Corrigendum: Does mosquito control by Bti spraying affect the phytoplankton community? A 5-year study in Camargue temporary wetlands (France)

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This article is a corrigendum of a former version by Fayolle et al., originally published in Ann. Limnol. - Int. J. Lim. 51:189–198, 2015. In its original form, the article contained portions of text that were identical to Duguma et al., J. Appl. Ecol. 52: 763–773, 2015, similarities that were not detected by our plagiarism detection software upon initial submission of Fayolle et al.’s manuscript. Based on independent investigations, the Editors of Ann. Limnol. - Int. J. Lim. and J. Appl. Ecol. established that Duguma et al.’s words found in Fayolle et al. were suggested to the latter authors during the review process (without citing any source), so that Fayolle et al. unintentionally reproduced sentences by Duguma et al. in their revised manuscript. After due consideration, authors and Editors concluded that a retractation of Fayolle et al. was not appropriate. The Editors of Ann. Limnol. - Int. J. Lim. have asked Fayolle et al. to prepare a corrigendum by rewriting those parts of the text that are identical to Duguma et al., and referencing the article in J. Appl. Ecol. Where Ann. Limnol. - Int. J. Lim. is concerned, plagiarism check remains a common practice at the time of first submission, but we will now systematically check revised manuscripts as well. Last, we wish to thank the authors of both articles as well as the Editor of J. Appl. Ecol. for helping us sorting out a very difficult situation.

The Editors

Abstract – Mosquitoes are both vectors of disease and a hindrance to outdoor activities. Since its discovery in 1976, the larvicide Bacillus thuringiensis var. israelensis (Bti) has proven its effectiveness in controlling mosquito populations, with negligible environmental impact. We performed an integrated, 5-year study of the direct and indirect effects of Bacillus thuringiensis var. israelensis (Bti applied as VectoBac®) on phytoplankton communities in the Camargue temporary wetlands of the French Mediterranean coast. Mosquito larvae are considered major algae predators, so a significant reduction in mosquito larval density (> 80%) in natural wetlands due to Bti treatment could indirectly affect phytoplankton communities. Physical parameters and phytoplankton were sampled in the water of three temporary oligohaline pools between 2006 and 2011 in the following order: T0 = 1 day before treatment (control), T2 = 2 days after treatment, T5 = 5 days after treatment, T11 = 11 days after treatment. We found no negative effects of Bti treatments on total phytoplankton density and community diversity (Shannon’s entropy and Pielou’s evenness). However, we observed changes in the density of some taxonomic groups; Diatoms were replaced by drought-resistant algae typical of temporary wetlands. These results suggest that changes in phytoplankton community structure are largely driven by natural environmental factors and by the ecological complexity of these temporary wetlands.

Key words: Bacillus thuringiensis / environmental management / food webs / mosquito control / wetlands

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Introduction

*Bacillus thuringiensis* var. *israelensis* serotype H14 (*Bti*) is a biological larvicide increasingly used worldwide for selective control of larval mosquito populations (Lacoursière and Boisvert, 2004; Becker, 2006; Wegner, 2006; Østman *et al.*, 2008; Després *et al.*, 2011). This bacterial larvicide is applied via aerial spraying over large areas in Northeastern Spain, along the French Mediterranean coast (Duchet *et al.*, 2010) and also in the French Atlantic coastal wetlands (Caquet *et al.*, 2011; Lagadic *et al.*, 2014). All these regions contain temporary aquatic ecosystems, habitats propitious to the massive emergence of mosquitoes with major nuisance potential (Becker *et al.*, 2003; Duchet *et al.*, 2010; Lagadic *et al.*, 2014). Living near wetlands often entails serious health hazards for humans and animals due to increased risk of mosquito-borne diseases (Russell, 1999; Walton, 2002; Kirkman *et al.*, 2011; Lagadic *et al.*, 2014). To date, *Bti* represents the best alternative to chemical insecticides for mosquito control (Després *et al.*, 2011) and is described as non-toxic to humans, mammals, birds, fish, plants and most aquatic organisms (Boisvert and Boisvert, 2000; Lacey and Merritt, 2004). *Bti* is the only larvicide used in Europe following implementation of the EU Biocidal Products Directive 98/8/EC (Lagadic *et al.*, 2014). Findings from numerous studies indicate that *Bti* can be considered as a larvicide with low environmental toxicity, even when used in repeated treatments from 3 to 7 years. Most of these long-term studies were conducted on invertebrate populations, under the assumption of direct effects. In a 4-year study (1998–2001) by Lagadic *et al.* (2002) in Morbihan, France, the health, number and abundance of non-target aquatic invertebrates present in mosquito breeding sites treated annually with *Bti* (VectoBac®12AS) were monitored. The authors found that the two sentinel species *Nereis diversicolor* and *Chironomus salinarius* were not impacted by long-term *Bti*-treatment, and that environmental fluctuations had greater influence on community structure than *Bti*. Other short- to long-term field studies (Caquet *et al.*, 2011; Lagadic *et al.*, 2014) aimed at analyzing the effects of *Bti*-treatments on non-target aquatic invertebrates in wetlands. These studies concluded that changes in invertebrate communities in terms of taxonomic richness and/or species abundance were mostly driven by spatial–seasonal changes in natural environmental parameters, rather than by *Bti*-treatments (see also Hershey *et al.*, 1998; Russell *et al.*, 2009). In summary, no study was able to reveal *Bti*-induced alterations of invertebrate community structure. *Bti* application at recommended rates (relative to the operational scale) further support the absence of adverse effects on non-target invertebrates and vertebrates living in the same habitats as mosquito larvae (Lacoursière and Boisvert, 2004; Lacey and Merritt, 2004; Després *et al.*, 2011; Lagadic *et al.*, 2014).

The Camargue temporary wetlands are ecological niches favorable to the development of mosquito species. Each *Bti*-treatment causes almost an 80% mortality of phytophagous mosquito larvae, with indirect effects on the microorganisms. For instance, Østman *et al.* (2008) demonstrated that a strong reduction in mosquito larvae density by *Bti* treatments in temporary flooded wetlands indirectly affected the protozoan community through predation release (4.5 times increase in protozoan density). Xu *et al.* (2008) observed an increase in the density of *Flavobacterium* species in tree holes after the eradication of *Aedes triseriatus* larvae by *Bti* treatment. These studies demonstrate that the local extinction of mosquito larvae by *Bti* treatments causes shifts in microbial communities, because there is a “top-down” control of food resource by the filter-feeding larvae. Other types of indirect effects are documented. Duguma *et al.* (2015) suggested that the inhibition of algal growth in aquatic microcosms treated with high *Bti* concentrations was associated with both the elimination of some bacterial taxa and the increase of bacterial diversity. Su and Mulla (1999) found that *Bti* applications in microcosms suppressed photosynthesis and algal productivity, and decreased the abundance of two unicellular green-algae. Accordingly, lower water turbidity was observed in the treated microcosms than in the control ones. These results suggest that, in addition to predation release (elimination of mosquito larvae), *Bti* can alter algal communities through complex interactions within the microbial communities.

Phytoplanktonic algae make a significant contribution to primary production and nutrient cycling in wetlands (Goldsborough and Robinson, 1996; Robinson *et al.*, 1997; Wu and Mitsch, 1998; Weilhoefer and Pan, 2007; Hagen, 2009). Phytoplankton cells are also a significant dietary resource for larvae of many species mosquito that feed opportunistically on microorganisms (i.e., bacteria, fungal, protozoa, algae) (Merritt *et al.*, 1992). The algae include phytoplankton filtered from the water column, periphyton grazed from the surface of various substrate types, or from the bottom of the pools. Gut content analyses of a variety of mosquito larvae showed that algae are well represented in proportion to their abundance among the microflora where mosquito larvae feed (Martén, 2007).

In this study, we monitored the effects of *Bti* treatment on the phytoplankton of wetlands, seeking to determine whether mosquito control could induce modifications in food web structure. The effects were tested on total phytoplankton density, on the density of different phytoplankton taxonomic groups, and on community structure (diversity and evenness). Phytoplankton might be subject either to direct toxic effects, or to indirect effects linked to decreased grazing pressure from mosquito larvae. Our hypothesis was that any decrease in mosquito larvae population would induce phytoplankton proliferation in our wetlands immediately after *Bti* treatment.

Materials and methods

Study sites

The French Mediterranean coast includes a large number of temporary wetlands characterized by large
Table 1. Causes of flooding, and sampling chronology before and after Bti-treatment (T0 = 1 day before treatment and considered as control – T2, T5 and T11, respectively, 2, 5 and 11 days after treatment) at three sites: Clos d’Armand = site 1; Belugue = site 2; Mourgues = site 3.

<table>
<thead>
<tr>
<th>Wetland</th>
<th>Sampling campaign</th>
<th>Cause of flooding</th>
<th>T0 Control</th>
<th>T2</th>
<th>T5</th>
<th>T11</th>
</tr>
</thead>
<tbody>
<tr>
<td>Site 1</td>
<td>August 2006</td>
<td>Irrigation</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>Dried</td>
</tr>
<tr>
<td></td>
<td>September 2006</td>
<td>Precipitation</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td></td>
<td>May 2008</td>
<td>Irrigation + precipitation</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>August 2008</td>
<td>Irrigation</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td></td>
<td>June 2009</td>
<td>Irrigation</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>September 2009</td>
<td>Precipitation</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td></td>
<td>April 2010</td>
<td>Precipitation</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>Dried</td>
</tr>
<tr>
<td></td>
<td>May 2010</td>
<td>Irrigation</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Site 2</td>
<td>May 2007</td>
<td>Irrigation</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td></td>
<td>June 2008</td>
<td>Irrigation</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>Dried</td>
</tr>
<tr>
<td></td>
<td>May 2009</td>
<td>Irrigation + precipitation</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>July 2010</td>
<td>Irrigation</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>April 2011</td>
<td>Irrigation + precipitation</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>June 2011</td>
<td>Precipitation</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Site 3</td>
<td>February 2010</td>
<td>Precipitation</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>May 2010</td>
<td>Precipitation</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>Dried</td>
</tr>
<tr>
<td></td>
<td>September 2010</td>
<td>Precipitation</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>Dried</td>
</tr>
<tr>
<td></td>
<td>April 2011</td>
<td>Precipitation</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>July 2011</td>
<td>Precipitation</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

Phytoplankton community and physico–chemical parameters

Analysis of phytoplankton dynamics was conducted over 19 campaigns spread over 5 years. For each campaign, total phytoplankton densities, densities of different phytoplankton taxonomical groups and community structure were analyzed before and after each treatment in the following order: T0 = one day before treatment and considered as control, T2, T5 and T11 = 2, 5 and 11 days, respectively, after the treatment. Samples at T11 were not systematically taken due to drying out of the wetlands (Table 1).

In the wetlands where phytoplankton was sampled, water depth was measured to the nearest millimeter using a graduated aluminum gauge at the same point on every sampling date. Concurrently, water temperature, conductivity and salinity were measured at 5 cm below the water surface, using a portable probe (Wissenschaftlich-Technische-Werkstätten – WTW). Measurements were always made between 9:00 a.m. and 12:00 noon to ensure consistency among data relative to possible circadian influence.

Nitrites (N–NO$_2^-$), nitrates (N–NO$_3^-$) and soluble reactive phosphate (SRP) were measured at the beginning of each campaign (control T0), because these parameters play a key role in eutrophication of surface waters (OECD, 1982; Reavie et al., 1995). High P concentrations have been linked to increasing rates of plant growth, changes in species composition and proliferation of planktonic and epiphytic and epibenthic algae, resulting
in shading of higher plants (Mainstone and Parr, 2002). Nitrites and nitrates were analyzed from water samples filtered in the laboratory using Whatman GF/C glass-fiber filters according to APHA (1989). SRP, a measure of monomeric inorganic phosphorus (orthophosphate) in solution, was measured spectrophotometrically following the formation of phosphomolybdic acid.

For each date and site, three phytoplankton samples (pseudo-replicates) were realized, making a total of 12 samples per mosquito control campaign. Three water samples were collected using PVC sterile 1 L bottles at the water sub-surface. These three phytoplankton samples were taken at points far enough apart to represent the entire wetland surface. All in all, 205 phytoplankton samples were preserved with formaldehyde solution (35%). Under an “Olympus” inverted microscope (×400 magnification), phytoplankton taxa were identified and the number of cells were counted for each species, allowing densities to be estimated for each species. Quantification of phytoplankton total cell number (density) was provided via standard counting techniques (APHA, 1989). Digested Bacillariophyta samples were mounted in a highly refractive medium, Naphrax, accentuating the frustular details used in taxonomy. Most taxa were identified using the diverse band of the Süßwasserflora von Mitteleuropa (Krammer and Lange-Bertalot, 1986, 1988, 1991a, 1991b; Komárek and Anagnostidou, 1999, 2005). Euglenophyta was identified using the atlas of Wotowski and Hindák (2005).

Statistical analysis

For each site and campaign, between-campaigns (n = 19) and between-dates comparisons were analyzed for total density values measured at T0 (control) and at T2, T5 and T11 (post-treatment dates), respectively. These values were log-transformed and compared using the Wilcoxon non-parametric test (R package pgirmess).

The response of each phytoplankton taxonomical group to Bti treatment was analyzed using generalized linear mixed models (GLMM), with time and sites as fixed effects (due to the low number of sites; Bolker et al., 2009) and dates and replicates nested in dates as random effects. To account for overdispersed count-data, we used negative binomial distribution as error distribution using the R software (version 3.1.2, R Core Team 2013) and the glmmADMB package (Fournier et al., 2012). These GLMMs estimated the average responses of phytoplankton abundance to Bti treatment over time, the between-dates variability of these responses and the variability among replicates.

Dissimilarity of phytoplankton communities was assessed using the Bray–Curtis distance calculation based on phytoplankton species cell counts. Between pseudo-replicates dissimilarities were calculated for each campaign and each date. Between-dates distances were calculated between T0 and T2, T0 and T5, T0 and T11, for each campaign. Between-sites differences were estimated for campaigns performed during the same season and for the same dates (T0, T2, T5 and T11). Finally, between-years changes were calculated between 2 years, respectively, 1, 2, 3 or 4 years of treatment apart.

Shannon’s entropy and Pielou’s evenness indices were calculated for the control (date T0) and after VectoBac® applications (mean values of post-treatment dates T2, T5 and T11). The calculations were performed with the R package Vegan, and were compared using the Wilcoxon non-parametric test (R package pgirmess).

Results

Water quality and nutrients

Water temperature varied from 9.4 to 25.9°C (February–July) and the mean depth of these sites never exceeded 56 cm (Table 2). Salinity varied slightly according to the source of flooding and the impact of the salt ground water during the decrease in water level (Table 2).

Chemical parameter values indicate the very limited effect of nutritive enrichment in the three study sites: nitrite (N–NO\textsuperscript{2}−), nitrate (N–NO\textsuperscript{3}−) and SRP values were very low, never exceeding 0.01 mg L\textsuperscript{−1}, 0.1 mg L\textsuperscript{−1} and 0.04 g L\textsuperscript{−1}, respectively. Nutrient values indicated oligotrophic conditions at all three study sites.

Phytoplankton communities

Total density

At sites 1 and 2, between-campaigns changes were observed in all total phytoplankton densities (Fig. 1(a) and (b). At site 1, total densities were similar in 2008 and

Table 2. Values and range of variation (minimum to maximum) of environmental parameters measured in water for the three wetlands from 2006 to 2011. (Site 1 n = 30; Site 2 n = 22; Site 3 n = 15). SRP are soluble reactive phosphate.

<table>
<thead>
<tr>
<th>Wetlands</th>
<th>T (°C)</th>
<th>Depth (cm)</th>
<th>Salinity (g.L\textsuperscript{−1})</th>
<th>N–NO\textsuperscript{2}− (mg.L\textsuperscript{−1})</th>
<th>N–NO\textsuperscript{3}− (mg.L\textsuperscript{−1})</th>
<th>SRP (μg.L\textsuperscript{−1})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Site 1</td>
<td>Mean</td>
<td>21.1</td>
<td>28</td>
<td>&lt;0.01</td>
<td>&lt;0.1</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td></td>
<td>Min</td>
<td>16.3</td>
<td>5</td>
<td>&lt;0.01</td>
<td>&lt;0.1</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td></td>
<td>Max</td>
<td>23.1</td>
<td>47</td>
<td>&lt;0.01</td>
<td>&lt;0.1</td>
<td>0.01</td>
</tr>
<tr>
<td>Site 2</td>
<td>Mean</td>
<td>21.3</td>
<td>35</td>
<td>&lt;0.01</td>
<td>0</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td></td>
<td>Min</td>
<td>17.4</td>
<td>6</td>
<td>&lt;0.01</td>
<td>0</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td></td>
<td>Max</td>
<td>25.2</td>
<td>56</td>
<td>&lt;0.01</td>
<td>&lt;0.1</td>
<td>0.04</td>
</tr>
<tr>
<td>Site 3</td>
<td>Mean</td>
<td>9.4</td>
<td>7</td>
<td>&lt;0.01</td>
<td>0</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td></td>
<td>Min</td>
<td>25.9</td>
<td>43</td>
<td>&lt;0.01</td>
<td>&lt;0.1</td>
<td>0.01</td>
</tr>
</tbody>
</table>
were significantly lower than densities registered in 2006, 2009 and 2010. These total densities were significantly higher for the two campaigns of 2009. At site 2, total phytoplankton densities were significantly higher in 2009 than in any other campaigns, and they were significantly lower in April 2011 than in any other campaigns. Algal cell densities at these two sites were highest in 2009. At site 3, a non-significant low temporal variation was observed between campaigns (Wilcoxon test, $P > 0.05$).

The dynamics of total phytoplankton densities (between T0 and T11) differed from site to site and campaign to campaign. Density patterns varied widely with cases both of increase (12 cases as follows: site 1: August and September 2006, May and August 2008 and April 2010; site 2: May 2007, June 2008, May 2009, June 2010; site 3: February and May 2010 and April 2011) and of decrease (seven cases) between the control date (T0) and the first post-treatment date (T2). Nevertheless, between-dates comparison (T0/T2, T0/T5 and T0/T11 for a single site and a single campaign) and of decrease (seven cases) between the control date (T0) and the first post-treatment date (T2). Nevertheless, between-dates comparison (T0/T2, T0/T5 and T0/T11 for a single site and a single campaign) revealed the differences to be non-significant (Wilcoxon test, $P$-value $> 0.05$). Thus, no tendency to phytoplankton proliferation was observed. Despite this inter-annual variability, total phytoplankton densities did not increase systematically after $Bti$-treatment.

Density of phytoplankton taxonomic groups

Five phytoplankton taxonomic groups were identified: Bacillariophyta (Diatoms), Chlorophyta, Cyanobacteria, Euglenophyta and Dinophyta. The GLMM showed Diatoms to be the only group with a significant average decrease in density over time (Wald test, $P$-value $< 0.001$). Diatoms group density showed a significant decrease after T0. No significant relationships between density and time were observed for the four other groups. The between-dates variability (random effect, Table 3) in the relationships between phytoplankton group density and times (intercept and slopes) was always significant (likelihood ratio test, $P$-value $< 0.001$). Notably, the between-dates variability on the slopes was always high (greater than the standard deviation on the fixed effect). No pattern emerged, since almost all possible responses of densities to time could be observed. In contrast, the variability

![Density of phytoplankton taxonomic groups](image)

**Table 3.** Effect of time (fixed effect) on algae abundances (slope on the link scale), and between-dates and between-replicates variability on intercept and slopes (random effect, SD). The significant effects are in bold (Wald test for the fixed effect and likelihood ratio test for random effects, $\alpha = 5\%$).

<table>
<thead>
<tr>
<th>Taxonomic Group</th>
<th>Intercept</th>
<th>Time</th>
<th>Date</th>
<th>Random effect</th>
<th>Replicates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diatoms</td>
<td>7.5426 (0.6662)</td>
<td>-0.3061 (0.0775)</td>
<td>1.459</td>
<td>0.2961</td>
<td>7.8 $\times 10^{-3}$</td>
</tr>
<tr>
<td>Chlorophyta</td>
<td>6.5817 (0.8877)</td>
<td>0.0674 (0.0977)</td>
<td>2.0157</td>
<td>0.3474</td>
<td>1.1 $\times 10^{-4}$</td>
</tr>
<tr>
<td>Cyanobacteria</td>
<td>9.5716 (0.4096)</td>
<td>-0.0288 (0.0437)</td>
<td>0.8533</td>
<td>0.1349</td>
<td>1.3 $\times 10^{-4}$</td>
</tr>
<tr>
<td>Euglenophyta</td>
<td>6.4881 (1.1538)</td>
<td>-0.0145 (0.0325)</td>
<td>2.7579</td>
<td>0.0985</td>
<td>2 $\times 10^{-4}$</td>
</tr>
<tr>
<td>Dinophyta</td>
<td>1.1515 (2.3225)</td>
<td>0.5078 (0.3283)</td>
<td>4.709</td>
<td>1.2814</td>
<td>2 $\times 10^{-4}$</td>
</tr>
</tbody>
</table>
Between pseudo-replicates dissimilarity was quite low, and did not change during a given campaign. The median varied between 17% (post-treatment T5) and 22.5% (control T0) (Fig. 2(a)), suggesting similar phytoplankton densities in the pseudo-replicates at all three sites. Between-dates dissimilarity values were quite high, and fluctuated during a given campaign (Fig. 2(b)). The median of the Bray–Curtis index varied between 67.4% (T0/T2) and 87.1% (T0/T11). The highest values were usually observed when the wetland began to dry up, suggesting that the impact of drought may have been greater than that of the VectoBac\textsuperscript{1} applications.

Between-sites dissimilarity (Fig. 2(c)) was very high, with the medians of the Bray–Curtis index varying between 77.9% (T2) and 96.7% (T0 control), confirming the hypothesis of higher phytoplankton community variability linked to inputs of water and origin of irrigation, and thus random phytoplankton colonization. Moreover, between-years dissimilarity (Fig. 2(d)) was high, the median varying from 53.3% (after 1 year of treatment) up to more than 80% (after, 2, 3 and 4 years of treatment), suggesting that each new water input engendered a new algal colonization leading to increasing between-years differences in communities.

A total of 88 phytoplankton taxa were identified over 19 treatment campaigns. Diatoms dominated, with 47 taxa. Chlorophyta (green algae) were represented by 21 taxa, Euglenophyta by 14 taxa, Cyanobacteria by 13 taxa and Dinophyta by four taxa.
Pielou’s evenness index (b) measured on the control (date T0, towards phytoplankton proliferation or density increase in the environmental conditions of each site. No trends in treated phytoplankton community variability (total density, taxonomic group density and community structure) in treated wetlands of our study area is driven by natural fluctuations. Our findings indicate that response to variable environmental stresses (Hutchinson, 1967; Lowe and Pan, 1996). Our findings indicate that high variability in physico-chemical parameters and hydrological conditions, including temperature, salinity, water depth, inputs of water, drought and seasonality, influenced phytoplankton colonization in these wetlands. Previous field studies failed to demonstrate short- or long-term effects of Bti on non-target species (Niemi et al., 1999; Duchet et al., 2008, 2010; Lündstrom et al., 2010a, 2010b; Caquet et al., 2011; Lagadic et al., 2014), reaching the same conclusions. Hydrological patterns play an important role in site heterogeneity in the Camargue wetlands. Phytoplankton dynamics are influenced by the duration of the hydroperiod and by seasonality in temporary wetlands, especially in a Mediterranean climate (Nuccio et al., 2003). Flooding and areal inundation were also shown to be decisive for annual heterogeneity of phytoplankton densities, with wetland landscape heterogeneity conditioned by water inflows due to areal inundation (Angeler et al., 2000).

In the Camargue wetland sites treated here, no phytoplankton proliferation was observed after the disappearance of mosquito larvae. Earlier research showed that a high-dosage Bti-treatment induced a significant reduction of algal biomass (proxied by total chlorophyll a) 2 weeks after Bti application (Su and Mulla, 1999; Duguma, 2013). Duguma (2013) monitored the algal biomass and showed that it ceased growing 9 days after strong Bti application. The high-dosage Bti treatments in Duguma (2013), Duguma et al. (2015), and in Su and Mulla (1999) were however significantly higher than that advised within the framework of mosquito control in our wetlands. Their findings revealed that the decrease in algal biomass noticed in the water column was not necessarily attributable to the chemical composition and application of VectoBac G. An alternative hypothesis is that application of Bti may have had an indirect effect mediated by the bacterial communities. Some research evidenced species-specific interactions between bacteria and phytoplankton, and concluded that bacteria contribute to the control of phytoplankton dynamics. Mayali and Azam (2004) suggested that bacterial-algal interactions (e.g., symbiosis, commensalisms) can shift to competition under stress conditions, and result to lysis and even cell death of algae by algicidal bacteria. According to Duguma (2013), Bti treatments may increase bacterial colonization and induce greater biochemical activities such as excretion of algicides (e.g., antibiotics). Several bacteria (genus Bacillus) specialized in antibiotics secretion and algicidal activities are known to cause the inhibition of Cyanobacterial growth (Reim et al., 1974).

In our study, the dominant phytoplankton taxonomic group is inoculated naturally by water input to wetland areas, and is composed of Diatoms and Cyanobacteria. During flooding by irrigation or precipitation, this Diatom inoculum is made of colonizer species (t-strategies) rather...
that a competitor species. Here, the Diatom group tended
to decrease, being replaced by species more typical of
temporary wetlands such as Euglenophyta and Dinophyta, whereas the Cyanobacteria remained.
Temporary pools do not provide very suitable ecological
niches for Diatoms, particularly in view of the hydro-
logical fluctuations characteristic of such pools. In these
temporary pools, therefore, the Diatom group did not
constitute a suitable bioindicator for monitoring the
effects of Bti treatment: decreasing depth and the
subsequent drought events contributed to their decline.
Another possible explanation for the decrease in Diatoms
may be low inorganic nutrient levels that they were
eliminated by other inorganics located very low in these
wetlands, such as silicium (Dixit et al., 1992). An
important question is whether Bti treatment may be the
cause. According to Goldsborough (2001) the short
generation time of algal cells means that changes in
taxonomic composition of an assemblage, due to toxicant
exposure for instance, can occur within a few days. Thus,
changes in the proportions of phytoplankton taxonomic
group, or local extinction, may be sensitive indicators of
autogenic or alloogenic stress. This hypothesis of a direct
effect of Bti treatment on the Diatoms group needs to be
tested within the framework of a study in batch culture.
Laboratory bioassays should be conducted with a limited
number of cultivable Diatom species. According to
Koskella and Stotzky (2002), the insecticidal toxins from
Bti did not affect the growth of a variety of algae
(primarily green and Diatoms) in pure and mixed culture.
The toxins from Bti were also non-inhibitory in dilution
tests to pure and mixed cultures of algae and Cyanobacteria. Although the results of these in vitro
studies agree with those observed in studies in soil, none
of these studies evaluated changes in the composition of
mixed cultures. The authors concluded that further studies
on the effects of these toxins on biodiversity are clearly
needed.

These hypotheses of allogenic stress and direct effect
linked to Bti application are not consistent with our
observations. Indeed, before Bti treatment (control T0),
the density of phytoplankton taxonomical groups varied
widely due to the coexistence of two phytoplankton
communities, the exogenous community dominated by
Diatoms and Cyanobacteria inoculated by inputs of water,
and the endogenous community at each site represented by
an algal crust (Euglenophyta, Dinophyta). The Diatom
group responds rapidly to environmental conditions,
so drought events may limit development of some species. The temporal dynamics of the phytoplankton
groups between T0 and T2, T5 and T11 in these temporary
wetlands can be described by the following colonization
dynamics. At T0: flooding (by irrigation or precipitation)
leads to a phytoplankton community composed of
“exogenous” algal and cyanobacteria groups represented
and dominated by Diatoms (colonizers) and Cyanobacteria (competitors). Water input engenders the
detachment of the algal community composed of “en-
dogenous” algal groups, plocon and metaphyton, as
described by Goldsborough (2001) in his work on
phytoplankton assemblages in wetlands. The detached
algae plocon and metaphyton are typical of encysted
Euglenophyta, Chlorophyta and Dinophyta. Chlorophyta
can be considered as competitors but this algal group is
weakly represented because calcareous conditions are
more marked and nutrient input lower. At dates T2 and
T5, fast-growing algae enhanced by water input and the
detachment of the algal crust increase, and recolonize the
water column. Cyanobacteria and Euglenophyta identified
in temporary wetlands possess wide ecological valency,
with a tolerance for drought expressed by forming an
algal crust and desiccation as well as fast recolonization
(2 days). At T11, evidence of the decrease in water
depth and inundation area is provided by the algal crust
formed at the surface, usually composed of mucilaginous
cyanobacterial trichomes, including motile Euglenophyta
cysts. Euglenophyta and Cyanobacteria are common
in these Mediterranean temporary wetlands, due to
their ability to tolerate variations in water level and
desiccation.

The changes in the density of phytoplankton taxonomical
groups observed here are therefore due to natural
conditions related to alternating flooding and drought
events, as phytoplankton responds to hydric stress.
Phytoplankton subjected to a variable moisture regime
must adapt, in order to tolerate extreme conditions
(Sheath and Wher, 2003). Other filamentous forms (Oscillatoria, Nostoc) may, during the open (flooded)
state, form thick mats that protect algal cells during
a later dry phase (Sheath and Wher, 2003). In the
Everglades wetlands, filamentous cyanobacteria, including
Scytonema, Schizothrix, Oscillatoria and Microcoleus, are
often abundant (Goldsborough and Robinson, 1996; Pan
and Stevenson, 1996). In our temporary wetlands, the
dynamics of the phytoplankton taxonomical groups
changed over time, from the beginning of water input to
water depletion in the pools, despite the very low nutrient
input. Phosphorus limitation has been suggested as a
factor structuring the taxonomic composition of phyto-
plankton communities in these wetlands (Beaver et al.,
1998). Diversity measured by the Shannon index con-
firmed our conclusions on phytoplankton densities, i.e.,
there was no evidence of change for all descriptors of
phytoplankton community structure after VectoBac application. Although changes in the abundance of
phytoplankton groups appeared during the monitoring of
Bti-treatment, phytoplankton community structure was
not impacted and the proportions of the different commu-
nities of the temporary wetlands remained the same after
Bti-treatment.

Conclusion

The reduction of mosquito larval density (> 80%) by
Bti treatment did not indirectly affect the overall phyto-
plankton density and community structure in our treated
temporary wetlands. Their elimination did not generate phytoplankton proliferation. It should be noted that the observed changes in phytoplankton taxonomic groups were due to natural conditions related to alternating flooding and drought events. We can thus conclude that the phytoplankton community was not under top-down control in our study systems. After investigating the indirect effects of Bti treatment we are able to confirm the hypothesis that this bioinsecticide does not affect phytoplankton density and diversity and does not constitute an anthropogenic stress to phytoplankton community.

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References


