Corrigendum: Using DNA metabarcoding for assessing chironomid diversity and community change in mosquito controlled temporary wetlands. MBMG 2: e21060. https://doi.org/10.3897/mbmg.2.21060

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Explanation regarding the background leading to this corrigendum

The main reasons for this corrigendum were discrepancies between the detected number of chironomids in a report from 2014 and the MBMG article, published in 2018. In the MBMG article from 2018, overall more chironomids were reported that were not detected in the report from 2014. The reasons for this are outlined below.

In general, the abundance data used in this study were part of a project conducted in 2013, which investigated the influence of mosquito control with Bti on the food web. This study was approved and financed by the town Neustadt an der Weinstrasse (Germany). The township requested a project report, which was due in March 2014. By this time, the data were not finally evaluated. In particular, a taxonomic training course for determining Chironomidae subfamilies was attended later in the year 2014 by some of the project participants. After this training we found out that many chironomids of the sub-family Orthocladiinae were wrongly determined as Cecidomyiidae. This had direct impacts on the results and created differences in comparison to the report that was published 2014. All detected differences between the report and this manuscript are listed below in Table S11.

Table S11. Explanation of detected differences in chironomid abundances between the report from 2014 and the manuscript Theissinger et al. (2018), listed by sampling site and sampling week (WAA).

<table>
<thead>
<tr>
<th>Sampling site</th>
<th>WAA</th>
<th>Report 2014</th>
<th>MBMG – manuscript</th>
<th>Explanation</th>
</tr>
</thead>
<tbody>
<tr>
<td>S</td>
<td>2–13 identical; WAA 12 is missing</td>
<td></td>
<td>This site was not sampled in WAA12.</td>
<td></td>
</tr>
<tr>
<td>G</td>
<td>2–13 identical; WAA 11 is missing</td>
<td></td>
<td>This site was not sampled in WAA11 due to weather conditions sampling in WAA11 was interrupted, and the few samples collected in WAA11 were pooled with the samples collected in WAA12.</td>
<td></td>
</tr>
<tr>
<td>M</td>
<td>2–11 identical; WAA 12 is missing</td>
<td></td>
<td>This site was not sampled in WAA12. In this context we detected a typo in Suppl. Material 1 and 5, where we accidently indicated WAA12 instead of WAA 13. This mistake has now been corrected. (i.e., sample “12UM” → “13UM” and sample “12TM” → sample “13TM”.</td>
<td></td>
</tr>
<tr>
<td>M</td>
<td>13</td>
<td>~20</td>
<td>~50</td>
<td>On the untreated site M in WAA13 some Orthocladiinae species had been wrongly identified as Cecidomyiidae instead of Chironomidae. After instructions of our chironomid specialist and co-author Susanne Michiels, this mistake was corrected.</td>
</tr>
<tr>
<td>CL</td>
<td>4</td>
<td>&lt;10</td>
<td>~95</td>
<td>In WAA4 some Orthocladiinae were previously wrongly determined (see above) and only later integrated into the data set.</td>
</tr>
<tr>
<td>CL</td>
<td>5</td>
<td>&lt;10</td>
<td>~35</td>
<td>In WAA5 some Orthocladiinae were previously wrongly determined (see above) and only later integrated into the data set.</td>
</tr>
<tr>
<td>CL</td>
<td>6</td>
<td>&lt;10</td>
<td>~20</td>
<td>In WAA6 some Orthocladiinae were previously wrongly determined (see above) and only later integrated into the data set.</td>
</tr>
<tr>
<td>CL</td>
<td>7</td>
<td>&lt;10</td>
<td>~15</td>
<td>In WAA7 some Orthocladiinae were previously wrongly determined (see above) and only later integrated into the data set.</td>
</tr>
<tr>
<td>CL</td>
<td>10</td>
<td>&lt;10</td>
<td>~50</td>
<td>In WAA10 some Orthocladiinae were previously wrongly determined (see above) and only later integrated into the data set.</td>
</tr>
<tr>
<td>CL</td>
<td>12</td>
<td>&lt;10</td>
<td>NA</td>
<td>From WAA11 onwards, 5 additional traps were deployed on this site for another side project. In these additional traps 10 chironomids were collected, which were included in the report, but not later for the manuscript (for comparability reasons).</td>
</tr>
<tr>
<td>CL</td>
<td>13</td>
<td>&lt;120</td>
<td>40</td>
<td>This discrepancy is due to the 5 additional traps (see above). The collected chironomids from these 5 traps were included for the report, but not in the manuscript.</td>
</tr>
</tbody>
</table>

12 is missing
11 is missing
10 is missing
95 is missing
85 is missing
75 is missing
65 is missing
55 is missing
45 is missing
35 is missing
25 is missing
15 is missing
0 is missing

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In this context of clarification, we came across some additional mistakes in the Microsoft Access data base query of the abundance data, where a filter was missing for excluding traps that fell dry during summer. Also, the project in 2013 was conducted over a longer period, namely 18 weeks after initial Bti application (WAA). In the MBMG article we only used the data until WAA13, because after this week too many traps fell dry, with only some data points for aquatic insects remaining. However, in the originally published MBMG article we falsely included emergence data of all individuals until WAA18, including terrestrial taxa from traps that fell dry. The re-calculation of the GLMM still resulted in a 65% significant chironomid reduction in the Bti-treated sites only until WAA4, but not across the entire study period. However, this 65% reduction has to be interpreted with caution because of the strong site heterogeneity and the potentially highly species specific Bti effect (see table below for further details). For full transparency, a new Supplementary Material 3 with all abundance data has now been added to the corrected version of our article.

Due to the strong within-site heterogeneity and the resulting highly variable numbers of chironomids detected per trap within each site, we reduced our overall statement of a clearly negative Bti effect on the chironomid community. In this context we have rearranged and added some text parts, which can be found with tracked changes in the new Supplementary Material 6.

The authors apologize for any inconveniences resulting from these changes and want to explicitly thank the editorial staff for the very thorough review of our study and support in correcting the mistakes.

Finally, we would like to point out that the mistake only affected the overall abundance data, but the metabarcoding part of the study was not concerned and has not been changed.

Below we point out the main changes to be considered by the readers:

**Abstract**

The re-calculation of the GLMM still resulted in a 65% significant chironomid reduction in the Bti-treated sites only until WAA4, but not across the entire study period. However, this 65% reduction has to be interpreted with caution because of the strong site heterogeneity and the potentially highly species specific Bti effect (see below for further details).

**Methodology – Study sites/ page 3, second paragraph**

Further information on discrepancies of Bti treatment on the respective sites has to be considered: The helicopter application took place on April 10, 2013 using IcyPearls (Verslob WG, Valenthosciences) at a concentration of 1.44 × 10^9 ITU ha for M and 2.38 × 10^9 ITU ha for S and G. Despite different Bti application rates, we subsequently categorized the further treated sites of the areas M, S and G as “Bti–treated”, the first season untreated sites of M, S, G as “untreated” and the control site CL as “never treated”. On the application day and over the following week, water depth at all sites varied only marginally (21 to 24 cm).

**Results – Emergence data – 2* paragraph/ Page 6**

High fluctuation “among traps” have to be highlighted. The term summer emergence peak has to be replaced by “increased emergence.”

**Results – Bti effects on chironomid community composition – 1* paragraph/ Page 7**

GLMM output values were corrected for the analysis of both time periods, with the total numbers of individuals found at the Bti–treated and Bti–untreated sites until WAA4 added. “The abundance of emergent chironomids until WAA 4 at the Bti–treated sites (N = 162, see Suppl. material 5) was significantly reduced (GLMM t = 9.63, p = 0.01, df = 2) by 65.0%, compared to the abundance in the Bti–untreated sites (N = 463), excluding the control site CL; see Suppl. material 3). However, chironomid emergence rates across WAA2 to WAA13 were similar between Bti–treated and untreated sites (GLMM, t = 0.19, p = 0.87, df = 2).”

**Discussion – Bti effects on chironomid community composition – 2* paragraph/Page 9**

The first sentence has to be changed to: “Our analysis revealed a negative effect of Bti on chironomid emergence within the first four weeks after application.” Moreover, it has to be pointed out in more detail that there was “no difference between Bti–treated and Bti–untreated sites regarding the chironomid abundance” over the whole study period.

**Discussion – Bti effects on chironomid community composition – 3* paragraph/Page 9**

Species composition and sensitivity of different chironomid species towards Bti has to be discussed in more detail: “Bti toxicity has been shown to be highly species specific, not only for mosquitoes but also for chironomids (reviewed in Wolfram & Wenzelhund 2018). Our data set was comprised of few very dominant and many rare chironomid taxa. On the one hand, some of these rare taxa, such as Limnophyes minutus, Dicrotendipes spec., Paratanytarsus spec., Procladius spec. and Tanytarsus spec., are known to be highly Bti sensitive (see Wolfram et al. 2018 for a review of studies). More importantly for the results of this study, however, P. uncinatum comprised almost 50% of all reads (see Suppl. material 5). Given the strong correlation of read numbers and specimen abundances (Suppl. material 4) P. uncinatum is likely the most abundant chironomid taxon in the wetlands studied. If P. uncinatum is a rather Bti–insensitive species, as reported for its sister species P. nigripalpis (Kondo et al. 1995), this could have overruled the overall signal of Bti treatment on chironomid abundances. However, broad tests for species specific Bti sensitivity in chironomids are widely lacking (Katol et al. 2017), making it difficult to make overall assumptions of Bti effects on abundance changes.”

**Discussion – Bti effects on chironomid community composition – 3* paragraph/Page 10**

The wording on the effect on communities has to be changed to “only prominent” shortly after application.

**Discussion – Bti effects on chironomid community composition – 4* paragraph/Page 10**

The last sentence highlighting the comparison of four study sites has to be deleted.

**Discussion – Bti effects on chironomid community composition – 5* paragraph/Page 10**

The wording of the assumption on the chironomid diversity has to be changed to “reduced diversity.”

**Conclusion**

The re-calculation of the GLMM still resulted in a 65% significant chironomid reduction in the Bti–treated sites only until WAA4, but not across the entire study period. However, this 65% reduction has to be interpreted with caution because of the strong site heterogeneity and the potentially highly species specific Bti effect (see above for further details).

**Supplementary material 3**

A new Suppl. Material 3 was added showing “all abundance data of aquatic emergence over 13 weeks after Bti application (WAA) across all sites (G, M, S, CL), treatment groups (C = Bti –untreated; T = Bti –treated) and traps (1 – 5). This updated Supplemental material 3 also includes a new Figure with Chironomid abundances across all traps per site, which have been log transformed for better readability.

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