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Cricotopus lebetis intraspecific competition and damage to hydrilla

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Abstract

Cricotopus lebetis Sublette (Diptera: Chironomidae) is an aquatic insect adventive in Florida. Evidence from previous studies suggest the insect may have value as an augmentative biological control agent for hydrilla, *Hydrilla verticillata* (L.f.) Royle (Hydrocharitaceae), but there are gaps in knowledge of the biology and life history traits. To increase understanding of this insect, this study was comprised of 3 experiments. In the first experiment, we investigated the productivity and survival rate of *C. lebetis* across 6 generations by analyzing our colony data. Then the effect of intraspecific competition on the rates of pupal and adult eclosion was studied by monitoring hydrilla tips in test tubes with varying numbers of larvae. Finally, the level of hydrilla stem tip damage caused by a *C. lebetis* larva was determined by monitoring tip damage following feeding of a known number of larvae on a standardized number of hydrilla tips. The first experiment revealed the average survival rate of *C. lebetis* from egg to adult was 16.4%. Approximately a third of the females (30.2%) oviposited. The egg masses had an average of 154.5 eggs per egg mass and an 83.7% fertility rate. Investigation of intraspecific competition revealed pupation and adult eclosion was highest with 1 *C. lebetis* larva per hydrilla stem tip. Evaluation of the impact of *C. lebetis* larvae feeding on hydrilla showed stem tips in treatments with *C. lebetis* larvae experienced 38% higher damage compared to stem tips in control treatments. Overall, this study provided valuable information useful in improving the mass rearing of *C. lebetis* and predicting the damage caused by *C. lebetis*. For example, for efficient mass rearing, an average of 1 larva per hydrilla tip should be maintained with the remainder of eggs being used for augmentative releases.

Key Words: biological control; Chironomidae; *Hydrilla verticillata*; aquatic plant; augmentative

Resumen

Cricotopus lebetis Sublette (Diptera: Chironomidae) es un insecto acuático adventicio en la Florida. La evidencia de estudios previos sugiere que el insecto puede tener valor como agente de control biológico aumentativo para hydrilla, *Hydrilla verticillata* (L.f.) Royle (Hydrocharitaceae), pero hay vacios en el conocimiento de las caracteristicas de su biología e historia de vida. Para aumentar el conocimiento de este insecto, se realizó este estudio que consta de 3 experimentos. En el primer experimento, investigamos la productividad y la tasa de sobrevivencia de *C. lebetis* en 6 generaciones por el análisis de los datos de nuestra colonia. Luego, se estudió el efecto de la competencia intraespecífica sobre las tasas de eclosión de pupas y adultos por el monitoreo de las puntas de las hojas de hidrilla en tubos de ensayo con un número variable de larvas. Finalmente, se determinó el nivel de daño en la punta del tallo de la hidrilla causado por una larva de *C. lebetis* al monitorear el daño de la punta después de la alimentación de un número conocido de larvas en un número estandarizado de puntas de la hidrilla. El primer experimento reveló que el promedio de la tasa de sobrevivencia de *C. lebetis* desde el huevo hasta el adulto fue del 16.4%. Aproximadamente un tercio de las hembras (30.2%) ovipositaron. Las masas de huevos tenían un promedio de 154.5 huevos por masa de huevo y una tasa de fertilidad del 83.7%. La investigación de la competencia intraespecífica reveló que la pupación y la eclosión de adultos fueron más altas con 1 larva de *C. lebetis* por punta del tallo de la hidrilla. La evaluación del impacto de las larvas de *C. lebetis* que se alimentan de la hidrilla mostró que las puntas del tallo en los tratamientos con larvas de *C. lebetis* experimentaron un 38% de daño mayor en comparación con las puntas del tallo en los tratamientos de control. En general, este estudio proporcionó información valiosa útil para mejorar la cría en masa de *C. lebetis* y predecir el daño causado por *C. lebetis*. Por ejemplo, para una cría en masa eficiente, se debe mantener un promedio de 1 larva por punta de hidrilla con el resto de los huevos utilizados para liberaciones aumentativas.

Palabras Clave: control biológico; Chironomidae; *Hydrilla verticillata*; planta acuática; aumentativo

Hydrilla, *Hydrilla verticillata* (L.f.) Royle (Hydrocharitaceae), is an aquatic, highly invasive weed in the USA. Several insects have been identified that feed on the weed both in its invasive and native range (Winston et al. 2014); one such insect is a small chironomid midge, *Cricotopus lebetis* Sublette (Diptera: Chironomidae). Commonly called the hydrilla tip mining midge, *C. lebetis* was first discovered in the USA in Louisiana (Epler et al. 2000), was later observed feeding on hydrilla in Crystal River, Florida, USA, and is currently found in several waterbodies in Florida (Cuda et al. 2002; Stratman et al. 2013a). Larvae mine into the apical meristems of hydrilla, inhibiting vertical growth and surface mat formation (Epler et al. 2000; Cuda et al. 2002, 2011). Since its discovery, *C. lebetis* has been evaluated for its potential as an augmentative biological control agent for hydrilla (Cuda et al. 2016). Previous studies have focused on biology and impact (Cuda et al. 2002, 2011), host range and searching behavior (Stratman et al. 2013b), distribution based on temperature (Stratman et al. 2014), colony rearing

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techniques (Cuda et al. 2002; Stratman et al. 2014; Baniszewski et al. 2015, 2016; Mitchell et al. 2018), and its compatibility with chemical control (Cuda et al. 2016).

Successful release of biological control organisms relies upon mass rearing of a laboratory colony. For a colony to be considered productive there should be maximal output (i.e., organisms for release) with minimal input (i.e., labor and costs). Research to date has established techniques for rearing, and has conducted life table analysis (Cuda et al. 2002), determined the effects of temperature on eggs and larvae (Stratman et al. 2014; Baniszewski et al. 2015), tested tools to reduce pest problems (Baniszewski et al. 2016), and proven the importance of timing in releases (Mitchell et al. 2018). Although these efforts have improved the productivity of the colony, further research is needed on ecological interactions, such as intraspecific and interspecific competition.

Understanding competitive interactions between species is important in predicting the level of control that a biological control agent, such as *C. lebetis*, may impose on its target, in this case hydrilla (van Veen et al. 2006). Additionally, these same interactions may impact the productivity of a colony when mass rearing for release of biological control agents. The carrying capacity, or the maximum population size that an environment can sustain, is limited by space, food, and other necessities for that species. Intraspecific competition is the struggle among individuals of the same species for those limited resources, which leads to density dependence, where the population growth is dependent upon density of the species. Intraspecific competition among larvae of *C. lebetis* in a mass rearing system or field population, where populations could be high, may have negative impacts on population growth and long-term control of hydrilla. High intraspecific competition for resources such as food or shelter may impede larval development, thus limiting full potential of a colony or field population. Previous work has found intraspecific competition to have negative effects on development and survival in other dipterans (Frouz et al. 2009; Reiskind & Lounibos 2009). Therefore, it is important to assess the impact of intraspecific larval competition on the success of *C. lebetis* colony rearing and potential effectiveness of *C. lebetis* as a biological control agent.

Cuda et al. (2002) has described the feeding habits and performance of *C. lebetis* on hydrilla strains from Florida, USA; New Delhi, India; and Burundi. Stratman et al. (2013b) described the performance of *C. lebetis* in monoecious and dioecious biotypes of hydrilla. Both the strain (Cuda et al. 2002) and biotype of hydrilla (Stratman et al. 2013b) had significant effects on the survival rate of *C. lebetis*. However, it has not been determined how much damage an individual *C. lebetis* can cause, nor how it can perform in resource-poor environments. If hydrilla is sparse, for example, due to extensive feeding in the previous generation, a drawdown, or drought, yet many eggs are laid and neonates hatch, then larvae will be more prevalent than hydrilla stem tips. Field studies have demonstrated tip damage due to natural populations to reach 72% in the peak season (Cuda et al. 2002). The maximum larval abundance recorded was approximately 35 larvae per m^2 during the same time period (Cuda et al. 2002). Consequently, there is likely to be intraspecific competition between larvae for the limited supply of apical meristems naturally, and even more so during a release. The extent of this competition has not been studied. It would be beneficial to understand these competitive interactions for colony rearing and estimating the number of eggs and neonates to release into the field for greatest impact in biological control efforts. Therefore, the objectives of this study were to (1) determine the limits on productivity in a *C. lebetis* colony, (2) study the effect of intraspecific competition on productivity, and (3) estimate the average number of tips damaged per *C. lebetis* larva.

Materials and Methods

HYDRILLA TIP MINING MIDGE REARING AND COLONY PRODUCTIVITY

The hydrilla tip mining midge, *C. lebetis*, was reared on hydrilla collected from the University of Florida, Institute of Food and Agricultural Sciences, Center for Aquatic and Invasive Plants (29.72639°N, 82.41778°W) in Gainesville, Florida, USA, or shipped from the University of Florida, Institute of Food and Agricultural Sciences, Indian River Research and Education Center (27.426081°N, 80.408043°W) in Fort Pierce, Florida, USA. Hydrilla was cleaned and rinsed using well water to wash off unwanted insects or eggs. Hydrilla stem tips approximately 12 cm long were obtained, rinsed again, and sonicated to kill any organisms remaining on the stem tips (Baniszewski et al. 2015). Stem tips were subsequently transferred and maintained in 11.4 L Sterilite® plastic trays (39.7 cm L × 31.4 cm W × 15.2 cm H; Sterilite Corp., Townsend, Massachusetts, USA) containing well water, which was aerated with a 20 L aquarium aeration pump. These hydrilla containing trays were confined within a mesh insect cage (61 cm L x 61 cm W x 61 cm H) as described by Cuda et al. (2002). The water depth in the trays was approximately 10 cm; daily monitoring of the trays was conducted to ensure that plant material remained fully submerged.

Cricotopus lebetis was reared in a University of Florida, Institute of Food and Agricultural Sciences, Entomology and Nematology Department greenhouse (21–38 °C, 14:10 h [L:D] photoperiod). Multiple egg masses comprising 1,200 to 1,500 eggs were added into a 11.4 L Sterilite® plastic tray (Towsend, Massachusetts, USA) containing approximately 2,000 hydrilla stem tips submerged in well water. Up to 5 trays were used per generation and each was monitored for adult eclosion. Water levels were maintained above the plant material. Eclosed adults were collected using a mouth aspirator, and transferred to an oviposition chamber in the laboratory (23 °C, 21% RH, 14:10 h [L:D] photoperiod). The oviposition chamber was a 500 mL stop-cock separatory funnel (Fisher Scientific, Waltham, Massachusetts, USA) with 200 mL of filtered hydrilla-treated well water (Mitchell et al. 2018). Adults mated and laid eggs in the oviposition chamber; eggs were collected and counted 24 h later (Cuda et al. 2002; Mitchell et al. 2018), and were enumerated for fecundity and fertility under a dissecting microscope (8×; Zeiss Stemi DV4; Carl Zeiss, Berlin, Germany).

At the beginning of the rearing process, the number of eggs and hydrilla stem tips placed into each rearing tray and the source of the hydrilla stem tips were recorded. Trays were monitored daily after 12 d, the earliest time insects were expected to start emerging (Cuda et al. 2002). The number of eclosing adult males and females per d, and the total number of d that adults were eclosing from plant material were recorded. Additionally, data were collected on egg production, including the number of egg masses collected per d, egg mass fecundity (number of eggs per egg mass), and fertility (number of fertile eggs per egg mass, observed by embryo development within the egg).

Adult eclosion colony data were used to investigate relationships between the dependent variables of total adults eclosed, male to female ratio, and number of adults eclosed per number of initial eggs (% eclosed adults) with the independent variables of generation, eggs used per number of hydrilla stem tips, hydrilla origin, and adult eclosion duration. Total number of adults eclosed and sex ratio data were log-transformed to achieve normality. Adults eclosed per number of eggs added per tray (% eclosed adults) and eclosion duration were square-root-transformed to achieve normality. Generalized linear models were conducted in R Statistical Software using Type II analysis of variance (ANOVA) (R Core Team 2018). Best fit models were selected using Bayesian Information Criterion values.

Egg production colony data (i.e., egg mass fecundity and fertility) were analyzed with Type II ANOVA in R Statistical Software (R Core Team, 2018). Egg mass fecundity and fertility data were considered for both the average number of eggs per egg mass, and the total number of eggs per generation. All data required log-transformation to achieve normality except for egg mass fertility. Independent variables considered generation and number of egg masses per d. Best fit models were selected by using Bayesian Information Criterion values.

INTRA-SPECIFIC LARVAL COMPETITION

For intraspecific larval competition experiments, egg masses were collected from the oviposition chamber (rearing described above), assessed for egg mass fecundity and fertility, and larvae were allowed to hatch. After hatching, individual larvae were carefully removed using a glass pipette under a 2.5× dissecting microscope (Zeiss Stemi DV4; Carl Zeiss, Berlin, Germany) placed in a 35 mL test tube with a single 3 to 5 cm long processed hydrilla stem tip and 30 mL of well water. Ten test tubes were set up for each treatment of 1, 2, 3, or 4 larvae added to 1 stem tip of hydrilla. Test tubes were placed in an incubation chamber (26 ± 0.5 °C, 14:10 h [L:D] photoperiod). Larval development, pupation, and adult eclosion were monitored over 21 d. This experiment was replicated 3 times with each replicate treated as a block in the statistical models.

Number of pupae and eclosed adults per treatment, as well as percent pupation and eclosion (emergence efficiency) calculated from the total number of larvae placed in the tube, were analyzed with Type II ANOVA using R Statistical Software (R Core Team, 2018). Both percent pupation and eclosion were log-transformed to achieve normality.

HYDRILLA TIP MINING MIDGE DAMAGE

From 22 Jun through 18 Aug 2015, hydrilla was collected as needed for experimentation from a man-made pond at the University of Florida, Institute of Food and Agricultural Sciences, Center for Aquatic and Invasive Plants, and processed as previously described. The entire experiment was conducted inside the greenhouse (environmental conditions described previously). After trimming away the excess plant biomass, five hundred 8-cm long hydrilla stem tips were added to a 17 L Sterilite® tray, then placed inside a mesh insect cage (61 × 61 × 61 cm). In total, 4 cages each containing 1 tray were set up per experiment; 2 treatment trays were inoculated with *C. lebetis* eggs, and 2 control trays that did not receive any *C. lebetis* eggs. Eggs were collected from a laboratory colony (rearing described above). Egg masses of about 100 fertile eggs were collected, then enumerated under a dissecting microscope (Zeiss Stemi DV4; Carl Zeiss, Berlin, Germany) for fertility. After inoculation, the treatments were monitored daily for the number of eclosed adult *C. lebetis*. After 30 d, 50 hydrilla stem tips were selected at random and analyzed for *C. lebetis* damage under a dissecting microscope (Zeiss Stemi DV4; Carl Zeiss, Berlin, Germany). *Cricotopus lebetis* damage is defined as feeding damage on the apical meristem (Mitchell et al. 2018). The experiment was replicated 3 times using *C. lebetis* from different generations.

The number of adult *C. lebetis* that eclosed was recorded. Each adult *C. lebetis* was confirmed to species and the sex was recorded. The number of stem tips damaged out of the 50-stem tip sample was recorded and analyzed by ANOVA using JMP (SAS 2010) after confirmation that data were following a normal distribution.

Results

COLONY REARING

Adult eclosion data shown are from generations 5 to 10, starting from Nov 2012 through Apr 2013 (Table 1). It should be noted that plant material collected from University of Florida, Institute of Food and Agricultural Sciences, Center for Aquatic and Invasive Plants occasionally contained larvae of the hydrilla leafcutter moth, *Parapoynx diminutalis* Snellen (Lepidoptera: Crambidae) (Winston et al. 2014), and herbivory by the moth interfered with generations 1 to 4. Most likely these moth larvae escaped the cleaning process as eggs. Consequently, adult eclosion from midge generations 1 to 4 was noticeably reduced and supplemented with eggs from cold storage from earlier generations. As refrigerated storage was later demonstrated to cause reduced hatching and survival of larvae (Baniszewski et al. 2015), these data have been excluded from the analysis.

An average of 4,592 eggs were used per generation for a total of 27,550 for maintenance of 6 generations (Table 1). In total, 4,779 adults were produced with 2,519 males and 2,260 females, which equates to a sex ratio of 1.26:1 males to females. Over the course of generations 5 to 10, 30.2% of *C. lebetis* females oviposited and produced a total of 683 egg masses, which had an average of 154.5 eggs per egg mass (fecundity), and 129.2 fertile eggs per egg mass (Table 2). The average percentage of fertile eggs was 83.7% but ranged between 77.5% and 87.1%.

In colony rearing, the total number of adults that eclosed and the number of adults eclosed per number of eggs (% eclosed adults) used to start the generation were significantly affected by generation and the ratio of eggs to hydrilla tips ($F = 8.327$; df = 18; $P < 0.05$; $F = 7.218$; df = 18; $P < 0.05$; Table 1). The percentage of eclosed adults varied across generations, ranging from 7% in F5 to 28% in F6, but on average, 16.4% of the eggs used to start a generation developed and eclosed into reproductive adults.

By increasing the number of eggs added per stem tip of hydrilla, we are increasing larval crowding in the colony over successive genera-

Table 1. Colony rearing data for adult eclosion in a colony of *Cricotopus lebetis* (generations 5–10) maintained at the University of Florida, Institute of Food and Agricultural Sciences, Entomology and Nematology Department.

Generation	No. eggs used in colony	Males eclosed	Females eclosed	Male:female ratio	Total adults eclosed	% eclosed adults
F5	2,000	89	57	1.56:1	146	7.3
F6	2,450	337	366	0.92:1	703	28.4
F7	3,600	326	288	1.13:1	614	17.1
F8	4,800	344	214	1.61:1	558	11.6
F9	6,000	302	229	1.32:1	531	8.9
F10	8,700	1,121	1,106	1.01:1	2,227	24.8
Average	4,592	420	377	1.26:1	797	16.4
Total	27,550	2,519	2,260	N/A	4,779	17.3

Table 2. Colony rearing data for egg production in a colony of *Cricotopus lebetis* (generations 5–10) maintained at the University of Florida, Institute of Food and Agricultural Sciences, Entomology and Nematology Department.

Generation	Females eclosed	No. egg masses oviposited	Egg mass fecundity	Egg mass fertility	% fertile eggs
F5	57	44	113.0	97.9	86.6
F6	366	111	245.9	199.8	81.3
F7	288	106	118.2	96.5	81.7
F8	214	97	89.6	69.4	77.5
F9	229	76	138.4	114.4	82.7
F10	1,106	249	170.0	148.0	87.1
Average	377	114	154.5	129.2	83.7
Total	2,260	683	N/A	N/A	N/A

tions. However, the relationship with adult eclosion remained positive, indicating that the number of stem tips of hydrilla (always more than the number of eggs, ranging from 0.38 to 0.73 eggs per hydrilla stem tip) was sufficient and did not reach a level that induced detectable negative impacts from intraspecific larval competition. Hydrilla origin and adult eclosion duration had no significant effect on adult eclosion dependent variables ($P > 0.05$). The ratio of males to females varied depending upon the number of eggs added per hydrilla stem tip ($F = 5.682$; df = 18; $P = 0.0383$); however, there was no clear trend. Origin of hydrilla, generation, and duration of eclosion had no significant effect on the ratio of males to females ($P > 0.05$).

Fecundity, the total number of eggs, increased by 165.6 eggs per egg mass added per d ($F = 22.13$; df = 65; $P < 0.0001$), but was not affected by generation ($P > 0.05$). Total number of fertile eggs also increased by 138.7 fertile eggs for each additional egg mass added per d ($F = 18.17$; df = 65; $P < 0.0001$, but not generation ($P > 0.05$). The total number of fertile eggs increased with increasing number of egg masses, as expected. Neither the number of eggs masses produced per d, nor generation affected the number of eggs per egg mass or number of fertile eggs per egg mass ($P > 0.05$). These results indicate that there is no trade-off for the number of egg masses with the number of eggs, or number of fertile eggs per egg mass. Total fecundity was 106,322 eggs, and the total number of fertile eggs was 89,007 from generations 5 to 10. The percentage of fertile eggs did not change, indicating the increased fertility was a result of the increase in fecundity.

INTRASPECIFIC LARVAL COMPETITION

The number of adults eclosing was not significantly affected by the number of larvae added per stem tip of hydrilla ($F = 1.302$; df = 117; $P = 0.115$; data not shown). The number of larvae that pupated significantly increased by 26% for each additional larva added per tip of hydrilla ($F = 6.648$; df = 111; $P < 0.001$; data not shown). For emergence efficiency, there was a decreasing trend of pupation and adult eclosion as the number of larvae per hydrilla stem tip increased (Fig. 1). The percentage adult eclosion from initial larvae added per stem tip of hydrilla decreased significantly for each additional larva ($F = 6.941$; df = 111; $P < 0.001$). Average percent pupation also significantly decreased for each additional larva added per tip ($F = 4.505$; df = 111; $P = 0.01$).

HYDRILLA TIP MINING MIDGE DAMAGE

In the assays measuring direct hydrilla damage, there was significant damage to hydrilla caused by *C. lebetis* ($F = 21.3238$; df = 1,10; $P = 0.0010$). The percent hydrilla damage observed in the control treatment was 36 ± 6%, whereas the percent damage of hydrilla in the treatment with *C. lebetis* was 74 ± 6%. In both treatments the damage was *C. lebetis*-specific stem damage; therefore, *C. lebetis* was already present at the field site causing a baseline level of damage in the control. The average adult eclosion from *C. lebetis* treated hydrilla was 21.9 ± 4.5% of the eggs inoculated. Of the eclosed adults, 100 *C. lebetis* in total, 63 were male and 37 were female, which is a 1.7:1 male to female ratio. No adult midges eclosed from the controls.

Discussion

The hydrilla tip mining midge, *C. lebetis*, has demonstrated effective biological control of hydrilla in both laboratory and field settings (Cuda et al. 2011). Mass rearing for augmentative releases, as well as the releases themselves, provide challenges such as the risk of intraspecific competition affecting development with adverse effects on colony productivity or management efforts. The overall goal of this research was to improve rearing and release efficiency of *C. lebetis* for hydrilla management through better understanding of competitive interactions between individuals of the same species. In this study, we (a) defined the productivity of a *C. lebetis* colony maintained at the University of Florida, Institute of Food and Agricultural Sciences, Entomology and Nematology Department; (b) determined the effect that intraspecific competition may be having on productivity in the colony

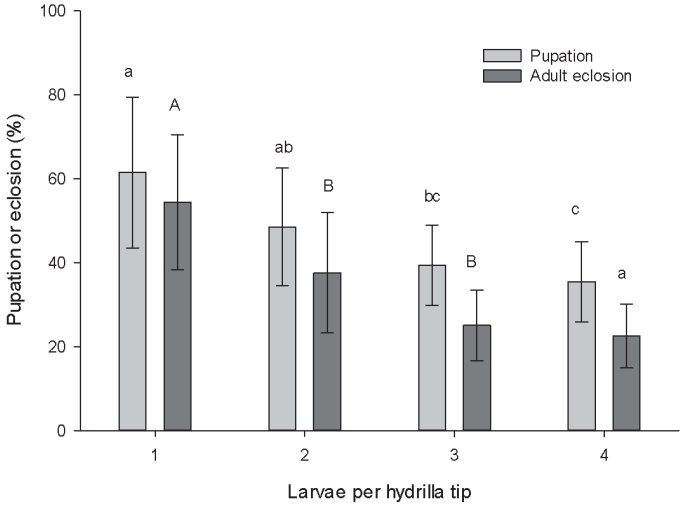

Fig. 1. Effect of intraspecific competition on adult eclosion and pupation of *Cricotopus lebetis*. Average percentage pupation and adult eclosion for 1, 2, 3, and 4 larvae per single tip of hydrilla (*Hydrilla verticillata* (L.f.) Royle). Different letters indicate significance between the number of larvae per tip of hydrilla using a least significant differences (LSD) test within groups. Capital letters indicate differences in adult eclosion, and lowercase letters indicate differences in pupation.

and perhaps during augmentative releases; and (c) we have estimated the average number of hydrilla tips that an individual *C. lebetis* larva can damage during its development.

With each additional larva added per tip of hydrilla, the percentage of adults eclosed decreased. Overall, the total number of pupae and adults produced did increase marginally when the number of larvae added increased. However, the decrease in the percentage of pupation and eclosion indicates intraspecific competition at the larval stage impacted adult eclosion. Comparison of these data to colony rearing data reveals a slightly higher adult eclosion of 22% when 4 larvae were added per tip of hydrilla, compared to 17% observed across the tested generations of colony rearing when less than 1 larva per tip was present. Although competition was not directly measured in the colony, it could be that even with greater hydrilla tips than larvae present, larvae still must compete for development sites. *Cricotopus lebetis* females lay eggs in a gelatinous tube that hatches synchronously, and it is not known how far these larvae are willing or able to disperse to locate a suitable development site, so even under field conditions with plenty of hydrilla tips, there is likely to be high densities of neonates within small areas. Although hydrilla attacked by *C. lebetis* will branch and produce additional hydrilla tips for feeding (Cuda et al. 2002, 2011), spreading the augmentative release of egg masses across a large area of hydrilla infested waters may be beneficial to minimize larval competition.

Limited research has been conducted on intraspecific competition in aquatic herbivorous dipterans. Although not herbivorous, density dependence and intraspecific interactions have been documented in other aquatic dipteran larvae (Bedhomme et al. 2003; Frouz et al. 2009; Reiskind & Lounibos 2009; Silberbush et al. 2014). Competition for resources such as food or shelter may detrimentally affect larval growth, thus limiting full potential of a colony or field population. Previous work, albeit on a detrivorous chironomid midge, has found intraspecific competition to have negative effects on larval development (Frouz et al. 2009). High larval densities and low food supplies have been shown to alter larval behavior, increase larval mortality, and reduce larval development and adult body size (Frouz et al. 2009).

Intraspecific competition among mosquito larvae at different developmental stages in a population has been shown to induce a longer developmental duration, and significantly reduce female pupation rates (Silberbush et al. 2014). This same effect was not seen in males when analyzing mosquito larvae in species-specific competition. This could induce a negative density-dependent environment that alters the sex ratios of adults (Silberbush et al. 2014). Similarly, Bedhomme et al. (2003) found that female mosquitoes took a longer time to complete development compared to males when intraspecific competition was imposed on the larval stage. However, it was shown that females seem to be superior competitors overall and males died sooner, weighed less, and developed shorter wings (as a proxy for fitness) when exposed to larval intraspecific competition (Bedhomme et al. 2003). Intraspecific competition between *C. lebetis* larvae may alter sex ratios if males are less fit and die sooner. Although not measured in larval competition experiments, the male to female ratio was 1.26:1 in the colony (averaged F5–F10 generations) and 1.7:1 in the hydrilla damage experiment. On average, the hydrilla tip to larvae ratio in the colony setting ranged from 1.33:1 to 1.67:1, whereas the tip to larvae ratio in the damage experiments was 5:1. In our study, exposing *C. lebetis* to less competition resulted in more males than females.

Additionally, although density-dependent competition between larvae may have negative impacts on adult eclosion, the exact causes are attributed to related factors, such as food supply (Legros et al. 2009) or individual fitness as assessed by wing length (Bedhomme et al. 2003). Food as a limiting factor has been tested by assessing starvation resistance between field and laboratory reared populations, suggesting that intraspecific competition may have detrimental effects due to competition over food (Arrivillaga & Barrera 2004; Legros et al. 2009). Rearing *C. lebetis* at a rate of 1 larva per tip is most efficient. Increasing the number of larvae per tip to 2 or more does not significantly increase total adult eclosion numbers, but it does significantly decrease emergence efficiency. This indicates that competition between larvae may ultimately reduce the number of eclosed adults per added larvae if they are competing for a tip. However, the chance of a single adult eclosing is increased compared to the probability of an adult emerging with just 1 larva. In other words, although the 4 larvae may compete, their survival is additive. Figure 1 shows approximately a 22.5% eclosion rate per larvae with 4 larvae added, or about 90% chance of a single adult eclosing compared to the 55% eclosion from a single larva. Ultimately, this indicates a better probability of a single adult, or possibly a higher chance of a healthier larva to survive and eclose when more larvae were added.

Applying the data of adult eclosion to augmentative releases, adding 3 or 4 times the number of larvae as there are hydrilla tips likely will not impact the final adult emergence numbers. Although this could be beneficial based on eclosion percentages, it is not the most efficient method because adding only one-third the number of larvae would most likely produce the same number of eclosing adults. Excess larvae may be wasted and could have been used at another field site or location. Furthermore, in the damage study, *C. lebetis* were able to damage more than 1 tip during development. *Cricotopus lebetis* damage was evident already in the field-collected hydrilla (although the larvae themselves would have been killed by sonication), so this experiment tested the impact of augmenting the natural population. The natural population resulted in 36% damage, which doubled to 74% when 1 *C. lebetis* per 5 tips was added to the hydrilla. The addition of 100 fertile eggs, therefore, resulted in the damage of 190 tips, or 38%. According to these data, each larva has the potential to damage approximately 2 tips during development. Therefore, adding approximately 1 larva per 2 tips should be sufficient in releases.

For augmentative biological weed control to be effective, intra- and interspecific competition should be considered. In this study, we obtained information to help ensure that releases of *C. lebetis* will not compete with its conspecifics for food or shelter. *Cricotopus lebetis* is unique in that it feeds on and damages the apical meristem, and limits growth rather than consuming leaves, stem tissue, or tubers. Therefore, *C. lebetis* likely would complement the effects of other hydrilla biological control agents that already have been field released, e.g., *Hydrellia pakistanae* Deonier (Ephydridae) (Center et al. 1997), which typically feeds on leaves and may enhance its effect. In previous studies, the combined action of multiple biological control agents feeding on different plant tissues reduced the ability of hydrilla to compete with the native plant vallisneria (*Vallisneria americana* Michx.; Hydrocharitaceae) (Van et al. 1998). However, despite this, future studies should focus on interspecific interactions, including competition for food and niches, as well as predator-prey relationships.

In conclusion, assessment of intraspecific larval competition, and its implications on mass rearing of *C. lebetis*, is important for understanding its effects on adult development. Intraspecific larval competition is not likely to play as large a role in augmentative releases of *C. lebetis* as it does in colony rearing. However, from the results presented herein, we conclude that in colony rearing it is best to maintain on average 1 larva per hydrilla tip, with the remainder of eggs being used for augmentative releases.

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