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## **Benthic fauna of extremely acidic lakes (ph 2-3)**

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Gilberto Goncalves Rodrigues

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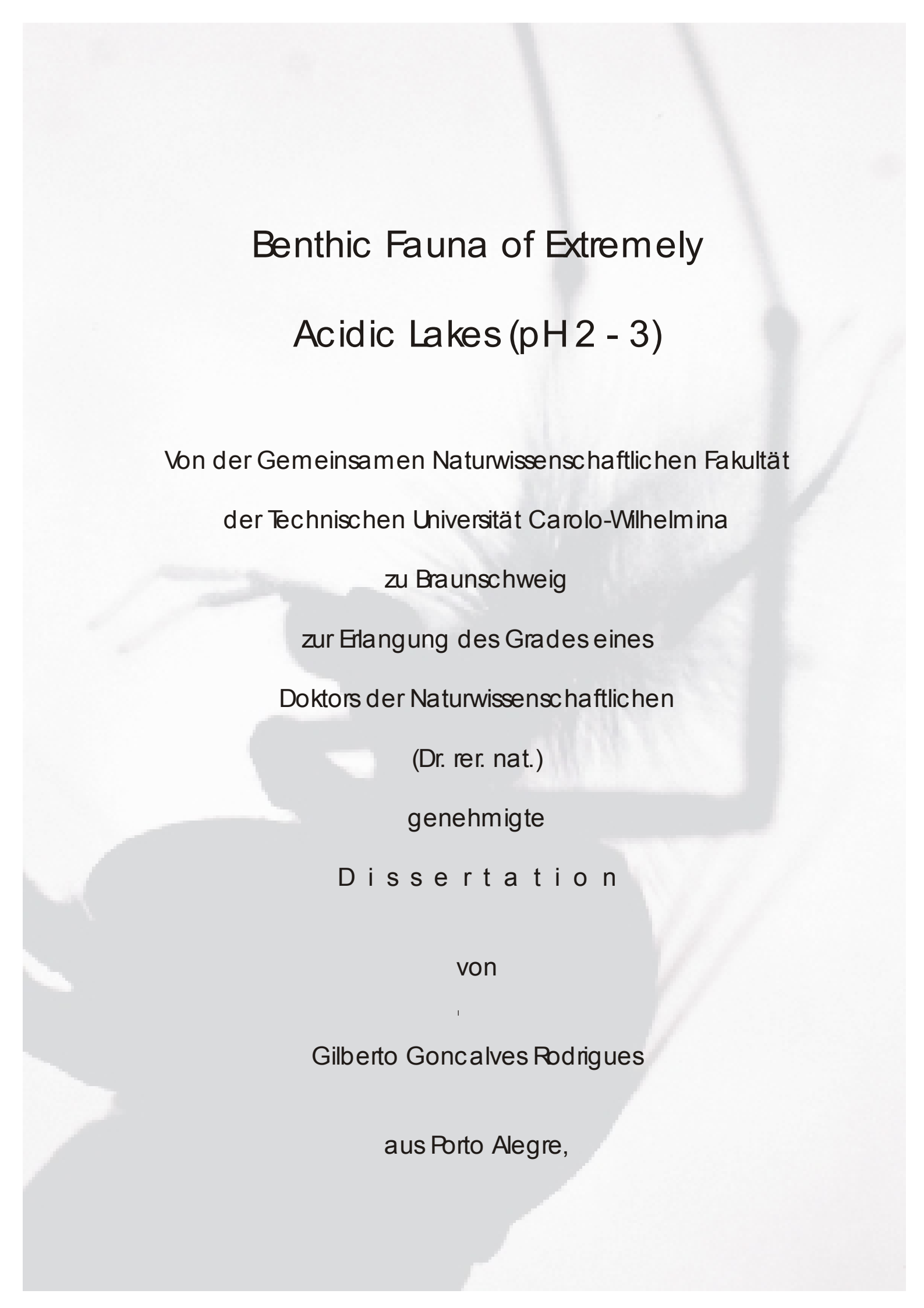
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# Benthic Fauna of Extremely Acidic Lakes (pH 2 - 3)

Von der Gemeinsamen Naturwissenschaftlichen Fakultät  
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zu Braunschweig

zur Erlangung des Grades eines  
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von

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aus Porto Alegre,

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## 1 Introduction

Remediation of extremely acidic environments is one of the largest problems in some parts of the world. As a consequence, studies were started to raise awareness the specific problems of the extremely acidic aquatic ecosystems (Smith & Frei 1971; Pietsch 1979; Klapper & Schultze 1995; Klapper *et al.* 1996; Schultze & Geller 1996; Geller *et al.* 1998).

These ecosystems exhibit very different chemical and physical conditions. High acidity with pH value from 2 - 3 is originated by a number of different geochemical process. Biological processes are also indispensable in these unique environments, which the flora and fauna are characterised by a very low species richness.

Lakes with pH up to 3.5 occur in Europe, North America and Scandinavia and some are atmospherically acidified (Schneider 1986; Heid & Schneider 1991; Steinberg & Wright 1994; Herinkson & Brodin 1995). Lakes of pH lower than 3.0 occur in other places of the world. The only parallels in nature with extremely acidic mining lakes are volcanic lakes in Japan, Indonesia, South America and bog lakes (Hutchinson 1957; Geller *et al.* 1998).

Species diversity is drastically reduced by low pH, but some vascular plants can occur at pH values near 3 (Pietsch 1965; Fyson 2000), as well as bryophytes (Hutchinson 1957, Satake 2000). Algae (Whitton & Diaz 1981; Nixdorf *et al.* 1998; DeNicola 2000; Lessmann *et al.* 2000; Olavesson & Nalewajko 2000) are especially important as the major primary producers in these ecosystems (Gross 2000). Microorganisms as fungi species (Suzuki 1961; Sparrow 1968; Gross & Robbins 2000) and bacteria (Wendt-Potthof & Neu 1998; Robbins 2000) also occur and play an important role in the biogeochemistry of sediments of these environments. These ecosystems are fish-free (Pietsch 1979; Fromm 1980; Havas & Hutchinson 1983) and high organism levels are rare. Aquatic insects are especially important as the major primary consumers in extremely acidic lakes, and also they occur as top-predators (Henrikson & Oscarson 1981; Wollmann 2000). In addition, rotifers, ciliates,

and cladocerans occur in these acidic environments with a number reduced of species (Woelfl *et al.* 1998, Deneke 2000; Packroff 2000; Packroff & Woelfl 2000).

Few studies have attempted to investigate benthic invertebrates from extremely acidic environments with pH under 3. Recently, some studies have been carried out on the biota of extremely acidic mining lakes from Germany. Studies such as those of Pietsch (1965, 1979, 1995), Geller *et al.* (1998) and the series by Nixdorf *et al.* (1998, 2000) have contributed to knowledge of these conditions. Biota of these ecosystems are quite reduced and investigations on benthic invertebrates regard only qualitative occurrence. For instance, a pioneer study of H. Müller in 1961 reported the occurrence of a *Chironomus* species in acidic mining lakes with pH value 4.7 – 4.8 from eastern Germany, but studies on extremely acidic lakes often lack process and interaction within the benthic invertebrate communities. These ecosystems offer a rather unique opportunity to study the adaptation mechanisms by which certain organisms do manage to survive in these extremely hostile environments, and the colonisation patterns, and interaction involving food webs and the interaction of prey – predator.

The present study “Benthic fauna in extremely acidic lakes with pH 2 – 3” seeks to integrate and emphasise the biological and ecological aspects closely related to extreme physical and chemical conditions found in acid body water with extremely pH values between 2 – 3. This study is divided into aspects which elucidate the natural occurrence of life in extreme conditions and provides information about the benthic fauna of extremely acidic mining lakes from Lusatian region in eastern Germany, as a contribution to management and restoration of these lakes ecosystems. The aim of this study was a) to characterise the benthic invertebrates species composition of the acidic mining lakes with pH 2 – 3; b) to investigate the colonisation of benthic invertebrates on leaves, and the autochthones deciduous leaf breakdown process in these ecosystems; and c) to describe the pupal exuviae of the acid-resistant *Chironomus crassimanus* species; and d) to investigate

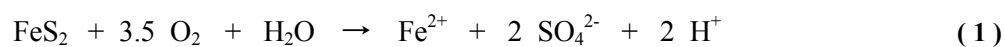
the diet of this key-species; and d) finally, to study the benthic food web of extremely acidic mining lakes (pH 2 – 3) from investigated Lusatian lakes from eastern Germany.

## 2 Study area and methods

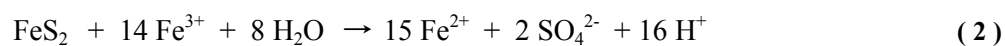
### 2.1 Study of area

Countries of central Europe such as The Czech Republic, Slovakia, Poland, and Germany are covered in the most reserve of lignite. Together with ex-Soviet Union these countries were responsible for more than 75 % of the coal production in the world (Petrascheck 1956; Pätz *et al.* 1989); which USSR occupied first position in statistics, followed by East and West Germany (Pätz *et al.* 1989). In these countries the coal mining activities occupied the largest areas and largest environmental problems (Schnoor *et al.* 1997).

During mining the soil is deposited in dumps. It contains iron sulfides, pyrite and marcasite, which are oxidized when the layers are removed and mixed, and thereby, aerated. The oxidation of these mineral leads a formation of  $\text{Fe}^{2+}$  and  $\text{SO}_4^{2-}$  and acidification (Silvermann 1967; Singer & Stumm 1970; Taylor & Wheeler 1984; Evangelous 1995):



Extremely acidic mining lakes are caused by oxidation of  $\text{FeS}_2$  in dissolved acidic products:



Also, acidic components  $\text{H}_2\text{SO}_4$  and iron species are formed when dumps are washed out by leaching of rainwater and/or by the fast-rising groundwater table of closed-down lignite pits (Geller *et al.* 1998).

In eastern Germany, all lignite deposits stem from the Tertiary period, which was a very active period in the earth's history (Schreck & Glässer 1998). They were formed from the Lower Eocene to the Upper Miocene, between 50 and 10 million years ago.

Lusatian lignite mining district is a centre of coal mining in eastern Germany. This vast area contains 13 billion tons of lignite (proven reserves), or one-quarter of the total German reserves. The Lusatian lignite mining district comprises two lithologically different areas: the economically more important area of Lower Lusatia, situated mainly in Brandenburg, and the area of Upper Lusatia in Saxony.

Lusatian region exhibited an area of about 760 km<sup>2</sup> of coal mining activities (LAUBAG 1995), and in connection with the water bodies originated by the fast-rising groundwater table of closed-down lignite pits corresponded until 1995 of about 2,500 km<sup>2</sup> (Landesumweltamt 1995). This region originated an area of mining lakes of 208 km<sup>2</sup> (Landesumweltamt 1995) which exhibited some lakes with pH from 2 to 3 (Pietsch 1979, Klapper & Schultze 1995, Schultze & Geller 1996).

The Lowerlausitz mining region comprises an area of south Brandenburg and the north-east of Saxony, 130 km south of Berlin and 100 km north of Dresden. It is situated in the south-eastern area of north Germany in central Europe (Fig. 1).

The studies were performed in areas that were contaminated after mining activities in Lusatia in the eastern part of Germany. Based on the results of a previous physical-chemical study, mining lakes were chosen for this study. The table 1 shows some general characteristics of the investigated lignite mining lakes in Lusatian region.

Studies were performed in four mining lakes. ML B was the control-lake. This lake has a pH-value of about 8.0 and it is situated in Schlabendorf in a less contaminated area (Fig. 2).

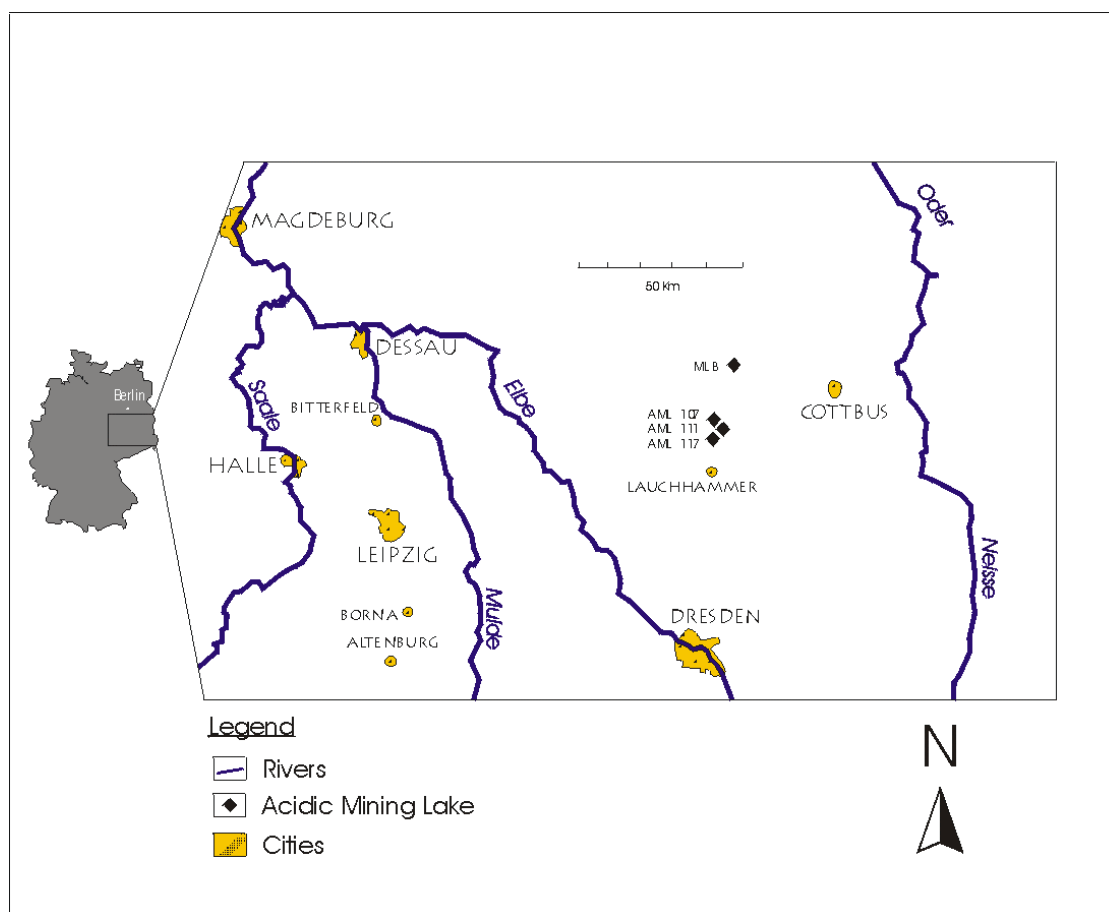
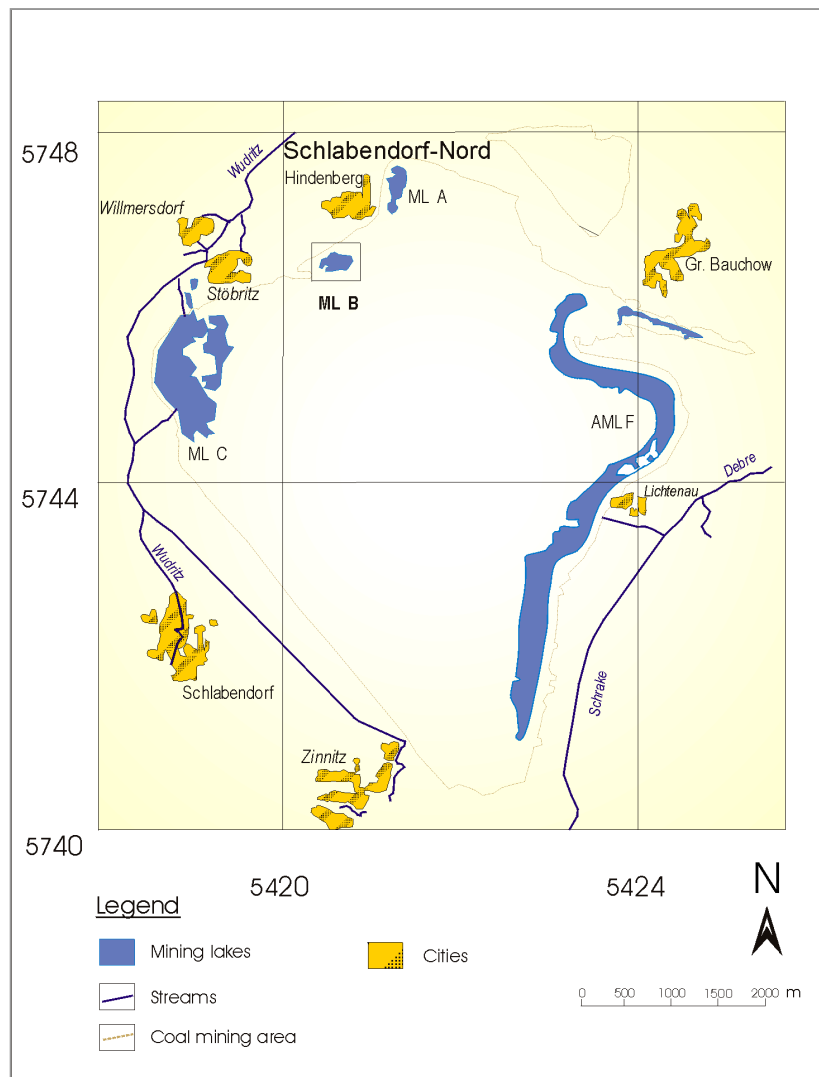


Fig. 1. Localisation map of the study area in south-eastern of north Germany (Modified map after Boehrer *et al.* 2000).

Table 1. General characteristics of acidic mining lakes from Lusatian region in Eastern Germany (Herzprung *et al.* 1998; Wiedermann 1994; Packroff *et al.* 2000).

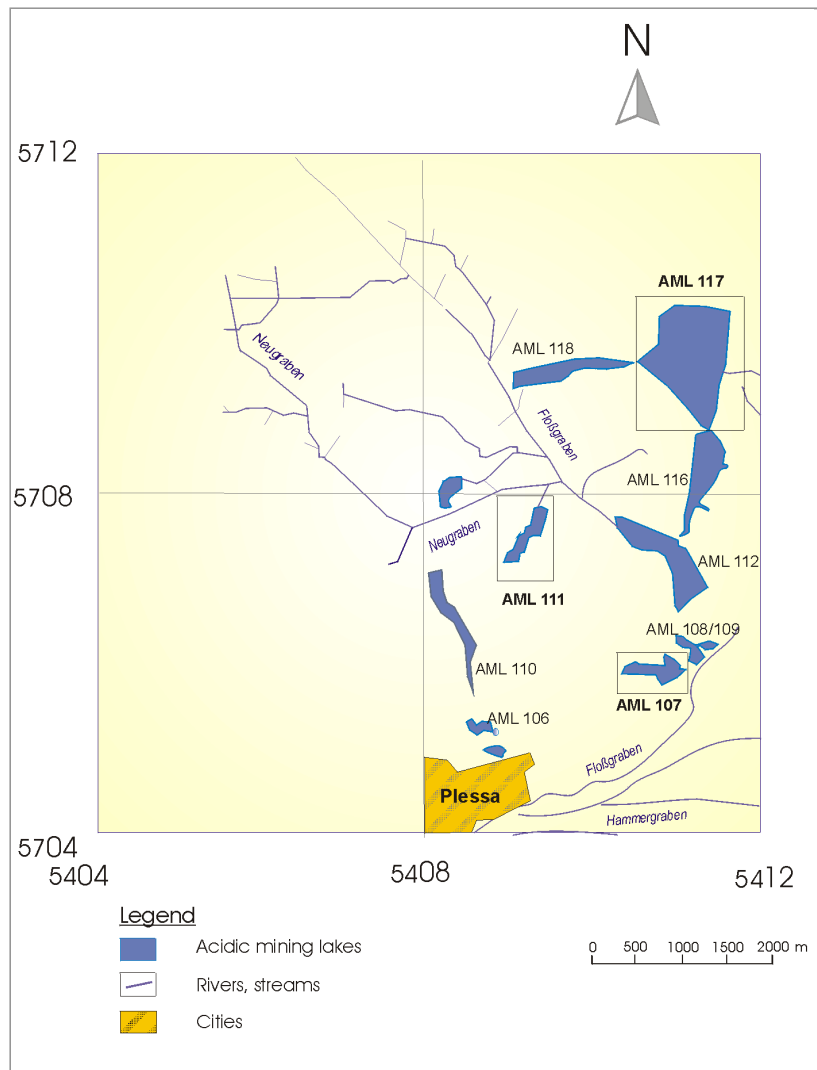
General characteristics	ML B	AML 117	AML 111	AML 107
Formation of the lake	1963/64	1966	1958	1928
Lake trophy	mesotrophic	oligotrophic	oligotrophic	oligotrophic
Water level (a.s.l.)	+ 55,0	+ 92.3	+ 94.1	+ 92.3
Area ( $10^3 \text{ m}^2$ )	100	950	107	122
$Z_{\text{max}}$ (m)	10	14	10,2	4.0
$Z_{\text{mean}}$ (m)	5 - 6	11	4,6	1,9
Volume ( $10^6 \text{ m}^3$ )	c. 0.6	10.4	0.5	0.23
pH-value *	8.00	2.97	2.61	2.31

(\* median)



**Fig. 2. Localisation of the lake ML B in the region Schlabendorf-Nord from Lusatian region, eastern Germany.**

The investigation of extremely acidic mining lakes were performed around Plessa (AML 111 and AML 107) and Grünewald (AML 117), which are located in the same lignite field. Figure 3 shows the localisation of acidic mining lakes AML 117, AML 111, and AML 107 that are located in the Lauchhammer-Plessa region. The surroundings of all studied lakes are dominated by a mixed forest of pioneer *Betula pendula*, *Robinia pseudoacacia*, and *Pinus silvestris*. Birch (*B. pendula*) is the most frequent pioneer tree and the biggest biomass produced in the littoral area of the mining lakes (Mutz *et al.* 2000).



**Fig. 3. Localisation of the mining lakes (AML 117, AML 111 and AML 107) in the Lauchhammer-Plessa region from Lusatia, eastern Germany.**

The investigated mining lakes AML 117, AML 111 and AML 107 are characterised by extremely low pH-values in the range of 2 – 3, which influence all other chemical parameters. Complete studies about chemical analysis were investigated from Lusatian mining lakes (Herzprung *et al.* 1998). The table 2 shows some physical and chemical characteristics of the investigated lignite mining lakes in Lusatian region during 1996 – 1998 (Herzprung *et al.* 1998; Wiedermann 1994; Packroff *et al.* 2000).

**Table 2. Some chemical parameters (1996 – 1998) from Lusatian mining lakes in eastern Germany (Herzprung *et al.* 1998; Wiedermann 1994; Packroff *et al.* 2000).**

Chemical parameters	ML B	AML 117	AML 111	AML 107
<b>K<sub>B</sub> 4,3</b> (mmol l <sup>-1</sup> )	0.02 – 0.2	2.41 – 2.73	13 – 25	27.40 – 55
<b>K<sub>S</sub> 4,3</b> (mmol l <sup>-1</sup> )	1.79 – 2.09	-	-	-
<b>Fe<sub>Tot</sub></b> (mg l <sup>-1</sup> )	0.01 – 0.067	15 – 25	120 – 190	400 – 800
<b>Al<sup>3+</sup></b> (mg l <sup>-1</sup> )	0.1	1.3 – 22	25 – 45	40 – 60
<b>SO<sub>4</sub><sup>2-</sup></b> (mg l <sup>-1</sup> )	400 – 433	374 – 410	1280 – 1890	2900 – 3410
<b>Ca<sup>2+</sup></b> (mg l <sup>-1</sup> )	188 – 204	94.7 – 105	222 – 242	337 – 402
<b>P<sub>Tot</sub></b> (mg l <sup>-1</sup> )	0.007 – 0.037	0.006 – 0.0018	0.008 – 0.016	0.012 – 0.053
<b>SRP</b> (mg l <sup>-1</sup> )	0.001 – 0.005	0.001 – 0.01	0.005 – 0.01	0.012 – 0.042
<b>NO<sub>2</sub>-N</b> (mg l <sup>-1</sup> )	0.001 – 0.005	0.001 – 0.002	0.001 – 0.002	0.001 – 0.002
<b>NO<sub>3</sub>-N</b> (mg l <sup>-1</sup> )	0.005 – 0.072	0.07 – 0.12	0.13 – 0.37	0.06 – 0.40
<b>NH<sub>4</sub>-N</b> (mg l <sup>-1</sup> )	0.014 – 0.190	1.4 – 2.0	1.4 – 2.0	2.2 – 11.57

Studies about limno-physical analysis have been investigated by Schimmele & Herzprung (2000) and Boehrer *et al.* (2000) from extremely acidic mining lakes in Lusatian region.

The figures 4, 5, 6 and 7 show some physical parameters as temperature, conductivity, oxygen concentration, pH-value, redox, and turbidity from ML B, AML 117, AML 111 and AML 107 between 1998 and 1999. The measurement are displaced in vertical profile in depth intervals of 10 cm. In all lakes was confirmed the annual cycle with epilimnion forming in summer, starting with a thickness of 2 meters in March and April and over 4 meters in May, June and July, and over 6 meters in September. From May to October, temperatures at 2 meters depth ranged between 10 – 24 °C. The temperatures values were between 0 – 5 °C at all levels in winter. Measurement of pH in acidic mining lakes ranged between 2.1 to 2.7 (AML 107) and 2.6 – 2.9 (AML 111), and 3.0 (AML 117). In ML B pH varied between 7.5 – 8.4.



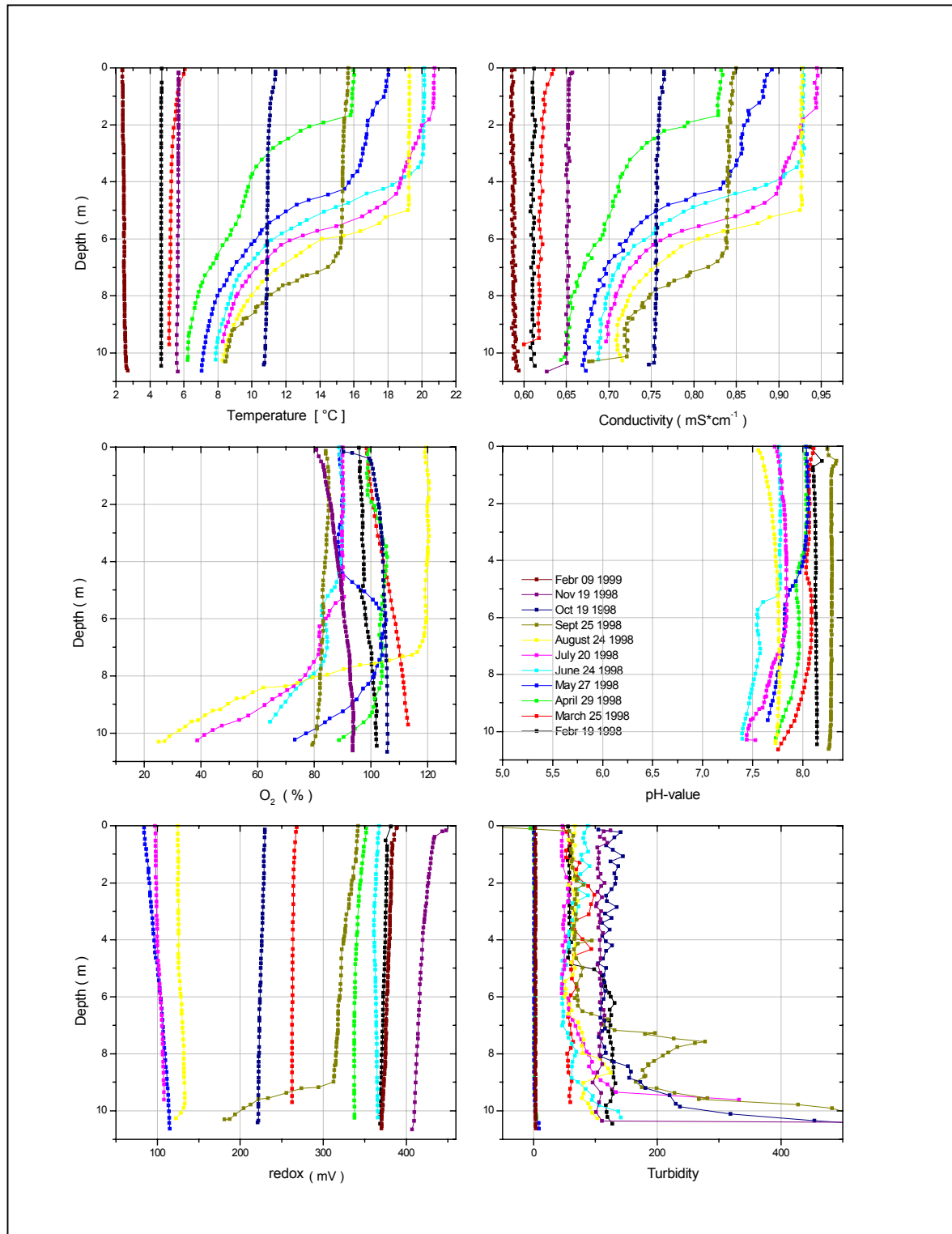


Fig. 4. Physical parameters: temperature, electric conductivity, oxygen concentration, pH-value, redox, and turbidity of ML B from Lusatian region in eastern Germany.

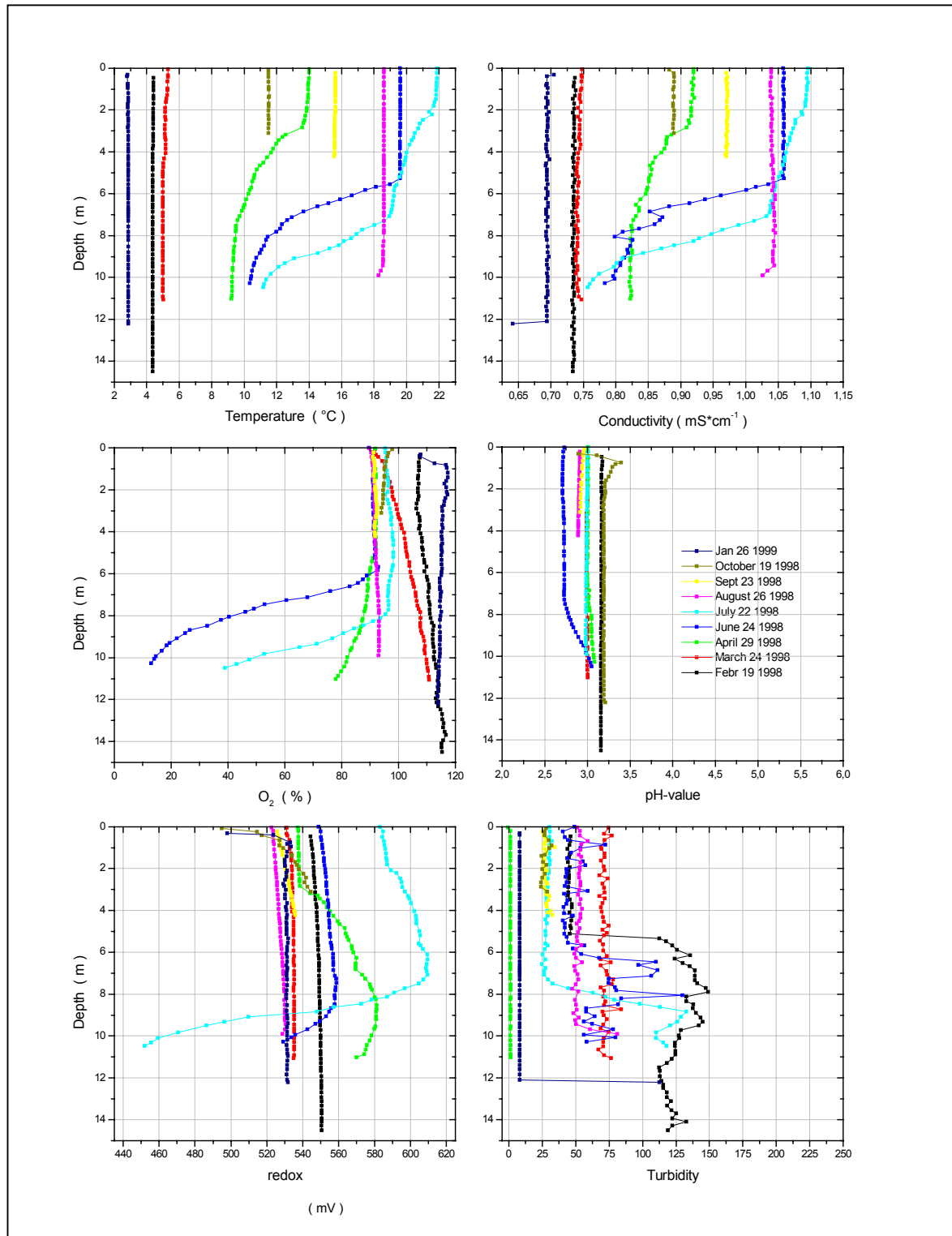


Fig. 5. Physical parameters: temperature, electric conductivity, oxygen concentration, pH-value, redox, and turbidity of AML 117 from Lusatian region in eastern Germany.

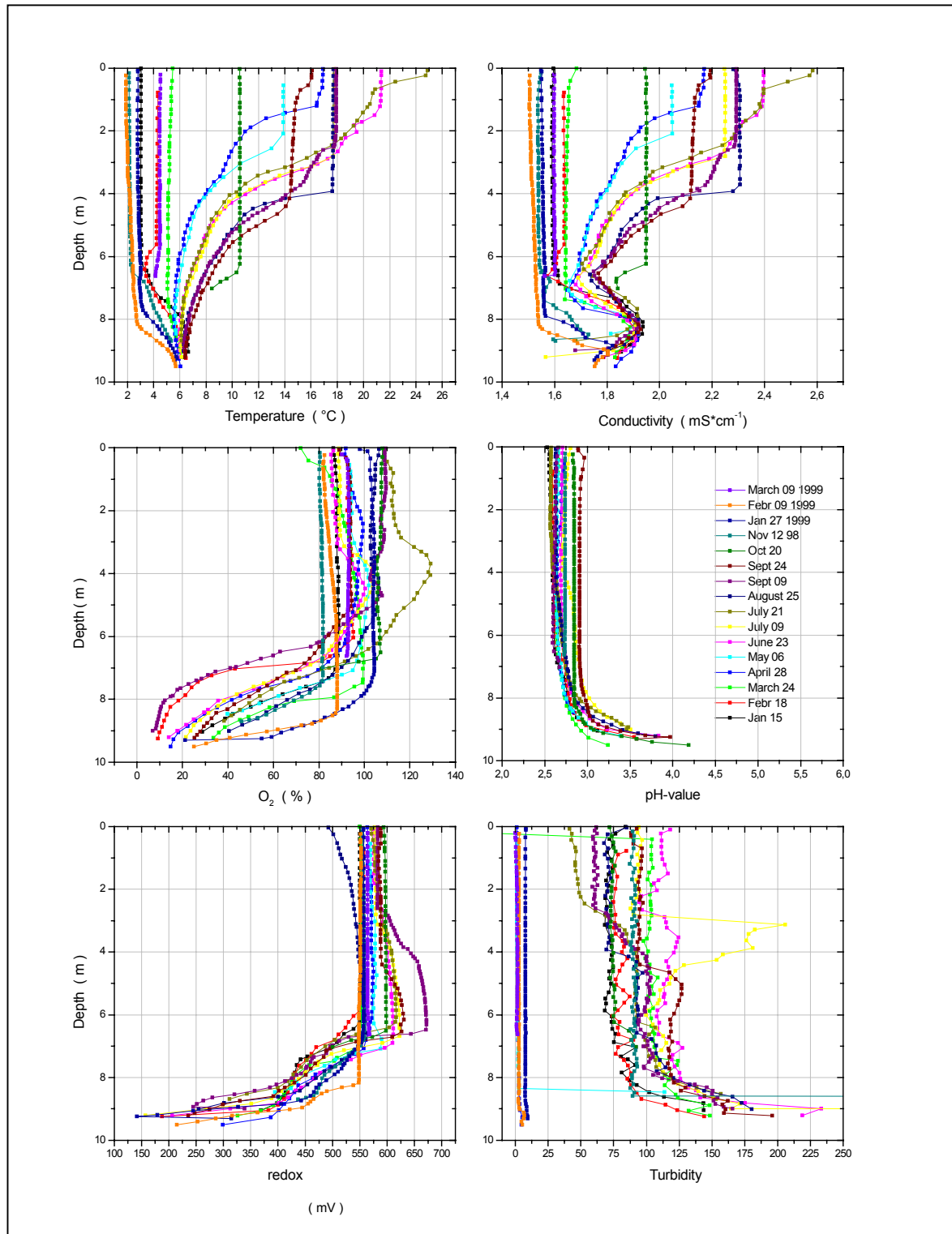


Fig. 6. Physical parameters: temperature, electric conductivity, oxygen concentration, pH-value, redox, and turbidity of AML 111 from Lusatian region in eastern Germany.

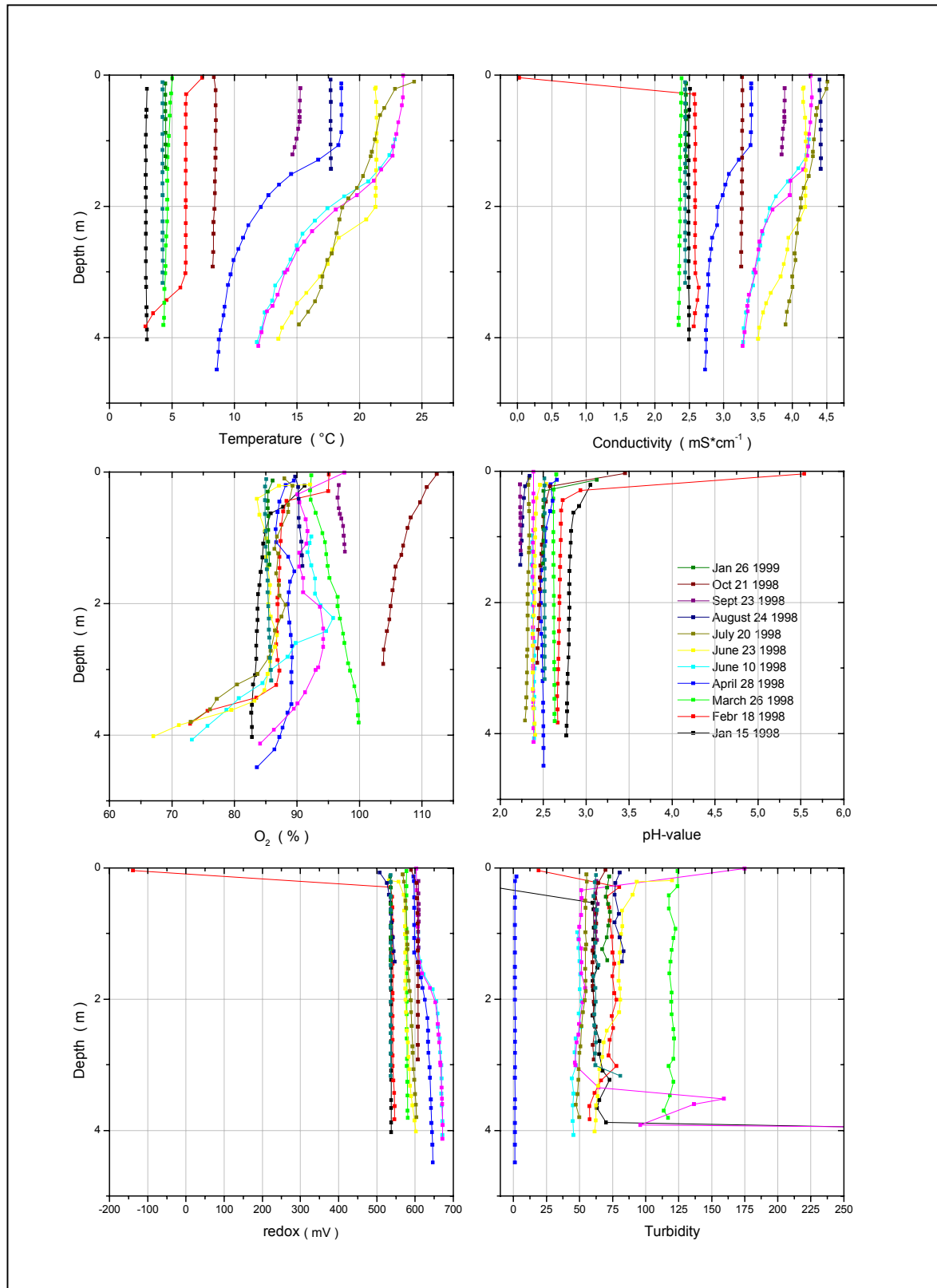


Fig. 7. Physical parameters: temperature, electric conductivity, oxygen concentration, pH-value, redox, and turbidity of AML 107 from Lusatian region in eastern Germany.

The most feature of these mining lakes from Lusatian region is their acidity with pH values between 2.0 to 3.0. In addition, the most striking physical characteristic of these lakes is their range of apparent colours – red-brown. These red hues are probably caused by a contamination of dissolved iron compounds ( $\text{FeS}_2$ ) stemming from pyrita and marcasita associated to the coal.

Some others characteristics are more conspicuous. For example, the electric conductivity was generally higher with value recorded to maximal  $4.5 \text{ mS.cm}^{-1}$  in AML 107, and  $2.4 \text{ mS.cm}^{-1}$  in AML 111 (Figs. 6,7). However, the range of conductivity values from AML 117 was about  $0.70$  to  $1.10 \text{ mS.cm}^{-1}$  (Fig. 5). Similar values ranged about  $0.6$  to  $0.95 \text{ mS.cm}^{-1}$  were found to ML B (Fig. 4). Furthermore, the range of conductivity values reported for freshwater lakes is about  $9 - 400$  micromhos ( $= 0.009 - 0.4 \text{ mS.cm}^{-1}$ ) (Hutchinson 1957), but some saline lakes my recorded  $60,000 \text{ }\mu\text{mS.cm}^{-1}$  ( $= 60 \text{ mS.cm}^{-1}$ ).

The investigated lakes (AML 107, AML 111, and AML 117) were characterised by high acidity, low pH and high concentrations of dissolved metal and sulphate (Table 2). Low nutrient concentrations were shown in all acidic lakes, especially phosphorous (max.  $0.053 \text{ mg P}_{\text{Tot}} \text{ l}^{-1}$ ) (Table 2), and chlorophyll *a* (max.  $13 \text{ }\mu\text{g l}^{-1}$ ) (Packroff *et al.* 1999). The relation of soluble reactive phosphorous (SRP) had a value of about 3:1 between the acidic mining lakes. (AML 107 with  $12 - 42 \text{ }\mu\text{g.l}^{-1}$ ; AML 111 with  $5 - 10 \text{ }\mu\text{g.l}^{-1}$ , and AML 117 with  $1 - 10 \text{ }\mu\text{g.l}^{-1}$ ). Nitrogen is available as ammonia ( $\text{NH}_4^+$ ) in excess in these extremely acidic lakes (Table 2). Inorganic and (dissolved) organic carbon were detectable at low concentrations only. The primary production rates are very low and may be due to low availability of anorganic carbon in low pH conditions (Kapfer 1998; Packroff 2000). The low total algal biomass contribute to describe these lakes as oligotrophic (Klapper & Schultze 1995). Algae occurred as primary colonisation (Deneke & Nixdorf 1997). The phytoplankton is dominated by highly mobile nanoflagellates belonging to the Chrysophyceae and the Chlorophyceae (Lessmann *et al.* 1999, 2000) in Lusatian mining lake. The zooplankton

consists of rotifers species, ciliates and heliozoans, and in some lakes cladoceran species (Lessmann *et al.* 1999; Deneke 2000; Packroff 2000). The phytobenthic compartment is dominated by *Euglena mutabilis* (Euglenophyta) and *Pinnularia acoricola* (Bacillariophyceae) (Lessmann *et al.* 1999).

The sediment-water interface plays a major role in the biological succession (Nixdorf & Kapfer 1998). Wendt-Potthoff & Neu (1998) regarded that the main biogeochemical process occurs in the level of surface sediments, such reduction of iron and manganese, bacterial sulphate reduction, and decomposition of organic matter. They are the key factors influencing sediment chemistry. Depending on the quality of the organic matter and the lake dynamics, hypolimnetic anoxia can lead to enhance microbial alkalinity production from anaerobic respiration, raising the pH in the sediment up to neutral pH. Figure 8 shows the view of the Lake 117 which exhibited pH value of 3.0.

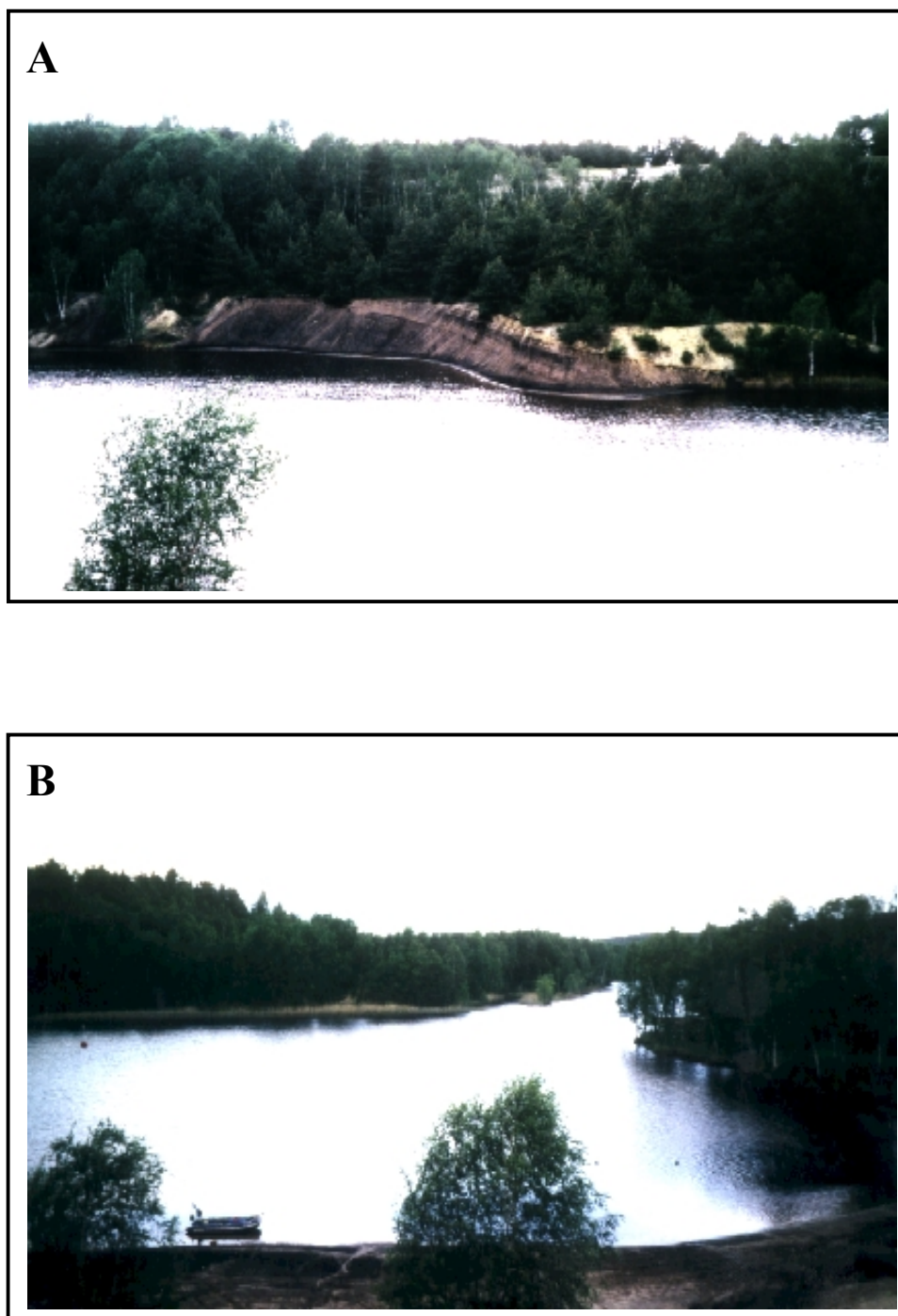


**Fig. 8.** View of Lake AML 117 (Grünewald) from Lusatian region (pH 3.0). It was a residual area of the lignite mining activities. The starting period of the mine was about 1956 and the mine was abandoned in 1966. Surrounding vegetation and aquatic vascular plants covered the littoral zone of this acidic lake. It is a recreational area, including a camping place. Lake AML 117 with acidity of  $2 \text{ mmol.l}^{-1}$  is thermally stratified during the summer months, and the epilimnion covered 0 – 8 m and the hypolimnion 10 – 12 m (Packroff 2000).

Lake AML 111 has been an experimental lake since 1996. This closed lake exhibits no inflow or outflow (Figs. 9 A,B). Normally, a thermal stratification develops in the summer, leading to oxygen consumption in the hypolimnion. During the winter, the lake is mixed across the entire water column, resulting in oxygen saturation (Friese *et al.* 1998).

In 1996 and 1997, AML 111 was permanently stratified in the deepest (> 7.5 m) part of the lake, which covers approx. 4% of the total area and the epilimnion was in the range 0-5 m (Packroff 2000). AML 111 has had a small monimolimnion below a 7-m depth since spring 1996. It formed when melting of the ice-cover diluted the surface water, thereby introducing additional stability. This shielded the heavier bottom water from mixing and allowed it to become even denser. The monimolimnion is considerably different from that of mixolimnion. This may be due to different redox conditions in the two layers. In the mixolimnion, ferric iron is the dominant iron species, whereas in the suboxic monimolimnion ferrous iron is in the same order of magnitude (Schimmele & Herzsprung 2000). The spring profile shows relatively low conductivity in the epilimnion, an increase in the hypolimnion and a minimum above the monimolimnion (Boehrer *et al.* 2000). This changes during summer, when electrical conductivity increases in the well mixed epilimnion. The zone of high electrical conductivity deepens with the deepening of the mixed layer in the fall. At the surface, ferric iron is reduced to ferrous iron photochemically (Herzsprung *et al.* 1998) and is redistributed in the epilimnion by a mixing process (Schimmele & Herzsprung 2000). The water of AML 111 was highly acidic (pH of 2.6) and highly turbid. AML 111 had been meromictic below a depth of 8 or 9 m for a few years. However, during the winter 1998/99, the chemocline might have been affected considerably by strong wind events during winter (Boehrer *et al.* 2000).

Figures 10 A,B show the view of the open pit lake AML 107 (Plessa) from Lusatian region in eastern Germany.



**Fig. 9 A, B.** View of Lake AML 111 (Plessa) from Lusatian region (pH 2.6 – 2.9). Lake AML 111 was a residual area of the mining activities. The starting period of this mine was about 1929. This mine was abandoned in 1956/58, and the inflowing groundwater was completed in 1969. A Surrounding forest is present around the lake.



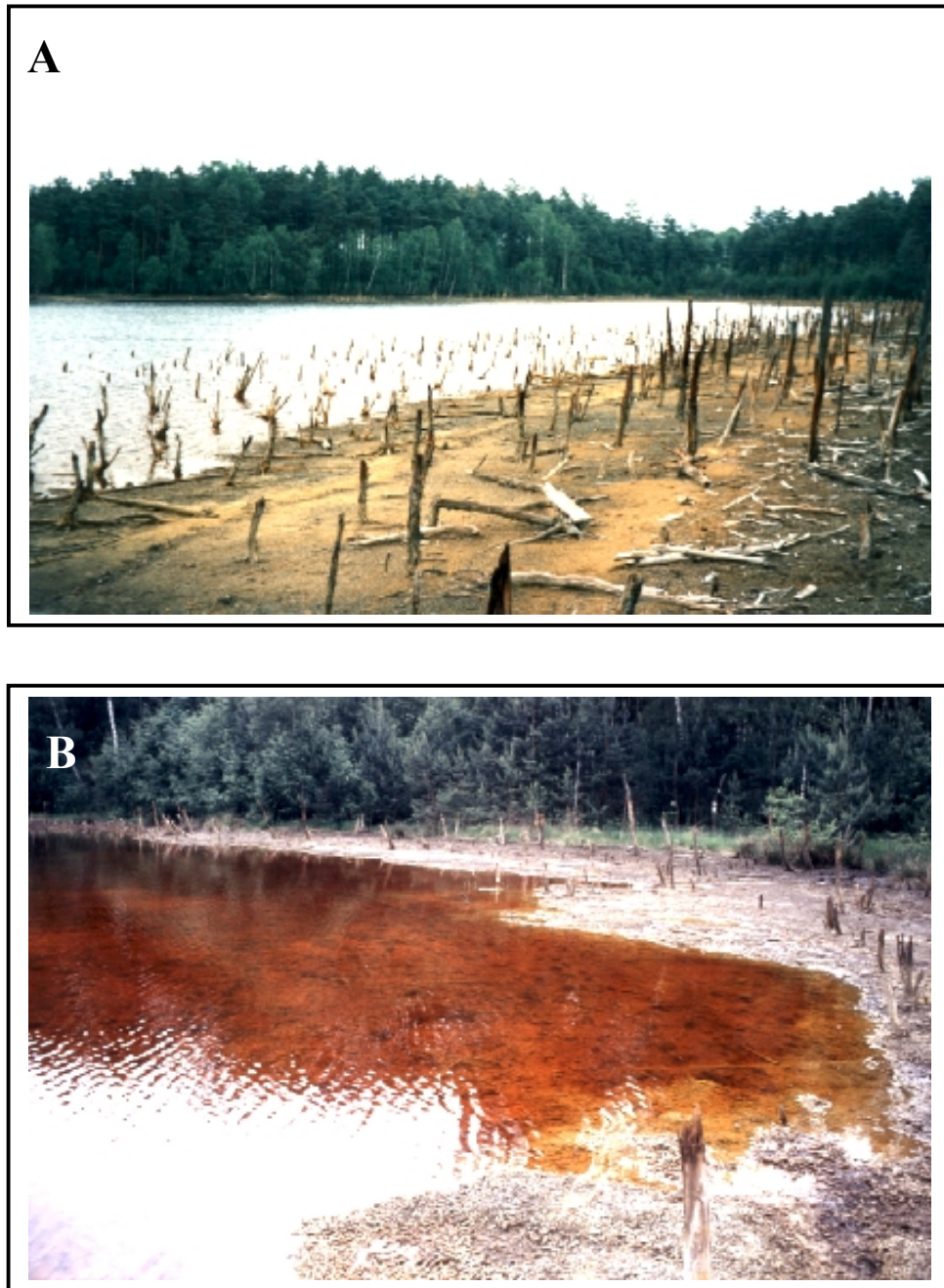


Fig. 10 A, B. View of open pit lake AML 107 (Plessa) from Lusatian region, which exhibited water with very low pH (min. pH 2.1). It was a residual area of the coal mine activities in the period of 1897 until 1928. Lake AML 107 is a closed lake with no inflow or outflow. The surrounding vegetation was removed during mining activities and had suffered from the falling or the recently recovering groundwater table. Stratification in Lake AML 107 lasted only from ice-cover (January – March 1996) until autumn 1996 (Herzprung *et al.* 1998) whereas a stable stratification was not detectable in 1997 (Packroff 2000). The epilimnion was in the ranges of 0 – 3 meters. The inflow of anoxic groundwater and the oxidation of  $\text{Fe}^{2+}$  are responsible for the oxygen decrease in the hypolimnion and above the sediment (Lessmann *et al.* 1999).

## 2.2 Sampling design

The sampling design comprised two phases: (  $\alpha$  ) the field work and (  $\beta$  ) the second phase of the laboratory experiments. The field work comprises: qualitative and semi-quantitative benthic invertebrates samples that were taken to evaluate the composition of the benthic invertebrate community; and the colonisation of deciduous leaves by benthic invertebrates was investigated. This study was performed between April 1997 and February 1999. The laboratory experiment comprised the ( 1 ) pupal description of *Chironomus crassimanus* (Insecta, Diptera, Chironomidae) and ( 2 ) predation effect of the *Chironomus crassimanus* on bacteria in microcosmos, and ( 3 ) gut content analysis of this species.

### 2.2.1 Field work

#### 2.2.1.1 Physical and chemical parameters

Vertical profile of temperature, conductivity, pH-value, redox, and oxygen concentration were measured with a Multiparameter Probe IDRONAUT Srl. (Brugherio, Italien). The oxygen sensor was calibrated to 100 % saturation at the beginning of each measuring day. The physical parameters were measured by my colleagues from the limno-physical working group within UFZ, section Gewässerforschung Magdeburg.

Water samples measuring 1.2 l were taken for chemical analysis. Sulphate ( $\text{SO}_4^{2-}$ ) and iron ( $\text{Fe}^{2+}$ ,  $\text{Fe}^{3+}$ ) and main trace-elements were analysed. The parameters total-phosphorous ( $\text{P}_{\text{Tot}}$ ), soluble reactive phosphorous (SRP), nitrite ( $\text{NO}_2^{-}\text{-N}$ ) and nitrate ( $\text{NO}_3^{-}\text{-N}$ ), and ammonia ( $\text{NH}_4^{+}\text{-N}$ ) were analysed next. The chemical parameters have been analysed since 1995 by chemical section by my colleagues of the chemical working group within section Gewässerforschung Magdeburg, UFZ.

The nutrients analysis ( $\text{NH}_4^+\text{-N}$ ,  $\text{NO}_2^-\text{-N}$ ,  $\text{NO}_3^-\text{-N}$ , SRP) and the determination of  $K_S$  and  $K_B$  values were measured by ÖHMI, Magdeburg in the period of July until September 1998.

The similarities and the standard deviation of the analysis results (ÖHMI / UFZ) were taken in September.

Samples of sulphate ( $\text{SO}_4^{2-}$ ), and calcium ( $\text{Ca}^{2+}$ ) and heavy metals were stabilised and kept cold and were analysed in October 1998. Other parameters (nutrients,  $K_S$  and  $K_B$  values) were measured in September 1998. The samples from October 1998 until February 1999 were analysed by UFZ.

### ***2.2.1.2 Sampling of benthic invertebrates***

Qualitative and semi-quantitative samples of invertebrates were taken from May 1997 until September 1997, from all lakes. The samples were taken with different methods: with Ekman grab ( $400\text{ cm}^2$ ) and hand nets with mesh of  $200\text{ }\mu\text{m}$  and though of benthic invertebrates colonisation experiments on leaf packs. The processing of leaf pack units were described in the section 2.2.2.4

### ***2.2.1.3 Leaf experiments in field***

The leaf colonisation experiments used wire-mesh containers ( $20\text{ x }20\text{ cm}$ ) with a mesh size of  $10\text{ mm}$  which allowed easy access by all potential benthic invertebrate colonists as aquatic insects, crustaceans and molluscs (Fig. 11).

Leaves from birch (*Betula pendula*), ash (*Fraxinus excelsior*) and walnut (*Juglans regia*) were selected for the experiment based on overall leaf colour and the absence of significant leaf damage. All air dried leaves were briefly soaked in distilled water until they were pliable, before the placement into the leaf packs. Exactly  $20\text{ g}$  of each leaf type were weighed and placed into individual leaf packs. Experiments were performed in water depths ranging

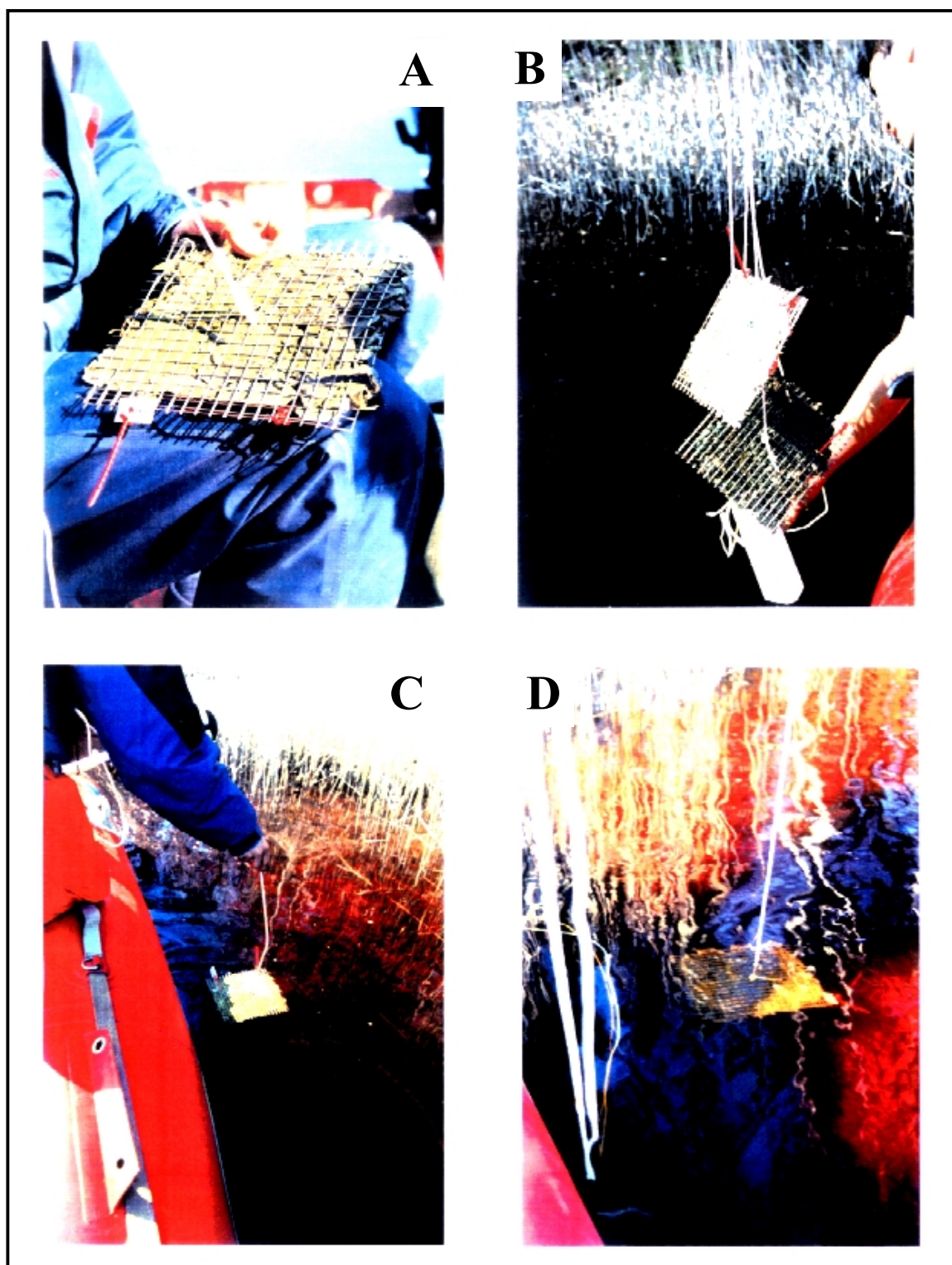


Fig. 11. Field experiments. A) Leaf pack unit for benthic invertebrate colonisation experiments; B) Leaf pack units (birch, ash, and walnut leaves); C and D) Exposure of leaf pack units in mining lakes.



from approximately 0.5 to 1.5 m in an area that was relatively homogeneous with respect to substrate types, and a bank of macrophytes was also present. Figures 12 and 13 show the localisation of this leaf packs units in AML 107 and AML 111.



**Fig. 12 . Localisation of the exposure site of the leaf pack units in the littoral zone from Lake AML 107 (Plessa, Lausitz, Germany).**



**Fig.13. Localisation of the exposure site of the leaf pack units in the littoral zone of the Lake AML 111 (Plessa, Lausitz, Germany).**

The individual leaf packs were placed in the littoral zone of the lakes, attached in groups of 9 (tree replicas of three leaf types). Deciduous leaf colonisation experiments were performed between March 1998 and February 1999 (Table 3).

**Table 3.** Leaf packs exposure experiments from Lusatia mining lakes (ML B, AML 117, AML 111 and AML 107).

**Data Set #1- Spring-Summer Experiment**

Experiment 1	ML B	AML 117	AML 111	AML 107	Time of exposure (days)
March-September 1998	24.03.98	23.03.98	23.03.98	24.03.98	180
April-September 1998	29.04.98	29.04.98	18.04.98	28.04.98	150
May-September 1998	27.05.98	28.04.98	09.06.98	28.05.98	120
June-September 1998	24.06.98	24.06.98	23.06.98	23.06.98	90
July-September 1998	20.07.98	22.07.98	21.07.98	20.07.98	60
August-September 1998	24.08.98	25.08.98	25.08.98	24.08.98	30
September 1998	24.09.98	23.09.98	24.09.98	23.09.98	Removed

**Data Set #2 -Summer Experiment**

Experiment 2	ML B	AML 117	AML 111	AML 107	Time of exposure (days)
June-July 1998	24.06.98	24.06.98	23.06.98	23.06.98	30
July-August 1998	20.07.98	22.07.98	21.07.98	20.07.98	30
August-September 1998	24.08.98	25.08.98	25.08.98	24.08.98	30

**Data Set #3- Autumn-Winter Experiment**

Experiment 3	ML B	AML 117	AML 111	AML 107	Time of exposure (days)
Oct.-Jan./Feb.1998	19.10.98	19.10.98	20.10.98	21.10.98	90 – (120 ML B)
Nov.-Jan./Feb.1998	19.11.98	17.11.98	18.11.98	17.11.98	60 – ( 90 ML B)
Jan/Feb. 1999	09.02.98	26.01.98	27.01.99	26.01.99	Removed

*Data set# 1 – Experiment*

The first experiment (March-September 1998) evaluated the leaf breakdown process of *Betula pendula* (birch), *Fraxinus excelsior* (ash) and *Juglans regia* (walnut) leaves in the time exposure of 180, 150, 120, 90, 60, and 30 days. Each colonisation experiment consisted of a total 216 individual leaf packs. All leaf packs were slowly removed simultaneously from 23<sup>th</sup> to the 24<sup>th</sup> of September 1998 (three replicas each of tree leaf types by 6 sampling times at four lakes). This resulted in maximal exposition periods of 180 days in the spring-summer season.

*Data set#2 -Experiment*

The second experiment investigated the leaf weight loss between birch, ash and walnut in the time of exposure of 30 days in summer season (June - September 1998). Birch, ash, and walnut leaf weight loss experiment consisted of a total 108 individual leaf packs exposed in each 30 days in the summer periods (3 replicas each of deciduous leaf types by 3 sampling times at 4 lakes). The leaf packs were removed every 30 days.

*Data set#3 – Experiment*

The third experiment (October 1998 – February 1999) evaluated the leaf breakdown process of leaves that had remained on the ground over winter. Birch, ash, and walnut leaf weight loss experiment consisted of a total 72 individual leaf packs (three replicas each of tree leaf types by 2 sampling times at four lakes). All leaf packs were slowly removed simultaneously from 26<sup>th</sup> to the 27<sup>th</sup> of January, and 9<sup>th</sup> of February 1999. In the autumn-winter experiment exposition periods were 100 days and 70 days respectively.

At the end of the experiment each leaf pack was brought to the surface using a rectangular fine-mesh net (200 µm). The leaf packs were collected from the water and placed separately into plastic bags and returned to the laboratory for processing. Physical and chemical parameters, were measured just before the leaf packs were retrieved.

**2.2.2 Laboratory experiments*****2.2.2.1 Pupal description of *Chironomus crassimanus****

Egg masses of chironomids were collected from acidic mining lakes and cultured in the laboratory for the identification of the species. *Chironomus* egg masses were attached to the littoral macrophytes from AML 117 and on the edge of the experimental platform (AML

111), or in the flotsam of the sampling station (AML 107). Egg masses were reared in batches in 150 ml water from the lake. The water was aerated continuously and leaves of *Urtica dioica* L. added for food.

Emerged adults were collected daily and 4<sup>th</sup> instar larvae were taken for karyological examination. The larvae were prepared by the author and identified by Prof. Dr. W. Wülker, Freiburg. Pupal exuviae were taken after emergence of the adults in the laboratory and collected in the littoral zone of AML 117 for description. Morphological nomenclature follows that of Langton (1991, 1994, 1995) and Langton & Armitage (1995). Pupal exuviae have been deposited in the Zoologische Staatssammlung München (ZSM), Germany.

#### **2.2.2.2 Predation effect of acid-resistant *Chironomus* on bacteria**

Laboratory-experiment was conducted to verify the predation effect of *Chironomus crassimanus* on bacteria under experimental condition. Sediment samples were taken from AML 111 with an Ekman grab and kept cold. In the laboratory the sediment was sieved with mesh size 0.2 mm and incubated in four batches.

The batches were kept in an aerated aquarium at a temperature of 8.5 °C. For 28 days vertical profiles of oxygen were measured with a microoptode (Presens, Neuburg, Germany) until stable oxygen concentration was achieved. Then 10 larvae of *Ch. crassimanus* were added to two batches and oxygen profiles were taken in the batches with and without chironomid larvae for 13 days. From the vertical profiles oxygen fluxes at these sediment surfaces were computed by the software PROFILE (Berg *et al.* 1998).

The number of iron-reducing bacteria (cells.ml<sup>-1</sup>) were determined by MPN (Most Probably Numbers) in the sediment of batches with and without *Ch. crassimanus*. The medium for Fe<sup>3+</sup> reducing bacteria (FeRB) was adapted from a medium for *Geobacter metallireducens* (DSM no. 579). It contained (in g l<sup>-1</sup>) 13.7 ferric citrate, 1.5 NH<sub>4</sub>Cl, 0.6 NaH<sub>2</sub>PO<sub>4</sub>, 0.1 KCl, 1.6 sodium acetate, 0.05 yeast extract, 0.25 mg l<sup>-1</sup> Na<sub>2</sub>SO<sub>4</sub>·H<sub>2</sub>O and 10



ml.l<sup>-1</sup> trace elements solution. The medium was buffered with 10 mM MES (2-morpholinoethanesulfonic acid) and posed to pH 6. Cultures were prepared in deep multiwell plates (8 parallels). Anoxic conditions for anaerobes were generated by placing cultures in sealed bags with gas generators (Merck Anaerocult A). Growth of FeRB was judged from accumulation of Fe<sup>2+</sup> determined in a microtiter format ferrozine assay. MPNs and their confidence limits were calculated with the program of Klee (1993).

#### **2.2.2.3 Gut content of the acid-resistant *Chironomus crassimanus***

For analysing the contents of the alimentary tract of *Ch. crassimanus* larvae, this species were collected out of AML 111 and 117 and fixed *in situ* with 10 %, 30 %, 50 %, 70% and 100 % ethanol for 10 minutes, and preserved in 70 % alcohol.

Both fresh and fixed larvae ( $n = 12$ ) were excised and the digestive tract were analysed. Confocal laser scanning (CLSM) microscopy was done on a TCS 4D (Leica, Heidelberg, Germany) equipped with Ar/Kr and UV laser.

The images were taken with the inverted microscope using the 63x 1.2 NA water immersion lens. Samples were transferred into coverslip chambers in which staining and imaging were performed. For staining the nucleic acid specific stain SYTO9 (Molecular Probes, Eugene, Oregon, USA) and the fungi specific cell stain Calcofluorwhite M2R (Sigma, Deisenhofen, Germany) were employed. Signals were detected in the reflection mode and the fluorescent mode taking advantage of the autofluorescence of the sample as well as after staining.

#### **2.2.2.4 Processing of leaf and benthic fauna samples in the laboratory**

- *Leaf weight loss*

In the laboratory, the wire-bags were opened. The content of each leaf pack was rinsed with tap water, so that the invertebrates and loosely attached fine sediments were removed. The

washed leaves (> 1.6 mm) were dried at 110 °C for 24 hrs and weighed to the nearest 0.001 g using an electronic scale balance SARTORIUS LC521P to determine the leaf weight loss (LW). To determine initial weight losses attributable to leaching, 20 g of dried leaves of each deciduous leaf species were immersed in a bucket of tap water at a constant temperature (10 °C). Leaves were removed after 24 hours, dried at 60 °C for 48 hours, and weighed.

- *Benthic invertebrate fauna*

In the laboratory, the leaves material were initially rinsed in tap water. The leaf material (>1.6 mm) was dried and weighed to determine the leaf weight loss (LW). The resulting material contained in sieves with mesh size of 200 µm were kept in 70 % ethanol. The benthic invertebrates were sorted at stereomicroscope ZEISS, counted and preserved for subsequent identification. After that they were dried at 105 °C for 24 hrs, and weighed to the nearest 0.001 g using an electronic scale balance SARTORIUS LC521P to determine the total biomass.

For the identification of chironomids the organisms were sorted and slide-mounted with Euparal. Slide-mounted larvae, and adult were identified with light of a microscope (ZEISS, AXIOKOP) using published keys. The pupal exuvie of *Ch. crassimanus* were described following nomenclature follows that Langton (1991, 1994, 1995) and Langton & Armitage (1995). Other groups of benthic invertebrates were identified according to general literature below (Table 4).

Benthic invertebrates were assigned into functional groups according to specialised literature, except *Chironomus crassimanus* were classified according the results of this study. The benthic organisms were classified into the guilds. Initial separation was based on: a) the major feeding strategies (herbivorous, detritivorous, carnivorous, and omnivorous). Later separation varied, with combinations of b) feeding habit or methods of feeding (shredders, collectors, scrappers (= grazers), piercers and predators), and c) food type being used.

**Table 4. General literature list used for the identification of benthic invertebrates.**

<b>Acari:</b> Gerecke (1994); Smith & Cook (1991); Viets (1987)
<b>Coleoptera:</b> Burmeister (1939); Fichtner, E. (1983, 1984); Klausnitzer, B. (1996); Richoux, P. (1982)
<b>Diptera:</b> Wiederholm (Eds.) (1983; 1986; 1989); Moller Pillot (1984); Vallenduk & Moller Pillot (1999); Webb & Scholl (1990) Müller (1995); Sæther (1970, 1972, 1990).
<b>Ephemeroptera:</b> Elliott <i>et al.</i> (1988)
<b>Hemiptera-Heteroptera:</b> Dethier (1985); Jansson (1969); Savage (1989); Cobben & Moller Pillot (1960)
<b>Megaloptera:</b> Dethier (1986); Elliott (1996); Kimmins (1962)
<b>Mollusca:</b> Glöer & Meier-Brook (1998)
<b>Odonata:</b> Askew (1988); Corbet (1955); Heidemann & Seidenbusch (1993)
<b>Ostracoda:</b> Meisch (2000)
<b>Oligochaeta:</b> Brinkhurst, R.O. (1986)
<b>Trichoptera:</b> Tobias & Tobias (1981); Pitsch (1993); Waringer & Graf (1997)

### 2.3 Statistical analysis

Following Benfield (1996), leaf processing was assumed to follow an exponential decay model of the form

$$W_t = W_0 e^{-kt} \quad (3)$$

It is the amount remaining after  $t$  of the initial amount  $W_0$  (after leaching) and  $k$  is the loss rate of the processing coefficient. Following  $\log_e$  transformation of the remaining percentage data (%R), linear regression was used to estimate the processing coefficient for each leaf species after excluding the leaching loss.

Leaf weights were corrected for initial leaching losses which were excluded from calculations of leaf decay rates. Processing coefficients -  $k$  ( $\text{day}^{-1}$ ) for weight loss of leaves were calculated as follows Petersen & Cummins (1974):

$$-k = \ln (\%R / 100) / t \quad (4)$$

where  $-k$  is the daily exponential rate of weight loss, %R is the percentage of leaf material remaining, and  $t$  is the duration of the experiment in days. Differences in processing coefficients and weight loss (log transformed) among leaf species were tested with ANOVA followed by Duncan's Multiple Range tests.

The amount of leaf material lost over a time is expressed by:

$$\%R = LW_t / LW_o \times 100 \quad (5)$$

where  $LW_o$  is the initial weight of leaf material and  $LW_t$  is the amount of material remaining after time  $t$  (Petersen & Cummins 1974).

Benthic invertebrates were converted to number ( $g^{-1}$ ) of remaining leaf matter for subsequent statistical analyses (cf. Benfield *et al.* 1977; Casas 1996; Dobson 1994; Dudgeon 1982; Hill & Perrote 1995; Petersen & Cummins 1974). The total abundance and total biomass of the benthic invertebrates were converted to remaining leaf weight loss in percentage (%R). The conversion adjust for the loss of leaf mass over time during the study periods as such proceeding provides a better estimate of invertebrate densities. Benthic invertebrate data were evaluated using two-way analysis of variance (ANOVA). A Sørensen similarity index (QS %) was calculated to verify the similarity of the species composition in twice data groups independent of the number of individuals in each groups sampled.

$$QS (\%) = \frac{2G}{SA + SB} * 100 \quad (6)$$

where:  $G$  = Number of same species in the places A and B.

$SA, SB$  = Species number in the place A respectively B.

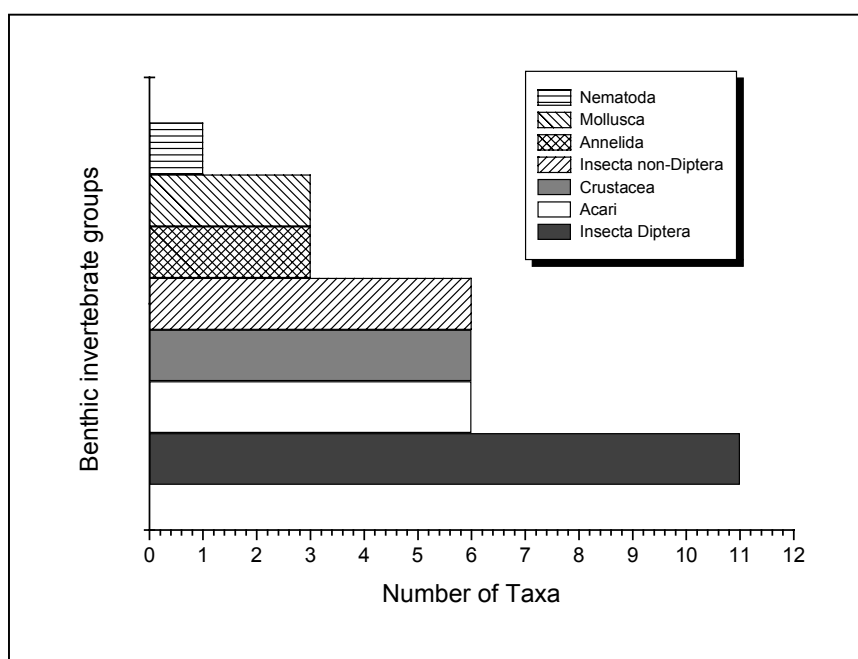
Pearson correlation were calculated between the mean of pH level and the species richness of the lakes ML B, AML 117, AML 111 and AML 107 (Zar 1984).

### 3 Results

#### 3.1 Fauna composition

##### 3.1.1 Benthic invertebrate fauna from control-lake ML B

In total 36 benthic invertebrate taxa were identified in the control lake (ML B). The most frequent group were aquatic insects-Diptera with 30.5 %, followed by Acari, Crustacea, and aquatic insects non-Diptera, each with 16.7 %. Mollusca and Annelida had each 8.3 %, and Nematoda 2.7 %. Together aquatic insects comprised 46.7 % of the benthic invertebrate fauna in ML B (Fig. 14).



**Fig. 14.** Taxa number of benthic invertebrate groups collected in the course of qualitative and semi-quantitative samples from Lake ML B.

The composition list of the benthic invertebrate fauna from Lake ML B is shown in the table 5. A total of 27 benthic invertebrate taxa were collected using traditional method (Ekman grab) for benthic invertebrate samples and a total of 21 taxa were found using a leaf colonisation experiment.

Chironomids (16 %) were found in greatest number of species by benthic samples and by leaf experiments. *Phaenopsectra flavipes*, *Microtendipes pedellus*, and *Dicrotendipes nervosus* are typical species from sublittoral, littoral zone and extreme marginal shallow habitats.

In both collecting methods ostracods, with 13.8 % (benthic samples) and 11.0 % (leaf experiment), occurred with greatest number of species. Also, Ephemeroptera occurred in a great results with 8.3 % and 5.5 %, respectively.

*Nehalennia speciosa* (Odonata) also occurred by both collecting methods. Halacaridae showed the most number of species of mites with 5 species, but were found only by benthic samples. Others benthic invertebrate taxa were variable from sample to sample but no one sample method appeared biased for any one group.

Some groups were not identified until species level as naidids (Oligochaeta, Naidade) and nematods. However, the highest number of these taxa were found in the benthic samples due also the samples were collected in the littoral and the profundal zone of the lake. For example, chaoborids were found only in the profundal zone, and leaf packs colonisation experiments were investigated only in the littoral zone of the lakes.

### 3.1.2 Benthic invertebrates from extremely acidic mining lakes

A total of 10 benthic invertebrate species were found in AML 107, AML 111 and AML 117 (Table 6). AML 117 showed the greatest number of species with 9 taxa, followed by AML 111 with 7 and AML 107 with 3 species. Aquatic insects were the most common group of benthic invertebrates from these acidic mining lakes.

Also, some chironomid species in the pupae stage and the pleustonic *Sigara nigrolineata* nymph were frequently found on the leaves during the experiment. The chironomids *Ablabesmyia longistyla* and *Corynoneura lobata* were poorly represented in the acidic mining lakes, but occurred in AML 111.

**Table 5. Composition of benthic invertebrates from Lake ML B. The taxa are given allowed x for qualitative and semi-quantitative benthic samples ( $\geq 200 \mu\text{m}$  net), and for leaf pack colonisation experiments.**

Species and taxa	Benthic samples	Leaf packs colonisation
Nematoda		x
Oligochaeta		
TUBIFICIDAE		
<i>Tubifex tubifex</i>	x	x
NAIDIDAE	x	
Hirudinea		
PISCICOLIDAE		
<i>Piscicola geometra</i> (L.)	x	
Mollusca		x
Planorbiidae	x	
Sphaeriidae		
<i>Pisidium tenuilineatum</i> STELFOX, 1918	x	
Hidrobiidae		
<i>Martonopsis scholtzi</i> (A. SMITH, 1856)	x	
Crustacea		
CYPRIDIDAE		
<i>Cypridopsis vidua</i> (O.F. MÜLLER, 1776)	x	x
<i>Isocypris beauchampi</i> (PARIS, 1920)	x	x
CANDONIDAE		
<i>Candona candida</i> (O.F. MÜLLER, 1776)	x	x
<i>Fabaeformiscandona caudata</i> (KAUFMANN, 1900)	x	
ILYOCYPRIDIDAE		
<i>Ilyocypris</i> sp.	x	x
GAMMARIDAE		
<i>Gammarus roeseli</i> (GERVAIS 1835)		x
Hemiptera-Heteroptera		
CORIXIDAE		x
Ephemeroptera		
CAENIDAE		
<i>Caenis pusila</i> Navas	x	x
LEPTOPHLEBIIDAE		
<i>Paraleptophlebia werneri</i> ULMER	x	x
SIPHONURIDAE		
<i>Ameletus inopinatus</i>	x	
Odonata		
COENAGRIONIDAE		
<i>Nehalennia speciosa</i> (CHARPENTIER, 1840)		x
Trichoptera	x	x
Diptera		
CERATOPOGONIDAE		
<i>Bezzia</i> sp.	x	x
CHABORIDAE		
<i>Chaoborus flavicans</i> (MEIGEN, 1818)	x	
CHIRONOMIDAE		
<i>Chironomus plumosus</i> MEIGEN	x	x
<i>Cricotopus</i> sp. v.d. WULP		x
<i>Corynoneura</i> sp.		x
<i>Dicrotendipes nervosus</i> STAEGER	x	x
<i>Limnophyes</i> sp. EATON	x	
<i>Microtendipes pedellus</i> KIEFFER		x
<i>Phaenopsectra flavipes</i> MEIGEN		x
<i>Procladius</i> s.str. SKUSE, 1899	x	
<i>Stictochironomus</i> sp. KIEFFER	x	
Acari		
ARRENURIDAE		
<i>Arrenurus (Arrenurus) crassicaudatus</i> KRAMER, 1875	x	
<i>A. (Micruracarus) biccissus</i> LEBERT, 1879	x	
<i>Forelia liliacea</i> (MÜLLER, 1776)	x	
LEBERTIDAE		
<i>Lebertia</i> sp. (Deutonymph) NEUMAN, 1980	x	
PIONIDAE		
<i>Piona</i> sp. (Deutonymph) KOCH, 1842	x	
ORIBATIDAE		x
<b>Taxa total</b>	<b>27</b>	<b>21</b>

**Table 6. Occurrence of benthic invertebrates on leaves colonisation from AML 107, AML 111, and AML 117. Frequency classes of benthic invertebrates were according to leaf colonisation experiment (ind . g leaf<sup>-1</sup>). The feeding habit and the feeding strategy were assigned according to Giani & Laville 1973; Griffiths 1973; Delettire 1978; Baker & McLachlan 1979; Pophan & Savage 1984).**

Taxa	Feeding habit/ mechanism	Feeding strategy	AML 107	AML 111	AML 117
<b>Diptera</b>					
Chironomidae					
<i>Chironomus crassimanus</i> Strenzke, 1959	collectors	detritivore	++++	++++	+++
<i>Ablabesmyia longistyla</i> Fittkau, 1962	collector/ predators	-		++	
<i>Limnophyes minimus</i> (Meigen 1818)	shredders	herbivore		+++	++
<i>Corynoneura lobata</i> (Edwards, 1924)	collectors	-		++	
<b>Coleoptera</b>					
Gyrinidae					
<i>Orectochilus villosus</i> (Müller, 1776)	predators	carnivore			+
<b>Odonata</b>					
Coenagrionidae					
<i>Coenagrion mercuriale</i> (Charpentier, 1840)	predators	carnivore			+
<b>Trichoptera</b>					
Phryganeidae	predators	carnivore			+
<b>Hemiptera-Heteroptera</b>					
Corixidae					
<i>Sigara nigrolineata</i> (Fieber, 1848)	predators	omnivore	+++	++	++
<b>Megaloptera</b>					
Sialidae					
<i>Sialis lutaria</i> L.	predators	carnivore			++
<b>Acari</b>					
Hydrozetidae					
<i>Hydrozetes lacustris</i> (Michael, 1882)	shredders	detritivore	+++	+++	++++
<b>Nematoda</b>				++	+
Total number of species			<b>3</b>	<b>7</b>	<b>9</b>

Frequency classes	Cod.	AML 107	AML 111	AML 117
Dominant	++++	1 – 10	10 – 100	> 100
Abundant	+++	0 – 1	1 – 10	10 – 100
Frequent	++		0 – 1	1 – 10
Rare	+			0 – 1

The relationship between the total species number and mean of pH-values in the studied mining lakes showed an abrupt gradient of decrease of species in relation to a very low pH-value. (Figure 15).



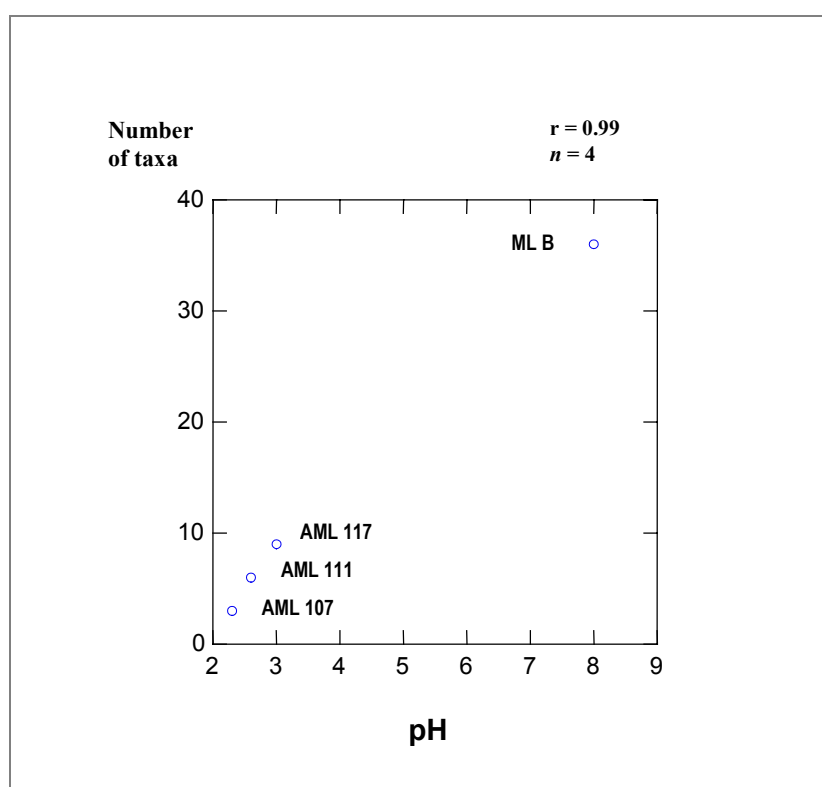


Figure 15. Pearson correlation between mean of pH-values and number of benthic invertebrate species from AML 107, AML 111, AML 117 and ML B.

The Sørensen similarity index was calculated using species richness for the mining lakes (Table 7). It showed that ML B has a lower similarity index in relationship to AML 107, AML 111, and AML 117. The acidic mining lakes had a higher similarities index in relation to the number of taxa.

Table 7. Sorensen similarity index (QS %) for the benthic invertebrate taxa between AML 107, AML 111, AML 117, and ML B.

QS (%)	AML 107	AML 111	AML 117
ML B	6.08	6.08	5.08
AML 117	60.1	71.0	-
AML 111	80.0	-	

High values of similarity index were found (60 – 80) for the benthic invertebrates from acidic mining lakes. However, low values of similarity index (5 – 6) were found between ML B and AML 107, AML 111 and AML117.

### 3.2 Abundance of benthic invertebrate fauna from leaf packs in acidic mining lakes

#### 3.2.1 Abundance of benthic invertebrate fauna from leaf packs in AML 107

Figures 16 – 25 show the abundance of the benthic invertebrates on birch, ash and walnut leaf packs colonisation experiment in the time of incubation comprised 30, 60, 90, 120, 150 and 180 days (Table 3, Data set#1).

*Chironomus crassimanus* larvae were found in all the time of incubation. Larvae exhibited the greatest abundance of benthic invertebrates from AML 107 ranged between  $1.75 \pm 0.6$  ind . g ash<sup>-1</sup> in 180 days of exposure until  $8.70 \pm 2.16$  ind . g walnut<sup>-1</sup> in 120 days. Pupae of *Ch. crassimanus* also were found in the walnut leaf packs colonisation experiments in the time of 60 days of incubation.

Nymphs of *Sigara nigrolineata* colonised the leaf packs in the time of incubation of 60 and 120 (birch leaves) and 30 days (ash leaves). Also, *Hydrozetes lacustris* colonised the leaf packs from AML 107 in the time of incubation of 60 and 150 days (birch leaf packs), and 120 days (walnut leaf packs) and 150 days (ash leaf packs). By ash leaf packs deutonymphs of *H. lacustris* were also found. The abundance of *S. nigrolineata* and *H. lacustris* was lower than the abundance of *Ch. crassimanus*.

#### 3.2.2 Abundance of benthic invertebrate fauna from leaf packs in AML 111

Figures 26 – 40 show the abundance of the benthic invertebrates in birch, ash and walnut leaf packs colonisation experiment, and the time of incubation comprised 30, 60, 90, 120, 150 and 180 days from AML 111 (Table 3, Data set#1).

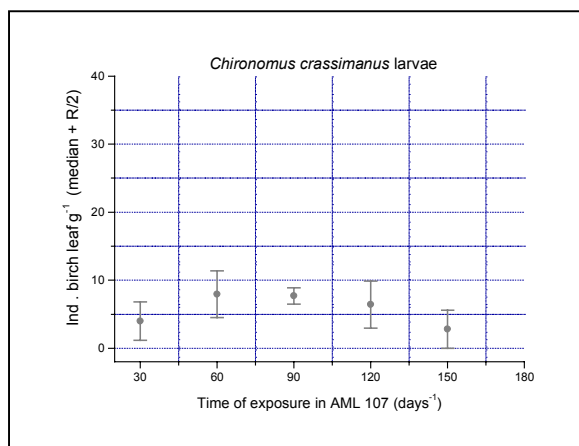


Fig. 16. Abundance of *Chironomus crassimanus* larvae on birch leaves in the time of incubation of 30, 60, 90, 120, 150 and 180 days in AML 107.

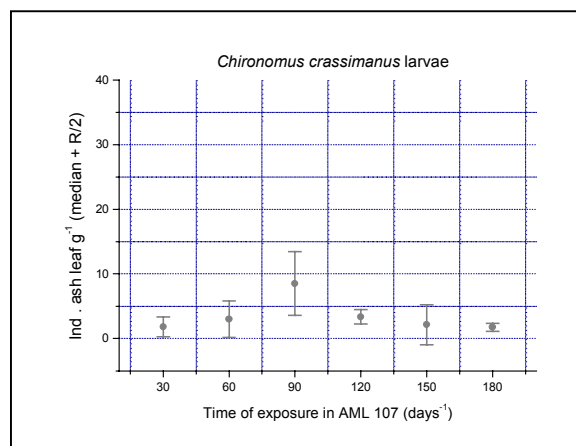


Fig. 17. Abundance of *Chironomus crassimanus* larvae on ash leaves in the time of incubation of 30, 60, 90, 120, 150 and 180 days in AML 107.

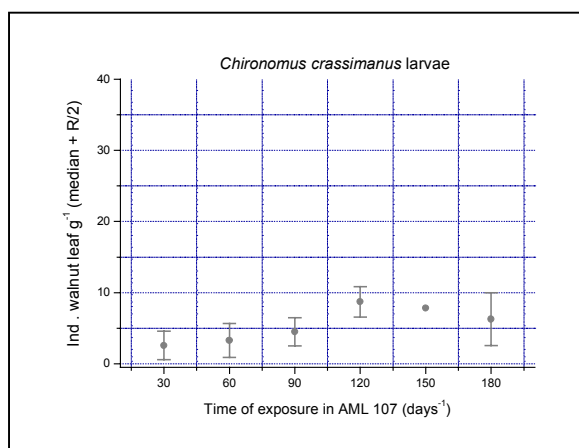


Fig. 18. Abundance of *Chironomus crassimanus* larvae on walnut leaves in the time of incubation of 30, 60, 90, 120, 150 and 180 days in AML 107.

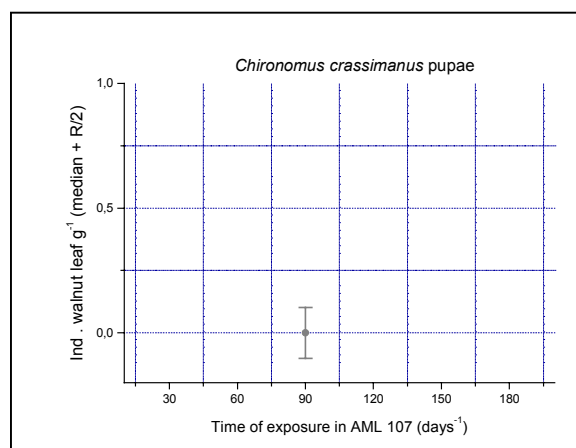


Fig. 19. Abundance of *Chironomus crassimanus* pupae on walnut leaves in the time of incubation of 30, 60, 90, 120, 150 and 180 days in AML 107.

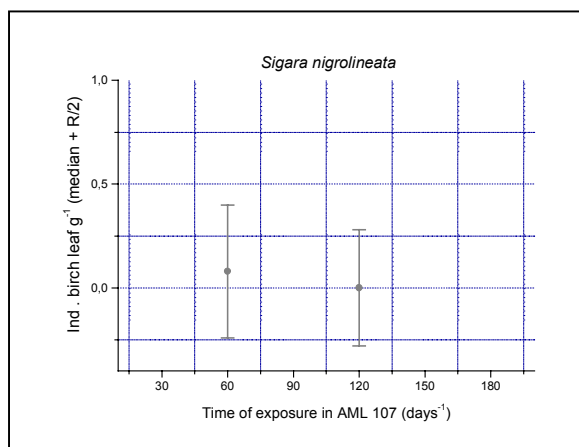


Fig. 20. Abundance of *Sigara nigrolineata* on birch leaves in the time of incubation of 30, 60, 90, 120, 150 and 180 days in AML 107.

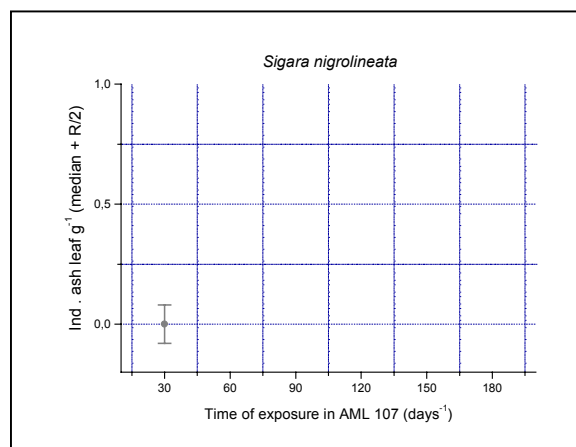


Fig. 21. Abundance of *Sigara nigrolineata* on ash leaves in the time of incubation of 30, 60, 90, 120, 150 and 180 days in AML 107.

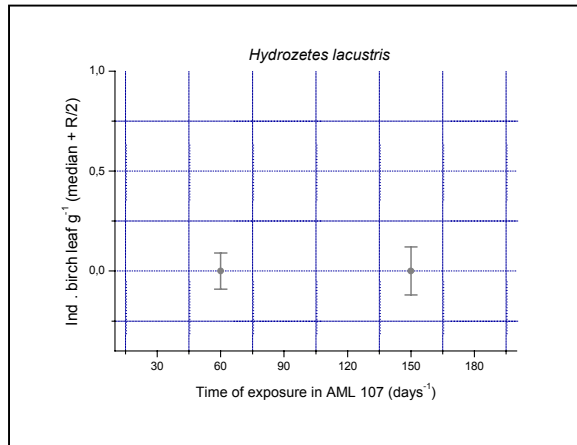


Fig. 22. Abundance of *Hydrozetes lacustris* on birch leaves in the time of incubation of 30, 60, 90, 120, 150 and 180 days in AML 107.

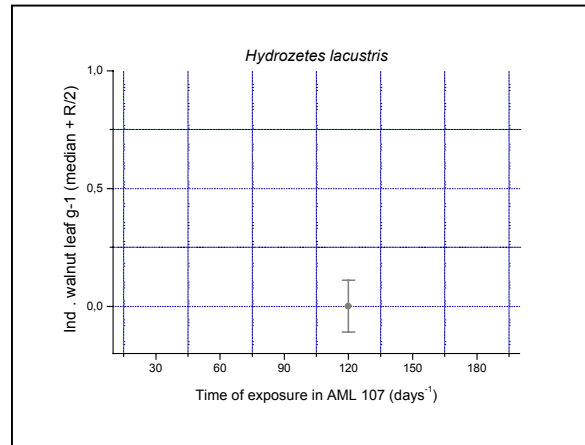


Fig. 23. Abundance of *Hydrozetes lacustris* on walnut leaves in the time of incubation of 30, 60, 90, 120, 150 and 180 days in AML 107.

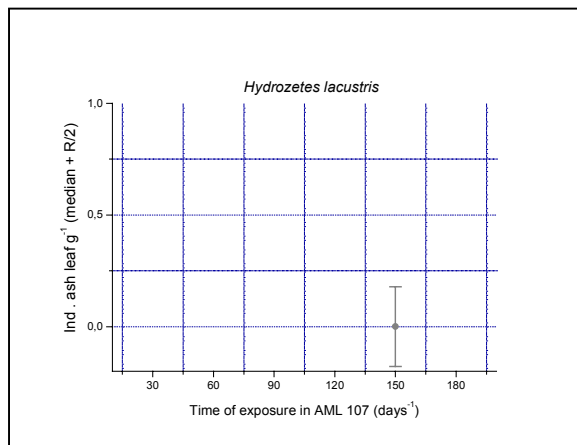


Fig. 24. Abundance of *Hydrozetes lacustris* on ash leaves in the time of incubation of 30, 60, 90, 120, 150 and 180 days in AML 107.

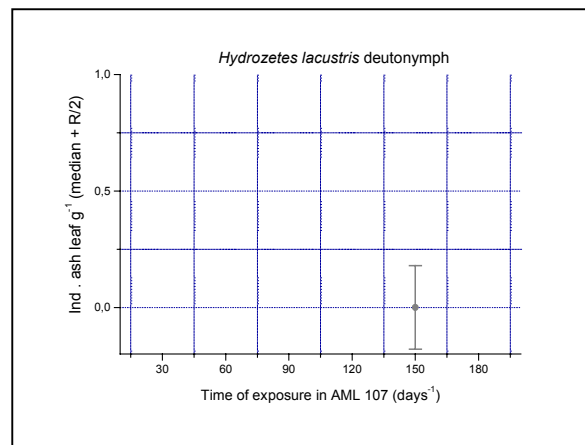


Fig. 25. Abundance of *Hydrozetes* deutonymph on ash leaves in the time of incubation of 30, 60, 90, 120, 150 and 180 days in AML 107.

*Chironomus crassimanus* larvae and pupae stages had colonised the birch, ash and walnut leaf packs. *Ch. crassimanus* exhibited the greatest abundance of benthic invertebrates with a median of  $0.72 \pm 0.72$  ind. g leaf<sup>-1</sup>. Also, the orthoclad *Limnophyes minimus* larvae and pupae stages had colonised the leaf packs (birch and walnut leaves) but with abundance lower than *Ch. crassimanus*. Both *Ch. crassimanus* and *L. minimus* colonised throughout the time of incubation experiments. Others taxa as *Hydrozetes lacustris*, *Sigara nigrolineata*, and Nematoda colonised the leaf pack experiments, but with low abundance.

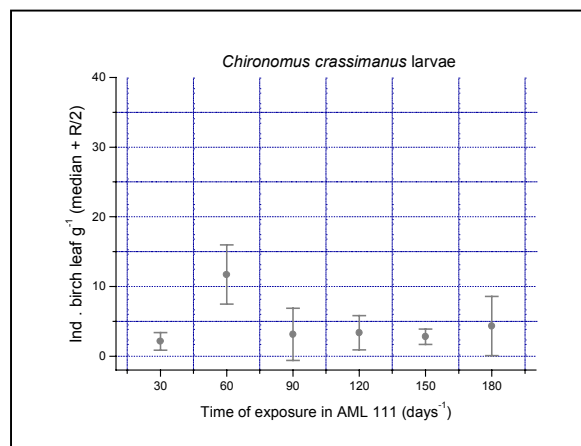


Fig. 26. Abundance of *Chironomus crassimanus* larvae on birch leaves in the time of incubation of 30, 60, 90, 120, 150 and 180 days in AML 111.

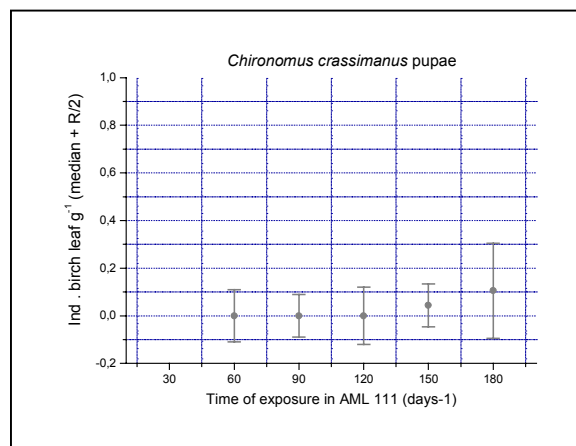


Fig. 27. Abundance of *Chironomus crassimanus* pupae on birch leaves in the time of incubation of 30, 60, 90, 120, 150 and 180 days in AML 111.

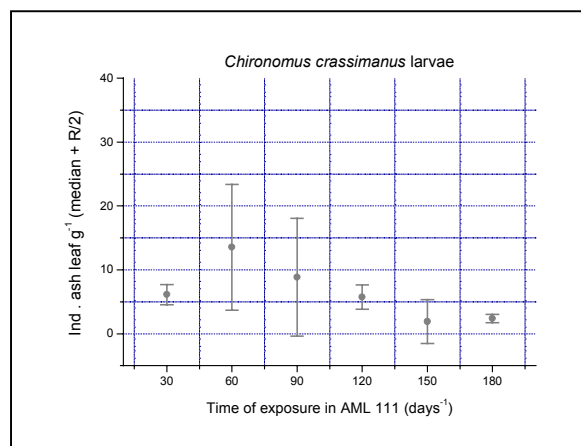


Fig. 28. Abundance of *Chironomus crassimanus* larvae on ash leaves in the time of incubation of 30, 60, 90, 120, 150 and 180 days in AML 111.

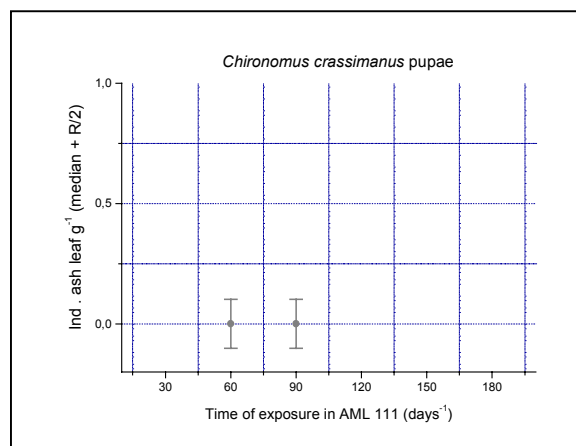


Fig. 29. Abundance of *Chironomus crassimanus* pupae on ash leaves in the time of incubation of 30, 60, 90, 120, 150 and 180 days in AML 111.

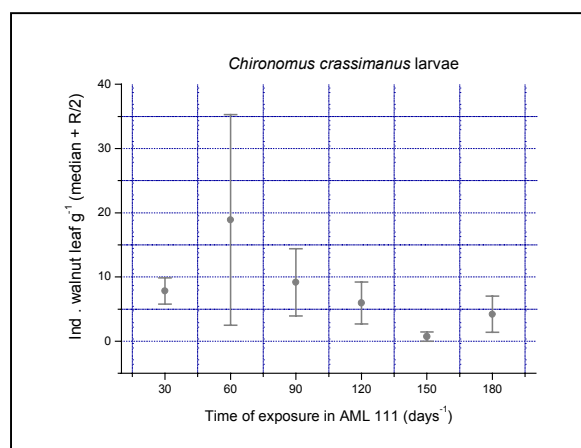


Fig. 30. Abundance of *Chironomus crassimanus* larvae on walnut leaves in the time of incubation of 30, 60, 90, 120, 150 and 180 days in AML 111.

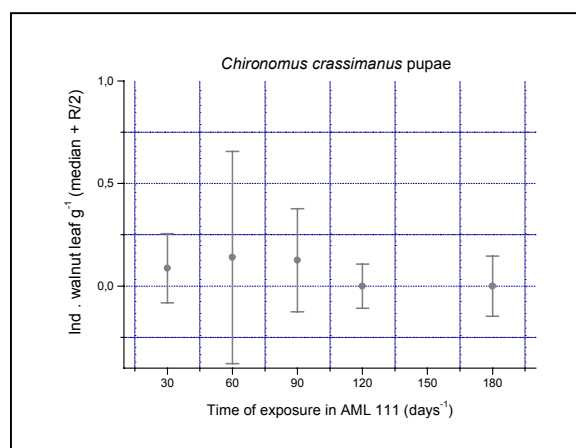


Fig. 31. Abundance of *Chironomus crassimanus* pupae on walnut leaves in the time of incubation of 30, 60, 90, 120, 150 and 180 days in AML 111.

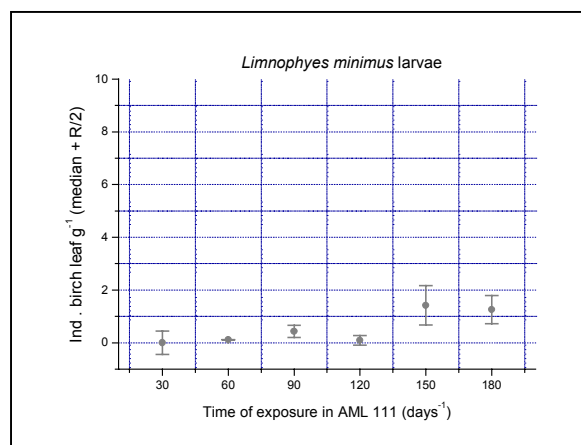


Fig. 32. Abundance of *Limnophyes minimus* larvae on birch leaves in the time of incubation of 30, 60, 90, 120, 150 and 180 days in AML 111.

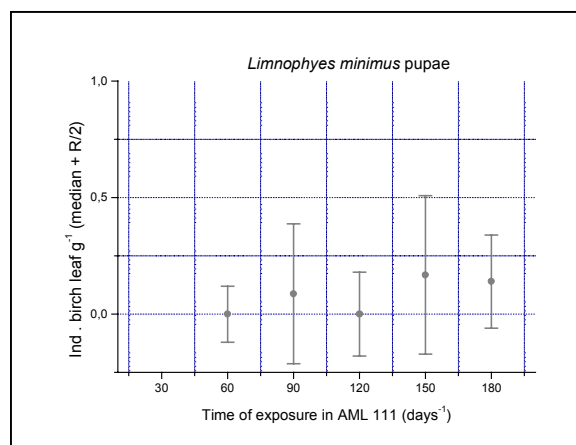


Fig. 33. Abundance of *Limnophyes minimus* pupae on birch leaves in the time of incubation of 30, 60, 90, 120, 150 and 180 days in AML 111.

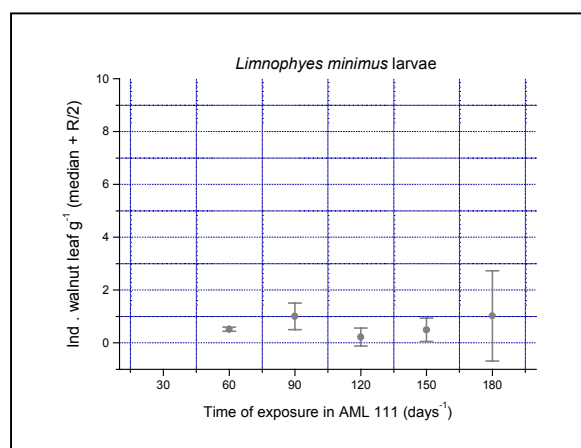


Fig. 34. Abundance of *Limnophyes minimus* larvae on walnut leaves in the time of incubation of 30, 60, 90, 120, 150 and 180 days in AML 111.

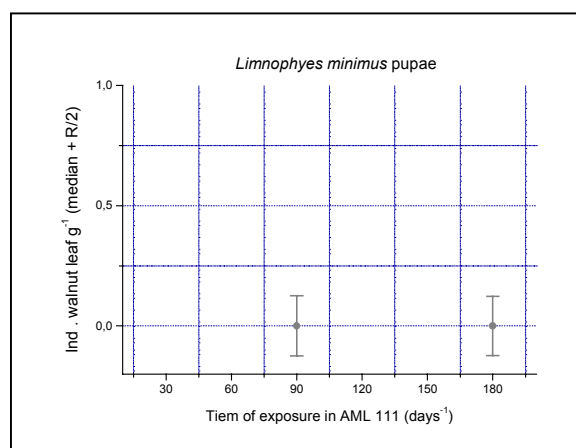


Fig. 35. Abundance of *Limnophyes minimus* pupae on walnut leaves in the time of incubation of 30, 60, 90, 120, 150 and 180 days in AML 111.

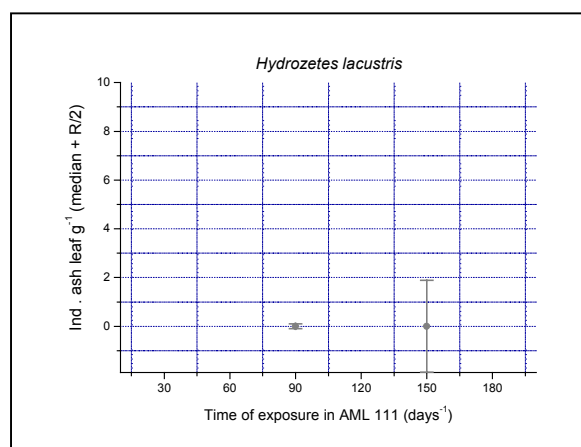


Fig. 36. Abundance of *Hydrozetes lacustris* on ash leaves in the time of incubation of 30, 60, 90, 120, 150 and 180 days in AML 111.

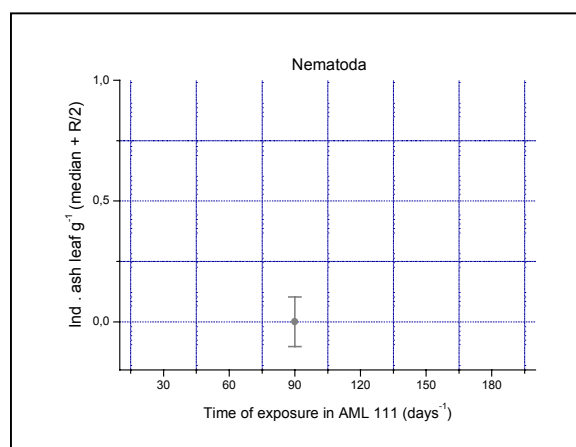


Fig. 37. Abundance of Nematoda on ash leaves in the time of incubation of 30, 60, 90, 120, 150 and 180 days in AML 111.

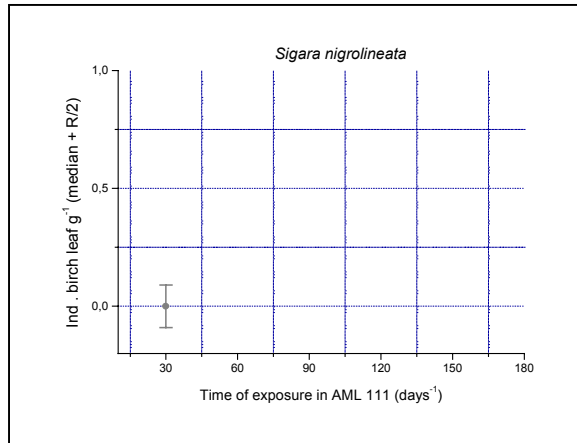


Fig. 38. Abundance of *Sigara nigrolineata* on birch leaves in the time of incubation of 30, 60, 90, 120, 150 and 180 days in AML 111.

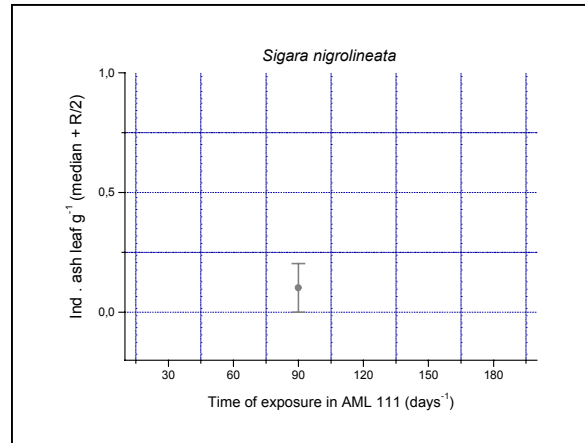


Fig. 39. Abundance of *Sigara nigrolineata* on ash leaves in the time of incubation of 30, 60, 90, 120, 150 and 180 days in AML 111.

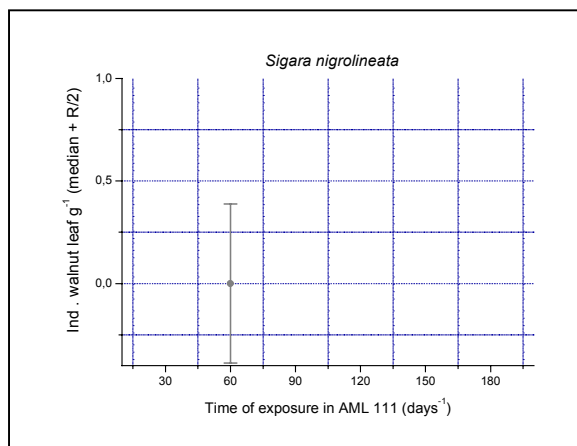


Fig. 40. Abundance of *Sigara nigrolineata* on walnut leaves in the time of incubation of 30, 60, 90, 120, 150 and 180 days in AML 111.

Table 8. Sorensen similarity index (QS %) calculated for benthic invertebrates colonisation on leaves from AML 111 and AML 107.

QS (%)	Deciduous leaves	
	<i>birch</i>	<i>ash</i>
AML 111		
<i>ash</i>	70.7	-
<i>walnut</i>	80.5	70.5
AML 107		
<i>ash</i>	80.5	-
<i>walnut</i>	80.5	100

*Chironomus crassimanus* made up more than 90% of numbers of the macroinvertebrate fauna in AML 111 and 107. *Ch. crassimanus* showed a great abundance in AML 111. The maximum abundance of *Ch. crassimanus* was  $18.88 \pm 16.39$  ind. g walnut<sup>-1</sup>.

The Sorensen similarity index for the abundance of benthic invertebrates on birch, ash and walnut leaves from AML 107 and 111 was calculated. It showed the greatest similarity between the abundance of benthic invertebrates colonisation on leaves which varied from 70.5 % – 100 % (Table 8).

### 3.2.3 Abundance of benthic invertebrate fauna leaf packs in AML 117

Figures 41 – 52 show the abundance of the benthic invertebrates on birch leaf packs colonisation experiments, and the time of incubation comprised 30, 60, 90, 120, 150 and 180 days from AML 117.

*Hydrozetes lacustris* species and deutonymph form colonised the birch leaf packs from AML 117. The median number of individuals reached  $103.6 \pm 145.3$  ind . g birch<sup>-1</sup> in exposure time of 180 days, and decreasing for 150 days ( $61 \pm 35.9$  ind . g birch<sup>-1</sup>), 120 days ( $146 \pm 27.9$  ind . g birch<sup>-1</sup>), and 90 days ( $14.58 \pm 24.4$  ind . g birch<sup>-1</sup>), and 60 days ( $4.46 \pm 5.36$  ind . g birch<sup>-1</sup>) and 30 days ( $0.27 \pm 0.4$  ind . g birch<sup>-1</sup>).

*Ch. crassimanus* larvae had still colonised species throughout the time of exposure with abundance in the range of  $3.24 \pm 14.0$  ind . g leaves<sup>-1</sup> to  $15.33 \pm 4.3$  ind . g leaves<sup>-1</sup>. *Ch. crassimanus* pupae were found in experiments with an exposure time of 90 days and 150 days.

*Limnophyes minimus* colonised the birch leaf packs in almost all the exposure times, except in 30 days. The abundance of *L. minimus* range maximum  $9.63 \pm 11.34$  ind . g birch<sup>-1</sup> at 150 days and  $5.95 \pm 6.65$  ind . birch<sup>-1</sup> at 180 days. The colonisation abundance on the birch leaves of this species was lower in the exposure time 60, 90 and 120 days. Adult species of *L. minimus* were found in the leaf packs experiment in the time of 150 and 180 days of incubation. The presence of the *L. minimus* imago is due to the emergence of adults at the moment the leaf packs were taken out.

In addition, *Silais lutaria* and the pleustonic *S. nigrolineata* colonised almost all exposure times, except in 150 and 180 days. The median abundance of *S. lutaria* reached maximal  $0.92 \pm 0.84$  ind . g birch<sup>-1</sup>. Also, the median abundance of *S. nigrolineata* reached maximal  $1.13 \pm 1.22$  ind. g birch<sup>-1</sup>.



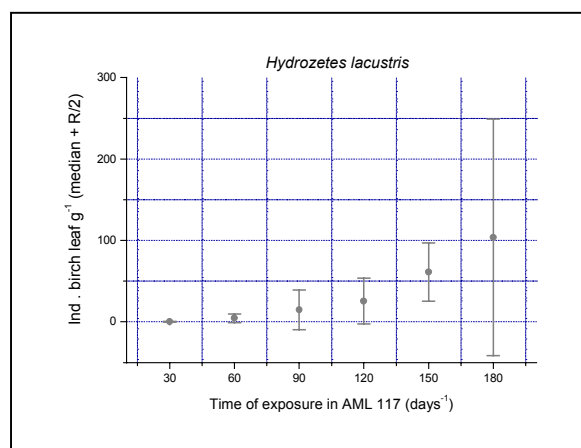


Fig. 41. Abundance of *Hydrozetes lacustris* on birch leaves in the time of incubation of 30, 60, 90, 120, 150 and 180 days in AML 117.

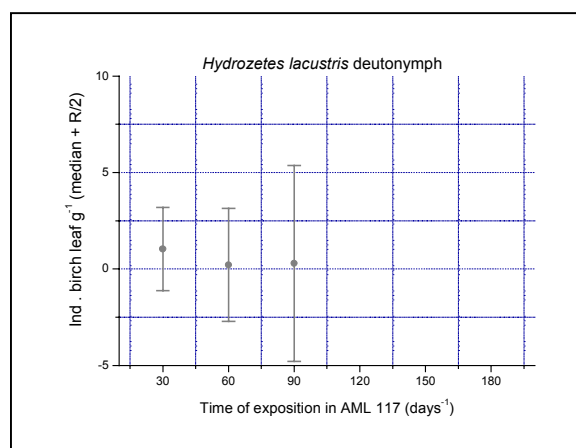


Fig. 42. Abundance of *Hydrozetes* deutonymph on birch leaves in the time of incubation of 30, 60, 90, 120, 150 and 180 days in AML 117.

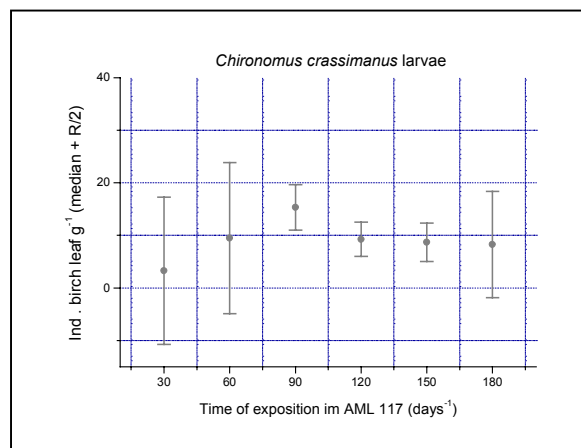


Fig. 43. Abundance of *Chironomus crassimanus* on birch leaves in the time of incubation of 30, 60, 90, 120, 150 and 180 days in AML 117.

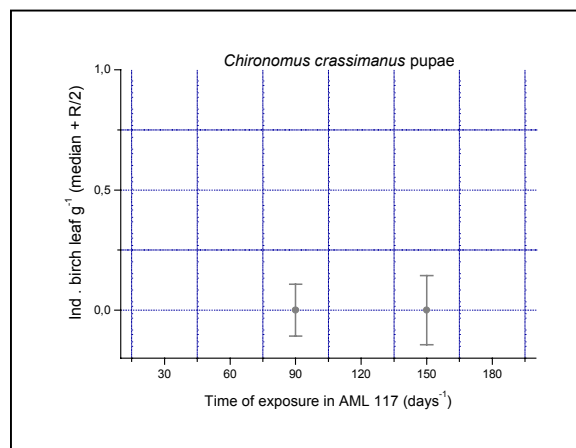


Fig. 44. Abundance of *Chironomus crassimanus* on birch leaves in the time of incubation of 30, 60, 90, 120, 150 and 180 days in AML 117.

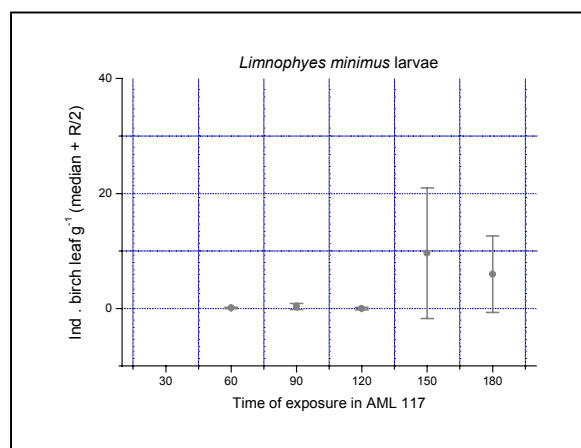


Fig. 45. Abundance of *Limnophyes minimus* larvae on birch leaves in the time of incubation of 30, 60, 90, 120, 150 and 180 days in AML 117.

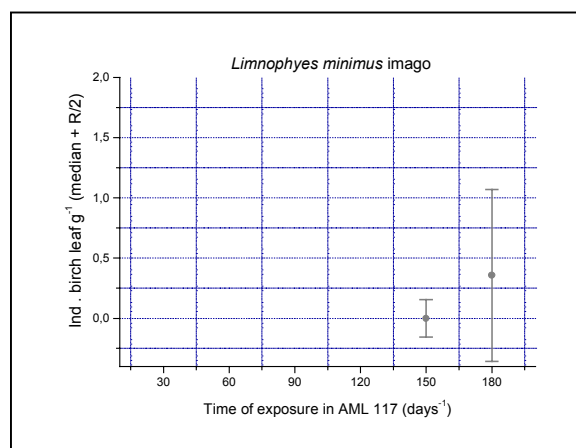


Fig. 46. Abundance of *Limnophyes minimus* imago on birch leaves in the time of incubation of 30, 60, 90, 120, 150 and 180 days in AML 117.

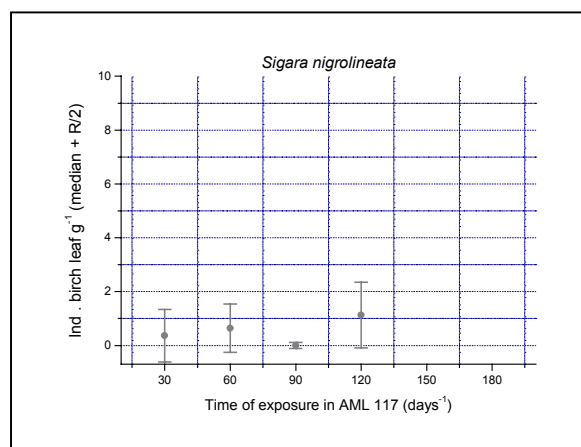


Fig. 47. Abundance of *Sigara nigrolineata* on birch leaves in the time of incubation of 30, 60, 90, 120, 150 and 180 days in AML 117.

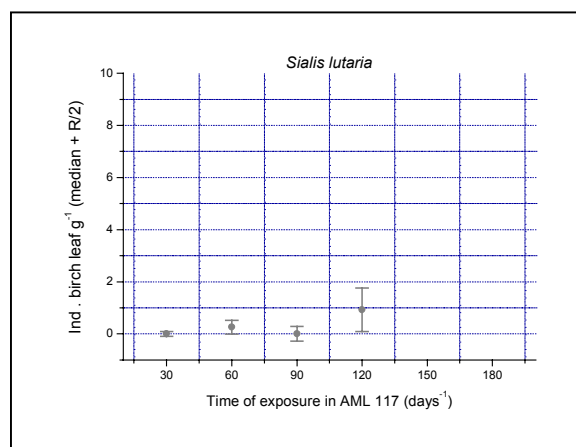


Fig. 48. Abundance of *Sialis lutaria* on birch leaves in the time of incubation of 30, 60, 90, 120, 150 and 180 days in AML 117.

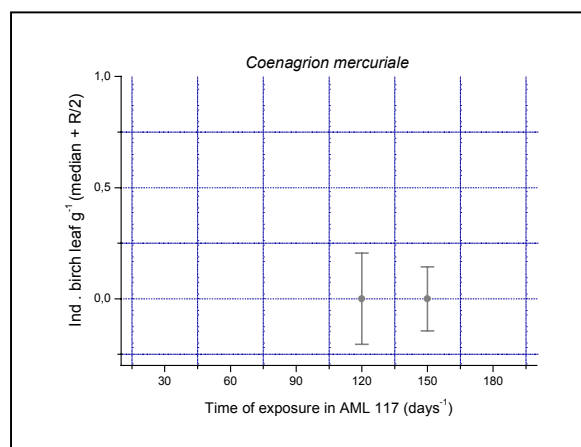


Fig. 49. Abundance of *Coenagrion mercuriale* on birch leaves in the time of incubation of 30, 60, 90, 120, 150 and 180 days in AML 117.

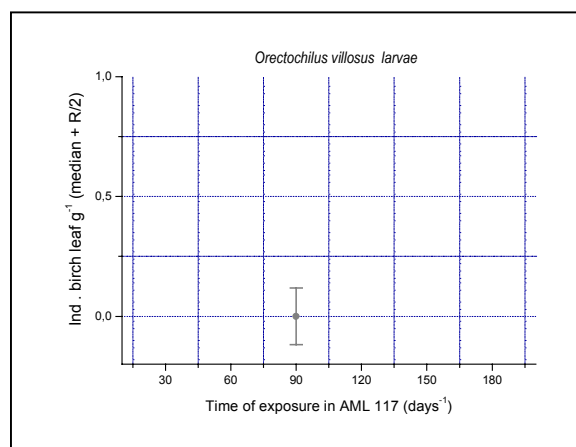


Fig. 50. Abundance of *Orectochilus villosus* larvae on birch leaves in the time of incubation of 30, 60, 90, 120, 150 and 180 days in AML 117.

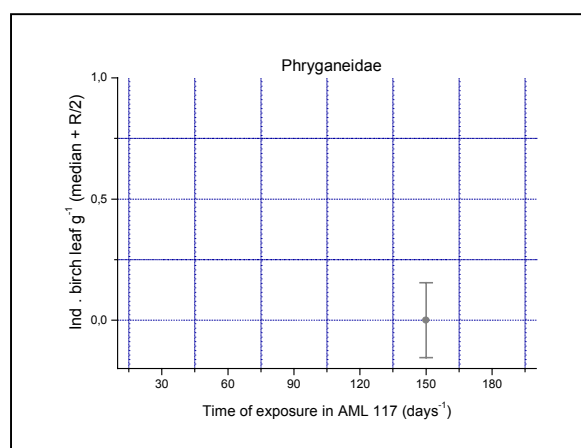


Fig. 51. Abundance of Phryganeidae on birch leaves in the time of incubation of 30, 60, 90, 120, 150 and 180 days in AML 117.

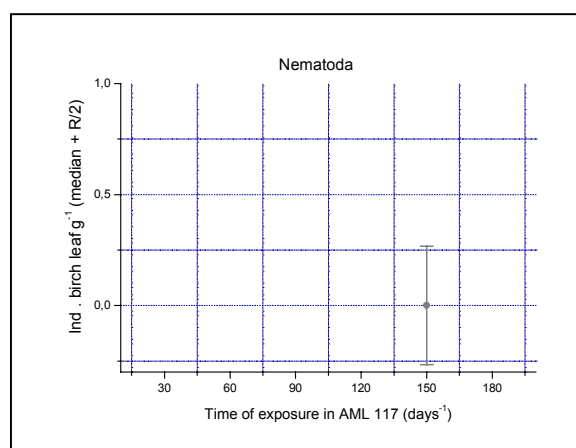


Fig. 52. Abundance of Nematoda on birch leaves in the time of incubation of 30, 60, 90, 120, 150 and 180 days in AML 117.

Furthermore, *Orectochilus villosus*, *Coenagrion mercuriale*, Phryganeidae and Nematoda occurred not in all the exposure time experiments, principally after 90 days of incubation (*O. villosus*) after 120 and 150 days (*C. mercuriale*), and after 150 days (Phryganeidae and Nematoda).

### 3.2.4 Abundance of benthic invertebrate fauna from leaf packs in ML B

The figures 53 – 69 show the abundance of benthic invertebrate groups colonisation on birch leaves from ML B.

As a control lake, the leaf pack colonisation by benthic invertebrates from ML B was also investigated. Ephemeroptera, ostracods, and oribatids showed the greatest abundance on birch colonisation from ML B.

The majority of individuals from ML B was the ephemeropteran *Caenis pusila* which reached median abundance maximum of  $79.85 \pm 30.51$  ind . g birch<sup>-1</sup> at 120 days. This species had colonised all the exposure time experiments. Also, ostracods had colonised all the exposure time experiments. The median abundance showed constant during all time of incubation. The median of abundance reached  $31.78 \pm 37.07$  ind . g birch<sup>-1</sup>. Oribatids had not colonised all the exposure times, only 60, 150 and 180 days. The median abundance of this taxa reached maximum to  $35.71 \pm 71.56$  ind . g birch<sup>-1</sup>.

The chironomids *Dicrotendipes nervosus*, and *Microtendipes pedellus*, and Pentaneurini, and the amphipod *Gammarus roeselli* had also colonised almost all the exposure time experiments, except 180 days. *D. nervosus* reached maximum median abundance of  $4.69 \pm 9.38$  ind . g birch<sup>-1</sup> at 90 days, while *M. pedellus* reached maximal median abundance  $5.51 \pm 6.05$  ind . g birch<sup>-1</sup> at 120 days. In contrast, *G. roeseli* and Pentaneurini median abundance reached maximum to  $2.20 \pm 2.75$  ind . g birch<sup>-1</sup> and to  $2.01 \pm 3.94$  ind . g birch<sup>-1</sup>, respectively.

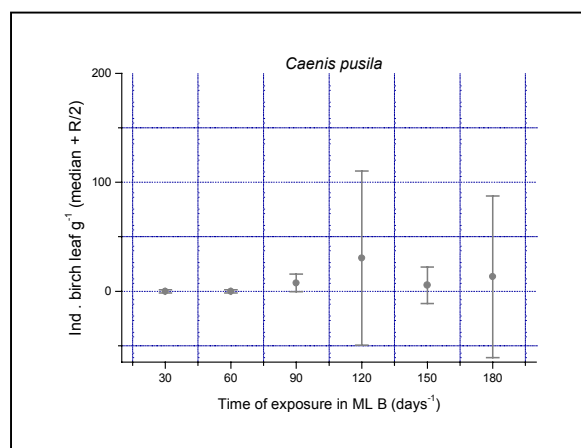


Fig. 53. Abundance of *Caenis pusila* on birch leaves in the time of incubation of 30, 60, 90, 120, 150 and 180 days in ML B.

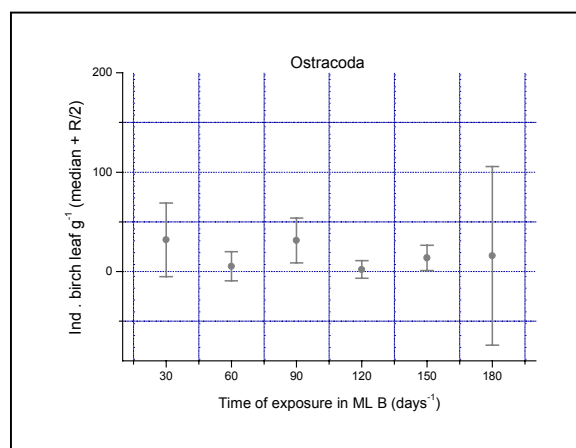


Fig. 54. Abundance of *Ostracoda* on birch leaves in the time of incubation of 30, 60, 90, 120, 150 and 180 days in ML B.

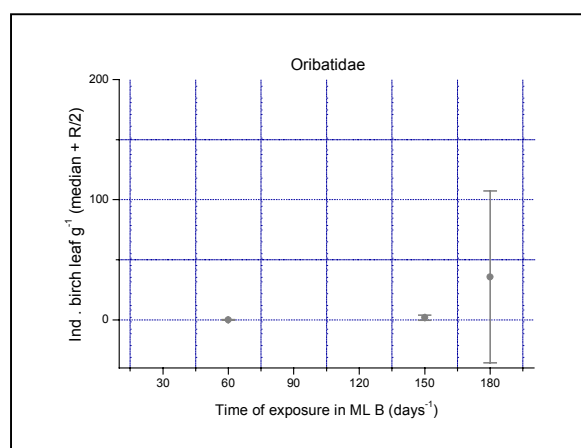


Fig. 55. Abundance of *Oribatidae* on birch leaves in the time of incubation of 30, 60, 90, 120, 150 and 180 days in ML B.

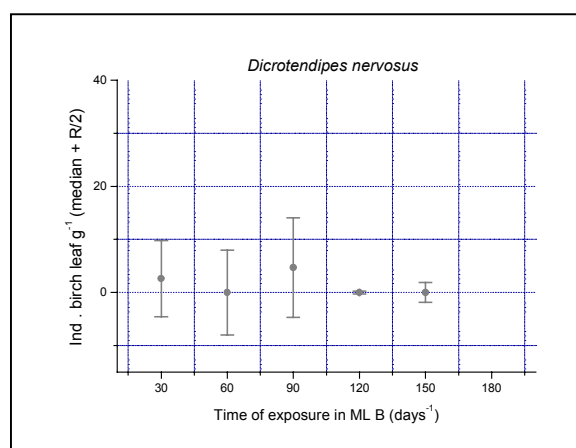


Fig. 56. Abundance of *Dicotendipes nervosus* on birch leaves in the time of incubation of 30, 60, 90, 120, 150 and 180 days in ML B.

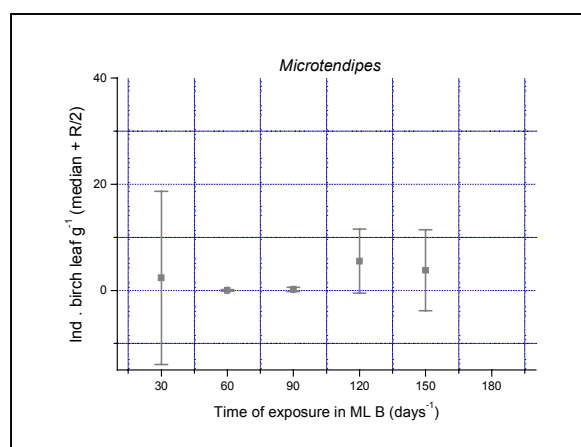


Fig. 57. Abundance of *Microtendipes* on birch leaves in the time of incubation of 30, 60, 90, 120, 150 and 180 days in ML B.

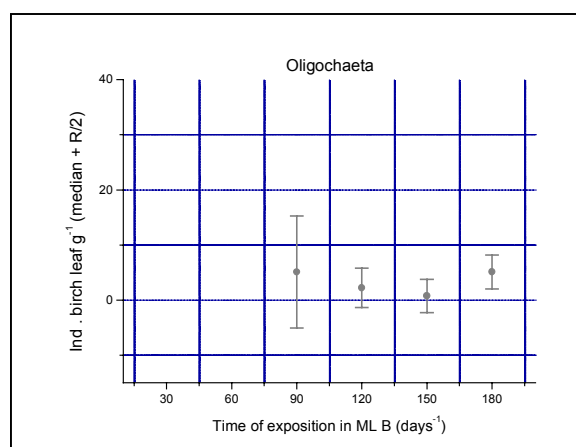


Fig. 58. Abundance of *Oligochaeta* on birch leaves in the time of incubation of 30, 60, 90, 120, 150 and 180 days in ML B.

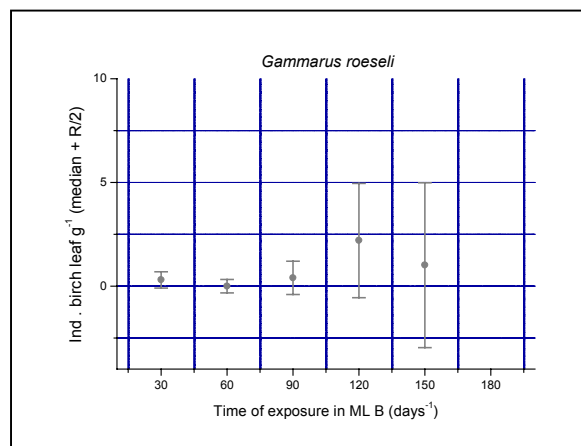


Fig. 59. Abundance of *Gammarus roeseli* on birch leaves in the time of incubation of 30, 60, 90, 120, 150 and 180 days in ML B.

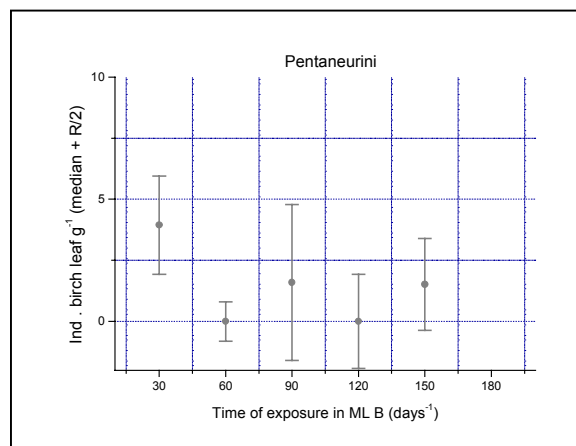


Fig. 60. Abundance of *Pentaneurini* on birch leaves in the time of incubation of 30, 60, 90, 120, 150 and 180 days in ML B.

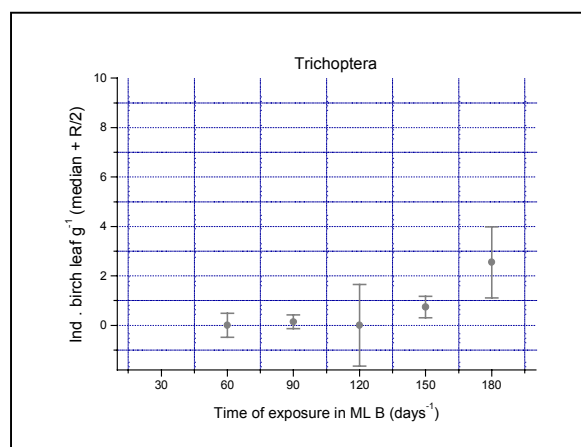


Fig. 61. Abundance of *Trichoptera* on birch leaves in the time of incubation of 30, 60, 90, 120, 150 and 180 days in ML B.

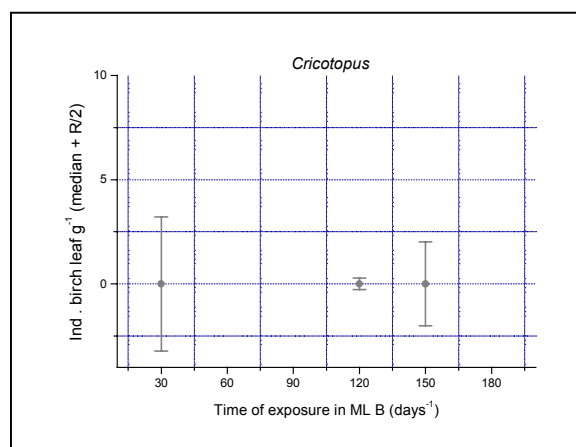


Fig. 62. Abundance of *Cricotopus* on birch leaves in the time of incubation of 30, 60, 90, 120, 150 and 180 days in ML B.

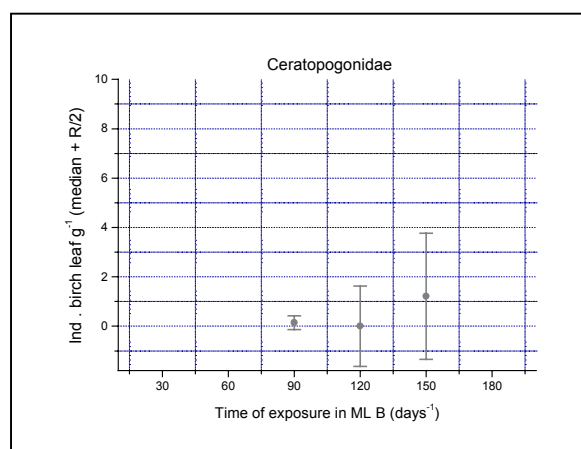


Fig. 63. Abundance of *Ceratopogonidae* on birch leaves in the time of incubation of 30, 60, 90, 120, 150 and 180 days in ML B.

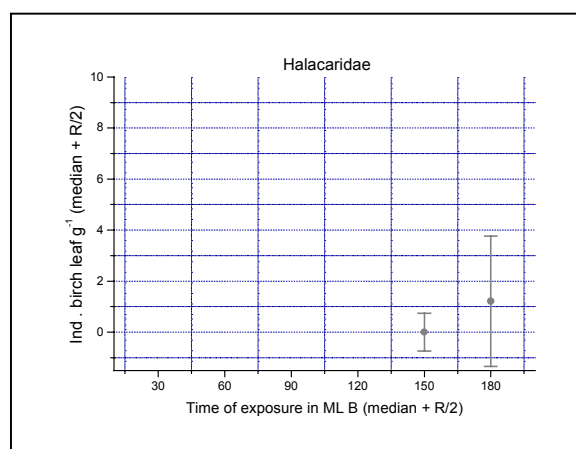


Fig. 64. Abundance of *Halacaridae* on birch leaves in the time of incubation of 30, 60, 90, 120, 150 and 180 days in ML B.

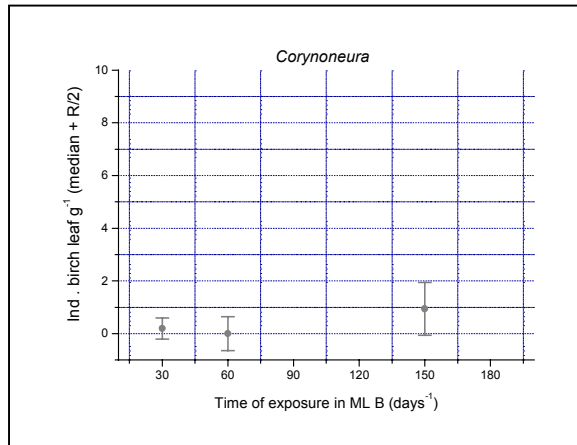


Fig. 65. Abundance of *Corynoneura* on birch leaves in the time of incubation of 30, 60, 90, 120, 150 and 180 days in ML B.

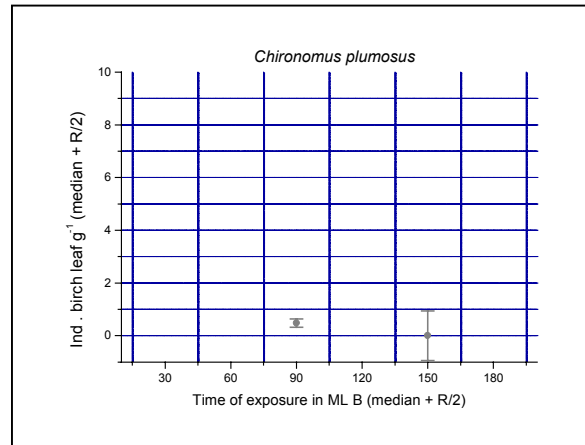


Fig. 66. Abundance of *Chironomus plumosus* on birch leaves in the time of incubation of 30, 60, 90, 120, 150 and 180 days in ML B.

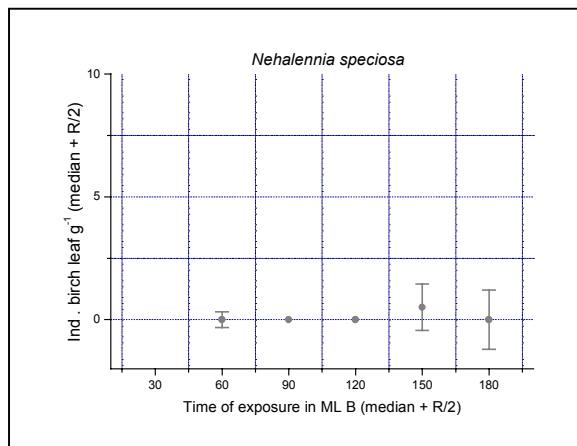


Fig. 67. Abundance of *Nehalennia speciosa* on birch leaves in the time of incubation of 30, 60, 90, 120, 150 and 180 days in ML B.

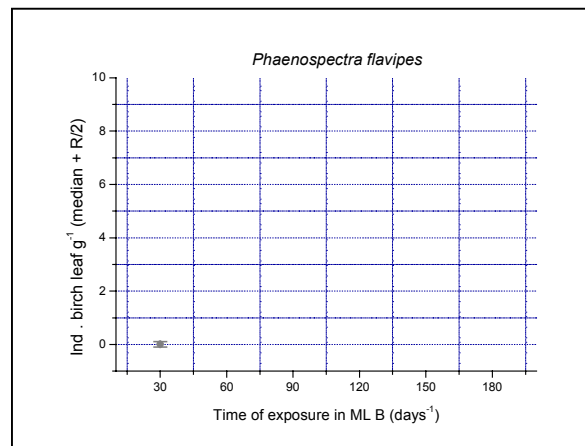


Fig. 68. Abundance of *Phaenospectra flavipes* on birch leaves in the time of incubation of 30, 60, 90, 120, 150 and 180 days in ML B.

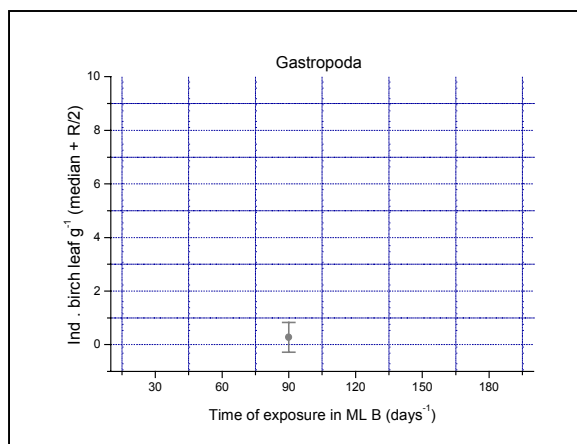


Fig. 69. Abundance of Gastropoda on birch leaves in the time of incubation of 30, 60, 90, 120, 150 and 180 days in ML B.

In addition, species of Oligochaeta colonised leaf packs at the time of exposure 90, 120, 150 and 180 days. The median abundance of the oligochaetes reached maximal of  $5.10 \pm 3.09$  ind. g birch<sup>-1</sup>.

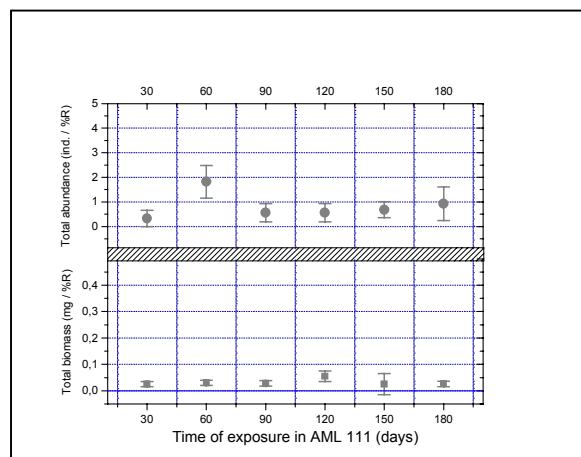
Furthermore, species of Trichoptera and *Nehalennia speciosa* colonised almost all the of exposure time experiments, except at 30 days. The maximal median abundance for Trichoptera reached  $2.55 \pm 1.43$  ind . g birch<sup>-1</sup>, and *N. speciosa* reached maximum  $0.5 \pm 0.94$  ind . g birch<sup>-1</sup>.

Other taxa that colonised the leaf pack experiments occurred not in all the exposure time. *Phaenospectra flavipes* (Chironomidae) and gastropods colonised only at 30 days and 90 days, respectively, while *Chironomus plumosus* (Chironomidae) and Halacaridae (Acari) colonised in the time of exposure for 90, and 150 days and 150, and 180 days, respectively. The dipterans Ceratopogonidae and *Corynoneura* sp. colonised more in time of exposure experiments at 90, 120, and 150 days and 30, 60, 150 days, respectively, but all taxa with low abundance.

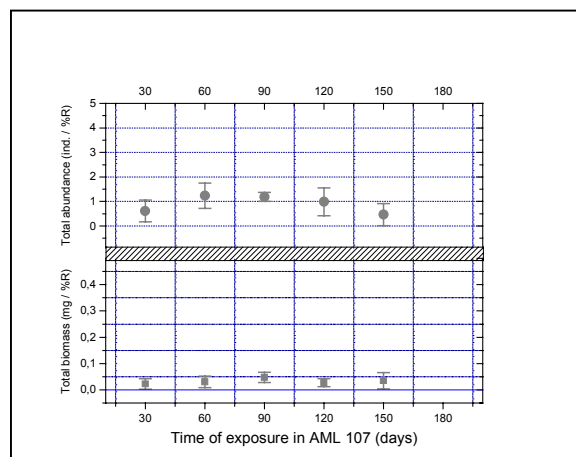
### 3.3 Total abundance and total biomass of benthic invertebrates on leaf packs

Figures 70 - 75 and 76 – 79 show the median of total abundance and the total biomass of benthic invertebrates fauna on percentage of remaining leaf weight loss (%R) of birch, ash and walnut leaves in exposure time of 30 days (August-September 1998), 60 days (April-September 1998), 90 days (May-September 1998), 120 days (June-September 1998), 150 days (July-September 1998), and 180 days (March-September 1998). The median of total abundance and total biomass are given followed the ranges (R/2).

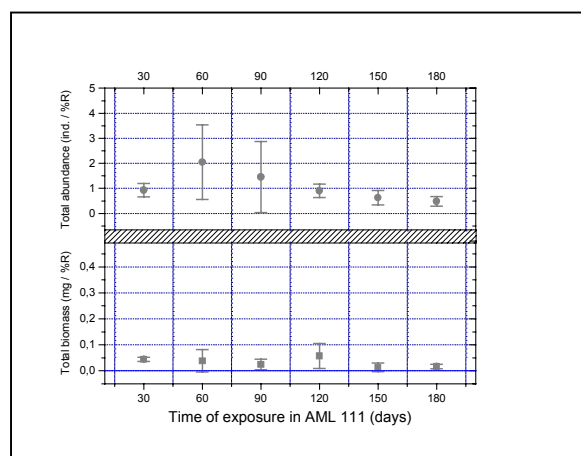
In AML 111, ash and walnut, and birch leaf colonisation by benthic invertebrates did not differ significantly ( $F = 1.216$ ;  $P = 0.324$ ) in terms of total abundance and total biomass in all the exposure time. In AML 107 the leaf colonisation experiment did not differ significantly ( $F = 1.612$ ;  $P = 0.23$ ) in terms of total benthic invertebrates abundance, but did vary significantly in terms of biomass ( $F = 8.108$ ;  $P = 0.017$ ) (Fig. 70 – 75).



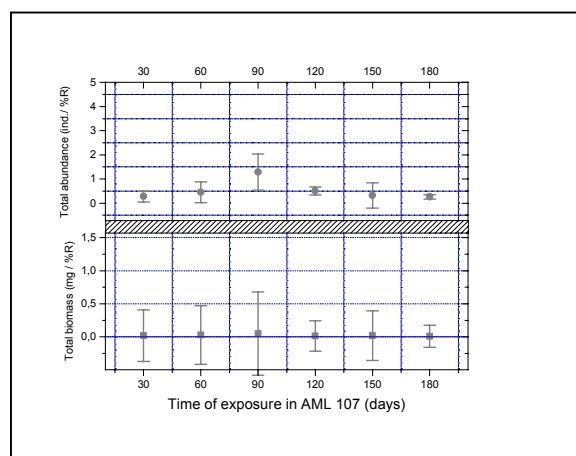
**Fig. 70.** Birch leaves colonisation from AML 111. Total abundance and total biomass of benthic invertebrates on percentage of remaining leaf weight loss (%R). The median are showing followed by ranges (median + R/2).



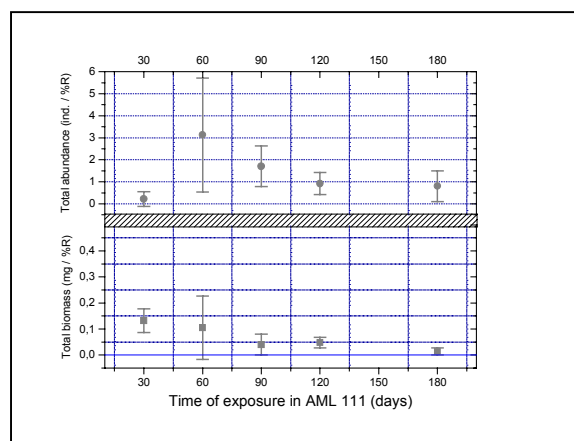
**Fig. 71.** Birch leaves colonisation from AML 107. Total abundance and total biomass of benthic invertebrates on percentage of remaining leaf weight loss (%R). The median are showing followed by ranges (median + R/2).



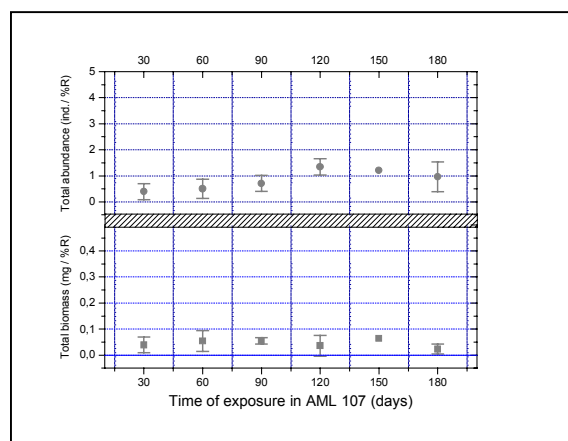
**Fig. 72.** Ash leaves colonisation from AML 111. Total abundance and total biomass of benthic invertebrates on percentage of remaining leaf weight loss (%R). The median are showing followed by ranges (median + R/2).



**Fig. 73.** Ash leaves colonisation from AML 107. Total abundance and total biomass of benthic invertebrates on percentage of remaining leaf weight loss (%R). The median are showing followed by ranges (median + R/2).



**Fig. 74.** Walnut leaves colonisation from AML 111. Total abundance and total biomass of benthic invertebrates on percentage of remaining leaf weight loss (%R). The median are showing followed by ranges (median + R/2).



**Fig. 75.** Walnut leaves colonisation from AML 107. Total abundance and total biomass of benthic invertebrates on percentage of remaining leaf weight loss (%R). The median are showing followed by ranges (median + R/2).



Total abundance and total biomass of benthic invertebrates on remaining leaf weight loss (%R) of birch leaves colonisation were performed from all mining lakes (Figures 76 - 79).

The lakes AML 107 and AML 111 ( $P = 0.698$ ;  $F = 0.369$ ), and AML 107 and AML 117 ( $P = 0.61$ ;  $F = 0.285$ ), and AML 111 and AML 117 ( $P = 0.49$ ;  $F = 0.52$ ) did not differ significantly between the abundance of the benthic invertebrates colonisation on birch-leaves.

In addition, we found significant differences between the abundance of benthic invertebrates on birch leaf between ML 107 and ML B ( $P = 8.83276E-4$ ;  $F = 23.719$ ), and AML 111 and ML B ( $P = 3.05225E-4$   $F = 29.064$ ); and AML 117 and ML B ( $P = 7.24702E-4$ ;  $F = 23.031$ ). Table 9 shows the analysis of variance (ANOVA) of benthic invertebrates colonisation on birch leaves from extremely acidic mining lakes (AML 107, AML 111 and AML 117) and a neutral control-lake (ML B). There was a great significant difference between the lakes.

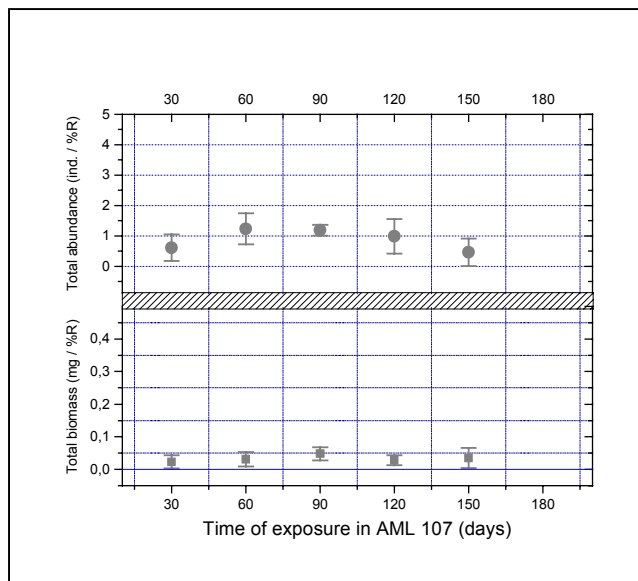
**Table 9.** Analyse of variance of benthic invertebrates colonisation on birch leaves from AML 107, AML 111, AML 117 and ML B. The median of total abundance are given followed by ranges (median + R/2).

<i>Birch</i> ( $n = 6$ )*	<i>AML 107</i>	<i>AML 111</i>	<i>AML 117</i>	<i>ML B</i>
<b>Median + R/2</b>	5.283	5.823	7.580	44.376
<b>Variance</b>	11.88	4.98	49.12	49.12
	$P=1.98E-6$ $F= 22.21$ $\alpha=0.05$			

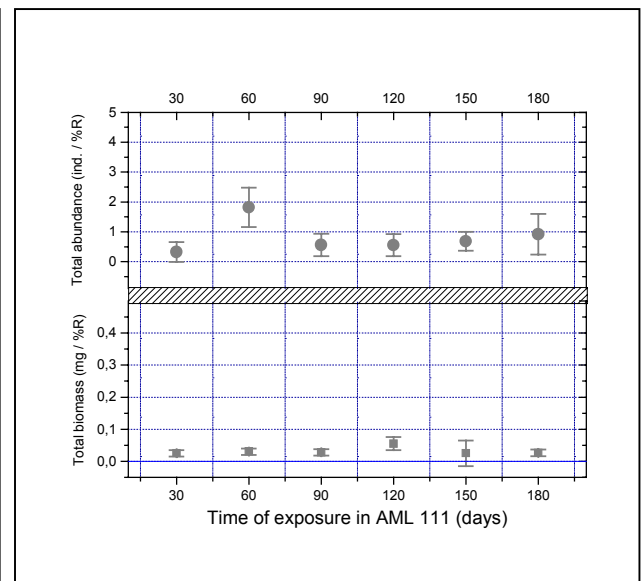
\*AML 111 ( $n = 5$ )

The total abundance and total biomass of benthic invertebrate colonisation on birch leaves did not differ significantly ( $F = 5,20$ ;  $P = 0.02$ ;  $n = 6$ ) in AML 107, AML 111, and AML 117. In 150 days and 180 days the total biomass of benthic invertebrates was higher in ML B than AML 117. The increase of biomass in AML 117 could be due to the presence of the *Sialis lutaria* and *Sigara nigrolineata* in the time of 30, 60, 90, and 120 days. *Sialis lutaria* occurred in the exposure time of 30 until 120 days. The abundance of *S. lutaria* ranged from  $0,92 \pm 0,84$

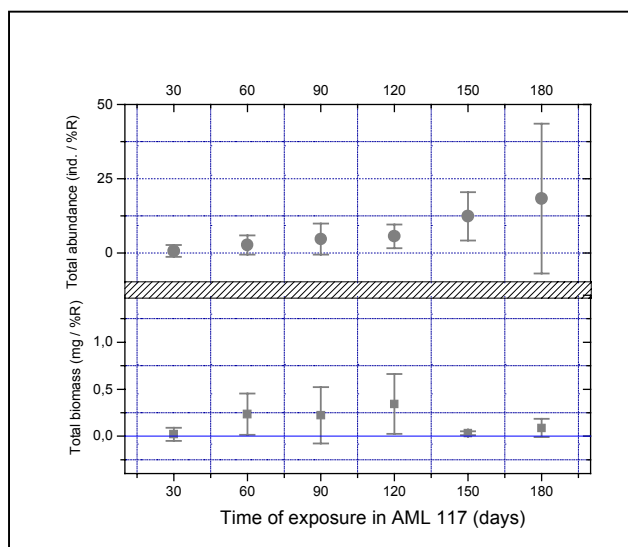
ind . g leaf<sup>-1</sup> (May-September);  $0,28 \pm 0,28$  ind . g leaf<sup>-1</sup> (June-September);  $0,25 \pm 0,26$  ind . g leaf<sup>-1</sup> (July-September), and  $0,09 \pm 0,09$  ind . g leaf<sup>-1</sup> (August-September).



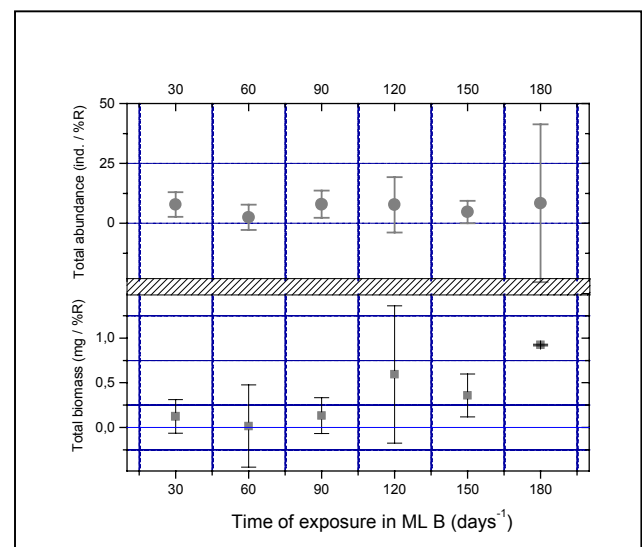
**Fig. 76.** Benthic invertebrates colonisation on birch leaves from AML 107 in exposure time of 30 days (August-September 1998), 60 days (April-September 1998), 90 days (May-September 1998), 120 days (June-September 1998), and 150 days (July-September 1998). Total abundance and total biomass (median + ranges/2) are given per remaining leaf weight loss in percentage (%R).



**Fig. 77.** Benthic invertebrates colonisation on birch leaves from AML 111 in exposure time of 30 days (August-September 1998), 60 days (April-September 1998), 90 days (May-September 1998), 120 days (June-September 1998), 150 days (July-September 1998), and 180 days (March-September 1998). Total abundance and total biomass (median + ranges/2) are given per remaining leaf weight loss in percentage (%R).



**Fig. 78.** Benthic invertebrates colonisation on birch leaves in AML 117 from exposure time of 30 days (August-September 1998), 60 days (April-September 1998), 90 days (May-September 1998), 120 days (June-September 1998), 150 days (July-September 1998), and 180 days (March-September 1998). Total abundance and total biomass (median + ranges/2) are given per remaining leaf weight loss in percentage (%R).



**Fig. 79.** Benthic invertebrates colonisation on birch leaves from ML B in exposure time of 30 days (August-September 1998), 60 days (April-September 1998), 90 days (May-September 1998), 120 days (June-September 1998), 150 days (July-September 1998), and 180 days (March-September 1998). Total abundance and total biomass (median + ranges/2) are given per remaining leaf weight loss in percentage (%R).

The abundance of *Sigara nigrolineata* ranged from  $0,36 \pm 0,97$  ind . g leaf<sup>-1</sup> (Ago-September);  $0,64 \pm 0,90$  ind . g leaf<sup>-1</sup> (July-September);  $0,11 \pm 0,11$  ind . g leaf<sup>-1</sup> (June-September),  $1,13 \pm 1,22$  ind . g leaf<sup>-1</sup> (Mai-September). In addition, *Coenagrion mercuriale* (120, and 150 days) and *Orectochilus villosus* (90 days), and Phryganeidae (150 days) also occurred after 90 days but in low abundance.

Control-lake (ML B) differed significantly ( $F = 22,502$ ;  $P = 1.803$ ) from AML 107, AML 111, and AML 117. The total biomass of benthic invertebrates from ML B was higher in all the exposure time than from AML 107 and AML 111.

### 3.4 Leaf weight loss

After 24 hrs, the leaching of leaves was calculated in the laboratory. Leaching leaf weight losses were for ash 24.8 % (1S.D.  $\pm 0.7$ ), birch 23.3 % (1S.D.  $\pm 0.6$ ) and walnut 23.4 % (1S.D.  $\pm 0.7$ ). Leaching did not vary for birch, ash and walnut leaves in the first 24 hrs water immersion. The amount of leaf material lost over a period time of 30, 60, 90, 120, 150, and 180 days was expressed by percentage remaining (%R) (Fig. 80).

Weight loss was most pronounced for ML B, with leaves losing about 30 - 40 % at 30 days, 60 - 70 % at 60 days, 80 % at 90 days, 90-96 % at 120, 150 and 180 days of their initial weight. Furthermore, in comparison with ML B, weight loss was less pronounced for AML 107 and AML 111, with leaves losing about 10 - 50 % of their initial weight. The remaining weight loss during 180 days was quite constant.

For AML 117 weight loss remaining after 30, 60 and 90 days was similar to AML 107 and AML 111. From 120 - 180 days the weight loss was quite gradual at 55 - 80 %. AML 117 shows similar leaf weight loss characteristics to the lakes AML 107 and AML 111 at the beginning of the experiment 30, 60, 90 days. After this incubation time, the leaf weight loss rose until the end of the experiment (180 days).

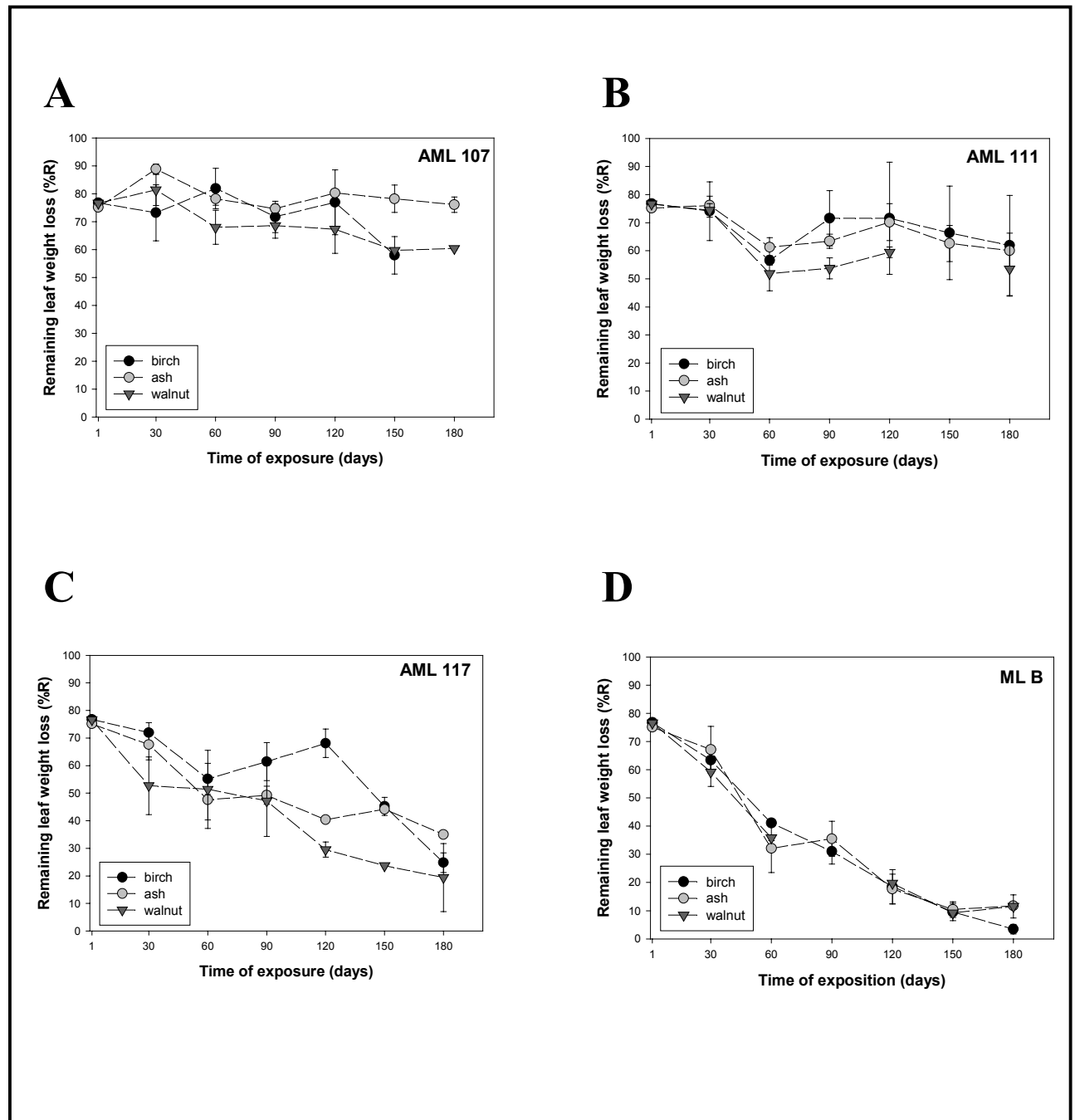


Fig. 80. Leaf weight loss remaining in percentage (%R) from mining lakes in Lusatian region, eastern Germany. A) Lake AML 107; B) Lake AML 111; C) Lake AML 117 and D) Control-lake ML B.

Following  $\log_e$  transformation of the remaining weight loss data, linear regression was used to estimate the processing coefficient for each leaf species after excluding the leaching loss. Both equations were significant ( $p < 0.05$ ), and the data fits well with the exponential model. All

leaves that were exposed in the lakes exhibited the same tendency of leaf weight loss over the experimental period. In general, leaf weight loss is gradual for the birch, ash and walnut in the periods until 180 days in AML 107 and AML 111.

Processing coefficient among deciduous leaf species (birch, ash and walnut) did not vary significantly (ANOVA,  $p < 0.05$ ;  $n = 36$ ) in the time of exposure of 30 days (Data set #2 - experiment) in the summer season (Table 10).

**Table 10. Analysis of variance among leaf weight loss of deciduous leaf species exposed in each 30 days in the summer period in mining lakes (Data set#2).**

<i>Lakes</i>	<i>Birch (n = 3)</i>	<i>Ash (n = 3)</i>	<i>Walnut (n = 3)</i>
<i>AML 107</i>	F = 0.049	P = 0.95	
<i>AML 111</i>	F = 0.180	P = 0.84	
<i>AML 117</i>	F = 0.087	P = 0.92	
<i>ML B</i>	F = 0.730	P = 0.52	

Also, the processing coefficient among deciduous leaf species (birch, ash, and walnut) did not vary significantly in the exposure time of 90 days in the winter season (Table 11).

**Table 11. Leaf weight loss analysis of variance among deciduous leaf species exposed in the winter period in mining lakes (Data set#3).**

<i>Lakes</i>	<i>Birch (n = 2)</i>	<i>Ash (n = 2)</i>	<i>Walnut (n = 2)</i>
<i>AML 107</i>	F = 0.494	P = 0.66	
<i>AML 111</i>	F = 1.347	P = 0.38	
<i>AML 117</i>	F = 2.475	P = 0.23	
<i>ML B</i>	F = 0.891	P = 0.50	

Leaf substrate did not mean a significant difference between the birch, ash and walnut leaves ( $F = 0.0137$ ;  $P = 0.9864$ ;  $p < 0.05$ ;  $n = 36$ ) in mining lakes ML B, and AML 117, AML 111 and AML 107.

After leaching the leaves showed the most low degradation in acidic lakes. In the control lake (ML B) the degradation occurred more rapidly. The loss rate coefficient ( $k$ ) is shown in the table 12. Leaf breakdown processing in AML 107, 111, and 117 did not show a significant difference ( $F = 4.57$ ;  $P = 0.06215$ ;  $p < 0.05$ ;  $n = 3$ ). Although, the processing rate coefficients for birch, ash and walnut in ML B were significantly greater than AML 107, 111 and 117 (ML B >>> AML) with  $p < 0.05$  between ML B and (F = 141,44;  $P = 2.86295E-4$ ); ML B and AML 111 (F = 867,34;  $P = 7.915$ ), and ML B and AML 107 (F = 575.904;  $P = 1.788E-5$ ).

**Table 12. Processing rate coefficient ( $k$ ) for birch, ash and walnut leaves in mining lakes from Lusatian region in eastern Germany (ML B, AML 117, AML 111 and AML 107).**

Mining lakes	Processing rate coefficient ( $k$ )		
	birch	ash	walnut
<b>ML B</b> (pH 8)	$k = .0137$ $r = 0.88$ ; $p < 0.05$	$k = .0101$ $r = 0.95$ ; $p < 0.05$	$k = .0097$ $r = 0.96$ ; $p < 0.05$
<b>AML 117</b> (pH 3)	$k = .0025$ $r = 0.76$ ; $p < 0.05$	$k = .0018$ $r = 0.89$ ; $p > 0.05$	$k = .0048$ $r = 0.93$ ; $p < 0.05$
<b>AML 111</b> (pH 2.6-2.9)	$k = .0004$ $r = 0.51$ ; $p < 0.05$	$k = .0004$ $r = 0.60$ ; $p < 0.05$	$k = .0007$ $r = 0.55$ ; $p < 0.05$
<b>AML 107</b> (pH 2.1-2.7)	$k = .0007$ $r = 0.67$ ; $p < 0.05$	$k = .0001$ $r = 0.35$ ; $p < 0.05$	$k = .0008$ $r = 0.91$ ; $p < 0.05$

Figure 81 shows the remaining birch leaf weight loss (%R) in lakes ML B, AML 117, AML 111 and AML 107 in the maximum exposure time of 180 days.

The leaf breakdown of ML B was faster than acidic mining lakes. The leaf breakdown of AML 107, AML 111 and AML 117 are similar until 120-days. After 150-days the leaf breakdown of AML 117 seems to be more intensive than AML 107 and AML 111.

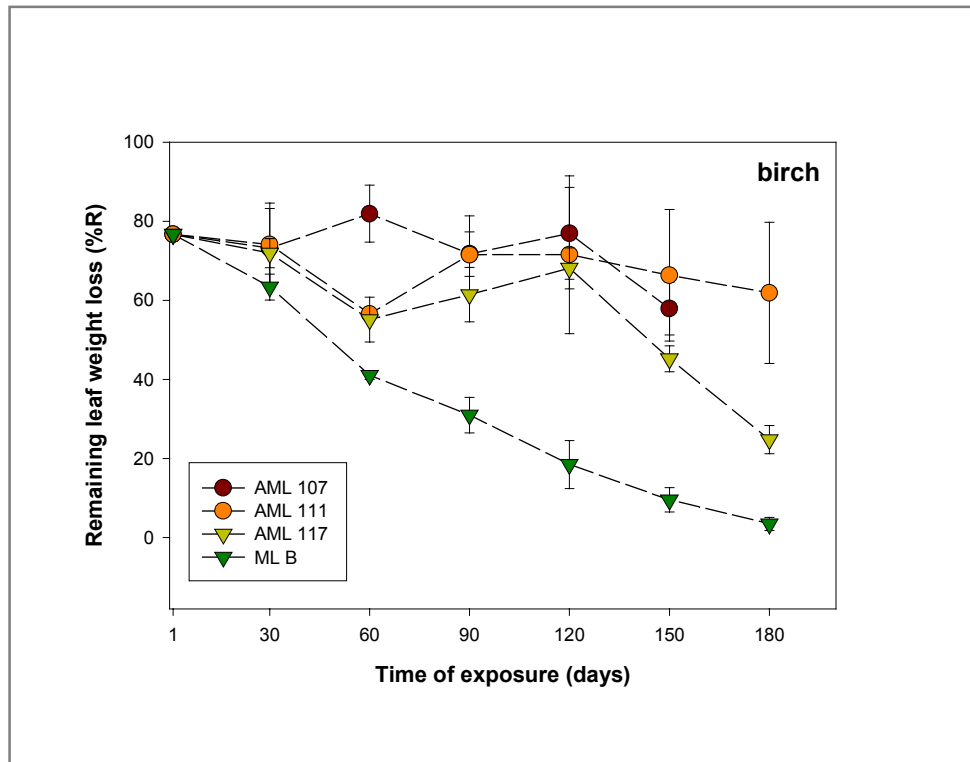


Fig. 81. Remaining leaf weight loss of birch in lakes (ML B; AML 117; AML 111 and AML 107) from Lusatian lakes in eastern Germany.

### 3.5 Acid resistant *Chironomus crassimanus*

#### 3.5.1 Pupal description of *Chironomus crassimanus*

Colour: Cephalothorax pale brown, darker brown anteriorly before and behind the oblique hinge line (Langton 1995, figs. 8.10) and posteriorly above the base of the wingsheath. Wingsheaths broadly streaked with pale brown and margined with darker brown. Abdomen colourless with lateral golden brown adhesion marks, diminishing in length from segment II to VI and a golden brown streak along the parasternites from segment V to VIII intensifying and becoming broader posteriad. Anal lobes golden brown, transparent at base and with a narrow pale streak along the insertion of the fringe.

Exuvial length 8.2 - 10.0 mm ( $n = 9$ ).

Cephalothorax. Cephalic tubercle (Fig. 82a) elongate conical, 70 - 120  $\mu\text{m}$  high ( $n = 8$ ); frontal seta 48 – 62  $\mu\text{m}$  long ( $n = 3$ ). Thorax distinctly small granulate anteriorly (Fig. 82b3, weakening posteriad, by mid thorax reticulate or smooth (Fig. 82b1). Basal ring of thoracic horn 140 x 60 - 180 x 90  $\mu\text{m}$  ( $n = 10$ ); tracheole diameter 2.7 - 3.3  $\mu\text{m}$  ( $n = 3$ ).

Abdomen. Point patches of tergites II-VI moderately developed for the genus (Figs. 83a1 and 83b), the points dense (Fig. 82c4), overlapping those of the previous row (Fig. 83a3); the patch on VI reduced in lateral extent in the posterior half of the tergite, to form a wine glass shaped pattern. Tergite VII with an anterior pair of patches of small points anterior to setae D1, narrowly separated medially or joined by shagreen. Tergite VIII with a large patch on each side of strong points, often some arranged in groups of two or three. Hook row of segment II about half segment width, of 68 - 108 hooks ( $n = 9$ ) (Fig. 83a6). Conjunctives IV/V, V/VI, VI/VII dorsally armed with small spinulate points. Paratergites V and VI with a longitudinal narrow band of spinulate points (Fig. 83a4). Pedes spurii B on pleuron II well developed. Pleuron IV armed with dense narrow spinules up to 14  $\mu\text{m}$  long (Fig. 83a3). Sternites (Fig. 82c): II nearly covered with small isolated points; III with lateral longitudinal bands of small points anteriorly spreading inwards, but not meeting medially; IV without lateral longitudinal point bands, though the anterior inward extensions may be developed; V as IV; VI and VII with antero-lateral patches of small points. Parasternite II shagreened; IV with well developed vortex, not extended anteriorly; V and VI armed with small points posteriorly. Spur of segment VIII with 1 – 4 robust long-acuminate teeth (Fig. 83c1, c2).



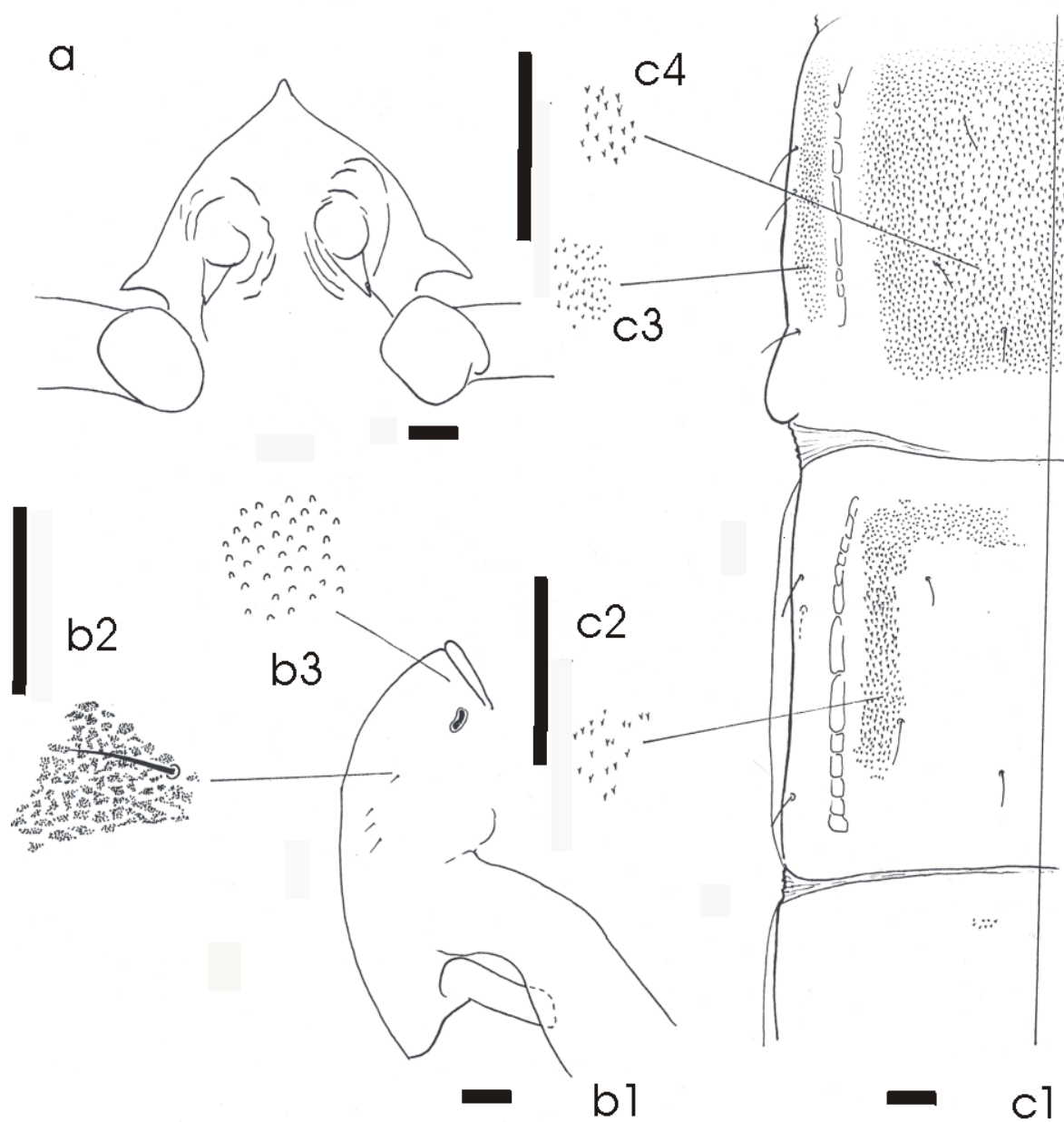


Fig. 82. *Chironomus crassimanus* Strenzke 1959; pupal exuvie, a. frontal apotome, b. thorax, lateral, c. segments II – IV, ventral (scale lines = 100 μm).

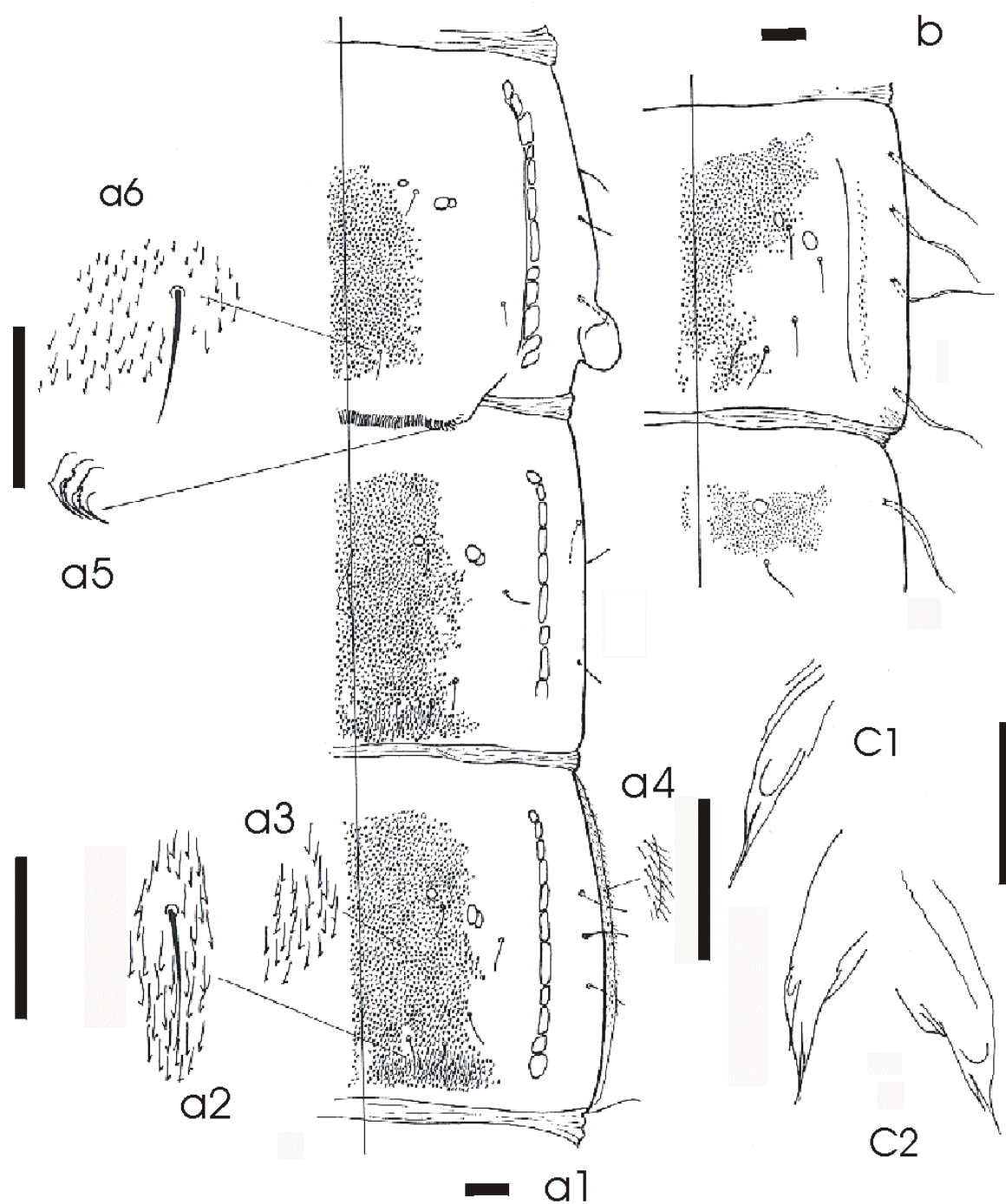


Fig. 83. *Chironomus crassimanus* Strenzke 1959 pupal exuviae, a. segments II – IV, dorsal, b. segments VI – VII, dorsal, c. spur of segment VIII. (scale lines = 100  $\mu$ m)

Chaetotaxy (D = dorsal, V = ventral, L = lateral setae; T = taeniae):

	I	II	III	IV	V	VI	VII	VIII	IX
D	2	3	5	5	5	5	5	2	
T									1
L	0	3	3	4					
T					4	4	(3)4	3-5	86-116 (n=10)
V	2	3	3	3	3	4	4	2	

- **Differential diagnosis**

The pupal exuviae of *Ch. crassimanus* may be distinguished from other species of *Chironomus* by the following combination of characters: moderate size (8-12mm length), thorax distinctly, but not densely, small granulate anteriorly only, reticulate elsewhere; sternite II nearly covered with small points, sternite III with lateral longitudinal bands of small points spreading inwards anteriorly to form a transverse band, broken medially, and sternite IV bare or with some small points forming a medially broken anterior band.

### 3.5.2 Predation of *Chironomus crassimanus* on iron-reducing bacteria

The most probably number of iron-reduced bacteria (cells.ml<sup>-1</sup>) were lower in the presence of *Ch. crassimanus* larvae by a factor of 2 – 5 (Table 13).

This method of estimating the number of bacteria considered the iron-reducing bacteria only. It was shown that *Ch. crassimanus* larvae had a significant effect on the bacteria population during the time of experiment. The substantial decrease of bacteria was probably due to the predation of chironomid larvae.

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**Note:** *Chironomus crassimanus* in Langton, 1991 was based on a slide in ZSM of three exuviae from Strenzke's original rearings. However, because the rearing was done from egg masses collected in the slides reveals one exuviae of *Ch. crassimanus* and two of an undescribed *Chironomus*. Neither species will run to *Ch. crassimanus* in the key, because the character set used in placing the taxon is a mosaic derived from both species.

**Table 13.** Number of iron-reducing bacteria in cells.ml<sup>-1</sup> in lake sediments from AML 111 incubated with (+) and without (-) *Chironomus crassimanus* larvae.

Sediment depth (mm)	(+) <i>Ch. crassimanus</i>			(-) <i>Ch. crassimanus</i>			Factor
	Average	Min.	Max.	Average	Min.	Max.	
0-4 mm	<b>99.12</b>	46.90	124.38	<b>193.4</b>	100.96	209.50	<b>2.0</b>
4-8 mm	<b>80.66</b>	37.89	105.04	<b>262.6</b>	138.20	324.10	<b>3.2</b>
8 mm	<b>49.44</b>	25.04	59.66	<b>247.3</b>	129.80	275.90	<b>5.0</b>

### 5.2.3 Gut content of acid-resistant *Chironomus crassimanus*

The analysis of the digestive tract content of *Ch. crassimanus* larvae showed that this species fed on microflora associated with detritus, principally fungi, bacteria, and phototrophic organisms, investigated in AML 111 and AML 117.

In AML 117 the gut content of the larvae consisted of mycelium of fungi (Fig. 84A) and short filamentous bacteria (Fig. 84B). This was detected using confocal laser scanning microscopy. In AML 111 *Ch. crassimanus* fed, principally, fungi and bacteria (Fig. 84C, D). The pigments (chlorophyll and maybe phycoeritrin) in the red (red) and for red (blue) channel result in an overlay of colours (pink).

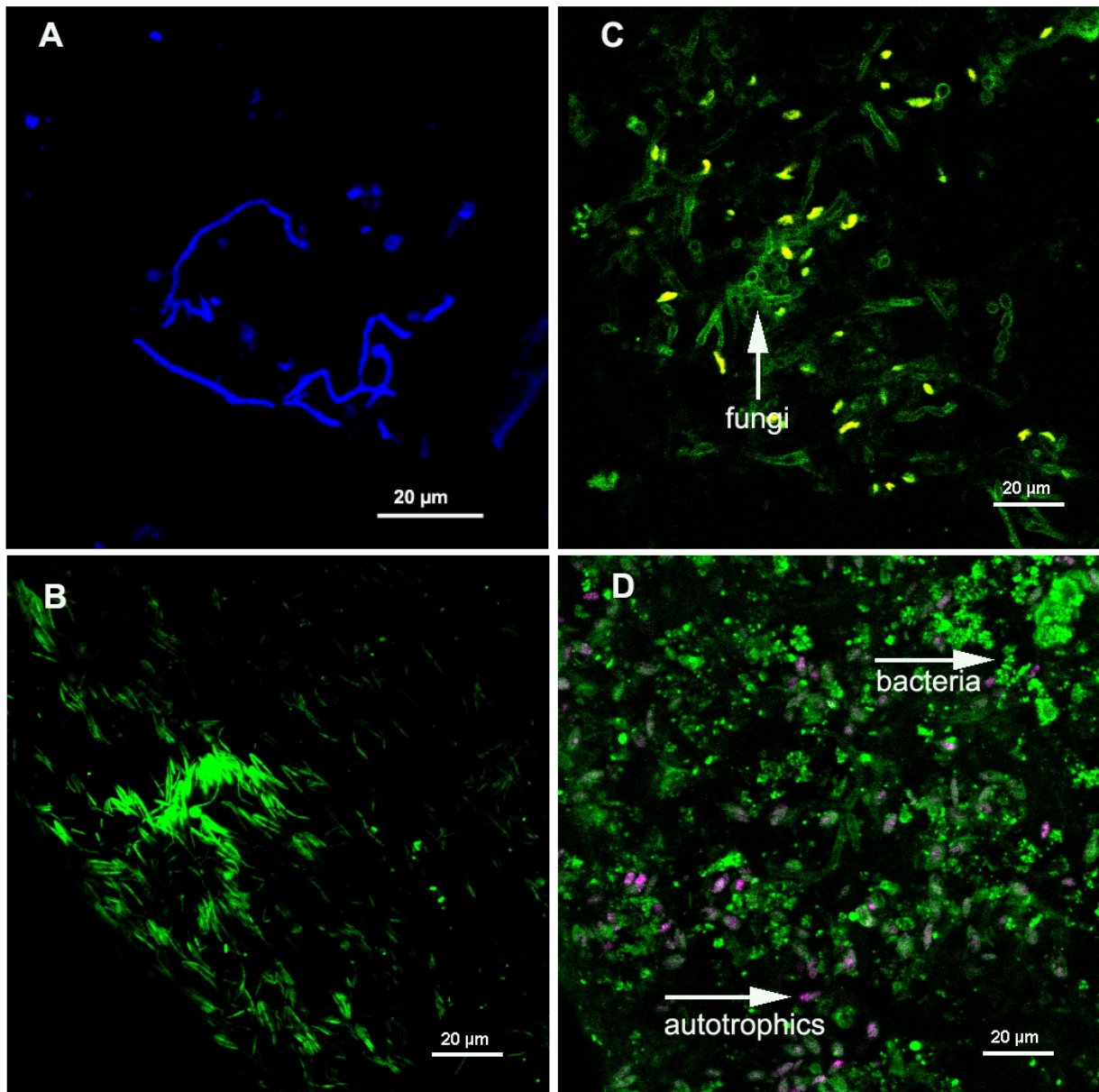


Fig. 84. Confocal laser micrograph of digestive content of the acid-resistant *Chironomus crassimanus* (Insecta, Diptera, Chironomidae) larvae from extremely acidic mining lakes. A) Confocal laser micrograph as maximum intensity projection of UV signal after staining with calcofluorwhite in AML 117. Different fungal cell structures can be identified. B) Confocal laser micrograph projected as maximum projection showing gut content after staining with SYTO9 in AML 117. Image shows filamentous bacteria arranged in dense bundles. C) Confocal laser micrograph as maximum intensity projection of the reflection signal without staining showing the gut content of the *Ch. crassimanus* in AML 111. Image shows cell labelling of filamentous fungi in the gut content of *Ch. crassimanus*. D) Three channel confocal laser micrograph presented as maximum intensity projection showing gut content of the *Ch. crassimanus* larvae after staining with SYTO9 in AML 111. Colour allocation: nucleid acid signal (green), autoflorescence of pigments in red channel (red) and far red channel (blue) was shown in pink.

## 4 Discussion

### 4.1 Benthic fauna of extremely acidic lakes

#### 4.1.1 General remarks

Extreme conditions are an abiotic environment factor which varies in space and time, and to which organisms respond differentially. But, it exerts a powerful influence on the distribution and abundance on the organisms, especially on benthic invertebrates that are non-mobile aquatic organisms. Furthermore, the habitat colonisation of these benthic organisms occurs principally by dispersion, and the choice of optimal sites depends on not only abiotic but also biotic conditions.

Reduction biodiversity, spatial and temporal variation of the benthic macroinvertebrate communities have been reported from acidic waters in some countries in connection with low pH values in aquatic ecosystems, most with pH up to 3.5, formed from soft-water and acid rain (e.g. Wiederholm & Eriksson 1977; Økland & Økland 1980, 1986; Schneider 1986; Hämäläinen & Huttunen 1990; Heij & Schneider 1993; Steinberg & Wtrite 1994; Henrikson & Brodin 1995; Reid & Somers 1995; Umweltministerium Baden-Wütterberg 1995).

Waters with low pH contain a high concentration of heavy metals and ions as to be severely toxic to most organisms. In extremely acidic mining lakes from Lusatian region both iron, aluminium, and sulphate are present in high concentrations. All the investigated acidic mining lakes are characterised by pH values in the range of 2 – 3, which influenced all other chemical parameters. Because of the low pH and the nature of the surrounding materials, a great variety of substances are dissolved in the water of acidic mining lakes in very high concentrations.

Total iron in these lakes varied between 400 – 800 mg.l<sup>-1</sup> (AML 107); 120 – 190 mg.l<sup>-1</sup> (AML 111), and 17 – 25 mg.l<sup>-1</sup> (AML 117). The high concentration of iron (pH 2.6 and 3.0; total iron 89 mg.l<sup>-1</sup> and 22 mg.l<sup>-1</sup>, respectively) influenced the zoobenthic structure in acid mine streams (pH 2.6 – 7.5) from Western Pennsylvania, USA (Koryak *et al.* 1972).

However, the direct impact of the toxicity of acidic lakes, e.g. high concentration of Fe<sup>2+</sup>, Fe<sup>3+</sup>, Al<sup>3+</sup>, and SO<sub>4</sub><sup>2-</sup> appears to be of major importance to explain the impoverishment of macroinvertebrate communities and also the reduction of quality and range of food sources available to benthic invertebrates. Aluminium reached 40 – 60 mg.l<sup>-1</sup> in AML 107, 25 – 45 mg.l<sup>-1</sup> in AML 111, and 1.8 – 2.2 mg.l<sup>-1</sup> in AML 117 (see Table 2). Aluminium is usually present in lake water in concentration less than 0.1 – 0.4 mg.l<sup>-1</sup> (Hutchinson 1957). The combination of low pH and high levels of certain forms of Al is probably the main reason why the number of animal species declines in acidified lakes (Dickson 1978). Recently, Baltes (2000) reported the influence of acidification on aquatic insects. Aluminium (max. 1.5 mg.l<sup>-1</sup>), pH > 3.5 and low alkalinity are the most relevant parameters for the identification of water acidification and these factors have a strong effect on benthic invertebrates from 22 streams in Northern Saarland in the western region of Germany. In addition, sulphate reached a maximum of 3.410 mg.l<sup>-1</sup> in AML 107, and 1.280 – 1.890 mg.l<sup>-1</sup> in AML 111. Conversely, AML 117 showed lower concentration (374 – 410 mg.l<sup>-1</sup>) nearest to the control lake ML B that present concentrations between 400 – 433 mg.l<sup>-1</sup> (see Table 2). Raddum (1980) reported that the excess of sulphate can be regarded as an index of acidification, and SO<sub>4</sub><sup>2-</sup> associated with the higher Ca<sup>2+</sup> content influenced on the benthic fauna from 34 oligotrophic lakes with pH-value 4.6 to 6.2 investigated in South Norway. This author emphasised that a high pH in lake water must not be regarded as an index of a healthy lake ecosystem in regions exposed to acid precipitation.

In the lakes investigated the major ions, calcium ( $\text{Ca}^{2+}$ ) is typically quite high with a maximum of 227 - 402  $\text{mg.l}^{-1}$  (AML 107); 222 – 242  $\text{mg.l}^{-1}$  (AML 111), and 94.7 – 105  $\text{mg.l}^{-1}$  (AML 117). These values are similar to acid strip lakes with pH about 3.0 from southern Indiana, USA, found by Smith & Frey (1971) that vary from about 120 – 500  $\text{mg.l}^{-1}$ . The range in natural surface water is from 0.13 or less to about 10.000  $\text{mg.l}^{-1}$  (Dead Sea) but most freshwater lakes contain 2.5 – 60  $\text{mg.l}^{-1}$  (Hutchinson 1957). The control lake (ML B), with an observed range of 188 - 203  $\text{mg.l}^{-1}$  was well within these limits. The high concentration of calcium may be one of reasons for the survival of aquatic organisms in these extreme conditions.

Conversely, the presence of specific benthic invertebrate species can indicate the acidity of habitats prevailing during the period of their residence, and a response to the change in nutrients and disruptions of food chains through decreasing pH. Braukmann (1995) categorised the macrozoobenthos from 32 acid streams and 17 non-acid streams in Black Forest in South Germany in four categories: acid-sensitive, acid-tolerant, acid-resistant, and non-indication taxa. The number of benthic macroinvertebrates in acid streams with pH values up to 3.5 corresponds to more than 10 taxa up to 50 taxa (Braukmann 1995; Fig. 85), while the number of taxa found in this study with pH values from 2.0 to 3.0 corresponding less to 10 taxa, varying from 3 (AML 107) to 7 (AML 111), and to 9 (AML 117) (Fig. 85). In addition, Smith & Frey (1971) reported about ten benthic invertebrate taxa for acid strip mining lakes with pH about 3.5 from southern Indiana in the USA.

The correlation between the number of taxa found in this study for AML 107, and AML 111, and AML 117 and a control lake (ML B) fits a Pearson correlation of  $r = 0.99$  ( $n = 4$ ) (Fig. 15).

Compared to macrozoobenthos species richness in environments at pH 4.5 – 5.0, there are many fewer taxa in environments pH < 3.0, suggesting a threshold between pH 4.5 and 3.5,



below which many species are unable to maintain a population. The major impact of acidic waters on benthic organisms has also been demonstrated principally with molluscs and crustaceans, of which most species are acid-sensitive ( $\text{pH} < 5.5$ ) (Økland & Økland 1986), because they need carbonate ( $\text{CO}_3$ ) to construct their shells and carapaces, respectively. However, Økland & Økland (1980) regarded that *Asellus aquaticus* (Crustacea: Isopoda) has its main distribution in parts of south-eastern Norway, where it is frequently found in lakes with pH down to 5.2, and rare in lakes with a pH up to 4.8.

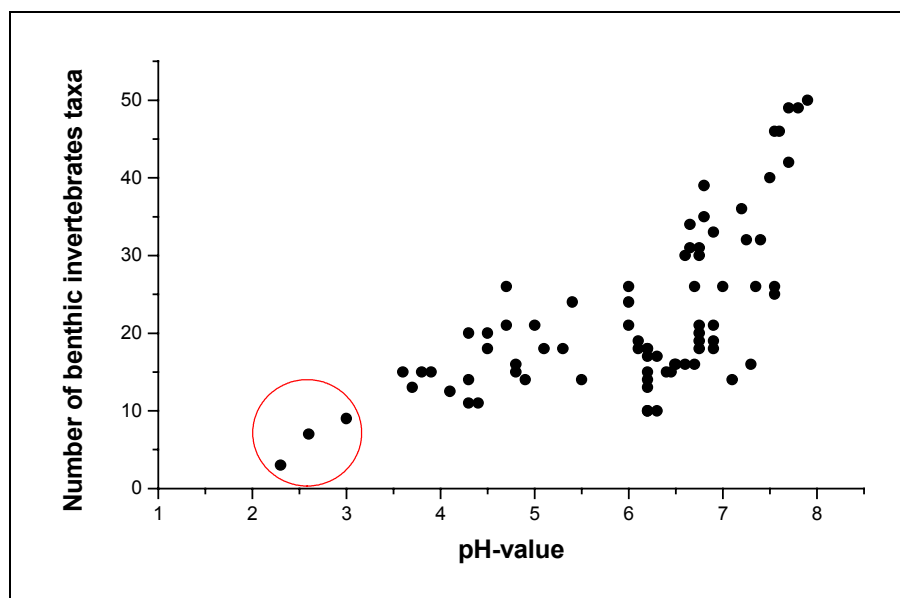


Fig. 85. Relation between number of benthic invertebrates taxa and pH-value up to 3.5 of 82 acid streams and non-acid streams, studied by Braukmann (1995). The circle shows the number of benthic invertebrates found in extremely acidic lakes (pH 2 – 3) from Lusatian lakes (AML 107, AML 111 and AML 117).

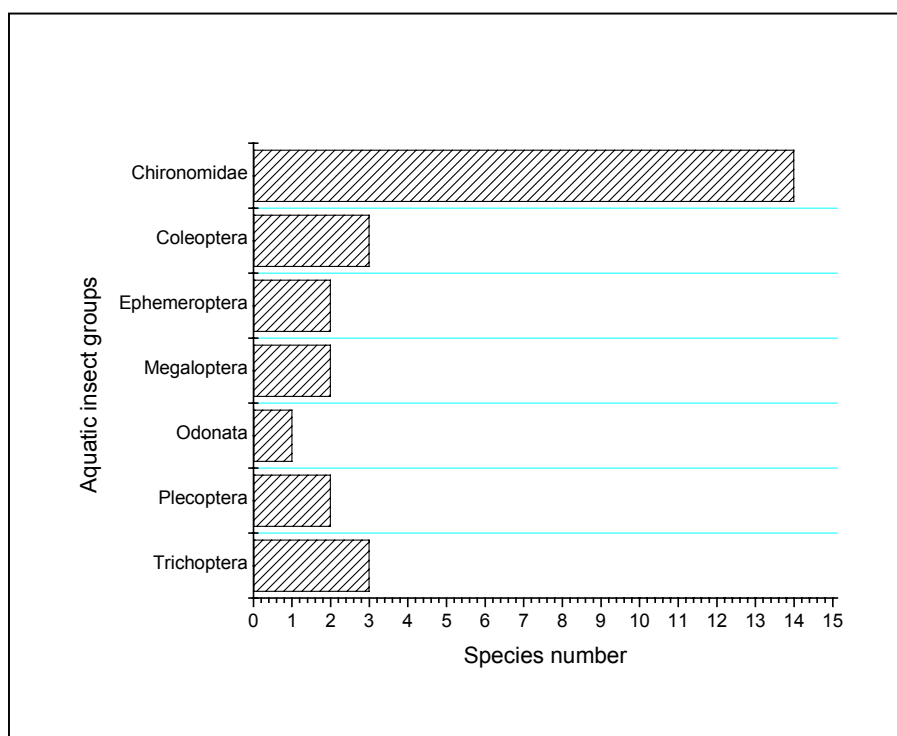
Tolerance limits for pH values vary among benthic invertebrate species, but only a minority are able to grow and reproduce at pH below about 3.5. However, some benthic invertebrate species are able to survive in extremely acidic waters, especially aquatic insects. More than 29 acid-resistant species were registered for some habitats with  $\text{pH} \leq 3$ , such as volcanic lakes, acid strip streams, and acidic mining lakes. Table 14 lists benthic invertebrates that occurred

in extremely acidic waters, with the corresponding pH-value, habitat type, recorded distribution, and references.

Benthic invertebrates are denominated as acid-resistant when the species can withstand very acidic water and survive in extreme ecological conditions.

Twenty nine taxa were carried out amongst acid-resistant species present in waters with pH below 3. Diptera comprised 48.3 %, followed by Coleoptera with 10.3 %, Trichoptera 10.3 %, Ephemeroptera, Megaloptera, and Plecoptera each with 6.9 %, and Odonata, Hirudinea and Acari each with 3.5 %.

In extremely acidic environments with pH below 3.0, aquatic insects (92 %) were the predominant group of benthic fauna. Chironomidae (Diptera) has the highest number of species. Figure 86 gives a representation of aquatic insects group, as well as the number of species recorded.



**Figure 86. Species number of aquatic insects carried out of the literature that occurred in extremely acidic water with pH under to 3.0.**

Table 14. List of benthic invertebrate taxa occurring in extremely acidic waters.

Taxon	pH	Habitat	Recorded Distribution	Reference
<b>INSECT-DIPTERA</b>				
<b>Chironomidae</b>				
<i>Ablabesmyia longistyla</i>	2.6-2.9	Acidic mining lake	Eastern Germany	(present study)
<i>Chironomus acerbiphilus</i>	1.4	Volcanic lake	Japan	Yamamoto (1986)
<i>C. decorus</i> group	2.3	Acid strip-mine	Central USA	Harp & Campbell (1967)
<i>C. (Chironomus) fusciceps</i>	≤ 3.0	Sulphur waters	Japan	Yamamoto (1990)
<i>C. crassimanus</i>	2.1-3.0	Acidic mining lakes	Eastern Germany	(present study)
<i>C. near maturus</i>	2.3-3.8	Acid strip-mine lake	Illinois, USA	Zullo & Stahl (1988)
<i>C. plumosus</i> group	2.3-2.8	Acid mine drainage stream; Strip-mine lakes	USA	Warner (1971); Harp & Campbell (1967)
<i>C. riparius</i>	2.8	Pond	North West Canada	Havas & Hutchison (1983)
<i>C. (Chironomus) sulfurosus</i>	≤ 3.0	Sulphur waters	Japan	Yamamoto (1990)
<i>C. zealandicus</i>	2.1	Acid thermal lake	New Zealand	Forsyth & McColl (1974); Forsyth (1977)
<i>Corynoneura lobata</i>	2.6-2.9	Acidic mining lakes	Eastern Germany	(present study)
<i>Limnophyes minimus</i>	2.1-3.0	Acidic mining lakes	Eastern Germany	(present study)
Orthoclaadiinae spp.	2.8-3.3	Acid mine drainage stream	New Zealand	Winterbourn & M'Diffett (1996)
<i>Tanytarsus dendyi</i>	2.3-3.8	Acid strip-mine lake	Illinois, USA	Zullo & Stahl (1988)
<b>INSECT NON-DIPTERA</b>				
<b>Coleoptera</b>				
Gyrinidae				
<i>Gyrinus aeratus</i>	2.8	Mining lake	Eastern Germany	(present study)
<i>Orectochilus villosus</i>	3.0	Mining lake	Eastern Germany	(present study)
<i>Ilybius fenestratus</i>	3.0	Mining lake	Eastern Germany	(present study)
<b>Ephemeroptera</b>				
<i>Zephlebia</i> sp.	2.9-6.1	Acid mine drainage stream	New Zealand	Winterbourn & M'Diffett (1996)
Leptophlebiidae				
<i>Deleatidium</i> sp.	2.9-3.9	Acid mine drainage stream	New Zealand	Winterbourn & M'Diffett (1996)
<b>Megaloptera</b>				
Sialidae				
<i>Sialis lutaria</i>	3.0	Mining lake	Eastern Germany	(present study)
<i>Sialis</i> sp.	2.8	Acid mine drainage stream	USA	Warner (1971); Tomkiewicz & Duson (1977)
<b>Odonata</b>				
Coenagrionidae				
<i>Coenagrion mercuriale</i>	3.0	Mining lake	Eastern Germany	(present study)
<b>Plecoptera</b>				
Notonemouridae				
	2.8-3.3	Acid mine drainage stream	New Zealand	Winterbourn & M'Diffett (1996)
Perlidae				
<i>Acroneuria carolinensis</i>	2.8-3.3	Acid mine drainage stream	North America	Whipple & Duncan (1993)
<b>Trichoptera</b>				
Limnephilidae				
<i>Hesperophylax</i> sp.	2.97	Mining lake	USA	Nelson (1994).
Kokiriidae				
<i>Kokiria miharo</i>	2.9	Acid mine drainage stream	New Zealand	Winterbourn & M'Diffett (1996)
Ptilostomatidae				
<i>Ptilostomis</i> sp.	2.6-3.0	Acid mine drainage stream	North America	Koryak <i>et al.</i> (1972)
<b>OTHER INVERTEBRATES</b>				
<b>Acari</b>				
Hydrozetidae				
<i>Hydrozetes lacustris</i>	2.1-3.0	Acidic mining lakes	Eastern Germany	(present study)
<b>Hirudinea</b>				
Glossifonidae				
<i>Helobdella</i> sp.	2.1	Acid thermal lake	New Zealand	Forsyth (1977)

Among the chironomids in extremely acidic environments, 64.3% of species belong to the Chironomini tribe, followed by Orthoclaudiini with 21.4 %, and Tanytarsini and Tanypodini each with 7.1 % of species. Mackay & Kersey (1985) reported the dominance of Chironomini and the abundance of Tanypodini typified most acid streams with pH value up to 3.5. However, in the investigated Lusatian lakes with pH 2 – 3 they were typified by the Chironomini and Orthoclaudiini.

Although, Zullo & Stahl (1988) reported that *Tanytarsus dendyi* was the only species that belongs to the Tanytarsini tribe, in acidic strip-mine lakes with pH 2.3 - 3.8 in Illinois, in the USA (Table 14).

*Chironomus* genus (Chironomidae, Chironomini) is a well-known spreading taxa in highly acidic waters (see table 14). *Chironomus zealandicus* was the only species of chironomids present in lake Rotokawa in New Zealand, and one of the species in the benthos that occurred in acidic mine drainage with pH 2.1 (Forsyth & McColl 1974; Zullo & Stahl 1988). Also *C. zealandicus* were found in lake Rottowhero (New Zealand), a natural, thermal and acidic lake of volcanic origin. In North America, *Chironomus attenuatus* might well be the same species of *C. maurus* complex that were reported in extremely acidic pit lakes, and was originally included in *C. decorus* (*C. attenuatus*) (Sublette & Sublette 1974). Harp and Campbell (1967) recorded the presence of *Tendipes plumosus* (Linné) (= *Ch. plumosus*) in 19 strip-mine lakes of varying pH. *T. plumosus* were present in pH between 2.3 to 7.3. Its distribution was not restricted by a mineral acid environment, but was related to the presence of leaf litter. Bates & Stahl (1985) demonstrated for *Ch. harpi* (as *Chironomus* nr. *maurus*) that growth rates were slower at pH 6.6, implying that the more acid environments are more optimal. There appears to be primitive facility in the *Chironomus* genome for adaptation to extreme acidic conditions not shared by other genera of the Chironomini.

It is also obvious that acid-resistant *Chironomus* have a high capacity to colonise some types of habitats. Their expansion is probably due to the elevated content of haemoglobin. This physiological adaptation seems to be relatively independent of the external pH concerning haemolymph, sodium and chloride balance (Jernelöv *et al.* 1981).

However, *Chironomus crassimanus* is a pioneer species in extremely acidic mining lakes with pH-value 2 - 3 in eastern Germany. *Ch. crassimanus* was described by Strenzke (1959) from a clay pool Tonteich near Reinbek, in northern Germany with a pH of 3.05. Adaptation to highly acid waters is known to have occurred repeatedly in the genus *Chironomus*.

In general, taxonomical information about benthic invertebrates in highly acidic environments is limited. Just a key to the species of *Chironomus* living in the highly acidic water in Japan was found (Yamamoto 1990). Three acid-resistant species have been reported from volcanic regions of Japan: *Ch. acerbiphilus* Tokunaga 1939 from Lake Katanuma (Fujimatsu 1938; Tokunaga 1938) with pH 1.4 (Yoshimura 1933), *Ch. fusciceps* Yamamoto 1990 and *Ch. sulfurosus* Yamamoto 1990 from hot sulphurous springs with pH 2.9 - 4.3 (Uéno instead of 1932, 1933, Itô 1937; reported by Yamamoto 1986) (Table 14).

According to Yamamoto (1986) these chironomids form a distinct species group, the male hypopygium having appendage 1 broad apically, and the gonostylus short and broad. *Ch. harpi* Sublette 1991 from acidic lakes (pH about 3, Zullo & Stahl 1988) in strip-mined areas of Illinois, USA, has appendage 1 long and narrow (Wülker *et al.* 1991), whereas in *Ch. crassimanus* it is short and somewhat swollen medially (Strenzke 1959). Although, these acid-resistant *Chironomus* species are not closely related according to the morphological characteristics of *Ch. crassimanus*. The description of the pupal exuvie of *Ch. crassimanus* in this study may facilitate the identification of this extremely acidic key-species (Figs. 82, 83).

#### 4.1.2 The chironomids in the extremely acidic lakes

In this study, egg-masses of chironomid were usually observed deposited around the buoy and the platform from AML 111, and AML 107 and AML 117. The egg masses were encased in a mucilaginous coating, and so large and conspicuous that they may be visible from a distance. In most cases the chironomid population is more or less stable and the females of many species lay over 1000 eggs (Langton 1991). The females were also usually collected near the egg masses from the investigated lakes. However, the females immediately die after the eggs are laid. *Ch. crassimanus* search to a specific sites for oviposition with defined ranges of pH value, where the females can deposit the egg masses. To the longevity of *Ch. crassimanus* I observed in the laboratory that the maturation takes approximately 2 weeks (14 – 17 days), and the laying of the last egg masses since the maturation takes at least two days. The first instar of the *Ch. crassimanus* larvae is mobil and planctonic (Fig. 87), and is responsible for finding an adequate substrate for the life cycle development. Food resources are necessary for the success of the species in the larval stage (I - IV instars).



Fig. 87. First instar of *Chironomus crassimanus* larvae from acidic mining lakes from Lusatian region with pH value from 2 to 3.

When the pupal chironomid is ready to eclose, it swims to the water surface enclosed in its pupal cuticle and it ecloses. This hatching process takes only a few seconds to complete. The pupal exuvie is left on the water surface and drifts downstream or is transported to the lee shore by the wind. The exuvie survive intact for only a few days before decomposition (Langton 1991). The imago flies after eclosion. Males formed swarms in which the females fly. Males are attracted to females by the sound produced by the female's wings (Pinder 1989). After copulation, the female searches for an oviposition site. The adult stage of Chironomidae is rather short, lasting from a few days to several weeks (e.g. Oliver 1971; Pinder 1986).

Another acid-resistant chironomid *Limnophyes minimus* (Meigen 1818) (Chironomidae, Orthocladiinae) inhabited extremely acidic lakes from Lusatian region with pH between 2 – 3. The rarely *Corynoneura lobata* (Edwards, 1924) (Chironomidae, Orthocladiinae) and *Ablabesmyia longistyla* Fittkau 1962 (Chironomidae, Tanypodinae) were also recorded for the Lusatia mining lakes with pH 2.6 - 2.9 (AML 111) in eastern Germany (see table 6), but with lower numbers compared to *Chironomus crassimanus* and *L. minimus*.

*Ablabesmyia longistyla* and *Corynoneura lobata* were rare species in these acidic mining lakes recorded only in AML 111, while *L. minimus* is considered to be a semi-aquatic species and the larvae may occur in wet soils or in benthic compartment (Hänel & Chown 1998). It is assigned as litter-feeder consumer (Delettre 1978) and was an important contributor to litter decomposition of Marion Island at least in some communities (Hänel & Chown 1998). It explains the capacity of this abundant species to colonise the leaf packs in the littoral zone of AML 111 and AML 117. Paleolimnological studies showed that *L. minimus* has been also colonised AML 107 (Scharf *et al.* in prep.)

*Ablabesmyia longistyla* was assigned in this study as deposit-feeder. The tribe Tanypodini

has been assigned as predator (Dugose 1980). But some authors, including Oliver (1971) and Baker & McLachlan (1979) had considered larvae of Tanypodini tribe as deposit-feeders in early larval instars. I had also observed benthic diatoms in the digestive tract of *A. longistyla* from AML 111. Kapfer (1998) and Lessmann *et al.* (2000) regarded the diatoms *Eunotia exigua* and *Nitzschia* sp. (Bacillariophyceae) have been found in Lusatian mining lakes with pH 2-3, especially in AML 111. *Corynoneura lobata* (Edwards, 1924) (Chironomidae, Orthocladiinae) were found in the qualitative samples from AML 111. Cranston (1982) reported that the larvae of this genus may have filter feeding habits, but further investigation of the unusual mode of feeding is necessary.

#### 4.1.3 Non-Diptera aquatic insects in the Lusatia extremely acidic lakes

Some others aquatic insects that do not belong to Diptera occurred also in extremely acidic waters. *Sialis lutaria* (Megaloptera, Sialidae) is an abundant acid-resistant species found at pH 3.0 in Lusatian lakes (AML 117). Kerekes *et al.* (1984) reported that the Megaloptera species were reported at pH 3.6 in aquatic ecosystems in Canada.

*Sialis lutaria* is a fairly widespread species through the whole of Europe, except the southeast (Vanhara 1970). They are often numerous in the benthos, specially in humic, acid lakes and ponds (Matthey 1971; McLachlan & McLachlan 1975). The life cycle usually takes about two years from egg to adult with 10 larval instars but a longer period of three years has been recorded in lakes at high altitudes in Switzerland (Geigy & Grobe 1958) and the French Pyrenees (Giani & Laville 1973), and a shorter period of one year in Denmark (Iversen & Throup 1987; Dall 1989). Lestage (1920) gives a detailed description of oviposition behaviour in *S. lutaria*. After the copulation the females searches for an oviposition site. The eggs are usually laid on the stems and leaves of plants overhanging the water but are occasionally found on stones, tree trunks and bridges. This species may colonise different



grades of trophic (Thienemann 1925). It is a predator which feeds principally on chironomids and oligochaetes (Griffiths 1973).

Furthermore, from AML 117 were also found larvae of *Orectochilus villosus* (Coleoptera: Gyrinidae). This species has been registered for still waters and slow flowing waters (Klausnitzer 1996). Kerekes *et al.* (1984) reported the occurrence of Coleoptera species at pH 3.6 in aquatic ecosystems in Canada. Some species in these extremely acidic environments do not depend on free acidity, but the increase of these potential predators may be due to the availability of food resource and the decrease of competition.

Henrikson & Oscarson (1981) reported the occurrence of species of pleustonic corixids (Hemiptera - Heteroptera) as top-predators in acidic waters. Nymphs of the pleustonic *Sigara nigrolineata* (Heteroptera: Corixidae) were found in all acidic mining lakes investigated. Pophan & Savage (1984) reported that the *S. nigrolineata* is an omnivorous organisms.

Wollmann (1998) supplied further data on Corixidae of acidic eastern German mining lakes and focussed principally on species composition in lakes with different pH values. This author found six species of corixids for AML 117 and eight species for AML 111. Ledger & Hildrew (2000) hypothesised that the non-predatory invertebrates of acid waters would feed on a broad range of resources, normally partitioned among a greater number of species in more species-rich neutral waters.

Hendrey & Wright (1976) considered that corixids species utilise the changed environment, the low pH, with a rapid increase in abundance. However, the pH changes not only favour the increase of the population of these predators in Lusatian extremely acidic mining lakes. Some others ecological implications have been involved in the biota from acidic mining lakes, as well as food resources, resources repartition, competition, and prey - predator interactions.

Odonata have been found to be moderately to very tolerant of low pH value water (e.g. Bell, 1971; Mossberg & Nyberg 1979; Kerekes *et al.* 1984; Schell & Kerekes 1989), but few studies were found that described the effects of pH at species or genus level (Lonergan & Rassmussen 1996). In Germany, Baltes (2000) found *Cordulegaster boltonii* (Odonata: Cordulegastridae) that occurred at pH 4.5 in acid streams in south Germany and Braukmann (1995) emphasised that this species has been considered as an acid-tolerant taxa. Furthermore, *Coenagrion mercuriale* were also found in AML 117 with pH-value of 3.0, but in low numbers. This species has a preference for habitats rich in calcium, and little streams rich in vegetation (Lehmann & Nüß 1998). Corbet (1955) reported that the *C. mercuriale* occurs typically in small streams draining acid peat-bogs in Britain. Appelberg (1995) reported that the number of the Odonata *Coenagrion* sp. increased in acidified Swedish Lake Stora Härsjön after the liming. The distribution of *C. mercuriale* is large in Upper Rhine Valley region in Germany, and rarely occurs in the Alps and in the South Palatinum in Germany too. Furthermore, they are very rare in Osnabrück, east of Lower Saxonia, Thüringen and Magdeburg (Lehmann & Nüß 1998).

Also, in Lusatian mining lakes Phryganeidae (Trichoptera) were rarely found in AML 117. In Middle Europe ten species are known (Botosaneanu & Malicky 1978; Tobias & Tobias 1981). They are large caddis flies, and have a large distribution and colonise principally aquatic lakes (Pitsch 1993). Also, the occurrence of a rarely recorded *Kokiria miharo* (Trichoptera, Kokiriidae) in acidic habitats from New Zealand where the pH-value was 2.9 (see Table 14) adds more information about the extreme environmental tolerance of some members of the Trichoptera. Warner (1971) and Tomkiewicz & Dunson (1977) reported that the very low pH and high concentration of many ions, including aluminium, were comparable to those tolerated in the field by larvae of *Ptilostomis* (Trichoptera, Ptilostomatidae) (Koryak *et al.* 1972) and *Hesperophylax* (Trichoptera, Limnephilidae) in North American streams

(Nelson 1994) (see table 14). In Germany, *Odontocerum albicorne* (Trichoptera: Odontoceridae) have been reported by Baltes (2000) at pH 3.7 in acid streams from the Black Forest.

In general, the species richness of Trichoptera, Plecoptera and Ephemeroptera decreasing when related to decreases the pH in soft waters (e.g. Økland & Økland 1986). However, Whipple & Duncan (1993) reported that both iron and aluminium are indispensable for the larvae of *Acroneuria carolinensis* (Perlidae, Plecoptera) at pH of 2.8 - 3.3 in acid mine drainage streams in North America (Table 14). Baltes (2000) reported the occurrence of *Leuctra inermis* (Plecoptera: Leuctridae) at pH-values at 3.7 in acid streams in south Germany. In addition, Winterbourn & McDiffett (1996) recorded the occurrence of *Deleatidium* (Ephemeroptera: Leptophlebiidae) at pH between 2.9 to 3.9, and also the occurrence of *Zephlebia* sp. at pH-values up to 2.9 and down to 6.1 (see Table 14). Nevertheless, Ephemeroptera group have been reported as extremely sensitive to a low pH-value. In Germany *Baetes vernus* (Baetidae) and *Ecdyonurus venosus* (Heptageniidae) were reported by Baltes (2000) at pH value of 4.5 in acid streams. Also, *Ameletus inospionatus* a frequent species from ML B have been reported as acid-tolerant species in acid rain streams in south Germany (Braukmann 1995) and in acidic lakes with pH value 4.4 in Sweden (Engblom & Lingdell 1984), but have not colonised the studied extremely acidic mining lakes from Lusatia.

#### **4.1.4 Aquatic invertebrates non-insects in extremely acidic lakes**

Amongst other aquatic invertebrates, non-insect *Hydrozetes lacustris* (Acari, Hydrozetidae) were found in Lusatian mining lakes with pH-value in the range 2.1-3.0 (Table 14). This species was the dominant species from AML 117 and may be an important contributor to litter decomposition (see item 4.2.2). Furthermore, others aquatic invertebrates have been

reported. Previous studies have suggested a strong relationship between the occurrence of leeches and water pH. Økland & Økland (1986) report that in Norway, leeches are a potentially valuable indicator for pH 5.0 - 6.0 range. However, the leech *Helobdella* sp. occurred in acid mine drainage with pH 2.1 in Lake Rotokawa in New Zealand (Forsyth 1977). Probably, the presence or absence of carnivorous leeches depends not only on the physiological characteristics of the leeches but also on the presence of available food. Leeches like to feed on gastropods that disappear below pH of about 5.5.

#### 4.1.5 Richness species and abundance of benthic invertebrates

Richness species of benthic invertebrates of leaf packs colonisation experiments from AML 117 with 9 taxa was greater than AML 111 (7 taxa) and AML 107 (3 taxa). The greatest similarity index (QS) (see table 7) verified, using number of taxa, comprising 80% between AML 107 and AML 111, followed by AML 111 and AML 117 with 71 %, and AML 107 and 117 with the lowest value of 60.1 % between the investigated acidic mining lakes. The QS between acidic lakes (AML 117, AML 111 and AML 107) and the control lake (ML B) ranged between 5.1 to 6.1 (Table 7).

However, AML 107 and AML 111 presented a similar composition of majority benthic invertebrate taxa. *Chironomus crassimanus* was the dominant species in AML 107 and AML 111. *Sigara nigrolineata* and *Hydrozetes lacustris* were abundant species in AML 107, while in AML 111 these species were frequent. However, *Limnophyes minimus* showed as the abundant species from AML 111. Furthermore, other rare chironomid species occurred in very low numbers in AML 111 as *Ablabesmyia longistyla* and *Corynoneura lobata*. In addition, other neustonic taxa as *Plea leachi* (Pleidae, Hemiptera-Heteroptera), *Hydromedrum stagnourum* (Hydrometridae, Hemiptera-Heteroptera), and *Gerris (Aquaticum) najas* (Gerridae, Hemiptera-Heteroptera), and *Hebrus pusillus* (Hemiptera-Heteroptera) were

observed in surface water from AML 107. Also, the hemipterous *H. stagnourum* and *G. (Aquaticum) paludum* were found in the neuston compartment from AML 111.

The total abundance and total biomass of benthic fauna from AML 107 and AML 111 were very low. Benthic invertebrates on leaves colonisation showed the total values of abundance varied not significantly between these acidic mining lakes (AML 107; AML 111), but the total biomass differ between AML 107 and AML 111. (Fig. 42 - 46). The dominant *Ch. crassimanus* larvae showed larger than those from AML 107. It may contribute to the increase of total benthic biomass from AML 111. Smith & Frey (1971) reported that in acid strip lakes, with pH values about 3.0, the larvae of the chironomid *Tendipes* sp. (= *Chironomus*) made up 85% and 99% of total biomass in two acid lakes from southern Indiana in the USA.

In AML 117 the leaf pack experiments were colonised by a great number of individuals of *Hydrozetes lacustris* being the dominant benthic invertebrate species in this lake, followed by the abundant chironomid *Chironomus crassimanus*. *Limnophyes minimus* and *Sialis lutaria* were the frequent species from this acidic lake. *Orectochilus villosus*, and *Coenagrion mercuriale*, and a Phryganeidae and Nematoda were found as rare species. Also, *Illybius fenestratus* (Dysticidae: Colymbetinae) was observed in the AML 117, but this species did not colonise the leaf packs. It was usually observed in the surface water (neuston) of extremely acidic mining lakes from Lusatia region. Therefore, aquatic insects are still a majority benthic group from AML 117 and the extremely acidic mining lakes. Smith & Frey (1971) also reported that the aquatic insects are the main group from acid strip lakes from southern Indiana, USA.

Also, the total values of abundance and biomass were higher in AML 117 than AML 107 and AML 111 (Figs. 76 - 78), although, the total abundance and total biomass of benthic

invertebrates did not differ significantly between the acidic mining lakes. When compared to control lake (ML B) only in the time of exposure 150 days and 180 days, the total biomass of ML B was higher than AML 117 (Fig 78, 79). The increase of total biomass could be due to the presence of the large organisms *Sialis lutaria* and *Sigara nigrolineata*. It can indicate the importance of the secondary production of this aquatic ecosystem. In addition, not only the species richness was higher in Lake AML 117, but also in terms of the number of individuals, mainly *Chironomus crassimanus* that showed greater abundance in AML 117 than in AML 107, and AML 111. Furthermore, the abundance of *Limnophyes minimus* increased in this lake principally in the time of leaf packs exposure of 150 days and 180 days (Fig. 45). Also, the abundance of *Hydrozetes lacustris* tends increase towards the large time of incubation of leaf packs (Fig. 41).

## **4.2 Benthic invertebrates and leaf breakdown process**

### **4.2.1 Leaf breakdown process**

Many studies suggest that the decomposition process in aquatic sediments appears to be relatively insensitive to acidification (e.g. Gahnström *et al.* 1980; Kelly *et al.* 1984; Hoeniger 1985).

Conversely, results showed the decomposition of allochthonous deciduous leaf were affected by acidification in Lusatian mining lakes. The leaf weight loss from AML 107 and AML 111 proceeds in a non-exponential fashion while in the control lakes (ML B) proceeds in a negatively exponential fashion. Grahn *et al.* (1974) emphasised that coarse detritus is accumulated in the littoral zone of acidified lakes. Gahnström (1995) reported that the decomposition of alder leaves measured in terms of weight loss was also low in the acidified Swedish lakes. Andersson (1985) reported that only 20 % weight lost occurred over a period

of seven months in Swedish acidic lakes. Traaen (1980) conducted laboratory experiments with litter bags using birch leaves in 16 acidic and less acidic softwater lakes in southern and western Norway. This author reported that the mean weight losses in acidic lakes after 1 and 2 years were respectively 31 and 23 % lower than in the less acidic lakes.

In the Lusatian lakes (AML 107 and AML 111) the weight loss for birch, ash and walnut leaves were at least 10 – 50 % lower than their initial weight, but that after the remaining weight loss was quite constant (Figs. 80 A,B). This leaf weight loss of AML 107 and AML 111 corresponding to leaf weight loss value found at 30 days exposure from a control lake (ML B). It is necessary to say, the leaching was calculated after 24 hrs between 23.3% to 24.8% for birch, ash and walnut leaves.

Furthermore, the leaf litter processing revealed the leaf packs processing rates for birch, ash and walnut leaves were lower in the investigated acidic mining lakes ( $\text{pH} \leq 3$ ) than in the control lake ( $\text{pH} 8$ ) (Fig. 80). Both processing rates and leaching of the leaves did not vary for birch, ash and walnut in these extremely acidic ecosystems. The breakdown of leaves may be classified as very slow for these acidic mining lakes and it was obvious that the decomposition process in aquatic sediments was affected by acidification. The classification of the leaf decomposition process as fast or slow has been criticised by Benfield *et al.* (1979). that emphasise the decomposition depends on methods used as bag or leaf packs, mesh size, etc.

However, the birch leaf processing rates from neutral control-lake (ML B) were within the reported values of other birch species found by Petersen & Cummins (1974). Hicks & Laboyrie (unpub. data *cit. in* Parkyn & Winterbourn 1997) found that silver birch (*Betula pendula*) broke down much faster than the other native species. The birch leaf processing ( $k$ ) from Lusatian acidic lakes exhibited rates of 0.0004 and 0.0007 for AML 107 and AML 111, respectively, and a rate of 0.0025 for AML 117 (Table 15).

**Table 15. Comparison of birch leaf processing coefficients ( $k$ ) found in this study with data reported by others authors.**

Species	$k$	pH	Habitat	Season	Reference
<i>Betula pendula</i>	.0144	8.0	Mesotrophic lake	Spring-summer	(Present study)
<i>Betula lenta</i>	.0116	-	*	*	Petersen & Cummins 1974
<i>Betula lenta</i>	.0036	-	*	*	Mayer & Johnson 1983
<i>Betula pendula</i>	.0025	<3	Acid-oligothrophic lake	Spring-Summer	(Present study)
<i>Betula pendula</i>	.0004 – .0007	<3	Acid-oligotrophic lake	Spring-Summer	(Present study)

\* in laboratory (mean temperature 18 °C)

The leaf breakdown processing in the studied acidic lakes was more intensive in AML 117 than in AML 107 and AML 111, although, the processing rate did not vary significantly for investigated lakes AML 107, and AML 111 and AML 117 (Table 9). The leaf breakdown from AML 117 occurred more intensively after 120 days of exposure. One reason for this is not only the chemical composition of this lake ecosystem, but the benthic invertebrate processing on the leaves (see item 4.2.2). Considering AML 107 alone, ash leaves showed a lower rate processing ( $k = 0.0001$ ) than birch ( $k = 0.0007$ ) and walnut ( $k = 0.0008$ ). In AML 111 ash and birch exhibited the same value of rate processing ( $k = 0.0004$ ) while walnut leaves exhibited  $k = 0.0007$ . Furthermore in AML 117 ash leaves presented  $k = 0.018$ , followed by birch ( $k = 0.0025$ ) and walnut ( $k = 0.0048$ ). In general the processing rate for ash tends to be more intensive than birch and walnut leaves, but in extremely acidic mining lakes it did not vary significantly.

#### 4.2.2 Processing of leaves breakdown by benthic invertebrates

Results showed the mites *Hydrozetes lacustris* had colonised all investigated acidic mining lakes. However, *H. lacustris* were found as a dominant species from AML 117. *H. lacustris* showed a tendency to increase their abundance in AML 117 with time of leaf packs



incubation, whereas they showed greater densities in the time of 180 days on birch leaf packs exposure. H. Schatz<sup>1</sup> (personal comm.) emphasised that the mite *H. lacustris* is a litter feeder that is common in freshwater ecosystems. Cummins *et al.* (1989) reported that the shredder consumers generally use plant litter as a food resource only after it has been conditioned. Also, Bueler (1984) *cit. in* Graca *et al.* (1993) and Arsuffi & Suberkropp (1984) reported that food preferences of benthic invertebrates are influenced by leaf incubation time.

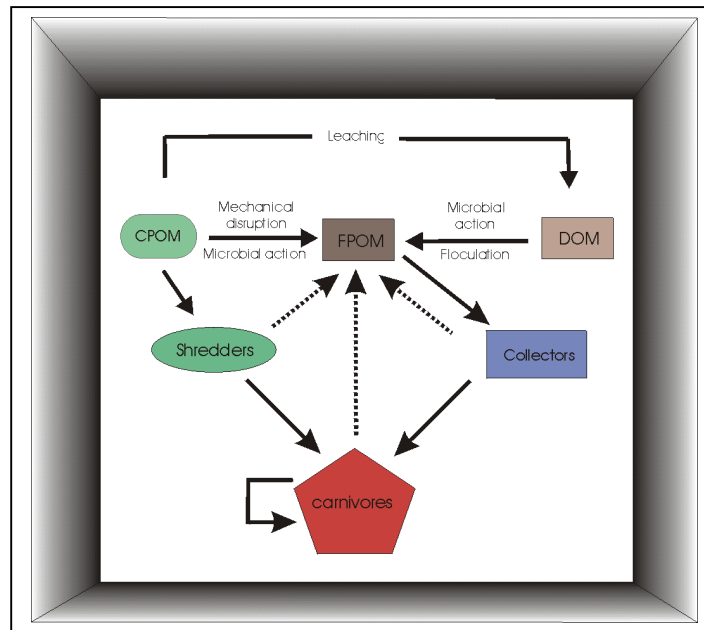
Conversely, Bärlocher & Kendrick (1973) and Suberkropp *et al.* (1983) reported that the food preferences are influenced also by leaf type. However, the density of benthic invertebrates in investigated acidic mining lakes was not significantly different between the three leaf species (birch, ash and walnut leaves) (Table 10), suggesting the three leaves were equally palatable or that the dominant benthic invertebrates were using the leaves primarily as habitat rather than as a food resource.

Furthermore, the collectors as *Chironomus crassimanus* were the main functional feeding group (see item 4.3) which colonised leaf packs in acidic mining lakes. In general, collectors are not considered to play an important role in the degradation of CPOM, but the act of feeding and moving over the leaf surface results in a reduction in particle size, which is important in the degradation of CPOM (Fig. 88). Bird & Kaushik (1985) demonstrated that collectors not only have the ability to process leaf material, but are able to assimilate it. Hence, it is highly likely that collectors use leaf packs not only as a substrate for attachment, but as a source of food as well as a fine particulate organic matter (FPOM).

This is consistent with previously reported findings for lotic systems (e.g. Maloney & Lamberti 1995; Short & Smith 1989; Grubbs & Cummins 1994; and Richardson 1992 *cit. in* Pope *et al.* 1999; and Dobson *et al.* 1992 and Casas 1996). It was suggested that increased colonisation of leaves by collectors was probably due to the accumulation of FPOM, and the

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<sup>1</sup> Henrich Schatz, University of Vienna, Austria. Personal communication, September 2000.



**Fig. 88. Hypothetical general model of energy flow in the extremely acidic lakes. CPOM: coarse particulate organic matter; DOM: dissolved organic matter; FPOM: fine particulate organic matter.**

collectors may depend on food of FPOM produced by the feeding activities of shredders (Cummins *et al.* 1973; Short & Maslin 1977; Meyer & O'Hop 1983; Cuffney *et al.* 1984).

Henrikson & Oscarson (unpubl. dates *cit. in* Gahnström 1995) studied the decomposition of birch leaves in Swedish lakes in bags with different mesh sizes (1 and 10 mm) in a total of 30 acidified, limed and unaffected lakes (neutral). After eight months the weight loss was greater in the bags with a large mesh, and larger in the unaffected and limed lakes than in the acidified ones. In the large-mesh bags, shredders such as *Asellus aquaticus* (Crustacea, Isopoda) and caddis fly larvae (Trichoptera) can fragment the leaf material (CPOM), while only micro-organisms can affect decomposition in the small-mesh bags. The decline in the number of *A. aquaticus* in acidic lakes certainly contributed to the decline in the rate of decomposition for the alder leaves in acidic Lake Gårdsjön in Sweden.

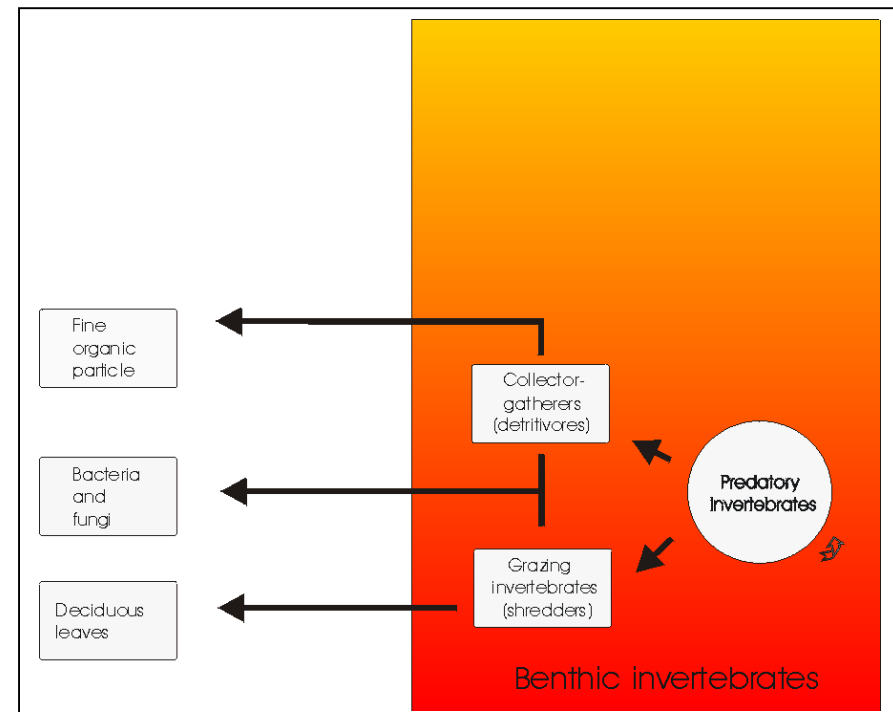
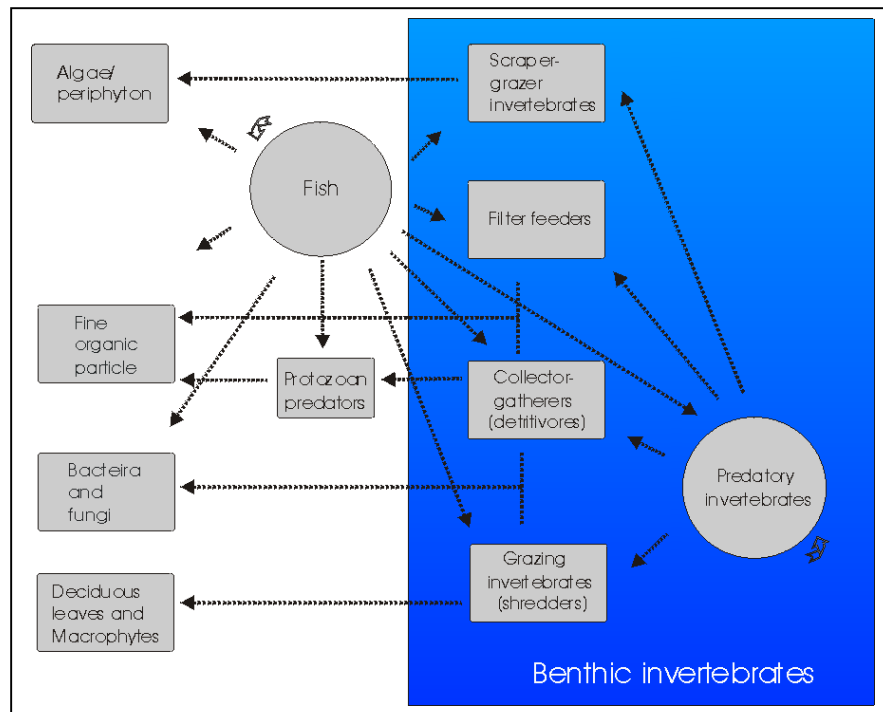
Fjellheim & Raddun (1988) studied the birch leaf processing and associated macroinvertebrates in an acidified lake (pH 4.3 – 4.7) in South Norway. They found that

shredders were the most common group within the chironomids, caddis larvae and stoneflies, and invertebrate predators played an important role as secondary consumers, and probably being an important factor in limiting the abundance of shredder populations. However, I found that the shredding *Hydrozetes lacustris* are likely to contribute most to ecosystems functioning via the processing of litter (decomposition of COPM), and also the primary consumers exert implication of prey – predator interactions (Fig. 88).

The increase of richness species, biomass and abundance from AML 117 may be due not only to the physical conditions (similar pH, redox, conductivity like in AML 111 and AML 107), but due to the low concentration of heavy metal and ions ( $\text{Fe}^{2+}$ ;  $\text{Fe}^{3+}$ ;  $\text{Al}^{3+}$ ;  $\text{SO}_4^{2-}$ ), and also principally due to the biotic condition. Odum (1963) emphasised that the biological community controls ecological succession and that the physical environment determines the patterns of succession but does not cause it.

#### 4.3 Benthic food web of extremely acidic mining lakes

Benthic fauna is directly influenced by acidification through physiological mechanisms and indirectly by changes in the trophic levels and feeding groups in an aquatic ecosystems (Lindegaard 1995). It is not possible to determine exactly the number of trophic levels in an ecosystem, since many species are omnivores or change their diet and behaviour during the course of their life cycles (e.g. larvae of Tanypodinae that can feed on microflora in the early larval stage and has a predator behaviour in the last instars). However, we can view the food web in terms of simple chains in which each link represents a trophic level (Schener 1971, Paine 1980; Pimm 1982) in order to compare the complexity of communities in different water ecosystems. The food chains of this sort can be used to describe the relationship between the trophic levels in a community. Figure 89 shows the diagrammatic representation of the food web in a neutral freshwater lake and in extremely acidic lakes with pH 2 – 3.



**Fig. 89.** General diagrammatic representation of the benthic food web in neutral freshwater lakes (left), and in extremely acidic mining lakes with pH 2 – 3 (right). The arrows display the resource pathways available in the aquatic systems.

The benthic food web of extremely acidic lakes AML 107, 111 and 117 was very short in terms of species richness, trophic level, feeding strategy, and guilds. Guild is defined according to Severinghaus (1981) as a group of species that exploit the same class of environmental resource in a similar way. Benthic invertebrates were separated into three guilds based on factors generally attributable to their feeding habits: a) collectors; b) shredders, and c) predators that reflected the major feeding strategies, and subsequent minor feeding strategies as habit preferences, behaviour patterns or, a combination of the three.

The number of trophic levels is also reduced to three. These levels consist of the: 1) detritus and microorganisms, 2) herbivores and detritivores as primary consumers; and 3) predatory invertebrates as secondary consumers. Therefore, as feeding strategies it comprised basically detritivorous, herbivorous and carnivorous consumers in these lakes (Fig. 88). In the studied acidic mining lakes only the pleustonic *S. nigrolineata* were assigned as a omnivorous/carnivorous. Lessmann *et al.* (1999) reported that the *S. nigrolineata* consume *Euglena mutalis* in the field, but feed on living chironomids in the laboratory. In general many aquatic insects are considered opportunistic omnivorous (Berg 1985).

The detritivore *Chironomus crassimanus* is generalist with collector-gathers feeding habit. It feed on microflora associated to detritus in the investigated acidic lakes. The gut content of *Ch. crassimanus* species from AML 117 consists of a lot of short filamentous bacteria building grass-like bushes, a few algae and fungi. In AML 111 *Ch. crassimanus* fed, principally, on hyphae of fungi, filamentous bacteria arranged in dense bundles and phototrophic organisms. Aquatic fungi and bacteria are likewise a part of the colonisation structure of extremely acidic waters (Suzuki 1961; Sparrow 1968; Wendt-Potthoff & Neu 1998). Fungal organisms are known to maintain a relatively neutral pH – internal pH regulation – by pumping protons out of the cell and by establishing a low proton membrane

permeability (Nicolay *et al.* 1987; Raven 1990). Gross & Robbins (2000) regarded a list with the morphological and chemical characteristics of 81 acidophilic and acid-tolerant fungi and yeast. Suzuki (1961) found aquatic phycomycetes in water of acidotrophic lakes derived from volcanoes with pH-value 2.9 in Japan. Fungi produce extensive branching, filamentous hyphae, which in many species are capable of penetrating organic matter like in deciduous leaves.

Bacteria contribute in many different ways to the acidic environments in which they cohabit. Some of them cause acid formation by excreting  $H^+$  ions directly, others precipitate minerals that release  $H^+$  ions (Ehrlich 1996 *cit. in* E. Robbins 2000), and some of them merely survive in acidic solution. Robbins (2000) emphasised that bacteria can be the major primary producers, some decomposers, and others are prey for phagotrophic acidophilic protozoa.

Bacteria and fungi destroy organic material and are an important segment for the food chain, and principally the feeding of acid-resistant *Chironomus crassimanus* species in extremely acidic mining lakes from Lusatian region. In addition, (see item 3.5.2) laboratory-experiment showed the importance of bacteria in the diet of *Ch. crassimanus* larvae in sediments of AML 111. *Ch. crassimanus* fed intensively on bacteria in the sediment depth up to 8 mm. It proved the feeding habits of the collector for this acid-resistant specie. Actually, the success of these collector-gather consumers depends on the supply of detritus food as fungi, bacteria, and algae, while the shredder consumers *Limnophyes minimus* and *Hydrozetes lacustris* depends on the coarse particulate organic matter (CPOM) (see item 4.2.2). However, the detritus and the microflora associated, and CPOM played a main pathway for benthic invertebrates from the extremely acidic mining lakes with pH 2 – 3.

In the investigated Lusatian acidic mining lakes a specialised benthic invertebrate scraper-grazer and filter-feeder were absent or occasional, either as a result of chemical toxicity (pH-

value; heavy metals concentration) or as a result of limitation by food quality or quantity. Studies by Sutcliffe (1978), Fromm (1980), Hall *et al.* (1980) support the idea that, chemical toxicity may be a limiting factor for the occurrence of the grazer consumers. Also, the absence of scraper-grazer benthic invertebrates observed during the colonisation experiment from AML 107, AML 111, and AML 117 is consistent with the findings of Dobson (1994), who noted that grazers are relatively rare or absent in detrital aggregation.

In Lusatian acidic mining lakes the scraper-grazing and the filter-feeder compartment were occupied by plankton organisms as pelagic rotifers, ciliates, heliozoans (AML 107, AML 111, and AML 117) and also cladoceran species (AML 117). Packroff (2000) regarded that AML 117 is inhabited by the rotifer *Branchionus urceolaris* and the cladoceran *Chydorus sphaericus*. The main group of ciliates found in the plankton were *Urotricha* sp. (Prostomatida) with a raptorial feeding, and Oxytrichidae (Hypotrichida) which ingested nanoalgae (e.g. *Chlamydomonas* and *Chrysophyceae*). The pleuston compartment consist of 5 corixids species with carnivore-feeding habit (*Actcorisa germari*, *Glaenocoprisa p. propinqua* and *Corixa dentipes*) and omnivore (*Sigara nigrolineata* and *S. concinna*) (Wollmann 1998). In AML 107 and AML 111 the ciliates were rarely found in the plankton and the main protozoans components were heliozoans. The pleuston compartment of AML 111 exhibited a great number of corixids species: *Actcorisa germari*, *Glaenocoprisa p. propinqua*, *Corixa dentipes*, *C. dentines*, *Callicorixa praeusta*, *Sigara nigrolineata*, *S. concinna*, *S. striata* and *S. falleni* (Wollmann 1998, 2000).

The benthic food web in the extremely acidic mining lakes from Lusatian is short and simple attributable primarily to the effects of low pH that affects the dynamics of the community. Lessmann *et al.* (1999) and Nixdorf *et al.* (2000) hypothesised that the pelagic

food web is not coupled to the benthic food web in AML 107 and AML 111. The pelagic food web consists of phytoflagellates (mainly *Ochnomonas* and *Chlamydomonas*), and rotifers (mainly *Cephalodella hoody* and *Rotaria rotatoria*), and ciliates species and heliozoans. In addition, the phytobenthic food web of AML 107 consists of diatom *Pinnularia acoricola* (Bacillariophyceae) and *Euglena mutabilis* (Euglenophyta). In AML 111 the phytobenthos are dominated by the acid-tolerant diatom *Eunotia exigua* (Bacillariophyceae).

Furthermore, the investigated acidic mining lakes are fish-free systems. The aquatic insects are the top-predators. Henrikson & Oscarson (1981) reported that in severely acidified lakes in which fish have died out, the actual top-predators are occupied by aquatic insects. AML 117 exhibited a larger spectrum of predatory invertebrate groups than AML 107 and AML 111. The aquatic insects that belong to the groups Megaloptera, Coleoptera, Odonata, and Trichoptera from lake AML 117 were assigned as carnivorous consumers (Giani & Laville 1973; Griffiths 1973; Delettire 1978; Baker & McLachlan 1979; Pophan & Savage 1984). *Sialis lutaria* (Megaloptera) showed the greatest abundance in AML 117. This acid-resistant species proved to play an important role as top-predators in this extremely acidic lake. Larvae of *Orectochilus villosus* (Gyrinidae) were rarely found. In addition, *Gyrinus aeratus* Stephens, 1835 were found in AML 117, but it did not colonise the leaf packs. Also, imagines of *Illybius fenestratus* (Fabricius 1792) (Dysticidae: Colymbetinae) were found and observed in all studied lakes (AML 117, AML 111, and AML 107), but did not colonise the leaf packs.

Hoy, O'Berg & Kauffman (1972), experimenting with larvivorous fish, have suggested that selective early season predation by fish on predaceous insects, particularly dysticids and hydrophilids, may cause the mosquito population to eventually increase when fish are absent. Furthermore, predatory invertebrates as *Coenagrion mercuriale* (Odonata) and Phryganeidae



(Trichoptera) were also found in AML 117. The successful reproduction of these species and the adaptation in extreme acidic conditions is not yet clear.

The presence of the top predator aquatic insects as *Sialis lutaria* (Megaloptera) *Orectochilus villosus* (Coleoptera, Gyrinidae), *Coenagrion mercuriale* (Odonata, Coenagrionidae) and Phryganeidae sp. (Trichoptera) did not depend essentially on the level of pH in the lakes, but on the availability of supplied food resources, principally collectors and shredder consumers (Fig. 90). However, the concentration of iron, aluminium, and sulphate are lower in AML 117 than in AML 111 and AML 107 (see table 2).

Hildrew & Townsend (1976) and Baker (1980) reported the numbers of some Sialidae and Coenagrionidae species are highest where prey populations are the most dense. Corbet (1980) and Tompson (1978b) *cit. in* Perchowsky (1982) reported that hungry damselfly nymphs of Coenagrionidae may abandon perches and search for prey at night.

- ***Donor controlled systems***

The primary consumers as chironomid larvae from Lusatian mining lakes proved to be a main source of food for the invertebrate predators. In these extremely acidic environments, the prey controls the density of the predator consumers and these aquatic communities are governed by species occurrence in which different species are assumed to have an equal opportunity to colonise and compete on equal terms (Begon *et al.* 1990). Appelberg (1995) reported that dominance is the most common controlling factor (dominance controlled) in Swedish limed lakes, where the recruitment of most organisms is strongly seasonal.

Aquatic systems formed principally to detritivores and the decomposers (fungi and bacteria), and their food resources in these extremely acidic mining lakes from Lusatia may be described as *donor – controlled system*. The donor as prey controls the density of the predators, but not the reserve (Pimm 1982). It is suspected that predator densities benefited

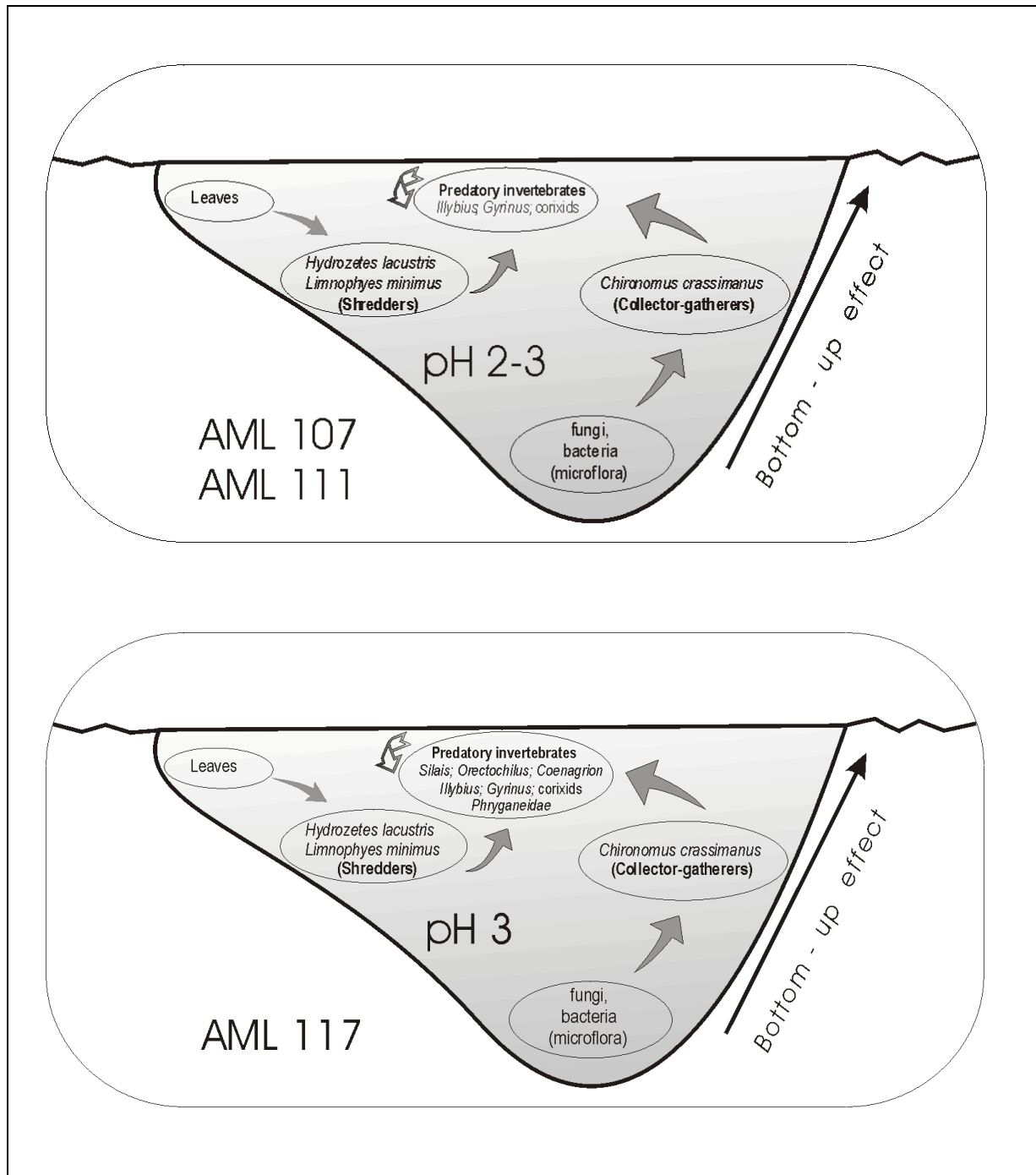


Fig. 90. General representation of the benthic fauna food web of extremely acidic mining lakes. The upper figure (Lake AML 107 and AML 111), and the lower (Lake AML 117) show the benthic invertebrate guilds (shredders; collector-gatherers, and predatory invertebrates), and available food resources (fungi and bacteria and deciduous leaves) for the benthic invertebrates. This acidic aquatic system is described as donor-controlled. The prey (detritivores and decomposer) controls the density of the predators (or consumers) and the strong link of the trophic relationships are governed by the bottom of the food chain (bottom – up effect).

indirectly benefited indirectly from the higher densities of prey (Dobson *et al.* 1994). Pope *et al.* (1999) regarded the same results for a leaf litter colonisation by invertebrates in the littoral zone of a small oligotrophic Scott lake in Canada. The dynamics of donor-controlled models differ in a number of ways from the traditional Lotka-Volterra model of predator – prey interactions, where the equilibrium density of the prey depends on that of the predator (Begon *et al.* 1986).

- ***Bottom – up effects***

Nevertheless, the benthic food webs of extremely acidic mining lakes may be described as detritus-based, due the dominance of detritivores consumers. Hildrew (1992) reported the acid streams are often described as being detritus-based, followed almost simultaneously by secondary consumers, mainly chironomid species and a large array of predator invertebrates that can also feed also upon one another. In the investigated lakes the increase in the food supply leads to a result of a higher level of microflora (bacteria and fungi) activity, and thus an increase in number of collectors, and increase in the decomposition of allochthonous material (CPOM) – that also is one explanation of shredding colonisation. In reality, the benthic food web from this ecosystem has a strong link with the trophic relationships that are governed by the bottom of the food chain. It suggested a *bottom – up effect* (Carpenter *et al.* 1985) for the extremely acidic ecosystems with pH-value from 2 to 3 from Lusatian region.

Comparisons between studies of communities in extremely acidic Lusatian mining lakes often yield considerable similarities. The structure of the benthic invertebrate communities of extremely acidic mining lakes exhibited a low species richness, a short food web with three level trophic and three guilds. In relation to the benthic invertebrates colonisation from Lusatian mining lakes with pH 2 - 3 it leads to some possible explanation for this:

- I. The benthic fauna development in extremely acidic lakes with pH 2 – 3 depends on the opportunities for colonisation of the “acid-resistant” species.
- II. Availability of supplied food resources in this ecosystems is indispensable to the development of the benthic invertebrate communities.
- III. Both the complexity of the benthic food web and the length of the food chain depend on the donor (prey) that controlled the densities of the consumer of higher levels (predators).
- IV. Detritus-base systems offer conditions for the development of the benthic fauna food web described as the bottom – up effect.

## 5 Conclusion

- (a) The benthic invertebrates community is very poor in extremely acidic lakes, comprising low number of species, abundance and biomass.
- (b) *Chironomus crassimanus* (Diptera, Chironomidae, Chironomini) can survive over an extreme range of conditions of pH-value from 2.1 to 3.0, and is being a key acid-resistant species. This pioneer and opportunist species has a large capacity to colonise extreme habitats, which permit their growth and reproduction in the extremely acidic mining lakes in Germany.
- (c) Decomposition of leaves were also affected by acidification. The leaf breakdown processing rates were very slow in extremely acidic Lusatian lakes.

- (d) The shredding *Hydrozetes lacustris* (Acari, Hydrozetidae) and *Limnophyes minimus* (Diptera, Chironomidae, Orthoclaadiinae) may be an important contributor to litter decomposition in extremely acidic mining lakes from Lusatian region.
- (e) The colonisation of litter leaves by benthic invertebrates does not depend on the quality of deciduous leaf species: *Betula pendula* (birch), *Fraxinus excelsior* (ash), and *Juglans regia* (walnut), but seems more related to the quality of associated microflora.
- (f) Detritus and the associated microflora play a main pathway for benthic invertebrates, especially for pioneer *Chironomus crassimanus* from the extremely acidic Lusatian mining lakes with pH 2 – 3. The availability of food is a principal factor for the increase of densities and species richness in these extreme ecosystems and has a strong link between the trophic relationships that are governed by the bottom of the food chain, described as bottom – up effect.
- (g) The benthic food web of extremely acidic lakes of Lusatian region in Germany (AML 107, AML 111 and AML 117) was very short in terms of species richness, guilds, feeding habits and functional feeding groups.
- (h) The presence of the top predator aquatic insects as *Sialis lutaria* (Megaloptera), *Orectochilus villosus* (Coleoptera, Gyrinidae), *Coenagrion mercuriale* (Odonata, Coenagrionidae) and Phryganeidae (Trichoptera) did not depend on the level of pH in the lakes, but on the availability of food resources. The chironomids may proved to be a main source of food for the invertebrate predators. These extreme environments may be described as a donor-controlled dynamic system in which the prey controls the density of the predator consumers.

## 6 Summary

Acidic mining lakes (open pit lakes) are peculiar environments, not only in terms of physical and chemical characteristics, but also principally in terms of biological and ecological interactions. The structure of the benthic invertebrate communities were investigated in terms of composition, abundance, and biomass from extremely acidic lakes with pH values from 2 to 3 in areas where coal was intensively mined in the Lusatian region in the eastern region of Germany. Benthic invertebrates colonisation on leaves and the breakdown rate processing of the three deciduous leaf: *Betula pendula* (birch), *Fraxinus excelsior* (ash), and *Juglans regia* (walnut) were investigated. Also, the main key-species of these acidic environments were investigated, in terms of description of pupal exuviae of *Chironomus crassimanus* and the feeding habit of this acid-resistant species through analysis of their gut content.

*Chironomus crassimanus* (Diptera, Chironomidae) is a pioneer and the most important benthic invertebrate species in the extremely acidic mining lakes in eastern Germany. *Ch. crassimanus* may be considered an opportunist species that have a large capacity to colonise in extreme habitats. They can survive over a specific range of conditions of pH-value from 2.1 to 3.0, and still grow and reproduce. It is a detritivore with collector- gatherers feeding habit, consuming mainly fungi, bacteria and algae associated to detritus in the lake sediments.

The benthic food web in extremely acidic mining Lusatian lakes is very short in terms of species richness, trophic relationship, guilds and functional feeding groups. Collector-filters and scraper-grazers were absent in extremely acidic mining lakes (AML 107, AML 111 and AML 117). Shredders as *Limnophyes minimus* (Diptera, Chironomidae, Orthocladiinae) and *Hydrozetes lacustris* (Acari, Hydrozetidae) occurred in low abundance in AML 107 and AML 111, and it may be in response to slow leaf breakdown process in these ecosystems, except in

AML 117 where the *H. lacustris* contributed most to ecosystems functioning via the processing of litter.

Aquatic insects as *Sialis lutaria* (Megaloptera, Sialidae), *Orectochilus villosus* (Coleoptera, Gyrinidae), *Coenagrion mercuriale* (Odonata, Coenagrionidae), and Phryganeidae (Trichoptera) are the top-predators of these ecosystems. They did not depend on the level of pH in the lakes, but on the availability of food resources. These fish-free systems are governed by detritus-base, that is the bottom of the food chain. Therefore this ecosystem can be considered as bottom – up effect. Detritivores, fungi and bacteria (decomposers), and their food resource are a main pathway for the benthic process in extremely acidic mining lakes. This system may be described as donor-controlled dynamics, in which the donor (prey) controls the density of the predators.

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## 8 Abbreviations

ANOVA	variance of analyse
AML	acidic mining lake (s)
a.s.l.	above sea level
CLSM	confocal laser scanning microscope
CPOM	coarse particulate organic matter
DOM	dissolved organic matter
e	exponential
e.g.	example given
°C	grades Celsius
cf.	conform
Fig.	figure (s)
FPOM	fine particulate organic matter
g	gram
G	number of same species in the place
h	hour
hrs	hours
ind.	individual (s)
$k$	rate processing coefficient
km	kilometre (s)
$K_S$	basic neutralisation capacity
$K_B$	acid neutralisation capacity
max	maximal
mg	milligram (s)
$m^2$	square meter (s)
$km^2$	square kilometre (s)
k	rate processing
l	litre
ln	natural logarithmic
LW	leaf weight loss
$LW_t$	amount weight of leaf material in determined time
$LW_0$	initial weight of leaf material
min	minute (s)

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ML	mining lakes (s)
mm	millimetre (s)
mmol	millilitre per molar
MPN	most probably numbers
N	north
n	number
ÖHMI	ÖHMI laboratory, Magdeburg
pH	potential hydrogenic
QS	qualitative similarity
%R	remaining percentage data of leaf weight loss
S	species number
S.D.	standard deviation
SRP	soluble reactive phosphorous
t	time
Tot	total
P <sub>Tot</sub>	total phosphor
vol.	volume
UFZ	Umweltforschungszentrum Leipzig-Halle GmbH
USA	United States of America
µm	micrometer (s)
W <sub>t</sub>	total weight
W <sub>0</sub>	initial weight

## 9 List of figures

**Fig. 1.** Localisation map of the study area in south-eastern of north Germany (Modified map after Boehrer *et al.* 2000).

**Fig. 2.** Localisation of the Lake ML B in the region Schalanberndorf-nord from Lusatian region, eastern Germany.

**Fig. 3.** Localisation of the mining lakes (AML 117, AML 111 and AML 107) in the Lauchhammer-Plessa region from Lusatia, eastern Germany.

**Fig. 4.** Physical parameters: temperature, electric conductivity, oxygen concentration, pH-value, redox, and turbidity of ML B from Lusatian region in eastern Germany.

**Fig. 5.** Physical parameters: temperature, electric conductivity, oxygen concentration, pH-value, redox, and turbidity of AML 117 from Lusatian region in eastern Germany.

**Fig. 6.** Physical parameters: temperature, electric conductivity, oxygen concentration, pH-value, redox, and turbidity of AML 111 from Lusatian region in eastern Germany.

**Fig. 7.** Physical parameters: temperature, electric conductivity, oxygen concentration, pH-value, redox, and turbidity of AML 107 from Lusatian region in eastern Germany.

**Fig. 8.** View of Lake AML 117 (Grünewald) from Lusatian region (pH 3.0). It was a residual area of the lignite mining activities. The starting period of the mine was about 1956 and the mine was abandoned in 1966. Surrounding vegetation and aquatic vascular plants covered the littoral zone of this acidic lake. It is a recreational area, including a camping place. Lake AML 117 with acidity of 2 mmol.l<sup>-1</sup> is thermally stratified during the summer months, and the epilimnion covered 0 – 8 m and the hypolimnion 10 – 12 m (Packroff 2000).

**Figs. 9 A, B.** View of Lake AML 111 (Plessa) from Lusatian region (pH 2.6 – 2.9). Lake AML 111 was a residual area of the mining activities. The starting period of this mine was about 1929. This mine was abandoned in 1956/58, and the inflowing groundwater was completed in 1969. A surrounding forest is present around the lake.

**Figs. 10 A, B.** View of open pit lake AML 107 (Plessa) from Lusatian region, which exhibited water with very low pH (min. pH 2.1). It was a residual area of the coal mine activities in the period of 1897 until 1928. Lake AML 107 is a closed lake with no inflow or outflow. The surrounding vegetation was removed during mining activities and had suffered from the falling or the recently recovering groundwater table. Stratification in Lake AML 107 lasted only from ice-cover (January – March 1996) until autumn 1996 (Herzprung *et al.* 1998) whereas a stable stratification was not detectable in 1997 (Packroff 2000). The epilimnion was in the ranges of 0 – 3 meters. The inflow of anoxic groundwater and the oxidation of  $\text{Fe}^{2+}$  are responsible for the oxygen decrease in the hypolimnion and above the sediment (Lessmann *et al.* 1999).

**Fig. 11.** Field experiments. A) Leaf pack unit for benthic invertebrate colonisation experiments; B) Leaf pack units (birch, ash and walnut leaves); C and D) Exposure of leaf pack units in mining lakes.

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