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Environmental Safety Review of Methoprene and Other Biorational Materials for Sustained Mosquito Control

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HIGHLIGHTS:

Sustained, biorational mosquito control protects public and environmental health.

Methoprene levels for mosquito control are low (≤10 ppb) and its motility is limited.

Methoprene is a low-risk larvicide; brief loss of small invertebrates is possible.

Sustained-release methoprene or Bti + Bs bacteria are effective, ecologically safe.

ABSTRACT

Several pesticides are applied directly to aquatic systems to control mosquitoes and thereby the pathogens they vector to humans and wildlife. Few biorational materials are available for sustained control larval mosquitoes in heavily organic waters (e.g., catchment basins, water treatment facilities, septic systems) and other habitats. These include the insect juvenile hormone mimic methoprene and several bacterially-derived larvicides. Health agencies, the public and environmental groups have debated methoprene's use because some studies have shown toxic effects on non-target organisms. However, data from other studies have demonstrated its apparent environmental safety. This review evaluates studies pertinent to the environmental safety of using methoprene to control mosquito larvae, and provides briefer assessments of bacterial larvicides that are used for sustained control of mosquitoes, especially in organically rich waters. First, the review outlines the ecological and health effects of mosquitoes, describes methoprene's mobility in soil and water, and distinguishes between laboratory toxicity and environmental effects. The article then interprets non-target toxicity findings in light of measured environmental concentrations of methoprene (as used in mosquito control) and field studies of its non-target effects. The final section reviews bacterially-derived formulations for sustained mosquito control. Results show that environmental concentrations of methoprene are usually 2-5 ppb (range 2-45 ppb) and that its motility is limited. These levels are not toxic to the vast majority of vertebrates and invertebrates tested in laboratories, except for a few species of crustacean larvae, zooplankton and small Diptera. However, studies in natural habitats have not documented population reductions except in small Diptera. Larvicides derived from the bacteria *Bacillus thuringiensis israelensis*, *Lysinibacillus sphaericus* and *Saccharopolyspora spinosa* showed similarly limited environmental effects, although recent studies in mesocosms and temporary pools showed broader effects on insects for the latter. These findings should be useful to a variety of stakeholders in informing decisions on larvicide use to protect public and environmental health.

INTRODUCTION

Mosquito abatement is needed because mosquito attacks and mosquito-borne diseases are detrimental to the health and well-being of humans, livestock, and wildlife (reviews: Foster and Walker 2009, Rey et al. 2012). The problem of vector-borne diseases and their control inspired the 'One Health' movement that seeks to simultaneously strengthen the health of humans, other taxa, and ecosystem functions (Cook et al. 2004, American Veterinary Medical Association 2008). The use of insecticides to control larval mosquitoes (larvicides) in aquatic systems is widespread due to the public health risks posed by mosquitoes. However, larvicide use is sometimes contentious due to the potential for non-target effects. Various stakeholders may debate which materials should be applied, including the public, vector control districts, environmental protection agencies, and public health agencies.

Larvicides can be categorized as biorational materials that are narrow-spectrum, affecting limited taxa and sometimes only immature stages (e.g., some bacterial toxins and insect growth regulators, a.k.a. biopesticides, Leahy et al. 2014), versus broad-spectrum insecticides that have toxic effects on most insects (e.g., pyrethroids, organophosphates). Biorational materials are important not only for environmental protection, but also because their use avoids harm to the beneficial predators that contribute greatly to mosquito control in many habitats. The goal of this review is to consolidate knowledge about the non-target effects and efficacy of biorational insecticides that offer sustained control of larval mosquitoes in organically-enriched waters. While the emphasis is on eutrophic waters because these are particularly problematic as mosquito sources, the non-target information also applies to other habitats where mosquitoes breed. Biopesticides (=biorational materials) are regarded by the United States Environmental Protection Agency (EPA) and others as of lower potential environmental impact than broad-spectrum materials, but there can still be controversy over their use, or choices within this category.

Only a few biorational materials are available for sustained control of larval mosquitoes in heavily organic waters such as catchment basins, water treatment facilities, and septic systems. These include methoprene, an insect juvenile hormone mimic, and toxins or mixtures of toxins contained in the bacterial taxa *Bacillus thuringiensis israelensis* (Bti), *Lysinibacillus sphaericus , or* Ls, (until recently known *as Bacilluls sphaericus* or Bs), and *Saccharopolyspora spinose* (spinosad). The bulk of this review focusses on methoprene, because it is both widely used and often more scrutinized due to studies that have shown that it can have toxic effects on some non-target animals. I provide briefer reviews of the bacteria-based methods, because Bti and Ls have been the subjects of recent reviews (e.g. Boisvert and Boisvert 2000, Lacey 2007), and there is less information available on spinosad due to its more recent registration as a larvicide. As background the review begins by distinguishing between laboratory toxicity and the environmental effects of pesticides, and it outlines the environmental and health effects of mosquitoes. I then review literature on the potential non-target effects of methoprene, beginning with a description of methoprene and its mobility in soil and water. This information is integrated with research on the toxicity of the specific application rates of methoprene used in mosquito control, expected environmental concentrations, and field research on methoprene's non-target effects. The final section discusses the bacterially-based alternatives for sustained mosquito control in organically rich environments.

Toxicity versus environmental effects

When evaluating the environmental safety of any compound, it is important to keep in mind the distinction between toxicity and environmental effects as these can be very different. The toxicity of a pesticide is defined by how much of it is required to cause morbidity and mortality when applied directly to an organism of interest. For aquatic organisms, the material is often added to its water container. Toxicity tests are often aimed at finding how much of a pesticide is necessary to produce a toxic effect or to understand possible physiological pathways for effects, whether or not the pesticide is expected to be applied at all levels used in an experiment. Many websites report that a variety of substances are 'toxic' without providing context about whether rates used in the laboratory are applied in the environment or whether laboratory toxicity is predictive of environmental effects. Some website users may assume that effects in the laboratory and the environment are equivalent.

Laboratory toxicity results may either over-estimate or under-estimate the environmental effects of a pesticide application (review: Sparling 2016). Pesticides sometimes break down more rapidly in the field than in the laboratory through the action of factors such as sunlight, environmental chemistry or microbial activity, or they may become less biologically available by adsorbing to materials. However, cold or dark environmental conditions could also sustain toxicity longer than is typical of lab tests. Some individual organisms may encounter more or less of the pesticide than expected depending on their location in the environment and their behavior. Other stressors in the environment could exacerbate the effects of a pesticide. Negative or positive indirect effects are also possible, for example a predator that is insensitive to a pesticide could be affected if a major prey type is depleted by the pesticide. Conversely, if the pest was a strong competitor or predator, its partners in those interactions could benefit. Therefore, when laboratory toxicity tests raise initial concerns about environmental safety, these should ideally be addressed by field experiments, environmental monitoring, and environmental modeling studies to determine actual environmental effects. Therefore this review puts toxicity results into a broader environmental context wherever possible.

Ecological and health effects of mosquitoes

Like non-target organisms, mosquitoes are a natural part of many terrestrial and aquatic ecosystems. They can occur in nearly all habitats except for the open ocean and permanently frozen environments (Wallace and Walker 2008). Mosquito biologists are sometimes asked about the ecological roles of mosquitoes and what might happen to food webs due to reducing mosquito abundances. The ecosystem effect of suppressing a species depends on whether its functions are distinct or redundant with other taxa in the community, taking into account the various roles it fills across both space and time (Loreau and De Manzancourt 2013). If a species filling an ecological role is either dominant or unique, larger effects of suppressing it are likely than if it has low abundance and its functions are redundant with other taxa (Hillebrand et al. 2008).

As larvae, most mosquito taxa filter or collect fine particulate organic material, some scrape at biofilms, and a few are predators of small invertebrates (Wallace and Walker 2008, Becker et al. 2010). Numerous other aquatic invertebrates share these roles, (Merritt et al. 2008), although mosquitoes occasionally dominate ephemeral habitats where aquatic predators are scarce, such as high salt marsh, puddles, and other floodwaters. Mosquito larvae are one of the food resources for generalist aquatic predators, but few if any predators specialize on mosquitoes. Even mosquitofish (Gambusia spp.), which are sometimes said to be specialists, consume many other types of prey (Blaustein 1992, Swanson et al. 1996). This allows them to maintain populations in habitats when mosquitoes are rare or absent.

As adults, females of many (but not all) species feed on animal blood. Females as well as males feed on plant fluids, especially nectar, and these may play a role in pollination (Becker et al.

2010, Wallace and Walker 2008). However, it is unclear if any plants have mosquitoes as a main pollinator, and mosquitoes might deplete nectar without effective pollination (Inouye 2010). Adults can be prey for a variety of terrestrial generalist predators (e.g., spiders, birds, bats). In some areas, mosquitoes periodically become very abundant and may represent an appreciable fraction of a predator's diet (e.g., salt marshes and arctic meltwaters). However, the prevailing view is that few if any predators are dependent on mosquitoes (Fang 2010). Both larvae and adults support generalist and specialist parasites and pathogens, and the role of adults in transmitting disease-causing organisms is well-known (Becker et al. 2010). It is primarily due to their role as vectors that the impact of mosquito control is difficult to project, because pathogens can regulate animal populations. While the literature has shown negative ecological effects of some pesticides used to control mosquitoes (generally older pesticides like organochlorines), there seem to be few if any papers demonstrating negative effects of decreased mosquito abundances *per se*.

Although few environmental benefits have been documented for mosquitoes, their environmental harms are well-known. Most female mosquitoes feed on the blood of one or more species to fuel egg development. Many take repeated blood meals, enabling them to vector pathogens between animals, including humans. This promote epidemics and epizootics of various diseases caused by viruses, protists, nematodes and more, causing morbidity and mortality in humans, domestic animals, and wildlife (reviews: Beaty and Marquardt 1996, Foster and Walker 2009). Examples of such pathogens include viruses that can cause encephalitis in humans, horses, birds and other animals, outbreaks of avian and human malaria, and nematodes that cause diseases of the cardiac and lymphatic systems in wildlife and humans (reviews: Wheeler et al., 2009, Foster and Walker 2009, Becker et al. 2010, Huang et al. 2013, CDC 2013). In addition to vectoring pathogens, mosquito problems may also affect the economy by discouraging recreation, tourism, and outdoor labor (Lawler and Lanzaro 2005).

Ideally, mosquito abatement programs will balance the protection of public and animal health with environmental protection, and promote sustainability of control methods via managing mosquito resistance to pesticides. Some mosquito populations have evolved such resistance where the same material was used to control them over a wide area and for many successive generations (review: Hemingway and Ranson 2000, Coleman and Hemingway 2007). Resistance can usually be forestalled by judicious alternation of control techniques or by combining insecticides (Coleman and Hemingway 2007). It is thus necessary to have several control options.

Sustained control of mosquitoes is desirable in organically-enriched environments that are highly productive of mosquitoes. In addition to tidal wetlands, these include catchment basins, wastewater ponds and wetlands, and improperly sealed septic systems, because these habitats have abundant organic resources and often, few predators (e.g., Lawler and Lanzaro 2005, Barrerra et al. 2008, Becker et al. 2010; review: Rey et al. 2012). Thus they can produce large numbers of mosquitoes if control lapses. Even worse, such habitats are usually situated in or near human settlements and livestock areas. Sustained-release control formulations can provide durable control and reduce abatement costs associated with frequent visits to the same sites. Therefore this review focuses on biorational materials expected to yield longer-term control.

METHODS

I found relevant scientific literature on methoprene by doing comprehensive Web of Science and Google Scholar searches for scientific articles under the term 'methoprene' and excluding patent applications. This yielded over 1,700 publications from 1974 -2015. Titles were individually screened to select all studies of mosquito larvicides that measured levels of methoprene released from pellets and briquettes, studies evaluating whether methoprene is toxic to non-target organisms, and field studies of whether methoprene has environmental effects. In addition, I did a Google search on 'methoprene' to find safety reports from the EPA, universities and similar academic and/or government research organizations. I excluded tests on methoprene in which it was applied solely at rates over 100X greater than in mosquito control due to low relevance to mosquito control applications; many of these were studies of animal physiology. I also excluded tests showing the apparent safety of acute dosages of methoprene (48 h follow-up or less) because methoprene is not expected to have a rapid effect. It acts by disrupting arthropod developmental pathways, therefore an absence of short term effects is misleading in terms of safety. EPA (2001) includes summaries of many of these studies.

To ease comparisons among methoprene concentrations used in the studies reviewed, I converted all units to parts per billion (ppb) or parts per million (ppm). Throughout, non-target effects of methoprene are compared to expected environmental levels from sustained-release briquette or pellet formulations of methoprene for mosquito control.

The briefer evaluations of Bti- and Bs-based larvicides summarize the conclusions from very comprehensive reviews (e.g., Boisvert and Boisvert 2000, Lacey 2007), and I also discuss more recent papers, especially if they showed contrasting results. The section on spinosad-based materials summarizes the findings of Hertlein (2010) and further reviews all available later publications on the efficacy and non-target effects of spinosad as it is used in mosquito control. I identified later papers by searching Web of Science by the scientific names of each material plus ‘mosquito’ and/or ‘non-target’. For this section I retained papers reporting efficacy and non-target effects at 24 and 48 hrs or more, because bacterial toxins can show toxic effects at shorter exposure durations than methoprene.

RESULTS AND DISCUSSION

Methoprene’s uses, and its persistence and expected amounts in mosquito abatement

Henrick (2007) and Ramaseshadri et al. (2012) provide comprehensive reviews of the development of methoprene and other juvenile hormone analogs as safer alternatives to neurotoxic insecticides (such as carbamates and organophosphates). Methoprene is a molecule that closely resembles insect juvenile hormone. Its full chemical name is 1-methylethyl (E,E)-11-methoxy-3,7,11-trimethyl- 2,4-dodecadienoate (Merck Index, 11th Edition). When used as a pesticide, methoprene acts by disrupting the molting cycle of some insects and other arthropods. It causes mosquitoes to die at the pupal stage when they cannot molt into adults.

Methoprene is widely applied to aquatic habitats to control larval mosquitoes, and in other formulations it is also commonly used to control fleas, mites, and flies (review: Struger et al. 2007). It is applied directly to many pets, livestock animals (including through the diet), and sometimes to habitations. Domestic and agricultural applications may at times exceed concentrations used in mosquito control. Methoprene can be applied directly to wetlands and waterways to control mosquitoes. Improper rinsing or disposal of containers or washing treated pets and livestock could contribute some methoprene to natural waters or to wastewater treatment sites.

According to the EPA fact sheet on methoprene (EPA 2001), it is ‘not expected to persist in soil or contaminate ground water’ because it degrades rapidly and binds to particles so that it does not leach. A California Department of Pesticide Regulation review reached the same conclusions (Csondes 2004). Methoprene and its breakdown products may be present in treated waters for up to a several weeks if liquid is used. If sustained-release pellets or ingots/briquettes are employed it will be present during and sometimes beyond the operational release period of 30 – 150 days. Sustained-release formulations may have longer than expected activity (beyond a year) (e.g., Lawler et al. 2000), especially in environments that are protected from sunlight (Ramaseshadri et al. 2012). Some reported half-lives of methoprene are 30 hrs in clean water and 60-70 hours in sewage (Csondes 2004). However, there have also been occasional reports of faster degradation of methoprene in brackish and polluted environments (review: Ramaseshadri et al. 2012). Degradation of methoprene and its metabolites is usually rapid in surface soils, with a half-life of approximately 10 days (Schooley et al. 1975).

Sustained-release methoprene products used for mosquito control are designed to produce low levels of methoprene in water (approximately 10 ppb or less, Ross et al. 1994), for 30 days (pellets) or 150 days (briquettes). The EPA Fact Sheet on methoprene products for mosquito control stated that data submitted to them showed that sustained-release products resulted in measured water concentrations less than or equal to 4 ppb, less than the manufacturer’s expected amount of 10 ppb (EPA 2001).

Field studies of mosquito treatments added directly to water since the 2001 EPA review found variable amounts of methoprene, but most reported low levels of methoprene in nearly all samples (5 ppb or less), with very rare higher readings of unknown cause (e.g, Hershey et al. 1995, Zulkosky et al. 2005, Lauriers et al. 2006, Li et al 2009, Butler et al. 2010, Kuo et al. 2010). Hershey et al.’s (1995) study of briquettes applied directly to wetlands showed an average of 0.5 ppb with occasional readings up to 45 ppb. Li et al. (2009) studied methoprene levels produced by both pellets and ingots in the water of Toronto storm water catch basins, finding levels ranging from 0.03-0.55 ppb with averages being 0.39 ppb in shallow basins and 0.13 ppb in deeper basins. Baker and Yan (2010) added 1 briquet per 5,500 liters to catch basins of two types: those containing a lot of organic debris, and ones that had been cleaned out. They detected methoprene in 44% of treated catch basins that contained debris and 17% of clean basins. Average concentrations were 2.2 ppb in debris basins and 0.047 ppb in clean basins (Note: amounts are drawn from the Results of the paper; a higher value mentioned in the Discussion were apparently a software unit conversion error on the Greek letter mu; the data graph and Results both gave lower figures). Levels of metabolites were lower still. Butler et al. (2010) found levels of 0.5 ppb or less within most Rhode Island catch basins after use of 30 day pellets, although some samples that also included organic particles ranged from 5.7 ppb to 15.4 ppb, likely due to the affinity of methoprene to bind to organics.

Butler et al. (2010) also measured methoprene in the water in outflow areas from catch basins; these had reliably low values of 2 ppb or less, indicating that methoprene did not move out of the treated area in amounts likely to affect non-target organisms. They reviewed prior studies of methoprene concentrations in field situations and found that levels were often not detectable. Methoprene movement is limited even in these flowing-water situations, likely because the methoprene rapidly adsorbs to any particles or surfaces it encounters and it also breaks down. Kuo et al. (2010) also found low levels of 0.04-0.14 ppb in tests of over 100 catch basin outflows. Generalizing to septic systems, if a treated septic system were to overflow or its waters were transferred to a waste treatment pond, levels of methoprene would not be expected to exceed 2 -10 ppb in receiving waters, with average levels far lower. The maximum field level of methoprene found to date in a natural system was 45 ppb (Hershey 1995), but overall, literature published since the EPA review (EPA 2001) agreed with its finding that realized environmental concentrations of methoprene due to mosquito control tended to be much less than 5 ppb and usually of 2 ppb or less. Therefore a possible realized environmental range of 2 to 45 ppb is useful to keep in mind when assessing the non-target studies reviewed, but the manufacturers' expected environmental level of 10 ppb is the standard used below for comparing laboratory toxicities to levels used in mosquito control.

Safety to humans, vertebrate pets, and livestock

Methoprene is of very low toxicity to humans and other vertebrates, and may be applied directly to pets, livestock and zoo animals for control of fleas, mites, and other parasites (reviews: Siddall 1976, EPA 2001, Extoxnet 1995). There have been no reported cases of adverse effects to humans after accidental exposures to methoprene (Extoxnet 1995). It has been safely used in veterinary contexts for decades, including on pregnant and nursing mammals. Extensive review showed that the veterinary literature is devoid of studies showing developmental effects of methoprene used on animals for parasite treatments. Methoprene and its breakdown products are sometimes used in studies attempting to disrupt the embryonic development of vertebrates, however levels necessary to produce developmental changes or other toxicities have been found to be over 100X that used in mosquito control or even greater (see, e.g., sections on amphibians and fishes, below).

Amphibians

Methoprene has very low toxicity to amphibians (EPA 2001). In a test with *Rana pipiens,* embryonic development was normal at levels of methoprene up to 50 ppb (Ankley et al. 1998). At very high exposures of 500 ppb, embryos were malformed and died (Ankley et al. 1998; intermediate levels were not tested). Degitz et al. (2003) demonstrated that methoprene had no toxic or developmental effects on African clawed frogs, *Xenopus laevis* at the highest level they tested, 2 ppm, or 200X the expected application rate of 0.01 mg/L. Its breakdown products were also non-toxic at that level. Similarly, La Clair (1998) found that excessive dosages of methoprene breakdown products in combination with UV exposure caused mortality of *X. laevis* embryos, however they concluded that in levels used in mosquito control, the breakdown products would not achieve toxic levels.

There has been little research on the environmental effects of methoprene on amphibians, possibly due to its known low toxicity for vertebrates. Pauley et al. (2015) tested the direct and indirect effects of methoprene on the survival and growth of tadpoles of the Gray Treefrog (*Hyla versicolor*). They employed a mesocosm system of artificial ponds created in cattle watering tanks. There were no detectable direct effects on tadpole growth or survival due to methoprene in the form of Mosquito Torpedo™ dunks (Wellmark International). However, in tanks where predatory dragonflies had also been added, tadpole survival dropped from about 35% to 2% in the presence of methoprene. The authors mentioned a possible physiological reaction to the combination of stress from predators and the insecticide, or a change in behavior of tadpoles due to the methoprene. No papers to date have reported stress or behavioral effects for methoprene but it is unclear whether such effects have been explicitly explored. Another possible cause of this drop could have been that the tank setup reduced recruitment of alternative prey. Methoprene caused depletion of cladoceran ‘water fleas’ as alternative prey for dragonflies. The mesocosms apparently lacked natural pond substrate that could have created hatches of other zooplankton, although whether these would have developed is unknown.

Screened lids on the tanks prevented colonization by insects as additional alternative prey. Further research would be needed to test this mechanism.

Methoprene was evaluated as a cause of limb deformities in amphibians because some deformities occurred in waters that had been treated against mosquitoes with methoprene, however, subsequent laboratory research showed that unrealistically high dosages of methoprene were required to induce malformations in amphibians, and the deformities so induced did not match those seen in field studies (Ankley et al. 2004). Therefore, some toxicologists and amphibian ecologists have concluded that methoprene is not an issue for amphibian decline (Ankley et al. 2004, Johnson et al. 2010).

Fishes

Studies to date show that methoprene applied at levels up to 100X of that expected in mosquito control were safe for the fishes that have been tested. McCague and Pridmore (1978) tested for sublethal effects of methoprene on the stress hormones of juvenile rainbow trout, *Salmo gairdneri*, and juvenile coho salmon, *Oncorhynchus kisutch*, and found no detectable effects until levels were two orders of magnitude greater than used in mosquito control. They also reviewed several earlier studies of the toxicity of methoprene to fishes, and these tests also showed no mortality until levels were two orders of magnitude above that used in mosquito control (tested animals included: trout (Onchorynchus spp), channel catfish (*Ictalurus punctatus*) bluegill sunfish (*Lepomis macrochirus*) and saltwater minnows (*Fundulus heteroclitus*)). Smith et al. (2003) were able to induce abnormalities in larval zebrafish (*Danio rerio*) using methoprene breakdown products, but only at levels 100X or greater than expected environmental levels. Several studies of Australian fishes also failed to detect adverse effects at levels used in mosquito control. Brown et al. (2002) showed that Australian juvenile rainbowfish (*Melanotaenia duboulayi*) were insensitive to methoprene at levels 10X or more the expected environmental concentration. Another study showed no effects of methoprene on swimming behavior of this species at 10X the expected environmental concentration (Hurst et al. 2007). Brown et al. (1998) showed that methoprene had no toxic effects on the Pacific Blue-Eye (*Pseudomugil signifier*) at 500 X levels used against mosquitoes.

Aquatic Invertebrates

Existing reviews show that levels of methoprene used for mosquito control have no detectable effects on a majority of the invertebrates tested, which include both freshwater and marine taxa (e.g., Wirth et al. 2001, EPA 2001, Csondes 2004, Henrick 2007). As mentioned above, short term studies (48 hr or less) that showed no effect of methoprene were omitted from this review, because in general effects of methoprene are seen when arthropods molt, and molting is not guaranteed over such short time frames.

Crustacea

A majority of papers published on methoprene and crustaceans have found no negative effects at levels used for mosquito control. The few crustaceans that did show effects were often smaller taxa (similar in size to mosquitoes or smaller), or occasionally the embryos or hatchling stages of larger taxa.

Blue crabs and a variety of other marine Crustacea only showed deleterious effects of methoprene at levels 100X in excess of that expected in mosquito control (0.1 or more ppm; review: Horst and Walker 1999). There is mixed evidence for sub-lethal effects of low levels of methoprene on fiddler crabs. Two studies have shown that most levels of methoprene ranging up to 200 ppb do not cause mortality or malformations of fiddler crabs (Stueckle et al. 2008 and references therein). However, levels of 10 ppb (the expected field level) or over slightly slowed limb regeneration in females and 0.1 ppb was associated with a greater frequency of limb malformations in males, although this effect vanished at higher levels (Stueckle et al. 2008).

Some smaller crustaceans, including freshwater zooplankton and larvae of marine crustaceans, showed toxic effects of methoprene in laboratory or mesocosm studies at relatively high levels (0.05 ppm) but sometimes also at the lower levels expected in mosquito control (Chu et al. 1997, Pauley et al. 2015). However, several existing studies of field applications have shown no effect on zooplankton abundances (e.g., Norland and Mulla 1975, Niemi et al. 1997, Davis and Peterson 2008).

Studies by Walker and colleagues have shown negative effects on lobster larvae of 2-50 ppb methoprene, which is in the range used in mosquito control, and also demonstrated that methoprene may become concentrated in some lobster tissues with chronic exposure (Walker et al. 2005). Mysid shrimp embryos showed small differences in hatching rate and development time at 1 ppb and larger changes at 100 ppb, and there were non-significant trends toward decreased survival of larvae (Ghekiere et al. 2007).

Occasional findings of methoprene toxicity to marine taxa such as these have caused concern. Local effects are possible; however environmental modeling and field data suggest that widespread effects are unlikely. Levels posing risks should not be approached in marine habitat receiving runoff due to the ocean's vast capacity to dilute (Miller et al. 2005). Zulkosky et al. (2005) provided evidence consistent with this model in that methoprene was not detectable in Long Island Sound waters in 2003, despite being used in many adjacent marshes and other waters on the island.

Not all Crustacea are sensitive to methoprene, even at early stages. For example, Su et al (2014a) demonstrated that relatively high levels of methoprene were non-toxic to hatchling tadpole shrimp that were followed through egg maturation at levels 90-900 times rates used in mosquito control, and field application rates at up to 4X label rate also showed no detectable effects. Wirth et al. (2001) found no significant effect of methoprene exposure on time to hatching of grass shrimp eggs from adults exposed for 35 days at 1 ppm; adults were also insensitive. Davis and Peterson (2008) may have detected a small and transient effect of methoprene on freshwater amphipod crustacean abundance, but this effect disappeared rapidly and they did not regard it as conclusive. Brown et al. (1999) found that methoprene was safe for the shrimp *Leander tenuicornis*. Lawler et al. (1999) found no effects of methoprene applied for mosquito control on amphipods (Talitridae) in a 4 day exposure in Florida mangrove swamps (this is admittedly a relatively short time frame). Russell et al. (2009) found no negative effects of methoprene applications on either terrestrial or aquatic salt marsh invertebrates, including insects and crustaceans. Some small related arthropods from other groups showed brief and inconsistent enhancements, and in one case a slight suppression that was non-significant after statistical tests were adjusted for multiple comparisons (mites and collembolans).

In summary, populations of some small crustaceans, including zooplankton and the larvae of some larger taxa, may show decreases in population size in waters deliberately treated for mosquito control. However, not all crustaceans show such effects and field studies have not detected negative impacts of methoprene on crustacean abundances.

Mollusca

There have been very few studies targeting the effects of methoprene on molluscs. Garcia et al. (2014) found that clam and oyster larvae were insensitive to levels of methoprene used in mosquito control. For both taxa, lethal concentrations were over an order of magnitude greater than those used for mosquito control (clams: 0.68 ppm; oysters: 1.32 ppm). Kikuchi et al. (1992) showed that the snail *Physa fontinalis* had a high LC 50 of 10.6 ppm. A long-term field study of freshwater aquatic invertebrates did not report any detectable effects of methoprene on freshwater Mollusca (mostly Gastropoda) (Hershey et al. 1998).

Aquatic Insects

Methoprene is toxic to insects, however a number of studies show that only some aquatic insects are affected by the relatively low concentrations that are used to control larval mosquitoes. Low levels can control mosquitoes because of their small size and relatively high sensitivity. For example, an early field experiment by Norland and Mulla (1975) in which methoprene was applied at 0.1 ppm showed reductions of small dipterans, mayflies, and predatory beetles, however dragonflies, and damselflies were unaffected. Breaud et al. (1977) believed they had found both positive and negative effects of repeated methoprene treatments on freshwater marsh invertebrates, however they only compared one treated site to one control site, therefore differences may have been due to natural variation between the marshes. Gelbic et al. (1994) found extra instars or delayed molting in predatory naucorid water bugs at levels twice that expected in mosquito control (e.g., 0.02 ppm and above). Lowe and Hershberger (2004) found that in the laboratory at methoprene levels expected from liquid methoprene sprays intercepted by vegetation, there was partial mortality of a small leaf beetle found on purple loosestrife in wetlands (*Galerucella calmariensis*). Hershey et al. (1998) followed the effects of methoprene applications onto freshwater marshes that were repeated every 3 weeks during the mosquito season for three years. Methoprene treatments had no detectable effects in 1991, the first year, but were associated with partial suppression of small dipterans and some predaceous insects part way through 1992 and 1993. However subsequent research in 1997 showed that reductions had been temporary and possibly associated with a drought (Schmude et al. 1998, Balcer et al. 1999). Breeding birds did not show effects of insect reductions in these marshes (Niemi et al. 1997). Lawler et al. (2000) studied the long-term effects of both liquid and sustained-release methoprene in salt marshes, and found that the species that comprised over 90% of insects were unaffected (the water boatman *Trichocorixa reticulata*); also, brine flies were able to grow and mature from the egg stage. Similarly, a field study by Russell et al. (2009) found no effects on non-target salt marsh insects, and Davis and Peterson (2008) did not detect depletion of non-target insects following experimental methoprene use for mosquito control on a freshwater pond margin, or on terrestrial insects.

Bacterially-derived, sustained-control options for organically-enriched waters

As mentioned above, several options for sustained mosquito control are desirable to forestall resistance, which can develop if populations are pressured with one material for many successive generations (review: Hemingway and Ranson 2000, Coleman and. Hemingway 2007). The other biorational methods available are based on naturally-occurring toxins produced by the bacteria *Bacillus thuringiensis israelensis* (Bti), *Lysinibacillus sphaericus or* Ls *(= Bacilluls sphaericus,* Bs), and *Saccharopolyspora spinosa*, or 'spinosad'. All are available in sustained-release formulations, either alone or sometimes in combination for Bti and Ls.

Bti and Ls bacteria contain toxic crystals that are activated by conditions in the mosquitoes' gut, and the larvae must consume enough to reach a toxic dose (review: Lacey 2007). This is sometimes a problem for Bti in organically-enriched waters; its activity drops off rapidly in such habitats and natural bacteria are so concentrated that mosquitoes do not need to filter much water to develop (Lacey 2007). Ls remains active in eutrophic waters for longer than Bti and has good efficacy, however, mosquitoes tend to become resistant to it with prolonged use (Lacey 2007). Combining toxins from Bti and Ls can strongly suppress the development of resistance (Lacey 2007), and so the mosquito control industry has produced combined formulations of Bti and Ls (Ferreira and Silva-Filha. 2013). One combined formulation (Vectomax® WSP, Valent BioSciences) gave good control of *Cx pipiens* larvae in septic tanks for 17 days (Cetin et al. 2015). The same study indicated good control for up to 24 days, however a general decline in mosquito numbers during the study, combined with a low number of control replicates, made later results less conclusive. Neither Bti nor Ls are expected to have significant adverse environmental effects at rates used in mosquito control, although some small flies (Diptera) may experience partial suppressions in areas where they are directly applied (Boisvert and Boisvert 2000, Lacey 2007). There have been a few reports of loss or drift of other benthic macroinvertebrates when Bti was used against black flies (Simuliidae) in stream systems, but applications for blackflies are usually pulsed through stream systems at concentrations five times higher or more than is used in mosquito control (Boisvert and Boisvert 2000, Lacey 2007, Duchet et al. 2015). Therefore Vectomax® or similar products may be a good option for sustained control of mosquitoes in enriched waters, given the generally favorable environmental profiles of Bti and Bs, plus the fact that combination of bacterial toxins is expected to forestall resistance.

Larvicides based on the bacteria *Saccharopolyspora spinosa*, or ‘spinosad’ contain toxins called spinosyns that are active against invertebrates both through the gut and cuticle (Hertlein et al. 2010). The latter action may decrease the necessity for insects to filter-feed enough of the material to get a toxic dose (Lawler and Dritz 2013). Spinosyns degrade rapidly in sunlight but are stable in water (Hertlein et al. 2010). Few studies exist on the motility of spinosyns, but the particles tend to adhere to substrates (Hertlein et al. 2010) and this could restrict movement outside of catch basins or septic systems in a manner similar to methoprene. A relatively recent review suggested that the efficacy of spinosad can decrease in highly organic waters, including sewage, although one study showed an opposite effect (Hertlein 2010). Formulations include sustained-release Natular® products that are OMRI certified for organic agriculture. Su et al. (2014b) showed sustained efficacy of spinosad in suburban catch basins that often contained considerable organic material. Natular® T30 tablets controlled mosquito larvae in storm drains as long as the tablets were attached to corks for floatation so that the toxin would distribute through the water column, instead of being immobilized in the sediment. More research is desirable on the efficacy and non-target effects of spinosad in enriched waters.

Spinosad-based products are active against a broader range of non-target invertebrates than the other bacterial larvicides and methoprene, but are considered low-risk for vertebrates. Notably, laboratory toxicity tests show that spinosad is not toxic to guppies (*Poecilia reticulata*) that are sometimes to control mosquitoes, in concentrations up to 1.5 ppm (Anogwi et al. 2015), nor to mosquitofish (Hertlein 2010; see also a table of non-target assessments herein). Several recent studies have found mortality of non-target insects and zooplankton after spinosad applications, including mayflies, dragonflies, beetles, aquatic bugs and midges (Lawler and Dritz 2013, Jones and Ottea 2013, Marina et al. 2014, Duchet 2015). With the exception of midges, such taxa are not expected to occur in septic systems but could be present in more moderately enriched waters.

CONCLUSIONS

Sustained-release formulations of methoprene and several bacterially-derived mosquito larvicides show good efficacy against mosquitoes in enriched waters as well as in other habitats. However, in enriched waters Bti tends to be less effective that the other materials reviewed if it is not used in combination with Ls, and the risk of resistance is greater. These materials have generally favorable environmental profiles at concentrations used in mosquito abatement, although more research is needed on spinosad because it may cause mortality in a wider variety of insects. Regarding methoprene, the vast majority of a large number of non-target taxa that have been tested do not show deleterious effects of either the expected field concentration (10 ppb) or levels detected in the field (usually 5 ppb or less). Laboratory and mesocosm trials show that it is possible for some species of small Diptera and zooplankton-sized Crustacea to show population reductions due to methoprene use. However, this has very rarely been seen in field studies. Both Bti and Ls have well-established records of environmentally safe mosquito control.

The limited non-target effects of most materials reviewed here supports their use in natural environments as well as in the enriched habitats that were a focus of this review due to the larger dangers of lapses in mosquito control in such waters. These findings may help inform discussions among government agencies and stakeholders with regard to which mosquito control methods to select in order to protect both public and environmental health (i.e., "One Health" sensu Cook et al. 2004, American Veterinary Medical Association 2008).

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