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Assess target and non-target effects of control operations on aquatic weeds, mosquitos, and other organisms to ensure efficacy while minimizing unwanted side effects

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Our goal is to develop knowledge that will inform aquatic weed managers, environmental resource managers, and mosquito control districts about how aquatic weeds and weed control affect mosquitoes and other elements of aquatic food webs. Public health workers need to understand how mosquito population dynamics respond to living and decaying weeds, so that they can target their efforts. Aquatic resource managers have a keen interest in the role of aquatic weeds as habitat for beneficial invertebrates that support fisheries and other wildlife, such as waterfowl and other riparian animals. Thus the project included mesocosm experiments on how living or herbicide-treated, decaying weeds influence mosquitoes and beneficial invertebrates, a large-scale experiment in the Delta that compared quantities of invertebrates in live and decaying water hyacinth beds, and we are currently executing toxicology tests on how herbicides affect mosquitoes, including their survival, growth, and resistance to pesticides used in public health.

Our first experiment in 2015 was performed with project leader Maribel Portilla. We tested the hypotheses that mosquito populations would differ between still water habitats that are either open, or with Brazilian water weed beds (*E. densa*), or with water hyacinth beds (*E. crassipes*), and that mosquitoes are expected to increase as a result of weed decay. We used artificial ponds constructed in 35, 1,325L tanks that were assigned to the treatments (5 replicates each): *E. densa, E. densa +* fluridone, *E. crassipes, E. crassipes +* glyphosate, substrate only, substrate +fluridone, substrate + glyphosate. Ponds included zooplankton from the Delta and insects colonized naturally for several weeks before herbicide application. Weed cover was at least 80%. We collected weekly samples for mosquito larvae and bi-weekly samples for other invertebrates. Mosquito larvae were present in all treatments. Repeated measures analysis of variance showed the results following (at statistical probabilities of 95% or better). Mosquito larvae were initially much more abundant in weed-free habitat than with either weed species. Herbicide treatment with glyphosate and an adjuvant (Agridex) initially suppressed mosquitoes. However, within four weeks mosquitoes rebounded in artificial ponds with decaying water hyacinth (Fig 1). Mosquitoes remained scarce in both treated and untreated *E. densa* tanks, likely because fluridone caused very slow mortality of the weed, and decay was not advanced enough to produce an effect by the end of the season. Beneficial invertebrates were generally scarcer in healthy water hyacinth treatments than in open water or with *E. densa*. This confirmed our hypothesis that decaying water hyacinth in low-flow areas could cause mosquito issues.

In 2016, we ran a second aquatic mesocosm experiment to assess the effects on mosquitoes of *Egeria densa* and its decomposition following herbiciding. Five mesocosms were assigned to each the following treatments: weedless controls, 80% *E. densa* cover, and 80% *E. densa* plus 0.137 ml fluridone sprayed after several weeks of insect and zooplankton population growth. We sampled mosquitoes and beneficial insects as in the earlier study. We also followed the survival and growth of *Cx. pipiens* mosquito larvae both inside of predator-exclusion cages following herbicide treatment. Results showed that there were initially fewer mosquitoes in healthy *E. densa* than in weedless controls, similar to the previous year's finding. Mosquito growth assays revealed that healthy *E. densa* dramatically suppressed mosquito growth, but there was a trend toward weakening of this effect in treated *E. densa* (Fig. 2). We are in the process of further data analysis.

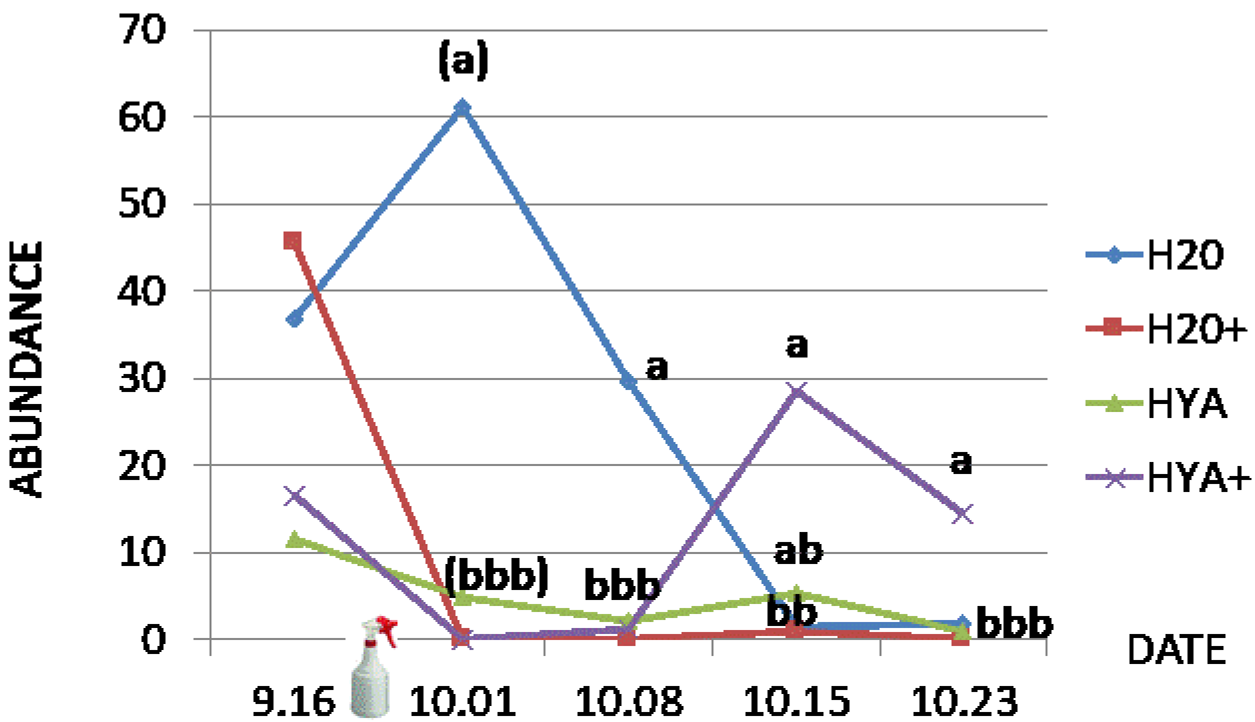
We collaborated with USDA on a large-scale field experiment in the Delta to assess the role of living and decaying water hyacinth as habitat for beneficial aquatic invertebrates. The project leader was former graduate student and USDA scientist Erin Donley Marineau. Five glyphosate-treated sites were paired with nearby untreated sites, and invertebrate densities were compared before treatment and 4 weeks after the date of treatment. Results showed that invasive water hyacinth provides habitat for macroinvertebrates and zooplankton. Suppression of water hyacinth with glyphosate herbicide did not cause an immediate loss of invertebrates. In fact, these increased substantially in decaying weeds during at least a month after treatment. The invertebrates likely benefited from the release of nutrients and edible detritus from the decaying water hyacinth. Results imply that judicious treatment of water hyacinth may not deplete invertebrates that serve as prey for fishes and other wildlife.

In current work we are following up on the finding that glyphosate plus an adjuvant caused mortality of larval mosquitoes. A question arising was whether glyphosate alone shows toxicity to mosquitoes. If so, it is possible that glyphosate could raise the mosquitoes' level of resistance to pesticides used in public health, as has been shown in *Aedes* mosquitoes. Project leader Maribel Portilla has confirmed that glyphosate does have some direct toxicity to larval *Culex pipiens* mosquitoes, albeit at rates over 10X higher than expected in most field applications. This raises the possibility that field levels may have sub-lethal effects that require exploration, because it is survivors of exposure that may ultimately promote resistance in the population. Ms. Portilla will expose replicated cohorts of second instar mosquitoes to glyphosate at realistic rates (1x or less of field rates), and unexposed mosquitoes will serve as controls. Half of the replicates will be subsequently be exposed to the larvicide B.t.i. in later instars, to test for upregulation of resistance.

Products to Date:

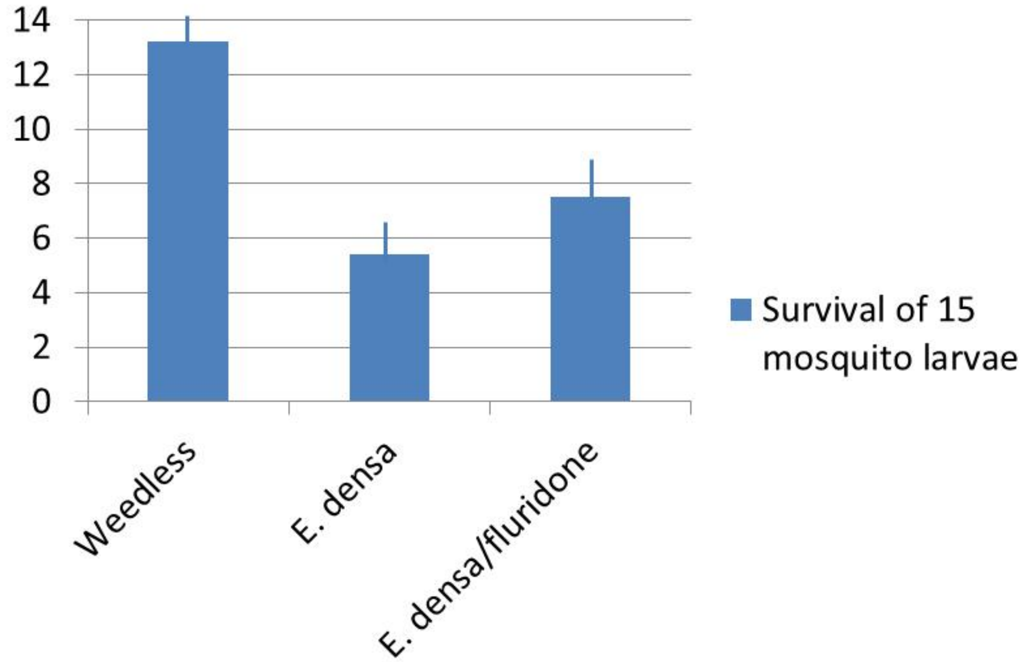
Dissertation: Marineau, Erin Elizabeth Donley. 2017. Aquatic Invertebrate Dynamics in Two Systems of Conservation Concern: Investigating the Roles of Macrophytes and Other Basal Resources. University of California, Davis.

Figure 1.



Abundance of mosquitoes in an experiment testing the effects of living and decaying aquatic weeds on mosquitoes, compared to weedless habitat. Bottle shows spray date. H_20 is weedless, + has glyphosate, HYA has water hyacinth. R-ANOVA on $L_{10}(n+1)$, Date X treatment F = 2.785, df 12, 64; $P_{GG} = 0.01$; single date ANOVAs/Tukey test significance shown by points with different letters.

Figure 2.



An experiment on *Culex pipiens* mosquito larvae held in predator exclusion cages in a mesocosm pond experiment showed that healthy *Egeria densa* inhibited their survival compared to weedless controls (ANOVA F 12.51, df 2, 12; P = 0.001). Bars show mean survival of 2 cages of 15 larvae $\pm$ s.e. There was a trend toward better survival in ponds with herbicided weeds than in healthy weeds.