



# Classical biological control and apparent competition: Evaluating a waterhyacinth invaded community module

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## Funding information

USDA National Institute of Food and Agriculture, Grant/Award Number: 2016-67013-24860

Handling Editor: Juan Corley

## Abstract

1. The scope and complexity of interactions within community food webs necessitates their simplification to a community module scale for conducting empirical studies. An outdoor mesocosm study in the USA quantified the strengths of direct and indirect interactions between two herbivore congeners that fed on two aquatic plant species while sharing a parasitoid.
2. *Kalopolynema ema* (Hymenoptera: Mymaridae) is a stenophagous native egg parasitoid that attacks the hemipteran species in this study, *Megamelus davisii* (Hemiptera: Delphacidae), a native herbivore that feeds on the native aquatic species *Nuphar advena*, and the introduced biological control agent *Megamelus scutellaris* (Hemiptera: Delphacidae) that feeds on the non-native aquatic species waterhyacinth, *Pontederia crassipes*.
3. The presence of *M. scutellaris* did not significantly increase parasitism of *M. davisii* indicating that apparent competition was not a factor in this community module. There was no evidence of any trophic cascades caused by these interactions based on the relative growth rates of biomass and leaf area for both plant species.
4. *Synthesis and applications.* The relative strengths of community interactions varied with herbivore densities suggesting that should negative indirect effects with biological control agents occur they would likely be transient and closely linked to population dynamics as influenced by abiotic factors like temperature. Despite identifying potential negative interactions using path analysis, we saw no apparent competition following the insertion of a weed biological control agent into a community that contains an ecological analog. In this example, the primary negative interaction was biotic resistance to *Megamelus scutellaris* from attack by *Kalopolynema ema*. Adopting weed management tactics that emphasize efficacy, sustainability and environmental benevolence argue for the use of classical biological control when appropriate.

## KEYWORDS

apparent competition, biological control, indirect effects, *Megamelus davisii*, *Megamelus scutellaris*, *Nuphar advena*, waterhyacinth

## 1 | INTRODUCTION

Indirect interactions in community food webs are ubiquitous, complicated and occur simultaneously with direct interactions (Menge, 1997; Miller, 1994). Their sheer number and degree of complexity render them functionally untestable at scales beyond the level of a community module that may serve as a useful proxy to elucidate processes found in whole communities (Holt, 1997). Given that there are currently few, if any, truly pristine uninvaded natural communities, novel direct and indirect interactions are increasingly common as human impacts promote more introductions of new species into food webs (Gallagher & Carpenter, 1997; McEvoy, 2002). Non-native insects and plants now account for 2% and 21% of North American species respectively (OTA – U.S. Congress Office of Technology Assessment, 1993; Rejmánek, 2000).

Although the mechanisms that enable plant species to invade communities are debatable, the consequences are frequently negative, including a common scenario whereby plant communities experience reductions in the abundance and diversity of natives following invasion by a dominant non-native (Vilá et al., 2011). The most frequently cited cause for this dominance is a lack of top-down regulation by natural enemies like co-evolved invertebrate herbivores that did not accompany them to the adventive range (Keane & Crawley, 2002; Shea & Chesson, 2002). Subsequent and deliberate introductions of these host-specific herbivores into these degraded communities may restore them to a degree by suppressing the now dominant non-native enough for natives to compete more successfully and increase their densities (Van Driesche et al., 2010). Such attempts to restore some degree of sustainable balance to these invaded communities is the goal of classical weed biological control programs, a tactic with a mixed record of landscape level successes in natural systems (Crawley, 1989; Van Driesche et al., 2010).

The unpredictability of these novel interactions has been cited as a concern for using classical biological control despite an overall lack of evidence of significant or permanent harm to communities (Holt & Hochburg, 2001). Those studies that have documented or posited negative direct and indirect effects have been limited in scope, duration and scale (Carvalho, Buckley, Ventim, Fowler, & Memmott, 2008; Willis & Memmott, 2005). The most rigorously documented interactions have been direct, involving interference or biotic resistance whereby the impact of the introduced herbivore is muted, allowing the target weed to escape greater levels of suppression than intended (Ciomperlik, Chandler, & Deloach, 1992; Goeden & Louda, 1976).

An example of a worse-case invasion scenario is the aquatic weed *Pontederia (Eichhornia) crassipes* L. (Pontederiaceae) which continues to dominate wetland communities throughout the southeastern U.S. following its initial introduction in 1884 (Center, Hill, Cordo, & Julien, 2002). In Florida, more than 18,000 ha of *P. crassipes* and waterlettuce, *Pistia stratiotes* L. (Araceae), were sprayed in 2014–2015 in public waters at a cost of more than

\$5 million US (Phillips, 2015). Four insect biological control agents have been developed with the most recent agent, *Megamelus scutellaris* (Berg) (Hemiptera: Delphacidae), first released in Florida in 2010 (Tipping, Gettys, Minter, Foley, & Sardes, 2017; Tipping et al., 2014). Three biological control species namely *Neochetina eichhorniae* Warner (Coleoptera: Curculionidae), *Neochetina bruchi* Hustache (Coleoptera: Curculionidae) and *Niphograptus albiguttalis* (Warren) (Lepidoptera: Crambidae) (Center et al., 2002) were introduced previously but are vulnerable to herbicides which, by killing the plants, eliminates large portions of the internally feeding immature stages of their populations. *Megamelus scutellaris* was developed specifically to deal with the wide-scale use of herbicides because nymphs and adults feed externally and can move readily from sprayed to unsprayed plants (Tipping, Center, Sosa, & Dray, 2011).

External feeding by *M. scutellaris* likely promotes more novel interactions by increasing their vulnerability to generalist predators and stenophagous parasitoids like *Kalopolynema ema* (Schauff and Grissell) (Hymenoptera: Mymaridae) that commonly attacks the eggs of hemipteran species including the native congener *M. davisii* Van Duzee (Hemiptera: Delphacidae), a specialist herbivore on the native aquatic plant *Nuphar advena* (Aiton) W. T. Aiton (Nymphaeaceae) (Minter et al., 2016). *Pontederia crassipes* readily invades and dominates communities with floating leaves like *N. advena* to the extent where *N. advena*, and presumably its herbivores, are often locally extirpated (Center et al., 2002).

Few biological control projects have quantitatively evaluated the presence or impact of positive or negative indirect effects (Denslow & D'Antonio, 2005). Although apparent competition, a mutual and negative indirect effect between prey species, is the most frequently cited potential outcome of biological control, the strongest evidence of negative indirect effects is the replacement of the target weed with another weed species (Suckling & Sforza, 2014). The potential certainly exists as native parasitoids are known to utilize introduced agents that share similarities with native hosts (Paynter et al., 2010). Other studies have confirmed the presence of apparent competition, but these experiments were limited in scope and of short duration. For example, Carvalho et al. (2008) documented negative correlations in species richness and abundance between native seed herbivores and their parasitoids with the abundance of a biological control agent in a study that lasted 3 months. During the same time, they also found an unexpected positive effect of the agent on native plant abundance (Carvalho et al., 2008).

The goal of this research was to characterize and quantify interactions over time using an experimental community module based on native and non-native members of an aquatic community that encompassed two plant species (*N. advena* and *P. crassipes*), two herbivores (*M. davisii* and *M. scutellaris*) and one shared parasitoid (*K. ema*). Parasitism was assumed to be an important process in regulating the dynamics of the module and thus we examined two general questions: (a) was the parasitism of a native species influenced by the presence of a non-native ecological analog, and (b) what was the impact of parasitism on growth rate metrics of the host plants.

## 2 | MATERIALS AND METHODS

### 2.1 | Study site and design

The experiment was conducted at the USDA-ARS Invasive Plant Research Lab (IPRL) in Davie, Florida in outdoor concrete mesocosms filled with well water (1.6 m<sup>2</sup> surface area, c. 868 L in volume) and equipped with hinged screened frames to prevent insect escape or entry. The fine mesh nylon screens permitted c. 600 photosynthetic photon flux densities at noon inside the mesocosms. On 7 February 2017, one to two plants of *N. advena* and three plants of *P. crassipes* were placed in opposite ends of mesocosms that were divided equally by a 0.5-m wide PVC barrier designed to prevent interspecific plant competition while not impeding insect movement. Each mesocosm received a mixture of 300 g of slow release 18-5-12 fertilizer plus 18 g of iron applied at the beginning and mid-way through the experiment. Aquashade (Lonza Ltd.) was added once at labelled rates at the beginning of the experiment to reduce algae. *Nuphar advena* plants were fertilized every 90 days with 13 g of 10-14-8 fertilizer applied as water soluble pellets (Plantabbs) that were inserted into the hydrosol of the pots.

There were five insect treatments arranged in a completely randomized design with 10 replications (Table 1). The initial fresh weight (FW) biomass of *P. crassipes* was recorded and converted to dry weight (DW) biomass using an estimate of 96% moisture that was based on previous sampling. Separating the spreading, fibrous rhizome of *N. advena* from the hydrosol made it challenging to accurately measure whole plant biomass, so an allometric equation was developed to estimate below-ground biomass using leaf area which was estimated at the beginning and end of the experiment using the following formula to first estimate leaf area:

$$\text{Leaf area} = \left( \left( \frac{L_1 \times W_1}{2} \times 0.505 \right) \times 0.9432 \right) + 12.346 \quad (R^2 = .889), \quad (1)$$

where  $L_1$  is the length from the connection of the petiole to the leaf tip,  $W_1$  is the width at the widest point of the leaf. Measurements of

whole plant leaf area were then used to estimate the below-ground dry weight (DW) biomass of the rhizome using the formula:

$$\text{Below ground DW biomass} = 0.0783(\text{Total leaf area}) + 86.84 \quad (R^2 = .21), \quad (2)$$

where rhizomes were washed to remove soil and debris then dried for 36 hr at 55°C.

The first releases of *M. davis* and *M. scutellaris* were conducted on 17 February 2017 by placing five females and five males of each species directly on plants within assigned mesocosms. Adults were 1–2 weeks old and were obtained from IPRL colonies raised for this purpose. A second release was conducted 21 days later on 10 March 2017 to ensure the establishment of both herbivore species and to promote overlapping generations to ensure that eggs would be continuously present for the parasitoid. The first release of *K. ema* was done on 30 March 2017 by releasing 20 unsexed adults from laboratory colonies, followed by another release 7 days later to promote overlapping generations. The sex ratio for this colony is normally 50:50 (P.W. Tipping, unpubl. data). Two estimates of herbivore and parasitoid density and percent parasitism were conducted 50 days apart on 6 June and 25 July 2017.

Densities of *M. scutellaris* and *K. ema* on *P. crassipes* were estimated by selecting a single plant without bias from each mesocosm, counting and removing all *M. scutellaris* adults and nymphs and *K. ema* adults and placing them back into the mesocosm on other *P. crassipes* plants. Parasitism was estimated using the same plant by first weighing it to obtain FW biomass, briefly submerging it in a label rate solution of Safer soap (Woodstream Corporation) to eliminate secondary pests, rinsing it with water and then placing it in a 2 L plastic container containing fertilized water as above and a screened lid. The container was transferred to a laboratory and held under grow lights (c. 100 μmol with a 12-hr photoperiod) and constant temperature (mean range 23.8–24.3°C). Plants were examined every 3–4 days and all emerged stages of all species were counted and removed until there was no new emergence for two consecutive sample dates.

Treatment	<i>M. davis</i>	<i>M. scutellaris</i>	<i>K. ema</i>	Question
1	–	–	–	Insect control—no herbivory
2	+	+	–	Parasitoid control—impact of unrestricted herbivory on plants
3	+	–	+	Impact of <i>K. ema</i> on <i>M. davis</i>
4	–	+	+	Impact of <i>K. ema</i> on <i>M. scutellaris</i>
5	+	+	+	Impact of <i>M. scutellaris</i> subsidy on parasitism of <i>M. davis</i>

**TABLE 1** List of treatments in mesocosms involving combinations of *Megamelus davis*, *Megamelus scutellaris* and *Kalopolytnema ema*

Note: –, absence; +, presence in mesocosms.

Densities of *M. davisii* and *K. ema* on *N. advena* were estimated by selecting a single leaf without bias, counting all nymphs and adults of *M. davisii* and *K. ema* adults that were present, then placing them on other *N. advena* plants in the mesocosm. Individual leaves of *N. advena* could not be removed from the plant without wilting rapidly and killing the *M. davisii* eggs within. Thus, parasitism was estimated in situ by enclosing the selected leaf in a floating cage (Figure 1) that was removed every 3–4 days at which time all emerged stages of all species were tallied and released into the mesocosm. Egg mortality was not included in any calculation because of the difficulties in removing eggs that were inserted into plant tissues without damaging them. The cage was then placed back over the leaf and sampling continued as before until there was no new emergence over two consecutive sample dates. Parasitism for both *Megamelus* species was estimated using the formula:

$$\text{Number of emerged } K. \textit{ema} / (\text{Number of emerged } K. \textit{ema} + \text{Number of emerged } \textit{Megamelus} \textit{ spp.}). \quad (3)$$

The experiment was ended on 20 September 2017 (225 days duration) after which five *P. crassipes* plants were selected without bias, weighed for FW biomass, then dried to a constant weight at 30°C to determine percent moisture to estimate DW biomass. The remaining plants from the mesocosms were weighed to obtain FW biomass which was then converted to DW biomass using the mean percent estimate of moisture per treatment obtained from the previously subsampled plants. Mean relative growth rate (MRGR; g DW biomass day<sup>-1</sup>) was calculated for *P. crassipes* biomass using the formula:

$$\text{MRGR} = (\ln W_2 - \ln W_1) / (t_2 - t_1), \quad (4)$$

where  $W_1$  and  $W_2$  are the DW biomass at the beginning ( $t_1$ ) and end ( $t_2$ ) of the sampling period, and  $\ln$  is the natural logarithm. The number of leaves and the total leaf area of *N. advena* was measured and compared to the initial leaf area and number to estimate percent change over time.



**FIGURE 1** Floating cage enclosing one *Nuphar advena* leaf used to determine percent parasitism of *Megamelus davisii* by *Kalopolytnema ema*

## 2.2 | Statistical analysis

Percent parasitism and population densities of *M. davisii* and *M. scutellaris*, and the density of *K. ema* were analysed with a GLM with insect treatment as a fixed effect and sample date as a random effect (SAS Institute, 2009). The effect of treatment on plant variables was analysed with ANCOVA with the starting FW biomass or leaf areas as covariates with relative growth rates and change in biomass and leaf area as response variables. Single degree of freedom contrasts were used to examine differences among means between individual or group plant responses to selected insect treatments (SAS Institute, 2009). The relative influence of *M. scutellaris* on percent parasitism of *M. davisii* was examined further using stepwise regression with a significance level of 0.15 for inclusion into the model (SAS Institute, 2009).

Interactions and their strengths (effect sizes) were also examined using path analysis (Proc CALIS SAS Institute, 2009) which analyses correlations and tests how well postulated causal pathways fit the observed data in a modelling context (Quinn & Keough, 2002). In this case, the measure for interaction strength was provided via correlations (Wootton, 1994). This type of analysis does not confirm or disprove causal links; causality can only be demonstrated through empirical experimentation (Petraitis, Dunham, & Niewiarowski, 1996). However, this type of analysis permits the evaluation of complex linear models while also providing useful graphical representations of relationships (Mitchell, 1993). It deals with more complicated schemes that often have more than one dependent variable and considers the effects of dependent variables on each other, a process that might serve as a proxy for quantifying interactions within food webs. Given specific hypothesized relationships among variables, this method estimates the relative strengths of direct and indirect effects on those variables (Wootton, 1994). A hypothesized path diagram was created using the densities of herbivores and the parasitoid as well as percent parasitism of the herbivores on their respective plants using standardized path coefficients, both direct and indirect, that were calculated according to Mitchell (1993). The direct effect was measured using the standardized partial regression coefficient between  $Y_i$  (percent parasitism of *M. davisii* on *N. advena*) and  $X_j$  (herbivore and parasitoid metrics), holding all other predictor variables constant, while indirect effects are measured using the correlations between  $X_j$  and all other predictor variables, including those which may have direct effects on  $Y_i$  (Table 2). For example, direct and indirect effects for predictor variable 1 (percent parasitism of *M. davisii*) were calculated by:

$$r_{1Y_i} = b_{1Y_i} + r_{12}b_2 + r_{13}b_3 + \dots + r_{16}b_6, \quad (5)$$

where  $r$  represents simple correlations and  $b$  represents standardized partial regression coefficients. The direct effect is calculated by the first term  $r_{1Y_i} = b_{1Y_i}$ , while indirect effects are calculated

**TABLE 2** Direct, indirect and total correlative effects of *Megamelus* sp. and *Kalopolynema ema* on the parasitism of *M. davisii* for two sample dates

Variable	Sample date 1			Sample date 2		
	DE	IE	TE	DE	IE	TE
Density of <i>M. davisii</i>	-0.0441	0.4269	0.3828	-0.1466	0.4465	0.2999
Density of <i>K. ema</i> on <i>Nuphar advena</i>	0.7392	0.0461	0.7853	0.8598	0.0499	0.9097
Density of <i>K. ema</i> on <i>Pontederia crassipes</i>	-0.1914	0.3937	0.2023	0.0815	-0.0444	0.0370
Parasitism of <i>M. scutellaris</i> (%)	0.2972	0.0780	0.3753	-0.1101	0.0605	-0.0496
Density of <i>M. scutellaris</i>	-0.0814	-0.0727	-0.1541	-0.0458	0.0958	0.0499

Note: Direct effects are path coefficients.

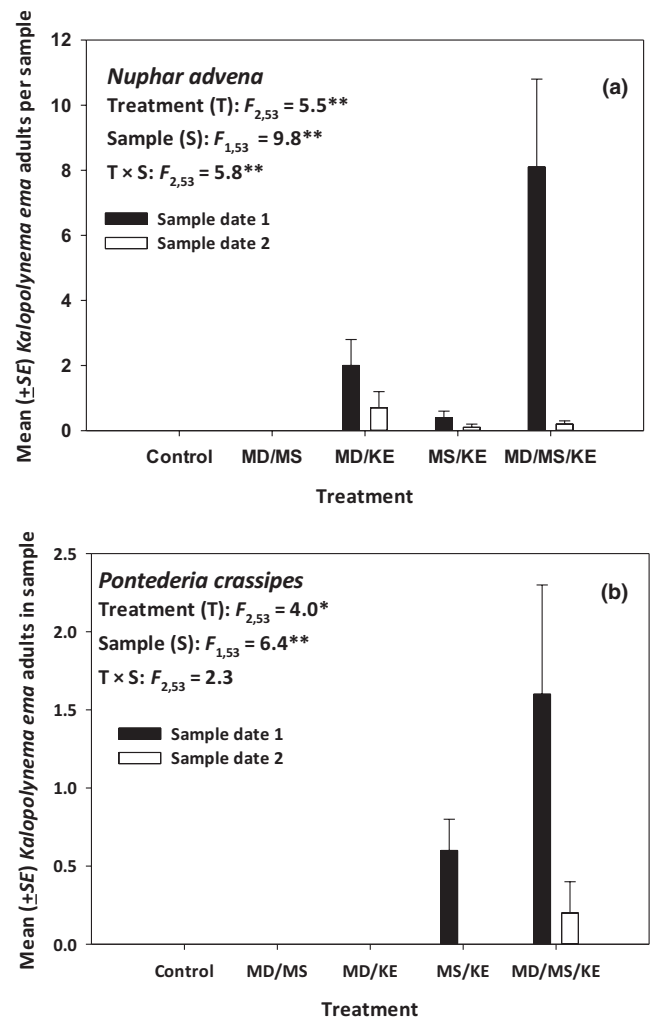
Abbreviations: DE, direct effect; IE, indirect effect; TE, total effect.

by the remaining terms in Equation (3). In this example, predictor variable 2 refers to the density of *M. davisii* on *N. advena*, predictor variable 3 to density of *M. scutellaris* on *P. crassipes*, and so on.

Several indices were used to assess the degree of agreement between the correlations in the data with those that would theoretically occur if the path diagram was correct. These indices included the comparative fit index (CFI) that measures the relative fit between a specified model and a baseline null model (Kline, 2005), the root mean square error of approximation (RMSEA) that evaluates the overall error in the population (Sun, 2005), and the standardized root mean square residual (SRMR) that calculates the standardized difference between observed and predicted correlations. Absolute fit indices like CFI determine how well the a priori model fits or reproduces the data. Relative fit indices like RMSEA compare the chi-square for the hypothesized model to one from a null or baseline model (MacCallum, Browne, & Sugawara, 1996). Standardized estimates of the covariances among herbivore and parasitoid metrics were calculated and *t* tests were used to test the null hypothesis that the corresponding covariances were equal to zero. In path analysis, the statistical significance of covariances among independent variables like herbivore and parasitoid metrics are usually of less interest than the predictive path coefficients but, in this case, these covariances may provide useful insight into the nature of these interactions and perhaps serve as a proxy metric for indirect effects within food webs, pending more empirical studies.

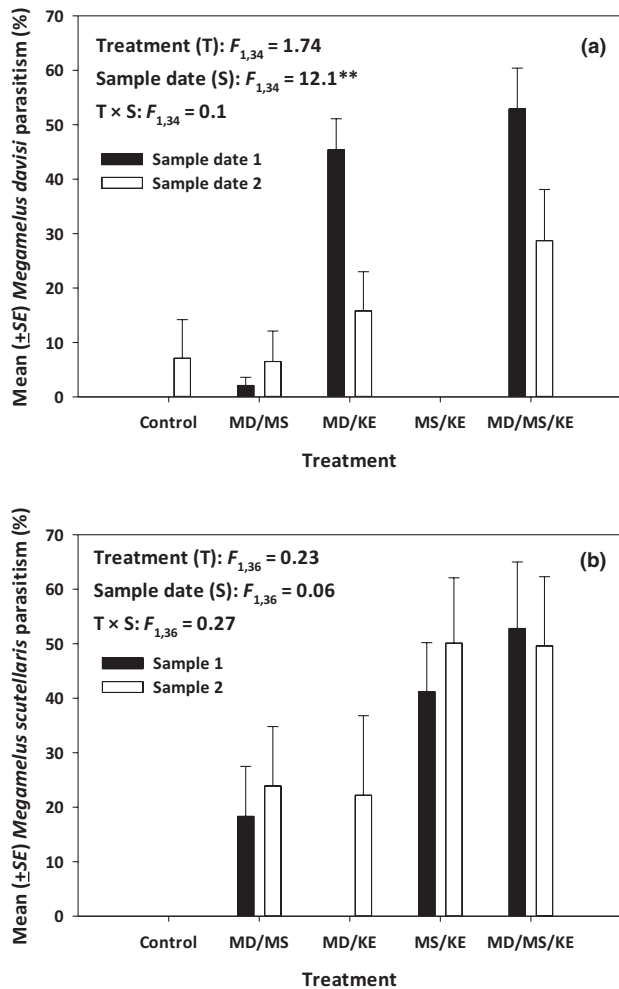
### 3 | RESULTS

The effect of sample date was significant for most variables, indicating that population densities of *M. davisii* (sample date:  $F_{1,52} = 20.6$ ,  $p = .0001$  [data not shown]), *M. scutellaris* (sample date:  $F_{1,54} = 18.5$ ,  $p = .0001$  [data not shown]) and *K. ema* (sample date:  $F_{1,53} = 9.8$ ,  $p = .002$  for *N. advena*;  $F_{1,53} = 6.4$ ,  $p = .01$  for *P. crassipes*) exhibited some degree of seasonality with their densities declining significantly from the first to the second sample date (Figure 2a,b). A few individuals of *M. davisii* or *M. scutellaris* were able to invade a few tanks near the end of the experiment (Figure 3a,b) but their influence on the specific treatment result was judged to be negligible.



**FIGURE 2** Mean ( $\pm$ SE) of number of *Kalopolynema ema* per sample on (a) *Nuphar advena* and (b) *Pontederia crassipes* in mesocosms. Treatments were: (1) Control—no herbivores; (2) MD/MS—*Megamelus davisii* and *M. scutellaris*; (3) MD/KE—*M. davisii* and *K. ema*; (4) MS/KE—*M. scutellaris* and *K. ema*; (5) MD/MS/KE—*M. davisii*, *M. scutellaris* and *K. ema*. The analyses are based on data from treatments 3, 4 and 5

The broader question of whether apparent competition occurred in this system found that the mean percent parasitism of *M. davisii* was not greater when *M. scutellaris* was present (treatment:  $F_{1,34} = 1.74$ ,



**FIGURE 3** Mean ( $\pm$ SE) percent parasitism of (a) *Megamelus davisii* and (b) *M. scutellaris* in mesocosms. Treatments were: (1) Control—no herbivores; (2) MD/MS—*M. davisii* and *M. scutellaris*; (3) MD/KE—*M. davisii* and *Kalopolyne ma*; (4) MS/KE—*M. scutellaris* and *K. ema*; (5) MD/MS/KE—*M. davisii*, *M. scutellaris* and *K. ema*. The analyses are based on data from treatments 3 and 5 for *M. davisii* and treatments 4 and 5 for *M. scutellaris*

$p = .19$ ) using data from treatments 3 and 5 (Figure 3a). Biotic resistance to *M. scutellaris* in the form of increased parasitism in the presence of *M. davisii* also did not occur (treatment:  $F_{1,36} = 0.23$ ,  $p = .63$ ) using data from treatments 4 and 5 (Figure 3b). Stepwise regression in the first sample date found that *M. davisii* density was the most important predictor of percent parasitism, followed by percent parasitism of *M. scutellaris*, and lastly by *M. scutellaris* density which was negative indicating that *M. davisii* parasitism declined when *M. scutellaris* parasitism increased (Table 3). There were no predictive variables for percent parasitism of *M. davisii* for the second sample date when overall densities were lower (Table 3).

The path analysis model illustrated in the path diagram (Figure 4a,b) provided a visualization of the interactions and their strengths observed for both sample dates. The CFI values of 0.99 for the first sample date and 0.95 for the second sample date, were greater than the value of 0.94 that Hu and Bentler (1999) considered to be suggestive of a good fit between the data and the hypothesized model

**TABLE 3** Stepwise regression of percent parasitism of *Megamelus davisii* by *Kalopolyne ma* with independent biotic variables at two sample dates

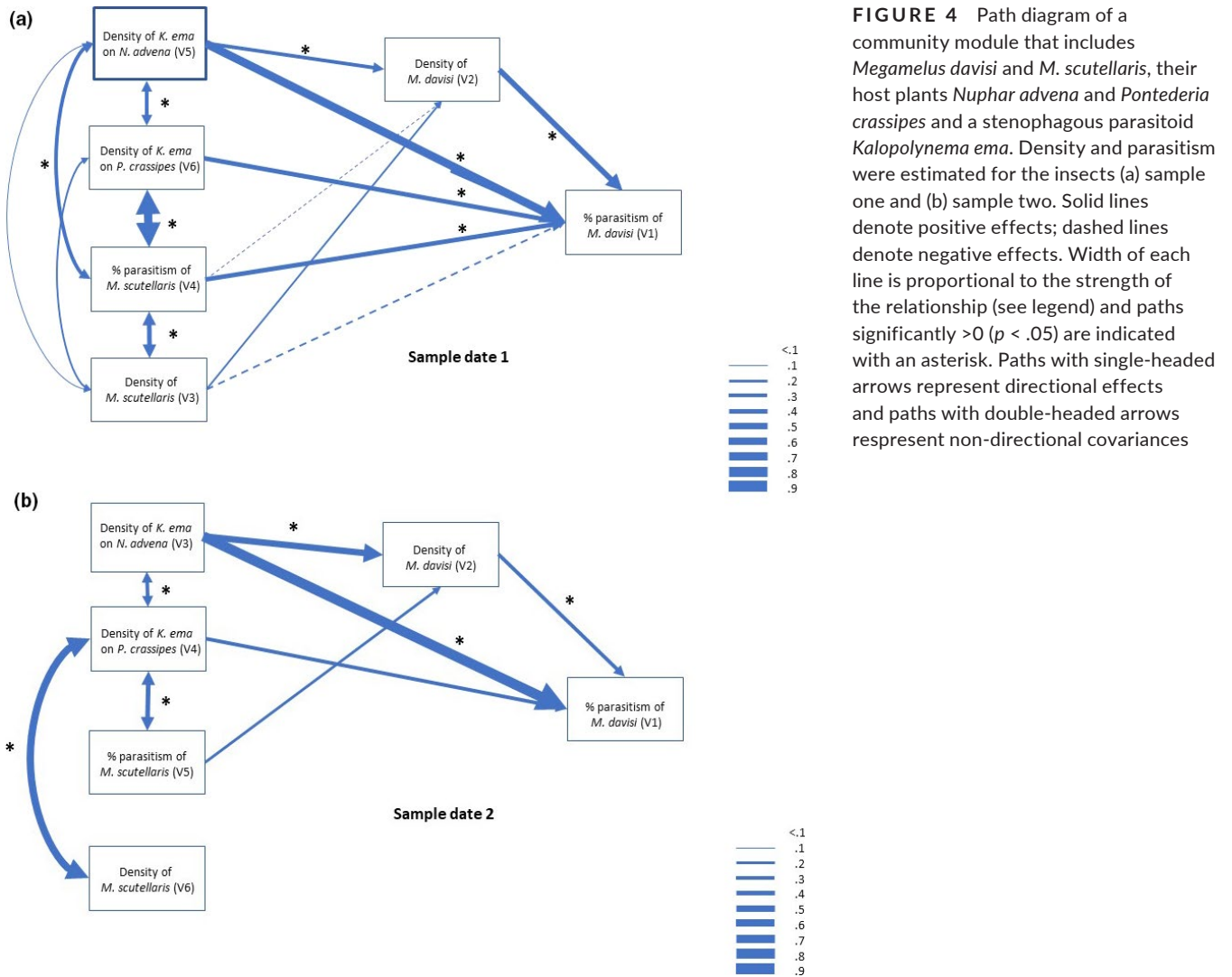
Dependent	Step	Independent variable	$r^2$	$p$	Slope
<i>M. davisii</i> parasitism					
Sample date 1	1	<i>M. davisii</i> density	.12	.015	0.004
	2	<i>M. scutellaris</i> parasitism	.22	.02	0.375
	3	<i>M. scutellaris</i> density	.331	.009	-0.023
Sample date 2	1	No variable met the 0.15 significance level for entry into the model			

Note: List of independent variables entered to select from: *M. davisii* parasitism, *M. scutellaris* parasitism, *M. davisii* density, *M. scutellaris* density, *K. ema* density on *Nuphar advena* and *K. ema* density on *Pontederia crassipes*. Presented are the explained cumulative variance ( $r^2$ ), the level of significance of each added variable and the slope.

(O'Rourke & Hatcher, 2013). The RMSEA of 0.043 was indicative of a good fit in the first sample date although RMSEA was 0.16 in the second sample date which indicated a poor fit (MacCallum et al., 1996). Finally, an absolute index, the standardized mean square residuals were 0.007 and 0.04 for the first and second sample dates respectively; values less than 0.055 suggest a good fit (Hu & Bentler, 1999). Taken together, these results indicated that the hypothesis that the path diagrams were a correct interpretation of the interactions and relative strengths present in this partial food web cannot be disproved (Mitchell, 1993). Although there were reasonably good fits between the models and the data with 63.4% and 69.1% of the variability in the percent parasitism of *M. davisii* explained by the variables in the regression equation for sample dates one and two, respectively, some caution is advised when assessing the magnitude of individual paths.

These assessments broadly corroborated calculated direct and indirect interactions with *M. davisii* density (Table 2). Disentangling the relative contributions of direct and indirect effects on percent parasitism of *M. davisii* indicated that, when densities of *M. scutellaris* were higher as in sample date one, there were increased interactions with *M. scutellaris* variables, despite the negative relationship that existed between *M. davisii* parasitism and *M. scutellaris* density. Further parsing direct and indirect effects using calculated values provided insight with the interaction between *K. ema* on *P. crassipes* and percent parasitism of *M. davisii* (Figure 4a) because, although the path diagram indicated a strong direct effect, the computed values consisted primarily of indirect rather than direct effects in the first sample date (Table 2).

Parasitoid interactions appeared to be more localized at both sample dates whereby *K. ema* densities were more likely to affect parasitism of herbivores on their respective host plants. Under the lower population densities present in sample date two, most of the direct and indirect effects on *M. davisii* parasitism were insignificant, the exception being the direct effects of *M. davisii* density and the density of *K. ema* on *N. advena* (Figure 4b). Although the links in the model were similar to those in the first sample date, the interactions in the

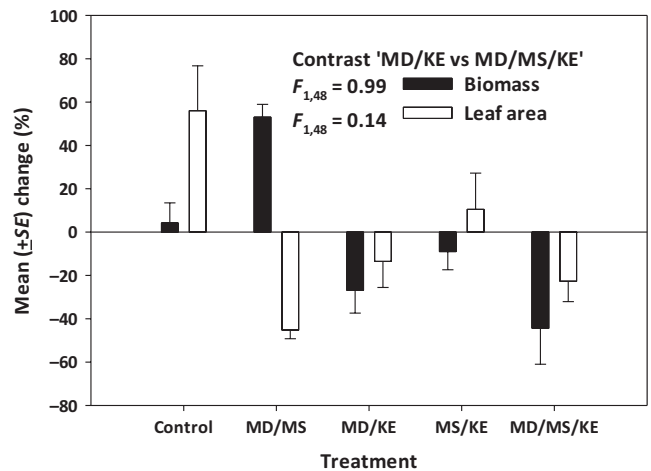


**FIGURE 4** Path diagram of a community module that includes *Megamelus davisi* and *M. scutellaris*, their host plants *Nuphar advena* and *Pontederia crassipes* and a stenophagous parasitoid *Kalopolynema ema*. Density and parasitism were estimated for the insects (a) sample one and (b) sample two. Solid lines denote positive effects; dashed lines denote negative effects. Width of each line is proportional to the strength of the relationship (see legend) and paths significantly >0 ( $p < .05$ ) are indicated with an asterisk. Paths with single-headed arrows represent directional effects and paths with double-headed arrows represent non-directional covariances

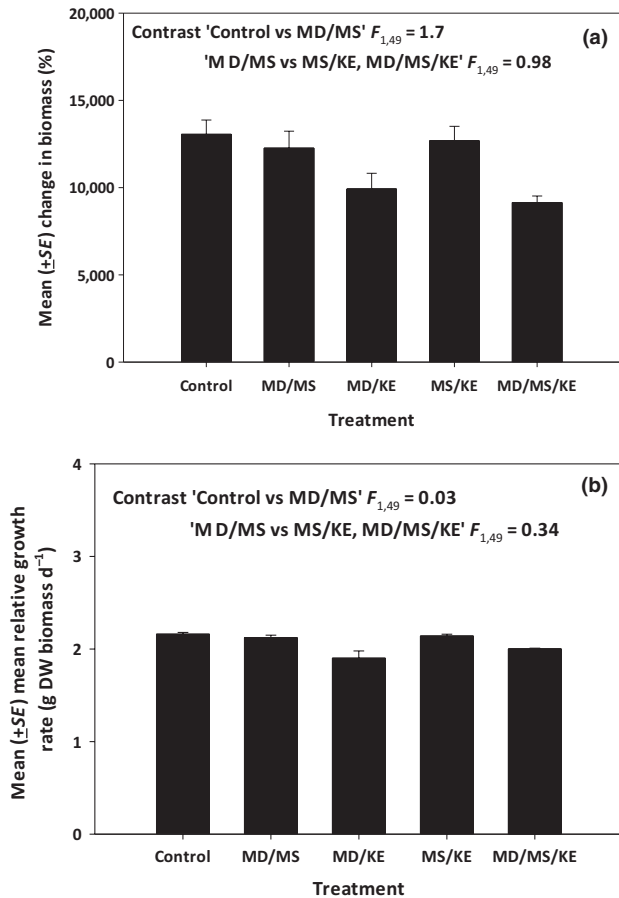
second sample date were weaker to the point of insignificance and the number of links was reduced. For example, there were 14 links in the first sample date, nine of which were significant versus eight links in the second sample date of which six were significant (Figure 4a,b).

There were no indications that trophic cascades were linked to potential apparent competition as evidenced by direct contrasts of the percent change in *N. advena* leaf area and biomass between treatments and groups of treatments (Figure 5), along with similar contrasts with *P. crassipes* (Figure 6). Although the presence of *M. davisi* with *K. ema* (contrast 'control vs. MD/MS, MD/KE, MD/MS/KE') ( $F_{1,48} = 15.7, p = .0003$  for *N. advena* leaf area) and without *K. ema* (contrast 'control vs. MD/MS') ( $F_{1,48} = 9.09, p = .004$  for *N. advena* biomass) did reduce leaf area and biomass, the addition of *M. scutellaris* (contrast 'MD/KE vs. MD/MS/KE') had no effect on either plant variable ( $F_{1,48} = 0.99, p = .32$  for leaf area;  $F_{1,48} = 0.14, p = .70$  for biomass) (Figure 5). Thus, any differences among the treatments in the percent change in *N. advena* leaf area and biomass were attributed primarily to the presence or absence of *M. davisi*, rather than the presence of *M. scutellaris*.

Unlike *M. davisi* on *N. advena*, the presence of *M. scutellaris* did not reduce *P. crassipes* biomass (contrast 'control vs. MD/MS')



**FIGURE 5** Mean ( $\pm$ SE) percent change in *Nuphar advena* biomass and leaf area following 225 days of exposure to different insect treatments in mesocosms. Treatments were: (1) Control—no herbivores; (2) MD/MS—*Megamelus davisi* and *M. scutellaris*; (3) MD/KE—*M. davisi* and *Kalopolynema ema*; (4) MS/KE—*M. scutellaris* and *K. ema*; (5) MD/MS/KE—*M. davisi*, *M. scutellaris* and *K. ema*. Specific contrasts examined the influence of *M. scutellaris* on biomass and leaf area



**FIGURE 6** Mean (±SE) percent change in *Pontederia crassipes* (a) biomass and (b) relative growth rate following a 225 days exposure to different insect treatments in mesocosms. Treatments were: (1) Control—no herbivores; (2) MD/MS—*Megamelus davisii* and *M. scutellaris*; (3) MD/KE—*M. davisii* and *Kalopolytnema ema*; (4) MS/KE—*M. scutellaris* and *K. ema*; (5) MD/MS/KE—*M. davisii*, *M. scutellaris* and *K. ema*. Contrasts examined the direct influence of the herbivore and the indirect influence of shared parasitism on plant metrics

( $F_{1,49} = 1.73$ ,  $p = .19$ ) (Figure 6a). The addition of *M. davisii* and *K. ema* with *M. scutellaris* (contrast 'MD/MS vs. MS/KE, MD/MS/KE') had no effect on *P. crassipes* MRGR ( $F_{1,49} = 0.34$ ,  $p = .56$ ) (Figure 6b), or with the change in biomass ( $F_{1,49} = 0.98$ ,  $p = .32$ ) (Figure 6a).

## 4 | DISCUSSION

Food web theory predicts that the non-random patterns of a few strong, along with many weak links within webs, will stabilize communities by buffering the effects of any one variable on the entire web and thus their cumulative impact at any one node is weak (Haydon, 2000; Neutel, Heesterbeek, & De Ruiter, 2002). The simple web within this module reflected this tenet and, coupled with the variable responses of the web constituents to temporal forces, may explain the lack of evidence of negative indirect interactions. This outcome provides additional evidence that despite the potential for unpredictable

novel interactions in assembled food webs, sufficient abiotic and biotic buffering may exist to restrain their ecological significance. The path model also provided additional insight by detailing reduced interaction strengths between the prey items during lower population densities, an outcome indicative of a more selective (stenophagous) parasitoid (Holt & Kotler, 1987). The declines in insect densities between sample dates were most likely caused by increasing temperatures over the course of the study; several members of the Delphacidae exhibit reduced survivorship at higher temperatures (Denno, 1994; Tsai & Wilson, 1986). During the early stages of the experiment, from mid-February through early June when the first sample was taken, the average daily temperature was 23.7°C. The mean daily temperature from the first sample date to the second sample date was 27.7°C.

We submit that the correlations documented in this study are analogous to indirect effects present within food webs. At the first sample date, the higher densities of *M. scutellaris* were negatively associated with percent parasitism of *M. davisii*, which argues against the presence of apparent competition between these herbivores (Figure 4a). However, *M. scutellaris* density was related to *M. scutellaris* parasitism that was, in turn, influenced by parasitoid density on its host plant *P. crassipes* (Figure 4a). Both of these latter two variables interacted positively with percent parasitism of *M. davisii*. These same interactions were not significant at the lower densities found at the second sample date (Figure 4b).

Mesocosm experiments generally inflate initial species densities, thereby causing larger effect sizes than do natural field experiments (Gurevitch, Morrow, Wallace, & Walsh, 1992). The densities and interaction strengths present in sample date one represented a version of the theoretic scenario of a high density, spill-over event following introductions of insect and weed biological control agents that is posited to cause transient to permanent damage to non-target species (Lynch, Ives, Waage, Hochberg, & Thomas, 2002). In this study, despite high densities of the non-native, there was neither damage to the native herbivore through increased parasitism via apparent competition, nor local extirpation. The lower densities at sample date two were closer to those found in the field, suggesting that the lack of negative indirect effects at this sample date would better predict outcomes in nature.

This study found no evidence of apparent competition despite the similarity of the congeners in this community module, an outcome that counters largely theoretical concerns of inevitable and permanent negative indirect effects on native organisms following the introduction of classical biological control agents for weeds. The most important finding of negative interactions was direct biotic interference from *K. ema* that may be reducing the negative direct effects of *M. scutellaris* on *P. crassipes*, although parasitism in the field is low (P.W. Tipping, unpubl. data) suggesting that indirect interactions between prey species may depend more on spatial scales. This might be influenced by patch type or size whereby different factors affect both prey choice between patches, and the choice of patches by parasitoids, resulting in interactions among prey species that are scale-dependent (Östman & Ives, 2003). The relative dominance of *P. crassipes* was very evident and, without the presence of a barrier, this plant would have likely overgrown and eliminated



both *N. advena* and *M. davisii*. Any significant biotic resistance to *M. scutellaris* at a community level will have negative implications for native species because it may ultimately increase the negative impacts of *P. crassipes* on both *N. advena* and *M. davisii*.

The lack of any trophic cascades to the producer level supports the theory that the effects of parasitoids become attenuated as they reach lower trophic levels (Shurin et al., 2002). Schmitz, Hambäck, and Beckerman (2000) posited that trophic cascades were dependent upon the strength of the direct effect of the herbivore on the plant, rather than the indirect effect of the parasitoid (predator) on the plant. Although only *M. davisii* significantly suppressed its host plant in this study, such impacts are likely dynamic and influenced by a host of biotic and abiotic factors that regulate their population dynamics.

Although classical weed biological control agents will always influence food webs to some degree, no single theoretical generalization predicts their community-level effects (Harmon & Andow, 2004). Apparent competition itself is theorized to lead to habitat partitioning, and thus ultimately to prey coexistence (Holt, 1984, 1997). The potential community-level outcomes from biological control programs should be scrutinized and evaluated but their ultimate effects, especially potential negative indirect effects, should be given their proper weight given the elemental direct and indirect effects created by both the wholesale transformation of ecosystems by invasive plants, as well as their subsequent management using tactics like the perpetual applications of herbicides. Indirect effects are widespread in natural systems, unique within communities and are dynamic processes whose ecological significance is debatable (Polis & Strong, 1996; Yodzis, 1988) and thus characterizing or quantifying interactions between species depends entirely upon spatial and temporal scales (Holt & Kotler, 1987). Given the need to manage already degraded natural plant communities, prioritizing the use of weed management tactics that emphasize efficacy, sustainability and environmental benevolence argue for the use of classical biological control when appropriate.

#### ACKNOWLEDGEMENTS

We thank Allissa Berro, Karen David, Eileen Pokorny, Brittany Knowles, Ryann Valmonte, Yuichi Sunni and Peter Martin for their assistance in establishing, maintaining and sampling in the experiment. This research was supported in part by the USDA National Institute of Food and Agriculture (2016-67013-24860 to P.W.T., M.C.S., E.C.L. and C.R.M.).

#### AUTHORS' CONTRIBUTIONS

All authors conceived the ideas and designed the methodologies; P.W.T., M.C.S., E.C.L., C.R.M., A.B.C.G. and J.R.F. collected the data; P.W.T. analysed the data and led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

#### DATA AVAILABILITY STATEMENT

Data are available via the USDA Ag Data Commons <https://doi.org/10.15482/USDA.ADC/1517659> (Tipping et al., 2020).

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**How to cite this article:** Tipping PW, Smith MC, Lake EC, et al. Classical biological control and apparent competition: Evaluating a waterhyacinth invaded community module. *J Appl Ecol.* 2020;57:926–935. <https://doi.org/10.1111/1365-2664.13593>