The Impact of Bti on the Survival of the Endangered Tadpole Shrimp *Lepidurus packardi*

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ABSTRACT: We investigated the effects of *Bacillus thuringiensis* var. *israelensis*, a bacterial mosquito control material, on the vernal pool tadpole shrimp *Lepidurus packardi* and larval mosquitoes. Field trials were conducted in ephemeral pools on a wildlife refuge to determine efficacy and residual activity in addition to non-target impacts. The VectoBac® 12AS formulation of Bti applied at 1169 ml/ha (16 fl. oz./A) killed 100% of target *Ochlerotatus dorsalis* mosquito larvae at 24 hours post-treatment, but had little effect at 48 hours. There were no detectable adverse effects on survival of the vernal pool tadpole shrimp.

INTRODUCTION

Previous investigators have been concerned with the effect that various vernal pool invertebrate species might have on the efficacy of *Bacillus thuringiensis* var. *israelensis* (Bti) against mosquitoes (Blaustein and Margalit 1991; Fry-O'Brien and Mulla 1996). However, increasing environmental awareness of the value of ephemeral habitats and the subsequent listing of some associated species as either threatened or endangered, has shifted the focus to the effects that contemporary mosquito control materials may have on the non-target organisms themselves. One such species of interest is the vernal pool tadpole shrimp (*Lepidurus packardi* Simon) which was listed as endangered on September 19, 1994.

Lepidurus packardi is found only in California's Central Valley between Shasta County to the north and Tulare County to the south. The only known populations of the tadpole shrimp outside the Central Valley are found in the complex of vernal pools on the Warm Springs Seasonal Wetlands unit of the Don Edwards San Francisco Bay National Wildlife Refuge in Alameda County and on the adjacent Catellus Development Corporation property (Arnold 1997a,b; Goettle 1997).

Prior to 1980, the Alameda County Mosquito Abatement District treated standing water on and around what is currently Refuge property with old style, broad spectrum pesticides to control mosquitoes which can cause discomfort and vector diseases of humans and animals in the surrounding urbanized areas (Hamersky, Rusmisel, pers. com). However, the commercial availability of biorationals like Bti in 1982 (Valent Biosciences, pers. com.) allowed the District to utilize more environmentally friendly, target specific materials to control mosquitoes within its jurisdiction. Although vernal pools have not been treated since the area was designated as a wildlife refuge in 1992, U.S. Fish and Wildlife officials question how this newer material might impact the tadpole shrimp if such applications were to become necessary today. The objective of our project was to investigate whether Bti (VectoBac® 12AS, Valent Biosciences, North Chicago, IL.) applied under operational conditions and rates adversely affects the survival of the vernal pool tadpole shrimp.

MATERIALS AND METHODS

A Section 7 permit request which would allow incidental take of an endangered species during the course of the research was submitted to the USFWS Endangered Species office in Sacramento in November 1997. Approval was obtained in time to conduct the study in March 1999. The project took place on the Warm Springs Seasonal Wetlands unit of the Don Edwards San Francisco Bay NWR. We found six pools with sufficient *L. packardi* numbers to perform the study, and randomly selected treated and control pools in paired sets (Fig. 1). Because vernal pool tadpole shrimp are endangered and may be declining at this location, we did a pre-experiment survival test to ensure that placing shrimp in sentinel containers

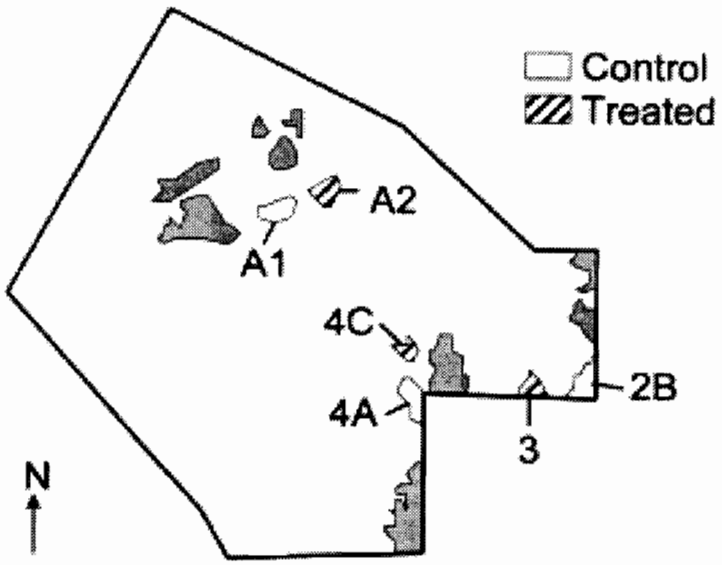

Figure 1. Diagram of Warm Springs Seasonal Wetlands in Alameda County, California. Labels indicate vernal pools used in study. Bti was applied at 1169 ml/ha (16 fl. oz./A) to treated pools.

would not kill them. Three 19 L (5 gal.) buckets with screened windows on the sides, bottom and lid were placed in one pool (A1) so that the container rested on the bottom and extended above the water surface. Five *L. packardi* were put in each container and counted daily for 4 days. There was no mortality for the first 3 days but one shrimp was found dead on day 4 which restricted our investigation to 3 days.

The experiment was set up by placing 2 sets of two sentinel containers in each of the 6 pools. Each set consisted of one, 19 L (5 gal.) bucket which contained five *L. packardi* and one, 4 L (1 gal.) floating bucket which contained 10 late 2nd instar *Ochlerotatus dorsalis* (Meigen) larvae. Both shrimp and mosquito larvae were placed in buckets 24 hours prior to the commencement of the experiment and were counted just before treatment and at 24 and 48 hours post-treatment. VectoBac® 12AS was applied by an environmental specialist from Alameda MAD at 1169 ml/ha (16 fl. oz./A) using a backpack sprayer to the 3 pools designated for treatment. Mosquito larvae were replaced daily as needed to document any residual activity the Bti may have had.

Data were subjected to analysis of variance (ANOVA) using Systat® 7 (SPSS Inc. 1997). Percent survivorship was the combined number in the two buckets per species per pool. Sites were treated as replicates for analysis.

RESULTS

Results of the experiments are summarized in Fig. 2. There was no apparent effect of Bti on tadpole shrimp. All *L. packardi* survived in every pond regardless of treatment and regardless of any mortality in mosquito larvae. However, Bti caused significant mortality of *Oc. dorsalis* (ANOVA $P<0.002$). All mosquitoes survived in all but one control site, but 100% died in the treated sites at 24 hours and 20% died at 48 hours. There was one control site with substantial mosquito mortality from unknown causes (100% at 24 hours and 55% at 48 hours) however, there was still a significant effect of treatment.

DISCUSSION

The *L. packardi* on the Warm Springs unit are of particular interest because they are geographically isolated from the remainder of the population in the Central Valley of California (Linder 1952, Longhurst 1955). Furthermore, refuge personnel are concerned because their surveys indicate that this population appears to be declining (Albertson, pers. com.) so it would be desirable to identify the reasons for this. The U.S. Fish and Wildlife Services' concern with mosquito abatement activities as a possible causative factor may stem from several published reports of adverse effects on another tadpole shrimp *Triops longicaudatus* LeConte (Notostraca: Triopsidae) which is found in fresh water agro-ecosystems like rice and is not endangered (Miura and Takahashi 1974; Walton et. al 1990). The aforementioned studies largely utilized classes of pesticides like synthetic pyrethroids, organophosphates and carbamates which are not currently used by the local mosquito

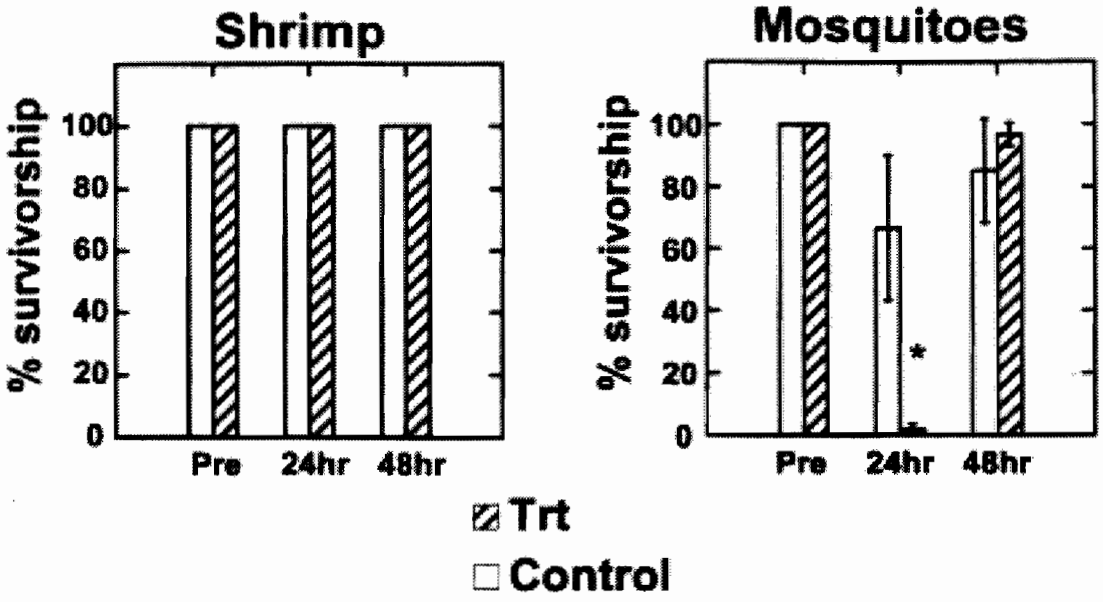

Figure 2. Mean percent survivorship of sentinel tadpole shrimp (*Lepidurus packardi*) and mosquito larvae (*Ochlerotatus dorsalis*) pre- and post- treatment with liquid Bti. Error bars indicate standard error; *indicates significant difference (ANOVA, F (Trt x Day) = 7.49, d.f. = 2,30, P<0.002).

abatement district. Biorationals such as Bti and methoprene are the preferred materials.

Our studies on the VectoBaC® 12 AS formulation of Bti indicate that there is no negative impact on the survival of the endangered vernal pool tadpole shrimp even when there is 100% control of the target organism. These results suggest that Bti is a good candidate material for use in this particular sensitive habitat.

Other factors besides liquid Bti may explain any declines in shrimp populations at this location. Habitat loss which has imperiled vernal pool tadpole shrimp elsewhere (Holland and Jain 1988; USFWS 1994) should not be a factor here since this population is within Refuge boundaries. However, degradation of the mud substrate in individual pools due to plant growth has been observed to decrease other vernal pool species like fairy shrimp (Belk, pers. com.) and should be considered. In addition, Ahl (1991) found the fecundity was drastically reduced in *L. packardi* individuals that were parasitized by an echinostome fluke. Direct predation of shrimp by waterfowl has been observed by Refuge biologists (Albertson, pers. com.) and may reduce *L. packardi* populations. Finally, since the Warm Springs unit is surrounded by industrial areas and is near a major transportation corridor, the presence of some sort of environmental contaminant is also a logical possibility. The unexplained mortality of sentinel mosquito larvae in one control site which was not contaminated from treatment but was adjacent to lifeless pools that contained submerged pipes, supports the latter theory. Our attempts to reproduce the mortality of *Oc. dorsalis* larvae in control pool A1 via a laboratory bioassay using soil and water samples from this particular pool failed, but more extensive chemical testing, which was beyond the scope of our work, merits further investigation.

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