COMAL SPRINGS RIFFLE BEETLE COTTON LURING ANALYSIS

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Executive summary

Biological monitoring of *Heterelmis comalensis* is mandated by the Edwards Aquifer Habitat Conservation plan (EAHCP) to determine if biological standards are met. A better understanding of the efficacy of the cotton lures used for monitoring is essential for meeting that goal. A field experiment and laboratory experiment were conducted to examine if adult and larval *H. comalensis* preferred conditioned cotton compared to other available resources. The field experiment compared the riffle beetle colonization on cotton lures to lures constructed of sycamore leaves of the same dimensions, placed side by side within springs. The laboratory experiment examined adult preference of resources presented to them within a closed setting, specifically, conditioned cotton, leaf, and wood lures.

Paired t-test of the field experiment showed no significant difference between the number of *H. comalensis* adults or larvae occurring on leaf or cotton lures. However, *M. pusillus* adults and larvae showed a preference for cotton lures during the same experiment.

The laboratory experiment resulted in a number of subjects “drifting” or leaving the area of the mesocosm where lure resources were available. A high level of variation was found to occur among replicates. No preference to a lure resource was detected with or within the inclusion of individuals that drifted from the system. Evidence showed that some individuals were actively moving within the mesocosm.

Results from this study suggest that cotton lures are no more or less effective at attracting *H. comalensis* compared to other resources within the same area.

Introduction

The Comal Springs riffle beetle (CSRB) *Heterelmis comalensis* (Bosse et al. 1988) is an aquatic beetle in the family Elmidae (Coleoptera) known primarily from Comal Springs, Comal County, Texas, but has also been collected from San Marcos Springs, in Hays County, Texas (Gibson et al. 2008). It is federally protected (USFWS 1997) and is a surrogate species for these spring habitats with 22 ha of designated critical habitat (USFWS 2013). Like many other species in the Edwards Aquifer, *H. comalensis* faces numerous threats to its ecosystem, especially, over pumping of water, pollution, and competition from the introduction of exotic species (Bowles and Arsuffi 1993). Biological monitoring of *Heterelmis comalensis* is mandated by the Edwards Aquifer Habitat Conservation plan (EAHCP) to determine if biological standards are met. A better understanding of the efficacy of the cotton lures used for monitoring is essential for meeting that goal.

In 2019, a small independent pilot study was conducted by BIO-WEST biologists to compare the traditional cotton lures with sycamore-leaf lures. In this experiment we followed the EAHCP CSRB standard operating procedure (SOP) and placed cotton lures and sycamore-leaf lures of approximate equal surface area in separate cages (**Fig. 1**). Cotton and sycamore-leaf lures were placed side by side within 16 tagged springs at Comal Springs. Lures were retrieved after 48-49 days and the numbers of beetles were enumerated for each lure. Paired t-tests were used to compare the numbers of individuals found among cotton lures and sycamore-leaf lures for each life stage.

Fig. 1. Sycamore leaf lure used for leaf and cotton lure efficacy pilot study. Left shows surface area of a standard cotton lure compared to sycamore leaves. Right image shows the sycamore lure.

The results of this study showed no significant difference (p = 0.96) among the number of *H. comalensis* larvae on cotton lures (44) and sycamore-leaf lures (45). The number of *H. comalensis* adults also did not vary statistically (p = 0.13) among cotton lures (115) and sycamore-leaf lures (38); however, higher numbers of adults on cotton lures was apparent, but no conclusions could be made due to high variation. Anecdotally, adults of *Microcylloepus pusillus* were found in greater numbers on cotton lures (111) compared to sycamore-leaf lures (17) (p = 0.006). Considering that this study was in line with gaining a better understanding of resource choice, it was of interest to replicate this study in order to see if additional sampling could produce more definitive results.

Goals and objectives

The goal of this project was to gain a better understanding of the applicability of cotton lures for the sampling of *H. comalensis* in the Comal springs ecosystem. The main objective was to test the efficacy of cotton lures in the presence of other resources commonly encountered at Comal Springs. This objective was tested by 1) conducting a laboratory experiment that provided adult *H. comalensis* with conditioned cotton, leaf, and wood lures, and 2) comparing beetle preference of cotton vs. sycamore leaf in the field.

Methods

Field lures

A cotton and sycamore lure (as described above) were placed side by side within 12 active springs located at Spring Runs 1, 2, 3 and the western shoreline and Spring Island areas of Comal Springs on 26 June, 2020. Lures were retrieved over two days to accommodate staggered launching of the 1st experimental run of the laboratory experiment on 19 and 20 August, 2020. A stereo scope was used in the field to determine riffle beetle species that were retrieved and to inspect the lure materials for larvae. All individuals that were not used for the laboratory experiment were returned to the spring from which they originated. The data was combined from the pilot study conducted in 2019 and analyzed with paired t-test to test if there was a difference between the number of individuals found on cotton vs. leaf lures for each species and life stage, separately.

Description of the experimental system

Experimental units were constructed at the BIO-WEST San Marcos office and transported to the US Fish and Wildlife Service San Marcos Aquatic Resources Center (SMARC). Five mesocosms were constructed

Fig. 2. The 30-gal cone stock tanks during preconstruction.

out of 30-gal cone-shaped stock tanks that were painted black to reduce light. Each mesocosm had a few pebble-sized limestone rocks placed at the bottom of the cone to help disperse water as it was pumped up through the bottom drain. Support screens consisted of a 250 µm mesh secured with hot glue to an egg-shell light fixture support and cut into 61 cm diameter rounds (**Fig. 2**). Each round was fit into a mesocosm and secured with silicone. A layer of large gravel to small pebble sized limestone rocks were placed on top of each support screen. Three 2.54 cm holes were cut equidistant from each other and fitted with bulkheads on the tank as drains to support ca. 75 L of water per tank. Each drain was fitted with ca. 10 cm PVC pipe on the inside with a 500 µm mesh screen, initially secured with a zip tie on the outside of the pipe; after the 1st experimental run, the screens were glued to the PVC pipe with hot glue. The outside of the drains was fitted with a 2.54 cm hose that led to a 50-gal stock tank that was used as a sump. Well water was used to fill the sump and was drained by a stand pipe to maintain 150 L while fresh well water was added at a rate of 0.09 L/sec. Water was pumped from the sump through two separate heating-chilling units set for a temperature of 23.3 ^{o}C and exited though separate flow bars. Each flow bar supplied water through ball-valves and hoses connected to the bottom of two or three mesocosms. Water then drained from the drains of the mesocosms back into the sump. In this fashion a partial recirculation system was created, where small amounts of freshwater were input to the system (**Fig. 3**). Tanks were run for several weeks to ensure that conditions could stabilize before test subjects were placed within them. A week before the experiment was initiated, a trial run with adult *H. comalensis* was implemented to ensure that individuals found these mesocosms as suitable habitats.

Fig. 3. Photograph of the entire system with four tanks.

Tank experiments

Tanks had conditioned cotton, leaf, and wood lures placed equidistant from each other and along the perimeter of the tank, between the drains. Cotton was pre-conditioned with well water in a flow-through container for 6-7 days to ensure that any toxins from the textiles would be removed. Leaves were conditioned for ca. 2-3 months. Wood materials consisted of poplar dowels that were conditioned for ca. 1 year and sycamore twigs that were conditioned for > 6 months; two twigs were placed on the sides of the lure with one dowel placed in the middle. Lures were assembled and placed in the tanks a day before experiments were initiated.

Test subjects were mainly acquired by placing lures in active springs at Comal Springs. Additional adults were hand collected from coarse wood known at specific springs. All individuals from the field experiment described above were utilized. Additional lures were placed at Comal Springs for the 2nd and 3rd experimental runs on 22 September, 2020 and 19 January, 2021, respectively. Due to federal permit limitations, some subjects were "recycled" for the 2nd or 3rd experimental run. All recycled test subjects were kept in a flow-through tube at SMARC with conditioned sycamore leaves and wood and conditioned poplar dowels as resources.

Experiments were initiated by placing 20 adult *H. comalensis* in the middle of each mesocosm. Individuals were observed to ensure that they attached to rocks or the support screen. Beetles that remained in suspension were gently guided to the bottom of the tank at the center until they attached to a substrate. Once subjects settled, the tanks were covered with a black tarp to reduce light. Each tank was checked once a week for individuals residing on cotton lures. The cotton was gently removed from its cage, unfolded in a glass dish and inspected on both sides for adults. Adults found on the cotton were left on the cotton, the lure was then folded back to its original configuration, placed back into its cage, returned to its place in the mesocosm. As lures were placed back in the tank, they were observed to ensure no beetles were shaken free from the lure during its replacement. Each drain screen was also inspected for stray individuals that floated after being placed in the tank. These individuals were removed from the drain screens and placed back in the middle of the tank, ensuring that they securely attached to the support screen or a rock.

All beetles were removed at the fourth week, noting their location, and were identified by gender following methods described by Kosnicki (2019). Individuals that were found on the drain screens were considered as "drifters" but were included as individuals recovered from the mesocosm. The total number of individuals recovered from the mesocosm was subtracted by the number of individuals that were originally placed within to estimate the number of individuals that may have left the system through the drains. These unaccounted individuals were also considered drifters and therefore the number of individuals exhibiting drift behavior were enumerated for each tank. Early instar larvae that were oviposited and had hatched during the experiment were also counted on each of the lure materials; however, the support screen was not inspected for early instar larvae.

Water quality was recorded during each weekly lure check except for a couple times during experimental run 2 when a meter was not available. Discharge was also measured before experimental runs 1 and 2 and after all three experimental runs.

<u>Run 1</u>

Five tanks were used for the 1st experimental run. All test subjects were acquired from lures placed at Comal Springs as described in the field study, or were retrieved from coarse woody material at a well-known spring during the time lures were retrieved. Tank 1 was initiated with seven individuals from leaf and 13 hand collected from wood. Tank 2 was initiated with 11 individuals found on cotton and nine found on leaf. Tank 3 was initiated with 20 individuals found on leaf. Tank 4 was initiated with nine individuals found on cotton and 11 found on leaf. Tank 5 was initiated with 10 individuals found on cotton and 10 found on leaf. Tanks 1 and 3 were initiated on 19 August, 2020 and concluded on 17

September, 2020. Tanks 2, 4, and 5 were initiated on 20 August, 2020 and concluded on 18 September, 2020.

Run 2

Four tanks were used for the 2nd experimental run. Tank 1 was initiated with 18 newly collected individuals and 2 that were used from experimental run 1. Tank 2 was not used. Tank 3 was initiated with newly collected individuals. Tank 4 was initiated with 20 individuals form experimental run 1 and Tank 5 was initiated with only 14 individuals from experimental run 1. All new subjects were collected on cotton lures. All tanks were initiated on 29 October, 2020. Tank 4 and 5 were concluded on 19 November, 2020 and Tanks 1 and 3 were concluded on 25 November, 2020 (see complications section in the results).

Run 3

Three tanks were used for the 3rd experimental run. Tank 1 was initiated with 20 newly collected individuals. Tank 2 was initiated with 10 newly collected individuals and 10 individuals from experimental run 2. Tank 3 was not used. Tank 4 was initiated with 20 newly collected individuals. All new subjects were collected on cotton lures. All tanks were initiated on 15 March, 2021 and concluded on 15 April, 2021.

Analysis of variance (ANOVA) was used to test for differences among treatments based on the proportion of individuals that were recovered from a resource or on a rock or the support screen. For example, if only 15 individuals were recovered from a tank, and 10 of those individuals were found on the cotton lure, then the proportion found on cotton for this replicate was 0.67. In this way, individual choice of a lure resource was directly tested among subjects that remained within the mesocosms. A second ANOVA was performed to test between these treatments that included the estimated number of “drifters” as described above. For the preceding example, the proportion found on cotton for that replicate would be 0.5 if 20 individuals originated in the tank at the start of the experiment.

Results

Field lures

Lures were retrieved from the field on 19 and 20 August, 2020 representing 54 and 55 days of conditioning and riffle beetle colonization; this was about one week longer than the pilot study. One lure set was potentially tampered with as a lure from another research group was found within the same spring location. Another lure set was completely lost and replaced by a lure of another research group and the cotton lure of another set was vandalized. These lure sets were not included for comparing cotton to leaf materials; however, any adults found on these lures were taken for the laboratory experiment. Paired t-test of both field trials combined showed no significant difference between the number of *H. comalensis* adults ($p = 0.268$) or larvae ($p = 0.360$). However, *M. pusillus* adults ($p = 0.021$) and larvae ($p = 0.048$) showed a preference for cotton lures (**Table 1**). Non parametric tests (Wilcoxon Rank Sign Test) were also evaluated but produced the same results.

Table 1. Comparison of number of riffle beetles found on cotton and sycamore leaves placed in springs at Comal Springs. Run 1 occurred from 13 August, 2019 to 30 September, 2019 or 1 October, 2019. Run 2 occurred from 26 June, 2020 to 19 or 20 August 2020. *H*.larvae = *Heterelmis comalensis* larvae; *H*.adult = *Heterelmis comalensis* adult; *M*.larvae = *Microcylloepus pusillus* larvae; *M*.adult = *Microcylloepus pusillus* adult.

	Cotton				Leaf			
Run	*H*.larvae	*H*.adult	*M*.larvae	*M*.adult	*H*.larvae	*H*.adult	*M*.larvae	*M*.adult
2	0	2	0	0	0	1	0	0
2	13	0	0	0	8	3	0	0
2	4	3	1	1	14	3	0	0
2	11	14	1	2	1	6	0	0
2	7	0	1	0	15	5	0	0
2	9	1	0	2	7	4	1	11
2	9	3	0	9	6	5	0	5
2	1	2	2	0	0	4	1	0
2	11	2	0	0	5	11	0	0
2	27	3	2	2	0	6	1	2
1	11	42	0	1	7	7	0	0
1	11	7	6	21	4	15	0	7
1	14	2	2	29	5	4	0	3
1	1	2	0	7	0	1	0	2
1	2	5	0	22	5	3	0	3
1	1	17	0	16	5	2	0	0
1	3	8	7	7	7	5	0	2
1	1	32	0	8	12	1	0	0
Totals	136	145	22	127	101	86	3	35

Tank experiments

The final check of each experimental run showed varying results with regard to resource selection by test subjects (**Table 2**). In general, there seemed to be a propensity for individuals to drift as individuals were almost always found on drain screens and a number of individuals were not recovered for most of the replicates. There were also a considerable number of early instar larvae noted on each of the resources provided, except for the cotton (see discussion below).

ANOVA of the proportion of adults on each lure type or not on a lure, excluding the number of individuals lost to drift found no difference in adult preference (*F-value* = 0.168; *p-value* = 0.197). Although cotton was slightly higher, there was a high amount of variation (**Fig. 4**).

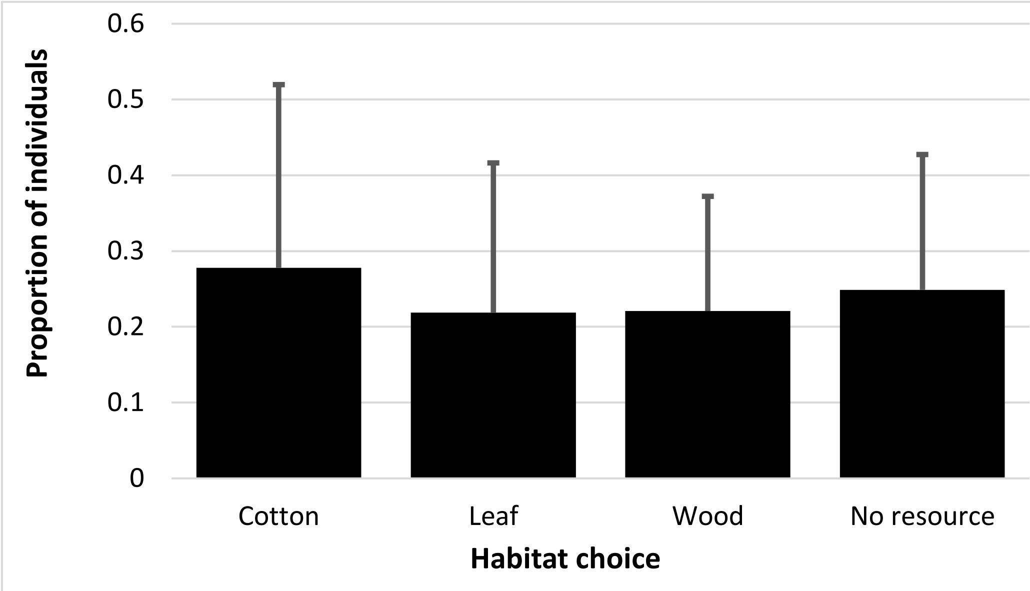

Fig. 4. The average proportion and standard deviation of adult *Heterelmis comalensis* found within experimental units. Proportions determined excluding drifting individuals.

Table 2. Results from the tank experimental runs comparing the preference of conditioned cotton, leaf, and wood lure resources by adult *Heterelmis comalensis*. Run is consistent with the initiation and conclusion dates given in the text and the Days represents the difference between those dates. Adults found “Not on lure” were found within the mesocosm either on the support screen or a rock not associated with a lure. Individuals found on the “Drain screen” were considered removed from the experimental unit and were counted as drift. Larvae found on lures were recent hatchlings (1st or 2nd instars). Larvae with "na" indicates that a search for larvae was not conducted. Drift* is based on the number of individuals found on the drain screens during the final check plus the estimated adults that left the mesocosms through the drain screens.

			Cotton			Leaf			Wood			Not on lure			Drain screen			
Run	Tank	Days	Female	Male	Larvae	Female	Male	Larvae	Female	Male	Larvae	Female	Male	NA dead	Female	Male	NA dead	Drift*
1	1	29	1	1	0	2	4	24	4	6	33	2	0	0	0	0	0	0
1	2	29	0	0	0	3	0	3	1	1	56	1	0	0	0	0	1	14
1	3	29	2	4	0	0	0	7	0	0	23	0	0	0	0	1	0	14
1	4	29	0	0	0	1	2	5	1	3	5	3	1	0	1	0	0	9
1	5	29	2	1	0	2	3	6	1	3	5	1	0	1	0	0	0	6
2	1	27	1	4	0	1	0	15	1	2	10	4	1	1	3	2	0	5
2	3	27	1	0	0	0	0	0	3	1	1	3	4	2	1	3	0	6
2	4	21	4	6	na	0	1	na	0	1	na	6	5	0	0	0	0	0
2	5	21	1	1	na	1	0	na	1	2	na	3	3	0	0	0	0	8
3	1	31	1	0	0	2	2	3	0	0	9	0	2	1	1	3	1	12
3	2	31	6	10	38	0	0	1	0	0	6	1	1	0	1	0	0	2
3	4	31	0	0	0	3	6	8	2	5	7	1	1	0	1	1	0	2

ANOVA of the proportion of adults on each lure type or not on a lure, or lost to drifting found no difference in adult preference (*F-value* = 1.541; *p-value* = 0.203). In this analysis, adults appeared to drift more than being found on any substrate; however, there was a high amount of variation (**Fig. 5**).

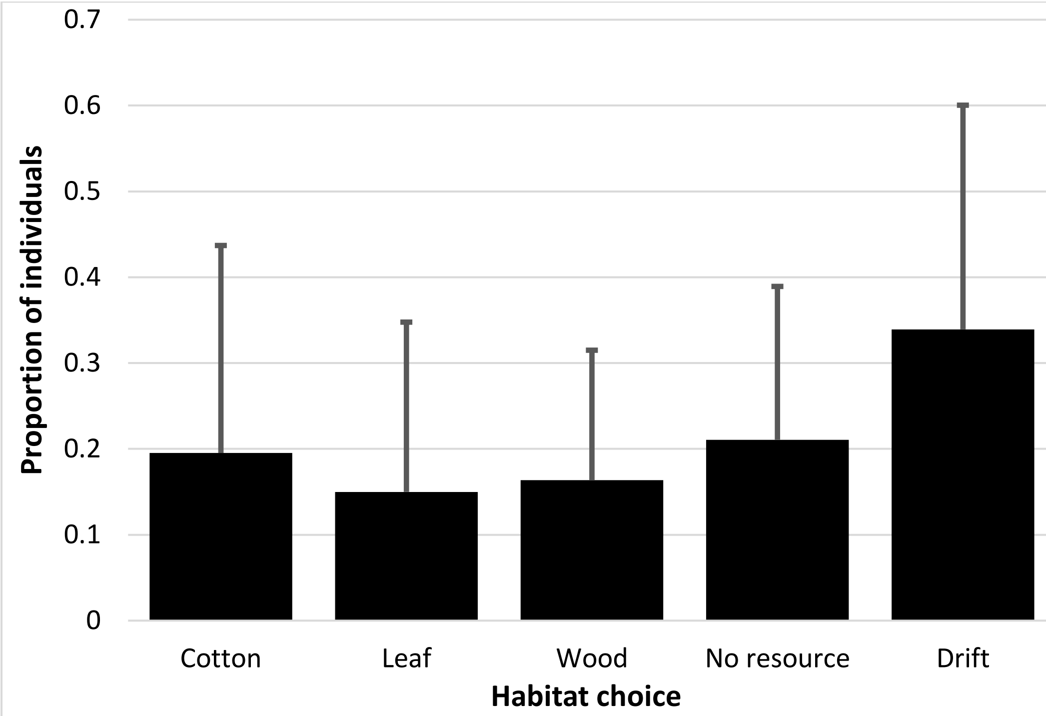

Fig. 4. The average proportion and standard deviation of adult *Heterelmis comalensis* found within experimental units. Proportions determined with the inclusion of drifting individuals.

Mean water quality recorded during the experiment and discharges estimated at the end of the experimental runs are given in **Table 3.**

Table 3. Water quality and discharge (Q) recorded for each of the experimental runs.

Run 1	Temp °C	DO%	DO mg/L	SPC -mS/cm	pH
1	22.6 ± 0.1	87.3 ± 1.1	7.5 ± 0.1	0.636 ± 0.010	7.9 ± 0.1
2	22.5 ± 0.3	80.4 ± 7.7	6.9 ± 0.6	0.646 ± 0.003	7.4 ± 0.4
3	22.6 ± 0.1	109.7 ± 1.3	9.5 ± 0.1	0.685 ± 0.003	7.3 ± 0.1

	Q (L/sec)				
Run 1	Tank 1	Tank 2	Tank 3	Tank 4	Tank 5
1	0.35	0.32	0.37	0.42	0.39
2	0.35	na	0.42	0.50	0.34
3	0.45	0.58	na	0.69	na

Complications

Each experimental run had its own set of complications. The entire system had multiple components that contributed to a balance of flow, incorporating 525 L of water (140 gal) with all 5 tanks involved. A system of this size, with a number of moving parts, can be expected to have some issues. An argument can be made that the complications experienced during each experimental run may draw question to their comparability. However, in the strictest sense, the ultimate objective was to test if adult *H. comalensis* found a preference to any of the lure resources provided and in this fashion the data was presented together. The complications from each run are described below.

<u>Run 1</u>

The final check and retrieval of the adults from these systems found that there were fewer individuals than were originally placed in them. Additionally, we found individuals underneath the support screen. On closer inspection, it appeared that beetles could wiggle underneath the 500 µm mesh secured by the zip-ties. Even more we acknowledge that the width of the average adult *H. comalensis* is about 500 µm and smaller ones could possibly fit through the mesh. We were relegated to using this mesh size since smaller mesh sizes did not allow the tanks to drain effectively enough with the designs that were implemented.

<u>Run 2</u>

Only four tanks were used for this run. There were only 74 adults available to conduct the 2nd experimental run; therefore, 20 individuals were placed into three tanks and 14 into a fourth tank. Half-way through the run a large amount of grey flocculation was noticed within the tanks accompanied by some type of slime. By the third week, breaches in the support screen were noticed in two tanks and the results from these tanks were recorded. Evidentially, sedimentation built up underneath the support screen so that pressure increased until a week point in the silicone seal was broken. Beetles from the other two tanks were retrieved at the end of the fourth week and by then the support screens from these were breached as well. Because of the breaches and because there were individuals underneath the support screens from the first run (and probably this one), one tank recovered more individuals than were originally placed into it. There were no foul smells during the experiment and in spite the flocculation and slime outbreaks, the beetles did not appear to be negatively affected as only 3 individuals were recovered dead.

After all data were collected, the support screens were pulled and inspected for adults that had drifted through the drains into the sump, pumped through the heating/chilling units, then pushed through the flow bars and ultimately into the tanks where they resided underneath the support screens. We found six adults living underneath the screens, apparently in good condition, and two dead adults. It was also evident that the adults were reproducing as well since 21 living larvae and 21 dead larvae were found. The adults were likely from the 1st experimental run, but could have been from the 2nd experimental run since adults can fit through the screens. In all, 30 adults were unaccounted from the first two runs and were presumed to be drifters that had resided somewhere else within the experimental system, or had exited through the sump stand pipe.

Run 3

These experimental units experienced the highest discharges and the oxygen levels compared to the other experimental runs (**Table 3**). The conditioning of the cotton lures appeared to progress faster and more intensely compared to the other experimental runs and a small amount of slime was noted in each of the tanks, though not nearly as much compared to the conclusions of experimental run 2. During the weekly checks, two individuals were found dead, stuck half-way through drain screens. One of the tanks experienced a high amount of drift (60%). Not only did this tank have four individuals found on the screen at the end of the experiment but evidentially, up to eight individuals left the tanks through the drain screens (not including an additional dead one found stuck in the drain screen during the experiment). After break-down of all tanks, four individuals were recovered underneath the support screens, indicating that they had to have left their mesocosms through a drain screen.

Discussion

Field lures

The 2019 lures hinted that *H. comalensis* may have had a preference for cotton over leaves, though not statistically significant. However, results from the 2020 lures found more adults on leaf than cotton lures (not statistically significant). T-tests of the combined data did not indicate that there was a preference to cotton or leaf for adult and larval *H. comalensis*. Previous comments surmised that conditioning rates may have different effects to varying materials. For instance, beetles may find leaves conditioned for longer periods of time more favorable than cotton conditioned for the same time, and that shorter time intervals beetles may find cotton more favorable. Furthermore, other factors such as spring flow may also influence conditioning rates. Interestingly, during this same experiment, *M. pusillus* adults and larvae were found to show a preference to cotton over leaf. Results of this experiment indicate that *H. comalensis* is not necessarily “lured” from its natural habitat to conditioned cotton; rather, these beetles are seeking whatever resources are available to them within their immediate area. However, it does not rule out the possibility that there are larger numbers of the population deeper within the aquifer and those that we find at the spring openings are “lured” by the abundance of resources closer to the surface.

Tank experiments

These results suggest that the test subjects were highly mobile and did not have a preference to any of the resources provided within the mesocosms. Furthermore, there was no difference in the proportions of adults found on lures or not on lures at all. In fact, the number of adults found on any lure was about the same as the number not found on a lure (excluding dead individuals outside of lures) was about 50:50 (117 and 121, respectively).

There were a number of early instar larvae recovered from leaf and wood lures, but only notable from one cotton lure. During the first experimental run, cotton lures were not inspected as carefully since the original goal was to observe the response of the adults. One of the reasons cotton was favored as a luring device is due to the ease of finding a little brown beetle on a mostly white piece of cloth, whereas it is much more challenging to find them on leave or wood, especially if they are borrowed in the wood. During the first experimental run, cotton lures were inspected mostly with the naked eye, all wood and leaf materials were carefully examined under magnification of a dissecting scope; thus, it is likely that more larvae were represented on cotton than is represented here. We adapted to the notion that the presence of larvae adds more information and we also inspected the cotton under magnification for the 2nd and 3rd experimental runs and so these numbers are more reflective of the larvae produced during the experiment.

The production of larvae is significant in giving information with regard to the movement of adults through the duration of the experiment. For example, Tank 2 from experimental run 1 had 23 recently hatched larvae on the wood lure, but no adults were found there. A single adult female can produce that many larvae within a month, especially if she had mated earlier (Kosnicki, 2020). However, it is likely that eggs are oviposited one at a time over a period of days or weeks, rather than all at once (Kosnicki, unpublished). Since newly hatched larvae have limited mobility and because it is unlikely that this number of larvae could randomly drift and settle on the same location, it seems highly probable that at least one gravid female adult had persisted at this location long enough to have deposited so many eggs. More likely, there may have been several gravid females at this location and not necessarily all at the same time, but moved on to other locations before the conclusion of the run.

Comments on drift

During the test run, one out of the 12 subjects was not recovered after a week and it is likely that it found a way into the drain, either through the mesh or under it. At the time of this test trial, finding > 90% of the subjects was seen as a positive as there are several explanations for why all individuals may not be recovered and so no additional caution was deemed necessary. However, in retrospect, 25% of the individuals were found on a drain screen, and these individuals were originally placed within a resource. It was presumed that all subjects would stay within a habitat once they found one, but some individuals were clearly not satisfied with staying within the resources provided.

Aquatic insects are known to actively drift as a result of unfavorable conditions and this action thereby enables them to seek out new habitats to colonize (Brittain and Eikeland, 1988). The force of flowing water can dislodge some individuals which could be seen as accidental drift; however, other individuals may actively choose to allow themselves to become suspended in the current and drift to a more favorable location. The review by Elliott (2008), indicates that drifting is not an uncommon phenomenon for several species of Elmidae and this may be their main mode of dispersion influenced by diel period and life-cycle, but is evidentially not due to density. Walton (1978) suggested that species may drift as a means of settling into specific substrates that favor that species. Therefore, it stands to reasons that the conditions within the mesocosms may have been unfavorable to some of the test subjects, which may explain why proportions of the beetles drifted.

Concluding remarks

Considering the propensity of drifting adults and from the evidence generated by the “orphaned” larvae in the mesocosms, it can be surmised that many of our test subjects were frequently on the move either by walking around the tank or becoming suspended in drift. This may be because test subjects found the experimental units unsatisfactory, or this may be a reflection of their natural behavior. Results of the field experiment complement the later notion. This species likely occurs at depths within springs. However, they may actively wander by drifting or walk to other areas, finding resources closer to the surface, those being biofilm conditioned cotton, leaf, wood, or other resources. In conclusion, the results from this study suggest that cotton lures are no more or less effective at attracting *H. comalensis* adults compared to other resources within the same area.

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