

Intra- and interspecific interactions between two biological control agents and the consequences on the invasive water hyacinth, *Eichhornia crassipes*

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ABSTRACT

There is potential for negative, additive, or synergistic consequences on invasive pest control when multiple biological control agents are released in the same habitat. Here, I examine the intra- and interspecific interactions among the weevil, *Neochetina bruchi*, and the plant hopper, *Megamelus scutellaris*, and the implications for the biological control of water hyacinth, *Eichhornia crassipes*, in the Sacramento-San Joaquin River Delta, California. With a factorial design of intra- and interspecific species interactions, I used experimental mesocosms with whole plants in a temperature-controlled incubator and measured plant growth, survival and insect reproduction and survival. I also conducted observational experiments and examined behavioral interactions among the intra – and interspecific treatments on both plant petioles and on excised leaves, each in dark and light phases. In the whole plant experiments, leaf mortality and overall plant survivorship differed among the treatments. The treatments with interspecific interactions had the highest loss of leaves and plant death. I found notable consequences on life-history parameters, as *M. scutellaris* produced more offspring, but had reduced survivorship in the interspecific treatments compared to the intraspecific treatments. Under laboratory conditions, it appears that although *M. scutellaris* does not negatively impact *N. bruchi*, there is potential that *N. bruchi* may have negative consequences on the survivorship of *M. scutellaris*. The results demonstrate that although presence of *N. bruchi* could potentially affect the population growth of *M. scutellaris*, the combination of these two herbivores could enhance the biological control of water hyacinth.

KEYWORDS

behavioral interactions, *Neochetina bruchi*, *Megamelus scutellaris*, competition, California San-Joaquin River Delta

INTRODUCTION

The Sacramento-San Joaquin River Delta in California is a crucial water resource comprised of a series of rivers, sloughs, and man-made waterways infested with a myriad of invasive aquatic weed species (Spencer and Ksander 2005). Invasive aquatic weeds have negative ecological and socio-economic impacts on the Delta (Toft et al. 2003, DBW 2012). Major municipalities and agricultural businesses rely heavily on the Delta as a drinking water source and for farmland (DBW 2012, Spencer and Ksander 2005). As an ecological and recreational resource, the Delta is constantly in high demand, containing over one hundred marinas, serving as a popular recreational site, and a source of fresh water for a number of endangered native species and 26 million people (Greenfield et al. 2007). However, invasive aquatic weeds can inhibit many of these ecosystem services provided by the Delta.

Water hyacinth, *Eichhornia crassipes* (Mart.) Solms-Laubach, is one of the most detrimental invasive aquatic weeds and is a major issue worldwide (Firehun et al. 2015). The weed has the ability to infest areas quickly, making it very difficult to manage. Water hyacinth infestations can disrupt water flow, interrupt navigation, increase water loss through evapotranspiration, and disrupt ecological balances (Spencer and Ksander 2005). Current management of the aquatic weeds in the Delta, including that of the invasive water hyacinth, involves: application for herbicides, mechanical removal and shredding (Greenfield et al. 2007), and classical biological control (Stewart et al. 1988). Traditional control techniques are prohibited in some areas due to the risk of harming endangered species, such as the Delta smelt (Greenfield et al., 2006). Thus, other sustainable and low environmental-risk means of control are necessary to continue the management of water hyacinth.

Classical biological control is a pest management technique that uses the introduction of a natural enemy (predator, parasitoid, or parasite) to control pest populations (Van Driesche et al. 2010). It is an established control method that does not carry the negative impacts of traditional methods (Van Driesche et al. 2010). The Army Corps of Engineers released biological control agents for water hyacinth in the early 1980s (Stewart et al. 1988). They released: two weevils, *Neochetina bruchi* Hustache (Coleoptera: Curculionidae); and *N. eichhorniae* (Warner) and a moth, *Niphograpta albiguttalis* (Warren).

Although the moth has not performed at acceptable levels in the Delta, *N. bruchi*

populations are currently well established with densities varying spatially and temporally; however, *N. eichhorniae* populations are low in abundance, located just south of the legal Delta boundary (Hopper *et al. In Press*). These two weevil species have successfully reduced the cover of water hyacinth in other regions such as, Lake Victoria in East Africa, Mexico, Florida, Australia, and China (Julien M.H. 2001), but have yet to make a notable impact in the Delta (Hopper *et al. In Press*). To aid in the control of water hyacinth, another biological control agent, *Megamelus scutellaris* Berg (Hemiptera: Delphacidae), a plant hopper, was released in 2011-2013 in Folsom, just north of the legal Delta boundary (Moran *et al.* 2016) and was still established in 2016 (Hopper *et al. In Press*). Although these biological control agents are safe, as they only feed and survive on water hyacinth, their ability to reduce the cover and/or biomass of water hyacinth below economic thresholds has yet to be demonstrated (Hopper *et al. In Press*).

The recent release of *M. scutellaris* in the Delta also raises the potential of interspecific interactions and potential cascades of these competitive interactions onto the host plant (Ehler and Hall 1982). Direct effects from species interactions are important in mandibulate herbivores such as beetle larvae that make use of concealed niches and can be strongly correlated to body size and aggression (Weyl and Hill 2012). Temporal partitioning within the microhabitat of the system determines the effects of interspecific interactions (Bergallo and Rocha 1994) and ultimately the consequences on water hyacinth control. Because *N. bruchi* is a nocturnal species, *M. scutellaris* theoretically should be able to coexist, as it is a diurnal species (Foley *et al.* 2016). Niche differentiation would permit for the coexistence of *N. bruchi* and *M. scutellaris*. However, there are possibilities that these two biological control agents may influence each other as current research is lacking on the direct and indirect effects of these interspecific interactions.

The objectives of this study is to qualify and quantify the interactions between *N. bruchi* and *M. scutellaris* in pairwise combinations in a factorial design on whole plants, leaf petioles, and excised leaves, to understand the implications for the biological control program in the Delta. The study aims to determine: 1) how *N. bruchi* and *M. scutellaris* interact, 2) how intraspecific and interspecific species interactions affect survivorship and fecundity, and 3) how intraspecific and interspecific species interactions affect water hyacinth growth and survival. Since each species feeds at different times during the day with niche differentiation, I hypothesize that these two species will not have negative consequences on one another, and that there will be additive or synergistic negative effects on the water hyacinth (resulting in increased

control). The USDA is currently requesting permits for the release of *M. scutellaris* in additional areas of the Delta; thus it is critical to understand more about the interactions between *N. bruchi* and *M. scutellaris* (Personal communication with Patrick Moran Ph.D. USDA).

METHODS

Plant and insect colonies and maintenance

The original stock of water hyacinth plants maintained in the greenhouse came from Whiskey Slough in Stockton, CA (collected February 2015), Willow Creek in Folsom, CA (collected March 2016), Riverdale Park and Fishing Access in Modesto, CA (collected April 2016), and Bacon Island Road in Stockton, CA (collected May, 2016). Plants from the field were held in greenhouse conditions in 100 gallon tanks for at least three months prior to use. I used subsequent daughter generations that grew under these greenhouse conditions for experiments. In the greenhouse, air temperatures were set for heating at 71°F and cooling at 90°F, monitored via a HOBO (Onset Computer Corporation, Bourne, MA). Photoperiod was 14 hours (supplemented with LED lights), with ambient humidity. The water in the tanks was maintained at 70°F using 300W submersible aquarium heaters (Aqueon Products, Franklin, WI). Water quality was monitored using YSI Professional Plus (YSI Incorporated, Yellow Springs, OH). Water pH was maintained at around ~6, and nutrients were maintained around 4-5ppm for each NO₃N and NH₄N using added fertilizers (General Hydroponics Flora Series, GH Inc., Sebastapol, CA; RAW Nitrogen, NPK Industries, Medford, OR) and supplemented with 10% iron chelate (Grow More Inc., Gardena, CA).

N. Bruchi was obtained from three sites in the Delta: Buckley's Cove in Antioch, Maize Overpass in the San Joaquin Refuge, and Riverdale Park and Fishing Access in Modesto, CA and *M. scutellaris* was sourced from Willow Creek in Folsom, CA. Following collections, *N. bruchi* and *M. scutellaris* were reared in outdoor tanks up until two weeks prior to experiments, upon which they were transferred to a 25°C incubator with 16:8 LD and >60% humidity maintained with water tanks, and fed leaves of greenhouse raised water hyacinth plants.

N. Bruchi is a nocturnal species and *M. scutellaris* is a diurnal species. Both herbivores have four stages in their life cycle: egg, larva, pupa, and adult. The larval stage of *N. bruchi* has

first, second, and third instar stages before pupation with a total life cycle time of fifty days (Deloach and Cordo 1976). *M. scutellaris* has five instar nymph stages with a generation time of five weeks (Sosa et al. 2005). I used the adult stages for all experiments and both the brachypterous, (short-winged) biotype of *M. scutellaris* for all experiments. I collected male and female insects of both herbivore species from the rearing tanks and separated according to sex (Cabrera Walsh and Maestro 2014). I standardized the size within each species of all herbivores selected for experiments.

Experimental design

I conducted whole plant experiments in mesocosms in a laboratory incubator (SHEL LAB™ Incubators) at the USDA West Regional Research laboratory in Albany, CA. I put 40 clean, vegetative daughter water hyacinth plants, with an average mass of $13.4 \text{ g} \pm 5.6 \text{ g}$, in separate 1025 ml plastic containers (Fabrikal™) filled with ~250 ml of distilled water and secured with ventilated lids with white nylon mesh fabric to allow the air to circulate in the containers, while prohibiting insects from escaping.

Using a factorial design with whole water hyacinth plants, 10 containers held two female and two male *M. scutellaris*, 10 containers held two female and two male *N. bruchi*, 10 containers held both herbivore species with two females and two males of each species together, and 10 containers held no herbivores as a control treatment. I ran 20 treatments for approximately three weeks from 11-21-16 to 12-9-16 and another 20 treatments from 2-4-17 to 2-25-17, with weekly water changes for all experiments. I randomized all containers and kept them in a 25°C incubator with a 16:8 LD light cycle and > 60% RH.

Herbivore interactions and impacts on water hyacinth:

Prior to introducing herbivores into the experimental containers, I measured several plant parameters to determine plant growth and health over the course of the experimental period. After drying plants with a paper towel, I measured fresh weight (mg), using a standard digital scale (Veritas™ analytical scale). Following this measurement, I marked three leaves with different colored tape to track changes in leaf surface area and leaf damage throughout the

experiment. I measured height and width (cm) of each plant; surface area of each marked leaf, maximum root length (cm), and counted the number of leaves on each plant. I replaced adult insects every four days if they died (only necessary for *M. scutellaris*).

To determine the impacts on water hyacinth, I measured the plant parameters again after the ~three-week period. I calculated the change in plant parameters and calculated healthy and damaged leaf area from the height and width of each leaf and the number of scars respectively. I determined the change in total leaf surface area by adding up the area of the marked leaves and subtracting the total scar area. To calculate total scar area I counted all weevil scars and measured a few scars per leaf and determined to average scar area to be 3.14 mm.

To determine the effect of intra- and interspecific interactions on the life-history performance of the two species, I recorded survivorship by counting and replacing dead adult insects weekly and I measured fecundity. To measure fecundity, I removed and counted the number of *M. scutellaris* nymphs and all *N. bruchi* eggs and larvae at the end of the ~three-week period.

Observation experiments on intraspecific and interspecific behavior:

To further determine the interspecific and intraspecific interactions between the species, I conducted observational behavioral studies. As *N. bruchi* is nocturnally active, I used a red light bulb to observe the 30 clear plastic containers (1025 ml) with water hyacinth petioles and both insects for three hour time periods. I observed five *N.bruchi* + *N.bruchi*, five *M.scutellaris* + *M.scutellaris*, and six *N.bruchi* + *M.scutellaris* treatments all containing two adults of each species for this experiment. In addition, I observed the containers in normal diurnal (fluorescent lighting) conditions to account for the active times of *M. scutellaris*. For each light cycle period, the observational records included: 1) the number of encounters between *N. bruchi* and *M. scutellaris*, 2) the number of times the herbivores retreated or fell off the plant because of an encounter, 3) the number of times the herbivores hid from one another, and 4) the number of times each herbivore fed during the observational period.

As there was a lack of interaction in the initial observational behavioral study using whole petioles and large containers, I conducted a second smaller scale observational behavioral experiment in smaller arenas using clear plastic petri dishes (6 cm diameter) with each petri dish

containing an excised undamaged water hyacinth leaf, standardized in size. I used a factorial design method with four dishes containing two male and two female *N. bruchi*, four dishes containing two male and two female *M. scutellaris*, and four containers containing one male and one female of each species. For the treatments containing *N. bruchi*, I additionally recorded the fresh weight (mg) of all *N. bruchi* individuals (as a size estimate) and tagged them on their dorsal side with a small dot of nail polish (red, orange, white, or blue) to track individuals prior to the experiment. Individuals were tracked to determine whether big males and females mated more frequently, and/or whether they were more aggressive toward each other or towards *M. scutellaris*.

I acclimated all insects for one hour in their respective petri dishes under natural light conditions and then observed the insects for one hour under fluorescent light conditions and then allowed a one hour acclimation in red light and observed insects in dark (red light) for one hour. For each light cycle period, I additionally recorded: 1) the number of direct contacts and the type of contact (head to head vs. body contact), 2) the frequency that the insects were within a ~ 1 cm² radius proximity of one another, 3) the number of mating incidences, and recording which individuals were mating for *N. bruchi* using the color identifiers, 4) the frequency of feeding acts, 5) any other unique interaction (hiding, feeding, etc.), and 6) the number of feeding scars after the end of both experimental periods (to measure the accuracy of observations for *N. bruchi* feeding frequency).

Statistical analysis

I used generalized linear models (GLMs) in R version 3.3 2015 to analyze all data. GLMs are similar to an ANOVA procedure that used a least squares regression approach to describe the statistical difference between the variables described above. I used standard link functions for the GLMs and selected error distributions to best represent the measurement variables analyzed (Poisson for counts, binomial for proportions, Gaussian for non normal data). I used Gaussian and binomial GLMs to compare plant parameters across all treatments with a 95% CI. I log transformed the plant biomass, plant height, and the change in root length data and used a Gaussian family GLM to compare across treatments. I used binomial GLMs to compare insect survivorship and leaf mortality, and Poisson GLMs to compare insect offspring among the intra

and interspecific treatments. Non-significant interactions and parameters are not presented in the results. I used the lsmeans package for an additional statistical summary of those parameters that were statistically significant. To create figures, I used Microsoft Excel 2011 for all statistically significant parameters.

RESULTS

Plant performance

I compared plant parameters after three weeks among four insect combination treatments. Overall, some of the plant growth parameters of water hyacinth were significantly affected by the different intra- and interspecific treatments. However, plant height, plant biomass, total leaf area, and root length was not significantly different among the intraspecific and interspecific treatment groups. Plant height did not significantly differ among any treatment group (GLM (Gaussian), $df = 3$, $F = 2.7$, $P = 0.072$). Plant biomass did not significantly differ between treatments either (GLM (Gaussian), $df = 3$, $F = 2.6226$, $p = 0.0738$). Total leaf area did not significantly differ between treatments (GLM (Gaussian), $df = 1$, $F = 0.6959$, $p = 0.4205$). Root length did not significantly differ between treatments (GLM (Gaussian), $df = 3$, $\chi^2 = 52.73$, $F = 1.097$, $P = 0.3698$).

Leaf mortality (Fig. 1a) was statistically significant across all treatment groups. Each plant started with an average of 11.4 ± 2.2 leaves and the leaf count decreased by an average of 0.4 leaves ± 2.9 leaves. The control treatment did not lose any leaves, whereas the *M.scutellaris* intraspecific treatment lost an average of 0.714 ± 0.70 leaves, the *N.bruchi* treatment lost an average of 2.0 ± 2.4 leaves, and the combined treatment lost the most leaves, with an average loss of 4.5 ± 3.04 leaves (GLM (Poisson), $\chi^2 = 68.253$, $df=4$, $P<0.001^{***}$). The overall plant survivorship in the interspecific treatment was significantly lower than the other treatments (GLM (binomial), $df=3$, $\chi^2 = 8.0477$, $p = 0.04504$ *). All of the plants survived in the other treatments.

Insect performance

I compared insect survivorship, and the number of viable offspring produced was across the interspecific, two intraspecific and control treatments. Survivorship of *M. scutellaris* (Fig. 1c) was marginally higher for the intraspecific treatment with *M. scutellaris* than the interspecific treatment with the presence of *N. bruchi* (GLM (binomial) $df=1$, $\chi^2= 3.4278$, $P = 0.06$). The survivorship for *N. bruchi* was not significantly different between the intraspecific and interspecific treatments (GLM (binomial) $df = 1$, $\chi^2 = 2.655$, $P = .1032$), for all insects survived the duration of the experimental period.

The number of viable offspring produced by *M. scutellaris* (Fig. 1d) was significantly higher in the intraspecific treatment than the interspecific treatment with *N. bruchi* present (GLM (Poisson), $\chi^2= 128.25$, $df=1$, $p < 0.001^{***}$). *M. scutellaris* produced on average 27.0 more offspring when paired with *N. bruchi* compared to the *M. scutellaris* treatment. The number of viable offspring produced by *N. bruchi* was not significantly different among any treatment (GLM (Poisson), $\chi^2= -2.88$, $df= -1$, $P = .08$).

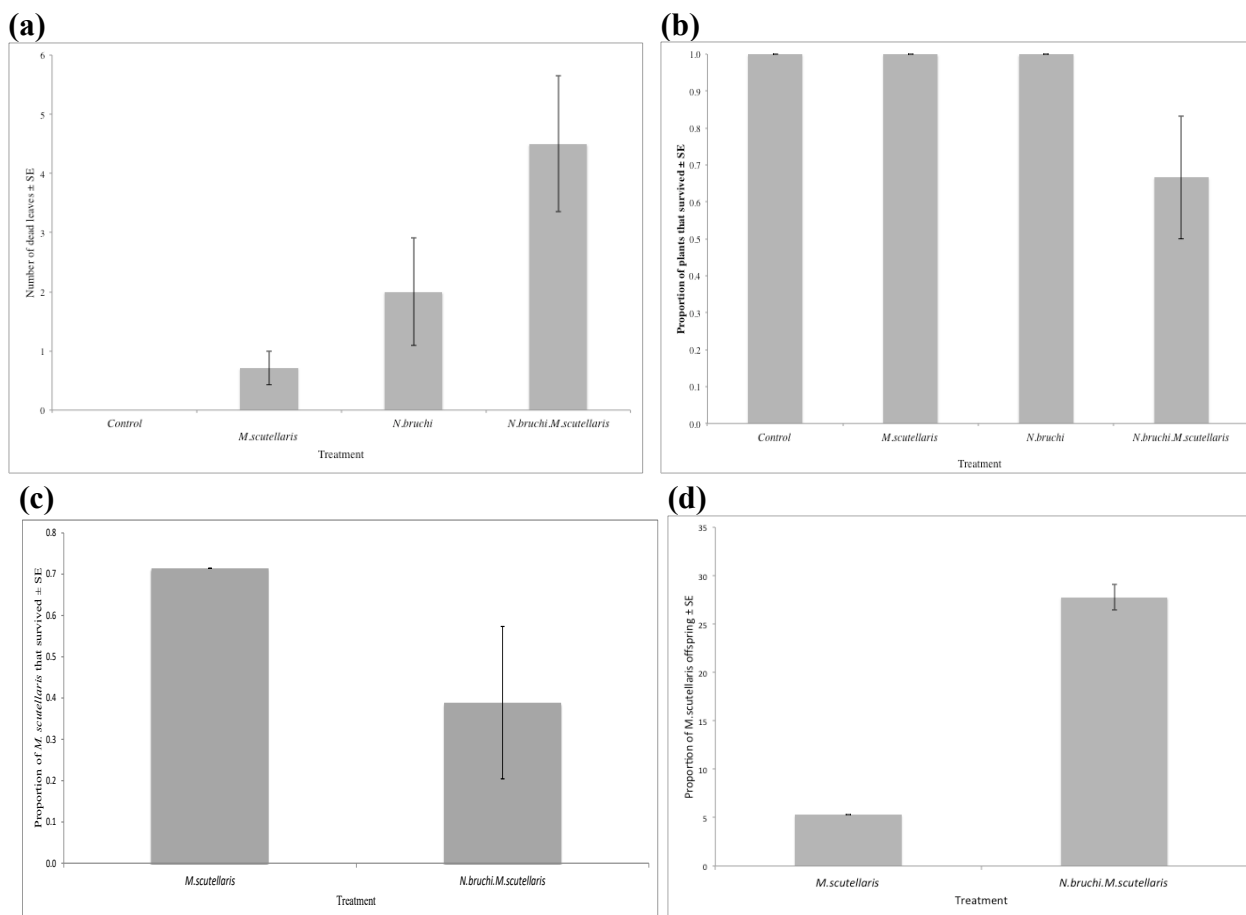


Figure 1. Bar charts of statistically significant parameters. (a) Number of dead leaves \pm SE compared between treatment groups after ~3-weeks. (b) The proportion of plants that survived \pm SE compared between treatment groups. (c) The proportion of *M. scutellaris* adults that survived \pm SE compared between the intraspecific and interspecific treatment. (d) The proportion of *M. scutellaris* offspring \pm SE compared between the intraspecific and interspecific treatments.

Behavioral observations of intraspecific interactions

In the first observational experiment using leaf petioles in medium-sized containers, the insects did not interact and they never shared a leaf surface at any point during the experimental period (N = 5). However, *N. bruchi* and *M. scutellaris* were within 5 cm proximity for 50% of the study period in both light phases. Both insects were more active during the red light phase than the fluorescent phase. *N.bruchi* had 2 more feeding occurrences during the red light phase than the fluorescent light phase. *M.scutellaris* insects moved around on the side of the mesocosm and on leaves together for 40% more time in the red light phase than the fluorescent light phase. *N.bruchi* individuals walked toward each other on 2 occasions in 2/5 *N.bruchi* intraspecific treatments and stayed within 1 cm proximity for the remainder (80%) of the observational period. *M.scutellaris* individuals walked toward each other on 3 occasions in 3/7 *M.scutellaris* intraspecific treatments and stayed within 2 cm proximity for the entire observation period during the red light phase. *N. bruchi* was not as active compared to *M. scutellaris* at either light period, except for a few instances of feeding during each of the lighting phases. There were an equal number of *N. bruchi* feeding occurrences in both lighting phases (2 feeding times, 2/7 intraspecific treatments).

Behavioral observations of interspecific interactions

In 90% of the time (N= 6), these species were observed at a distance of 5 cm from the other species in both light phases. There was only one interaction between *N. bruchi* and *M. scutellaris* during the red (dark) lighting phase. In 100% of the time, neither *N. bruchi* nor *M. scutellaris* shared the same side of the leaf. On the 2 occasions that they were both present on the leaf, they were on the opposite sides of the leaf. Occasionally I observed that *M. scutellaris* would advance toward *N. bruchi* on the opposite side of the leaf, but *M. scutellaris* would retreat immediately upon the visual cue of *N. bruchi*, and remained on the opposite side of the leaf for the rest of observational time period.

DISCUSSION

Plant parameters

In this study, I examined intra- and interspecific interactions among *N. bruchi* and *M. scutellaris* and the consequences of these interactions on the life history performance of these biological control agents, and on the growth, health and survivorship of the invasive water hyacinth, *Eichhornia crassipes*. I found significant effects of intra- and interspecific species interactions on plant growth and plant quality. Specifically, an average of 2.5 more leaves died in the interspecific treatment with both *M. scutellaris* and *N. bruchi* than in the control treatments without herbivores. Furthermore, the entire plant died in the interspecific treatment four times, whereas all plants survived in the intraspecific and control treatments. However, I did find an effect of interspecific interactions on the performance of *M. scutellaris*, as 213 more nymphs were present in the single species treatments compared to the interspecific treatments with *N. bruchi*. These negative consequences were limited to *M. scutellaris* as the number of *N. bruchi* eggs; however, did not significantly differ between treatments.

As the growth, health, and survivorship of water hyacinth was significantly lower in the interspecific treatment compared to the control and intraspecific treatments, biological control of water hyacinth control may potentially be enhanced if both species are present at the same time in the Delta. The results of this study suggest that combining the two agents may be better for the overall control project. This study also demonstrates that the presence of *M. scutellaris* does not affect the performance of *N. bruchi* as a biological control agent. As *M. scutellaris* is a diurnal species and *N. bruchi* is a nocturnal species (Heard et al. 2014), these two species are also unlikely to frequently interact or affect the feeding behaviors of one another in the field. However, I am unable to conclude whether *N. bruchi* affected the performance of *M. scutellaris* as a biological control agent since numerically one weevil results in considerably more damage to water hyacinth than one plant hopper. Furthermore, *M. scutellaris* is a small insect that does not eat as much as *N. bruchi*, but when it does, the feeding scars are not as harmful to water hyacinth as the feeding scars of *N. bruchi* (Weyl and Hill 2012).

Insect performance

Both *M. scutellaris* and *N. bruchi* were able to produce viable offspring by the end of the experimental period in both of the interspecific and intraspecific treatments. The number of *N. bruchi* eggs and larvae did not differ among the single species and the interspecific treatments. *N. bruchi* lays its eggs within the petiole and the larvae emerge and feed inside the plant, thus offering protection from predators and herbivores outside of the plant (Deloach and Cordo 1976).

M. scutellaris populations frequently died during the course of the experiment, and survivorship was lowest in the interspecific treatment. The insects also had an equal amount of time to reproduce in all treatments. It is likely that populations of *M. scutellaris* crashed because they are more fragile during handling (Tipping et al. 2011) and the laboratory conditions were not ideal for survival. It is unlikely; however, that the low survivorship impacted the interspecific interactions as *M. scutellaris* individuals were constantly replaced and consistently across all treatments (with and without weevils present).

The number of offspring produced by *M. scutellaris* was unexpectedly higher in the interspecific treatment with the presence of *N. bruchi*. The presence of *N. bruchi* may have affected the reproduction of *M. scutellaris*. It is possible that *M. scutellaris* eggs were placed in weevil feeding scars, so there is more overall area for plant hopper adults to lay eggs. It is also possible that *M. scutellaris* was stressed in the presence of *N. bruchi* and this could have caused the plant hoppers to reproduce more frequently.

Behavioral observations

N. bruchi and *M. scutellaris* interacted with one another; however, contact was infrequent, as the two species never shared the same leaf surface at any point in time during the observational periods. This could suggest a possible inhibition of performance as control agents for water hyacinth. Unexpectedly, *M. scutellaris* was more active during the dark (red light) phases than the fluorescent periods. This is contrary to current information, which states that *M. scutellaris* is a diurnal species (Foley et al. 2016). Although I did not find any adverse effects on water hyacinth plant growth or health, the behavioral observations imply that observed avoidance behavior may contribute to decreased performance of these biological control agents

when present together.

Study limitations

Study limitations included: 1) the negative effects of laboratory conditions on *M. scutellaris* survivorship, 2) the confinements of the small plastic containers and petri dishes which may have affected the results that differ from interactions occurring in the Delta, 3) the lack of variable abiotic conditions, 4) the time restrictions of the study, 5) the simplified plant measurements, and 6) insufficient replicates for robust data analysis. In the laboratory, the insects were confined to one plant/leaf as a food resource, whereas in the field it is likely that the insects would move to a new plant in the field instead of hiding from one another on the same plant. Furthermore, as the experimental set-up had consistent temperature conditions, abiotic factors may affect intra and interspecific interactions occurring in the Delta. Due to the short time frame of this study, the larva or nymphs, respective to the species, did not have a substantial amount of time to reach adulthood, which may have underestimated interactions that occur over time from differences in population growth of the two species in a longer time span. Limitations of plant performance parameters were apparent, as these parameters may not reflect water hyacinth growth under field conditions. In particular, plant biomass, and plant height were difficult to measure and these variables were not consistent in the results. It would be more useful to use dry-weight biomass as a parameter; however, this method was not feasible, as living plants were needed for species interactions to take place. Lastly, the entire experiment was also limited by the lack of sufficient replicate, so more data is necessary for more accurate conclusions.

Future directions

This study would benefit from further replication of the insect and plant performance experiments to make affirmative conclusions. In addition to more replication, a field experiment would be beneficial to determine how the interactions of these species affect the control of water hyacinth. This style of experiment would eliminate the effect of insect confinement and lack of variable conditions. Additionally, as the data demonstrates that the presence of *N. bruchi*

potentially affects *M. scutellaris* offspring survivorship, additional replicates of this experiment would be useful.

Broader implications

Understanding how *M. scutellaris* and *N. bruchi* interact is crucial for the future release of *M. scutellaris* in the Sacramento-San Joaquin River Delta. The results demonstrate that although presence of *N. bruchi* could potentially affect the population growth of *M. scutellaris*, the combination of these two herbivores could enhance the biological control of water hyacinth. The project concerning the biological control of water hyacinth continues in the Delta and the USDA plans to release more control agents soon. It is important to understand the implications of this project because it could be counterintuitive to release more plant hoppers in areas where the weevil is already well established; however, the results suggest otherwise. These results are useful for water hyacinth control projects worldwide that already are using or are considering the release of *M. scutellaris* and *N. bruchi* as control agents together. This study is another small step towards reducing the cover and biomass of the invasive water hyacinth and may lend valuable knowledge toward the sustainable control of additional invasive weeds.

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