



Megamelus scutellaris (Berg) (Hemiptera: Delphacidae) biology and population dynamics in the highly variable landscape of southern Florida

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HIGHLIGHTS

- *M. scutellaris* has established, dispersed, and is impacting control of *P. crassipes*.
- Wild populations of *M. scutellaris* are producing macropters and dispersing via flight.
- Dispersal has been documented from herbicide treated sites and across terrestrial habitat.
- New populations have reached an equilibrium density that does not change with more releases.

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ABSTRACT

Changes to the historical flow and nutrient levels of freshwater bodies in Florida have made control of waterhyacinth difficult. Biological control agents were introduced to augment herbicidal control of this plant. The newest insect agent, *Megamelus scutellaris*, was released in 2010, has established, and has been documented dispersing more than 6 kms away from release locations, across aquatic and terrestrial habitats. These insects were able to successfully disperse away from herbicide treated areas and across terrestrial habitats to reach new mats of waterhyacinth. Macropterous individuals were the first *M. scutellaris* documented at three non-release sites, indicating that the established wild populations are producing flighted individuals and are dispersing actively. Established populations appear to have reached an equilibrium density of 10.74 ± 19.74 *M. scutellaris*/m² and this does not fluctuate with additional releases.

1. Introduction

The quantity, quality, timing, and distribution of water is tightly controlled across the transformed Everglades landscape in Florida, especially in the region from Lake Okeechobee to the south. The management of this water provides irrigation, flood control, nutrient management, and preserves drinking water for metropolitan areas. Management was accomplished through an extensive system of canals, water control structures, pumps, culverts, and other structures that can move water from Lake Okeechobee to the Atlantic Ocean or Gulf of Mexico. Water can also be moved within the system to agricultural areas

and stormwater treatment areas (STAs) to water conservation areas, tribal lands, then to Everglades National Park, and finally Florida Bay (Izuno et al., 1999). Aside from large scale land use changes caused by agriculture and metropolitan development, water management is the primary abiotic restoration issue facing the Everglades (Sklar et al., 2005). Historically, water spilled from the southern edge of Lake Okeechobee and flowed slowly south through the original Everglades with seasonally changing levels and rates (Engstrom et al., 2006). In 1930, the Rivers and Harbors Act authorized the construction of more than 129 kms of dike around Lake Okeechobee, primarily on the southern edge, cutting off the original flow patterns. By the 1960s, this

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had expanded to over 225 kms of dikes and levees surrounding the lake (USACE). Now water flows in controlled canals with levels and rates that change frequently causing unseasonably high or low water levels across Lake Okeechobee and other connected bodies of water and resulting in significant modifications to the native ecosystem (Perry, 2008).

Human activities have also transformed the historically low nutrient greater Everglades environment into a more eutrophic system (Engstrom et al., 2006). This has posed a serious obstacle for the restoration of the Everglades and was the impetus for the 1994 Everglades Forever Act (EFA) with the goal of reducing phosphorus in the water to 10 ppm system wide (FDEP). The EFA made it mandatory for agricultural areas to mitigate nutrients being released into the canal systems, it also mandated the creation of STAs to clean the water before it is released south into water conservation areas and then to Everglades National Park (Izuno et al., 1999, Light, 2010).

Artificial water level changes and eutrophic water have exacerbated the challenges posed by invasive aquatic plants (Center and Durden, 1981, Reddy and DeBusk, 1984). One of the most problematic non-native invasive species is waterhyacinth (*Pontederia* [Eichhornia] *crassipes* (Mart.) Solms Commelinales: Pontederiaceae) which was introduced to Florida in the 1880s. *Pontederia crassipes* spread rapidly through the waterways and by the mid-1900s was a costly problem for shipping and transportation activities (Center, 1994). This species grows quickly in nutrient-rich water creating dense mats that can impede or block boat traffic and obstruct water control structures (Center et al., 2002). It became such a nuisance that the U.S. Army Corps of Engineers was given the permission to control it by any means necessary (Izuno et al., 1999, Rivers and Harbors Appropriation Act of 1899 [33 U.S.C. 403, Chapter 425, March 3, 1899; 30 Stat. 1151]). Effective herbicides approved for aquatic use became available in the 1940s and 1950s and have since been the primary management method for this species (Schmitz et al., 1993, Gettys et al., 2014).

Despite the repeated herbicide treatments that now commonly occur, *P. crassipes* populations rebound quickly and can double in biomass in less than two weeks (Reddy and DeBusk, 1984). In addition to vegetative growth, this species also reproduces from seed, which can remain viable in the hydrosol for up to twenty years (Matthews et al., 1977). In order to find a more sustainable solution other than the perpetual applications of herbicides, a classical biological control project was started in the 1960s (Center, 1996) resulting in the release of three species of insects, two beetles and one moth, that have established in the U.S. The first biological control agents were two weevils: *Neochetina eichhorniae* Warner (Coleoptera: Curculionidae) and *Neochetina bruchi* Hustache (Coleoptera: Curculionidae) which were released in the early 1970s (Perkins, 1973, Center, 1994), followed by a moth, *Niphograptus* (*Sameodes*) *albiguttalis* (Warren) (Lepidoptera: Crambidae), in 1977 (Center and Durden, 1981). Currently, the *Neochetina* spp. are common everywhere *P. crassipes* is found in Florida while *N. albiguttalis* is found primarily in the edges of expanding mats of *P. crassipes* (Tipping et al., 2014a). After these introductions, research on developing new agents for this weed largely ceased because of a lack of financial resources and the prioritization of new weed species.

Interference from herbicides has prevented the first three agents from achieving their maximum impact on *P. crassipes* because spraying rapidly reduces plant populations over a wide area. Both *Neochetina* spp. and *N. albiguttalis* have juvenile stages that remain within the petioles and roots of *P. crassipes* which die along with the plants, thereby preventing the buildup of insect densities necessary for suppression (Haag, 1986, Wilson et al., 2006). Despite this interference, these earlier agents do provide significant management benefits by decreasing biomass and seed production while increasing the susceptibility of the plant to herbicides (Tipping et al., 2014a, Tipping et al., 2017). Active integration is possible; Haag (1986), Haag et al. (1988), and Haag and Habeck (1991) modified the patterns and timing of herbicide applications to enhance the herbivory impacts from *Neochetina* spp. including varying the herbicide spray coverage and treating smaller areas sequentially. These

integrated strategies allowed for untreated *P. crassipes* to act as refuges for the insects, thus maintaining their populations at levels that could significantly impact the remaining, untreated plants and impede regrowth.

Research on new biological control agents resumed in 2007 and focused on *Megamelus scutellaris* (Berg) (Hemiptera: Delphacidae), a delphacid planthopper found in Argentina and Paraguay. All life stages of *M. scutellaris* beyond the egg stage are vagile, allowing them to disperse from herbicide treated areas as plants become unsuitable. Females predominantly oviposit on the isthmus between the petiole and the lamina of *P. crassipes* (Sosa et al., 2005) and eggs can continue to develop even after the plant begins to degrade due to leaf senescence or herbicide mortality (pers. obs.). Following oviposition, nymphs emerge 1–2 weeks later and are mobile enough to move between plants (Sosa et al., 2005) which allows them to abandon sprayed, dying plants and disperse to healthier ones. *Megamelus scutellaris* is also wing dimorphic whereby adults can be either macropterous (flighted) or brachypterous (non-flighted). However, the overwhelming majority of *M. scutellaris* that have been produced for field release in Florida were brachypterous (Fitzgerald and Tipping, 2013). Since its release in 2010, *M. scutellaris* has established persistent populations and dispersed into nearby areas (Tipping et al., 2014b). The objectives of the present study were to monitor the *M. scutellaris* population densities, dispersal rates, and to quantify its impact on *P. crassipes* populations across a highly managed landscape. Secondarily, experimental plots were established to assess the potential for an integrative plant management strategy using herbicides and biological control. (See Table 1)

2. Methods

2.1. Monitoring of release and control sites

Megamelus scutellaris were mass reared under laboratory conditions at the USDA-ARS Invasive Plant Research Laboratory (IPRL) in Ft. Lauderdale, FL to support the release of almost 3 million insects from 2011 through 2020. Planthoppers were deployed at release sites using both direct releases of adults on plants and by placing egg-laden plants within existing *P. crassipes* populations (Tipping et al., 2014b, Goode et al., 2019; Fig. 1). Experimental control sites where no releases occurred were located at varying distances from the release sites (indicated by white squares in Fig. 1). During 2017–2020, 12 release sites were established and paired with 12 control sites located 1–2 km from the paired release site. Control sites were monitored regularly for the presence of *M. scutellaris* following their assumed dispersal from release sites. Sites were chosen based on accessibility and plant species present and were monitored from boats or by wading in from the bank. To reduce the movement of the deployed egg-laden *P. crassipes*, sites were chosen with intermingled populations of non-floating plants such as cattail (*Typha domingensis* Pers.), dollarweed (*Hydrocotyle umbellata* L.), or alligator flag (*Thalia geniculata* L.). Two additional release sites without paired control sites were added in 2017. These sites were monitored for population fluctuations of both the *M. scutellaris* and plant community, persistence during *P. crassipes* control measures (herbicide treatments and water level changes), as well as local dispersal of *M. scutellaris* within existing mats of *P. crassipes*.

Monitoring surveys consisted of either ten samples of five plants, where five individual, adjacent *P. crassipes* plants were visually examined for the presence of *M. scutellaris* or counts were made of numbers in ten bucket samples (Fig. S1). The type of survey conducted was based on the site characteristics at the time of sampling, primarily water level, and they are considered to provide comparable estimates of insect numbers (pers. obs.). Bucket samples were used when water levels were sufficient to submerge the *P. crassipes* plants; if the water was too shallow, individual plants were sampled. Bucket samples were taken using a 24 L plastic bucket (39 × 26 × 26 cm) with the solid bottom removed and replaced with a 2.5 cm mesh screen (26 × 26 cm). The

Table 1

Primary *Megamelus scutellaris* release and control sites monitored. Native marsh (NM) – no significant population of *P. crassipes*; Invaded Marsh (IM) – significant population of *P. crassipes*; Terrestrial (T) – site became terrestrial habitat due to water level changes; Marsh/Open Water (M/OW) – site cycles between open water and marsh habitat due to water level changes, occasionally will contain *P. crassipes*. P = Present at last survey, A = Absent at last survey. The Lock 7 Pier site became open water after Hurricane Irma in 2017. The Clewiston site had dispersal early on and so an additional control location was added. “Days Till Dispersal” was not documented for the Lake Okeechobee locations because *P. crassipes* movement is frequent and the sites did not retain persistent populations.

Location	Site #	Release Location				Control Location				Distance	Days Till Dispersal
		Latitude	Longitude	Final Habitat	<i>M. scutellaris</i>	Latitude	Longitude	Final Habitat	<i>M. scutellaris</i>		
STA-1E	1	26.6681	–80.3315	IM	A	26.65953	–80.31538	IM	P	1.88 km	2219 days
STA-1W	2	26.6552	–80.4007	NM	A	26.65710	–80.39629	OW	A	0.48 km	
STA-2	3	26.4144	–80.5551	T	A	26.42023	–80.50010	IM	P	5.52 km	58 days
STA-3/4 West	4	26.385	–80.6362	IM	P	26.39244	–80.63617	IM	P	2.34 km	1377 days
STA-5 Central	5	26.4615	–80.9073	IM	P	26.46149	–80.89889	NM	A	0.8 km	861 days
Corkscrew Swamp Sanctuary	6	26.4246	–81.5786	IM	P	26.42084	–81.64294	IM	P	6.4 km	1013 days
Nubbin Slough	7	27.2137	–80.7234	IM	P	27.21364	–80.72559	IM	P	0.17 km	25 days
Lake Okeechobee, Clewiston Lock	8	26.7637	–80.9169	NM	A	26.76355	–80.89766	NM	A	1.91 km	
Lake Okeechobee, King's Bay	9	27.09290	–80.8443	NM	A	27.08685	–80.83228	NM	A	1.26 km	
Lake Okeechobee, Torry Island	10	26.7328	–80.70070	NM	A	26.71598	–80.70947	NM	A	2.05 km	
Lake Okeechobee, Lock 7 Pier	11	26.19560	–80.829	M/OW	A	27.16182	–80.85490	NM	A	4.53 km	
Lake Okeechobee, Clewiston	12	26.7606	–80.917	NM	A	26.81282	–80.99480	M/OW	A	3.2 km	
						26.81742	–81.02140	M/OW	A	6.39 km	

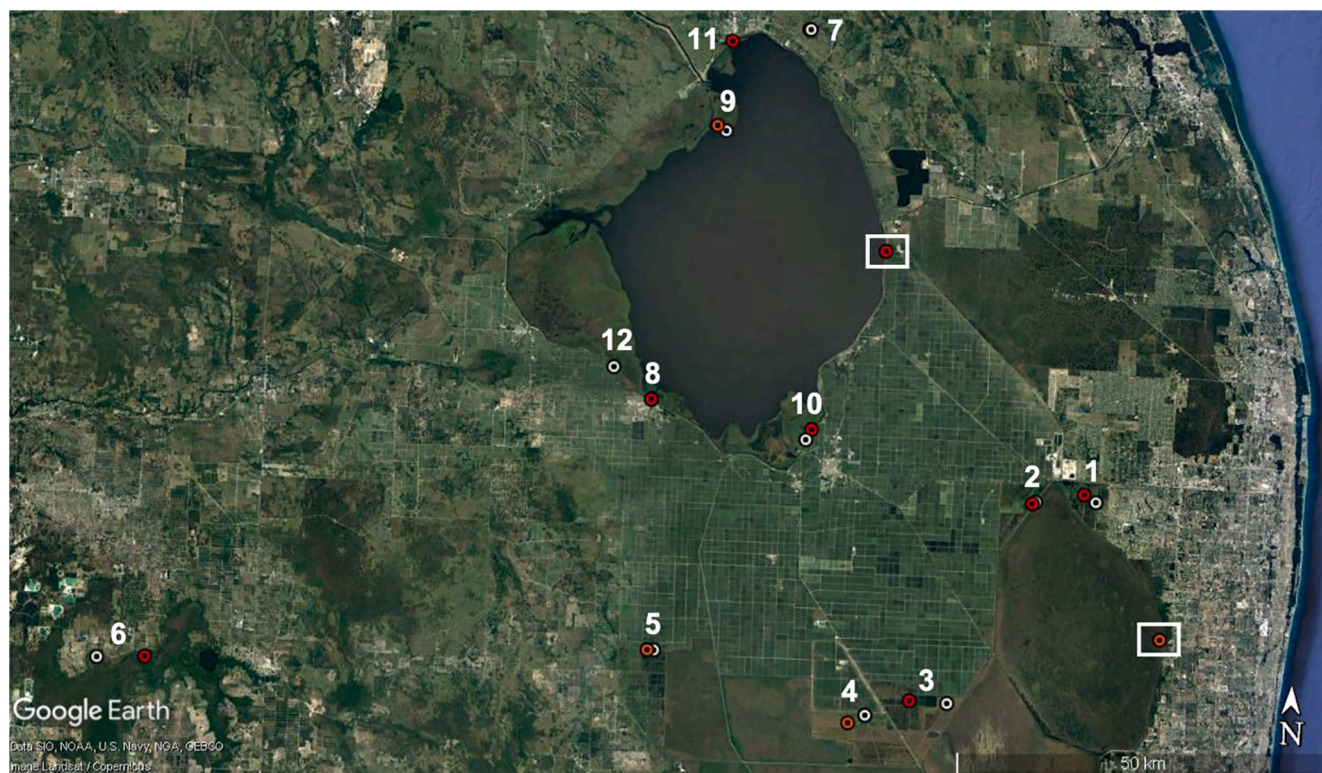


Fig. 1. Map of release and control locations. Sites identified by number in Table 1. Red points are release sites; white points are control sites. In some cases, points overlap due to proximity of release / control sites. White squares indicate release sites with no associated control site. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

bucket was then pressed mesh side down onto the plants. The screen submerged a known area of *P. crassipes* (0.07 m^2 , ~ 5 plants) forcing the insects off the plants and onto the sides of the bucket where they were identified and counted. Individual plants were sampled when water levels were too shallow for bucket samples. When possible, *P. crassipes* were sampled ~ 2 m apart. Otherwise, they were sampled evenly across

the available *P. crassipes* at the site.

With both of the direct sampling methods described above, the number of brachypterous and macropterous adults and nymphs were recorded as well as site characteristics such as plant community composition and herbicide treatment of *P. crassipes*. If no *M. scutellaris* were found at a site an indirect sampling method was used where

2–3P. *crassipes* plants were collected and returned to the lab. Plants were cleaned with insecticidal soap (Safer Insect Killing Soap, Safer Inc., Lititz, PA, USA) at label rate, rinsed with fresh water, placed in 24 L clear plastic containers with screened lids filled with 4 L fertilized water (Osmocote Plus 15–9–12 [ICL Fertilizers, Dublin, Ohio; 0.31 g per liter] and chelated iron [Sequestrene 330 Fe, BASF Corporation, Research Triangle Park, North Carolina; 0.02 g per liter]), and maintained under ambient lab humidity and temperature (range: 23–30.5 °C, average: 24.5 °C, 12:12 L:D). Plants were checked every 2–3 days for emergence of *M. scutellaris*, which were counted and removed, and sampling ended when no new emergence occurred over two consecutive sample dates. This indirect method was useful in detecting low densities of *M. scutellaris* or in cases where most of the population existed as eggs. It also detected *Kalopolytnema ema* (Schauff and Grissell) (Hymenoptera: Mymaridae), a parasitoid of *M. scutellaris* (Minteer et al., 2016) which is stenphagous on *Megamelus* species (Triapitsyn & Berezovskiy 2002) and whose presence is indicative of a *M. scutellaris* population.

Most of the original releases sites from 2011 to 2017 no longer contained *P. crassipes* by 2017 when the intensive monitoring period began because of water level changes or construction of berms in the areas. Although all 12 release/control site pairs monitored contained populations of *P. crassipes* and *M. scutellaris* initially, only five sites (Sites 1, 4, 5, 6, and 7) maintained persistent *P. crassipes* and *M. scutellaris* populations, while populations of *P. crassipes* in six other sites (Sites 2, 8, 9, 10, 11, and 12) disappeared. The last site (Site 3) dried out and was replaced by an upland plant community. The areas around the sites were frequently treated with herbicides and water levels were highly variable. These perturbances caused unpredictable and wide fluctuations in the *P. crassipes* populations. Analysis was conducted on data from six of the release/control pairs that were monitored from 2017 to 2020 and the two additional release only sites (indicated by white squares in Fig. 1). The other release/control site pairs (Sites 2, 8, 9, 10, 11, and 12) were not used in the analysis because they transitioned to habitats without *P. crassipes* within the first year of the study.

All statistical tests were conducted in R (version 3.6.1; R Core Team, 2019). The differences in density of *M. scutellaris* between sites was analyzed using generalized linear mixed models fit by the Adaptive Gauss-Hermite Quadrature (glmer function in R, lme4 package, Bates et al., 2015) using the logarithmic link function and location as a random effect. A generalized linear model was built using type of site (release or control), date, number of *M. scutellaris* released at that specific point on the sampling date (“released”), number of days since the last release event at that specific point (“days since last release”), number of *M. scutellaris* released on the sampling date immediately prior (“released prior”), and the grand total of *M. scutellaris* released at that point as predictive variables (“total released”). Total *M. scutellaris* density was log transformed to fit the model assumptions. The model was then fitted using backwards selection (drop1 function in R, lme4 package, Bates et al., 2015), which removes the variables which contribute the least to the model one at a time, until the lowest AIC is reached. The model was developed using the full complement of release data starting in 2011, then rerun for a subset from 2017 to 2020, during the routine monitoring period. Kruskal-Wallis rank sum tests were used to determine the impact of *P. crassipes* density on the presence of macropterous individuals (using the 2017–2020 dataset only) and to determine differences in *M. scutellaris* density based on site type.

2.2. Monitoring of refuge areas on Lake Okeechobee

An assessment of a potential integrated management strategy was conducted by creating insect refuges within treated areas of Lake Okeechobee using floating squares of 0.2 m diameter PVC pipe (1 m² in area) that were anchored into persistent mats of *P. crassipes* (Fig. S2). Squares in the north (N = 4) and south (N = 2) ends of Lake Okeechobee were placed in January and February of 2018. Locations were chosen away from popular boat channels to avoid collision risk, reduce the

effect of wave action on the squares, and to prevent vandalism. Squares were marked with 1 m tall PVC posts with flags to identify them to cooperating herbicide treatment crews who were usually able to avoid spraying them. The refuges and the adjacent areas were sampled every 4–6 weeks from February 2018 through February 2019 for densities and biomass of *P. crassipes*, and population densities of *Neochetina* spp. and *M. scutellaris*.

Initially, refuge squares contained exclusively *P. crassipes* and were set within larger, contiguous mats consisting primarily of *P. crassipes*. Over the course of the year, the contents of the refuges shifted to a mix of *P. crassipes*, *H. umbrellata*, and *Polygonum* spp. By December 2018, refuges were completely devoid of *P. crassipes* with only a few plants in the adjacent areas. Most sites were treated with herbicide twice during the monitoring period of February – December 2018, although not on the same day for all sites. Different herbicides and concentrations were used depending on the vegetation present including 2,4-D, penoxsulam, and flumioxazin. Although the refuges were generally left untreated, two of the sites and the refuges within were directly affected by an aerial herbicide treatment of flumioxazin in May 2018. Additionally, while all herbicide treatments were applied foliarly, there is the possibility that plants within the refuges were affected systemically, as has been seen in tank experiments (Goode et al. 2019). By February 2019, all sites were dry due to a very low lake stage (less than 4 m) and no longer contained any *P. crassipes* in or adjacent to the refuges.

Pontederia crassipes density was measured using a randomly placed ¼ m² PVC square and counting the number of plants within and *M. scutellaris* density was measured using two bucket samples. To determine *Neochetina* spp. density, five *P. crassipes* plants were collected, weighed, and placed in Berlese funnels for one week to obtain adults and larva. Plant material from the Berlese funnels was then dried to a constant weight to obtain dry weight (DW) biomass for use in statistical analyses and estimation of the final DW biomass within the refuges. After measurements were taken, adult *M. scutellaris* and egg-laden *P. crassipes* plants were released into the refuge (average = 2,355 *M. scutellaris*, range = 200–10,763 insects per release event). Efforts were made to ensure that the number of insects released were approximately equal for each release event across locations.

Data were analyzed using Kruskal-Wallis rank sum tests to look at differences between insect and plant populations inside and outside of the refuges. Additionally, Dunn multiple comparisons using the Benjamini-Hochberg method of *p*-value adjustment for multiple comparisons were used to indicate specific differences between sites. Paired Wilcoxon signed-rank tests were used to compare insect and plant densities within each location.

3. Results

3.1. Monitoring of release and control sites

Megamelus scutellaris dispersed from release sites and was detected at all control sites with persistent populations of *P. crassipes*. While most release/control site pairs were connected by water, in one instance, *M. scutellaris* was able to disperse to the control site that was separated from the release site by ≈6 kms of terrestrial habitat at the Corkscrew location. The fastest observed dispersal rate was 6.8 m day⁻¹. Macropterous adults were seen at all sites at least once. At three control sites, indirect sampling of *P. crassipes* detected the insects 29, 68, and 256 days before direct sampling did.

Megamelus scutellaris population densities across all sites and years ranged from 0 – 458.6 *M. scutellaris* m⁻² (mean ± SE = 23.0 ± 40.9 *M. scutellaris* m⁻²), with release sites routinely containing higher densities (38.1 ± 51.0 *M. scutellaris* m⁻²) than control sites (10.7 ± 19.7 *M. scutellaris* m⁻²; Kruskal-Wallis $\chi^2 = 17.99$, df = 1, *p* < 0.001). The model describing the population density of *M. scutellaris* was influenced by date, days since last release, number of insects released on the prior sampling date, the grand total released at each point, and site type

Table 2

Selected model for the density of *M. scutellaris* at field locations 2011–2020 (conditional $R^2 = 0.81$, marginal $R^2 = 0.57$).

Variable	Estimate	SE	z value	P
Intercept	1.03	0.12	8.74	<<0.0001
Date	-0.0006	0.0003	-2.23	0.026
Released	0.058	0.039	1.51	0.13
Days Since Last Release	-0.076	0.028	-2.76	0.0059
Prior	0.065	0.033	2.00	0.046
Total Released	0.43	0.055	7.74	<<0.0001
Site - Control	-0.39	0.07	8.75	<<0.0001

(Table 2). Further efforts to refine the model by restricting the model to 2017–2020, when routine monitoring occurred, found that removing the “released” variable improved the model (Table 3).

Reductions in *P. crassipes* densities caused by herbicide treatments during 2017–2020 influenced *M. scutellaris* nymph densities (Kruskal-Wallis $\chi^2 = 5.24$, $df = 1$, $p = 0.022$), with higher nymph densities found where *P. crassipes* density had been reduced (21.3 ± 58.6 *M. scutellaris* nymphs m^{-2}) compared to where density had not changed (17.1 ± 26.4 *M. scutellaris* nymphs m^{-2}). Reduction of *P. crassipes* density did not significantly influence the presence of macropterous *M. scutellaris* (Kruskal-Wallis $\chi^2 = 3.68$, $df = 1$, $p = 0.055$), with macropterous individuals occurring in 6.9% of surveys where *P. crassipes* density was reduced compared to 7.4% of surveys where *P. crassipes* density was unchanged.

3.2. Monitoring of refuge areas on Lake Okeechobee

When the refuges contained plants, *P. crassipes* density was higher inside ($44.3 \pm 39.7P. crassipes m^{-2}$) compared to outside ($35.1 \pm 28.7P. crassipes m^{-2}$) of the refuges (Wilcoxon signed rank test: $V = 264.5$, $p = 0.025$). *Megamelus scutellaris* density was also higher inside ($318.2 \pm 593.3 M. scutellaris m^{-2}$) compared to outside ($15.6 \pm 38.1 M. scutellaris m^{-2}$) of the refuges (Wilcoxon signed rank test: $V = 199$, $p = 0.0005$). Average densities of *M. scutellaris* on Lake Okeechobee at other release locations prior to extirpation of *P. crassipes* were lower still ($4.0 \pm 12.0 M. scutellaris m^{-2}$).

Pontederia crassipes densities within the refuges varied by date (Kruskal-Wallis $\chi^2 = 23.12$, $df = 13$, $p = 0.04$), with highest densities in the spring (March–June) along with *M. scutellaris* densities that peaked in the mid to late spring (April–June) (Kruskal-Wallis $\chi^2 = 26.17$, $df = 13$, $p = 0.016$). *Megamelus scutellaris* density per g DW biomass of *P. crassipes* g^{-1} varied by date (Kruskal-Wallis $\chi^2 = 28.88$, $df = 13$, $p = 0.018$), with highest densities in late winter to late spring (February–June).

Neochetina spp. densities (including adults and larva) were similar inside the refuges ($179.2 \pm 210.5 Neochetina spp. m^{-2}$, $1.1 \pm 2.5 Neochetina spp. DW biomass of P. crassipes g^{-1}$) and outside of the refuges ($184.0 \pm 153.6 Neochetina spp. m^{-2}$, $3.7 \pm 7.3 Neochetina spp. DW biomass of P. crassipes g^{-1}$) and did not vary by date. *Neochetina* spp. density adjacent to the refuges varied by site (*Neochetina* spp. m^{-2} : Kruskal-Wallis $\chi^2 = 14.75$, $df = 5$, $p = 0.011$; *Neochetina* spp. DW biomass of *P. crassipes* g^{-1} : Kruskal-Wallis $\chi^2 = 15.21$, $df = 5$, $p = 0.009$). The number of *Neochetina* spp. m^{-2} varied between sites 1 and 2 ($Z = 3.30$, $p adj. = 0.014$) and sites 3 and 2 ($Z = -2.73$, $p adj. = 0.047$). When

Table 3

Selected model for total density of *M. scutellaris* at field locations 2017–2020 (conditional $R^2 = 0.68$, marginal $R^2 = 0.60$).

Variable	Estimate	SE	z value	P
Intercept	1.14	0.067	16.93	<<0.0001
Date	-0.0006	0.0002	-2.31	0.021
Days Since Last Release	-0.14	0.027	-5.08	<<0.0001
Prior	-0.054	0.028	-1.94	0.053
Total Released	0.098	0.044	2.23	0.026
Site - Control	-0.49	0.057	-8.63	<<0.0001

adjusted for host plant biomass (computed on DW biomass of *P. crassipes* g^{-1}), *Neochetina* spp. densities also varied between sites, specifically, between sites 1 and 5 ($Z = -3.01$, $p adj. = 0.039$), 2 and 5 ($Z = -2.70$, $p adj. = 0.035$), and 6 and 5 ($Z = 2.9$, $p adj. = 0.027$). *Neochetina* spp. densities were not affected by *P. crassipes* densities adjacent to the refuges, which varied by site (Kruskal-Wallis $\chi^2 = 11.24$, $df = 5$, $p = 0.047$), specifically between sites 1 and 5 ($Z = 3.07$, $p adj. = 0.032$), with all others falling in between. Site 5 initially had a low *P. crassipes* density and, after an aerial treatment of the area on 24 May 2018, *P. crassipes* never returned to the site but was replaced by *Pistia stratiotes* L.

4. Discussion

Rigorously documenting the population dynamics of *M. scutellaris* on a landscape scale was challenging given the backdrop of intensive and confounding management activities, coupled with the significant environmental variation that occurred during this study. Despite these obstacles, it is clear that the most recent biological control agent released against *P. crassipes* in Florida has both persisted at field locations where *P. crassipes* is found (Tipping et al., 2014b) and has dispersed from those original locations across the landscape. This dispersal predominantly occurred in continuous water bodies with the insects either moving between patches or within continuous mats of *P. crassipes*, or by insects travelling with plants transported by water flow or boat traffic. However, in at least one location, dispersal occurred without any direct water connection between the locations, confirming the ability of *M. scutellaris* to disperse over or through terrestrial habitats.

The dynamic nature of the study sites was evident; many sites changed from invaded marsh habitats that were dominated by *P. crassipes*, to more native marsh communities, with one transforming into an upland plant community because of water level changes. The reasons for the decline of *P. crassipes* at these sites was not determined because of a lack of true insect presence or absence control plots, where insects could have been excluded using insecticide. The frequent herbicide applications would also have made it difficult to differentiate the effect of the insects in the system. However, the decline in densities of *P. crassipes* may have resulted from a combination of the herbivory effects of *M. scutellaris* and *Neochetina* spp. coupled with an intensive herbicide treatment program. The biocontrol agents have been shown to reduce both the biomass and reproduction in *P. crassipes*, while rendering the weakened plants more susceptible to herbicides (Tipping et al., 2017).

While adults have been documented moving several meters a day (Goode et al., 2019), and have been observed moving 15–20 m in the weeks following an herbicide application at a site on Lake Okeechobee, nymphs are unlikely to have the ability to move long distances quickly. When plants are treated and begin to degrade, adults tend to disperse to new mats, whereas nymphs tend to migrate to viable (untreated) plants within the same mat, increasing their local density. An indicator of metapopulation dispersal was provided by the refuge monitoring on Lake Okeechobee whereby *M. scutellaris* densities within the refuges were much higher than normally seen across the sites sampled on the lake. The PVC squares successfully contained the *P. crassipes*, resulting in higher plant density inside the refuges. The square also kept the released egg-laden plants from floating away, likely accounting for the higher density of *M. scutellaris*, and specifically nymphs. Reduction of local *P. crassipes* populations also affected the presence of macropterous individuals; fewer were detected in mats that had been reduced than those that had not changed. Other delphacid planthopper species have been known to produce more macropterous individuals at high densities, however in this study the differences were probably due to macropters dispersing away from impacted areas. This is another indicator of functional metapopulation dispersal, as herbicide treatments are constantly occurring across the range of *P. crassipes* in Florida.

The models developed from the release-control site data all indicate that *M. scutellaris* density decreases over the course of the year, and

while this and other studies (Foley et al., 2016) have shown that adult lifespan and fecundity change with temperature, the decrease seen in this analysis is more likely caused by *P. crassipes* fluctuations. Variation in *P. crassipes* density among refuge sites highlights the differences seen across the landscape where, within a small area, *P. crassipes* presence can vary widely. This variation in space was also seen in the *Neochetina* spp. density estimates. *Neochetina* spp. are very sensitive to plant quality. Adults are highly mobile and will disperse to other locations when the quality of the plants declines (Haag, 1986). The estimated *Neochetina* spp. density from this experiment did correspond with previous estimates for some locations in Florida from Tipping et al. (2014a) and Haag and Habeck (1991). The higher densities within the refuges indicate that these refuges may be useful in helping maintain biological control agent populations in locations where herbicide treatments occur regularly. The higher densities outside the squares at the same sites indicated that it is effective to release *M. scutellaris* at point locations since they will readily disperse throughout the mats.

Macropterous individuals were the first *M. scutellaris* documented at three control sites, and more were detected at control sites overall compared to release sites. This indicated that the established wild populations were producing macropters and dispersing via flight. Dispersal is an inherently risky endeavor as insects are exposed to increased predation and other risks which explains, in part, why densities at control sites were low. Macropterous individuals in other delphacid species are less fecund than brachypterous adults (Denno et al., 1989), so the F₁ generation at the new location is likely to be small, especially if only one mated female colonizes a new site.

Both the model that included all years (2011–2020) and the one that was limited to the intensively monitored years (2017–2020) had a similar trend of increased density as the total number of *M. scutellaris* released increased. The release strategy for this insect has been to deploy large numbers of adults and egg-infested plants together at a site, which creates waves of propagule pressure, maintaining higher planthopper densities over time and increasing dispersal (Goode et al., 2019). Additionally, the model from 2017 to 2020 did not contain the “released” variable and was significantly impacted by all other variables. When site types are compared, release site densities continue to increase as the total number released increased, whereas control site densities leveled off and were less affected by additional releases. Furthermore, as more *M. scutellaris* are released at a site, more disperse to surrounding areas.

Megamelus scutellaris was chosen as a biological control agent because of its mobility and its suspected ability to survive in a heavily sprayed environment (Sosa et al., 2005). Despite the disorderly nature of the local environments, *M. scutellaris* has established and dispersed. The control sites may provide a useful conservative estimate of the current field densities of *M. scutellaris* in Florida and may provide realistic guidance on the impact of their contribution to the overall biological suppression of *P. crassipes*. Additionally, since *M. scutellaris* has been released in other states (Grodowitz et al., 2014; Moran et al., 2016) and South Africa (Hill and Coetzee, 2017), these densities could be used as a benchmark for other monitoring programs to indicate establishment.

CRediT authorship contribution statement

A.B.C. Goode: Conceptualization, Methodology, Formal analysis, Investigation, Data curation, Writing - original draft, Writing - review & editing, Visualization, Supervision. **P.W. Tipping:** Conceptualization, Methodology, Formal analysis, Investigation, Data curation, Writing - review & editing, Supervision, Funding acquisition. **C.R. Minter:** Conceptualization, Methodology, Investigation, Data curation, Writing - review & editing. **E.N. Pokorny:** Investigation, Writing - review & editing. **B.K. Knowles:** Investigation, Writing - review & editing. **J.R. Foley:** Conceptualization, Methodology, Investigation, Writing - review & editing. **R.J. Valmonte:** Investigation, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.biocontrol.2021.104679>.

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