

# High species richness of Chironomidae (Diptera) in temporary flooded wetlands associated with high species turn-over rates

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## Abstract

Species richness and species turn-over of Chironomidae was studied in irregularly flooded wetlands of the River Dalälven flood-plains in central Sweden. The chironomid fauna, sampled with emergence traps in six wetlands over six summers, contained as much as 135 species, and the cumulative species curves indicated that the regional species pool contain several more species. Recurrent irregular floods may have induced this high chironomid species richness and the high species turn-over in the temporary wetlands, as the dominance between terrestrial and aquatic species shifted between years. Half of the wetlands were treated with *Bacillus thuringiensis* var. *israelensis* (*Bti*) against larvae of the flood-water mosquito *Aedes sticticus*. These treatments had no significant effect on chironomid species richness, but there was a higher species turn-over between years of primarily low abundance species in the treated wetlands. The cumulative number of species was also higher in the *Bti*-treated experimental wetlands than in the untreated reference wetlands. Thus, *Bti* treatment against mosquito larvae seemed to have only small effects on chironomid species richness but seemed to increase the colonisation-extinction dynamics.

**Keywords:** Chironomidae, species richness, species turn-over, *Bti*, wetlands

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## Introduction

Temporary flooded wetlands can be species-rich and contain several species in need of protection (Balla & Davies, 1995; Tocker *et al.*, 1998; Gopal & Junk, 2000) and are ecosystems desired to conserve (cf. European Community Water Framework Directive). The Chironomidae (chironomids) is often a species-rich organism family in temporarily

flooded areas and other kinds of wetlands (Moller Pillot & Buskens, 1990; Batzer & Wissinger, 1996; Leeper & Taylor, 1998), and many species also thrive well under strongly changeable conditions in flooded wetlands (Armitage *et al.*, 1995). They often contribute strongly to animal wetland biomass production (Delettre, 1989; Leeper & Taylor, 1998; Batzer *et al.*, 2006) and are thus important food items for birds (Laursen, 1978; Buchanan *et al.*, 2006), bats (Vaughan, 1997; Encarnação & Dietz, 2006) and frogs (Vignes, 1995).

Here, we have studied the temporal and spatial variation in chironomid species richness over six years in temporary flooded wetlands of the River Dalälven flood-plains, in central Sweden. However, flooded wetlands are also

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characterized by recurrent massive production of flood-water mosquitoes; and, in this region, *Aedes sticticus* (Meigen, 1838) is the most prevalent flood-water mosquito causing massive nuisance problems (Schäfer *et al.*, 2008). Since 2002, the biological mosquito larvicide *Bacillus thuringiensis* var. *israelensis* (*Bti*) is used in some of the temporary wetlands of the River Dalälven flood-plains to reduce the abundance of these nuisance mosquitoes. Chironomid larvae are the only non-target organisms sometimes affected by *Bti* in dosages used against mosquito larvae (Boisvert & Boisvert, 2000). Our evaluation of chironomid production in irregularly flooded wetlands of the River Dalälven flood-plains showed that *Bti* used in the dosage for flood-water mosquito control has no detectable negative effect on chironomid abundance (Lundström *et al.*, 2009). However, *Bti* may still have an effect on Chironomidae species richness, and especially the sub-family Chironominae has been shown to be sensitive to *Bti* (Boisvert & Boisvert, 2000).

Our aims are to investigate chironomid species richness, and annual turn-over rates of species in temporary wetlands exposed to recurrent but irregular floods, and to evaluate the potential effects on these non-target insects of *Bti* treatments against mosquitoes in the wetlands.

## Material and methods

### *Study areas and insect sampling*

The temporary wetlands in the River Dalälven flood-plains are irregularly flooded both for natural reasons and due to water regulation. The hydrological character changes between extremes, from terrestrial conditions during most of the year to aquatic conditions with several decimetres of water for several months of the year.

Six wetlands were selected for the study of chironomid species richness and diversity. Each wetland had four emergence traps, Mundie's cone-formed trap (Service, 1993) modified as described in Lundström *et al.* (2009), in operation from early May (week 19) to late September (week 37) 2002–2007. These modified Mundie's emergence traps float on the water during floods and settle on the ground during periods without surface water. Sampling of insects was continuous for the whole vegetation season each year, and the insect samples were collected once a week. During each weekly visit, the water level under each trap was measured; and, based on these measurements, we classified the local environment as aquatic (mean water depth above 1 cm) or terrestrial (mean water depth below 1 cm).

Three of the temporary wetlands (Laggarbo, Nordmyra and Valmbäcken) were dedicated as experimental wetlands, and in these areas *Bti* was used against larvae of *Aedes sticticus* and other flood-water mosquitoes when needed to reduce mosquito emergence and, thus, reduce nuisance. Three temporary wetlands (Lusmyren, Fågle and Koversta) were dedicated as reference wetlands, and no mosquito control activities were performed in these areas. A more detailed description of the wetlands is given in Lundström *et al.* (2009).

### *Species identification*

Insects sampled with emergence traps were collected in ethylene glycol and transferred to 70% alcohol for storage until sorting. Around 21,000 chironomids were picked out from the general insect samples and transferred to new vials

for latter species identification. The majority of males and some of the females were identified to species based on descriptions in the published literature. For most females, literature does not provide enough information for species identification. These females were identified by association with safely identified males from the same sample. Microscope slide preparation was necessary for safe identification of several species. All identifications were done by the author YB.

### *Statistical analyses*

Chironomid species richness was measured as the number of recorded species for each wetland for each vegetation season (week 19 in early May to week 37 in late September). Thus, the annual species richness figure for each wetland is based on 19 weekly samples from four emergence traps, or 72 trap-weeks.

The number of chironomids collected differed widely between wetlands and years, and large abundance variation is known to influence the observed number of species. Rarefaction analysis, in which the expected number of species is simulated at a given number of individuals sampled (typically the number of individuals in the smallest sample size), is one method to correct for differences in sample size on species richness (Krebs, 1999). In addition to performing statistical analysis on the original chironomid data set, we also performed the statistical analysis on the same data set after rarefaction analyses to investigate if the 'among wetlands' variation in number of individuals sampled caused or masked variation in species richness. For each wetland and year, we rarefied species richness 1000 times to the abundance of the wetland with the lowest number of chironomids sampled each year. The results of rarefactions are dependent on the relative species abundances; a more skewed abundance distribution will cause a lower rarefied number of species than from a more even distribution. To minimize the effect of variation in abundance of common species, we excluded the three most abundant species (*Limnophyes asquamatus*, *L. minimus* and *Pseudosmittia angusta*, constituting 43% of all sampled individuals) that were present in all wetlands all years. The abundance level (i.e. the number of individuals randomly sampled from each wetland) for the rarefaction analyses each year was set to the minimum number of individuals sampled (excluding the three species above) in a wetland for each year, which was 22 in 2002, 42 in 2003, 126 in 2004, 133 in 2005, 152 in 2006 and 41 in 2007. However, we did not rarefy data when only analysing the subfamily Chironominae, as the variation in number of individuals sampled per wetland was too high (Appendix 1) and the abundance at some occasions was so low that rarefying to such abundance levels is meaningless.

To calculate the chironomid species turn-over rate within wetlands over the years, we used both the Jaccard's similarity index based on species presence-absence and the Bray Curtis similarity index based on species abundances (Colwell, 2006). When calculating the Jaccard's similarity index of species turn-over between years, variation in number of individuals trapped between years can confound the results. We, therefore, also calculated Jaccard's indices after data had been rarefied to the minimum number of individuals sampled (excluding the three species above) in a wetland for each year. For the analysis of variation between

Table 1. Chironomidae species richness, the total number of individuals sampled per wetland and year, and the numbers after rarefaction, for six temporary flooded wetlands of the River Dalälvens flood-plains, central Sweden, during the years 2002–2007. The experimental wetlands (Exp) were treated with *Bacillus thuringiensis* var. *israelensis* (*Bti*) against flood-water mosquitoes in 2002, 2003, 2005 and 2006 to reduce nuisance, while the reference (Ref) wetlands were untreated.

Wetland	Year	<i>Bti</i> treatment	Species richness	Ind. sampled	After rarefaction
Fågle Ref	2002	no	22	157	8.4
Fågle Ref	2003	no	28	473	8.6
Fågle Ref	2004	no	18	413	5.9
Fågle Ref	2005	no	19	232	6.1
Fågle Ref	2006	no	24	265	8.1
Fågle Ref	2007	no	12	316	4.1
Laggarbo Exp	2002	yes	51	1301	22.6
Laggarbo Exp	2003	yes	42	889	22.8
Laggarbo Exp	2004	no	27	1068	15.7
Laggarbo Exp	2005	yes	18	184	14.7
Laggarbo Exp	2006	yes	29	422	20.5
Laggarbo Exp	2007	yes	24	408	15.7
Koversta Ref	2002	no	13	281	10.0
Koversta Ref	2003	no	16	668	13.0
Koversta Ref	2004	no	24	734	12.5
Koversta Ref	2005	no	19	636	6.4
Koversta Ref	2006	no	18	2101	2.1
Koversta Ref	2007	no	16	247	10.1
Valmbäcken Exp	2002	yes	14	180	9.8
Valmbäcken Exp	2003	no	19	614	10.2
Valmbäcken Exp	2004	no	30	1429	10.4
Valmbäcken Exp	2005	yes	24	256	11.7
Valmbäcken Exp	2006	yes	27	296	12.1
Valmbäcken Exp	2007	no	22	82	19.0
Lusmyren Ref	2002	no	22	103	19.0
Lusmyren Ref	2003	no	31	702	20.5
Lusmyren Ref	2004	no	30	575	17.3
Lusmyren Ref	2005	no	27	528	16.1
Lusmyren Ref	2006	no	22	415	13.7
Lusmyren Ref	2007	no	31	800	16.0
Nordmyra Exp	2002	yes	28	140	25.0
Nordmyra Exp	2003	yes	20	1726	13.8
Nordmyra Exp	2004	no	11	1123	6.0
Nordmyra Exp	2005	yes	19	553	10.4
Nordmyra Exp	2006	yes	26	514	15.5
Nordmyra Exp	2007	no	14	563	6.3

\* no treatment with *Bti* in the experimental wetlands during 2004 and 2007.

consecutive years, we calculated the mean Jaccard's index from 1000 rarefied communities each year. The variation in sample size is not as large a problem for Bray-Curtis indices, as these are calculated from abundances and are rather insensitive to rare species.

All statistical analyses were done using the SAS statistical software, version 9.1 (SAS Institute, 2004). Analysis of variance (ANOVA, using PROC GLM) was used for evaluating differences in species richness and species turn-over. 'Wetlands' 'years' and 'experimental/reference wetlands' were category variables. To evaluate differences in relation to drought intensity and sample size, drought intensity and sample size were covariates in an analysis of co-variance (ANCOVA, using PROC GLM) with 'year' and 'experimental/reference wetlands' as class variables. Year was added as a category variable in all ANOVAs and ANCOVAs to account for differences between years. Also, the interaction terms for 'year × experimental/reference wetlands' and

'year × drought frequency' were included in the analysis, except when  $P < 0.1$ .

## Results

### *Species richness*

A total of 135 species of Chironomidae were identified from the emergence trap sampling 2002 to 2007 in the temporary wetlands (Appendix 1). The species richness differed significantly and consistently between wetlands over the years (ANOVA:  $F = 3.30$ ,  $P = 0.020$ ,  $df = 5$ ,  $N = 36$ ) and also after rarefaction (ANOVA:  $F = 8.00$ ,  $P < 0.001$ ,  $df = 5$ ,  $N = 36$ ). The reference wetlands provided between 12 and 31 species each year with an average of 21.8 species per year over the six-year period, and the experimental wetlands provided between 11 and 51 species per year with an average of 24.7 species per year (table 1). Species richness

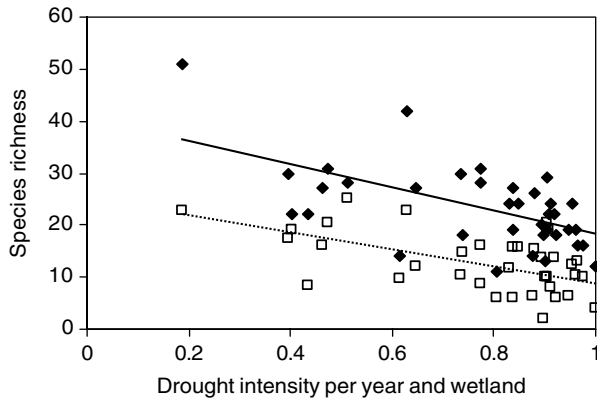


Fig. 1. The species richness (♦, solid line) of Chironomidae in temporary flooded wetlands of the River Dalälven flood-plains, central Sweden, in relation to drought intensity, i.e. proportion of occasions with terrestrial conditions in the wetlands. □ and dashed line show species richness after rarefaction.

did not evidently differ consistently between experimental and reference wetlands (ANOVA:  $F=1.50$ ,  $P=0.200$ ,  $df=1$ ,  $N=36$ ); but, after rarefaction, more species were found in experimental than reference wetlands (ANOVA:  $F=4.50$ ,  $P=0.043$ ,  $df=1$ ,  $N=36$ ). The drought intensity, the proportion of sampling occasions when there was less than one centimetre of water, was 0.31 for 2002, 0.63 for 2003, 0.63 for 2004, 0.66 for 2005, 0.75 for 2006 and 0.81 for 2007. The drought intensity differed between wetlands (ANOVA:  $F=5.25$ ,  $P=0.002$ ,  $df=1$ ,  $N=36$ ), but there was a clear year effect (ANOVA:  $F=6.20$ ,  $P<0.001$ ,  $df=1$ ,  $N=36$ ), meaning that differences between years were similar between wetlands. Chironomid species richness decreased with increasing drought intensity (ANCOVA:  $F=20.0$ ,  $P<0.001$ ,  $df=1$ ,  $N=36$ ; fig. 1), which was evident also after rarefaction (ANCOVA:  $F=12.0$ ,  $P=0.002$ ,  $df=1$ ,  $N=36$ ; fig. 1).

For the subfamily Chironominae, the number of species also differed consistently between sites over the years (ANOVA:  $F=4.20$ ,  $P=0.006$ ,  $df=5$ ,  $N=36$ ) but was strongly positively correlated to sample size ( $r_p=0.79$ ,  $P<0.001$ ,  $N=36$ ). However, neither species richness (ANOVA:  $F=3.01$ ,  $P=0.100$ ,  $df=1$ ,  $N=36$ ) nor the number of Chironominae sampled (ANOVA:  $F=0.90$ ,  $P=0.400$ ,  $df=1$ ,  $N=36$ ) differed consistently between experimental and reference wetlands.

Each year, new species of chironomids occurred in the samples; and, therefore, we constructed diagrams for the accumulated number of species by year for all experimental wetlands combined and for all reference wetlands combined (fig. 2). These results show a continuous annual addition of new species in samples from both experimental and reference wetlands. Interestingly, the accumulated species curve for the reference wetlands is constantly lower than the accumulated species curve for the experimental wetlands.

#### Species turn-over

The Jaccard's index, based on species presence-absence data, showed higher between-year turn-over rates in species composition for the experimental wetlands, than for the reference wetlands (ANOVA:  $F=25.0$ ,  $P<0.0001$ ,  $df=1$ ,

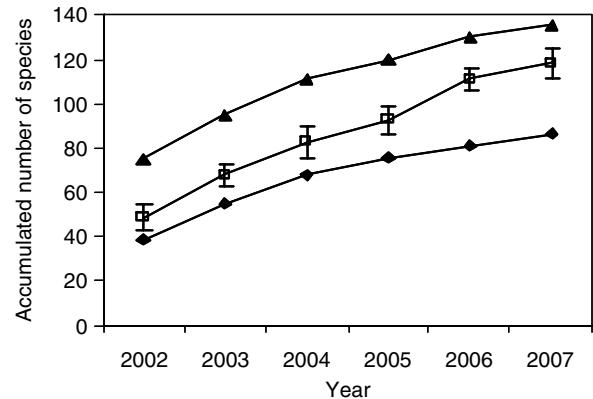


Fig. 2. Accumulated number of Chironomidae species in all six temporary flooded wetlands (▲) of the River Dalälven flood-plains, central Sweden, over six years. The experimental wetlands (□) were treated with *Bacillus thuringiensis* var. *israelensis* (*Bti*) against flood-water mosquitoes in 2002, 2003, 2005 and 2006 to reduce nuisance, while the reference wetlands (♦) were untreated. Due to more individuals being sampled in experimental wetlands, the species richness was rarefied to the same number of individuals as in reference wetlands. The error bars on experimental wetlands show the 99% confidence interval from 1000 randomisations.

$N=30$ , fig. 3). Also, after rarefaction, the species turn-over was significantly higher in experimental than in reference wetlands (ANOVA:  $F=5.10$ ,  $P=0.030$ ,  $df=1$ ,  $N=30$ ; fig. 3). The species turn-over rates were not coupled to the difference in drought intensity between years, neither when analysed for the original data set (ANCOVA:  $F=1.00$ ,

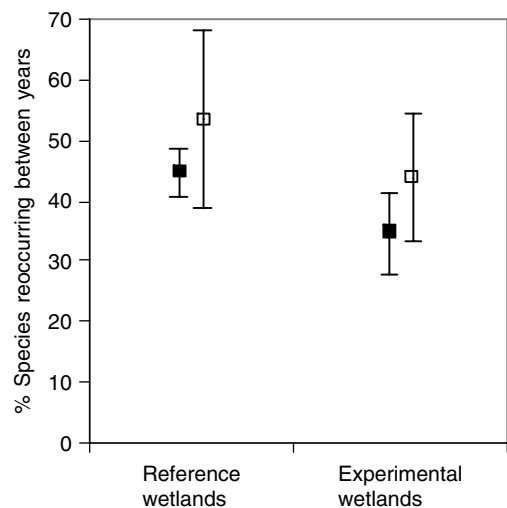


Fig. 3. The average (squares) and standard deviation (error bars) of annual Chironomidae species turn-over in temporary flooded wetlands of the River Dalälven flood-plains, central Sweden. The experimental wetlands were treated with *Bacillus thuringiensis* var. *israelensis* (*Bti*) against flood-water mosquitoes in 2002, 2003, 2005 and 2006 to reduce nuisance, while the reference wetlands were untreated. ■ represent calculated Jaccard's indices from all observed species, whereas □ represent Jaccard's indices calculated after rarefaction.

Table 2. Annual abundance ranking of the eight most abundant Chironomidae species in temporary flooded wetlands of the River Dalälven flood-plains, central Sweden, during the years 2002–2007. Low numbers show high abundance ranking, bold figures show the eight most high-ranking species each year and (–) show that the species was not present in the annual sample. Species with mainly aquatic larvae (Aq) and mainly semi-terrestrial or terrestrial larvae (Te) are indicated under category, while others are blanks.

Species	Category	2002	2003	2004	2005	2006	2007
<i>Ablabesmyia longistyla</i>	Aq	<b>6</b>	33	36.5	–	21.5	48
<i>Corynoneura minuscula</i>		–	27	29	11	10	<b>6</b>
<i>Limnophyes asquamatus</i>		<b>5</b>	<b>3</b>	11	13	<b>6</b>	<b>8</b>
<i>Limnophyes difficilis</i>	Te	13	<b>4</b>	<b>5</b>	<b>4</b>	<b>3</b>	<b>3</b>
<i>Limnophyes margaretae</i>		17	<b>6</b>	36.5	32	20	–
<i>Limnophyes minimus</i>	Te	<b>4</b>	<b>2</b>	<b>2</b>	<b>3</b>	<b>4</b>	<b>2</b>
<i>Macropelopia notata</i>		–	47.5	13.5	<b>8</b>	12	–
<i>Micropsectra notescens</i>	Aq	67	–	<b>3</b>	32	–	–
<i>Paramerina cingulata</i>		<b>7</b>	12	40.5	46	24	24.5
<i>Paraphaenocladus intercedens</i>	Te	–	14	<b>7</b>	<b>7</b>	24	13.5
<i>Paratendipes subaequalis</i>		–	–	–	23	16	<b>7</b>
<i>Polypedilum trigonus</i>		<b>8</b>	<b>7</b>	21	14	28.5	35.5
<i>Psectrocladius oxyura</i>		53.5	41	10	<b>2</b>	<b>1</b>	<b>9</b>
<i>Pseudorthocladus curtistylus</i>	Te	25.5	<b>5</b>	<b>4</b>	<b>1</b>	<b>5</b>	<b>1</b>
<i>Pseudosmittia angusta</i>	Te	<b>1</b>	<b>1</b>	<b>1</b>	<b>6</b>	<b>2</b>	<b>4</b>
<i>Tanytarsus curticornis</i>	Aq	<b>2</b>	17	54.5	–	–	48
<i>Tanytarsus medius</i>	Aq	<b>3</b>	13	34	19	42.5	48
<i>Tanytarsus verralli</i>	Aq	–	–	<b>6</b>	<b>5</b>	<b>8</b>	11
<i>Tavastia yggdrasilia</i>		14	<b>8</b>	<b>8</b>	9	7	<b>5</b>

$P=0.300$ ,  $df=1$ ,  $N=30$ ) nor when analysed after rarefaction (ANCOVA:  $F=0.40$ ,  $P=0.600$ ,  $df=1$ ,  $N=30$ ). The quantitative Bray Curtis similarity index did not show any significant difference in diversity changes over the years between experimental and reference wetland (ANOVA:  $F=0.33$ ,  $P=0.570$ ,  $df=1$ ,  $N=30$ ).

#### Species dominance

The actual species that dominated numerically in the individual wetlands varied strongly over the six-year study period. This can be illustrated by *Micropsectra notescens* and *Psectrocladius oxyura*, which were the markedly most frequent species in a wetland one year but not found another year in the same wetland. The rapid turn-over of the numerically dominating species is also seen when considering the most abundant chironomid species for all six wetlands together (table 2). Only two species, the mainly terrestrial *Pseudosmittia angusta* and *Limnophyes minimus*, occurred among the eight most abundant species every year over the six-year period studied. Species among those eight most frequently occurring one year could not be found in another year. This was, for example, the case for the mainly aquatic *Tanytarsus curticornis*, which was the second most frequently found species in 2002 but for the next five years was absent or rare.

#### Discussion

The temporary flooded wetlands of the River Dalälven flood-plains, in central Sweden, had a remarkably rich chironomid fauna with a total of 135 species recorded. In addition, the accumulated chironomid species curves show that more species are to be expected, and we anticipate that several more years of sampling is required to provide an almost complete list of the species in the regional species pool. The 135 species recorded in the temporary flooded wetlands, is higher than reported from any other wetland

study known to us and seems, in fact, only to be surpassed by a few rather large or very large European lakes (Reiss, 1968; Tuiskunen & Lindeberg, 1986). These lakes probably have relatively stable and predictable environmental conditions, whereas the results of our study of the chironomid fauna in the temporary flooded wetlands of the River Dalälven flood-plains show that the high species richness is due to a high degree of species turn-over between years. The importance of the temporal aspect for high chironomid species richness and high diversity is also seen in other markedly unstable habitats, such as newly created lentic waters (Titmus, 1979; Koskenniemi & Paasivirta, 1987; Dettinger-Klemm, 2003) and rivers experiencing strong water level fluctuations (Reckendorfer *et al.*, 1996).

At least 95% of the identified chironomid species sampled in temporary flooded wetlands of the River Dalälven flood-plains are opportunists, judging from their frequent presence in strongly unstable habitats (e.g. Wiederholm *et al.*, 1977; Koskenniemi & Paasivirta, 1987; Fillinger, 1998; Dettinger-Klemm, 2003). Furthermore, they have been found in more than half of European countries (De Jong *et al.*, 2008), including Nordic countries (Schnell & Aagaard, 1996; Lindegaard, 1997; Paasivirta, 2009; Brodin & Paasivirta, unpublished), and in many different ecosystems including wetlands, lakes and running waters (Fittkau *et al.*, 1978).

There was a significant difference in species richness between wetlands, and variation in hydrological conditions (drought intensity) explained, in large part, the variation in species richness. Hydrological conditions in the wetlands can suddenly and unpredictably change from terrestrial to aquatic (with several decimetres of water) and back to terrestrial with more or less moist soils. In the present study, increasing drought intensity seemed to cause a decline in chironomid species richness. The wetland with by far the most chironomid species found over a year was Laggårbo in 2002, which had by far the lowest drought intensity. This can be explained by a large sample size and a high inflow of aquatic species from the adjacent river (table 1). But, after

rarefaction, species richness was relatively high here, like in other years. Thus, variation in hydrological condition seemed to explain most variation in species richness.

There was no evident support for a negative *Bti* treatment effect on chironomid species richness, not even for the subfamily Chironominae, which has been shown to be sensitive to *Bti* (Boisvert & Boisvert, 2000). After rarefaction, there was actually a significantly higher species richness in experimental than reference wetlands in the present study. Thus, the *Bti* treatments, rather, were associated with increased chironomid richness, maybe as an effect of reduced competition from mosquito larvae. Furthermore, species turn-over between years was larger in experimental wetlands than in reference wetlands, and more chironomid species were found in experimental wetlands than in reference wetlands. However, there were no differences between experimental and reference wetlands when using the quantitative Bray-Curtis similarity index, suggesting it is mainly low abundant species that repeatedly colonise and disappear. Also, chironomid species turn-over between years was generally high in all wetlands; and, on average, only 40% of the species in a wetland were sampled in consecutive years. Partly because of a random sampling effect, it is unlikely that all species could be sampled in a wetland in a given year. However, also after controlling for differences in the number of chironomids sampled with rarefactions, there was a significant difference in species turn-over between experimental and reference wetlands. In conclusion, this suggests that there is a higher random colonisation from a regional species pool in the experimental wetlands, rather than some species actually responding directly to the *Bti* treatment. What causes this larger dynamic among experimental wetlands is not clear. Treatment with *Bti* against mosquito larvae may have some direct negative effects on chironomids (Boisvert & Boisvert, 2000), which may increase (pseudo)extinction of low abundant species from *Bti*-treated wetlands. We, however, do not have evidence of direct effects on chironomid species in the studied wetlands and no overall negative effect on the production of chironomids (Lundström *et al.*, 2009).

There also can be indirect effects from *Bti* treatments against mosquito larvae on extinctions and colonisations of chironomids. The *Bti* treatment managed to prevent mosquito mass-emergence by an almost 100% reduction of the mosquito larval populations (Martina L. Schäfer & Jan O. Lundström, unpublished observations). Many of the chironomid species in the temporary flooded wetlands of the River Dalälven flood-plains probably are filter-feeders that utilize about the same food resource as mosquitoes, and this may increase the probability of more chironomid species to successfully emerge to adults in experimental wetlands in comparison with reference wetlands. In line with this assumption, we have shown that abundance of the protozoan prey of mosquito larvae increased fivefold after removal of mosquito larvae by *Bti* treatment (Östman *et al.*, 2008). How the potentially reduced competition from mosquito larvae may have affected colonisations and extinctions of chironomids, however, is not clear.

We have shown that the chironomid fauna is highly diverse in the studied wetlands. This high regional diversity of chironomids depends on very dynamic communities over time, probably driven by recurrent but unpredictable flooding of the wetlands. However, it is important to note that chironomids may not be appropriate indicators for the

overall animal diversity of the studied wetlands (Batzer *et al.*, 2006). Although species richness differed between sites, this could not be attributable to *Bti* treatments in the wetlands, not even for the most *Bti*-sensitive subfamily Chironominae. However, a larger part of the regional species pool was found in the experimental wetlands than in the reference wetlands, and species turn-over of generally low abundant species was generally higher in the experimental wetlands.

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Appendix 1. Chironomidae species and abundances, in temporary flooded wetlands of the River Dalälven flood-plains, during 2002–2007. The experimental wetlands were treated with *Bacillus thuringiensis* var. *israelensis* (*Bti*) against flood-water mosquitoes in 2002, 2003, 2005 and 2006 to reduce nuisance, while the reference wetlands were untreated.

Chironomidae species	reference			experimental			Total
	FÅGLE	LUSMYREN	KOVERSTA	LAGGARBO	NORDMYRA	VALMBÄCKEN	
<i>Lasiodiamesa gracilis</i> (Kieffer 1924)		1					1
<b>subfamily</b>		<b>1</b>					<b>1</b>
<b>Podonominae total</b>							
<i>Ablabesmyia longistyla</i> Fittkau 1962	1	1		121	1		124
<i>Ablabesmyia monilis</i> (Linnaeus 1758)	1	18		42	1		62
<i>Ablabesmyia phatta</i> (Egger 1863)				6			6
<i>Guttipeloplia guttipennis</i> (van der Wulp 1861)			1	4			5
<i>Krenopeloplia binotata</i> (Wiedemann 1817)	2	14	12			60	88
<i>Macropeloplia notata</i> (Meigen 1818)						151	151
<i>Monopeloplia tenuicalcar</i> (Kieffer 1918)					2		2
<i>Natarsia punctata</i> (Fabricius 1805)	2	23	1	4	2	22	54
<i>Paramerina cingulata</i> (Walker 1856)	56			109	1	1	167
<i>Procladius crassinervis</i> (Zetterstedt 1838)	1				1		2
<i>Procladius longistilus</i> Kieffer 1916				9			9
<i>Procladius</i> sp. A			1	6			7
<i>Psectrotanypus varius</i> (Fabricius 1787)	1						1
<i>Telmatopeloplia nemorum</i> (Goetghebuer 1921)	50	20	26	56	16	95	263
<i>Thienemannimyia carnea</i> (Fabricius 1805)			1			1	2
<i>Xenopeloplia nigricans</i> (Goetghebuer 1927)	19	27		14	7		67
<i>Zavrelimyia barbatipes</i> (Kieffer 1911)	6	3				3	12
<i>Zavrelimyia hirtimana</i> (Kieffer 1918)	4						4
<i>Zavrelimyia melanura</i> (Meigen 1804)	4			1			5
<b>subfamily</b>	<b>147</b>	<b>108</b>	<b>42</b>	<b>372</b>	<b>31</b>	<b>333</b>	<b>1033</b>
<b>Tanypodinae total</b>							
? <i>Acamptocladius</i> sp. A				1			1
<i>Bryophaenocladius</i> sp. A						1	1
<i>Bryophaenocladius</i> sp. B	3	7	14			2	26
<i>Bryophaenocladius</i> sp. C			5			1	6
<i>Bryophaenocladius</i> sp. D						1	1
<i>Bryophaenocladius</i> ? <i>xanthogyne</i> (Edwards 1929)					1		1
<i>Corynoneura celeripes</i> Winnertz 1852	9	113		13	9	11	155
<i>Corynoneura coronata</i> Edwards 1924	5	5			1	1	12



## Appendix 1. Continued

Chironomidae species	reference			experimental			Total
	FÅGLE	LUSMYREN	KOVERSTA	LAGGARBO	NORDMYRA	VALMBÄCKEN	
<i>Corynoneura edwardsi</i> Brundin 1949				1			1
<i>Corynoneura ? lacustris</i> Edwards 1924			1				1
<i>Corynoneura lobata</i> Edwards 1924			1	2		1	4
<i>Corynoneura ? minuscula</i> Brundin 1949	5	178		4	1	1	189
<i>Cricotopus annulator</i> Goetghebuer 1927					1	1	2
<i>Cricotopus bicinctus</i> (Meigen 1818)			1	3		2	6
<i>Cricotopus ? suspiciosus</i> Hirvenoja 1973					1		1
<i>Diplocladius cultriger</i> Kieffer 1908						15	15
<i>Eukiefferiella</i> sp. A			1				1
<i>Gymnometriocnemus</i> <i>brumalis</i> (Edwards 1929)		1				6	7
<i>Heterotrissocladius</i> <i>marcidus</i> (Walker 1856)	1	8		1		3	13
<i>Limnophyes aagaardi</i> Sæther 1990	9	31	7	23	19	1	90
<i>Limnophyes asquamatus</i> Søgaard Andersen 1937	116	183	80	279	344	97	1099
<i>Limnophyes difficilis</i> Brundin 1947	512	291	98	271	308	99	1580
<i>Limnophyes habilis</i> (Walker 1856)	3	1	4	1	2	15	26
<i>Limnophyes margaretae</i> Sæther 1975	42	56		76	39	3	216
<i>Limnophyes minimus</i> (Meigen 1818)	330	264	804	338	243	511	2490
<i>Limnophyes natalensis</i> (Kieffer 1914)	5	7	8	13	12	12	57
<i>Limnophyes pentaplastus</i> (Kieffer 1921)	1	1		7	5	35	49
<i>Limnophyes</i> sp. A	2		1			38	41
<i>Limnophyes</i> sp. B	6	25		11	1	1	44
<i>Limnophyes</i> sp. C		1					1
<i>Limnophyes</i> sp. D			1				1
<i>Metriocnemus albolineatus</i> (Meigen 1818)	36	2	8	3		17	66
<i>Metriocnemus eurynotus</i> (Holmgren 1883)	1		2			62	65
<i>Nanocladius balticus</i> (Palmén 1959)	1						1
<i>Nanocladius dichromus</i> (Kieffer 1906)				1			1
? <i>Orthocladius</i> sp. A			1				1
<i>Parakiefferiella bathophila</i> (Kieffer 1912)				1			1
<i>Paralimnophyes longiseta</i> (Thienemann 1914)	1	1	3			5	10
<i>Parametriocnemus ?</i> <i>stylatus</i> (Spärck 1923)		10					10

## Appendix 1. Continued

Chironomidae species	reference			experimental			Total
	FÅGLE	LUSMYREN	KOVERSTA	LAGGARBO	NORDMYRA	VALMBÄCKEN	
<i>Paraphaenocladius impensus</i> (Walker 1856)	8	4	26	2	4	23	67
<i>Paraphaenocladius intercedens</i> Brundin 1947	2	272		1	20	1	296
<i>Parasmittia carinata</i> Strenzke 1950						1	1
<i>Psectrocladius oxyura</i> Langton 1984	8	13	2109	17	4	20	2171
<i>Psectrocladius psilopterus</i> (Kieffer 1906)		6		3	1		10
<i>Pseudorthocladius curtistylus</i> (Goetghebuer 1921)	96	382	51	337	1163	6	2035
<i>Pseudorthocladius filiformis</i> (Kieffer 1908)					4		4
<i>Pseudosmittia angusta</i> (Edwards 1929)	403	68	1310	1185	1935	709	5611
<i>Pseudosmittia forcipata</i> (Goetghebuer 1921)	20	2	39	11	5	46	123
<i>Pseudosmittia</i> sp. A		3			19		22
<i>Pseudosmittia</i> sp. B		38		3	8		49
<i>Rheocricotopus effusus</i> (Walker 1856)	2		15			30	47
<i>Smittia edwardsi</i> Goetghebuer 1932				1			1
<i>Smittia nudipennis</i> (Goetghebuer 1913)	13	2	5	2	4	2	28
<i>Smittia</i> sp. A		1		1		1	3
<i>Tavastia yggdrasilia</i> (Brodin, Lundström & Paasivirta 2008)	52	94	8	171	336	3	664
<i>Thienemanniella</i> sp. A		2		5	1		8
<i>Tokunagaia</i> sp. A						1	1
<b>subfamily Orthoclaadiinae total</b>	<b>1692</b>	<b>2078</b>	<b>4605</b>	<b>2789</b>	<b>4491</b>	<b>1785</b>	<b>17440</b>
<i>Chironomus pseudothummi</i> Strenzke 1959		3			2	1	6
<i>Chironomus</i> sp. A				1			1
<i>Chironomus</i> sp. B						1	1
<i>Cladopelma edwardsi</i> (Kruseman 1933)				3			3
<i>Cryptochironomus</i> sp. A				1			1
<i>Endochironomus tendens</i> (Fabricius 1775)				2			2
<i>Glyptotendipes cauliginellus</i> (Kieffer 1913)				2			2
<i>Harnischia curtilamellata</i> (Malloch 1915)				1			1
<i>Kiefferulus tendipediformis</i> (Goetghebuer 1921)				30			30
<i>Micropsectra junci</i> (Meigen 1818)		5				42	47
<i>Micropsectra klinki</i> Stur & Ekrem 2006		2				83	85

## Appendix 1. Continued

Chironomidae species	reference			experimental			Total
	FÅGLE	LUSMYREN	KOVERSTA	LAGGARBO	NORDMYRA	VALMBÄCKEN	
<i>Micropsectra notescens</i> (Walker 1856)		3	1			550	554
<i>Micropsectra pallidula</i> (Meigen 1830)						5	5
<i>Micropsectra recurvata</i> Goetghebuer 1928						11	11
<i>Micropsectra roseiventris</i> (Kieffer 1909)						28	28
<i>Micropsectra</i> sp. A						1	1
<i>Micropsectra</i> sp. B		1					1
<i>Microtendipes pedellus</i> (De Geer 1776)	1		1	7			9
<i>Parachironomus digitalis</i> (Edwards 1929)				28	2	2	32
<i>Parachironomus frequens</i> (Johannsen 1905)				1	2	1	4
<i>Parachironomus parilis</i> (Walker 1856)				3			3
<i>Paralauterborniella nigrohalteralis</i> (Malloch 1915)		2		5			7
<i>Paratanytarsus dissimilis</i> (Johannsen 1905)	1	1	1	19	5	1	28
<i>Paratanytarsus</i> sp. A					1	1	2
<i>Paratendipes subaequalis</i> (Malloch 1915)		100		1	3		104
<i>Phaenopsectra flavipes</i> (Meigen 1818)				15	2		17
<i>Phaenopsectra punctipes</i> (Wiedemann 1817)	1			1	1		3
<i>Polypedilum cultellatum</i> Goetghebuer 1931					6		6
<i>Polypedilum ? scirpicola</i> (Kieffer 1921)					1		1
<i>Polypedilum sordens</i> (van den Wulp 1874)	2	1		2		4	9
<i>Polypedilum</i> sp. A				2			2
<i>Polypedilum trigonus</i> Townes 1945	4	98		122	47		271
<i>Polypedilum tritum</i> (Walker 1856)	2	5		4	7		18
<i>Rheotanytarsus photophilus</i> (Gethghebuer 1921)			1	1			2
<i>Stempellina bausei</i> (Kieffer 1911)		1		4			5
<i>Stempellina subglabripennis</i> (Brundin 1947)				1			1
<i>Stempellinella edwardsi</i> Spies & Sæther 2004		1	3	8	1		13
<i>Stenochironomus fascipennis</i> (Zetterstedt 1838)				14			14
<i>Stenochironomus gibbus</i> (Fabricius 1794)				2			2
<i>Synendotendipes dispar</i> (Meigen 1830)		1			2		3

## Appendix 1. Continued

Chironomidae species	reference			experimental			Total
	FÅGLE	LUSMYREN	KOVERSTA	LAGGARBO	NORDMYRA	VALMBÄCKEN	
<i>Synendotendipes impar</i> (Walker 1856)	1	71		4	1		77
<i>Synendotendipes lepidus</i> (Meigen 1830)		2		2	8		12
<i>Tanytarsus curticornis</i> Kieffer 1911			2	401		1	404
<i>Tanytarsus dibranchius</i> Kieffer 1926				3			3
<i>Tanytarsus ejuncidus</i> (Walker 1856)				4			4
<i>Tanytarsus eminulus</i> (Walker 1856)				1		1	2
<i>Tanytarsus medius</i> Reiss & Fittkau 1971		19	7	270		2	298
<i>Tanytarsus nemorosus</i> Edwards 1929				40			40
<i>Tanytarsus occultus</i> Brundin 1949		1		1			2
<i>Tanytarsus signatus</i> (van der Wulp 1959)				4			4
<i>Tanytarsus smolandicus</i> Brundin 1947					1	1	2
<i>Tanytarsus telmaticus</i> Lindeberg 1959	1	2	1	3	1		8
<i>Tanytarsus usmaensis</i> Pagast 1931			1	7			8
<i>Tanytarsus verralli</i> Goetghebuer 1928	1	483	1	61		3	549
<i>Virgatanytarsus</i> <i>arduennensis</i> (Goetghebuer 1922)			1				1
<i>Zavrelia pentatoma</i> Kieffer 1921	3	136		28	3		170
<i>Zavreliella marmorata</i> (van der Wulp 1859)					1		1
<b>subfamily</b>	<b>17</b>	<b>938</b>	<b>20</b>	<b>1109</b>	<b>97</b>	<b>739</b>	<b>2920</b>
<b>Chironominae total</b>							
<b>Grand total</b>	<b>1856</b>	<b>3124</b>	<b>4667</b>	<b>4270</b>	<b>4619</b>	<b>2857</b>	<b>21393</b>