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Diversity of Groundwater Crustaceans in Wells in Various Geologic Formations of Southern Poland

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Abstract: Data on Crustacea from underground waters accessed through wells are limited in Poland. A recent study was undertaken to determine diversity and factors influencing the crustacean communities inhabiting wells drilled in three bedrocks, Jurassic limestone, Cretaceous marls and flysch. A total of 23 crustacean species and subspecies were recorded belonging to Copepoda, Ostracoda, Amphipoda and Bathynellacea. Only four species of low abundance, however, were stygobionts. Our studies showed that abundance and species number of Copepoda and Ostracoda were affected by bedrock geology (with higher abundances and species richness in wells of Cretaceous marls), and in the case of copepods, also by sampling season. Furthermore, this paper lists all species of Crustacea recorded from inland groundwater habitats of Poland based published over the last 133 years. The most species-rich group was Copepoda with 43 representatives (four stygobites), followed by Ostracoda and Amphipoda with a total of 37 and 12 species, respectively (each with nine stygobites). In addition, two species of Isopoda (one stygobite) and one Bathynellid appear in the checklist. The checklist identifies geographical (and environmental) gaps which require further research.

Keywords: copepods; ostracods; subterranean crustacean checklist; ecology

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1. Introduction

The subterranean aquatic environment represented by cave waters, dug or drilled wells, interstitial waters and hypotelminorheal [1] is the habitat of a range of invertebrates. Among them, crustaceans arprovude the largest number of stygobiontic species [1–3] often accompanied by epigean species.

Wells are a source of drinking water in African countries which is why their fauna has been frequently studied and contains numerous stygobiontic Crustacea [4]. In European countries, studies on crustacean fauna were undertaken in dug wells in former Czechoslovakia [5–7], in boreholes in Germany [8] and in both types in Ireland [9].

In Poland, studies on aquatic subterranean crustaceans started in wells, when Wrześniowski [10] described *Niphargus tatrensis* and Jaworowski [11] published the results of his invertebrate investigations in Kraków and Lvov.

More recently, other researchers from Poland studied particular crustacean groups in this habitat: Isopoda [12,13], Copepoda [14–20] and Ostracoda [21–28]. The Amphipoda of wells (beside Wrześniowski [10]) were studied by Haeckel [29], Micherdziński [30] and Skalski [31,32] who also summarised the state of knowledge concerning the distribution of this group in Poland [33–35]. Studies on more than one crustacean group in wells are rare [36,37]. The mentioned studies were restricted to one region of Poland and to single wells, and the knowledge on crustaceans inhabiting subterranean waters in Polish wells therefore remained poor (except for the genus *Niphargus*—see Dumnicka and Galas [38]).

In rural areas situated in southern Poland, there are numerous old dug wells, mainly unused presently. Access to them gives the possibility to study subterranean aquatic invertebrates in regions of different geological character, even in areas without caves. During the macroinvertebrate studies from 26 wells in southern Poland, only one crustacean species *Niphargus tatrensis* was determined [3].

The biodiversity of groundwater in Poland remains poorly known compared to that of freshwater surface habitats, so the aim of our studies, conducted in 2010–2016 was to fill this gap in information on the diversity of crustaceans inhabiting groundwater. We also tested the hypothesis that geological bedrock type (Jurassic limestone, Carpathian flysch and Cretaceous marls) in which wells were dug as well as the sampling season (month) influence abundance, diversity and composition of crustacean assemblages.

2. Materials and Methods

2.1. Study Area

Our study included 33 wells distributed in three geologically different regions viz. (1) the Jurassic limestone area of the Kraków-Częstochowa Upland (14 wells in Szklary and Witkowice villages), (2) the Cretaceous marls of the Miechów Upland (7 wells in Prandocin village) and (3) the flysch areas of the Pogórze Wiśnickie foothills (7 wells in Kawec) and the Beskid Mały mountains (5 wells in Jaszczurowa village) (Figure 1, Table 1).

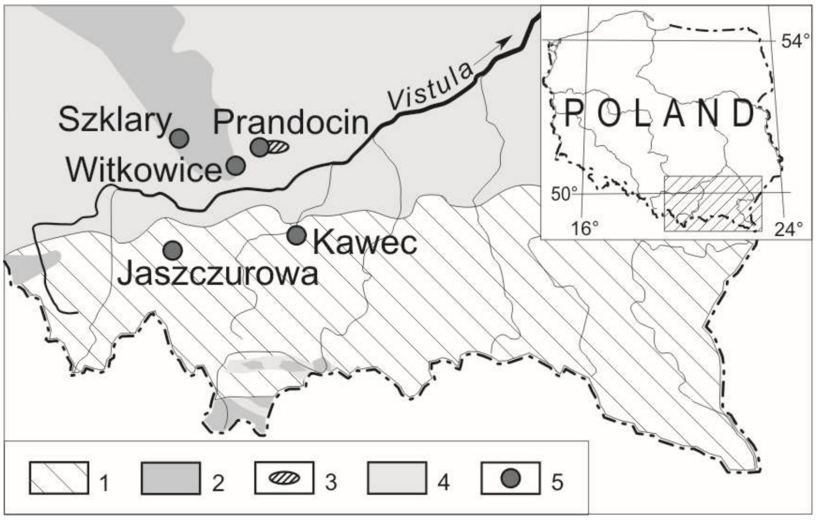

Figure 1. The study area with locations of villages where the studied wells are situated.

Bedrock includes flysch rocks of the Carpathians (1), Jurassic limestone sedimentary rocks (2), Cretaceous marls (3) and other sedimentary rocks of various ages (4). The dots (5) indicate villages with the sampled wells.

Table 1. Localization of villages with data on the sampled wells.

Localization	Coordinates	Geology Type	No of Studied Wells	Dates of Samplings	Depth (m)
Szklary (Sz)	50°10′ N; 19°42′ E	Jurassic limestone	7	June, August, October 2010	1.5–20
Witkowice (Wi)	50°10′ N; 19°94′ E	Jurassic limestone	7	May, July, October 2012	4.5–20
Jaszczurowa (Ja)	49°47′ N; 19°30′ E	Carpathian flysch	5	June, August, November 2012	1–4
Kawec (Ka)	49°84′ N; 20°22′ E	Carpathian flysch	7	May, July, October 2013	1.9–12.5
Prandocin (Pr)	50°15′ N; 20°06′ E	Cretaceous marls	7	May, August 2016	6–14

2.2. Sampling and Measurements of Water Properties

Samples of invertebrate fauna and water for chemical analyses were collected from 5–7 wells in each of the five particular regions (33 wells in total, see Figure 1) in 2010–2016 (Table 1). In each locality, wells were sampled seasonally three times a year (from May to November), except for wells in the Prandocin village where samples were taken on two occasions (Table 1). Thus, the sampling effort included a total 92 samples. The depth of the studied wells differed in particular regions ranging from 1.5 to 20 m. Only in the Jaszczurowa village were all studied wells relatively shallow, i.e., not exceeding 4 m (Table 1). Most of the studied wells were located in agricultural areas in small farms where non-intensive farming methods were used. Except for wells in the Witkowice village, they were all situated in gardens. The water in the studied wells is not drinking water, farmers use the water from the wells for garden watering and irrigation, which causes fluctuations in the water level, and some of them are not used at all.

Methods used for analyses of physical and chemical water feature analyses of the investigated wells have been described in Dumnicka et al. [3]. The results of studies on benthic invertebrates other than Crustacea (Copepoda and Ostracoda) from the same wells have already been published [3], while the data on plankton samples from wells from the area of Jaszczurowa village were presented as a conference poster [39].

Qualitative samples of Copepoda were taken by a plankton net (50 µm mesh size), using vertical hauls from the well bottom. Benthic samples were taken by an Ekman sampler (20 × 20 cm) and filtered through 0.3-mm net mesh. All samples were preserved in 4% formaldehyde, and fauna was determined using selected keys: for Copepoda e.g., [18,40–42] and Ostracoda [23,43–45]. Prior to the identification, ostracods (intact complete specimens with limbs as well as empty carapaces and valves) were rinsed in water, transferred to 96% ethanol and then analysed following Namiotko et al. [46]. Investigated specimens were identified to the species or the lowest possible taxonomic level (genus).

2.3. Statistical Analyses

To evaluate if the sampling effort was sufficient to represent biodiversity of the crustacean assemblages in the studied area, we performed accumulation curves of the observed and estimated species number by the Chao 1 index using PRIMER 7 software [47].

Other statistical analyses were performed with XLSTAT Ecology (Addinsoft). We used two-way unbalanced ANOVA to determine (i) the effect of physical and chemical variables, and (ii) geology with sampling season (month) on the Copepoda and Ostracoda communities (abundance, number of species). We used the same approach to test the effect of the above factors on the dominant species/genus. To find out if the two or more variables, and their interaction, provide the same amount of information we used Type I SS (sum of squares). For pairwise differences between means, we used Tukey's HSD (honestly significantly different) test. The most important differences were presented by box plots with basic descriptive statistics. The samples without Copepoda and Ostracoda were excluded from the statistical analysis.

3. Results

3.1. Physical and Chemical Water Properties of the Studied Wells

Water of the wells was circumneutral to alkaline, and mean values ranged from 7.0 in the Ka wells to 7.5 in the Sz wells. The other parameters of water such as conductivity, Ca^{2+}, NO_3^-, O_2, SO_4^{2-} and Cl^- in the studied wells differed strongly (Figure 2). The mean value of conductivity in Jurassic limestone varied from 606 to 1006 µS/cm, in flysch regions from 286 to 706 µS/cm, and in Cretaceous marls 917 µS/cm. The mean values of calcium concentrations in Jurassic limestone and Cretaceous marls areas were higher than in flysch areas, though in water in Ka wells this parameter reached high concentration values. The mean oxygen concentration was high in all wells in Pr, whereas in Wi it was mostly relatively low (Figure 2). In the remaining regions, the mean value of this parameter varied from well to well e.g., in Sz from 9.76 in well no 6 to 3.36 in well no 3 whereas in Ja from 8.27 in well no 1 to 0.96 in Well No. 4. Similar fluctuations were observed for nitrates, especially in the water of Wi wells (Figure 2), with the lowest values recorded in the flysch area. Chloride concentrations were low and constant in Ja, while most variable in Wi (Figure 2). In Pr and Ja, the content of sulphates was leveled but in Pr it was relatively high while and in Ja, low. In the remaining three villages the values of this parameter varied strongly. Polluted wells influenced by antropogenic factor occurred in several studied regions, but most often in Wi and Pr.

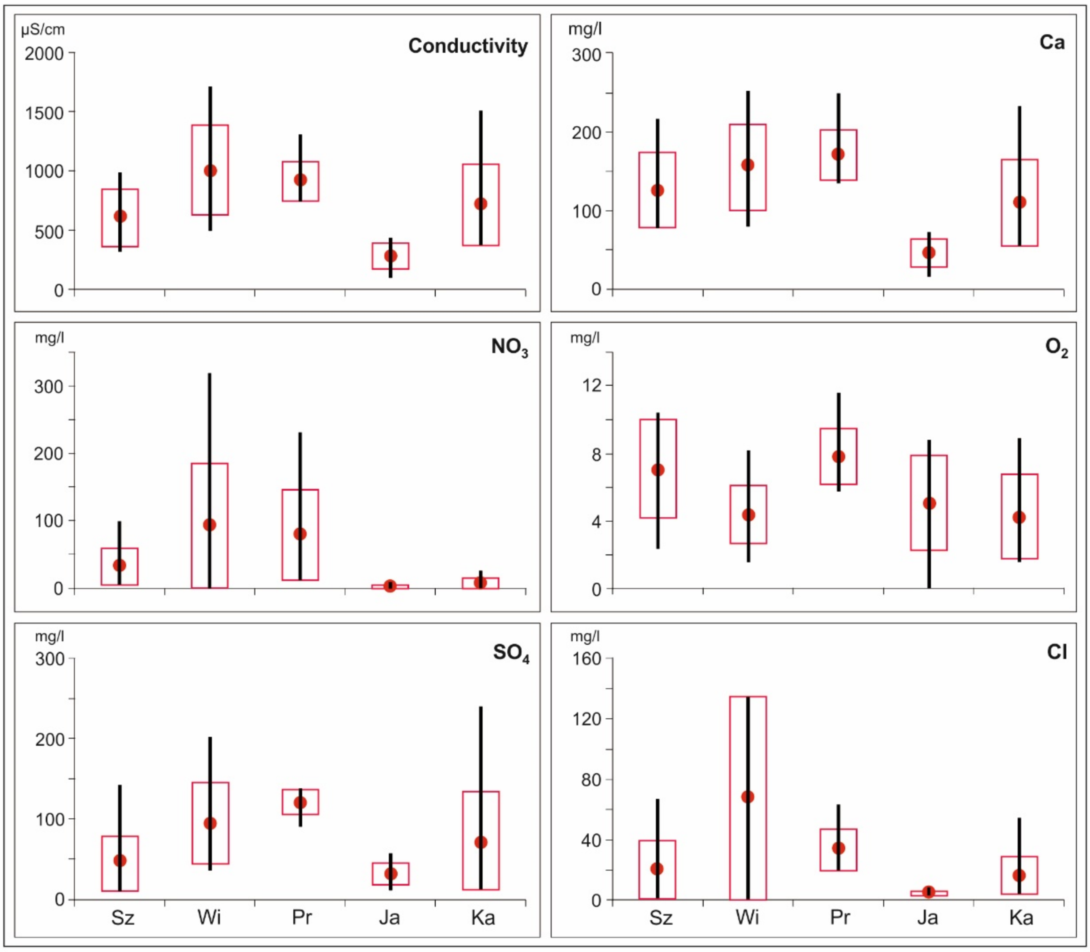

Figure 2. Selected water physical and chemical parameters: mean (in the middle of box), ±SD (box), max and min–whiskers. Wells in Sz and Wi are in Jurassic limestone bedrock, those in Pr are located in Cretaceous marls, whereas wells in Ja and Ka are in flysch (see Table 1 for village codes and other details).

3.2. Crustaceans in the Studied Wells

A total of 23 crustacean taxa of the ranks of species and subspecies were recorded (some were left in open nomenclature). They belonged to Copepoda, Ostracoda, Amphipoda and Bathynellacea. Although the accumulation plot of the observed species number did not reach asymptotic levelling-off (Figure 3), the total observed crustacean species richness was 73.5% of the species number estimated by the Chao 1 index (mean ± standard deviation SD = 32.7 ± 10.27).

In the studied wells, 13 copepod taxa were stated. Cyclopoida were represented by ten taxa of the species group (Table 2). Only three species were most abundant: *Diacyclops bisetosus* (from 7 to 34 individuals in the wells of Sz and Pr villages), *Acanthocyclops vernalis* (from 1 to 24 ind.-Ja, Pr), and *Megacyclops viridis* (from 1 to 15 ind.-Ja) and these species were also the most frequent. Only three Harpacticoida species were determined including stygobiontic species *Elaphoidella elaphoides*. In four wells, only cyclopoid nauplii and/or copepodites was identified, and in one well, only harpacticoid copepodites were presented (Table 2).

Altogether, eight species of Ostracoda were recorded during this survey (Table 2, *Cryptocandona* sp. is considered to represent juveniles of *Cryptocandona matris*), of which three (and the mentioned *Cryptocandona* sp.) remained in open nomenclature due to a poor preservation state and/or juvenile stage preventing certain identification. *Cryptocandona matris* (including *Cryptocandona* sp.) and *Typhlocypris* cf. *eremita* (both belonging to family Candonidae) can be regarded as stygobiontic species. The latter species and *Cavernocypris subterranea* were the most common, both with records from five wells, while six other species were found only in one well. The maximum number of species reported in a single well (Ka6) was four (*C. matris*, *Cyclocypris ovum*, *Cypria ophtalmica* and *Fabaeformiscandona brevicornis*), whereas the most abundant ostracod samples were taken from Pr wells.

Among amphipods, singular specimens of *Niphargus tatrensis* were found in two wells. Finally, one species of bathynellaceans, *Bathynella natans*, was recorded in the Prandocin wells (Table 2).

Table 2. Crustacea recorded in the studied wells.

Crustacean Groups	Order	Taxa and Life Stages of Invertebrates	Wells				
			Szklary	Jaszczurowa	Kawec	Witkowice	Prandocin
Copepoda	Cyclopoida	nauplii Cyclopoida	+	+			+
		copepodids Cyclopoida	+	+		+	+
		Acanthocyclops kieferi (Chappuis, 1925)				+	
		Acanthocyclops vernalis (Fischer, 1853)		+			+
		Acanthocyclops robustus (Sars G.O., 1863)				+	
		Diacyclops sp.				+	
		Diacyclops bisetosus (Rehberg, 1880)	+				+
		Diacyclops crassicaudis (Sars G.O., 1863)	+				+
		Diacyclops crassicaudis brahycercus (Kiefer, 1927)					+
		Megacyclops viridis (Jurine, 1820)		+			
		Paracyclops imminutus Kiefer 1929		+			
		Tropocyclops prasinus (Fischer,1860)		+			
	Harpacticoida	copepodids Harpacticoida					+
		Elaphoidella elaphoides (Chappuis, 1923)					+
		Elaphoidella cf. *elaphoides*			+		
		Mesochra sp. (Schmeil, 1894)					+
Ostracoda		*Cavernocypris subterranea* (Wolf, 1920)	+				+
		Cryptocandona matris (Sywula, 1976)			+		
		Cryptocandona sp.			+		
		Cyclocypris ovum (Jurine, 1820)			+		
		Cyclocypris cf. *serena* (Koch, 1838)				+	
		Cypria ophtalmica (Jurine, 1820)			+		
		Fabaeformicandona brevicornis (Klie, 1925)			+		
		Potamocypris cf. *pallida* Alm, 1914					+
		Typhlocypris cf. *eremita* (Vejdovsky, 1882)	+				+
Bathynellacea		*Bathynella natans* Vejdovsky, 1882					+
Amphipoda		*Niphargus tatrensis* Wrześniowski, 1888			+		+
Sum of taxa			4	4	7	4	11

+ —presence taxon confirmation.

3.3. Statistical Analyses

The physical and chemical variables did not significantly affect the abundance of Copepoda (F = 0.63; $p = 0.81$) and Ostracoda (F = 1.57; $p = 0.22$), as well as the number of Copepoda (F = 0.96; $p = 0.54$) and Ostracoda species (F = 2.56; $p = 0.06$). The abundance of dominant Copepoda and Ostracoda species also was not significantly affected by water properties. Only the abundance of the *Acanthocyclops* species was affected by these variables (F = 11.3; $p < 0.0001$). The Type I SS analysis indicated that *Acanthocyclops* abundance was affected by temperature ($p < 0.0001$), phosphates ($p < 0.0001$), nitrates ($p < 0.0001$), dissolved oxygen ($p = 0.003$), electrical conductivity ($p = 0.022$), and sulphates ($p = 0.048$).

We found that both the geology and the sampling season (month) significantly affected the abundance of Copepoda (F = 4.15; $p = 0.0003$) and Ostracoda (F = 5.54; $p < 0.001$), number of species of Copepoda (F = 3.58; $p = 0.001$) and Ostracoda (F = 5.72; $p < 0.001$), as well as the abundance of dominant species (Figures 4 and 5). Only Ostracoda abundance (Figure 4B) and number of species (Figure 4D) were not affected by months. Copepoda highest abundance (Figure 4A) and number of species (Figure 4C) were found in September. The Cretaceous marls and Carpathian flysch had a higher abundance of Copepoda, and a higher number of species than Jurassic limestone (Figure 5A,C). Ostracoda had the highest abundance and number of species in Cretaceous marls (Figure 5B,D).

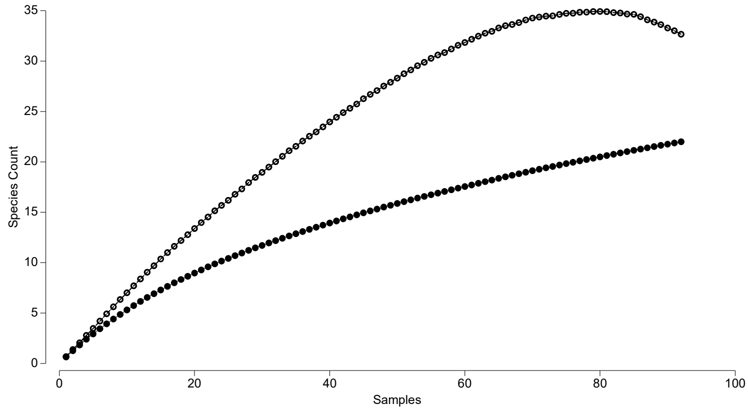

Figure 3. Species accumulation plot based on all 92 studied samples. Closed circles—number of observed species, open circles—number of estimated species by the Chao 1 index.

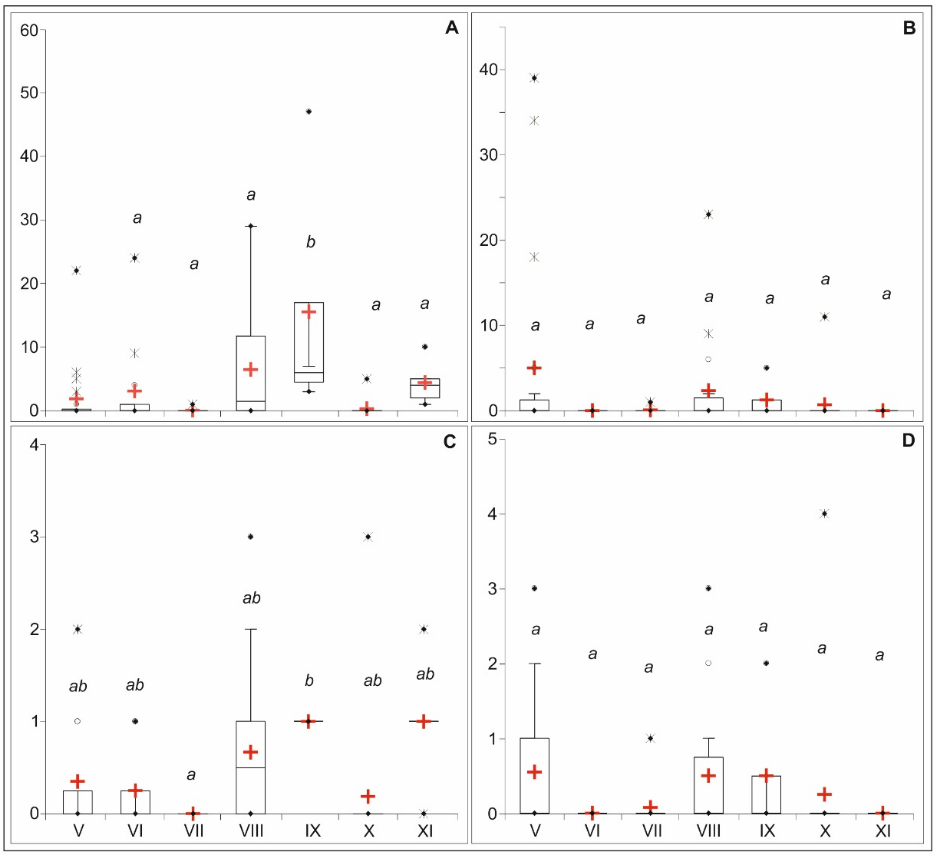

Figure 4. Monthly differences in Copepoda abundance (**A**), Ostracoda abundance (**B**), number of Copepoda species (**C**), number of Ostracoda species (**D**) in the studied wells. The different letters (*a*, *b*) above the box plots denote significantly different values at $p < 0.05$ and the same letters denote no statistically significant differences. The limits of the boxes are the first and third quartiles, crosses represent the means, central horizontal bars are the medians, points above or below are outliers, and the whiskers represented min and max.

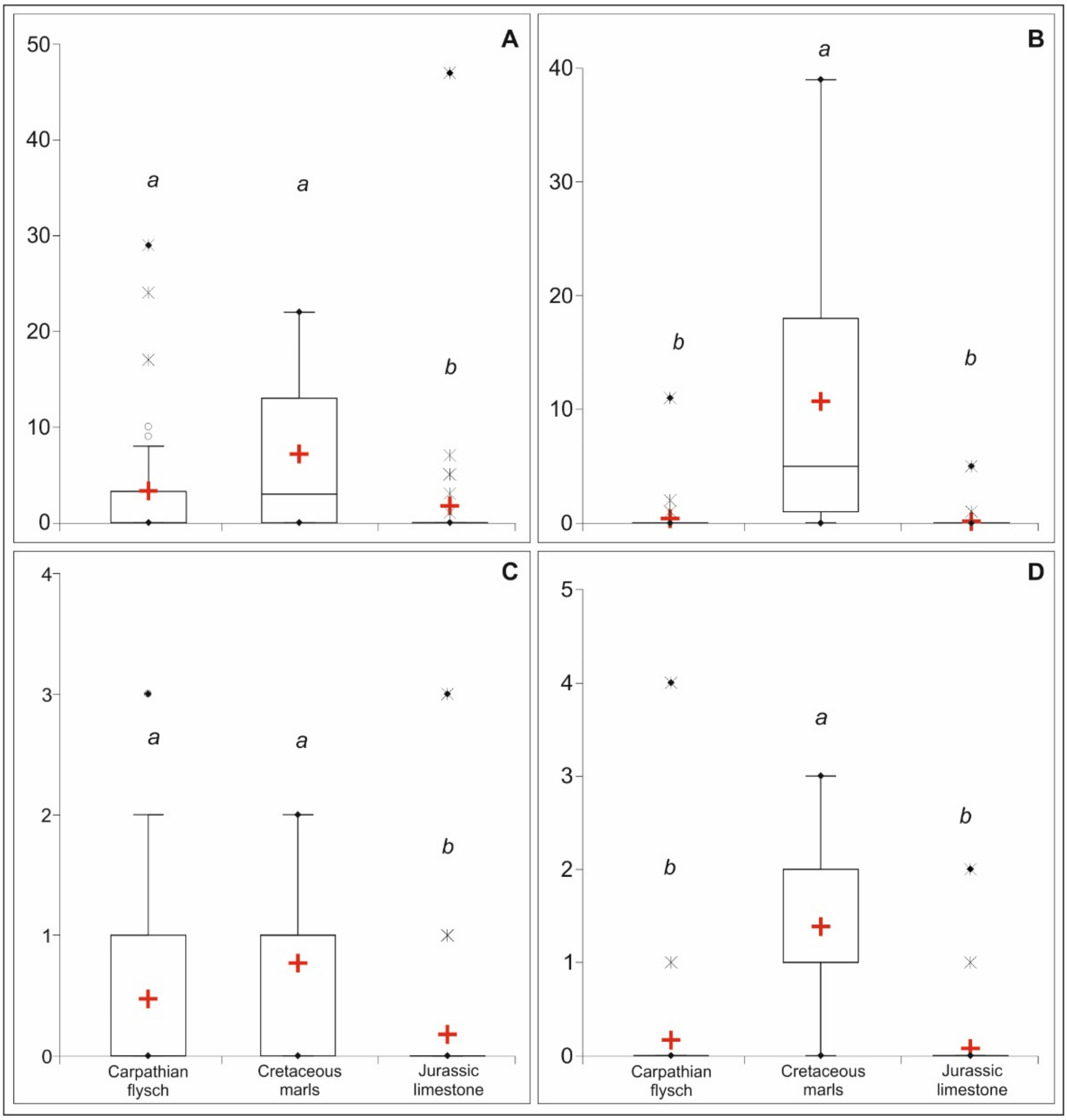

Figure 5. The effect of geology on Copepod abundance (**A**), Ostracoda abundance (**B**), number of Copepoda species (**C**), number of Ostracoda species (**D**) in the studied wells. The different letters (*a*, *b*) above the box plots denote significantly different values at $p < 0.05$ and the same letters denote no statistically significant differences. The limits of the boxes are the first and third quartiles, crosses represent the means, central horizontal bars are the medians, points above or below are outliers, and the whiskers represent min and max.

The geology and months also significantly affected the abundance of dominant species/genus. *Diacyclops* was affected by months ($p = 0.001$) and geology ($p = 0.044$), with the highest abundance in September and Cretaceous marls. *Megacyclops viridis* was affected by geology ($p = 0.031$) with the highest abundance in Carpathian flysch. We did not find a significant effect of geology and month on the abundance of *Acanthocyclops* genus. Concerning ostracods, *Cavernocypris subterranea* and *Typhlocypris* cf. *eremita* abundances were significantly affected by the geology ($p < 0.0001$) with the highest abundance of both species in Cretaceous marls.

3.4. Crustacea in Subterranean Waters of Poland-State of Current Knowledge

The literature concerning Crustacea recorded from subterranean waters (including caves, wells and interstitial waters) of Poland is limited. Since Wrześniowski's classic work [10], in which two amphipod species new to science (*Niphargus tatrensis* and *Synurella tenebrarum)* were described, 38 papers have been published on subterranean Crustacea fauna of Poland (Table 3). Among all crustaceans (95 taxa) found in caves, wells and interstitial (inland) waters in Poland, only 24 taxa are stygobionts (nine Amphipoda; one Isopoda; four Copepoda; nine Ostracoda and one Bathynellid) (Table 3).

Based on the literature, the occurrences of crustaceans in subterranean waters of Poland were classified in Table 3 by habitat types (caves, wells and inland interstitial waters). The most diverse fauna was found in wells (59 taxa), then in interstitial waters (44) and in caves (31). Among Ostracoda, unfortunately for seven taxa there are no data available on habitat type and region where they were found (Table 3).

Table 3. List of Crustacean species recorded from subterranean aquatic habitats in Poland along with the data on ecology (subterranean habitat type) and geographical distribution (regions according to Catalogus Faunae Poloniae). Abbreviations of geographic names: Mts—mountains; Upl.—upland; Low.—lowland.

Group/Taxa	Habitat (No of Object)			Region	Refrences
	Caves	Wells	Interstitial Waters		
AMPHIPODA					
Crangonyx paxi # Schellenberg, 1935	1			Sudety Mts	[48]
Gammarus balcanicus Schäferna, 1922		1		Pieniny Mts	[30]
Gammarus fossarum Koch, 1836		1		Beskid Zach. Mts	[30]
Gammarus pulex polonensis # Karaman and Pinkster, 1977			+	Wielkopolsko-Kujawska Low.	[49]
Niphargus aquilex # (?) Schiödte, 1856		+		Wielkopolsko-Kujawska Low.	[29]
Niphargus casimiriensis # Skalski, 1980		11		Małopolska Upl.	[36]
Niphargus leopoliensis #* Jaworowski, 1893		3		Kraków-Wieluń Upl., Małopolska Upl., Bieszczady Mts	[36]
Niphargus tatrensis #* Wrześniowski, 1888	13	+	+	Kraków-Wieluń Upl., Małopolska Upl., Sudety Mts, Beskid Zach. Mts, Beskid Wsch. Mts, Bieszczady Mts, Tatra Mts, Pogórze Wiśnickie foothills,.	see [38], orginal data
Niphargellus arndti # (Schellenberg, 1933)	3			Sudety Mts	[48,50,51]
Synurella ambulans (Müller, 1846)		1		Małopolska Upl.	[52]
Synurella coeca # Dobreanu and Manolache, 1951		4		Małopolska Upl.	[36]
Synurella tenebrarum # (Wrześniowski, 1888)		+		Beskid Zach. Mts	[10]
BATHYNELLACEA					
Bathynella natans # Vejdovsky, 1882		1	5	Sudety Mts., Beskid Zach. Mts, Miechów Upl.	[13], orginal data
ISOPODA					
Asellus aquaticus (L.)	2	8		Kraków-Wieluń Upl., Małopolska Upl., Swiętokrzyskie Mts, Beskid Wsch. Mts	[12,37,53,54]
Proasellus slavus # (Remy, 1948)		5	1	Beskid Zach. Mts	[13]
COPEPODA CYCLOPOIDA					
Acanthocyclops kieferi (Chappuis, 1925)	7	3	1	Kraków-Wieluń Upl., Małopolska Upl., Świętokrzyskie Mts, Sudety Mts, Beskid Zach. Mts, Pieniny Mts, Tatra Mts	[20], orginal data
Acanthocyclops rhenanus # Kiefer, 1936			+ !	Małopolska Upl.	[14]
Acanthocyclops robustus (Sars G.O.,1863)	4	2	1	Sudety Mts, Kraków-Wieluń Upl., Małopolska Upl., Pieniny Mts	[20,55], orginal data
Acanthocyclops venustus (Norman and Scott, 1906)	3	1	2	Kraków-Wieluń Upl., Małopolska Upl., Pieniny Mts	[20]
Acanthocyclops vernalis (Fischer, 1853)	1	4	1	Małopolska Upl., Upper Silesia, Beskid Mały.	[14,20], orginal data
Cyclops abyssorum (Sars G.O.,1863)	1			Tatra Mts	[20]
Cyclops bohater Koźmiński, 1933		1		Tatra Mts	[20]
Cyclops pulchellus Koch, 1838 (1)		1		Kraków-Wieluń Upl.	[11]
Cyclops strenuus Fischer, 1851		2		Kraków-Wieluń Upl., Małopolska Upl.	[15,17]
Cyclops vicinus Uljanin, 1875	1	1		Kraków-Wieluń Upl., Małopolska Upl.	[20]
Diacyclops sp.		1		Kraków-Wieluń Upl.	[orginal data]
Diacyclops abyssicola (Lilljeborg, 1901)	1	1		Upper Silesia, Małopolska Upl.	[20]
Diacyclops bicuspidatus (Claus, 1857)	7	2	2	Kraków-Wieluń Upl., Małopolska Upl., Lubelska Upl., Pieniny Mts	[14,15,20,37,54]
Diacyclops bisetosus (Rehberg, 1880)		2	1	Kraków-Wieluń Upl., Małopolska Upl.	[17], orginal data
Diacyclops clandestinus # (Yeatman, 1964) (2)	1	1		Upper Silesia, Tatra Mts	[20,56]
Diacyclops crassicaudis (Sars G.O., 1863)	1	+		Małopolska Upl., Kraków-Wieluń Upl.	[14,20,37], orginal data

Table 3. *Cont.*

Group/Taxa	Habitat (No of Object)			Region	Refrences
	Caves	Wells	Interstitial Waters		
Diacyclops crassicaudis brachycercus (Kiefer, 1927)		1		Małopolska Upl.	[orginal data]
Diacyclops disjunctus (Thallwitz, 1927) (3)	2			Sudety Mts.,	[57]
Diacyclops languidoides (Lilljeborg, 1901)	5	1	1	Upper Silesia, Małopolska Upl., Beskid Zach. Mts, Pieniny Mts	[15,20]
Diacyclops languidus (Sars G.O., 1863)		1		Małopolska Upl.	[15]
Diacyclops nanus (Sars G.O., 1863)	1	1		Kraków-Wieluń Upl., Małopolska Upl.	[20]
Eucyclops macruroides (Lilljeborg, 1901)		2	2	Upper Silesia, Małopolska Upl.	[14,20]
Eucyclops serrulatus (Fischer, 1851)	2	6	3	Kraków-Wieluń Upl., Małopolska Upl., Świętokrzyskie Mts, Pieniny Mts	[14,15,20]
Eucyclops speratus (Lilljeborg, 1901)		1		Małopolska Upl.	[14]
Graeteriella unisetigera # (Graeter, 1908)		1		Wielkopolsko-Kujawska Low.	[16]
Macrocyclops albidus (Jurine, 1820)	1	1	3	Upper Silesia, Kraków-Wieluń Upl., Małopolska Upl., Lubelska Upl., Sudety Mts	[14,17,20]
Macrocyclops fuscus (Jurine, 1820)		1		Małopolska Upl.	[14]
Megacyclops gigas (Claus,1857)			1	Pieniny Mts	[20]
Megacyclops viridis (Jurine, 1820)	4	2		Kraków-Wieluń Upl., Małopolska Upl., Sudety Mts, Beskid Mały	[14,20,54,57], orginal data
Metacyclops sp.	1			Kraków-Wieluń Upl.	[55]
Paracyclops affinis (Sars G.O., 1863)	1	1		Kraków-Wieluń Upl., Małopolska Upl.	[20,37]
Paracyclops fimbriatus (Fischer, 1853)	1	4	1	Kraków-Wieluń Upl., Małopolska Upl., Beskid Zach. Mts	[15,17,37]
Paracyclops imminutus Kiefer 1929		1		Beskid Mały	[orginal data]
Paracyclops poppei (Rehberg, 1880)	2	2		Kraków-Wieluń, Małopolska Upls, Sudetes, Beskid Zach. Mts	[20]
Thermocyclops crassus (Fischer, 1853)	1	1		Małopolska Upl.	[20]
Tropocyclops prasinus (Fischer, 1860)				Beskid Mały	[orginal data]
CALANOIDA					
Eudiaptomus graciloides (Lilljeborg, 1888)		1		Małopolska Upl.	[15]
HARPACTICOIDA					
Attheyella wierzejskii (Mrázek, 1893)		1	1	Małopolska Upl., Sudety Mts	[20]
Bryocamptus cuspidatus (Schmeil, 1893)		1		Małopolska Upl.	[58]
Bryocamptus dacicus (Chappuis, 1923)	2			Sudety Mts	[57]
Bryocamptus echinatus (Mrázek, 1893)		1		Małopolska Upl.	[58]
Bryocamptus typhlops (Mrázek, 1893)	1			Sudety Mts	[57]
Canthocamptus microstaphylinus (Wolf, 1909)	1		1	Małopolska Upl., Lubelska Upl.	[19]
Canthocamptus staphylinus (Jurine, 1820)	1		1	Małopolska Upl., Lubelska Upl.	[19]
Elaphoidella elaphoides # (Chappuis, 1924)		4		Podlasie Low., Małopolska Upl.	[18,58], orginal data
Elaphoidella cf. *elaphoides* #		1		Pogórze Wiśnickie foothills	[orginal data]
Epactophanes richardi Mrázek, 1893	1			Sudety Mts.,	[57]
Nitokra hibernica hyalina Jakubisiak, 1929			+	Wielkopolsko-Kujawska Low.	[59]
Mesochra sp. (Schmeil, 1894)		1		Małopolska Upl.	[orginal data]
OSTRACODA					
Bradleystrandesia reticulata (Zaddach, 1844) (4)	no details on habitats			no details on sites	[24]
Candona candida (O.F. Müller, 1776)		+	+	Kraków-Wieluń Upl., Sudety Mts, Beskid Zach. Mts, Bieszczady Mts, Małopolska Upl. Wielkopol.-Kujawska Low, Lublin Upl..	[24,26,36,60,61]
Cavernocypris subterranea (Wolf, 1919)		+, 2	+	Sudety Mts, Beskid Zach. Mts, Bieszczady Mts, Kraków-Wieluń Upl., Małopolska Upl.	[24,26,60,61], orginal data
Cryptocandona sp.		1		Pogórze Wiśnickie foothills	[orginal data]
Cryptocandona matris # (Sywula, 1976)		+		Małopolska Upl., Beskid Zach. Mts, Beskid Wsch. Mts, Bieszczady Mts, Pieniny Mts, Lublin Upl., Pogórze Wiśnickie foothills	[23–25,27,28,36], orginal data
Cryptocandona reducta (Alm, 1914)		+	+	Sudety Mts, other sites	[24,27,60,61]
Cryptocandona vavrai Kaufmann, 1900		+	+	Sudety Mts, Beskid Zach., Tatra Mts	[24,27,60,61]
Cyclocypris ovum (Jurine, 1820)	no details on habitats	1		no details on sites, Pogórze Wiśnickie foothills	[24], orginal data
Cyclocypris serena (Koch, 1838)	no details on habitats			no details on sites	[24]
Cyclocypris cf. *serena* (Koch, 1838)		1		Kraków-Wieluń Upl.	[orginal data]
Cypria ophtalmica (Jurine, 1820)		+	+	Sudety Mts, Beskid Zach. Mts., Pogórze Wiśnickie foothills	[24], orginal data
Cypria reptans Bronstein, 1928			+	no details on sites	[24]
Darwinula stevensoni (Brady and Robertson, 1870)			+	no details on sites	[24]
Eucypris pigra (Fischer, 1851)			+	no details on sites	[24]
Fabaeformiscandona breuili #* (Paris, 1920) (5)		+	+	Sudety Mts, Bieszczady Mts, Pieniny Mts, Beskid Zach. Mts, Wielkopolsko-Kujawska Low.	[22,24,25,28]
Fabaeformiscandona brevicornis (Klie, 1925) (6)		1	+	no details on sites, Beskid Mały	[24], orginal data
Fabaeformiscandona latens #* (Klie, 1940)			+	Sudety Mts, Beskid Zach. Mts.	[22,24]

Table 3. *Cont.*

Group/Taxa	Habitat (No of Object)			Region	Refrences
	Caves	Wells	Interstitial Waters		
Fabaeformiscandona wegelini #* (Petkovski, 1962)		+	+	Sudety Mts, Beskid Zach. Mts, Małopolska Upl., Wielkopolsko-Kujawska Low., Lublin Upl..	[22,24,36]
Herpetocypris sp.	no details on habitats			no details on sites	[24]
Ilyocypris bradyi Sars, 1890			+	no details on sites	[24]
Limnocythere inopinata (Baird, 1843)			+	no details on sites	[24]
Mixtacandona sp. #		+		Małopolska Upl.	[24,36]
Nannocandona faba Ekman, 1914		+	+	Sudety Mts	[24]
Nannocandona stygia # Sywula, 1976		+	+	Sudety Mts, Beskid Zach. Mts.	[23,24]
Neglecandona lindneri (Petkovski, 1969)	no details on habitats			no details on sites	[24]
Neglecandona neglecta (Sars, 1887)			+	no details on sites	[24]
Notodromas monacha (O.F. Müller, 1776)	no details on habitats			no details on sites	[24]
Physocypria kraepelini G.W. Müller, 1903) (7)			+	no details on sites	[24]
Potamocypris fulva (pallida) (Brady, 1868)		+	+	Sudety Mts, Beskid Zach, Bieszczady Mts	[24]
Potamocypris cf. *pallida* (Alm, 1914)		1		Małopolska Upl.	[orginal data]
Potamocypris zschokkei (Kaufman, 1900) (8)			+	no details on sites, except Kraków-Wieluń Upl.	[24,26]
Pseudocandona albicans (Brady, 1864) (9)		+	+	Małopolska Upl., Kraków-Wieluń Upl., Sudety Mts, Beskid Zach. Mts	[21,22,24–26,36]
Pseudocandona compressa (Koch, 1838)		+		no details on sites	[24]
Pseudocandona mira # (Sywula, 1976)		+		Beskid Zach. Mts	[23]
Pseudocandona pratensis (Hartwig, 1901)		+		Sudety Mts and other sites	[24,60]
Pseudocandona sarsi (Hartwig,1899)	+	+	+	Małopolska Upl., Kraków-Wieluń Upl., Sudety Mts, Beskid Zach. Mts	[24,37,54,60]
Pseudocandona semicognita (Schafer, 1934)	no details on habitats			no details on sites	[24]
Pseudocandona triquetroides (Sywula, 1974)			+	Sudety Mts, Beskid Zach. Mts	[24]
Typhlocypris eremita # * (Vejdovsky, 1882)		+		Sudety Mts, Małopolska Upl., Wielkopolsko-Kujawska Low., Beskid Zach. Mts, Lublin Upl..	[24,36], Sywula-pers. notes
Typhlocypris cf. *eremita* (Vejdovsky, 1882)		2		Kraków-Wieluń Upl., Małopolska Upl.	[orginal data]
Typhlocypris szoecsi # (Farkas, 1958)				Małopolska Upl., Beskid Zach. Mts, Lublin Upl..	[24,36]

+—presence taxon confirmation. #—stygobiontic species. *—for stygobionts only: found also in surface waters. !—found in peat bog forest Reservoirs 1, 2, and 3—"original" names of species used in cited papers. (1)—*C. pulchellus* Koch, 1838 is currently regarded as nomen dubium; in the past some carcinologists used this name as a senior synonym of *Diacyclops bicuspidatus* (Claus, 1857). (2)—as *Diacyclops clandestinus* (Kiefer, 1926) in [56]. (3)—as *Diacyclops languidus disjunctus* (Thallwitz, 1927) in [57]. (4)—as *Cypricercus affinis* (Fischer, 1851) in [24]. (5)—as *Candona hertzogi beskidana* (Sywula 1974) in [22,24,25,28]. (6)—as *Candona limnocrenica* (Sywula 1971) in [24]. (7)—as *Physocypria fadeewi* (Dubovsky 1926) in [24]. (8)—as *Potamocypris foxi* (Sywula 1972) in [24,26]; *P. wolfi* (Brehm 1920) in [24]. (9)—as *Candona parallela* (G.W. Muller) 1900 in [21,22,24–26,36].

4. Discussion

The subterranean fauna dwells in underground waters such as caves, interstitial waters, wells, as well as other man-made subterranean habitats such as adits, shafts or mines. It also occurs in springs [3].

Although several samples did not yield any crustaceans, the results suggest that the sampling effort was adequate to represent crustacean communities in the wells. The recorded 23 (sub-)species of Crustacea in the wells amounted to 73.5% of the estimated species richness (Figure 3), a value within the range (50–75%) which Heck et al. [62] consider an adequate approximation.

The values of physical and chemical parameters of water in wells located in different bedrocks (including flysch rocks of the Carpathians, Jurassic limestone sedimentary rocks and Cretaceous marls) differed. The mean values from wells located in Jurassic limestone (SZ, Wi) and cretaceous marls (Pr) bedrock were usually similar, but wells located on flysch bedrock (Ja, Ka) had mostly lower means that may be related to lower mineralization. It should be emphasized that the water in the studied wells was neutral to slightly alkaline. The increased levels of nitrates, chlorides and sulfates in some wells indicate a significant human impact on the quality of the groundwater. Similar results concerning quality of groundwater were observed earlier in two regions, in the Kraków-Częstochowa upland [63,64] and in the Wiśnickie foothill [65,66]. In regions where polluted wells predominate, the species richness and abundance of the crustecean fauna is much smaller [3].

The copepod stygobiont *Elaphoidella elaphoides*, previously was found only in wells in the villages of Ogrodniczki and Ciasne in Poland (Uplands of the Podlaskie Plain) [18,58]. In Europe, this stygobiont is widely distributed in underground waters, including caves, hyporheic and phreatic waters and often occurs in epigean waters as well [18].

Two stygobiontic ostracod species, *Cryptocandona matris* in Kawec and *Typhlocypris* cf. *eremita* occurred in Szklary and Prandocin wells. The former species was originally described by Sywula [23] from a well at Cisna village in the Bieszczady Mts. and further recorded in wells and interstitial habitats of the Lublin Upland and Carpathian Mountains (in Poland) (Table 3) and in north-eastern Romania [27]. *Typhlocypris eremita* is the type and the most-widespread species of the genus, occurring in groundwaters of Central and South-Eastern Europe [46], mainly as all-female (parthenogenetic) populations. As the male genital morphology offers better characteristics than that of female on which to define the species in the genus *Typhlocypris*, it is not unlikely that some of recorded populations could represent different species, as documented by Iepure et al. [67]. Thus, a re-examination of records identified as *Typhlocypris eremita* is required to better understand the extent of variation of this and closely related species. In Poland, *T. eremita* is known mostly from an area south of the maximum limit of the Vistulian glaciation, with the most significant exception of two surface-water sites in the Vistula fens in northern Poland (Table 3). These are the northern-most localities of this species, which were most probably reached by this species via the alluvial groundwaters of the Vistula River [68,69].

The amphipod stygobiont *Niphargus tatrensis* previously was reported from the wells situated in the Kraków-Czestochowa upland [3], and is common in southern Poland [38]. It was found in the Prandocin well (Pogórze Wiśnickie foothill) and is the first record of this species from this region. Other species of crustaceans found in the studied wells were non-obligate groundwater inhabitants occurring mainly in surface inland waters. Considering ostracods, all the remaining non-stygobite species collected during this study, have been already recorded in groundwaters of Poland [24] (Table 3). Two of these, *Cavernocypris subterranea* and *Fabaeformiscandona brevicornis* may qualify as stygophiles or crenobionts, as they inhabit both groundwaters and surface waters associated with springs [24,44].

Statistical analyzes showed that the species richness and abundance of crustacean fauna in the studied wells depended especially on the bedrock in which the wells are located, and not on the measured chemical and physical parameters of water. For Ostracoda growth and survival may be greatly affected by the solute composition and concentration of major ions in water. In waters depleted in calcium and magnesium ostracod shell calcification at moulting may be disturbed, resulting in development of not fully calcified, soft carapaces [70]. It seems that wells localized in the geological formation of the Cretaceous marls and characterized by the highest average calcium concentration in water, proved to represent the habitat successful for ostracoda populations. Ostracods in the Prandocin wells had indeed the highest abundances and species richness. The low abundance and number of copepods species in Jurassic wells could be influenced by pollution of some of them (especially in Wi). Results based on various groups of benthic fauna (excluding microcrustaceans) studied in the wells located in the flysch and limestone regions showed that the parameters of water chemistry related to the pollution and depth of the studied wells influenced the diversity, composition and abundance of the fauna [3]. However, it was possible that other constrains, including water properties (including that not measured during this survey) and/or sediment type and some biological factors might also play important role in determining the demonstrated differences in crustacean alpha diversity and abundances between subterranean waters of the studied geological formations. Groundwater invertebrates in Poland have been studied from different perspectives, including a focus on regions, habitats and finding stygobiontic species. The south of Poland is the best studied region, the north is the weakest. In Poland, up to now among the total species number of crustaceans (95 taxa) found in groundwater (caves, wells and interstitial waters), only 24 obligate stygobiont species were recorded (nine species of amphipods, one bathynellacean, one isopod, four copepods and nine ostracods) (Table 3). The most diverse groups are copepods and ostracods. Most stygobionts have a narrow range, so the risk of species extinction is particularly high in the face of the increase in multiple anthropogenic pressures [71,72]. Our study showed that the literature concerning Polish crustacean fauna from subterranean waters (including stygobiotnic species) is still limited and thus provides an opportunity for further study. Especially the crustaceans fauna in interstitial water has been weakly studied, resulting in a small number of species known from this habitat. Knowledge on the diversity of faunal communities that live in wells can be used to monitor, protect, and manage the environment and can be useful for public health by indicating local water pollution.

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