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Assessment of genetic variability in common whitefish from the catchment area of the Oder river using microsatellite markers

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Keywords *Coregonus maraena*, SSR, genetic variability

Abstract Common whitefish (*Coregonus maraena*) in Poland belongs to the endangered species. The degradation of the environment causes common whitefish to lose its natural reproduction sites. The natural genetic structure of whitefish has been compromised by anthropogenetic activities involving eutrophication, river regulation, the introduction of non-native species and as well as excessive exploitation of the species. The genetic variability of common whitefish (*Coregonus maraena*) from 2 sites: Pomeranian Bay and the lower Oder river, was assessed using microsatellite markers. A total of 45 caught individuals were analysed (26 from Pomeranian Bay and 19 from the Oder river). Polymorphism at nine loci, Str60INRA, Str73INRA, Strutta 12, OmyFgt1TUF, Str85INRA, Str591INRA, Ssa85, Ssa197, T3-13 was assessed. The results indicated that all the investigated populations showed a high level of genetic variability. The level of genetic variability was determined using the F_{ST} parameter and was high investigated populations (0.215). Microsatellite analysis demonstrated a higher observed heterozygosity as compared with the expected heterozygosity in all the investigated populations. The F_{IS} coefficient values below zero in all the investigated populations of common whitefish indicate the excess of heterozygotes. The high number of heterozygotes may be related with a more intense influx of genes from outside of the local population. The study demonstrated that microsatellite markers (SSR) are very useful in the assessment of the genetic variability of common whitefish (*Coregonus maraena*). Our results characterize the selected populations of whitefish and may be useful for further research on this endangered species.

Ocena zmienności genetycznej siei ze zlewni Odry z wykorzystaniem markerów mikrosatelitarnych

Słowa kluczowe *Coregonus maraena*, SSR, zmienność genetyczna

Streszczenie Sieja (*Coregonus maraena*) w Polsce zaliczana jest do gatunków zagrożonych. Degradacja ich naturalnego środowiska spowodowała, że gatunek ten traci miejsca naturalnego rozrodu. Naturalna struktura genetyczna siei została naruszona przez działania antropogeniczne z udziałem eutrofizacji, regulacji rzek, wprowadzaniem gatunków obcych, jak również nadmierną eksploatację tego gatunku. Zmienność genetyczną dwóch populacji siei (*Coregonus maraena*) z Zatoki Pomorskiej i Dolnej Odry oceniano za pomocą markerów mikrosatelitarnych. W sumie analizowano 45 pozyskanych osobników (26 z Zatoki Pomorskiej i 19 z Dolnej Odry). W pracy oceniano polimorfizm dziewięciu loci: Str60INRA, Str73INRA, Strutta 12 OmyFgt1TUF, Str85INRA, Str591INRA, Ssa85, Ssa197, T3-13. Wyniki wskazują, że obie badane populacje wykazywały wysoką zmienność genetyczną. Stopień zmienności genetycznej ustalono za pomocą parametru F_{ST} i była ona wysoka dla badanych populacji – wynosiła 0,215. Analiza sekwencji mikrosatelitarnych wykazała wyższą obserwowaną heterozygotyczność w porównaniu z heterozygotycznością oczekiwaną wszystkich badanych populacji. Wartości współczynników F_{IS} poniżej zera we wszystkich badanych populacjach siei ukazuje nadmiar heterozygot. Wysoka liczba heterozygot może być związana z bardziej intensywnym napływem genów spoza lokalnej populacji. Niniejsze badania ukazały, że markery mikrosatelitarne (SSR) markerów są bardzo przydatne w ocenie zmienności genetycznej siei (*Coregonus maraena*). Nasze wyniki ukazały charakterystykę dwóch wybranych populacji siei i mogą być przydatne do dalszych badań dotyczących tego zagrożonego gatunku.

Introduction

Common whitefish (*Coregonus maraena*) in Poland belongs to the endangered species (Witkowski et al., 2009). Natural populations of this species in Poland occur in Pomeranian Bay and Lake Łebsko (Heese, 1999). Moreover, common whitefish inhabits reservoirs and coastal waters of the Baltic Sea, as well as lakes, e.g., Lake Miedwie and Lake Wigry (Szczerbowski, 2000). The species inhabits clean, cool and well-aerated water. The degradation of the environment causes common whitefish to lose its natural reproduction sites. In the recent years, the number of Polish lakes inhabited by common whitefish has decreased significantly, which is caused by the deterioration of spawning sites (Wilkonska, Zuromska, 1982), as well as excessive exploitation of the species (Witkowski et al., 2009). Currently, large-scale species restoration programmes are being carried out in many lakes. Mass introduction of the fish has been conducted without any preliminary identification of individuals (Szczerbowski, 2000). Introducing closely related individuals derived from a small number of spawners may lead to the impoverishment of the gene pool (Fraser, 2008). However, the restoration of endangered species should be accompanied by genetic monitoring (Foop-Bayat, Wiśniewska, 2010). Such studies are conducted in common whitefish inhabiting Lake Łebsko (Wiśniewska et al., 2010). Common whitefish observed in the Oder mouth constitutes a large stable population used as a source for introductions. Therefore, it is vital to perform genetic analysis of the species, which will permit a more effective fisheries management. The aim of this study was to assess the genetic variability of common whitefish (*Coregonus maraena*) from 2 sites, i.e. Pomeranian Bay and the lower Oder river, using microsatellite markers.

Material and methods

Research subject

The analysis was performed in common whitefish from 2 sites: Pomeranian Bay and the lower Oder river (Figure 1), in which the fish putatively form a local population. A total of 45 caught individuals were analysed (26 from Pomeranian Bay and 19 from the Oder river). The fish were weighed and measured, and samples of muscle tissue were taken. The samples were subsequently frozen at –70°C.

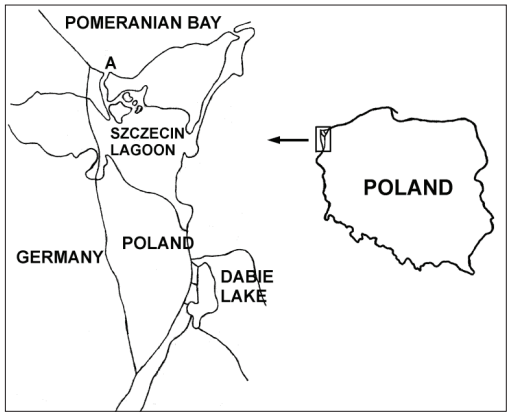

Figure 1. Map of the Poland selected sampling location. Research area: Pomeranian Bay and Oder River (A)

Isolation of genomic DNA

DNA was extracted from 0.2 g muscle tissue taken from every caught individual of the investigated fish population. The material was placed in 1.5 ml tubes and 1 ml extraction buffer (100 mM Tris-HCl, 200 mM NaCl, 0.2% SDS, 5 mM EDTA and 100 µg/ml proteinase K) was added. The mixture was incubated at 55°C for 12 h and then centrifuged at 6000 × g for 15 min. The supernatant was transferred into a new tube and 700 µl isopropanol was added. The mixtures were centrifuged again at 6000 × g for 15 min. The supernatant was discarded and the remaining pellet was resuspended in 400 µl 70% EtOH. The mixtures were centrifuged again at 6000 × g for 5 min, the alcohol was discarded and the samples were dried. The pellet was dissolved in 20 µl TE buffer. The extracts were stored at –20°C. The quantity and the purity of DNA was determined using the BioRad SmartSpec^{TM}Plus spectrophotometer.

PCR-SSR analysis

Nine microsatellite sequences were analysed (Table 1): Str60INRA, Str73INRA (Estoup et al., 1993), Strutta 12 (Poteaux et al., 1995), OmyFgt1TUF (Slettan, 1995), Str85INRA, Str591INRA (Presa et al., 1996), Ssa85, Ssa197 (O'Reilly et al., 1996), T3-13 (Estoup et al., 1998). The reactions were performed in 20 µl mix containing: 120 ng/µl DNA, 1 × PCR buffer (Promega,

USA), 2.5 mM $MgCl_2$, 0.2 mM dNTPs (Fermentas, Lithuania), 0.5 mM primers (provided by the Institute of Biochemistry and Biophysics, Pol. Acad. Sci.), 0.2 U GoTaq™ DNA Polymerase (Promega, USA). The following PCR thermal profiles were used for each set of primers: initial denaturation at 95°C for 4 min followed by 30 cycles of DNA denaturation at 95°C for 30 s, primer annealing at 50–60°C for 45 s and DNA chain elongation at 72°C for 2.5 min. The final elongation was conducted at 72°C for 5 min. Electrophoretic separation of the PCR products was conducted on 3% agarose gel (High Resolution, Sigma-Aldrich, St. Louis, USA) and visualized under UV using ethidium bromide. The size of the PCR products was assessed via comparison with a standard molecular weight marker (MassRuler™DNA Ladder Mix – 80–10,000 bp, Fermentas, Lithuania).

Table 1. List of SSR primer sequences and their annealing temperatures (Ta)

Primer	Ta	Repeat motif	Primer sequence
Str60INRA	60°C	GT	5'-CGGTGTGCTTGTCAGGTTTC-3' 5'-GTCAAGTCAGCAAGCCTCAC-3'
Str73INRA	58°C	GT	5'-CCTGGAGATCCTCCAGCAGGA-3' 5'-CTATTCTGCTTGTAACTAGACCTA-3'
Str85INRA	55°C	CT	5'-GGAAGGAAGGGAGAAAGGT-3' 5'-GGAAAATCAATACTAACAA-3'
Str591INRA	55°C	CT	5'-CTGGTGGCAGGATTTGA-3' 5'-CACTGTCTTTCGTTCTT-3'
T3-13	54°C	GT	5'-CCAGTTAGGGTTCATTGTCC-3' 5'-CGTTACACCTCTCAACAGATG-3'
Strutta 12	56°C	GT	5'-AATCTCAAATCGATCAGAAG-3' 5'-AGCTATTTCAGACATCACC-3'
Ssa197	60°C	GTGA (+GT)	5'-GGGTTGAGTAGGGAGGCTTG-3' 5'-TGGCAGGGATTTGACATAAC-3'
Ssa85	60°C	GT	5'-AGGTGGGTCCTCCAAGCTAC-3' 5'-ACCCGCTCCTCACTTAATC-3'
OmyFgt1TUF	60°C	GT	5'-AGATTTACCCAGCCAGGTAG-3' 5'-CATAGTCTGAACAGGGACAG-3'

Statistical analysis of the results

The results were stored on a BioRad gel documentation system and analysed using the Quantity-One® software (BioRad, USA). The number of alleles per locus (Na) and the number of effective alleles per locus (Ne) were calculated for each investigated population and for all populations at the same time. Genetic information was determined for nine SSR loci in 2 populations using the following indices: number of private alleles per population (Np), Shannon diversity index (I) (Shannon, Weaver, 1949), observed heterozygosity (H_O), expected heterozygosity (He), unbiased expected heterozygosity (uHe), inbreeding coefficient (F_{IS}), fixation index (F_{ST}). Variability per locus was measured using the coefficient of Polymorphic Information Content (PIC) (Anderson et al., 1993).

$$PIC = 1 - \sum_{i}^{n} p_i^2,$$

where: p – band frequency.

The genetic similarity between populations was expressed using Nei's genetic distance (Nei et al., 1983). All calculations were done with GenStat 15th Edition and GenAlEx v. 6.5b4.

Results

The investigated populations were characterized by a high level of genetic variability (Tables 2 and 3). In all of them, the mean number of alleles per locus (Na) was higher than or equal to the mean number of effective alleles per locus (Ne) (Table 3). Shannon diversity index (I) was insignificantly lower in the lower Oder population compared with the other investigated population. The observed heterozygosity (Ho) was lower than the expected heterozygosity (He) in all populations and most heterozygotes were observed in the Oder river population, while the least were observed in the Pomeranian Bay population (Tables 2 and 3). Private alleles were observed exclusively in common whitefish inhabiting Pomeranian Bay (Table 3). Statistical analyses

Table 2. Genetic coefficients of variation of the investigated populations based on the 9 SSR primers

Population	Sample size (N)	Number of alleles per locus (Na)	Number of effective alleles per locus (Ne)	Shannon diversity index (I)	Number of private alleles (Np)	Observed heterozygosity (Ho)	Expected heterozygosity (He)	Unbiased expected heterozygosity (uhe)	Inbreeding coefficient (F_{IS})
Pomeranian Bay	26	1.889	1.630	0.463	0.540	0.450	0.308	0.316	–0.412
Lower Oder	19	1.667	1.661	0.460	0.00	0.652	0.332	0.398	–0.922

Table 3. Summary statistics of the results for microsatellite data

Locus	No. Alleles	Polymorphic Information Content (PIC)	Expected heterozygosity (He)	Observed heterozygosity (Ho)	Fixation index (F_{ST})	Inbreeding coefficient (F_{IS})
Ssa197	3	0.75	0.53	0.80	0.05	–0.52
Str85INRA	2	0.70	0.50	1.00	0.00	–1.00
OmyFgt1TUF	2	0.81	0.00	0.00	1.00	–
Str60INRA	2	0.78	0.49	0.90	0.01	–0.83
Str73INRA	3	0.90	0.05	0.03	0.06	0.46
T3-13	1	0.85	0.00	0.00	–	–
Strutta12	2	0.58	0.49	0.94	0.01	–0.91
Ssa85	3	0.47	0.38	0.75	0.43	–1.00
Str591INRA	2	0.82	0.50	1.00	0.00	–1.00
Mean	2.22	0.74			0.20	–0.68

Table 4. Genetic variability between the investigated populations (F_{ST})

	Pomeranian Bay	Lower Oder
Pomeranian Bay	0.000	
Lower Oder	0.215	0.000

revealed that the investigated whitefish populations are characterized by inbreeding coefficient (F_{IS}) below zero, which indicates a high excess of heterozygotes (Tables 2 and 3). The coefficient of Polymorphic Information Content (PIC) values for each of the SSR (Simple Sequence Repeats) primers ranged from 0.47 to 0.90. The mean value of this index was 0.74 (Table 3). The observed heterozygosity values generated by each SSR primer were between 0 and 1, while the values of expected heterozygosity ranged from 0 to 0.53 (Table 3). The level of genetic variability between the populations was determined using the F_{ST} parameter. The genetic distance between the investigated whitefish populations is great (0.215) (Table 4). The percentage of polymorphic loci was identical in the two investigated populations (66.67%).

Discussion

In the region of the Baltic Sea, the reduction of the common whitefish population is a result of intensive fishing and environmental pollution. The rapid loss of genetic diversity leads to decreased adaptation capabilities and an increased risk of extinction of the species (Frankel, Soul, 1981; Frankham, 1995). If restoration of an endangered species is performed, genetic monitoring should be conducted in parallel to avoid changes in the genetic structure and the impoverishment of the gene pool of the population (Fraser, 2008; Fopp-Bayat, Wiśniewska, 2010). The morphological and genetic variability of *Coregonus maraena* was assessed using microsatellite sequences (Bernatchez et al., 1999; Lu, Bernatchez, 1999; Østbye et al., 2004; Hansen et al., 2008) and mitochondrial DNA (Kohlmann et al., 2007; Kempter et al., 2010).

In this study, polymorphisms among common whitefish populations were determined using the microsatellite markers, successfully employed in the assessment of genetic variability and the degree of similarity between fish species (Fopp-Bayat, Wiśniewska, 2010; Säis et al., 2008; Winkler, Weiss, 2008; Dierking et al., 2014).

The values of expected heterozygosity (He), obtained by employing microsatellite sequences in the study of *Coregonus maraena* populations, ranged from 0.308 to 0.332. The level of heterozygosity of the investigated populations of common whitefish is lower than in the Alpine populations from Austria (He = 0.37 – 0.95) (Winkler, Weiss, 2008) and three naturally reproductively isolated whitefish taxa in Germany (He = 0.66 – 0.76) (Dierking et al., 2014), but similar to that of Norwegian populations (Østbye et al., 2004). The high number of heterozygotes may be related with a more intense influx of genes from outside of the local population. Various studies indicate that many fish populations, such as *Culter erythropterus* (Wang et al., 2007), *Engraulis encrasicolus* (Zarraonaindia et al., 2009), or the investigated *Coregonus lavaretus* (McCairns et al., 2012), face the problem of a reduced frequency of heterozygotes due to inbreeding (O'Reilly et al., 1996). It is therefore a very positive signal that no such phenomenon is observed in the two investigated populations of common whitefish. Surprising is the fact that the presence of private alleles (Np), occurring exclusively in a given population, was observed only in the common

whitefish population of Pomeranian Bay. This means that the other populations are characterized by a lower genetic variability. It may be a result of the disappearance of those alleles due to genetic drift or due to the failure to fully detect the polymorphism of these populations. The high genetic variability of common whitefish inhabiting Pomeranian Bay, compared with the lower Oder population, is also demonstrated by the genetic variability coefficient (F_{ST} = 0.215). The population of Pomeranian Bay did not reveal such genetic homogeneity. Low F_{ST} values are usually characteristic of the *Coregonus* populations, despite the morphological and ecological diversity of the genus. The F_{ST} values of the common whitefish populations from 8 Alpine lakes, averaging at 0.049 (Douglas et al., 1999), or those of the populations from Norwegian lakes, ranging from 0 to 0.0143 (Østbye et al., 2004), are examples of this phenomenon.

The high level of genetic variability retained by the investigated common whitefish populations indicates that despite the significant reduction of the number of individuals, no effect of genetic drift has occurred. As a result of the long-term effect of the process, a reduction in the intra-population variability occurs along with an increase in the inter-population variability.

The conducted study demonstrated a high genetic variability between the analysed common whitefish populations, which may be explained by the mass introduction of the species into waterbodies without proper identification (Szczerbowski, 2000). It is even hypothesized that finding a pure form of common whitefish is very unlikely (Witkowski et al., 2009).

Continuous genetic monitoring is necessary in the process of renewal of endangered species in order to prevent the disruption of the genetic structure of the population (Fopp-Bayat, 2010). The need to know and to characterize this structure as many populations also indicate other researchers (Pamminger-Lahnsteiner et al., 2009).

Our results characterize the selected populations of whitefish and may be useful for further research on this species.

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Syntomus pallipes (Dejean, 1825) (Coleoptera: Carabidae) – ground beetle new to Belarus

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Keywords: Coleoptera, Carabidae, *Syntomus pallipes* (Dejean, 1825), new record, SE Belarus

Abstract: *Syntomus pallipes* (Dejean, 1825) was recorded for the first time in Belarus from Gomel city (SE Belarus).

Syntomus pallipes (Dejean, 1825) – nowy gatunek dla fauny Białorusi z rodziny biegaczowatych (Coleoptera: Carabidae)

Słowa kluczowe: Coleoptera, Carabidae, *Syntomus pallipes* (Dejean, 1825), pierwsza rejestracja, południowo-wschodnia Białoruś

Streszczenie: *Syntomus pallipes* (Dejean, 1825) został odnotowany po raz pierwszy na Białorusi w Homlu (Białoruś południowo-wschodnia).

Introduction

The genus *Syntomus* Hope 1838 includes 52 wide dispersed mainly tropical species (Lorenz, 2005). There are 38 species known in Palaearctic region (Kabak, 2003), in Europe – 16 species, and 4 – in Middle Europe (Persohn, 2004). Nowadays 2 species only was known in Belarus (Aleksandrowicz, 2014).

S. pallipes occurs in Eurasia and North Africa. Its range takes whole temperate and steppe zones of Eurasia: from Netherlands to the Russian Far East. In the forest zone, it is very seldom, in the steppe – more common (Persohn, 2004).

Based on the author's research conducted in the south-eastern Belarus *Syntomus pallipes* was found to occur in the country.

Material and methods

Locality: Belarus – Gomel city, (UTM UD61) (Figure 1).

The species new for the Belarus fauna: *Syntomus pallipes* (Dejean, 1825): Gomel, Auerbah str., 11.03.2016, 9 ex., in dry plant remains in the garden; ad lucem, 16.04.2016, 11 ex.; Gomel, Central City Park, sand beach of Sozh river, 28.05.2017, 2 ex., leg. A. Ostrovsky. Two specimens were with fully development wings, 20 – wingless. A material was collected by the second author by hand in 2016–2017.

Discussion

According to Kabak (2003) *S. pallipes* is distributed from North Africa and southern Europe, through the Balkans and Asia Minor to Central Asia, East Siberia and Far East.

In Central Europe occurs, but in most cases only single, in the south and south-east. From Germany, in addition to the current guidelines of Saxony, there are only old or questionable findings (Persohn, 2004). There are only old dates in Poland – more than 100 years ago (Burakowski et al., 1974; http://baza.biomap.pl/en/taxon/species-syntomus_pallipes/mapb). *S. pallipes* not found yet in north Ukraine (Putchkov, 2011).

It is interesting, that *S. pallipes* was known from large cities: Prague (Veselý, 2002) and Vienna (Hepner et al., 2008). We found all specimens in Gomel – a comparatively large city too.

Environmental requirements of *S. pallipes* are poorly known. According Persohn (2004) it prefers dry steppe habitats or light forests from lowlands to foothills. Koch (1989) considers it as an eurybiont.

According Persohn (2004) the wings are predominantly reduced. In our material 91% of specimens were wingless.

It is very rare everywhere. In Poland and Germany it has been placed on red lists of endangered species (Pawłowski et al., 2002; Schmidt et al., 2016). In Upper Silesia it is known as the extinct (Kuśka, 2007).

Conclusion

Nowadays, there are three species of the genus *Syntomus* Hope, 1838 known from Belarus, namely *S. foveatus* (Geoffroy in Fourcroy, 1785), *S. pallipes* (Dejean, 1825), *S. truncatellus* (Linnaeus, 1761).

S. pallipes is the next steppen species found in the south-eastern Belarus last decades. There were *Calosoma investigator* (Illiger, 1798), *C. denticolle* (Gebler, 1833), *Harpalus subcylindricus* (Dejean, 1829), *H. honestus* (Duftschmid, 1812), *Zabrus tenebrioides* (Goeze, 1777) (Aleksandrowicz, 2011) and *Lebia marginata* (Geoffroy, 1785) (Halinouski et al., 2015).

Figure 1. Distribution map of *Syntomus pallipes* in West Palaearctic region (according Vigna Taglianti 2013) with new date from Belarus (gray – species occurs, white – absent, black dot – new date)

Source: https://fauna-eu.org.

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Preliminary data on the epigeic beetle fauna (Coleoptera) of the Golczewskie Uroczysko Nature Reserve

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Keywords epigeic beetles, Coleoptera: Catopidae, Carabidae, Elateridae, Geotrupidae, Silphidae, Staphylinidae, Tenebrionidae, Golczewskie Uroczysko Nature Reserve, West Polish Pomeranian

Abstract A study was conducted in May–August 2006 in the Golczewskie Uroczysko Nature Reserve, UTM WV06, using 10 Barber traps containing ethylene glycol. A total of 2,141 beetles were collected, belonging to 58 species from 7 families. The epigeic beetle fauna of the reserve comprised mainly forest, grassland and peatland species. Stable populations of three ground beetle species under partial protection, *Carabus convexus*, *Carabus coriaceus* and *Carabus glabratus*, are present in the forest habitats. The most ecologically valuable species include hygrophilous peatland species: *Agonum ericeti* (VU), *Pterostichus rhaeticus*, *Agonum hypocrita*, *Limodromus krynickii*, *Oodes helopioides* (VU), and the rare click beetle *Paraphotistus impressus*, all of which have poorly known ecological preferences.

Wstępne dane do poznania fauny chrząszczy epigeicznych (Coleoptera) Rezerwatu przyrody „Golczewskie Uroczysko"

Słowa kluczowe chrząszcze epigeiczne, Coleoptera: Catopidae, Carabidae, Elateridae, Geotrupidae, Silphidae, Staphylinidae, Tenebrionidae, Rezerwat przyrody „Golczewskie Uroczysko"

Streszczenie Badania były prowadzone w maju–sierpniu 2006 roku na terenie Rezerwatu przyrody „Golczewskie Uroczysko", UTM WV06, za pomocą 10 pułapek ziemnych z glikolem etylenowym. Zebrano 2178 chrząszczy, należących do 71 gatunków z 15 rodzin. Fauna chrząszczy epigeicznych rezerwatu ukształtowana główne przez gatunki leśne, łąkowe i torfowiskowe, W siedliskach leśnych występują stabilne populacje 3 gatunków biegaczy objętych ochroną częściową: *Carabus convexus, Carabus coriaceus* oraz *Carabus glabratus*. Do najcenniejszych

przyrodniczo można zaliczyć hygrofilne gatunki torfowiskowe: *Agonum ericeti* (VU), *Pterostichus rhaeticus*, *Agonum hypocrita, Limodromus krynickii, Oodes helopioides* (VU), oraz rzadki sprężyk *Paraphotistus impressus* ze słabo poznanymi preferencjami ekologicznymi.

Introduction

Raised peat bogs in their natural state are currently still found in Ireland, Great Britain, Scandinavia, countries of the former Soviet Union, and Poland (Sjörs, 1983). Marshland and swamps are characteristic of northern and north-eastern Poland. They occupy about 4% of the area of the country, and raised peat bogs make up 0.2% (Ilnicki, 2002). According the European Union Natura 2000 directive, raised bogs and marshland are especially valuable habitats (Council Directive 92/43/EEC).

Marshes are destroyed during agricultural practices, drainage and peat extraction. Particularly dangerous for peatlands is the decline in the groundwater level, this is a consequence of dehydration. In Poland it is the main cause of the disappearance of peatlands (Żurek, 1987). For this reason there is a need for intensive research on the fauna of these rare and threatened habitats.

Uroczysko Golczewskie is an ecologically valuable peatland complex containing forest habitats, lakes, raised bogs and transitional bogs (Figure 1).

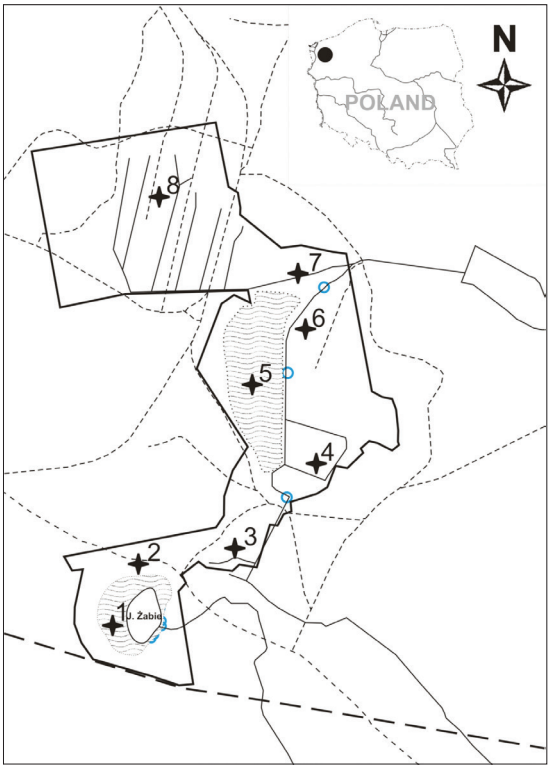

Figure 1. Map of the area with sites where insects were obtained

In northern and central Europe, research on the ground beetles of raised bogs has a fairly long tradition. It is worth recalling some of the work on this subject: Peus (1928–1932) in north-western Germany (Mossakowski, 1970), in eastern Prussia (Skwarra, 1929; Främbs et al., 2002; Mossakowski et al., 2003), in the Czech Republic (Roubal, 1934), in Finland (Renkonen, 1938; Krogerus, 1960), and in Belarus (Sushko, 2006; Aleksandrowicz, 2014).

In Poland, the epigeic beetles of raised peat bogs are little known; only the ground beetles (Carabidae) of the "Torfiaki" peatland complex in the vicinity of Olsztyn have been studied (Aleksandrowicz et al., 2017).

Study area

The Golczewskie Uroczysko nature reserve was established on 5 May 2004 to protect the raised peat bog and the dystrophic Lake Żabie with its surrounding transitional bog and adjacent forest complexes containing valuable plants. It is located on the Gryfice Plain, near the village of Rokita, in a moraine upland area in the Kamień Pomorski anticline, and occupies two shallow depressions in the ground moraine landscape, separated by a small hill, and a flat moraine plain in the northern part of the reserve, with a moraine kame at 40 m a.s.l. situated on the north-western border of the reserve (Kondracki, 2004).

The southern depression is occupied by Lake Żabie, a small dystrophic lake located in a transitional, topogenous peat bog. The depression occupying the central part of the reserve is entirely filled with peat. In the eastern part it is a transitional, topogenous bog, while in the western part it is a raised, ombrogenous bog.

The research area was divided into eight sites, to more fully show the species richness of the entire reserve. The sites were classified as follows: sphagnum phytocoenoses with fragments of pine forest, *Sphagno squarrosi-Alnetum, Ribeso nigri-Alnetum,* ash and alder riparian forest, sphagnum peat bog, *Vaccinio uliginosi-Betuletum pubescentis,* phytocoenoses mainly dominated by sub-Atlantic lowland oak and hornbeam forest, phytocoenoses mainly dominated by *Vaccinio uliginosi-Betuletum pubescentis.*

Methods

The study was carried out from 28 May to 15 August 2006. Due to the fact that the studies were conducted in the nature reserve, the duration of the study and the number of traps used were limited.

The beetles were caught using Barber traps, each consisting of a plastic 500 ml container buried in the earth so that its rim did not protrude above the surface, making it much easier for insects to fall in. About 15 ml of a 10% solution of ethylene glycol was poured into the container. A plastic lid was placed about 2 cm over the trap to protect it from rain and falling leaves that could block the opening. This is a standard method that is widely used in this type of research (Thiele, 1977).

The habitat preferences of each species were based on a work by Koch (1989) and Aleksandrovich (2004).

Results

In the Golczewskie Uroczysko Nature Reserve 2,141 beetles were captured and identified, comprising 58 species from 7 families (Table 1).

The most abundant families were Carabidae, with 34 species and 1,108 specimens, Silphidae, with 8 species and 631 specimens, Staphylinidae 4 species and 18 specimens, and Geotrupidae, with 3 species and 315 specimens (Table 1).

Table 1. List of families of epigeic beetles

Family	Number of species	Number of specimens
Carabidae	34	1,108
Catopidae	2	45
Elateridae	6	22
Geotrupidae	3	315
Silphidae	8	631
Staphylinidae	4	18
Tenebrionidae	1	2
Total	58	2,141

Table 2. List of families and species of epigeic beetles caught in Barber traps

Family	Species	Number of specimens	Habitat preferences
1	2	3	4
Carabidae	*Agonum ericeti* (Panzer, 1809)	1	peatland
	Agonum fuliginosum (Panzer, 1809)	2	forest
	Agonum hypocrita (Apfelbeck, 1904)	2	peatland
	Amara plebeja (Gyllenhal, 1810)	2	grassland
	Calathus micropterus (Duftschmid, 1812)	5	forest
	Carabus arcensis (Herbst, 1784)	44	forest
	Carabus auratus (Linnaeus, 1761)	2	field
	Carabus convexus (Fabricius, 1775)	18	forest
	Carabus coriaceus (Linnaeus, 1758)	17	forest
	Carabus glabratus (Paykull, 1790)	152	forest
	Carabus granulatus (Linnaeus, 1758)	138	eurybiont
	Carabus hortensis (Linnaeus, 1758)	120	forest
	Carabus nemoralis (O.F.Müller, 1764)	1	eurybiont
	Carabus violaceus (Linnaeus, 1758)	116	forest
	Chlaenius nitidulus (Schrank, 1781)	1	grassland
	Cychrus caraboides (Linnaeus, 1758)	7	forest
	Harpalus rufipes (Degeer, 1774)	1	field
	Leistus terminatus (Hellwig, 1793)	1	peatland

1	2	3	4
	Limodromus assimilis (Paykull, 1790)	8	forest
	Limodromus krynickii (Sperk, 1835)	10	peatland
	Loricera pilicornis (Fabricius, 1775)	4	peatland
	Nebria brevicollis (Fabricius, 1792)	1	forest
	Oodes helopioides (Fabricius, 1792)	1	peatland
	Oxypselaphus obscurus (Herbst, 1784)	2	forest
	Patrobus atrorufus (Strom, 1768)	10	peatland
	Pterostichus anthracinus (Illiger,1798)	6	peatland
	Pterostichus diligens (Sturm, 1824)	115	peatland
	Pterostichus melanarius (Illiger, 1798)	5	eurybiont
	Pterostichus minor (Gyllenhal, 1827)	163	peatland
	Pterostichus niger (Schaller, 1783)	19	forest
	Pterostichus nigrita (Paykull, 1790)	83	forest
	Pterostichus oblongopunctatus (Fabricius, 1787)	19	forest
	Pterostichus rhaeticus (Heer, 1838)	31	peatland
	Pterostichus strenuus (Panzer, 1797)	1	forest
Catopidae	*Catops fuliginosus* (Erichson, 1837)	2	forest
	Sciodrepoides watsoni (Spence, 1815)	43	forest
Elateridae	*Actenicerus sjaelandicus* (O.F .Müller, 1764)	3	peatland
	Athous haemorrhoidalis (Fabricius, 1801)	1	forest
	Athous subfuscus (O.F.Müller, 1764)	9	forest
	Dalopius marginatus (Linnaeus, 1758)	3	forest
	Denticollis linearis (Linnaeus, 1758)	2	forest
	Paraphotistus impressus (Fabricius, 1792)	4	forest
Geotrupidae	*Anoplotrupes stercorosus* (Hartmann in L.G. Scriba, 1791)	227	forest
	Geotrupes stercorarius (Linnaeus, 1758)	80	grassland
	Trypocopris vernalis (Linnaeus, 1758)	8	forest
Silphidae	*Nicrophorus humator* (Gleditsch, 1767)	8	forest
	Nicrophorus investigator (Zetterstedt, 1824)	10	grassland
	Nicrophorus vespillo (Linnaeus, 1758)	91	grassland
	Nicrophorus vespilloides (Herbst, 1783)	416	forest
	Nicrophorus vestigator (Herbst, 1807)	17	grassland
	Oiceoptoma thoracica (Linnaeus, 1758)	86	forest
	Phosphuga atrata (Linnaeus ,1758)	1	forest
	Thanatophilus sinuatus (Fabricius, 1775)	2	grassland
Staphylinidae	*Philonthus decorus* (Gravenhorst, 1802)	1	forest
	Scaphidium quadrimaculatum (Olivier, 1790)	1	forest
	Staphylinus erythropterus (Linnaeus, 1758)	15	grassland
	Tachinus rufipes (Linnaeus, 1758)	1	grassland
Tenebrionidae	*Lagria hirta* (Linnaeus, 1758)	2	grassland
Total specimens		2,141	

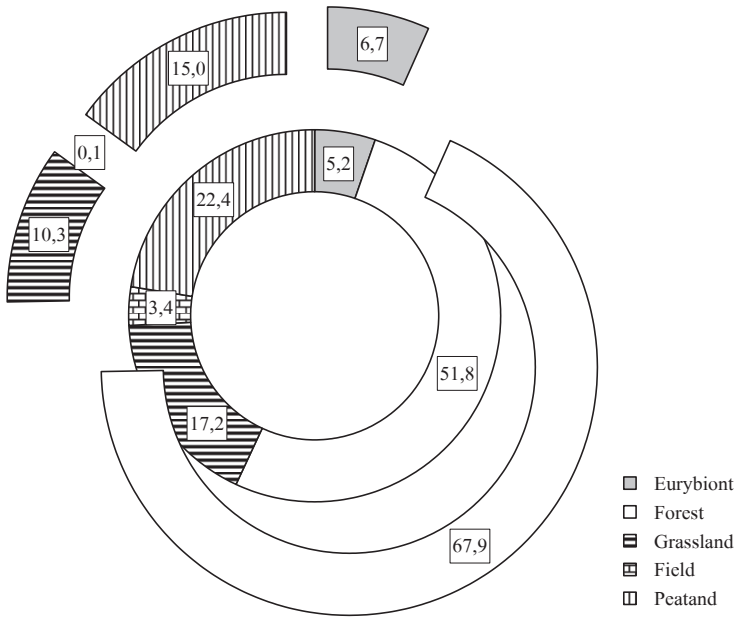

Figure 2. Habitat preferences of epigeic beetles of the Uroczysko Golczewskie Nature Reserve (inner ring – number of species, outer ring – number of specimens)

The epigeic beetle fauna of the Uroczysko Golczewskie Nature Reserve is not very rich, consisting primarily of forest, grassland and peatland species (Figure 2).

The presence of three ground beetle species under partial protection was established: *Carabus convexus*, *Carabus coriaceus* and *Carabus glabratus* (Dz.U. [Journal of Laws] of 2016, item 2183). These species are fairly abundant in forest habitats (phytocoenoses dominated by sub-Atlantic lowland oak and hornbeam forests) and their populations are not endangered.

Three species are on the Polish Red List of endangered species (Pawłowski et al., 2002): the near threatened (NT) *Carabus convexus* and the vulnerable (VU) *Agonum ericeti* and *Oodes helopioides.*

The most ecologically valuable are stenobiontic hygrophilous species typical of raised bogs: ground beetles *Agonum ericeti*, *Pterostichus diligens* and *Pterostichus rhaeticus* and of transitional bogs and fens ground beetles *Agonum hypocrita* and *Limodromus krynickii*, the click beetle *Paraphotistus impressus.*

Also included among peatland species were hygrophilous species which, apart from peatlands, are also found in other wetlands: *Actenicerus sjaelandicus*, *Leistus terminatus*, *Loricera pilicornis*, *Oodes helopioides*, *Patrobus atrorufus*, *Pterostichus anthracinus* and *Pterostichus minor.*

Agonum ericeti – a stenobiontic hygrophilous species of raised bogs. One species caught 1 July 2006 in a raised peat bog. Recorded the first time for West Pomerania.

Agonum hypocrita – a stenobiontic hygrophilous species of lowland and carbonate fens. Rarely encountered in Pomerania (http://baza.biomap.pl/pl/taxon/order-coleoptera/default/tlm/checklist). Two specimens caught 9 August 2006 in a peat bog with fragments of pine forest.

Limodromus krynickii – a stenobiontic hygrophilous species of lowland fens. Rarely encountered in Poland (Burakowski et al., 1974). Recorded for the first time for Pomerania. One specimen, caught 28 May 2006 in a *Vaccinio uliginosi-Betuletum pubescentis* association.

Pterostichus rhaeticus – a stenobiontic hygrophilous species of raised bogs. 21 species caught on 9 August 2006 in a raised peat bog, 6 specimens in a peat bog with fragments of pine forest, and 4 in a *Sphagno squarrosi-Alnetum* association.

Oodes helopioides – a stenobiontic hygrophilous species of peatlands and other wetlands. Common in Poland (http://baza.biomap.pl/pl/taxon/species-oodes_helopioides_helopioides/default/tr/y). Caught on 28 May 2006, one specimen in a *Vaccinio uliginosi-Betuletum pubescentis* association.

Paraphotistus impressus – rare and sporadic species associated with coniferous forests (Burakowski et al., 1985). Four specimens caught on 9 August 2006, in a peat bog with fragments of pine forest.

Discussion

Peatlands are considered very demanding, extreme environments (Främbs et al., 2002). Species strictly associated with these biotas are called tyrphobionts, while those occurring in the highest numbers, but with a broader ecological spectrum, have been classified as tyrphophiles (Peus, 1932). The tyrphobiontism of numerous taxa occurring in marshes and peatlands is the result of regional limitations. Most of these species are eurytopes which have spread in the continental regions of Eurasia, while in Central and Western Europe their occurrence is limited to oligotrophic peatlands (Thiele, 1977). In the Uroczysko Golczewskie reserve, *Agonum ericeti* and *Pterostichus rhaeticus* belong to this group.

In the study area we can observe that the species occurring in the highest numbers are representatives of the Carabidae and Silphidae families. The first of these are predatory species which actively seek prey, while the latter are scavengers (apart from the predatory *Phosphuga atrata* and the polyphagous *Thanatophilus sinuatus*).

Tews et al. (2003) found that species richness is most affected by habitat structure in periodically flooded wetlands. It is likely that the mosaic character of the habitats in the Uroczysko Golczewskie reserve had a significant effect on species diversity.

Comparison of the species composition of the epigeic beetles of the Uroczysko Golczewskie reserve indicates a fairly high degree of similarity with the fauna of peatlands of NW Germany (Peus, 1932, Mossakowski, 1970), NE Poland (Aleksandrowicz et al., 2017) and northern Belarus (Sushko, 2006).

Conclusions

The study was conducted in May–August 2006 in the Golczewskie Uroczysko Nature Reserve, UTM WV06. Ten Barber traps with ethylene glycol were used to catch 2,141 beetles belonging to 58 species from 7 families.

The most abundant families were Carabidae, with 34 species and 1,108 specimens, Silphidae, with 8 species and 631 specimens, and Geotrupidae, with three species and 315 specimens.

The epigeic beetle fauna of the reserve consisted mainly of forest, grassland and peatland species.

The presence of three species of ground beetles under partial protection was established: *Carabus convexus*, *Carabus coriaceus* and *Carabus glabratus* (Dz.U. [Journal of Laws] of 2016, item 2183). These species are fairly abundant in forest habitats (phytocoenoses dominated by sub-Atlantic lowland oak and hornbeam forests) and their populations are not endangered.

Three species are on the Polish Red List of endangered species (Pawłowski et al., 2002): the near threatened (NT) *Carabus convexus* and the vulnerable (VU) *Agonum ericeti* and *Oodes helopioides*.

The most ecologically valuable species include hygrophilous peatland species: *Agonum ericeti*, *Pterostichus rhaeticus*, *Agonum hypocrita*, *Limodromus krynickii*, *Oodes helopioides*, the rare click beetle *Paraphotistus impressus*, all of which have poorly known ecological preferences.

Comparison of the species composition of the epigeic beetles of the Uroczysko Golczewskie reserve indicates a fairly high degree of similarity with the fauna of peatlands of north-western Germany and northern Belarus.

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Epigeic beetles (Coleoptera) of the Lake Świdwie nature reserve

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Keywords Coleoptera, fauna, species composition, Świdwie nature reserve

Abstract

The data presented concern preliminary results of faunistic research carried out on the epigeic beetle fauna in the Lake Świdwie nature reserve, NW Poland (UTM VV53). Fourteen pitfall traps were placed in four different habitats in the reserve. A total of 957 specimens were collected belonging to 83 species and 16 families: Byrrhidae, Carabidae, Catopidae, Curculionidae, Dermestidae, Dryopidae, Elateridae, Eucinetidae, Hydrophilidae, Geotrupidae, Leiodidae, Limnichidae, Silphidae, Staphylinidae, Scarabaeidae, Tenebrionidae. Representatives of Staphylinidae, Hydrophilidae and Curculionidae were determinate to the family level only.

Chrząszcze epigeiczne (Coleoptera) rezerwatu przyrody „Jezioro Świdwie"

Słowa kluczowe Coleoptera, chrząszcze, fauna, skład gatunkowy, rezerwat przyrody „Jezioro Świdwie"

Streszczenie

Przedstawiono wstępne wyniki badań faunistycznych chrząszczy epigeicznych w rezerwacie przyrody „Jezioro Świdwie" w Polsce północno-zachodniej (UTM VV53). Czternaście pułapek umieszczono w czterech różnych siedliskach. Odłowiono łącznie 957 okazów należących do 83 gatunków i 16 rodzin: Byrrhidae, Carabidae, Catopidae, Curculionidae, Dermestidae, Dryopidae, Elateridae, Eucinetidae, Hydrophilidae, Geotrupidae, Leiodidae, Limnichidae, Silphidae, Staphylinidae, Scarabaeidae, Tenebrionidae. Przedstawiciele Staphylinidae, Hydrophilidae i Curculionidae oznaczone tylko do poziomu rodziny.

Introduction

The protected areas of Western Pomerania are relatively well known, but only for selected families of beetles. A study by Wolender (2013) summarizes the results of many years of research on one of the largest families of epigeic beetles, the Carabidae. This paper, however, presents material from forest, grassland and dune habitats. The little information available on carabids of marshland is presented in a faunistic study of the fauna of the island of Wolin (Radawiec et al., 2015).

The best known are the carabids of wetlands (rushes and sedges) of the buffer zone of the Leon Wyczółkowski Cisy Staropolskie reserve (Stachowiak, Wilcz, 2001).

Other families of epigeic beetles in the wetlands of Western Pomerania have not been the subject of special studies. There has been only preliminary research on the fauna of the epigeic beetles of the Lake Szare nature reserve (Aleksandrowicz, Dąbkowski, 2007) and a study on the fauna of Gmina Tuczno, where Gutowski and Ruta (2004) recorded 38 and 49 beetle species in peat bogs and wet grassland. However, Gutowski and Ruta (2004) did not use pitfall traps.

Study area

Lake Świdwie is located in West Pomerania, about 20 km northwest of Szczecin, in the Puszcza Wkrzańska forest (E14°21'41'' N53°33'50''). This shallow lake, together with the surrounding wetlands, is an ecologically valuable area, constituting one of the most important areas in the country on the bird migration path and under protection as the Świdwie nature reserve, which was added to the list of wetlands of international importance in 1978 under the Ramsar Convention.

At present, the reserve covers 891.28 ha, and the lake together with complexes of rushes covers a total area of 358 ha (Pienkowski, Kupiec, 2001). The surface area of the open water of the lake constitutes only 5.56% of its original area. The reserve area is fed by the Upper Gunica River and has a well-developed hydrographic network in the form of numerous natural watercourses and artificial canals (Kowalski, Bacieczko, 1993). The dominant communities having the most important role in the terrestrialization of the area are *Phragmitetum australis*, *Typhetum angustifolie*, *Typhetum latifolie* and *Sparganietum erecti* (Bacieczko, Kowalski, 1993). Dominant among grassland vegetation are *Arrhenatheretum elatioris*, *Deschampsietosum caespitosae*, *Potentillo Festucetum arundinaceae*, *Calamagrostietum epigeji*, *Urtico Calystegietum sepium*, *Caricetum gracilis* and *Caricetum ripariae* (Bacieczko, Kowalski, 1993). The forests in the reserve belong to the classes *Alnetea glutinosae* and *Vaccinio piceetea* (Kowalski, Bacieczko, 1993).

The study area was divided into 5 sites. The site numbers correspond to the locations marked in Figure 1.

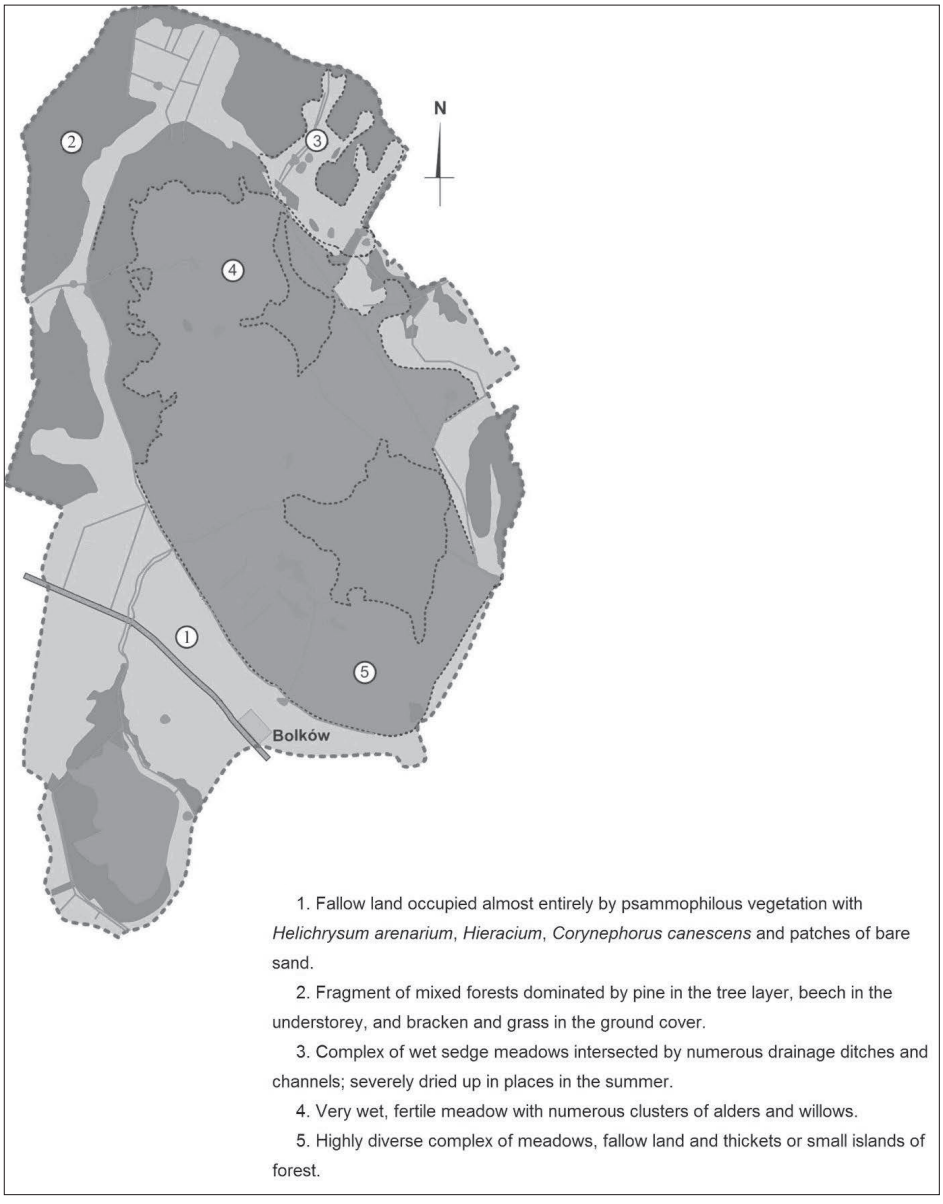

Figure 1. Map showing the distribution of the study sites in the Świdwie reserve

Research methods

Three research cycles were carried out in 2010 in the Świdwie nature reserve on 15–21 June, 10–15 July and 17–22 August.

Epigeic beetles were caught using 500 ml plastic cups. The upper rim of the cup, which was also the entrance to the trap, was 10 cm in diameter. The trap was placed in the ground so that the rim was level with the surface, to ensure that beetles penetrating the soil surface would fall in easily. To kill and preserve the insects entering the traps, they were filled with 50 ml of ethylene glycol (25%). The traps were arranged in a line, about 10 metres apart. Ten traps per habitat were set up in the line.

This is a standard, commonly used method in this type of research (Thiele, 1977).

The beetles were preserved in 70% ethyl alcohol and identified in the laboratory.

Works by Koch (1989, 1991) were used to determine species habitat preferences.

Similarity of species composition was evaluated using the Jaccard index and PAST software (Hammer et al., 2013).

Results and conclusions

During the study season a total of 957 epigeic beetles were caught, belonging to 16 families and 83 species (Table 1). Representatives of the families Staphylinidae, Hydrophilidae and Curculionidae were not identified to species.

There were 51 Carabidae species, 7 species each assigned to the Elateridae and Silphidae families, 3 each to Tenebrionidae and Leiodidae, 2 species each to Catopidae, Dryopidae, Scarabaeidae and Geotrupidae, and one each to Byrrhidae, Dermestidae, Eucinetidae and Limnichidae (Table 1).

Table 1. Species composition and number of specimens of selected epigeic beetle families caught in the Świdwie reserve

Familia and species	Fallow land	Fragment of mixed forest	Complex of wet sedge meadows	Very wet, fertile meadow	Highly diverse complex of meadows	Total
1	2	3	4	5	6	7
1. Byrrhidae (Latreille, 1806)						
Cytilus sericeus (Forster, 1771)	1					1
2. Carabidae (Latreille, 1802)						
Abax parallelepipedus (Piller et Mitterpacher, 1783)		4				4
Agonum emarginatum (Duftschmid, 1812)				7		7
Agonum fuliginosum (Panzer, 1809)			12	46	6	64
Agonum viduum (Panzer, 1796)				1		1
Amara aenea (Degeer, 1774)	1					1
Amara bifrons (Gyllenhal, 1810)	2					2

1	2	3	4	5	6	7
Amara familiaris (Duftschmid, 1812)					1	1
Amara littorea (Thomson, 1857)	1					1
Amara lunicollis (Schiødte, 1837)		2		1	4	7
Amara spreta (Dejean, 1831)	1					1
Badister sodalis (Duftschmid, 1812)				1		1
Bembidion assimile (Gyllenhal, 1810)			2	3		5
Bembidion gilvipes (Sturm, 1825)				12		12
Bembidion guttula (Fabricius, 1792)				1	1	2
Bembidion mannerheimii (C. Sahlberg, 1827)			8	11	6	25
Calathus erratus (Sahlberg, 1827)	2					2
Calathus fuscipes (Goeze, 1777)	7	1	1			9
Calathus melanocephalus (Linnaeus, 1758)	3					3
Carabus arcensis (Herbst, 1784)		1				1
Carabus granulatus (Linnaeus, 1758)			5	7	2	14
Carabus violaceus (Linnaeus, 1758)		4				4
Clivina fossor (Linnaeus, 1758)				3	1	4
Dyschirius globosus (Herbst, 1784)			8	4	2	14
Elaphrus uliginosus (Fabricius, 1792)					1	1
Harpalus latus (Linnaeus, 1758)	1	1	8		2	12
Harpalus luteicornis (Duftschmid, 1812)			1		1	2
Harpalus pumilus (Sturm, 1818)	1					1
Harpalus rufipes (Degeer, 1774)	1		1			2
Harpalus smaragdinus (Duftschmid, 1812)	3					3
Harpalus tardus (Panzer, 1797)	2		2			4
Leistus ferrugineus (Linnaeus, 1758)		1				1
Microlestes minutulus (Goeze, 1777)	7	1	1			9
Olisthopus rotundatus (Paykull, 1790)			1			1
Oodes helopioides (Fabricius, 1792) – VU				23	1	24
Oxypselaphus obscurus (Herbst, 1784)				4	1	5
Panagaeus cruxmajor (Linnaeus, 1758)					2	2
Poecilus lepidus (Leske, 1785)		1				1
Poecilus versicolor (Sturm, 1824)			8		14	22
Pterostichus diligens (Sturm, 1824)				7	1	8
Pterostichus minor (Gyllenhal, 1827)				4		4
Pterostichus niger (Schaller, 1783)		16	3		3	22
Pterostichus nigrita (Paykull, 1790)			7			7
Pterostichus oblongopunctatus (Fabricius, 1787)		8				8
Pterostichus rhaeticus (Heer, 1838)				2		2
Pterostichus strenuus (Panzer, 1797)				2		2
Pterostichus vernalis (Panzer, 1796)				7	2	9
Stenolophus mixtus (Herbst, 1784)				2		2
Stomis pumicatus (Panzer, 1796)			1			1
Syntomus foveatus (Fourcroy, 1785)	2					2
Trechus obtusus (Erichson, 1837) – LC			2			2
Trechus quadristriatus (Schrank, 1781)			1			1

1	2	3	4	5	6	7
3. Catopidae (Thomson, 1862)						
Catops fuliginosus (Erichson, 1837)	1	5	1		1	8
Sciodrepoides watsoni (Spence, 1815)	3	2				5
4. Curculionidae (Latreille, 1802)	19	16	8	5	7	55
5. Dermestidae (Latreille, 1807)	4	4	2			10
Dermestes laniarius (Illiger, 1801)	4	4	2			10
6. Dryopidae (Fleming, 1821)						
Dryops ernesti (Des Gozis, 1886)				2	2	4
Dryops nitidulus (Heer, 1841)					2	2
7. Elateridae (Leach, 1815)						
Agriotes lineatus (Linnaeus, 1767)					2	2
Agriotes obscurus (Linnaeus, 1758)				4	6	10
Agrypnus murinus (Linnaeus, 1758)	2	1			12	15
Hemicrepidius niger (Linnaeus, 1758)					3	3
Hypnoidus riparius (Fabricius, 1792)				28		28
Prosternon tesselatum (Linnaeus, 1758)		2				2
Selatosomus aeneus (Linnaeus, 1758)		4				4
8. Eucinetidae (Lacordaire, 1857)						
Eucinetus haemorrhoidalis (Germar, 1818)	1					1
9. Geotrupidae (Latreille, 1806)						
Anoplotrupes stercorosus (Hartmann in L.G.Scriba, 1791)		2	1			3
Trypocopris vernalis (Linnaeus, 1758)	3	22	7			32
10. Limnichidae (Erichson, 1846)						
Limnichus sericeus (Duftschmid, 1825)				8	6	14
11. Scarabaeidae (Latreille, 1802)						
Aphodius coenosus (Panzer, 1798)	1					1
Phyllopertha horticola (Linnaeus, 1758)		3				3
12. Silphidae (Latreille, 1807)						
Nicrophorus investigator (Zetterstedt, 1824)	1	1				2
Nicrophorus vespillo (Linnaeus, 1758)	10		1	2	5	18
Nicrophorus vespilloides (Herbst, 1783)	1	10				11
Phosphuga atrata (Linnaeus, 1758)		5				5
Silpha carinata (Herbst, 1783)		54	12			66
Silpha tristis (Illiger, 1798)					1	1
Thanatophilus sinuatus (Fabricius, 1775)					1	1
13. Staphylinidae (Latreille, 1802)	22	16	113	67	68	286
14. Tenebrionidae (Latreille, 1802)						
Crypticus quisquilis (Linnaeus, 1761)	5					5
Opatrum sabulosum (Linnaeus, 1761)	2					2
Lagria hirta (Linnaeus, 1758)		2				2
Total specimens	110	189	217	264	167	957
Total spesies	29	27	26	27	31	83

Species richness was relatively even: the most species (31) were found in the highly diverse complex of meadows and the fewest (26) in the complex of wet sedge meadows. The number of individuals caught varied within a fairly large range: from 110 individuals in the fallow land with psammophilous vegetation to 264 individuals in the very wet, fertile meadow near Lake Świdwie Małe (Tabble 1).

No species common to all habitats were identified. The species composition of the families was highly varied (Figure 2). The highest similarity (about 36%) was noted for the assemblages of wet meadows. These assemblages are very different from the others – diverse meadow, mixed forest and psammophilous vegetation, with a similarity index of only about 10%.

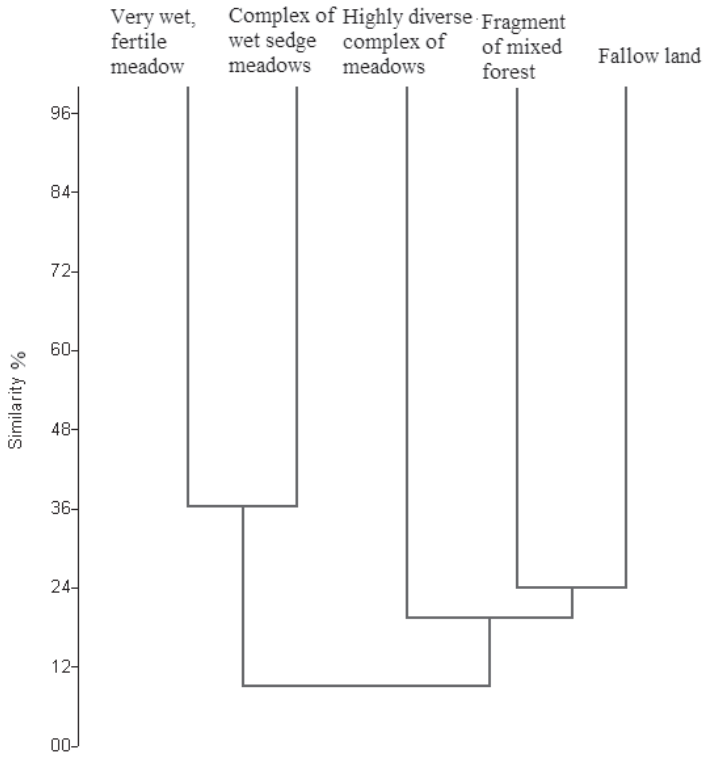

Figure 2. Similarity of the species composition of the habitats based on Jaccard's index

This indicates a great diversity of species composition and its evident dependence on the high moisture level at the site. Assemblages typical of wet habitats are on the left side of the dendrogram and assemblages of dry habitats are on the right (Figure 2).

Widely represented and fairly abundant stenobiontic hygrophilous species were noted in wet meadows: *Agonum emarginatum*, *A. fuliginosum*, *Bembidion gilvipes*, *B. guttula*, *B. mannerheimii*, *Carabus granulatus*, *Oodes helopioides*, *Oxypselaphus obscurus*, *Panagaeus cruxmajor*, *Pterostichus rhaeticus*, *P. vernalis*, *Stenolophus mixtus*, *Dryops ernesti*, *D. nitidulus*, *Hypnoidus riparius* and *Limnichus sericeus*.

Mesophilic grassland species are fairly abundant on the meadow composed of diverse habitats: *Poecilus versicolor*, *Dyschirius globosus* and *Harpalus latus*.

Mesophilic, stenobiontic forest species are dominant in the mixed forest: *Carabus violaceus*, *Pterostichus niger*, *P. oblongopunctatus*, *Abax parallelepipedus*, *Silpha carinata*, *Phosphuga atrata*, *Nicrophorus vespilloides* and *Trypocopris vernalis*.

Mesoxerophilic and xerophilic open-area species were noted in the psammophilous vegetation: *Amara aenea*, *Amara bifrons*, *Amara littorea*, *Amara spreta*, *Calathus erratus*, *Harpalus pumilus*, *H. smaragdinus*, *H. tardus*, *Microlestes minutulus*, *Syntomus foveatus*, *Crypticus quisquilis* and *Opatrum sabulosum*.

Ecologically valuable species with varying conservation status included *Oodes helopioides* (VU), and *Trechus obtusus* (LC). *Oodes helopioides* was most abundant in the wet meadow by Lake Świdwie Małe. *Trechus obtusus* was represented by single specimens.

The research is preliminary; the list of epigeic beetle species will increase considerably when the seasonal study period has been extended and other research methods have been applied.

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QS – systems communication of Gram-negative bacterial cells

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Keywords interspecies communication, *quorum sensing* history, *quorum sensing* in *Vibrio* and *Pseudomonas aeruginosa*, synthesis of AI-2 autoinducer

Abstract *Quorum sensing* (QS) is a communication mechanism used by bacteria to recognize cell population density fluctuations and control gene expression, which is a critical role both in intra- and interspecies communication and controls microbe-host interactions. *QS* is the process in which the bacterial cells detect threshold concentration of signaling molecules in the external environment, and then after having exceeded this allowable threshold, they respond accordingly and modify their behavior by altering the expression of their genes. Regulation of gene expression in response to the density of bacterial cells in a population is a key phenomenon in the mechanism of *QS* and it is used by both Gram-negative and Gram-positive bacteria. In Gram-negative bacteria LuxR protein plays a key role in *QS* system as a type of transcription regulators and participates in a variety of biological behaviors with LuxI protein and signal molecules, including those encoding virulence factors and antibiotics biosynthesis, plasmid transfer, bioluminescence, and biofilm formation. New researches which highlight the unusual signaling molecules, novel regulatory components and heterogeneity in the *QS* system of Gram-negative bacteria are presented in this paper.

Systemy komunikacji *QS* w komórkach bakterii Gram-ujemnych

Słowa kluczowe komunikacja międzygatunkowa, historia *quorum sensing*, *quorum sensing* u *Vibrio* i *Pseudomonas aeruginosa*, synteza autoinduktora AI-2

Streszczenie *Quorum sensing* (QS) jest mechanizmem komunikacji używanym przez bakterie do rozpoznawania zmian w zagęszczeniu populacji i kontroli ekspresji genów, który jest ważny zarówno w komunikacji wewnątrz jak i pomiędzy gatunkowej oraz kontroluje interakcje bakteria-gospodarz. *QS* jest procesem, w którym komórki bakteryjne wykrywają progową koncentrację cząsteczek sygnałowych w środowisku zewnętrznym, a następnie, po przekroczeniu tego progu, odpowiadają swoiście i modyfikują swoje zachowanie przez zmiany w ekspresji genów. Regulacja ekspresji genów w odpowiedzi na gęstość komórek bakteryjnych w populacji jest kluczowym fenomenem w mechanizmie *QS* i jest stosowana zarówno przez bakterie Gram-ujemne, jak i Gram-dodatnie. U bakterii Gram-ujemnych białko LuxR odgrywa kluczową rolę w systemie *QS* jako rodzaj regulatora transkrypcji oraz wspólnie z białkiem LuxI i cząsteczkami sygnałowymi uczestniczy w różnych procesach biologicznych takich jak kodowanie czynników wirulencji i biosyntezie antybiotyków, w transferze plazmidów, bioluminescencji

i formowaniu biofilmu. W pracy zaprezentowano nowe badania, które przybliżają te niezwykłe cząsteczki sygnałowe, nowe komponenty regulatorowe i heterogenność QS-systemu u bakterii Gram-ujemnych.

Introduction

Conventionally, *quorum sensing* (QS) was defined as cell-cell communication among bacteria that effects in changes in transcription factor activity, and consequently, changes in gene expression. *QS*-directed behaviors were defined as those that need all of the bacteria in the population to act in harmony to make the behaviors successful (Fuqua et al., 1994; Bassler, 2002). Newer research extends these definitions by showing inter-kingdom communication (Pacheco, Sperandio, 2009), responses by intracellular small-molecule chemical signals (Srivastava, Waters, 2012), and heterogeneity in gene expression that is controlled by *QS* (Grote et al., 2015). Regulation of gene expression in response to the density of bacterial cells in a population is a key phenomenon in the mechanism of *QS* and it is used by both Gram-negative and Gram-positive bacteria (Schauder et al., 2001).

The general scheme of functioning of the *QS* in Gram-positive bacteria is similar to the systems of intercellular communication in Gram-negative bacteria and is based on four main elements which include: synthesis of biochemical signaling molecules (autoinducers) within the bacterial cell, active or passive release of signaling molecules into the environment, recognition of autoinducers by specific receptors after exceeding the threshold concentration and cell response reflected in changes in the regulation of gene expression (Sifri, 2008). Autoinducers accumulate in the environment as bacterial population density increases. Bacteria monitor changes in the concentration of autoinducers to introduce modifications in their cell numbers and to collectively change total forms of gene expression. Processes that are controlled by *QS*, such as bioluminescence, the production of biofilms or the secretion of virulence factors are unproductive when undertaken by a single bacterial cell, but become effective when undertaken by the group (Bassler, Losick, 2006).

Short history of *QS* discovery

The mechanism of recognizing the quantity of bacteria was first observed in Gram-negative bacteria *Vibrio fischeri* and later in *V. harveyi*. Because these two species are present in the marine environment for a long time, it was believed that the mechanism is unique and concerns only the two species of the *Vibrio* genus. However, in the 90s it was found that the phenomenon of *QS* also relates to other Gram-negative bacteria (Suárez-Moreno et al., 2012).

The first information about the ability to communicate bacterial cells in populations appeared in literature in 1970, thanks to research conducted by two scientists, Nealson and Hastings (O'Toole, 2016). In their publication in the "Journal of Bacteriology" for the first time they described the phenomenon of bioluminescence in marine bacterium *V. fischeri*.

V. fischeri lives in symbiosis with Hawaiian squid *Euprymna scolopes*, which uses the ability of bioluminescence of bacteria as a camouflage for defense against predators. Squid has the ability to control the intensity of the emitted light thanks to the special organ situated at its bottom side, called a light organ, which is the "home" for the *V. fischeri* cells (Verma, Miyashiro, 2013). *E. scolopes* is nocturnal and lives in shallow marine waters off the coast of Hawaii. During starry nights, when the intensity of light coming from the atmosphere through the water column is

high, thanks to the light receptors located on the back, the squid can receive and record the light, then using special shutter, according to the needs, it can close or open the light organ filled with bacteria. *E. scolopes* opens or closes the shutter so that the amount of light produced by bacteria is perfectly the same as intensity and wavelength of light reaching the back of the squid, which causes that its shade and contours are unseen and as a result it is invisible. Using the light of bacteria, the squid lights up itself and defends against attacks of potential predators, which without seeing the squid's shadow cannot trace it. At dawn, it pumps out of its body to the environment about 95% of the bacterial cells, which being dispersed in water are incapable to generate light.

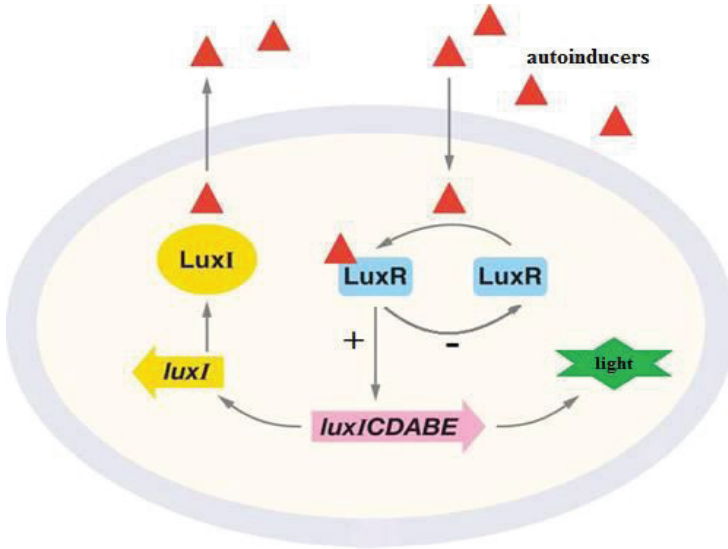
Interesting for scientists was not the fact of production of light by *V. fischeri*, but when the light was produced. It was noted that the bacteria show the ability to emit light only inside the squid's organ, but they lose it in a dilute suspension as free-living cells in marine waters. One question was bothering, how bacteria, such primitive organisms are able to detect a situation in which they are alone (no luminescence) from those when they are in the community (the appearance of luminescence) (Bassler, Losick, 2006).

The presence of small, chemical signaling molecules, called anutoinducers was recorded very quickly. After exceeding the threshold concentration of these molecules in the bacterial growth medium, which also indicates that the bacterial cells reach a suitable number, there is a synchronized reaction of whole population - in the case of bacteria *V. fischeri* – the production of light (Taga, Bassler, 2003). It was concluded that the entire population by using chemical signals inform all organisms that constitute it about the need for proper change of certain behaviors.

Thus, there are two conditions at the basis of the phenomenon of bioluminescence of *V. fischeri*. The first one is the availability of carbon and energy sources in the specialized light organs of the host, presenting favorable conditions for rapid growth and development of bacteria, so that the population can achieve a high density (Jaworski et al., 2005). The second condition is the ability to achieve high levels of the released signaling molecules only in the colonized squid's organ. It has been shown that the number of cells of *V. fischeri* in 1 ml of sea water does not exceed 100, while in the light organs of *E. scolopes*, it can reach a value of 10^{10}–10^{11}/1 ml (Fuqua et al., 1996). Low availability of nutrients in the sea water hampers the development of both the population and the accumulation of secreted autoinducers in the environment of growth, which results in their leaving and no light emission by bacteria. Once it has been discovered how the bacterium *V. fischeri* produces light, the next step was to use the tools of molecular biology to know better the mechanism (Bassler, Losick, 2006). It has been found that the diffusive signal molecules are molecules of N-acyl-L-homoserine lactone. These autoinducers demonstrate species specificity, due to the presence of various substituents attached to the side of the N-acyl chain. *V. fischeri* bacteria secrete synthesized by themselves molecules of N-(2-Oxohexanoyl) homoserine lactone, which accumulate in the medium of cells' growth. After acquiring the appropriate number of cells in a population, and thus the proper concentration of autoinducers in the environment and threshold value will become exceeded, the production of light by bacteria will start (Kołodyński, Jankowski, 2005).

The genetic studies found that the system responsible for regulating the process of bioluminescence in *V. fischeri* includes two genes: *luxI* and *luxR* (Kołodyński, Jankowski, 2005). These two genes encode regulatory proteins, LuxI and LuxR, the first one acts as the synthase of autoinducer and the second one is a receptor of this autoinducer. LuxR cytoplasmic protein recognizes and binds to a signaling molecule, specific to itself, and forms a complex that induce the transcription of genes responsible for the synthesis of the light emitting luciferase enzyme complex (Waters, Bassler, 2005). Luciferase enzyme complex is encoded by five structural genes *luxCDABE*, being

a part of the operon *luxICDABE*. Connection of LuxR protein with the autoinducer changes the spatial conformation of the LuxR protein, and as a result it comes to the exposition of its binding domain. The LuxR protein, amended in this way recognizes and binds to a promoter of the *luxICDABE* operon, allowing for the transcription of genes of this operon (Figure 1). What is important, the complex AHL/LuxR not only initiates the synthesis of proteins of luciferase complex (LuxCDABE), but also the synthesis of LuxI protein, responsible for the production of signaling molecules. The same complex has also the ability to connect to the promoter of the *luxR* gene and on the basis of the negative control it may inhibit synthesis of LuxR regulatory protein (Engebrecht, Silverman, 1984; Shadel, Baldwin, 1991). This repression is required, for example in the case of strong induction when proteins of *luxICDABE* operon are produced in excess. It shows that the production of light by *V. fischeri* is actually a precisely controlled process, regulated by the concentration of signaling molecules, synthesized and released into the environment by the bacterial cells living in the population (Shadel, Baldwin, 1991).



LuxR/autoinducer complex activates (+) transcription of *luxICDABE* operon encoding expression of luciferase enabling luminescence and initiating synthesis of LuxI protein responsible for the production of autoinducers. LuxR/autoinducer complex simultaneously inhibits (–) the production of LuxR protein

Figure 1. *Quorum sensing* regulation system in bacterium *Vibrio fischeri*

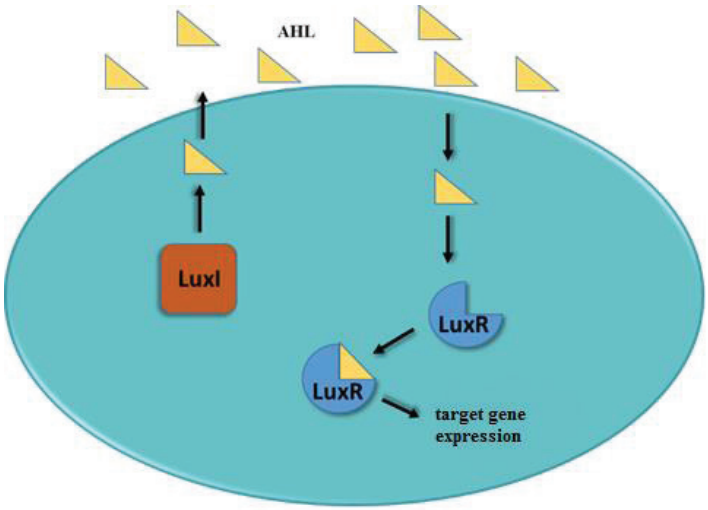
Source: Waters, Bassler (2005).

QS communication system

As it has been already mentioned above, the sensing mechanism of bacteria was first observed in bacteria *Vibrio fischeri* and later in *V. harveyi*. Because these two species are present in the marine environment, it was believed for a long time that the mechanism is unique and is restricted

only to the two species of the *Vibrio* genus. However, in the 90s it was found that the phenomenon of *QS* is much more widespread, and also relates to other Gram-negative bacteria (Súarez-Moreno et al., 2012). Gram-negative bacteria usually use a system LuxI/LuxR *QS* homologous to the system responsible for the bioluminescence of *V. fischeri* (Rutherford, Bassler, 2012).

This mechanism is compounded with four common features that are found in nearly all known Gram-negative *QS* systems (Bassler, 2010) and these are: signal molecules acyl-homoserine lactones (AHLs), proteins from the family of autoinducer synthase (LuxI), proteins from the family of transcriptional regulators (LuxR) and target genes. The function of LuxI protein as the synthase of autoinducer comes down to catalyze the reaction of joining S-adenosylmethionine (SAM) with an acylated, carrier protein, ACP which results in the formation of AHL molecule (Rutherford, Bassler, 2012). The autoinducers AHLs or other molecules that are synthesized from S-adenosylmethionine (SAM) are able to diffuse passively through the bacterial membrane and to accumulate in bacterial environment until they reach the critical concentration. Autoinducers are bound by specific receptors that reside either in the inner membrane or in the cytoplasm. Then, the sensor of LuxR signal detects the autoinducer and connects to it, interacting with the promoter sequence of an operon which leads to the induction of transcription of selected genes. The specific structure of LuxR allows for the dual role of this protein – N-terminal fragment of the polypeptide chain is responsible for the recognition and binding with the AHL, and the C-terminal fragment of the chain interacts directly with DNA. In the presence of autoinducer molecule, LuxR protein is activated and changes its conformation, and so induces the transcription of target genes (Figure 2) (Rutherford, Bassler, 2012). The newly-discovered enzyme system has been described to degrade efficiently different AHLs of various Gram-negative pathogens (Zhang et al., 2017). The enzymatic degradation of *QS* molecules is called *quorum quenching* (QQ) and it has been considered as a promising anti-virulence therapy to treat biofilm-related infections and battle antibiotic resistance.



AHL – acyl-homoserine lactone, LuxI – protein responsible for AHL production, LuxR – signal sensore

Figure 2. General scheme of *quorum sensing* system in Gram-negative bacteria

Source: Siepka, Gładkowski (2012).

Homologs of LuxI/LuxR system have been identified so far in over 100 species of Gram-negative bacteria (Rutherford, Bassler, 2012). Many bacterial pathogens using *QS* mechanism controls the production of virulence factors – for example such systems as: LasI/LasR and RhlI/RhlR in *Pseudomonas aeruginosa*, SmaI/SmaR in *Serratia marcescens* or CviI/CviR in *Chromobacterium violaceum* (Rutherford, Bassler, 2012). However, it should be noted that genes controlled by *QS* encode not only the classical virulence factors, but also proteins involved in basic metabolic processes of cells (Sifri, 2008). It is estimated that a significant part of the bacterial genome (4–10%) and proteome (20% or more) may be regulated by mechanisms of mutual cell communication.

Then, it is known that Gram-negative bacteria often use several autoinducers, however now new studies are highlighting the molecular factors that bring the receptors strange specificity in individuating between closely related molecules. *QS* information is often joined by small RNAs that control target gene expression and that also function in reaction loops. (Papenfort, Vogel, 2010).

QS system in *Pseudomonas aeruginosa*

P. aeruginosa, the Gram-negative bacteria is a bacterium that causes chronic lung infections in patients suffering from cystic fibrosis created on biofilm formation (Høiby et al., 2010). This pathogenic phenotype is particularly serious in patients with HIV co-infection. Selective pressure applied by anti-infective treatments positively selects multidrug-resistant *P. aeruginosa* strains. Moreover, this effect challenges the antibiotics treatment of this pathogen (Pesci et al., 1999). Resistance is acquired either by joining plasmid-encoded resistance genes or by spontaneous resistance mutations of *P. aeruginosa* (Lister et al., 2009).

P. aeruginosa uses two recognized AHL autoinducers as well as non-AHL autoinducers for *QS*. Precisely, cyclic dipeptides (2,5-diketopiperazines, DKPs) are produced by tRNA-dependent on cyclodipeptide synthases (Campbell et al., 2009) and 2-(2-hydroxyphenyl)-thiazole-4-carbaldehyde (IQS) is produced by proteins that are encoded by gene cluster *ambBCD,* the non-ribosomal peptide synthase (Lee et al., 2013). In addition, a quinolone (2-heptyl-3-hydroxy-4-quinolone, known as PQS) is used as an autoinducer, which is synthetised by proteins that are encoded by the *pqsABCDH* genes, and with the two AHLs it controls the construction of biofilms and the production of virulence factors (Lee, Zhang, 2015). Quinolones are generally recognized for their antibiotic and anticancer activities (Heeb, 2011), which reveals the multi-functionality of particular autoinducers (Papenfort, Bassler, 2016).

P. aeruginosa utilises *QS* for cell-to-cell communication to regulate the expression of virulence factors and for biofilm formation what allows disrupting the host immune systems and causes chronic infections. Examples of virulence factors, as wrote above, are LasA, LasB, and also Exotoxin A (ToxA) which is a transferase that is allied with cellular death (Ballok, O'Toole, 2013). The elastases LasA and LasB were shown to have an effect on cell wall elasticity and in consequence delay the therapeutic process (de Bentzmann et al., 2000). *P. aeruginosa* also produces, for example, hydrogen cyanide, which is a powerful inhibitor of cellular respiration and is associated with compromised lung function in patients (Ryall et al., 2008). *P. aeruginosa* in contrast to *V. fischeri* that uses only one *QS* circuit, displays the three *QS* routes named *Las*, *Rhl*, and *Pqs* that are interconnected with each other. These signalling routes are hierarchically regulated: the *Las* system activates both the *Rhl* and *Pqs* systems (Jimenez et al., 2012), while *Rhl* can destroy *Pqs* and *Pqs* activates *Rhl* (Welsh et al., 2015). Gallagher et al. (2002) suggested the

involvement of protein PqsE in *Pqs* signaling, rather than *Pseudomonas* quinolone signal (PQS) biosynthesis.

Interspecies communication

Bacteria usually co-occur with other bacterial species in multi-species communities in external environment or inside the host (e.g. in the gastrointestinal system or in the oral cavity). Both Gram-negative and Gram-positive bacteria are capable to recognize and consider autoinducing signaling molecules of other species (Jayaraman, Wood, 2008). It was also revealed that pathogenic bacteria can interact with eukaryotic host cells with operating each other's autoinducing signals (Jayaraman, Wood, 2008).

The concept of bacterial interspecies communication was first introduced in 1997 by research conducted by the American team led by Bassler et al. (1997). In their work, the authors have demonstrated the existence of a signaling molecule (called AI-2 autoinducer) responsible for inducing bioluminescence in free-living marine bacteria, *Vibrio harveyi*. This molecule, often referred as "universal" (Xavier et al., 2007) is used to communicate bacterial cells in mixed populations living together in the same environment (Federle, 2009). Since the discovery of the AI-2 autoinducer, numerous studies have been conducted in which the mechanisms for controlling the emission of light by *V. harveyi* were deeply analysed. Currently, the model of regulation of luminescence in cells of this bacterium is well known and described in many publications.

The ability of *V. harveyi* to recognize AI-2 autoinducer produced by its own cells as well as by other bacterial species allows it to monitor not only the density of its own population, but also populations of other species occupying the same niche. This results in a coordinated change in the expression of selected genes and consequently, the appropriate response of the entire population, allowing for effective adaptation to the prevailing environmental conditions (Pereira et al., 2013). The production of the AI-2 autoinducer is not only limited to the *V. harveyi* species. The activity of this molecule has been demonstrated in over 100 different Gram-positive and Gram-negative bacteria (Hirakawa, Tomita, 2013). Detailed studies are ongoing for better understanding the role that AI-2 autoinducer plays in the *QS* mechanism, and to know all the biological activities of bacteria controlled by this molecule. The ability to produce a universal signaling molecule such as the AI-2 autoinducer by various species of bacteria allows us to claim that this is the oldest evolutionary autoinducer in the world of microorganism, which was developed before the bacteria split into two living Gram-positive and Gram-negative (Schauder, Bassler, 2001).

Synthesis of the molecule of AI-2 autoinducer

As it was described above, the chemical signaling molecules biosynthesized by Gram-positive and Gram-negative bacteria differ in the type and number of amino acids and the length of the acyl chain. Different structure of each molecule makes them species-specific, which means that they can be recognized and transmitted only by cells that belong to a particular species but are not recognized by other species of bacteria, even those closely related (Schauder et al., 2001). Unlike acylated homoserine lactones and oligopeptides, AI-2 autoinducer is non-species-specific and chemical structure of its molecules and its synthesis pathway are identical, regardless of the species of bacteria.

Analysis of the AI-2 biosynthesis pathway indicates that the autoinducer is generated as a result of transformation of basic for the metabolism compound, S-adenosylmethionine (SAM) (Matejczyk, Suchowierska, 2011). SAM is the main donor of methyl groups for methylation of both DNA, RNA as well as many proteins in the cell (Schauder et al., 2001). It is also the donor of other function groups necessary for the proper course of many cellular processes such as the synthesis of phospholipids or vitamins (Pereira et al., 2013). It should be noted that SAM is a substrate for both biosynthesis of AI-2 autoinducer and for the synthesis of species-specific acyl-HSl molecules. Transferring the methyl group from SAM to the various terminal acceptors catalysed by methyltransferases leads to the formation of intermediate product in cells, S-adenosylhomocysteine (SAH). Since SAH is cytotoxic to the cell, it is rapidly transformed by the Pfs nucleosidase, which acts to remove adenine to produce S-ribosyl homocysteine (SHR) (Schauder et al., 2001). Next, the LuxS enzyme, the expression product of the *luxS* gene, catalyses the fission of SHR to homocysteine and 4,5-dihydroxy-2,3-pentandione (DPD). Organisms with the *sahH* enzyme can decompose SAH directly into adenosine and homocysteine (Pereira et al., 2013).

DPD is a highly reactive molecule that is convertible and able to form complexes with other compounds, what suggests that different molecules derived from DPD conversion may be signals recognized by different bacterial species as AI-2 autoinducer (Waters, Bassler,2005). Two DPD-derived signaling molecules have been identified in *Vibrio harveyi* and *Salmonella typhimurium* on the basis of analysis of crystal structure of the complexes that form these molecules with corresponding AI-2 receptor proteins (in *V. harveyi* the function of the AI-2 receptor performs LuxP protein, in *S. typhimurium* – LsrB protein). It has been found that in *V. harveyi* the AI-2 autoinductor is (2S, 4S)-2-methyl-2,3,3,4-tetrahydroxytetrahydrofuran borate produced by cyclization and complexation of boron by DPD. In turn, in *S. typhimurium*, the function of AI-2 performs (2R, 4S)-2-methyl-2,3,3,4-tetrahydroxytetrahydrofuran, which does not contain boron (Waters, Bassler, 2005).

The discovery of boron in the AI-2 signaling molecule synthesized in *V. harveyi* cells is surprising because only few known functions of this element in nature have been known so far (Waters, Bassler, 2005). The presence of boron in AI-2 autoinducer in *V. harveyi* is probably due to the high concentration of this element in the aquatic environment in which the bacteria live. In the terrestrial environment, the concentration of boron is much lower, which makes this element an unlikely component of AI-2 molecules in bacteria found in those ecosystems (for example in *S. typhimurium*). On the basis of these early observations concerning the AI-2 molecule, we can put forward a thesis that bacteria use a conservative pathway for biosynthesis of intermediate product DPD, whereas the final form of AI-2 is determined by the chemical composition of the specific environment (Waters, Bassler, 2005).

The existence of other biologically active derivatives of DPD is not excluded. Moreover, there is a possibility of presence in the bacterial cells, two or more receptor proteins recognizing various DPD derivatives. This would allow the bacteria to alter the expression level of particular genes depending on the information transferred by each signal (Waters, Bassler, 2005).

Conclusions

QS is a signaling machinery that is common in bacteria and includes the exchange of small molecules between bacteria and they are able to adapt the activity of gene expression to the population density in the close environment. This allows bacteria to recognize who their neighbours are and create phenotypes that are helpful for the group and that are useful for their survival.

New discovery shows how *QS* systems work using similar rules of action, which are rooted in the physical and chemical properties of the AIs, the matching receptors and their downstream regulators. The enzymatic degradation of *QS* molecules called *quorum quenching*, *QQ* has been considered as a promising anti-virulence therapy to treat biofilm-related infections and battle antibiotic resistance. Better understanding of *QS* details gives chance to combat bacterial infections by the attenuation of their *QS* communication systems and virulence.

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QS – systems communication of Gram-positive bacterial cells

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Keywords *Quorum sensing*, Gram-positive bacteria, signaling molecules, gene expression, competence of *Streptococcus pneumoniae* and *Bacillus subtilis,* virulence of *Staphylococcus aureus*

Abstract In Gram-positive bacteria, cell-to-cell communication, also called *quorum sensing* (QS) mainly is dependent on extracellular signaling oligopeptide pheromones, which stimulate a response either indirectly, by activating a two-component phosphorelay, or directly, by binding to cytoplasmic effectors. The oligopeptide pheromones production and secretion are initiated in response to specific environmental stimuli or stresses. These pheromones are biosynthesized through different pathways and some have unusual functional chemistry as a result of post-translational modifications. In the cells of *Bacillus subtilis* and *Streptococcus pneumoniae* this system controls the acquisition of the state of competence, while in *Staphylococcus aureus* it regulates virulence. The review aims at giving an updated overview of these peptide-dependant communication pathways.

Systemy komunikacji *QS* w komórkach bakterii Gram dodatnich

Słowa kluczowe *quorum sensing,* bakterie Gram dodatnie, cząsteczki sygnałowe, ekspresja genów, kompetencja *Streptococcus pneumoniae* i *Bacillus subtilis,* wirulencja *Staphylococcus aureus*

Streszczenie U bakterii Gram dodatnich komunikacja od komórki do komórki, zwana także *guorum sensing* (QS) jest przede wszystkim zależna od zewnątrzkomórkowych sygnałowych oligopeptydowych feromonów, które stymulują odpowiedź także pośrednio poprzez aktywowanie dwuskładnikowych fosforanów lub bezpośrednio przez wiązanie efektorów cytoplazmatycznych. Wytwarzanie i wydzielanie feromonów oligopeptydowych jest inicjowane w odpowiedzi na specyficzne czynniki środowiskowe lub stres. Feromony są syntetyzowane różnymi drogami a niektóre mają jeszcze dodatkową funkcję chemiczną jako wynik modyfikacji potranslacyjnej. W komórkach *Bacillus subtilis* i *Streptococcus pneumoniae* ten system kontroluje nabycie stanu kompetencji, natomiast u *Staphylococcus aureus* reguluje wirulencję. Celem pracy był przegląd aktualnych danych dotyczących peptydozależnych dróg komunikacji.

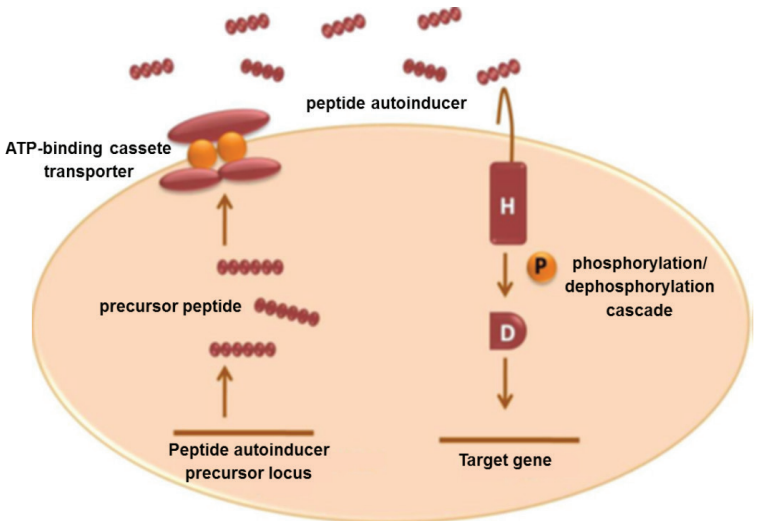
Introduction

Bacterial cell-cell communication systems also called *quorum sensing* (QS) are based on the secretion of signal molecules. Within Gram-positive bacteria, the expression of target genes

is controlled at the population level via signaling peptides, also known as pheromones (Cook, Federle, 2014; Monnet et al., 2016). Differences in the *QS* systems between Gram-positive and Gram-negative bacteria are seen in the mechanisms of synthesis of signal peptides and in the way they are transmitted from the sensor proteins to the cell effectors. Also, different molecules act as the signaling molecules in both groups of bacteria, and the differences in these chemicals being used in the sensing of quorum result primarily from differences in the construction of structures of the cell wall of bacteria that produce them. Gram-positive system usually uses secreted oligopeptides and two-component systems, which consist of membrane-bound sensor kinase receptors and cytoplasmic transcription factors that direct fluctuations in gene expression. Gram-negative bacteria often use several autoinducers, but new studies reveal unusual signaling molecules, novel regulatory components and heterogeneity in *QS* responses (Papenfort, Bassler, 2016).

QS – system communication

AI molecules produced by Gram-negative bacteria diffuse passively into and out of cells, whereas AIs synthesized by Gram-positive bacteria are actively transported (Miller, Bassler, 2001). Gram-positive bacteria communicate with each other using the two-component system of detection and respond to the presence of autoinducer (Li, Tian, 2012). Inside the bacterial cell, the oligopeptides are generated and then transported to the outside environment via the ABC transport protein (ATP-binding cassette transporter) (Siepka, Gładkowski, 2012). The mechanism of signal transmission occurs on a basis of cascade of phosphorylation and dephosphorylation (Kleerebezem et al., 1997). The signal oligopeptides released outside after reaching a threshold concentration are detected by transmembrane protein kinase that acts as a receptor protein.



H – transmembrane protein kinase, P – phosphate group, D – regulatory protein

Figure 1. The general scheme of the *quorum sensing* system in Gram-positive bacteria

Source: Vijayalakshmi (2013).

The interaction of protein kinase with the ligand leads to its autophosphorylation thereby initiating the cascade of reactions that result in a phosphorylation of regulatory protein. The phosphorylated form of regulatory protein is able to recognize and bind to the suitable promoters of target genes involved in *QS*, thereby initiating their expression (Li, Tian, 2012). A schematic model showing the system of *quorum sensing* in Gram-positive bacteria has been presented in Figure 1.

In different species of Gram-positive bacteria *QS* system has a different biological role. The examples can be here the cells of *Bacillus subtilis* and *Streptococcus pneumoniae*, in which this system controls the acquisition of the state of competence, while in *Staphylococcus aureus* it regulates virulence and in *Enterococcus faecilis* process of conjugation (Vijayalakshmi, 2013). In recent years, it has been noticed a large increase in studies discovering new pheromone pathways among Gram-positive bacteria that improve our understanding of peptide signaling described for model organisms like *Bacillus subtilis*, *Staphylococcus aureus*, and *Streptococcus pneumoniae* (Cook, Federle, 2014).

QS in acquiring competence of *Streptococcus pneumoniae*

Streptococcus pneumoniae was the first species of bacteria in which in 1928 the genetic transformation was described (Lorenz, Wackernagel, 1994). The transformation process occurs only in the competent cells, which have the ability to collect the DNA from the surrounding environment. The bacterium *S. pneumoniae* reaches the state of competence spontaneously (in a natural way). The acquisition of competence depends on many complex physiological processes, many of which are under control of *QS* (Lee, Morrison, 1999).

In the *S. pneumoniae* system the signaling molecule is oligopeptide CSP (competence stimulating peptide) (Vijayalakshmi, 2013). It is built of 17 amino acids and is formed from the precursor peptide, called ComC. The oligopeptide CSP is transported out of the bacterial cell by the transporter protein, ABC. The protein of ComD kinase is a sensor detecting the accumulated extracellularly autoinducer CSP. At a high concentration of CSP in the environment of bacterial growth, ComD undergoes autophosphorylation and via the phosphorylation and dephosphorylation reactions pathway it transmits the signal to the final acceptor, which is a regulatory protein, ComE. The phosphorylated protein ComE activates the transcription of the *comX* gene encoding alternative sigma factor ComX required for the expression of structural genes involved in the acquisition of competence (Vijayalakshmi, 2013).

QS in acquiring competence of *Bacillus subtilis*

The process of acquiring the status of competence in bacteria of the genus *Bacillus subtilis* has been understood in details (Wolska, 2012). It is known that cells of these bacteria synthesize two types of signal molecules, one of which, ComX controls the process of acquiring the competence and the second, CFS regulates sporulation (Solomon et al., 1996). This allows the *B. subtilis* cells for precise response to external factors and appropriate adaptation to changing environmental conditions (Solomon et al., 1996).

When the population of cells reaches the high density in its growth environment, a ComP kinase, acting as a sensor for the autoinducer ComX, receives a cumulated signal and passes it through a cascade of reactions of phosphorylation and dephosphorylation to form a regulatory ComA protein (Vijayalakshmi, 2013). The phosphorylated ComA protein activates the expression

of the *comS* gene and the protein ComS inhibits proteolytic degradation of the second regulatory protein, ComK (thereby increasing the intercellular level of this protein), which is an activator of transcription that regulates expression of structural genes involved in the development of competence of *B. subtilis* (Vijayalakshmi, 2013).

The second of the above-mentioned signaling molecules – CFS, responsible for the stimulation of sporulation processes in adverse conditions, for example in reduced access to food sources, is imported into the cell by the bacterial ABC transporter called Opp (Jaworski et al., 2005). At high extracellular concentrations of CFS autoinducer, it comes to the inhibition of ComS protein, which in consequence inhibit the expression of competence genes and leads to activation of the metabolic pathways associated with cell sporulation. Conversely, in the case of low intercellular concentrations of CFS, it binds with RapC phosphatase and inhibits its activity, increasing this way the level of phosphorylated ComA protein and directs the cells of *B. subtilis* on the way of acquiring the status of competence (Vijayalakshmi, 2013).

QS in virulence of *Staphylococcus aureus*

S. aureus is a bacterium responsible for infections of skin and soft tissues, bacteraemia, endocarditis, sepsis, and toxic shock syndrome (Cheung et al., 2011). Treatment of *S. aureus* is complicated due to the devolvement of multidrug-resistant *S. aureus* strains, known as methicillin-resistant *S. aureus* (MRSA) and these virulence factors are a crucial part in the pathogenesis of that bacterial infections (Lowy, 2003). Virulence factors involve a wide range of various enzymes and exotoxins that enable the avoidance of the immune system and tissue adhesion damage to the host cell or enterotoxin release the toxic shock syndrome. Finally, α-hemolysin, which causes the destruction of membrane structures and can cause pneumonia (Gordon, Lowy 2008). The expression of different virulence factors is determined by external influences and is regulated by the cell-density-dependent *QS* accessory gene regulator (*agr*) system of *S. aureus* (Painter et al., 2014). The *agr* locus includes five genes *agrA*, *agrB*, *agrC*, *agrD*, and *hld* that are organized in one operon. The *agr* operon and *hld* are controlled by different promoters, named P2 and P3. Each of these proteins takes over different functions in the *QS* system, the last one is converted into the autoinducing peptide (AIP) that is used as cellular signaling molecule and with the *agr* system regulates the expression of virulence factors. However, many hospital-isolated strains of *S. aureus*, as the most frequently isolated pathogens in intensive care units, are *agr* defective and deficiency the main QS-controlled virulence regulatory system. Paulander et al. (2013) showed that *agr*-negative strains have an advantage over *agr*-positive strains what may be an explanation of so frequently isolated *agr*-defective *S. aureus* strains in hospitals. As peptide signaling systems remain to be discovered, there is a rising need to understand the details of these communication mechanisms. This information will deliver understanding how Gram-positives coordinate cellular events and help to board these pathways for infection treatments. The global emergence of antibiotic-resistance amongst infectious bacteria requires detection not only of new antimicrobials but also alternative therapeutic strategies to control pathogen (Jimenez, Federle, 2014; Aggarwal et al. 2015). One of those strategies is to control the virulence gene expression, mainly through the manipulation of *QS* systems in a process of *quorum sensing* inhibition (QSI) also discussed as "*quorum quenching*". Due to the importance of peptide systems in pathogenesis, there is emerging interest in *quorum-quenching* methods for therapeutic intervention (Cook, Federle, 2014). The quenching strategies that have successfully blocked signal biosynthesis are also covered. As new peptide systems are discovered and characterized, and if found to influence

pathogenic attributes of host-microbe interactions, then *QS*Is may become an increasingly important source of novel therapeutics against bacterial infections, while providing treatments that are less likely to impose the evolutionary constraints associated with the development of antibiotic resistance.

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Water quality and ecological role of urban lake: a case study of Słoneczne Lake in Szczecin (NW-Poland)

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Keywords water quality, urban lake, CA, FA

Abstract This paper presents the differentiation of water quality in urban flowing water reservoir on the example of Słoneczne Lake in Szczecin along the water runway through the lake on a basis of 21 selected water quality indices at intervals of approximately one month. Water quality was determined on the basis of current Legal Acts in Poland. Based on the collected data and the use of chemometric techniques, an attempt was made to determine the role of the Słoneczne Lake in the hydrological network of Szczecin.

Jakość wody i ekologiczna rola jeziora śródmiejskiego na przykładzie Jeziora Słonecznego w Szczecinie (NW-Polska)

Słowa kluczowe jakość wody, jezioro miejskie, analiza skupień, analiza czynnikowa

Streszczenie W niniejszej pracy przedstawiono zróżnicowanie jakości wody w miejskim przepływowym zbiorniku wodnym na przykładzie Jeziora Słonecznego w Szczecinie wzdłuż drogi spływu wód przez zbiornik wodny, oznaczając 21 wybranych wskaźników jakości wody w odstępach ok. jednomiesięcznych. Określano jakość wody na podstawie obecnie obowiązujących przepisów prawnych w Polsce. Na podstawie zebranych danych i wykorzystaniu technik chemometrycznych podjęto próbę określenia roli, jaką pełni Jezioro Słoneczne w sieci hydrologicznej miasta Szczecina.

Introduction

Water reservoirs in cities, which are usually flowable, of natural or artificial origin, fulfill a number of important functions in urban agglomerations. First of all – a recreational function,

because the areas located near the reservoirs are often park areas with walking trails or other recreational functions. At the same time, very often, or indeed always, they are receivers of water from the urban sewage system, in which they act as retention reservoirs, as well as sedimentation ponds and even biological sewage treatment plants. Increase in environmental pollution and constantly emerging new sources of pollution make it necessary to regularly carry out studies aimed at determining the quality of the water in the tanks and the inner-discernment – if changes occur in the quality of the way along the run-off – which biogeohydrochemical processes changes affect their quality. The Słoneczne Lake in Szczecin, located in the Gumieńce district, through which the Bukowa stream flows, is an example of such an urban reservoir (Angyual et al., 2016; Gutches et al., 2016; Hill et al., 2017; Huser et al., 2016; Miller et al., 2016; Olguin et al., 2017; Song et al., 2016).

The purpose of this work was to determine – based on the research conducted in the period from January to June 2015 of the selected water quality indices of the Słoneczne Lake – the quality of the water in this Lake and to determine whether this urban lake acts as a pond for decontamination and biological treatment of waste water – as long as there will be changes in the quality of the water flowing through this reservoir – what biogeohydrochemical processes have caused changes in the quality of water in the reservoir.

Characteristic of Słoneczne Lake

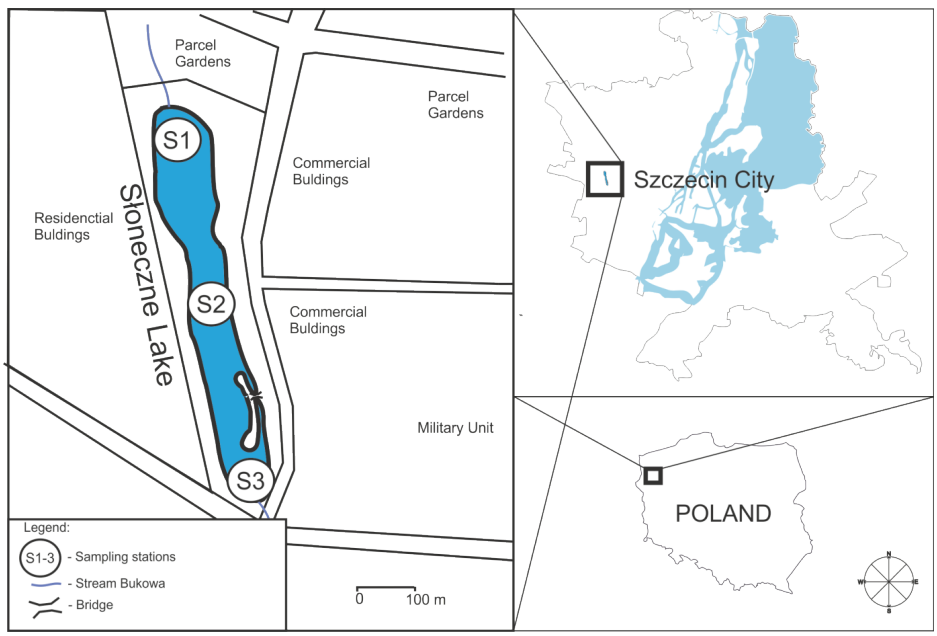


Figure 1. Słoneczne Lake in Szczecin (NW-Poland)

Słoneczne Lake (Figure 1) is a flowing water reservoir in the western part of the city of Szczecin in the Gumieńce district. It is an artificial reservoir created in the 30s of the last century on the marshy riverbeds adjoining the Bukowa River, during regulation of its runoff road. The Bukowa flows from the Bezrzecze district and is additionally supplied with the waters of Stobnica and Wierzbak. Bukowa river water flows after passing through Słoneczne Lake and further through Szczecin's urban areas – to the Western Odra River (Białecki et al., 1991; Hłyńczak et al., 1998; Niedźwiecki et al., 2007; Tadajewski et al., 1993). The surface of the tank is approx. 4.8 ha. The length is about 1.3 km and the max depth is almost 2m. The average retention time – based on surface runoffs – is from 14 to 33 days. Detailed lake morphometric indicators are included in Table 1 (Białecki et al., 1991; Hłyńczak et al., 1998; Niedźwiecki et al., 2007; Tadajewski et al., 1993).

Table 1. Morphometric characteristic of Słoneczne Lake in Szczecin

Geographical coordinates	Latitude	53°25′29″N
	Longitude	14°29′54″E
Morphometric data		
Morphometric indicator	Units	Słoneczne Lake
Water level	[m asl]	16,9
Area	[10^4 m^2]	5,4
Capacity	[10^3 m^3]	59,4
Depth – max	[m]	1,3
Depth – average	[m]	1,1
Length max	[m]	640,0
Width max	[m]	90,0
Length of coastline	[m]	1320

Material and methods

Samples of water for the study were taken from January to June 2015 from a depth of about 25 cm below the water mirror at a distance of about 2 m from the shore at the stations indicated in Figure 1, and in particular at the stations: No. 1 (S1) – inflow area of Bukowa River to Słoneczne Lake, station No. 2 (S2) – in the central part of the lake and station No. 3 (S3) – in the area of the outflow of water from the reservoir. Samples were collected on 11.01, 23.02, 22.03, 25.04, 25.05 and 17.06.2015 according to APHA (2012).

The 21 selected water quality indices were determined, in particular: physical parameters such as temperature (TEMP), pH, redox potential (Eh), electrical conductivity of water at 20°C (EC) and chemical – chemical oxygen demand (COD-Cr, COD-Mn), dissolved oxygen (DO) water saturation by O_2 (WS), concentrations of NO_3^-, NO_2^-, NH_4^+, PO_4^{3-} (soluble reactive orthophosphates (V) – SRP), total nitrogen (TN) and phosphorus, concentrations of Ca^{2+}, Cl^-, SO_4^{2-}, general hardness (TH), general alkalinity (Alk) and total concentration of iron (Fe_{tot}). All analysis, storage and transport of samples for testing were performed as recommended by APHA (2012).

Chemometric procedures

The results of the research on the quality parameters of the solar lake were analyzed using selected chemometric procedures and in particular the cluster analysis (CA), Spearman coefficients were calculated between successive water quality indices and the factor analysis (FA). The CA method was used to detect and visualize the similarities and differences between the variability of individual water quality indices, examining the nature of variations for different sampling sites (station numbers) and individual sampling dates

Table 2. Statistical characteristics of selected water quality indices on all sampling stations in the period from January to June in 2015 year

Detail	Units	Descriptive statistics	S1	S2	S3
1	2	3	4	5	6
Temperature	°C	Mean ±SD Range CV	10.8 ±6.6 3.9–20.1 0.61	11.0 ±8.7 1.2–22.0 0.79	10.9 ±8.2 1.4–21.0 0.75
pH	jedn. pH	Mean ±SD Range CV	7.50 ±0.31 7.11–7.77 0.04	7.59 ±0.45 7.03–8.12 0.06	7.63 ±0.42 7.16–8.17 0.05
EC	$\mu S \cdot cm^{-1}$	Mean ±SD Range CV	729 ±248 393–1031 0.34	625 ±128 453–768 0.20	604 ±170 329–787 0.28
Eh	mV	Mean ±SD Range CV	448 ±12 429–458 0.03	453 ±17 434–480 0.04	448 ±27 407–490 0.06
COD-Mn	$mg\ O_2 \cdot dm^{-3}$	Mean ±SD Range CV	11.9 ±1.2 11.1–14.2 0.10	11.2 ±1.1 10.4–13.5 0.10	11.1 ±1.0 10.2–13.0 0.09
COD-Cr	$mg\ O_2 \cdot dm^{-3}$	Mean ±SD Range CV	303.0 ±141.6 150.0–573.0 0.47	79.7 ±32.7 52.0–135.0 0.41	66.4 ±19.4 40.0–93.0 0.29
BOD_5	$mg\ O_2 \cdot dm^{-3}$	Mean ±SD Range CV	7.7 ±2.3 5.0–10.0 0.29	5.3 ±0.8 4.0–6.0 0.15	4.7 ±1.4 3.0–7.0 0.29
DO	$mg\ O_2 \cdot dm^{-3}$	Mean ±SD Range CV	10.2 ±4.4 6.0–18.0 0.43	13.3 ±4.5 8.0–20.0 0.34	12.8 ±3.7 9.0–19.0 0.29
WS	%	Mean ±SD Range CV	88.1 ±26.8 65.4–137.0 0.30	114.3+18.2 90.4–141.2 0.16	110.8 ±12.4 99.8–134.9 0.11
NO_3^-	$mg\ N–NO_3 \cdot dm^{-3}$	Mean ±SD Range CV	1.37 ±0.37 1.01–1.97 0.27	0.91 ±0.21 0.63–1.23 0.23	0.86 ±0.22 0.60–1.15 0.25
NO_2^-	$mg\ N–NO_2 \cdot dm^{-3}$	Mean ±SD Range CV	0.147 ±0.137 0.040–0.400 0.93	0.052 ±0.057 0.009–0.130 1.09	0.064 ±0.054 0.004–0.130 0.84

1	2	3	4	5	6
NH_4^+	mg N–NH_4 · dm^{-3}	Mean ±SD Range CV	1.23 ±0.48 0.71–1.95 0.39	0.63 ±0.09 0.53–0.78 0.14	0.45 ±0.15 0.29–0.70 0.33
TN	mg N · dm^{-3}	Mean ±SD Range CV	3.580.53 2.70–4.21 0.15	1.72 ±0.58 1.00–2.50 0.34	1.56 ±0.43 1.11–2.30 0.28
SRP	mg P– PO_4 · dm^{-3}	Mean ±SD Range CV	0.37 ±0.24 0.08–0.68 0.65	0.36 ±0.21 0.10–0.63 0.58	0.37 ±0.18 0.19–0.60 0.48
TP	mg P– PO_4 · dm^{-3}	Mean ±SD Range CV	1.02 ±0.54 0.35–1.68 0.53	1.04 ±0.53 0.47–1.68 0.51	1.04 ±0.53 0.46–1.70 0.51
TH	mg $CaCO_3$ · dm^{-3}	Mean ±SD Range CV	269 ±123 124–437 0.46	215 ±52 183–321 0.24	196 ±75 112–337 0.38
Ca^{2+}	mg Ca · dm^{-3}	Mean ±SD Range CV	83 ±36 387–136 0.43	6 ±7 57–76 0.10	96 ±73 62–112 0.76
Cl^-	mg Cl · dm^{-3}	Mean ±SD Range CV	50 ±26 28–86 0.52	50 ±22 27–81 0.44	52 ±27 28–98 0.51
SO_4^{2-}	mg SO_4 · dm^{-3}	Mean ±SD Range CV	60 ±13 46–80 0.22	58 ±13 44–78 0.22	57 ±13 43–77 0.23
Alkalinity	mmoli HCl · dm^{-3}	Mean ±SD Range CV	3.72 ±1.47 1.70–5.00 0.39	3.82 ±1.51 1.60–5.50 0.40	3.75 ±1.68 1.40–5.60 0.45
Fe_{tot}	mg Fe · dm^{-3}	Mean ±SD Range CV	0.29 ±0.21 0.07–0.62 0.72	0.12 ±0.17 0.01–0.45 1.41	0.17 ±0.17 0.05–0.51 1.00

(sampling dates) and to compare the variability of all identified water quality indices in relation to each other.

The Ward method was used to determine the distance between the clusters – equal squares of the Euclidean distance (Badillo-Camacho et al., 2015; Li et al. 2015; Kari et al., 2009; Mustapha et al., 2013; Miller et al., 2016; Najar et al., 2012). The calculation of Spearman correlation coefficients was to determine the relationship between the water quality indexes so as to distinguish independent indices that characterize water quality changes during the research period. Factor analysis (FA) was used to determine which water quality indices could show the hidden dependencies between its own variability and significant changes in water quality (Affum et al., 2015; Kumar et al., 2014; Kumarasamy et al., 2014; Longanathan et al., 2015; Miller et al., 2016; Wang et al., 2013).

Prior to statistical analysis, standardization of measurement data was carried out to avoid discrepancies between different units of individual water quality indices. To test the usage of collected data for multivariate statistical techniques, the Kaiser-Meyer-Olkin measure of sample

adequacy test was performed and Bartlett's test of sphericity was performed (Chow et al., 2016; Gao et al., 2015; Taoufim et al., 2017; Singh et al., 2016).

Statistica 12.0 PL and Statgraphics Centurion XVII software were used for the calculations.

Results

Results of the 21 selected water quality indices of the Słoneczne Lake in period January – July 2015 are presented in Table 2 and in the graphs (Figures 2–3), where water quality indicators for all three measurement stations and all sampling dates were compiled.

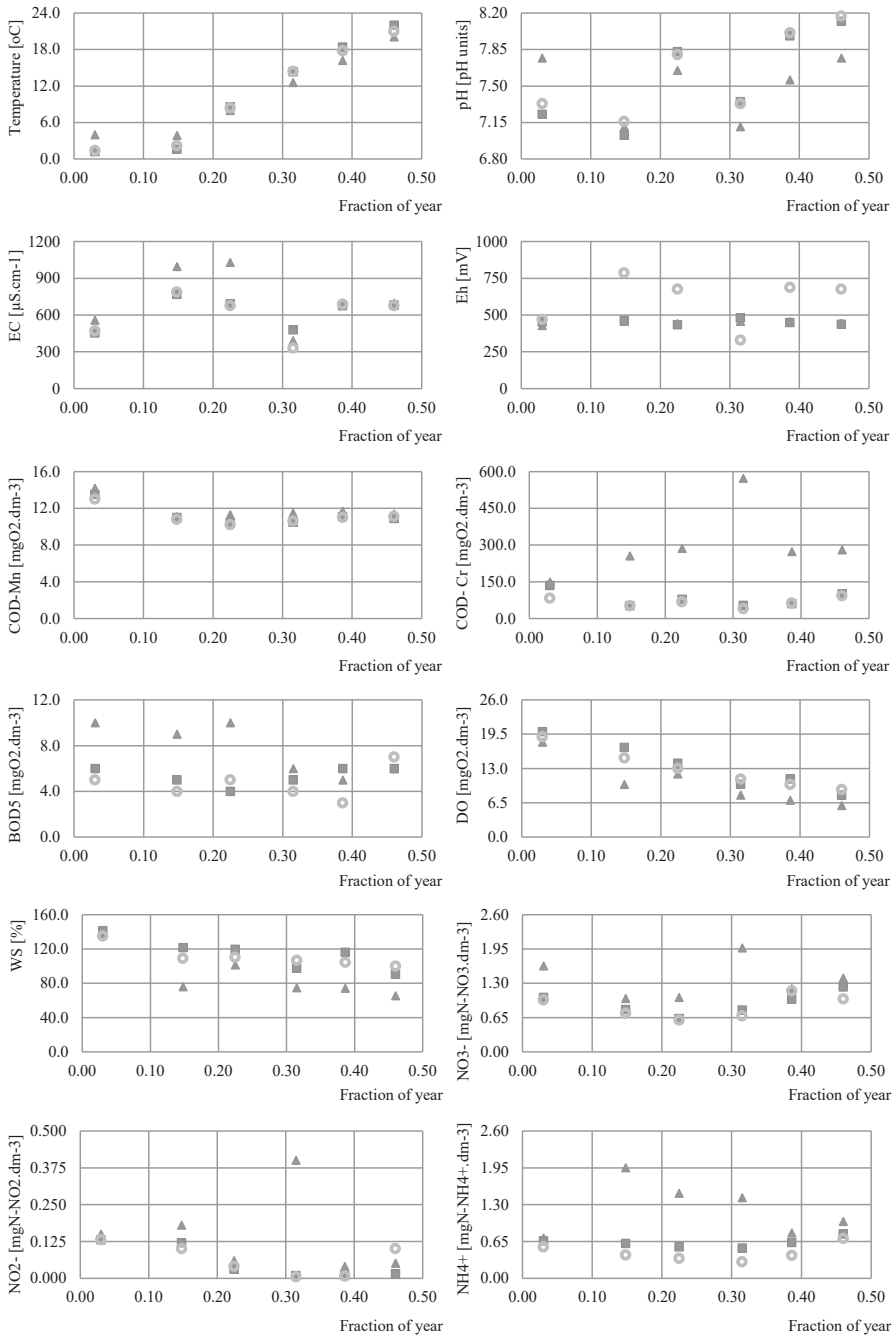
Water temperature changes in the reservoir during the study period were typical for the lake ecosystem with significant water retention in the temperate zone, where the gradual rise in temperature occurs along with seasons. The pH of the waters of the Słoneczne Lake fluctuated slightly in particular months. The results of the electric conductivity (EC), as measured during the study period, also changed slightly, remaining stable at all measuring stations at all sampling terms. The value of the oxidation-reduction potential (Eh) index was high and varied slightly between 450–490 mV.

Concentration of organic compounds in water characterized by COD-Cr, COD-Mn, and BOD_5 had relatively high values, decreasing progressively with the movement of the lake waters towards the drain zone. Results of dissolved oxygen concentration showed a relatively high degree of oxygenation of water. In turn, the values of concentrations of biogenic substances in the waters of the Słoneczne Lake were very diverse. The values of nitrate (V), which were highest in the inflow area of the Bukowa River and were decreasing as the water flowed into the outflow zone from the reservoir, was remarkably changed. Nitrate (III) concentrations generally had stabilized values. Concentrations of ammonium ions and total nitrogen in the studied waters decreased as the water flowed through the lake. Conversely, the concentration of total and soluble reactive orthophosphates (V), whose concentration increased as water flowed through the reservoir, was reversed. Total hardness reached the highest values in the area of inflow zone of Bukowa River. Occasionally, the concentration of calcium ions changed, increasing to more than 100 mg $Ca \cdot dm^{-3}$. Typically, the value of this indices did not exceed 80 mg $Ca \cdot dm^{-3}$. Cl^- concentrations were usually low, which gradually decreased during the period from January to June. The concentration of SO_4^{2-} in the waters of the Słoneczne Lake was relatively low and stabilized. Total alkalinity values generally were above 3.75 mmol $HCl \cdot dm^{-3}$. Total iron concentrations were always very low.

Discussion

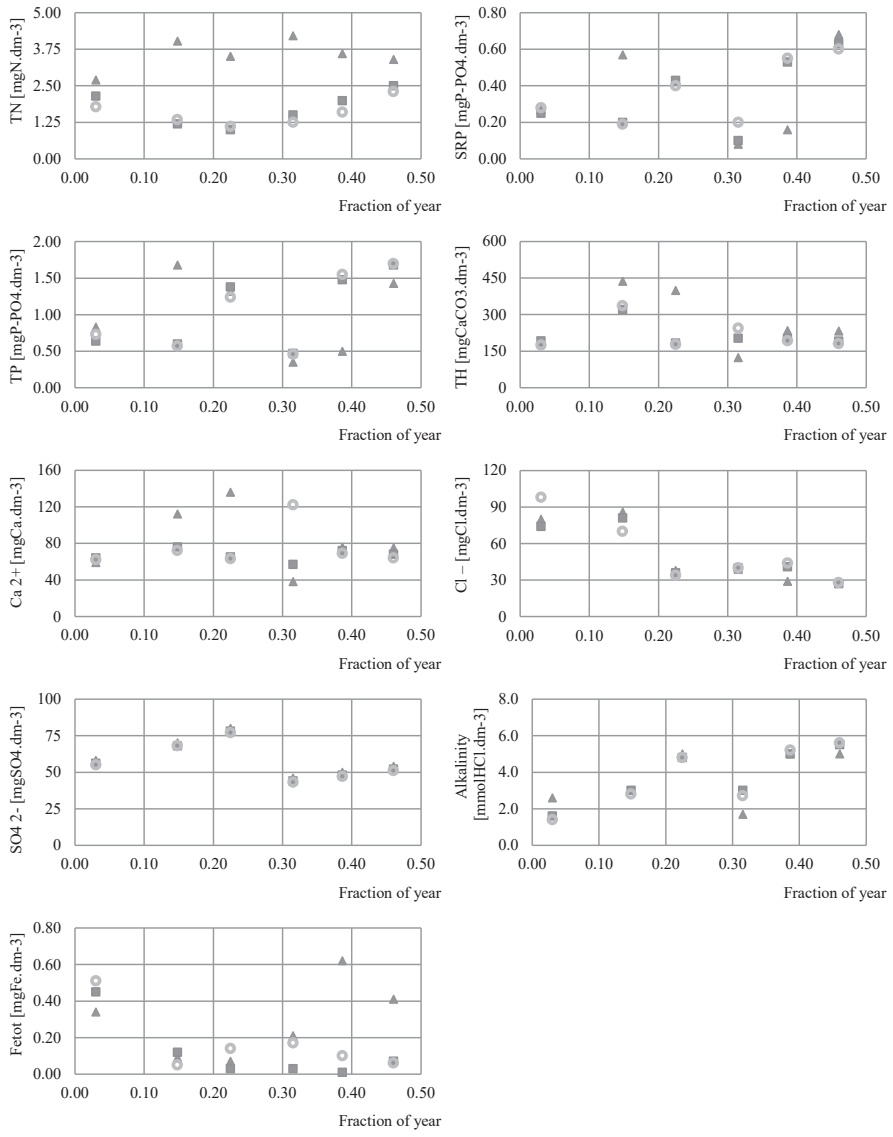
Assessment of the Słoneczne Lake water quality according to currently valid Polish criteria for assessing the quality of lake waters

In Table 3 shows the classification of the tested waters currently binding in Poland (Regulation, 2016) criteria for assessing the quality of lake water. From the data presented, it is clear that the assessment of water quality based only on physical and chemical indices should be made only on the basis of electrical conductivity in 20°C, dissolved oxygen, total concentrations of nitrogen and phosphorus among the indices investigated in this paper. From the presented data it is clear that the lowest quality had waters at the S1 station (the zone of water inflow to the lake), where water – actually regardless of the time – were III and lower class water (criteria for III, IV and V



▲ – inflow area, ■ – middle of the Lake, ○ – outflow area.

Figure 2. Changes in selected water quality indices in Słoneczne Lake in 2015 year



▲ – inflow area, ■ – middle of the Lake, ○ – outflow area.

Figure 3. Changes in selected water quality indices in Słoneczne Lake in 2015 year

are not given in the Regulation). Out of the criteria indicating a very low class of water quality, the indices were: the total nitrogen concentration and the total concentration of phosphorus, and also during the melting snow waters inflow – electric conductivity of water. Dissolved oxygen concentrations generally corresponded to I and II water quality classes, as were the other stations.

Table 3. Classification of water quality based on selected water quality indices of Słoneczne Lake during study period from January to July (according to Regulation 2016 J.L. of 2016, item 1187)

No.	Indices	Water Quality Class																	
		T1			T2			T3			T4			T5			T6		
		S1	S2	S3	S1	S2	S3	S1	S2	S3	S1	S2	S3	S1	S2	S3	S1	S2	S3
1	Temp[1)]	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
2	pH[1)]	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
3	EC	I, II[A]	I, II[A]	I, II[A]	≤III[3)]	I, II[A]	I, II[A]	≤III[3)]	I, II[A]	I, II[A]	I, II[A]	I, II[A]	I, II[A]	I, II[A]	I, II[A]	I, II[A]	I, II[A]	I, II[A]	I, II[A]
4	Eh[1)]	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
5	COD-Mn[1)]	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
6	COD-Cr[1)]	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
7	BOD[1)]	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
8	DO	I, II[A]	I, II[A]	I, II[A]	I, II[A]	I, II[A]	I, II[A]	I, II[A]	I, II[A]	I, II[A]	I, II[A]	I, II[A]	I, II[A]	I, II[A]	I, II[A]	I, II[A]	I, II[A]	I, II[A]	I, II[A]
9	WS [2)]	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
10	NO_3^- [1)]	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
11	NO_2^- [1)]	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
12	NH_4^+ [1)]	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
13	TN	≤III[3)]	≤III[3)]	I, II[A]	≤III[3)]	I, II[A]	I, II[A]	≤III[3)]	I, II[A]	I, II[A]	≤III[3)]	I, II[A]	I, II[A]	≤III[3)]	I, II[A]	I, II[A]	≤III[3)]	≤III[3)]	≤III[3)]
14	SRP [1)]	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
15	TP [1)]	≤III[3)]	≤III[3)]	≤III[3)]	≤III[3)]	≤III[3)]	≤III[3)]	≤III[3)]	≤III[3)]	≤III[3)]	II	≤III[3)]	II	≤III[3)]	≤III[3)]	≤III[3)]	≤III[3)]	≤III[3)]	≤III[3)]
16	TH [1)]	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
17	Ca^{2+}[1)]	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
18	Cl^- [1)]	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
19	SO_4^{2-} [1)]	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
20	Alkalinity[1)]	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
21	Fe_{tot} [1)]	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–

[A] Water quality limit value common for I and II water quality class
[1)] water quality indices not included in w Regulation of the Minister of the Environment of 21 July 2016 On how to classify the state of surface water bodies and environmental standards of priority substances (J.L. of 2016 item, 1187)
[2)] Water quality parameters included in the above. Regulation but not applicable to the classification of lakes of type 3b
[3)] For class below II water quality class – the limits not determined

From the compiled data it shows that the water in the Słoneczne lake flowing from inflow area gradually improved its quality, which concerns in particular the total nitrogen concentrations and total phosphorus (in a small extent). In general it can be stated that the indices that "disqualified" tested waters during the research period was the total phosphorus, which concentrations in the waters of the Słoneczne Lake were always very high at all sampling stations in the whole research period.

Biogehydrogchemical processes occurring in the Słoneczne Lake ekosystem

Based on data presented in Tables 2–3 and Figures 2–6, it can be concluded that the quality of water flowing from the Bukowa River inflow area to the Słoneczne Lake to the outflow area of the lake has changed considerably. In particular, such indicators as pH, Eh, COD-Cr, TN, NO_3^- and NH_4^+. These changes have shown a gradual improvement in the quality of the water as it flows towards the outlet of the drainage channels from the reservoir. Particularly clear are the changes between the station No. 1 (area of the water inflow) and the station No. 2 (center of the lake). At this point, the concentration of organic matter in the tank was clearly reduced with a drop in value of COD-Cr, TP, BOD_5, NO_3^-, NH_4^+, connected with a decrease in total hardness, total alkalinity and additionally simultaneously with the increase in concentration of dissolved phosphorus and reactive orthophosphate – although this effect was less clear. This clearly demonstrates that between the stations S1 and S2 sedimentation of the suspensions took place – independently of the time of the sampling. Also on the way from station No. 1 to station No. 2 increased the oxygenation of water. At the same time, the increase in the redox potential (Eh) of the Słoneczne Lake waters was noticeable on the flow from Station 2 to Station 3. All of the above mentioned indices were correlated respectively, as is evident in Table 4.

Very well it is documented also on Figure 4, where the dendrogram showing the similarities and differences of variation of determined water quality parameters. At the same time, the similarity of variability is specifically varied. This is illustrated in Figure 5, showing variation in the variability of the indicators at stations S1 and S2, showing that the greatest variation in the waters of Słoneczne Lake was between S1 and S2. Then the quality of water between the S2 and S3 stations has changed slightly.

Very specifically, the variability of water quality is shown in Figure 6. It follows that the particular similarity of water quality variability was marked by February and March and February and June – which is completely understandable due to climate change. Water quality in the month of January – probably by low temperatures – changed completely.

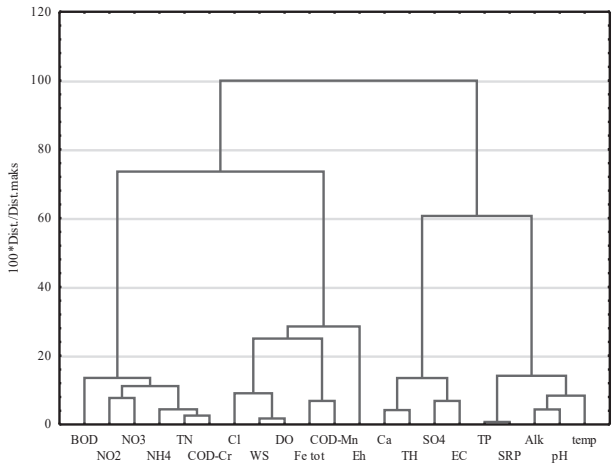


Figure 4. Similarities and differences in variation of selected water quality indices

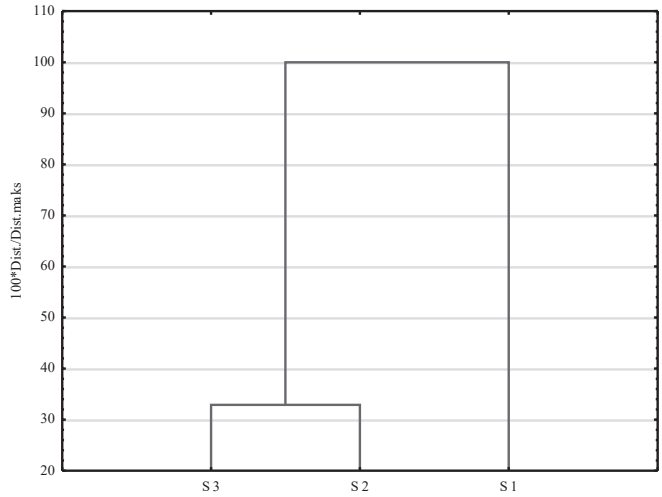


Figure 5. Differentiation of the quality of the waters of the Słoneczne Lake during the research period in the sampling stations

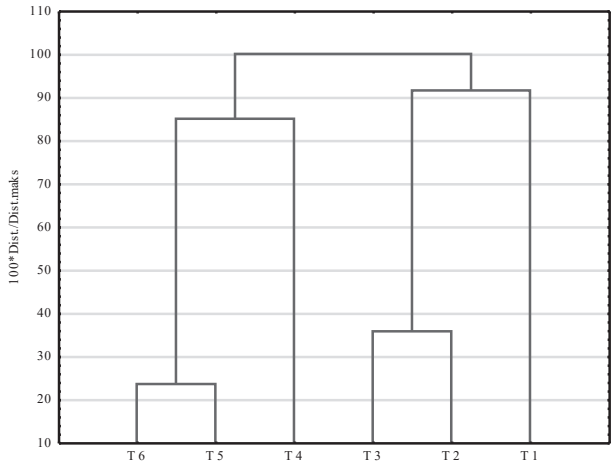


Figure 6. Differentiation of the water quality of the Słoneczne Lake during the study period from January to June 2015 in subsequent months from T1 to T6

Otherwise, as in the month of April, which is a "transitional" month between winter and warmer seasons. Very specific changes in the redox potential that occurred between S2 and S3 stations, which do not coincide with changes in oxygenation of water, can be easily explained on the basis of knowledge about redox potential measurements since the redox potential in the

Table 4. Spearman correlation coefficients for a set of measurement data on all stations on Słoneczne lake in study period

	Temp.	pH	EC	Eh	COD-Mn	COD-Cr	BOD_5	DO	WS	NO_3^-	NO_2^-	NH_4^+	TN	SRP	TP	TH	Ca^{2+}	Cl^-	SO_4^{2-}	Alk.	Fe_{tot}
Temp.	1.00																				
pH	0.72	1.00																			
EC	–0.04	0.00	1.00																		
Eh	–0.23	–0.74	–0.13	1.00																	
COD-Mn	–0.22	–0.19	–0.14	–0.12	1.00																
COD-Cr	0.07	0.01	0.15	–0.32	0.66	1.00															
BOD_5	0.01	0.05	0.09	–0.37	0.57	0.68	1.00														
DO	–0.82	–0.30	–0.15	–0.07	0.07	–0.37	–0.11	1.00													
WS	–0.61	–0.07	–0.28	–0.18	0.06	–0.44	–0.14	0.94	1.00												
NO_3^-	0.26	0.07	0.00	–0.13	0.73	0.75	0.55	–0.43	–0.39	1.00											
NO_2^-	–0.61	–0.55	0.06	–0.05	0.62	0.52	0.54	0.28	0.13	0.34	1.00										
NH_4^+	0.08	–0.11	0.34	–0.14	0.61	0.87	0.80	–0.40	–0.48	0.72	0.54	1.00									
TN	0.18	–0.09	0.12	–0.04	0.71	0.86	0.72	–0.51	–0.57	0.84	0.47	0.90	1.00								
SRP	0.44	0.64	0.35	–0.61	–0.04	0.20	0.32	–0.26	–0.15	0.16	–0.16	0.25	0.13	1.00							
TP	0.39	0.66	0.40	–0.64	–0.05	0.17	0.35	–0.20	–0.09	0.13	–0.09	0.25	0.13	0.95	1.00						
TH	–0.16	–0.24	0.81	0.22	0.05	0.11	0.19	–0.09	–0.24	0.11	0.08	0.38	0.24	0.10	0.17	1.00					
Ca^{2+}	0.08	–0.14	0.59	0.27	–0.12	–0.09	–0.06	–0.16	–0.23	–0.10	–0.26	0.14	0.05	0.23	0.14	0.54	1.00				
Cl^-	–0.77	–0.61	–0.09	0.40	0.27	–0.27	–0.06	0.68	0.58	–0.13	0.42	–0.16	–0.13	–0.30	–0.22	0.16	–0.02	1.00			
SO_4^{2-}	–0.55	–0.10	0.61	–0.43	–0.04	0.18	0.23	0.49	0.34	–0.23	0.40	0.19	–0.12	0.24	0.29	0.36	0.17	0.15	1.00		
Alk.	0.70	0.76	0.48	–0.46	–0.41	0.02	–0.05	–0.50	–0.36	–0.03	–0.54	0.08	–0.07	0.61	0.66	0.19	0.26	–0.68	0.09	1.00	
Fe_{tot}	–0.27	–0.33	–0.26	0.07	0.64	0.42	0.06	0.05	0.01	0.42	0.39	0.18	0.33	–0.22	–0.35	–0.23	–0.05	0.14	–0.08	–0.51	1.00

solar lake was shaped by the redox pair NO_3^-/NH_4^+, as a result of the data on concentration values mentioned above ions (Schuring et al., 2000).

The factor analysis presented in Tables 5–7 shows that indices whose variability in a statistically significant way – as the studies showed – characterize the variability of water in the examined tank were: TEMP, pH, EC, Eh, COD-Cr, DO, WS, NO_3^-, NH_4^+, TN, SRP, TP, TH, Cl^-.

Table 5. Factor Analysis for Słoneczne Lake in study period

Indices	VF1	VF2	VF3	VF4
Temp	**0.9262**	0.0022	0.2791	0.1989
pH	0.4792	–0.1996	0.2543	**0.7803**
EC	0.0379	0.2483	**–0.7814**	0.4756
Eh	0.0295	–0.0971	–0.1553	**–0.8795**
COD-Mn	–0.6772	0.3614	0.5566	0.1422
COD-Cr	0.1391	**0.8895**	0.0877	–0.1876
BOD_5	–0.2834	0.6892	–0.1594	0.3713
DO	**–0.9306**	–0.3244	0.0597	0.0196
WS	**–0.7394**	–0.5331	0.2049	0.1522
NO_3^-	0.0769	**0.7726**	0.5218	0.0013
NO_2^-	–0.3273	0.6186	0.1673	–0.3187
NH_4^+	0.0134	**0.8732**	–0.3630	0.0493
TN	0.1507	**0.9683**	0.0215	0.0369
SRP	0.3621	0.0404	–0.1631	**0.7901**
TP	0.3348	0.0134	–0.2543	**0.8140**
TH	–0.1665	0.2508	**–0.8735**	–0.0452
Ca^{2+}	0.0240	0.1496	–0.6986	0.0545
Cl^-	**–0.8729**	0.0364	–0.0871	–0.1664
SO_4^{2-}	–0.4090	0.0029	–0.6964	0.3736
Alkalinity	0.6150	–0.1749	–0.2232	0.6444
Fe_{tot}	–0.3100	0.3320	0.5251	–0.0703
T1	**–0.7965**	0.0507	0.5342	0.1214
T2	–0.3013	0.0315	–0.6636	–0.2545
T3	–0.0486	–0.1413	–0.4296	0.3683
T4	0.2874	0.0260	0.1880	**–0.7893**
T5	0.3599	–0.0750	0.1658	0.0756
T6	0.4990	0.1080	0.2052	0.4785
S1	0.0134	**0.9354**	–0.0752	0.0142
S2	–0.0131	–0.3581	0.0187	0.0065
S3	–0.0002	–0.5773	0.0565	–0.0207

In summary, based on the collected data from the first half of the year 2015, it was stated that in the Słoneczne Lake self-purification processes based on sedimentation of suspensions, changes in concentrations of suspended and dissolved organic matter related to oxidation of organic matter

and changes were documented in this work. The redox potential of the studied waters, which only became apparent after the water has reached the outflow area of the lake.

Table 6. Eigenvalues for factor analysis conducted after varimax rotation Słoneczne Lake in study period for all sampling stations

No.	Eigenvalue	Per cent total variation	Cumulated Eigenvalue	Cumulated per cent of total variance
1	6.667039	22.22346	6.66704	22.22346
2	6.345121	21.15040	13.01216	43.37387
3	5.176954	17.25651	18.18911	60.63038
4	3.821286	12.73762	22.01040	73.36800

Table 7. Results of the validity test of the use of FA for selected water quality indices of Słoneczne Lake

Kaisera-Mayer-Olkin		0.6430
Bartlett's Sphericity test	Chi-square	383
	df	136
	Significance level	0.0001

Thus, in the study period Słoneczne Lake was a settling pond and relatively efficiently operating biological pond for water Bukowa stream flowing through the Słoneczne reservoir.

Conclusions

1. The quality of water flowing through the Słoneczne Lake during the research period, i.e. January to June 2015, improved to a certain extent – along the course of the lake's water flow, especially between station S1 (Bukowa river inflow area to the lake) and the station S2 (center of the lake). However, the quality of the tested waters according to the official lake quality assessment criteria – which should be used for the assessment of the quality of the waters of the Słoneczne Lake was III or lower quality class at all measurement points due to the high values of total phosphorus concentrations in the waters.

2. Słoneczne Lake during the research period acted as a settling pond and a biological pond, especially the zone from the inflow area to central part of the lake, where suspended sedimentation and oxidation of suspended and dissolved organic matter occurred, and these effects were quantitatively assessed as moderate.

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Assemblages of stonefly larvae (Plecoptera) in the area of small hydrological structures, in the streams of the Kamienica Nawojowska river basin (The Beskid Sądecki)

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Keywords Plecoptera, macroinvertebrates, regulation of streams, hydrological structures

Abstract The study of larval stoneflies was carried out in the years 2010–2011 in the Kamienica Nawojowska river basin. The study involved three left-bank tributaries of the Kamienica Nawojowska River: the Kryściów, Łabowczański Potok and Homerka streams, in the course of which there were present small transverse hydrological structures. In the streams, within the investigated facilities, a large taxonomic richness of stoneflies was discovered. There were no significant differences in taxonomic richness and abundance of organisms forming stonefly clusters between stations situated upstream and downstream from the small transverse hydrological structures, which do not change the volume of the flow. The shape of the clusters in the studied streams and their individual sections were not as much influenced by the presence of small transverse hydrological structures as by other conditions such as: seasonal occurrence of taxa, similar type and structure of the bottom substrate, the way in which the territory of the basin is used. Assemblages of stoneflies were not significantly depleted after regulatory works, which is confirmed by the comparison with the results of previous years.

Kształtowanie się zgrupowań larw widelnic (Plecoptera) w rejonie występowania obiektów małej zabudowy hydrotechnicznej, w potokach zlewni Kamienicy Nawojowskiej (Beskid Sądecki)

Słowa kluczowe Plecoptera, makrobezkręgowce, regulacja potoków, zabudowa hydrotechniczna

Streszczenie Badania larw widelnic prowadzono w latach 2010–2011, w zlewni Kamienicy Nawojowskiej. Objęto nimi trzy lewobrzeżne dopływy Kamienicy Nawojowskiej, potoki: Kryściów, Łabowczański i Homerkę, w biegu których obecne były obiekty małej poprzecznej zabudowy hydrotechnicznej. W potokach, w obrębie badanych obiektów stwierdzono duże bogactwo taksonomiczne widelnic. Nie wykazano znaczących różnic w bogactwie taksonomicznym i liczebności organizmów tworzących zgrupowania widelnic pomiędzy stanowiskami położonymi powyżej i poniżej małej poprzecznej zabudowy hydrotechnicznej, która nie zmienia objętości przepływu. Na kształt zgrupowań w badanych ciekach i na poszczególnych ich odcinkach większy wpływ niż obecność małej poprzecznej zabudowy hydrotechnicznej

miały inne uwarunkowania, takie jak: sezonowość występowania taksonów, podobny rodzaj i struktura substratu dennego, charakter zagospodarowania terenu zlewni. Zgrupowania widelnic nie uległy znaczącemu zubożeniu po wykonaniu prac regulacyjnych, za czym przemawia porównanie z wynikami badań z lat wcześniejszych.

Introduction

Mountain streams are subject to a variety of hydromorphological processes. One of them is erosion: lateral, bottom and channel erosion. It is supposed to be counteracted by hydrological structures such as river bars, drop hydraulic structures and anti-debris dams (Radecki-Pawlik, 2012). However, they have an influence on a number of abiotic and biotic factors, e.g. the volume and velocity of flow, debris transport, bottom/channel shape (Wyżga et al., 2011). These changes affect in various ways particular assemblages of organisms, including benthos (Dukowska, Grzybkowska, 2007; Kukuła, Bylak, 2011) and constitute, among other things, an obstacle to the migration on aquatic organisms, cause depletion of biocenoses (Wyżga et al., 2008; Błachuta et al., 2011) and affect the drifting of invertebrates (Brittain, Eikeland, 1988).

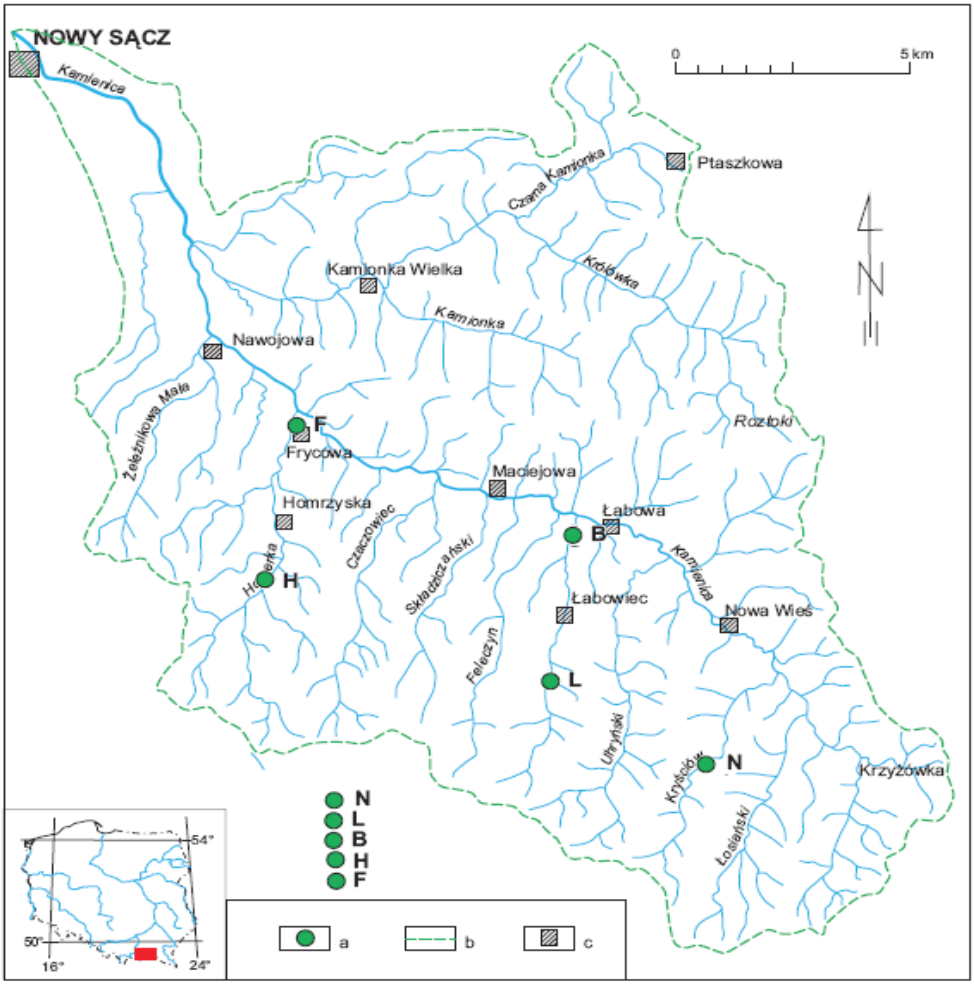
The aim of this study was to analyze the structure of stonefly taxocoenoses (Plecoptera) which are formed in the area of small hydrological structures in the streams of the Beskid Sądecki. It was also attempted to determine whether and to what extent the impact of these structures on the fauna of stonefly larvae is evident.

Study area and methodology

The research was conducted in the years 2010–2011 in the Kamienica Nawojowska River basin: the Kryściów, Łabowczański Potok and Homerka streams. These are streams of the fourth category and they are characterized by deeply cut valleys which are typical of the Beskids. The Kamienica Nawojowska River is a right-bank tributary of the Dunajec, into which it flows in Nowy Sącz. The length of the river is 33.079 km and its basin covers 237.83 km^2 (according to The Regional Water Management in Krakow 2010 – RWM Krakow 2010).

The term „facility" in order to describe the hydrological structures located in the studied streams. The following facilities were designated: one on the Kryściów stream (Nowa Wieś – facility N, basin covered with forest), two on the Łabowczański Potok (Łabowiec – facility L and Łabowa – facility B, basin with rural buildings and used for agricultural purposes), two on the Homerka (Homrzyska – facility H, basin covered with forest, Frycowa – facility F, basin with rural buildings and used for agricultural purposes). Three study sites were designated within each facility, in the longitudinal profile of the stream:

- site of type 1, situated approx. 20 metres upstream from the hydrological structure,
- site of type 2, situated approx. 2–3 metres downstream from the structures, depending on the size of the structure as well as the presence and the size of evorsion potholes,
- site of type 3, situated approx. 20 metres downstream from the site of type 2.



a – sampling sites, b – river basin, c – townes/villages.

Figure 1. Distribution of research facilities a) in the Kamienica Nawojowska basin, b) with the distribution of towns/villages, c) according to Niechwiej 2013, RWM Cracow 2010

Basic hydrological and morphological parameters of the studied streams: depth, width, water flow rate, shading of sites, coverage of the substrate by periphyton and coarse particulated organic matter (CPOM) were measured six times each year; in March, May, June, August, September and November (Bajkiewicz-Grabowska et al., 1993). Each time the physical parameters of water were measured: temperature, electrolytic conductivity, level of oxygen saturation and pH, as well as basic chemical parameters: the concentration of ammonium, nitrates, total phosphorus and phosphates. The standardized Multi-Habitat Sampling (AQEM-STAR, 2002) method was used to collect samples of benthic fauna. Stonefly larvae were preserved in 70% ethanol and determined to the genus according (Zwick, 2004).

Figure 2. The typical hydrological structure in the Kamienica Nawojowska river basin

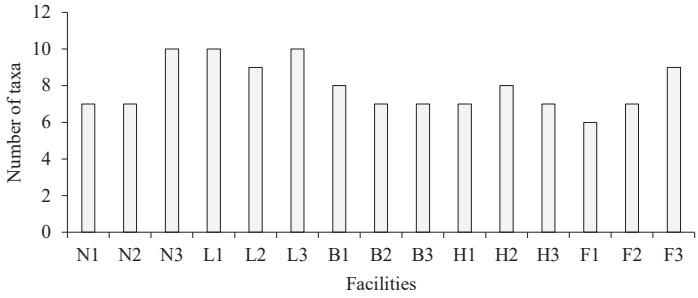
During the analysis of the results were determined: taxonomic richness, the numer of genera, the density of larvae, the structure of domination (the Szujecki occurrence stability factor, Shannon-Wiener index), faunictick similarities, the share of representatives of food guilds.

Results

Based on the measurement results, it was found out that the average depth of the surveyed streams varied in the season from 0.15 m (Nowa Wieś) to 0.40 m (Frycowa), while the average width of the flow varied from 1.5 m (Nowa Wieś) to 4.5 m (Frycowa). The flow rate of water, as determined by the floater method, ranged from 0.1 m/s (autumn, Kryściów) to 1.1 m/s (Homerka). The periphyton coverage of the substrate, which was determined visually, was at its lowest in March (less than 5%), at its highest in June – to 70%, whereas the coverage by CPOM was the lowest (5%) in May and the highest in November (50%).Within all studied objects physical and chemical parameters of water were similar and showed small fluctuations in the annual cycle, with the exception of temperature, which ranged from 2 to 12,5°C. The degree of oxygen saturation at each site was greater than 90%, the electrolytic conductivity ranged between 244 and 331 μScm^{-1}, and the pH was within the range between 8 to 9. The content of nitrate exceeded 1.0 $mgNO_3dm^{-3}$ (1,010 only at the facility B – Łabowa), whereas the highest value of nitrogen – 0.134 $mgNO_3dm^{-3}$ – was recorded at the facility F (Frycowa). The highest concentration of total phosphorus was in Homrzyska (facility H) – 0,037 $mgPdm^{-3}$, the level of phosphates was the highest n Łabowa – 0.093 $mgPO_4dm^{-3}$.

General characteristics of stonefly taxocoenoses

It was discovered that in streams, within the investigated objects there is a large taxonomic richness of stoneflies. There was a total of 15 genera: two of the family Taeniopterygidae, four of the Nemouridae family, one of the Leuctridae family, two of the Perlidae and Chloroperlidae family and four belonging to the Perlodidae family.



N1, N2, N3 – sites in Nowa Wieś; L1, L2, L3 – sites in Łabowiec; B1, B2, B3 – sites in Łabowa; H1, H2, H3 – sites in Homrzyska; F1, F2, F3 – sites in Frycowa

Figure 3. Taxonomic richness of stoneflies (genera) at the tested sites

The highest taxonomic richness of Plecoptera was stated at sites N3, F3 and at all sites within the facility L – more than 8 genera were found there. The smallest number, 6 genera, were discovered at the site F1 (Figure 3). There were no regularities in the differentiation of the number of types of stoneflies between different types of sites (types 1, 2 and 3).

Seasonal an spatial variation in the richness of stonefly taxocoenoses

The number of stonefly taxa, recorded in the studied streams, fluctuated throughout the year. The highest taxonomic richness was recorded in spring and autumn (especially in November), the lowest in summer (Figure 4).

The spring, especially May, was the period when then the largest number of stonefly taxa were recorded. The presence of species of the genus *Brachyptera, Rabdiopteryx, Nemurella, Xantoperla, Chloroperla, Dictyogenus, Dinocras* was found primarily in the spring. During the summer and early autumn (September) the number of genera was lower, and in some cases (B1 and B3 in June, L2 in August) there were no representatives of Plecoptera at all. In the summer it was the primarily the presence of the larvae of the families Nemouridae and Lauctridae which was demonstrated and which was observed throughout the season. The occurrence of representatives of the genera *Perlodes* and *Acrynopteryx* was recorded only in this period.

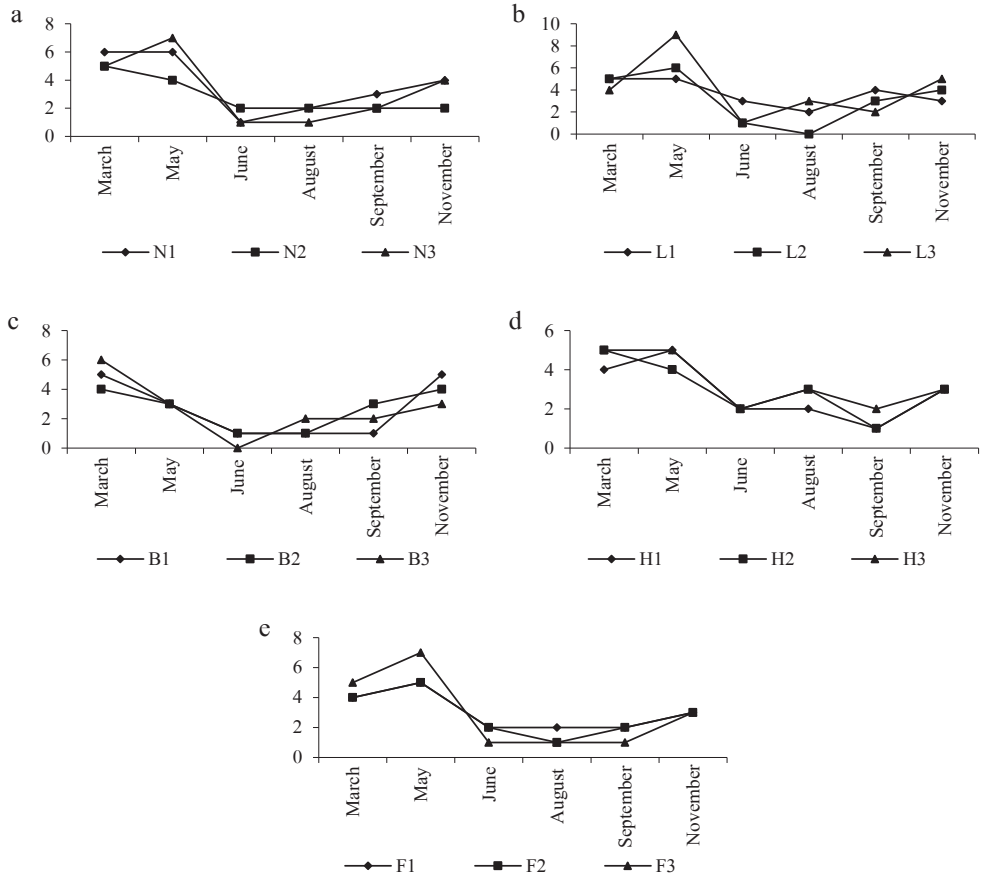


Figure 4. The number of stonefly genera observed at the studied sites in individual months

Seasonal an spatial variation in the density of stonefly taxocoenoses

The density of larvae in the studied sections of streams was subject to large fluctuations during the year. The highest density was shown in the spring, followed by a sharp decline in the summer and rise again in the autumn (Figure 5).

The highest density of larvae occurred mainly in March – to more than 600 individuals/ m^2 (the site directly downstream from the structure in Łabowiec – L2). In addition, the highest density was shown at sites located upstream from the structure, although apart from the facility in Frycowa, the differences were not significant compared with the sites downstream from the structure. These were especially representatives of the families Taeniopterygidae (*Brachyptera sp.*), Nemouridae (*Nemours sp.*) and Leuctridae (Table 1). The period from June to September was marked by a sharp decline in the number of stoneflies, and in some cases (eg. in June at the site B1) they were not reported at all. In this period these were mostly representatives of *Protonemura sp.* and *Leuctra sp.* that were found. November was a time of density re-growth, not as significant as

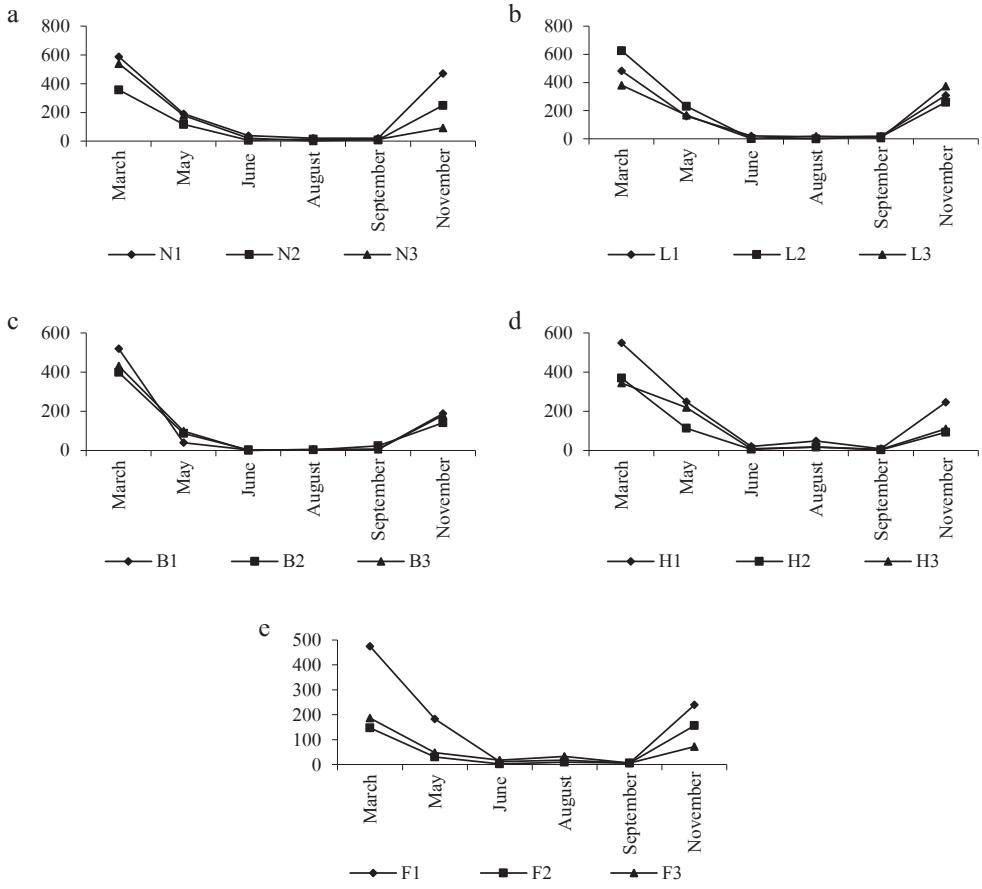


Figure 5. Changes in the density of larvae of stoneflies at different sites in individual months

Table 1. Numbers of larvae at particular posts

List	N1	N2	N3	L1	L2	L3	B1	B2	B3	H1	H2	H3	F1	F2	F3
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Rhabdiopteryx sp.	0	0	3	0	3	3	0	0	0	0	0	0	0	0	0
Brachyptera sp.	141	105	27	243	276	213	120	213	198	240	129	219	81	15	102
Nemoura sp.	591	291	357	498	531	354	352	174	210	465	216	207	393	123	96
Protonemoura sp.	138	39	75	21	3	57	30	84	93	120	6	24	48	6	9
Amphinemoura sp.	0	0	0	3	3	6	3	3	0	12	6	3	0	0	0
Nemurella sp.	3	3	3	0	6	0	0	0	0	0	0	0	0	3	0
Leuctra sp.	369	252	354	168	228	231	195	132	147	270	222	237	324	156	120
Chloroperla sp.	0	3	9	0	0	0	0	0	0	0	3	0	0	0	3
Xantoperla sp.	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0
Perla sp.	0	0	3	3	6	6	9	3	6	3	12	3	6	3	9

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Dinocras sp.	3	0	3	0	0	0	0	0	0	0	0	0	0	0	3
Perlodes sp.	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0
Dictyogenus sp.	0	0	0	0	0	0	0	0	3	0	0	0	0	0	3
Isoperla sp.	87	63	15	66	69	75	57	87	52	9	9	15	81	39	18
Acrynopteryx sp.	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0

in early spring, although eg. at the site N1 there were 471 individuals/m^2. It was shown that the most numerous genera of larvae were *Namoura, Lauctra*, and *Isoperla* (Table 1). In November, the same as in March, the highest density of stoneflies was recorded at sites of type 1, with the exception of the facility L.

The number of larvae at different sites

The structure of dominance

Several genera clearly dominated in the studied clusters of stoneflies. The following genera can be included in the group of eudominanta (over 10% share), or dominanta (5–10% share) on an annual basis: *Brachyptera, Nemours, Protonemura, Leuctra* and *Isoperla*. This was evident at different sites, their types (1, 2, 3) and in individual months (Figures 6–8).

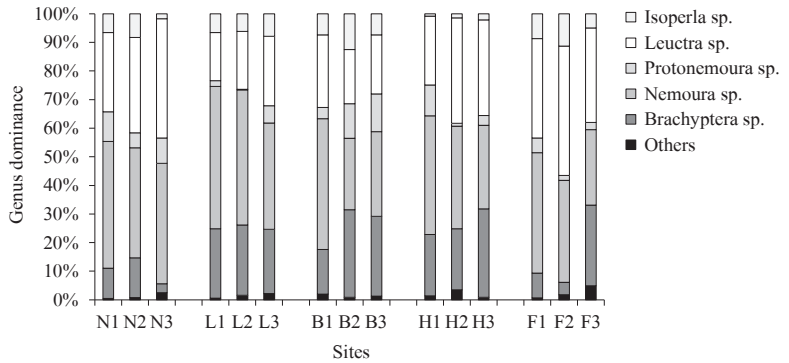


Figure 6. The share of eudominanta and dominanta at different sites during the year

All sites were clearly dominated by *Nemours sp.* and *Leuctra sp.* (Figure 6). Their share reached nearly 50% at the sites L1 (*Nemours sp.*) and F2 (*Leuctra sp.*). Representatives of *Brachyptera* genus had a 30% share in all assemblages (B2 and H3), although at some sites they did not reach the status of eudominanta (N1, F1, F2). Representatives of *Protonemura sp.* and *Isoperla sp.* had a significantly smaller share in the assemblages.

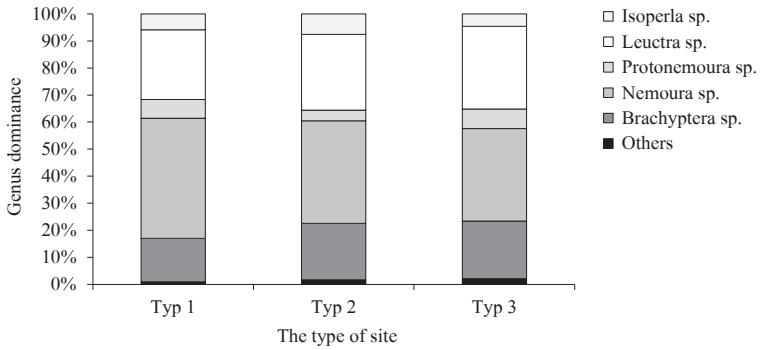


Figure 7. The share of eudominanta and dominanta at different types of sites on an annual basis

No clear trends are visible concerning the structure of dominance at individual types of sites (Figure 7). At the sites of type 1 there was the greatest share of representatives of *Nemoura sp.* and the smallest share of representatives of *Brachyptera sp.*, in comparison with other types. These are not, however, significant differences.

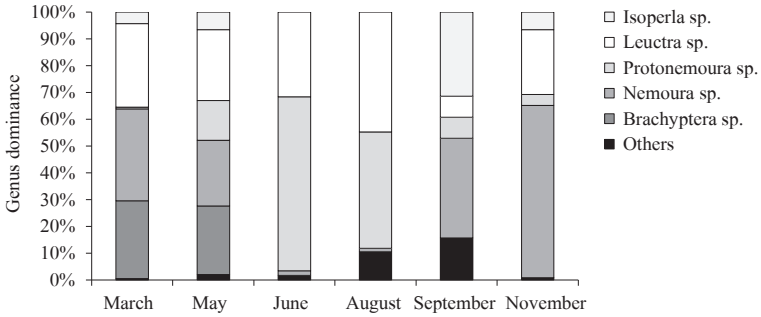


Figure 8. The share of edominanta and dominanta per year in each month

Differences in the structure of domination become apparent more clearly over time (Figure 8). The genera which dominate clearly in the spring are *Brachyptera*, *Nemoura* and *Leuctra*. However, they manifest themselves more visibly in early spring (March). During the summer the larvae of *Leuctra sp.* codominate with *Protonemura sp.* In September the biggest percentage is constituted by *Nemoura sp.* and *Isoperla sp.* November is a time of overwhelming dominance of *Nemoura* larvae (over 50%), with a significant share of *Leuctra sp.*

The stability of occurrence of dominant taxa fluctated during the year. The Szujecki occurrence stability factor ranged from 0 to 100 (Table 2).

Table 2. The stability factor representing the occurrence of eudominanta and dominanta

	March	May	June	August	September	November
Brachyptera sp.	100	80	0	0	0	7
Nemoura sp.	100	87	7	7	60	100
Protonemura sp.	27	87	73	60	20	40
Leuctra sp.	100	100	60	87	20	93
Isoperla sp.	93	60	0	0	47	53

Representatives of *Brachyptera sp., Nemoura sp.* and *Leuctra sp.* were noticed in early spring at all tested sites (*Leuctra sp.* also in May). During the summer no larvae of the genera *Brachyptera* and *Isoperla* were recorded. In November only representatives of *Nemoura sp.* were present at all sites. The smallest fluctuations in the occurrence stability were observed in case of *Lauctra sp.*

Taxonomic diversity

Taxonomic diversity of individual clusters of stoneflies was characterized by considerable volatility over the year, as evidenced by the values of Shannon-Wiener index (Table 3). There were months (usually the summer ones) when no specimens or only a single taxon (0 value in the table) were identified at part of the sites. In turn, the highest values of the index were recorded in spring months – up to 1.75 at the site L3.

Table 3. Values of te Shannon-Wiener index for stonefly clusters at tested sites

Facilities	Posts	Values		
		min.	max.	average value
N	N1	0	1.33	0.97 ±0.49
	N2	0.64	1.26	0.94 ±0.30
	N3	0	1.36	0.67 ±0.58
L	L1	0.59	1.43	1.03 ±0.34
	L2	0	1.48	0.72 ±0.63
	L3	0	1.75	0.85 ±0.57
B	B1	0	1.28	0.49 ±0.57
	B2	0	1.17	0.53 ±0.50
	B3	0	1.29	0.63 ±0.43
H	H1	0	1.46	0.67 ±0.54
	H2	0	1.10	0.73 ±0.39
	H3	0.64	1.20	0.93 ±0.23
F	F1	0.56	1.35	0.94 ±0.31
	F2	0	1.42	0.77 ±0.46
	F3	0	1.71	0.54 ±0.73

There are no significant differences in the index values between particular watercourses. There are also no clear patterns in case of site types.

Trophic functional groups:
Various food guilds were represented by the following taxa:
Scrapers: Taeniopterygidae
Shredders: Leuctridae and Nemouridae
Predators: Chloroperlidae, Perlidae and Perlodidae.

There were no significant differences in the percentage of representatives of trophic functional groups between different sites and between different watercourses. As for the types of sites, there was a greater share of shredders at the sites of type 1 (upstream from the structures) (Figure 9).

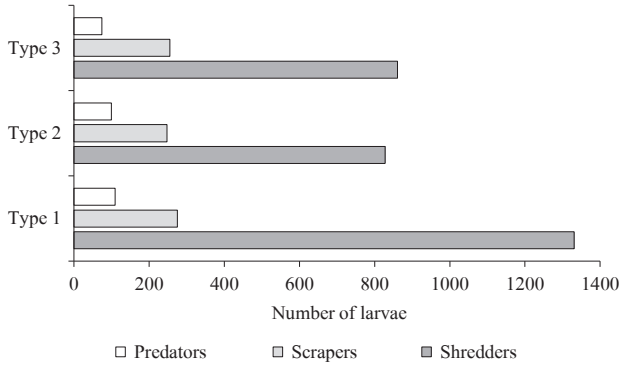


Figure 9. The share of representatives of food guilds at particular types of sites

Faunistic similarities

The tested groupings of stoneflies showed considerable faunistic similarities. This was probably due to a similar hydromorphological character of studied sections of streams and their location in a relatively small area of the same river basin. In case of sites of individual types, the distribution of faunistic similarities could be presented as Figure 10.

There is a slight difference between sites situated upstream and downstream from the hydrological structures. At the sites of the first type there were no larvae of the family Chloroperlidae or stoneflies of the genus *Rhabdiopteryx* (Taeniopterygidae). In addition, upstream from the structures there was a higher number of representatives of families which were present in large densities: Nemouridae, Leuctridae, Perlodidae (Table 1).

Figure 10. Diagram representing faunistic similarities by site types

Discussion

The results of the studies of stonefly fauna in the Kamienica Nawojowska river basin from the years 2010–2011 can be compared with the results of research conducted by Zaćwilichowska (1968). Zaćwilichowska collected samples in the summer and early autumn, in the period when most of the streams in Kamienica Nawojowska river basin were not yet regulated. During the comparative analysis of the results it was assumed that the sections of streams with hydrological facilities designated for research (including three sites located not far from each other) corresponded approximately to one site designated on a particular watercourse by Zaćwilichowska. Therefore, the site set out in the years 1964–1965 on the Łabowczańskim Potok corresponded to the location of the facility in Łabowiec (L), whereas the location of the site on the Homerka corresponded to the facility in Homrzyska (H). In case of Kryściów stream the sites in both studies were designated at similar sections of the watercourse. Taxonomic richness in the tested period of summer and early autumn (the years 1964–1965 and 2010–2011) was similar, but the taxonomic structure of stonefly assemblages was different. In the years 2010–2011, especially in the Kryściów and Homerka streams, the most numerous were larvae of *Protonemura sp.* In addition, in the Homerka stream there was a large number of stoneflies of the genus *Leuctra*, whereas the Krysciów stream abounded in the representatives of the genus *Nemoura*. Also the studies conducted in the 1960's demonstrated that there were numerous representatives of *Protonemura sp.* at all sites, and their largest number was recorded on the Łabowczanski Potok stream. In contrast, there were no stoneflies of the genus *Nemoura*. Zaćwilichowska also observed a significant share of the larvae of *Perlodes* (*Perlodes dispar* accounted for 7% of the total number of benthic fauna in the Kryściów stream) in the clusters of stoneflies in the Kryściów and Homerka streams, whereas in the years 2010–2011 those stoneflies were not noticed in the area of facilities N, L and H. The present study of sections of streams in the area of selected hydrological facilities revealed the presence of representatives of 15 stonefly genera belonging to 6 families, of which the most numerous were Nemouridae, Taeniopterygidae and Leuctridae. These are taxa reported at other sites in other Beskid river basins characterized by a great taxonomic richness of stoneflies (Sowa, Szczęsny, 1970; Zasępa et al., 2006). The stoneflies observed during the previous studies of Beskid river

basins were represented mostly by the Nemouridae and Leuctridae families and also the less numerous Perlidae and Chloroperlidae (Sowa, Szczęsny, 1970; Dratnal et al., 1979; Dratnal et al., 1982; Szczęsny, 1995). However, in the higher-lying parts of those basins there were almost no representatives (with the exception of *Brachyptera sp.*) of the Taenipterygidae family. This is partially confirmed by the results of research conducted in the Kamienica Nawojowska river basin in the years 2010–2011, that is large numbers of representatives of this family were recorded in spring in the studied parts of the basin which were lying below the altitude of 600 metres above sea level. The common larvae, however, were those of the Nemouridae and Leuctridae, but not of the Perlidae. The abiotic factors which most affect the taxonomic wealth of stonefly clusters in Beskid streams are: the velocity and volume of flow, the type and structure of the substrate, the nature of river basin management, as well as water temperature and its oxygenation (Hawkins et al., 1981; Donehy et al., 1999; Jowett, 2003). In the studied streams the environmental abiotic factors mentioned above were similar, favoring the presence of reophilic organisms, which resulted in a significant similarity of stonefly assemblages. There were no radical differences in taxonomic wealth between facilities (the greatest one was observed in Łabowiec); some differences occurred only in the taxonomic structure of assemblages. The bottom substrate of the examined sections of streams consisted mainly of stones and gravel, as well as coarse particulate organic matter (CPOM) and deposits of sand and silt. This was conducive to a high diversity of habitats and influenced the density and distribution of benthos (Thorp, Covich, 2001). The presence of rock material with high granularity, as well as wood debris and fallen leaves create good conditions for many species (Wyżga et al., 2002). The low water temperature, alkalinity and high oxygen saturation of water contributed to the development of cryophilic species and species living in highly-oxygenated water as well as acid-sentitive ones, such as representatives of the families Leuctridae, Perlidae, Perlodidae and Chloroperlidae (Thomsen, Friberg, 2002; Bogdanowicz et al., 2007; Kozačekova et al., 2009). The taxonomic wealth of stoneflies and larval abundance are influenced by the trophic qualities of water, which are shaped, among other things, by the management of the river basin and coastal area (Kopacz, Twardy, 2006). According to Törnblom et al. (2011) stoneflies prefer watercourses of wooded river basins. Hence, the highest densities of larvae were recorded in the sections located in the wooded river basin and in midstream of the watercourses, especially in Nowa Wieś (facility N) and Łabowiec (facility L). The type of river basin cover and the position of the site in the longitutinal profile of the watercourse also influence the trophic structure of macroinvertebrate groupings (Törnblom et al., 2011). Medhurst et al. (2010) report that shredders and gatherers prevail in watercourses of wooded basins. Representatives of those trophic guilds, which were identified on the tested sections of streams, were larvae from the families Leuctridae and Nemouridae (shredders). The highest density of Leuctridae was recorded in Nowa Wieś, where there was a typically wooded river basin. Shredders also prevail in midstream of watercourses (facilitie N, L, H). The changes in the number of stonefly taxa and in the density of specimens were mainly related to their life cycles (Pastuchova, 2006; Bogdanowicz et al., 2007; Alibozek, Ganger, 2008; Błaszak et al., 2012). This was true of the Taenipterygidae family, whose representatives were recorded primarily in spring, as well as the Nemouridae family (especially *Nemoura sp.*) and, to a lesser extent, the Leuctridae family, but also in this case the largest numbers were recorded in spring and autumn. The velocity and volume of water flow in the stream influence the deposition of organic matter, bottom substrate structure, and thus the nutrient base of many substrate invertebrates and the microhabitat (Thorp, Covich, 2001, Small et al., 2008). The presence of transverse hydrological structure in the longitudinal profile of the watercourse influences the benthos (Fleituch, 2003; Santucci et al., 2005; Tiemanna et al., 2005;

Vallania, Corigliano, 2007; Brown et al., 2010; Bellucci et al., 2011). The hydrological structures on the tested sections of streams did not lead to any change in the flow volume, but only in the flow velocity. Hence there is no clear difference or regularity of changes in stonefly assemblages downstream and upstream from the structures in terms of taxonomic richness or density of specimens. Aassemblages of larvae at the sites of type 2 and 3 are more similar to each other in faunistic terms than to groupings at the sites of type 1, but these are not so big differences. The similarity of clusters for three types of sites is about 75%. There were also no differences in percentages of representatives of particular trophic guilds. The settlement of different types of habitats within the river channel is due, among other things, to the preferences of taxa concerning the flow velocity and drift susceptibility (Möbes-Hansen, Waringer, 1998; Armitage, Cannan, 2000). In the studied streams there were taxa preferring a fast flow and showing tolerance to high values of shear stress. These were, among other, stoneflies of the *Brachyptera sp.* (chosing habitats with the fastest flow), as well as *Nemoura sp., Leuctra sp., Isoperla sp.*

Conclusions

There were no a big differences in the taxonomic wealth and number of organisms forming stonefly clusters between sites situated upstream and downstream small transverse hydrological structures, which do not change the flow volume. The shape of assemblages in the investigated streams and on their particular sections was more influenced by other factors, e.g. the seasonality of taxa, the similar type and structure of the bottom substrate, the nature of river basin development. Stonefly assemblages were not significantly impoverished after regulatory works, which can be supported by the comparison with reserach from previous years.

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Body satisfaction versus anthropometric and lifestyle characteristics in secondary school youth in a three-year study

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Keywords body satisfaction, anthropometry, high-school youth, lifestyle, eating habits, eating behaviours

Abstract

Background. The current trend for a slim body has spread to all age groups and social strata. It is necessary to monitor health- and eating-related behaviours among young people to counteract bad eating habits.
Aim. To examine body satisfaction taking into account anthropometrics and physical activity data in high-school youth over the course of a three-year study.
Material & Methods. Anthropological examinations on the same high-school students were conducted in: 2014, 2015, 2016 – always in September. The parameters measured included: body weight, body height (B-v), waist and hip circumference. Somatic indices were calculated: BMI, WHR, Rohrer's index. The study also included a questionnaire with questions about the number of hours of physical activity per week, the length of time in training in a given sports discipline, self-assessment of body satisfaction and eating habits.
Results. For both girls and boys the intensity of physical exercise declined year by year. The potential reason may be that the young people got to know one another after the first year and did not feel compelled to work on their body (to appear more attractive). As many as 56% of girls were not satisfied with their body in first grade and 71% of boys. In the two subsequent years, dissatisfaction rates dropped for both sexes.
Conclusions. There is a need for health-promoting programmes so that young people can learn about the principles of nutrition and not experiment with excessive dieting.

Zadowolenie z własnej sylwetki wobec charakterystyki antropometrycznej i trybu życia u młodzieży licealnej w ciągu trzech lat badań

Słowa kluczowe zadowolenie z własnej sylwetki, antropometria, młodzież licealna, tryb życia, nawyki żywieniowe, zachowania żywieniowe

Streszczenie

Tło. Współczesna moda na szczupłą sylwetkę przenika do wszystkich grup społecznych w każdym wieku. Monitorowanie zachowań zdrowotnych i żywieniowych wśród młodzieży jest niezbędne, aby przeciwdziałać złym nawykom żywieniowym.
Cel. Zbadanie zadowolenia z własnej sylwetki przy uwzględnieniu antropometrii i aktywności ruchowej u młodzieży licealnej w ciągu trzech lat badań.

Materiał & Metoda. Badania antropologiczne u tej samej młodzieży licealnej zostały wykonane w 2014, 2015, 2016 roku – zawsze we wrześniu. Zmierzono: masę ciała, wysokość ciała (B-v), obwód pasa i bioder. Wyliczono wskaźniki somatyczne: BMI, WHR, wskaźnik Rohrera. Pytania ankietowe dotyczyły liczby godzin aktywności fizycznej w tygodniu, jak długo trenuje uczeń podaną dyscyplinę sportową, ocena zadowolenia z własnej sylwetki, wywiad dotyczący nawyków żywieniowych.
Wyniki. U dziewcząt i chłopców intensywność ćwiczeń fizycznych z roku na rok spadała. Być może dlatego, że młodzież się poznała po pierwszym roku i już nie chciała pracować nad sylwetką (dla większej atrakcyjności). Aż 56% dziewcząt nie było zadowolonych w I klasie ze swojej sylwetki i 71% chłopców. W dwóch kolejnych latach nauki odsetek niezadowolonych obniżył się u obu płci.
Podsumowanie. Konieczne jest wdrażanie programów prozdrowotnych, aby młodzi ludzie poznali zasady żywieniowe i nie eksperymentowali z przesadnym kontrolowaniem masy ciała.

Introduction

In the contemporary world appearance plays a very significant role in people's lives. Body shape is of particular importance to young people, who are prone to body insecurities and because of that liable to cause considerable damage to themselves and their health. Young people tend to pursue the ideal look, often inspired by the mass media (Kakeshita, Almeida, 2006). For instance, it was demonstrated that young women with poor eating habits are seven times more likely to develop eating disorders than women who observe the rules of healthy nutrition (Patton et al., 1999). Eating disorders form a group of psychosomatic disorders, characterised by strong deviations from healthy eating patterns. Patients suffer mostly from symptoms related to distorted body image, which makes them see themselves as having excess weight even though their actual weight is normal or even extremely low (Myszkowska-Ryciak et al., 2015).

However, teenagers are not always aware of the fact that their appearance-related problems are rooted in bad nutrition and unhealthy lifestyles. Often, in spite of knowing the theory on good nutrition, young people do not adhere to the principles of healthy eating, and as a result perpetuate the bad eating habits developed in childhood. Physical inactivity and poor eating habits persistent from childhood have a serious impact on health in later life, including such problems as iron-deficiency anaemia, obesity, eating disorders (*anorexia nervosa*, *bulimia nervosa*) (Woynarowska, 2003). They often contribute to the development in adult life of many chronic metabolic diseases "of civilisation", like e.g. cardiovascular disease caused by excessive consumption of animal fats (US Public Health Service, 1988) or insufficient calcium levels in bones leading to osteoporosis in later life, especially in women (Sandler at al., 1985). On the other hand, low body weight associated with protein-energy malnutrition as well as deficiencies of minerals and vitamins may disrupt the processes of physical and sexual growth in adolescence and later ontogenetic stages leading to problems with fertility, and in extreme cases even to cachexia (Szponar, Respondek, 2000).

Many of those young people who are unhappy about their body shape fail to take into account the fact that puberty brings dramatic changes in the body. During this period, the body may for some time look disproportionate, awkward, while skin and hair may be far from perfect. In a large percentage of dissatisfied teenagers, their body weight self-assessment differs from BMI-based standards of normal nutrition (Wojtyła-Buciora, Marcinkowski, 2010), and they often regard themselves as too slim or too fat. Still, discrepancies between reality and self-perception tend to be more common among people who are actually overweight or obese and who, having lived since childhood among people with excess weight, consider it to be the norm (Maximowa et al., 2008). To bridge this gap between real and self-perceived body weight, awareness campaigns should be

organised for young people on healthy lifestyles, correct assessment and healthy self-perception of own appearance. Interestingly, children/teenagers are often very similar, also in terms of weight, to their parents as they were at the same calendar age (Sorensen et al., 1983), which proves that body shape predisposition is to a large degree genetically determined. On the other hand, environmental factors and health-oriented behaviours play a significant role in the prevention of lifestyle diseases (diseases of civilisation), so it would be unwise to dismiss them and attribute extra kilograms solely to genetic factors.

In the case of *bulimia* and *anorexia,* unrealistic perceptions of a fat, stocky body give rise to tension and strong emotions, causing stress (Schneider et al., 2009). Prolonged stress may contribute to anxiety disorders, depression, neuroses, and under special circumstances may lead to personality disorders. Long-term studies show that depressed moods and depression are predisposing factors for actual weight gain – overweight and obesity – during puberty and in later life (Tanofsky-Kraff et al., 2006).

Therefore, it seems necessary to monitor health- and eating-oriented behaviours among youth and to develop health-promoting programmes to counteract unhealthy practices related to body weight management (Wojtyła-Buciora, Marcinkowski, 2010).

Materials and methods

Young people were examined in three consecutive years between 2014 and 2016, always at the beginning of the school year. Examinations included one class from high school no 7 (*Liceum Ogólnokształcące nr VII*) in Szczecin, majoring in biology with elements of forensic science. The collected material yielded anthropological information on students. The analysis covers a group of $n = 34$ individuals born in 1998. In the final year of the study, 2016, the students reached the age of 18. Examinations were non-invasive, and the form master, school principal and students' parents had given their consent to the anthropological research. Participation in the study was voluntary, and there were no consequences for refusing to take part. The students were measured for: body height (B-v) [cm], waist circumference and hip circumference [cm] as well as body weight [kg]. To examine body build, the following somatic indices were calculated:

1. Body Mass Index BMI = body weight [kg]/(B-v) $[m]^2$.
   Results were interpreted based on WHO classification, where BMI ≤ 18.49 corresponds to underweight; BMI 18.50–24.99 normal (healthy weight) range; and BMI 25.00–29.99 overweight; BMI 30.00–34.99 obesity 1°; BMI 35.00–39.99 obesity 2°; BMI ≥ 40 obesity 3° (Physical Status 1995).
2. WHR = Waist-to-Hip Ratio = waist circumference [cm]/hip circumference [cm], which describes the distribution of subcutaneous adipose tissue, and identifies the android and gynoid type of fat distribution.
   Results were interpreted based on the following classification: WHR ≥ 0.8 in women indicates visceral fat accumulation, i.e. the android body type (the "apple"), whereas WHR < 0.8 is characteristic of the gynoid body type (the "pear"). Among men, the android type corresponds to WHR ≤ 1.0 and gynoid fat distribution – WHR > 1.0 (Drozdowski, 1998).
3. Rohrer's Index = body weight [g]/(B-v)^3 [cm], which indicates the degree of leanness or corpulence.
   Rohrer's index of ≤1.36 indicates a lean figure for 16-year-old girls, the value of ≤1.39 indicates a lean figure for 17-year-old girls, and ≤1.41 indicates a lean figure for 18-year-old girls. Values above the thresholds listed above indicate a corpulent figure for each of the

respective age categories. Likewise, for boys Rohrer's index scores of ≤1.23, ≤1.25 and ≤1.27 indicate a lean figure for: 16-year-old, 17-year-old and 18-year-old boys respectively. Results above these thresholds indicate a corpulent figure (Drozdowski, 1998).

Questionnaire data were used to classify the intensity of physical activity according to the following categories: sedentary (no physical activity); minimally active (exercise once a week); active (exercise twice a week); and highly active lifestyle (exercise 3 ≥ times a week). A unit of physical activity was taken to amount to 45 minutes of physical activity (e.g. swimming, jogging, dancing, pilates, walking, cycling, etc.), engaged in consciously in pursuit of health benefits and leisure. As an additional question, the respondents were asked to declare the duration (number of months) of the given activity.

Moreover, each respondent was asked whether they were guided by health benefits in their choice of food. Each respondent described their body satisfaction level according to the following categories: yes; rather yes; no.

Information about the students' date of birth was used to determine their calendar age on the day of the examination. Calendar age was measured according to the following procedure: age in years and decimal parts of the year (Łaska-Mierzejewska, 1997).

The students included in this anthropological research were examined on three occasions, that is in the three consecutive years of the research project. Most students ($n = 25$) were examined three times and their data lend themselves to a detailed analysis of changes in anthropometrics and survey responses over the course of three years. Other students ($n = 4$) were examined twice, due to their absence at school on the examination day. Arithmetic mean values of anthropometric measurements also include the measurements of students who were only examined once ($n = 5$). These students were only present at school on one of the three examination days.

The analyses for significant relationships between measurements and survey responses from the three years of study took into account only $n = 21$ young women, seen as only this number of female students participated in the study in three consecutive years. The research material collected among young men was too small to merit statistical analysis. The analysis was performed using Statistica 12 (StatSoft, Poland). Body measurements and somatic indices were subject to analysis to calculate the arithmetic mean (x), standard deviation (SD), and range (min-max), and the obtained indices were compared to pre-determined categories (standards). Calculations were performed separately for each year of study. To compare the arithmetic means of the measurements, indices and survey data collected according to the year of study, the Kruskal-Wallis test was used, one-way ANOVA by ranks, the non-parametric equivalent of one-way analysis of variance. A non-parametric test was used because some of the measurable variables failed to follow a normal distribution and due to categorisation of somatic indices and survey data. Post-hoc tests (multiple comparison procedures) were performed in all cases. To examine the associations of measurable features and dichotomous data, Pearson and Spearman correlation analysis was performed respectively.

Results

The anthropometric measurements and survey data from the three-year study of high-school students were used to calculate the results for each year of study.

Table 1. Arithmetic means of body measurements and somatic indices in female participants over the course of the 3-year study

Measure/index	2014 (1st grade, n = 23)			2015 (2nd grade, n = 24)			2016 (3rd grade, n = 23)		
	x	SD	min–max	x	SD	min–max	x	SD	min–max
Calendar age	16.3	0.47	15.8–18.1	17.4	0.53	16.8–19.1	18.3	0.54	17.8–20.4
Body height	166.2	6.49	154.7–180.4	165.7	5.74	154.5–178.4	165.9	5.94	155.0–178.1
Waist circumference	76.2	9.33	63.0–93.0	76.0	12.1	63.5–106.0	76.3	11.21	63.0–100.0
Hip circumference	99.2	9.68	86.0–119.0	98.2	10.63	86.0–122.0	100.0	10.92	88.0–127.0
Body weight	62.7	13.57	46.0–93.0	61.2	14.26	47.8–102.0	62.6	14.94	47.6–102.0
BMI	22.5	3.74	16.8–30.3	22.2	4.24	16.7–33.2	22.6	4.40	16.9–33.4
WHR	0.8	0.05	0.7–0.9	0.8	0.06	0.7–0.9	0.8	0.05	0.7–0.9
Rohrer's	1.4	0.20	1.0–1.7	1.3	0.24	1.0–1.9	1.4	0.24	1.0–1.9

Table 2. Arithmetic means of body measurements and somatic indices in male participants over the course of the 3-year study

Measure/index	2014 (1st grade, n = 7)			2015 (2nd grade, n = 4)			2016 (3rd grade, n = 6)		
	x	SD	min–max	x	SD	min–max	x	SD	min–max
Calendar age	16.32	0.29	15.97–16.69	17.25	0.36	16.90–17.60	18.43	0.310	17.96–18.71
Body height	179.57	7.38	171.5–191.1	178.85	8.21	172.4–190.7	180.77	7.570	173.00–178.20
Waist circumference	80.71	7.85	69.0–93.0	83.25	5.25	76.00–88.00	84.50	4.950	77.00–90.00
Hip circumference	99.0	8.64	90.0–112.0	100.50	7.05	92.00–109.00	99.50	4.230	93.00–106,00
Body weight	72.71	8.46	60.0–85.0	76.78	9.84	63.10–86.2	76.82	6.720	64.80–84.10
BMI	22.58	2.60	19.17–25.66	23.95	1.99	21.23–25.50	23.49	1.210	21.65–24.82
WHR	0.80	0.03	0.77–0.84	0.83	0.02	0.81–0.84	0.85	0.001	0.81–0.89
Rohrer's	1.26	0.17	1.00–1.44	1.34	0.12	1.23–1.45	1.30	0.090	1.19–1.40

Comparative analysis of arithmetic means of the measurements and somatic indices demonstrated that the differences in the results of female students in 1st, 2nd and 3rd grade were not statistically significant. This finding suggests that questionnaire responses and body measurements did not differ sufficiently for it to be borne out by significance calculations. It needs to be emphasised that only $n = 21$ girls were included in significance calculations, i.e. only those respondents who were examined three times.

Women's somatic indices

Table 3. Results of young women's somatic indices with categorisation

Index	Category	2014 (1st grade, n = 23)		2015 (2nd grade, n = 24)		2016 (3rd grade, n = 23)	
		n	%	n	%	n	%
BMI	underweight	2	9	2	8	2	9
	normal	17	74	18	75	17	74
	excess kilograms	4	17	4	17	4	17
WHR	gynoid type	15	65	18	75	18	78
	android type	8	35	6	25	5	22
Rohrer's	lean figure	14	61	16	67	15	65
	corpulent figure	9	39	8	33	8	35

Men's somatic indices

Table 4. Results of young men's somatic indices with categorisation

Index	Category	2014 (1st grade, n = 7)		2015 (2nd grade, n = 4)		2016 (3rd grade, n = 6)	
		n	%	n	%	n	%
BMI	underweight	0	0	0	0	0	0
	normal	5	71	2	50	6	100
	excess kilograms	2	29	2	50	0	0
WHR	gynoid type	2	29	0	0	0	0
	android type	5	71	4	100	6	100
Rohrer's	lean figure	4	57	2	50	3	50
	corpulent figure	3	43	2	50	3	50

The activity levels for young women over the course of the three years of study were relatively stable. In first grade, 83% of girls were active or highly active, while 17% were minimally active. Moreover, as many as 65% of all young women engaged in additional sports activities in their free time. First grade was characterised by more physical activity than school years two and three among women. In second grade, active and highly active women accounted for 58%, and minimally active women for 42%, whereas in third grade these figures were 56% and 44% respectively, with additional sports activity pursued by 61% of the female respondents. The intensity of physical exercise declined year by year.

A similar percentage distribution was observed in women's responses to the question about their body satisfaction. The highest degree of dissatisfaction was visible in first grade (56% dissatisfied), going hand in hand with the highest degree of physical activity (83%). One of the explanations may be that the girls exercised because they wanted to lose weight. In second and third grade of high school, female students tended to be happier about their bodies (54%), even

though a decline in physical activity was observed in the last two years of school. This may reflect a growing acceptance of own body among the studied women. This finding goes against the grain of many other studies on this subject, where girls with age get increasingly dissatisfied and undertake more measures to improve the appearance of their bodies, often resorting to drastic methods, such as for instance using restrictive and unhealthy diets and engaging in excessively strenuous exercise.

The level of physical activity among the studied men was more satisfactory. In first grade, 100% of men were active or highly active, in second grade this number dropped to 75%, and a sedentary lifestyle was declared by 25%. In turn, in third grade active and highly active men accounted for 80% and minimally active men for 20%, which means that the studied young men were physically active. All third-grade students declared that they engaged in additional sports other than physical education classes at school.

The percentage distribution of body satisfaction among men shows that in first grade 71% were dissatisfied, 29% were rather satisfied, and there was no-one fully satisfied with his body. Second grade brought about a significant change, because those fully and partly satisfied accounted for 75% in total, and only 25% were dissatisfied (a drop from 71 to 25%), whereas in third grade the level of dissatisfaction went up again to 50%.

These results show that young men were more critical of their bodies than women, especially so in first and third grade, with the following dissatisfaction trend in girls: 56% → 46% → 46%, and in boys: 71% → 25% → 50%.

BMI scores show that a strong majority of the class included in the study, both sexes, fell in the normal range. Among women, in the course of the three years 74–75% of the group fell in the normal range, 8–9% were underweight, and 17% had excess kilograms. According to Rohrer's index, 61–67% of the girls had lean figures in the space of three years. These numbers show that the young women were too critical in their body self-assessment. On the other hand, WHR results for women show that such strong body dissatisfaction among female students may have been caused by the pattern of body fat distribution, as well as too much or too little body fat. The android type (the apple shape – typical of men) was found in 22–35% of the young women in the space of three years. The android type in women may be caused by too much visceral fat or too narrow hips and means that the female figure loses its characteristic hour glass shape.

Among the young men, no-one was underweight, but two individuals in first grade represented the gynoid type of fat distribution (the pear shape – characteristic for women). These results may suggest that body satisfaction among men goes up together with body weight (particularly muscle mass) and in correlation with body shape, which is a reflection of today's male body trends.

Discussion

The analysis of the above results reveals little change in terms of body height for both young men and women involved. Body weight, waist circumference and hip circumference also remain almost unchanged in women. In young men, however, these three parameters paint a different picture, whereby over the course of three years significant changes can be seen, in terms of both body weight, which gradually went up by an average of about 4 kg, and waist circumference, which went up by about 4 cm on average, with negligible fluctuations in hip circumference.

The small differences in body build of the students included in the study are probably due to the fact that high-school age coincides with the final stage of puberty, when growth rate is not as spectacular as the growth spurts in middle school. Greater differences in body measurements

and weight among the studied young men may on the one hand be attributed to the later onset of puberty, which continues in men up to more or less 20 years of age, where in women it is over by the age of approx. 18. On the other hand, faster and greater growth of muscle mass is natural for men at this age. Another factor at play may be that the young men in this study were involved in much more physical activity than women, which translates into muscle growth, and consequently weight gain. It is a common practice among young men to put on weight deliberately, so as to sculpt a muscular physique later. Nearly all men included in the study declared high physical activity, which is related to a keen interest in sports, including working out at the gym, team games and martial arts, among men. Some studies show that more and more women are interested in "sculpting" their bodies, but this aspiration is much more common for men (McCreary, Saucier, 2009).

Physical activity is a very important factor facilitating weight loss, whose effects are more durable if the individual (particularly an overweight person) is more disciplined about keeping up regular physical exercise. The type of physical activity chosen may be very different and, like with the choice of diet, there is no one right way that will achieve the same therapeutic effect in everyone. Besides, any form of activity has a profound impact on improving psychosomatic well-being (Romanowska-Tołłoczko, Kałwa, 2014).

Weight loss should not exceed 0.5 kg per week, and the optimal rate of weight loss is 0.25 kg per week, seen as at this pace it will not slow down metabolism (Levitsky, 2005). Rapid weight loss is conducive to (just as rapid) weight gain after coming off the diet, and the pace of both these processes is proportionate (McGuire, 1999).

To sum up, while studies show that women generally want to reduce their body weight (Lipowska, Lipowski, 2006), two trends can be identified with regard to men: one is to get rid of surplus kilograms, but the other, much more common, is to increase muscle mass – which inevitably leads to an increase in overall body weight (Tylka, 2011).

In recent years, there has been a growing number of empirical reports indicating that more and more men are dissatisfied with many different aspects of their bodies (Frederick et al., 2007). Studies on the attractiveness of male body to the opposite sex show that men tend to overestimate the role of a muscular physique as an important determinant in the choice of a partner (Diedrichs, Lee, 2010). Own research shows clearly that when students engaged in additional sports activity, their body satisfaction increased.

However, body measurements and weight may not be treated as the only determinants of men's increasing dissatisfaction. Men attach equal importance to physical fitness, which is a guarantee of self-confidence and attractiveness to the opposite sex (Lipowski, Lipowska, 2015).

The very small number men in the study group generally prohibits far-reaching conclusions from statistical analysis. The class included in the study was predominantly female.

Studies show that the most common nutritional errors among teenagers are related to the insufficient intake of fruit and vegetables in everyday diet, coupled with excessive consumption of fats and sweets. The favourite food products named by young people include calorie-rich fast-food type meals (Wojtyła-Buciora, Marcinkowski, 2010).

When it comes to the choice of food for its health benefits, women fare better than men, but their results are far from satisfactory. In first grade 52% of young women declared they take note of what they eat. Otherwise, 22% of female students said they do it sometimes, and 26% did not pay attention to the health properties of the food they eat, so in other words their food choices were not health-oriented. The second year at high school was different, with the following respective percentage distribution: 46% (yes), 46% (sometimes) and 8% (do not pay attention to the choice of

food). There was a noticeable drop in second grade in the number of girls who paid no attention whatsoever (26% → 8%), and an increase among those who sometimes paid attention to food choices (22% → 46%). In third grade the picture gets brighter because the choice of healthy food was always an issue for 52% of female respondents, sometimes – for 44%, and only 4% ignored the health properties of what they ate. This may be a sign of a growing awareness among the young women as to the positive impact of nutrition on one's body and appearance, pointing to the students' growing knowledge about the disastrous effects of junk food.

Among the studied men, the majority unfortunately did not attach much weight to what they eat. In first grade, 43% of the studied young men responded that they do not pay attention to the health benefits of food, the same percentage said they sometimes paid attention, and only 14% chose their meals consciously. Responses in second grade were more varied, with 50% of male respondents declaring they do not pay attention to the quality of food, 25% that they take note sometimes, and 25% that they chose their meals consciously. Third grade revealed the greatest diversity in survey responses because as many as 67% of respondents did not pay attention to the choice of food products, but on the other hand, the greatest percentage of all three years, 33%, declared that they chose food products according to their health properties.

In the final year at high school a clear division becomes visible between conscious eaters and those who do not pay attention to the nutritional value of their meals. This may be a token of the growing knowledge about healthy nutrition in the Polish society. It may be assumed that awareness of healthy eating, nutritional value of food products and the role of physical activity for shaping a healthy body will continue to grow. It is encouraged by the growing trend for "being fit", which is increasingly promoted in social media and on television, by celebrities, presenters as well as professional trainers and chefs.

Conclusions

The analysis of results has led to the following final conclusions:

1. Only half of young women made their food choices based on health benefits. The young men included in the analysis were less likely to pay attention to healthy food choices.
2. The strong body criticism among first-grade female students may be explained by the fact that finding themselves in a new peer group made them conscious of the shortcomings in their bodies and awoke aspirations for a slimmer figure.
3. The differences in survey responses and body measurements in the young women included in the analysis over the course of three years of observation did not have statistical significance. It means that changes in body measurements and responses provided by female students in the questionnaire were rather small.
4. A lower degree of body satisfaction among the young men corresponded to an increased interest in additional sports activity.
5. The interest in physical activity among the young women was relatively stable over the three years of study, peaking in first grade. Among the young men physical activity remained high throughout high school, and was especially so in first and third grade.
6. In the space of three years of high school, as many as four girls and two boys (in the first two years of study) had dangerously elevated BMI scores. Excess weight at such an early stage of adulthood may lead to the development of metabolic disorders and result in diseases of civilisation.
7. Satisfaction levels in young men increased together with body weight.

8. There is a visible need for the development of programmes promoting body awareness and self-acceptance among school youth.

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Growth hormone and growth hormone gene of the American mink (*Neovison vison*) – the current state of knowledge of one of the key hormones in one of the most intensively economically exploited species

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Abstract Despite the fact, that the American mink (*Neovison vison*) is one of the most intensively economically exploited and problematic, from the ecological point of view, fur-bearing animal, it remains one of the least studied livestock species. It can be proven by the fact, that the research on one of the key hormones, which has systemic effects, that is the growth hormone, are rather poorly advanced. The purpose of this review is to present the summary and the critical analysis of the current state of knowledge on the topic of the growth hormone in the American mink, its biological function, production in physiological conditions, regulation of secretion and transduction of the hormone's signal. The article contains also an up to date information on the Amrican mink growth growth hormone gene and its protein product.

Hormon wzrostu i gen hormonu wzrostu norki amerykańskiej (*Neovison vison*) – stan wiedzy na temat kluczowego hormonu u jednego z najintensywniej eksploatowanych gospodarczo gatunków zwierząt

Słowa kluczowe Norka amerykańska, hormon wzrostu, gen hormonu wzrostu, funkcje fizjologiczne, wydzielanie, transdukcja sygnału

Streszczenie Mimo że norka amerykańska (*Neovison vison*) jest jednym z najintensywniej eksploatowanych gospodarczo, a także problematycznych, z ekologicznego punktu widzenia, gatunków zwierząt futerkowych, pozostaje jednym z najsłabiej zbadanych gatunków zwierząt gospodarskich. Dowodzi tego, między innymi fakt, że badania nad jednym z kluczowych hormonów o działaniu systemowym, jakim jest hormon wzrostu, są słabo zaawansowane. Celem niniejszego artykułu jest przegląd i krytyczna analiza aktualnego stanu wiedzy na temat hormonu wzrostu u norki amerykańskiej, jego funkcji biologicznej, produkcji w warunkach fizjologicznych, regulacji wydzielania oraz transdukcji sygnału. Artykuł zawiera również aktualne informacje o genie hormonu wzrostu oraz jego produkcie białkowym.

Introduction

The history of the systematic farm breeding of the American mink (*Neovison vison* Schreb., 1777) has only began in the sixties of the XIX c. when, due to the fear of the excessive depletion of the wild population and difficulties in obtaining fur in quantities to meet the growing market demand, the first permanent farms were established (Shackelford, 1949; Bowman et al., 2007). The dynamic development of the farm breeding of *N. vison* can be evidenced by the fact that only 90 years from the initiation of the domestication process, the number of animals kept on farms around the world was approx. 10–11 million (Thompson, 1968). Today, this dynamics is much greater – in 2001 the world production of mink fur was approx. 29.5 million furs, while in 2012 already approx. 59.1 million were produced (Kopenhagen Fur, 2013). At the same time, the value of the global market for the American mink furs increased from over 9.8 to over 14.0 billion US dollars (Ward, 2011).

The cited data indicate that the American mink is today one of the most intensively economically exploited livestock species. All the more surprising is the fact that the research on one of the key hormones, which has systemic effects, that is the growth hormone (GH, somatotropin), are rather poorly advanced (Sereikaite et al., 2006). The summary and the critical analysis of the current state of knowledge on the topic of somatotropin in the American mink, which is the aim of the present paper, seems particularly important from this point of view.

The first international standard of the growth hormone was developed in 1955 for the bovine somatotropin, while in 1982 for the human growth hormone (Bristow, 1999). Such a standard is lacking for somatotropin of *N. vison* (Sereikaite et al., 2007).

The amino acid sequence of the growth hormone of the American mink (*mGH*) was for the first time reported in 1990 (Shoji et al.). Two efficient methods of a recombinant mink somatotropin production were developed in the cells of *Escherichia coli* – the first one in 1992 (Harada et al., 1994), while the second one in 2006 (Sereikaite et al., 2006; Sereikaite et al., 2007). Previously, this hormone has only been obtained in small amounts, by extraction from the mink pituitary glands (Harada et al., 1994).

It should be noted that a few studies on the mink somatotropin polypeptide are limited substantially to the development and improvement of methods for the obtaining the recombinant *mGH* in bacterial cells (Harada et al., 1994; Sereikaite et al., 2006; Sereikaite et al., 2007), as well as by optimizing the conditions of its storage and processing (Bajorunaite et al., 2007; Borromeo et al., 2008; Cirkovas, Sereikaite, 2010; Cirkovas, Sereikaite, 2011a,b; Zilinskas, Sereikaite 2011). This fact explains the scarcity of the available literature data on the specificity of the growth hormone molecule of *N. vison* (Sereikaite et al., 2006).

Hormone of growth only?

A characteristic feature of the growth hormone is the functional and systemic multidirectional impact (Waters et al., 1999; Sirotkin, 2005). Somatotropin is the major non-genetic factor stimulating the postnatal body growth, by induction of growth and differentiation of cells of the mesenchyme-derivative tissues. However, its action is not limited to the stimulation of growth, regeneration, differentiation and development of cells, tissues and organs, but it also includes the metabolic interaction on proteins, carbohydrates and fats, participation in the mineral economy and in processes connected with reproduction and immune functions of the body (Chawla et al.,

1983; Hull, Harvey, 2000a; Okada, Kopchick, 2001; Li et al., 2005; Huising et al., 2006; Giustina et al., 2008; Breederveld, Tuinebreijer, 2012; Skottner 2012).

The primary function of the growth hormone is the postnatal stimulation of the bone, cartilage, muscle and fat tissue development, taking place both directly – by the presence of specific receptors on the cells of these tissues, as well as indirectly – by the insulin-like growth factor-1 (IGF-1) (Okada, Kopchick, 2001; Li et al., 2005; Huising et al., 2006). It is particularly important to stimulate the growth of bones at length, by the direct interaction with receptors present on the surface of cells of the proliferative layer of the growth plate of long bones (Giustina et al., 2008; Skrzypczak et al., 2011). It has also been shown that the GH has an effect on the activation of growth and differentiation of chondrocytes, osteoblasts and osteoclasts, leading to the increase of the bone mass (Sims et al., 2000; Giustina et al., 2008). This process is promoted by the stimulation of the collagen synthesis, as well as the effect of the hormone on the mineralisation of the bone tissue, by the regulation of the activity of the renal 1α-hydroxylase 25(OH)D (EC 1.14.13.13), and thus the calcium-phosphate metabolism (Roy et al., 1997; Vestergaard et al., 2012).

The GH impact on the muscles refers to the hypertrophy of muscle fibres, creation of new fibres and their regeneration (Iida et al., 2004; West et al., 2010). The growth hormone also conditions the proper composition of the body, mostly within the volume and topography of the fat tissue, as well as it regulates the proper development and functioning of many organs and organ systems, like heart and circulatory system, brain and nervous system, stomach and digestive system, kidneys and excretory system, as well as lungs and respiratory tract (Nass et al., 1995; Merola et al., 1996; Parks et al., 1998; Waters et al., 1999; Frago et al., 2002; Napoli et al., 2003; Dattani, Preece, 2004; Biller, 2007; Krysiak, Okopień, 2007; Harvey, 2010).

The crucial role of somatotropin was proven in the stimulation of proliferation and regeneration of trophoblast, blastocyst, cells of the immune system, pancreas and endothelial cells (Ikeo et al., 2001; Nielsen et al., 2001; Jeay et al., 2002; Markham, Kaye, 2003; Lacroix et al., 2005; Breederveld, Tuinebreijer, 2012). It was stated that GH, next to the promotion of the proliferative activity, shows the ability to inhibit apoptosis (Kölle et al., 2003; Bogazzi et al., 2004). The growth hormone also has a great meaning in the regeneration of nervous fibres and in wound healing (Breederveld, Tuinebreijer, 2012; Devesa et al., 2012). GH stimulates migration and proliferation of neural stem cell (NSC), as well as induction of differentiation of progenitor nervous cells (Pathipati et al., 2011). On the other hand, it has been proven that this hormone stimulates, in an autocrine path, the cancer transformation, being able to participate in the process of carcinogenesis in some tissues (Perry et al., 2006; Harvey, 2010).

It is very important that promoting growth through GH takes place indirectly, by inducing the synthesis of IGF-1 in the liver, muscles, lungs, bones, or cartilage (Okada, Kopchick, 2001). In fact, distinction of functions and involvement of GH and IGF-1 in particular physiological processes is often very difficult (Mauras, Haymond, 2005). Although IGF-1 is a major systemic factor mediating in the growth hormone action, it has been proven that this hormone stimulates synthesis of many other growth factors and receptors specific for them, e.g. Fibroblast Growth Factor (FGF), Hepatocyte Growth Factor Receptor (HGFR), Epidermal Growth Factor Receptor (EGFR), Nerve Growth Factor Receptor (NGFR) (Ekberg et al., 1989; Ekberg et al., 1992; Scharfmann et al., 1994; Izumi et al., 1995). It should be noted that the synthesis of certain growth factors, induced by somatotropin, shows clear tissue-organ specificity (Waters et al., 1999).

In the American mink the most intense growth of the body takes place during the first eight months of life. This is evident in the body weight growth in the time function, while the top and

stabilization of the body weight of the pups born in April takes place in November (Ahlstrøm et al., 2006; Liu et al., 2011).

The growth hormone participates in the metabolism of basic organic compounds for the body functioning – proteins, carbohydrates, lipids and vitamins (Feld, Hirschberg, 1996; Chen et al., 1997; Møller, Jørgensen, 2009; Vijayakumar et al., 2010). Metabolic effects exerted by somatotropin are frequently matched with those caused by insulin and, therefore, divided into the insulin-like effects (anabolic – metabolism of proteins) and against-insulin effects (catabolic, diabetogenic – metabolism of fats and carbohydrates) (Renaville et al., 2002; Dominici et al., 2005; Mauras, Haymond, 2005; Vijayakumar et al., 2010). Generally speaking, metabolic effects of the growth hormone manifests in synthesis and increase of the protein amounts, lowering the use and consumption of carbohydrates and mobilization of fat reserves (Renaville et al., 2002; Vijayakumar et al., 2010).

In the scope of the systemic protein metabolism GH increases the uptake and trans-membrane transport of amino acids into the cell (synergistic effect with respect to insulin) and stimulation of translation (Breier, 1999; Hossner, 2005). Protein synthesis relates primarily to the muscle tissue (Fryburg, Barrett, 1993; Hossner, 2005). Participation of the growth hormone in the protein metabolism is connected with the retention of nitrogen compounds in the blood and the limitation of their urinary excretion, as well as the decrease of catabolic transformations of proteins, their increased assimilation and utilisation (Breier, 1999; Hossner, 2005).

With regard to the carbohydrate metabolism, the growth hormone shows the insulin-like and antagonistic effects towards insulin at the same time (Renaville et al., 2002; Mauras, Haymond, 2005). This hormone also affects the increased uptake of glucose in the muscles and the decrease of its uptake in the adipose tissue, as well as stimulates gluconeogenesis and glycogenolysis in the liver and its increased release from this gland, causing the increase of the glucose level in the blood (diabetogenic effect) (Dominici et al., 2005; Hossner, 2005; Møller, Jørgensen, 2009; Kim et al., 2012). GH affects the reduction of sensitivity of the cells to insulin (especially adipocytes), and also stimulates its release (Manson, Wilmore, 1986; Nam et al., 2001; Renaville et al., 2002; Dominici et al., 2005; Hossner, 2005; Freda et al., 2008). This is mainly done by increasing the level of sugar in the blood and stimulation of the hypertrophy of pancreatic β-cells (Nielsen, Serup, 1998; Nielsen et al., 2001; Dominici et al., 2005). The sustained hyperglycemia associated with the persistence of the elevated levels of somatotropin, again stimulates the pancreatic islets to secrete insulin and can lead to the depletion of β-cells of Langerhans islets (Hellerström et al., 1984; Dimitriadis et al., 1985). In the glucose metabolism GH can be considered the "hunger hormone", promoting lipolysis, hyperglycemia and insulin resistance (Desborough, 2000; Clemmons, 2004; Freda et al., 2008).

Also in the case of the lipid economy the growth hormone shows the effect opposite to the insulin interaction, demonstrating lipolytic effects and affecting the reduction of lipogenesis (Etherton, 2000; Hossner, 2005; Freda et al., 2008; Bergman et al., 2012). Somatotropin causes the hydrolysis of triacylglycerol of the fat tissue, releasing free fatty acids (FFA) and glycerol to the blood (Manson, Wilmore, 1986; Møller et al., 1990; Richelsen, 1997). Mobilization of FFA from the adipose tissue contributes to the reduction of body weight and decrease of the adipose tissue volume (Etherton, 2000; Nam et al., 2001; Pasarica et al., 2007). GH also causes the reduction of cholesterol concentration in the plasma, stimulates the conversion of fatty acids to acetyl coenzyme A, exhibits ketogenic effects, leading to the increase of the ketone bodies concentration in the blood (ketosis) and their increased excretion in the urine (Manson, Wilmore, 1986; Møller et al., 1990; Nam et al., 2001; Møller et al., 2007; Vijayakumar et al., 2010; Palakawong, Arakaki,

2012). Somatotropin also conditions the proper differentiation and maturation of adipocytes (Nam, Lobie, 2000; Farnier et al., 2003).

The growth hormone participates in the systemic mineral metabolism, affecting the positive balance of nitrogen, phosphorus, potassium, calcium, sodium and magnesium (Manson, Wilmore, 1986; Pointillart et al., 1994; Baum et al., 1996; Dimke et al., 2007; Kamenicky et al., 2008; Auriemma et al., 2010). This is done, among others, by the increase of the alkaline phosphatase content (EC 3.1.3.1) and inorganic phosphorus in the plasma, reduction of the content of nitrogen in urea, increase of absorption of calcium in the gastrointestinal tract (more efficient than the secretion of this element in the urine, under the influence of GH), increase of resorption of electrolytes in renal tubules and their retention (Gertner et al., 1979; Dahms et al., 1989; Marcus et al., 1990; Yeh, Aloia, 1990; Baum et al., 1996; Dimke et al., 2007; Kamenicky et al., 2008; Auriemma et al., 2010). By participating in the systemic electrolyte economy GH plays an important role in maintaining water homeostasis of the body (Dimke et al., 2007; Auriemma et al., 2010).

The growth hormone plays an important role in many physiological functions related to reproduction, both in females and males (Woliński, 1964; Sirotkin, 2005). In both sexes this hormone participates in sexual maturation and conditions the sexual dimorphism (Hull, Harvey, 2001; Low et al., 2001). It has been shown that somatotropin stimulates fertility and animal fecundity (Sirotkin, 2005).

In females, the growth hormone, together with gonadotropins, stimulates folliculogenesis and the luteinisation process, as well as participates in the regulation of ovulation (Eckery et al., 1997; Hull, Harvey, 2001). GH also participates in the regulation of the secretory function of follicle, stimulating the synthesis of steroid hormones (steroidogenesis) in granulosa cells and ovarian theca (Gregoraszczuk et al., 2000; Hull, Harvey, 2001). Somatotropin here acts both directly and through the IGF-1 produced locally in ovary (Hull, Harvey, 2001). It is also suggested that the synergistic effect of the growth hormone and gonadotropins, consisting of the mutual increase of the number of specific receptors in the follicle cells by these hormones (Adashi et al., 1994). It should be noted that in the case of the reproductive system of females, GH can show the effects on the endocrine path, through pituitary somatotropin, as well as auto-, para- or intracrine, through the hormone produced locally in the ovary (Schwärzler et al., 1997). The presence of receptors for GH was shown in the luteal tissue in many mammal species, where it stimulates secretion of progesterone (P_4) and maintains the function of the corpus luteum (Carlsson et al., 1993; Liebermann, Schams, 1994; Juengel et al., 1997). The stimulation of the increase of proliferation and uterine cells has also been shown (Gunin, 1997).

Although, the growth hormone is of secondary importance to the foetus, it is essential for the proper development in the prenatal period – it conditions the implantation of blastocyst, regulates trophoblast growth and development of placenta, as well as participates in the pre-birth regulation of metabolism of liver in mammal foetuses (Labastie et al., 1998; Markham, Kaye, 2003). In the body of the pregnant female, GH conditions the repartition of nutrients between the mother's body and the foetus (Hull, Harvey, 2001).

GH plays a very important role in the metabolic adaptation of the female's body to pregnancy (Handwerger, 2009). Somatotropin also shows outstanding lactogen effects, thanks to the trophic and mitogene impact to the cells of the mammary gland (Mulhall et al., 2005). GH also induces the expression of key genes of milk proteins – casein and lactabumin (Sakamoto et al., 2005; Zhou et al., 2008; Johnson et al., 2010).

In males, GH conditions the proper growth and development of testes (Ohyama et al., 1999; Hull, Harvey, 2000b). Somatotropin takes part in the differentiation of Leydig cells (Kulin et al.,

1981; Kanzaki, Morris, 1999). This hormone is involved in the regulation of steroidogenesis on the endocrine path, by sensitizing the Leydig cells to the effects of the luteinizing hormone (LH), in turn leading to the increase of testosterone secretion and induction of expression of genes for the crucial enzymes and regulatory proteins in the steroidogenesis process (Chatelaine et al., 1991; Ohyama et al., 1995; Kanzaki, Morris, 1999; Mani et al., 2000). By acting on the Sertoli cells, GH stimulates the differentiation of germ cells at all stages of spermatogenesis (Swanlund et al., 1995). The growth hormone is therefore an important factor regulating both spermatocytogenesis and spermiogenesis (Hull, Harvey, 2000b). It has also been shown that GH conditions the proper development of Wolff ducts in males, and in the post-natal period the proper functioning of their derivative structures – prostate and vas deferens (Reiter et al., 1992; Ghosh, Bartke, 1993; Nguyen et al., 1996).

The growth hormone participates in the proper functioning of the immune system, among others, by regulation of the functioning of thymus and spleen (de Mello-Coelho et al., 1998; Dialynas et al., 1999). This takes place by stimulation of proliferation of thymus cells, inducing the thymulin production, conditioning the blast transformation of T lymphocytes and participation in the regulation of their apoptosis (Timsit et al., 1992; de Mello-Coelho et al., 1998; Dobashi et al., 2001). It was also shown that GH conditions the survival of thymus-dependent lymphocytes in the stress conditions (Murphy et al., 1999). Receptors of the growth hormone are present on the surface of the immune system cells and the production of GH was stated by the lymphoid tissue (Jeay et al., 2002). Somatotropin also stimulates the production of interleukin 6 (Saggese et al., 1993).

In addition to these physiological functions, the growth hormone is characterised, through the signal path of the insulin-like growth factor-I, with the notably systemic effects on the process of the body aging and conditioning of the length of life of the individual (Okada, Kopchick, 2001; Holzenberger, 2004). It has been shown that the reduced level of somatotropin greatly increases the life span of animals (Holzenberger et al., 2004; Masternak, Bartke, 2012). A clear positive correlation between the systemic level of GH and the progress of the body aging processes is connected, most probably, with the great energy and substrate absorption of processes conditioned by this hormone (mainly the stimulation of growth and development and regulation of the body composition) (Masternak, Bartke, 2012). It was also shown that the increase of the hormone level in mature individuals results in the reduction of resistance to oxidative stress, and consequently may lead to metabolic complications and the increase of the risk of occurrence of cancerous changes (Hoffman, Ceda, 2004; Janssen, Lamberts, 2004).

The growth hormone, prolactin and placental lactogen show considerable structural and functional similarities, among others, in the stimulation of proliferation and differentiation of cells of many tissues (Wallis, 1992). Functionally, the growth hormone belongs to the so-called somatotropic axis, which also includes somatoliberin (GHRH), prolactin (PRL), receptors of the growth hormone (GHR) and prolactin (PRLR), insulin-like growth factor-I, transcription factor Pit-I and transcription factor STAT5 (Parmentier et al., 1999; Katoh et al., 2007; Bideci, Çamurdan, 2009).

Production in physiological conditions

The growth hormone in American mink, like in other mammals, is produced and secreted by the somatotropic cells and somatomammotropic cells of the frontal lobe of pituitary (*adeno-hypophysis*) (Machnik, Lechniak, 2000; Borromeo et al., 2008; Harvey, 2010). In addition to the

pituitary, the growth hormone is produced in, among others, gonads, uterus, placenta, mammary gland and by leukocytes, while the growth hormone receptor (GHR) is present on the surface of cells of most tissues (Kelly et al., 1991; Hull, Harvey, 2000a, 2000b; Hull, Harvey, 2001; Harvey, 2010).

Somatotropic cells of *N. vison* have the spherical or polygonal shape, and GH is stored and released from their electron-thick granules, with a diameter of approx. 210 nm (Vidal et al., 1995). It has been shown that the number of somatotropic cells considerably varies, depending on the age and physiological state of minks – the number of cells producing GH decreases during lactation, for the prolactin-secreting cells (PRL) (Vidal et al., 1995). An intermediate form between the somatotropic cells and the ones producting PRL mammotropic cells are the mammosomatotropic cells, which produce both the growth hormone and prolactin (Vidal et al., 1995, 1997).

The GH concentration in the mink blood is approx. 0.38 ng/ml in males and approx. 0.53 ng/ml in females (Ryökkynen et al., 2003). These are values lower than those stated in rats (2.4–2.8 ng/ml) and people (1.1–1.9 ng/ml) and higher from the concentration of GH marked in the raccoon dog plasma (0.1–0.3 ng/ml) (Mustonen et al., 2001; Nieminen et al., 2002; Barkan et al., 2003). It has also been proven that there exists a positive correlation between the concentration of the growth hormone in the blood and the body weight, as well as between the concentration of leptin and ghrelin in the blood plasma of American mink (Ryökkynen et al., 2003).

Mink growth hormone's protein

The growth hormone is included to the group of peptide hormones. The mGH particle (accession code *UNIPROT: P19795 SOMA_MUSVI*) has the structure of a simple polypeptide chain, composed of 190 amino acids (in the largest amount there is leucine, consisting 13.2% of all amino acids, while in the smallest one – tryptophan, consisting 0,5%), with the molecular weight of 21,717.84 Da (Harada et al., 1990; Rice et al., 2000). The mature hormone is formed from the precursor molecule, consisting of 216 amino acids, of which 26 form a signal peptide proteolytically cleaved during the post-translational processing (Shoji et al., 1990). The molecular formula of mGH is $C_{973}H_{1519}N_{263}O_{285}S_8$, and the theoretical value of the isoelectric point (pI) is 7.38 (Rice et al., 2000; Artimo et al., 2012). It was also found that the growth hormone is rapidly metabolised – the period of its half-life in the blood is approx. 20–50 min. (Goya et al., 1987; Faryna 2009; de Graaf-Roelfsema et al., 2011).

According to the system of structural classification of proteins SCOP, GH polypeptide has the *all-α* over-secondary structure type and belongs to super-family of 4-helical cytokines and the family of long-chain cytokines (Murzin et al., 2009). In the spatial structure of the growth hormone we can distinguish four left α-helix in the system of a bundle and spatial orientation of the "*up-up-down-down"* type, covering up to 70% of the polypeptide. The remaining part are the turns and random structures (Kopchick et al., 2002; Borromeo et al., 2008; Murzin et al., 2009). The GH particle also contains the hydrophobic core, consisting of approx. 20 amino acids (Kopchick et al., 2002).

The growth hormone of American mink, like in case of other mammals, contains in its structure of the polypeptide chain two internal disulfide bonds (disulfide bridges) between cysteines in the position of Cys78-Cys189 and Cys206-Cys214 (Watahiki et al., 1989; Shoji et al., 1990). Also the presence of two zinc binding sites in the amino acid sequence of the GH polypeptide of *N. vison* was shown – in the position of His45 and Glu198 (The UniProt Consortium, 2012).

The analysis of the primary structure of the growth hormone protein of American mink, for the presence of characteristic functional amino acids motifs, proves the existence of 14 such motifs (program PPSearch PROSITE, www.ebi.ac.uk/Tools/ppsearch). These include protein kinase C phosphorylation site (EC 2.7.11.13), casein kinase II phosphorylation site (EC 2.7.11.1), N-myristoylation site, leucine zipper pattern, somatotropin, prolactin and related hormones signature 1 and the motif of somatotropin, prolactin and related hormones signature 2.

The growth hormone has a high heterogeneity, i.e., multitude of structural forms (structural polymorphism); so far we approx. 30 isoforms of the human GH were identified (Baumann, 2009). Heterogeneity of the macro-molecule of somatotropin is the result of the alternative splicing, post-translation modifications, oligomerisation and polymerisation, existence of different variants of the quaternary structure and binding to the growth hormone binding protein (GHBP) (Junnila et al., 2008).

The alternative splicing of the translation product of the primary GH gene transcript in human leads to the synthesis of five isoforms of somatotropin, differing in size (from 7.4 to 22.0 kDa), binding to other binding places on the growth hormone receptor and showing the different biological activity (Takahashi, 2002; Longhi, 2003; Piekiełko-Witkowska, Nauman, 2011). So far also a series of post-translation modifications of the GH particle were described, like glycosylation, acetylation, deamidation, phosphorylation, proteolysis and oxidation (Lewis, 1984; Baumann, 1991; Diaz, 1993; Haro, Lewis, 1996; Baumann, 1999; Garcia-Barros, 2000; Baumann, 2009). Also the occurrence of different mer forms was observed (monomers, dimmers, oligomers and heteropolymers) of the variants of the growth hormone particle, however, their biological significance is not fully explained (Baumann, 1991; Junnila et al., 2008).

The growth hormone circulating in the blood is present partially in the form connected with GH proteins (Baumann, 2001; Baumann, 2009). Two types of these proteins were identified – GHBP with high-affinity, being the extracellular domain of the growth hormone receptor or the product of the alternative splicing of the GHR gene product, and GHBP with low affinity, being α2-macroglobulin or its modified form (Baumann, 2001). It is estimated that even 55% of the GH present in the human blood occurs in the form connected with GHBP with high-affinity, while with GHBP with low affinity – to 8% (Baumann et al., 1990; Veldhuis et al., 1993). It is also known that the amount of GH connected with GHBP subjects to the very dynamic fluctuations, constituting a significant reserve of the free hormone in the system (Veldhuis et al., 1993). This binding is reversible, and the growth hormone in the form of the GH-GHBP complex loses the ability to bind with the specific trans-membrane receptor, what results in the functional inactivation of the hormone (Baumann, 2009). On the other hand, the said complex protects the growth hormone from degradation and secretion from the system (Baumann, 1994).

The growth hormone of American mink exhibits a high level of homology of the primary structure with GH of other mammalian species (Morozov, Malchenko, 1993). For example, there was demonstrated the 97% relative similarity of the amino acid mGH sequence with somatotropin of the fox (Li et al., 1989) and dog (Queiroga et al., 2008), 96% with GH of the cat (Wallis, 2008) and pig (Seeburg et al., 1983), 94% with the growth hormone of the rat (Bailey-Downs, 2012), 88% with the domestic cattle (Heidari et al., 2012) and 66% – human (Martial et al., 1979). Despite the relatively small differences in the structure of polypeptide of somatotropin of different species of vertebrate, this hormone exhibits an excellent specificity of the species in relation to the biological activity (Lindholm, 2006). Such high species functional specificity is mainly connected to the different amino acid sequence of receptors for GH in different animals (Allan et al., 1999).

Studies, conducted in the recent years, on the biological activity of the purified mGH showed that it is equivalent only in 8% of activity proper for the growth hormone of cattle and in 63% for somatotropin of pigs. The concentration value of mGH causing the 50% of the maximal response (EC_{50}) in mink is approx. 103 ng/ml (Sereikaite et al., 2007).

Regulation of secretion

The concentration of the growth hormone in the blood is subject to fluctuations in the circadian rhythm (Veldhuis et al., 1991). Secretion of GH is pulsating, while its episodic nature depends on the gender and age (Veldhuis et al., 1991; Jaffe et al., 1998; Skottner, 2012). In humans approx. 10 secretions, lasting for about 30 minutes, of somatotropin in men and approx. 20 in women a day are observed, while the total amount of the released hormone in a day is greater in men (Cauter et al., 1992; Jaffe et al., 1998). With regard to the degree of GH secretion per year, no changes in the level of GH were stated in the pituitary of minks in different seasons, and only its reduction in feeding mothers was reported (Vitale et al., 2001).

The hormone content decreases with age – the decrease begins after the end of puberty, it is strictly correlated with the aging process and is determined in mature individuals as somatopause (Anawalt, Merriam, 2001; Krysiak et al., 2009).

The increased GH concentration in the blood is observed in gigantism (young individuals) and acromegaly (mature individuals), as well as liver cirrhosis, renal failure, anorexia nervosa, type 1 diabetes, hyperthyroidism, some cancerous changes of pituitary and in normal physiological conditions in pregnant females (Scheithauer et al., 1995; Barkan et al., 1997; Kuol et al., 2002; Grottoli et al., 2003; Pedersen et al., 2010). While the reduction of the growth hormone level usually proves the diseases damaging the pituitary gland or hypothalamus, caused for example by inflammation or degeneration, cancers and mechanical injuries of the head (Diaz-Espiñeira et al., 2008; Gardner et al., 2013; Hirohata et al., 2013). The low level of GH in the blood also occurs in dwarfism, obesity, hypothyrodism, hyperthyroidism and adrenal hypogonadism (Giustina et al., 1997; Kohn, Kopchick, 2002; Arnaldi et al., 2003; Diaz-Espiñeira et al., 2008; Alvarez-Castro et al., 2013).

The regulation of secretion of growth hormone is a highly complex and comprehensive process, including the hormonal factors, as well as environmental and behavioural ones, and the proliferation of somatotroph cells (Müller et al., 1999; Bideci, Çamurdan, 2009). The important meaning in the regulation of GH secretion is played by hypothalamic neurohormones and neurotransmitters, interacting with adrenergic receptors, dopaminergic, serotonergic, gabaergic or cholinergic (Ginalska-Malinowska, Malinowska, 2009). Release of the growth hormone in the pituitary gland primarily subjects to the double regulation by the hypothalamus – the hypothalamic growth hormone releasing hormone (GHRH, somatoliberine) stimulates, while growth hormone release inhibiting hormone (GHRIH, somatostatine) inhibits the secretion of the growth hormone, what is connected with the increased or reduced sensitivity of somatotroph cells to these neurohormones (Gianotti et al., 1999; Tannenbaum et al., 2007). Both of these neurohormones remain in the mutual feedback (Skoczylas, Więcek, 2006).

Among the peripheral hormones of endocrine glands the synthesis and GH secretion are stimulated by estrogens and androgens (especially testosterone, which acts here in a systemic way), gherlin, leptin, motilin, luteinizing hormone (LH), corticosterone (CORT), progesterone and vasopressin (Dean, Porter, 1999; Watanobe, Habu, 2002; Peeters, 2003; Katoh et al., 2005; Veldhuis et al., 2005; Tannenbaum et al., 2007). There were conducted studies on the associations

of leptin with fluctuations of the body weight in *N. vison*, which were indirectly affected by the growth hormone (Tauson et al., 2004).

A separate group of factors regulating the growth hormone secretion are the biologically active peptides and synthetic compounds. The first ones include somatotropin stimulating secretion, polypeptide activating pituitary adenylate cyclase activating polypeptide (PACAP) and the group, so-called growth hormone secretagogue (GHS) (McDowell et al., 1995; Montero et al., 2000; Smith et al., 2004; Skoczylas, Więcek, 2006). The most important synthetic chemical compounds stimulating the GH secretion are apomorphine (APO), propranolol, clonidine and synthetic growth hormone releasing peptide (GHRP), e.g. GHRP-6 and hexarelin (Low, 1991; Scacchi et al., 1999; Lengyel, 2006). The GH level also increases under the influence of glucogenic amino acids (arginine, glycine, glutamine), L-Doop, niacin, opioid peptides, galanin, vitamin A, or glucagon (Morita et al., 1989; Johnson et al., 1993; Zdrojewicz et al., 2000; Gómez et al., 2002; Arwert et al., 2003; Ginalska-Malinowska, Malinowska, 2009; Skottner, 2012; Vught et al., 2012).

Environmental factors, stimulating the growth hormone secretion, include the stressful stimuli (Desborough, 2000). Studies conducting on the impact of photoperiod on the secretion of somatotropin in American mink showed the lack of connection between the length of the light day and changes of GH content in the blood (Meunier et al., 1988).

The most important behavioural factor connected with the regulation of growth hormone secretion is the dream (Kim et al., 2010). The peak of GH secretion takes place within two hours from the occurrence of deep sleep (Born, Wagner, 2009). The behavioural factors stimulating the GH secretion are the states of deficiency of energy substrates (starvation, hypoglycaemia, presence of 2-deoxyglucose, physical effort) and high-protein diet (Daughaday, 1989; Sato et al., 1995; Kanaley et al., 1997; Nørrelund, 2005; Matthiesen et al., 2008; Goldstein et al., 2011). The stimulating effect of starvation on the secretion of the growth hormone in American mink is confirmed by the results of experiments by Rouvinen-Watt et al. (2010). However, at the same time, studies on the impact of the winter deficiency of food on the GH content in the blood of *N. vison* indicates the significant drop of the concentration of this hormone already after five days of starvation (Mustonen et al., 2005).

An important element of regulation of the production and secretion of GH is the stimulation of proliferation and differentiation of somatotrophic and somatomammotropic cells (Dean, Porter, 1999). The most important role here is played by glucocorticoids, thyroid hormones, corticosterone, cAMP, as well as CXC chemokines (Nogami et al., 1999; Dean, Porter, 1999; Lee et al., 2008; Lania et al., 2012). The direct cause of the growth hormone secretion, from granulates produced by eosinophils, is the increase of cytoplasmic level of cAMP and Ca^{2+} ions (Strobl, Thomas, 1994).

The factors inhibiting the growth hormone secretion include hyperglycaemia, cortisol, chronic lack of sleep, free fatty acids, neuropeptide Y (NPY), vitamin D, thyroid hormones and melatonin (Rettori et al., 1990; Barb et al., 1995; Giustina, Wehrenberg, 1995; Thompson et al., 1995; Seoane et al., 2002; Karasek et al., 2007; Kim et al., 2010; Skrzypczak et al., 2011). An important, inhibitory effect on the secretion activity of acid-absorbant cells of the pituitary gland is shown, in the mechanism of the negative feedback, by GH and IGF-1 present in the peripheral circulation (Yamasaki et al., 1991; Grilli et al., 1997; Skottner, 2012).

Transduction of the signal

The growth hormone affects the target tissues (cells) both directly, like hormone or cytokine, or indirectly, by stimulating the production and secretion of *insulin-like growth factor-I* (IGF-1),

mostly by the liver (Waters et al., 1999; Borst, 2004; Krysiak et al., 2009; Frystyk, 2010; Skottner, 2012). As a hormone, GH acts on the target cells on the classic endocrine way, like cytokine, on the about-crine path, paracrine and autocrine (Waters et al., 1999; Jeay et al., 2002; Soares, 2004; Harvey, 2010). The para- and autocrine impact most of all is shown by the locally produced somatotropin outside pituitary, while the pituitary nature of GH is rather systemic and endocrine (Harvey, 2010).

The mechanism of the direct effect of somatotropin is based, most of all, on its interaction with growth hormone receptor (GHR) and induced by this interaction intracellular transduction of the signal (Lanning, Carter-Su, 2006; Zych et al., 2006).

GHR belongs to the super-family of cytokine receptors of IA class and shows great structural-functional resemblance to the prolactin receptor (Goffin, Kelly, 1996; Zych et al., 2006). The somatotropin receptor is a transmembrane protein consisting of three domains: 1 – extracellular, binding ligands (GH molecule), 2 – transmembrane, anchoring receptor in the plasma, 3 – cytoplasmic, responsible for the transduction of the signal (Goffin, Kelly, 1997; Zych et al., 2006). Extracellular domain (ECD, identical to GHBP) contains two conservative evolutionary sub-domains – D1, containing six cysteines connected with disulfide bridges, and D2, with a characteristic motif YXXFS (tyrosine-glycine, serine, lysine or glutamic acid-phenylalanine-serine) (Moutoussamy et al., 1998; Zych et al., 2006; Conway-Campbell et al., 2008). Transmembrane domain (TMD) consists of a short polypeptide chain with the α-helical structure (Michalik, Bartoszewicz, 2002). While the characteristic elements of the cytoplasmic fragments of GHR (ICD, intracellular domain) is the box1 region, containing numerous prolines, and box2 region, consisting of the hydrophobic and acidic amino acids alternating in arrangement (Argetsinger, Carter-Su, 1996; Bole-Feysot, 1998; Moutoussamy et al., 1998). There were described several isoforms of the growth hormone differentiating in size and resulting from the alternative splicing (Moutoussamy et al., 1998).

GHR is activated by the dimerization induced by the binding of a receptor with the GH particle (Wells, 1996). The growth hormone in its particle has two receptor binding sites – high-affinity binding site 1 and low-affinity binding site 2 (Waters et al., 1999). Binding the growth hormone to the receptor particle with high-affinity binding site 1, results in the formation of the hormone-receptor complex (GH-GHR), activating low-affinity binding site 2 (Waters et al., 1999). Only binding of the second receptor particle (GHR homodimerization) by one GH particle gives an active complex GHR-GH-GHR (GH-GHR_2), which activates tyrosine kinases JAK2 (Janus kinase 2, EC 2.7.10.2), constitutively connected to the region box1 of both monomers GHR (Dinerstein et al., 1995; Wells, 1996; Zych et al., 2006). The activated kinases JAK2 phosphorylate themselves mutually and the tyrosine residues of the intracellular parts of the receptor itself (Ceseña et al., 2007). In such state receptor initiates the transduction of a signal into the cell, which results in the induction of the specific biological effect (Moutoussamy et al., 1998; Ceseña et al., 2007).

The transduction of the signal, inuced by the activation of the complex GH-GHR-JAK2, can take place through the engagement of the STAT1, STAT3, STAT5a and STAT5b proteins (Signal Transducer and Activator of Transcription) (Argetsinger, Carter-Su, 1996; Lanning, Carter-Su, 2006). Phosphorylated tyrosine residues of GHR enable the phospohorylation of the STAT protein particle, which then undergoes homodimerisation (Lanning, Carter-Su, 2006). The resultant STAT-STAT dimmers get into the nucleus, where they connect with specific nucleotide sequences of promoters of the target genes and activate them (Herrington et al., 2000; Waxman, O'Connor, 2006). STAT5a and STAT5b proteins exhibit strong affinity to specific sequences of the IGF-1 gene promoter, what proves the somatotropin dependent activation of transcription of this gene

and the crucial role of these proteins in the realization of the basic physiological functions of the growth hormone (Herrington et al., 2000; Waxman, O'Connor, 2006). On the other hand, it has been proven that the growth hormone and IGF-1 interact synergistically with respect to transduction of the induced intracellular signal and that this last one can efficiently strengthen the signal transmitted through GHR (Huang et al., 2004).

Signal paths of the growth hormone receptor can also be connected to phosphorylated cytoplasmic domain of proteins with the SH2 domain (Src Homology 2) (VanderKuur et al., 1995a). This happens in the case of activation of the signal path by the mitogen activated protein kinase (MAPK). In this case, the phosphorylated tyrosine residues of GHR enable the connection to the GHR-GH-GHR complex of the adaptor proteins Shc/Grb2/SOS, which in turn activate the cascade Ras/Raf/MAPK, responsible for the activation of many transcription factors and enzymes (VanderKuur et al., 1995b, 1997). The growth hormone can also use the signal path of the protein A kinase (EC 2.7.11.1), dependent on cAMP (Vijayakumar et al., 2011).

It is believed that the growth hormone can stimulate the phosphorylation of tyrosine residues of particles proper for the signal paths of insulin – insulin receptor substrates IRS-1, IRS-2 and IRS-3 (Insulin Receptor Substrate) and kinase 3'-phosphatidylinositol PI-3K (Tsuruzoe et al., 2001; Lanning, Carter-Su, 2006; Xu, Messina, 2009). The last one participates in the transmembrane transport of glucose, DNA synthesis and activation of ribosomal kinase $p70^{rsk}$, involved in the protein biosynthesis (Argetsinger, Carter-Su, 1996). Also important is the mediation of kinase 3'-phosphatidylinositol in GH-dependent re-arrangements of the actin and micro-tubular cytoskeleton (Goh et al., 1997; Goh et al., 1998). Through its receptor, the growth hormone can also activate the intracellular signal paths independent of kinase JAK2 (Lanning, Carter-Su, 2006). An example is the participation of the growth hormone in the cellular calcium economy, taking place through the tyrosine kinase Src (Zhang et al., 2006). Transduction of the signal induced by GH is sometimes connected with the intracellular changes of the concentration of calcium ions and takes place through the calcium channels dependent on kinase PI3-K and activation of protein kinase C (PKC, Protein Kinase C) (Moutoussamy et al., 1998).

In addition to the most important, above mentioned paths regulating the transcription of target genes, the growth hormone acts on many other levels of modulation of expression of the selected genes (Ceseña et al., 2007). The best known contain:

- epigenetic control of expression, by affecting the methylation and demethylation of DNA and the reversible conversion of euchromatin to heterochromatin (Waxman, O'Connor, 2006),
- GH participation in post-translational modifications (phosphorylation, acetylation, methylation, ubiquitination, sumoylation) of nuclear proteins included in the transcription complexes (Ceseña et al., 2007),
- the effect of somatotropin on the composition of nucleo-protein complexes (Ceseña et al., 2007),
- regulation of intracellular translocation of elements of the GH signal paths (Ceseña et al., 2007).

There was described the signal path independent of the membrane growth hormone receptor, through which GH directly regulates the metabolic activity of mitochondria, demonstrating the direct inhibiting effect on the succinate dehydrogenesis (EC 1.3.99.1) and cytochrome c oxidase (EC 1.9.3.1) (Ardail et al., 2010). The growth hormone also shows the ability to bind with the prolactin receptor, thus being the competitive antagonist of this hormone (Rose et al., 1986; Kelly et al., 1991).

Also the silencing factors of this signal are involved in the regulation of the transduction signal induced by GH, such as the suppressor of cytokine signalling (SOCS) protein and tyrosine phosphatase (EC 3.1.3.48) (Greenhalgh et al., 2002; Lanning, Carter-Su, 2006; Martinez et al., 2012). These molecules are involved in the molecular mechanism of ubiquitination, internalisation and degradation of GHR and dephosphorylation of the phosphorylated elements of the signal paths of the growth hormone (van Kerkhof et al., 2000; Rico-Bautista et al., 2004; Lanning, Carter-Su, 2006). Of fundamental importance is also the reduction of the number of free receptors and reduction of their affinity to GH, with the increase of its concentration (Deng et al., 2007).

In desensitization of intracellular signal mechanisms of GH there is also involved the phospholipase C (EC 3.1.4.3) and the reversible process of GHBP formation, through the GHR proteolysis, leading to the split of its extracellular domain (Fernández et al., 1998; Rui et al., 2000; Guan et al., 2001). This process takes place through the protein kinase path C (EC 2.7.11.13), involving the platelet-derived growth factor (PDGF) and lysophosphatidic acid (LPA) (Rui et al., 2000).

Recently, it has been proven that an important role in the regulation of the GH transduction signal is played by the environmental factors, such as body temperature – its decrease under the optimal value for the given species results in the prominent limitation of the response to the signal induced by the growth hormone (Nespital, Strous, 2012).

Growth hormone gene

The growth hormone gene, the prolactin gene (*PRL*), the placental lactogen (*PL*, chorionic sommatomammotropine, *CS*), the somatolactine (*SL*) occurring in fish and genes of a series of the GH-like proteins and prolactine-like proteins show great structural-functional similarity and belong to one, monophyletic family of the somatomammotrophic genes (Wallis, 1992; Goffin et al., 1995; Lin et al., 1997; Soares, 2004; Huising et al., 2006; Fukamachi, Meyer, 2007). Numerous data indicate that these genes evolved from the ancestral gene through duplication, deletion and insertion, resulting in its divergent evolution (Owerbach et al., 1981; Nicoll et al., 1986; Vidal et al., 1995). It is assumed that the closest to this original gene is currently the growth hormone gene, and the formation of a separate gene for prolactin probably took place not earlier than 400 million years ago (Miller, Eberhardt, 1983; Cooke et al., 1988; Kawauchi et al., 2002).

In most mammals (except for primate and some members of the Caprinae family) the growth hormone gene occurs in one copy (Chen et al., 1989; Cooke, Liebhaber 1995; Wallis et al., 1998; Krawczak et al., 1999). In American mink it is located in chromosome 5 (pp. 25–23) (Malchenko et al., 1994; Serov, Rubtsov, 1998). This location was specified thanks to the comparative genomics (Serov, 1998). Characteristic, also for *N. vison*, is the synteny of *GH*, *ALDC* (aldolase C gene), *HOXB* (homeotic B gene), *GALK* (galactokinase gene), *TK1* (thymidine kinase gene 1) and *UMPH2* (phosphohydrolasis 2 gene uridine-5'monophosphate) genes (Koroleva et al., 1996; Serov, 1998).

cDNA for the GH gene of American mink (*mGH*) was sequenced and described for the first time by Shoji et al. (1990) (GenBank: X56120.1). The sequence deposited in GenBank was then verified and supplemented by Harada et al. (1990) (GenBank: E04303.1) and Perelygina et al. (1991) (GenBank: E04303.1). The total length coding sequence (CDS) was specified on 648 bp (Shoji et al., 1990). There is also known the sequence of untranslated regios (UTR) in position 5' (fragment) and 3', enabling the localisation of the poliadenylation signal (5'-AATAAA-3') in position c.*84 –*89, as well as the flanking region 3' (Harada et al., 1990; Shoji et al., 1990). The complete sequence of the growth hormone gene in American mink was described by Skorupski (2017).

The *mGH* gene consists of 5 exons (10 bp, 161 bp, 117 bp, 162 bp, 201 bp) and 4 introns (245 bp, 171 bp, 176 bp, 290 bp), and its total length is 1745 bp (Skorupski, 2017).

Skorupski (2017) identified fourteen polymorphic variable sites – 12 SNP substitutions (g.616G>C, g.703G>A, g.742G>A, g.748T>C, g.775G>A, g.778G>A, g.837G>C, g.846A>G, g.931C>T, g.1156A>G, g.1219C>G, g.1329T>C), one single nucleotide deletion (g.885delC) and one ins/del polymorphism (18-nucleotide motif – g.1219_1236delCTCTTGCAGGGGCAGGGG) . It was shown that the biological effect of the American mink growth hormone gene is related to changes in a splicing regulatory sequences and sequence motifs, different reading of codons and on an influence on the mRNA secondary structure (Skorupski, 2015).

Haplotype analysis of polymorphisms in the American mink growth hormone gene revealed exsistence of four haplotype blocks, with a correlation coefficient ranging from 50 to 94% (Skorupski, 2016). The described LD proves a relatively low frequency of recombination events between variable sites of the *mGH* (Skorupski, 2016).

The expression on the level of growth hormone gene transcription regulates, most of all, a specific transactivator of the POU domain specific for the pituitary gland – transcription factor Pit-1 (Li et al., 1990; Gil-Puig et al., 2005). The key role in promoting the expression of the *GH* gene in somatotropic and somatomammotropic cells, as well as silencing of this expression in lactotropic cells, plays a supersensitive place for deoxyribonuclease I (HSI, deoxyribonuclease I hypersensitive site I), located from several to several dozens of kb under the gene promoter (Aizawa et al., 1995; Su et al., 2000; Ho et al., 2011). In some species HSI is located in the *locus* control region (LCR) of the somatotropin gene, also grouping other transcription regulatory genes (Su et al., 2000; Ho et al., 2011). In addition, the epigenetic regulation of the GH gene transcription was reported, connected to the acetylation of chromatin domains in the LCR region (Ho et al., 2002).

The coding sequence of the *mGH* shows a strong resemblance to the cDNA of the growth hormone gene of other mammalian species. The number of synonymous substitutions per nucleotide, compared to the gene of somatotropin of domestic goat, pig, rat, mouse, human and cattle is, respectively, 0.44, 0.33, 0.52, 0.48, 0.53 and 0.44. While the number of non-synonymous substitutions on average per nucleotide, compared to those species, is, respectively, 0.05, 0.01, 0.03, 0.03, 0.18 and 0.05 (Ohta 1993). At the same time, it is estimated that the *mGH* gene is divided, from ancestral growth hormone gene for mammals, by three non-synonymous substitutions and nine synonymous substitutions (Adkins et al., 2001).

It is worth mentioning that the growth hormone gene is characterised in mammals with low basic evolutionary rate, estimated at 0.25 substitutions/codon/year x 10^9 (Wallis, 2001). However, characteristic for the evolution of this gene are the periods of discrete, rapid changes, during which the evolution rate increases even 50 times in relation to the basic rate (Wallis, 1994; Wallis, 2001). Two similar cases of evolutionary acceleration in Mammalia group were described – in the evolutionary line of even-toed ungulates and primate (Wallis, 2001). Currently, it is estimated that approx. 58% of substitutions in the nucleotide sequence of the growth hormone gene of different mammalian groups is the result of the above described evolutionary changes of a step (punctualistic) nature and constitutes the confirmation of the hypothesis of the so-called episodic nature of the somatotropin gene evolution (Ohta, 1993; Wallis, 2001).

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Molecular basis of *quorum sensing* signal-response systems in bacteria

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Abstract Bacteria use *quorum sensing* (QS) to conduct gene expression programmes connected with collective behaviors. *QS* indicates on the capacity of bacteria to monitor their population density and regulate gene expression. *QS* activates from tens to hundreds of genes that underlie different biological processes. The *QS*-regulated processes organize horizontal gene transfer, the formation of biofilms, multicellular behaviors, microbe–host and microbe–microbe relations. The *QS* signaling requires the production, release, detection, exchange and perception of bacterial compounds, known as autoinducers or *QS* signals. Recently, new autoinducers have been discovered in bacteria and it has been shown how these molecules are recognized by the respective receptors. Autoinducers belong to three major classes: acyl-homoserine lactones (AHLs) used by Gram-negative bacteria, specific oligopeptides used by Gram-positive bacteria and universal autoinducers. The aim of this paper is to provide an overview of molecular basis of the *QS* phenomenon, characterization of intra- and interspecies *QS* signaling molecules and biological processes regulated by these molecules.

Molekularne podstawy *quorum sensing* systemów odpowiedzi sygnałowej u bakterii

Słowa kluczowe autoinduktory 2 i 3, chinolonowe cząsteczki sygnałowe, cząsteczki sygnałowe bakterii Gram-dodatnich i Gram-ujemnych, zjawisko *QS*

Streszczenie Bakterie używają *quorum sensing* (QS) do przeprowadzania programów ekspresji genów związanych z zachowaniami grupowymi. *QS* oznacza zdolność bakterii do monitorowania gęstości swojej populacji i regulacji ekspresji genów. *QS* aktywuje od dziesiątek do setek genów, które są związane z różnymi procesami biologicznymi. Procesy regulowane przez *QS* są związane z horyzontalnym przepływem genów, tworzeniem biofilmów, zachowań wielokomórkowych, oraz relacji bakteria–żywiciel i bakteria–bakteria. Sygnalizacja *QS* wymaga wytwarzania, uwalniania, wykrywania, wymiany i percepcji komponentów nazywanych autoinduktorami lub sygnałami *QS*. Ostatnio u bakterii zostały odkryte nowe autoinduktory oraz wykazano jak te cząsteczki są rozpoznawane przez odpowiednie receptory. Autoinduktory należą do trzech głównych klas: do acylowanych laktonów homoseryny (AHLs) używanych przez bakterie Gram-ujemne, specyficznych oligopeptydów używanych przez bakterie Gram dodatnie i do

autoinduktorów uniwersalnych. Celem artykułu jest przegląd bazy molekularnej zjawiska *QS*, w tym wewnątrz i międzygatunkowych cząsteczek sygnałowych *QS* oraz procesów biologicznych regulowanych przez te cząsteczki.

Introduction

Successful survival in nature of all organisms is dependent on their ability of perception and adaptation to the surrounding environment. Bacteria spread throughout the natural world, inhabit a variety of ecosystems – from the thermal springs to the highly acidic environment of the human stomach. An indispensable component of a successful colonization and exploitation of such niches is the ability of microorganisms to sense the environment and the appropriate modification of their behavior, which will allow them to survive under certain conditions (Pereira et al., 2013). Adaptation of microorganisms to the prevailing conditions results from their interrelationships through a complex network of signaling pathways which activation leads to changes in bacterial gene expression and generate optimal phenotypes under certain conditions (Pereira et al., 2013).

The discovery of the phenomenon of *QS*, otherwise called as phenomenon of sensing the amount by Nealson and Hastings, announced a revolution in the field of microbiology, transforming current thinking about microorganisms (O'Toole, 2016). This discovery was a huge surprise to many researchers, who believed for a long time that such action is limited only to multicellular organisms (Li, Tian, 2012), and microorganisms in contrast to higher organisms do not have any system to enable them "smart" communication with each other (Matejczyk, Suchowierska, 2011).

In the mechanism of *QS*, bacteria communicate with each other through generated by themselves and excreted to the external environment chemical, relaying signals called autoinducers (Suárez-Moreno et al., 2010). The concentration of signaling molecules in the environment is strictly correlated with the density of a growing population of bacteria, so that the response of individual cells is dependent on the behavior of the entire living population in a particular niche (Suárez-Moreno et al., 2010). In practice, the system is used by the bacteria to activate processes which would be ineffective in the case of acting alone, for example in biofilm formation (Bassler, Losick, 2006). In short, the molecular mechanism of this phenomenon is based on the production of signaling compounds by cells, their accumulation in the environment of bacterial growth, then the recognition of produced signals by specific receptor proteins acting as sensors of the signal, and in consequence, coordinated, global response of cells to these signals, resulting in a change in the expression of genes that control essential metabolic pathways and processes of life (Jaworski et al., 2005).

There are four functional groups among the genes that control the *QS* (Grandclement et al., 2016). The first group includes genes encoding proteins involved in the basic life functions, the second one includes genes related to the behavior of cells in a given environment, in the third group there are genes which subject to a horizontal transfer, and in the fourth one – genes that encode proteins that enable interaction with other organisms, e.g. proteins associated with virulence.

The term "*QS*" comes from the Latin word "*quorum*" which means the number of votes that must be cast during the election or referendum, so that the election was considered as valid (ScienceDaily, 2009). It is believed that the transfer of this concept on the microbiological grounds best captures the essence of sensing mechanism of the number of bacteria.

In microbiology this term is defined as a process in which the bacterial cells detect the minimum, threshold concentration of signaling molecules in the external environment and then

after exceeding the allowable minimum, they respond accordingly and modify their behavior by altering the expression of their genes (Bassler, Losick, 2006).

A wide range of biological processes is regulated through the mechanism of *QS*, such as: bioluminescence, mobility, synthesis of virulence factors, competence, horizontal gene transfer, biofilm formation, production of antibiotics or swariming growth (Lixa, 2015).

The phenomenon of *QS* has been discovered in different species of bacteria, but similar systems have been also reported in the case of other organisms (Suárez-Moreno et al., 2010). It should be noted that the various sensing systems of bacteria are very similar to each other, which is due to the fact that the ability of intercellular communication is common in the world of bacteria, because it increases the chance of survival in a specific environment. However, types of chemical signals, their receptors, mechanism of signal transmission and purpose of each system of *QS* show the uniqueness for each species of bacteria (Papenfort, Bassler, 2016).

Autoinducers of *QS*

Intercellular communication of microbes is based on the synthesis, secretion and response to small diffusive signaling molecules called autoinducers, which are signalers of cell density (Jamuna Bai, Ravishankar Rai, 2011). Their concentration in the environment for bacterial growth is closely correlated to the number of bacterial cells. These compounds are secreted and released into the environment either by diffusion or by active transport. After reaching a certain concentration of signaling molecules, it comes to the induction of the expression of certain genes under the control of *QS* system (Baranowska, Rodziewicz, 2008), and as a consequence to simultaneous metabolic changes in all cells of the population (Kołwzan, 2011). According to Wizner et al. (2002), diffusive signaling molecules have some characteristic features that distinguish them from secondary metabolites. The four basic criteria that must be fulfilled by a molecule so that it is recognized as autoinducer by *QS* system are:

1. Production of this molecule must occur at specific stages of growth of the population in certain physiological conditions or in response to environmental changes.
2. Such compound should be accumulated extracellularly and recognized by specific receptors.
3. The accumulation of such molecules should generate a coordinated response of all cells of the population after reaching a certain critical threshold concentration.
4. The cell response of the population should go beyond the metabolic and physiological changes which comprise only the use of this compound as a source of carbon and energy.

Chemical structure of autoinducers and mechanism of their action is different, according to the species of the microorganism (Kołwzan, 2011). Most often, the examined autoinducers belong to one of three classes: acyl-homoserine lactones (AHLs) used by Gram-negative bacteria, specific oligopeptides used by Gram-positive bacteria and universal autoinducers (autoinducers-2) used by both gram-negative and gram- positive bacteria (LaSarre, Federle, 2013). However, it should be noted that there are also other signaling molecules of *QS* system not belonging to any of these classes and these include quinolone molecules used by *Pseudomonas aeruginosa* (PQS) or autoinducer-3 (AI-3). There is no doubt that the new signal molecules will be discovered at the time when the studies on *QS* are expanded with bacterial species which have been unexplored yet (LaSarre, Federle, 2013). The previous observations of phenomenon of communication among microorganisms have shown that bacteria are capable to send both specific communication signals

within their own species, and non-specific, among different species (Matejczyk, Suchowierska, 2011).

Signal molecules of Gram-negative bacteria

The four common features are found in almost all known *QS* systems in Gram-negative bacteria (Ng, Bassler, 2009). Firstly, autoinducers in such systems are mentioned above acyl-homoserine lactones (AHLs) or other molecules which are synthesized from S-adenosylmethionine (SAM), and they are able to diffuse freely through the bacterial membrane. Secondly, autoinducers are associated with specific receptors, which are located both in the inner membrane and in the cytoplasm. Thirdly, *QS* usually activates from tens to hundreds of genes that underlie different biological processes. Fourthly, in a process called autoinduction, activation of *QS*, led by autoinducers stimulates the increased synthesis of autoinducers, which establishes the so-called feed-forward loop which supports synchronous expression of genes in the population. The feed-forward loop is a common motif in the regulatory network of biological pathways. The loop is composed of two factors (usually the regulators of transcription), one regulates the other so that both of these factors together regulate target genes. The recent studies on *QS* in Gram-negative bacteria have demonstrated the existence of new autoinducers in these bacteria and have shown how these molecules are recognized by the respective receptors; new regulatory elements have been also revealed, which are included in the basic signaling circuits, and there have been identified new regulatory networks (Papenfort, Bassler, 2016).

Gram-negative bacteria often use several autoinducers, and new studies reveal the molecular determinants that provide a unique receptor specificity in distinguishing closely related molecules. The *QS* information is often integrated by small RNA molecules (sRNAs) (Papenfort, Vogel, 2010) that control the expression of target genes, and also act in feedback loops. The network architecture of *QS* supports the accuracy of signaling, time control and flexible dynamics of flows.

As mentioned above, the role of signal molecules in Gram-negative bacteria play acyl-homoserine lactones (AHLs) (Gera, Srivastava, 2006). A common feature of all AHLs, also referred as type 1 autoinducers (AI-1) is a lactone ring of homoserine. The difference in the chemical structure of each of the homoserine lactones in different bacteria species concerns the side acyl substituent. Various substituents may have different length depending on the number of carbon atoms in chain and may differ in further modifications (Siepka, Gładkowski, 2012). Additionally, it was found that a significant number of AHLs molecules synthesized by Gram-negative bacteria include an even number of carbon atoms in their side chain (Gera, Srivastava, 2006). Differences in the structure of the side chain give each AHL molecule unique specifications by which each species of bacteria has its own, characteristic for each other "language of communication" incomprehensible for other species (Federle, Bassler, 2003). Depending on the length of the acyl chain, transport of the AHL through the membrane and cell wall to the external environment may take place without energy input, in a passive way based on the phenomenon of diffusion (autoinducers having substituents with short chains), or with the involvement of energy by active transport (long-chain AHLs) (Siepka, Gładkowski, 2012).

As substrates for the production of signaling molecules, Gram-negative bacteria use S-adenosylmethionine (SAM) and acyl group being the product of transformation of fatty acids (Baranowska, Rodziewicz, 2008). Transfer of the acyl group on the lactone molecule is performed by the carrier protein, ACP (*acyl carrier protein*) (Kołwzan, 2011). The role of LuxI protein in the autoinducer synthesis covers only connection of SAM with the specific for the bacteria species

ACP by formation of amide linkage (Waters, Bassler, 2005). Then comes to the formation of an ester linkage in the homoserine molecule and as a result of those reactions, 5'-methylthioadenosine product occurs, and after its disconnection, the finished AHL is formed (Czajkowski, Jafra, 2006).

Signal molecules of Gram-positive bacteria

In the evolution process, Gram-positive bacteria have developed different processes of synthesis of signal molecules as well as methods of transmitting signals from the sensor proteins of a cell to the effectors. Mechanisms and proteins involved in *QS* in Gram-positive bacteria are best known in *Streptococcus pneumoniae, Bacillus subtilis* and *Staphylococcus aureus* (Grossman, 1995). Systems of gram-positive bacteria normally use secreted oligopeptides and two-component systems, consisting of receptors for a sensor kinase associated with membrane and cytoplasmic transcription factors that determine changes in gene expression (Novick, Geisinger, 2008).

Gram-positive bacteria, in contrast to Gram-negative ones do not produce AHLs, and as signaling molecules, essential for intercellular communication use oligopeptide autoinducers AIPs (*autoinducing peptides*) (Kołodyński, Jankowski, 2005). The AIPs result from digestion of larger protein precursors (Kołwzan, 2011). They consist of 5–17 amino acids and modified side chains (Siepka, Gładkowski, 2012). A large part of Gram-positive bacteria belonging to phylum *Firmicutes* use thiolactone/lactone peptides as AIPs communication signals to coordinate *QS* circuit. In particular, *QS* of staphylococci, enterococci, and clostridia have been intensively examined in terms of alternative target of anti-pathogenic therapy independent of bactericidal antibiotics (Singh et al., 2016). Unlike AHLs, signaling oligopeptides of Gram-positive bacteria are not able to diffuse freely through the cell membrane (Kołwzan, 2011). They are produced in the cytoplasm in the form of precursors and they are modified and separated from the cell (Siepka, Gładkowski, 2012) with the participation of ABC transporting protein, dependent from ATP (*ATP – binding cassete*) (Jaworski et al., 2005).

Autoinducers-2

It has not been established yet why Gram-negative bacteria use acylated homoserine lactones as signaling molecules, while Gram-positive ones – oligopeptide autoinducers (Kołodyński, Jankowski, 2005). The use of various autoinducers and communication mechanisms results probably from different construction of their cell walls (Matejczyk, Suchowierska, 2011). However, it has been proved that both groups of bacteria are capable to produce molecules non-specific for any species. It means that with the help of these autoinducers, called autoinducers-2 (AI-2) or universal autoinducers, the microorganisms may communicate interspeciesly (Sztajer et al., 2008). The discovery of AI-2, by which different species and types of bacteria can communicate with each other, was a breakthrough in research on *QS* system and allowed for a better knowing and understanding of interactions between microorganisms in the environment. Chemical structure and biosynthesis of these autoinducers is described in further part of this paper.

Quinolone signal molecules

As mentioned before, except the three main categories of autoinducers, there are also other signaling molecules, which are not included in any of the above classes (LaSarre, Federle, 2013).

The quinolone molecules are examples of such autoinducers, used by *Pseudomonas aeruginosa* in the intercellular communication (*Pseudomonas quinolone signal molecule* – PQS) (Myszka, Czaczyk, 2010). 3,4-dihydroxy-2-heptylquinoline (PQS) and its precursor 4-hydroxy-2-heptylquinoline (HHQ) are the products of expression of the operon *pqsABCDE*. Both molecules act as autoinducers of *QS* system and the only difference in their chemical structure is the presence of an additional hydroxy group located in the 3' position of PQS. Although only *P. aeruginosa* produces PQS, other species of *Pseudomonas* and *Burholderia* spp. may use HHQ as a *QS* signal (LaSarre, Federle, 2013).

Autoinducer-3

Autoinducer-3 (AI-3) is the least known signal molecule of *QS* system. AI-3 is produced by the commensal bacteria constituting the physiological intestinal microflora of humans as well as certain species of intestinal pathogens. The molecular structure of this molecule or gene responsible for its synthesis have not been known so far (LaSarre, Federle, 2013). In enterohemorrhagic strain of *Escherichia coli* (EHEC), mechanism of communication with the use of AI-3 is responsible among other things for the regulation of motility or activation of transcription of genes involved in virulence (Kendall, Sperandio, 2014). But in enteropathogenic *E. coli* (EPEC), this system is related in part to the formation of biofilm, because it controls the expression of the protein EspA, which is involved in the creation of micro-colonies in the initial phase of development of the biofilm of this strain (Kendall, Sperandio, 2014). AI-3 molecule is recognized by a protein kinase QseC, which due to autophosphorylation, initiates a cascade of reactions leading to the phosphorylation of the regulator of QseB response and in consequence, to activation of transcription of target genes. Because the same two-component regulatory system QseC/B is also used for the detection of adrenaline and noradrenaline, it is believed that AI-3 is structurally similar to these hormones (LaSarre, Federle, 2013). This is confirmed by the fact that the effects induced by AI-3 may be inhibited by antagonists of adrenergic receptors (Kendall, Sperandio, 2014).

Conclusions

Diffusion chemical communication among bacteria through *QS* is a central system of bacteria's life that facilitates bacteria to recognise synergistic or antagonistic population in environment and enables them to control collective behaviors. Synthesis of *QS* modulators is being dynamically pursued to modify bacterial behavior on request. These and other new research raise our knowledge about the bacterial *QS* networks which enable them to survive and actively colonize new ecological niches and increase their expansion using new nutrient substrates.

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Lichens and lichenicolous fungi of the "Golczewskie Uroczysko" nature reserve (NW Poland)

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Keywords lichens, nature reserve, Poland, Pomerania

Abstract Lichens of the "Golczewskie Uroczysko" nature reserve were studied in 2007–2008 and 2015–2016. Within the examined area, 68 species of lichens and 5 lichenicolous fungi were observed. Eleven species are included in the red list of threatened lichens in Poland, six as vulnerable (VU) (*Bryoria fuscescens*, *Buellia disciformis*, *Calicium viride*, *Ochrolechia androgyna*, *Pertusaria pertusa* and *Tuckermannopsis chlorophylla*) and five as near threatened (NT) (*Alyxoria varia*, *Chaenotheca furfuracea*, *Evernia prunastri*, *Graphis scripta* and *Hypogymnia tubulosa*).

Porosty rezerwatu „Golczewskie Uroczysko" (NW Polska)

Słowa kluczowe porosty, rezerwat, Polska, Pomorze

Streszczenie Badania prowadzono nad biotą porostów rezerwatu „Golczewskie Uroczysko". Stwierdzono w sumie 68 gatunków porostów i 5 grzybów naporostowych. Ponad 56% bioty porostów stanowiły gatunki nadrzewne, wsród których występowały taksony rzadkie i zagrożone w skali całego kraju. Blisko połowę wszystkich gatunków stanowiły porosty o plesze skorupiastej. Charakterystyczna cechą lichenobioty badanego rezerwatu jest duży udzial gatunków występujących na pojedynczych stanowiskach.

Introduction

The "Golczewskie Uroczysko" nature reserve is located in West Pomerania Province, in Golczewo Commune. It was created in 2004, covers 101.05 ha, and comprises woodlands and peatlands. The reserve includes patches of nearly natural, old coniferous and mixed bog forest, with several-hundred-year-old trees. The dominant trees are oaks (*Quercus* sp.), as their contribution to the tree layer is up to 40%. Also birches (*Betula* sp.) and pine trees (*Pinus sylvestris*) are frequent there. Another valuable characteristic of the landscape are peat deposits in the bog in the central part of the reserve (Zawal, Stępień, 2006).

So far, lichens have not been studied in the reserve. The aim of this study was to present the current biota of lichens and lichenicolous fungi in the reserve.

Material and methods

Field research was conducted in the years 2007–2008 and 2015–2016. The random point method was used, which enabled us to explore the area objectively and evenly. Easily identifiable species of lichens were recorded in the field, while all the others were collected and identified in the laboratory of the Department of Ecology and Environmental Protection of the University of Szczecin. Species names follow Fałtynowicz and Kossowska (2016) and Czyżewska and Kukwa (2009).

The list of species is ordered alphabetically. Types of substrate are given for each species. Names of lichenicolous fungi are marked with asterisks(*). The list of threatened species, with their threat category, is based on the Polish red list (Cieśliński et al., 2006).

Results

As a result of the research at 53 sites, 481 records were collected (herbarium specimens or records in the field) (Figure 1). In total, in the "Golczewskie Uroczysko" nature reserve, 68 species of lichens and 5 lichenicolous fungi were found. The contribution of lichens to the landscape is remarkable, although it varies between habitats.

Epiphytic lichens

The most numerous were epiphytic lichens, which accounted for 56% of the lichen biota of the study area (Figure 2). Among the morphological types of this group of species, crustose lichens were most numerous, accounting for nearly half (23 taxa) of the total number of epiphytic species (Figure 3). The base of tree trunks was most often colonized by *Coenogonium pineti, Porina aenea* and *Parmeliopsis ambiqua*, while higher parts of tree trunks, by *Hypogymnia physodes, Lecanora conizaeoides*, *Parmelia sulcata* and *Phlyctis argena.*

The largest number of species was recorded on bark of *Betula* sp. (32 species) including 6 exclusive species, which were not recorded on other plant hosts (Figure 4). These include *Bryoria fuscescens*, *Calicium viride*, *Cladonia floerkeana*, *C. macilenta*, *Ochrolechia androgyna* and *Usnea dasopoga*.

Bark of *Quercus* sp. ranked second (29 species), and some species were exclusive to this microhabitat: *Alyxoria varia*, *Melanelixia fuliginosa, Pseudevernia furfuracea,* and *Tuckermanopsis chlorophylla* (Figure 4). They were supplemented with the rich flora of lichens associated with the bark of *Pinus sylvestris* (23 species), *Acer* sp., and *Fagus sylvatica* (10 species). Most of the taxa are frequent or common in the western part of Polish Pomerania (Fałtynowicz, 1992). Some of the recorded epiphytic species are protected by Polish law and/or threatened in Poland (Cieśliński et al., 2006; Fałtynowicz, Kukwa, 2007) (Table 1). These include 10 exclusively epiphytic species, found on very old trees. The sites of protected and threatened species are scattered all over the reserve.

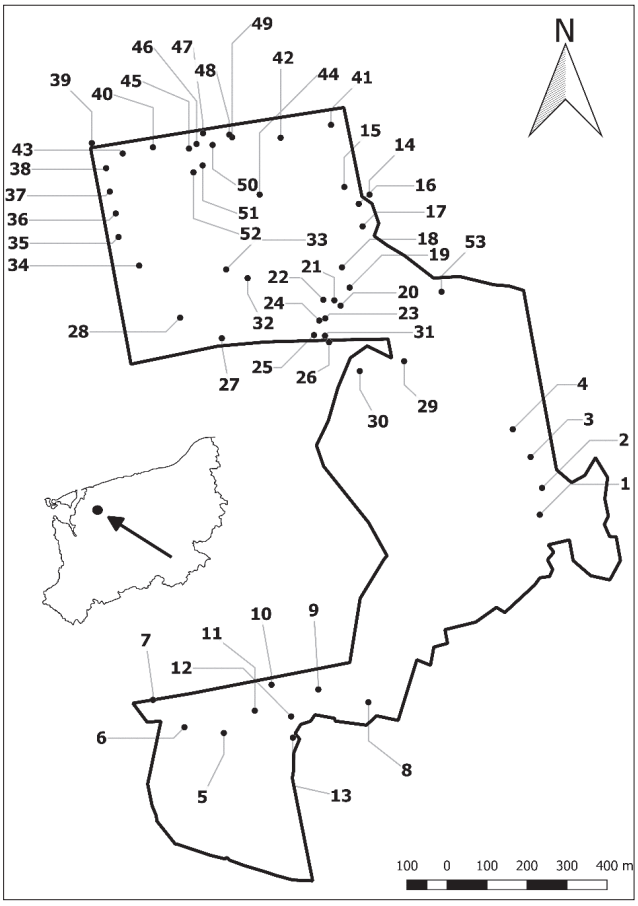

Figure 1. Map of the studied sites

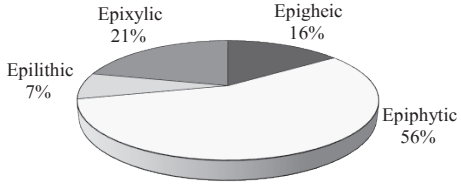

Figure 2. Percentages of lichen species from various ecological groups

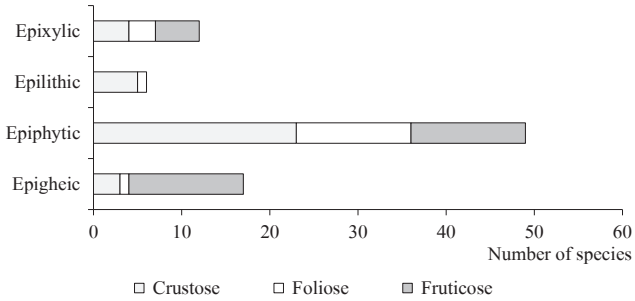

Figure 3. Spatial distribution of lichen species and contributions of morphological forms to each ecological group

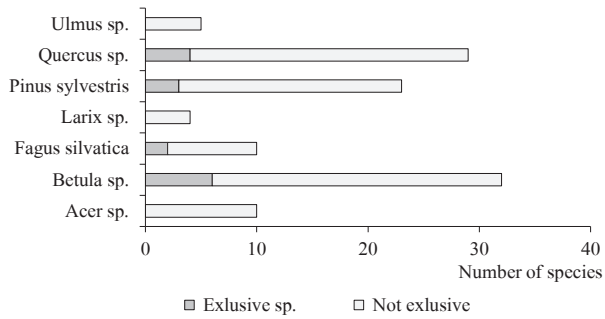

Figure 4. Contribution of exclusive and non-exclusive species in epiphytic groups of lichens

Table 1. Lichen species of the" Golczewskie Uroczysko" nature reserve included in the red list of threatened lichens in Poland, red list of threatened lichens in Pomerelia (Gdańsk Pomerania)

Species	Protection status	Threat category (Cieśliński et al., 2006)	Threat category (Fałtynowicz, Kukwa, 2007)
Alyxoria varia	–	NT	–
Bryoria fuscescens	Partly protected	VU	VU
Buellia disciformis	–	VU	VU
Calicium viride	–	VU	–
Chaenotheca furfuracea	–	NT	–
Evernia prunastri	–	NT	–
Graphis scripta	–	NT	–
Hypogymnia tubulosa	Partly protected	NT	–
Ochrolechia androgyna	–	VU	VU
Pertusaria pertusa	–	VU	–
Tuckermannopsis chlorophylla	Partly protected	VU	VU

Explanations: VU – vulnerable; NT – Near Threatened.

Epixylic lichens

Lichens found on decaying or rotting wood of logs and trunks were classified as epixylic. On such substrates we recorded 18 species, which accounted for 21% of the lichen flora of the study area (Figure 2). They were mostly frequent and common lichens of the genera *Cladonia*, *Micarea*, *Placynthiella*, and *Trapeliopsis.* In moist patches near the bog, young stumps were most often colonized by crustose lichens, such as *Lecanora conizaeoides*, *Micarea denigrata*, *Placynthiella uliginosa*, and *Trapeliopsis flexuosa*. On more strongly decomposed wood, they were succeeded by fruticose lichens, mostly of the genus *Cladonia. Hypocenomyce scalaris* and *Lepraria* sp. were also abundant there.

Epigeic lichens

Epigeic lichens accounted for nearly 16% of the total number of species recorded in the "Golczewskie Uroczysko" nature reserve (Figure 2). They were found at the edges of the bog and of forest glades, and were represented most frequently by lichens of the genus *Cladonia.* Less frequently, species associated with thermophilous sandy pine forests were present, such as *Cladonia arbuscula* subsp. *mitis* or *Cladonia uncialis*. Only sporadically we recorded *Bacidia bagliettoana,* and *Peltigera rufescens.*

Epilithic lichens

This group of lichens was represented exclusively by species colonizing anthropogenic substrates, such as concrete poles or walls. In this study it included only 6 taxa, which are common calcicolous lichens: *Flavoplaca citrina*, *Calogaya decipiens*, *Candelariella aurella*, *Myriolecis albescens*, *M. dispersa*, and *Lepraria* sp.

Discussion

Natural bogs and poor fens are ecosystems characterized by a low abundance of lichens. This is due to the high moisture content of the substrate. Lichens in such communities grow mostly on tree bark and at the drier crests of hummocks. However, in the "Golczewskie Uroczysko" nature reserve, its protection succeeded to preserve the natural vegetation and safeguard valuable ecological systems, which constitute a refuge for many threatened and protected species.

In comparison with other nature reserves in northern Poland (Fałtynowicz, 1983, 1996), species diversity in our study area is relatively high. The total number of species (68) is close to that observed in similar study areas. For example, in the "Bagno Biel" nature reserve, protecting peatland covering a 3-fold larger area, as many as 81 species were recorded, while in the "Bagnisko Niedźwiady" nature reserve, which is 4-fold larger, only 52 species. The large number of species in the "Golczewskie Uroczysko" nature reserve, in spite of its smaller area, is linked with the richness of plant hosts. Our study area comprises not only pine forest, which is poor in lichens, but also patches of nearly natural, old forest, with several-hundred-year-old oak trees.

In all the 3 nature reserves, epilithic lichens were the rarest, as suitable natural sites were absent there. Epilithic species were found only sporadically, on concrete electricity poles or small poles marking forest sections.

It can be concluded that the study area is valuable from a lichenological point of view and is an important nature reserve protecting lichen diversity in the western part of Polish Pomerania.

List of species

For each species, the type of substrate on which it is found and numbers sites are given.

Symbols: * = lichenicolous fungus, VU = vulnerable, NT = near threatened; PR = strict or partial protection.

Alyxoria varia (Pers.) Ertz & Tehler – [NT], on oak tree bark: 18.
Amandinea punctata (Hoffm.) Coppins & Scheid. – on birch, maple, and pine tree bark: 2, 3, 6, 14, 15, 18, 32.
**Athelia arachnoidea* (Berk.) Jülich – on thallus of *Lecanora conizaeoides* on pine tree bark: 1.
Bacidia bagliettoana (A. Massal. & De Not.) Jatta – on soil: 3.
Bryoria fuscescens (Gyeln.) Brodo & D. Hawksw. – [VU, PR], on birch tree bark: 32.
Buellia disciformis (Fr.) Mudd – [VU], on tree bark: 15, 18, 21, 33.
B. griseovirens (Turner & Borrer ex Sm.) Almb. – on birch, and oak tree bark: 15, 18.
Calicium viride Pers. – [VU], on birch tree bark: 52.
Calogaya decipiens (Hoffm.) Arup, Frödén & Søchting – on concrete posts: 13, 39.
Candelariella aurella (Hoffm.) Zahlbr. – on concrete posts: 13, 39.
Chaenotheca ferruginea (Turner ex Sm.) Mig. – on pine tree bark: 4, 11, 15, 18, 19.
C. furfuracea (L.) Tibell – [NT], on pine, birch, oak, beech, elm tree bark, and wood: 1, 2, 11, 13–16, 18–20, 34, 44, 50, 51, 53.
Cladonia arbuscula (Wallr.) Flot. em. Ruoss subsp. *mitis* (Sandst.) Ruoss – on soil on the peat: 4, 29.
C. chlorophaea (Flörke ex Sommerf.) Spreng. – on soil on the peat, rarely on tree bark: 6, 21, 42, 51, 52.
C. coccifera (L.) Willd. – on soil: 4.
C. coniocraea auct. – on soli, wood, and oak, pine, elm, and birch tree bark: 2, 4, 5, 6, 11, 13–18, 21–26, 28, 33, 36–41, 45, 46, 48–53.
C. cornuta (L.) Hoffm. – on oak, and birch tree bark: 25, 51.
C. deformis (L.) Hoffm. – on soil: 6.
C. digitata (L.) Hoffm. – on soil, wood, and elm, birch, pine, and oak tree bark: 1, 4, 5, 13–15, 21, 23–26, 29, 30, 32–38, 40, 46–49, 51.
C. fimbriata (L.) Fr. – on soil, and birch tree bark: 2, 6, 21.
C. floerkeana (Fr.) Flörke – on birch tree bark, and wood: 2, 24.
C. furcata (Huds.) Schrad. – on soil: 2, 4, 8, 9, 29.
C. glauca s.l. Flörke – on birch, pine, and oak tree bark, rarely on wood: 4, 6, 14, 39, 45, 51.
C. macilenta Hoffm. – on birch tree bark: 2, 6, 15, 17, 21, 22, 25, 26, 32, 38, 47, 49.
C. ochrochlora Flörke – on wood: 23.
C. squamosa (Scop.) Hoffm. – on soil: 6.
C. subulata (L.) Weber – on soil, and pine, oak, and birch tree bark: 4–6, 14, 15, 18–21, 29, 44, 47.
C. uncialis (L.) F.H. Wigg. – on soil: 4.
**Clypeococcum hypocenomycis* D. Hawksw. – on thallus of *Hypocenomyce scalaris* on pine tree bark: 4, 11, 20, 41, 53.
Coenogonium pineti (Schrad.) Lücking & Lumbsch – on pine, and birch tree bark: 4, 5, 7, 11–16, 18–23, 27, 29, 30, 33, 40, 43, 45, 50–52.
Evernia prunastri (L.) Ach. – [NT], on oak, and elm tree bark, and wood: 14, 30, 43, 44.
Flavoplaca citrina (Hoffm.) Arup, Frödén & Søchting. – on concrete posts: 13, 39.

Graphis scripta (L.) Ach. – [NT], on beech tree bark: 6, 9.
Hypocenomyce scalaris (Ach.) Choisy – on pine tree bark, and wood: 4, 5, 11, 12, 19, 20, 29, 41, 46–48, 52, 53.
Hypogymnia physodes (L.) Nyl. – on oak, pine, birch, beech, and maple tree bark, and wood: 3–7, 14, 17, 18, 20–23, 27–33, 35, 36, 38, 39, 41, 43–48, 51, 52.
H. tubulosa (Schaer.) Hav. – [NT, PR], on pine tree bark, and wood: 31, 47.
Lecanora conizaeoides Nyl. – on pine tree bark: 3–5, 11–13, 15, 16, 19, 20, 29, 41, 46, 47.
L. expallens Ach. – on pine, beech, oak tree bark: 3, 5, 7, 14, 17, 18, 20, 23, 28, 34, 44.
L. pulicaris (Pers.) Ach. – on beech, oak, and pine tree bark: 5, 8, 9, 13, 27, 30, 39, 43, 45, 50.
Lecidella elaeochroma (Ach.) Choisy – on beech, oak, pine, birch, and maple tree bark: 1, 2, 5, 7, 20, 27, 30, 33, 40, 43, 45, 47, 52.
Lepraria sp. – on beech, oak, pine, birch, and maple tree bark, and wood: 1–5, 7–19, 24, 25, 28, 35, 42, 43, 45, 49–52.
**Lichenoconium erodens* M. S. Christ. & D. Hawksw. – on thallus of *Lecanora conizaeoid*es: 5, 13, 15, 16, 29, 46, 47.
**L. lecanorae* (Jaap) D. Hawksw. – on apothecium of *Lecanora conizaeoides*: 3, 4, 12, 13, 19, 47.
Melanohalea exasperatula (Nyl.) O. Blanco, A. Crespo, Divakar, Essl., D. Hawksw. & Lumbsch – on oak, and beech tree bark: 18, 27, 33.
Melanelixia fuliginosa (Fr. ex Duby) O. Blanco, A. Crespo, Divakar, Essl., D. Hawksw. & Lumbsch – on oak tree bark: 2.
Micarea denigrata (Fr.) Hedl. – on wood: 21, 43, 48.
M. prasina Fr. – on wood: 5, 14, 31, 48.
Myriolecis albescens (Hoffm.) Śliwa, Zhao Xin & Lumbsch – on concrete posts: 39.
M. dispersa (Pers.) Śliwa, Zhao Xin & Lumbsch – on concrete posts: 13, 39.
Ochrolechia androgyna (Hoffm.) Arnold – [VU], on birch tree bark: 22.
Parmelia sulcata Taylor – on birch, and oak tree bark: 2, 6, 15, 17, 18, 21, 22, 24, 27, 30, 32, 33, 38–40, 43–45, 47, 49, 52.
Parmeliopsis ambiqua (Wulfen) Nyl. – on pine, oak, and birch tree bark: 2, 6, 11, 17, 18, 25, 30, 33, 38, 44, 47.
Peltigera rufescens (Weiss) Humb. – on soil: 6.
Pertusaria albescens (Huds.) Choisy & Werner – on oak, and beech tree bark: 5, 9, 30.
P. amara (Ach.) Nyl. – on oak, and beech tree bark: 3, 5, 9, 30, 39, 44, 50.
P. pertusa (Weigel) Tuck. – [VU], on oak, and beech tree bark: 5, 9, 27, 39, 42, 44, 51.
Phaeophyscia nigricans (Flörke) Moberg – on birch tree bark, and on concrete post: 3, 39, 46, 49, 52.
Phlyctis argena (Ach.) Flot. – on oak, pine, and birch tree bark: 3, 5, 9, 16, 18, 21, 22, 27, 30, 39, 42, 44, 50, 51, 52.
Physcia tenella (Scop.) DC. – on wood: 14, 43.
Placynthiella uliginosa (Schrad.) Coppins & P. James – on humus: 4, 6, 14, 28, 31,43.
Platismatia glauca (L.) W.L. Culb. & C.F. Culb. – on birch, and oak tree bark: 6, 17, 25, 30, 33, 45.
Polycauliona polycarpa (Hoffm.) Frödén, Arup & Søchting – on birch, and pine tree bark: 17, 25, 45.
Porina aenea (Wallr.) Zahlbr. – on beech tree bark: 1, 3, 6, 45, 49, 51.
Pseudevernia furfuracea (L.) Zopf – on oak tree bark: 39.
Scoliciosporum chlorococcum (Graeve ex Stenh.) Vězda – on birch, and pine tree bark: 2, 4, 12, 15, 18, 25.
Trapeliopsis flexuosa (Fr.) Coppins & P. James – on wood, and pine tree bark: 4–6, 14, 23, 28, 43, 48.

T. granulosa (Hoffm.) Lumbsch – on wood: 5, 35, 48, 53.
**Tremella cladoniae* Diederich & M. S. Christ. – on thallus of *Cladonia coniocraea*: 2, 13, 23, 49.
Tuckermannopsis chlorophylla (Willd.) Hale – [VU, PR], on oak tree bark: 2, 18, 45.
Usnea dasopoga (Ach.) Nyl. – on birch tree bark: 32.
Violella fucata (Stirt.) T. Sprib. – on maple, and oak tree bark: 3, 6, 18, 27, 30, 33, 39, 43, 44, 45, 50.
Xanthoria parietina (L.) Th. Fr. – on wood: 43, 48, 53.
Xylopsora caradocensis (Nyl.) Bendiksby & Timdal – on pine wood: 47.

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Lichens and lichenicolous fungi of the "Wrzosowisko Sowno" nature reserve (NW Poland)

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Keywords lichens, nature reserve, Poland, Pomerania

Abstract Lichens of the "Wrzosowisko Sowno" nature reserve in the western part of Polish Pomerania were studied in 2006 and 2014. Within the examined area, 90 species of lichens were observed. Eighteen species are included in the red list of threatened lichens in Poland, eight as vulnerable (VU) (*Bacidia rubella*, *Bryoria fuscescens*, *Buellia disciformis*, *Calicium viride*, *Ochrolechia androgyna*, *Pertusaria pertusa*, *Pseudoschismatomma rufescens*, *Ramalina farinacea*, *R. pollinaria* and *Tuckermannopsis chlorophylla*), seven as near threatened (NT) (*Chaenotheca furfuracea*, *Evernia prunastri*, *Graphis scripta*, *Hypogymnia tubulosa*, *Pertusaria coccodes*, *Vulpicida pinastri* and *Zwackhia viridis*), two as endangered (EN)(*Melanelixia glabra* and *Pleurostica acetabulum*) and one as critically (CR) (*Melanohalea exasperata*).

Porosty rezerwatu „Wrzosowisko Sowno" (NW Polska)

Słowa kluczowe porosty, rezerwat, Polska, Pomorze

Streszczenie Badania nad biotą porostów rezerwatu „Wrzosowisko Sowno" przeprowadzono w latach 2006–2014. Na badanym obszarze stwierdzono 85 gatunków porostów i 5 grzybów naporostowych. Ponad 52% bioty porostów stanowiły gatunki nadrzewne, wśród których występowały taksony rzadkie i zagrożone w skali całego kraju. Blisko połowę gatunków stanowiły porosty o plesze skorupiastej. Najmniej licznie reprezentowane są epility, co zwiazane jest z brakiem odpowiednich siedlisk dla tej grupy porostów.

Introduction

The floristic reserve "Wrzosowisko Sowno" is located in West Pomerania Province, within Gryfice County, 2 km NW of the town of Płoty. Although the reserve created in 1977 covers 39.3 ha, the peatland covers over 26 ha. It is a transitional mire composed of *Sphagnum* mats (typical of raised bogs) with *Scheuchzeria palustris*, but their upper layers in the course of time were partly dried and overgrown by birch woods. *Salix* thickets and patches of vegetation dominated by mosses and sedges (Janowska et al., 2003−2004) have developed at its edges.

The habitat is an Atlantic wet heath specific to the nature of the Baltic coast in the western part of Polish Pomerania, with the characteristic *Erica tetralix* and *Ledum palustre*. They are accompanied by other Atlantic species, especially of *Sphagnum* mosses and boreal species of the family Ericaceae. This study aimed to investigate the species composition of lichens and lichenicolous fungi in the reserve.

Material and methods

The analysis of lichen flora is based on materials collected during field research conducted in the years 2006–2014. The random point method was used, which enabled us to explore the area objectively and evenly. Part of the reserve, especially the innermost portion, was not explored because of difficult access (waterlogged area, thickets).

Easily identifiable species of lichens were recorded in the field, while all the others were collected and identified in the laboratory of the Department of Ecology and Environmental Protection of the University of Szczecin. As a result of the research at 238 sites, 1909 records were collected (herbarium specimens or records in the field) (Figure 1).

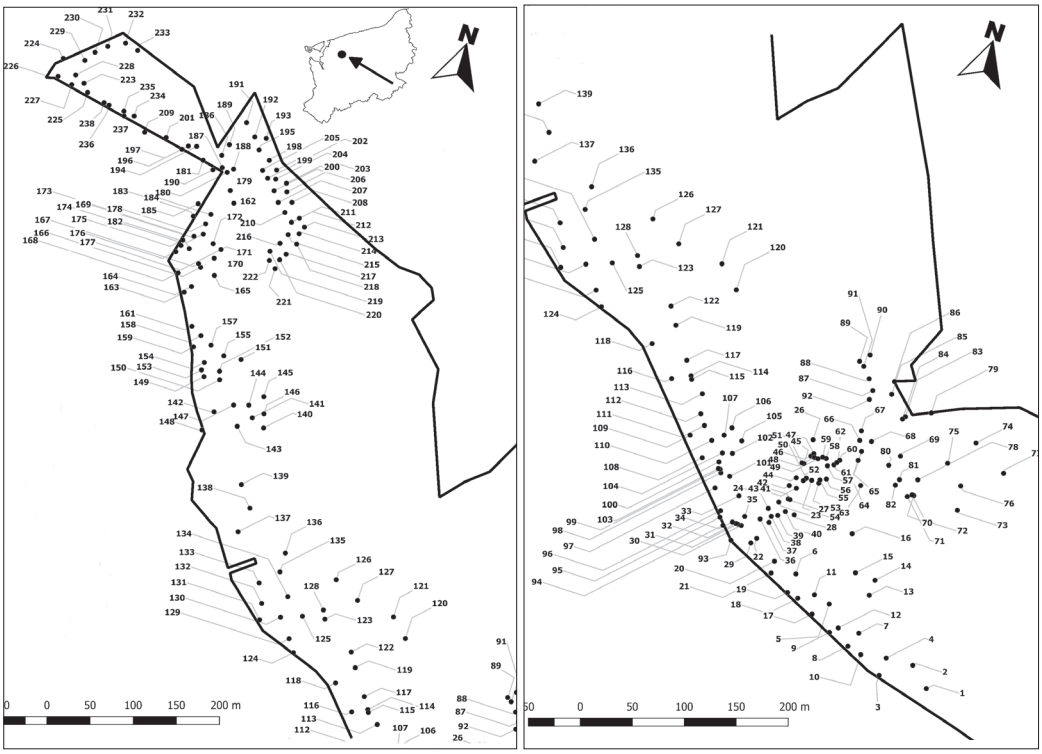

Figure 1. Study area and location of points

Species names follow Fałtynowicz and Kossowska (2016), and Czyżewska and Kukwa (2009).

The list of threatened species, with their threat status, is based on the Polish red list (Cieśliński et al., 2006).

The list of species is ordered alphabetically. Types of substrate are given for each species. Names of lichenicolous fungi are marked with asterisks (*).

Results

In the "Wrzosowisko Sowno" nature reserve, 85 species of lichens and 5 lichenicolous fungi were found.

Epiphytic lichens

Epiphytic species are the dominant group, accounting for 52% of the total lichen biota of the study area (Figure 2). The largest number of species was found on bark of *Betula* sp. (36 species), which accounted for 20% of epiphytic lichens. Slightly smaller numbers were found on the bark of *Quercus* sp. (31 species, 18%), *Fagus sylvatica* (26 species, 15%), and *Populus* sp. (22 species, 12%). They are accompanied by the lichen biota of *Corylus* sp. (14 species, 8%), *Pinus sylvestris* (13 species, 7%), *Salix* sp. (12 species, 7%), *Frangula alnus* (9 species, 5%), and *Acer* sp. (6 species, 3%) (Figure 3). Most of the recorded epiphytic lichens are not exclusive but found on bark of various tree species (Figure 4). Exclusive species, found on bark of only one plant host, include: *Arthonia radiata* (on *Fagus sylvatica*), *Bryoria fuscescens* (*Quercus* sp.), *Chaenotheca furfuracea* (*Quercus* sp.), *Cladonia cornuta* (*Betula* sp.), *Graphis scripta* (*Fagus sylvatica*), *Melanelixia fuliginosa* (*Betula* sp.), *M. glabra* (*Quercus* sp.), *Ochrochelia androgyna* (*Betula* sp.), *Zwackhia viridis* (*Fagus sylvatica*), *Pertusaria albescens* (*Fagus sylvatica*), *P. coccodes* (*Quercus* sp.), *Platismatia glauca* (*Populus* sp.), *Ramalina pollinaria* (*Betula* sp.), and *Vulpicidia pinastri* (*Betula* sp.).

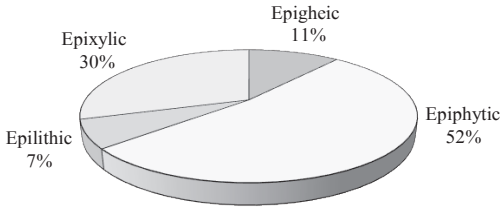

Figure 2. Percentages of lichen species from various ecological groups

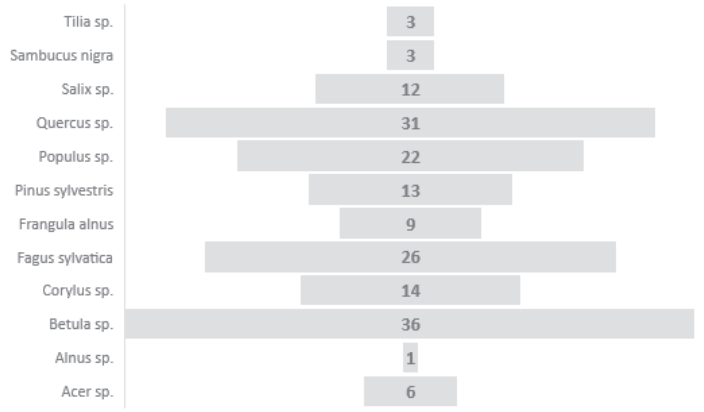

Figure 3. Contribution of epiphytic lichens on examined trees

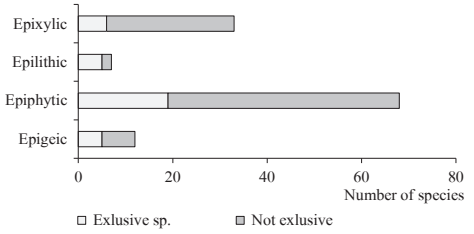

Figure 4. Contribution of exclusive and non-exclusive species in ecological groups of lichens

Most of epiphytic lichens have crustose thalli, accounting for over 50% of the total number of species of epiphytic lichens (Figure 5).

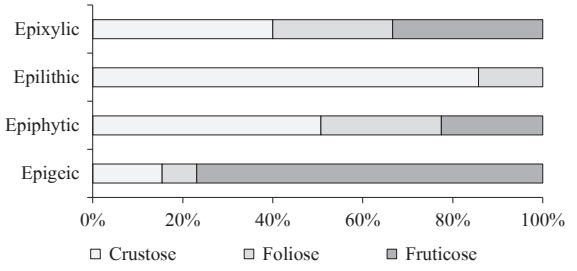

Figure 5. Spatial distribution of lichen species and contributions of morphological forms to each ecological group

The group of epiphytes is dominated by taxa that are frequent or common in the western part of Polish Pomerania (Fałtynowicz, 1992). Only few are protected by Polish law and/or threatened in Poland (Cieśliński et al., 2006). These include *Bacidia rubella* (VU = vulnerable), *Bryoria fuscescens* (partly protected, VU), *Buellia disciformis* (VU), *Chaenotheca furfuracea* (NT = near threatened), *Evernia prunastri* (NT), *Graphis scripta* (NT), *Hypogymnia tubulosa* (partly protected, NT), *Melanohalea exasperata* (CR = critically endangered), *Melanelixia glabra* (EN = endangered), *Ochrolechia androgyna* (VU), *Pseudoschismatomma rufescens* (VU), *Pertusaria coccodes* (NT), *P. pertusa* (VU), *Pleurosticta acetabulum* (partly protected, EN), *Ramalina farinace*a (partly protected, VU), *R. pollinaria* (partly protected, VU), *Zwackhia viridi*s (VU), and *Vulpicida pinastri* (partly protected, NT).

Epixylic lichens

On decaying or rotting wood, 35 lichen species were found, accounting for 30% of the lichen flora of the study area (Figure 2). They were mostly frequent and common lichens of the genera *Cladonia*, *Micarea*, *Placynthiella*, and *Trapeliopsis.* A vast majority of epixylic species were not exclusive to one host species (82%) (Figure 4). In moist patches near the mire, young stumps were usually colonized by crustose lichens, accounting for 40% of the total number of epixylic species (Figure 3). These include *Lecanora conizaeoides*, *Micarea denigrata*, *Placynthiella uliginosa*, and *Trapeliopsis flexuosa*. On stumps with more strongly decomposed wood, they were succeeded by fruticose lichens, mostly of the genus *Cladonia*, frequently also *Hypocenomyce scalaris* and *Lepraria* sp.

Epigeic lichens

In the "Wrzosowisko Sowno" nature reserve, epigeic lichens accounted for nearly 11% of the total number of species (Figure 2). Most of them were not exclusive but grew at the bases of many tree species (Figure 4). Fruticose lichens prevailed among them (Figure 5). Epigeic lichens were found at the edges of the mire and of forest glades. They were represented most often by lichens of the genus *Cladonia*. Species associated with thermophilous sandy pine forests, such as *Cladonia arbuscula* subsp. *mitis*, *Cladonia uncialis*, were less frequent. Only sporadically we recorded *Bacidia bagliettoana*, *Peltigera rufescens, Cladonia rangiferina* (partly protected).

Epilithic lichens

This group of lichens was represented exclusively by species found on anthropogenic substrates, such as concrete poles or walls, and accounted for only 7% of the lichen flora of the study area (Figure 2). The group consists of only 8 species, with crustose thalli, including common calcicolous lichens: *Flavoplaca citrina*, *Calogaya decipiens*, *Candelariella aurella*, *Myriolecis albescens*, *M. dispersa*, and *Lepraria* sp.

Discussion

The species composition of lichens of "Wrzosowisko Sowno" nature reserve is conditioned primarily by specific environmental conditions associated with the presence of the mire. Many species were found at sites that are not accessible to average tourists, so it can be assumed that they are not subject to strong human pressure. The presented results of the research on the biota of

the peatland reserve are consistent with data from other small nature reserves in northern Poland (Fałtynowicz, 1996). The dominant species are epiphytic, common or frequent in the western part of Polish Pomerania. Also the lack of epilithic species is characteristic in the nature reserves, which is associated with the absence of suitable substrates.

Nearly 30% of the recorded epiphytic taxa were rare and threatened, reported from 1–2 localities in reserve Their presence in the reserve attests to the high species diversity of the lichen biota of the reserve and it is an important refuge for rare and threatened species.

List of species

For each species, the type of substrate on which it is found and numbers sites are given. Symbols: * = lichenicolous fungus CR = critically endangered, EN = endangered, VU = vulnerable, NT = near threatened; PR = strict or partial protection.

Acarospora fuscata (Nyl.) Arnold – on concrete posts: 86, 93, 148, 187.
Amandinea punctata (Hoffm.) Coppins & Scheid. – on birch, and poplar tree bark, and wood: 6, 8, 11, 20, 22, 41, 51, 54, 63, 72, 99, 100, 111, 127, 129, 131−134, 144, 145, 176, 181, 207, 226.
Arthonia radiata (Pers.) Ach. – on beech tree bark: 25, 30, 67.
Athalia holocarpa (Hoffm.) Arup, Frödén & Søchting – on concrete posts: 3, 79, 148.
**Athelia arachnoidea* (Berk.) Jülich – on thallus of *Lecanora conizaeoides* on pine tree bark: 11, 23, 25, 45, 80, 71, 82, 92, 98, 118, 119, 129, 132, 181, 184, 185, 193, 226, 230, 232, 237.
Bacidia rubella (Hoffm.) A. Massal. – [VU], on wood: 28.
Bryoria fuscescens (Gyeln.) Brodo & D. Hawksw. – [PR, VU], on oak, beech tree bark: 8, 27, 50,101, 130, 180, 187, 190, 195, 207.
Buellia disciformis (Fr.) Mudd – [VU], on oak tree bark, and wood: 45, 74, 75, 84, 153, 162, 198.
B. griseovirens (Turner & Borrer ex Sm.) Almb. – on oak, birch, maple, poplar, and beech tree bark: 4, 15, 16, 27, 56, 59, 80, 88, 106, 120, 136, 140, 145, 221.
Calogaya decipiens (Hoffm.) Arup, Frödén & Søchting – on concrete posts: 3, 93, 148, 190.
Candelariella xanthostigma (Ach.) Lettau – on birch, poplar, and linden tree bark: 6, 90, 111, 156.
Chaenotheca chrysocephala (Ach.) Th. Fr. – on wood: 214.
C. ferruginea (Turner ex Sm.) Mig. – on birch, pine, oak, and poplar tree bark, and wood: 2, 10, 12, 27, 32, 58, 61, 62, 64, 66, 71, 73, 79, 81, 84, 88, 91, 154, 191, 209.
C. furfuracea (L.) Tibell – [NT], on oak tree bark: 88.
Chrysothrix candelaris (L.) J.R. Laundon – on oak tree bark: 78.
Cladonia cenotea (Ach.) Schaer. – on wood: 36.
C. chlorophaea (Flörke ex Sommerf.) Spreng. – on birch, poplar, oak tree bark, and wood: 7, 8, 10−12, 15, 19, 23, 30−32, 48, 51, 58, 63, 64, 68, 71−73, 78, 80, 92, 94, 98, 99, 102, 108, 111, 114, 116, 122, 126, 127, 132, 135, 138, 139, 145, 156, 157, 160, 171, 177, 179, 185, 188, 191, 196, 202, 208, 212, 219.
C. cervicornis (Ach.) Flot. subsp. *verticillata* (Hoffm.) Ahti – on soil: 36.
C. coniocraea auct. – on poplar, birch, linden, beech, and hazel tree bark, and wood: 1, 2, 6, 10−22, 25−29, 32, 34, 37, 39, 42, 58, 63, 64, 69, 72, 81,108, 109, 114, 119, 133, 152, 173, 219, 225, 233.
C. cornuta (L.) Hoffm. – on birch tree bark: 71,
C. deformis (L.) Hoffm. – on wood, soil, and rare birch tree bark: 13, 18, 24, 30, 34, 39, 41, 43, 48, 53, 57, 58, 60, 64, 66, 68, 71, 72, 74, 78, 83, 88, 98, 103, 105, 111, 116, 126, 127, 132, 140, 146, 157, 160, 162, 167, 173, 184, 185, 191, 196, 198, 200, 202.

C. digitata (L.) Hoffm. – on birch, oak, and pine tree bark, and wood: 7, 12, 15, 16, 26, 30, 32, 34, 48, 52, 58, 60, 62, 64, 69, 71, 79, 88, 89, 91, 95, 102, 104, 118, 121, 123, 126, 128, 135, 139, 141, 146, 171, 173, 177, 184, 188, 192, 208, 221.

C. fimbriata (L.) Fr. – on birch, oak, and poplar tree bark, and wood: 3, 10, 13, 15, 16, 18–21, 23, 24, 28, 33, 41, 51, 55, 57, 59, 60, 62–64, 68–71, 73, 74, 78, 79, 83–85, 87, 89, 90, 92–94, 96, 99, 100, 102, 106, 108, 109, 113, 119, 123, 125, 126, 130, 133, 135–141, 145, 146, 152, 154, 160–162, 165, 171, 223, 232, 233.

C. floerkeana (Fr.) Flörke – on wood, and soil: 7, 49, 56, 86, 110, 132, 173, 220.

C. furcata (Huds.) Schrad. – on soil: 13–16, 70, 71–73, 75–78, 105, 106, 114, 115, 117, 119–121, 126, 127, 136, 162, 165, 170, 172, 200, 202, 203, 205–208, 210, 211, 215–217.

C. glauca s.l. Flörke – on birch, and oak tree bark, soil, and wood: 4, 6, 12, 14, 22, 23, 27, 38, 39, 73, 76, 80–82, 86, 87, 92, 99, 108, 110, 119, 120, 127, 136, 139, 140, 143, 145, 151, 162, 165, 179, 198, 202, 206, 208, 211, 215, 220.

C. macilenta Hoffm. – on soil, wood, and birch, poplar tree bark: 3, 5, 6, 11–16, 20, 22–24, 27, 28, 32–35, 44, 49, 54–56, 58–60, 63–65, 71–74, 76–78, 80, 83–85, 90, 91, 98–100, 104–107, 114, 115, 119, 122, 123, 126–128, 139–141, 144, 146, 150–152, 155–157, 164, 165, 170–173, 179, 188–193, 195, 198, 200–205, 211–215, 218–221, 230–233, 235, 236.

C. rangiferina (L.) Weber – on soil: 13, 16, 73.

C. subulata (L.) Weber – on soil, wood, and birch tree bark: 5, 8, 12, 15, 16, 19, 23, 26, 32, 34, 36, 43, 47–49, 55, 58, 61, 62, 64, 79, 81, 88, 95, 108, 115, 121, 126, 146, 157, 185, 219.

C. uncialis (L.) F.H. Wigg. – on soil: 15, 221.

**Clypeococcum hypocenomycis* D. Hawksw. – on thallus of *Hypocenomyce scalaris* on pine tree bark: 5, 13, 32, 36, 81, 100, 102, 111, 126, 192.

Coenogonium pineti (Schrad.) Lücking & Lumbsch – on oak, and beech tree bark, and wood: 1, 2, 11, 12, 15, 17, 19, 21, 23, 24, 30, 39, 43, 47, 63, 78, 95, 112–114, 121–123, 125, 135, 173, 192, 196, 199, 223, 224, 232, 235.

Evernia prunastri (L.) Ach. – [NT], on willow, poplar, and oak tree bark, and rare wood: 3, 11, 19, 27, 33, 36, 41, 43, 44, 47, 49, 68, 72, 80, 94, 95, 109, 127, 152, 174, 186, 187, 190, 192, 195, 210, 219, 220, 222, 235, 236, 237.

Flavoplaca citrina (Hoffm.) Arup, Frödén & Søchting – on concrete posts: 3, 79, 93, 148, 190.

Graphis scripta (L.) Ach. – [NT], on beech tree bark: 51, 53.

Hypocenomyce scalaris (Ach.) Choisy – on pine, and oak tree bark, and wood: 5, 12, 13, 15, 16, 23, 26, 32, 36, 38, 71, 81, 100, 102, 108, 111, 113, 119, 126, 128, 192, 236.

Hypogymnia physodes (L.) Nyl. – on pine, oak, willow, poplar, beech, and maple tree bark, and wood: 1–17, 19, 22–25, 27, 29, 30, 33, 34, 36, 37, 39, 40, 41, 42, 44–47, 49–52, 54–59, 61, 63, 65, 68–70, 72–74, 77, 78, 80–85, 87, 88–100, 102, 106–110, 114–119, 121, 125–127, 129, 130, 133, 135–139, 141–143, 146, 154, 158, 160–163, 165, 168, 169, 171, 173–177, 186, 187, 195, 196, 200–202, 212, 220, 222, 224, 226–232, 234–236.

H. tubulosa (Schaer.) Hav. – [PR, NT], on birch, oak, willow, and pine tree bark, and wood: 14, 16, 27, 72, 111, 123, 126.

**Illosporiopsis christiansenii* (B. L. Brady & D. Hawksw.) D. Hawksw. – on thallus of *Physciatenella* on birch tree bark: 8, 34, 74, 129.

Lecanora carpinea (L.) Vain. – on poplar beech, and hazel tree bark, and wood: 24, 25, 43, 60, 66, 191.

L. chlarotera Nyl. – on wood: 26.

L. conizaeoides Nyl. – on birch, poplar, beech, hazel, and oak tree bark, and wood: 1, 11, 16, 19, 20, 22, 23, 25, 45, 46, 56, 57, 63, 80, 71, 72, 74, 75, 78, 82, 84, 85, 92, 98, 103, 107, 109, 118, 119, 128, 129, 132, 150, 163, 181, 184, 185, 193, 195, 197, 200, 208, 221, 226, 227, 228, 230, 232, 233, 237, 238.

L. expallens Ach. – on birch, poplar beech, and oak tree bark: 7, 21, 34, 55−58, 89, 99, 112, 140, 157, 185, 187,190−196, 210−214.

L. pulicaris (Pers.) Ach. – on pine, birch, poplar, beech, and oak tree bark, and wood: 8, 23, 25, 33, 38, 56, 70, 78, 99, 101, 104, 110, 143, 156−159, 170, 182, 196, 203, 206.

L. saligna (Schrad.) Zahlbr. – on oak, beech tree bark: 36, 57, 77, 101.

Lecidella elaeochroma (Ach.) Choisy – on willow, and pine tree bark: 15, 34, 67, 88, 121, 193.

Lepraria sp. (L.) Ach. – on poplar, oak, maple, hazel tree bark, and wood: 1−3, 5−13, 15−28, 30−85, 87−99, 101−104, 106−133, 135−177, 179−181, 183, 184, 186−196, 198−208, 210−215, 219−227, 229−236, 238.

**Lichenoconium erodens* M. S. Christ. & D. Hawksw. – on thallus of *Hypocenomyce scalaris, Hypogymnia physodes* and *Lecanora conizaeoides*: 25, 30, 33, 34, 72, 84, 197, 238.

**Lichenoconium lecanorae* (Jaap) D. Hawksw. – on apothecia of *Lecanora conizaeoides*: 11, 16, 25, 45, 80, 71, 72, 84, 118, 197, 238.

Melanohalea exasperata (De Not.) O. Blanco, A. Crespo, Divakar, Essl., D. Hawksw. & Lumbsch – [CR], on birch, and hazel tree bark, and wood: 28, 73, 93.

M. exasperatula (Nyl.) O. Blanco, A. Crespo, Divakar, Essl., D. Hawksw. & Lumbsch – on birch tree bark: 7, 96, 174.

Melanelixia fuliginosa (Fr. ex Duby) O. Blanco, A. Crespo, Divakar, Essl., D. Hawksw. & Lumbsch – on birch tree bark: 84.

M. glabra (Schaer.) O. Blanco, A. Crespo, Divakar, Essl., D. Hawksw. & Lumbsch – [EN], on oak tree bark: 19.

Micarea denigrata (Fr.) Hedl. – on wood: 7, 9, 123.

Myriolecis albescens (Hoffm.) Śliwa, Zhao Xin & Lumbsch – on concrete posts: 79, 93, 148.

M. dispersa (Pers.) Śliwa, Zhao Xin & Lumbsch – on concrete posts: 3, 79, 93, 148, 190.

Ochrolechia androgyna (Hoffm.) Arnold – [VU], on birch tree bark: 62, 78.

Parmelia sulcata Taylor – on birch, pine, poplar, willow, oak, beech, hazel, and linden tree bark, and wood: 1−6, 8−12, 17, 19, 20, 22−24, 27−29, 31, 36, 39, 41, 43, 44, 47, 49, 50−53, 55−57, 59, 61, 64, 65, 67−73, 75−77, 79, 80, 82, 83, 86−91, 94−96, 101, 103, 105, 106, 108, 109, 111−113, 115, 116, 118, 121, 122, 124, 125, 127, 129, 132−135, 137, 139, 143−145, 151, 152, 154, 155, 157−163, 166, 168, 169, 173, 174, 179, 186, 187, 190, 196, 200, 210, 221, 222, 227, 232, 235−238.

P. saxatilis (L.) Ach. – on birch and alder tree bark: 6, 37, 94, 165.

Parmeliopsis ambigua (Wulfen) Nyl. – on birch, beech, willow, and poplar tree bark: 2, 3, 6, 11, 13, 19, 73, 85, 154.

Peltigera rufescens (Weiss) Humb. – on soil: 72, 76, 126, 140.

Pertusaria albescens (Huds.) Choisy & Werner – on beech tree bark: 76, 163.

P. amara (Ach.) Nyl. – on hazel, and beech tree bark: 9, 55, 135.

P. coccodes (Ach.) Nyl. – [NT], on oak tree bark: 24, 77, 162.

P. pertusa (Weigel) Tuck. – [VU], on hazel, and beech tree bark: 9, 72, 87, 104, 125, 148, 181, 222.

Phaeophyscia nigricans (Flörke) Moberg – on linden, and birch tree bark, and on concrete posts: 93, 148, 190, 197, 234.

P. orbicularis (Neck.) Moberg – on poplar tree bark: 235.

Phlyctis argena (Ach.) Flot. – on beech, hazel, and oak tree bark: 7, 26, 31, 67, 85, 91, 94–96, 165, 174, 175, 237.

Physcia adscendens (Fr.) H. Olivier – on hazel, and willow tree bark: 1, 196, 231.

P. tenella (Scop.) DC. – on birch, tree poplar, hazel, willow bark, and wood: 1, 3, 4, 6–10, 12, 17–24, 26–30, 32, 34, 36, 37, 40, 41, 43–45, 49–53, 55, 57–61, 65, 67, 68, 70–80, 82, 83, 85–89, 91–101, 103–116, 119–122, 125–130, 132, 134–139, 141–170, 176, 178–180, 184, 189, 190, 196, 199, 200, 201, 202, 206, 208, 212, 217–219, 223, 226, 232, 233.

Placynthiella icmalea (Ach.) Coppins & P. James – on hazel tree bark, and wood: 8, 48, 53, 57, 68, 71, 86, 92, 94, 96, 112, 1124, 184, 162, 164, 185, 233.

P. uliginosa (Schrad.) Coppins & P. James – on soil: 8, 75, 86, 94, 108, 111, 132, 143, 185, 192, 196, 198, 200.

Platismatia glauca (L.) W.L. Culb. & C.F. Culb. – on poplar tree bark: 17, 103, 107.

Pleurostica acetabulum (Neck.) Elix & Lumbsch – [PR, EN], on oak, birch maple tree bark: 11, 24, 36, 40, 50, 55, 62, 72, 78, 85, 96, 106, 117, 132, 149, 170, 210.

Polycauliona candelaria (L.) Frödén, Arup & Søchting – on oak, willow, and beech tree bark: 3, 9, 44.

P. polycarpa (Hoffm.) Frödén, Arup & Søchting – on willow, birch, maple, poplar, and oak tree bark and wood: 3–6, 9, 10, 17, 19, 24, 29, 31, 34, 37, 41, 48, 51, 52, 54, 57, 59, 60, 61, 63, 65, 68, 74, 76, 77, 83, 84, 92, 94, 96, 97, 100, 101, 105–107, 112, 113, 116, 124, 127, 144, 146, 157, 169, 195, 215, 216, 231.

Porina aenea (Wallr.) Zahlb. – on beech tree bark: 8, 44, 51, 53, 55.

Pseudevernia furfuracea (L.) Zopf – on oak, beech, and birch tree bark: 11, 85, 90, 135.

Pseudoschismatomma rufescens (Pers.) Ertz & Tehler – [VU], on beech, and hazel tree bark: 8, 87.

Ramalina farinacea (L.) Ach. – [PR, VU], on oak, maple, willow tree bark: 6, 16, 24, 51, 65, 112, 184.

R. pollinaria (Westr.) Ach. – [PR, VU], on birch tree bark: 64, 52.

Scoliciosporum chlorococcum (Graeve ex Stenh.) Vězda – on pine, hazel tree bark: 22, 43, 50, 61, 68, 88, 111, 135.

Trapeliopsis flexuosa (Fr.) Coppins & P. James – on wood, and birch tree bark: 7, 10, 15, 18, 24, 30, 32, 33, 36, 43, 49, 56, 60, 68, 75, 86, 92, 99, 111, 116, 124, 132, 154, 162, 185, 191, 198, 200, 209, 219, 233.

T. granulosa (Hoffm.) Lumbsch – on soil, wood, and birch tree bark: 9, 12, 16, 19, 24, 29, 30, 33, 51, 62, 63, 72, 78, 88, 108, 109, 113, 119, 132, 135, 146, 184.

Tuckermannopsis chlorophylla (Willd.) Hale – on oak, and beech tree bark: 7, 48, 54, 79, 124, 167, 213.

Usnea dasopoga (Ach.) Nyl. – on birch tree bark: 51, 94, 109, 132, 184, 223.

Violella fucata (Stirt.) T. Sprib. – on beech tree bark: 67.

Vulpicida pinastri (Scop.) J.-E. Mattsson & M.J. Lai – [PR, NT], on birch tree bark: 3, 64, 160.

Xanthoria parietina (L.) Th. Fr – on willow, birch, oak, poplar, pine, and beech tree bark, and wood: 3–6, 8, 10, 17–28, 30–36, 39–41, 43–48, 50–53, 57, 63, 64, 66, 68, 70, 72–78, 80, 82–86, 89, 92, 94, 97–100, 102, 103, 105, 108, 109, 111, 112, 114–116, 127, 129, 131, 132, 134, 137, 143, 144, 146, 149, 150, 157, 158, 160, 164, 167, 173–175, 177, 179, 182–193, 195–200, 203, 207, 208, 210, 212, 217, 218, 221–224, 226–229, 232–236, 238.

Zwackhia viridis (Ach.) Poetsch & Schied. – [PR, NT], on beech tree bark: 8.

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Lichen biota of the "Wrzosowiska Cedyńskie im. inż. Wiesława Czyżewskiego" nature reserve in the Cedynia Landscape Park (NW Poland)

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Keywords xerothermic lichens, rare lichens, protected species, threatened lichens, nature reserve, NW Poland

Abstract Lichens of the "Wrzosowiska Cedyńskie im. inż. Wiesława Czyżewskiego" nature reserve were studied in 2005 and 2011. Within the examined area, 103 species of lichens were observed. These include 23 species that are new to this area, some of them calciphilous, e.g. *Agonima gelatinosa* and *Collema crispum*. Many of them are rare in the Polish lowlands, e.g. *Cladonia stellaris*, *Rhizocarpon geographicum*, *R. polycarpum*, *Stereocaulon condensatum*, and *S. incrustatum.*

Biota porostów rezerwatu "Wrzosowiska Cedyńskie im. inż. Wiesława Czyżewskiego" w Cedyńskim Parku Krajobrazowym

Słowa kluczowe porosty kserotermiczne, porosty rzadkie, gatunki chronione, porosty zagrożone, rezerwat przyrody, NW Polska

Streszczenie Porosty rezerwatu "Wrzosowiska Cedyńskie im. Wiesława Czyżewskiego" badano w 2005 i 2011 roku. Na badanym obszarze zaobserwowano 103 gatunki porostów. Wśrod nich 23 taksony to gatunki nowe na tym obszarze, niektóre z nich to porosty kalcyfilne np. *Agonima gelatinosa* i *Collema crispum*. Wiele z nich jest rzadkich na polskich nizinach, np. *Cladonia stellaris*, *Rhizocarpon geographicum*, *R. polytropa*, *Stereocaulon condensatum* i *S. incrustatum*.

Introduction

Xerothermic grasslands in Pomerania are the northern most relict steppe communities in Poland. They develop in the places where edaphic and microclimatic conditions are similar to those characteristic of the plant formations of steppes and forest steppes. They are found

primarily on S- and W-facing slopes of valleys of the lower Oder (Odra), Vistula, and in the Toruń-Eberswalde Proglacial Valley (Pradolina Toruńsko-Eberswaldzka).

Soils overgrown by xerothermic vegetation are fertile and rich in calcium carbonate. Such conditions are favourable for development of lichens of two groups: xerothermophytes and calciphiles, i.e. species found on limestones or on other substrates containing calcium (Czyżewska, 1986). Xerothermic species of lichens are floristic curiosities in the western part of Polish Pomerania, as they are usually found in mountains and uplands. Some xerothermic lichens have been reported from the lowlands of Poland (e.g. Tobolewski, 1962; Glanc, 1964; Ceynowa-Giełdon, 1993, 2001; Ceynowa-Giełdon, Glazik, 1994; Ceynowa-Giełdon et al., 2004; Czyżewska, 1986; Wójciak, 1987), but the most characteristic species of xerothermic grasslands are threatened in the central part of the European Lowland (Aptroot et al., 2011; Dolnik et al., 2010; Hauck, de Bryun, 2010; Litterski, Schiefelbein, 2007; Otte, Ratzel, 2004; Søchting, Alstrup, 2008). This is due to the usually small size of patches of such habitats and their geographic division, but also to less frequent use of the land, especially stopping sheep grazing.

Here, the importance of habitats of this type for the biota of lichens of north-western Poland is discussed on the basis of the current species composition of lichens in this reserve, with particular reference to the earlier disregarded xerothermic lichens.

Study area

The "Wrzosowiska Cedyńskie im. inż. Wiesława Czyżewskiego" nature reserve, covering an area of 72.02 ha, is situated in the western part of the Cedynia Landscape Park, about 3 km south-west of the town of Cedynia. It is composed of patches of heaths of the alliance *Pohlio-Callunion* (Matuszkiewicz, 2001) and xerothermic grasslands of the class *Festuco-Brometea*, which are rare in Central and Western Europe. The reserve was created in 1985, based on documents prepared by Ćwikliński (1976). Its northern boundaries run along the Cedynia-Osina road, eastern along the valley bottom at the forest fringe, southern along the plateau, and western along the valley bottom.

The reserve lies on moraine hills made of postglacial, slightly loamy sands, reaching up to 50 m above sea level. The protected area covers the hillocks distinguished by their varied shape and vegetation. It is an almost woodless area, dominated by common heather *Calluna vulgaris* (Figure 1) and xerothermic plants. Herbaceous plants within the heather patches include German greenweed *Genista germanica*, carline thistle *Carlina vulgaris*, mouse-ear hawkweed *Hieracium pilosella*, and field wood-rush *Luzula campestris* (Ćwikliński, 1976; Ziarnek et al., 2006). Among the grasses, there are many stones and boulders, which are microhabitats for epilithic lichens.

In total, 89 species of lichens were reported from the reserve in 1976–2013, including 41 epigeic, 26 epiphytic, and 20 epilithic species. (Ćwikliński, 1976; Ziarnek et al., 2006; Ciaciura et al., 2008; Wieczorek, Schiefelbein, 2013). Considering the specific microclimatic conditions, special attention should be paid to xerothermic lichens found in the reserve. High temperatures of the top layer of the soil and high concentrations of calcium carbonate create favourable conditions for development of xerothermic lichens, which are rare in Pomerania. In the study area they were represented by e.g. *Bacidia bagliettoana, Blenothallia crispa, Cladonia foliacea, C. pocillum, Enchylium tenax,* and *Placidium squamulosum*.

Figure 1. Sandy grassland with *Calluna vulgaris*

Material and methods

The study was conducted in 2007–2010. A map used in the fieldwork was divided into a grid of 100 m × 100 m squares, numbered as in Figure 2. Each square (100 m × 100 m = 10 000 m^2) was regarded as one locality. In total, 365 lichen specimens were collected and deposited in the Lichen Herbarium of the Department of Ecology and Environmental Protection of the University of Szczecin. The species are listed alphabetically, with information on their localities (square numbers) and type of substratum. Terminology was adopted from Fałynowicz and Kossowska (2016). Threat categories (EN, VU, NT) are given according to the "Red List of extinct and threatened lichens in Poland" (Cieśliński et al., 2006).

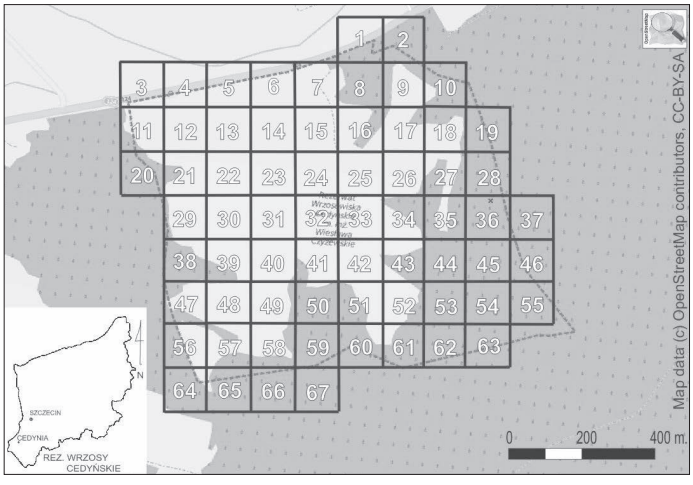


Figure 2. Distribution of grid squares

Results

In the study area, 103 lichen species were recorded as a result of field research. The most frequently represented ecological group were epigeic lichens, which account for 42% of the total number of species. This is understandable because of the small number of woody plants in the reserve. About 26% of the total number of species are epiphytic lichens, growing on the bark of various trees growing on the fringe of the study area. The least numerous ecological groups are epilithic lichens occurring on rocks (19%) and epixylic lichens found on dead wood of tree trunks and branches (10%), because of a lack of substrata suitable for the development of that ecological group (Figure 3).

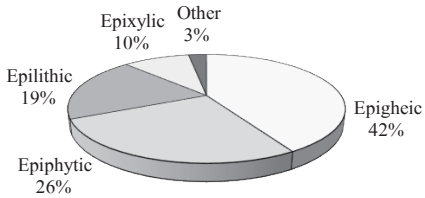
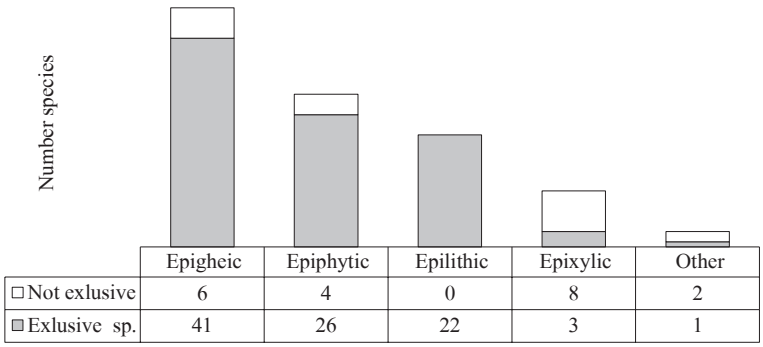


Figure 3. Percentage of species from various ecological groups

Epigeic lichens constitute an important component of the plant cover of the reserve, as 47 epigeic lichen species were recorded within the study area. A vast majority of them (41 taxa) are typical of dry and acidic sandy grasslands (Figure 4). Species with fruticose thalli prevail among epigeic lichens (Figure 5) and are represented first of all by common taxa of the genus *Cladonia*. Out of the 29 recorded species of that genus, most are typical of sandy grasslands. The most frequently recorded psammophilous species are *C. fimbriata*, *C. furcata*, *C. macilenta*, *C. foliacea*, *C. subulata*, *C. chlorophaea* s.l., and *C. uncialis*. Among crustose epigeic lichens, constituting barely 15% of the soil-growing species (Figure 3), the most frequently recorded ones include *Placynthiella oligotropha*, *P. uliginosa*, and *Trapeliopsis granulosa*.



	Epigheic	Epiphytic	Epilithic	Epixylic	Other
□ Not exlusive	6	4	0	8	2
▨ Exlusive sp.	41	26	22	3	1

Figure 4. Shares of ecological groups in exclusive and non-exclusive species lichen biota

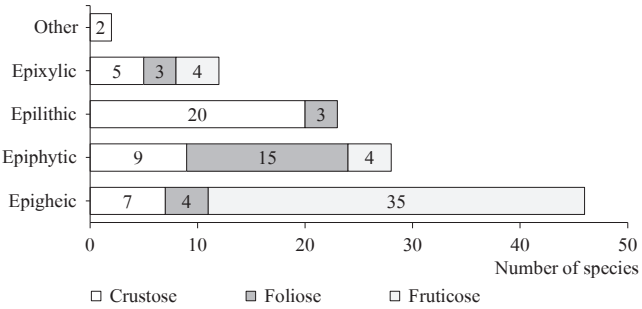


Figure 5. Spatial distribution of lichen species and contribution morphological forms in each

Out of the 103 recorded lichen species, 30 were epiphytic, found on the bark of various tree species. They were usually common taxa, widespread in Poland, e.g. *Hypocenomyce scalaris*, *Hypogymnia physodes*, *Lecanora conizaeoides*, *Physcia tenella*, *Parmelia sulcata*, *Parmeliopsis ambigua*, *Scoliciosporum chlorococcum*, *Xanthoria parietina*, and *X. polycarpa.* However, species like *Evernia prunastri*, *Hypogymnia tubulosa*, *Lecidella elaeochroma*, *Melanelixia fuliginosa*, *Physcia adscendens*, *Physconia grisea*, and *Platismatia glauca*, were only sporadically recorded in Pomerania. Out of all the morphological forms of that group of lichen species, decidedly most abundant ones are those with foliose thalli, constituting 53% of the epiphytic lichens (Figure 5). Special attention should be paid to *Usnea hirta* and *Pleurosticta acetabulum*, found in single locations, as they are red-listed in Poland. Although they occur in single locations in the reserve, they are quite frequent in other parts of the West Pomerania Province.

Discussion

Out of the 103 species recorded in this study, 24 were not reported earlier (Ćwikliński, 1976; Ziarnek et al., 2006; Ciaciura et al., 2008; Wieczorek, Schiefelbein, 2013).

Populations of lichens typical of sandy grasslands are very numerous within the area under study and form extensive carpets interspersed with heather shrubs and tufts of moss and flowering plants. The bare sandy soil is overgrown with *C. arbuscula*, *C. ciliata*, *C. coccifera*, *C. glauca*, *C. phyllophora*, *C. pleurota*, *C. polydactyla*, *C. portentosa*, *C. pyxidata*, and *C. cervicornis* subsp. *verticillata*. Among the epigeic lichen species, only a few are forest acidophytes, occurring not only on sandy grasslands but also quite frequent in dry pine forests and thickets on sandy soils, e.g. *C. cornuta*, *C. deformis*, *C. digitata*, *C. rangiferina*, and *C. squamosa*. Between those lichens, small thalli of *Cetraria aculeata*, *C. islandica*, and *Peltigera didactyla* can be found sporadically. Within the group of epigeic lichen species, special attention should be paid to the lichens that are very rare in Western Pomerania (Fałtynowicz, 1992). They include *Cladonia stellaris*, *Cetraria muricata*, *Stereocaulon condensatum*, and *S. incrustatum*, which in the study area form very small populations. Despite repeated searches, their single thalli were observed in single locations only.

The most interesting lichens are calciphilous species, as xerothermic grasslands are rare in Pomerania. Species of this group are most abundant in Poland in mountains and uplands within the belt of limestones. In other areas of our country, xerothermic grasslands are observed on steep slopes of river valleys, particularly of the Oder and Vistula, or on the moraine hills

exposed to the south. In the "Wrzosowiska Cedyńskie im. inż. Wiesława Czyżewskiego" nature reserve, 10 xerothermic lichen species were recorded. The most frequent among them is *Bacidia bagliettoana*. It is found between tall grasses, on bare soil. In Poland it is one of the most widespread xerothermic lichens (Fałtynowicz, 1992; Ceynowa-Giełdon, 2001; Wieczorek, Schiefelbein, 2013). Apart from this species, small foliose thalli of *Enchylium tenax*, *Placidium squamulosum* and slightly larger ones of *Peltigera rufescens* occur with a similar frequency. Out of the xerothermic lichens with fruticose thalli, the most frequent is *Cladonia pyxidata*. Apart from it, the photophilous and calciphilous species *Agonimia gelatinosa*, *Blenothallia crispa*, and *Diploschistes muscorum* are observed sporadically. A species very rarely occurring on uncovered limestone hillsides in the higher parts of slopes and tops is *Rufoplaca arenaria*, with characteristic orange ascocarps (apothecia), known in Western Pomerania from several locations (Fałtynowicz, 1992). Xerothermic species include also *Cladonia foliacea* and *C. rangiformis* according to some authors (Fałtynowicz, 1992).

The group of epiphytic lichens, despite a relatively small area being occupied by trees and shrubs in the reserve, is very rich. On the one hand, it is connected with the presence of extensive forest areas around the reserve, but on the other hand, with the forest-heath ecotone on the border with extensive woodless areas of the reserve. As a result, photophilous and dust-loving (pollution-tolerant) lichen species, e.g. *Xanthoria polycarpa* or *X. parietina*, are observed here, apart from the typical forest lichens, like *Bryoria* spp.

The occurrence of rare lichen taxa of natural bedrocks in Pomerania also deserves attention. A substratum for epiphytic lichens in the reserve are stones of various size and shape, scattered between heather and grass patches, but also small concrete border posts. The richest lichen patches are observed on stones in the open ground. Among the various morphological forms, crustose lichens prevail, constituting 83% of all epilithic species. Characteristic species growing on stones are *Acarospora fuscata*, *Lecanora polytropa*, *Protoparmeliopsis muralis*, *Lecidea grisella*, and *Rusavskia elegans*, while *Lecania erysibe*, *Rhizocarpon polycarpum*, and *R. geographicum* were very rare, on single stones. The latter group of taxa are very rare in the lowlands, but more frequent in higher, mountainous locations. In shady places, between tall grasses, substantially fewer lichen species were observed on stones, those including *Porpidia crustulata*, *Trapelia placodioides*, and *Verrucaria nigrescens*. On concrete posts, calciphilous lichens typical for that habitat were observed, e.g. *Myriolecis dispersa* or *Candelariella aurella*.

Within the "Wrzosowiska Cedyńskie im. inż. Wiesława Czyżewskiego" nature reserve, the occurrence of 13 red-listed species (Cieśliński et al., 2006) was observed, and they constitute 13% of the total number of lichens recorded in this study (Figure 6). Particularly valuable are endangered species (EN), such as *Cladonia stellaris* (occurring in a small fragment of pine forest), *Pleurosticta acetabulum* (observed on the bark of a lonely birch by the road), and *Stereocaulon incrustatum* (found between other lichen taxa on bare soil). Other red-listed lichen species in the reserve are: *Bryoria fuscescens*, *Cetraria islandica*, *Stereocaulon condensatum*, *Tucermanopsis chlorophylla*, and *Usnea hirta*, representing the category of vulnerable taxa (VU), and *Cetraria muricata*, *Evernia prunastri*, *Hypogymnia tubulosa*, *Placidium squamulosum*, and *Vulpicidia pinastris*, representing the category of near-threatened taxa (NT). All of these lichens were observed in single locations. No signs of degeneration, such as discolouration or missing reproduction organs, were observed on the thalli of these species but it is difficult to predict transformations and the direction of changes in the lichen due to the lack of earlier detailed lichenological data from this area.

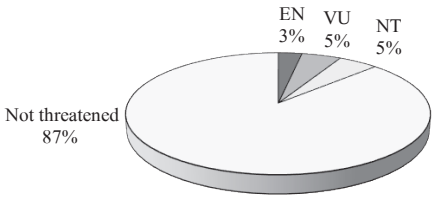


Figure 6. Numbers of lichen species in particular threat categories (Cieśliński et al., 2006): EN = endangered; VU = vulnerable; NT = near threatened

Within the area of the reserve, the occurrence of 23 species protected by Polish law was recorded (marked with PR in the species list below).

Despite the impact of many anthropogenic factors, including trampling in particular, the diversity of lichens in the reserve is very high, with many interesting and rare species. They represent the taxa mostly specialised and adapted to specific habitat conditions, so good prospects for their preservation are created by the local ecological conditions.

List of species

For each species, the type of substrate on which it is found and square numbers are given.

Symbols: ! = new species for the "Wrzosowiska Cedyńskie im. inż. Wiesława Czyżewskiego" nature reserve: CR = critically endangered, EN = endangered, VU = vulnerable, NT = near threatened; PR = strict or partial protection.

Acarospora fuscata (Nyl.) Arnold – on stones: 35, 41.
!*Agonimia gelatinosa* (Ach.) Brand & Diederich – on soil: 26.
Amandinea punctata (Hoffm.) Coppins & Scheid. – on birch tree bark: 1, 39, 50.
Aspicilia cinerea (L.) Körb. – on stone: 41.
Athalia holocarpa (Hoffm.) Arup, Frödén & Søchting – on a concrete post: 7.
Bacidia bagliettoana (A. Massal. & De Not.) Jatta – on soil: 8, 12, 21, 35, 40, 43.
!*Blenothallia crispa* (Huds.) Otálora, P.M. Jørg. & Wedin – on soil: 33, 35.
Bryoria fuscescens (Gyeln.) Brodo & D. Hawksw. – [PR, VU], on birch tree bark: 29.
Calogaya decipiens (Hoffm.) Arup, Frödén & Søchting – on a concrete post: 7.
!*C. pusilla* (A. Massal.) Arup, Frödén & Søchting – on a concrete post: 7.
Candelaria concolor (Dicks.) Stein – on birch, and oak tree bark: 45, 50.
Candelariella aurella (Hoffm.) Zahlbr. – on stones: 13, 26.
C. coralliza (Nyl.) H. Magn. – on stones: 7, 13.
C. vitellina (Hoffm.) Müll. Arg. – on stones and on a concrete post: 16, 35, 38.
!*C. xanthostigma* (Ach.) Lettau – on birch tree bark: 5.
Cetraria aculeata (Schreb.) Ach. – on soil: 4, 6–8, 12, 14, 16, 17, 21, 24–26, 29–33, 39, 40, 43.
C. islandica (L.) Ach. – [PR, VU], on soil: 21, 39.
C. muricata (Ach.) Eckfeldt – [PR, NT], on soil: 12, 30.
Cladonia arbuscula (Wallr.) Flot. em. Ruoss subsp. *mitis* (Sandst.) Ruoss – on soil: 12, 32, 33.
C. arbuscula (Wallr.) Flot. subsp. *squarrosa* (Wallr.) Ruoss – on soil: 12, 21, 29–31, 40.

C. cenotea (Ach.) Schaer. – on soil: 40.
C. cervicornis (Ach.) Flot. subsp. *verticillata* (Hoffm.) Ahti – on soil: 12–15, 21, 27, 29, 36.
C. chlorophaea s.l. (Flörke ex Sommerf.) Spreng. – on soil, and wood: 4–9, 12–18, 21–27, 29–36, 40–43.
C. ciliata Stirt. Harm. var. *tenuis* (Flörke) Nimis – [PR], on soil: 4, 12, 21, 32, 33.
C. coccifera (L.) Willd. – on soil: 32, 41, 57.
C. coniocraea auct. – on soil, and at the base of a pine tree trunk: 1, 5, 8, 11, 26, 28, 29, 39, 44, 45, 48, 50–66.
C. cornuta (L.) Hoffm. – on soil: 16, 17, 39, 40.
C. deformis (L.) Hoffm. – on soil: 6, 25, 30, 36.
C. digitata (L.) Hoffm. – on soil: 4, 5, 8, 13, 15, 16, 30, 33, 34, 49.
C. fimbriata (L.) Fr. – on soil, and wood: 4–19, 21–23, 25, 28, 29, 31, 32, 35–45, 48–51, 53–60, 62, 63.
C. floerkeana (Fr.) Flörke – on soil: 5, 6, 9, 11–13, 21, 23, 27, 30, 36, 39, 49.
C. foliacea (Huds.) Willd. – on soil: 4–9, 11–17, 21–26, 29–35, 40, 42.
C. furcata (Huds.) Schrad. – on soil: 4–9, 12–18, 21, 22, 24–27, 29–36, 39–43,48, 49, 57.
C. glauca s. l. Flörke – on soil: 5, 12, 35, 43.
C. gracilis (L.) Willd. – on soil: 4, 12.
C. macilenta Hoffm. – on soil, and wood: 4, 6, 7, 9, 10, 12–18, 21–24, 26–29, 32–34, 39–43, 47–49, 51, 57.
C. phyllophora Hoffm. – on soil: 22, 26, 31, 35, 40.
C. pleurota (Flörke) Schaer. – on soil: 11, 33, 36.
C. polydactyla (Flörke) Spreng. – on soil: 5, 7, 8, 12, 13.
C. portentosa (Dufour) Coem. – [PR], on soil: 7–9, 16, 34.
C. pocillum (Ach.) O.-J. Rich. – on soil: 17, 18, 21, 30, 40.
C. pyxidata (L.) Hoffm. – on soil: 7, 8, 12, 13, 17, 23, 26, 30, 36, 48, 49.
C. rangiferina (L.) Weber – on soil: 5, 8, 9, 12, 14, 21, 29, 39.
C. rangiformis Hoffm. – on soil: 4, 7.
C. scabriuscula (Delise) Nyl. – on soil: 4, 9, 12.
C. squamosa (Scop.) Hoffm. – on soil: 22, 23.
C. stellaris (Opiz) Pouzar & Vězda – [PR, EN], on soil: 21.
C. subulata (L.) Weber – on soil, and wood: 4–9, 11–17, 21–26, 29–35, 40, 42.
C. uncialis (L.) F.H. Wigg. – on soil: 5–9, 11–17, 2123, 25, 26, 29–35, 40, 42.
Coenogonium pineti (Schrad.) Lücking & Lumbsch – on oak tree bark: 50, 54.
!*Diploschistes muscorum* (Scop.) R. Sant. – on soil: 32, 34.
Enchylium tenax (Sw.) Gray – on soil: 32–34, 41, 42.
Evernia prunastri (L.) Ach. – [NT], on birch tree bark: 1.
!*Fellhanera subtilis* (Vězda) Diederich & Sérus. – on small branches of *Calluna vulgaris*: 15.
Hypocenomyce scalaris (Ach.) M. Choisy – on pine, and birch tree bark, and wood: 1, 2, 11, 18, 23, 38, 45, 48, 50–55, 57–63, 65.
Hypogymnia physodes (L.) Nyl. – on pine, and birch tree bark: 1–5, 7, 9–11, 18, 19, 29, 38, 39, 43–48, 50–67.
H. tubulosa (Schaer.) Hav. – [PR, NT], on birch tree bark: 5, 8, 9, 43.
!*Lecania erysibe* (Ach.) Mudd – on stones: 16, 21.
Lecanora conizaeoides Nyl. – on pine, and birch tree bark: 1, 2, 8, 9, 29, 38, 47, 50, 51, 53, 55.
L. polytropa (Ehrh. ex Hoffm.) Rabenh. – on stones: 28, 30.

L. pulicaris (Pers.) Ach. – on birch, and poplar tree bark: 43, 44, 59.
Lecidea fuscoatra (L.) Ach. – on stones: 22, 24, 25.
!*Lecidella elaeochroma* (Ach.) Choisy – on birch tree bark: 3, 8.
Lepraria incana (L.) Ach. – on tree bark, and wood: 1, 2, 8–11, 29, 38, 43–47, 50–66.
!*Melanelixia fuliginosa* (Fr. ex Duby) O. Blanco & al. – on birch tree bark: 2.
!*Micarea denigrata* (Fr.) Hedl. – on wood: 18.
Myriolecis dispersa (Pers.) Śliwa, Zhao Xin & Lumbsch – on concrete posts: 8.
Parmelia sulcata Taylor – on pine, and birch tree bark: 1–3, 7, 28, 57, 58, 62, 63.
Parmeliopsis ambigua (Wulfen) Nyl. – on pine, and birch tree bark, and wood: 1–3, 7, 9, 10, 11, 44.
Peltigera didactyla (With.) J.R. Laundon – [PR], on soil: 7, 12, 29.
P. rufescens (Weiss) Humb. – on soil: 11.
!*Phaeophyscia nigricans* (Flörke) Moberg – on concrete posts: 57.
!*P. orbicularis* (Neck.) Moberg – on concrete posts: 57.
Phlyctis argena (Ach.) Flot. – on oak tree bark: 6, 10.
Physcia adscendens Fr. H. Olivier – on birch tree bark: 2.
P. tenella (Scop.) DC. – on birch tree bark: 2, 7.
!*Physconia grisea* (Lam.) Poelt – on birch tree bark: 7.
Placidium squamulosum (Ach.) O. Breuss – [NT], on soil: 16, 21, 24, 25.
!*Placynthiella icmalea* (Ach.) Coppins & P. James – on soil: 5, 6, 9.
!*P. oligotropha* (Vain.) Coppins & P. James – on soil: 5, 8, 9, 11, 12, 15, 17, 30, 34.
!*P. uliginosa* (Schrad.) Coppins & P. James – on soil, and wood: 3, 6–8, 22, 27, 39, 40.
Platismatia glauca (L.) W.L. Culb. & C.F. Culb. – on birch tree bark: 1, 3, 50.
!*Pleurosticta acetabulum* (Neck.) Elix & Lumbsch – [PR, EN], on birch tree bark: 11.
Polycauliona candelaria (L.) Th. Fr. – on wood: 7.
P. polycarpa (Hoffm.) Th. Fr. ex Rieber – on birch tree bark: 3, 7.
Porpidia crustulata (Ach.) Hertel & Knoph – on stones: 16, 25.
Protoparmeliopsis muralis (Schreb.) Choisy – on stones: 16, 20.
Pseudevernia furfuracea (L.) Zopf – on pine tree bark: 1, 11, 29.
Rhizocarpon geographicum (L.) DC. – on stone: 16.
!*R. polycarpum* (Hepp) Th. Fr. – on stone: 16.
!*Rufoplaca arenaria* (Pers.) Arup, Søchting & Frödén – on soil: 24.
Rusavskia elegans (Link) Th. Fr. – on stones: 16, 20.
Scoliciosporum chlorococcum (Graeve ex Stenh.) Vězda – on pine, and birch tree bark: 1, 3, 53.
!*Stereocaulon condensatum* Hoffm. – [PR, VU], on soil: 6.
!*S. incrustatum* Flörke – [PR, EN], on soil: 6.
!*Trapelia placodioides C*oppins & P. James – on stone: 16.
Trapeliopsis flexuosa (Fr.) Coppins & P. James – on wood: 6, 10.
T. granulosa (Hoffm.) Lumbsch – on soil, and dead bark: 6, 7, 10, 39, 41, 46, 57.
Tuckermanopsis chlorophylla (Willd.) Hale – [PR, VU], on birch, and oak tree bark: 11, 48.
Usnea hirta (L.) Weber ex F.H. Wigg. – [PR, VU], on birch tree bark: 2.
!*Verrucaria nigrescens* Pers. – on stones, and on concrete posts: 16.
Vulpicida pinastri (Scop.) J. E. Mattson & M. J. Lai – [PR, NT], on birch tree bark: 11.
Xanthoparmelia loxodes (Nyl.) O. Blanco & al. – [PR], on stone: 40.
X. parietina (L.) Th. Fr. – on birch tree bark: 3, 7, 18, 20, 46, 47, 49.

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