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Indirect effects of mosquito control using *Bti* on dragonflies and damselflies (Odonata) in the Camargue

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Abstract. 1. *Bacillus thuringiensis var. israelensis (Bti)* has become the most commonly used larvicide to control mosquitoes worldwide. *Bti* is considered non-toxic to most organisms, except some Diptera such as chironomids, which are a major prey in wetland food webs.

2. Although Odonata are important predators of mosquitoes and chironomids at the larval and adult stages, no study has ever considered the potential indirect effects of *Bti* on Odonata abundance through trophic interactions. We addressed this topic in the Camargue where 2500 of the 25 000 ha of mosquito larval biotopes are *Bti*-sprayed (aqueous solution of VectoBac 12AS at 2.5 L ha^{-1}) whenever mosquito larvae appear in water bodies (i.e. 30–50 aerial treatments overall annually).

3. Adult Odonata were surveyed along a 100-m line transect in spring, summer and autumn at three control and three treated sites over a 5-year period.

4. Mean number of species (9.9 vs. 5.2) and of individuals (100 vs. 50) detected per year were significantly higher in control areas compared to *Bti*-sprayed areas. *Bti* treatment contributed to 87.3% of the explained variance in Odonata richness, compared to 2.9% for site, 6.8% for year and 3.0% for salinity effects.

5. These results are coherent with other studies carried out in the same area and time period highlighting a lower abundance of chironomids, and a lower intake of odonates by breeding birds in treated areas.

6. We conclude that mosquito control using *Bti* should be acknowledged as a potential threat to Odonata.

Key words. Anisoptera, *Bacillus thuringiensis israelensis*, Camargue, insecticide impact, mosquito control, Odonata, trophic interactions, wetland conservation, Zygoptera.

Introduction

Since the discovery of its selective acute toxicity against mosquitoes and black flies in Goldberg and Margalit (1977), the microbial agent *Bacillus thuringiensis var. israelensis (Bti)* has become the most commonly used larvicide to control these pest species worldwide (Lacey, 2007; Rowe *et al.*, 2008), and the only one authorised in Europe since 2007. *Bti* is considered as non-toxic to most aquatic organisms (Boisvert & Boisvert, 2000; Lacey & Merritt, 2004) and its direct effects on the non-target fauna are largely limited to other Nematocera, such as non-biting midges (Chironomidae) (Ali, 1981; Liber *et al.*, 1998) and crane flies (Tipulidae) (Oestergaarda *et al.*, 2006). In spite of the high selectivity of *Bti*, indirect effects through food web perturbations have yet received little attention, accounting only for 4% of 311 studies on *Bti* environmental use (Poulin, 2012). Odonata are no exception, being specifically considered in only two studies addressing direct effects (Painter *et al.*, 1996; Davis & Peterson, 2008). Within the non-target fauna, odonates are particularly likely to be affected by a decrease in *Bti*-sensitive insects, since small dipterans represent a major food

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source at both larval and adult stages (reviewed in Corbet, 1999). This is especially true in the Camargue where availability of chironomids and mosquitoes are considered as potentially limiting Odonata populations (Aguesse, 1961).

The Rhône delta or Camargue in southern France (43.54°N, 4.55°E) consists of a mosaic of natural and human-modified ecosystems including salt marshes, reed beds, temporary ponds, lagoons, agricultural fields (mostly rice) and salt pans spread over 145 000 ha. It is located within the biodiversity hotspot of the Mediterranean basin (Myers *et al*., 2000) and has over 1000 vascular plant and 400 bird species. To reduce the nuisance caused by biting mosquitoes to local populations and tourists, a control programme encompassing 2500 of the 25 000 ha of potential larval biotopes was launched in August 2006. Impact studies on the non-target fauna were then initiated by independent research scientists under the aegis of the Parc Naturel Régional de Camargue. Ever since, *Bti* spraying (aqueous solution of VectoBac 12AS® at 2.5 L ha^{-1}) is systematically carried out whenever *Ochlerotatus caspius* and *Oc. detritus* larvae appear in natural or semi-natural water bodies that are regularly monitored by technicians from the Entente Interdépartementale de la Démoustication (EID), a public service in charge of mosquito control operations in France since 1965 (www.eid-med.org/). Treated areas are located within a maximal distance of 8.5 km from the targeted zone where mosquito nuisance is expected to be reduced by 90%. Frequency of *Bti* spraying depends upon meteorological conditions and water management, with approximately a third of mosquito production being related to human intervention on marsh hydrology. In any year, the same water body can be sprayed more than once, depending on the monitoring results. Overall, from 30 to 50 aerial treatments, representing a cumulated sprayed area of 5000–8000 ha were carried out annually during the study period, in addition to ground spraying, which accounts for 30% of treated areas.

This study, which is part of a larger research programme, investigates the trends in richness and abundance of adult Odonata monitored through observation transects carried out within *Bti*-sprayed and control areas over a 5-year period. Our hypothesis is that a reduction in mosquitoes and chironomids caused by *Bti* spraying might affect the survival of Odonata at the larval and/or adult stages through food deprivation, translating into a reduction in abundance and richness of adult Odonata at *Bti*-treated sites. Owing to their larger dispersal capabilities and feeding range (Corbet, 1999), potentially encompassing the treated zone, we would expect Anisoptera to be proportionally less reduced than Zygoptera at treated sites relative to controls. We also present the results from an unpublished independent experiment comparing chironomid relative abundance at treated and untreated wetlands in 2012 as evidence of food reduction to Odonata. No data are available on mosquito abundance, but we assume that *Bti*-spraying is efficient for reducing their numbers as well.

Methods

Study sites

The location of the six transects used for the Odonata survey and of the eight marshes where chironomids were captured are indicated relative to mosquito producing habitats and *Bti*-sprayed areas on Fig. 1. Transect location was selected based on their ecological similarity in terms of wetland configuration, using a balanced sample of *Bti*-sprayed ($n = 3$) and control areas ($n = 3$). *Bti* spraying was partially interrupted at one of the sites in 2012 (Belugue), so that only two treated sites were included in the study design in the last 2 years (Table 1). All transects were located next to temporary marshes of relatively low salinity (<10 g L^{-1}) with bulrush (*Bolboschoenus maritimus*) as the dominant species. Irrigation canals lined with reed (*Phragmites australis*) are common in the Camargue and occurred within less than 2 km from all transects. Chironomids were sampled by passive traps located in the centre of large areas of reed (*P. australis*) and Salicornia marshes. There is an obvious geographical bias related to the southern location of the treated sites compared to the control sites, the former targeting the protection of coastal population and tourists, so that sea distance and mosquito control are intrinsically linked. Although treated sites are from 1 to 6 km from sea, and control sites are from 13 to 18 km from sea, proportion of different land cover types is relatively well balanced according to treatment (Table 2).

Sampling methodology

The sampling protocol for Odonata was defined based on a cost-effective methodology to be maintained in the long term and inspired from the British national scheme for dragonfly survey (Smallshire & Beynon, 2010). A 100-m long transect, located nearby bulrush ponds providing good breeding and foraging opportunities to odonates, was walked slowly (30–45 min) by the same observer (C. Jakob) in spring, summer and autumn. To reduce phenological bias, all transects were surveyed within a single week, with at least one control and one *Bti*-sprayed areas surveyed within the same day. Sampling was carried out 2 h before or after midday, under low wind conditions (<10 km h^{-1}) and sunny weather when Odonata activity is expected to be highest (Corbet, 1999; Jakob & Suhling, 1999). All adult Odonata observed flying or resting on the vegetation within five metres from each side of the transect line were counted and identified to species or genus, using binoculars and insect net when necessary. When individuals could not be trapped or showed doubtful colours (e.g. *Ischnura elegans*), we occasionally used photographs and in some rare cases, such as for Aeshnidae, fresh exuviae found on the transect to confirm species identification. Some flying *Sympetrum* sp. could be identified to genus only.

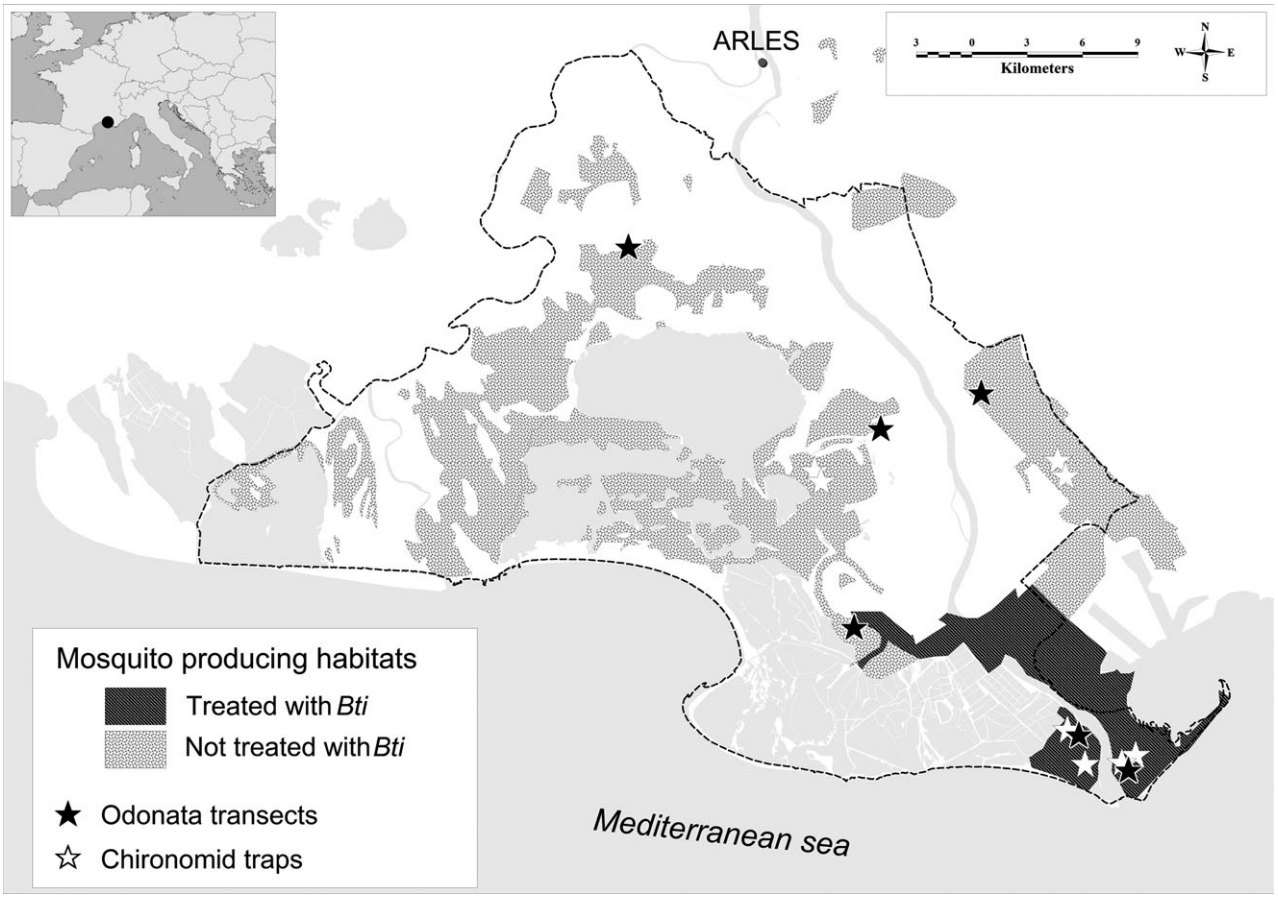


Fig. 1. Location of the study sites relative to mosquito producing habitats and mosquito-controlled areas within the limits of the Natural Regional Park of the Camargue.

Chironomids were sampled with two yellow pan traps of 30-cm diameter distant by 200 m located in four reed marshes and four *Salicornia* marshes. Traps were located in the centre of the marsh, slightly below the top of the vegetation in order to reduce edge effects. The traps were in operation during 4 days (96 h) in May, June and September 2012, avoiding periods of rain. All captured insects were preserved in ethanol and kept in the freezer before identification under the stereoscope. In order to reduce phenological biases, at least one control and one treated site were sampled simultaneously during each of the three sampling periods.

Statistical analyses

The cumulated number of species and individuals of Odonata observed on a transect each year was submitted to a generalised linear model using nested-ANOVA designs. We combined data from all three seasons because they involve different Odonata individuals (adult lifespan <60 days) and that large differences can occur in period of Odonata emergence according to years due to weather and marsh flooding conditions (Aguesse, 1961). Three descriptive variables were nested in treatment (*Bti* spraying): year, site and water salinity. In other words, we used generalised linear mixed models (GLMM) with *Bti* treatment as a fixed factor. Salinity values from the water surface of the ponds were grouped into three classes (<2 g L^{-1}, 2–5 g L^{-1}, 5–10 g L^{-1},) and could vary for a same site according to seasons or years. A generalised regression model (GRM) including the distance from the sea and *Bti* treatment as explaining variables was used to test the impact of sea proximity on the richness and abundance of Odonata, given the geographical bias of treated sites versus control sites. All analyses were made with all taxa and by distinguishing Anisoptera from Zygoptera. In all cases, the data were normally distributed (Kruskall–Wallis tests, $P > 0.2$).

The number of chironomids captured in each trap was submitted to a generalised linear model using a nested-design ANOVA. Four descriptive variables were nested in treatment (*Bti* spraying): habitat type, site, sampling period and trap.

Table 1. Mean number of individuals observed by year from each Odonata species or genus at the six transects for the whole study period.

	Control areas			*Bti*-sprayed areas			
Species	Boutardière (n = 5)	Fangouse (n = 5)	Rousty (n = 5)	Belugue (n = 3)	Clos Armand (n = 5)	They de Roustan (n = 5)	Total
Lestidae							
Lestes barbarus	0	1.6	0.4	0	0.4	0	2.8
Lestes sponsa	4.4	3.2	0.2	0	0	0	7.8
Lestes macrostigma	0	2.0	0.4	1.0	0.2	0	3.8
Sympecma fusca	0	0.6	0	0	0	0.2	0.8
Platycnemidae	0	0	0	0	0	0	0
Platycnemis acutipennis	0	0.6	0	0	0	0	0.6
Platycnemis latipes	0.2	0	0.4	0	0	0	0.6
Coenagrionidae	0	0	0	0	0	0	0
Ischnura pumilio	0.8	0.6	2.0	0	0	0	3.4
Ischnura sp.	0.2	0	0	0	0	0	0.2
Erythromma sp.	0	0	0.4	0	0	0	0.4
Coenagrion sp.	0.4	0	0	0	0	0	0.4
Aeshnidae	0	0	0	0	0	0	0
Aeshna affinis	0	1.0	1.2	0	0.2	0.2	2.6
Aeshna mixta	1.8	2.0	6.2	3.0	1.2	1.2	13.8
Anax ephippiger	0.2	0	3.4	0	0	0	3.6
Anax imperator	0.2	0	1.4	0	0	0.4	2.0
Anax parthenope	3.2	1.6	1.6	1.3	0.6	1.2	9.2
Anax sp.	0.8	0.4	0	0	0	0	1.2
Libellulidae	0	0	0	0	0	0	0
Orthetrum cancellatum	8.4	7.0	4.6	7.0	6.6	2.6	33.4
Orthetrum albistylum	3.4	1.4	2.6	0	0	0	7.4
Orthetrum coerulescens	0.8	1.0	0	0	2.4	0	4.6
Crocothemis erythraea	9.6	10.8	26.0	24.7	1.8	9.8	76.6
Sympetrum sanguineum	1.0	6.4	0	5.0	0.0	0	10.4
Sympetrum striolatum	3.4	7.0	5.8	15.3	1.8	4.4	34.6
Sympetrum meridionale	0	3.0	0.6	0	0.8	2.2	6.6
Sympetrum fonscolombii	9.4	12.8	19.4	9.7	0.6	1.2	52.2
Sympetrum sp.	11.6	10.6	5.4	0.7	1.0	8.0	39.4
Total	83.2	86.6	130.4	86.7	30.4	49.4	432.0

Table 2. Proportion (%) of land cover types at control and *Bti*-spayed sites within a 1-km radius (943 ha) of the Odonata transects.

	Control areas		*Bti*-sprayed areas	
Land cover type	Min-max	Mean	Min-max	Mean
Urban areas/roads	4–6	5	0	0
Agricultural areas	6–19	13	0–7	2
Natural dry areas	4–22	12	7–31	17
Natural wet areas	64–79	71	62–93	81

Results

Effect of sampling effort on estimation of species richness for Odonata

A total of 23 species were detected during the 5-year survey (Table 1). Species accumulation curves for *Bti*-sprayed and control sites reached an asymptote in the second year of sampling, except for the control site Rousty, which reached an asymptote on the third year of sampling (Fig. 2).

Effect of sea proximity on Odonata richness and abundance

Distance of the sampling sites from the sea cannot be added as an explaining variable in the analyses on species richness and abundance because mosquito control is targeting the protection of coastal population and tourists, so both are intrinsically linked. Considering that a 5-km gradient exists within both treated and control sites, we conducted a GRM using sea distance and *Bti* treatment to test whether our results were reflecting an environmental gradient (sea distance) rather than a treatment effect. For all variables tested (Odonata richness and abundance, Anisoptera and Zygoptera abundances), the factor sea distance did not contribute significantly ($P > 0.19$) to the GRM models.

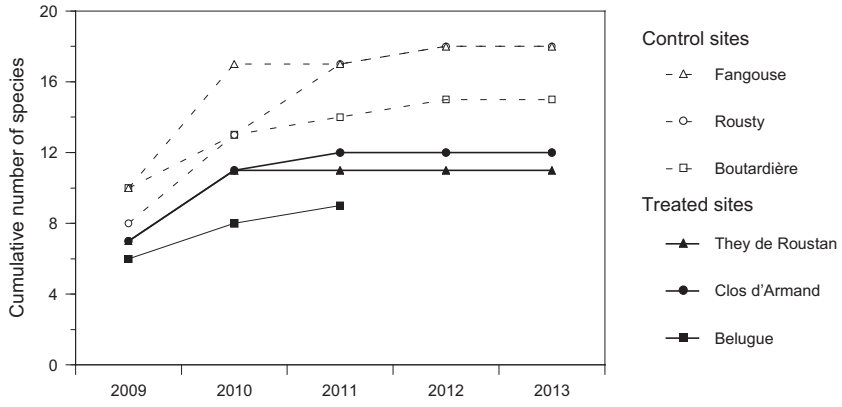


Fig 2. Cumulative number of species detected along each transect over the 5-year study period.

Effect of Bti on species richness of Odonata

Species richness per transect varied from 2 to 13 annually, with a mean value of 5.2 (±1.9 SD) species at treated sites and 9.9 (±1.8 SD) species at control sites. *Bti* spraying accounted for 87.3% of the explained variance by the nested-ANOVA designed GLM, compared to 6.8% for year, 3.0% for salinity and 2.9% for site effects. Discrepancies between control and treated sites tend to increase overtime ($F_{(8,11)} = 2.72$, $P = 0.06$), with a significantly higher species richness at control sites ($F_{(1,11)} = 35.09$, $P < 0.0001$) (Fig. 3).

Effect of Bti on Odonata abundance

Total number of individuals observed each year varied from 271 to 670, totalling 2160 individuals over the 5-year study period (Table 1). Mean annual abundance per transect was 49.9 (±39.0 SD) individuals for treated sites and 99.9 (±41.7 SD) individuals for control sites. *Bti* spraying accounted for 81.0% of the explained variance by the nested-ANOVA designed GLM, compared to 13.0% for year, 3.6% for site and 2.4% for salinity effects. Odonata abundance was significantly higher at control sites ($F_{(1,11)} = 11.2$, $P = 0.006$), with larger discrepancies between control and treated sites by the third year of the study (Fig. 4). Significant differences between control and treated sites were also observed when testing separately Anisoptera ($F_{(1,11)} = 4.7$, $P = 0.05$) and Zygoptera ($F_{(1,11)} = 10.6$; $P = 0.007$) abundances. The effect of *Bti* treatment contributed to explain, respectively, 77.0% and 68.6% of the variance observed in the number of individuals observed for these two groups.

Relative abundance of chironomids relative to Bti spraying

Some 10 839 insects were captured in yellow pan traps, including 3913 chironomids. This group was significantly

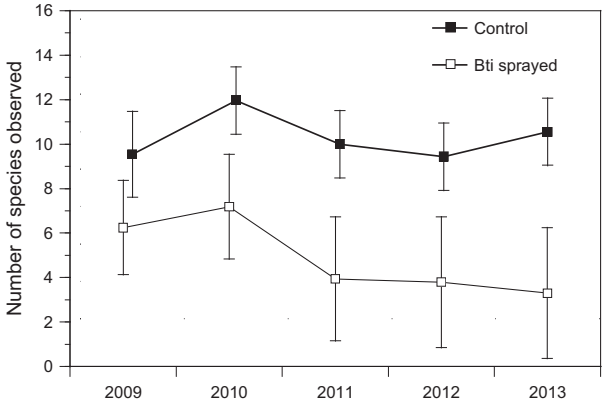


Fig. 3. Annual trends in Odonata richness (95% CI) estimated along the transect at *Bti*-sprayed and control areas over the 5-year period according to the nested-ANOVA designed GLM.

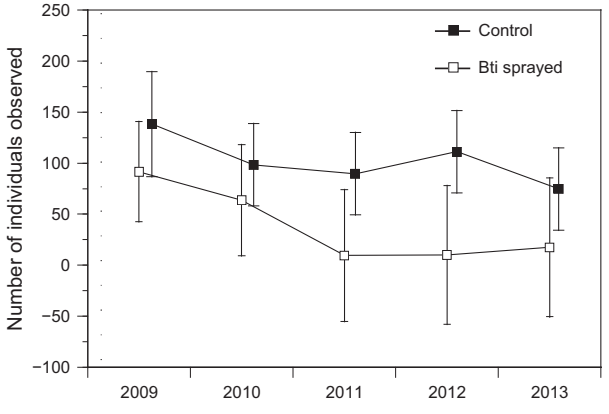


Fig. 4. Annual trends in mean Odonata abundance (95% CI) estimated along the transects at *Bti*-sprayed and control areas over the 5-year study period according to the nested-ANOVA designed GLM.

more abundant in reed marsh than in Salicornia marshes ($F_{(1,4)} = 12.4$, $P = 0.001$). In both habitat types, chironomids showed a significantly lower abundance at treated ($n = 1341$) versus control ($n = 2572$) sites ($F_{(17,1)} = 79.9$, $P < 0.001$) over the three sampling periods (Fig. 5). *Bti*-spraying contributed to 63% of the explained variance by the nested-ANOVA design. All other effects, except habitat, were significant and overall accounted for 35% of the remaining variance.

Discussion

No study has ever addressed the indirect impacts of mosquito control on richness and abundance of Odonata to our knowledge. Painter *et al*. (1996) estimated the effect of repeated external and internal contacts of *Bti* for nymphs of a common dragonfly, Erythemis simplicicollis (Say), during an entire life cycle in a controlled laboratory environment and did not document any mortality. Davis and Peterson (2008), when testing in experimental pools acute impacts of *Bti,* did not detect significant effects on non-target aquatic organisms after a single application. Our results suggest that *Bti*-spraying affects locally the number of species and individuals of adult Odonata through food depletion. Most of the studies on indirect *Bti* effects found no significant difference in abundance of non-target insects, based on the monitoring of sites before and after mosquito control or the comparison of treated and untreated sites (Colbo & Undeen, 1980; Merritt *et al*., 1989, 1991; Charbonneau *et al*., 1994; Wipfli & Merritt, 1994a,b; Hanowski *et al*., 1997a,b; Niemi *et al*., 1999; Russell *et al*., 2009; Vinnersten *et al*., 2009). Among the few exceptions are the study of Purcell (1981) documenting a reduced abundance of water bugs in salt marshes, and that of Hershey *et al*. (1998) showing a 57–83% reduction in insect density and a 50–83% decrease in biomass in the second and third year after initiation of *Bti* spraying. Odonata accounted for only 0.1% (26/23 031) of all insects sampled in Hershey's study, which was based on the comparison of 18 wetlands randomly assigned to an equal number of control and *Bti*-treated sites.

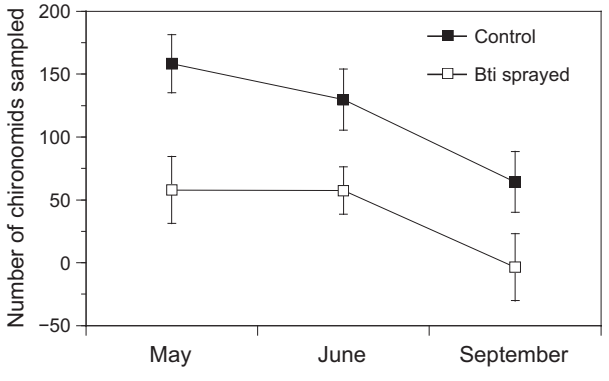


Fig. 5. Mean seasonal relative abundance of chironomids (95% CI) captured at *Bti*-sprayed and control marshes dominated by reed ($n = 4$) and Salicornia ($n = 4$) vegetation, according to the nested-ANOVA designed GLM.

Although our sampling was not exhaustive, species accumulation curves reached an asymptote on nearly all transects on the second year of sampling. The number of species detected along our six transects ($n = 23$) represents 80% of the number of species censused in the last Camargue inventory (Papazian, 1992). These results suggest that the sampling effort is sufficient to detect differences across sites, especially considering the annual fluctuations that characterised the Odonata assemblage in the Camargue: an intensive 4-year study showed that 75% of the species present are detected every year (Aguesse, 1961).We attempted to reduce biases by applying a standardised protocol executed by the same observer under optimal weather conditions at six sites of similar habitat configuration. Unfortunately, no data exist prior to August 2006 to confirm that values in number of species and individuals were similar on all transects prior to the initiation of *Bti*-spraying.

Considering that *Bti*-sprayed sites are located in the southeast end of the Rhone delta close to the sea, wetland salinity would appear as a potential explaining factor for the lower values observed at treated sites (Cannings & Cannings, 1987). This environmental factor accounted for only 2–3% of the variance observed within our dataset. Besides, the species considered among the most tolerant to salinity (e.g. *Lestes barbarus and L. macrostigma;* Aguesse, 1955; Geijskes & van Tol, 1983) were predominantly (73%) observed in control areas. Alternatively, genera typically associated with freshwater habitats (Sympetrum, Crocothemis, Orthetrum, Aeshna and Anax; Aguesse, 1955), were well represented at the treated sites.

Differences in land cover types around the transects could also potentially explain the difference observed between treated and control areas. The higher proportion of wetlands, along with the lower proportion of urban and agricultural landscapes in *Bti*-treated areas would presumably lead to a higher Odonata occurrence at mosquito-controlled sites, while we observed the opposite pattern.

Certainly, other biological or physical factors (e.g. phenology in Odonata emergence, vegetation composition and density, water levels, etc.) could potentially affect locally the observed species assemblage independently from *Bti*-spraying. Considering that there was no management modification at any site, it seems unlikely that factors other than *Bti* spraying could account for the negative trends observed from 2009 to 2011 at *Bti*-treated sites only. In addition to the expected lower abundance of mosquitoes at treated sites, the significantly lower abundance of chironomids at eight marshes located in the proximity of the four Odonata transects in 2012, further support the hypothesis of a lower food availability to odonates at *Bti*-treated sites. In contrast to mosquitoes, chironomids are considered as a major component of

wetland food webs (Ali, 1995; Batzer & Wissinger, 1996), and owing to their benthic habits, are exposed to *Bti* over periods well extending those of *Bti* efficacy against mosquitoes, especially in standing water bodies (Dupont & Boisvert, 1985; Ohana *et al*., 1987). The observed decrease in Odonata and Chironomidae at treated sites are further consistent with the results from a study carried out on House martin (*Delichon urbicum)* breeding colonies in the same area (Poulin *et al*., 2010). According to this study, adult birds feed their chicks with a significantly lower proportion of chironomids, mosquitoes and odonates when the colony is surrounded by *Bti*-sprayed marshes, resulting in a 33% decrease in chick survival. Sampling of aerial plankton around the bird colonies in residential areas revealed a 78% reduction in both chironomid and non-chironomid nematocerans in *Bti*-sprayed areas relative to control areas (Poulin B. & Lefebvre G., unpublished data).

After 5 years of Odonata survey, we observe a five-fold difference in abundance and a three-fold difference in richness of Odonata between control and treated sites. Odonata are generalised, obligate carnivores at both the larval and adult stage. They feed on any animal that is small enough to be captured, soft enough to be swallowed and always tend to eat what is most easily available (Corbet, 1962). The diversity of Camargue wetlands in terms of hydrology certainly contributes to provide a fairly constant and high abundance of small dipterans (Poulin, 2012), which are likely to represent a major part of Odonata diet. *Sympetrum fonscolombii*, one of the most commonly observed species at the study site, has been reported to feed mainly on small dipteran, especially chironomids during its larval stage in the Camargue (Aguesse, 1961). This species suffer a six-fold decrease at treated sites compared to control sites. As for breeding House martins, it appears that no alternative prey can numerically compensated for the reduced abundance of small dipterans at treated sites.

Trends observed in Odonata richness and abundance are consistent with a population size adjusting to the lower carrying capacity of the treated sites caused by a reduction in food availability. We would then expect the currently low population levels and species richness at treated sites to remain unchanged under a similar mosquito control pressure (Johnson & Crowley, 1992). This hypothesis does not exclude the possibility that Odonata at *Bti*-treated sites persist as sink populations, which are renewed by immigration of individuals from neighbouring untreated water bodies.

Conclusions

Although *Bti* is currently the most selective and least toxic agent to control mosquitoes, its deleterious effect on mosquitoes, chironomids and potentially other small Diptera can affect significantly the non-target fauna through alteration of the food web. *Bti* can persist for several months under certain environmental conditions (Tilquin *et al*., 2008; Tétreau *et al*., 2012; Duchet *et al*., 2014). For instance, up to 8 500 000 spores of *Bti* per gram of soil were found 4 months after the last treatment of the year in a Camargue marsh close to one of the Odonata transect (Poulin B., Lefebvre G. & Després L., unpublished data). Hence, *Bti* effects on benthic chironomids can extend well over the period of efficiency against water-filtering mosquitoes, with cascading effects at several trophic levels. Odonata is an insect group of high interest for biodiversity conservation (Clausnitzer *et al*., 2009), as ecosystem indicators (Sahlén & Ekestubbe, 2001; Acquah-Lamptey *et al*., 2013), and for environmental education (Niba & Samways, 2006). Odonata declines in Western Europe are mainly attributed to habitat loss and fragmentation, changes in farm management practices, and water pollution/eutrophication caused by use of agrochemical pesticides/fertilisers (Raebel *et al*., 2012). Mosquito control using *Bti* should be acknowledged as a non-negligible potential threat to these insects in relatively pristine wetland ecosystems.

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References

Acquah-Lamptey, D., Kyerematen, R. & Owusu, E.O. (2013) Using odonates as markers of the environmental health of water and its land related ecotone. *Biodiversity Conservation*, **5**, 761–769.

Aguesse, P. (1955) Note préliminaire sur les odonates de Camargue. *Revue d'Écologie (Terre Vie)*, **102**, 287–308.

Aguesse, P. (1961) *Contribution à l'étude écologique des Zygoptères de Camargue*. PhD thesis, Université de Paris.

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

Ali, A. (1995) A concise review of Chironomid midges (Diptera: Chironomidae) as pests and their management. *Journal of Vector Ecology*, **21**, 105–121.

Batzer, D.P. & Wissinger, S.A. (1996) Ecology of insect comunities in nontidal wetlands. *Annual Review of Entomology*, **41**, 75–100.

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.

Cannings, R.A. & Cannings, S.G. (1987) The Odonata of some saline lakes in British Columbia, Canada: ecological distribution and biogeography. *Advances in Odonatology*, **3**, 7–22.

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

Clausnitzer, V., Kalkman, V.J., Ram, M., Collen, B., Baillie, J.E.M., Bedjanič, M., Darwall, W.R.T., Dijkstra, K.-D.B., Dow, R., Hawking, J., Karube, H., Malikova, E., Paulson, D., Schütte, K., Suhling, F., Villanueva, R.J., von Ellenrieder, N. & Wilson, K. (2009) Odonata enter the biodiversity crisis debate: the first global assessment of an insect group. *Biology Conservation*, **142**, 1864–1869.

Colbo, M.H. & Undeen, A.H. (1980) Effect of *Bacillus thuringiensis var. israelensis* on non-target insects in stream trials for control of Simuliidae. *Mosquito News*, **40**, 368–371.

Corbet, P.S. (1962) *A Biology of Dragonflies*. H.F.&G. Witherby Ltd, London, UK.

Corbet, P.S. (1999) *Dragonflies. Behaviour and Ecology of Odonata*. Harley Books, Colchester, UK.

Davis, R.S. & Peterson, R.K.D. (2008) Effects of single and multiple applications of mosquito insecticides on non-target arthropods. *Journal of the American Mosquito Control Association*, **24**, 270–280.

Duchet, C., Tetreau, G., Albane, M., Rey, D., Besnard, G., Perrin, Y., Paris, M., David, J.-P., Lagneau, C. & Després, L. (2014) Persistence and recycling of bioinsecticidal *Bacillus thuringiensis* subsp. *israelensis* spores in contrasting environments: evidence from field monitoring and laboratory experiments. *Microbial Ecology*, **67**, 576–586.

Dupont, C. & Boisvert, J. (1985) Persistence of *Bacillus thuringiensis serovar. israelensis* toxic activity in the environment and interaction with natural substrates. *Water, Air, & Soil Pollution*, **29**, 425–438.

Geijskes, D.C. & van Tol, J. (1983) *De Libellen van Nederland (Odonata)*. Konijkle Nederlandse Natuurhistorische Vereniging, Hoog-woud, Netherlands.

Goldberg, L.J. & Margalit, J. (1977) A bacterial spore demonstrating rapid larvicidal activity against *Anopheles sergentii, Uranotaenia unguiculata, Culex univitattus, Aedes aegypti* and *Culex pipiens* complex. *Mosquito News*, **37**, 355–358.

Hanowski, J.M., Niemi, G.J., Lima, A.R. & Regal, R.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 *Bacillius thuringiensis israelensis (Bti)* and methoprene on nontarget macroinvertebrates in Minnesota wetlands. *Journal of Applied Ecology*, **8**, 41–60.

Jakob, C. & Suhling, F. (1999) Risky times? Mortality during emergence in two species of damselflies. *Aquatic Insects*, **21**, 1–10.

Johnson, P.H. & Crowley, D.M. (1992) Variability and stability of a dragonfly assemblage. *Oecologia*, **90**, 260–269.

Lacey, L.A. (2007) *Bacillus thuringiensis* serovariety *israelensis* and *Bacillus sphaericus* for mosquito control. *Journal of American Mosquito Control Association*, **23**, 133–163.

Lacey, L.A. & Merritt, D.L. (2004) The safety of bacterial microbial agents used for black fly and mosquito control in aquatic environments. *Environmental Impacts of Microbial Insecticides: Need and Methods for Risk Assessment* (ed. by H.M.T. Hokkanen and A.E. Hajek), pp. 151–168. Kluwer Academic Publishers, Dordrecht, The Netherlands.

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

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 American Mosquito Control Association*, **5**, 397–415.

Merritt, R.W., Wipfli, M.S. & Wotton, R.S. (1991) Changes in feeding habits of selected nontarget aquatic insects in response to live and *Bacillus thuringiensis* var. *israelensis* de Barjac killed black fly larvae (Diptera: Simuliidae). *The Canadian Entomologist*, **123**, 179–185.

Myers, N., Mittermeier, R.A., Mittermeier, C.G., da Fonseca, G.A.B. & Kent, J. (2000) Biodiversity hotspots for conservation priorities. *Nature*, **403**, 853–858.

Niba, A. & Samways, M. (2006) Development of the concept of 'core resident species' for quality assurance of an insect reserve. *Biodiversity Conservation*, **15**, 4181–4196.

Niemi, G.J., Hershey, A.E., Shannon, L., Hanowski, J.M., Lima, A., Axler, P. & Regal, R.R. (1999) Ecological effects of mosquito control on zooplankton, insects and birds. *Environmental Toxicology and Chemistry*, **18**, 549–559.

Oestergaarda, J., Belaua, C., Strauchb, O., Esterc, A., van Rozenc, A. & Ehlersa, R.-O. (2006) Biological control of *Tipula paludosa* (Diptera: Nematocera) using entomopathogenic nematodes *(Steinernema spp.)* and *Bacillus thuringiensis* subsp. *israelensis*. *Biological Control*, **39**, 525–531.

Ohana, B., Margalit, J. & Barak, J. (1987) Fate of *Bacillus thuringiensis* subsp. *israelensis* under simulated field conditions. *Applied and Environmental Microbiology*, **53**, 828–831.

Painter, M.K., Tennessen, K.J. & Richardson, T.D. (1996) Effects of repeated applications of *Bacillus thuringiensis israelensis* on the mosquito predator *Erythemis simplicollis* (Odonata: Libellulidae) from hatching to final instar. *Environmental Entomology*, **25**, 184–191.

Papazian, M. (1992) Inventaire des odonates de Camargue: essai de cartographie et constitution d'une collection de référence. Pp. 103-109 in Compte rendu scientifique 1992 - SNPN/Réserve de Camargue.

Poulin, B. (2012) Indirect effects of bioinsecticides on the nontarget fauna: the Camargue experiment calls for future research. *Acta Oecologica*, **44**, 28–32.

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.

Purcell, B.H. (1981) Effects of *Bacillus thuringiensis var. israelensis* on *Aedes taeniorhynchus* and some nontarget organisms in the salt marsh. *Mosquito News*, **41**, 476–484.

Raebel, E.M., Merckx, T., Feber, R.E., Riordan, P., MacDonald, D.W. & Thompson, D.J. (2012) Identifying high-quality pond habitats for Odonata in lowland England: implications for agri-environment schemes. *Insect Conservation and Diversity*, **5**, 422–432.

Rowe, G.E., Margaritis, A. & Wei, N. (2008) Specific oxygen uptake rate variations during batch fermentation of *Bacillus thuringiensis* subspecies *kurstaki* HD-1. *Biotechnology Progress*, **19**, 1439–1443.

Russell, T., Kay, B. & Skilleter, G. (2009) Environmental effects of mosquito insecticides on saltmarsh invertebrate fauna. *Aquatic Biology*, **6**, 77–90.

Sahlén, G. & Ekestubbe, K. (2001) Identification of dragonflies (Odonata) as indicators of general species richness in boreal forest lakes. *Biodiversity Conservation*, **10**, 673–690.

Smallshire, D. & Beynon, T. (2010) *Dragonfly Monitoring Scheme Manual*. British Dragonfly Society, <http://www.british-dragonflies.org.uk/sites/british-dragonflies.org.uk/files/images/Dragonfly%20Monitoring%20Scheme%202010%20manual%20draft%20v2_0.pdf> 24th January 2016.

Tétreau, G., Alessi, M., Veyrenc, S., Périgon, S., David, J.-P., Reynaud, S. & Després, L. (2012) Fate of *Bacillus thuringiensis* subsp. *israelensis* in the field: evidence for spore recycling and differential persistence of toxins in leaf litter. *Applied Environmental Microbiology*, **78**, 8362–8367.

Tilquin, M., Paris, M., Reynaud, S., Despres, L., Ravanel, P., Geremia, R.A. & Gury, J. (2008) Long lasting persistence of *Bacillus thuringiensis* subsp. *israelensis Bti* in mosquito natural habitats. *PLoS ONE*, **310**, e3432. doi:10.1371/journal.pone.0003432.

Vinnersten, T.Z.P., Lundström, J.O., Petersson, E. & Landin, J. (2009) Diving beetles assemblages of flooded wetlands in relation to time, wetland type and *Bti*-based mosquito control. *Hydrobiologia*, **635**, 189–203.

Wipfli, M. & Merritt, R. (1994a) Effects of *Bacillus thuringiensis* var. *israelensis* on nontarget benthic insects through direct and indirect exposure. *Journal of the North American Benthological Society*, **13**, 190–205.

Wipfli, M. & Merritt, R. (1994b) Disturbance to a stream food web by a bacterial larvicide specific to black flies: feeding responses of predatory macroinvertebrates. *Freshwater Biology*, **32**, 91–103.

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