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MONITORING OF AQUATIC BIOTA IN INTENSIVE FISH FARMING SYSTEM

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Abstract

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The aim of the study was to conduct a detailed research of aquatic invertebrates in the intensive breeding system of salmonid fish in order to determine the taxonomic composition of the community and its seasonal dynamics, and to identify those species that may be hosts of parasitic disease agents. To date, this issue has not been studied at all in the Czech Republic. Monitoring was conducted on the Danish type recirculation system near the municipality of Pravíkov in the Highlands of the Czech Republic from April till November 2015. A total of 9 series of samples were taken. Macrozoobenthos was evaluated in terms of taxonomic composition and abundance. Basic physicochemical properties of water (temperature, oxygen concentration, pH and conductivity) were also measured. In total, 69 taxa of aquatic invertebrates were noted, with the wall being the richest with a mean abundance of 756 pcs/m^2. Permanent groups predominated; the most numerous group was the subphylum Crustaceae, represented by a single species, *Asellus aquticus.*

Keywords: intesive fish farming, macroinvertebrates, fish parasites

INTRODUCTION

In many countries of the world, aquaculture and fish breeding are the fastest growing branch of animal production (FAO 2014). Aquaculture in the Czech Republic is mainly connected with breeding in fish ponds; however, at present, there is an increasing number of specialized modern systems that are mainly designed for the intensive breeding of salmonid fish (MZe ČR 2014). Modern aquaculture systems are built for intensive fish breeding usually without the presence of other organisms. Via the water sources, the stocking of fish of various origin but also by natural pathways, the facility often gets inhabited by various species of invertebrates whose occurrence is mainly affected by local conditions.

The function of invertebrates within the system and their possible effect on the facility are mainly determined by the ecological and biological properties of the particular species. Some species have no effect on the facility, some invertebrates may affect the system significantly, and their presence may be undesirable.

Aquatic invertebrates are among the first animals to colonize a newly created aquatic biotope (Milner 1994). The colonization of aquatic biotope is faster, if another aquatic biotope is close to it (Fairchild *et al.* 2000). The presence of some species of aquatic invertebrates may be undesirable due to the fact that they can be hosts of a wide range of parasites that cause disease in fish. Parasites may cause serious problems in fish breeding, especially if there are conditions suitable for fulfilling their whole development cycle (Palíková *et al.* 2014). Aquaculture conditions are suitable for relatively few parasitic species, however, their effect is manifold higher compared to open waters. Besides, they may also occur at the inflows into the system. Apart from mortality, parasites may cause limiting of feed intake, growth reduction, increased susceptibility to bacterial and fungal diseases, and cause morphological deformities (Meyer 1991).

The most serious parasitic diseases whose agents have their development cycle connected with invertebrates and that may occur in intensive systems of salmonid fish breeding include the proliferative kidney disease (PKD). This disease is a threat to salmonid fish breedings in Europe and North America (Okamura and Wood 2002). The agent is the myxozoan *Tetracapsuloides bryosalmonae,* and its invertebrate hosts are bryozoans (Bryozoa) (Tops and Okamura 2005). Serious problems are also caused by the myxobolosis of salmonids whose agent is the myxozoan *Myxobolus cerebralis*; part of the development cycle happens in Oligochaeta (Tubificidae) (Svobodová *et al.* 2007). In intensive systems of salmonid fish breeding we further encounter the nematode *Raphidascaris acus* (Palíková *et al.* 2014) whose intermediate host can be the amphipod *Gammarus fosarum* (Moravec 2004a). Production of salmonids is also influenced by trematodes, mainly *Diplostomum spathaceum* and *Crepidostomum* sp. (Svobodová *et al.* 2007) whose development cycle is bound to freshwater snails (Brassard *et al.* 1982, Sous 1992, Svobodová *et al.* 2007).

Detailed research of aquatic invertebrates in the intensive system of salmonid fish breeding was conducted within this study with the aim to firstly, determine the taxonomic composition of the community and its seasonal dynamics, and secondly, to specify those species that might be hosts of parasitic disease agents. This issue has not been studied yet in the Czech Republic.

MATERIALS AND METHODS

Samples of aquatic biota were taken at the recirculation facility of the company Biofish Ltd. which is located near the municipality of Pravíkov in the Highlands in the Czech Republic. Samples were taken in the field at a monthly interval from April till November 2015; a total of 9 series of samples were taken. Three sampling sites with different conditions were selected within the system, namely, the bottom of the outflow canal from the breeding trough (outflow), the bottom of the inflow canal into the breeding troughs (inflow), and the wall at the inflow into the system (the wall).

Considering the specific character of the recirculation system, the sampling device needed to be appropriately adjusted. To the rim of the benthic net of the dimensions 23×30 cm, a solid and sharp cutting edge was welded which allowed for sampling from a solid surface (Fig. 1). At the same time, a long handle was fastened to the net because of the approximately 2 m depth of water in the system. In cases of outflow and inflow, the sampling was done by traction along the bottom; the length of traction was 3 m in total. Samples from the wall were taken by scratching off the bryophyte growth. The substrate containing the animals was then caught into the net with a mesh size of 0.5 mm. Fixed samples were processed and evaluated during the year 2016 according to standard methodology (ČSN 757703). Apart from the aquatic biota sampling, selected physicochemical properties of water (temperature, pH, conductivity, dissolved oxygen concentration) in the system were measured. Conductivity was measured using the conductometer HANNA Combo HI 98129; other properties were measured using the oxymeter HACH HQ40d.

Individual samples were evaluated on the basis of data on the taxonomic composition and abundance of macrozoobenthos. Microsoft Excel program was used for depicting the abundance and numbers of species of individual groups of macrozoobenthos in graphs. The species determined were compared with data in literature on invertebrate hosts of fish parasites (Lom *et al.* 1986, Olsen 1986, Sous 1992, Buchar *et al.* 1995, Overath *et al.* 1999, Ondračková *et al.* 2004, Svobodová *et al.* 2007).

1: *Adjusted sampling net with a cutting edge for taking samples from solid surfaces.*

RESULTS AND DISCUSSION

Abiotic factors

The abundance and taxonomic composition of the macrozoobenthos community are mainly influenced by the abiotic conditions in the system and by the life cycle of aquatic invertebrates. The abiotic conditions for the monitored period are summarized in the Tab. I. Each sampling point was monitored to eliminate the influence of abiotic factors. Because of the minimal differences in the obtained results, the system is evaluated as a whole. Oxygen concentration in the monitored period ranged between 6.5–11.8 mg/l, pH ranged from 5.7 to 7.7. Water temperature in the system did not drop below 5.0 °C; the highest temperature measured was 18.3 °C. Conductivity ranged between the values of 237–405 µS/cm. Bregnballe (2015) reports the optimum values of hydrochemical properties for Danish type recirculation systems to be 70–250 % for water saturation with oxygen, and 6.5—7.5 for pH, and Svobodová *et al.* (2007) mentions the optimum temperature for salmonid fish species to be 8—16 °C. All measured values are in accordance with the ones recommended, except for pH measured at the beginning of the month of June when it dropped to 5.7, while it is recommended that it should not drop below 6.2.

In recirculation systems, macrozoobenthos community may be affected also by technological interventions. Frequently, pH values are adjusted using sodium carbonate; since pH is adjusted in order to approach the value of 7, it does not represent a major intervention into the lives of invertebrates. There may be also therapeutic interventions conducted within the system that have a fundamental effect on the function of the biofilter. For example, the use of antibiotics can completely kill the biofilter bacteria (Palíková *et al.* 2015) which causes fundamental changes in the water chemistry.

Macrozoobenthos community composition

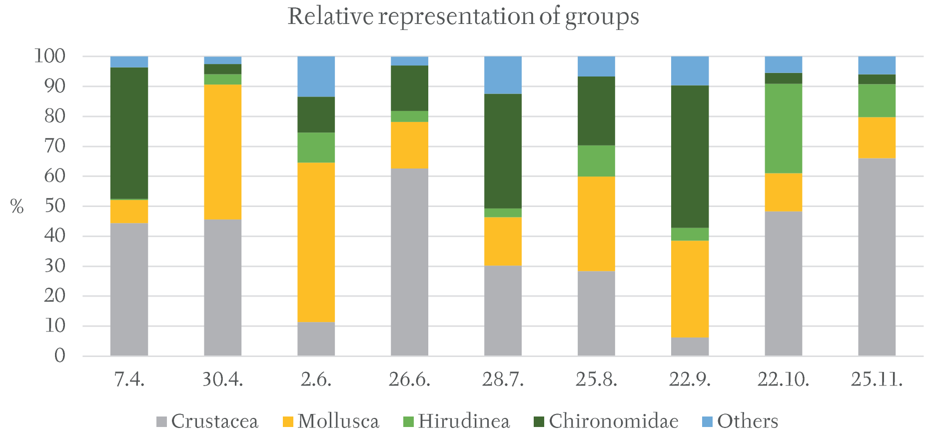
Inhabitation of the fish breeding facility in Pravíkov by aquatic invertebrates is possible by several pathways. Since the facility has no roof, the first possibility for some species is the colonization of imagoes from a nearby pond (within a distance of approximately 20 m). Invertebrates capable of colonizing new habitats in this way include, e.g. representatives of Odonata, Ephemeroptera, Diptera, Heteroptera, and Coleoptera (Anderson and Smith 2003). Another possibility is the brook that serves as the water source for the system. Water is supplied to the system primarily from a borehole; in the summer months, due to increased evaporation this source is insufficient and water is supplied from a nearby brook. This supply is not secured by a mesh dense enough to prevent the penetration of invertebrates. Invertebrates may enter the system also together with the fish stock.

Permanent groups of aquatic invertebrates unequivocally predominated at the monitored fish breeding facility; i.e. those representatives of the macrozoobenthos that are permanently bound to the aquatic environment. Tab. II presents an overview of the taxa of aquatic invertebrates found at the monitored intensive fish breeding facility and the figure (Fig. 2) shows the relative representation of groups of invertebrates. The most abundant group was the subphylum Crustacea with the highest percentage in most samplings. Crustaceans were represented by a single species, *Asellus aquaticus* which at the end of the month of June and in November represented up to 62.6 and 66 %, respectively, of all the aquatic invertebrates. This species is not sensitive to water pollution, feeding on organic remains, detritus or fallen leaves and due to the high intensity of fish breeding, it has enough food here. *A. aquaticus* is intermediate host of Acanthocephala, rainbow trout is host of species *Acanthocephalus anguillae* or *A. lucii* (Moravec 2004b). *A. aquaticus* may present a problem once it enters the biofilter space. It feeds on the bacterial growth there, thus decreasing its efficacy.

The efficacy of biological filtration of the bacterial growth may also be decreased by aquatic molluscs. At the monitored facility they occurred as the second most abundant group, and at the beginning of the month of June and in August they were recorded as the most abundant with a relative representation of 53.3 a 31.6 %, respectively. The most numerous species was the mollusc *Lymnaea peregra*. Molluscs of the genus *Lymnaea* can be intermediate hosts of several species of trematodes. In cyprinid fish breeding, it is mainly *Posthodiplostomum cuticola* whose metacercariae parasitize in the fish cutis and subcutis (Svobodová *et al.* 2007). This trematode matures sexually in the intestines of aquatic birds (Ondračková *et al.* 2004); those are prevented from entering the fish breeding facility thanks to the use of nets. *Lymnaea peregra* can be an intermediate host of the trematodes *Crepidostomum metoecus* and *Diplostomum spathaceum*. Hosts of *Crepidostomum*

I: *Minimum, maximum, and the mean abiotoic properties of water in the system over the monitored period.*

	inflow, the wall			outflow		
	min	max	Mean	Min	max	mean
dissolved oxygen [mg/l]	7.8	11.8	9.3	6.5	11.3	8.4
pH	5.7	7.4	7.0	5.7	7.7	7.1
temperature [°C]	5.0	18.1	12.7	5.1	18.3	12.9
conductivity [µS/cm]	237.0	403.0	278.0	239.0	405.0	280.0



2: *Relative representation of individual groups of macrozoobenthos at the monitored facility.*

farionis can be pea clams *Pisidium* sp. (Olsen 1986, Sous 1992), which have been also found in the system.

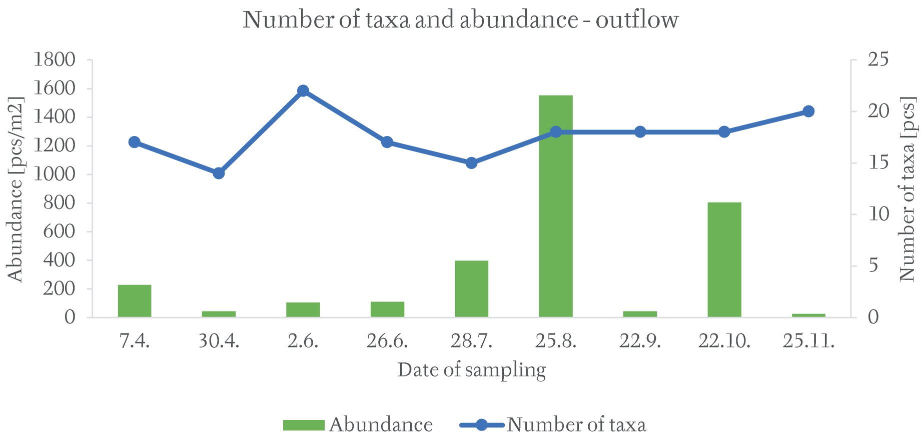
Temporary fauna, i.e. one that undergoes only part of its life cycle in the aquatic environment, was represented most abundantly by the midges that commonly occur in all types of surface waters. They were the third most abundant group at the monitored facility, and during two samplings in the months of July and September they were the most abundant, making 38.3 and 47.5 %, respectively, of all the invertebrates. In most stagnant waters they represent the dominant component of all living organisms (Armitage *et al.* 1995).

The fourth most abundant group was the class Hirudinea; in the month of October, leeches represented up to 29.8 %, thus being the second most abundant group. Reproduction of blood flagellates of the genera *Trypanosoma* and *Trypanoplasma* (Lom *et al.* 1986, Overath *et al.* 1999, Svobodová *et al.* 2007) occurs in the leech digestive system. These flagellates live in the blood circulation of cyprinid fish species mainly, often causing massive infections (Svobodová *et al.* 2007). Only two species of leeches were found at the monitored facility, neither of which is a fish ectoparasite and neither of which serves as a host of blood flagellates. *Erpobdella octoculata* feeds on small aquatic invertebrates and *Glossiphonia complanata* feeds by sucking on the haemolymph of molluscs and annelids (Buchar *et al.* 1995).

Evaluation of individual sampling sites

Outflow

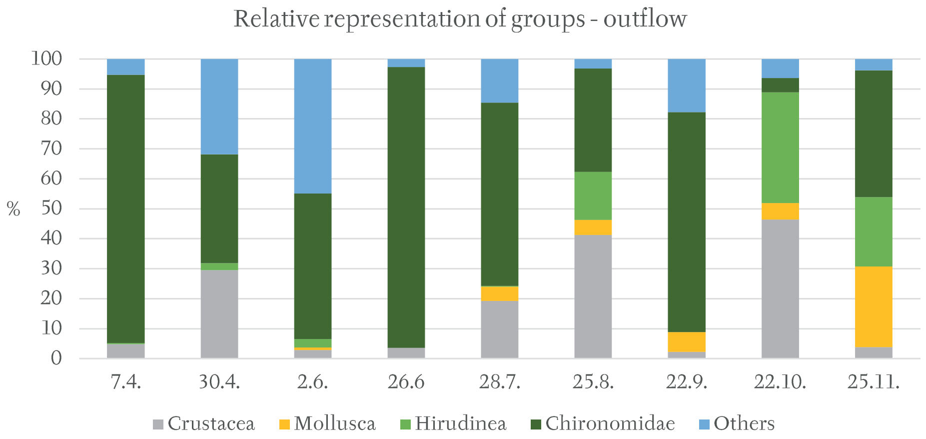
At the bottom of the outflow canal, 44 taxa of aquatic invertebrates were found during the research. The highest number of macrozoobenthos taxa (22) was found at the beginning of the month of June, the lowest at the end of the month of April. Macrozoobenthos abundance reached high values in the months of August and October; the lowest at the end of April, in September, and November (Fig. 3). The mean value over the monitored period was 369 pcs/m^2. The increase of abundance of organisms in the month of August was caused mainly by the increase of numbers of water lice and leeches, and the high numbers of midges were also notable. The increase in the month of October was caused mainly by the increase of numbers of water lice and leeches.



3: *Outflow – number of taxa and abundance of macrozoobenthos*

II: *Comparison of the taxonomic composition of individual sampling sites.*

Taxon/sampling site	outflow	inflow	the wall	Taxon/sampling site	outflow	inflow	the wall
Turbellaria				**Coleoptera**			
Polycelis sp.	+	+	+	*Brychius* sp. LV.			+
Cnidaria				*Colymbetes* sp. LV.			+
Hydra sp.	+	+	+	*Dytiscinae*			+
Crustacea				**Diptera**			
Asellus aquaticus	+	+	+	*Limnophora* sp.	+		+
Mollusca				*Psychoda* sp.			+
Ancylus fluviatilis		+	+	*Simulium* sp.		+	+
Bathyomphalus contortus		+	+	*Tipula* sp.			+
Gyraulus sp.	+		+	**Chironomidae**			
Lymnaea peregra	+	+	+	*Brillia bifida*	+		
Pisidium sp.		+	+	*Brillia longifurca*		+	+
Oligochaeta				*Cardiocladius* Cf. *capucinus*	+	+	+
Nais barbata			+	*Conchapelopia* sp.	+	+	+
Nais communis	+	+	+	*Cricotopus bicinctus*	+	+	+
Nais christinae	+		+	*Cricotopus* gr. *tremulus*	+	+	+
Nais pseudobtusa			+	*Cricotopus* sp.	+		+
Stylaria lacustris			+	*Dicrotendipes* sp.	+	+	+
Hirudinea				*Eukiefferiella* sp.	+	+	+
Erpobdella octoculata	+	+	+	*Eukieferiella* gr. *brevicalcar*			+
Glossiphonia complanata	+	+	+	*Glyptotendipes* sp.	+		
Ephemeroptera				*Heterotrissocladius marcidus*		+	
Baetis rhodani	+	+	+	*Holotanypus* sp.	+		
Baetis sp.	+	+	+	*Chironomus* sp. *juv.*	+		
Caenis horaria		+		*Metriocnemus* sp.			+
Caenis sp.		+		*Micropsectra* sp.	+	+	
Heptagenia sp.			+	*Microtendipes gr. chloris*	+	+	+
Plecoptera				*Nanocladius* sp.	+	+	+
Nemoura sp.			+	*Orthocladius* sp.			+
Trichoptera				*Paratanytarsus* sp.	+		
Hydropsyche angustipennis	+	+	+	*Parametriocnemus stylatus*	+	+	
Hydropsyche incognita	+			*Paratendipes albimanus*	+		
Hydropsyche sp.		+		*Paratrichocladius rufiventris*			+
Chaetopteryx villosa			+	*Polypedilum* gr. *laetum*	+	+	+
Limnephilus rhombicus	+			*Polypedilum* sp.	+	+	
Neureclipsis bimaculata	+	+	+	*Prodiamesa olivacea*	+		
Polycentropus flavomaculatus	+	+	+	*Rheocricotopus fuscipes*			+
Polycentropus kingi	+			*Rheotanytarsus* sp.	+	+	+
Sericostoma sp.	+			*Tanytarsus curticornis*	+	+	
				Tanytarsus sp.	+	+	
				Tvetenia discoloripes/verralli	+	+	+



4: *Outflow – relative representation of individual groups of macrozoobenthos*

Overall, the most abundantly represented at the outflow canal was the family Chironomidae whose presence was noted during all samplings, when mainly the taxa *Cricotopus bicinctus* and *Rheotanytarsus* sp. were found. The second most abundant group was the subphylum Crustacea, represented only by the water louse. The class Hirudinea was mainly represented by the species *Erpobdella octoculata* (Fig. 4).

Inflow

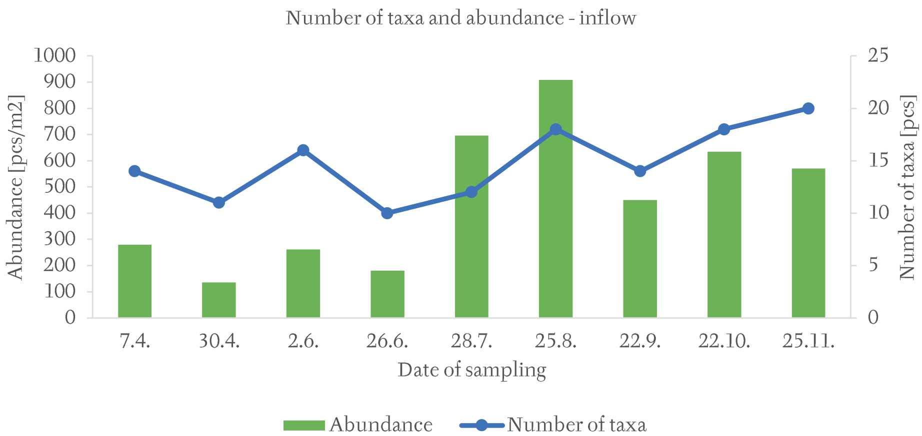
At the inflow, a total of 37 macrozoobenthos taxa were found over the monitored period. The highest number of macrozoobenthos taxa (20) was found in November, and the lowest number was found in June. High abundance of aquatic invertebrates was noted during the months of July, August and October. In contrast, the lowest abundance was noted in April and June (Fig. 5). The mean abundance over the monitored period was 458 pcs/m^2. The increased abundance in July was caused mainly by the midges, followed by the water louse and molluscs. In August it was caused mainly by the high numbers of molluscs; also blackflies and hydras were found. In October it was also leeches, beside the water louse and molluscs.

Overall, the most abundant group of invertebrates found was the phylum Mollusca with the strongly dominating species *Lymnaea peregra*. The second most abundant group was the subphylum Crustaceae, represented only by the water louse. A significant part was also made by the family Chironomidae, mainly *Cricotopus bicinctus*, and by leeches, mainly *Erpobdella octoculata* (Fig. 6).

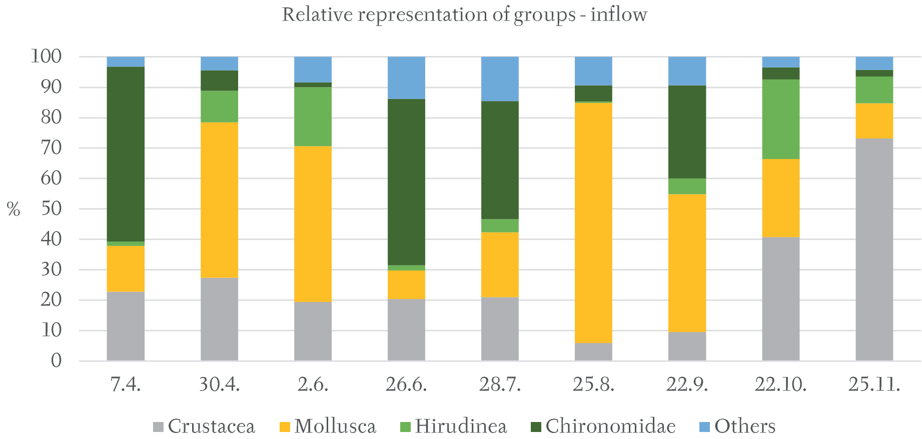
The wall

In samples taken from the wall, the highest number of taxa of aquatic invertebrates was found, a total of 48. High abundance was found at the beginning of the month of April and at the end of June; the lowest was found in the months of August, September, and October (Fig. 7). The mean abundance over the monitored period was 756 pcs/m^2. High abundance in the months of April was found mainly due to the increase of the numbers of water lice and molluscs; a high number of midges was also noted. Similarly, in the month of June a high number of water lice and molluscs was found.

The most numerous group of macrozoobenthos at the wall was the subphylum Crustaceae. The mean abundance of *Asellus aquaticus* over the monitored



5: *Inflow – number of taxa and abundance of macrozoobenthos*

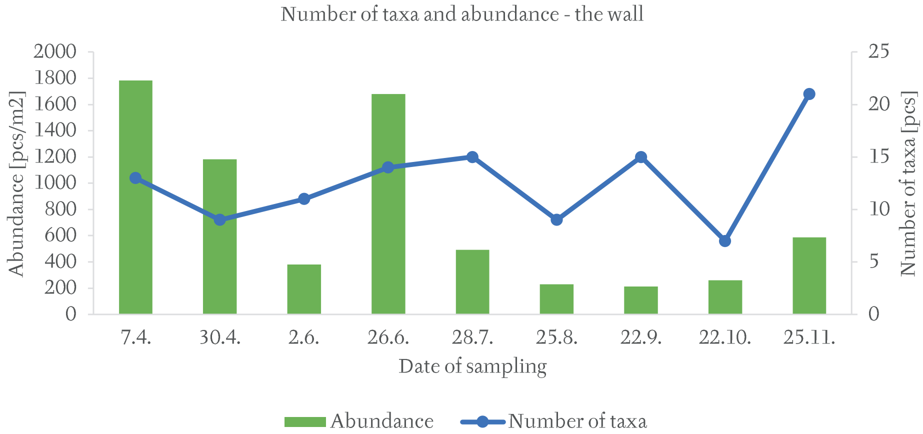


6: *Inflow – relative representation of individual groups of macrozoobenthos*

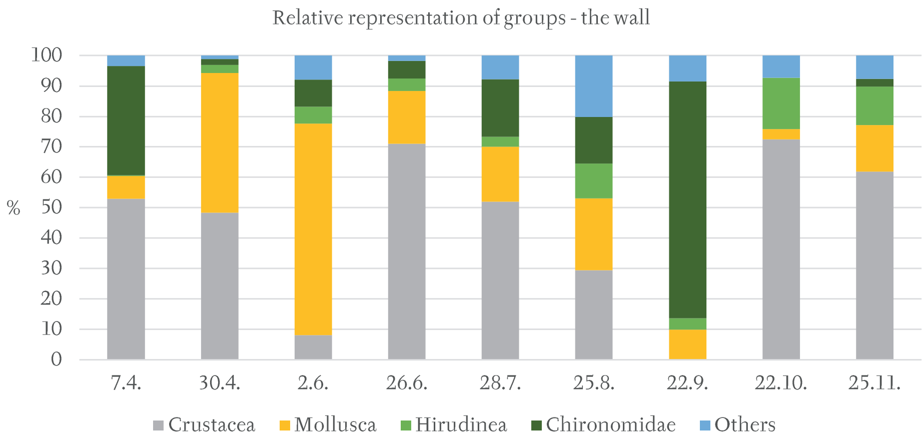
period was 452 pcs/m²; the highest abundance found was 1,193 pcs/m². The second most abundant group was the phylum Mollusca, represented mainly by the species *Lymnaea peregra*. The least abundant group was the family Chironomidae, mostly represented by the taxon *Cricotopus bicinctus*. Another important component was the class Hirudinea, especially *Erpobdella octoculata* (Fig. 8). In contrast to the other sampling sites, the order Ephemeroptera was noted here, represented mainly by the species *Baetis rhodani*.

Comparison of individual sites

The abundance of aquatic invertebrates varied markedly at the individual sampling sites in the system. The highest abundance of



7: *The wall – number of taxa and abundance of macrozoobenthos*



8: *The wall – relative representation of individual groups of macrozoobenthos*

macrozoobenthos was found on the wall. The mean abundance in the year 2015 was 756 pcs/m^2 at the sampling site wall, 458 pcs/m^2 at the inflow, and 369 pcs/m^2 at the outflow. The highest abundance at the wall was most probably due to the occurrence of extensive growth of bryophytes. These provided an ideal environment for the aquatic invertebrates both by giving shelter and by catching particles of organic material that served as food. Comparison of obtained results with results of other authors is very problematic, because the topic correlated to similar conditions is difficult to find in scientific literature. Because of the mentioned, study was focused on evaluation of invertebrate occurrence and comparison of individual sampling sites of the system.

The taxonomic composition of the community differed at the individual sampling sites. A total of 69 taxa of invertebrates were caught, of which 22 were recorded at all the sampling sites. In terms of species, the richest site was the wall where a total of 48 taxa of invertebrates were noted compared to 44 at the outflow and only 37 at the inflow.

Comparing the relative representation of groups of macrozoobenthos at the individual sampling sites, most samplings at the wall were dominated by the crustaceans. Exceptions were in the months of June, when the most significant part was made by the molluscs, and September, when it was the midges. Furthermore, the groups Hirudinea, Ephemeroptera and Coleoptera were also noted.

The inflow was dominated by the molluscs, other abundant groups were the midges and crustaceans. Also the subclass Hirudinea was noted at the inflow in all samplings. Furthermore, hydras and planarians were found at the inflow.

The outflow was dominated in most samplings by the midges. Compared to the other sampling sites, also representatives of the order Trichoptera occurred at the outflow, mainly the species *Polycentropus flavomaculatus*. In most samplings, the subclass Hirudinea was noted; at the beginning of June, a significant part was also made by the class Oligochaeta; in other samplings its share was negligible.

CONCLUSION

Colonization of Danish type recirculation facilities by aquatic invertebrates is not being studied at all in the Czech Republic at present. As presumed, macrozoobenthos at the monitored facility occurred at high abundance and large numbers of taxa. Representatives of permanent fauna prevailed; temporary fauna representatives made approximately one third of all the invertebrates.

The most abundant group was the subphylum Crustacea, followed by the molluscs and midges. Other groups noted included Hirudinea, Cnidaria, Oligochaeta, Trichoptera, Ephemeroptera, Diptera, Coleoptera etc.

Upon evaluation of the effect of the found taxa of invertebrates on the function of the system and health condition of fish, several potentially threatening species were determined. The crustacean *Asellus aquticus* devours bacterial growth and if it enters the biofilter space, it might decrease its efficacy, same as the abundant water molluscs. The mollusc *Lymnaea peregra* is the intermediate host of the trematodes *Crepidostomum metoecus* and *Diplostomum spathaceum*; the genus *Pisidium* sp. is the intermediate host of *Crepidostomum farionis*. Other species do not pose a potential threat to the system because they are not intermediate hosts of fish parasites and occur at very small numbers.

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