



## Benthic communities in inland salinized waters with different salinities and nutrient concentrations and the ecology of *Chironomus aprilius* (Diptera: Chironomidae) in the Czech Republic

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**Abstract.** The macrozoobenthos in saline pools at dumps in a former coal mining area was studied over a period of two years. Due to specific environmental conditions these pools are unique in the Czech Republic. Extremely high values of salinity (up to 11‰) along with a low concentration of dissolved phosphorus (0.01–0.1 mg.l<sup>-1</sup>) are typical of some of the water in this area. The pools were grouped into three categories based on their conductivity values and treated using cow dung, municipal wastewater treatment sludge and inorganic NPK (nitrogen-phosphorus-potassium) fertilizer at doses recommended for carp ponds. The application of fertilizer had a positive effect on the density and biomass of all the groups in the macrozoobenthos. The highest and the lowest increases in macrozoobenthos biomass were recorded after the application of NPK and cow dung, respectively. However, the application of fertilizer had no effect on the diversity of macrozoobenthos. *Chironomus aprilius*, recorded in the Czech Republic for the first time, inhabited all pools with conductivity ranges of between 5,000–16,000 µS.cm<sup>-1</sup>. The density of *C. aprilius* larvae increased with increasing salinity reaching a maximum of about 17,083 ind.m<sup>-2</sup> (biomass – 82 g.m<sup>-2</sup>). Analysis of *C. aprilius* phenology revealed a bivoltine pattern with the summer generation of larvae reaching a maximum in June–July and the overwintering generation in October to November.

### INTRODUCTION

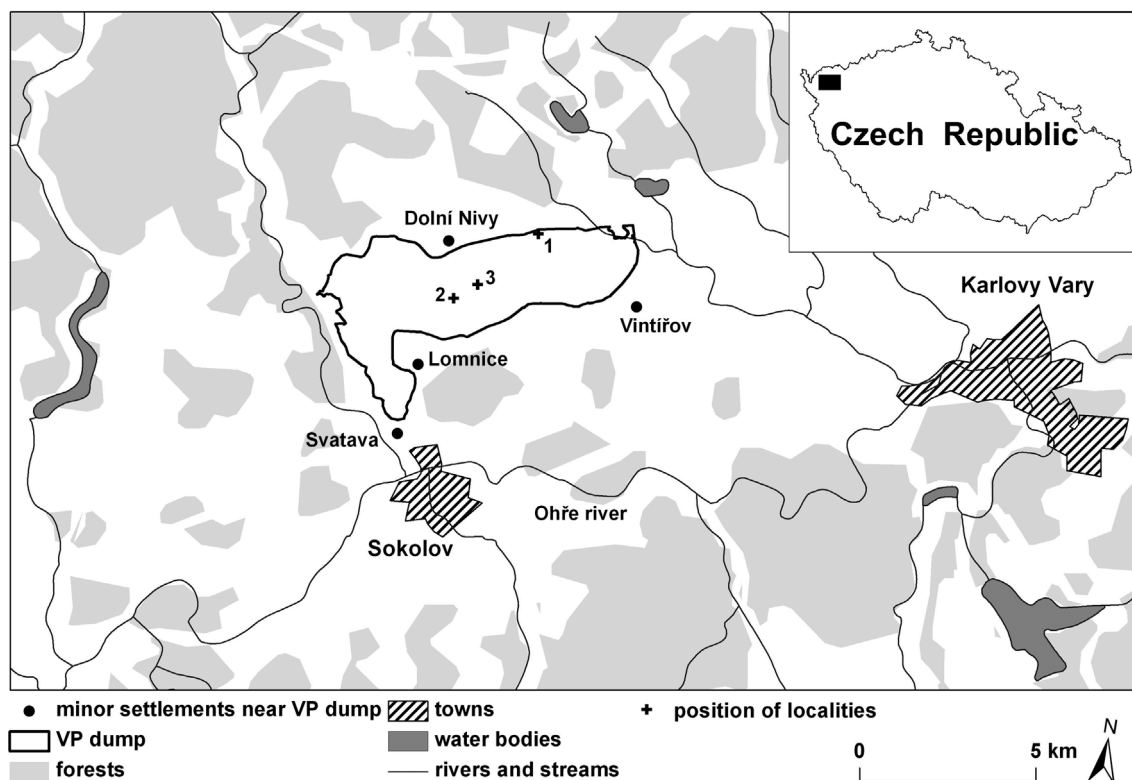
The majority of aquatic diptera are restricted to freshwater and only a few species tolerate high salinities. Inland saline waters are unique habitats with typical communities of organisms tolerant of adverse and fluctuating environmental conditions. These systems provide a unique opportunity to study the composition of their biological communities and how they respond to changes in their physico-chemical characteristics. The species diversity in the benthic communities in these waters is very low and often dominated by a few tolerant species, which can however occur in high numbers.

*Chironomus aprilius* Meigen, 1818 (syn. *Chironomus halophilus* Kieffer, 1913, *Chironomus obscurus* Goetghebuer, 1921) is a halophilous species regularly inhabiting coastal biotopes. It occurs in the shallow southern part of the Baltic Sea (Thienemann, 1954; Palmén & Aho, 1966; Zettler et al., 1996), the Aral Sea (Aladin et al., 2005), coastal marshes in Spain (Fuentes et al., 2005) and flooded brackish river lagoons in England (Rehfish, 1994) and Ireland (Healy, 1997). *C. aprilius* together with *C. salinarius* (Kieffer) and *Microchironomus deribae* (Kieffer)

are tolerant of a wide range of saline conditions (Parma & Krebs, 1977). *C. aprilius* is also found together with *C. riparius*, *C. plumosus* and *C. piger* along the coast of the Mediterranean in North Africa in sediments in brackish lagoons (Ramdani et al., 2001).

All the above mentioned localities are mesohaline (salinity 3–8‰) to polyhaline (salinity 18–30‰) waters located close to marine ecosystems. There are only a few records of *C. aprilius* in saline inland waters and brackish salt-marshes (5–29‰) (Thienemann, 1954; Yadav, 2003). *C. aprilius* is able to cope with brackish water conditions < 30‰ because of its ability to tolerate an increase in the osmotic concentration of its blood (Cheng, 1976).

The aims of this study were: (i) to describe the benthic communities in saline pools at a coal mining dump in western Bohemia, Czech Republic and their response to gradients in salinity and nutrients, with special attention to *C. aprilius*, a halophilous species, which is here recorded in the Czech Republic for the first time, (ii) to explain the factors affecting its distribution and phenology and (iii) to follow the effect of applying fertilizer on the biotic communi-



**Fig. 1.** Map of the Velká Podkrušňohorská (VP) dump with an inset showing its location within the Czech Republic. Numbers 1 to 3 indicate the position of localities studied.

ties and the use of fertilizer for mitigating and accelerating the revitalization of abandoned mining areas.

## MATERIAL AND METHODS

### Study area

This study was carried out in the Sokolov basin, a brown-coal mining region in the west of the Czech Republic. Macrozoobenthos communities were sampled in aquatic habitats created for restoring habitats in the open-cast mine or in areas outside the mining area where overburden from the open-cast mine was dumped (Pecharová et al., 2001).

The Podkrušňohorská dump covers an area of 2,000 ha and is located 3 km north-west of the city of Sokolov (50°12'–50°14'N, 12°38'–12°42'E) (Fig. 1). It is the largest dump in the Czech Republic and consists of overburden from the Jiří, Družba and Lomnice brown coal mines. The overburden consists mostly of montmorillonite kaolinite-illite clays and claystones (Kříbek et al., 1998). Many pools developed spontaneously at this dump or are newly established as part of the hydric restoration of this former coal-mining region.

Waters in mining areas are often highly acidic due to the leaching by drainage water of compounds present in mine tailings. Thanks to the high neutralization ability of clay, acidification by acid mine drainage (hereafter AMD) water does not play as important a role in the area studied as it does in other coal mining areas throughout the world (Nixdorf et al., 1998; Geller et al., 2000). The pH values of the water bodies we studied were close to neutral (Table 1). High concentrations of dissolved solids (conductivity up to 16,000  $\mu\text{S}\cdot\text{cm}^{-1}$ ), high alkalinity (8.5–15.5  $\text{mmol}\cdot\text{l}^{-1}$ ) and low concentrations of dissolved phosphorus (0.06–0.7  $\text{mg}\cdot\text{l}^{-1}$ ) are typical of water at the Podkrušňohorská dump. Salinity of 3–11‰ corresponds to slightly brackish (mesohaline) water (Healy, 1997; Pitter, 2009). Unlike in seawater, the pre-

vailing ions include the bivalent ions of calcium, magnesium and sulphates (70% of total dissolved solids) together with carbonates, instead of the monovalent ions of sodium and chloride in sea water, which contains up to 70% sodium chloride. Other indicators were within the range of surface waters in the Czech Republic, see Table 1.

The pools were grouped into three categories according to their locality, conductivity and presence of littoral and submerged vegetation (Table 1). The conductivity recorded in the pools at the different localities did not overlap. The pools had a surface area of 15–45  $\text{m}^2$  at locality 1; 20  $\text{m}^2$  at locality 2 and 250–1000  $\text{m}^2$  at locality 3. The depth was about 30 cm at localities 1 and 2 and 50 cm at locality 3.

Application of fertilizers was used to determine the possibility of accelerating the biotic recovery of the pools studied. Cow dung, municipal wastewater treatment sludge or inorganic NPK fertilizer (commercial CERERIT, N – 15%,  $\text{P}_2\text{O}_5$  – 15%,  $\text{K}_2\text{O}$  – 15%) was added to particular pools in early August 2001. NPK fertilizer was also added to pools at locality 2 in early September as shown in Table 1. The dose of fertilizers was chosen so that the final concentration of TP was 0.8  $\text{mg}\cdot\text{l}^{-1}$  in the pools to which fertilizers were added. The wastewater treatment sludge and cow dung were applied at the maximum dose of 5  $\text{t}\cdot\text{ha}^{-1}$ , which is the dose applied to semi-intensively managed carp ponds.

### Environmental and biological sampling

Water samples, 100 ml each, were collected monthly from each pool. The pH, conductivity and oxygen concentration were measured in situ using a Multiline P4 device (WTE, Germany). Salinity ( $\text{mg}\cdot\text{l}^{-1}$ ) was calculated from the conductivity measurements using conversion factor 8 as per Pitter (2009). In the laboratory,  $\text{NH}_4^+$ ,  $\text{NO}_3^-$ ,  $\text{NO}_2^-$ ,  $\text{PO}_4^{3-}$ ,  $\text{SO}_4^{2-}$ ,  $\text{Cl}^-$ , total N and total P concentrations were measured after filtration (GF/C filters), using a flow injection analyses method and a Tecator FIA 5042 Star instrument

**Table 1.** Characteristics and chemical parameters recorded at the localities studied and carp ponds in the Czech Republic (highest values are in bold).

	Unit	Locality 1	Locality 2	Locality 3	Average values of carp ponds in the Czech Republic (Hartman et al., 1998)
Status		artificial	artificial	natural	
Number of pools		12	11	4	
Age of pools (years)		2	1	10	
Vegetation		<i>Chara</i> sp. <i>Typha angustifolia</i> <i>Potamogeton</i> sp.	no vegetation artificial substrate in water column	<i>Chara</i> sp. <i>Typha angustifolia</i> <i>Potamogeton</i> sp.	
Treatment/ number of pools		NPK/4 Sludge/4 Control/4	NPK/2 Sludge/2 Cow dung/2 Control/5	NPK/3 Control/1	
Salinity	mg.l <sup>-1</sup>	3200–5120	7200–9200	9840–13040	
pH		7.4–8.7	7.8–8.4	8.2–8.3	5.5–9.5
Alkalinity ANC <sub>4.5</sub>	mmol.l <sup>-1</sup>	8.5–12.6	6.4–8.8	11.2–15.8	0.25–6
Conductivity	μS.cm <sup>-1</sup>	4000–6400	9000–11500	<b>12300–16300</b>	100–600
N-NH <sub>4</sub>	mg.l <sup>-1</sup>	0.06–0.79	0.18–0.99	0.3–2.3	0.01–1.2
N-NO <sub>3</sub>	mg.l <sup>-1</sup>	0.14–0.63	0.44–1.15	0.08–0.25	0.05–3
TP	mg.l <sup>-1</sup>	0.06–0.15	0.08–0.15	0.08–0.77	0.025–1.4
Na <sup>+</sup>	mg.l <sup>-1</sup>	130–670	1700–2520	<b>2200–2900</b>	4–85
K <sup>+</sup>	mg.l <sup>-1</sup>	20–30	20–30	<b>40–50</b>	1–35
Ca <sup>2+</sup>	mg.l <sup>-1</sup>	30–130	90–120	<b>160–250</b>	10–100
Mg <sup>2+</sup>	mg.l <sup>-1</sup>	80–240	330–400	<b>740–910</b>	1–60
Cl <sup>-</sup>	mg.l <sup>-1</sup>	1.8–2.9	0.7–1.2	0.5–0.8	5–90
SO <sub>4</sub> <sup>2-</sup>	mg.l <sup>-1</sup>	1700–4100	5200–9800	<b>13800–15800</b>	30–250
Fe	mg.l <sup>-1</sup>	0.2–0.5	0.1–0.7	0.2–0.7	0.05–0.6

(Parsons et al., 1984). The concentrations of Ca, Mg, K, Na and Fe cations were determined using the AAS method and a Varian SpectraAA-640 instrument (Varian Inc., <http://www.varianinc.com>). Alkalinity was measured by potentiometric titration with 0.1 M HCl.

Samples of macrozoobenthos were collected monthly from July until November 2001 (except August) and from February till October 2002 (except August). Each sample consisted of 10 subsamples pooled together. Individual quantitative subsamples were collected using a plastic cylindrical corer (working area 96 cm<sup>2</sup>). Samples were washed through a sieve (1.0 mm mesh size) and fixed using 6% formaldehyde. The remaining benthic invertebrates on the sieve were sorted by hand in the laboratory. Biomass was assessed as formalin wet weight. In each sample, the biomass and species composition of the macrozoobenthos were determined. The larvae of *C. aprilius* were identified morphologically (Webb & Scholl, 1985) and cytotoxonomically using the polythene chromosomes in the salivary glands (Keyl & Keyl, 1959; Keyl, 1962) of a sample of unpreserved material. Size data for all the *C. aprilius* larvae were assessed in terms of body length (measured from the anterior margin of the labrum to the end of the abdomen) and larval instar based on head width (at the widest point of head capsula) (Saether, 1980).

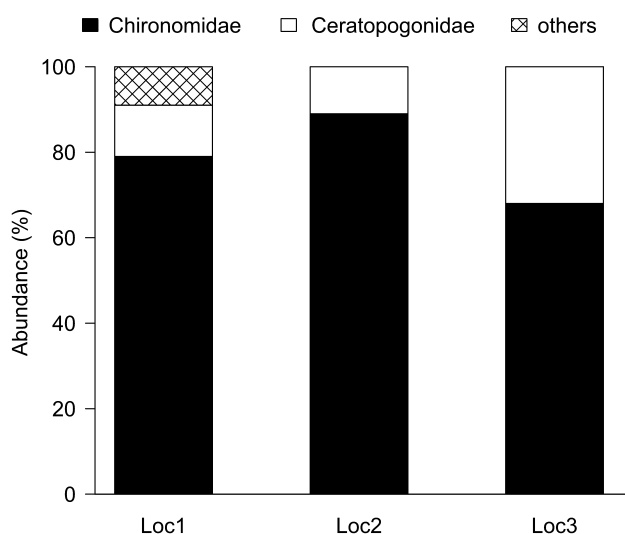
### Statistical analyses

Direct gradient redundancy analysis (RDA) in Canoco for Windows 4.5 software (Ter Braak & Šmilauer, 1998) was used to evaluate the association of all the environmental variables recorded (vegetation cover, alkalinity, type of fertilization, chlorophyll a, conductivity, Cl<sup>-</sup>, NH<sub>4</sub><sup>+</sup>, PO<sub>4</sub><sup>3-</sup> and seasonality as individual month of observations) with the macrozoobenthos community [total abundance of zoobenthos, abundance and biomass of *C. aprilius*, the percentage of *C. aprilius*, Shannon-Wiener index  $H' = -\sum p_i \ln p_i$  ( $H'$  – calculated using natural logarithms of relative densities of particular taxa)] and equitability index  $E = H'/\ln S$ , where  $S$  is the number of taxa in a sample. Statistical significance of each analysis was tested using the Monte Carlo

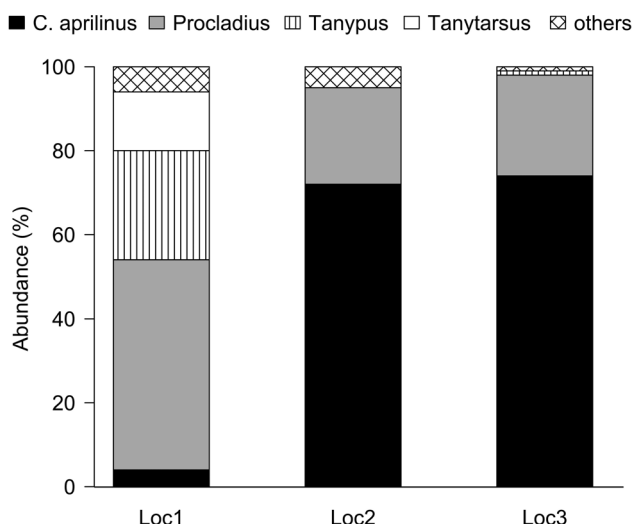
permutation test with 499 permutations as a time series and pools set as covariates. The analysis was visualized using CanoDraw software (Ter Braak & Šmilauer, 2002). Level of significance was set to  $\alpha \leq 0.05$  in all statistical comparisons.

For analysis of differences in species abundance/numbers between localities two-way ANOVA and for analyses of statistical comparison of average *C. aprilius* abundance in different treatments one-way ANOVA in STATISTICA software version 12.0 (Statsoft Inc., 2008) was used.

The association between presence/absence of *C. aprilius* and environmental factors (vegetation cover, age of reservoirs, water electric conductivity) and their interactions was tested using a generalized linear model with a binomial error distribution and a logit link.



**Fig. 2.** Percentage composition of macrozoobenthos at localities 1–3. Mean percentage abundance for all the pools at each locality is presented.



**Fig. 3.** Percentage composition of chironomid larvae at localities 1–3. Mean percentage abundance for all pools at each locality is presented.

## RESULTS

### Occurrence of macrozoobenthos at the different localities

Chironomid larvae dominated the benthic communities' at all three localities, making up 70–90% of the total macrozoobenthos abundance (Fig. 2). At localities 2 and 3, the percentage made up of *Chironomus* larvae exceeded 70% (Fig. 3). Larvae of *C. aprilinus* made up on average 72% of the total chironomid abundance at locality 2 (conductivity 9,000–11,500  $\mu\text{S}\cdot\text{cm}^{-1}$ ) and 74% at locality 3 (conductivity > 12,000  $\mu\text{S}\cdot\text{cm}^{-1}$ ).

The abundance of particular groups of macrozoobenthos (Chironomidae, Ceratopogonidae and others) differed significantly between localities (Two-way ANOVA,  $F_{(4, 849)} = 4.824$ ,  $p = 0.00075$ ). The highest species richness was recorded at locality 1 where the other categories (Ephemeroptera, Odonata, Coleoptera, Diptera: Stratiomyidae) made up about 10% of total numbers. At the other two localities they made up less than 1% of the total numbers (Fig. 2). Similarly the number of chironomid taxa differed significantly between localities (Two-way ANOVA,  $F_{(8, 1415)} = 18.890$ ,  $p < 0.00001$ ). At localities 2 and 3 *C. aprilinus* made up about 70% of all chironomids and at locality 1 only 5%. On the other hand, larvae of *Procladius* made up 50% of all chironomids at locality 1 and only 23% at localities 2 and 3.

The highest species diversity was recorded in pools with the lowest conductivity at locality 1 (4,000–6,400  $\mu\text{S}\cdot\text{cm}^{-1}$ ). Larvae of *Procladius*, *Tanypus* and *Tanytarsus* made up more than 90% whereas *Chironomus* larvae made up only 4% of all chironomid larvae and were found in only 5 of the 12 pools studied (Fig. 3). An exceptionally high density of 10,417 ind. $\cdot\text{m}^{-2}$  (biomass 21 g. $\cdot\text{m}^{-2}$ ) of *C. aprilinus* was recorded in June 2002 at one pool with a conductivity of 5,700  $\mu\text{S}\cdot\text{cm}^{-1}$ . The numbers were lower by an order of magnitude in the other pools at locality 1. The diversity index  $H'$  was significantly positively correlated with the presence of aquatic vegetation (Fig. 6).

### Factors affecting abundance and biomass

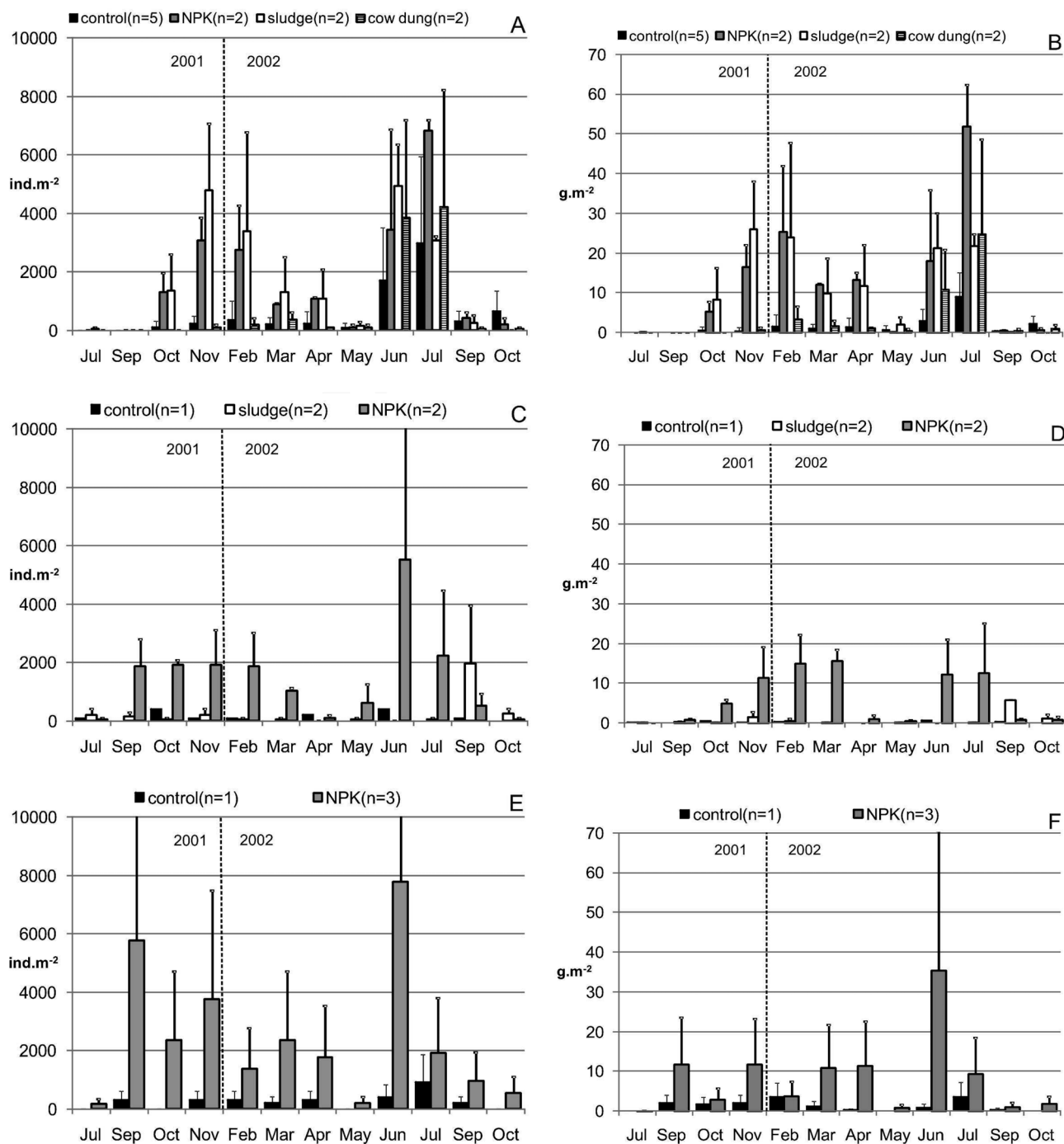
The application of fertilizers had a positive effect on zoobenthos at all three localities. The highest average abundance of *C. aprilinus* was recorded in pools fertilized with NPK at all three localities in 2002 (Fig. 4). The difference between the densities of *C. aprilinus* recorded in pools fertilized with NPK and those treated with sludge and the control pools that were not treated with fertilizers was significant (One-way ANOVA,  $F_{(3, 272)} = 11.22$ ;  $p < 0.001$ ) (Fig. 5).

The highest average densities of *C. aprilinus* were recorded in pools treated with inorganic NPK in 2002 (Fig. 4). The only exception were the pools at locality 2 in the first two months after the application of wastewater treatment sludge (October and November 2001). Maximum densities were recorded in some pools at locality 2 in November 2001 (7,083 ind. $\cdot\text{m}^{-2}$ , 38 g. $\cdot\text{m}^{-2}$ ) and June, July 2002 (6,875 ind. $\cdot\text{m}^{-2}$ , 35.8 g. $\cdot\text{m}^{-2}$ ), (7,188 ind. $\cdot\text{m}^{-2}$  (62.4 g. $\cdot\text{m}^{-2}$ ), respectively. At locality 3, *C. aprilinus* reached peaks in abundance in September 2001 (10,208 ind. $\cdot\text{m}^{-2}$ , 23.7 g. $\cdot\text{m}^{-2}$ ) and June 2002 (17,083 ind. $\cdot\text{m}^{-2}$ , 66.4 g. $\cdot\text{m}^{-2}$ ). All these high values were recorded in pools fertilized with NPK. In control pools without fertilization at this locality, *C. aprilinus* made up between 10–20% of total abundance of macrozoobenthos and 80% of the larvae belonged to the family Ceratopogonidae.

Factors affecting the abundance and biomass of *C. aprilinus* are presented in Fig. 6. The closer the explanatory arrows and explained variables are the closer they are positively correlated. Opposite direction of arrows indicates a negative correlation. The variability in the abundance and biomass of *C. aprilinus* and total diversity ( $H'$ ) is best explained by the time of year – June (4% of total variance explained by all factors,  $F = 11.99$ ,  $p = 0.002$ ). After including this variable in the model, most of the residual variance

**Table 2.** GLM analysis of the relationship presence / absence of *Ch. aprilinus* and the age of the pools, conductivity and presence of vegetation (\*\* $p < 0.001$ , \* $p < 0.01$ , \* $p < 0.1$ , n.s. –  $p > 0.5$ ).

	Df	Dev.Resid.	Df Resid.	DevPr	p level
vegetation	1	15.586	284	380.54	***
age	1	44.295	283	336.25	***
conductivity	1	1.541	282	334.71	n.s.
vegetation × age	1	5.987	281	328.72	*
vegetation × conductivity	1	4.034	280	324.69	*
age × conductivity	1	0.033	279	324.66	n.s.
vegetation × age × conductivity	1	9.825	279	314.83	**



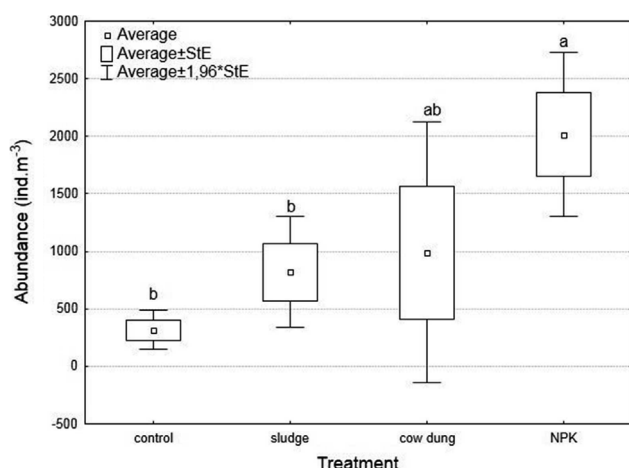
**Fig. 4.** Effect of fertilizers on the abundance (left column) and biomass (right column) of *C. aprilius*; locality 1 (A–B), locality 2 (C–D), locality 3 (E–F); n = number of pools with *C. aprilius*.

is explained by the presence of vegetation (3%,  $F = 9.53$ ,  $p = 0.002$ ), November (2%,  $F = 7.21$ ,  $p = 0.002$ ), chlorophyll a (2%,  $F = 8.88$ ,  $p = 0.008$ ), application of NPK (2%,  $F = 7.02$ ,  $p = 0.002$ ) and one percent of the variability can be explained by the following factors: July, alkalinity, February, March, control. Further variables proved to be insignificant.

The results of the GLM (Table 2) revealed a negative correlation between the occurrence of *C. aprilius* and the presence of vegetation in pools but, this relationship is probably indirect as the presence of vegetation is primarily correlated with the salinity of habitats.

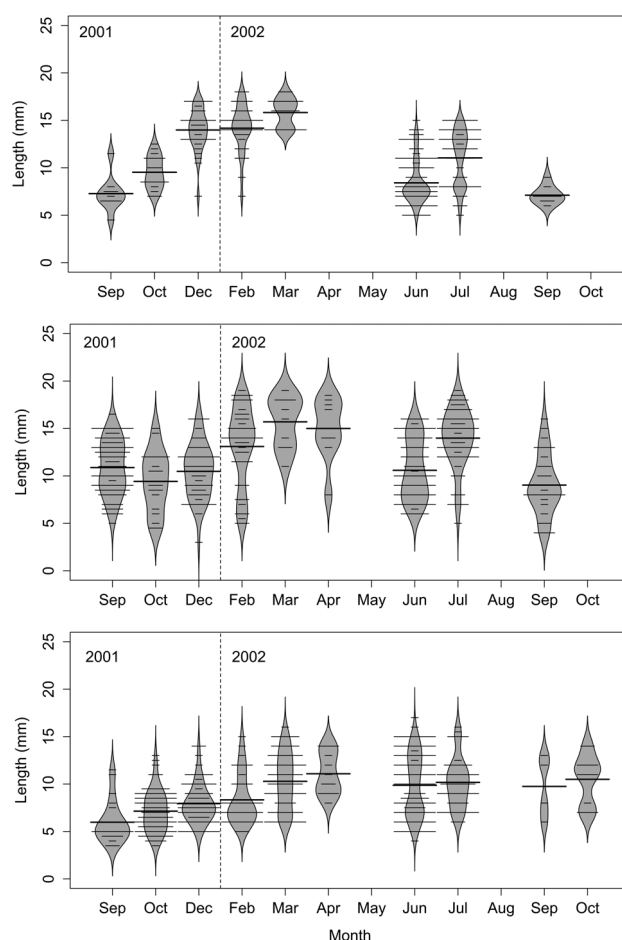
### Seasonal dynamics

Average densities and biomass of *C. aprilius* larvae changed seasonally. Maxima were recorded during autumn (September to November) and summer (June to July) (Figs 4 and 6). The summer maximum biomass was higher than that recorded in autumn. Length analysis (Fig. 7) and changes in abundance and biomass (Fig. 4) indicated a bivoltine cycle in the population. Larvae of the 3<sup>rd</sup> instar (length 3–9 mm) dominated in September to October. They grew during the winter and the highest occurrence of 4<sup>th</sup> instar larvae was recorded in February and March. The emer-



**Fig. 5.** Statistical comparison of average abundance of *C. aprilius* in the different treatments. One way ANOVA, different letters above boxes indicate significant differences ( $p < 0.001$ ).

gence of the overwintering generation started in March/April at locality 1, depending on weather. Mass emergence took place in May at localities 2 and 3. After that low numbers of *C. aprilius* larvae were recorded at all localities in May (Fig. 4). Larvae of the 1<sup>st</sup> and 2<sup>nd</sup> instars were regularly found in zooplankton samples taken at all three localities in March to July. The emergence of the summer generation was more protracted and occurred during August and



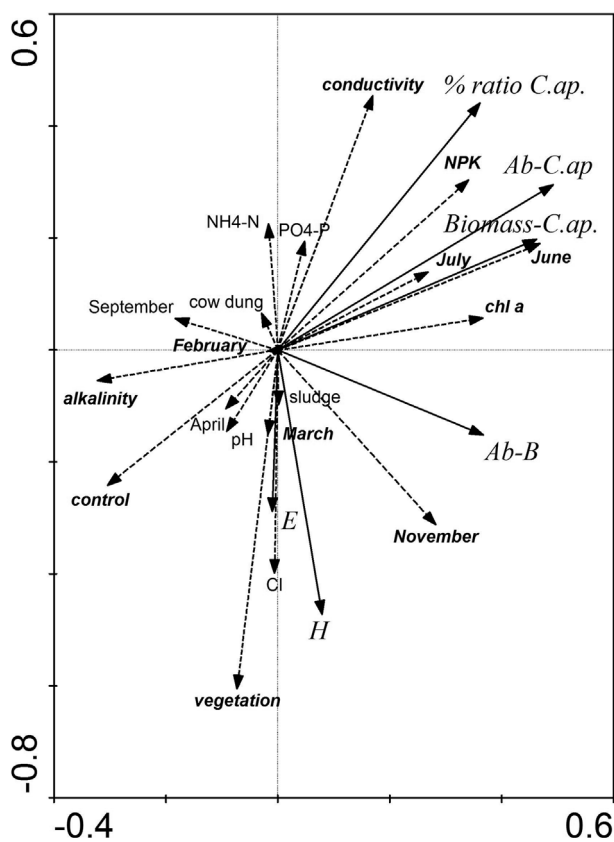
**Fig. 7.** Length frequency distributions of *C. aprilius* larvae over time. Upper panel – locality 1, middle – locality 2, bottom panel – locality 3. Bold horizontal bars indicate the average length for a given month, narrow bars indicate numbers of larvae in a given length class. Each bean plot shows the length frequency recorded in each month and is smoothed using a Gaussian kernel.

September. In September the larvae of the 4th instar disappear from the benthos and the population was dominated by 3<sup>rd</sup> instar larvae of the overwintering generation (Fig. 7). The two generations were well separated at localities 1 and 2, whereas they overlapped at locality 3.

## DISCUSSION

Saline pools at the dumps in a former coal mining area are unique habitats with a fauna found nowhere else in the Czech Republic. The inland saline pools differed from coastal waters in their water chemistry. The salinity was due mainly to sulphates and corresponds to mesohaline conditions. *Chironomus aprilius* was recorded at these localities in the Czech Republic for the first time. Besides *C. aprilius*, other halophilous organisms were also recorded, e.g., the rotifer *Hexarthra fennica* (Levander) and larvae of *Ephydra* cf. *riparia* (Diptera: Ephydriidae).

The macrozoobenthos was dominated by chironomids in both recent and old pools, and the occurrence of *C. aprilius* was positively correlated with salinity. Strenzke (1960) reports females only depositing eggs in brackish water with a chloride content of 4000–13700 mg l<sup>-1</sup> under experimental conditions. The same author found this species in natu-



**Fig. 6.** Results of RDA analysis of selected variables. First axes explain 16.5% of variability, second 4.1%. (Marks in bold and italics are statistically significant.) *Ab-C.ap* – abundance of *C. aprilius*, *Ab-B* – abundance of total benthos, *H* – Shannon-Wiener index, *E* – equitability index.

ral conditions in Germany at salinities of 1,200–16,100 mg l<sup>-1</sup>. Tourenq (1975) reports incidental occurrence in fresh water but gave 1,500–6,000 mg l<sup>-1</sup> as the normal range. He found, however, larvae of this species in places where the chloride content was as high as 15,000–22,000 mg l<sup>-1</sup>. Steenbergen (1993) cites a mean of 5,800 mg l<sup>-1</sup> and rarely recorded larvae below 1,000 mg l<sup>-1</sup>. Vallenduuk in Eggermont et al. (2008) reports conductivity limits of 3,000–5,000 µS.cm<sup>-2</sup> for the occurrence of *C. aprilius*.

These results are in good agreement with the salinity range recorded at the pools studied (3,200–13,040 mg l<sup>-1</sup>). However, at the locality with the lowest salinity (3,200–6,400 mg l<sup>-1</sup>) not all pools were inhabited by *C. aprilius*.

The biomass of benthic and planktonic communities depends on the primary production of autotrophic organisms, which is limited mainly by the availability of phosphorus and nitrogen in the water column. The availability of nutrients, along with high conductivity, is the limiting factor of productivity in water biotopes. As a result, poor development of phytoplankton and littoral macrophytes was recorded at the different localities studied. The application of fertilizers is the fastest method, but not the most long-lasting, to support primary producers in these ecosystems and subsequent development of the macrozoobenthos. The genus *Chironomus* is tolerant of eutrophic conditions (Marquez et al., 1999). This was confirmed in our study. Larvae of the sub-family Chironominae showed a significant increase in abundance in response to organic enrichment. The larvae of *Chironomus* dominated in fertilized pools at all salinities.

Chironomid larvae play a pivotal role as potential drivers of nutrient dynamics. The role of chironomids is not only as an important source of food for benthic fish, but also as a major contributor to the phosphorus flux from sediments in ponds with a residual organic load (Biswas et al., 2009), especially in pools without fish.

The analysis of the phenology of *C. aprilius* revealed a bivoltine cycle with possibly a partial third generation. These findings are in agreement with the results of other studies. Palmén & Aho (1966) report two generations of *C. aprilius* in southern Finland, but only one in cold environments and possibly a partial third generation in warm years. In the delta region of the Netherlands, Krebs (1982) recorded two generations a year (sometimes three), emerging from the end of March until early November. Larvae overwinter in the third or fourth instar. Spring and autumn emergence of Chironomidae is typical in temperate wetlands (Armitage et al., 1995; Moeller-Pillot, 2009). In warmer climatic conditions there are likely to be more generations per year and the emergence of the overwintering generation can take place earlier. Fuentes et al. (2005) found high densities of 3<sup>rd</sup> and 4<sup>th</sup> instar larvae in May, which may correspond to the first summer generation. Similarly, Rajabipour et al. (2011) show that May, June and July are the best months for collecting egg clutches of *C. aprilius*. At our localities we recorded larval minima in May. Due to the sampling method used, the small larvae of the first two instars passed through the sieve. Moreover, it

is difficult to sample the first two instars as they only take a few days to complete their development (Matěna, 1989).

The total absence of another halophilous to halobiont species *C. salinarius* Kieffer is interesting. This species is regularly reported as syntopic with *C. aprilius* along seashores worldwide (Parma & Krebs, 1977; Drake & Arias, 1995; Kondo, 1998). According to Cartier et al. (2006) *C. salinarius* can tolerate variation in salinity with very few individuals surviving in environments with a salinity level over 35 g l<sup>-1</sup>, high survival at low salinities (0 and 5 g l<sup>-1</sup>) and moderately high salinities (20, 25 and 30 g l<sup>-1</sup>) and poor survival at intermediate levels of salinity (10 and 15 g l<sup>-1</sup>). So the salinities of the pools investigated were within the range tolerated by this species.

We can suggest two reasons for the absence of *C. salinarius* in the pools examined in this study. The species is either bound to habitats close to the seashore where the salinity is determined by a high content of chlorides and is unable to cope with high concentrations of sulphates or only *C. aprilius* is capable of dispersing from coastal areas because it can tolerate freshwater habitats and so use such places as stepping stones to find more suitable habitats. These hypotheses require further study.

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