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## RESEARCH ARTICLE

# Host range and searching behaviour of *Cricotopus lebetis* (Diptera: Chironomidae), a tip miner of *Hydrilla verticillata* (Hydrocharitaceae)

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A chironomid midge, *Cricotopus lebetis* Sublette (Diptera: Chironomidae), was discovered feeding on *Hydrilla verticillata* (L.f.) Royle (Hydrocharitaceae) in Crystal River, Citrus, Co., Florida, in the 1990s. Larvae of the midge mine the apical meristems of hydrilla, causing terminal branching and stunting of the plant. We investigated the fundamental host range of the midge by conducting a series of no-choice and paired-choice tests. No-choice developmental tests with neonate larvae revealed that the fundamental host range of *C. lebetis* included not only on hydrilla but also several other aquatic plants in different families, suggesting that this insect is not a hydrilla specialist. In paired-choice bioassays, larval colonisation of *Elodea canadensis* Michx. (Hydrocharitaceae) and *Najas guadalupensis* (Spreng.) Magnus (Najadaceae) was greater than colonisation of *H. verticillata*. Behavioural bioassays in a Y-tube olfactometer and in Petri dishes suggested that neonate larvae were not able to locate host plant material, whereas older larvae were successful in finding hosts. In paired-choice oviposition tests, adult females discriminated between potential oviposition sites, with greater numbers of eggs laid on *E. canadensis* and *N. guadalupensis* than on *H. verticillata*. This study is the first detailed account of host searching and oviposition behaviour of a phytophagous chironomid midge. The results will be used to assess the potential value of *C. lebetis* as a biological control agent of hydrilla.

**Keywords:** weed biological control; host finding; random search; aquatic olfactometer

## 1. Introduction

*Hydrilla verticillata* (L.f. Royle) (Hydrocharitaceae) (hereafter hydrilla) is one of the most serious aquatic invasive plants in the world (Haller, 2009). A dioecious form of hydrilla was imported into the United States through the aquarium trade in the early 1950s and quickly spread throughout Florida and into other areas of the southern United States (Langeland, 1996; Madeira, Jacono, & Van, 2000; Schmitz, Nelson, Nall, & Schardt, 1991). A monecious form of the plant was found in the Washington, DC, area in 1982 and has since spread to areas of the north-east,

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midwest, Atlantic coast and California (Madeira et al., 2000; Steward, Van, Carter, & Pieterse, 1984). Hydrilla has many negative impacts, including displacement of native vegetation (Haller & Sutton, 1975), impediment of boat traffic, recreational and commercial losses, clogging of water intake pipes and canals and reductions in tourism and real estate values (Langeland, 1996; Schmitz et al., 1991). Managing hydrilla is both time consuming and expensive, and there are few effective options (Hoyer, Netherland, Allen, & Canfield, 2005). Long-term use of the herbicide fluridone against hydrilla has resulted in the selection of resistant populations, further limiting management options and resulting in a decreased ability to control large infestations (Michel et al., 2004).

In 1992, the chironomid midge *Cricotopus lebetis* Sublette (Diptera: Chironomidae) was discovered damaging apical meristems of hydrilla in Crystal River, FL (Cuda, Coon, Dao, & Center, 2002). This midge is a member of the *Cricotopus sylvestris* species group, in the subgenus *Isocladius* of the subfamily Orthocladiniiae (Epler et al., 2000). The first record of *C. lebetis* in the United States was from Natchitoches, LA, in 1957 (Sublette, 1964), and it was later found at two other locations in Louisiana (Sublette, 1964; Epler et al., 2000). In Florida, the midge was first collected in 1976 in 'south-west Florida' (specific location not available) and has since been found at several other locations in the state (Cuda et al., 2002; John Epler, personal communication). The native range of the midge is unknown (Epler et al., 2000) and difficult to predict because *C. sylvestris* group species are widely distributed in the Nearctic and Palearctic regions (Gresens et al., 2012).

Information about the life history of *C. lebetis* was non-existent until Cuda et al. (2002) described its biology and rearing methods. Females lay gelatinous strings of about 150 eggs on the surface of the water. The eggs hatch after 2 days, and early instar larvae burrow into and destroy the apical meristems of hydrilla. Mature larvae bore into stems in preparation for pupation and, in doing so, cause tip abscission. The feeding damage results in stunting and prolific apical branching of plants and maintains hydrilla below the water surface. A greenhouse study revealed that *C. lebetis* larvae could reduce the biomass of hydrilla in glass tanks by as much as 99% over a 2-month period (Cuda, Coon, Dao, & Center, 2011).

Because of the midge's demonstrated ability to severely damage hydrilla plants, there is interest in exploring its potential as a biological control agent (Cuda et al., 2002, 2011). However, the host range of *C. lebetis* has not been investigated, and this information is critical in determining its value for biological control. With the exception of one individual found associated from *Potamogeton illinoenis* Morong (Dana Denson, personal communication), all field records associated with plants are from hydrilla, suggesting that the midge may be a specialist (Cuda et al., 2002, 2011; Stratman, unpublished data). Moreover, *Cricotopus myriophylii* Oliver, a closely related species in the same *silvestris* species group as *C. lebetis*, is a specialised herbivore of *Myriophyllum* spp. (Macrae, Winchester, & Ring, 1990). Based on field records and its close taxonomic relationship to a known specialist, we hypothesised that *C. lebetis* was a specialist herbivore of hydrilla. The purpose of this study was to test this hypothesis by conducting a series of studies to evaluate the midge's host finding behaviour and host range and use this information to gauge its potential for use as a biological control agent of hydrilla.

## 2. Materials and methods

A series of experiments was conducted to examine the host range and host finding behaviour of *C. lebetis*. First, a no-choice larval development and survival test was performed using dioecious hydrilla and 11 other plants to estimate the midge's fundamental host range. Next, a paired-choice test was conducted to compare larval colonisation of hydrilla with three other plant species, which supported high survival in the no-choice test. Third, the host finding behaviour of larvae in an aquatic Y-tube olfactometer was examined to evaluate the response of larvae to water soluble host plant compounds. Fourth, we monitored the movement of larvae in a Petri dish to determine whether movement was directed towards host plant tissue or random. Next, we examined host finding in two types of water (well and distilled) because some of our assays were conducted in each water type and we wanted to make sure that water source did not influence the results. Likewise, we tested host finding in light and dark conditions because some assays were conducted over time periods encompassing both light and dark cycles, whereas others were conducted only under lighted conditions. Finally, we examined the ability of adult females to discriminate between plant species in a paired-choice oviposition experiment.



### 2.1. *Source and culturing of* H. verticillata *and* C. lebetis

Hydrilla was collected in Lake Tohopekaliga, Osceola, Co., FL (28.2° N, 81.4° W), and *C. lebetis* was collected in Lake Rowell, Bradford, Co., FL (29.9° N, 82.1° W). Hydrilla was propagated in a greenhouse from stems (10–30 cm) collected at the field site and planted in 10.2 cm (diameter) pots containing a layer of potting soil (~5 cm) covered by a layer of sand (~3 cm). The pots were placed in large plastic livestock watering tanks (378 l) filled to a depth of 50 cm with well water, and the tanks were covered with 60% shade cloth to suppress algal growth. After about 1 month, apical stem tips were harvested as needed. *Cricotopus lebetis* was reared by placing hydrilla tips in a plastic tub (34 × 28 × 15 cm, L × W × H) held inside a cage (50 × 50 × 50 cm) constructed from PVC tubing covered with fine nylon mesh cloth. The plastic tubs were filled with well water and aerated with an aquarium pump, and *C. lebetis* masses were placed in the containers. Emergent adults were collected using an aspirator and transferred to a 250-ml separatory funnel filled with approximately 15 ml of well water. Females oviposited on the water surface, and egg masses were collected by opening the stopcock on the separatory funnel.

### 2.2. *No-choice survival and development test*

Healthy, undamaged plant tips, 4–6 cm in length, were placed individually in 35 ml test tubes filled with well water. Each test included three non-target plants and hydrilla as a control (Table 1). There were 10 test tubes per plant species, and the tubes were placed randomly in a rack that held 40 tubes. The experiment was replicated three times. Two newly hatched *C. lebetis* larvae were transferred by pipette to each tube. Once the larvae were introduced, tubes were capped with a perforated plastic lid and placed in an environmental growth chamber maintained at 25°C and a 14:10 (L:D) photoperiod. Tips were checked daily to monitor feeding and replaced as necessary to allow complete development to adulthood. The number of

Table 1. Plants tested for no-choice larval development of *Cricotopus lebetis*.

| Family | Species | Origin | Common name |
|---|---|---|---|
| Hydrocharitaceae | *Elodea canadensis* Michx. | Native | Canadian waterweed |
| | *Egeria densa* Planch. | Exotic | Brazilian elodea |
| | *Vallisneria americana* Michx. | Native | American eelgrass, tapegrass |
| | *Hydrilla verticillata* (monoecious) (L.f. Royle) | Exotic | Hydrilla |
| | *Najas guadalupensis* (Spreng.) Magnus | Native | Southern naiad |
| Potamogetonaceae | *Potamogeton illinoensis* Morong | Native | Illinois pondweed |
| Ceratophyllaceae | *Ceratophyllum demersum* L. | Native | Coontail |
| Alismataceae | *Sagittaria kurziana* Glück | Native | Strap-leaf sagittaria |
| Cyperaceae | *Eleocharis baldwinii* (Torr.) Chapm | Native | Road-grass |
| Lentibulariaceae | *Utricularia macrorhiza* Leconte | Native | Bladderwort |
| Characeae | *Chara vulgaris* L. | Native | Muskgrass |

larvae that completed development to the adult stage and the developmental time were recorded for each plant species.

### *2.3. Paired-choice larval colonisation test*

Plant tips of hydrilla and three other species that supported high survival in the no-choice test (*Elodea canadensis* Michx., *Egeria densa* Planch. and *Najas guadalupensis* [Spreng.] Magnus.) were used in a paired-choice experiment. Plant tips were placed in water in a plastic container ($34 \times 28 \times 15$ cm, $L \times W \times D$) that was divided into two sections using wire screen mesh with a grid size of approximately $1 \times 1$ cm. Hydrilla tips (40) were placed in a randomly selected side of the container, and 40 tips of one of the other plants were placed in the other side. Neonates (100) were then released in the centre of the container, and after 10 days, plant tips were removed and dissected under a microscope to determine the presence or absence of larvae. The experiment was replicated three times with each plant species.

### *2.4. Paired-choice olfactometer test*

An aquatic olfactometer similar to the one used by Van Gool and Ringelberg (1996) for *Daphnia* was constructed using a Y-tube and a low flow peristaltic pump (Model No. 13-876-1; Fisher Scientific, Waltham, MA). The Y-tube was glass, with an inside diameter of 4 mm, having two arms and a stem 5.3 and 5.5 cm in length, respectively. Distilled water was pumped at a rate of 0.575 ml/min into each arm of the Y-tube and was measured by collecting water from each arm into a graduated cylinder for 20 minutes. A plant tip was placed in one arm of the Y-tube and the other arm remained empty. The Y-tube was placed on a flat translucent surface that was lighted from the back. A camera attached to a microscope was used to project the image onto a computer screen, and screen recording software (CamStudio v2.6; TechSmith, Corp., Okemos, MI) was used to record all trials. A neonate was released into the stem of the Y-tube and given a maximum of 10 minutes to move towards one of the

olfactometer arms. Larvae that had not moved into one of the arms after 10 minutes were recorded as no response. A larva was recorded as having made a decision once it entered an arm of the Y-tube. This process was replicated 10 times using hydrilla, *E. canadensis* and distilled water only. The Y-tube arms were reversed after five replications to avoid any directional bias of movement.

### *2.5. Petri dish host finding behaviour bioassay*

A Petri dish, 3.5 cm in diameter, was divided into quadrants using an indelible marker, and a small circle was drawn in the middle of the dish to serve as the starting point. Two holes were drilled into opposite ends of the Petri dish, and plastic tubing, 2 mm inside diameter, was inserted into each hole. Distilled water was pumped through the Petri dish using a peristaltic pump at a rate of 0.25 ml/min to create a constant gentle flow. A single plant tip of either hydrilla or *E. canadensis* was placed in the quadrant with the water inlet so that the current was flowing across the plant tip to the other side of the dish. These two plants were selected because hydrilla was the target species and *E. canadensis* was shown to be one of the best hosts for survival in the no-choice test. A pipette was used to release a neonate into the centre of the dish, and the larva was given 60 minutes to locate the plant tip. Video imagery was captured through a dissecting microscope and recorded on a computer. The path of the larva was traced on an acrylic sheet, and the time spent in each quadrant was recorded. This process was replicated 10 times using hydrilla, *E. canadensis* and a control with no plant material. The position of the insect was determined every 3 minutes to examine its movement pathway.

### *2.6. Host finding test in two types of water in light and dark conditions*

A Petri dish (3.5 cm in diameter) was filled with either well water or distilled water. The two types of water were compared to determine whether the source of water influenced host finding behaviour because some bioassays had been conducted with well water (no-choice survival and development and paired-choice larval colonisation) and others with distilled water (olfactometer, Petri dish host finding). A single hydrilla tip, 1 cm in length, was placed in the centre of the dish, and dishes were maintained at ambient light conditions in the laboratory or in a completely darkened room. A larva was placed at the edge of the dish and given 60 minutes to locate the host plant. This test was replicated 20 times using neonates and medium-sized larvae (second-third instars). After 60 minutes, the host plant was removed from the Petri dish and inspected to determine whether the larva had colonised the hydrilla tip.

### *2.7. Paired-choice oviposition test*

A plastic divider, 2.6 cm in height, was glued to the base of a cubic cage (Bugdorm model DP 1000; Megaview Science, Co., Ltd., Taichung, Taiwan; 29.8 cm on each side) to divide the bottom of the container in two sections. Each section was filled to a depth of 1.5 cm with distilled water and received 20 plant tips (ca. 5–8 cm long) of hydrilla, *N. guadalupensis*, *E. canadensis* or tips of an artificial plastic aquarium plant that resembled hydrilla (leaves in whorls of four, 1 cm long, arranged along a central stem). Four *C. lebetis* pairs were released into the cage and left for 48 hours, after

which the containers were examined for the presence of egg masses. The following paired-choice combinations were tested:

(1) Hydrilla vs. distilled water;
(2) Artificial hydrilla vs. distilled water;
(3) Hydrilla vs. artificial hydrilla;
(4) Hydrilla vs. *E. canadensis*; and
(5) Hydrilla vs. *N. guadalupensis.*

### *2.8. Data analysis*

Means of larval survival and developmental time in the no-choice larval study and the time spent by larvae in each quadrant of the host finding test were compared with analysis of variance (ANOVA) and means separated with Student-Newman-Keuls test when ANOVAs were significant (PROC GLM, SAS Institute, 2008). The numbers of larvae found in different plants in the paired-choice larval colonisation study were compared with *t* tests. Data from the aquatic olfactometer bioassay were not statistically analysed because of low response. The movement pathways of neonate larvae in the host finding behaviour test were examined by fitting the data to a correlated random walk model (CRW), which describes movement in two-dimensional space where there is persistence in the direction of movement (autocorrelation in direction) (Brouwers & Newton, 2010, 2011; Kareiva & Shigesada, 1983; Turchin, 1998). The CRW model is constructed as follows:

$$R_n^2 = nL_2 + \left(2L_1^2\right)(c/1-c)(n-(1-c^n)/(1-c)), \quad (1)$$

where $R_n^2$ = mean squared displacement, $L_1$ = mean move length (in cm); $L_2$ = mean squared move length ($cm^2$); $n$ = number of consecutive moves; and $c$ = mean cosine of the turning angle between 3-minute steps. The observed mean square distances travelled in each step were compared to model predicted values with linear regression, and slopes were tested for equality to one (PROC REG, SAS Institute, 2008). The mean step lengths (distance moved in 3 minutes) were compared between treatments with ANOVA (PROC GLM, SAS Institute, 2008). G tests of independence adjusted with William's correction were used to analyse differences in host finding ability of neonates and medium-sized larvae (second and third instars) in distilled and well water and in light and dark conditions. The results from the paired-choice adult oviposition tests were compared with G tests of independence (Sokal & Rohlf, 1995).

## 3. Results

### *3.1. No-choice survival and development test*

Larvae were able to complete development on the majority of plants tested (Figure 1a). Plants that supported the highest survival were in the same family as hydrilla (Hydrocharitaceae). Survival was highest on monoecious hydrilla (100%), *E. canadensis* (96.7%), *N. guadalupensis* (83.3%) and *Egeria densa* (80%). Plants in families more distantly related to hydrillda, such as Potamogetonaceae, Ceratophyllaceae and Cyperaceae, were generally poorer hosts for survival than more closely

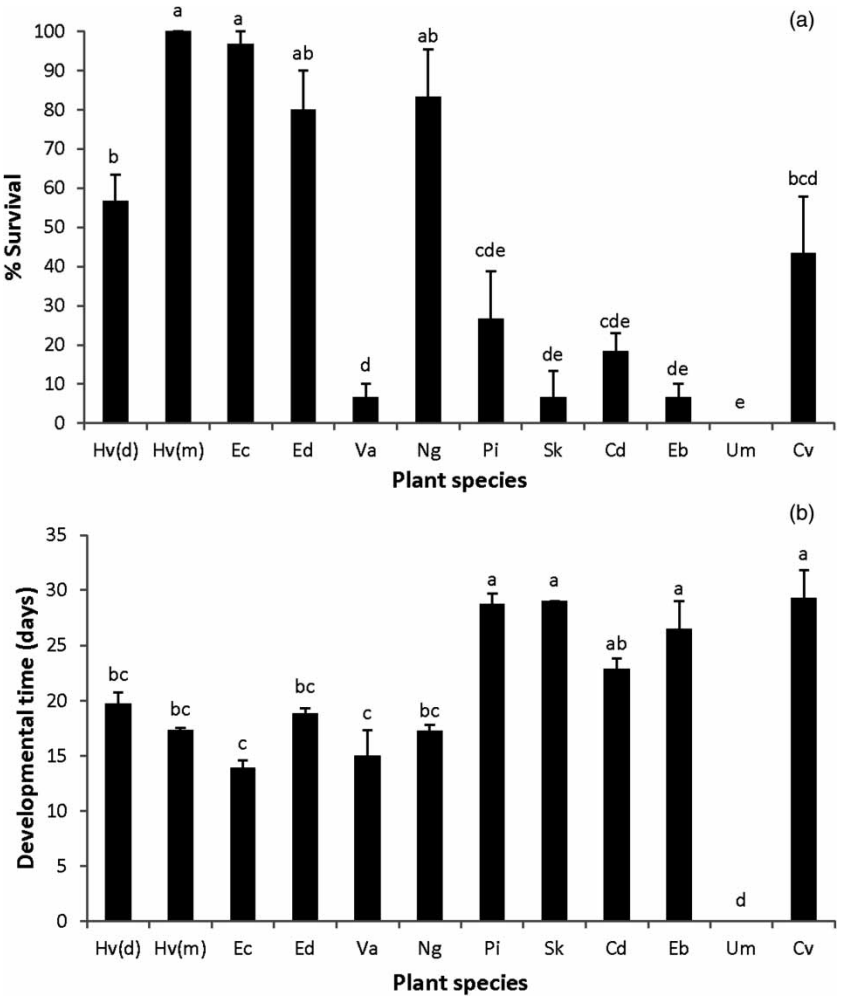


Figure 1. Survival (a) and developmental time (b) of *Cricotopus lebetis* larvae on selected aquatic plants under no-choice conditions. Hv (d), dioecious *Hydrilla verticillata*; Hv (m), monecious *H. verticillata*; Ec, *Elodea canadensis*; Ed, *Egeria densa*; Va, *Vallisnaria americana*; Ng, *Najas guadalupensis*; Pi, *Potamogeton illinoensis*; Sk, *Sagattaria kurziana*; Cd, *Ceratophyllum demersum*; Eb, *Eleocharis baldwinii*; Um, *Utricularia macrorhiza*; Cv, *Chara vulgaris*. Plants ordered from left to right based on their taxonomic relatedness to dioecious *H. verticillata*.

related plants. Survival on *Chara vulgaris* L. (Characeae), a multicellular green algae, was 43.3%. *Utricularia macrorhiza* Leconte (Lentibulariaceae), a carnivorous bladderwort, supported some larval feeding but was the only plant tested that did not allow complete development to adulthood. Developmental rate also varied between hosts and was faster on *E. canadensis* (13.9 days) than either of the hydrilla types (Figure 1b). Interestingly, developmental time was short (15.2 days) on *Vallisneria americana* Michx., a member of the Hydrocharitaceae family, although it was a very poor host for survival (6.7%). Dioecious hydrilla, the target plant,

supported moderate performance of *C. lebetis* in comparison with the other plants tested with an average survival of 56.6% and a developmental time of 19.7 days.

### 3.2. Paired-choice larval colonisation test

More *C. lebetis* larvae were found in *E. canadensis* compared to hydrilla ($t = 3.44$, $P = 0.026$) (Figure 2a). When hydrilla was compared with *N. guadalupensis*, the number of larvae was significantly higher in *N. guadalupensis* ($t = 2.98$, $P = 0.041$) (Figure 2b). Hydrilla and *E. densa* did not differ in the proportion of plant tips infested with larvae ($F = 0.96$, $P = 0.392$) (Figure 2c).

### 3.3. Paired-choice olfactometer test

The majority of larvae did not respond by entering either of the arms of the aquatic olfactometer. In the test with hydrilla and distilled water, one larva entered the olfactometer arm with distilled water, and in the test with *E. canadensis* and distilled water, one larva moved into the arm with *E. canadensis.*

### 3.4. Petri dish host finding behaviour bioassay

Regressions of observed mean square distance traversed on CRW model predicted values were significant for hydrilla, *E. canadensis* and the distilled water control (hydrilla: $F = 10.8$, $df = 1,18$, $P = 0.004$; *E. canadensis:* $F = 17.5$, $df = 1,18$, $P = 0.0006$; control: $F = 9.5$, $df = 1,18$, $P = 0.006$) (Figure 3), and the slopes of the regressions were not different from one for *E. canadensis* ($F = 3.28$, $df = 1, 18$, $P = 0.08$) or the control ($F = 0.53$, $df = 1, 18$, $P = 0.48$). In arenas with *E. canadensis* or water only, movement conformed to a theoretical model of CRW, in which the direction of movement is correlated with the direction taken in the previous step (i.e., individuals tended to continue moving in the same direction) (Figure 3). However, with hydrilla, the slope of the regression of observed values on model predicted values was less than one ($F = 4.9$, $df = 1,18$, $P = 0.04$). The mean step length (distance moved in 3-minute intervals) was greatest when there was only water in the Petri dish ($0.68 \pm 0.04$ cm) (mean $\pm$ SE), intermediate with *E. canadensis* ($0.50 \pm 0.3$ cm) and lowest when hydrilla ($0.25 \pm 0.02$ cm) was in the Petri dish ($F = 38.6$, $df = 2,587$, $P < 0.0001$).

Times spent in the quadrants of the Petri dishes with hydrilla and *E. canadensis* were not different ($F = 2.7$, df 3,36, $P = 0.06$; $F = 1.8$, df 3,36, $P = 0.16$, respectively), and in the Petri dish with only water, more time was spent in the quadrant with plant material than in the opposite quadrant and one of the adjacent quadrants ($F = 3.7$, df 3,36, $P = 0.02$) (Figure 4). In one replication with hydrilla, the larva was able to locate the plant in 10.4 minutes, but in the remainder of replications, the larvae had not settled on the host plant after 60 minutes. Larvae did not locate the plant tip in the trials with *E. canadensis.* Examination of the movement pathways indicated that larvae often swam very close to the host plant, but their movement was not arrested.

### 3.5. Host finding test in two types of water in light and dark conditions

There was no difference in the response of larvae in well and distilled water regardless of lighting conditions or larval size (neonates in light: $G_{adj} = 0.0$, $P > 0.05$,

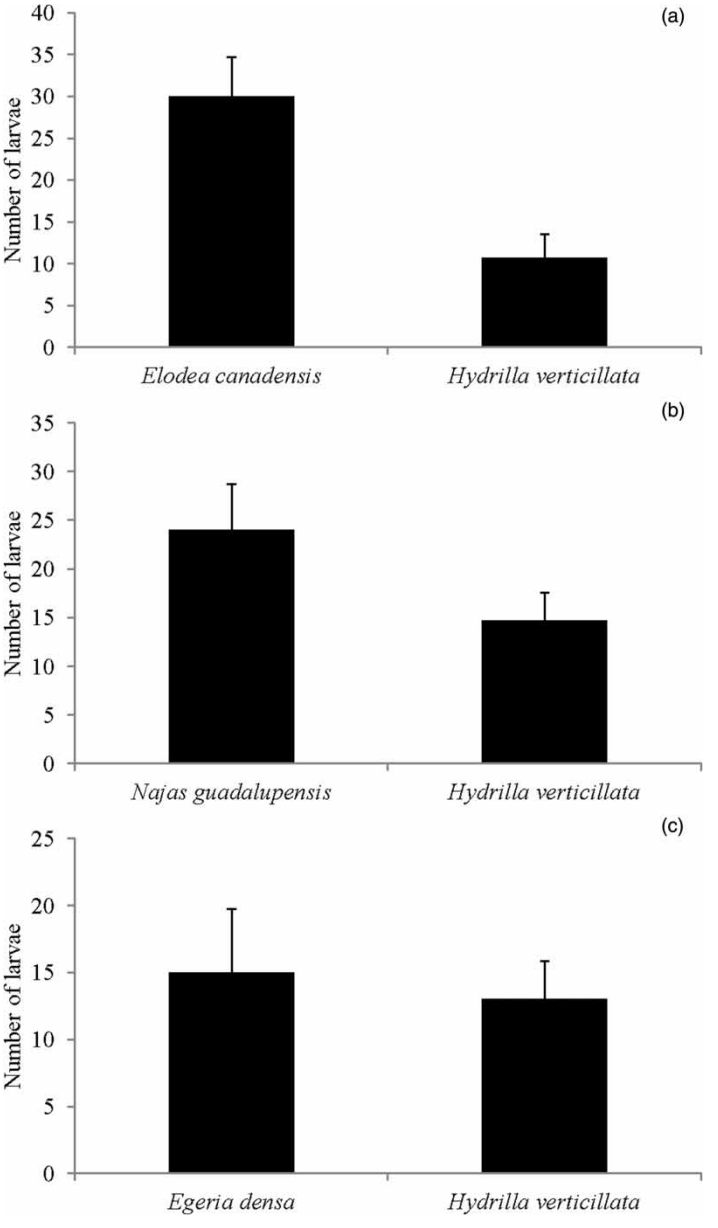


Figure 2. Number of *Cricotopus lebetis* larvae recovered in paired-choice tests with (a) *Hydrilla verticillata* and *Elodea canadensis*, (b) *H. verticillata* and *Najas guadalupensis* and (c) *H. verticillata* and *Egeria densa*.

medium-sized larvae in light: $G_{adj} = 2.5$, $P > 0.05$), neonates in dark: $G_{adj} = 0.0$, $P > 0.05$), medium-sized larvae in dark, $G_{adj} = 0.0$, $P > 0.05$), and therefore data from the two types of water were pooled for further analyses. There were differences in the host finding ability of the two larval size classes in lighted conditions ($G_{adj} = 36.7$, $P < 0.001$), with 17/20 medium-sized larvae, but no neonates, finding the host.

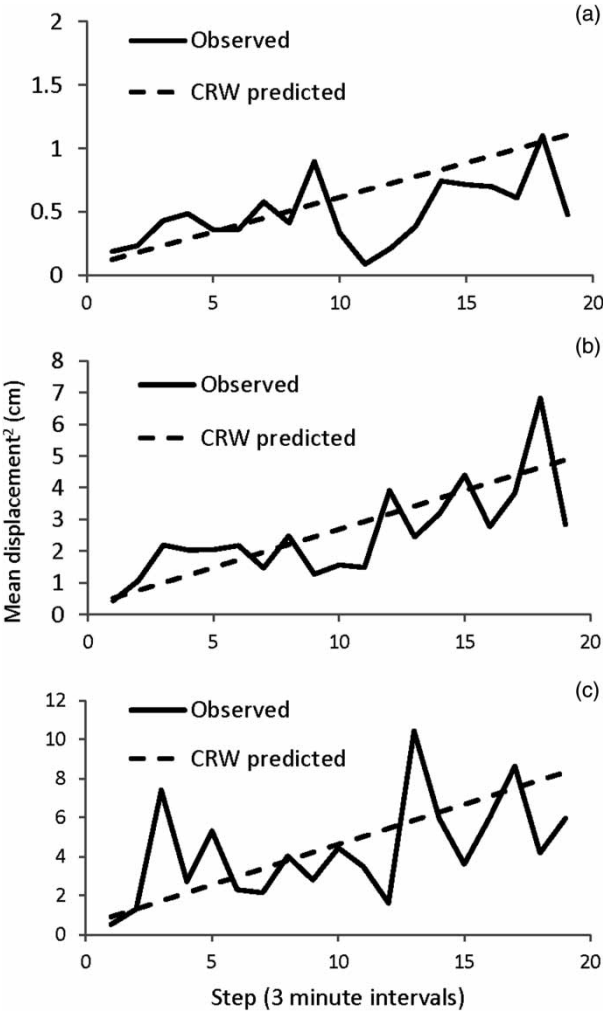


Figure 3. Relationship between mean squared displacement of *C. lebetis* larvae and time in Petri dish arenas with (a) *H. verticillata*, (b) *E. canadensis* and (c) no plant material. CRW, correlated random walk.

In dark conditions, neither neonates nor medium-sized larvae were able to find the host ($G_{adj} = 0.0$, $P > 0.05$) (Figure 5).

### *3.6. Paired-choice oviposition test*

Females preferred to lay eggs on or near hydrilla when given a choice between hydrilla and artificial hydrilla ($G_{adj} = 13.01$, $P < 0.001$) or hydrilla and distilled water ($G_{adj} = 16.76$, $P < 0.001$) (Figure 6). There was no difference in ovipositional preference when females were given a choice between hydrilla and *N. guadalupensis* ($G_{adj} = 0.517$, $P > 0.3$). Females preferred to lay eggs on *E. canadensis* over hydrilla ($G_{adj} = 5.1$, $P < 0.05$). There was also a preference to lay eggs on artificial hydrilla compared to distilled water ($G_{adj} = 4.69$, $P < 0.05$) (Figure 6).

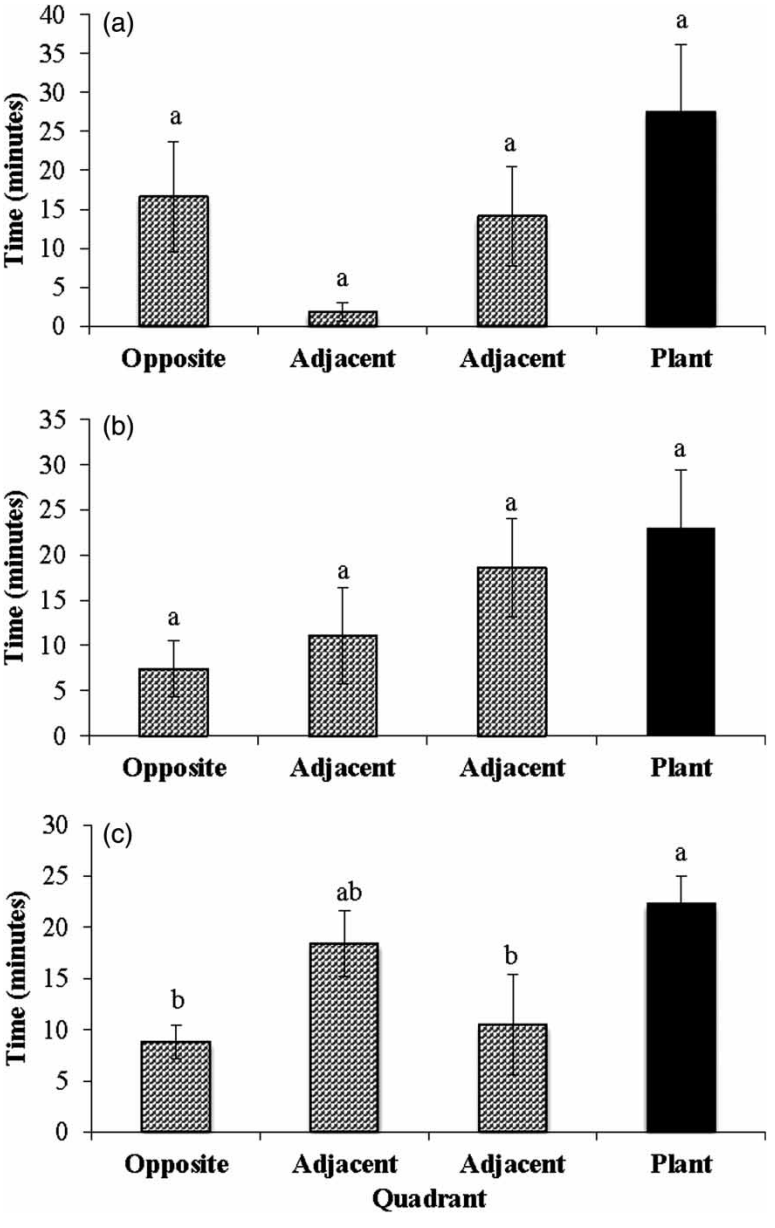


Figure 4. Time spent in four quadrants of Petri dishes containing pieces of (a) *H. verticillata*, (b) *E. canadensis* or (c) no plant material. The plant material was in quadrant four in tests with *H. verticillata* and *E. canadensis.*

## 4. Discussion

The native range of *C. lebetis* is unknown, but because it was not discovered in the United States until 1957 (Sublette, 1964), after the introduction of hydrilla in the early 1950s (Schmitz et al., 1991), Epler et al. (2000) speculated that it may be an adventive species that arrived in the United States with hydrilla. Supporting the

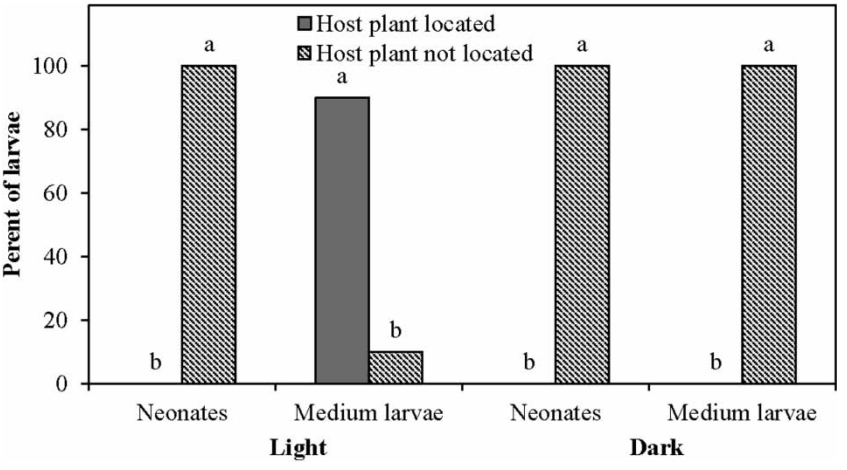


Figure 5. Percent of neonate and medium-sized larvae that located or did not locate hydrilla tips in Petri dishes under light and dark conditions.

contention of recent introduction are the results of an extensive 2-year (1978–1980) survey of insects associated with hydrilla in the southeastern United States, Texas and California (Balciunas & Minno, 1984). Among the 191 species found at 72 locations were several species of *Cricotopus* but not *C. lebetis* (Epler et al., 2000). Additionally, all records of *C. lebetis* in the United States (Cuda et al., 2002; Epler et al., 2000; Sublette, 1964; Epler, personal communication; Stratman, unpublished data) are coincident with the current distribution of hydrilla (Madeira et al., 2000), although hydrilla was not reported in Louisiana, where *C. lebetis* was first found, until 1969 (Sanders, Johnson, & Kelso, 2010). The lack of records of hydrilla in Louisiana prior to the discovery of *C. lebetis* provides circumstantial evidence that the midge feeds on plants other than hydrilla but is not definitive evidence because hydrilla may have been present for several years prior to its discovery. In Florida, hydrilla is thought to have been introduced in the early 1950s but was not correctly identified until many years later in 1969 (Schmitz et al., 1991). If *C. lebetis* were shown to be host specific to hydrilla, then the midge would almost certainly be an exotic species inadvertently introduced into North America with its host.

However, contrary to our hypothesis that *C. lebetis* was a specialist on hydrilla, the no-choice larval developmental study suggested that the midge is a generalist. *Cricotopus lebetis* was able to successfully develop on a taxonomically diverse group of plants, including a non-vascular, multicellular algae, *Chara vulgaris*. The only plant on which the midge did not complete development was *Utricularia macrorhiza*, a carnivorous blatterwort, which probably consumed the larvae. With the exception of *V. americana*, survival was highest on plants in the family to which hydrilla belongs, Hydrocharitaceae. *Vallisneria americana* has a very different architecture than the other three members of the Hydrocharitaceae tested, which may have influenced the ability of larvae to feed and pupate. Both *E. canadensis* and monoecious hydrilla were more suitable for the development of *C. lebetis* than dioecious hydrilla. These plants occur further north in the United States than

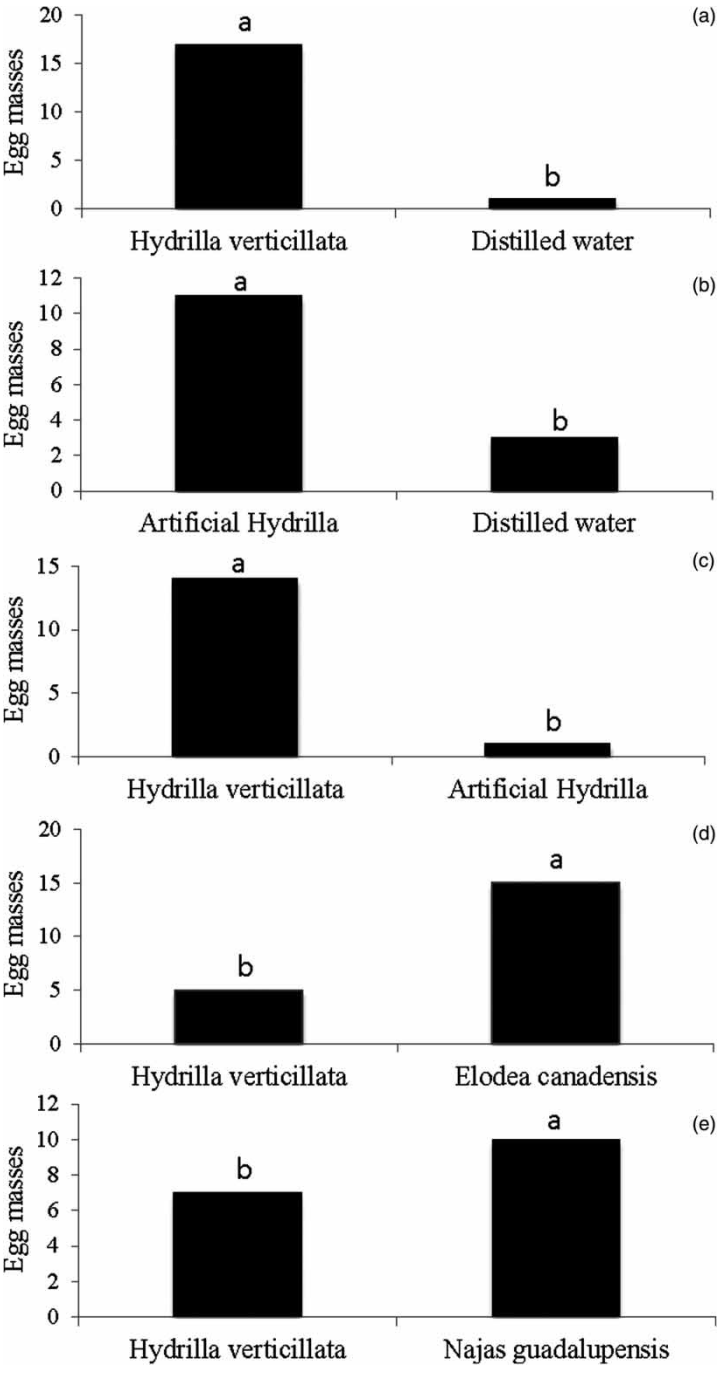


Figure 6. Number of egg masses laid in paired-choice adult oviposition trials with (a) *Hydrilla verticillata* vs. distilled water, (b) artificial hydrilla vs. distilled water, (c) *H. verticillata* vs. artificial hydrilla, (d) *H. verticillata* vs. *Elodea canadensis* and (e) *H. verticillata* vs. *Najas guadalupensis.*

Florida. *Elodea canadensis* was reported in Jackson County in the northern panhandle of Florida in 1937 (University of Florida Herbarium record), but recent surveys suggest that it may no longer occur there (Raymond Hix, personal communication). Monoecious hydrilla is found in the United States in the north-east and in the Atlantic coastal areas as far south as South Carolina (Madeira et al., 2000). Based on the high suitability of these plants for survival of *C. lebetis*, it would be interesting to determine whether *C. lebetis* occurs with these plants in more northern areas of the United States.

In the paired-choice test, *C. lebetis* larvae colonised *E. canadensis* and *N. guadalupensis* more successfully than it colonised hydrilla, which agrees with the results of the no-choice test. There were no differences in colonisation of hydrilla and *E. densa*, which is also consistent with the no-choice test where performance on those two plants did not differ. The experimental design of the paired-choice test does not allow the separation of two processes; success in locating hosts and larval survival once hosts are found, so the greater number of larvae found in *E. canadensis* and *N. guadalupensis* compared to hydrilla could have been due to either factor. However, the results of the no-choice assay suggest that the greater colonisation of these plants was due, at least in part, to increased survival since both of these hosts supported higher survival than hydrilla.

As far as we are aware, the host range of only one other phytophagous chironomid midge, *C. myriophylii,* has been investigated. *Cricotopus myriophylii* is closely related to *C. lebetis* and a member of same *silvestris* species group. Based on feeding studies, Macrae et al. (1990) concluded that *C. myriophylii* was a specialised herbivore of *Myriophyllum* spp., feeding only on the invasive *M. spicatum* L. and the native *M. sibiricum* Kom. Similar to *C. lebetis*, *C. myriophylii* feeds in apical meristems and prevents plants from reaching the water surface (Kangasniemi & Oliver, 1983). Thus, the breadth of the host ranges of these two closely related species appears to be quite different, with *C. myriophylii* having a much greater degree of specialisation than *C. lebetis.*

Neonate larvae were used in three host finding bioassays; the Y-tube aquatic olfactometer, the Petri dish host finding behaviour bioassay and the test of host finding under light and dark conditions. Neonates were also used in the pair-choice larval colonisation test, but that assay was conducted for 10 days, during which time the larvae matured to third and fourth instars. The results of the three bioassays conducted entirely with neonates strongly suggest that neonates are not able to locate hosts. Neonates did not respond to host plant cues in the olfactometer, and in the Petri dish experiment, only one larva (of 10) found the piece of hydrilla, and none found the piece of *E. canadensis.* There did appear to be a trend towards larvae spending more time in the quadrant where the host plant was located, but the same trend was observed when there was no plant material in the Petri dish. The quadrant with the plant tip was also the quadrant with the water inlet, suggesting that neonates may have rheotactic tendencies. The fit of the neonate movement data to the CRW model suggests that larval movement was not directed when *E. canadensis* or no host material was in the arena. Interestingly, when hydrilla was in the Petri dish, movement did not conform to the model. Examination of the data shows that displacement was less than predicted when hydrilla was present. When observed values fall below model predicted values (i.e., when the slope $< 1$), there is less autocorrelation in turning angles (Brouwers & Newton, 2010). Difference in

behaviours of larvae in the three treatments was also evident in step length. In arenas with no plant material, step length was highest, and in arenas with hydrilla, it was lowest. Thus, even though only one larva successfully located a plant tip in this assay, there was evidence that the presence of plant material affected larval movement, with hydrilla possibly having a stronger effect than *E. canadensis*.

Initially, we thought that the lack of evidence for host finding of neonates may have been related to the experimental set-up. In both the olfactometer and Petri dish bioassays, the searching arenas were lighted from the underside, which was necessary to allow video recording of larval movement. Lighting from underneath, which would be the opposite of that encountered in nature, could conceivably have affected host-finding behaviour. Moreover, the water currents in the bioassays may have been too strong, or too weak, to allow expression of typical host finding behaviour. However, the bioassay conducted with two different larval age classes (neonates and medium-sized larvae) in light and dark conditions demonstrated that neonates were unable to find host material in an arena with no water flow, regardless of lighting conditions, during a 60-minute period, whereas older larvae successfully located plants, but only under light conditions. We speculate that older larvae may need to search for additional host plant tissue when they have depleted an available resource and perhaps to search for suitable pupation sites.

The apparent lack of host finding ability of neonates implies that larval survival depends to a large extent on the ability of adult females to select appropriate oviposition sites. Like other chironomids (Williams, 1982), the gelatinous eggs masses of *C. lebetis* are sticky and adhere to substrates they contact (Cuda et al., 2011). If an egg mass makes contact with a suitable host plant, larval colonisation of the plant will follow. However, neonates emerging from egg masses stuck to non-hosts, or freely floating in the water, will depend on currents and larval movement to locate a host. In the oviposition study, females overwhelmingly laid their eggs in the vicinity of plant material, whether it was a real or artificial. When given a choice between real and artificial plants, real plants were selected. Studies have shown that visual cues affect the oviposition behaviour of some chironomid species (Lerner et al., 2008). *Cricotopus lebetis* females may initially respond to visual cues when selecting oviposition sites but later rely on tactile or olfactory signals, if available. When plants have reached the surface, females may contact them and discriminate between plants, but when the plants are below the surface, it seems likely that plant cues would be difficult to detect. It would be interesting to examine the ability of females to discriminate between plants, which have not reached the water surface.

The greater number of eggs deposited on *E. canadensis* and *N. guadalupensis* compared to hydrilla, coupled with the higher success of larvae in colonising the same two plants in the paired-choice test, suggest that ovipositional preference coincides with success of larval development and, thus, conforms to the 'optimal oviposition theory' (also known as the 'preference-performance hypothesis' or the 'mother knows best principle'), first proposed by Jaenike (1978) and since then investigated in numerous studies (see Gripenberg, Mayhew, Parnell, & Roslin, 2010 for a recent review). Typically, specialist insects behave in agreement with the preference-performance hypothesis to a greater extent than generalists (Janz & Nylin, 1997; Liu, Scheirs, & Heckel, 2012). Conformation of *C. lebetis* to the preference-performance hypothesis, coupled with a tendency towards superior performance in

Hydrocharitaceae compared to plants in other families, suggests that *C. lebetis* is neither highly specialised nor a broad generalist but rather occupies an intermediate position on the specialist-generalist continuum.

Laboratory bioassays conducted to delineate the fundamental host range of *C. lebetis* indicate that the midge is not a specialist of hydrilla. However, the ecological (=realised) host range may differ from the fundamental host range (Briese, 2005; Haye, Goulet, Mason, & Kuhlmann, 2005). All field records of *C. lebetis* that are associated with host plants, with the exception of one record from *Potamogeton illinoensis* (Dana Denson, personal communication), have been from hydrilla (Cuda et al., 2002, 2011; Stratman, unpublished data). This could be due to a sampling bias, because hydrilla has undoubtedly been sampled more intensely than other submersed aquatic plants in Florida or reflect an actual field preference for hydrilla. If the latter is true, then releases of *C. lebetis* may result in minimal damage to non-target plants. Moreover, hydrilla typically grows in monospecific patches (Van, Wheeler, & Center, 1999). Egg masses released in these patches would likely have a very low probability of moving outside the release area and attaching to non-targets. Finally, *C. lebetis* does not kill hydrilla plants but rather stunts vertical growth and results in profuse branching below the surface. This feeding damage prevents the formation of surface mats and therefore greatly reduces the weed's negative effects (Cuda et al., 2011). The effect of the midge on non-target plants may also be sub-lethal, although that has not been examined. Sub-lethal stunting of non-targets and the low mobility of immature midges may greatly reduce the possibility of population level impacts to native vegetation.

A next step in assessing the potential value of *C. lebetis* as an augmentative biological control (or as a classical biological control agent for release in areas where it does not occur) would be to evaluate the host range and impact in the field. If field tests reveal that *C. lebetis* prefers hydrilla over other aquatic plants or that releases can be designed in ways that minimise effects to non-targets, *C. lebetis* may have use as an augmentative biological control agent against dioecious hydrilla in Florida and possibly in more northern areas of the United States where monecious hydrilla occurs.



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