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EFFICACY AND NONTARGET EFFECTS OF A SPINOSAD-BASED LARVICIDE IN MINNESOTA VERNAL POOLS AND CATTAIL MARSHES

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ABSTRACT. Larvicides that contain spinosad, a bacterial metabolite, are used to control mosquitoes in diverse aquatic habitats. These same habitats are home to other invertebrates, including Crustacea—fairy shrimp, isopods, and amphipods—and mollusks—fingernail clams and freshwater snails. A double-blind study evaluated the effects of Natular® G, a granular treatment containing spinosad, on spring *Aedes* spp. and nontarget invertebrates in vernal wetlands. Within 14 days after application, Natular G controlled larvae of spring *Aedes* by 53–84%, depending on species, but had no significant effects on numbers of fairy shrimp, fingernail clams, or freshwater snails. A second double-blind study evaluated effects on *Coquillettidia perturbans* and nontarget isopods and amphipods in cattail marshes. Treatment reduced emergence of *Cq. perturbans* by 25% but did not change numbers of isopods or amphipods. The 2 experiments indicate Natular G could be effective against spring *Aedes* in vernal wetlands, less so against *Cq. perturbans* in cattail marshes, and yet pose minimal risk to crustaceans and mollusks in either vernal wetlands or cattail marshes.

KEY WORDS Spinosad, nontarget, larval control, wetlands

INTRODUCTION

Preventing mosquito-borne disease and preserving outdoor quality of life for urban residents are primary justifications for mosquito control (Spielman and D'Antonio 2001). Integrated mosquito management methods include habitat modifications and chemical and biological control. In Minnesota, the Metropolitan Mosquito Control District (MMCD) uses integrated management methods comprising mostly larval control using the most mosquito-specific products available, including *Bacillus thuringiensis israelensis* De Barjac (VectoBac®) and methoprene (Altosid®, MetaLarv®) (MMCD 2019). Integrating mosquito-specific products containing new actives such as spinosad strengthens the management program and fosters product rotation.

The bacterium *Saccharopolyspora spinosa* Mertz and Yao (Actinomycetales) secretes macrocyclic lactones with potent insecticidal activity. These fermentation-derived natural products are called spinosyns and have been commercialized as control agents for insect pests of importance in agriculture, forests, turf, and medical/veterinary applications (Sparks et al. 2001).

A mixture of the A and D isomers of spinosyns is marketed as spinosad, which is the active ingredient in the Natular® series of granular mosquito larvicides (Clarke Inc., St. Charles, Illinois). The use of spinosad for mosquito control has been previously documented (Bond et al. 2004, Darriet et al. 2005, Romi et al. 2006, Hertlein et al. 2010). The label rate for Natular G is 3.9 to 7.3 kg/ha (3.5 to 6.5 lb/acre) for woodland and snowmelt ponds. As formulated, treatment with 5.6 kg/ha (5 lb/acre) would deliver a concentration of 19 parts per billion (ppb) in 15 cm of water.

Aquatic habitats that support mosquito larvae also support other nontarget macroinvertebrates that are consumed by waterfowl and other vertebrates. Thus, nontarget effects of spinosad on nontarget macroinvertebrates are of interest. The US Environmental Protection Agency (EPA) classified spinosad as a reduced risk pesticide, based on testing that includes a few surrogate species of freshwater fish and crustaceans (EPA 2019). Other tests, both lab and field, raised questions about the sensitivity of other species to spinosad. Jones and Ottea (2013) evaluated efficacy of spinosad against larvae of *Culex quinquefasciatus* Say in Louisiana and, in parallel, evaluated effects on naiads of a mayfly (Ephemeroptera: Caenidae), a damselfly (Odonata: Coenagrionidae), and a dragonfly (Odonata: Libellulidae) in the laboratory. Mayfly naiads were far more susceptible to spinosad than were damselfly and dragonfly naiads. Treatment of wetland mesocosms in California with single-brood and extended-release formulations of spinosad controlled larvae of *Culex tarsalis* Coquillett and chironomid midges for over 4 wk, but toxic effects on mayfly naiads were undetectable by 3 wk after treatment (Lawler and Dritz 2013).

Marina et al. (2014) found that treating temporary pools in Mexico with 10 parts per million (ppm) of spinosad, a rate $> 500 \times$ current label rate, controlled larvae of *Anopheles albimanus* Wiedemann, *Cx. coronator* Dyar and Knab, *Cx. (melanoconion)* sp., and *Uranotaenia lowii* Theobald for 15–20 wk but also reduced numbers of nontarget predatory Coleoptera, Hemiptera, and Odonata.

Earlier studies of nontarget effects of spinosad on invertebrates focused on insects and the planktonic crustacean *Daphnia magna* Straus and *D. pulex* Leydig (Duchet et al. 2008, 2010). Duchet et al.

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(2008) found that 8 ppb of spinosad reduced populations of *D. pulex* initially, but they recovered 1 wk after treatment. Other common macroinvertebrates in vernal North American wetlands include a fairy shrimp, *Eubranchipus bundyi* Forbes, an amphipod, *Hyalella azteca* Saussure, and mollusks such as fingernail clams (Sphaeriidae: *Musculium*, *Pisidium*, and *Sphaerium*) and freshwater snails (Physidae: *Physella*) (Voshell 2002, Batzer et al. 2004). Those taxa commonly inhabit wetlands in Minnesota, and their susceptibility to spinosad has not been evaluated in operational settings, so these groups were chosen for the study.

The 7-county MMCD in Minnesota considered the use of Natular G as part of their resistance management program and to diversify their larvicide options. Moreover, because spinosad is a reduced risk pesticide (EPA 2019), its inclusion as part of an integrated mosquito management program is consonant with MMCD's mission. In a 1-yr study, MMCD evaluated operational treatments to control mosquito larvae and nontarget effects of spinosad in vernal wetlands. In a second year, MMCD examined effects in cattail marshes. Both studies employed a double-blind design to minimize biases.

MATERIALS AND METHODS

Vernal wetland study

Ten vernal wetlands, each less than 1.2 ha, were chosen for study based on previous productivity of spring-hatching *Aedes* species: *Aedes excrucians* (Walker), *Ae. stimulans* (Walker), *Ae. fitchii* (Felt and Young), and others. A double-blind method was used to determine which sites were treated with blank or active granules, 5 with blank granules and 5 with active Natular G granules at a midlabel rate of 5.6 kg/ha. Sites were treated on May 5, 2014. All sites were sampled before and after treatment to assess changes in abundance of mosquitoes and nontarget macroinvertebrates. Packets of granules for each site in the study were double-blind coded by a collaborator not involved in selecting test sites, sampling, or treating the sites. Field applicators, samplers, and sample processors did not know which sites received active or blank granules until all samples were processed.

Mosquitoes and other macroinvertebrates were sampled with a heavy-handled D-frame aquatic net with a 500-μm mesh size, based on Wen (1992), Genet and Bourdaghs (2006), Anteau and Afton (2008), and Merritt et al. (2008). Three days before and 7 and 14 days after treatment, each site was sampled along 2 randomly selected 2-m transects: 1 transect at the water surface and 1 just above the wetland bottom. Each separate sample was placed in a concentrator bucket to remove plant debris and excess water; contents were then preserved in 100% ethanol with a small amount of wetland water. Samples were processed in the laboratory by filtering through a 200-μm sieve. Invertebrates were identified at least to family, counted, and stored in 80% ethanol as voucher specimens. Identifications were based on keys in Burch (1982), Voshell (2002), Merritt et al. (2008), Smith (2008), and Perez and Sandland (2014).

Cattail marsh study

No amphipods were detected in the vernal sites in 2014, so a second study was conducted in 2015 to assess the effects of Natular G in cattail marshes where amphipods were expected to occur. Using double-blind assignment, 5 marshes received Natular G at 5.6 kg/ha, and 5 received blank granules. Thereafter, adult mosquitoes emerging from each marsh were collected at 3–5 days intervals with five 1×1 m basal area pyramidal emergence traps placed over randomly chosen cattail clumps. Both sexes of *Cq. perturbans* were counted and totaled on each collection date.

Effects on amphipods in each marsh were measured with a 20-μm mesh screened dipper developed for sampling *Cq. perturbans* larvae (Batzer 1993). At 5 points around 2 independent locations, the dipper was placed at the bottom of the marsh and then scooped up through the water column and vegetation. Contents were combined and washed into a concentrator bucket, and then the pooled sample was preserved in 100% ethanol diluted with a small volume of marsh water. Samples were processed in the laboratory by filtering through a 200-μm sieve, and amphipods and isopods were identified at least to family, counted, and stored in 80% ethanol as voucher specimens. Isopods were included in study because fewer than expected amphipods were collected.

Pretreatment samples were collected on May 28, 3 days before treatment; treatments occurred on June 1, and post-treatment samples were collected on June 4, 3 days after treatment, and June 26, 25 d after treatment. Water temperatures and depths were measured on each date at each location.

Statistical analysis

Counts of mosquito larvae and nontarget taxa from the vernal wetland study were averaged across transect halves, and then analyzed by fitting generalized linear mixed models using the *glmer* function of the *lme4* package (Bates et al. 2015) in R (R Core Team 2019). Fixed effects were treatment (blank or active), with dates as repeated measures within sites. Random effects were sites, and errors were Poisson.

Counts of *Cq. perturbans* in the pyramid traps on the cattail marsh study were averaged across traps within days, and then analyzed with the same model and error structure, plus offset for numbers of days in the different trapping intervals. Counts of nontarget isopods and amphipods in the scoop samples were averaged across scoops within the same wetland and date, and then analyzed with the same fixed and random effects, plus fixed covariates of water

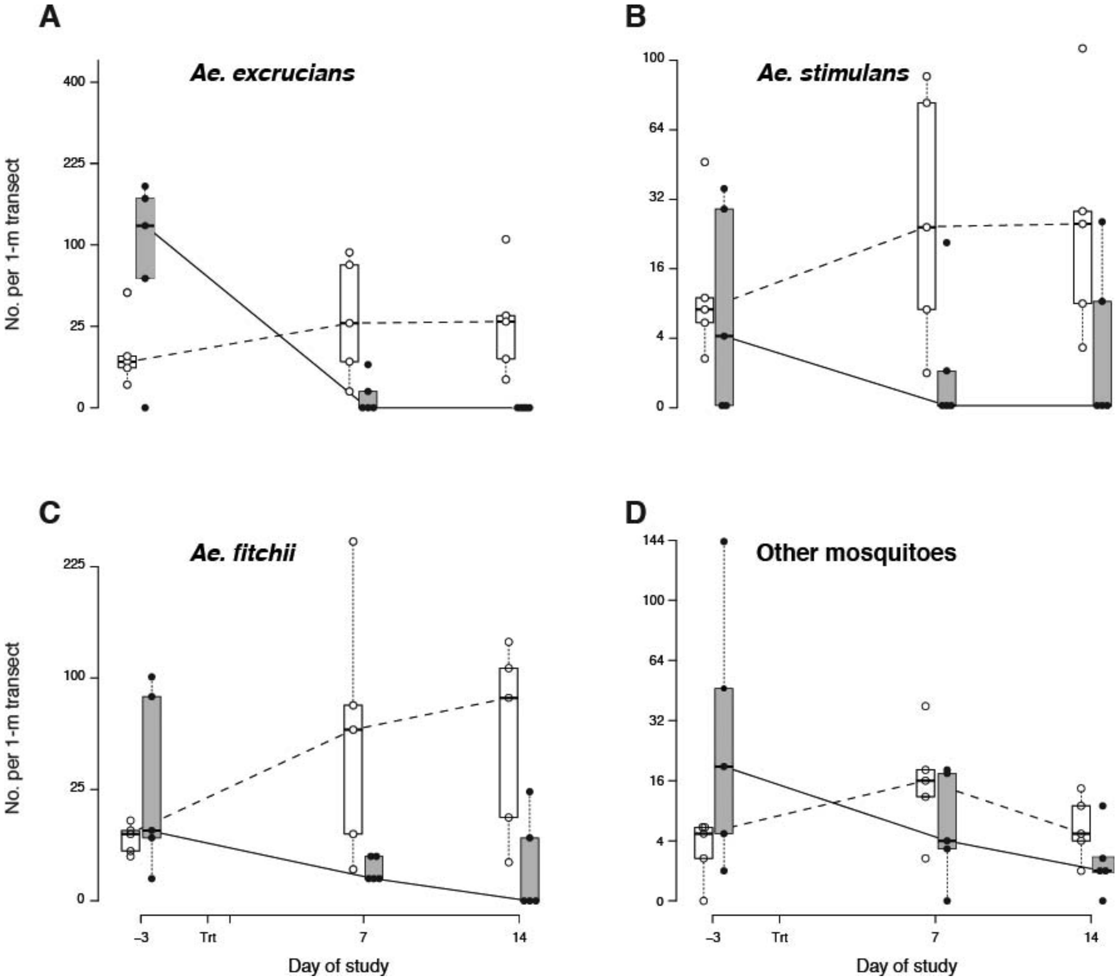

Fig. 1. Tukey box and whisker plots of counts of mosquito larvae and pupae in 5 vernal wetlands treated with blank corn cob granules (open circles, open interquartile ranges), or active granules containing spinosyns (filled circles and shaded ranges), $n = 5$ wetlands each. Each wetland sampled with a D-frame net along a 2-m transect, 3 days before and 7 and 14 days after treatment (Trt). Treatment groups offset horizontally around indicated sampling dates to avoid overlap. Horizontal lines connect group medians. Points outside boxes not connected with fine dotted lines are outliers. Vertical axes (in square root scale) differ among panels.

temperature and depth on each sampling day. Type I significance levels for multiple effects considered in both studies were adjusted for corresponding numbers of main effect tests using Bonferroni correction, $0.05/9 = 0.006$ for the vernal wetland study, and $0.5/4 = 0.013$ for the cattail marsh study.

RESULTS

Vernal wetland study

A total of 4,072 mosquito larvae and pupae were obtained in the D-frame net samples collected along 2-m transects in the vernal wetlands (Fig. 1). Identifiable late instars of *Ae. excrucians*, *Ae. stimulans*, and *Ae. fitchii* were abundant enough to analyze separately, whereas specimens of less abundant mosquito species and life stages, including first instars and pupae that could not be identified to species, were combined and analyzed as "other mosquitoes."

From 3 days before to 14 days after treatment, counts of the 4 mosquito groups increased slightly in wetlands receiving blank granules, but decreased more substantially in sites treated with active granules (Fig. 1). Interactions between treatment and day were statistically significant in all 4 cases (Type II Wald $X^2 > 75.5$, df $= 2$, $P < 0.001$); spinosad reduced mosquito abundance for at least 14 days after application. Compared to the untreated controls 7 and 14 days after treatment, the average percent reduction ranged from 53% for *Ae. excrucians* to 84% or greater for *Ae. stimulans*, *Ae. fitchii*, and other spring *Aedes* species.

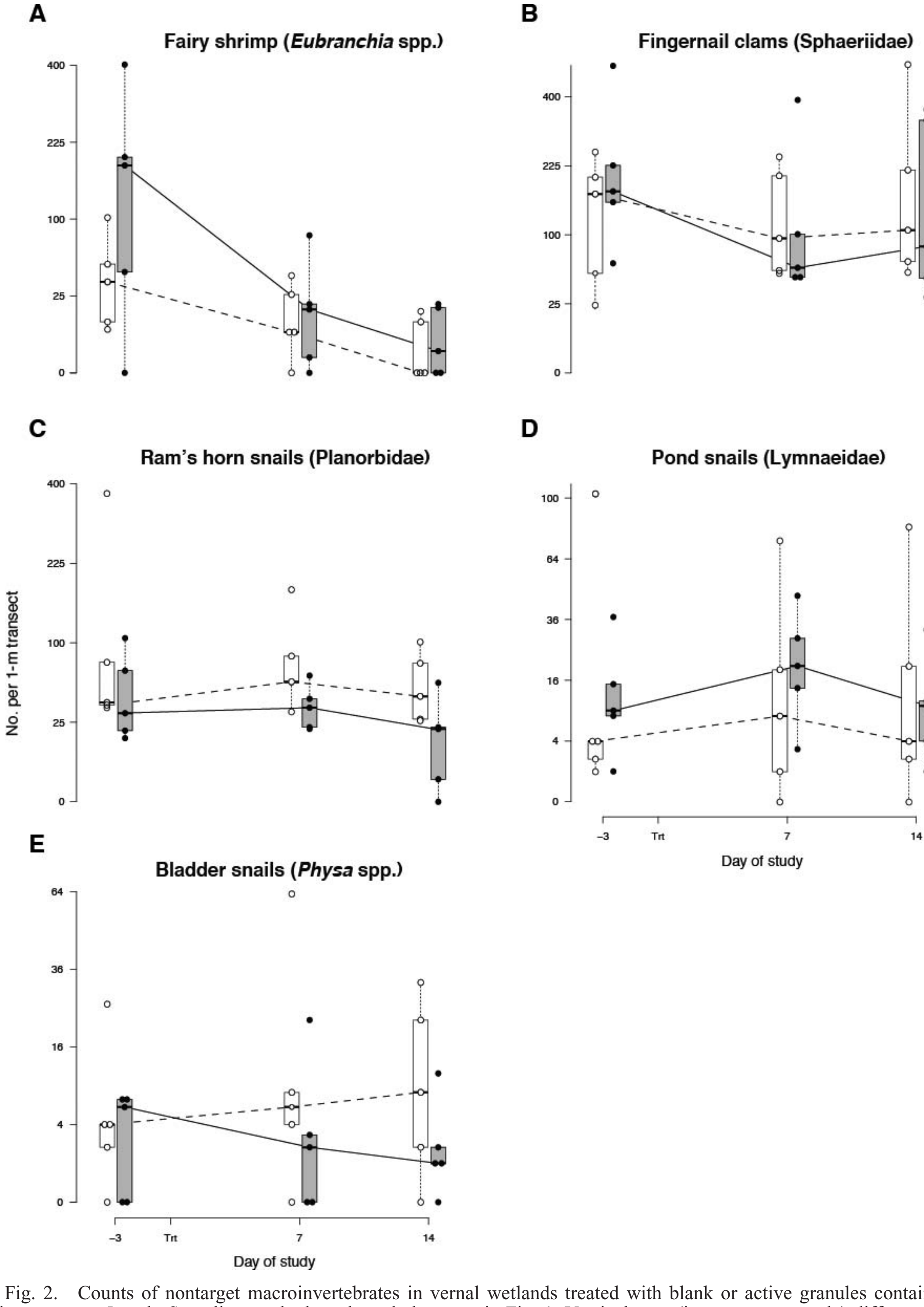

Fig. 2. Counts of nontarget macroinvertebrates in vernal wetlands treated with blank or active granules containing spinosyns, $n = 5$ each. Sampling methods and symbols are as in Fig. 1. Vertical axes (in square root scale) differ among panels.

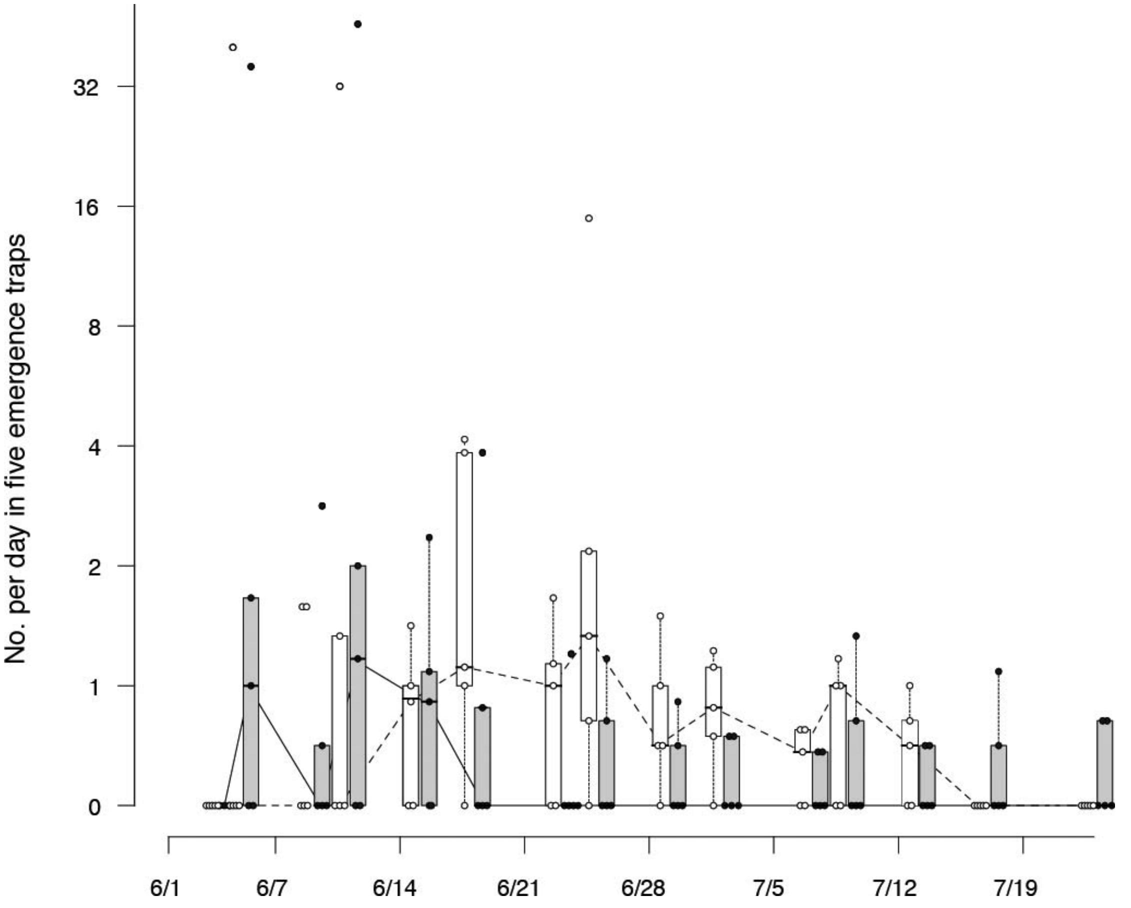

Fig. 3. Counts of adult *Coquillettidia perturbans* that emerged per day in sets of 5 randomly placed traps in each of 5 cattail marshes treated with blank granules (open circles and ranges) and 5 others treated with active granules containing spinosyns (filled circles and ranges). Marshes treated on June 1, 2015. Symbols are as in Fig. 1.

A total of 9,001 nontarget invertebrates were obtained in the D-net samples over the same 3 dates (Fig. 2). Specimens counted included fairy shrimp (Branchiopoda: Anostraca, *Eubranchia* spp.), fingernail clams (Mollusca: Sphaeriidae), ram's horn snails (Planorbidae), pond snails (Lymnaeidae), and bladder snails (Physidae, *Physa* spp.). Amphipods were not detected in any of the 10 vernal wetlands.

The abundance of fairy shrimp declined from 3 days before to 7 and 14 days after treatment in both treated and nontreated sites (Fig. 2A). Interaction between treatment and day was significant ($X^2 = 45.9$, df $= 2$, $P < 0.001$), due to a steeper decline from –3 to 7 days in the wetlands receiving active granules. Numbers of fingernail clams (Sphaeriidae) also declined from 3 days before to 7 days after treatment, more so in sites treated with active granules than blank (Fig. 2B). Interaction between treatment and day for fingernail clams was significant ($X^2 = 74.4$, df $= 2$, $P < 0.001$). Nevertheless, differences between numbers at 7 and 14 days in blank and active sites were not significant ($P > 0.27$). Numbers of ram's horn snails (Planorbidae), pond snails (Lymnaeidae), and bladder snails (*Physa* spp.) all remained comparatively steady from 3 days before to 14 days after treatment (Fig. 2C–E). With each kind of snail, pairwise differences between treatment groups over dates were neither large nor significant ($P > 0.02$). All combined, active granules reduced the numbers of spring *Aedes*, but they caused small and largely insignificant reductions in the numbers of nontarget fairy shrimp, clams, and snails in the vernal wetlands.

Cattail marsh study

Totals of 342 and 255 *Cq. perturbans* were obtained in emergence traps on the 5 marshes treated with blank and active granules, respectively, for a 25% reduction over the ~8-wk study (Fig. 3). Date by date, comparisons between the two treatment groups were significant only on June 25, but not on any of the other post-treatment dates.

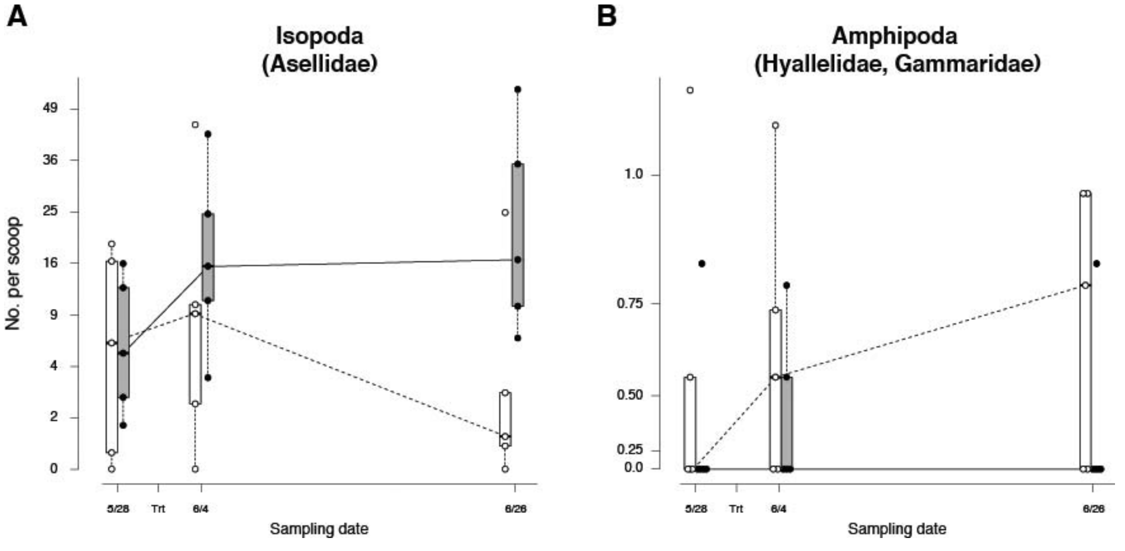

Fig. 4. Counts of isopods (A) and amphipods (B) per vertical scoop with a screen-bottomed dipper in cattail marshes 3 days before and 3 and 25 days after treatment (Trt) with blank (open) or active (filled) granules. Vertical axes (in square root scale) differ between panels. Symbols are as in Fig. 1.

Scoop samples for nontarget Crustacea before and after treatments yielded 3,877 isopods (Asellidae), and 73 amphipods (*H. azteca* and *Gammarus* sp.). The numbers of isopods per scoop increased significantly after treatments, and increases were greater in marshes treated with active granules than with blanks (interaction $X^2 = 202$ with 2 df, $P < 0.001$) (Fig. 4A). Over a range of 11–45 cm depths, numbers per scoop in both treatment groups increased modestly with depth ($X^2 = 22.5$, 1 df, $P < 0.001$) and decreased modestly with water temperature ($X^2 = 74$, 1 df, $P < 0.001$); the deeper and the warmer the water, the greater the count of isopods. While numbers of the rarer amphipods (Fig. 4B) tended to be lower in marshes treated with active granules, numbers overall were insufficient to make any judgements about the effect of treatment ($X^2 = 1.48$, df = 1, $P = 0.22$). Active granules did not have clear adverse impacts on crustaceans encountered in the study's 10 cattail marshes.

DISCUSSION

Freshwater testing is classically limited to a few surrogate species of freshwater fish and crustaceans, from which predictions of effect are made about environmental exposures to the pesticide on fish, aquatic amphibians, and mollusks and other aquatic invertebrates. *Daphnia* as surrogates are assumed to predict toxicological effects on other organisms, although Milam et al. (2005) suggest these traditional toxicity evaluations may miss harmful effects on mollusks. Although spinosad is of moderate acute toxicity to *Daphnia* ($LC_{50} = 1.5$ ppm), its acute toxicity to a marine mollusk, the eastern oyster (*Crassostrea virginica* Gmelin), is higher ($LC_{50} = 0.3$ ppm) (DPR 1995). One goal of the present study was to determine what these acute toxicities indicated about the risk of adverse impacts on mollusks and crustaceans in vernal wetlands including fairy shrimp, *E. bundyi*, an amphipod, *H. azteca*, and mollusks such as fingernail clams (Sphaeriidae: *Musculium*, *Pisidium*, and *Sphaerium*) and freshwater snails (Physidae: *Physella*) due to operational treatments for larval mosquito control. Demonstrating a lack of impacts caused by operational larval mosquito control treatments to a wide range of nontarget invertebrates in these habitats provides confidence that mosquito control will not harm unintended organisms.

Spinosad applied to vernal wetlands at the midlabel rate of 5.6 kg/ha effectively controlled spring *Aedes* larvae but had no effect on the numbers of nontarget fairy shrimp, fingernail clams, and freshwater snails. In addition, spinosad provided minimal control of *Cq. perturbans* in separate cattail marshes but had no clear adverse effects on Crustacea. These studies provide more confidence that Natular G can be an effective part of an integrated mosquito management program, consonant with the MMCD's mission. The MMCD will proceed with the use of Natular G to control *Aedes* mosquitoes in vernal wetlands but will not use this formulation in cattail marshes. Spinosad has a mode of action that is different from other larvicides, which will help with resistance management, and it satisfies the public demand for treatments with minimal impact on freshwater ecosystems. Our study of operational treatments may help others determine how products containing spinosad could add effectiveness and environmental sensitivity to their integrated mosquito management programs.

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