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Bti sprays do not adversely affect non-target aquatic invertebrates in French Atlantic coastal wetlands

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Summary

1. Both the increase in human mobility and climate change contribute to the globalization of vector-borne diseases. Some mosquito species are efficient disease vectors in Europe, thus increasing the risk of epidemic (re)emergence.

2. *Bacillus thuringiensis* var. *israelensis* (*Bti*) is considered as the most efficient larvicide to control mosquito populations with negligible environmental impacts. However, repeated field applications of *Bti* over many years raise the question of possible long-term effects on non-target invertebrates with putative subsequent alterations of food webs.

3. Environmental effects of *Bti* have mainly been studied in continental freshwater wetlands. Much less is known for brackish water coastal wetlands. We investigated whether repeated treatments with *Bti*, applied as VectoBac® WG over seven consecutive years, may affect non-target invertebrate communities in wetlands of the French Atlantic coast. Particular attention was devoted to invertebrates potentially used as food sources by shorebirds and wading birds.

4. Invertebrates were sampled in the water and sediment of control and VectoBac®-treated saltmarsh pools between 2006 and 2012. Taxa abundance data were used to calculate community descriptors and to analyse the potential structural changes due to VectoBac® using the principal response curve method and similarity analysis. Physicochemical parameters were measured in the same pools so that homogeneity of the environmental conditions between the control and treated areas could be tested.

5. We demonstrated that long-term use of VectoBac® WG in French Atlantic coastal wetlands had no influence on the temporal evolution of the taxonomic structure and taxa abundance of non-target aquatic invertebrate communities, which is highly driven by abiotic factors. In addition, over the long term, the amount of invertebrates that could be used as food resources by birds is maintained in VectoBac®-treated areas.

6. *Synthesis and applications*. Reduced application rate and targeted spraying of VectoBac® WG in mosquito breeding sites minimize potential environmental impacts of *Bacillus thuringiensis* var. *israelensis* (*Bti*). Even so, surveillance of its possible primary side effects is needed, which requires comparable control and treated areas. Indeed, systematic temporal trends and subtle differences in the range of variation of abiotic factors result in discrepancies between control and treated area in terms of invertebrate abundance, which could be wrongly attributed to VectoBac®. Management decisions and mitigation measures may therefore benefit from (i) extending surveillance to a time frame that allows for coverage of the immense temporal variation in taxa abundance and diversity and (ii) the inclusion of environmental variables in the monitoring of non-target animal communities potentially exposed to *Bti*.

Key-words: *Bacillus thuringiensis israelensis*, bird food resources, brackish water pools, larvicide, long-term field biomonitoring, mosquito control, non-target invertebrates, saltmarshes, taxonomic richness and diversity

Introduction

Over the past thirty years, *Bacillus thuringiensis* var. *israelensis* (*Bti*) has been increasingly used for selective

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control of larval populations of mosquitoes in continental aquatic ecosystems such as the floodplains of the River Rhine in Germany (Becker 2006), River Vistula in Poland (Wegner 2006) or River Dalälven in Sweden (Östman, Lundström & Persson Vinnersten 2008). Coastal wetlands are also subjected to *Bti* applications because these regularly flooded areas are highly suitable habitats for mosquitoes (Giberson, Bilyj & Burgess 2001). *Bti* is thus aerially sprayed in large areas in Ebro Delta, north-eastern Spain, and along the French Mediterranean coast. This bacterial larvicide is also used in land-based treatments of French Atlantic coastal wetlands (Fourcy *et al.* 2002; Caquet *et al.* 2011), where both high tides and frequent rainfalls contribute to the massive emergences of mosquitoes.

Mosquito nuisance may contribute to non-negligible money losses in regions where tourism contributes to the economy (Breeland & Mulrennan 1983). Improved human comfort and health from control activities that reduce the nuisance caused by mosquitoes also concern resident populations (Worobey *et al.* 2013). Around the world, allergic reactions to mosquito bites are an increasing clinical concern (Viniaker & Lavaud 2005; Peng & Simons 2007). Health concerns are not only due to allergy. Mosquitoes are the only vectors of human pathogenic viruses occurring in Europe including the recurrent outbreaks of Sindbis virus in Sweden and Finland, the reoccurrence of outbreaks of West Nile virus in several south European countries, the recent outbreaks of the exotic Chikungunya virus in Italy and the sporadic transmission of Chikungunya and dengue virus infection in Southern France (Lundström 1999; Rezza *et al.* 2007; Kurkela *et al.* 2008; Sainz-Elipe *et al.* 2010; Butler 2012; Vega-Rua *et al.* 2013). Mosquitoes are also the only vectors of human malaria, and there is a distinct risk of malaria resurgence in southern Europe, which shows that the predicted globalization of vector-borne diseases has become a reality, and climate change may enhance this process (Erickson *et al.* 2012). In such a context, mosquito control is of primary importance for human and animal health.

Many different methods have been developed to act directly on larval mosquito populations, as it is easier and more effective to control mosquitoes where they breed. Currently, *Bti* is the only larvicide used in Europe as the result of the implementation of the EU Biocidal Products Directive 98/8/EC. *Bti* is usually applied as the liquid (AS), granule (G) or water-dispersible granule (WG) formulations of the commercial product VectoBac® (Després, Lagneau & Frutos 2011). Expressed as International Toxic Units (ITU), the recommended application rate of VectoBac® G ($0{\cdot}75\ 10^9$–$6\ 10^9$ ITU ha^{-1} corresponding to 3·75–30 kg ha^{-1}) is approximately twofold higher than those of VectoBac® WG and 12AS ($0{\cdot}375\ 10^9$–$3\ 10^9$ ITU ha^{-1} corresponding to 0·125–1 kg ha^{-1} and $0{\cdot}371\ 10^9$–$1{\cdot}59\ 10^9$ ITU ha^{-1} corresponding to 0·29–1·24 L ha^{-1}, respectively). With respect to environmental impacts, controversial results have been obtained for *Bti*-containing larvicides. A number of laboratory, semi-field and field studies showed that *Bti* can be considered as the environmentally safest larvicide due to its selectivity towards mosquitoes. However, even if we exclude laboratory experiments, where unrealistic exposure concentrations were generally used, there are indications that non-target aquatic invertebrates may be impacted by *Bti* in the field. In particular, the implementation of repeated *Bti* treatments during several years raises the question of their possible long-term effects on these animal communities. A field study conducted between 1991 and 1993 in Minnesota freshwater wetlands found that over the last 2 years, *Bti* (aerially applied as VectoBac® G) induced a significant decrease in the abundance of non-target invertebrates, especially Nematocera, associated with a reduction in insect genera richness and an increase in dominance indices (Hershey *et al.* 1998; Niemi *et al.* 1999). In contrast, in a 6-year survey performed in the River Dalälven floodplain, no significant effect of mosquito control using aerial application of VectoBac® G was shown on the production of emerging insects (Persson Vinnersten *et al.* 2010), nor on chironomid species richness and production (Lundström *et al.* 2010a,b).

However, most of the available information about long-term effects of repeated *Bti* applications concerns continental wetlands and is not applicable to saltwater/brackish water coastal wetlands due to differences in terms of hydrology, invertebrate community composition and food web structure (Russell, Kay & Skilleter 2009). The present study was therefore implemented to investigate whether repeated treatments with VectoBac® WG over seven consecutive years may have long-term effects on the abundance and diversity of non-target aquatic invertebrates in wetlands of the Atlantic coast of southern Brittany. This region is registered as a Ramsar site (no. 517) and benefits from legal protection status according to the Natura 2000 network policy. It is also an important feeding and breeding ground for birds (Ganter 2000; Butler, Davidson & Morrison 2001; Sirot, Maes & Gélinaud 2012). Therefore, a particular attention has been devoted herein to non-target invertebrates that represent a food source for birds. To date, no study has shown that birds can be directly affected by the exposure to *Bti* (SPRP 1996; US EPA 1998; MMCD 1999; WHO 1999; Boisvert & Boisvert 2000), and indirect effects have not been demonstrated either. Field investigations that indicate changes in bird reproduction or breeding success were not able to identify *Bti* as responsible for the observed indirect effects (Hanowski *et al.* 1997a,b; Poulin, Lefebvre & Paz 2010). This is primarily due to environmental discrepancies between treated and control areas (e.g. they were 20–35 km apart, the treated area being along the Mediterranean coast, whereas the control sites were located inland, much closer to urban areas in the Poulin *et al.* study) so that birds could have been affected by any environmental factor that would have altered their habitat, not only the

nature of their food resources. Our hypothesis is that aquatic invertebrates would be the first trophic level affected by applications of *Bti* with significant changes in their abundance and diversity. Some invertebrates (e.g. Nematocera) may be directly impacted by the larvicide, as mentioned above, leading to a reduction in their abundance. Other invertebrate taxa may be indirectly affected, either positively or negatively, due to alterations in competition and/or predation relationships. Overall, changes in the invertebrate community may putatively affect secondary consumers, including birds.

Invertebrates were repeatedly sampled in control and *Bti*-treated saltmarsh pools in coastal wetlands of Morbihan (southern Brittany) from 2006 to 2012. Water depth, temperature, pH, salinity and dissolved oxygen saturation were measured in the pools where invertebrates were sampled so that the similarity of the environmental conditions between control and treated areas and the community responses could be tested.

Materials and methods

STUDY SITE

The study was performed from February 2006 to October 2012 (Table S1 in Supporting Information) in a 5-ha saltmarsh close to a tidal estuary (Locoal-Mendon, Morbihan, France; 47°42′30″ N–03°07′48″W). This is a former saltern where hundreds of pools (10–30 m² surface area) are regularly flooded by incoming estuary water at high tide (coefficient >90) and by rainfall. Part of this wetland (*c*. 1 ha) was delimited as an untreated, reference area before the beginning of the mosquito control programme in January 1998 (Fourcy *et al*. 2002). Next to the control area, a zone of *c*. 2 ha was delimited, where VectoBac® WG has been applied since April 2006 to control *Ochlerotatus* (*Aedes*) *caspius* and *O*. *detritus* larvae. Control and treated areas are adjacent, but separated by a small channel corresponding to a former saltmarsh work, and care is taken to preserve a 50- to 100-m wide buffer zone between the two areas in order to prevent VectoBac® spray drift. Within each area, five pools were randomly chosen before the treatments started in 2006.

TREATMENTS

VectoBac® WG (37·4% *Bti*; 3000 ITU mg^{-1}; Valent Biosciences, Libertyville, IL, USA) was applied at 300 g ha^{-1} (0·9 10^9 ITU ha^{-1}) from April 2006 to June 2011. From July 2011, dosage has been reduced to 220 g ha^{-1} (0·66 10^9 ITU ha^{-1}). The actual applied dose was thus 3·3- to 4·5-fold lower than the application rate currently authorized (1 kg ha^{-1}, 3000 10^6 ITU ha^{-1}). VectoBac® was diluted into tap water in portable sprayers for application at the water surface, thus reducing spray drift. Treatments were performed by qualified personnel from the Etablissement Interdépartemental de Démoustication du littoral Atlantique (Rochefort-sur-Mer, France). The treatment procedure requires that VectoBac® is applied only if the actual abundance of mosquito larvae in pools exceeds a given threshold (usually 5 larvae per litre; Carron *et al*. 2003). Between April 2006 and October 2012, 47 VectoBac® applications occurred.

INVERTEBRATE COMMUNITY SAMPLING AND ANALYSIS

Thirty-eight sampling campaigns were performed from March 2006 to October 2012 (Table S1, Supporting Information). Invertebrates were sampled in water and sediment of control and treated pools (five replicates in each type of pool), as described elsewhere (Caquet *et al*. 2011). Water and sediment samples were immediately preserved using neutral aqueous formaldehyde (10%, v/v). In the laboratory, the samples were passed through a series of sieves of decreasing mesh size (8, 4, 2, 1, 0·5, 0·25 mm). The invertebrates were then enumerated and identified at the lowest practical taxonomic level under a stereomicroscope.

Abundance data for the various taxa were used to compute several quantitative descriptors of community structure (Clarke & Warwick 2001): taxonomic richness (*S*), total density (*N*), Shannon's diversity index (*H′*) and Pielou's evenness (*J*). The abundance of annelids, molluscs, crustaceans and insects, known as major food resources for waterfowl and wading birds (Le Drean Quenec'hdu & Mahéo 1997; MacKenzie 2005; Santos, Granadeiro & Palmeirim 2005; Pedro & Ramos 2009; Viain *et al*. 2011), were analysed in more detail, considering only the individuals that were retained in sieves with mesh size ≥2 mm.

In control and treated pools, at each location where invertebrates were sampled, water depth, temperature, dissolved oxygen saturation, salinity and pH were measured as previously described (Caquet *et al*. 2011). Measurements were always made between 10:00 AM and noon to ensure consistency among data relative to possible circadial influence.

STATISTICAL ANALYSES

Since the same pools were repeatedly sampled, all data were analysed using mixed models to account for pseudoreplication. Environmental parameters, *H′* and *J* values were analysed using linear mixed models (*lmer* function of R package *lme4*), with time and area (control or treated) as fixed factors and pool identity nested in area as a random factor. Before analysis, normality of the data was checked using Shapiro–Wilk's test. When necessary, Box–Cox transformation was used to normalize data distribution. In addition, on each sampling date, mean values of *H′* and *J* were compared using one-way ANOVA (*glht* function from R package *multcomp*) to detect point differences between control and treated areas. Count-based data (e.g. *S*, *N* and abundance of the various taxa) were analysed using a generalized linear mixed model (O'Hara & Kotze 2010). Analyses were performed using the *glmer* function of R package *lme4*, with time and area as fixed factors and pool identity nested in area as a random factor, and using a quasi-Poisson distribution to account for data overdispersion. In addition, on each sampling date, mean values of the different variables were compared using a quasi-Poisson generalized linear model to detect point differences between control and treated areas.

Potential changes in the structure of the invertebrate community due to VectoBac® exposure were analysed using the principal response curve (PRC) method (van den Brink & ter Braak 1999). For each pool and date, invertebrate abundance data in water and sediment were grouped, and the resulting values were ln (100x + 1)-transformed before analysis (van den Brink *et al*. 2000). To take into account the possible influence of environmental parameters other than VectoBac® applications on invertebrate community dynamics, water depth, salinity and dissolved oxygen

saturation were included as covariables in the analysis (Lepš & Šmilauer 2003). The corresponding values were standardized before analysis. Statistical tests of significance were carried out using Monte Carlo simulations (999 permutations).

Temporal changes in the dissimilarity of invertebrate communities between control and treated areas were assessed using the Bray–Curtis index calculation based on fourth-root transformed abundance data. The first analysis was performed between the two areas. On each sampling date, Bray–Curtis distance was calculated for all the pairs of control and treated pools, generating a maximum of 25 distance values (this number was lower when some pools dried up). The mean and standard error of these values were then computed. The second analysis was performed within each area according to Collins, Micheli & Hartt (2000). On each sampling date, the mean abundance of each taxonomic group in control and treated pools, respectively, was calculated. Mean abundance values were then used to compute Bray–Curtis distances between sampling dates for a given area. These values were finally linearly regressed against the square root of the time-lag values. The slopes of the regression lines obtained for the two areas were compared using analysis of covariance (ANCOVA).

Multivariate analyses were performed with CANOCO for Windows software package, version 4.55 (Center for Biometry-Wageningen, the Netherlands). The other tests were performed using R for Windows, version 2.8.0 (R Development Core Team 2008). Statistical threshold was $\alpha = 0{\cdot}05$ for all tests, using the Benjamini and Hochberg false discovery rate (FDR) multiple testing correction (Benjamini & Hochberg 1995) for date-by-date analyses to account for the high number of analysis performed on the data set.

Results

ENVIRONMENTAL PARAMETERS

All pools fell dry on 25 July 2006, and partial drying was observed on 14 June 2006 (two control and three treated pools), on 05 September 2006 (one treated pool) and on 26 June 2008 (three control and four treated pools). Environmental conditions varied between the control and treatment area on many sampling dates (Fig. 1, Table S2, Supporting Information). For all parameters, a highly significant interaction between time and area was shown ($P < 0{\cdot}001$). Mean differences between the two areas were of the same order of magnitude as the natural range of variation of these parameters within each area. Overall, except for water depth, the environmental parameters showed larger variation ranges in pools of the treated area. For most sampling dates, temperature and salinity were significantly lower and higher, respectively, in the control area than in the treated one (Table S3, Supporting Information). Water depth and pH were systematically lower in VectoBac®-treated pools, as compared to the controls, although significant differences only occurred in 10·8 and 8·1% of the sampling dates, respectively. Finally, when dissolved oxygen saturation was significantly different (13·5% of the sampling dates), differences were balanced between control and treated pools. A principal component analysis performed on physicochemical parameters showed that salinity was positively correlated with temperature, whereas depth was positively correlated with pH (Fig. S1, Supporting Information). Therefore, to avoid effects of collinear variables, temperature and pH were omitted from the statistical models; only water depth, salinity and dissolved oxygen saturation were included as covariables in the analysis of the variations of non-target invertebrate community structure.

NON-TARGET INVERTEBRATE COMMUNITY STRUCTURE

Irrespective of the sampling area, 61 invertebrate taxa were identified for the whole study period. Grouping of data was performed for some taxa, according to their taxonomic relationship, when their occurrence frequency was below 5%; this avoided giving too much weight to rare taxa. The resulting list of 27 taxonomic groups is given in Table S4 (Supporting Information). Crustaceans (*Corophium volutator*, *Palaemonetes varians* adult and larvae, *Lekanesphaera* sp., Copepods and Ostracods), annelids (*Nereis diversicolor*, Capitellidae and Spionidae), insects

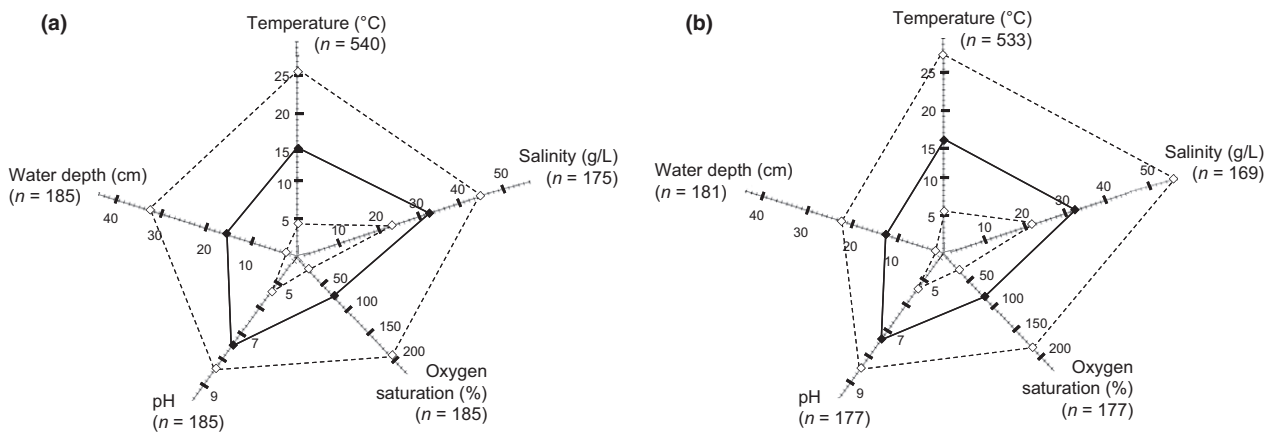

Fig. 1. Radar-type diagrams representing the mean values of the environmental parameters (*n* indicated in brackets for each parameter in each area) measured from 2006 to 2012 in the control (a) and VectoBac®-treated (b) areas of the study site of Locoal-Mendon, France. Maximal and minimal values are represented by the outer and inner dotted lines, respectively.

(larvae of Chironomini from the *Chironomus salinarius* group, and of Orthocladiinae) and water mites were the most prevalent taxonomic groups in the community. *Ochlerotatus* sp. larvae and pupae were always found in low abundance, especially in the treated pools.

Important temporal variations were observed for all descriptors of the community structure, and patterns were almost identical in the two areas (Fig. 2). Time had a highly significant effect on all descriptors (Table 1), and droughts in the summers of 2006 and 2008 had strong negative impacts on taxonomic richness and an even stronger effect on total abundance. Most taxa were severely impacted, although highly mobile groups such as copepods, shrimps (*Palaemonetes varians*) and insects (especially midge larvae) were unaffected or weakly affected (Fig. S2, Supporting Information).

Area had no significant effect on S values; there was only a significant effect of time, and there was no significant interaction between the two factors. In contrast, time × area interaction was significant for N, H' and

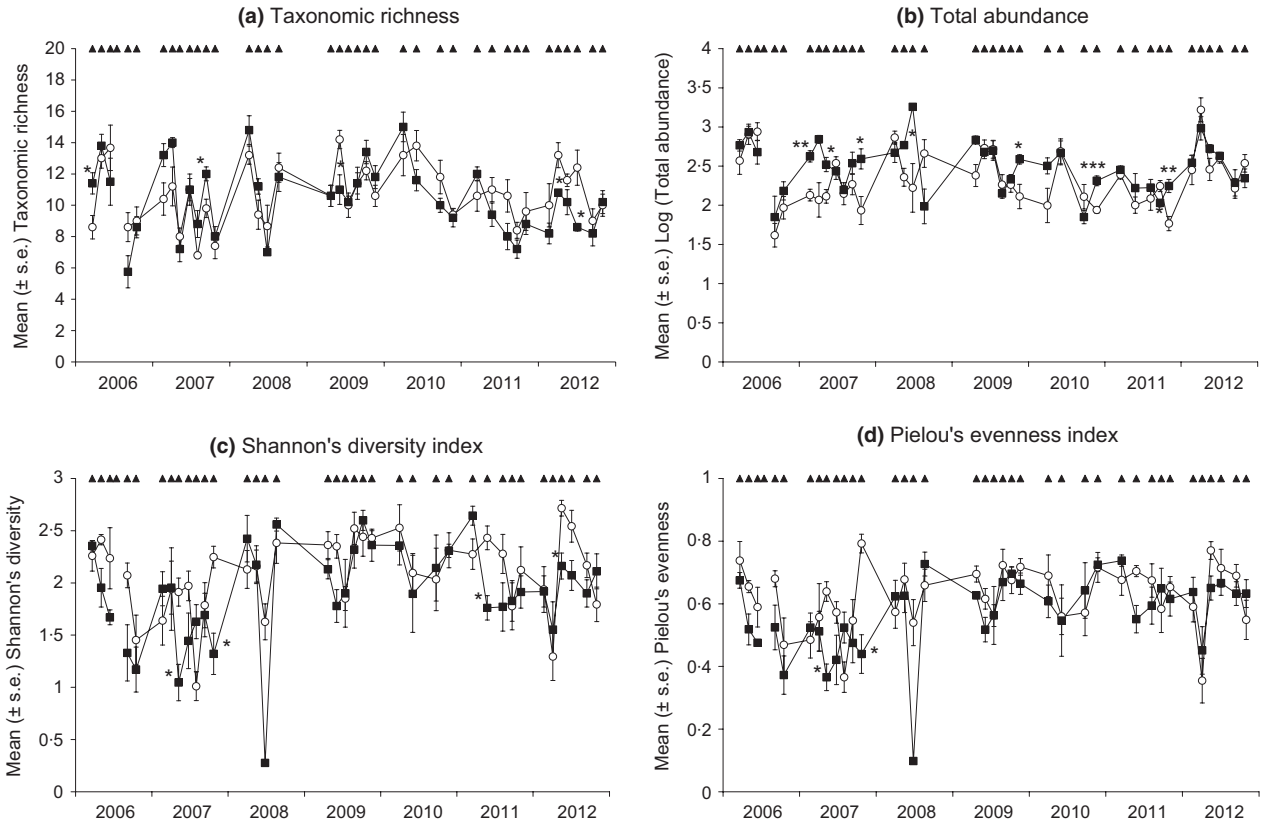

Fig. 2. Changes in mean ± SE (n = 5) values of descriptors of the invertebrate communities in control (empty circles) and VectoBac®-treated pools (filled squares). (a) taxonomic richness; (b) total abundance; (c) Shannon's diversity index; (d) Pielou's evenness index. Statistically significant difference according to GLM analysis or ANOVA with FDR correction: *: $0{\cdot}01 < P < 0{\cdot}05$; **: $0{\cdot}001 < P < 0{\cdot}01$; ***: $P < 0{\cdot}001$. Interruption of the lines in 2006 corresponds to a drought period. The triangles indicate the dates of VectoBac® applications in the treated area.

Table 1. Mean ± SE and range of variation of descriptors of the invertebrate communities in the control and VectoBac®-treated pools during the whole study period (2006–2012) and results of the statistical analysis using linear mixed models

Invertebrate community descriptor	Control area	VectoBac®-treated area	Time effect χ^2; d.f. – F; d.f. P	Area effect χ^2; d.f. – F; d.f. P	Time × area χ^2; d.f. – F; d.f. P
Total density (N)	358·5 ± 34·0 [13·5–3769]	407·0 ± 25·5 [12·4–2067·2]	59 932; 36 <0·001	1·3; 1 0·25	12 989; 36 <0·001
Taxonomic richness (S)	10·7 ± 0·2 [5–16]	10·5 ± 0·2 [4–18]	114·4; 36 <0·001	0·26; 1 0·61	27·0; 36 0·86
Shannon's diversity index (H')	2·10 ± 0·04 [0·53–3·19]	1·95 ± 0·04 [0·28–3·13]	174·7; 36 <0·001	3·1; 1 0·076	75·1; 36 <0·001
Pielou's evenness (J)	0·62 ± 0·01 [0·16–0·97]	0·58 ± 0·01 [0·1–0·86]	140·5; 36 <0·001	3·0;1 0·085	75·2; 36 <0·001

χ^2, chi-square test statistics; F, Fisher statistics; d.f., degrees of freedom; P, corresponding probability.

J. Significant point differences between control and treated areas were only observed on 1, 3 and 2 dates for *S*, *N* and *J*, respectively (Fig. 2), with no systematic trend.

Invertebrate abundance exhibited important temporal changes in the two areas for all taxa (Fig. S2, Supporting Information). Detailed results of the statistical analysis are provided in Table S5 (Supporting Information). There was a significant time × area interaction effect ($P \leq 0{\cdot}001$) on abundance for 19 of the 27 groups, suggesting the existence of different temporal dynamics for these groups between the two areas. Date-by-date analysis (Table S6, Supporting Information) detected statistically significant differences on less than one-third of the sampling dates except for *C. volutator* for which differences were observed on nearly 60% of the dates. When differences occurred, higher abundances were systematically observed in the treated area, as compared to the control, for *C. volutator*, *Lekanesphaera* sp., Orthocladiinae larvae, Talitridae and Chironomidae pupae. It was the opposite for *S. plana*, *S. shrubsolii*, Sabellidae, Hydrobiidae and Ostracods.

Principal response curve analysis of invertebrate abundance data with water depth, salinity and dissolved oxygen saturation as covariables (Fig. 3) showed significant differences between control and treated pools ($P = 0{\cdot}001$). Monte Carlo permutation tests for the whole data set indicated that a significant amount of the variance explained by the treatments is displayed in the diagram of the first PRC ($P = 0{\cdot}001$). Of all the variance in the abundance data, 44·5% could be attributed to the sampling date, and only 13·9% could be attributed to the treatment regime (i.e. area × time interaction; 25·2% of this variance is displayed on the vertical axis of the first PRC). Tests for each sampling date indicated differences between control and treated pools on only 4 sampling dates out of 37. Analysis of the distribution of species weights (b_k) confirms that hydrozoans, *C. volutator*, Sabellidae, *Lekanesphaera* sp. and oligochaetes were more abundant in VectoBac®-treated pools, as compared to the controls. In contrast, *S. shrubsolii*, *S. plana*, Hydrobiidae, *C. capitata,* Nematods and Platyhelminthes were less abundant in the treated pools than in the control ones. These results are in accordance with those obtained for the individual species (Fig. S2 and Table S6, Supporting Information). It is noteworthy that *S. plana* was found in the samples only since 2009; from then, its abundance increased more rapidly in the control pools as compared to the treated ones.

Mean value of Bray–Curtis dissimilarity between the two areas fluctuated during the study period (Fig. 4). Highest values were usually observed when the pools were dry (e.g. Summer 2006 and 2008), suggesting that the impact of drought may have been different in the two areas. There was no significant relationship between mean Bray–Curtis dissimilarity values and elapsed time since the beginning of the study (Spearman $r = -0{\cdot}028$, $P = 0{\cdot}87$), showing that VectoBac® applications did not increase invertebrate community dissimilarity between control and treated areas. The relation between Bray–Curtis dissimilarity and time lag between sampling dates

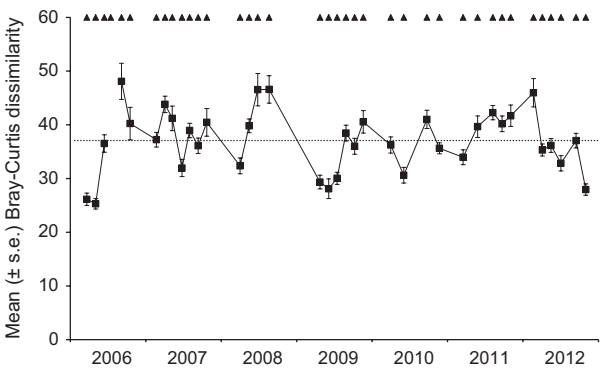

Fig. 4. Changes in mean ± SE (n = 10–45, depending on the date) value of Bray–Curtis dissimilarity between the control and VectoBac®-treated areas. Interruption of the lines in 2006 corresponds to a drought period. The triangles indicate the dates of VectoBac® applications in the treated area.

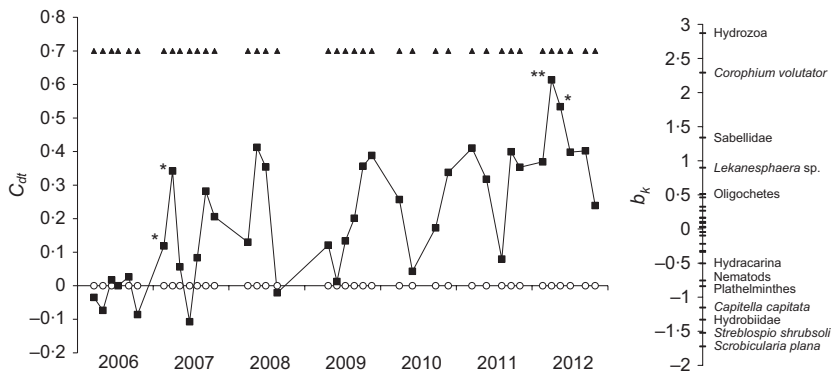

Fig. 3. Principal response curves (PRC) resulting from the analysis of invertebrate abundance dataset for the whole study period. The vertical axis represents the difference in community structure between the control (empty circles) and VectoBac®-treated (filled squares) areas expressed as regression coefficients (C_{dt}) of the PRC model. The species weights (b_k) can be interpreted as the affinity of the taxon to the principal response. Only species with a weight greater than 0·5 or less than −0·5 are shown. Results for statistical comparison for each sampling date: **: $0{\cdot}001 < P < 0{\cdot}01$; *: $0{\cdot}01 < P < 0{\cdot}05$. The triangles indicate the dates of VectoBac® applications in the treated area.

was highly significant ($P < 0{\cdot}001$) for both control and treated areas (Fig. 5), indicating that the structure of the communities evolved with time in both areas. ANCOVA showed that the slopes of the relations were not statistically different ($P = 0{\cdot}40$), suggesting similar rates of community changes in the two areas.

INVERTEBRATE PREYS FOR BIRDS

GLMER analysis showed that interaction between time and area had a highly significant effect on the density of the four prey categories for waterfowl and wading birds (Table S7, Supporting Information). The abundance of annelids showed high intra- and interannual variability with parallel patterns in the control and treated areas (Fig. 6a), and no significant between-area difference was observed when considering the whole study period ($\chi^2 = 0{\cdot}29$, d.f. = 1, $P = 0{\cdot}59$). Significant differences between control and treated areas were found for molluscs and insects (χ^2 values of 5·08 and 4·3, d.f. = 1, $P = 0{\cdot}024$ and 0·039, respectively). Date-by-date analysis (Table S6, Supporting Information) showed that molluscs were more abundant in the control than in the treated area in 2011 and 2012 (Fig. 6b). This corresponds to the increase in *S. plana* in the control area (Fig. S2, Supporting Information). The abundance of crustaceans exhibited important seasonal variations with peak values usually occurring in mid-spring and summer and lower values by the end of the year (Fig. 6c). Insects were more abundant in the treated pools in 2006 and 2010 (Fig. 6d). The abundance patterns of larvae of the two midge groups were relatively similar, with lower values for Orthocladiinae than for Chironomini (Fig. S2, Supporting Information). Mean density of midge larvae dramatically decreased by the end of the study period in both control and treated areas.

Discussion

This study provides further evidence that long-term changes in the diversity and abundance of non-target aquatic invertebrates in coastal wetlands are driven by natural fluctuations in the environmental conditions, and VectoBac® spraying only plays a negligible role, if any, in the evolution of these animal communities. Between-area dissimilarity did not change during the study period (Fig. 4), showing that repeated VectoBac® applications did not cause any divergence in non-target invertebrate community structure. Furthermore, our analyses revealed significant changes in the structure of the invertebrate community over 7 years, but the intensity of this evolution was nearly identical in both control and treated areas (Fig. 5). Our results thus support contentions that to fully understand the changes in invertebrate communities induced by VectoBac® in such variable environments, trends should be assessed at a time scale sufficient to capture habitat variation. Given that the environmental conditions were very similar between the control and treated areas (Fig. 1), it would be tempting to ascribe changes in the abundance of invertebrate taxa to the presence of VectoBac®. However, the number of dates when taxa were significantly more abundant in the control area is equivalent to the number of dates when taxa were significantly more abundant in the treated area, and overall, total abundance of invertebrates was more often significantly higher in the treated area than in the control one (Table S6, Supporting Information). This obviously requires scrutinizing the respective roles of natural environmental factors and repeated VectoBac® spraying as local drivers of non-target invertebrate community.

Aquatic habitats associated with saltmarshes are usually highly variable with respect to temperature, salinity, water depth and other environmental parameters. This was confirmed by our data. In the study areas, water temperature, salinity and pH were associated with water depth, which, in turn, is directly influenced by the balance between evaporation and inputs of water by tide and rainfall. Local topography and hydrology (i.e. the way tide water flooded the marsh) at our study site resulted in systematically higher water level in the control pools, as compared to the treated ones. Consequently, temperature and salinity were

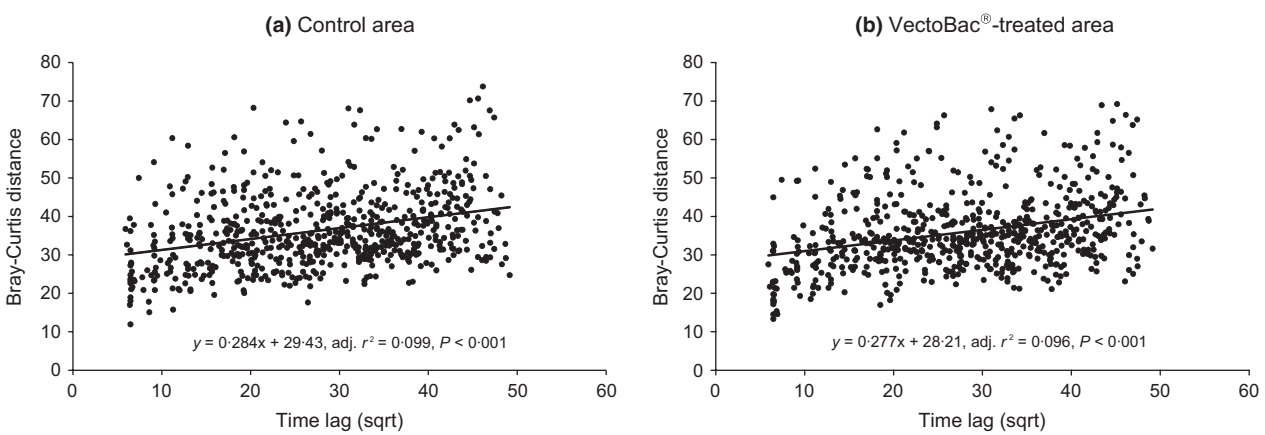

Fig. 5. Time-lag analysis of invertebrate community dynamics in the control (a) and VectoBac®-treated (b) pools.

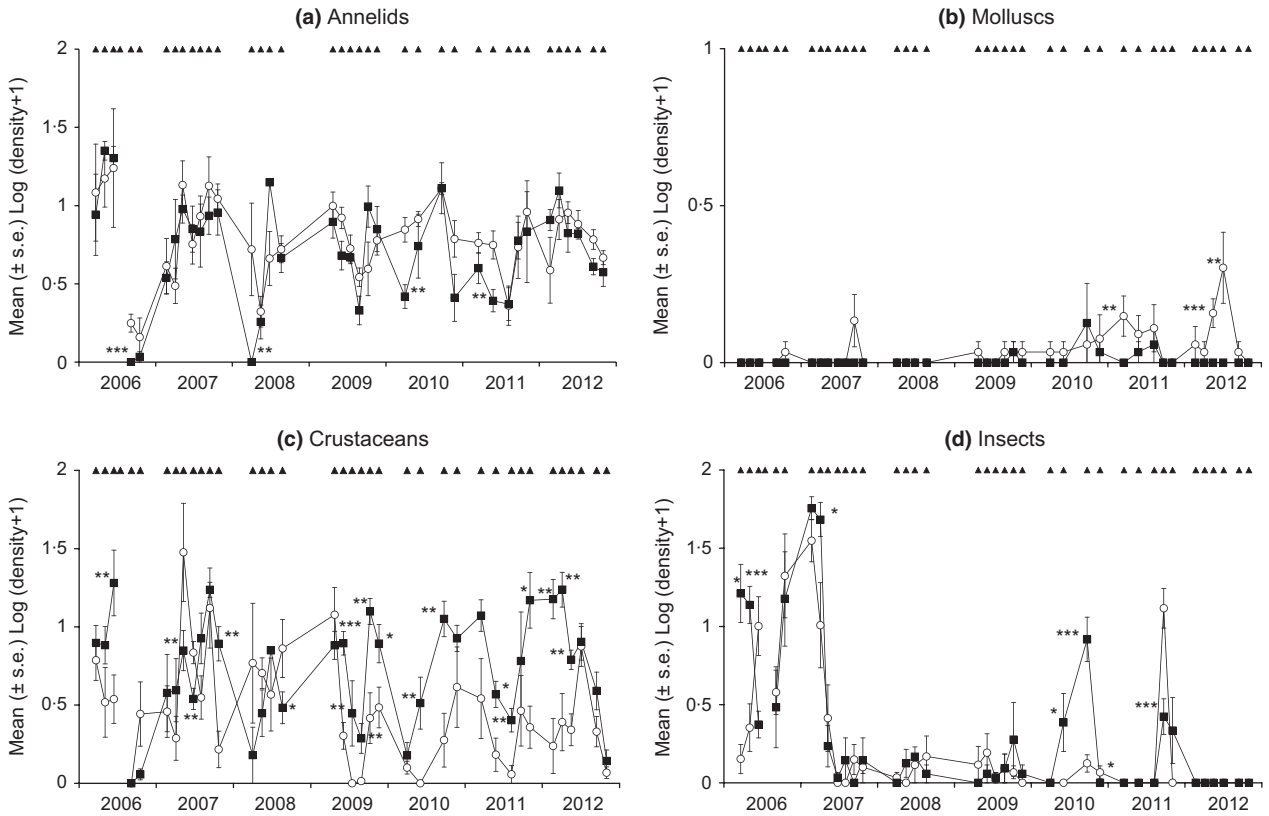

Fig. 6. Changes in mean ± SE (n = 5) abundance of the various groups of invertebrates considered as major food resources for waterfowl and wading birds in control (empty circles) and VectoBac®-treated areas (filled squares). (a) annelids; (b) molluscs; (c) crustaceans; (d) insects. Statistically significant difference according to GLM analysis with FDR correction: *: 0·01 < P < 0·05; **: 0·001 < P < 0·01; ***: P < 0·001. Interruption of the lines in 2006 corresponds to a drought period. The triangles indicate the dates of VectoBac® applications in the treated area.

more often lower and higher, respectively, in the control area than in the treated one. Another essential consequence of higher water level in the control area is that drought occurred more frequently in the treated area. Although such systematic discrepancies between control and treated pools were occasional for most environmental factors (e.g. partial drought in the treated area only occurred in 8·1% of the sampling dates, and significantly lower water depth values in treated pools were only observed in 10·8% of the sampling dates), they were more regularly observed for water temperature and salinity (48·6 and 32·4% of the sampling dates, respectively; Table S3, Supporting Information). In addition, extreme values of water temperature, salinity, dissolved oxygen saturation and pH were higher in the treated pools than in the control ones (Fig. 1 and Table S3 in Supporting Information). Altogether, these systematic trends may explain why molluscs (*S. plana* and Hydrobiidae) and worms (*S. shrubsolii*, *C. capitata* and Platyhelminthes) were found in increasing abundance in the control area. Conversely, environmental factors in VectoBac®-treated pools may provide suitable habitat conditions for Malacostraca (*Lekanesphaera* sp. and *C. volutator*), Sabellidae and Hydrozoa (Fig. 3 and Fig. S2 in Supporting Information). A common feature of *S. shrubsolii*, *C. capitata*, *S. plana* and *Hydrobia* species is their preference for muddy sediments (see e.g. Warren 1977; Sardá & Martin 1993; Kevrekidis, Gouvis & Koukouras 1996; Santos *et al.* 2011). Such conditions exist in the control area, which retains water for longer period than the treated area. For *S. plana*, which is more tolerant to low salinities than most common estuarine bivalves, settlement can be prevented by coarser sediment and high temperature (Santos *et al.* 2011). With consistently low salinity and temperature, and soft sediment, control pools at our study site may therefore provide a fairly suitable habitat for *S. plana*. Conversely, in the VectoBac®-treated pools where the range of variation of temperature and salinity was larger than in the control pools, favourable habitat conditions may exist for *Lekanesphaera* sp. and *C. volutator*. *Lekanesphaera* species are able to tolerate a wide range of temperature (Castañeda & Drake 2008), and they are very good osmoregulators (Vignes *et al.* 2012). *Corophium volutator* populations show huge abundance variations as a result primarily of environmental factors such as temperature, salinity, oxygen availability and sediment properties (Mills & Fish 1980, Murdoch, Bärlocher & Laltoo 1986; Raffaelli *et al.* 1991). Both species may thus tolerate the wider ranges of variation of most environmental parameters in the treated pools, as compared

to the control ones. Interspecies interactions between mudflat invertebrates may also result in the patchy distribution observed for most taxa. Interestingly, comparisons of small-scale spatial distribution patterns of brackish water invertebrates indicated a negative correlation between *C. volutator* and *Hydrobia* sp. (Lawrie 1996). This is consistent with the fact that these two species appear on the opposite parts of the b_k axis in the PRC analysis (Fig. 3). Indeed, between-species interactions and competition for food resources are a key feature of the distribution and abundance of brackish water invertebrates. However, investigation of the feeding biology of the numerous species found at our study site was out of the scope of our study. Instead, we focussed on the sensitivity of those species to *Bti*.

Clearly, if we exclude laboratory data, where high *Bti* dosages are most frequently used (nearly 70% of the published studies according to Boisvert & Boisvert 2000), and field situations where overdosage occurred, none of the aquatic invertebrate taxa encountered in our study is known as sensitive to *Bti*, except Chironominae and, to a lesser extent, Orthocladiinae (Boisvert & Boisvert 2000; Boisvert & Lacoursière 2004). This is consistent with the fact that epithelial cells which are vulnerable to *Bti* toxins, are present at high density in the anterior midgut of dipteran larvae (Culicidae, Chironomidae and Simuliidae). In contrast, crustaceans are likely to be insensitive to the larvicide because their *Bti*-sensitive epithelial cells have a rapid turnover and are scarce and patchily distributed along the midgut (Rey *et al.* 1998). However, our results show that chironomids were not adversely affected by repeated VectoBac® applications. In fact, when significant effects occurred in Chironomidae (Chironomini larvae, Orthocladiinae larvae, other Chironomidae larvae and Chironomidae pupae), they were more frequently associated with higher abundances in the treated area (Table S6, Supporting Information). In field studies where *Bti* is applied at recommended levels (i.e. not overdosed), effects on chironomids are unusual (WHO 1999; Boisvert & Boisvert 2000; Boisvert & Lacoursière 2004) and generally only detected when *Bti* is used in lotic systems to control black fly larvae. However, *Bti*-related changes in the abundance of chironomids have no effects on food webs in rivers (Merritt *et al.* 1989; Molloy 1992; Jackson, Horwitz & Sweeney 2002) where predator and detritivorous invertebrates and fish are not directly or indirectly affected by larvicide applications, so that functional impacts on the ecosystem are unlikely (Boisvert & Lacoursière 2004).

In lentic systems, the effects of *Bti* on chironomids, among other aquatic invertebrates, have mainly been assessed in experimental conditions (e.g. *in situ* enclosures or outdoor artificial ponds; Miura, Takahashi & Mulligan 1980; Ali 1981; Charbonneau, Drobney & Rabeni 1994; Liber, Schmude & Rau 1998; Pont, Franquet & Tourenq 1999; Russell, Kay & Skilleter 2009), which do not necessarily reflect the actual use of the larvicide according to the recommended practices for mosquito control. In particular, these studies were conducted over the short term (one treatment season, i.e. <1 year), generally after a single application of *Bti*. Nevertheless, they consistently show transient effects of *Bti* on chironomids at dosages which were several folds higher than the recommended application rates for mosquito control. At recommended levels, *Bti* had no effects on the abundance of chironomids in short-term semi-field trials (Ali 1981; Charbonneau, Drobney & Rabeni 1994; Liber, Schmude & Rau 1998; Russell, Kay & Skilleter 2009), and this was confirmed in field studies conducted over one to 3 years (Ali 1981; Mulla, Federici & Darwazeh 1982; Mulligan & Schaefer 1982).

The only long-term survey of the effects of operational use of *Bti* on chironomids in lentic ecosystems has been conducted over 6 years in the River Dalälven floodplain (Sweden). In this study, aerial applications of VectoBac® G (13–15 kg ha^{-1} ≡ 2·6 10^9–3 10^9 ITU ha^{-1}) had no effect on chironomid species richness and production (Lundström *et al.* 2010a,b). These results are fully consistent with our data. However, they contradict previous findings from a study conducted in Minnesota freshwater wetlands, which showed that when aerially applied for 3 years (1991–1993) according to standard mosquito control practices, VectoBac® G (11·72 ± 0·64 kg ha^{-1} ≅ 2·34 10^9 ITU ha^{-1}) caused a 60–80% reduction in chironomid abundance during the last 2 years (Hershey *et al.* 1998). Total richness of insects was also reduced by 33–66%, and insect biomass showed a similar trend of significant reduction during the 1992 and 1993 treatment seasons (Niemi *et al.* 1999). Further samplings in 1997 and 1998 showed, however, that after intensive and continuous VectoBac® G application, the only difference between treated and untreated sites was lower population levels of some groups within the Chironomidae in treated sites, but other chironomid groups were more abundant, resulting in no difference in chironomid numbers or biomass as a whole (Balcer *et al.* 1999). On the other hand, in spite of the reductions in insect density and biomass observed in 1992 and 1993, integration of the data obtained for zooplankton, insects and breeding birds indicated no significant effect on food web in these wetlands (Niemi *et al.* 1999). In particular, the data collected for the bird community and 19 individual bird species showed that the reduction in aquatic insects (including mosquitoes) was unlikely to depress food available to birds during the breeding season (Hanowski *et al.* 1997a,b). Our results support the fact that this is also true for the French Atlantic coastal wetlands where VectoBac® WG is used to control mosquito populations.

We analysed the availability of invertebrate groups most frequently used as food resources by shorebirds and wading birds, which are regularly observed in Atlantic coastal wetlands. Literature data indicate that annelids, molluscs and crustaceans are common prey items for these birds, whereas chironomids and mosquitoes, among other insects, are rarely mentioned (Le Drean Quenec'hdu

& Mahéo 1997; MacKenzie 2005; Santos, Granadeiro & Palmeirim 2005; Goss-Custard *et al.* 2006; Granadeiro *et al.* 2007; Pedro & Ramos 2009; Viain *et al.* 2011). As bird foraging behaviour is influenced not only by prey abundance but also by prey size and biomass (Santos, Granadeiro & Palmeirim 2005; Goss-Custard *et al.* 2006; Pedro & Ramos 2009), we compiled the abundance data of all taxa belonging to these four invertebrate groups considering, for each of them, the individuals found in sieves with mesh size ≥2 mm. Abundance patterns of annelids, molluscs, crustaceans and insects were very similar between the control and VectoBac®-treated areas (Fig. 6). Nevertheless, significantly higher mollusc abundances were found in the control area, whereas insects were significantly more abundant in the treated area. However, date-by-date analysis showed that significant differences between the two areas were occasional for insects, annelids and molluscs (18·9, 10·8, 8·1% of the sampling dates, respectively) and much more frequent for crustaceans (48·6% of the sampling dates; Table S6, Supporting Information). Annelids and molluscs (mainly *S. plana*) were more often found at significantly higher abundances in the control area, whereas crustaceans (mainly *C. volutator*) and insects were significantly more abundant in the treated area. Overall, these results indicate that over the long term, the amount of invertebrates that could be used as food resources by birds is maintained in areas treated with VectoBac® WG. In such conditions, alterations in the food web in Atlantic coastal wetlands where this larvicide is used for mosquito control are very unlikely.

This study represents the largest investigation ever conducted over the long term to analyse the effects of *Bti*-based mosquito control on non-target aquatic invertebrates in wetlands. Our results further support the conclusion that the evolution of invertebrate communities in terms of taxa abundance, richness and diversity is highly driven by natural environmental factors (e.g. Hershey *et al.* 1995; Russell, Kay & Skilleter 2009; Caquet *et al.* 2011). Due to the wide range of variation of these factors in such ecosystems, temporal fluctuations in numbers of invertebrate taxa within a given area are often much greater in magnitude than any difference between control and treated pools. Nevertheless, our results contribute to fill the knowledge gap on unwanted effects of *Bti* in saltmarshes. Indeed, as compared to freshwater wetlands, data on the impact of *Bti* on saltmarsh invertebrate communities are scarce. Barnes & Chapman (1998) found no effect of VectoBac® 12AS on the abundance of chironomid larvae, crustaceans or molluscs in sediments from temperate New South Wales (Australia) saltmarshes. Similarly, Russell, Kay & Skilleter (2009) showed that VectoBac® 12AS did not affect the abundance and composition of non-target arthropod assemblages in subtropical saltmarshes in south-east Queensland, Australia. Our study fully supports these findings. We demonstrated that long-term use of VectoBac® WG, whose potency is equivalent to that of VectoBac® 12AS, had no influence on the temporal evolution of the taxonomic structure and taxa abundance of non-target aquatic invertebrates in French Atlantic coastal wetlands. Our results have practical implications in terms of improvement in the environmental safety of mosquito control practices. *Bti* is used world-wide for larval mosquito control, raising common concerns about unwanted effects. However, only a few countries are dealing with such effects over the long term, so that there is no general guidance for sustainable mosquito control programmes and associated environmental monitoring. Current practices (e.g. land-based treatments with portable sprayers, the use of formulations with a low potency in terms of ITU, substantial reduction in the applied dosage as compared to the recommended rate) to control mosquitoes along the French Atlantic coast minimize potential environmental impacts of VectoBac® (e.g. reduction in the application rate down to 150 g ha^{-1} ≡0·45 10^9 ITU ha^{-1} has been achieved without loss of efficiency on mosquito larvae). From this experience, improvement in mosquito control practices such as dosage reduction, timely application and targeted spraying of *Bti*-based larvicides should be promoted as preventive environmental protection measures. Even so, surveillance of possible primary side effects of these larvicides is needed. For such a purpose, the choice of study sites and, within each study site, of comparable control and treated areas, is of primary importance. Indeed, systematic temporal trends and subtle differences in the range of variation of abiotic factors between control and treated areas may result in discrepancies in taxa abundance and diversity, which could be wrongly attributed to *Bti* spraying. Such misleading situations, which may result in irrational management decisions and inappropriate mitigation measures, are more likely to occur when environmental conditions in control and treated areas are dissimilar. In any case, environmental management will benefit from long-term surveillance based on improved sampling strategies, consistent monitoring of abiotic factors and expert knowledge as this is the only way to identify the respective roles of mosquito control and natural environmental changes on the evolution of non-target fauna in wetlands where *Bti* is used.

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References

Ali, A. (1981) *Bacillus thuringiensis* serovar. *israelensis* (ABG-6108) against chironomid midges and some nontarget invertebrates. *Journal of Invertebrate Pathology*, **38**, 264–272.

Balcer, M.D., Schmude, K.I., Snitgen, J. & Lima, A.R. (1999) Long-term effects of the mosquito control agents *Bti* (*Bacillus thuringiensis israelensis*) and methoprene on non-target macro-invertebrates in wetlands in Wright County, Minnesota (1997–1998). Report to Metropolitan Mosquito Control District, St. Paul, Minnesota. 76 pp. plus appendices.

Barnes, P.B. & Chapman, M.G. (1998) *Effects of the Larvicide VectoBac on Assemblages of Benthic Invertebrates in Bicentennial Park*. Centre for Research on Ecological Impacts of Coastal Cities, Sydney.

Becker, N. (2006) Microbial control of mosquitoes: Management of the Upper Rhine mosquito population as a model programme. *An Ecological and Societal Approach to Biological Control* (eds J. Eilenberg & H.M.T. Hokkanen), pp. 227–245. Springer Verlag, Berlin.

Benjamini, Y. & Hochberg, Y. (1995) Controlling the false discovery rate: a practical and powerful approach to multiple testing. *Journal of the Royal Statistical Society Series B*, **57**, 289–300.

Boisvert, M. & Boisvert, J. (2000) Effects of *Bacillus thuringiensis* var. *israelensis* on target and nontarget organisms: a review of laboratory and field experiments. *Biocontrol Science and Technology*, **10**, 517–561.

Boisvert, J. & Lacoursière, J.O. (2004) Le *Bacillus thuringiensis israelensis* et le contrôle des insectes piqueurs au Québec. Envirodoq no ENV/2004/0278, Ministère de l'Environnement, Québec.

Breeland, S.G. & Mulrennan, J.A. Jr (1983) Florida mosquito control. *Pest Control*, **51**, 16–24.

van den Brink, P.J. & ter Braak, C.J.F. (1999) Principal response curves: analysis of time-dependent multivariate responses of biological community to stress. *Environmental Toxicology and Chemistry*, **18**, 138–148.

van den Brink, P.J., Hattink, J., Bransen, F., van Donk, E. & Brock, T.C.M. (2000) Impact of the fungicide carbendazim in freshwater microcosms. II. Zooplankton, primary producers and final conclusions. *Aquatic Toxicology*, **48**, 251–264.

Butler, D. (2012) Europe on alert for flying invaders. *Nature*, **489**, 187–188.

Butler, R.W., Davidson, N.C. & Morrison, I.G. (2001) Global scale shorebird distribution in relation to productivity of near-shore ocean waters. *Waterbirds*, **24**, 224–232.

Caquet, T., Roucaute, M., Le Goff, P. & Lagadic, L. (2011) Effects of repeated field applications of two formulations of *Bacillus thuringiensis* var. *israelensis* on non-target saltmarsh invertebrates in Atlantic coastal wetlands. *Ecotoxicology and Environmental Safety*, **74**, 1122–1130.

Carron, A., Duchet, C., Gaven, B. & Lagneau, C. (2003) An easy field method for estimating the abundance of culicid larval instars. *Journal of the American Mosquito Control Association*, **19**, 353–360.

Castañeda, E. & Drake, P. (2008) Spatiotemporal distribution of *Lekanesphaera* species in relation to estuarine gradients within a temperate European estuary (SW Spain) with regulated freshwater inflow. *Ciencias Marinas*, **34**, 125–141.

Charbonneau, C.S., Drobney, R.D. & Rabeni, C.F. (1994) Effects of *Bacillus thuringiensis* on nontarget benthic organisms in a lentic habitat and factors affecting the efficacy of the larvicide. *Environmental Toxicology and Chemistry*, **13**, 267–279.

Clarke, K.R. & Warwick, R.M. (2001) *Change in Marine Communities: An Approach to Statistical Analysis and Interpretation*, 2nd edn. PRIMER-E, Plymouth, MA.

Collins, S.L., Micheli, F. & Hartt, L. (2000) A method to determine rates and patterns of variability in ecological communities. *Oikos*, **91**, 285–293.

Després, L., Lagneau, C. & Frutos, R. (2011) Using the bio-insecticide *Bacillus thuringiensis israelensis* in mosquito control. *Pesticides in the Modern World – Pests Control and Pesticides Exposure and Toxicity Assessment* (ed. M. Stoytcheva), pp. 93–126. InTech, Rijeka, Croatia.

Erickson, R.A., Hayhoe, K., Presley, S.M., Allen, L.J.S., Long, K.R. & Cox, S.B. (2012) Potential impacts of climate change on the ecology of dengue and its mosquito vector the Asian tiger mosquito (*Aedes albopictus*). *Environmental Research Letters*, **7**, 034003.

Fourcy, D., Jumel, A., Heydorff, M. & Lagadic, L. (2002) Esterases as biomarkers in *Nereis* (*Hediste*) *diversicolor* exposed to temephos and *Bacillus thuringiensis* var. *israelensis* used for mosquito control in coastal wetlands of Morbihan (Brittany, France). *Marine Environmental Research*, **54**, 755–759.

Ganter, B. (2000) Seagrass (*Zostera* spp.) as food for brent geese (*Branta bernicla*): an overview. *Helgoland Marine Research*, **54**, 63–70.

Giberson, D.J., Bilyj, B. & Burgess, N. (2001) Species diversity and emergence patterns of Nematocerous flies (Insecta: Diptera) from three coastal salt marshes in Prince Edward Island, Canada. *Estuaries*, **24**, 862–874.

Goss-Custard, J.D., West, A.D., Yates, M.G., Caldow, R.W.G., Stillman, R.A., Bardsley, L. *et al.* (2006) Intake rates and the functional response in shorebirds (Charadriiformes) eating macro-invertebrates. *Biological Reviews*, **80**, 501–529.

Granadeiro, J.P., Santos, C.D., Dias, M.P. & Palmeirim, J.M. (2007) Environmental factors drive habitat partitioning in birds feeding in intertidal flats: implications for conservation. *Hydrobiologia*, **587**, 291–302.

Hanowski, J., Niemi, G., Lima, A. & Regal, R. (1997a) Response of breeding birds to mosquito control treatments of wetlands. *Wetlands*, **17**, 485–492.

Hanowski, J.M., Niemi, G.J., Lima, A.R. & Regal, R.R. (1997b) Do mosquito control treatments of wetlands affect red-winged blackbird (*Agelaius phoeniceus*) growth, reproduction, or behavior? *Environmental Toxicology and Chemistry*, **16**, 1014–1019.

Hershey, A.E., Lima, A.R., Niemi, G.J. & Regal, R.R. (1998) Effects of *Bacillus thuringiensis israelensis* (*Bti*) and methoprene on non-target macroinvertebrates in Minnesota wetlands. *Ecological Applications*, **8**, 41–60.

Hershey, A.E., Shannon, L., Axler, R., Ernst, C. & Mickelson, P. (1995) Effects of methoprene and *Bti* (*Bacillus thuringiensis* var. *israelensis*) on non-target insects. *Hydrobiologia*, **308**, 219–227.

Jackson, J.K., Horwitz, R.J. & Sweeney, B.W. (2002) Effects of *Bacillus thuringiensis israelensis* on black flies and nontarget macroinvertebrates and fish in a large river. *Transactions of the American Fisheries Society*, **131**, 910–930.

Kevrekidis, T., Gouvis, N. & Koukouras, A. (1996) Bionomy of macrobenthic molluscs in Evros Delta (North Aegean Sea). *Internationale Revue der gesamten Hydrobiologie und Hydrographie*, **81**, 455–468.

Kurkela, S., Rätti, O., Huhtamo, E., Uzcátegui, N.Y., Nuorti, J.P., Laakkonen, J. *et al.* (2008) Sindbis Virus infection in resident birds, migratory birds, and humans, Finland. *Emerging Infectious Diseases*, **14**, 41–47.

Lawrie, S.M. (1996) *Spatial pattern of the amphipod* Corophium volutator *on the Ythan Estuary* PhD Thesis, University of Aberdeen, 204 p.

Le Drean Quenec'hdu, S. & Mahéo, R. (1997) Régime alimentaire des limicoles dans les traicts du Croisic et impact sur les populations de mollusques bivalves. *Spatule*, **3**, 19–24.

Lepš, J. & Šmilauer, P. (2003) *Multivariate Analysis of Ecological Data Using CANOCO*. Cambridge University Press, Cambridge, UK.

Liber, K., Schmude, K.L. & Rau, D.M. (1998) Toxicity of *Bacillus thuringiensis* var. *israelensis* to chironomids in pond mesocosms. *Ecotoxicology*, **7**, 343–354.

Lundström, J.O. (1999) Mosquito-borne viruses in Western Europe: a review. *Journal of Vector Ecology*, **24**, 1–39.

Lundström, J.O., Brodin, Y., Schäfer, M.L., Persson Vinnersten, T.Z. & Östman, Ö. (2010a) High species richness of Chironomidae (Diptera) in temporary flooded wetlands associated with high species turn-over rates. *Bulletin of Entomological Research*, **100**, 433–444.

Lundström, J.O., Schäfer, M.L., Petersson, E., Persson Vinnersten, T.Z., Landin, J. & Brodin, Y. (2010b) Production of wetland Chironomidae (Diptera) and the effects of using *Bacillus thuringiensis israelensis* for mosquito control. *Bulletin of Entomological Research*, **100**, 117–125.

MacKenzie, R.A. (2005) Spatial and temporal patterns in insect emergence from a Southern Maine salt marsh. *The American Midland Naturalist*, **153**, 257–269.

Merritt, R.W., Walker, E.D., Wilzbach, M.A., Cummins, K.W. & Morgan, W.T. (1989) A broad evaluation of *B.t.i.* for black fly (Diptera: Simuliidae) control in a Michigan river: efficacy, carry and nontarget effects on invertebrates and fish. *Journal of the American Mosquito Control Association*, **5**, 397–415.

Mills, A. & Fish, J.D. (1980) Effects of salinity and temperature on *Corophium volutator* and *C. arenarium* (Crustacea: Amphipoda), with particular reference to distribution. *Marine Biology*, **58**, 153–161.

Miura, T., Takahashi, R.M. & Mulligan, F.S. III (1980) Effects of the bacterial mosquito larvicide, *Bacillus thuringiensis* serotype H-14 on selected aquatic organisms. *Mosquito News*, **40**, 619–622.

MMCD – Metropolitan Mosquito Control District (1999) A program evaluation report. Program Evaluation Division, Office of the Legislative Auditor, State of Minnesota. 118 p.

Molloy, D.P. (1992) Impact of the black fly (Diptera: Simuliidae) control agent *Bacillus thuringiensis* var. *israelensis* on chironomids (Diptera: Chironomidae) and other non target insects: results of ten field trials. *Journal of the American Mosquito Control Association*, **8**, 24–31.

Mulla, M.S., Federici, B.A. & Darwazeh, H.A. (1982) Larvicidal efficacy of *Bacillus thuringiensis* serotype H-14 against stagnant-water mosquitoes and its effects on nontarget organisms. *Environmental Entomology*., **11**, 788–795.

Mulligan, F.S. III & Schaefer, C.H. (1982) Integration of a selective mosquito control agent *Bacillus thuringiensis* serotype H.14, with natural

predator populations in pesticide-sensitive habitats. *Proceeding of the California Mosquito Vector Control Association*, **49**, 19–22.
Murdoch, M.H., Bärlocher, F. & Laltoo, M.L. (1986) Population dynamics and nutrition of *Corophium volutator* (Pallas) in the Cumberland Basin (Bay of Fundy). *Journal of Experimental Marine Biology and Ecology*, **103**, 235–249.
Niemi, G.J., Hershey, A.E., Shannon, L., Hanowski, J.M., Lima, A., Axler, R.P. & Regal, R.R. (1999) Ecological effects of mosquito control on zooplankton, insects, and birds. *Environmental Toxicology and Chemistry*, **18**, 549–559.
O'Hara, R.B. & Kotze, D.J. (2010) Do not log-transform count data. *Methods in Ecology and Evolution*, **1**, 118–122.
Östman, Ö., Lundström, J.O. & Persson Vinnersten, T.Z. (2008) Effects of mosquito larvae removal with *Bacillus thuringiensis israelensis* (Bti) on natural protozoan communities. *Hydrobiologia*, **607**, 231–235.
Pedro, P. & Ramos, J.A. (2009) Diet and prey selection of shorebirds on salt pans in the Mondego estuary, Western Portugal. *Ardeola*, **56**, 1–11.
Peng, Z. & Simons, F.E. (2007) Advances in mosquito allergy. *Current Opinion in Allergy and Clinical Immunology*, **7**, 350–354.
Persson Vinnersten, T.Z., Lundström, J.O., Schäfer, M.L., Petersson, E. & Landin, J. (2010) A six-year study of insect emergence from temporary flooded wetlands in central Sweden, with and without *Bti*-based mosquito control. *Bulletin of Entomological Research*, **100**, 715–725.
Pont, D., Franquet, E. & Tourenq, J.N. (1999) Impact of different *Bacillus thuringiensis* variety *israelensis* treatments on a chironomid (Diptera: Chironomidae) community in a temporary marsh. *Journal of Economic Entomology*, **92**, 266–272.
Poulin, B., Lefebvre, G. & Paz, L. (2010) Red flag for green spray: adverse trophic effects of *Bti* on breeding birds. *Journal of Applied Ecology*, **47**, 884–889.
R Development Core Team (2008) *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org.
Raffaelli, D., Limia, J., Hull, S. & Pont, S. (1991) Interactions between the amphipod *Corophium volutator* and macroalgal mats on estuarine mudflats. *Journal of the Marine Biological Association of the United Kingdom*, **71**, 899–908.
Rey, D., Long, A., Pautou, M.P. & Meyran, J.C. (1998) Comparative histopathology of some Diptera and Crustacea of aquatic alpine ecosystems, after treatment with *Bacillus thuringiensis* var. *israelensis*. *Entomologia Experimentalis and Applicata*, **88**, 255–263.
Rezza, G., Nicoletti, L., Angelini, R., Romi, R., Finarelli, A.C., Panning, M. *et al*. (2007) Infection with Chikungunya virus in Italy: an outbreak in a temperate region. *Lancet*, **370**, 1840–1846.
Russell, T.L., Kay, B.H. & Skilleter, G.A. (2009) Environmental effects of mosquito insecticides on saltmarsh invertebrate fauna. *Aquatic Biology*, **6**, 77–90.
Sainz-Elipe, S., Latorre, J.M., Escosa, R., Masià, M., Fuentes, M.V., Mas-Coma, S. & Bargues, M.D. (2010) Malaria resurgence risk in southern Europe: climate assessment in an historically endemic area of rice fields at the Mediterranean shore of Spain. *Malaria Journal*, **9**, 221.
Santos, C.D., Granadeiro, J.P. & Palmeirim, J.M. (2005) Feeding ecology of Dunlin *Calidris alpina* in a southern European estuary. *Ardeola*, **52**, 235–252.
Santos, S., Luttikhuizen, P.C., Campos, J., Heip, C.H.R. & van der Veer, H.W. (2011) Spatial distribution patterns of the peppery furrow shell *Scrobicularia plana* (da Costa, 1778) along the European coast: a review. *Journal of Sea Research*, **66**, 238–247.
Sardá, R. & Martin, D. (1993) Population of *Streblospio* (Polychaeta: Spionidae) in temperate zones: demography and production. *Journal of the Marine Biological Association of the United Kingdom*, **73**, 769–784.
Sirot, E., Maes, Ph. & Gélinaud, G. (2012) Movements and conflicts in a flock of foraging black-tailed godwits (*Limosa limosa*): the influence of feeding rates on behavioural decisions. *Ethology*, **118**, 127–134.
SPRP – Scientific Peer Review Panel (1996) An assessment of non-target effects of the mosquito larvicides, *Bti* and methoprene, in metropolitan area wetlands. Report to the Metropolitan Mosquito Control District, 61 p.
US EPA – United States Environmental Protection Agency (1998) *Reregistration Eligibility Decision (RED) – Bacillus thuringiensis*. United States Environmental Protection Agency, Office of Prevention, Pesticides and Toxic Substances, Washington, DC, 157 p.
Vega-Rua, A., Zouache, K., Caro, V., Diancourt, L., Delaunay, P., Grandadam, M. & Failloux, A.-B. (2013) High efficiency of temperate *Aedes albopictus* to transmit Chikungunya and dengue viruses in the Southeast of France. *PLoS ONE*, **8**, e59716.
Viain, A., Corre, F., Delaporte, P., Joyeux, E. & Bocher, P. (2011) Numbers, diet and feeding methods of Common Shelduck *Tadorna tadorna* wintering in the estuarine bays of Aiguillon and Marennes-Oléron, western France. *Wildfowl*, **61**, 121–141.
Vignes, F., Fedele, M., Pinna, M., Mancinelli, G. & Basset, A. (2012) Variability of *Lekanesphaera monodi* metabolic rates with habitat trophic status. *Acta Oecologica*, **41**, 58–64.
Viniaker, F. & Lavaud, F. (2005) Allergie aux piqûres de moustiques/Mosquito bite allergy. *Revue Française d'Allergologie et d'Immunologie Clinique*, **45**, 620–625.
Warren, L.M. (1977) The ecology of *Capitella capitata* in British waters. *Journal of the Marine Biological Association of the United Kingdom*, **57**, 151–159.
Wegner, E. (2006) Mosquito control programme implemented in Wilanów (Warsaw, Poland) in summer 2002. *European Mosquito Bulletin*, **21**, 14–18.
WHO (1999) *Microbial Pest Control Agent Bacillus thuringiensis*. World Health Organization, Geneva (Environmental Health Criteria; 217), 105 p.
Worobey, J., Fonseca, D.M., Espinosa, C., Healy, S. & Gaugler, R. (2013) Child outdoor physical activity is reduced by prevalence of the Asian tiger mosquito, *Aedes albopictus*. *Journal of the American Mosquito Control Association*, **29**, 78–80.

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Supporting Information

Additional Supporting Information may be found in the online version of this article.

Fig. S1. Results of the Principal Component Analysis performed on standardized values of the environmental parameters measured in water of the control and VectoBac®-treated pools.

Fig. S2. Changes in mean log-transformed density of the different groups of invertebrates sampled in control and VectoBac®-treated pools.

Table S1. Dates of VectoBac® applications and aquatic invertebrate sampling.

Table S2. Mean ± SE and range of variation (minimum to maximum) of the environmental parameters measured in water of the control and VectoBac®-treated pools.

Table S3. Synthesis of the date-by-date analysis of the environmental parameters measured in water of the control and VectoBac®-treated pools.

Table S4. List of the 27 taxonomic groups of invertebrates found in water and sediment samples from the control and VectoBac®-treated pools.

Table S5. Results of the GLMER analysis of abundance data for the different invertebrate taxa sampled in control and VectoBac®-treated pools.

Table S6. Synthesis of the date-by-date analysis of descriptors of the invertebrate communities in the control and VectoBac®-treated areas.

Table S7. Results of the GLMER analysis on the density of invertebrates potentially used as food resources by waterfowl and wading birds in control and VectoBac®-treated areas.