Ecological Indicators 116 (2020) 106525

Contents lists available at ScienceDirect

Ecological Indicators

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

The weighted Groundwater Health Index (wGHI) by Korbel and Hose (2017) in European groundwater bodies in nitrate vulnerable zones

Tiziana Di Lorenzo[a,*], Barbara Fiasca[b], Agostina Di Camillo Tabilio[b], Alessandro Murolo[b], Mattia Di Cicco[b], Diana Maria Paola Galassi[b]

[a] Research Institute on Terrestrial Ecosystems (IRET-CNR), Via Madonna del Piano 10, 50019 Sesto Fiorentino, Florence, Italy
[b] Department of Life, Health and Environmental Sciences, University of L'Aquila, Via Vetoio 1, Coppito 67100, L'Aquila, Italy

ARTICLE INFO

Keywords:
Unconsolidated aquifer
Stygofauna
Ecological status
European Directives
Nitrates

ABSTRACT

The weighted Groundwater Health Index (wGHI), introduced by Korbel and Hose (2017), is a multimetric, two-tiered framework for measuring and identifying the ecological status of groundwater ecosystems using biotic and abiotic indicators. The wGHI was conceived and tested in unconsolidated alluvial aquifers in Australia. In this study we applied and tested the index in European unconsolidated aquifers located in nitrate vulnerable zones (NVZ). A refinement of the wGHI was necessary to ensure the compliance with the requirements of the European Directives. We called the refined index $wGHI^N$ where N stands for nitrates. We tested the $wGHI^N$ in an unconsolidated aquifer (VO_EU_GWB) in the River Vomano catchment (central Italy) that was designated NVZ in 2005 and has since been subject to management measures pursuant to the Nitrate Directive. We also monitored a complex of minor confined unconsolidated aquifers (VO_CON_GWB) located in the same catchment. The $wGHI^N$ highlighted extensive nitrate contamination in both the VO_EU_GWB and VO_CON_GWB aquifers. Despite the widespread contamination, most of the monitoring sites showed only minor deviations from good ecological status. The index also highlighted the biodiversity of the aquifers which happened to be among the most diverse in Europe. The $wGHI^N$ proved to be theoretically sounding, concrete, numerical and easily understandable by the public and policy-makers. Finally, the index was economically sustainable. The $wGHI^N$ has certain limitations that have to be resolved, such as the low correlation of some indicators to the index overall score in the aquifers of the River Vomano and the fact that the index is not “operationally simple” since it requires taxonomic and ecological skills.

1. Introduction

Groundwater hosts about 5000 species of stygobiotic invertebrates, of which 70% are crustaceans (Botosaneanu, 1986; Stoch and Galassi, 2010). Stygobiotic invertebrates are those that carry out their entire life cycle in groundwater habitats and are unable to survive and reproduce in surface water habitats (Gibert et al., 1994). In Europe there are approximately 2000 stygobiotic invertebrate species (Gibert et al., 2009), of which 1570 are crustaceans (Zagmajster et al., 2014). This number is surely underestimated and constantly increasing (about 10–20 new species per year; Stoch and Galassi, 2010) due to the high number of narrow endemics (i.e., species with a distribution restricted to a single aquifer or a portion of it, or to a single sampling site; Boulton, 2020). However, despite the noteworthy taxonomic richness, there is still no European regulation for the protection and conservation of the stygofauna. In fact, the Habitat Directive (92/43/EEC; EC, 1992), concerning the conservation of habitats and species of wild fauna and flora of interest at the European scale, only marginally deals with groundwater ecosystems. The two main European water Directives, the Water Framework Directive (WFD; EC, 2000) and the Groundwater Daughter Directive (GDD; EC, 2006), define groundwater in many ways (e.g., “natural resource”, “water which is below the surface of the ground”) but never as an ecosystem hosting specialized fauna.

At present, the only way to protect the stygobiotic diversity in Europe is through the management measures that Member States must put in place to protect the groundwater quality and quantity pursuant the WFD and the GDD. It is hoped (but not mandated or planned) that these measures will also protect the specialized fauna. For surface water things are different. According to Annex V of the WFD, the ecological status of a surface water body must be periodically monitored and considered good when both its biological and chemical elements show no, or only very minor, evidence of distortion from the reference

* Corresponding author.
E-mail address: tiziana.dilorenzo@cnr.it (T. Di Lorenzo).

https://doi.org/10.1016/j.ecolind.2020.106525
Received 8 March 2020; Received in revised form 6 May 2020; Accepted 11 May 2020
Available online 21 May 2020
1470-160X/

conditions (EC, 2000). In contrast, the ecological status of groundwater bodies is not even defined by the WFD and GDD, despite various indicators, indices and approaches being proposed for this purpose over recent years (e.g., Malard et al., 1996; Hahn, 2006; Korbel and Hose, 2011, 2017; Marmonier et al., 2013; Mermillod-Blondin et al., 2013; Griebler et al., 2014). A framework based on a two-tiered approach using biotic and abiotic indicators, and culminating in an index called the weighted Groundwater Health Index (wGHI), was proposed by Korbel and Hose (2011; 2017). With respect to the previous approaches, the wGHI had the merit of considering both biotic and abiotic indicators of a groundwater body and classifying its overall ecological health based on the deviation from reference conditions. The wGHI approach was conceived and first applied in several unconfined alluvial aquifers located in eight Australian catchments, where agriculture was the main anthropic pressure in all catchments.

In Europe, a specific type of unconsolidated alluvial aquifer in agricultural catchments is that of nitrate vulnerable zones (NVZ; EC, 1991). An NVZ aquifer is a groundwater body with a significant groundwater flow (> 10 m^3/day) and a volume of abstraction sufficient to serve 50 people, that is also extensively polluted by nitrates from agriculture, with concentrations exceeding the European regulatory threshold of 50 mg/L NO_3^- in $> 20\%$ of the sampling sites (EC, 2000, 2006). This study aimed at: i) applying the wGHI approach in European NVZ unconsolidated aquifers and assessing its performance; ii) refining the approach through incorporating a Tier 3 consisting of five site selection criteria (this refined wGHI is called $wGHI^N$ hereafter; *N* stand for nitrates); iii) highlighting the applicability of the $wGHI^N$ as a management and communication tool for local authorities in charge of NVZ in Europe. To this end, we investigated an unconsolidated aquifer in central Italy (VO_EU_GWB) that was designated NVZ in 2005 and has since been subject to management measures by the local authority pursuant the Nitrates Directive. We also monitored a series of minor confined unconsolidated aquifers (hereafter cumulatively indicated as VO_CON_GWB complex), not designated NVZ, albeit surrounding VO_EU_GWB and within the same catchment.

In this study, the following adjustments to terminology were made to adapt the wGHI jargon to that of the two European Water Directives: 1) “groundwater ecosystem” was replaced by “groundwater body”, which is the management groundwater unit according to the European Directives; 2) “groundwater ecosystem health” was replaced by “ecological status of a groundwater body”; 3) ” similar to reference health” was replaced by “good ecological status”; “mild deviation from reference” by “mild deviations from good ecological status” and “major deviation from reference” by “major deviations from good ecological status”.

2. Study area

2.1. Vo_eu_gwb

VO_EU_GWB is an unconsolidated aquifer located in the catchment (485 km^2) of the River Vomano (central Italy; Fig. 1). The VO_EU_GWB outcrop is 30 km^2 (Desiderio et al., 2003). The aquifer substrate consists of very low permeable lithotypes (marly clays and clayey marls). The overlaying permeable deposits are distributed in four orders of terraces, consisting of gravelly and sandy-gravelly thick layers (max 28 m) and, subordinately, silty-clayey lenses of a few meters (Desiderio et al., 2003). The hydraulic conductivity is high and ranges between 1×10^{-4} m/s and 2×10^{-3} m/s. The analysis of the unconsolidated deposits showed that VO_EU_GWB can be considered a monolayer aquifer (Regione Abruzzo, 2010). The aquifer is mainly fed by the River Vomano waters, with minor recharges due to rainfall (about 800 mm/y). The groundwater flow is directed toward the Adriatic coast with the main drainage axes corresponding to the paleo-riverbeds (Desiderio et al., 2003). The land use is mainly agricultural with two small industrial areas of limited extension. VO_EU_GWB meets the requirements of the WFD (i.e., groundwater flow > 10 m^3/day and a volume of abstraction sufficient to serve 50 people) and was, therefore, identified as a significant groundwater body and, as such, subject to a chemical and quantitative monitoring by the local environmental protection agency every six months since 2000 (Regione Abruzzo, 2010). Due to the persistent nitrate contamination affecting $> 50\%$ of the aquifer volume, VO_EU_GWB has been designated as a NVZ since 2005 and subject to restrictive measures regarding the use of synthetic and organic fertilizers and of the number of cattle head per hectare (Regione Abruzzo, 2010).

2.2. Vo_con_gwb

The territory that extends outside VO_EU_GWB, though within the same catchment, is a hilly area mainly consisting of Plio-Pleistocene clayey and clayey-marly layers, with arenaceous deposits of decametric thickness alternating in sequence (Desiderio et al., 2003). In the arenaceous layers, several narrow confined aquifers occur, with a groundwater flow rarely higher than 1 L/s each (Fig. 1). Since none of these aquifers can serve at least 50 people, they were not considered significant groundwater bodies according to the WFD and, as such, they have never been monitored by the local environmental agency. These minor aquifers (overall indicated with VO_CON_GWB in this study) are exploited for irrigating small vegetable gardens (Regione Abruzzo, 2010). The VO_CON_GWB outcrops cover an area of about 120 km^2, cumulatively. The area is mainly used for agricultural purposes, with large zones of uncultivated pasture.

3. Materials and methods

3.1. Sampling survey

In this study, we monitored 40 bores used for irrigation in VO_EU_GWB and 26 in the VO_CON_GWB complex, in Autumn 2014 and in Spring and Autumn 2015. It was not always possible to make three sampling surveys per each bore due to unforeseen events related to the availability of the owners. The bores were located in areas with $> 95\%$ of land use devoted to agriculture. The bores' depth was in the range 2–32 m in VO_EU_GWB and 2–100 m in VO_CON_GWB with two bores (PV7 and PV32) located in deep aquifers. The bores' characteristics (coordinates, altitude above sea level, depth) are reported in the BORES sheet of the Supplementary File and in Table 1.

The basic chemico-physical parameters (temperature, pH, dissolved oxygen and electrical conductivity) were measured in each bore on each occasion using a WTW 3430 SET G multi-parameter probe prior to stygofauna collection. The piezometric level was measured by a phreatimeter. Afterwards, stygofauna was collected using two different methods depending on the bore type. A Cvetkov net (Cvetkov, 1968), with a mesh of 60 µm, was used in hand-dug shallow bores and in bores with a diameter > 50 cm. The net, equipped with a weight anchored to one end, was lowered into the bores down to the bottom and subsequently hauled in order to filter the entire column of groundwater. Ten hauls were performed in each bore (Hancock and Boulton, 2009). The volume (V) of the filtered water column was calculated as $V = \pi r^2 \cdot h^3$ where *r* was the bore radius in meters and *h* was equal to the difference between the piezometric level and the depth of the bore in meters. In the bores with a diameter < 50 cm, stygofauna was collected by pumping 50 (when replenishment of the bore was slow) to 500 L of water and passing it through a 60-µm mesh sieve (Malard et al., 2002). Pump filters were unhooked before using so as not to damage the animals. The biological samples (153 in total) were preserved by adding alcohol up to 70% in solution in 500 mL plastic vials. Bores were purged after stygofaunal collection by pumping three bore volumes of groundwater. Afterwards, two liters of bore water were taken in order to analyze 56 agrochemicals (sulphates, N-compounds, heavy metals, fertilizers and pesticides). Volatile organic compounds were also

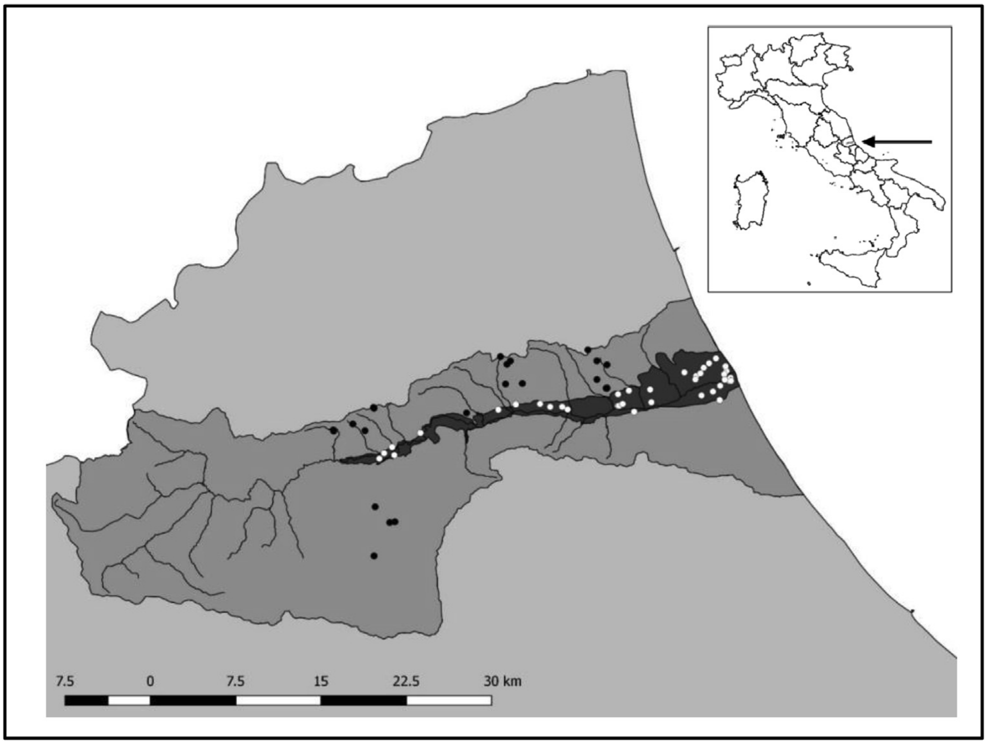


Fig. 1. Location of VO_EU_GWB (black) and VO_CON_GWB (dark gray) in Abruzzo region (light gray) and Italy. Sampling sites are represented by white dots in VO_EU_GWB and black dots in VO_CON_GWB. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

analyzed in order to ascertain that the two small industrial areas and the narrow urban areas had not contaminated the aquifers.

Biological samples were sorted in the laboratory using a Leica M205C stereomicroscope at 16x magnification. The specimens were then classified to the lowest taxonomic level possible, and the taxa were attributed to two ecological categories (Gibert et al., 1994): stygobites (SB; species that spend the whole life cycle in groundwater habitats) and non-stygobites (nSB; epigean species that accidentally occur in groundwater habitats but that do not have any adaptation to stably live there). Copepods were identified to species level according to Dussart and Defaye (2006) and the more recent literature available. Taxonomic keys were used to identify the taxa to different hierarchical levels belonging to the following groups: Acari (Di Sabatino et al., 2002), Crustacea (Kiefer, 1960; Amoros, 1984) and Diptera Chironomidae instars (Schmid, 1993). Each collected SB specimen was inspected under the stereomicroscope at 12X magnification and i) juveniles and adults were identified, ii) sex (M or F) was assigned to adult individuals.

3.2. Preliminary characterization

The aquifers were characterized to provide preliminary information. First, the biological sampling effort was assessed through a species richness estimation, using non-parametric estimators, namely Chao 1, Chao 2, Jackknife 1 and Jackknife 2, Bootstrap, MM and UGE, all described in Magurran and McGill (2011) (2010 and references therein). Values were estimated by means of 999 randomizations without replacement. Secondly, environmental and biological differences between VO_EU_GWB and the VO_CON_GWB complex were ascertained by using one-way permutational analysis of variances (PERMANOVA; Anderson, 2008). The environmental PERMANOVA was performed retaining the 22 variables (out of 116) that showed a standard deviation different from zero at least in one of the two aquifers. In fact, variables with standard deviations equal to zero must be excluded from the analyses to avoid multi-collinearity (Anderson et al., 2008). The abiotic variables were normalized before PERMANOVA. Levene's test was performed prior to PERMANOVA to verify the homogeneity of the variances. Unrestricted permutation of raw data and Type I of sum of squares on a Euclidean similarity matrix were applied as they provide an exact test for an unbalanced one-way design (Anderson et al., 2008). The significance level (α) was set at 0.05. The environmental variables were also explored by univariate statistics, using independent samples t-tests when both the assumptions of normality and homoscedasticity were valid, or with a Wilcoxon rank sum test. The Shapiro's test was used to test the normality of the data while the Levene's test were used to verify the homoscedasticity of the variances. The biological PERMANOVA was performed on the basis of a Bray–Curtis dissimilarity matrix computed on taxa densities (ind/L) under the same settings as for the environmental variables. A dummy variable equal to 1 was added to all biological samples to allow the inclusion of otherwise empty cells. The biological data were log (x + 1)-transformed prior to the analyses. All multivariate analyses were performed with E-PRIMER and PERMANOVA + software (Anderson et al., 2008). Univariate analyses were performed using R software v. 2.15.0 (R Development Core Team, 2008).

4. The wGHI^N theory

The wGHI^N consists of the wGHI approach followed by a site selection procedure. The selection procedure is necessary for wGHI meeting the requirements of the European Directives (EC, 1991, 2000, 2006). The original wGHI was applied to shallow aquifers only. In this study, we applied the wGHI^N to shallow aquifers and also to two deep aquifers belonging to the VO_CON_GWB complex. We discussed the results for these aquifers in a separate section of the results.

4.1. The wGHI rational

The wGHI is based on two tiers of assessment of functional, organizational and stressor indicators. The overall weighted score (OWS) of the wGHI must be calculated for each temporal replicate (hereinafter referred to as "site replicate") of each site in a groundwater body (Korbel and Hose, 2017). The indicators of the two tiers of the wGHI were identified and described by Korbel and Hose (2011, 2017), to which reference is made for information. In brief, Tier 1 (Table 2)

Table 1
Mean values, standard deviations (SD), maximum and minimum values of 22 environmental parameters occurring with concentrations above the detection limits in VO_CON_GWB and VO_EU_GWB aquifers. Alt: altitude (m a.s.L.); W.t.: water table (m b.g.L.); T: temperature (°C); EC: electrical conductivity (µS/cm); DO: dissolved oxygen (mg/L); POM: particulate organic matter (mg/L); TOC: total organic carbon (mg/L); DOC: dissolved organic carbon (mg/L); NO_2^-, NO_3^-; NH_4^+, SO_4^{2-}; Cl^-, PO_4^{3-}, Ca^{2+}, K^+, Na^+ are in mg/L; DIC: *cis*-1,2-dichloroethylene (µg/L); TCE: 1,1,2,2-tetrachloroethylene (µg/L); CHL: trichloromethane (µg/L); THC: total hydrocarbons (expressed as n-hexane in µg/L). Values exceeding the European and Italian legal threshold values are indicated in bold.

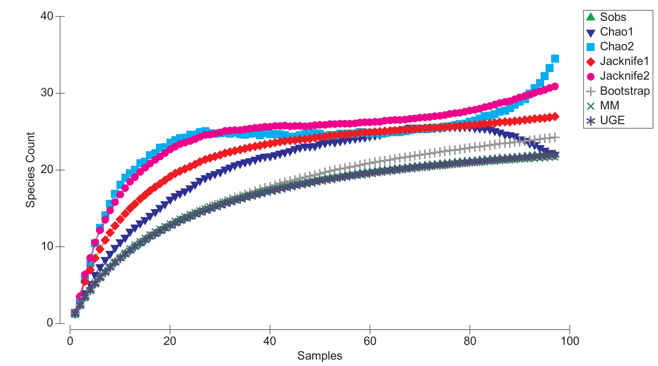
	Alt	W.t.	T	EC	pH	DO	POM	TOC	DOC	NO_2^-	NO_3^-	NH_4^+	SO_4^{-2}	Cl^-	PO_4^{3-}	Ca^{2+}	K^+	Na^+	DIC	TCE	CHL	THC
Quality standards										0.5	50	0.5	250						0.05	1.1	1.5	3.5
VO_CON_GWB																						
Mean	320	17	15.5	1555	7.29	3.74	1.19	10.9	10.1	**3.11**	**57.56**	**1.56**	219.78	109	0.39	149	14.2	98	0.025	0.025	0.025	25
SD	126	21	1.8	887	0.2	1.97	3.55	17.2	17	10.36	51.16	3.73	204.93	204.93	0.49	98.7	16.2	177.7	0	0	0	0
Max	530	90	19	4795	7.85	7.84	28.1	88.3	87.3	39.51	200	16.53	1400	1062.5	2.65	591	78	935.5	0.025	0.025	0.025	25
Min	128	1	13.2	610	6.97	0.67	0	1.1	0.9	0.02	0.38	0.02	28	13	0.14	33.6	2.6	19.5	0.025	0.025	0.025	25
VO_EU_GWB																						
Mean	73	8	16.9	1167	7.28	4.69	0.74	3.02	2.18	**10.25**	**61.6**	0.23	103.75	56	0.27	145	5.2	60	0.003	0.071	0.037	26
SD	72	5	1.7	232	0.18	1.56	2.32	2.4	1.7	11.19	30.07	1	51.97	37.49	0.42	30.1	2.3	33.3	0.004	0.132	0.046	4
Max	240	26	20.9	1480	7.74	7.16	14.1	10.05	8.85	34.51	122.67	2.17	281	172.67	2.84	218.5	11.1	162.7	0.3	0.8	0.293	53
Min	0	1	13.8	260	7	1.19	0.01	0.7	0.6	0.025	2	0.02	5.27	6.37	0.11	63	1.2	5	0.025	0.025	0.025	25

Table 2
The two tiers of the wGHI by Korbel and Hose (2017). Tier 1 and 2 indicators are shown together with their respective benchmarks. MA: microbial activity. ESP: Environmental Sample Processor.

Tier 1		
Indicator type	Indicator	Benchmark
Functional	Dissolved Organic Carbon	< 4 mg/L
Organizational	Total abundances of crustaceans	> 50%
Organizational	Total abundances of oligochaetes	< 10%
Organizational	Stygoxenes	Absent
Stressors	Pesticides	Absent
Stressors	Nitrate-N	< 2 mg/L
Tier 2		
Functional	MA – number of sources used, assessed through BiologTM Ecoplates	0.12–0.97
Functional	MA ESP – loss of tensile strength assessed through cotton strip essay	24.1 ± 6
Functional	Abundance of stygobiotic species	> 1
Functional	Dissolved organic carbon	0–3.7
Organizational	Microbial diversity, assessed through BiologTM Ecoplates	1–21
Organizational	Stygobiotic species richness	1–8
Organizational	% crustaceans (abundances)	50–100
Organizational	% oligochaetes (abundances)	0–36
Organizational	% stygoxenes (abundances)	0–14
Stressors	Agrochemicals	Absent
Stressors	Nitrates (mg/L)	0–2
Stressors	Reactive phosphorous (mg/L)	0.03–0.57

allows for a preliminary assessment of the ecological status of a site replicate based on 6 indicators, namely: i) dissolved organic carbon (functional type); ii) total abundances of crustaceans, total abundances of oligochaetes, presence/absence of non-stygobiotic taxa (organizational type); iii) presence/absence of pesticides and nitrate (stressor type). If one of the site replicates fails even only one of the benchmarks of Tier 1, then the site shall go through a Tier 2 assessment (Table 2). What happens if, instead, all the site replicates pass all the benchmarks of Tier 1, is explained later in the text.

Tier 2 (Table 2) deepens on the ecological conditions of the site replicates through 12 indicators (4 of the functional type, 5 of the organizational type and 3 of the stressors type). The number of failures (hereafter indicated with “F”) first must be added per type of indicator (respectively, organizational, functional and stressor) and then multiplied by a pre-set weighting factors that, in the case of the stressor indicators, is equal to 2. The weighting factors for the organizational and functional indicators were determined by Korbel and Hose (2017) by considering the number of environmental variables (e.g., sediment types, percentage of total organic matter, prevalence of trees or dissolved oxygen concentration) influencing the stygofaunal assemblages. The overall average ‘failure’ score for both functional and organizational indicator groups must be multiplied by 0.5 if three or more environmental influencing factors are present at the sites, by 0.75 if there are two environmental factors and by 0.875 if there is one environmental factor influencing the site (Korbel and Hose, 2017). The OWS is calculated for each site replicate as the average of the weighted number of failures of each indicator type, rounding to the nearest whole number. The interpretation of the OWS is as following: good ecological status for OWS = 0 or 1; mild deviation from good status when OWS = 2 or OWS = 3; major deviation from good ecological status when OWS ≥ 4. OWS must be assessed for each site temporal replicate to take into account the variability of the sites (Korbel and Hose, 2017). A diagram of Tier 2 is shown in Fig. 2 of Korbel and Hose (2017). In this study, the microbiological indicators were not examined for lack of funds. The missed application of three microbiological indicators (two functional and one organizational) prevented the OWS from assuming values > 3. For this reason, the ranges of OWS values were re-modulated as follows: good ecological status for OWS = 0 or 1; mild deviation from good ecological status when OWS = 2; major deviation



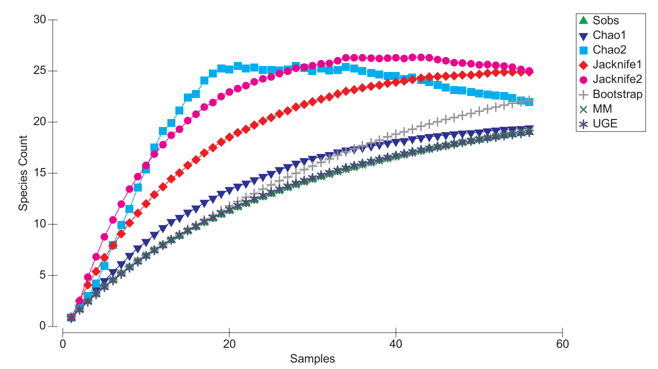


Fig. 2. Species rarefaction curves and estimators' curves for the taxa collected in VO_EU_GWB (up) and VO_CON_GWB (bottom) at increasing sample size. S (obs): species rarefaction curve of observed species richness. Other lines represent the estimated species richness using mean values obtained by the non-parametric estimators. Estimated species richness after 97 samples in VO_EU_GWB: Chao1: 56; Chao2: 43; Jacknife1: 46; Jacknife2: 48; Bootstrap: 42; MM: 38; UGE: 38. Estimated species richness after 56 samples in VO_CON_GWB; Chao1: 31; Chao2: 37; Jacknife1: 40; Jacknife2: 42; Bootstrap: 35; MM: 31; UGE: 31.

from good ecological status when OWS $>$ 2. The remodeling is allowed as "*it is possible to choose different subsets of indicators and methods for Tier 2 assessment depending on the situation or resources available"* (Korbel and Hose, 2017)".

To assess the performance of the wGHI in VO_EU_GWB and VO_CON_GWB complex, we considered how the quality of each individual indicator affected the performance of the resulting OWS (Moriarty et al., 2018). Quality was defined as the ability of an indicator of Tier 2 to track the OWS and was assessed by computing the indicator's correlation to the OWS, according to Moriarty et al. (2018). We defined high-quality indicators those showing Spearman's correlation $\geq$0.70 to the OWS, medium-quality indicators those with correlation in the range 0.30–0.69 and low-quality indicators those showing correlation $<$ 0.30. The metric used to evaluate the ability of the wGHI to track the OWS (performance) was the average Spearman's correlations, computed at regional (n = 153) and aquifer scale (n = 97 in VO_EU_GWB; n = 56 in VO_CON_GWB), where n is the number of site replicates.

4.2. The site selection procedure

The Directives require that the Member States report an overall verdict of the ecological status of a groundwater body as either good or poor, on the basis of the results of two to three sampling surveys in a number of sampling sites sufficient to represent most of its volume. To this purpose, we introduced a Tier 3, following Tier 2, that made the necessary refinement to the wGHI. Tier 3 consists of five site selection criteria.

Criterion#1 (*Tier 1 refinement*): if a site meets all the indicators' benchmarks of Tier 1 in all its replicates and shows at least one SB species in at least one of its replicates, it shall be considered having an OWS = 1 and passed to Criterion#5. Otherwise, it shall be inspected through Tier 2.

Criterion#2 (*Tier 2 refinement*): each site shall be attributed the highest OWS value among those of its replicates.

Criterion#3 (*Tier 2 refinement*): the sites that, following Criterion#2, are in good ecological status (OWS = 0–1), but never hosted SB species, shall be excluded from Criteria#4 and #5 and reported with a gray color and an "undetermined ecological status" interpretation in the map. The absence of SB species in sites where no functional, organizational and stressor deviations from the respective benchmarks occur, can be due to factors not considered in the wGHI, such as the alteration of the aquifer level due to excessive pumping (Di Lorenzo and Galassi, 2013), unsuitable sediments (Korbel et al., 2019; Piccini et al., 2019), presence of chemical compounds not related to the agricultural practice and, therefore, not monitored, such as pharmaceutical compounds or mixtures of compounds with synergistic toxic effects (Di Marzio et al., 2018; Di Lorenzo et al., 2018, 2019). The sites with an "undetermined ecological status" must be subject to a specific monitoring, of which details will be given in the discussion paragraph.

Criterion#4 (*Tier 2 refinement*): the sites that, following Criteria#2 and 3, are in good ecological status (OWS = 0–1) but that do not have at least one SB species with, at least, three individuals, one male, one female and one juvenile, shall be excluded from Criterion#5 and reported with a "good ecological status but with potentially impaired population dynamics" interpretation and a yellow color in the map. Criterion#4 is to be intended as an early warning principle indicating possible alterations of the populations' dynamics. The rationale of Criterion#4 was based on the evidence that: i) stress factors can affect population dynamics of some species of freshwater crustaceans (e.g., Peschke et al., 2014; Cifoni et al., 2017;); ii) the sex ratio of some populations of freshwater copepods were skewed in favor of female individuals in impaired water bodies (e.g., Krupa, 2015); iii) the development of juvenile copepods was slowed down in populations exposed to ammonium nitrate (Di Marzio et al., 2013); iv) juveniles of some species of stygobiotic copepods, such as *Diacyclops belgicus*, were more sensitive to ammonium nitrate than adults (Di Marzio et al., 2018).

Criterion#5 (*Tier 2 refinement*): following Criteria#1–4, a groundwater body shall be considered in a good ecological status, and reported with a green color in the map, if > 80% of its sites is in good ecological status (OWS = 0–1). Otherwise, the groundwater body shall be considered in a poor ecological status and reported with a red color in the map. The 80% is suggested as default criterion by WFD (CIS-EU, 2009).

5. Results

5.1. Preliminary characterization

The values of the 116 environmental variables of VO_EU_GWB and VO_CON_GWB, per sampling survey, are shown in the Supplementary File (CHEM). Only 22 out of 116 variables occurred with concentrations above the detection limits and were used in the statistical analyses (Table 1). Nitrites contamination was detected in 35% of sites in VO_CON_GWB and 60% of sites in VO_EU_GWB. Nitrate contamination (concentrations $\geq$50 mg/L) was detected in 65% of sites in VO_CON_GWB and 75% in VO_EU_GWB. Nitrate concentrations were > 2 mg/L in 97% of the sites in both aquifers. Ionized ammonium contamination was detected in 31% of sites in VO_CON_GWB and none in VO_EU_GWB. The variances of the 22 variables were heteroskedastic (PERMDISP: F = 4.13, p = 0.0462). Although this does not constitute a specific violation of PERMANOVA's assumptions, we preferred not to perform this analysis on the whole set of variables to avoid errors of interpretation. The number of variables was, therefore, reduced to 20 by excluding altitude and piezometric level. The variances of the remaining 20 variables were homoscedastic (PERMDISP: F = 2.81, p = 0.0986). The PERMANOVA analysis showed that the hydrochemistry was significantly different between VO_CON_GWB and VO_EU_GWB (PERMANOVA, pseudo-F = 3.71, p = 0.0001, perm = 9898), VO_CON_GWB being higher in the concentrations of calcium, sulphates, ammonium, potassium, sodium, chlorides, TOC, DOC and electrical conductivity (Table 1). Univariate statistics were significant for temperature (W = 282, p = 0.0018), dissolved oxygen (t = −2.09, p = 0.0426), TOC (W = 768.5, p = 0.0011), DOC (W = 802.5, p = 0.0002), NO_2^- (W = 301, p = 0.0034), PO_4^{3-} (W = 753, p < 0.0001), K^+ (W = 727.5, p = 0.0066), 1,1,2,2-tetrachlorethylene (W = 325, p = 0.0005) and altitude (W = 714, p = 0.0111). Temperature (W = 282, p = 0.0026), NO_2^- (W = 243, p = 0.0001), PO_4^{3-} (W = 585.5, p = 0.0056) and K^+ (t = 2.6013, p = 0.0124) were also significant after outliers were removed. VO_EU_GWB, which is located at lower altitudes respect to VO_CON_GWB, had higher values of temperature, dissolved oxygen, nitrites and phosphates and lower values of TOC, DOC and TCE compared to VO_CON_GWB (Table 1).

Overall, 5724 individuals belonging to 38 taxa (22 SB and 16 nSB) were collected in VO_EU_GWB and 10,733 individuals belonging to 31 taxa (19 SB and 11 nSB) in VO_CON_GWB (Supplementary File, BIO). Crustaceans, insects and acari were collected in VO_EU_GWB; crustaceans, insects, acari and nematomorphs were collected in VO_CON_GWB. All SB taxa were crustaceans in both VO_CON_GWB and VO_EU_GWB. Copepods (Crustacea Copepoda) accounted for > 97% of the abundances in both VO_EU_GWB and VO_CON_GEB. Four SB species new to science were found, one in VO_EU_GWB and three in VO_CON_GWB. In VO_EU_GWB, three non-parametric estimators out of seven reached the asymptotes, indicating the exhaustiveness of the sampling effort (Chao1, MM and UGE; Fig. 2a). The remaining estimators indicated that the sampling effort was such to unveil from a minimum of 63% (Chao 2) to a maximum of 91% (Bootstrap) of the expected biodiversity. Similarly, in VO_CON_GWB, three non-parametric estimators out of seven reached the asymptotes (Chao1, MM and UGE; Fig. 2b). The remaining estimators indicated that the sampling effort was such to unveil from a minimum of 79% (Chao 2) to a maximum of 86% (Bootstrap) of the expected biodiversity. Thirteen species were exclusive of VO_EU_GWB, while 6 species occurred in VO_CON_GWB only (Supplementary File, BIO), however the PERMANOVAs returned no significant differences in the biological assemblages of the aquifers (pseudo-F = 1.76, p = 0.0986, perm = 9937).

5.2. The wGHI^N

The environmental variables considered to be associated with high values of stygobiotic species richness and abundances in the aquifers of the River Vomano were: 1) sand and coarse sand/gravel and 2) dissolved oxygen concentrations > 30%. The numbers of failures of the functional and organizational indicators were multiplied by 0.875 in twenty-one site replicates where dissolved oxygen concentrations were < 30% and by 0.75 in the remaining site replicates. All the site replicates in VO_EU_GWB presented at least one failure of the Tier 1 benchmarks (Supplementary File, Tier 1), likewise for all the site replicates in VO_CON_GWB. In detail, 95% of the VO_EU_GWB site replicates and 91% of VO_CON_GWB's failed the stressor benchmarks because of nitrates (Table 3). In addition, functional indicator failures occurred in 13% of the VO_EU_GWB site replicates and in 30% of VO_CON_GWB's. Finally, 52% of the VO_EU_GWB site replicates and 64% of the VO_CON_GWB's failed the organizational benchmarks. The results of Tier 2 for each site replicate are shown in the Supplementary File (Tier 2). Overall, 87% of the VO_EU_GWB site replicates and 89% of VO_CON_GWB's failed the functional benchmarks. The organizational benchmarks were failed in 61% of the site replicates of VO_EU_GWB and in 71% of VO_CON_GWB's. In both aquifers, most of the failures were due to deviations in the percentages of nSB and SB abundances. The stressors benchmarks were failed in 97% of VO_EU_GWB site replicates and 98% of VO_EU_GWB site replicates. The failure rate for the nitrate benchmark was > 90% in both aquifers.

Finally, 54% of the site replicates of VO_EU_GWB were in good ecological status, 44% showed only mild deviation from good ecological status and 2% major deviations. As for the VO_CON_GWB complex, 46% of the site replicates were in good ecological status, 50% showed mild deviations and 4% major deviations (Table 3). The values of OWS were variable from one survey to another (Table 3) meaning that most of the sites passed from a good ecological status to mild/major deviations, and vice versa, during the survey.

The performance of the wGHI was medium at all scales (r = 0.36, n = 153; r = 0.44, n = 97; r = 0.44, n = 56). Overall, 7 out of 9 indicators of Tier 2 showed significant correlation to OWS (p < 0.05), however the quality was high for the agrochemical indicator only, being low for nitrate and medium for the remaining indicators (Table 4). At the aquifer level, the agrochemical indicator showed the highest quality in both the two aquifers, being the quality of the remaining indicators medium/low (Table 4).

The whole wGHI cost 34,435 € in VO_EU_GWB and 19,880 € in VO_CON_GWB, with the overall cost per sample about 355 €, that is about 55 € more per site replicate (at the current exchange rate) than in Australia (Korbel and Hose, 2017). The difference in cost of the wGHI^N over the routine monitoring carried out for the purposes of the Water Directives in Europe was equal to 5200 € in VO_CON_GWB and 8000 € in VO_EU_GWB. Details of the costs per sample are provided in the Supplementary File (COSTS).

As for Critetion#1, in both VO_EU_GWB and VO_CON_GWB, all site replicates failed Tier 1 and were inspected through Tier 2. 'Following Criterion#2, 5 out of 40 sites in VO_CON_GWB and 7 out of 26 sites in VO_EU_GWB had good ecological status (Supplementary File, Tier3). Following Criterion#3, two sites were excluded from both VO_EU_GWB and VO_CON_GWB because SB species did not occur in any of their site replicates. Following Criterion#4, only 2 sites in good ecological status in VO_CON_GWB and 3 in VO_CON_GWB presented populations with one male, one female and one juvenile individuals for at least one SB species (Supplementary File, MFJ_SB) and were, therefore, retained in Criterion#5. Finally, following Criterion#5, only 7% of the VO_EU_GWB sites and 8% of the VO_CON_GWB's were found to be in good ecological status. The remaining sites showed only mild deviation from the good ecological status, except for two sites in VO_EU_GWB and two in VO_CON_GWB, which showed major deviation. Both aquifers were considered in a poor ecological status overall. A representation of the ecological status of the individual sites is represented in Fig. 3.

No additional costs were required for the application of Tier 3 as the distinction in males, females, juveniles and adults was performed while identifying individuals at the species level.

5.3. The wGHI^N in deep aquifers

The bores PV7 (depth: 82 m) and PV32 (depth: 100 m), which intercepted two deep aquifers in the complex VO_CON_GWB, did not show anomalies with respect to the functional and organizational indicators, i.e. the values recorded in these bores were in the range of those observed for the sites in the shallow aquifers. In particular, the number of SB species in PV7 (=3) and PV32 (=2) was equal to that showed by 33% of the samples.

6. Discussion

Despite the terms indicator and index are often used interchangeably in the literature, they are different concepts involving, respectively, only one directly observable data stream and a quantitative aggregation of two or more variables. An index is mainly used in decision analysis to evaluate the impacts of alternative management strategies, or to define thresholds and goals for management. Through collapsing functional, organizational and stressor indicators into a

Table 3
Percentage of site replicates that failed (“F_”) the benchmarks of Tier 1 and percentage of site replicates in the 0–1 and 2–3 OWS classes. DOC: dissolved organic carbon; TAC: total abundances of crustaceans; TAO: total abundances of oligochaetes; nSB: non-stygobiotic taxa; SB_ABB: total abundances of stygobiotic taxa; SSR: species richness of stygobiotic taxa; C: % of crustaceans; O: % of oligochaetes; Agro: occurrence of agrochemicals with concentrations above the detection limits; Surveys: I: Autumn 2014; II: Spring 2015; III: Autumn 2015. OWS: overall weighted score.

Tier 1	Surveys	Replicates	Functional		Organizational				Stressors		
Aquifer			F_DOC		F_TAC	F_TAO	F_nSB		F_pesticides	F_Nitrates	
VO_EU_GWB	I,II,III	97	13		18	0	52		0	95	
VO_CON_GWB	I,II,III	56	30		16	0	64		0	91	
Tier 2			Functional		Organizational				Stressors		
Aquifer	Surveys	Replicates	F_SB_ABB	F_DOC	F_SSR	F_C	F_O	F_nSB	F_Agro	F_Nitrate	F_Phosphate
VO_EU_GWB	I,II,III	97	80	15	39	18	0	43	49	95	2
VO_CON_GWB	I,II,III	56	93	32	57	16	0	57	52	91	7
Aquifer	Surveys	OWS = 0,1	OWS = 2	OWS = 3							
VO_EU_GWB	I	69	31	0							
VO_EU_GWB	II	60	39	1							
VO_EU_GWB	III	40	59	1							
VO_EU_GWB	I,II,III	54	44	2							
VO_CON_GWB	I	68	30	2							
VO_CON_GWB	II	32	68	0							
VO_CON_GWB	III	22	76	2							
VO_CON_GWB	I,II,III	46	50	4							

single value, the $wGHI^N$ can serve as a management and communication tool for the local authority in charge of the NVZ in the River Vomano catchment.

The first indication that the $wGHI^N$ provided is that nitrate contamination extensively affected both VO_EU_GWB and the minor aquifers of the VO_CON_GWB complex. Both VO_EU_GWB (75% of sites) and VO_CON_GWB (65% of sites) exceeded the European threshold value of 50 mg/L NO_3^-. The contamination did not only concern nitrate but also nitrites, ionized ammonium and phosphates which were detected in most areas of each aquifer. No pesticides were found with concentrations above the limit of instrumental quantification, nevertheless, the stressors indicators of Tier 2 of the $wGHI^N$ highlighted that, from a chemical point of view, the conditions were unfavorable for the resident stygobiotic community.

Despite the widespread and persistent agricultural contamination, only 4 out the 66 sites of VO_EU_GWB and VO_CON_GWB, cumulatively, showed major deviation from good ecological status. This result, although surprising, is reliable. In fact, the stygobiotic community of these aquifers was dominated by crustaceans, a typical condition of groundwater bodies in a good status (Hancock and Boulton, 2009; Galassi et al., 2009, 2014, 2014). In addition, oligochaetes, which are often collected from aquifers polluted by organic compounds (Lafont et al., 1996; Malard et al., 1996), never occurred in either VO_EU_GWB or VO_CON_GWB. Furthermore, the stygobiotic diversity detected in the two aquifers (22 species in VO_EU_GWB and 19 VO_CON_GWB; 4 species new to Science) is truly remarkable.

A dataset of stygobiotic species richness (SSR) in European unconsolidated porous aquifers was provided by Malard et al. (2009). It includes the SSR data from 51 porous aquifers, characterized by the best possible environmental conditions for the resident stygofauna from 8 European regions (Fig. 4). The SSR detected in the aquifers of this study fell in the first quartile of the distribution of SSR data for European porous aquifers, that is VO_EU_GWB and VO_CON_GWB both ranked among the most biodiverse European aquifers (Fig. 4). Considering the SSR at the catchment level (SSR = 26 in 150 km^2 in this study), it can be noted that the stygobiotic diversity of the aquifers of the River Vomano catchment is second only to that of the Upper Right Rhone alluvial aquifer (SSR = 27 in 129 km^2; Malard et al., 2009) out of the 51 analyzed aquifers. In VO_CON_GWB, the two sites in good ecological status (mapped in green in Fig. 3), cumulatively presented an SSR = 7, a value which is higher than those of 14 aquifers out of 51 in the database used by Malard et al. (2009). The three sites in good ecological status of VO_EU_GWB showed an SSR = 9, representing 41% of the biodiversity of the whole aquifer. In both VO_EU_GWB and VO_CON_GWB, the high stygobiotic species richness was often associated with the occurrence of non-stygobiotic species in almost half of the sites.

The collection of stygoxene species in unconsolidated aquifers has been observed frequently (e.g., Galassi et al., 2009; Di Lorenzo and Galassi, 2013; Di Lorenzo et al., 2015) and it is not, *per se*, a dangerous condition for the resident stygofauna. In most cases, in fact, the occurrence of stygoxenes is due to connections with the surface aquatic environments (Lafont et al., 1996; Malard et al., 1996, Dumas et al., 2001; Schmidt et al., 2007; Di Lorenzo et al., 2012). Stygoxenes become a negative impact on the resident stygofauna only when conditions favorable to their survival occur, such as a constant and abundant presence of organic matter in groundwater. Under these conditions, the stygoxene organisms, which have metabolic rates (e.g., Di Lorenzo et al., 2015) and fertility higher than those of their surface relatives, manage to take the greatest advantage of the trophic resource and end up outnumbering the resident species (Malard et al., 1996). In the aquifers of the River Vomano catchment, the occurrence of stygoxenes was likely favored by the recharge of the aquifers through the infiltration of the water of the River Vomano. However, the amount of trophic resource in the sampling sites (see DOC values) were in line with that of unpolluted aquifers (e.g., Galassi et al., 2014) and were, in average, not such as to favor the long permanence of the voracious stygoxenes. Therefore, the map of Fig. 3, which showed dots mainly colored in orange, indicates that the groundwater bodies of the River Vomano presented, after all, only moderate deviations with respect to good ecological status.

It still remains to be understood how the stygobiotic community of VO_EU_GWB and VO_CON_GWB can cope with such a marked and persistent contamination of nitrogen compounds. What we know is that some stygobiotic crustacean species are highly affected by ionized ammonium (Di Lorenzo et al., 2014; Di Marzio et al., 2018) and moderately by nitrates (Fakher el Abiari et al., 1998; Mösslacher and Notenboom, 2000). We also know that crustacean juvenile stages, such as copepodids, are much more sensitive than the adults (Di Marzio et al., 2013, 2018). Based on the above observations, it is plausible to

Table 4
Spearman's correlation at regional and aquifer scale. SB_ABB: abundances of stygobiotic speceis; DOC: dissolved organic carbon; SB_SR: species richness of stygobiotic taxa; C: % of crustaceans; O: % of oligochaetes; nSB: % of non-stygobiotic taxa; AGRO: agrochemicals; NIT: nitrates; PHOS: phosphates. M: medium; L: low; H: high. Quality was defined as the ability of an indicator of Tier 2 to track the OWS and was assessed computing the indicator's correlation to the OWS.

Regional				
Variable 1	Variable 2	r	p-values	Quality
SB_ABB	OWS	−0.48	0.0001	M
DOC	OWS	0.33	0.0001	M
SB_SR	OWS	−0.43	0.0001	M
C	OWS	−0.17	0.0329	L
O	OWS	0	1	
nSB	OWS	0.19	0.0185	L
AGRO	OWS	0.79	0.0001	H
NIT	OWS	0.16	0.0424	L
PHOS	OWS	−0.14	0.0766	
VO_CON_GWB				
Variable 1	Variable 2	R	p-values	Quality
SB_ABB	OWS	−0.46	0.0008	M
DOC	OWS	0.28	0.035	L
SB_SR	OWS	−0.42	0.0019	M
C	OWS	−0.07	0.5909	
O	OWS	0	1	
nSB	OWS	0.22	0.1016	
AGRO	OWS	0.75	0.0001	H
NIT	OWS	0.24	0.0796	
PHOS	OWS	0.29	0.0254	L
VO_EU_GWB				
Variable 1	Variable 2	R	p-values	Quality
SB_ABB	OWS	−0.46	0.0001	M
DOC	OWS	0.30	0.0041	L
SB_SR	OWS	−0.40	0.0002	M
C	OWS	−0.22	0.0277	L
O	OWS	0	1	
nSB	OWS	0.13	0.2084	
AGRO	OWS	0.82	0.0001	H
NIT	OWS	0.12	0.231	
PHOS	OWS	−0.41	0.0002	M

expect that the biodiversity of the aquifers of the River Vomano catchment prior to contamination should have been higher than it is now, and that, probably, the most sensitive species have now disappeared. This result suggests that the indicators' benchmarks for stygobiotic species richness and abundances of Tier 2 may need to be adjusted to account for the disappeared biodiversity. This could have been done by sampling reference sites to establish what the biodiversity (in terms of richness) should be and adjusting the reference thresholds accordingly. However, this approach is impossible since there are no true reference sites left to sample in these aquifers.

It is not unlikely that some of the stygobiotic species currently occurring in the aquifers will soon end up the same way. For instance, in 18 months of monitoring, the juveniles of the SB species *Parapseudoleptomesochra italica* were never collected in the VO_CON_GWB complex (data not shown), which was contaminated by ionized ammonium in addition to nitrate. Finally, we know that when a N-compound, such as ionized ammonium is mixed up with a pesticide, such as the herbicides Imazamox, the toxicity of the individual chemicals to groundwater copepods increases because of a synergic effect (Di Marzio et al., 2018). At the moment, this is a condition that has not occurred in the investigated aquifers, but must be a warning to the local authority to continue limiting the use of pesticides in the River Vomano catchment.

The $wGHI^N$ showed a medium performance in both VO_EU_GWB and VO_CON_GWB and, therefore, the index should be applied to additional aquifers before being fully validated. The $wGHI^N$ validation is challenging at the moment due to the lack of large datasets that can be used to test the index accuracy. However, the $wGHI^N$, as well as the mother-index wGHI, fulfills many of the criteria used to score existing indices against (Moriarty et al., 2018). First, the index is theoretically sound, since scientific, peer-reviewed findings demonstrated that the indicators are reliable surrogates for groundwater ecosystem key attributes. Secondly, the spatial and temporal variation of the scores in the $wGHI^N$ (and wGHI) are understood. Thirdly, the index is "concrete" (i.e. that the indicators are directly measurable) and "numerical" (i.e. quantitative measurements were preferred over categorical – presence/absence – measurements). Since the site scores can be reported in color-coded maps, the results of the $wGHI^N$ are also easily understood by the public and policy-makers. Finally, the index is economically sustainable. However, it is worth mentioning some issues that have yet to be addressed and resolved. In fact, many indicators responded ambiguously to the variation of the ecosystem (i.e., the correlation to OWS was medium/low for most of the indicators in the River Vomano aquifers). In addition, the index portability, that is the extent of repeatability and reproducibility in different contexts, have to be tested yet. The $wGHI^N$ is not exactly "operationally simple". In fact, the methods for sampling, measuring, processing and analyzing the indicator data are feasible, though requiring taxonomic and ecological skills. Finally, the application of the $wGHI^N$ to deep aquifers did not show evident indicator anomalies. Although the number of sites in the deep aquifers was really too small to draw any conclusions, future studies could be aimed at evaluating the performance of the indicators in aquifers deeper than 30–40 m.

The indications that the results of this study provides to the local government are as follows: 1) the delimitation of the NVZ, and the application of the relative restrictive measures in terms of the use of natural or synthetic fertilizers, as well as of the number of livestock per hectare, should be extended to the whole catchment of the River Vomano and not to be limited to the outcrop of VO_EU_GWB, as it is presently. 2) The management measures should be more restrictive at sites that were in good ecological status and in a "good ecological status but with potentially impaired population dynamics", in order to safeguard their biodiversity. 3) The sites that were in an "undetermined ecological status" must be further investigated to identify the presence of non-agricultural factors affecting the stygobiotic community. 4) Local authority should invest in monitoring the microbiological component of the aquifers in order to complete the assessment of the ecological status of the aquifers.

7. Conclusions

The weighted Groundwater Health Index, conceived by Korbel and Hose (2017) to assess the ecological status of unconsolidated alluvial aquifers in Australia, was successfully applied to unconsolidated aquifers widely contaminated by nitrogen compounds of agricultural origin in central Italy. We observed that, following a simple refinement that led to the formalization of $wGHI^N$, the index satisfactorily described the ecological status of the aquifers. The refined wGHI provided results that can be easily understood and used by the local government for the management of nitrate vulnerable zones pursuant to the European Nitrates Directive and the Water Directives. Specifically, the $wGHI^N$ provides an unequivocal assessment of the ecological status of a groundwater body, that can be either good or poor. The study also highlighted the relevant biodiversity of the aquifers of the River Vomano catchment which happened to be among the most biodiverse in Europe. Despite the fact that the performance of the index in VO_EU_GWB and VO_CON_GWB was medium, its application in other contexts is encouraged in order to better tune its indicators.

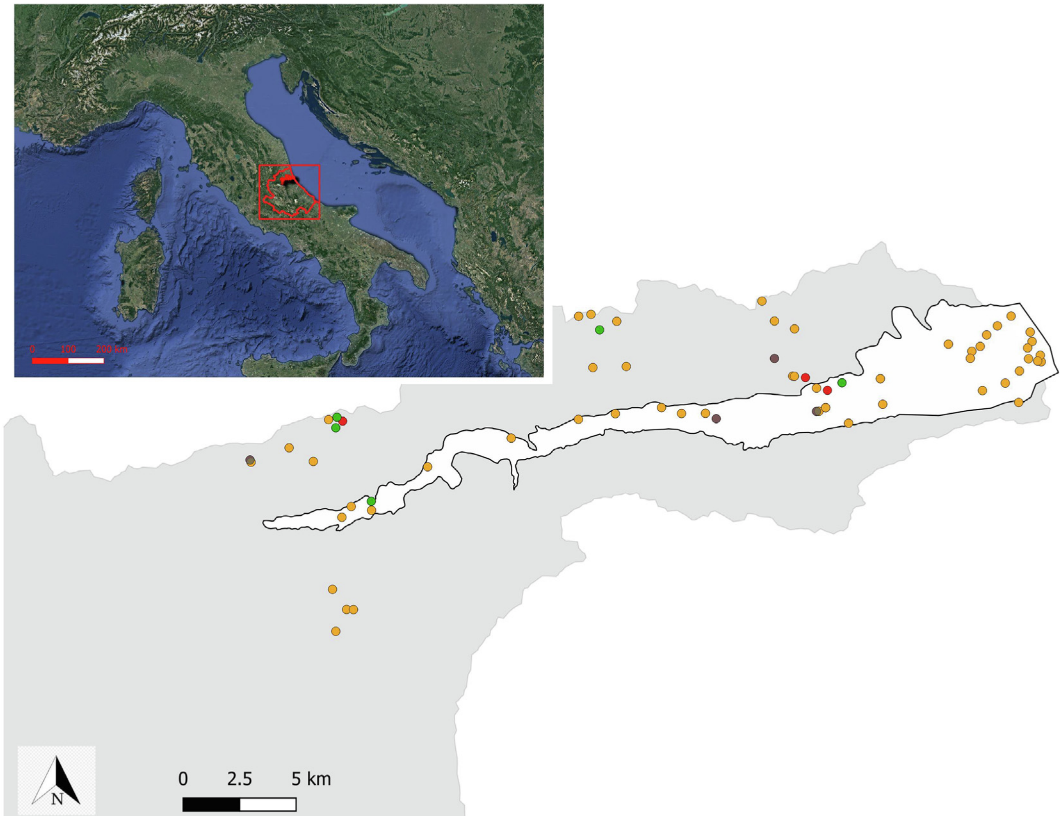


Fig. 3. Representation of the sites (points) of VO_EU_GWB (delimited white area) and VO_CON_GWB (gray area) and their location in Abruzzo region and in Italy. The colors represent the ecological status: green = good ecological status; yellow: good ecological status but with potentially impaired population dynamics; gray: undetermined ecological status; orange: mild deviation from good ecological status. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

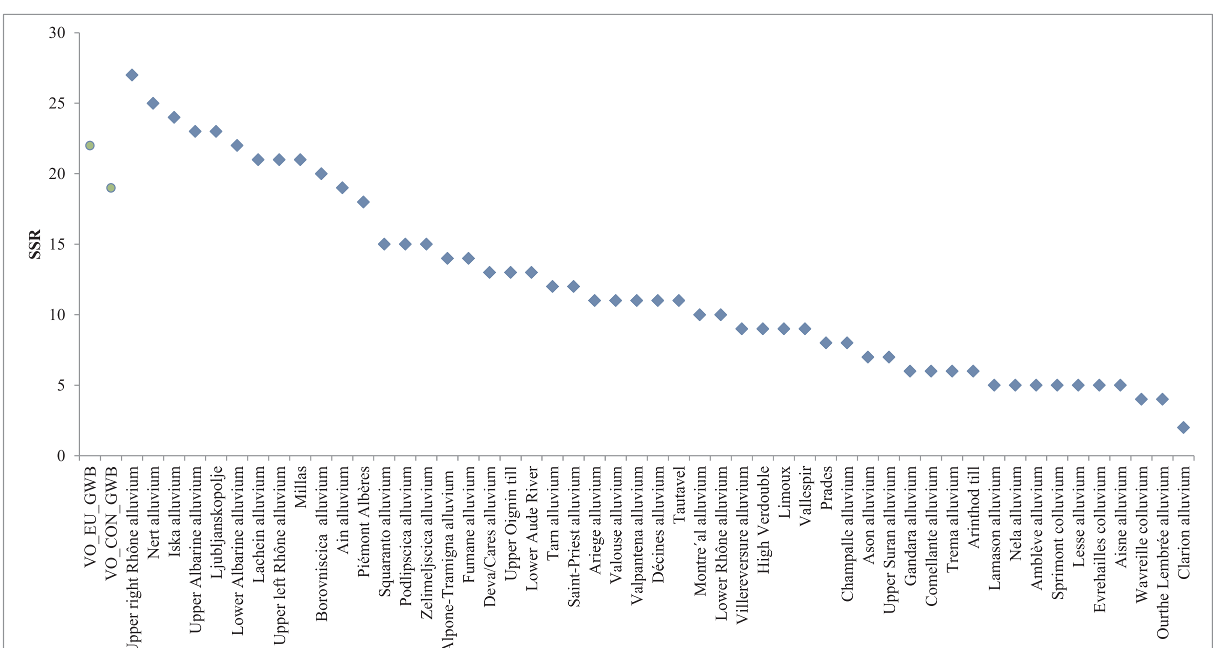


Fig. 4. Stygobiotic species richness (SSR) data from 51 porous aquifers (blue diamonds) characterized by the best possible environmental conditions for the resident stygofauna, from eight European regions after Malard et al. (2009) and SSR (stygobiotic species richness) data from VO_EU_GWB and VO_CON_GWB (green dots). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

CRediT authorship contribution statement

Di Lorenzo Tiziana: Conceptualization, Methodology, Validation, Writing - original draft, Writing - review & editing. **Fiasca Barbara:** Validation, Investigation, Data curation, Project administration. **Tabilio Di Camillo Agostina:** Formal analysis, Investigation, Data curation. **Murolo Alessandro:** Formal analysis, Investigation, Data curation. **Di Cicco Mattia:** Formal analysis, Data curation. **Galassi Diana Maria Paola:** Conceptualization, Validation, Resources, Funding acquisition.

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.

Acknowledgments

We thank the students of the Laboratory of Stygobiology at the University of L'Aquila (IT) for the support in the sampling and sorting activities. This research was funded by the European Commission - LIFE12 BIO/IT/000231 AQUALIFE "Development of an innovative and user-friendly indicator system for biodiversity in groundwater dependent ecosystems". We are grateful to two anonymous reviewers who improved the manuscript with their comments.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ecolind.2020.106525.

References

Amoros, M., 1984. Introduction pratique à la systématique des organismes des eaux continentales françaises - 5. Crustacés Cladocères. Bullet. Société Linnéenne de Lyon 53, 72–107.

Anderson, M.J., Gorley, R.N., Clarke, K.R., 2008. PERMANOVA + for PRIMER: Guide to software and statistical methods. PRIMER–E Ltd, Plymouth.

Botosaneanu, L. Stygofauna Mundi: A faunistic, distributional, and ecological synthesis of the World fauna inhabiting subterranean waters (including the marine interstitial). E. J. Brill/W. Backhuys: Leiden. ISBN 90-04-07571-2, 740 pp (1986).

Boulton, A.J., 2020. Editorial: Conservation of groundwaters and their dependent ecosystems: Integrating molecular taxonomy, systematic reserve planning and cultural values. Aquat. Conserv. Mar. Freshwater Ecosyst. 30, 1–7. https://doi.org/10.1002/aqc.3268.

Cifoni, M., Galassi, D.M.P., Faraloni, C., et al., 2017. Test procedures for measuring the (sub)chronic effects of chemicals on the freshwater cyclopoid *Eucyclops serrulatus*. Chemosphere 173, 89–98. https://doi.org/10.1016/j.chemosphere.2016.12.151.

CIS-EU (Common Implementation Strategy for the Water framework Directive of the European Commission). Guidance on groundwater status and trend assessment. Luxembourg: Office for Official Publications of the European Communities (2009), pp. 1-82.

Cvetkov, L., 1968. Un filet phréatobiologique. Bulletin de l'Institut de Zoologie et Musée Sofia 22, 215–219.

Desiderio, G., Nanni, T., Rusi, S.La., 2003. pianura del fiume Vomano (Abruzzo): Idrogeologia, antropizzazione e suoi effetti sul depauperamento della falda. Bollettino della Società Geologica Italiana 122, 421–434.

Di Lorenzo, T., Di Cicco, M., Di Censo, D., et al., 2019. Environmental risk assessment of propranolol in the groundwater bodies of Europe. Environ. Pollut. 255, 113189. https://doi.org/10.1016/j.envpol.2019.113189.

Di Lorenzo, T., Cifoni, M., Fiasca, B., et al., 2018. Ecological risk assessment of pesticide mixtures in the alluvial aquifers of central Italy: Toward more realistic scenarios for risk mitigation. Sci. Total Environ. 644, 161–172. https://doi.org/10.1016/j.scitotenv.2018.06.345.

Di Lorenzo, T., Borgoni, R., Ambrosini, R., et al., 2015. Occurrence of volatile organic compounds in shallow alluvial aquifers of a Mediterranean region: Baseline scenario and ecological implications. Sci. Total Environ. 538, 712–723. https://doi.org/10.1016/j.scitotenv.2015.08.077.

Di Lorenzo, T., Di Marzio, W.D., Sáenz, M.E., et al., 2014. Sensitivity of hypogean and epigean freshwater copepods to agricultural pollutants. Environ. Sci. Pollut. Res. 21 (6), 4643–4655. https://doi.org/10.1007/s11356-013-2390-6.

Di Lorenzo, T., Galassi, D.M.P., 2013. Agricultural impact in Mediterranean alluvial aquifers: Do groundwater communities respond? Fundamental Appl. Limnol. 182 (4), 271–282. https://doi.org/10.1127/1863-9135/2013/0398.

Di Lorenzo, T., Brilli, M., Del Tosto, D., et al., 2012. Nitrate source and fate at the catchment scale of the Vibrata River and aquifer (central Italy): An analysis by integrating component approaches and nitrogen isotopes. Environ. Earth Sci. 67 (8), 2383–2398. https://doi.org/10.1007/s12665-012-1685-0.

Di Marzio, W.D., Cifoni, M., Sáenz, M.E., et al., 2018. The ecotoxicity of binary mixtures of Imazamox and ionized ammonia on freshwater copepods: Implications for environmental risk assessment in groundwater bodies. Ecotoxicol. Environ. Saf. 149, 72–79. https://doi.org/10.1016/j.ecoenv.2017.11.031.

Di Marzio, W.D., Castaldo, D., Di Lorenzo, T., et al., 2013. Developmental endpoints of chronic exposure to suspected endocrine-disrupting chemicals on benthic and hyporheic freshwater copepods. Ecotoxicol. Environ. Saf. 96, 86–92. https://doi.org/10.1016/j.ecoenv.2013.06.029.

Di Sabatino, A., Boggero, A., Miccoli, F., et al., 2002. Diversity, distribution and ecology of water mites (Acari: Hydrachnidia and Halacaridae) in high Alpine lakes (Central Alps, Italy). Exp. Appl. Acarol. 34, 199–210. https://doi.org/10.1023/B:APPA.0000045251.44202.58.

Dumas, P., Bou, C., Gibert, J., 2001. Groundwater macrocrustaceans as natural indicators of the Ariège alluvial aquifer. Int. Rev. Hydrobiol. 86, 619–633 doi.org/10.1002/1522-2632(200110)86:6 < 619::AID-IROH619 > 3.0.CO;2-P.

Dussart, B., Defaye, D., 2006. World Directory of Crustacea Copepoda of Inland Waters. II—Cyclopiformes. Backhuys Publishers: Leiden, The Netherlands, pp. 276.

EC (European Commission). Directive 2006/118/EC of the European Parliament and of the Council of 12 December 2006 on the protection of groundwater against pollution and deterioration. Official Journal L 372, 27.12.2006, pp. 19-31 (2006).

EC (European Commission). Directive 2000/60/EC of the European Parliament and of the Council establishing a framework for the Community action in the field of water policy. Official Journal L 327, 22/12/ 2000, pp. 1-73 (2000).

EC (European Commission). Council Directive 92/43/EEC of 21 May 1992 on the conservation of natural habitats and of wild fauna and flora. Official Journal L 206, 22/07/1992, pp. 7-50 (1992).

EC (European Commission). Council Directive 91/676/EEC of 12 December 1991 concerning the protection of waters against pollution caused by nitrates from agricultural sources. Official Journal L 375, 31.12.1991, pp. 1-8 (1991).

Fakher el Abiari, A., Oulbaz, Z., Yacoubi-Khebiza, M., et al., 1998. Etude expérimentale de la sensibilité comparée de trois crustacés stygobies vis-à-vis de diverses substances toxiques pouvant se rencontrer dans les eaux souterraines. Mémoires de Biospéologie 25, 167–181.

Galassi, D.M.P., Lombardo, P., Fiasca, B., et al., 2014. Earthquakes trigger the loss of groundwater biodiversity. Sci. Rep. 4, 6273. https://doi.org/10.1038/srep06273.

Galassi, D.M.P., Stoch, F., Fiasca, B., et al., 2009. Groundwater biodiversity patterns in the Lessinian Massif of northern Italy. Freshw. Biol. 54 (4), 830–847. https://doi.org/10.1111/j.1365-2427.2009.02203.x.

Gibert, J., Stanford, J.A., Dole-Oliver, M.J., 1994. Basic attributes of groundwater ecosystems and prospects for research. Academic Press, California, CA, USA, pp. 7–40.

Gibert, J., Culver, D.C., Dole-Olivier, M.-J., et al., 2009. Assessing and conserving groundwater biodiversity: synthesis and perspectives. Freshwater Biol. 54, 930–941. https://doi.org/10.1111/j.1365-2427.2009.02201.x.

Griebler, C., Malard, F., Lefébure, T., 2014. Current developments in groundwater ecology – From biodiversity to ecosystem function and services. Curr. Opin. Biotechnol. 27, 159–167. https://doi.org/10.1016/j.copbio.2014.01.018.

Hahn, H.J., 2006. The GW-Fauna-Index: A first approach to a quantitative ecological assessment of groundwater habitats. Limnologica 36 (2), 119–137. https://doi.org/10.1016/j.limno.2006.02.001.

Hancock, P.J., Boulton, A.J., 2009. Sampling groundwater fauna: Efficiency of rapid assessment methods tested in bores in eastern Australia. Freshw. Biol. 54, 902–917. https://doi.org/10.1111/j.1365-2427.2007.01878.x.

Kiefer, F., 1960. Beiträge zur Copepodenkunde (XX). Zool. Anz. 165, 37–45.

Korbel, K.L., Stephenson, S., Hose, G.C., 2019. Sediment size influences habitat selection and use by groundwater macrofauna and meiofauna. Aquatic Sci. 81, 39. https://doi.org/10.1007/s00027-019-0636-1.

Korbel, K.L., Hose, G.C., 2017. The weighted groundwater health index: Improving the monitoring and management of groundwater resources. Ecol. Ind. 75, 164–181. https://doi.org/10.1016/j.ecolind.2016.11.039.

Korbel, K.L., Hose, G.C., 2011. A tiered framework for assessing groundwater ecosystem health. Hydrobiologia 661, 329–349. https://doi.org/10.1007/s10750-010-0541-z.

Krupa, E.G., 2015. Population densities, sex ratios of adults, and occurrence of malformations in three species of cyclopoid copepods in waterbodies with different degrees of eutrophy and toxic pollution. J. Mar. Sci. Technol. 13, 226–237.

Lafont, M., Camus, J., Rosso, A., 1996. Superficial and hyporheic oligochaete communities as indicators of pollution and water exchange in the River Moselle France. Hydrobiologia 334, 147–155. https://doi.org/10.1007/BF00017364.

Magurran, A.E., McGill, B.J., 2011. Biological Diversity: Frontiers in Measurement and Assessment. University Press, Oxford, UK, pp. 368.

Malard, F., Boutin, C., Camacho, A.I., et al., 2009. Diversity patterns of stygobiotic crustaceans across multiple spatial scales in Europe. Freshw. Biol. 54, 756–776. https://doi.org/10.1111/j.1365-2427.2009.02180.x.

Malard, F., Dole-Olivier, M.-J., Mathieu, J., et al. Sampling manual for the assessment of regional groundwater biodiversity. In: European Project PASCALIS (Protocols for the Assessment and Conservation of Aquatic Life in the Subsurface). Fifth Framework Programme Key Action 2: Global Change, Climate and Biodiversity 2.2.3 Assessing and Conserving Biodiversity Contract n° EVK2–CT–2001–00121. Viewed 19/02/2019. http://umr5023.univ-lyon1.fr/productions/bases-de-donnees.

Malard, F., Plenet, S., Gibert, J., 1996. The use of invertebrates in ground water monitoring: A rising research field. Groundwater Monitor. Remed. 16, 103–113. https://doi.org/10.1111/j.1745-6592.1996.tb00130.x.

Marmonier, P., Maazouzi, C., Foulquier, A., et al., 2013. The use of crustaceans as sentinel

organisms to evaluate groundwater ecological quality. Ecol. Eng. 57, 118–132. https://doi.org/10.1016/j.ecoleng.2013.04.009.
Mermillod-Blondin, A., Foulquier, C., Maazouzi, S., et al., 2013. Ecological assessment of groundwater trophic status by using artificial substrates to monitor biofilm growth and activity. Ecol. Ind. 25, 230–238. https://doi.org/10.1016/j.ecolind.2012.09.026.
Moriarty, E.E., Hodgson, H.E., Froehlich, S.M., 2018. The need for validation of ecological indices. Ecol. Ind. 84, 546–552. https://doi.org/10.1016/j.ecolind.2017.09.028.
Mösslacher, F., Notenboom, J., 2000. Groudwater Biomonitoring. [Uetikon-Zuerich]: Trans Tech Publications, Switzerland, pp. 119–139.
Peschke, K., Jonas, G., Kohler, H.-R., et al., 2014. Invertebrates as indicators for chemical stress in sewage-influenced stream systems: Toxic and endocrine effects in gammarids and reactions at the community level in two tributaries of Lake Constance, Schussen and Argen. Ecotoxicol. Environ. Saf. 106, 115–125. https://doi.org/10.1016/j.ecoenv.2014.04.011.
Piccini, L., Di Lorenzo, T., Costagliola, P., et al., 2019. Marble Slurry's impact on groundwater: The case study of the Apuan Alps Karst Aquifers. Water 11 (12), 2462. https://doi.org/10.3390/w11122462.
Regione Abruzzo. Piano di Tutela delle Acque. Relazione Generale e Allegati. 2010. Available online: http://www.regione.abruzzo.it/pianoTutelaacque/index.asp?modello = elaboratiPiano&servizio = lista&stileDiv = elaboratiPiano (accessed on 08 March 2020).
Schmid, P.E., 1993. A key to the larval Chironomidae and their instars from Austrian Danube region streams and rivers with particular reference to a numerical taxonomic approach Part I. Diamesinae, Prodiamesinae and Orthocladiinae. Wässer und Abwässer 3 (93), 1–514.
Schmidt, S.I., Hahn, H.J., Hatton, T.J., et al., 2007. Do faunal assemblages reflect the exchange intensity in groundwater zones? Hydrobiologia 583, 1–19. https://doi.org/10.1007/s10750-006-0405-8.
Stoch, F., Galassi, D.M.P., 2010. Stygobiotic crustacean species richness: A question of numbers, a matter of scale. Hydrobiologia 653, 217–234. https://doi.org/10.1007/s10750-010-0356-y.
R Core Team. R: A Language and Environment for Statistical Computing; R Foundation for Statistical Computing:Vienna, Austria (2008). Available online: http://www.R-project.org/ (accessed on 25 February 2020).
Zagmajster, M., Eme, D., Fišer, C., et al., 2014. Geographic variation in range size and beta diversity of groundwater crustaceans: Insights from habitats with low thermal seasonality. Glob. Ecol. Biogeogr. 23, 1135–1145. https://doi.org/10.1111/geb.12200.