Ecological Engineering 57 (2013) 118–132

Contents lists available at SciVerse ScienceDirect

Ecological Engineering

journal homepage: www.elsevier.com/locate/ecoleng

The use of crustaceans as sentinel organisms to evaluate groundwater ecological quality

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ARTICLE INFO

Article history:
Received 16 November 2012
Received in revised form 19 February 2013
Accepted 4 April 2013

Keywords:
Groundwater evaluation
Gammarus
Niphargus
Glycogen
Triglycerides

ABSTRACT

Criteria for the evaluation of groundwater quality are essentially based on the physical and chemical characteristics of the water, but biological and ecological indicators are needed to estimate groundwater ecosystem disturbance correctly. Such ecological evaluations may use communities (of micro- or macro-organisms) as disturbance indicators, but the density and diversity of groundwater fauna can be too low to permit effective evaluation. In these cases, the use of sentinels (i.e., caged organisms in situ) may complement physical and chemical indicators in the assessment of subterranean ecosystems. We tested the use of aquatic crustaceans (Amphipoda and Isopoda) as sentinel organisms by caging and exposing them in piezometers. In a first step, four species were tested in six piezometers located in the east Lyon aquifer, located upstream and downstream of three urban storm-water infiltration basins. In a second step, we used two species: the epigean Amphipoda *Gammarus pulex* for a short-duration exposure (one week) and the stygobite *Niphargus rhenorhodanensis* for a long-duration exposure (one month). Sentinels were tested in four infiltration basins, using upstream (control) and downstream (impacted) piezometers, on three occasions in 2010 and 2011 and in the laboratory using three types of water with increasing pollution. Infiltration of storm water induced a decrease in dissolved oxygen (DO) and an increase in dissolved organic carbon (DOC) between control and impacted piezometers. We therefore proposed a Water-Quality Index (WQI) based on the ratio of DO to DOC concentrations in groundwater. We measured the survival rates and the levels of body stores (glycogen and triglyceride) at the end of the exposure period. The survival rates of both species, when significantly different, were lower in impacted than in control piezometers, but body-store levels did not change with location. We propose an Ecophysiological Index (EPI) that combines the survival rate and the state of body stores. The EPI of sentinels at the end of each exposure period was negatively correlated with DOC concentrations and positively correlated with WQI for both species; this measure was also positively correlated with DO concentrations for *N. rhenorhodanensis*. Short-term exposure (i.e., one week) of an epigean species (such as *G. pulex*) may be used to assess acute toxic disturbance, while a longer exposure (i.e., one month) of a stygobite organism (here *N. rhenorhodanensis*) may be used to assess diffuse organic pollution and for a global evaluation of groundwater ecological quality if the appropriate ecophysiological indicators are used to estimate stress during exposure.

1. Introduction

Groundwater is a major source of potable and irrigation water in several countries (Zektser and Everett, 2004), but these water resources are under threat due to agriculture (Bohlke, 2002; Legout et al., 2005, 2007: Martin et al., 2006), industry and urbanisation (Datry et al., 2005; Foulquier et al., 2010, 2011; Lerner and Barrett, 1996; Trauth and Xanthopoulos, 1997). All of these

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0925-8574/$ – see front matter © 2013 Published by Elsevier B.V.
http://dx.doi.org/10.1016/j.ecoleng.2013.04.009

human activities may endanger groundwater ecosystem health, i.e., a system that can sustain its ecological structure and function (biodiversity, ecological processes) while sustainably providing ecosystem services (Korbel and Hose, 2011). An extensive evaluation of groundwater quality is needed to develop a coherent and efficient protection strategy and to plan restoration programs (Boulton, 2005). Evaluation criteria are principally based on the physical and chemical characteristics of the water (e.g., EU-GWD, 2006; Moura et al., 2011; Wendland et al., 2005, 2008), but many authors and environmental agencies advocate the development of biological and ecological indicators to assess groundwater quality in an ecosystem context (Danielopol et al., 2004, 2006a, 2008; EPA, 2003; Griebler et al., 2010; Stein et al., 2010; Korbel and Hose, 2011). Ecological evaluations of groundwater ecosystems may use communities (of micro- or macro-organisms) as disturbance indicators (Boughrous et al., 2007; Danielopol et al., 2006b, Griebler et al., 2010; Hahn, 2006), but in several cases, the density and diversity of groundwater fauna are too low for efficient evaluation (Deharveng et al., 2009). This difficulty is especially true in areas where freezing in the last glacial period led to depopulation of the groundwater fauna (Castellarini et al., 2007; Dole-Olivier et al., 2009; Gibert et al., 2009). In these cases, sentinels (i.e., caged organisms in situ) may complement the use of physical and chemical indicators in assessing groundwater ecosystems.

Sentinel organisms caged in rivers and streams are regularly used to monitor surface water (Maltby et al., 1990, 2002; Xuereb et al., 2009) post-evaluation. The methods consist of exposing caged organisms for a defined period of time to local physical and chemical stressors in real-life exposure assessments (Schmitt et al., 2010) that evaluate the environment quality after the exposure period. These indicators are generally based on several health status criteria: survival rate (Brown, 1980, Gust et al., 2010), feeding activity (Coulaud et al., 2011; Crane et al., 1995; Forrow and Maltby, 2000), physiological rates (e.g., respiration, Gerhardt, 1996; vitellogenesis, Xuereb et al., 2011) and life-history traits (e.g., reproduction, Gust et al., 2011; Schmitt et al., 2010). The organisms used in monitoring surface water in situ are very diverse and include molluscs (Schmitt et al., 2010; Taleb et al., 2009), crustaceans (Coulaud et al., 2011; Debourge-Geffard et al., 2009; Maltby, 1995; Maltby and Crane, 1994) and fish (Hanson, 2009). Macro-faunal communities in groundwater ecosystems are dominated in most cases by crustaceans (Gibert and Culver, 2009). If caged sentinels are used to monitor groundwater, macrocrustaceans are the most appropriate species to sample and manipulate for field exposure.

The main objectives of this work were (1) to establish a new method for the evaluation of groundwater ecological quality based on organisms caged in situ, (2) to test the efficiency of this method in an urban aquifer disturbed by storm-water infiltration, and (3) to propose an ecophysiological index based on sentinel survival rate and ecophysiological state (body store levels). We tested four different crustacean species, of which we used two upstream and downstream of four storm-water infiltration basins in the eastern Lyon aquifer (France). In this area, infiltration basins are widely used to drain storm water from urban areas because of the lack of superficial watercourses or nearby sewer networks and the high hydraulic conductivity of the soil. In France, they are most often used to reduce peak flows and volumes of downstream surface waters or sewers (limitation of flood effects) and/or to favour groundwater recharge. They are also used for their proposed role in the efficient removal of pollutants, especially heavy metals and some hydrocarbons (Barraud et al., 1999; Dechesne et al., 2004; Le Coustumer et al., 2007). However, the impact of infiltration systems on groundwater quality over time remains to be verified, and monitoring systems have yet to be developed. Addressing this question is one of the challenges of this work.

2. Site description

Four infiltration basins (artificial recharge systems) were used in this study. They are located in the eastern and southern parts of the city of Lyon, France (Fig. 1). The first infiltration basin (IUT, 45.7870 N, 4.8825 E) is located near the University Technological Institute of the University Lyon 1 Campus; it has an unsaturated zone of 2.8 m depth and a catchment area of 2.5 ha, with teaching and research buildings, parking lots, roads and lawns. The infiltration surface is 0.08 ha, with an annual recharge of 9.7 $m^3\ m^{-2}$. The Django–Reinhardt infiltration basin (DjR, 45.7366 N, 4.9577 E) has an unsaturated zone 13 m in depth and a catchment area of 185 ha, which is composed of industrial buildings and roads; the infiltration surface is approximately 0.8 ha, and the annual recharge is 73.5 $m^3\ m^{-2}$. The Minerve infiltration basin (MIN, 45.7153 N, 4.9154 E) has an unsaturated zone 3.2 m in depth and a catchment area of 270 ha. It is dominated by urban land use (commercial centres, lawns and large roads), and its infiltration surface is 0.39 ha, with an annual recharge of 202 $m^3\ m^2$. Finally, the Granges Blanches infiltration basin (noted as GB, 45.6581 N, 4.8954 E) has an unsaturated zone 1.7 m in depth and a catchment area of 100 ha, which is mostly covered by intensive cultures (corn and wheat), small houses and roads. Its infiltration surface is 0.4 ha, and the annual recharge is 73.1 $m^3\ m^{-2}$. All of the catchments are drained by a separate storm-water sewer network, the outlets of which are the infiltration basins.

3. Material and methods

3.1. Groundwater quality

To evaluate groundwater quality, water was sampled using a hand-pump technique based on the Bou–Rouch pumping method (Bou, 1974; Bou and Rouch, 1967). After 40 L was pumped to wash the piezometer completely, the temperature, electric conductivity, dissolved oxygen, and pH were measured (HQ20, HACH™, Dusseldorf, Germany) and 1 L of groundwater was sampled, stored in a cooler box and returned to the laboratory for chemical analyses. We measured seven chemical parameters: alkalinity (by potentiometry, Radiometer), chloride, sulphate, nitrate (ionic chromatography, Dionex DX120), ammonium, soluble reactive phosphorus (SRP, by spectrometry, Smartchem 200) and dissolved organic carbon (DOC, Pyrolysis and InfraRed detection, Bioritech OIA 1010 and Shimadzu TOC V). In addition, 42 volatile organic compounds (VOCs, head-space gas chromatography/mass spectrometry (HS-GC/MS), Agilent Technologies) and 24 polycyclic aromatic hydrocarbons (PAHs, GC/MS after solid-phase extraction (SPE), Agilent Technologies) were measured in the groundwater in triplicate (using three different piezometers) at the beginning and at the end of each exposure period ($n = 6$ for each mean value), except for the DjR basin where only one (upstream location) or two piezometers (downstream location) were available ($n = 2$ and $n = 4$, respectively).

Pesticides are difficult to detect in groundwater, especially in urban areas, because of their low concentrations (Gonzalez et al., 2008); we thus decided to test an integrative sampling technique (Polar Organic Chemical Integrative Sampler, POCIS, Pharm-sorbent, Exposmeter SA, Tavelsiö, Sweden) during the last study period, upstream and downstream of the four infiltration basins. This method allows a wide screening of targeted pesticides below standard detection limits (Kot et al., 2000). Two successive exposure periods of two weeks each were used (i.e., from the

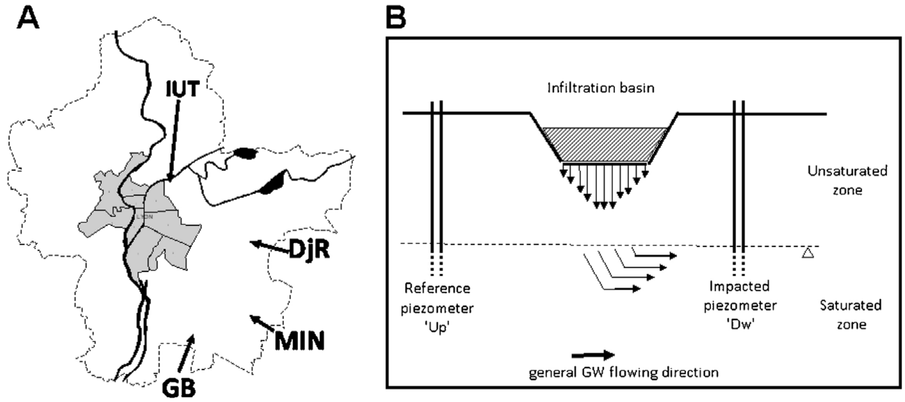

Fig. 1. Location of the four infiltration basins (A), showing the limits of the city of Lyon (in grey) and of the urban area (dotted line). Theoretical cross-section of an infiltration basin (B) with the direction of storm water and groundwater fluxes (arrows) and the location of the reference (upstream) and impacted (downstream) piezometers.

3rd to the 17th of May and from the 24th of May to the 7th of June 2011). After exposure, each POCIS was opened, and the sorbent was recovered from the polyethersulfone (PES) membranes with ultrapure water, transferred into a 1-mL empty solid-phase extraction (SPE) tube with a polyethylene frit and packed under vacuum conditions using a Visiprep SPE manifold. The sorbent was dried for 30 min in a vacuum. Prior to extraction, 75 μL of atrazin-d5 (0.5 mg/L) was added during the sequestering phase. Pesticides were extracted by elution under vacuum conditions with 10 mL of acetonitrile. The eluate was evaporated under a gentle stream of nitrogen, and the volume of the extract was reduced to 1 mL. Prior to analysis, all sample extracts were spiked with 50 μL of deuterated internal standard simazine-d10 (2 mg/L) and analysed using ultra performance liquid chromatography–tandem mass spectrometry (UPLC–MS/MS). Liquid-chromatography separations were performed in a Waters ACQUITY UPLC system (Waters, Guyancourt France) using a 150 mm × 2.1 mm × 1.7 μm ACQUITY BEH C18 column. Mass-spectrometry detection was performed using a Quattro Premier XE MS/MS (Waters, Guyancourt, France).

3.2. Enclosure units for sentinel organisms

Cages consisted of cylinders 6 cm long and 1.5 cm in diameter, made of iron stainless net and closed by two plastic caps. Three mesh sizes (1 mm, 500 μm and 200 μm) were tested in the laboratory to evaluate any potential increase in mortality caused by limited water exchange between the inside and outside of the cage. The results showed high survival rates (> 85%) at all mesh sizes (no significant differences, $F_{2,102} = 1.38$, $p = 0.26$). The mesh size retained for use in the field was 200 μm to avoid animal escape.

3.3. First step: the choice of sentinel species

We tested four crustacean species from the 4th to the 31st of March 2010, upstream and downstream of three infiltration basins (i.e., IUT, DjR and MIN), to compare their responses to groundwater exposure. These species were (1) *Gammarus pulex* (Linnaeus), an epigean amphipod species found in rivers and streams that is frequently used as a sentinel to assess river pollution (e.g., Khan et al., 2011); (2) *Gammarus roeselii* (Gervais), an epigean amphipod species found in slow-flowing rivers (Roux, 1969) that was not previously used in pollution surveys but that is potentially highly sensitive to pollutants (Dehédin et al., 2013); (3) *Asellus aquaticus* (Linnaeus), an epigean isopod species found in wetlands and slow-flowing streams that is considered resistant to stress (Maltby, 1995; Tachet et al., 2000); and (4) *Niphargus rhenorhodanensis* Schellenberg (from the Dombes population, haplotype I in Lefebure et al., 2007), a hypogean species that is very resistant to environmental stress, with well-known ecophysiological responses to hypoxia (Hervant et al., 1995), starvation (Hervant et al., 1997) and pollution (Canivet and Gibert, 2002; Plénet, 1995, 1999). These four species were selected to combine assessment of short-term (one week, *G. pulex* and *G. roeselii*) and long-term (one month, *A. aquaticus* and *N. rhenorhodanensis*) exposure periods because of significant differences in resistance to starvation among these species (Hervant et al., 2000).

The three epigean species were sampled in three Rhone River wetlands upstream of Lyon (*G. pulex* in the Chaume wetland, *G. roeselii* in the Meant wetland and *A. aquaticus* in the Iles Nouvelles wetland), and the hypogean *N. rhenorhodanensis* was collected in the forest of Chalamont (Lefebure et al., 2007). After two weeks in the laboratory, the four sentinel species were exposed in six cages containing five individuals each (for the two species of *Gammarus*), or in three cages containing 10 individuals each (for *A. aquaticus* and *N. rhenorhodanensis*). The number of animals per cage was reduced for *Gammarus* species because of their predatory behaviour at high population densities (Bollache et al., 2008) and because the animals were not fed during exposure.

3.4. Second step: field testing of the method

To test the method in the field, we exposed 30 individuals of two selected species in six (*G. pulex*) or three cages (*N. rhenorhodanensis*), in piezometers located upstream and downstream of the four infiltration basins (Fig. 1). We repeated this experiment three times: in March 2010, October/November 2010, and May/June 2011. During the first experimental period, a total of 47 to 59 mm of rainwater (according to the location of the infiltration basins) was recorded over 10 rainy days; during the second period, 54 to 57 mm of water fell over 9 rainy days; and during the third period, 42 to 47 mm of water fell over 11 days.

We measured three biological parameters after retrieval of organisms: (1) survival rates, (2) glycogen and (3) triglyceride concentrations in sentinels. For each period and each site, caged animals that had survived the exposure were freeze-dried, and three pools of three organisms were used for each biochemical analysis. Glycogen and total triglyceride concentrations were measured by standard enzymatic methods using specific test combinations, according to Hervant et al. (1995, 1996). Glucose HK and GPO Trinder kits specific to glycogen and triglycerides, respectively, were purchased from Sigma-Aldrich (France). Glycogen concentrations were expressed in mg of glycosyl, and triglyceride concentrations were expressed in mg of glycerol. All assays were performed in an Uvikon 940 recording spectrophotometer (Kontron Inc., Germany) at 25 °C.

Finally, we tested the method in the laboratory. Three pools of *G. pulex* and three pools of *N. rhenorhodanensis* (each with 100 to 150 individuals) were exposed at 11 °C to three different waters: (1) groundwater from the control piezometer located upstream of the DjR basin, (2) groundwater from the impacted piezometer located downstream of the same basin, and (3) polluted groundwater sampled above an industrial area close to DjR basin (resulting in a total of 1074 and 1467 individuals for *G. pulex* and *N. rhenorhodanensis*, respectively). Chemical characteristics (including the 42 volatile organic compounds and the 24 polycyclic aromatic hydrocarbons) were measured in these three waters. Survival rates (expressed in %), glycogen (in mg glycosyl/g dry weight) and triglyceride (in mg glycerol/g dry weight) concentrations were measured in the organisms after one month of exposure.

3.5. Third step: calculation of water quality and ecophysiological indexes

A Water-Quality Index (WQI, dimensionless) was calculated using the following equation:

$$\mathrm{WQI} = [\mathrm{DO}]/[\mathrm{DOC}] \quad (1)$$

where [DO] is the concentration of dissolved oxygen and [DOC] is the concentration of dissolved organic carbon in mg/L. This index has a high value in undisturbed and well-aerated groundwater, where DO is not consumed by microbial activity and the DOC inputs are low, while the value decreases with storm water infiltrations that bring poorly oxygenated water rich in organic compounds into the aquifer (Datry et al., 2003, 2004; Foulquier et al., 2010, 2011).

An Ecophysiological Index (EPI, dimensionless) was calculated for sentinels that survived the exposure using the following equation:

$$\mathrm{EPI} = \text{Survival rate} * ([\mathrm{Gly}]/([\mathrm{Tri}] + [\mathrm{Gly}])) \quad (2)$$

where the sentinel survival rate (as a %) is combined with the state of their energetic stores as a ratio between glycogen content (noted as [Gly]), which is the first energetic store used in response to acute stress (Maazouzi et al., 2011), and their total body stores, as estimated from the sum of triglyceride (noted as [Tri]) and glycogen concentrations. This index is high when the sentinels survive at high rates despite exposure and are able to maintain their body stores, but the value decreases with a reduction in sentinel survival rate or in glycogen levels (Maazouzi et al., 2011). It theoretically varies between 0 (when all animals die during exposure) and nearly 100 (when all animals survive and their glycogen content is maintained at a high level). It is not possible to achieve a value of 100 because these species naturally store some of their body stores as triglycerides.

To test the potential effect of temperature on the physiological response of the sentinels to exposure, we included a correction for temperature (in Celsius degree-days, T°d) in the EPI calculation:

$$\mathrm{EPI} = \text{Survival rate} * (([\mathrm{Gly}]/([\mathrm{Tri}] + [\mathrm{Gly}])) * \mathrm{T}^{\circ}\mathrm{d}) \quad (3)$$

3.6. Statistical analysis

Analysis of variance (ANOVA) was used to test for the effect of artificial groundwater recharge on the physicochemistry, survival rates and body stores. For each exposure period, we used a nested design with infiltration basins (n = 4) and "upstream–downstream" conditions nested in infiltration basins (n = 2) as the main effects to avoid any effect of between-basin differences on the upstream-downstream conditions. Tukey's HSD tests were used for multiple comparisons whenever a significant effect was observed. For body stores, conditions without surviving animals were excluded from the analyses.

Regression analyses (both linear and logarithmic) were used to determine the significance of the relationship between survival rates, body stores or the EPI and the physicochemical characteristics of the water (DO, DOC, WQI). On three occasions, the biomass of crustaceans at the end of the exposure was not large enough for body store analyses (i.e., IUT downstream for *G. pule*x and MIN downstream and IUT downstream for *N. rhenorhodanensis*, during the first experiment). These samples were excluded from the regression analyses.

4. Results

4.1. Infiltration basins and urban groundwater quality

Very small differences were observed in the variability in the piezometric levels between the upstream and downstream piezometers of the infiltration basins (Table 1). In two of the studied basins (MIN and IUT), mean temperatures differed between locations, with lower mean temperatures downstream of the basin in early spring and fall 2010 (when air temperature was low) and higher mean temperatures downstream of the basins in late spring 2011 (when air temperature was high). The temporal variability in groundwater temperature was always higher downstream of the four infiltration basins than upstream (Table 1); however, the recorded temperature never exceeded 16.2 °C at DjR, 18.3 °C at MIN, 19.5 °C at IUT and 20.7 °C at GB (for only a few hours after a storm event in June 2011).

The chemical characteristics of the groundwater significantly differed between the locations upstream and downstream of the basins (Table 1). Electrical conductivity, alkalinity and the major solutes (i.e., chloride and sulphate) were always lower downstream than upstream of the infiltration basins ($F_{4,98} = 69.05$, $p < 0.001$ for electric conductivity; $F_{4,98} = 83.27$, $p < 0.001$ for alkalinity; $F_{4,98} = 67.48$, $p < 0.001$ for chloride; and $F_{4,98} = 174,48$, $p < 0.001$ for sulphate). Dissolved oxygen also decreased downstream of the basins ($F_{4,98} = 16.97$, $p < 0.001$), but this decrease was low at DjR (where the unsaturated zone was 13 m) and high in the three other basins (with an unsaturated zone thinner than 3.5 m). Nitrogen consisted of nitrate, which reached very high values upstream of the basins, with these values being generally lower downstream ($F_{4,98} = 6127.52$, $p < 0.001$). This decrease in nitrate levels between the upstream and downstream locations was very similar to the decrease in chloride at DjR, IUT and GB, but was markedly higher at MIN, where nitrate was five- to 40-fold lower downstream than upstream of the basin. In contrast, SRP and DOC increased downstream of the basins ($F_{4,98} = 8.17$, $p < 0.001$ for DOC and $F_{4,98} = 47.64$, $p < 0.001$ for SRP). This difference was particularly notable at IUT and GB for SRP and at DjR and MIN for DOC (Table 1).

For organic pollutants, very low concentrations of VOCs were observed both upstream and downstream of the four basins (Table 2), except at IUT during the second and the third study periods, where high amounts of methyl tertiary-butyl ether (MTBE) were measured. In the same way, very low concentrations of PAHs were measured during the three sampling periods both upstream and downstream (Table 2).

Using POCIS, several pesticides (32 compounds) were observed in the groundwater of the studied area (Table 3), showing differences between basins and between locations (upstream versus downstream). A weak spatial pattern was observed in the number

Table 1
Physicochemical characteristics of groundwater upstream (up) and downstream (dw) of the four infiltration basins (Django Reinhardt-DjR, Minerve-MIN, IUT, and Granges Blanches-GB) and in the three sampling periods (1st semester: March-April 2010, 2nd semester: October–November 2010, and 3rd semester: May–June 2011). 'Control', 'Impact' and 'Polluted' correspond to waters used in the laboratory experiment. dNP: variation in the piezometric level, T: mean temperature (and total range), EC: Electric Conductivity, DO: Dissolved Oxygen, Alk: Alkalinity, SRP: Soluble Reactive Phosphorus. DOC: Dissolved Organic Carbon. All parameters, except dNP and T, are expressed as mean values (± standard deviation, n = 6 for all basins but for DjR, where n = 2 upstream and n = 4 downstream).

Station		dNP (m)	T (°C)	EC (µS/cm)	pH	DO (mg/L)	Alk (°F)	NH_4^+ (µg/L)	Cl (mg/L)	SO_4^- (mg/L)	NO_3^- (mg/L)	SRP (µg/L)	DOC (mg/L)
DjR up	1st	0.34	13.72 (0.01)	692 (±8)	6.9 (±0.3)	7.88 (±0.24)	27.3 (±0)	0	24.7 (±0.4)	22.2 (±0.1)	48.1 (±1.3)	25 (±21)	1.51 (±0.57)
	2nd	0.65	13.76 (0.01)	691 (±13)	7.2 (±0)	7.65 (±0.45)	26.8 (±0.3)	35 (±49)	24.3 (±0.6)	22.2 (±0.1)	46.8 (±0.6)	15 (±7)	1.31 (±0.16)
	3rd	0.86	13.84 (0.07)	704 (±7)	7.3 (±0.1)	8.30 (±0.0)	27.0 (±0.2)	0	24.1 (±0.1)	22.3 (±0.1)	45.7 (±0.1)	15 (±21)	0.9 (±0.1)
DjR dw	1st	0.37	12.90 (0.9)	647 (±78)	7.0 (±0.2)	7.16 (±0.47)	25.5 (±2.9)	0	25.1 (±5.6)	34.9 (±7.9)	24.9 (±7.6)	58 (±55)	2.93 (±2.11)
	2nd	0.51	14.3 (0.68)	542 (±27)	7.3 (±0.3)	6.80 (±0.5)	21.7 (±1.2)	43 (±10)	18.7 (±1.5)	24.1 (±1.6)	20.9 (±1.9)	50 (±42)	2.55 (±0.48)
	3rd	0.24	13.72 (2.69)	629 (±124)	7.2 (±0.1)	7.49 (±0.53)	24.7 (±3.5)	0	22.7 (±8.8)	28 (±8)	23.1 (±8.5)	98 (±76)	1.9 (±0.42)
MIN up	1st	0.09	11.67 (1.37)	669 (±108)	7 (±0.1)	5.8 (±1.45)	23.6 (±3.6)	0	35.6 (±3.4)	30.9 (±7.1)	41.5 (±14.5)	10 (±9)	1.40 (±0.6)
	2nd	0.32	15.01 (2.97)	587 (±300)	7.4 (±0.4)	6.45 (±0.36)	23.3 (±4)	0	28.8 (±4.8)	30.9 (±7.7)	41.5 (±14.8)	10 (±9)	1.65 (±0.37)
	3rd	0.20	12.98 (0.88)	776 (±12)	6.9 (±0.3)	7.29 (±0.86)	26.7 (±0.4)	0	32.4 (±0.5)	38.4 (±0.7)	55.7 (±1.2)	22 (±12)	0.73 (±0.26)
MIN dw	1st	0.67	7.19 (5.44)	355 (±59)	7.6 (±0.2)	1.49 (±0.56)	13.1 (±1.3)	0	29.3 (±14)	9.6 (±2.1)	0.6 (±0.8)	20 (±23)	2.18 (±0.15)
	2nd	1.26	13.57 (5.73)	243 (±25)	8.0 (±0.3)	4.91 (±0.73)	10.2 (±1)	0	10.5 (±1.2)	5.7 (±1.2)	1 (±0.8)	32 (±21)	2.17 (±0.36)
	3rd	0.97	16.58 (4.58)	452 (±43)	7.5 (±0.4)	2.45 (±0.32)	16.2 (±2.6)	0	30.2 (±17.2)	15.1 (±2.5)	7.9 (±5.7)	32 (±19)	2.2 (±0.85)
IUT up	1st	0.56	15.08 (0.30)	561 (±9)	7.3 (±0.2)	4.54 (±1.89)	22.1 (±0.3)	0	15.9 (±0.8)	47.6 (±0.3)	11.2 (±0.3)	10 (±11)	1.14 (±0.5)
	2nd	0.38	15.83 (0.20)	572 (±7)	7.5 (±0.1)	4.38 (±1.09)	21.5 (±0.1)	0	17.5 (±0.5)	48 (±0.4)	11.5 (±0.3)	13 (±8)	2.81 (±0.5)
	3rd	1.42	14.55 (0.71)	599 (±16)	7.4 (±0.2)	4.62 (±1.51)	22.0 (±0.2)	0	23.2 (±3.6)	46.6 (±0.9)	12.2 (±0.9)	8 (±10)	0.55 (±0.10)
IUT dw	1st	0.69	8.07 (3.61)	230 (±94)	7.4 (±0.6)	3.37 (±2.01)	10.3 (±3)	0	4.4 (±4.0)	8.7 (±9.9)	2.7 (±2.8)	350 (±134)	1.90 (±0.62)
	2nd	0.70	13.71 (5.73)	213 (±48)	7.9 (±0.2)	3.6 (±0.76)	9.3 (±2.5)	27 (±65)	3.2 (±0.7)	6.9 (±1.8)	5.5 (±2.4)	412 (±187)	2.86 (±1.29)
	3rd	1.45	16.79 (5.47)	326 (±94)	7.7 (±0.4)	4.30 (±0.57)	14.6 (±4.6)	10 (±24)	9.2 (±6.5)	16.7 (±13.9)	9.4 (±2.6)	370 (±108)	1.86 (±0.87)
GB up	2nd	0.10	14.91 (0.46)	1064 (±129)	7.3 (±0.1)	5.6 (±0.8)	30.9 (±1.4)	0	80.3 (±17.0)	88.7 (±17.2)	29.1 (±1.6)	20 (±0)	2.20 (±0.3)
	3rd	0.65	16.95 (2.48)	1107 (±63)	7.1 (±0.2)	5.41 (±0.53)	31.3 (±0.9)	0	93.3 (±9.4)	99.3 (±13.1)	31.1 (±1.2)	3 (±8)	1.23 (±0.18)
GB dw	2nd	0.13	15.19 (1.15)	628 (±78)	7.6 (±0.3)	5.53 (±2.03)	21.0 (±5.0)	0	23.1 (±9.4)	29.8 (±4.6)	15.2 (±4.0)	673 (±420)	2.81 (±0.88)
	3rd	0.13	16.12 (6.24)	768 (±66)	7.1 (±0.5)	4.07 (±0.95)	28.8 (±1.9)	0	38.5 (±5.6)	38.2 (±4.2)	25.6 (±6.4)	678 (±431)	1.07 (±0.29)
Control		–	11	676	7.1	–	27.2	0	25.6	21.2	42.4	60	0.40
Impact		–	11	684	7.4	–	26.8	0	30.1	30.6	29.9	170	0.70
Polluted		–	11	934	6.9	–	33.9	0	53.0	76.0	22.7	60	0.90

Table 2
Polycyclic Aromatic Hydrocarbons (PAHs) and Volatile Organic Compounds (VOCs) contents in groundwater upstream (up) and downstream (dw) of the four infiltration basins (Django Reinhardt-DjR, Minerve-MIN, IUT, and Granges Blanches-GB), in the three sampling periods (1st: March-April 2010, 2nd: October–November 2010, and 3rd: Mai–June 2011). 'Control', 'Impact' and 'Polluted' correspond to waters used in the laboratory experiment. Mean concentrations (mean conc.) and mean numbers of compounds (mean numb.) are calculated by combining all samples for VOCs and PAHs. No value (–): below the detection limit. Mean values (± standard deviation, n = 6, except for DjR where n = 2 upstream and n = 4 downstream).

Stations		MTBE (μg/L)	1,1,1-Trichloro ethane (μg/L)	Cis-1,2-Dichloro ethylen (μg/L)	Tetra chloro ethylen (μg/L)	Tri chloro ethylen (μg/L)	VOCs mean conc. (μg/L)	VOCs mean numb. (n)	Fluoren (ng/L)	Phenanthrem (ng/L)	PAHs mean conc. (ng/L)	PAHs mean numb. (n)
DjR up	1st	–	1.3 (±0.9)	–	2 (±2.8)	–	3.29 (±1.82)	1.5 (±0.7)	6.5 (±9.2)	19 (±27)	25.5 (±36.1)	1.0 (±1.4)
	2nd	–	0.4 (±0.5)	–	2.2 (±0)	–	2.55 (±0.49)	1.5 (±0.7)	–	9 (±13)	9 (±12.7)	0.5 (±0.7)
	3rd	–	–	–	1 (±1.3)	–	1 (±1.3)	0.5 (±0.7)	–	–	–	–
DjR dw	1st	–	1.6 (±0.1)	0.1 (±0.3)	2.6 (±1.1)	–	4.3 (±1)	2.25 (±0.5)	–	–	–	–
	2nd	–	1.3 (±0.4)	0.1 (±0.3)	2 (±1)	–	3.35 (±1.57)	2.25 (±0.5)	–	8.0 (±10)	8.0 (±10)	0.5 (±0.6)
	3rd	–	0.6 (±0.8)	0.2 (±0.3)	1.2 (±1.3)	0.1 (±0.3)	2.1 (±2.5)	1.5 (±1.9)	–	–	–	–
MIN up	1st	–	–	–	0.1 (±0.2)	–	0.08 (±0.2)	0.17 (±0.41)	–	12.0 (±15)	12.3 (±15.3)	0.5 (±0.5)
	2nd	–	–	–	0.2 (±0.3)	–	0.2 (±0.3)	0.33 (±0.52)	–	4.2 (±6.5)	4.2 (±6.5)	0.33 (±0.52)
	3rd	–	–	–	0.1 (±0.3)	0.1 (±0.3)	0.22 (±0.34)	0.33 (±0.52)	–	–	–	–
MIN dw	1st	–	–	–	–	–	–	–	–	9.3 (±10)	9.3 (±10)	0.5 (±0.5)
	2nd	–	–	–	–	–	–	–	–	4.3 (±6.8)	4.3 (±6.3)	0.33 (±0.5)
	3rd	–	–	–	–	–	–	–	–	–	–	–
IUT up	1st	–	0.2 (±0.3)	–	–	–	0.2 (±0.3)	0.5 (±0.8)	2 (±4.9)	8.5 (±15)	10.5 (±19.3)	0.5 (±0.8)
	2nd	–	0.3 (±0.4)	–	0.6 (±0.4)	–	0.82 (±0.45)	1 (±0.6)	–	6.2 (±9.6)	6.2 (±9.6)	0.33 (±0.5)
	3rd	–	0.4 (±0.4)	–	0.5 (±0.5)	–	0.84 (±0.94)	1 (±1.1)	–	–	–	–
IUT dw	1st	–	–	–	0.2 (±0.4)	–	0.15 (±0.37)	0.17 (±0.4)	3.7 (±5.7)	11.0 (±14)	14.8 (±19.2)	0.83 (±0.98)
	2nd	17.3 (±21)	–	–	0.3 (±0.6)	–	17.6 (±20.7)	0.6 (±0.5)	–	5.7 (±8.8)	5.7 (±8.8)	0.33 (±0.52)
	3rd	616.7 (±743.5)	–	–	0.5 (±0.8)	–	617. 1 (±744.3)	0.83 (±0.98)	–	–	–	–
GB up	2nd	–	0.4 (±0.3)	–	–	–	0.4 (±0.3)	0.33 (±0.58)	–	6.0 (±10)	6.0 (±10)	0.33 (±0.58)
	3rd	–	–	–	–	–	–	–	–	–	–	–
GB dw	2nd	–	0.18 (±0.31)	–	–	–	0.18 (±0.31)	0.33 (±0.58)	–	–	–	–
	3rd	–	–	–	–	–	–	–	–	2.2 (±5.3)	2.2 (±5.3)	0.17 (±0.41)
Control		–	–	–	1.6	–	1.6	1	–	–	–	–
Impact		–	1.3	–	1.9	0.6	3.8	3	–	–	–	–
Polluted		–	5.7	2.2	18.0	2.6	30.9	7	–	–	–	–

Table 3
Pesticides concentrations (in ng.L^{-1}, means ± standard deviation, n = 2) calculated from POCIS (82 compounds targeted) in the groundwater upstream (up) and downstream (dw) of the four infiltration basins (Django Reinhardt-DjR, Minerve-MIN, IUT, and Granges Blanches-GB) during the last sampling period (Mai–June 2011). n.d.: not detected in the sample.

	DjR up	DjR dw	IUT up	IUT dw	GB up	GB dw	MIN up	MIN dw
Number of detected compounds (first and second exposure)	21 (20-11)	15 (14-14)	6 (6-6)	18 (11-15)	15 (15-14)	11 (10-11)	9 (9-7)	23 (22-20)
Atrazine	3.61 (±1.64)	11.25 (±8.08)	1.54 (±1.03)	0.48 (±0.37)	3.55 (±0.35)	3.15 (±0.45)	4.67 (±0.37)	0.65 (±0.47)
Desethylatrazine	8.98 (±4.23)	17.47 (±8.85)	4.53 (±2.15)	1.89 (±0.79)	6.44 (±2.27)	6.65 (±1.02)	5.24 (±0.65)	1.92 (±0.91)
Desethylterbutylazine	0.62 (±0.27)	0.57 (±0.38)	0.25 (±0.16)	2.80 (±2.25)	0.66 (±0.08)	0.35 (±0.01)	0.25 (±0.06)	2.33 (±1.54)
Desisopropylatrazine	1.56 (±1.21)	1.77 (±0.24)	0.20 (±0.06)	0.61 (±0.22)	0.54 (±0.03)	0.47 (±0.07)	0.23 (±0.33)	0.42 (±0.16)
Dimethenamide	0.09 (±0.13)	n.d.	n.d.	0.16 (±0.22)	0.14 (±0.20)	n.d.	n.d.	0.36 (±0.21)
Diuron	1.24 (±0.47)	1.02 (±0.92)	0.19 (±0.16)	6.91 (±5.85)	1.55 (±0.80)	2.46 (±0.15)	0.28 (±0.11)	1.09 (±0.93)
Hexazinon	0.61 (±0.28)	0.57 (±0.39)	n.d.	n.d.	2.01 (±0.69)	0.75 (±0.03)	0.84 (±0.06)	0.06 (±0.09)
Isoproturon	0.18 (±0.07)	0.93 (±0.78)	n.d.	n.d.	0.29 (±0.04)	0.13 (±0.01)	n.d.	0.21 (±0.12)
Metolachlor	1.57 (±0.75)	1.63 (±1.13)	n.d.	0.48 (±0.67)	0.50 (±0.22)	0.59 (±0.10	0.27 (±0.14)	4.89 (±2.26)
Propanil	0.34 (±0.48)	0.30 (±0.43)	n.d.	0.30 (±0.42)	n.d.	0.39 (±0.55)	n.d.	0.26 (±0.37)
Simazine	1.39 (±0.70)	3.89 (±2.50)	0.39 (±0.26)	0.21 (±0.10)	2.76 (±0.43)	1.85 (±0.06)	0.44 (±0.02)	0.24 (±0.01)
Terbuthylazine	0.32 (±0.46)	0.64 (±0.52)	n.d.	n.d.	0.57 (±0.11)	0.26 (±0.01)	n.d.	0.07 (±0.10)

of detected compounds that increased (i.e., IUT and MIN basins) or decreased (i.e., DjR and GB) downstream of the infiltration basins, while concentrations of the dominant compounds generally increased downstream: in DjR, three compounds increased from the upstream to downstream locations (atrazine, desethylatrazine and simazine), as did two compounds at IUT (desethylbutylazine and diuron), one compound in GB (diuron) and two in MIN (desethylterbutylazine and metolachlore). In the last basin, a reversed pattern (a decrease from the upstream to the downstream locations) was found for atrazine and desethylatrazine.

4.2. First step: the choice of the sentinel species

Significant differences in survival rates of the two *Gammarus* species (Fig. 2) were detected only between downstream (impacted) and upstream (control) locations of the infiltration basins, with lower values downstream: at the IUT station for *G. pulex* (Tukey HSD, $p < 0.001$) and at the IUT and MIN stations for *G. roeselii* (Tukey HSD, $p < 0.001$). In the same way, body glycogen concentrations at the end of the exposure period, when significantly different, were lower downstream than upstream of the MIN infiltration basin (i.e., Tukey HSD, $p < 0.05$ for both *G. pulex* and *G. roeselii*); surprisingly, however, they were increased at DjR for *G. roeselii* (Tukey HSD, $p < 0.05$).

Asellus aquaticus showed very low survival rates downstream of the three studied infiltration basins (decreasing from MIN to IUT and DjR, Tukey HSD, $p < 0.05$, Fig. 2) and, surprisingly, a higher body glycogen concentration downstream of MIN than upstream (Tukey HSD, $p < 0.05$; data for DjR and IUT were not available due to insufficient availability of biomass for glycogen analysis).

Finally, *N. rhenorhodanensis* survival rates decreased downstream of the three infiltration basins (Tukey HSD, $p < 0.001$, decreasing from MIN to IUT and DjR, Fig. 2) and body glycogen concentrations (when measurable) were similar (i.e., IUT, Tukey HSD, $p = 0.51$) or significantly lower downstream than upstream (i.e., MIN, Tukey HSD, $p < 0.05$). According to these results from this first round of experiments and to the biogeography of the species and their ecological requirements (see Discussion), *G. pulex* and *N. rhenorhodanensis* were selected for further experiments.

4.3. Step 2: Reaction of the selected sentinel organisms

G. pulex was used for short and *N. rhenorhodanensis* for long exposures to evaluate the effect of storm water infiltration on groundwater ecological quality. Survival rates of *G. pulex*, when significantly different, were always lower downstream (impacted) than upstream (control) of the infiltration basins (Fig. 3), but they differed between the study periods; the rate decreased significantly at IUT during the first and second experiments (Tukey HSD, $p < 0.001$, for both dates), as well as at DjR during the second (Tukey HSD, $p < 0.001$) and at MIN during the third (Tukey HSD, $p < 0.001$) experiment. Glycogen concentrations usually did not change noticeably with location; when the difference was significant, the levels decreased (at MIN during the first experiment and at DjR and MIN during the second experiment, Tukey HSD, $p < 0.05$) or slightly increased (at IUT during the third experiment, Tukey HSD, $p < 0.05$) downstream of the infiltration basins. Triglyceride concentrations were never significantly different between the upstream and downstream regions of the infiltration basins. Few significant correlations were observed between the survival rates of *G. pulex* and the physicochemical characteristics of the groundwater; survival increased with electrical conductivity ($r^2 = 0.20$, $p = 0.018$) and with alkalinity ($r^2 = 0.25$, $p = 0.009$) because of lower survival rates downstream of the infiltration basins where storm water diluted the groundwater. The survival rates and glycogen and triglyceride concentrations in *G. pulex* were not significantly correlated with DO concentrations, DOC or with the Water-Quality Index (WQI, Table 4).

Survival rates of *N. rhenorhodanensis*, when significantly different, were always lower downstream than upstream of the infiltration basins (Fig. 4): at DjR, IUT and MIN during the first

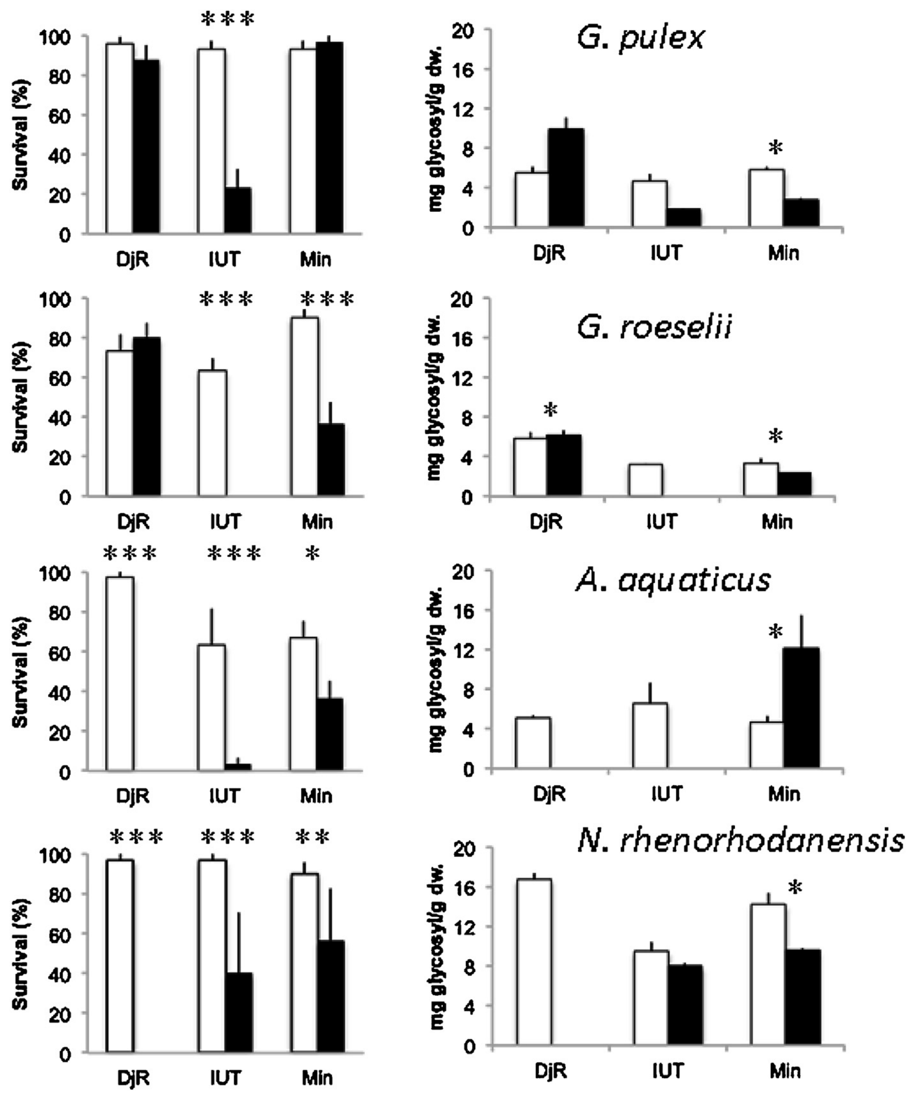

Fig. 2. Survival rate and body stores (mean ± SE) of *Gammarus pulex*, *Gammarus roeseli*, *Asellus aquaticus* and *Niphargus rhenorhodanensis* exposed in piezometers located upstream (white bars) and downstream (black bars) of three infiltration basins (DjR, IUT and MIN) during the first experiment. Significant differences are noted by * for $p < 0.05$, ** for $p < 0.01$ and *** for $p < 0.001$. Glycogen is expressed in mg of glycosyl. Note that the glycogen content scale is four-fold higher for *Niphargus rhenorhodanensis* than the other species.

Table 4
Correlations between ecophysiological indicators (survival rates, glycogen, triglyceride concentrations and EPI values) and physico-chemical characteristics of groundwater (Oxygen, DOC concentrations and WQI values) in *G. pulex* and *N. rhenorhodanensis*. (+)Correlation coefficients were calculated for a logarithmic regression curve when the resulting p-values were lower than for the linear regression.

	Oxygen		DOC		WQI	
	r^2	p	r^2	p	r^2	p
G. pulex						
Survival rates (n = 22)	0.10	n.s.	0.09	n.s.	0.12+	n.s.
Glycogen (n = 19)	0.12	n.s.	0.03	n.s.	0.13+	n.s.
Triglycerides (n = 19)	0.016+	n.s.	0.11+	n.s.	0.10+	n.s.
EPI (n = 20)*	0.022+	n.s.	**0.25+**	**0.012**	**0.21+**	**0.019**
N. rhenorhodanensis						
Survival rates (n = 22)	**0.17**	**0.02**	0.01	n.s.	0.11+	n.s.
Glycogen (n = 19)	0.11	n.s.	**0.24**	**0.016**	**0.28**	**0.009**
Triglycerides (n = 17)	0.02+	n.s.	**0.25+**	**0.028**	**0.26**	**0.018**
EPI (n = 20)*	**0.178+**	**0.032**	**0.336+**	**0.004**	**0.509+**	**0.0002**

* The higher number of available samples for EPI than for the glycogen or the triglycerides is linked to the 100% mortality samples (where EPI = 0).

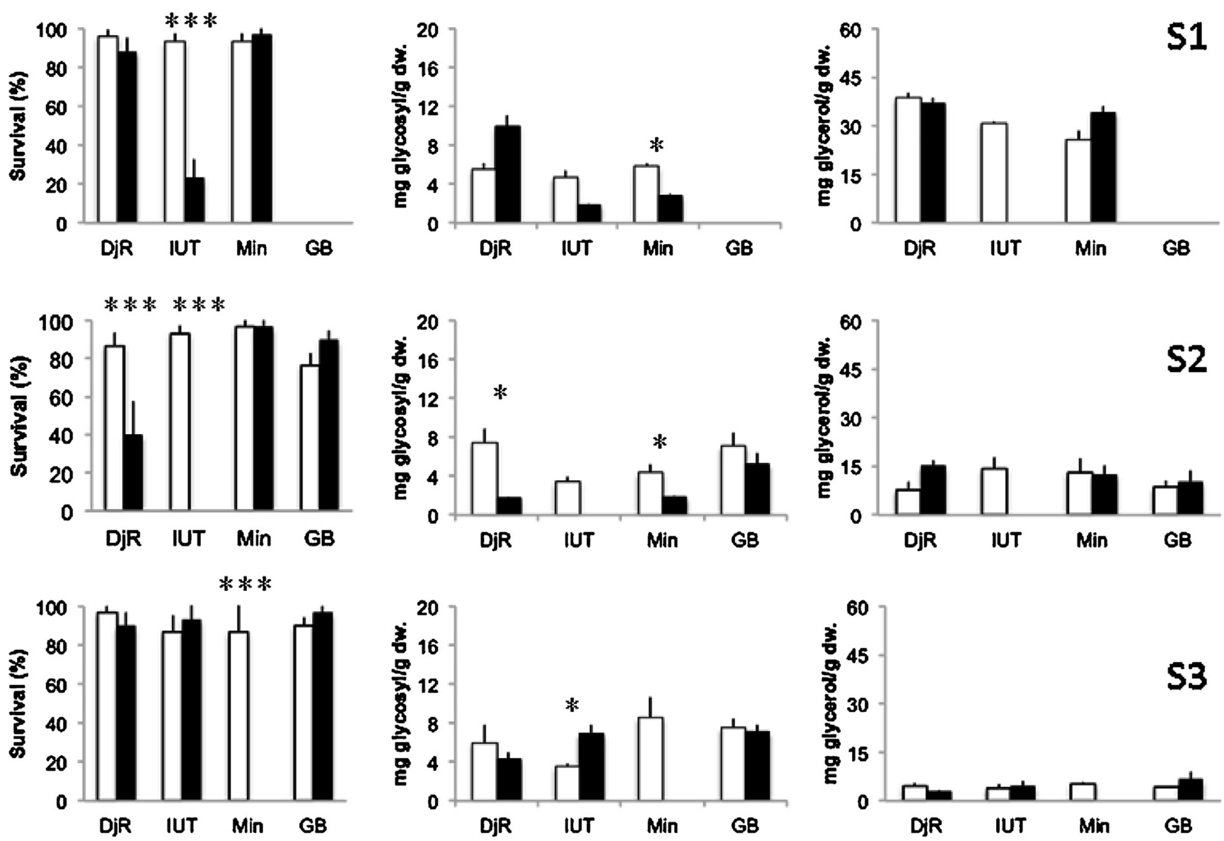

Fig. 3. Survival rate and body stores (mean ± SE) of *Gammarus pulex* in piezometers located upstream (white bars) and downstream (black bars) of four infiltration basins (DjR, IUT, Min and GB) during the three experiments (S1, S2, and S3). Significant differences are noted by * for $p < 0.05$, ** for $p < 0.01$ and *** for $p < 0.001$. Glycogen is expressed in mg of glycosyl, and triglycerides are in mg of glycerol.

experiment (Tukey HSD, $p < 0.001$, $p < 0.001$ and $p < 0.01$, respectively) and at MIN and GB during the third experiment (Tukey HSD, $p < 0.001$ for both sites). Glycogen concentrations decreased significantly downstream of the infiltration basins on two occasions: at MIN during the first experiment (Tukey HSD, $p < 0.05$) and at IUT during the second experiment (Tukey HSD, $p < 0.05$). Triglyceride concentrations did not change notably with location: no significant differences between upstream and downstream locations were observed. Survival rates of *N. rhenorhodanensis* were not significantly correlated with electrical conductivity or alkalinity ($p > 0.05$ in both cases), but they were positively correlated with DO concentrations. The glycogen and triglyceride contents of *N. rhenorhodanensis* were significantly correlated with DOC (increasing and decreasing with DOC concentrations, respectively) and with the Water-Quality Index (decreasing and increasing with WQI, respectively, Table 4).

The one month exposure experiment in the laboratory was performed on unpolluted (Control) or lowly polluted waters (Impacted and Polluted, Tables 1 and 2). Despite these weak differences in pollution levels, we measured decreases in survival rates and body stores with decreasing water quality in both species (Table 5). After 30 days in the polluted groundwater sampled above an industrial area, survival rates of the two sentinel species were lower than after exposure to water sampled in the control piezometer and intermediate values were measured for animals exposed to water sampled in the impacted piezometer (downstream of the DjR basin).

For both species, no significant correlation was found during field exposures between survival rates, glycogen and triglyceride concentrations and pollutants measured in the groundwater (VOCs and PAHs expressed in concentrations or in number of compounds). Few significant correlations were observed between *G. pulex* survival rates and pesticides measured with the POCIS during the third experiment; survival rates were negatively correlated with concentrations of dimethenamide ($r^2 = 0.67$, $p = 0{,}007$) and metolachlore ($r^2 = 0.82$, $p = 0.001$). In contrast, no significant correlations were found for *N. rhenorhodanensis*.

4.4. Step 3: Combining survival rates and ecophysiology

When the survival rate was combined with the state of the body stores (using the EPI calculation, Eq. (2)), a positive correlation

Table 5
Mean survival rates, mean EPI values (± standard deviation, n = 3) and range of EPI values for *Gammarus pulex* and *Niphargus rhenorhodanenesis* exposed for one month in the laboratory to three types of water. "Control": piezometer located upstream of DjR basin. "Impacted": piezometer located downstream of DjR basin. "Polluted": piezometer located in an industrial area close to DjR basin.

	G. pulex	N. rhenorhodanensis
Control		
Survival rates (%)	91.7 ± 3.5	92.3 ± 2.3
EPI	26.5 ± 7.4	29.3 ± 4.7
Range	18.8–36.3	19.0–37.0
Impacted		
Survival rates (%)	80.3 ± 1.5	85.0 ± 2.2
EPI	23.1 ± 7.3	26.5 ± 8.3
Range	9.0–30.9	13.8–39.3
Polluted		
Survival rates (%)	70.7 ± 2.2	76.7 ± 1.2
EPI	22.7 ± 7.0	26.6 ± 8.5
Range	10.1–31.4	16.0–41.2

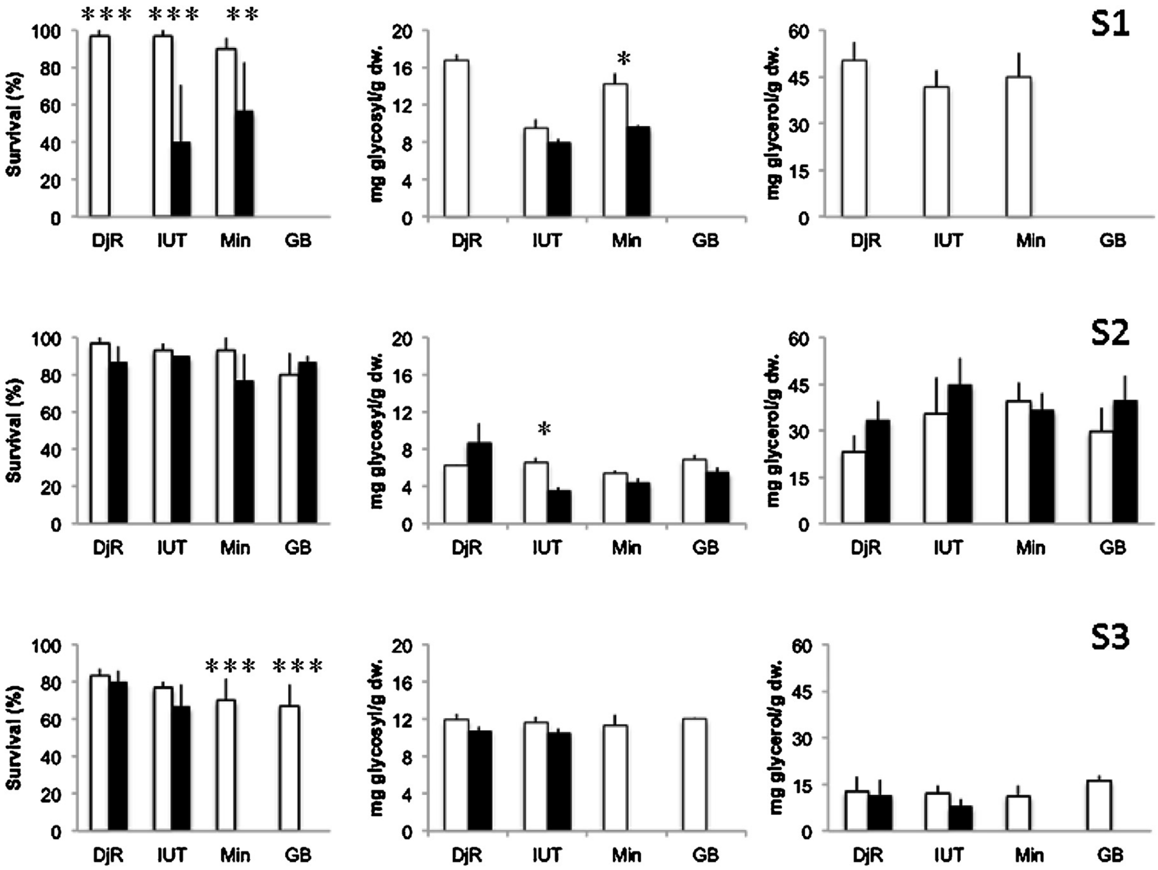

Fig. 4. Survival rate and body stores (mean ± SE) of *Niphargus rhenorhodanensis* in piezometers located upstream (white bars) and downstream (black bars) of four infiltration basins (DjR, IUT, MIN and GB) during the three experiments (S1, S2, and S3). Significant differences are noted by * for $p < 0.05$, ** for $p < 0.01$ and *** for $p < 0.001$. Glycogen is expressed in mg of glycosyl, and triglycerides are in mg of glycerol.

was observed with the DO concentrations for *N. rhenorhodanensis* and negative correlations were found with DOC and WQI for both *G. pulex* and *N. rhenorhodanensis*: the ecophysiological state of both sentinel species significantly improved with the quality of groundwater (Fig. 5). The logarithmic relationship between EPI and WQI was less consistent for *G. pulex* ($r^2 = 0.21$, $p = 0.019$) because of a strong variability in EPI for intermediate water quality values (reaching up to 50 and 60 for low and high WQI values, Fig. 5). The results were more consistent for *N. rhenorhodanensis* ($r^2 = 0.509$, $p = 0.0002$), for which EPI regularly increased with WQI and reached maximal values (approximately 40) for the highest WQI values. When temperature was included in the calculation of EPI (Eq. (3)), the ordination of samples changed only slightly, and the correlation coefficients did not increase significantly ($r^2 = 0.274$, $p = 0.009$, and $r^2 = 0.523$, $p < 0.001$ for *G. pulex* and *N. rhenorhodanensis*, respectively; r^2 comparison tests with and without temperature were not significant, $p > 0.05$).

In the laboratory experiment (Table 5), where dissolved oxygen concentrations were always high and the temperature constant, the EPI calculation gave intermediate values compared to field experiments (Fig. 5). Animals exposed to the water sampled in the "Control" piezometer showed mean EPI values of approximately 26.5 and 29.3 for *G. pulex* and *N. rhenorhodanensis*, respectively (with minimal EPI values of approximately 20 for both species), which correspond to intermediate water quality in field experiments. Mean EPI values slightly decreased for the animals exposed to waters sampled in the "Impacted" and "Polluted" piezometers with very low minimal values (approximately 10 for *G. pulex* and 15 for *N. rhenorhodanensis*), which correspond to low water quality in field experiments (Fig. 5).

5. Discussion

5.1. The use of sentinel organisms in groundwater (objective 1)

Our experiments highlighted differences in survival rates of crustaceans between piezometers located upstream (control) and downstream (impacted) of infiltration basins, and we therefore propose crustaceans as potential sentinel organisms for evaluating groundwater ecosystem health.

Preliminary experiments showed that cage mesh size had a low effect on survival rates, and we therefore proposed using the finest mesh size (i.e., 200 μm) to prevent any animal escape. The choice of the sentinel species was more complex. For short exposure periods (one week), very similar results were obtained for the two epigean *Gammarus* species (i.e., a similar decrease in survival rates and lower body energy reserves downstream than upstream of the infiltration basins), with a slightly higher sensitivity for *G. pulex* than for *G. roeselii*. For example, we observed a slight increase in the body glycogen content downstream of one of the studied basins (i.e., DjR site) for *G. roeselii*. For the following experiments, we chose *G. pulex* as a short-duration sentinel species, as (1) this species has a wide geographical distribution compared to *G. roeselii* (Pinkster, 1972), making it useful for this purpose in several different countries; (2) it exists at high density in several wetlands, streams and rivers, making it easy to collect; and (3) its use in

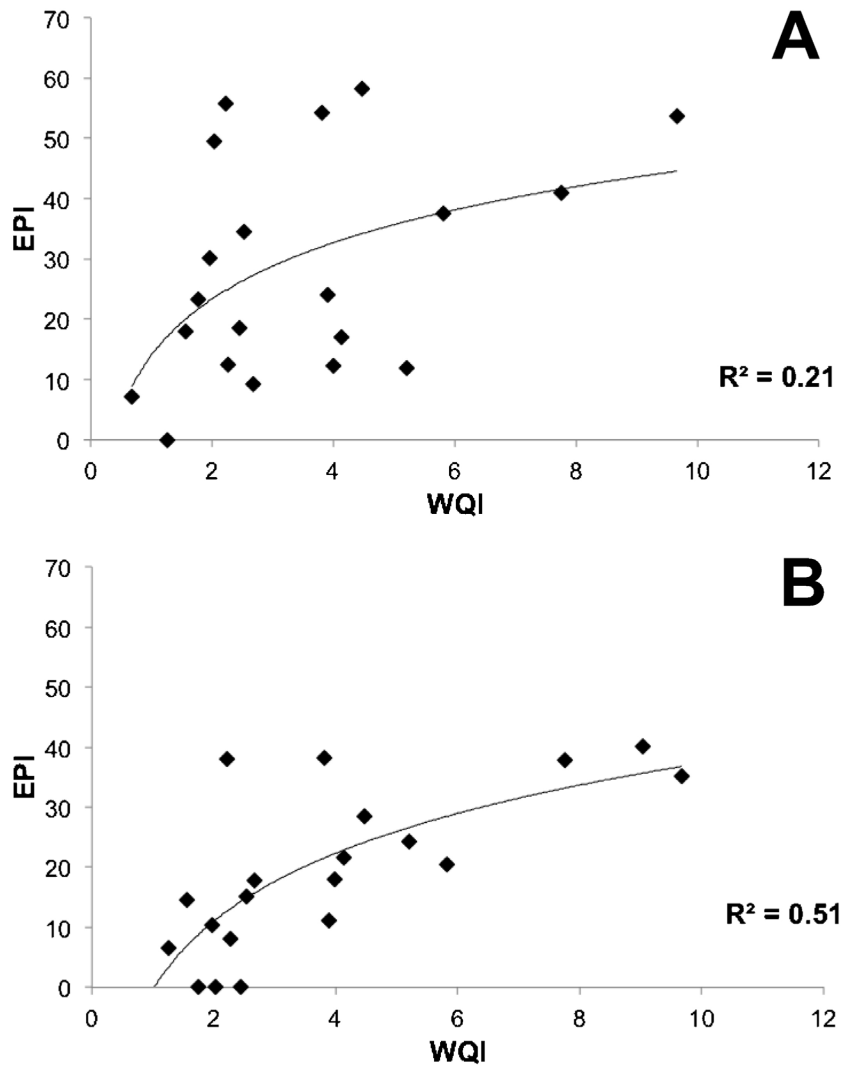

Fig. 5. Logarithmic correlations between the Water Quality Index (WQI, dimensionless) and the Ecophysiological Index (EPI, dimensionless) of *Gammarus pulex* (A) and *Niphargus rhenorhodanensis* (B).

the evaluation of surface-water quality (Maltby et al., 1990, 1994) makes cross-system comparison possible.

For sentinels used during long exposure periods (one month), similar results for survival rates were obtained for *A. aquaticus* and *N. rhenorhodanensis*, with lower rates downstream than upstream of the infiltration basins; however, body glycogen concentrations in *N. rhenorhodanensis* showed more consistent changes (i.e., a significant decrease downstream of the MIN basin) than in *A. aquaticus*. For the following experiments, we therefore chose *N. rhenorhodanensis* as the sentinel species. This amphipod was also chosen because its starvation point and oxygen needs are well known (Hervant et al., 1997, 2000), because of its sensitivity to pollutants (Canivet and Gibert, 2002; Plénet, 1995, 1999) and because groundwater is the natural habitat for this stygobite species.

5.2. Testing the method in a disturbed urban aquifer (objective 2)

5.2.1. Changes in the physicochemical groundwater characteristics linked to artificial recharge by storm water

The observed changes in groundwater chemistry downstream of the infiltration basins confirmed the results of previous studies, showing a decrease in solute concentrations (electrical conductivity, alkalinity, chloride, sulphate and nitrate) due to dilution of the groundwater by storm water (Barraud et al., 1999; Datry et al., 2004; Foulquier et al., 2010). This decrease had weak effects on the ecological quality of the groundwater ecosystem. In contrast, significant decreases in DO concentrations and significant increases in temperature variability, SRP and DOC can have strong effects on groundwater processes and quality (Chapelle, 1993; Datry et al., 2005; Foulquier et al., 2011; Griebler et al., 2010; Korbel and Hose, 2011). Few differences were observed in the concentrations of dissolved PAHs between the upstream and downstream sections of the infiltration basins. The low concentrations of dissolved PAHs found in the groundwater are not surprising and corroborate other works. Storm water (more specifically, runoff water that is drained by infiltration systems) generally has a high concentration and high variability of PAHs, but the PAHs are mostly in particulate form (Gaspéry et al., 2005; Gonzalez et al., 2000) and are adsorbed during percolation of the water through the top soil layers (Dechesne et al., 2004; Nogaro et al., 2007). Additionally, only a few differences were observed in dissolved VOC levels between the upstream and downstream locations. A punctual measure of these toxic compounds is certainly not the most efficient strategy by which to evaluate groundwater pollution (Alvarez et al., 2007; Dougherty et al., 2010). This problem of punctual sampling was highlighted by our experimental use of an integrative sampling technique (i.e., the POCIS) for polar pesticides. These compounds had been considered absent from the urban groundwater of the Lyon area because pesticides rarely reached the detection limit when punctually measured in water. In contrast, using an integrative technique, we were able to detect 32 polar pesticides in groundwater. The number of detected compounds tended to increase (IUT and MIN) or decrease (DjR and GB) downstream of the infiltration basins, while their concentrations generally increased downstream of the basins, suggesting an input of pesticides with runoff. These inputs have already been observed in several studies that focused on rural areas where pesticides are used for agriculture; some studies, however, also observed the same phenomenon in urban areas (Blanchoud et al., 2007; Fischer et al., 2003). Atrazine, simazine and terbuthylazine are no longer authorised for use in France (since 2002, 2003 and 2004, respectively). The observed changes between upstream and downstream areas may emerge from a combination of dilution, degradation and remobilisation processes (Baran et al., 2007).

5.2.2. Responses of the two selected sentinel species

The survival rates of the two sentinel species generally decreased downstream of the infiltration basins (and with water quality in laboratory experiments), but they differed according to the site and the experimental period. These variations between experimental periods may be linked to local variability in pollutant inputs. When all sites and experimental periods were grouped together, no significant relationship was observed between the survival rates of *G. pulex* and groundwater quality. In contrast, survival rates for *N. rhenorhodanensis* showed a significant positive correlation with the DO concentrations. The decrease in the survival rates of in situ sentinels is seen in many surface water studies (e.g., Maltby et al., 1990, 1994) and may be the only descriptor considered in 40% of these studies (Schmolke et al., 2010). The lack of significant correlation between the survival rates of the two sentinel species and other groundwater characteristics (e.g., DOC or the WQI) in our study clearly showed that the survival rate per se is not efficient enough as a measure of the intensity of disturbance in groundwater ecosystems.

The variations in survival rates observed between the reference and impacted piezometers may also be due to changes in groundwater temperature induced by storm water infiltrations; however, this effect is certainly weak in these experiments performed during spring and autumn. The introduction of temperature in the EPI calculation did not modify the results, and the groundwater temperature measured downstream of the basins never reached lethal values for the selected species: it never exceeded 20.7 °C during the three exposure periods, far below the physiological limits of the species (e.g., D50% at 21 °C is 111 days for *N. rhenorhodanensis*, Issartel et al., 2005a). Even if temperature remained low during the three exposure periods, temperature variability may have a combined effect with toxic compounds during summer. An interaction between temperature and toxic compounds was described for Zn on an Isopod species (Donker et al., 1998) and for ammonia on an Isopod and two *Gammarus* species (Dehédin et al., 2013). Future laboratory experiments must include the combination of pollutant and temperature variability to highlight their potential additive effect.

In relation to toxic inputs to groundwater, no correlation was observed for *N. rhenorhodanensis*, while significant negative correlations were observed for *G. pulex* with two compounds (i.e., dimethenamide and metolachlor). Significant differences in sensibility to pesticides between surface and groundwater fauna were already observed for Australian organisms (Hose, 2005). The differences between the two sentinel species observed in our study may be explained by their different sensitivities, which in turn depend on the duration of exposure. *N. rhenorhodanensis* shows very high resistance and can survive in extreme environmental conditions. For example, this species can survive severe hypoxia and starvation for at least six months (Hervant et al., 1995, 1997, 1999a) and tolerates toxic exposure well (Canivet et al., 2001, Canivet and Gibert, 2002). The higher resistance of *N. rhenorhodanensis* to toxic compounds has been ascribed to its lower metabolic and assimilation rates, meaning that the lethal internal contaminant concentration will be reached more slowly than for epigean species (Canivet and Gibert, 2002; Plénet, 1995, 1999). Consequently, these two species represent two different indicators for both the nature of the disturbance and the exposure duration: *G. pulex* may be a good short-term indicator of acute toxic pollution, while *N. rhenorhodanensis* would be an efficient indicator for long-term monitoring of persistent organic pollution and the resulting decrease in DO concentrations. A combination of these two complementary indicators may improve the reliability of groundwater assessment.

5.3. The use of the ecophysiological characteristics (objective 3)

Previous studies demonstrated that ecophysiological characteristics of amphipods changed with starvation and environmental constraints (e.g., Hervant et al., 1999). For example, Maazouzi et al. (2011) showed that the glycogen content was a good indicator of the optimal thermal window for two amphipods, including *G. pulex*. Similarly, Hervant et al. (1995, 1996, 1999a, 1999b) and Issartel et al. (2005a, 2005b) found that body reserves become depleted in *Gammarus fossarum* (an epigean species) and *N. rhenorhodanensis* under harsh environmental conditions, such as long-term starvation, severe hypoxia or variations in temperature. However, in the present study body stores seem poorly impacted by water quality (i.e. little changes between the upstream and downstream locations). Triglyceride concentrations, in particular, never significantly decreased at downstream sites. Body glycogen concentrations decreased on a few occasions (three times for *G. pulex* and two times for *N. rhenorhodanensis*). While body reserves in *N. rhenorhodanensis* were significantly correlated to DOC concentrations and the WQI, no significant correlations were observed between body stores and water quality for *G. pulex*. The measure of body stores alone is thus not sufficient to evaluate groundwater quality.

When body stores and survival rates were combined in an ecophysiological index (EPI, Eq. (2)), several significant correlations with groundwater characteristics were observed: EPI increased with available DO (for *N. rhenorhodanensis*) and WQI (for both species) and decreased with increasing DOC (for both species). The EPI can be calculated only when there was enough biomass at the end of the exposure period, and this is a major limit to its use in strongly polluted groundwater where only few animals survive. This index highlighted the fast response to acute environmental stresses because glycogen is used rapidly to maintain homeostasis. To the best of our knowledge, this is the first report using body stores as ecophysiological indicators of stress in aquatic invertebrates being used as in situ sentinel species. The combination of survival rates and changes in body store must thus be tested in different types of habitats (karstic systems, fissured aquifers), different pollution contexts (agriculture), and in laboratory experiments with major types of pollutants (pesticides, metals). The first test in the laboratory was performed on groundwater sampled in the Lyon area, with a low level of pollution, constant temperature (11 °C) and no limitation in dissolved oxygen availability. Future experiments must combine contrasting levels of toxic compounds with increases in temperature and decreases in oxygen concentrations.

Differences observed between the two species suggested that a stygobite species (such as the amphipod *N. rhenorhodanensis*) may be of greater interest than an epigean organism for evaluating global groundwater quality (diffuse organic pollution and DO depletion), even though epigean species are widely used in surface water. The high efficiency of stygobite species as sentinels for evaluating disturbances in a groundwater ecosystem is directly linked to their physiological strategies: lower metabolic rate (Roux, 1982), low growth and reproductive rates and increased longevity (Ginet, 1960). Such life-history traits make possible the observation of longer periods of stress than can be used for epigean species (e.g., thermic stress, Issartel et al., 2005a, 2005b, 2007; metal pollution, Plénet, 1995, 1999; starvation, Hervant et al., 1997).

6. Conclusions

In conclusion, the results of the present study suggested that sentinel crustaceans may be an efficient tool with which to evaluate groundwater quality. Short-term exposure (one week) of an epigean species (such as *G. pulex*) may be used for acute toxic disturbances, while the exposure of a stygobite species (here *N. rhenorhodanensis*) for a long period (one month) may be the most efficient indicator of diffuse organic pollution and global groundwater ecosystem quality. The first results presented here also suggest that the survival rates of the sentinels are insufficient for correct characterisation of groundwater disturbance; integrated ecophysiological parameters are needed for a more precise evaluation of the disturbance. Future studies must focus on the ecophysiological characteristics that may be measured after exposure and used for other EPI calculations. The present study was based on a small number of experiments and focused only on an urban aquifer disturbed by artificial storm water infiltrations. Other types of aquifers (e.g., karstic aquifers) and other types of human activities (e.g., agriculture) must be considered in future research to increase the number of case studies and to optimise the use of sentinel organisms in groundwater quality evaluations.

Acknowledgements

The authors thank OTHU (Field Observatory in Urban Water Management, especially L. Bacot), the Greater Lyon (especially R. Visiedo and L. Campan) and the Rhône-Alpes Region for scientific and technical support. This study benefited from the scientific background and exchanges linked to the ZABR (LTER 'Zone Atelier Bassin du Rhône') and the Water Agency Rhône Méditerranée & Corse networks. We thank Yannick Negrutiu and Chloé Legros for early experiments on sentinel organisms.

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