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Review

Environmental and socioeconomic effects of mosquito control in Europe using the biocide *Bacillus thuringiensis* subsp. *israelensis* (Bti)

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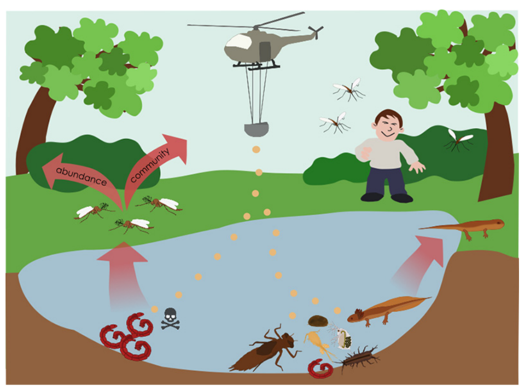
HIGHLIGHTS

- *Bacillus thuringiensis subsp. israelensis* (Bti) is widely used for mosquito control.
- Risk of resistance to Bti is limited despite spores and toxins persistence.
- Reported effects on non-target organisms challenge environmental safety of Bti.
- Monitoring should be performed by independent bodies devoid of conflicts of interest.
- Alternative mosquito control methods should be considered in conservation areas.

GRAPHICAL ABSTRACT



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ABSTRACT

Bacillus thuringiensis subsp. *israelensis* (Bti) has been used in mosquito control programs to reduce nuisance in Europe for decades and is generally considered an environmentally-safe, effective and target-specific biocide. However, the use of Bti is not uncontroversial. Target mosquitoes and affected midges represent an important food source for many aquatic and terrestrial predators and reduction of their populations is likely to result in food-web effects at higher trophic levels. In the context of global biodiversity loss, this appears particularly critical since treated wetlands are often representing conservation areas. In this review, we address the current large-scale use of Bti for mosquito nuisance control in Europe, provide a description of its regulation followed by an overview of the available evidence on the parameters that are essential to evaluate Bti use in mosquito control. Bti accumulation and toxin persistence could result in a chronic expose of mosquito populations ultimately affecting their susceptibility, although observed increase in resistance to Bti in mosquito populations is low due to the four toxins involved. A careful independent monitoring of mosquito susceptibility, using sensitive bioassays, is mandatory to detect resistance development timely. Direct Bti effects were documented for non-target chironomids and other invertebrate groups and are discussed for amphibians. Field studies revealed contrasting results on possible impacts on chironomid abundances. Indirect, food-web effects were rarely studied in the

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environment. Depending on study design and duration, Bti effects on higher trophic levels were demonstrated or not. Further long-term field studies are needed, especially with observations of bird declines in Bti-treated wetland areas. Socio-economic relevance of mosquito control requires considering nuisance, vector-borne diseases and environmental effects jointly. Existing studies indicate that a majority of the population is concerned regarding potential environmental effects of Bti mosquito control and that they are willing to pay for alternative, more environment-friendly techniques.

Contents

1. Introduction

1.1. Mosquitoes and the human population

Mosquitoes affect the health and well-being of human populations for two main reasons: the transmission of mosquito-borne diseases and the nuisance associated with mosquito bites (Becker et al., 2010). On a global scale, the greatest concern about mosquitoes is their vector competence for transmitting diseases such as malaria, dengue, or West Nile Virus (WNV). Although mainly restricted to tropical and subtropical areas, few autochthonous transmission cases of dengue and Chikungunya by the invasive Asian tiger mosquito *(Aedes albopictus)* were recently recorded in Southern Europe (Succo et al., 2016; Calba et al., 2017). However, most Central- and North-European countries are free of autochthonous transmission since the elimination of mosquito-borne diseases in the 1950s, which was mainly achieved by socio-economic improvements (Zhao et al., 2016; Falkenhorst et al., 2018).

In temperate regions, seasonal outbreaks of mosquitoes cause nuisance in recreational and residential areas. Mosquitoes are widely considered incompatible with human life quality as they prevent people from enjoying outdoor activities and can also negatively affect the economy by discouraging tourism and outdoor labor (von Hirsch and Becker, 2009; Halasa et al., 2014). For example, along the lower Dalälven River in Central Sweden, dense populations of the mosquito *Aedes sticticus* occasionally occur after inundation of wetlands during high flood events (Schafer et al., 2008), resulting in reduced amount of outdoor activities, increased level of stress, decreased well-being and sleep disturbance among local residents (Hallberg, 2013). In Europe, nuisance is the most frequent reason for large-scale mosquito control. Effects of Bti nuisance mosquito control in Europe are therefore the focus of this paper.

1.2. Mosquitoes and their control

Mosquito control has a long history: control trials in the 1910s, involving screening houses, oiling water, draining standing water and distributing larva-eating minnows followed by spraying “Paris Green” (a copper and arsenic salt) in the 1920s, and using DDT (Dichlorodiphenyltrichloroethane) afterwards (Becker and Ludwig, 1993; Stapleton, 2004). In Europe, large-scale mosquito control programs were introduced in the 1960's to improve human comfort and promote tourism, especially along the Mediterranean coast (Majori, 2012; Parrinello and Bécot, 2019). After DDT was banned from many countries in the 1970s, it was replaced by other chemicals while searches for biodegradable botanical compounds continued in parallel (Sukumar et al., 1991). Other classes of chemical insecticides that have been or are still used for mosquito control include organophosphates, carbamates and pyrethroids (Becker et al., 2010; N'Guessan et al., 2010; van den Berg et al., 2012). However, due to human health and environmental effects of these chemical insecticides, especially by direct water applications, as well as development of high levels of resistance in mosquito populations (Hemingway and Ranson, 2000; Coetzee and Koekemoer, 2013; van den Berg et al., 2015), efforts have been made to develop alternative and more environmental-friendly control methods.

Mosquitoes breed in a variety of aquatic habitats with stagnant water. For instance, vector species such as *Ae. albopictus* and *Ae. aegypti* are typical container-inhabiting mosquitoes associated with human habitats where they utilize tires, bins but also knotholes as breeding sites (Medlock et al., 2012; Becker and Lüthy, 2017; Vega and Okech, 2019). Conversely, floodwater species such as *Ae. vexans* and *Ae. sticticus* hatch simultaneously in massive numbers after flooding events along rivers, which poses nuisance for people living next to inundated areas (Becker, 2006; Schafer et al., 2008). Control strategies depend on the target species: nuisance control of the floodwater mosquitoes often requires extensive, large-scale spatial treatments of mosquito larvae in wetlands (e.g. by helicopter), while vector control of the container breeding mosquito species is mostly performed locally around urban breeding sites (van den Berg et al., 2012).

Preserving simultaneously human health and comfort, as well as the environment has always been a major challenge in large-scale mosquito control. In 1976, the bacterium *Bacillus thuringiensis* subsp. *israelensis* (*Bti*) was isolated (Goldberg and Margalit, 1977). Its insecticidal properties are Diptera-specific and the acute toxicity to other animals, so called non-target organisms, is low. Bti therefore presented a seeming potential for mosquito control with reduced effects on other fauna. Since the early 1980s, Bti-based biocides have been available commercially (Lacey, 2007). Bti was rapidly implemented in mosquito control programs all over the world and is currently used in Europe, Canada, the USA, and tropical areas in South East Asia, Africa and South America (Schäfer and Lundström, 2014). Germany was one of the early users, and between 1981 and 2016, up to 5000 tons of Bti formulations were applied to >400,000 ha in the Upper Rhine Valley (Becker et al., 2018).

1.3. Mosquito control with Bti and the environment

Bti is considered to be an environmentally-safe, effective and target-specific biocide (Despres et al., 2011). Most organisms tested so far, except for target mosquitoes (Culicidae) and black flies (Simuliidae) and non-target midges (Chironomidae), did not reveal mortality even at high, unrealistic Bti concentrations. However, the use of Bti is not uncontroversial. Mosquitoes are a substantial part of the biomass in a wide range of wetlands and represent food sources for many aquatic and terrestrial predators (Shaalan and Canyon, 2009; Becker et al., 2010). In addition, adult mosquitoes play an underestimated role in pollination (Peach and Gries, 2016; Lahondère et al., 2020).

Chironomids usually constitute a major proportion of invertebrate biomass in lotic and lentic systems (Leeper and Taylor, 1998; Williams, 2006; Lundstrom et al., 2010b; Allgeier et al., 2019a) and contribute considerably to species diversity (Lundstrom et al., 2010a; Theissinger et al., 2018; Wolfram et al., 2018; Theissinger et al., 2019). Their high protein content and digestibility make them a quality food resource for both aquatic (amphibians, fish, insects) and terrestrial (birds, bats, spiders, insects) predators (De La Noüe and Choubert, 1985; Armitage et al., 1995; Arnold et al., 2000; Poulin et al., 2010; Jakob and Poulin, 2016; Quirino et al., 2017). Adult chironomids form huge swarms and can dominate insect emergence in wetlands with over 90% of the emerging individuals (Leeper and Taylor, 1998) resulting in up to 100 g dry weight biomass per year and square meter (Armitage et al., 1995). Therefore, chironomids represent important links between the aquatic and terrestrial food web (Hoekman et al., 2011). Negative effects on mosquito and chironomid populations leading to lower abundances are therefore likely to result in effects at higher trophic levels (Poulin et al., 2010; Schulz et al., 2015; Jakob and Poulin, 2016).

While Bti use has increased exponentially worldwide, studies monitoring environmental effects have remained relatively scarce. In the United States, Sweden and France, field studies addressing this sensitive issue were conducted when control programs were introduced, leading to contrasting observations regarding environmental effects of Bti (see below). The potential of Bti to cause food-web related effects is particularly important as many of the treated wetlands are conservation areas of national (bird sanctuaries, nature conservation areas, national parks), European (Fauna-Flora-Habitat Network) or global (RAMSAR) status. For example, in Sweden, approximately 40% of the endorsed mosquito control area is within Natura 2000 areas. Around 90% of the Bti-treated area of Rhineland-Palatinate in Germany is situated in nature protection areas. In France, one of the last large marshes in Western Europe (Marais de Lavours) is a national protected area since 1984, treated with Bti since the eighties, as are the smaller lowland protected mashes still persisting along the French Atlantic coast and in the Rhone-Alpes region (Duchet et al., 2014; Lagadic et al., 2016). In the UNESCO man and biosphere reserve Camargue, containing many Natura 2000 sites, Bti treatment was introduced lately in 2006 (Poulin et al., 2010).

The ongoing global loss of biodiversity is one of the most critical environmental issues that threatens ecosystem processes and services (Diaz et al., 2006; Cardinale et al., 2012; Mace et al., 2012). The continued growth of human population, which is accompanied by habitat destruction, release of pollutants, transport of invasive species and climate disruption, further intensifies species losses leading to an accelerated human-induced sixth mass extinction crisis (Butchart et al., 2010; Ceballos and Ehrlich, 2010; Ceballos et al., 2015). Most attention was previously given to worldwide population declines of vertebrates (Ceballos et al., 2015; Ceballos et al., 2017), but entomofauna is also heavily affected and roadmaps for their conservation were recently formulated (Imperatriz-Fonseca et al., 2016; Powney et al., 2019; Harvey et al., 2020). A substantial decline of >70% in flying insect biomass was recorded over a span of 27 years in German nature reserves and its effect on higher trophic levels, including birds feeding their nestlings with insects, are discussed (Hallmann et al., 2017). Mosquito control for nuisance should therefore also be considered in the context of observed biodiversity declines since insects are target species of large scale operations.

1.4. Rationale and methodology of the review

To date, no comprehensive synthesis of the peer-reviewed published literature is available to summarize the current knowledge on mosquito control using Bti and its associated effects. In this review, we first provide a description of the regulation of Bti use in Europe (part I). Then, we continue with an overview of the available evidence on the parameters that are essential to evaluate the use of Bti in mosquito control. This includes Bti persistence in the environment (part II), the risk of resistance development to Bti in mosquito populations (part III), direct and indirect environmental effects (part IV), as well as socio-economic aspects and public perception of mosquito control using Bti (part V).

Although this is not a systematic review, we used elements of this methodology (Tranfield et al., 2003). We conducted a literature search using the ISI Web of Knowledge database with the search terms (Bti* OR *Bacillus thuringiensis israelensis*) AND (persistence OR resistance Or environment OR socio-econom*). In addition, various terms were searched via Google Scholar (e.g., "Bti environment effect," "Bti persistence sediment Europe"). To limit the number of hits in the socioeconomic area, we focused on EconLit, the leading database of scientific economic literature, using search terms like "mosquito control", "Bti" and "vector-borne disease". We carefully evaluated the resulting publications by reading title, abstract and conclusion. Citation tracing was used in key publications and recent papers. While this review lacks the narrow focus and comprehensive searches of a systematic review, our ambition is to be critical, objective and transparent and present the retrieved studies that we believe to be essential, in a concise form. We believe that this review is suitable for decision makers to rationally conclude on the suitability of mosquito control options with Bti. This review is addressing the current large-scale Bti use for mosquito nuisance control in Europe.

1.5. Regulation of Bti use in Europe

Since 2012, insecticidal products that are not used in an agricultural context are addressed in the biocide regulation EU 528/2012 and before in directive 98/8/EC (European Parliament and Council, 1998; European Parliament and Council, 2009). According to the regulation, the active substance within a biocidal product, which may further contain formulation chemicals, needs to be assessed according to its impact on humans, animals and the environment. In the environmental risk assessment (ERA), all available data are summarized and effect data (sensitivity of organisms) are compared to exposure data (concentrations in the environment). The Bti Serotype H-14 Strain AM65–52 was assessed as an insecticide in 2010 and market access was granted although *"the need for long-term data to evaluate food web effects"* was expressed due to ambiguous results (European Parliament and Council, 2010). According to the procedure, the formulated products Vectobac 12AS, WG, G and GR were assessed by member states. Regulation is based on mutual recognition of the rapporteur member states decisions and therefore formulations became available for mosquito control in the market of other European countries, namely Romania, Sweden, Hungary, Italy, France, the Netherlands, Germany, Czech Republic, Bulgaria, Austria, Switzerland, Spain and Portugal (European Chemicals Agency, 2019). Application of Bti products is permitted by aircraft, specifically for ice granules of Vectobac WG, and on the ground by spray or hand. However, the application of Bti products from air is prohibited in some member states (e.g. the Netherlands) as a result of the national implementation of the Sustainable Use Directive (2009/128/EC, (European Parliament and Council, 2009)) whereas in others it is allowed (e.g. Germany) or can be performed under annual prefectural derogation (e.g. France). One post-authorization requirement is to report effects on biodiversity every two years to authorities (KEMI, 2015). For the application of Bti products in the different countries, the authorised control operator needs to obtain permission from water authorities, since a biocide is applied directly into the water, and from nature conservation authorities, as many treated areas are protected by law and management aims include the conservation of threatened fauna and biodiversity. The regulation and authorisation processes vary widely between countries and they can be difficult to understand for authorities and communities that consider the introduction of mosquito control or already implement a mosquito control program. In Europe, detailed information on treated areas, on the concentration and nature of the Bti products used as well as the number of treatments per year is neither centralised nor easily accessible. Yet, this information is fundamental to monitor Bti exposure of wetlands and acknowledge potential resulting effects.

2. Persistence of Bti in the environment

Bti is generally applied as a formulated suspension of spores and crystals of toxins. Therefore, persistence of Bti is considered separately for insecticidal activity (1.), toxins (2.) and spores (3.) and its impact on sediment biomes (4.) where Bti accumulates over time.

2.1. Persistence of the insecticidal activity

Bti is applied in water bodies and its insecticidal activity directly depends on its availability to mosquito larvae. Biological, operational and environmental factors can affect the duration of insecticidal (residual) activity of Bti.

Biological factors encompass all mosquito-related parameters, such as the mosquito species or the larval stage. For example, the surface-feeding *Anopheles* larvae are less exposed to Bti than *Culex* and *Aedes* larvae who actively collect the food in the water column and in the bottom, because Bti quickly falls down to the bottom after treatment (Amalraj et al., 2000). As a consequence, the residual activity of Bti is less important for *Anopheles* than other genera. Last instar larvae strongly reduce their feeding activity and are much bigger than first instars, and therefore require ingestion of more Bti for the same toxic effect (Wraight et al., 1981).

Different formulations (operational factor) have been developed to adapt to the different treatment sites (Vilarinhos and Monnerat, 2004). To increase residual activity, some formulations allow a slow release of Bti over time, while others delay Bti sedimentation (Becker, 2003; Mulla et al., 2004; Ritchie et al., 2010). Interestingly, increasing the operational dose of Bti does not seem to extend the duration of the mosquito control (Mulla et al., 1993).

Finally, many environmental factors affect Bti persistence. Water turbidity and/or pollution increase toxin degradation and/or adsorption to organic matter particles in suspension and reduce its availability to mosquitoes (Margalit and Bobroglo, 1984; Karch et al., 1991; Sheeran and Fisher, 1992; Srivastava et al., 1998; Tetreau et al., 2012c). Moreover, UV light, high temperature and low vegetation cover are all parameters that can also reduce the duration of Bti toxicity (Boisvert et al., 2001; Christiansen et al., 2004). A comprehensive analysis of the target ecosystem to be treated and knowledge of the ecology of the target mosquito species are prerequisites for adapting mosquito control.

2.2. Persistence of toxins

During bacterial sporulation, four toxins are produced as crystals, including three different Cry toxins (Cry4Aa, Cry4Ba and Cry11Aa) and one Cyt toxin (Cyt1Aa) (Ben-Dov, 2014). It was shown that the toxic crystals can be present in the environment from weeks up to years after a treatment, depending on the environment (Dupont and Boisvert, 1986; Boisvert and Boisvert, 1999). Crystals immobilized in sediments or trapped in algae can conserve up to 90% of their insecticidal activity, up to 22 days after Bti application (Ohana et al., 1987; Sheeran and Fisher, 1992; Tousignant et al., 1993; Boisvert et al., 2001). However, toxins do not equally persist in the environment: Cyt1Aa toxins were shown to exhibit the lowest persistence when in contact with leaf litter with a half-life of 2–4 days, while Cry4Aa and Cry4Ba toxins showed half-lives of up to 3 weeks (Tetreau et al., 2012a). This differential toxin persistence could result in a chronic expose of mosquito populations to a changing toxin cocktail, ultimately affecting their susceptibility to Bti (Paris et al., 2011b). However, there is no evidence that such accumulation of Bti and differential persistence of toxins in different compartments of the ecosystem alter the efficacy of mosquito treatments.

2.3. Persistence of spores

Spores are persistent forms of bacteria that can be detected in the environment months after treatment (Hajaij et al., 2005; De Respinis et al., 2006; Duchet et al., 2014). However, Bti spore load does not seem to significantly increase after continuous treatments over the years (Guidi et al., 2011). As an entomopathogen, Bti proliferates in mosquito cadavers (Aly et al., 1985; Khawaled et al., 1990; Raymond et al., 2010; Duchet et al., 2014) and independent studies reported spore recycling in different environments, such as forest temporary ponds (Tilquin et al., 2008) and containers (de Melo-Santos et al., 2009). These events remain rarely documented and seem to depend on mosquito presence and density (Duchet et al., 2014).

Sterilization of Bti by gamma-radiation before application to remove viable spores and prevent *de novo* sporulation and recycling of spores is resulting in a 20–30% decreased toxicity (Becker, 2002). To our knowledge, the use of commercial formulations based on sterilized Bti spores is currently restricted to Germany. Implementing such sterilization procedure embraces the precautionary principle but appears counter-intuitive for a product claimed to be environmentally-safe.

2.4. Bti in the sediment

Like any insecticide, Bti toxins, spores and formulation ingredients (of unknown composition) are likely to affect ecosystem health by interacting with biological communities (Duguma et al., 2015). They could alter ecosystem function, as it is observed with natural and anthropogenically-induced disturbances in soil (Griffiths et al., 2000). Studies on the route and rate of degradation in soil, mobility in soil and degradation in water and water sediment are usually critical for the approval of pesticides by the regulatory authority (European Food Safety Authority, 2013). However, soil function studies are scarce for Bti because compared to other pesticides, fate assessment cannot be performed by using already established chemical analytical methods. However, recently, liquid chromatography-mass spectrometry (LC-MS) based methods have been developed to detect Bt toxins (Yang et al., 2015) as well as Bti-specific metabolic changes in sediment samples (Salvia et al., 2018). The application of environmental metabolic footprinting (EMF) (Patil et al., 2016), which consists of the analysis of the sediment meta-metabolome (SMM) as a function of time, is sensitive enough to detect sediment metabolic perturbation induced by commercial Bti formulations (Fig. 1) (Salvia et al., 2018). However, defining sediment recovery, that is the return of a metabolic footprint to its initial (or close to) operating state after exposure to an environmental stressor, is an important endpoint in ERA (Environmental Risk Assessment) assuming variations with time and between different sediment ecosystems (Vighi and Rico, 2018). To date, recovery times of sediment ecosystem after Bti exposure are not available. However, changes in SMM after Bti application could be monitored over time using an EMF approach (Salvia et al., 2018). Thus, to obtain robust sediment recovery values after Bti exposure, a long-term experimental monitoring with different sediment types combined with latest metabolomic tools is required.

3. Mode of action of Bti toxins and resistance development in mosquitoes

3.1. Mode of action of Bti

The toxicity and specificity of Bti to mosquito larvae is related to its multiple Cry and Cyt toxins (Fig. 2). The mode of action of Cry toxins for mosquitoes has been extensively studied during the last decades (Vachon et al., 2012). After ingestion, crystals are solubilized in the alkaline gut of the mosquito larvae, releasing protoxins that are activated by proteases of mosquito gut and bacteria to toxins (Rukmini et al., 2000). Cry toxins then bind to specific protein receptors present on the outer membrane of gut cells, allowing them to oligomerize and to form pores in the cell membrane, ultimately leading to gut disruption and larval death (Vachon et al., 2012). The spores then reach the hemolymph where they germinate and the bacteria proliferate. Cyt toxins also require crystal solubilization and protoxin activation, but directly bind to lipids from the cell membrane for their cytolytic activity (Butko et al., 1997). While the exact mechanism of toxicity of Cyt1Aa has long been debated (Soberon et al., 2013), a recent work reconciled the two hitherto proposed models (i.e., "pore forming" or "detergent-like"), revealing the formation of two oligomeric forms, including one porous perforating the gut cells membrane (Tetreau et al., 2020). Furthermore, Cyt toxins act as membrane receptors for Cry toxins, thereby increasing Cry toxicity (synergist) (Soberon et al., 2013).

3.2. Bti resistance in mosquitoes

Many cases of high levels of resistance (thousand fold) against individual Cry toxins from Bt subspecies other than *israelensis* against different insect orders have been documented (Tabashnik et al., 2009). Simultaneous resistance to multiple Cry toxins from other Bt subspecies was also observed in laboratory and field studies (Janmaat and Myers, 2003; Brévault et al., 2013), rising concerns about a potential development of resistance in mosquitoes to the Bti four-toxins mixture. Bti resistance studies were conducted in the laboratory on *Culex pipiens* (Saleh et al., 2003), *Cx. quinquefasciatus* (Georghiou and Wirth, 1997; Mittal et al., 2005), and *Aedes aegypti* (Goldman et al., 1986; Tetreau et al., 2012b). After up to 30 generations of exposure with Bti and its four toxins in the laboratory, 3.5-fold resistance was obtained, meaning that a 3.5 higher dose of Bti was necessary to kill resistant mosquitoes as efficiently as susceptible ones. In contrast, it is possible to obtain high resistance levels (hundreds to thousands fold) to the individual Cry toxins from Bti when selection is performed with each toxin separately (Wirth et al., 2010; Wirth et al., 2012; Stalinski et al., 2014). The observed low level of resistance to Bti is partly attributed to the different gut receptors for the three Cry toxins and mostly associated with the presence of Cyt toxin (Soberon et al., 2013). Its capacity to serve as a receptor for Cry toxins seems to bypass any target-based resistance, which is generally responsible for high levels of resistance to Cry toxins (Pardo-Lopez et al., 2013). Laboratory experiments revealed that selecting for resistance to Cry toxins in the presence of Cyt toxin is strongly impeded and that the presence of Cyt toxin is able to revert the phenotype of resistance to Cry toxins (Wirth et al., 2004; Wirth et al., 2005), even in non-mosquito insects (Federici and Bauer, 1998).

Hundreds of studies, mostly performed by mosquito control operators, investigated resistance to Bti in the field as part of mosquito control programs by performing bioassays following World Health Organization guidelines (WHO, 2005). They all concluded that no resistance was detected, with the exception of one single report by Cornell University scientists of a 32 fold increased tolerance to Bti in a population of *Cx. pipiens* collected from sewers in upstate New York (USA) in 2005 (Paul et al., 2005). However, no resistance has been reported in the region since then. While resistance is routinely evaluated by mosquito control operators themselves, monitoring should be conducted by independent authorities and such information should be made available.

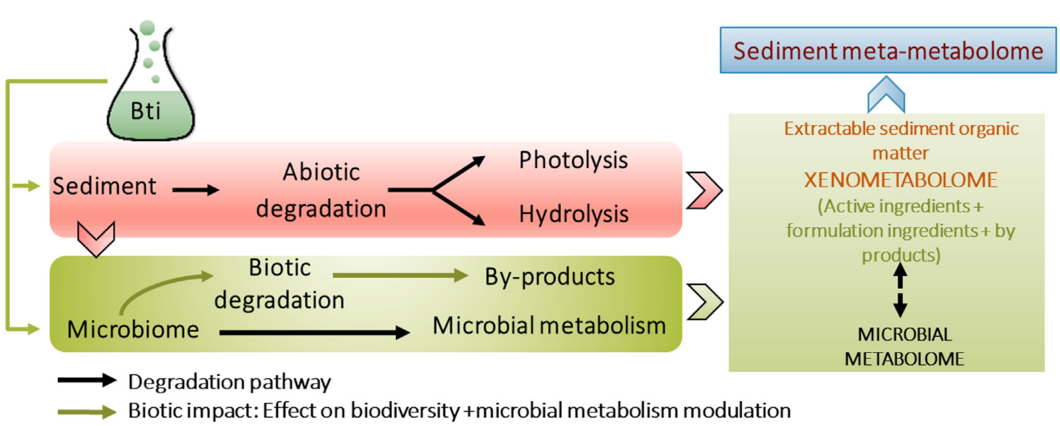

Fig. 1. Bti is degraded in the sediment by abiotic and biotic processes that lead to the formation of the Xenometabolome and the microbial metabolome. Together they form the Sediment Meta-Metabolome (SMM).

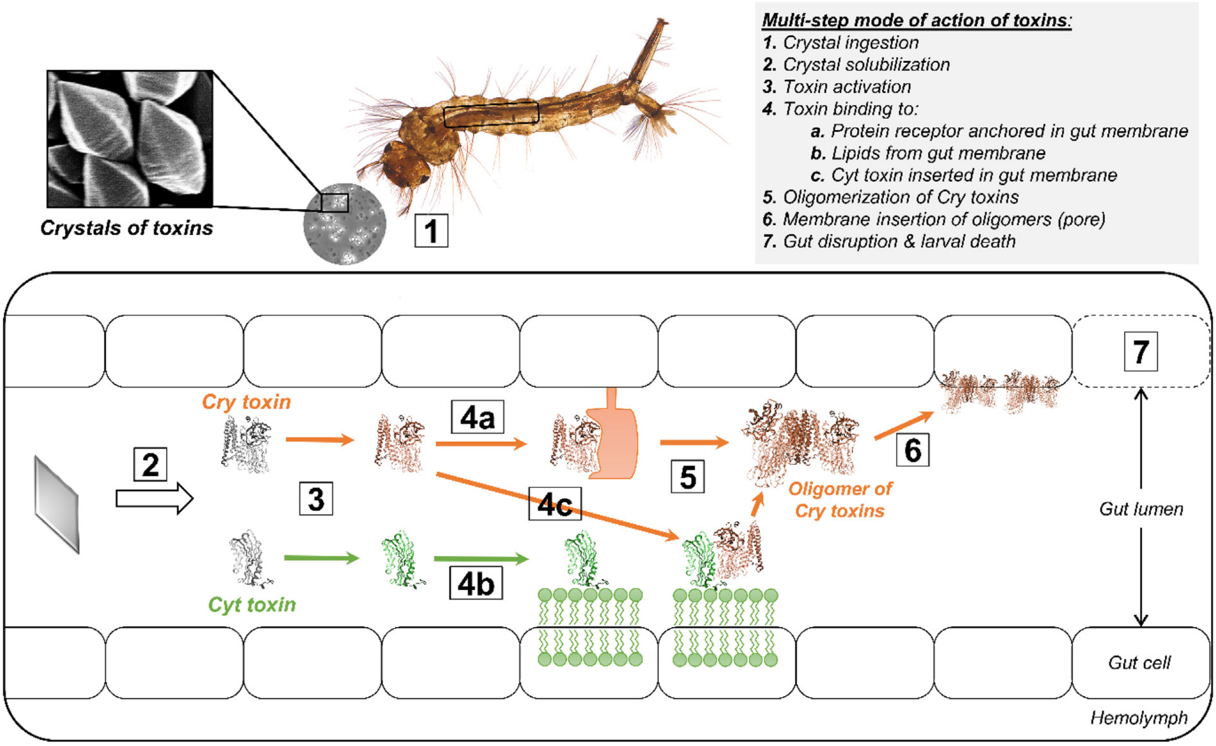

Fig. 2. Schematic mode of action of Cry (orange) and Cyt (green) toxins from *Bacillus thuringiensis* subsp. *israelensis* within the larval mosquito gut. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

The lack of resistance to Bti in field populations of mosquitoes despite intensive use over decades could also be explained by high associated fitness costs. In laboratory strains of *Cx. pipiens* and *Ae. aegypti*, Bti-resistant individuals exhibited extended larval development, lower female fecundity and decreased egg survival in the absence of Bti as compared to the parental strains (Saleh et al., 2003; Paris et al., 2011a). Moreover, resistance disappeared after only three (*C. pipiens*) and six (*A. aegypti*) generations without selection (Saleh et al., 2003; Paris et al., 2011a).

Therefore, the development of resistance significantly reducing mosquito control efficacy in the field seems unlikely, and even if it developed, the fitness costs associated with Bti resistance would limit its spreading in mosquito populations. However, a careful monitoring of mosquito susceptibility, notably using sensitive bioassay tools, is mandatory to allow an early detection of a potential reduced efficacy of the treatments. This could be an early indicator of a resistance under development that could be easily countered within a year by relaxing Bti treatments and/or by using formulations combining Bti with *Lysinibacillus sphaericus* (Caprio, 1998).

4. Environmental effects

For environmental effects, a distinction has to be made between direct and indirect (food-chain) effects. Direct effects refer to the toxicity of Bti to organisms leading to mortality or sublethal effects such as changes in behaviour, reproduction, fertility or development. Indirect effects are changes in food-web interactions affecting organisms at higher trophic levels. In the case of Bti, this is relevant for species that feed on mosquito or midges as larvae or adults, as well as for species that prey upon these first-level predators.

4.1. Direct effects

Due to the specific mode of action of its toxins, the direct effect of Bti leading to mortality is largely limited to larvae within the suborder Nematocera (Boisvert and Boisvert, 2000; Lacey, 2007). Thus, Bti is used to control mosquitoes, black flies but also chironomids that are considered pests or a nuisance for the local human population. Chironomids represent non-target organisms in case of mosquito control but they are also directly targeted, for example in Cardiff Bay in South Wales where their swarms can be massive, covering the walls of houses (Vaughan et al., 2008). Several acute toxicity studies assessed the efficiency of Bti towards chironomids as the target species, allowing calculation of EC_{50} (effective concentration where 50% of the individuals are immobile) for different Bti products (Boisvert and Boisvert, 2000). For instance, during its late larval stage, when it causes most crop damage, the rice midge *Chironomus tepperi* was shown to be 15 to 75 times less sensitive to Bti than mosquitoes (Boisvert and Boisvert, 2000; Becker and Lüthy, 2017).

However, chironomids found in aquatic habitats subject to mosquito control can be exposed to Bti as first instar larvae shortly after hatching. Although *C. riparius* is routinely tested for ERA of pesticides (Weltje et al., 2010; OECD, 2011), no sensitivity data for first instar larval stages of chironomids and Bti were available until recently (Kästel et al., 2017). The laboratory study revealed that sensitivity towards Bti is two orders of magnitude higher in first-instar larvae compared to the frequently tested fourth instar larvae. The ERA performed for the regulation of Bti in Europe considered *Daphnia magna* as the most sensitive aquatic invertebrate species (European Commission, 2011). Comparing field exposure data (environmental concentrations during mosquito control) with recent sensitivity data for first-instar *C. riparius* larvae (Kästel et al., 2017), it became evident that the assessment approval based on *D. magna* toxicity data is not protective.

The acute toxicity test is a worst-case scenario and in the field, Bti effects on chironomids could be reduced by the presence of sediment, sunlight and other abiotic and biotic factors that reduce Bti availability (see part II). However, the EC_{50} value of first instar larvae of *C. riparius* was >200 times below the lowest recommended field application concentrations in Europe (Kästel et al., 2017; Bordalo et al., 2020). This large difference between laboratory effect and field exposure concentrations, that could be even higher in other European control programs (Ostman et al., 2008; Poulin et al., 2010; Lagadic et al., 2016), indicates that effects on non-target chironomids in mosquito control areas are likely.

Due to the low sensitivity of different chironomid species in their older larval stage, it was previously assumed that field populations are not affected by Bti application rates regularly used for mosquito control (WHO, 1999; Lundstrom et al., 2010a). However, several semi-field and field studies focusing on chironomids revealed contrasting results on possible impacts on chironomid abundances but also on species richness (Table 1).

Results reported varied from no negative effects observed (Lundstrom et al., 2010b; Lagadic et al., 2014; Duchet et al., 2015) up to 41 to 100% decrease in chironomid abundance (Miura et al., 1980; Jakob and Poulin, 2016; Allgeier et al., 2018). While semi-field approaches often applied Bti over-dosages, field studies were implemented at recommended application rates (RAR) of Bti, which vary for different European countries and products, leading to slightly different ITU (International Toxic Units) application rates and application frequencies. Of 15 semi-field and field studies, five studies revealed chironomid abundance reductions by 41 to 84% at RAR. Sampling time ranged from 4 to 105 days after Bti application to observations between 1 and 7 years of regular Bti treatment. Particularly in long-term studies, annual variations in hydrology and habitat-related factors seem to be more important in explaining chironomid abundances than Bti treatments (Lundstrom et al., 2010b; Lagadic et al., 2016). Bti is applied in various ecosystems: in inundation forests along large streams in Northern and Central Europe, along lakes in alpine areas, and in coastal saltmarshes along the Mediterranean and Atlantic coastlines. Due to the differences in invertebrate community composition and chironomid species presence, general conclusions from the reviewed studies on direct effects in the field appear to be challenging. Nevertheless, several suggestions can be made to reliably predict effects on chironomids for wetland types. Firstly, only six out of 15 studies explicitly mentioned or assessed the magnitude of Bti effect on the target organism mosquito (Table 1). Unaffected mosquito populations after Bti treatment may

Table 1
Published semi-field (A.) and field (B.) studies of non-target effects on chironomid abundances. Details on application (Formulation, rate (RAR = Recommended Application Rates, ITU (International Toxic Units) content of formulation, sampling time, maximum number of applications are given. Mosquito reduction (if measured: n.e. = not examined) and chironomid reduction (in %) are provided (in bold).

Semi-field studies

Study area	Wetland type	Formulation	Toxicity	Treatments	Application rate	$\times 10^9$ ITU/ha	Max. sampling time	Max. applica-tions	Mosquito reduction	Chironomid reduction	Reference
Bakersfield, USA	lentic freshwater	SAN 402 I WDC	1.3 × 10^3 spores/ml	1	0.25 kg/ha	–	4 days	1	n.e.	**100%**	Miura et al., 1980
Minnesota, USA	lentic freshwater	VectoBac G	200 ITU/mg	1	5.6 kg/ha (RAR)	1.12	58 days	3	**97–100%**	no	Charbonneau et al., 1994
				2	28 kg/ha	5.6					
Minnesota, USA	lentic freshwater	VectoBac G	200 ITU/mg	1	9 kg/ha (RAR)	1.8	53 days	2	n.e.	no	Liber et al., 1998
				2	45 kg/ha	9				**55–75%**	
				3	90 kg/ha	18				**70–90%**	
Camargue, France	oligohaline marsh	VectoBac 12AS	1200 ITU/mg	1	2 L/ha (RAR)	2.56	12 days	1	n.e.	no	Pont et al., 1999
				2	4 L/ha	5.26				no	
				3	8 L/ha	10.2				**62–88%**	
Camargue, France	oligohaline marsh	VectoBac 12AS	1200 ITU/mg	1	0.8 L/ha	1.02	21 days	1	n.e.	no	Duchet et al., 2015
				2	2.5 L/ha	3.2					
Upper Rhine Valley, Germany	lentic freshwater	VectoBac WG	2400 ITU/mg	1	0.6 kg/ha (RAR)	1.44	15 weeks	2	**24%**	**41%**	Allgeier et al., 2019a

Field studies

Study area	Wetland type	Formulation	Toxicity (ITU/mg)	Application rate	$\times 10^9$ ITU/ha	Max. sampling time	Max. applications	Mosquito reduction	Chironomid reduction	Reference
Minnesota, USA	lentic freshwater	VectoBacG	200	11.72 kg/ha (RAR)	2.34	3 years	18	**77–83%**	**63–84%**	Hershey et al., 1998
River Dalälven, Sweden	freshwater river floodplain	VectoBacG	200	13–15 kg/ha (RAR)	2.6–3	6 years	3 to 5	n.e.	no	Lundstrom et al., 2010b
Atlantic coast, France	saltmarsh	VectoBacWG; VectoBac12AS	3000; 1200	0.4 kg/ha; 0.5 L/ha	0.64–1.2	2 years	11	n.e.	no	Caquet et al., 2011
Atlantic coast, France	coastal saltmarsh	VectoBacWG	3000	0.22–0.3 kg/ha	0.66–0.9	7 years	47	n.e.	no	Lagadic et al., 2014
Coastal and Continental, France	saltmarsh and freshwater wetlands	VectoBacWG; VectoBac12AS	3000; 1200	0.125–0.5 kg/ha; 0.35–2.5 L/ha	0.38–3.2	4 years	4 to 25	n.e.	no	Lagadic et al., 2016
Camargue, France	*meso*−/oligohaline marsh	VectoBac12AS	1200	2.5 L/ha	3.2	1 year	30 to 50	n.e.	**48%**	Jakob and Poulin, 2016
Upper Rhine Valley, Germany	lentic freshwater	VectobacWG	2400	0.6 kg/ha (RAR)	1.44	13 weeks	1	**97%**	**65%**	Theissinger et al., 2018
Floodplains, Austria	lentic freshwater	VectoBacWG; VectoBac12AS; VectoBacG	3000; 1200; 200	0.5-1 L/ha; 400 g/ha; 10–12 kg/ha	0.64–2.4	3–4 days	1	**100%**	no	Wolfram et al., 2018
Upper Rhine Valley, Germany	lentic freshwater	VectobacWG	2400	0.6–1.8 kg/ha (RAR)	1.44–2.88	14 weeks	1	**92–99%**	**68–77%**	Allgeier et al., 2019a

indicate an inappropriate sampling protocol, or that applied Bti concentrations do not display effective mosquito control concentrations, possibly due to various interacting environmental parameters, which may lead to underestimated effects on chironomid populations. The validity of studies that do not report effects on the target-organisms (mosquitoes) is therefore questionable. Additionally, short-term studies might not capture the effect of Bti on sensitive, first larval instars, as they need considerable time to develop and emerge and/or are too small for in-field sampling. To comprehensively cover, the entire community and the developmental times of several chironomid species, adult emergence should be monitored during at least three months after the first Bti application (Allgeier et al., 2019a).

As Bti sensitivity varies between chironomid species and between larval stages (Liber et al., 1998; Kästel et al., 2017), the effect on species composition of chironomid communities in regularly treated wetlands is also of importance to minimize adverse effects on biodiversity. Four field studies explicitly addressed chironomid species composition in freshwater wetlands using manual identification at the larval (Wolfram et al., 2018) or adult stage (Lundstrom et al., 2010a) or by using state-of-the-art metabarcoding (Theissinger et al., 2018). Results concerning species richness were again highly variable, some reporting a modification of chironomid community composition due to reduction in species richness (Theissinger et al., 2018), while others found no effect after four days (Wolfram et al., 2018) and even increasing chironomid larval richness after years of Bti treatments (Lundstrom et al., 2010a). Theissinger et al. (2019) compared species richness in a temporary flooded meadow left untreated after 20 years of regular Bti treatment to a meadow with continued treatment. While the difference after one year was minor, four years of Bti intermittence seemed to favor the recolonization of new species that did not occur in the continuously treated site.

Besides the high sensitivity of chironomids, Bti is assumed to have no adverse effect on other non-target organisms (NTO) at recommended application rates. The first and most detailed review on direct effects of Bti on non-target organisms was conducted by Boisvert and Boisvert (2000) and included 75 published studies until the year 1999 that dealt with mosquito control in stagnant waters as well as black fly control in slowly flowing water bodies. As the current review only includes studies on mosquito control in laboratory experiments and lentic habitats, we cumulated the results of 35 relevant studies from the review of Boisvert and Boisvert (2000) (9 conducted in lentic environments, 25 laboratory/artificial, 1 both) in Table 2.

These studies revealed negative effects in laboratory tests on some taxa within Chlorophyta, Diptera (outside the target group Nematocera), Lepidoptera and Plecoptera (Table 2). Studies conducted after the year 1999 focused on various invertebrates, insects, annelids, fish and amphibians (Table 3).

A recent laboratory study on zooplankton (two copepods and three cladocerans) from mosquito control regions in Spain concluded that negative effects at the community level are likely as some species were affected at concentrations close to field applications (Olmo et al., 2016). However, several other studies did not find any effect on zooplankton in more realistic semi-field or field approaches with a longer sampling period (Duchet et al., 2010b; Lagadic et al., 2014; Lagadic et al., 2016). Although amphibians develop in temporary water bodies targeted by mosquito control operations, information on adverse effects is scarce. First assessments were performed with some non-commercial formulations by mosquito control operators and did not find direct effects (Boisvert and Boisvert, 2000). However, one laboratory study observed a shorter time to metamorphosis and higher weights in the European common frog *Rana temporaria* after exposure to small quantities of Bti (Paulov, 1985). Mortality recorded after exposing tadpoles of the South American common frog *Leptodactylus latrans* to environmentally-relevant concentrations of a commercial liquid Bti formulation (Introban) was most likely related to formulation byproducts (Lajmanovich et al., 2015). Two formulations primarily applied in

Table 2
Bti toxicity assessed in various organism groups (direct effects). Number of taxa studied and percentage showing direct acute effects given. (* Range of mortality given for taxonomic groups where 50% or more taxa showed effects, in bold). Diptera taxa were not mosquitoes, black flies or midges. All studies were evaluated from the review of Boisvert and Boisvert, 2000.

Study type	Taxonomic group	Number of taxa studied	Direct effects	in % of taxa
	Chlorophyta	2	**90–99% mortality***	100
	Hydra	1	no	100
	Diptera	18	**42–100% mortality***	50
	Hemiptera	18	no	94.4
	Lepidoptera	3	**mortality***	100
Laboratory	Trichoptera	7	no	71.4
	Plecoptera	2	**40% mortality***	50
	Crustacea	35	no	91.4
	Turbellaria	3	no	100
	Annelida	5	no	100
	Amphibia	16	no	100
	Pisces	20	no	65
	Collembola	1	no	100
	Diptera	10	no	100
	Hemiptera	20	no	95
Field	Lepidoptera	1	no	100
	Crustacea	14	no	100
	Annelida	3	no	66.6
	Pisces	1	no	100
	Mollusca	12	no	100
Laboratory+field	Odonata	26	no	100
	Ephemeroptera	10	no	100
	Coleoptera	67	no	100

Europe (VectoBac WG and 12AS) were not acutely toxic to *R. temporaria*, even at 10 x RAR (Allgeier et al., 2018). Nevertheless, Bti induced several sublethal effects in form of subcellular alterations of biomarkers indicating detoxification, oxidative stress and genotoxicity (Lajmanovich et al., 2015; Allgeier et al., 2018) and behavioral changes resulting in affected swimming behavior (Junges et al., 2017). A recent study did not confirm sublethal effects and concluded that water temperature might be a co-stressor (Schweizer et al., 2019).

4.2. Indirect (food web) effects

Indirect effects of Bti used in mosquito control programs, affecting the food web and organisms at higher trophic levels, were suspected by environmental organizations since the beginning of Bti use and were also acknowledged by control operators (Becker and Ludwig, 1983). Indirect effects can be caused by a reduction of populations of mosquitoes and/or non-target chironomids. Effective mosquito predators like cyprinid fish can consume more than one thousand larvae within 12 h (Becker and Ludwig, 1983). Crested newt larvae *(Triturus cristatus)* have been recorded to consume around 900 mosquito larvae in 10-day feeding experiments (Günther, 1996). Bats (e.g. *Myotis daubentonii*) and swallows (*Delichon urbica* and *Hirundo rustica*), as well as predatory insects such as water beetles and striders feed on mosquito and chironomid larvae and pupae (Becker and Ludwig, 1983; Vaughan, 1997; Vinnersten et al., 2009; Gutierrez et al., 2017). Odonata (dragonflies and damselflies) also consume mosquitoes and chironomids at both the larval and adult stages (Corbet, 1999; Pfitzner et al., 2015). Gut flushing of amphibians in the Upper Rhine valley showed only a minor contribution of mosquitoes in the food of different amphibian species (Blum et al., 1997). The feces of two bat species (*M. daubentonii* and *Pipistrellus nathusii*) contained 3–8% mosquitoes but >80% chironomid remains (Arnold et al., 2000). The reported low proportion of mosquitoes in the diet contrasts with other studies, where mosquitoes represent the major food items for bats (Sullivan et al., 1993; Beck, 1995). Since some studies revealed a low proportion

Table 3
Laboratory (A.), semi-field (B.) and field (C.) studies on direct effects of Bti after 1999 (not included in Boisvert and Boisvert, 2000). Formulation, application rate or concentration, treatment numbers and study duration are given. Effects on the specific groups are described (effects in bold).

Laboratory studies

Taxonomic group	Taxa	Formulation	Rate/Concentration	No. of treatments	Study duration	Effects	Reference
Cladocera	*Daphnia magna, Daphnia pulex*	VectoBac12AS	2.5 L/ha	1	14 d	no effect	Duchet et al., 2010b
Zooplankton	*Tropocyclops prasinus, Acantocyclops americanus, Ceriodaphnia reticulata, Chydorus sphaericus, Daphnia pulex*	VectoBac12AS	5–500 mg/L	1	15 d	**increasing mortality with concentration and time**	Olmo et al., 2016
Notonectidae	*Buenoa tarsalis*	Bt-HorusSC	25 mg ai/L	1	16 d (2 h)	no mortality (enhanced predatory abilities)	Gutierrez et al., 2017
	Leptodactylus latrans	Introban	2.5–40 mg/L	1	48 h	**mortality, sublethal effects (GST, CAT), genotoxicity**	Lajmanovich et al., 2015
Amphibians	*Rhinella arenarum, Rhinella fernandezae, Physalaemus albonotatus*	Introban	1.5–40 mg/L	1	48 h	**effects on swimming behaviour of *R. arenum*, mortality at high concentrations**	Junges et al., 2017
	Rana temporaria	VectoBacWG; Vectobac12AS	0.6–6 kg/ha; 2–20 L/ha	3	60 d	**sublethal effects (GST, GR, AChE)**	Allgeier et al., 2018
	Rana temporaria	VectoBacWG	1 mg /L, 10 mg/L, 100 mg/L	1	11 d	no sublethal effects (Hsp70, A ChE)	Schweizer et al., 2019
	Melanotaenia duboulayi	VectoBac12AS	12 L/ha	1	20 min	no effects on swimming performance	Hurst et al., 2007
Fish	*Melanotaenia duboulayi*	Teknar	1 L/ha	1	24 h	no toxicity	Brown et al., 2002
	Danio rerio, Oreochromis niloticus	isolated strains	10^{8}–10^{10} spores/ml	1	30d (72 h)	no mortality/genototxicity, increased frequency of necrotic cells in *O. niloticus*	Grisolia et al., 2009

Semi-field studies

Habitat	Taxonomic group	Taxa	Formulation	Rate/Concentration	No. of treatments	Study duration	Effects	Reference
Freshwater	Aquatic invertebrates		VectoBacWG	1.2 kg/ha	1 to 2	7 w	**reduced chironomid abundances**, no other treatment effects	Allgeier et al., 2019a,b
		Daphnia pulex	VectoBac12AS	(0.8) 2.5 L/ha	1	21 d	no effect on abundance	Duchet et al. 2008
Marsh	Cladocera	*Daphnia magna*	VectoBac12AS	2.5 L/ha	1	21 d	**negative effect on density at Day 21**	Duchet et al. 2010a
		Daphnia magna, Daphnia pulex	VectoBac12AS	2.5 L/ha	2	2 y	no effect	Duchet et al., 2010b

Field studies

Habitat	Taxonomic group	Taxa	Formulation	Rate/Concentration	No. of treatments	Study duration	Effects	Reference
	Zooplankton		VectoBacG	11.72 kg/ha (RAR)	18	3 y	no effect	Niemi et al., 1999
	Aquatic+terrestrial arthropods		VectoBac	302.6 g/ha	1	4 w	no overall treatment effect	Davis and Peterson, 2008
Freshwater	Emerging insects		VectoBacG	13–15 kg/ha (RAR)	3 to 5	6 y	no effects on insect production, with exception of less Coleoptera and more Ceratopogonidae	Vinnersten et al., 2010
	Aerial insects		–	–	–	3 y	no effect on abundances	Timmermann and Becker, 2017
Saltmarsh and freshwater	Aquatic invertebrates		VectoBacWG; VectoBac12AS	0.125–0.5 kg/ha; 0.35–2.5 L/ha	4 to 25	4 y	no effect on taxonomic structure and abundances	Lagadic et al., 2016
	Aquatic invertebrates		VectoBacWG	0.22–0.3 kg/ha	47	7 y	no effect on taxonomic structure and abundances	Lagadic et al., 2014
	Arthropods		VectoBac12AS	2.5 L/ha	30 to 50/ year	9 y	**reduced abundances of Diptera, Aranaea, Coleoptera, Hymenoptera**	Poulin and Lefebvre, 2016
Saltmarsh	Aquatic +terrestrial invertebrates		VectoBac12AS	1.2 L/ha	1	20 d	no effect (inconsistent, short term)	Russell et al., 2009
	Invertebrate community	*Nereis diversicolor, Corophium volutator*	VectoBacWG; Vectobac12AS	0.4 kg/ha; 0.5 L/ha	5 to 6 / year	2 y	no effect on abundances of annelids, crustacean, midge larvae	Caquet et al., 2011
	Annelidae	*Nereis diversicolor*	VectoBac12AS	1 L/ha	14	3 y	variation of esterase activity	Foucry et al., 2002
Flooplain soil	Bacillus	*Bacillus cereus group*	VectoBacG	13–15 kg/ha	0 to 2/ year	11 y	no effect on Bcg abundances, **higher Bti abundances in soil**	Schneider et al., 2017

of mosquitoes in animal diets, mosquito control operators in Germany assumed that Bti mosquito control had no negative food web effects and, together with the absence of direct mortality observed in organisms at higher trophic levels, coined Bti use as "environmentally friendly". However, at the beginning of Bti mosquito control, the operators mentioned that *"it is very important when applying Bti against early instar mosquito larvae to take into consideration that you will remove the food source necessary to maintain a population of specific mosquito predators. Therefore, it is necessary in this case to use Bti only against late instar larvae"* (Becker and Ludwig, 1983). Today, 35 years later, control measures usually start while the larvae are in early developmental stages due to their higher sensitivity and operations use ice formulations and helicopter application (Becker and Margalit, 1993; Becker, 2003). The first field study that reported a negative effect on non-target taxa was from an assessment of mosquito treatment with Bti in a salt marsh in Florida (Purcell (1981); Table 4).

Non-target species were sampled with a dip net before and one day after treatment in one brackish water pond. The authors observed a decline of individuals in a backswimmer species as an indirect Bti effect and speculated on food depletion causing their migration to other ponds. However, the study was limited to one site and data were not statistically analyzed (Purcell, 1981). A thorough study program on ecological effects was established when Bti was introduced as mosquito control agent in Minnesota, USA (Hanowski et al., 1997; Hershey et al., 1998; Niemi et al., 1999). Several wetlands were selected in Western Wright County to study the effect of 20 Bti applications over a 3–4 year period on zooplankton, benthic macroinvertebrates and a bird species (Table 4). No effect was observed in Bti-treated wetlands on zooplankton compared to untreated sites, although macroinvertebrate populations were reduced (Nematocera, including chironomids 63–84%). No Bti-related effects were observed in the red-winged blackbird *(Agelaius phoeniceus)*. However, its nesting season was already completed when decreases of emerging aquatic insects became prominent. Additionally, *A. phoeniceus* forages both within and off the wetland for insects to feed their young. Landscape context, feeding ecology of study species and time are important factors to consider for assessing mosquito control effects. The group of researchers in Minnesota presumed that *"ecological effects of applying these materials for decades is unknown"* (Niemi et al., 1999), and concluded that long-term studies were needed. Unfortunately, no follow-up of this research program was implemented.

A second set of studies was conducted in the River Dalälven floodplains in central Sweden where Bti was introduced in 2002 to control the floodwater mosquito *Ae. sticticus* in temporary wetlands (Ostman et al., 2008; Vinnersten et al., 2009). The monitoring program included three treated and three untreated wetlands for comparison of direct and indirect Bti effects over six years. The control program used 13–15 kg/ha of Vectobac G in an aerial application that reduced female adult mosquito population close to 100% (Vinnersten et al., 2010). One study focused on predatory diving beetles (Dytiscidae) for indirect effect assessment. An analysis of >6000 beetles belonging to 61 species showed increases in medium-sized adult diving beetles in treated wetlands (Vinnersten et al., 2009). The authors concluded that hydrology was the most important factor for structuring the water beetle community, irrespective of the presence and abundance of prey taxa. The density of protozoans, which form the food of mosquito larvae, was 4.5 higher after mosquito removal and taxonomic richness increased by 60% two weeks after a Bti application (Ostman et al., 2008) (Table 4). No other group of organisms interacting with mosquitoes or chironomids was studied in Sweden and the monitoring was discontinued.

The most comprehensive, long-term study on food web related effects of Bti mosquito control was conducted in Camargue (Southern France). In 2006, Bti mosquito control was initiated and the associated monitoring program evaluated effects on reed invertebrates, dragonflies and birds (house martins, *Delichon urbicum*) (Table 4). Dragonflies were sampled over six years and species richness as well as abundance were significantly reduced (−50%) in Bti-treated compared to untreated sites. The authors concluded that mosquito control using Bti should be acknowledged as a potential threat to Odonata (Jakob and Poulin, 2016). Reedbeds in the Camargue support a specific avifauna of conservation concern. A study carried out in 1998–1999 showed that abundance of breeding reed passerines was strongly correlated with that of their invertebrate prey (Poulin et al., 2002). The comparison of treated ($n = 5$) and untreated ($n = 10$) reed marshes revealed a significant reduction (33%) in invertebrates serving as food to passerines birds, with spiders being particularly affected (Poulin and Lefebvre, 2016). The house martin *(D. urbicum)* is a good biological model to assess indirect effects of Bti because 35% of food items given to chicks

Table 4
Studies on indirect, food-web related effects of Bti. Formulation, application rate, treatment numbers and study duration are given. Effects on the specific groups are described (effects in bold).

Study type	Taxonomic group	Taxon	Formulation	Application rate	No. of treatments	Duration	Effects	Reference
Mesocosm	Amhibians	*Hyla versicolor*	Mosquito Dunks, Mosquito Bits	1.275 g Bti	>2	–	**reduced survival in presence of predator**	Pauley et al., 2015
	Aquatic invertebrates		VectoBacWG	1.2 kg/ha	1	7 w	**decreased chironomid abundances**	Allgeier et al., 2019a
Field	Protozoans		VectoBacG	15 kg/ha	2	2 w	**increasing (heterotrophic) protozoan richness (60%) and densities (4.5 times)**	Ostman et al., 2008
	Backswimmer	*Notonecta indica*	PM50 (Biochem)	3–13.5 ITU/ml	1	1 d	**abundance decline**	Purcell, 1981
	Benthic macroinvertebrates		VectoBacG	11.72 kg/ha (RAR)	18	3 y	**decreased abundances of predominantly Nematocerans (63–84%)**	Hershey et al., 1998
	Diving beetles		VectoBacG	13–15 kg/ha	1 to 5	5 y	**slight abundance increase in medium-sized dytiscids**	Vinnersten et al., 2009
	Dragonflies		VectoBac12AS	2.5 L/ha	30–50/year	5 y	**effect on species richness, abundance**	Jakob and Poulin, 2016
	Birds	*Agelaius phoeniceus*	VectoBacG	11.72 kg/ha (RAR)	19	4 y	no effect	Niemi et al., 1999
			VectoBacG	11.72 kg/ha (RAR)	18	3 y	no effect on bird community	Hanowski et al., 1997
		Delichon urbicum	VectoBac12AS	2.5 L/ha	30–50/year	3 y	**lower breeding success**	Poulin et al., 2010
		Delichon urbicum	VectoBac12AS	2.5 L/ha	30–50/year	3 y	**lower intake of Nematocera and large prey, smaller clutch size and fledging success**	Poulin, 2012

are small chironomids and mosquitoes (Poulin et al., 2010). House martins, together with other swallows and bats, were also mentioned as important mosquito predators by mosquito control operators in the 1980s, and increasing their nesting sites as secondary control options was suggested (Becker and Ludwig, 1983). Comparison of chick diet based on feces analysis at six house martin colonies, three of which were surrounded by Bti-treated wetlands, revealed diet modifications related to Bti treatments. Intake of nematocerans (mosquitoes and chironomids) and their predators (odonates, neuropterans and spiders) was significantly lower at treated sites. Dietary shift had consequences on breeding success, resulting in significant reductions of fledglings by up to 36% at treated sites due to increased mortality by starvation, showing Bti effects at two trophic levels (Poulin, 2012). This finding provided the first compelling evidence of Bti application indirectly affecting vertebrate populations.

Following the food web effects revealed in the Camargue, aerial insect trapping data from 1989 to 1991 were reanalyzed in 2017 by German mosquito control operators in context of diet observations in nestlings of house martins from two broods in 1991 (Timmermann and Becker, 2017). Chironomids were among the most frequently trapped insects during the study period. House martin fledglings of the first brood were mostly fed with aphids (80% of individuals). Chironomids, the most frequently trapped insects, reached >5% of individuals in the diet. Unfortunately, the number of nestlings studied was not provided. Until now, these study sites have been treated multiple times per year with Bti from 1980 onwards. It seems likely that insect communities and feeding preferences of house martins have changed due to a chronic Bti-induced effect and a repetition of this study would be timely. Further studies in the Upper Rhine Valley are especially needed since a 43-year long monitoring of the breeding bird community at an oxbow lake within the mosquito control area showed significant changes (Schrauth and Wink, 2018). For 74% of the insectivorous birds, decreasing populations were found in the long-term trend, especially for species breeding in wetland areas. Among other factors, the authors also considered the possibility "*that mosquito control at 'Lampertheimer Altrhein' with Bti could lead to additional loss of food resources for insectivorous birds*".

In addition to field studies, where the control of environmental factors is difficult, mesocosm studies were performed on Bti effects on reconstructed aquatic food webs. Pauley et al. (2015) examined the interaction between predation and Bti formulations on amphibians. Survival of tadpoles of the Gray Treefrog *(Hyla versicolor)* was significantly reduced by 80% in the presence of predators (dragonfly larvae) and a Bti formulation (Mosquito dunk) in pond mesocosms. In a similar approach, Allgeier et al. (2019a) assessed the indirect Bti effects on the availability of food resources on predatory newt larvae (*Lissotriton helveticus* and *L. vulgaris*). A dragonfly larva *(Aeshna cyanea)*, acting as a predator on newts but also on chironomids, was 27% more lethal to larval newts in Bti-treated mesocosms with lower chironomid abundances. However, unaffected densities of chironomids as alternative prey organism favored their coexistence with newt larvae in control mesocosms.

Interestingly, no food-web study with fish was ever performed although especially fish brood is feeding on mosquito and chironomid larvae. Ecological food web-related effects were analysed in parallel to the introduction of Bti mosquito control in three areas in the USA, Sweden and France but were missing in in the historically oldest European-treated area, the Upper Rhine Valley in Germany.

With the on-going debate on environmental effects of human activities, long-term studies are still needed for each Bti mosquito control area to include potential habitat specific system properties in the analysis. It is therefore recommended to establish sound monitoring with enough control sites to evaluate potential long-term food web perturbations and resulting declines in biodiversity in mosquito control areas. Only a thorough evaluation of such data can confirm if mosquito control with Bti is "environmentally friendly".

5. Socio-economic assessment and public perception

The socio-economic relevance of mosquito control in the temperate Northern hemisphere has changed over time. Mosquitoes were considered a nuisance and mosquito control programs were seen to contribute to human well-being due to a reduction of mosquito bite incidences. The recent invasion of tropical mosquitoes in temperate regions, potential vectors of diseases, add public health to the socio-economic relevance of mosquito control (von Hirsch and Becker, 2009). Additionally, potential environmental effects of mosquito control and its link with biodiversity decline is a rising concern in Europe (Schwarz et al., 2017; Langhans et al., 2019). Socio-economic relevance of mosquito control is therefore multi-faceted and involves trade-offs among conflicting objectives like avoidance of nuisance and environmental harm. A comprehensive assessment of socio-economic relevance requires considering the three dimensions "nuisance", "vector-borne diseases" and "environmental effects" jointly.

5.1. Nuisance

A survey of local authorities in the UK showed evidence of more than a two-fold increase in nuisance reports between 1999 and 2009 (Medlock et al., 2012). Mosquito nuisance in New Jersey (USA) was perceived equal to living with up to two (out of potential five) health risk factors for diabetes or equal to women experiencing menstrual disorders (Halasa et al., 2014). Further, enjoying outdoor activities without mosquitoes was rated as important as neighborhood safety and more important than a clean neighborhood. An economic cost-benefit analysis in the Upper Rhine Valley, Germany used a method resembling contingent valuation to assess the benefits due to nuisance reduction resulting from Bti mosquito control (von Hirsch and Becker, 2009). Based on their assessment of household willingness to pay to achieve the current mosquito reduction rate through a campaign using Bti they found a benefit/cost ratio of 1.8, similar as observed for mosquito control programs in the USA (John et al., 1987; Farmer et al., 1989) and Sweden (Soutukorva et al., 2013). However, potential environmental effects on non-target species were so far ignored. Also, the selected method implementation is prone to hypothetical bias so that these results have only limited validity.

5.2. Vector-borne diseases

Economic choice experiments to assess the monetary benefit of mosquito control programs in Madison, Wisconsin (USA) reported a higher willingness to pay for mitigating nuisance than for reducing the risk of being infected with WNV (Dickinson and Paskewitz, 2012). A similar choice experiment in Athens (Greece) showed a 48% higher mean willingness to pay for reducing diseases induced by the Asian tiger mosquito than for WNV alone (Bithas et al., 2018). A significantly positive willingness to pay for reducing nighttime – but not daytime – nuisance was also shown.

5.3. Environmental effects

Respondents in the Madison survey stated their concern for avoiding adverse environmental effects in follow-up questions (Dickinson and Paskewitz, 2012). However, environmental effects were explicitly taken into account in other economic valuation studies. An early study by Lichtenberg (1987) assesses the cost-efficiency of integrated or ecologically-sensitive mosquito control programs compared to those relying on chemical agents in the San Joaquin Valley in California. They find that the cost of integrated programs relying predominantly on biological pest control with mosquitofish can be as low as a quarter of the cost of using chemical agents. While these results cannot be generalized they show the potential of environment-friendly methods and the concern regarding the environmental effects of chemical mosquito treatment. John et al. (1992) show that people value ecologically-sensitive mosquito control programs more than those relying on chemical agents. More recently, in a non-comparative rating between the two control programs, only one third of the surveyed population in the Marais des Baux wetland in Southern France attributed a positive value to the established Bti control program while the natural control program, including water table management and fish as predators, was always valued positively (Westerberg et al., 2010). This study, as well as one on governance and decision making about the Camargue Bti experiment, showed that it is not sufficient to evaluate the cost of only one type of program (Guillet and Mermet, 2013). Instead, Bti mosquito control should be compared to alternative and equally effective programs relying on other techniques, like nature management or mosquito traps.

Although people perceive considerable benefits through a decrease in nuisance and disease risk levels, the relative importance of these two benefits seems to depend on the context. Existing studies indicate that a majority of the population is skeptical regarding the environmental side effects of mosquito control with Bti and that there exists a substantial willingness to pay for alternative, more environment-friendly techniques, if they are available and effective.

6. Conclusion

Even though Bti is currently the most selective and least toxic agent available to control mosquitoes, control programs should integrate non-biased awareness campaigns and mitigation measures balancing the social demands for mosquito reduction with the factors involved in mosquito proliferation and dispersion. Novel and eco-friendly strategies that are not based on the use of insecticides are increasingly investigated (Benelli, 2015; Benelli et al., 2016; Benelli and Mehlhorn, 2016). These methods include the usage of repellents (Sharma, 2001; Park et al., 2005; Semmler et al., 2009), natural predators (Brodman and Dorton, 2006; Meyabeme Elono et al., 2010; Soumare and Cilek, 2011; Acquah-Lamptey and Brandl, 2018), natural ecological traps influencing oviposition (Gardner et al., 2018), mechanical traps for adults (Jackson et al., 2012; Englbrecht et al., 2015; Poulin et al., 2017), nanoparticles (Govindarajan et al., 2016), and active citizen participation (Johnson et al., 2018). Measures for nuisance control of mosquitoes could also consist of improved wetland management, reduction in area and periods of Bti spraying, use of alternative methods such as mosquito traps (Poulin et al., 2017) and suspension of mosquito control in environmentally sensitive wetlands where nature preservation is a priority. Monitoring should not only include the obligatory mosquito resistance evaluation but also Bti exposure as well as environmental and food-web related effects on ecosystems. There is currently an inadequate number of studies available to unequivocally conclude that Bti used for nuisance control of mosquitoes can be considered environmentally safe. Persistence, resistance and environmental effect assessments should be conducted by independent bodies in complement to mosquito control operators to generate public trust in the studies' outcomes. To understand the scale of exposure of wetlands, we also recommend publishing treatment areas, Bti formulations and rates as well as frequencies of applications in a transparent way, especially since Bti mosquito control is funded directly and/or indirectly by the public.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

Acquah-Lamptey, D., Brandl, R., 2018. Effect of a dragonfly (*Bradinopyga strachani* Kirby, 1900) on the density of mosquito larvae in a field experiment using mesocosms. Web Ecology 18, 81–89.

Allgeier, S., Frombold, B., Mingo, V., Bruhl, C.A., 2018. European common frog *Rana temporaria* (Anura: Ranidae) larvae show subcellular responses under field-relevant *Bacillus thuringiensis* var. *israelensis* (Bti) exposure levels. Environ. Res. 162, 271–279.

Allgeier, S., Kastel, A., Bruhl, C.A., 2019a. Adverse effects of mosquito control using Bacillus thuringiensis var. israelensis: reduced chironomid abundances in mesocosm, semi-field and field studies. Ecotoxicol. Environ. Saf. 169, 786–796.

Allgeier, S., Friedrich, A., Brühl, C.A., 2019b. Mosquito control based on Bacillus thuringiensis israelensis (Bti) interrupts artificial wetland food chains. Sci. Total Environ. https://doi.org/10.1016/j.scitotenv.2019.05.358.

Aly, C., Mulla, M.S., Federici, B.A., 1985. Sporulation and toxin production by *Bacillus-thuringiensis* var *israelensis* in cadavers of mosquito larvae (Diptera, Culicidae). J. Invertebr. Pathol. 46, 251–258.

Amalraj, D.D., Sahu, S.S., Jambulingam, P., Doss, P.S.B., Kalyanasundaram, M., Das, P.K., 2000. Efficacy of aqueous suspension and granular formulations of *Bacillus thuringiensis* (Vectobac) against mosquito vectors. Acta Trop. 75, 243–246.

Armitage, P.D., Pinder, L.C., Cranston, P., 1995. The Chironomidae: Biology and Ecology of Non-biting Midges. Springer, Netherlands.

Arnold, A., Braun, M., Becker, N., Storch, V., 2000. Zur Nahrungsökologie von Wasser-und Rauhautfledermaus in den nordbadischen Rheinauen. Carolinea 58, 257–263.

Beck, A., 1995. Fecal analyses of European bat species. Myotis 32, 109–119.

Becker, N., 2002. Sterilization of *Bacillus thuringiensis israelensis* products by gamma radiation. J. Am. Mosq. Control Assoc. 18, 57–62.

Becker, N., 2003. Ice granules containing endotoxins of microbial agents for the control of mosquito larvae - a new application technique. J. Am. Mosq. Control Assoc. 19, 63–66.

Becker, N., 2006. Biological control of mosquitoes: management of the Upper Rhine mosquito population as a model programme. In: Eilenberg, J., Hokkanen, H.M.T. (Eds.), An Ecological and Societal Approach to Biological Control. Springer Netherlands, Dordrecht, pp. 227–245.

Becker, N., Ludwig, H.W., 1983. Mosquito control in West Germany. Bull. Soc. Vector Ecol. 8, 85–93.

Becker, N., Ludwig, M., 1993. Investigations on possible resistance in *Aedes vexans* field populations after a 10-year application of *Bacillus thuringiensis israelensis*. J. Am. Mosq. Control Assoc. 9, 221–224.

Becker, N., Lüthy, P., 2017. Chapter 26- Mosquito control with entomopathogenic bacteria in Europe. In: Lacey, L.A. (Ed.), Microbial Control of Insect and Mite Pests. vol. 2017. Academic Press, pp. 379–392.

Becker N., Margalit J., 1993. Use of *Bacillus Thuringiensis Israelensis* against mosquitoes and black flies. In: Entwistle P.F., Corry J.S., Balley M.J., Higgs S., editors. *Bacillus thuringiensis*, an Environmental Biopesticide: Theory and Practice. John Wiley, Chichester, UK, 1993.

Becker, N., Zgomba, M., Petric, D., Dahl, C., Boase, C., Lane, J., et al., 2010. Mosquitoes and their Control. Springer US, New York.

Becker, N., Ludwig, M., Su, T., 2018. Lack of resistance in *Aedes vexans* field populations after 36 years of *Bacillus thuringiensis* subsp. *Israelensis* applications in the upper Rhine Valley, Germany. J. Am. Mosq. Control Assoc. 34, 154–157.

Ben-Dov, E., 2014. *Bacillus thuringiensis* subsp. *israelensis* and its dipteran-specific toxins. Toxins 6, 1222–1243.

Benelli, G., 2015. Plant-borne ovicides in the fight against mosquito vectors of medical and veterinary importance: a systematic review. Parasitol. Res. 114, 3201–3212.

Benelli, G., Mehlhorn, H., 2016. Declining malaria, rising of dengue and Zika virus: insights for mosquito vector control. Parasitol. Res. 115, 1747–1754.

Benelli, G., Jeffries, C.L., Walker, T., 2016. Biological control of mosquito vectors: past, present, and future. Insects 7.

Bithas, K., Latinopoulos, D., Kolimenakis, A., Richardson, C., 2018. Social benefits from controlling invasive Asian tiger and native mosquitoes: a stated preference study in Athens, Greece. Ecol. Econ. 145, 46–56.

Blum, S., Basedow, T., Becker, N., 1997. Culicidae (Diptera) in the diet of predatory stages of anurans (Amphibia) in humid biotopes of the Rhine Valley in Germany. Journal of vector ecology: journal of the Society for Vector Ecology 22, 23–29.

Boisvert, M., Boisvert, J., 1999. Persistence of toxic activity and recycling of *Bacillus thuringiensis* var. *israelensis* in cold water: field experiments using diffusion chambers in a pond. Biocontrol Sci. Tech. 9, 507–522.

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

Boisvert, M., Boisvert, J., Aubin, A., 2001. Factors affecting residual dosages of two formulations of *Bacillus thuringiensis* subsp *israelensis* tested in the same stream during a 3-year experiment. Biocontrol Sci. Tech. 11, 727–744.

Bordalo, M.D., Gravato, C., Beleza, S., Campos, D., Lopes, I., Pestana, J.L.T., 2020. Lethal and sublethal toxicity assessment of *Bacillus thuringiensis* var. *israelensis* and *Beauveria bassiana* based bioinsecticides to the aquatic insect *Chironomus riparius*. Sci. Total Environ. 698, 134155.

Brévault, T., Heuberger, S., Zhang, M., Ellers-Kirk, C., Ni, X., Masson, L., et al., 2013. Potential shortfall of pyramided transgenic cotton for insect resistance management. Proc. Natl. Acad. Sci. U. S. A. 110, 5806–5811.

Brodman, R., Dorton, R., 2006. The effectiveness of pond-breeding salamanders as agents of larval mosquito control. J. Freshw. Ecol. 21, 467–474.

Brown, M.D., Carter, J., Thomas, D., Purdie, D.M., Kay, B.H., 2002. Pulse-Exposure Effects of Selected Insecticides to Juvenile Australian Crimson-Spotted Rainbowfish (Melanotaenia duboulayi). J. Econ. Entomol. 95, 294–298 https://doi.org/10.1603/0022-0493-95.2.294.

Butchart, S.H., Walpole, M., Collen, B., van Strien, A., Scharlemann, J.P., Almond, R.E., et al., 2010. Global biodiversity: indicators of recent declines. Science 328, 1164–1168.

Butko, P., Huang, F., Pusztaicarey, M., Surewicz, W.K., 1997. Interaction of the delta-endotoxin CytA from *Bacillus thuringiensis* var. *israelensis* with lipid membranes. Biochemistry 36, 12862–12868.

Calba, C., Guerbois-Galla, M., Franke, F., Jeannin, C., Auzet-Caillaud, M., Grard, G., et al., 2017. Preliminary report of an autochthonous chikungunya outbreak in France, July to September 2017. Euro surveillance: bulletin Europeen sur les maladies transmissibles = European communicable disease bulletin 22.

Caprio, M.A., 1998. Evaluating resistance management strategies for multiple toxins in the presence of external refuges. J. Econ. Entomol. 91, 1021–1031.

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. Ecotoxicol. Environ. Saf. 74, 1122–1130.

Cardinale, B.J., Duffy, J.E., Gonzalez, A., Hooper, D.U., Perrings, C., Venail, P., et al., 2012. Biodiversity loss and its impact on humanity. Nature 486, 59–67.

Ceballos, G., Ehrlich, P., 2010. The sixth extinction crisis loss of animal populations and species. Journal of Cosmology 8, 1821–1831.

Ceballos, G., Ehrlich, P.R., Barnosky, A.D., Garcia, A., Pringle, R.M., Palmer, T.M., 2015. Accelerated modern human-induced species losses: entering the sixth mass extinction. Sci. Adv. 1, e1400253.

Ceballos, G., Ehrlich, P.R., Dirzo, R., 2017. Biological annihilation via the ongoing sixth mass extinction signaled by vertebrate population losses and declines. Proc. Natl. Acad. Sci. U. S. A. 114, E6089–e6096.

Charbonneau, C.S., Drobney, 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. Environ. Toxicol. Chem. 13, 267–279 https://doi.org/10.1002/etc.5620130211.

Christiansen, J.A., McAbee, R.D., Stanich, M.A., DeChant, P., Boronda, D., Cornel, A.J., 2004. Influence of temperature and concentration of Vectobac((R)) on control of the saltmarsh mosquito, *Ochlerotatus squamiger*, in Monterey County, California. J. Am. Mosq. Control Assoc. 20, 165–170.

Coetzee, M., Koekemoer, L.L., 2013. Molecular systematics and insecticide resistance in the major African malaria vector *Anopheles funestus*. Annu. Rev. Entomol. 58, 393–412.

Corbet, P.S., 1999. Dragonflies. Behaviour and Ecology of Odonata. Harley Books, Colchester.

Davis, R.S., Peterson, R.K.D., 2008. Effects of Single and Multiple Applications of Mosquito Insecticides on Nontarget Arthropods. J. Am. Mosq. Control Assoc. 24, 270–280 https://doi.org/10.2987/5654.1.

De La Noüe, J., Choubert, G., 1985. Apparent digestibility of invertebrate biomasses by rainbow trout. Aquaculture 50, 103–112.

de Melo-Santos, M.A.V., de Araujo, A.P., Rios, E.M.M., Regis, L., 2009. Long lasting persistence of *Bacillus thuringiensis* serovar. *israelensis* larvicidal activity in *Aedes aegypti* (Diptera: Culicidae) breeding places is associated to bacteria recycling. Biol. Control 49, 186–191.

De Respinis, S., Demarta, A., Patocchi, N., Luthy, P., Peduzzi, R., Tonolla, M., 2006. Molecular identification of *Bacillus thuringiensis* var. *israelensis* to trace its fate after application as a biological insecticide in wetland ecosystems. Lett. Appl. Microbiol. 43, 495–501.

Despres, L., Lagneau, C., Frutos, R., 2011. Using the bio-insecticide *Bacillus Thuringiensis Israelensis* in mosquito control. In: Stoytcheva, M. (Ed.), Pesticides in the Modern World. IntechOpen, Rijeka, p. 2011.

Diaz, S., Fargione, J., Chapin 3rd, F.S., Tilman, D., 2006. Biodiversity loss threatens human well-being. PLoS Biol. 4, e277.

Dickinson, K., Paskewitz, S., 2012. Willingness to pay for mosquito control: how important is West Nile virus risk compared to the nuisance of mosquitoes? Vector-Borne and Zoonotic Diseases 12, 886–892.

Duchet, C., Larroque, M., Caquet, Th., Franquet, E., Lagneau, C., Lagadic, L., 2008. Effects of spinosad and Bacillus thuringiensis israelensis on a natural population of Daphnia pulex in field microcosms. Chemosphere 74, 70–77 https://doi.org/10.1016/j.chemosphere.2008.09.024.

Duchet, C., Coutellec, M., Franquet, E., Lagneau, C., Lagadic, L., 2010a. Population-level effects of spinosad and Bacillus thuringiensisisraelensis in Daphnia pulex and Daphnia magna: comparison of laboratory and field microcosm exposure conditions. Ecotoxicology 19, 1224–1237 https://doi.org/10.1007/s10646-010-0507-y.

Duchet, C., Coutellec, M.A., Franquet, E., Lagneau, C., Lagadic, L., 2010b. Population-level effects of spinosad and *Bacillus thuringiensis israelensis* in *Daphnia pulex* and *Daphnia magna*: comparison of laboratory and field microcosm exposure conditions. Ecotoxicology 19, 1224–1237.

Duchet, C., Tetreau, G., Marie, A., Rey, D., Besnard, G., Perrin, Y., et al., 2014. Persistence and recycling of bioinsecticidal *Bacillus thuringiensis* subsp. *israelensis* spores in contrasting environments: evidence from field monitoring and laboratory experiments. Microb. Ecol. 67, 576–586.

Duchet, C., Franquet, E., Lagadic, L., Lagneau, C., 2015. Effects of *Bacillus thuringiensis israelensis* and spinosad on adult emergence of the non-biting midges *Polypedilum nubifer* (Skuse) and *Tanytarsus curticornis* Kieffer (Diptera: Chironomidae) in coastal wetlands. Ecotoxicol. Environ. Saf. 115, 272–278.

Duguma, D., Hall, M.W., Rugman-Jones, P., Stouthamer, R., Neufeld, J.D., Walton, W.E., et al., 2015. Microbial communities and nutrient dynamics in experimental microcosms are altered after the application of a high dose of Bti. J. Appl. Ecol. 52, 763–773.

Dupont, C., Boisvert, J., 1986. Persistence of *Bacillus-thuringiensis* serovar *israelensis* toxic activity in the environment and interaction with natural substrates. Water Air and Soil Pollution 29, 425–438.

Englbrecht, C., Gordon, S., Venturelli, C., Rose, A., Geier, M., 2015. Evaluation of BG-sentinel trap as a management tool to reduce Aedes albopictus nuisance in an urban environment in Italy. J. Am. Mosq. Control Assoc. 31, 16–25.

European Chemicals Agency, 2019. List of Authorized Biocidal Products (November 2019).

European Commission, 2011. Annex I Assessment report: *Bacillus thuringiensis* subsp. *israelensis* Serotype H-14 Strain AM65-52. Product-type 18 (Insecticide) Directive 98/8/EC Concerning the Placing Biocidal Products on the Market.

European Food Safety Authority, 2013. Conclusion on the peer review of the pesticide risk assessment of the active substance Aureobasidium pullulans (strains DSM 14940 and DSM 14941). EFSA J. 11, 3183.

European Parliament and Council, 1998, Directive 98/8/EC of the European Parliament and of the Council of 16 February 1998 concerning the placing of biocidal products on the market. (31998L0008).

European Parliament and Council, 2009, Directive 2009/128/EC of the European Parliament and of the Council of 21 October 2009 establishing a framework for community action to achieve the sustainable use of pesticides (text with EEA relevance). (32009L0128).

European Parliament and Council, 2010, *Bacillus thuringiensis* subsp. *israelensis* serotype H-14 strain AM65-52 product-type 18 (insecticide), directive 98/8/EC concerning the placing biocidal products on the market.

Falkenhorst, G., Enkelmann, J., Frank, C., Stark, K., 2018. Zur Situation bei wichtigen Infektionskrankheiten Reiseassoziierte Krankheiten. p. 2017.

Farmer, F.L., Redfern, J.M., Meisch, M.V., Inman, A., 1989. An evaluation of a community based mosquito abatement program: residents' satisfaction, economic benefits and correlates of support. J. Am. Mosq. Control Assoc. 5, 335–338.

Federici, B.A., Bauer, L.S., 1998. Cyt1Aa protein of *Bacillus thuringiensis* is toxic to the cottonwood leaf beetle, *Chrysomela scripta*, and suppresses high levels of resistance to Cry3Aa. Appl. Environ. Microbiol. 64, 4368–4371.

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). Mar. Environ. Res. 54, 755–759 https://doi.org/10.1016/S0141-1136(02)00153-8.

Gardner, A.M., Muturi, E.J., Allan, B.F., 2018. Discovery and exploitation of a natural ecological trap for a mosquito disease vector. Proceedings. Biological sciences/The Royal Society 285.

Georghiou, G.P., Wirth, M.C., 1997. Influence of exposure to single versus multiple toxins of *Bacillus thuringiensis* subsp. *israelensis* on development of resistance in the mosquito *Culex quinquefasciatus* (Diptera: Culicidae). Appl. Environ. Microbiol. 63, 1095–1101.

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*. Mosquito News 37, 355–361.

Goldman, I.F., Arnold, J., Carlton, B.C., 1986. Selection for resistance to *Bacillus thuringiensis* subspecies *israelensis* in field and laboratory populations of the mosquito *Aedes aegypti*. J. Invertebr. Pathol. 47, 317–324.

Govindarajan, M., Hoti, S.L., Benelli, G., 2016. Facile fabrication of eco-friendly nano-mosquitocides: biophysical characterization and effectiveness on neglected tropical mosquito vectors. Enzym. Microb. Technol. 95, 155–163.

Griffiths, B.S., Ritz, K., Bardgett, R.D., Cook, R., Christensen, S., Ekelund, F., et al., 2000. Ecosystem response of pasture soil communities to fumigation-induced microbial diversity reductions: an examination of the biodiversity ecosystem function relationship. Oikos 90, 279–294.

Grisolia, C.K., Oliveira-Filho, E.C., Ramos, F.R., Lopes, M.C., Muniz, D.H.F., Monnerat, R.G., 2009. Acute toxicity and cytotoxicity of Bacillus thuringiensis and Bacillus sphaericus strains on fish and mouse bone marrow. Ecotoxicology 18, 22 https://doi.org/10.1007/s10646-008-0252-7.

Guidi, V., Patocchi, N., Luethy, P., Tonolla, M., 2011. Distribution of *Bacillus thuringiensis* subsp *israelensis* in soil of a Swiss wetland reserve after 22 years of mosquito control. Appl. Environ. Microbiol. 77, 3663–3668.

Guillet, F., Mermet, L., 2013. L'expertise, composante essentielle mais insuffisante des stratégies pour la biodiversité: le cas de la démoustication en Camargue (France). Vertigo 13.

Günther, R., 1996. Die Amphibien und Reptilien Deutschlands. Gustav Fisher Verlag, Jena.

Gutierrez, Y., Ramos, G.S., Tome, H.V.V., Oliveira, E.E., Salaro, A.L., 2017. Bti-based insecticide enhances the predatory abilities of the backswimmer *Buenoa tarsalis* (Hemiptera: Notonectidae). Ecotoxicology 26, 1147–1155.

Hajaij, M., Carron, A., Deleuze, J., Gaven, B., Setier-Rio, M.L., Vigo, G., et al., 2005. Low persistence of *Bacillus thuringiensis* serovar *israelensis* spores in four mosquito biotopes of a salt marsh in southern France. Microb. Ecol. 50, 475–487.

Halasa, Y.A., Shepard, D.S., Fonseca, D.M., Farajollahi, A., Healy, S., Gaugler, R., et al., 2014. Quantifying the impact of mosquitoes on quality of life and enjoyment of yard and porch activities in New Jersey. PLoS One 9, e89221.

Hallberg, J., 2013. Myggen som folkhälsoproblem (in Swedish). . 23. Länstyrelsen i Gävleborg Gävle, Sweden.

Hallmann, C.A., Sorg, M., Jongejans, E., Siepel, H., Hofland, N., Schwan, H., et al., 2017. More than 75 percent decline over 27 years in total flying insect biomass in protected areas. PLoS One 12, e0185809.

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

Harvey, J.A., Heinen, R., Armbrecht, I., Basset, Y., Baxter-Gilbert, J.H., Bezemer, T.M., et al., 2020. International scientists formulate a roadmap for insect conservation and recovery. Nat Ecol Evol https://doi.org/10.1038/s41559-019-1079-8.

Hemingway, J., Ranson, H., 2000. Insecticide resistance in insect vectors of human disease. Annu. Rev. Entomol. 45, 371–391.

Hershey, A.E., Lima, A.R., Niemi, G.J., Regal, R.R., 1998. Effects of *Bacillus thuringiensis israelensis* (*BTI*) and methoprene on nontarget macroinvertebrates in Minnesota wetlands. Ecol. Appl. 8, 41–60.

Hoekman, D., Dreyer, J., Jackson, R.D., Townsend, P.A., Gratton, C., 2011. Lake to land subsidies: experimental addition of aquatic insects increases terrestrial arthropod densities. Ecology 92, 2063–2072.

Hurst, T.P., Kay, B.H., Ryan, P.A., Brown, M.D., 2007. Sublethal Effects of Mosquito Larvicides on Swimming Performance of Larvivorous Fish Melanotaenia duboulayi (Atheriniformes: Melanotaeniidae). J. Econ. Entomol. 100, 61–65 https://doi.org/10.1093/jee/100.1.61.

Imperatriz-Fonseca, V.L., Potts, S., Baste, I., Yeboah, A., Joly, C., Bartuska, A., et al., 2016. The Assessment Report of the Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services on Pollinators, Pollination and Food Production. Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services (IPBES).

Jackson, M.J., Gow, J.L., Evelyn, M.J., McMahon, T.J., Howay, T.J., Campbell, H., et al., 2012. An evaluation of the effectiveness of a commercial mechanical trap to reduce abundance of adult nuisance mosquito populations. J. Am. Mosq. Control Assoc. 28, 292–300.

Jakob, C., Poulin, B., 2016. Indirect effects of mosquito control using Bti on dragonflies and damselflies (Odonata) in the Camargue. Insect Conservation and Diversity 9, 161–169.

Janmaat, A.F., Myers, J., 2003. Rapid evolution and the cost of resistance to *Bacillus thuringiensis* in greenhouse populations of cabbage loopers, *Trichoplusia ni*. Proceedings of the Royal Society of London Series B-Biological Sciences 270, 2263–2270.

John, K.H., Stoll, J.R., Olson, J.K., 1987. An economic assessment of the benefits of mosquito abatement in an organized mosquito control district. J. Am. Mosq. Control Assoc. 3, 8–14.

John, K.H., Walsh, R.G., Moore, C.G., 1992. Comparison of alternative nonmarket valuation methods for an economic assessment of a public program. Ecol. Econ. 5, 179–196.

Johnson, B.J., Brosch, D., Christiansen, A., Wells, E., Wells, M., Bhandoola, A.F., et al., 2018. Neighbors help neighbors control urban mosquitoes. Sci. Rep. 8, 15797.

Junges, C.M., Maglianese, M.I., Lajmanovich, R.C., Peltzer, P.M., Attademo, A.M., 2017. Acute toxicity and etho-toxicity of three insecticides used for mosquito control on amphibian tadpoles. Water Air Soil Pollut. 228, 143.

Karch, S., Manzambi, Z.A., Salaun, J.J., 1991. Field trials with Vectolex (*Bacillus-sphaericus*) and Vectobac (*Bacillus-thuringiensis* (H-14)) against *Anopheles-gambiae* and *Culex-quinquefasciatus* breeding in Zaire. J. Am. Mosq. Control Assoc. 7, 176–179.

Kästel, A., Allgeier, S., Brühl, C.A., 2017. Decreasing *Bacillus thuringiensis israelensis* sensitivity of *Chironomus riparius* larvae with age indicates potential environmental risk for mosquito control. Sci. Rep. 7, 13565.

KEMI, 2015. Product assessment report related to product authorisation under regulation (EU) no 528/2012- VectoBac G and VectoBac GR. Kemikalieinspektionen Swedish Chemicals Agency.

Khawaled, K., Bendov, E., Zaritsky, A., Barak, Z., 1990. The fate of *Bacillus-thuringiensis* var *israelensis* in *Bacillus-thuringiensis* var *israelensis*-killed pupae of *Aedes-aegypti*. J. Invertebr. Pathol. 56, 312–316.

Lacey, L.A., 2007. *Bacillus thuringiensis* serovariety *israelensis* and *Bacillus sphaericus* for mosquito control. J. Am. Mosq. Control Assoc. 23, 133–163.

Lagadic, L., Roucaute, M., Caquet, T., 2014. Bti sprays do not adversely affect non-target aquatic invertebrates in French Atlantic coastal wetlands. J. Appl. Ecol. 51, 102–113.

Lagadic, L., Schafer, R.B., Roucaute, M., Szocs, E., Chouin, S., de Maupeou, J., et al., 2016. No association between the use of Bti for mosquito control and the dynamics of non-target aquatic invertebrates in French coastal and continental wetlands. Sci. Total Environ. 553, 486–494.

Lahondère, C., Vinauger, C., Okubo, R.P., Wolff, G.H., Chan, J.K., Akbari, O.S., et al., 2020. The olfactory basis of orchid pollination by mosquitoes. Proc. Natl. Acad. Sci. 117, 708–716.

Lajmanovich, R.C., Junges, C.M., Cabagna-Zenklusen, M.C., Attademo, A.M., Peltzer, P.M., Maglianese, M., et al., 2015. Toxicity of *Bacillus thuringiensis* var. *israelensis* in aqueous suspension on the South American common frog *Leptodactylus latrans* (Anura: Leptodactylidae) tadpoles. Environ. Res. 136, 205–212.

Langhans, S.D., Jähnig, S.C., Lago, M., Schmidt-Kloiber, A., Hein, T., 2019. The potential of ecosystem-based management to integrate biodiversity conservation and ecosystem service provision in aquatic ecosystems. Sci. Total Environ. 672, 1017–1020.

Leeper, D.A., Taylor, B.E., 1998. Insect emergence from a South Carolina (USA) temporary wetland pond, with emphasis on the Chironomidae (Diptera). J. N. Am. Benthol. Soc. 17, 54–72.

Liber, K., Schmude, K.L., Rau, D.M., 1998. Toxicity of Bacillus thuringiensis var. israelensis to Chironomids in pond mesocosms. Ecotoxicology 7, 343–354 (Ftxt: Full Text).

Lichtenberg, E., 1987. Integrated versus chemical pest management: the case of rice field mosquito control. J. Environ. Econ. Manag. 14, 304–312.

Lundstrom, J.O., Brodin, Y., Schafer, M.L., Vinnersten, T.Z.P., Ostman, O., 2010a. High species richness of *Chironomidae* (Diptera) in temporary flooded wetlands associated with high species turn-over rates. Bull. Entomol. Res. 100, 433–444.

Lundstrom, J.O., Schafer, 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. Bull. Entomol. Res. 100, 117–125.

Mace, G.M., Norris, K., Fitter, A.H., 2012. Biodiversity and ecosystem services: a multilayered relationship. Trends Ecol. Evol. 27, 19–26.

Majori, G., 2012. Short history of malaria and its eradication in Italy with short notes on the fight against the infection in the mediterranean basin. Mediterranean journal of hematology and infectious diseases 4, e2012016.

Margalit, J., Bobroglo, H., 1984. The effect of organic materials and solids in water on the persistence of *Bacillus-thuringiensis* var *israelensis* serotype-H-14. J. Appl. Entomol. 97, 516–520.

Medlock, J.M., Hansford, K.M., Schaffner, F., Versteirt, V., Hendrickx, G., Zeller, H., et al., 2012. A review of the invasive mosquitoes in Europe: ecology, public health risks, and control options. Vector-Borne and Zoonotic Diseases 12, 435–447.

Meyabeme Elono, A.L., Liess, M., Duquesne, S., 2010. Influence of competing and predatory invertebrate taxa on larval populations of mosquitoes in temporary ponds of wetland areas in Germany. Journal of vector ecology: journal of the Society for Vector Ecology 35, 419–427.

Mittal, P.K., Adak, T., Subbarao, S.K., 2005. Laboratory selection to investigate the development of resistance to *Bacillus thuringiensis* var. *israelensis* H-14 in *Culex quinquefasciatus Say* (Diptera: Culicidae). National Academy Science Letters-India 28, 281–283.

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

Mulla, M.S., Chaney, J.D., Rodchareon, J., 1993. Elevated dosages of *Bacillus thuringiensis* var. *israelensis* fail to extend control of *Culex* larvae. Bulletin of Society For Vector Ecology 18, 125–132.

Mulla, M.S., Thavara, U., Tawatsin, A., Chompoosri, J., 2004. Procedures for the evaluation of field efficacy of slow-release formulations of larvicides against *Aedes aegypti* in water-storage containers. J. Am. Mosq. Control Assoc. 20, 64–73.

N'Guessan, R., Asidi, A., Boko, P., Odjo, A., Akogbeto, M., Pigeon, O., et al., 2010. An experimental hut evaluation of PermaNet((R)) 3.0, a deltamethrin-piperonyl butoxide combination net, against pyrethroid-resistant Anopheles gambiae and *Culex quinquefasciatus* mosquitoes in southern Benin. Trans. R. Soc. Trop. Med. Hyg. 104, 758–765.

Niemi, G.J., Hershey, A.E., Shannon, L., Hanowski, J.M., Lima, A., Axler, R.P., et al., 1999. Ecological effects of mosquito control on zooplankton, insects, and birds. Environ. Toxicol. Chem. 18, 549–559.

OECD, 2011. Test No. 235: Chironomus sp., Acute Immobilisation Test.

Ohana, B., Margalit, J., Barak, Z., 1987. Fate of *Bacillus-thuringiensis* subsp *israelensis* under simulated field conditions. Appl. Environ. Microbiol. 53, 828–831.

Olmo, C., Marco, A., Armengol, X., Ortells, R., 2016. Effects of *Bacillus thuringiensis* var. *israelensis* on nonstandard microcrustacean species isolated from field zooplankton communities. Ecotoxicology 25, 1730–1738.

Ostman, O., Lundstrom, J.O., Vinnersten, T.Z.P., 2008. Effects of mosquito larvae removal with *Bacillus thuringiensis israelensis* (*Bti*) on natural protozoan communities. Hydrobiologia 607, 231–235.

Pardo-Lopez, L., Soberon, M., Bravo, A., 2013. *Bacillus thuringiensis* insecticidal three-domain cry toxins: mode of action, insect resistance and consequences for crop protection. FEMS Microbiol. Rev. 37, 3–22.

Paris, M., David, J.P., Despres, L., 2011a. Fitness costs of resistance to *Bti* toxins in the dengue vector *Aedes aegypti*. Ecotoxicology 20, 1184–1194.

Paris, M., Tetreau, G., Laurent, F., Lelu, M., Despres, L., David, J.-P., 2011b. Persistence of *Bacillus thuringiensis israelensis* (*Bti*) in the environment induces resistance to multiple *Bti* toxins in mosquitoes. Pest Manag. Sci. 67, 122–128.

Park, B.S., Choi, W.S., Kim, J.H., Kim, K.H., Lee, S.E., 2005. Monoterpenes from thyme (*Thymus vulgaris*) as potential mosquito repellents. J. Am. Mosq. Control Assoc. 21, 80–83.

Parrinello, G., Bécot, R., 2019. Regional planning and the environmental impact of coastal tourism: the Mission Racine for the redevelopment of Languedoc-Roussillon's Littoral. Humanities 8, 13.

Patil, C., Calvayrac, C., Zhou, Y., Romdhane, S., Salvia, M.V., Cooper, J.F., et al., 2016. Environmental metabolic footprinting: a novel application to study the impact of a natural and a synthetic beta-triketone herbicide in soil. Sci. Total Environ. 566-567, 552–558.

Paul, A., Harrington, L.C., Zhang, L., Scott, J.G., 2005. Insecticide resistance in *Culex pipiens* from New York. J. Am. Mosq. Control Assoc. 21, 305–309.

Pauley, L.R., Earl, J.E., Semlitsch, R.D., 2015. Ecological Effects and Human Use of Commercial Mosquito Insecticides in Aquatic Communities. vol 49. BIOONE.

Paulov, S., 1985. Interactions of *Bacillus thuringiensis* var. *israelensis* with developmental stages of amphibians (*Rana temporaria*). Biologia (Bratislava) 40, 133–138.

Peach, D.A.H., Gries, G., 2016. Nectar thieves or invited pollinators? A case study of tansy flowers and common house mosquitoes. Arthropod Plant Interact. 10, 497–506.

Pfitzner, W.P., Beck, M., Weitzel, T., Becker, N., 2015. The role of mosquitoes in the diet of adult dragon and damselflies (Odonata). J. Am. Mosq. Control Assoc. 31, 187–189.

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. J. Econ. Entomol. 92, 266–272 https://doi.org/10.1093/jee/92.2.266.

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

Poulin, B., Lefebvre, G., 2016. Perturbation and delayed recovery of the reed invertebrate assemblage in Camargue marshes sprayed with *Bacillus thuringiensis israelensis*. Insect Sci 25, 542–548.

Poulin, B., Lefebvre, G., Mauchamp, A., 2002. Habitat requirements of passerines and reedbed management in southern France. Biol. Conserv. 107, 315–325.

Poulin, B., Lefebvre, G., Paz, L., 2010. Red flag for green spray: adverse trophic effects of *Bti* on breeding birds. J. Appl. Ecol. 47, 884–889.

Poulin, B., Lefebvre, G., Muranyi-Kovacs, C., Hilaire, S., 2017. Mosquito traps: an innovative, environmentally friendly technique to control mosquitoes. Int. J. Environ. Res. Public Health 14.

Powney, G.D., Carvell, C., Edwards, M., Morris, R.K.A., Roy, H.E., Woodcock, B.A., et al., 2019. Widespread losses of pollinating insects in Britain. Nat. Commun. 10, 1018.

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

Quirino, B.A., Carniatto, N., Guglielmetti, R., Fugi, R., 2017. Changes in diet and niche breadth of a small fish species in response to the flood pulse in a Neotropical floodplain lake. Limnologica 62, 126–131.

Raymond, B., Johnston, P.R., Nielsen-LeRoux, C., Lereclus, D., Crickmore, N., 2010. *Bacillus thuringiensis*: an impotent pathogen? Trends Microbiol. 18, 189–194.

Ritchie, S.A., Rapley, L.P., Benjamin, S., 2010. *Bacillus thuringiensis* var. *israelensis* (*Bti*) provides residual control of *Aedes aegypti* in small containers. Am J Trop Med Hyg 82, 1053–1059.

Rukmini, V., Reddy, C.Y., Venkateswerlu, G., 2000. *Bacillus thuringiensis* crystal delta-endotoxin: role of proteases in the conversion of protoxin to toxin. Biochimie 82, 109–116.

Russell, T., Kay, B., Skilleter, G., 2009. Environmental effects of mosquito insecticides on saltmarsh invertebrate fauna. Aquat. Biol. 6, 77–90 https://doi.org/10.3354/ab00156.

Saleh, M.S., El-Meniawi, F.A., Kelada, N.L., Zahran, H.M., 2003. Resistance development in mosquito larvae *Culex pipiens* to the bacterial agent *Bacillus thuringiensis* var. *israelensis*. Journal of Applied Entomology-Zeitschrift Fur Angewandte Entomologie 127, 29–32.

Salvia, M.V., Ben Jrad, A., Raviglione, D., Zhou, Y., Bertrand, C., 2018. Environmental Metabolic Footprinting (EMF) vs. half-life: a new and integrative proxy for the discrimination between control and pesticides exposed sediments in order to further characterise pesticides' environmental impact. Environ. Sci. Pollut. Res. Int. 25, 29841–29847.

Schäfer, M.L., Lundström, J.O., 2014. Efficiency of *Bti*-based floodwater mosquito control in Sweden – four examples. Journal of the European Mosquito Control Association 32.

Schafer, M.L., Lundstrom, J.O., Petersson, E., 2008. Comparison of mosquito (Diptera: Culicidae) populations by wetland type and year in the lower river Dalalven region, Central Sweden. Journal of vector ecology: journal of the Society for Vector Ecology 33, 150–157.

Schneider, S., Tajrin, T., Lundström, J.O., Hendriksen, N.B., Melin, P., Sundh, I., 2017. Do Multi-year Applications of Bacillus thuringiensis subsp. israelensis for Control of Mosquito Larvae Affect the Abundance of B. cereus Group Populations in Riparian Wetland Soils? Microb. Ecol., 1–9 https://doi.org/10.1007/s00248-017-1004-0.

Schrauth, F., Wink, M., 2018. Changes in species composition of birds and declining number of breeding territories over 40 years in a nature conservation area in Southwest Germany. Diversity 10, 97.

Schulz, R., Bundschuh, M., Gergs, R., Brühl, C.A., Diehl, D., Entling, M.H., et al., 2015. Review on environmental alterations propagating from aquatic to terrestrial ecosystems. Sci. Total Environ. 538, 246–261.

Schwarz, N., Moretti, M., Bugalho, M.N., Davies, Z.G., Haase, D., Hack, J., et al., 2017. Understanding biodiversity-ecosystem service relationships in urban areas: a comprehensive literature review. Ecosystem Services 27, 161–171.

Schweizer, M., Miksch, L., Köhler, H.-R., Triebskorn, R., 2019. Does *Bti* (*Bacillus thuringiensis* var. *israelensis*) affect *Rana temporaria* tadpoles? Ecotoxicol. Environ. Saf. 181, 121–129.

Semmler, M., Abdel-Ghaffar, F., Al-Rasheid, K., Mehlhorn, H., 2009. Nature helps: from research to products against blood-sucking arthropods. Parasitol. Res. 105, 1483–1487.

Shaalan, E.A., Canyon, D.V., 2009. Aquatic insect predators and mosquito control. Trop. Biomed. 26, 223–261.

Sharma, V.P., 2001. Health hazards of mosquito repellents and safe alternatives. Curr. Sci. 80, 341–343.

Sheeran, W., Fisher, S.W., 1992. The effects of agitation, sediment, and competition on the persistence and efficacy of *Bacillus-thuringiensis* var *israelensis* (*Bti*). Ecotoxicol. Environ. Saf. 24, 338–346.

Soberon, M., Lopez-Diaz, J.A., Bravo, A., 2013. Cyt toxins produced by *Bacillus thuringiensis*: a protein fold conserved in several pathogenic microorganisms. Peptides 41, 87–93.

Soumare, M.K., Cilek, J.E., 2011. The effectiveness of *Mesocyclops longisetus* (Copepoda) for the control of container-inhabiting mosquitoes in residential environments. J. Am. Mosq. Control Assoc. 27, 376–383.

Soutukorva, A., Johansson, K., Hasselström, L., Cole, S., Remvig, H., Kristöm, B., 2013. Samhällsekonomisk analys av myggproblemets kostnader (in Swedish). 16. Länsstyrelsen i Gävleborg, Gävle, Sweden.

Srivastava, R., Bhalwar, R., Tilak, V.W., 1998. A mathematical model for predicting the persistence and efficacy of *Bacillus thuringiensis* var. *israelensis* in relation to water pollution. Medical Journal Armed Forces India 54, 107–110.

Stalinski, R., Tetreau, G., Gaude, T., Despres, L., 2014. Pre-selecting resistance against individual *Bti* Cry toxins facilitates the development of resistance to the *Bti* toxins cocktail. J. Invertebr. Pathol. 119, 50–53.

Stapleton, D.H., 2004. Lessons of history? Anti-malaria strategies of the International Health Board and the Rockefeller Foundation from the 1920s to the era of DDT. Public Health Rep. 119, 206–215.

Succo, T., Leparc-Goffart, I., Ferre, J.B., Roiz, D., Broche, B., Maquart, M., et al., 2016. Autochthonous dengue outbreak in Nimes, South of France, July to September 2015. Euro surveillance: bulletin Europeen sur les maladies transmissibles = European communicable disease bulletin 21.

Sukumar, K., Perich, M.J., Boobar, L.R., 1991. Botanical derivatives in mosquito control: a review. J. Am. Mosq. Control Assoc. 7, 210–237.

Sullivan, C.M., Shiel, C.B., McAney, C.M., Fairley, J.S., 1993. Analysis of the diets of Leisler's *Nyctalus leisleri*, Daubenton's *Myotis daubentoni* and pipistrelle *Pipistrellus pipistrellus* bats in Ireland. J. Zool. 231, 656–663.

Tabashnik, B.E., Van Rensburg, J.B.J., Carriere, Y., 2009. Field-evolved insect resistance to *Bt* crops: definition, theory, and data. J. Econ. Entomol. 102, 2011–2025.

Tetreau, G., Alessi, M., Veyrenc, S., Périgon, S., David, J.P., Reynaud, S., et al., 2012a. Fate of *Bacillus thuringiensis* subsp. *israelensis* in the field: evidence for spore recycling and differential persistence of toxins in leaf litter. Appl. Environ. Microbiol. 78, 8362–8367.

Tetreau, G., Bayyareddy, K., Jones, C.M., Stalinski, R., Riaz, M.A., Paris, M., et al., 2012b. Larval midgut modifications associated with *Bti* resistance in the yellow fever mosquito using proteomic and transcriptomic approaches. BMC Genomics 13.

Tetreau, G., Stalinski, R., Kersusan, D., Veyrenc, S., David, J.P., Reynaud, S., et al., 2012c. Decreased toxicity of *Bacillus thuringiensis* subsp. *israelensis* to mosquito larvae after contact with leaf litter. Appl. Environ. Microbiol. 78, 5189–5195.

Tetreau, G., Banneville, A.S., Andreeva, E.A., Brewster, A.S., Hunter, M.S., Sierra, R.G., Teulon, J.M., Young, I.D., Burke, N., Gruenewald, T., Beaudouin, J., Snigireva, I., Fernandez-Luna, M.T., Burt, A., Park, H.W., Signor, L., Bafna, J.A., Sadir, R., Fenel, D., Boeri-Erba, E., Bacia, M., Zala, N., Laporte, F., Després, L., Weik, M., Boutet, S., Rosenthal, M., Coquelle, N., Burghammer, M., Cascio, D., Sawaya, M.R., Winterhalter, M., Gratton, E., Gutsche, I., Federici, B., Pellequer, J.L., Sauter, N.K., Colletier, J.P., 2020. Serial femtosecond crystallography on in vivo-grown crystals drives elucidation of mosquitocidal Cyt1Aa bioactivation cascade. Nat. Commun. 11, 1153.

Theissinger, K., Kästel, A., Elbrecht, V., Makkonen, J., Michiels, S., Schmidt, S.I., et al., 2018. Using DNA metabarcoding for assessing chironomid diversity and community change in mosquito controlled temporary wetlands. Metabarcoding and Metagenomics 2.

Theissinger, K., Roder, N., Allgeier, S., Beermann, A.J., Bruhl, C.A., Friedrich, A., et al., 2019. Mosquito control actions affect chironomid diversity in temporary wetlands of the Upper Rhine Valley. Mol. Ecol. 28, 4300–4316.

Tilquin, M., Paris, M., Reynaud, S., Despres, L., Ravanel, P., Geremia, R.A., et al., 2008. Long lasting persistence of *Bacillus thuringiensis* subsp. *israelensis* (*Bti*) in mosquito natural habitats. PLoS One 3, e3432.

Timmermann, U., Becker, N., 2017. Impact of routine *Bacillus thuringiensis israelensis* (Bti) treatment on the availability of flying insects as prey for aerial feeding predators. Bull. Entomol. Res. 107, 705–714.

Tousignant, M.E., Boisvert, J.L., Chalifour, A., 1993. Loss of Bacillus-thuringiensis var israelensis larvicidal activity and its distribution in benthic substrates and hyporheic zone of streams. Can. J. Fish. Aquat. Sci. 50, 443–451.

Tranfield, D., Denyer, D., Smart, P., 2003. Towards a methodology for developing evidence-informed management knowledge by means of systematic review. Br. J. Manag. 14, 202–222.

Vachon, V., Laprade, R., Schwartz, J.L., 2012. Current models of the mode of action of *Bacillus thuringiensis* insecticidal crystal proteins: a critical review. J. Invertebr. Pathol. 111, 1–12.

van den Berg, H., Velayudhan, R., Ebol, A., Catbagan Jr., B.H.G., Turingan, R., Tuso, M., et al., 2012. Operational efficiency and sustainability of vector control of malaria and dengue: descriptive case studies from the Philippines. Malar. J. 11, 269.

van den Berg, H., Yadav, R.S., Zaim, M., 2015. Setting international standards for the management of public health pesticides. PLoS Med. 12, e1001824.

Vaughan, I.P., Newberry, C., Hall, D.J., Liggett, J.S., Ormerod, S.J., 2008. Evaluating large-scale effects of *Bacillus thuringiensis* var. *israelensis* on non-biting midges (*Chironomidae*) in a eutrophic urban lake. Freshw. Biol. 53, 2117–2128.

Vaughan, N., 1997. The diets of British bats (Chiroptera). Mammal Rev. 27, 77–94.

Vega, Rúa A., Okech, B.A., 2019. The spread of mosquito-borne diseases: A major and global public health problem. In: Picimbon, J.-F. (Ed.), Olfactory Concepts of Insect Control-Alternative to Insecticides: Volume 1. Springer International Publishing, Cham, pp. 1–27.

Vighi, M., Rico, A., 2018. The concept of resilience in ecological risk assessment: scientific and regulatory issues. Integr. Environ. Assess. Manag. 14, 581–585.

Vilarinhos, P.T.R., Monnerat, R., 2004. Larvicidal persistence of formulations of *Bacillus thuringiensis* var. *israelensis* to control larval *Aedes aegypti*. J. Am. Mosq. Control Assoc. 20, 311–314.

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

Vinnersten, T.Z.P., Lundstrom, J.O., Schafer, 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. Bull. Entomol. Res. 100, 715–725.

von Hirsch, H., Becker, N., 2009. Cost-benefit analysis of mosquito control operations based on microbial control agents in the upper Rhine valley (Germany). European Mosquito Bulletin 27, 47–55.

Weltje, L., Rufli, H., Heimbach, F., Wheeler, J., Vervliet-Scheebaum, M., Hamer, M., 2010. The chironomid acute toxicity test: development of a new test system. Integr. Environ. Assess. Manag. 6, 301–307.

Westerberg, V.H., Lifran, R., Olsen, S.B., 2010. To restore or not? A valuation of social and ecological functions of the Marais des Baux wetland in southern France. Ecol. Econ. 69, 2383–2393.

WHO, 1999, Microbial pest control agent: Bacillus thuringiensis; environmental health criteria 217.

WHO, 2005. Guidelines for Laboratory and Field Testing of Mosquito Larvicides. World Health Organization, Geneva.

Williams, D.D., 2006. The Biology of Temporary Waters.

Wirth, M.C., Jiannino, J.A., Federici, B.A., Walton, W.E., 2004. Synergy between toxins of *Bacillus thuringiensis* subsp *israelensis* and *Bacillus sphaericus*. J. Med. Entomol. 41, 935–941.

Wirth, M.C., Park, H.W., Walton, W.E., Federici, B.A., 2005. Cyt1A of *Bacillus thuringiensis* delays evolution of resistance to Cry11A in the mosquito *Culex quinquefasciatus*. Appl. Environ. Microbiol. 71, 185–189.
Wirth, M.C., Walton, W.E., Federici, B.A., 2010. Inheritance patterns, dominance, stability, and Allelism of insecticide resistance and cross-resistance in two colonies of *Culex quinquefasciatus* (Diptera: Culicidae) selected with cry toxins from *Bacillus thuringiensis* subsp. *israelensis*. J. Med. Entomol. 47, 814–822.
Wirth, M.C., Walton, W.E., Federici, B.A., 2012. Inheritance, stability, and dominance of cry resistance in *Culex quinquefasciatus* (Diptera: Culicidae) selected with the three toxins of *Bacillus thuringiensis* subsp. *israelensis*. J. Med. Entomol. 49, 886–894.
Wolfram, G., Wenzl, P., Jerrentrup, H., 2018. A multi-year study following BACI design reveals no short-term impact of Bti on chironomids (Diptera) in a floodplain in Eastern Austria. Environ. Monit. Assess. 190, 709.
Wraight, S.P., Molloy, D.P., Jamnback, H., McCoy, P., 1981. Effects of temperature and Instar on the efficacy of *Bacillus thuringiensis* var. *israelensis* and *Bacillus sphaericus* strain 1593 against *Aedes stimulans* larvae. J. Invertebr. Pathol. 38, 78–87.
Yang, Q., Tang, S., Rang, J., Zuo, M., Ding, X., Sun, Y., et al., 2015. Detection of toxin proteins from *Bacillus thuringiensis* strain 4.0718 by strategy of 2D-LC-MS/MS. Curr. Microbiol. 70, 457–463.
Zhao, X., Smith, D.L., Tatem, A.J., 2016. Exploring the spatiotemporal drivers of malaria elimination in Europe. Malar. J. 15, 122.