivity of groundwater invertebrates (Castano-Sanchez et al., 2020b), which may also be true for long-term contaminated urban aquifers.

5. Further perspectives and conclusions

It is worth mentioning that there are no established standard methods for sampling and toxicity testing or representative groundwater test organisms, as critically discussed by Di Lorenzo et al. (2019b). Due to the difficulties in proper feeding and cultivation of stygobionts in the laboratory, they worked out ten recommendations for toxicological testing of stygobionts including, for example, acclimation times of at least 48 h before testing, test durations including the standard times but also exceeding 96 h, no feed throughout short term testing, or species identification of tested stygobionts.

Compared to the wide range of toxicants and pollution sources in urban areas, only a small number of contaminants was intensively tested until now. Especially the effect of microplastics on groundwater organisms is still unclear. Microplastics are already found widely in groundwater with amounts of 16 pieces/L (Campos and Pestana, 2020) or 15.2 pieces/L in karst groundwater systems (Panno et al., 2019). Despite the fact that microplastic were found in 93% of 260 drinking water sources in 11 countries (Marsden et al., 2019), their impact on the environment and organisms is rarely discovered (Chia et al., 2021). The destructive effect on soil organisms (earthworms, mites, nematodes, snails) includes metabolism disruption, increased mortality, decreased growth rate, or damage of gut (Chia et al., 2021; Huang et al., 2021). Microplastic can originate from landfills, sewage sludge, fertilizer (covering), plastic covering, littering or dumping. In the future, more research is needed to identify sources, transport pathways, incorporation in organisms, and adsorption of toxic substances (Chia et al., 2021). Also, typical CECs in urban groundwater like a variety of drugs, pesticides, food additives, and personal care products need more research regarding effective sampling and analysing techniques and their effects on stygofauna (Tang et al., 2020). In particular studies observing the effects of contaminant mixtures and other superpositioning factors typical for urban subsurface are rare. Furthermore, increasing temperatures revealed to affect the sensitivity to the possible pollutants.

Due to observed differing bioaccumulation rates of contaminants and sensitivities as well as lower oxygen consumption rates of stygobionts (Canivet et al., 2001; Di Lorenzo et al., 2014; Di Marzio et al., 2009; Di Marzio et al., 2018; Schäfers et al., 2001; Wilhelm et al., 2006) in comparison with surface relatives, but also within stygobiontic species, it may be important to measure a variety of metabolic processes and by-products for a better comparison and understanding of organisms′ sensitivities.

For improvement of knowledge, field surveys must include the sampling of fauna, routinely record of physio-chemical and microbiological parameters. In general, the knowledge about the origin, fate and distribution of urban contaminants and hydraulic properties of urban aquifers needs to be improved by constant monitoring and modelling of current and possible future conditions (Miller et al., 2020). Until now, only one study has tried to acquire an absolute understanding of urban groundwater characteristics and their influence on the groundwater ecosystem. By this, Koch et al. (2021) found no significant correlations between physico-chemical characteristics and biodiversity patterns of the stygofaunal community. Contrastingly, differences in spatial distributions were supported. The future goal is to increase the number of such studies to gain knowledge about the effects of urban pollution and if biological parameters are suitable for groundwater quality monitoring even in urban areas. One main question is also if existing thresholds are needed to improve, or if new thresholds for example for temperature pollution have to be defined. For this, detailed assessments and validation of existing thresholds of different contaminants based on intensive field studies and toxicity tests have to be carried out. Also, research and management strategies need to consider the interactions between different realms and that pressures occurring in one realm can have impacts on another. For example, groundwater extraction or surface sealing can result in a loss or change of connectivity of water bodies and with that recharge rates, groundwater quality, biodiversity, or biomass in groundwater ecosystems can be affected. Effluents from industrial facilities contaminated with different chemicals can affect physico-chemical and ecological (habitat loss, biodiversity) conditions of the aquifer. Furthermore, such local pressures concerning groundwater bodies (chemical contamination, heat pollution, water extraction, surface sealing) affecting the urban groundwater overlap with regional and global pressures like climate change or land-use changes (Bugnot et al., 2019).

In conclusion, tolerance breadths seem to be very species-specific as well as location dependent and forms of pre-adaptations to high pollution concentrations must be considered. In spite of this, it is unclear if these observations are just valid for the short-term or will last for a longer period of time periods. Especially since pollution in groundwater can remain for several years and decades, the water quality only recovers slowly even after elimination of the source of pollution and remediation are very expensive and often not possible – prevention is the recommended strategy (Evanko and Dzombak, 1997; Hashim et al., 2011; Hose, 2005). To ensure a sustainable use of groundwater while conserving a diverse and functional ecosystem, it will be important to limit metal, salt and organic contamination pollution as well as temperature rises in cities and develop an inclusive assessment strategy that is based on stygofaunal indicator species.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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