

Macroinvertebrate communities on various microhabitats of a saline coal mine settling pond

by

Agnieszka Sowa*, Mariola Krodkiewska,
Dariusz Halabowski

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*Department of Hydrobiology,
University of Silesia, ul. Bankowa 9,
40-007 Katowice, Poland*

Abstract

To date, no studies have been conducted on macroinvertebrate communities in coal mine settling ponds used for temporary retention of saline mine waters. The objective of the research was to evaluate which habitat – *Ruppia maritima*, *Phragmites australis* or sediments without macrophytes – is the most favorable for the abundance and biomass of macroinvertebrate communities. The study was carried out in a hyposaline settling pond located in a mining and urban area in southern Poland. At this time, it is the only inland locality of *R. maritima* in Poland.

In the studied coal mine settling pond, the non-native, euryhaline amphipod *Gammarus tigrinus* dominated in the communities on all the habitats. The abundance of other taxa was small and similar on each type of substrate; only Corixidae were much more abundant on the widgeongrass beds. The highest abundance and biomass of macroinvertebrates was recorded at the sites with *R. maritima*. Our study highlights the importance of coal mine settling ponds as a substitute habitat for salt-tolerant invertebrates.

Key words: fauna, *Ruppia maritima*, *Phragmites australis*, sediments, industrial pond, mining area

* Corresponding author: agsowa@us.edu.pl

Introduction

Anthropogenic water bodies that are located in urban and industrial areas can be very important for aquatic fauna and flora, especially in areas where there are no natural water bodies (Gee et al. 1997; Gledhill et al. 2008; Vermonden et al. 2009; Chester & Robson 2013; Hassall 2014; Hill et al. 2016; Martínez-Abraín & Jiménez 2016; Rewicz et al. 2016; Hill et al. 2017). These anthropogenic habitats may be particularly important for rare and threatened species. For example, Lewin & Smoliński (2006) reported the presence of the rare gastropod species *Anisus vorticulus* (Troschel) and *Valvata naticina* Menke in clay pit ponds; the former is Near Threatened according to the European Red List of Non-marine Molluscs (Cuttelod et al. 2011) and the Red List of Threatened Animals in Poland (Głowaciński & Nowacki 2004), and the latter is Critically Endangered in Poland (Głowaciński & Nowacki 2004). Pakulnicka (2008) found a rare species of water beetles, *Ochthebius hungaricus* Endrödy-Younga, in a gravel pit pond, and Nowak et al. (2007) noted rare and threatened pondweed communities that had been formed by species from the genus *Potamogeton* (L.).

Some anthropogenic water bodies are associated with various technological processes including coal, brown coal, nickel, salt or sand mines (Echols et al. 2009; Jaruchiewicz 2014; Luek & Rasmussen 2017). The activity of mines is connected, among others, with the formation of settling ponds. These water bodies are used for temporary retention of saline mine waters before they are discharged into rivers, and therefore their waters are, among others, contaminated with salt (Echols et al. 2009).

It should be emphasized that until recently research on macroinvertebrate communities in settling ponds has rarely been undertaken. The research focused on the effects of drainage from surface coal mining operations on zoobenthos and indicated that settling ponds may create favorable conditions for various taxa of macroinvertebrates (Canton & Ward 1981). However, research on the saline settling ponds that are associated with underground coal mining has never been undertaken.

Anthropogenic salinization is one of the most important factors responsible for biological changes in aquatic biota (Bäthe & Coring 2011; Kang & King 2012; Arle & Wagner 2013), thus it is particularly important in the case of coal mine settling ponds whose waters are often very saline. It should be emphasized that saline inland water bodies can be unique habitats. Only a few submerged aquatic plants can grow and reproduce in conditions of high salinity. Among the submerged macrophytes, *Ruppia* (L.) has the highest

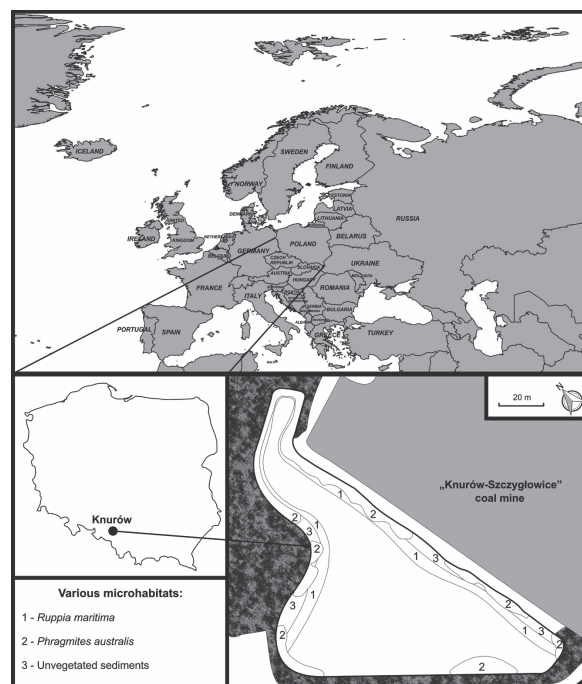


Figure 1

Location of the study area and investigated microhabitats

tolerance to salinity (Triest & Sierens 2009). A preliminary study carried out in saline coal mine settling ponds in southern Poland resulted in the discovery of a *Ruppia maritima* (L.) locality in one of them. To the best of our knowledge, this is currently the only inland site of this species in Poland. *R. maritima* occurs both in the coastal zone of the seas and estuaries (Verhoeven 1979) as well as in freshwater environments (Lazar & Dawes 1991).

To date, research on invertebrates that inhabit widgeongrass beds has been carried out in estuaries (e.g. Fredette et al. 1990; Heck et al. 1995; Keats & Osher 2007; Henninger et al. 2009; Barnes & Ellwood 2012), lagoons (e.g. Por 1971; Casagrande et al. 2006), coastal habitats (e.g. Montague & Ley 1993; Boström & Bonsdorff 2000; Leopardas et al. 2014; Bartolini-Rosales et al. 2016) and various inland saline habitats such as streams (Velasco et al. 2006), ponds (Verhoeven 1978, 1980; Guerrini et al. 1998) and lakes (Brock & Shiel 1983; Wollheim & Lovvorn 1995; 1996; Herbst et al. 2013), while such research has never been undertaken in saline coal mine settling ponds.

The objective of the study was to verify whether various microhabitats affect the abundance and composition of macroinvertebrates in a saline settling pond. To test this, the research was carried out on microhabitats that occur in a settling pond such as *R. maritima*, *Phragmites australis* (Cav.) Trin. ex Steud and sediments with no macrophytes.

Materials and methods

Study site

The studies were conducted in a settling pond located in the center of the mining town of Knurów in Upper Silesia, southern Poland (Fig. 1). Upper Silesia is one of the most industrialized and urbanized regions in Europe and one of the largest coal basins in the world (Jaruchiewicz 2014). The investigated water body (50°13'09"N, 18°40'11"E) was created in 1974 to drain the salt waters from the "Knurów-Szczygłowice" coal mine (Bielańska-Grajner & Cudak 2014). The waters from the settling pond flow into the Bierawka River through small streams, which in turn flows into the Oder River, the second largest river in Poland.

The water body surface area is 7185.75 m². Both the shoreline of the settling pond and its bottom are built of post-mining waste with the size ranging from 0.2 cm to 2.0 cm. Only two species of macrophytes were recorded – *Phragmites australis* on the edges of the settling pond and *Ruppia maritima*, which inhabits the coastal zone of the pond, at a short distance from the shore at a depth of about 0.5–2.0 m.

Sampling procedure

The studies were carried out once a month from June to October in 2016. Three types of habitats in different parts of the pond were selected for the sampling of macroinvertebrates, i.e. the bottom overgrown with *R. maritima*, the bottom overgrown with *P. australis* and bottom sediments with no macrophytes (Figs 1, 2). Samples of macrozoobenthos were collected only in the coastal zone up to a depth of 0.7 m due to the morphometry of the pond (rapid increase in depth to several dozen meters). Samples were collected according to a quantitative method using a 0.25 × 0.25 m quadrat wooden frame with a height of 0.5 m, which was placed randomly at three locations in each microhabitat, which were about 2 m in size. In the case of substrates overgrown with *R. maritima* and *P. australis*, the frame was placed on plants, which were then put into a plastic container to prevent the escape of invertebrates. During the study period, five samples of invertebrates were collected from each microhabitat. The biological samples were transported to the laboratory and then sieved using a 0.5 mm mesh sieve, sorted and preserved in 80%



Figure 2

Three microhabitats occurring in the studied settling pond

ethanol. The collected material was identified to the family (Diptera, Lepidoptera, Heteroptera) and species level (Odonata, Araneae, Amphipoda, Oligochaeta and Gastropoda). All of the invertebrates were counted and weighed on laboratory scales with an accuracy of 0.001 g.

The data on macroinvertebrate communities were processed according to dominance (%), frequency (%) and abundance (individuals m^{-2}). During the sampling of macroinvertebrates, samples of water and bottom sediments were also collected for analyses. Water parameters such as conductivity, pH, total dissolved solids (TDS), dissolved oxygen and temperature were measured in the field using Hanna Instruments portable meters in each month of the survey. Other parameters such as alkalinity, iron, chlorides, nitrates, nitrites, ammonium, phosphates, potassium and sulfates were analyzed in the laboratory according to the standard methods by Hermanowicz et al. (1999). The total organic matter (%) from the place where *R. maritima* and *P. australis* grow and from the unvegetated bottom sediments was determined according to the loss-on-ignition method by combusting the sediments at 550°C for 7 h (Myślińska 2001).

Data analyses

One-way analysis of variance (ANOVA) was applied to assess any differences in the mean abundance and biomass of macroinvertebrates and the mean abundance of taxa between the studied habitats. The statistical analyses were performed using Statistica version 12.0. Factors with $p < 0.05$ were considered significant.

Results

Environmental conditions of the investigated coal mine settling pond

The water in the settling pond was well oxygenated and slightly alkaline. It was characterized by a very high conductivity as well as a high content of sulfates, potassium and chlorides (Table 1). The total dissolved solids ranged from 12.9 to 17.1 $g\ dm^{-3}$. According to the Hammer scale (1990), the water is classified as hyposaline (TDS = 3.0–20.0 $g\ dm^{-3}$).

The organic matter content in the sediments of all the habitats was small. It ranged from 0.08% to 0.17% in the bottom overgrown with *R. maritima*, from 0.02% to 0.32% in the bottom overgrown with *P. australis* and from 0.09% to 0.14% in the sediments with no vegetation.

Abundance, diversity and biomass of macroinvertebrates on different microhabitats

A total of 15 191 individuals representing 11 macroinvertebrate families were collected. The abundance of invertebrates was higher on *R. maritima* (from 2203 ind. m^{-2} in June to 28 128 ind. m^{-2} in October, mean – 12 204 ind. m^{-2}) and on the bottom sediments with no macrophytes (from 2384 ind. m^{-2} in June to 20 144 ind. m^{-2} in September, mean – 12 458 ind. m^{-2}) compared to the fauna abundance that was associated with *P. australis* (from 6352 ind. m^{-2} in June to 6907 ind. m^{-2} in October, mean – 4494 ind. m^{-2}). The highest number of taxa was recorded on *P. australis* (eleven families) and the lowest one on *R. maritima* (eight families).

Table 1

Parameters of the water in the investigated settling pond

Parameter	n	Minimum	Maximum	Mean	Standard deviation
Conductivity ($\mu S\ cm^{-1}$)	5	26 000.0	34 400.0	30 944.0	2948.1
pH	5	7.1	8.5	7.7	0.5
Alkalinity ($mg\ CaCO_3\ dm^{-3}$)	5	150.0	250.0	185.8	45.1
Total dissolved solids ($g\ dm^{-3}$)	5	12.9	17.1	15.4	1.5
Dissolved oxygen ($mg\ O_2\ dm^{-3}$)	5	10.2	17.6	13.4	2.9
Chlorides ($mg\ Cl^- dm^{-3}$)	5	7840.0	14 650.0	10 782.5	2463.8
Iron ($mg\ dm^{-3}$)	5	0.07	0.26	0.14	0.1
Nitrates ($mg\ NO_3^- dm^{-3}$)	5	3.1	11.96	6.2	3.6
Nitrites ($mg\ NO_2^- dm^{-3}$)	5	0.0	1.04	0.67	0.4
Ammonium ($mg\ dm^{-3}$)	5	1.08	6.97	2.4	2.3
Phosphates ($mg\ PO_4^{3-} dm^{-3}$)	5	0.001	1.2	0.24	0.5
Potassium ($mg\ dm^{-3}$)	5	40.8	68.0	54.3	13.6
Sulfates ($mg\ dm^{-3}$)	5	902.0	1530.0	1197.3	315.7
Temperature ($^{\circ}C$)	5	13.8	25.1	21.1	4.7

The most dominant invertebrate species on all the habitats was the amphipod *Gammarus tigrinus* Sexton. It represented 94.5% of the total fauna from the common reed, 94.6% from widgeongrass and 96.5% from the bottom sediments. In addition, it was present in every month of the study period. The abundance of *G. tigrinus* fluctuated throughout the survey period and ranged from 560 to 20 086 ind. m⁻² on *R. maritima*, from 75 to 6896 ind. m⁻² on *P. australis* and from 1621 to 20 064 ind. m⁻² on the unvegetated bottom (Fig. 3).

Although the total abundance and biomass of the family Corixidae were not significantly different between the habitats ($p > 0.05$), the values were much higher on *R. maritima* (mean abundance – 333 ind. m⁻²) compared to *P. australis* (mean abundance – 7 ind. m⁻²) and the bottom sediments (mean abundance – 44 ind. m⁻²) (Fig. 4). The contribution of other taxa in the fauna was small and similar on each type of substrate. Moreover, dragonfly larvae *Enallagma cyathigerum* (Charpentier) were found only on widgeongrass beds, while the oligochaete species *Limnodrilus hoffmeisteri* Claparède, the spider species *Argyroneta aquatica* (Clerck) and larvae of Lepidoptera were found only on the common reed, whereas the dipteran larvae of the family Stratiomyidae were found only on the bottom with no vegetation (Table 2).

The biomass of macroinvertebrates associated with *R. maritima* (from 21.02 g m⁻² in June to 57.99 g m⁻² in October, mean – 37.87 g m⁻²) and the bottom sediments with no vegetation (from 15.24 g m⁻² in June to 34.98 g m⁻² in July, mean – 23.03 g m⁻²) was higher compared to the biomass on *P. australis* (from 2.65 g m⁻² in June to 43.32 g m⁻² in October, mean

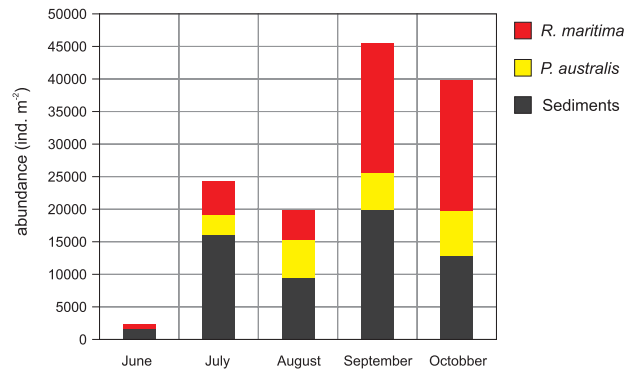


Figure 3

Seasonal changes in the abundance of *Gammarus tigrinus* on different microhabitats

17.05 g m⁻²). Most of the invertebrate biomass was provided by the great abundance of *G. tigrinus*.

The results of one-way ANOVA indicated that the impact of the microhabitat type on the abundance and biomass of macroinvertebrates and the abundance of particular taxa was insignificant ($p > 0.05$).

Discussion

Because there are no data on the fauna of anthropogenic saline ponds, it is impossible to compare our results. It should be emphasized that the studies to date have focused primarily on the influence of water salinity on benthic invertebrates in rivers and streams. These studies have indicated that increased

Table 2

Structure of macroinvertebrate communities on the microhabitats in the coal mine settling pond

Higher taxa	Family	Species	<i>Ruppia maritima</i>		<i>Phragmites australis</i>		Bottom sediments	
			D (%)	F (%)	D (%)	F (%)	D (%)	F (%)
Diptera	Chironomidae		1.8	80	2.3	80	2.5	100
	Stratiomyidae		0	0	0	0	0.03	20
Lepidoptera	Crambidae		0	0	0.02	20	0	0
Heteroptera	Corixidae		3.2	100	0.2	80	0.4	80
	Veliidae		0.1	40	1.9	20	0.1	40
Odonata	Coenagrionidae	<i>Ischnura elegans</i> (Vander Linden, 1820)	0.04	20	0.1	20	0.03	20
		<i>Enallagma cyathigerum</i> (Charpentier, 1840)	0.01	20	0	0	0	0
	Libellulidae	<i>Orthetrum albistylum</i> (Selys, 1848)	0.03	20	0	0	0.01	0
Araneae	Cybaeidae	<i>Argyroneta aquatica</i> (Clerck, 1758)	0	0	0.02	20	0	0
Amphipoda	Gammaridae	<i>Gammarus tigrinus</i> Sexton, 1939	94.7	100	94.5	100	96.5	100
Oligochaeta	Naididae		0	0	0.02	20	0.03	20
		<i>Limnodrilus hoffmeisteri</i> Claparède, 1862	0	0	0.1	20	0	0
		<i>Paranais litoralis</i> (Müller, 1780)	0.02	20	0.7	60	0.5	80
Gastropoda	Hydrobiidae	<i>Potamopyrgus antipodarum</i> (Gray, 1843)	0	0	0.1	20	0.1	60
Total number of individuals			9683		4213		1295	
Total number of families			8		11		10	

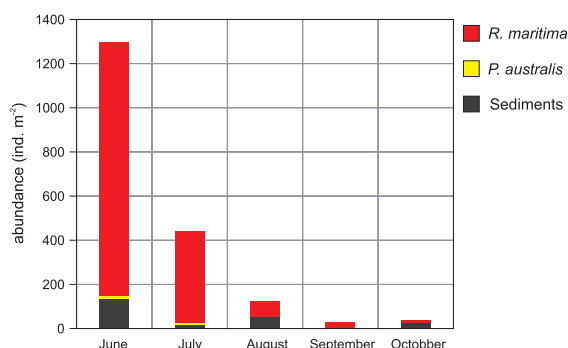


Figure 4

Seasonal changes in the abundance of Corixidae on different microhabitats

salinization results in the elimination of freshwater taxa and the replacement of sensitive species by eurytopic species and species that are resistant to high salt concentrations, including invasive non-native species (e.g. Braukmann & Böhme 2011; Petruck & Stöffler 2011; Arle & Wagner 2013; Cañedo-Argüelles et al. 2014).

Our studies have shown that the euryhaline amphipod *Gammarus tigrinus*, which is an alien species in Polish waters, dominated in all the microhabitats. It is possible that the species migrated into the studied settling pond from the Bierawka River and the Oder River where it was previously observed (Grabowski et al. 2009). Its great abundance in the investigated pond confirms the finding that anthropogenic salinization may cause the colonization and establishment of non-native species (Piscart et al. 2005; 2011). The high salinity tolerance (up to 25 PSU) and the high reproductive potential of *G. tigrinus* compared to other gammarids enables the species to colonize the most saline habitats in Europe (Bousfield 1969; Piscart et al. 2005; 2006; Grabowski et al. 2009; Braukmann & Böhme 2011; Petruck & Stöffler 2011). It often dominates the fauna of salt-polluted rivers, for example it reached 98.4% of the total number of all taxa in the Werra River (Braukmann & Böhme 2011). The colonization success of *G. tigrinus* in saline habitats may also be supported by the lack of native species that are excluded by salinity stress (Piscart et al. 2005). As a result, alien species often become the dominant groups in non-native regions (Alonso & Castro-Díez 2008; Ba et al. 2010; Bätke & Coring 2011). However, Petruck & Stöffler (2011) indicated that *G. tigrinus* can inhabit environments with both the highest and the lowest chloride concentrations. The authors suggested that the correlation between its occurrence and water salinity preferences may not be very strong.

In addition to *G. tigrinus*, we also found the high salt-tolerant oligochaete species *Paranais litoralis* (O. F. Müller). It has been described as a cosmopolitan

inhabitant of brackish waters and waters that receive saline pollution (van Haaren et al. 2017). Previously, it was reported only from the Polish coastal waters and the Baltic Sea (Marszewska et al. 2017). Our finding is the first record of this species in an inland habitat. *P. litoralis* might have been introduced into the settling pond by waterfowl and other migratory birds, which are a likely vector for the long-distance dispersal of oligochaetes (Milbrink & Timm 2001).

In the studied settling pond, we also observed the non-native gastropod species *Potamopyrgus antipodarum* (Gray), insect larvae from the genus *Enallagma* Charpentier (Odonata, Coenagrionidae) as well as the families Ceratopogonidae, Chironomidae (Diptera) and Corixidae (Heteroptera) that are considered halotolerant (Verhoeven 1980; Hammer et al. 1990; Williams & Williams 1998; Keats & Osher 2007). We also found other widely distributed damselfly species such as *Ischnura elegans* (Vander Linden) and *Orthetrum albistylum* (Selys), which have a high tolerance to salinity (Sato & Riddiford 2008). Our study highlights the importance of saline coal mine settling ponds as habitats for halotolerant invertebrates.

The results of our study are consistent with the findings of Hutchinson (1937), who stated that salt-tolerant species of the family Corixidae were much more abundant on widgeongrass beds compared to other aquatic macrophytes. We observed that their abundance was the highest in the first month of the study (June) and decreased by the end of the survey (Fig. 4). The reverse trend was determined for *G. tigrinus* (Fig. 3). Savage (1981) showed that due to the shortage of vegetable organic matter in sediments, *G. tigrinus* becomes carnivorous and may eat the nymphs of Corixidae. Furthermore, it may eliminate Corixidae by using the same food resources. It is possible that this phenomenon also occurs in the studied coal mine settling pond. The study by Wollheim & Lovvorn (1995, 1996) also showed much higher predator biomass than primary consumer biomass in a mesosaline lake (Lake Hutton, Wyoming, USA).

An unexpected result of our study was the relatively low seasonal variability of the macroinvertebrate richness on all the habitats. Similar results were observed by Wenner & Beatty (1988), Heck et al. (1995) and Boström & Bonsdorff (2000). The lack of taxa associated with a particular habitat suggests that the type of substrate is not a major determinant in the diversity of benthic assemblages and that these taxa showed no specific habitat preferences. One reason may be the mobility of the species (dominant taxa are good swimmers) and the degree of their aggregation (Wilson & Koutsagiannopolou 2014).

The results of our research on the macroinvertebrate biomass are consistent with the study by Kornijow et al. (1990), who noted that the biomass of zoobenthos was lower on *P. australis* than on other studied macrophytes. The biomass and abundance of the macrofauna associated with *R. maritima* in the investigated settling pond were low compared to previous studies of widgeongrass meadows (Verhoeven 1980; Boström & Bonsdorff 2000; Keats & Osher 2007). However, much lower fauna abundance on *R. maritima* was estimated in a New England estuary by Heck et al. (1995). Murkin et al. (1992), who found lower abundance of invertebrates in submerged rather than emergent vegetation, suggested that this could be caused by stressful dissolved oxygen levels and fish predation as well as the fact that emergent plants produce more organic matter. In our study, the total dissolved oxygen was high (never lower than 10.2 mg O₂ dm⁻³) and no aquatic vertebrate predators were found. Thus, the oxygen levels and predation had little effect on the abundance and biomass of macroinvertebrates. Similar results were reported by Wollheim & Lovvorn (1996) in saline lakes in the USA. They also indicated that macrophytes supported the greatest total biomass of invertebrates compared to unvegetated sediments and open water. Our results showed both the highest abundance and biomass on the widgeongrass beds compared to the two other microhabitats. This may be caused by the fact that vegetated habitats create heterogeneity and represent more valuable food resources such as epiphytic algae (Fredette et al. 1990; Guerrini et al. 1998). This indicates that widgeongrass meadows can be convenient habitats for fauna, not only in coastal waters but also in saline inland waters such as settling ponds.

Because there are no data on the fauna of saline coal mine settling ponds, it is important that the future research will focus on the biodiversity of macroinvertebrates, the rate of colonization by alien species and the relationships between taxa and habitat conditions.

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