Comal Springs Riffle Beetle Laboratory Study: Evaluation Under Variable Flow Conditions

Final Report
Variable Flow Study: Project 802, Task 18
San Marcos National Fish Hatchery & Technology Center, San Marcos, Texas

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EXECUTIVE SUMMARY

BIO-WEST conducted laboratory experiments to evaluate the habitat use of the endangered Comal Springs riffle beetle, *Heterelmis comalensis*. A paucity of data regarding physiological and ecological needs of the insect limits ones understanding of its habitat and necessitates basic research of the species. This study expanded upon recent field efforts by BIO-WEST, which revealed an expansion of the known range of the Comal Springs riffle beetle. Previously it had been found primarily in spring runs feeding Landa Lake, New Braunfels, Texas, but individuals have recently been collected in seeps along the shoreline and around upwellings on the bottom of this lake.

Because the Comal Springs riffle beetle is found outside of the spring runs, it may be able to occupy more habitat than previously believed; this may also help explain the persistence of the species during periods of drought, particularly in 1956 when Comal Springs ceased flowing for 5-months. Nevertheless, the tendency of the species to congregate in areas of spring upwelling raises questions about how individuals react to a significant reduction or cessation of flow. It has been suggested that individuals retreat into springheads during such periods of low-flow. Under these conditions, water movement will attenuate and springflow may only be detected in subterranean habitats. It is also possible that a portion of the population regularly occupies interstitial spaces below the uppermost layer of gravel and rock that has been sampled exclusively to date.

To examine this issue, an experimental representation of the natural habitat was designed and the response of the Comal Springs riffle beetle to water flow of varying intensities and with different combinations of horizontal and vertical flow assessed. Observations were made of depth and orientation toward current under the different treatments applied with these variables. Barriers to restrict vertical movement and flow were added in the later trials. Initial trials were conducted with a surrogate species, *Microcylloepus pusillus*, to evaluate the design of the enclosures and assess several artificial substrates.

Although results revealed no statistically significant conclusions, the beetles displayed tendencies for movement downward and towards current. All trials with the surrogate and the first two trials with the Comal Springs riffle beetle had similar results; the beetles all congregated on or near the bottom regardless of conditions. To account for a potential oversimplification of the natural habitat (very large interstitial spaces), barriers were placed between every other layer. The barriers were installed to restrict downward movement and simulate the increasingly small spaces likely to be encountered below the upper layer of substrate in the wild. The barriers may have also improved lateral flow across layers such that beetles were more able to detect flow direction more readily; more beetles were found in the front of each panel, where flow originated. The results of different barrier/flow intensity/flow direction combinations were for beetles to be found in the higher layers with horizontal flow only across the top layers, to display a more even vertical distribution when horizontal flow was allowed throughout, and to congregate on the bottom in upwelling-only treatments.

The tendency of the Comal Springs riffle beetles to orient toward flow confirms field observations that the species associates with flowing water. The overwhelming tendency for the beetles to move downward during the first two trials was hardly conclusive, but trials with highly restrictive barriers (i.e., 1-5% passable) provide evidence to suggest that individuals will search for moving water

when it cannot be detected near the surface. With the barriers, upwelling flow would have been severely restricted and likely detected only by individuals once they had moved to the lower levels, yet most beetles were found there. In contrast, trials with only lateral flow across the upper layers had more beetles in those layers; the beetles presumably did not move to lower layers in search of the requisite flow. Finally, a trial with flow decreasing to zero over time and barriers in place resulted in a higher frequency of beetles in the lower layers in the trial with horizontal flow throughout. If these observations translate to behavior in the natural habitat, it is likely that individuals would respond to decreasing flow by retreating into more subterranean habitats. Further, it is possible that individuals may inhabit areas deeper in the gravel and sediment around spring orifices than have been previously sampled.

It is important to note that these results may be affected by a number of factors that remain unknown about the species and were unaccounted for in this experiment. Dietary needs, reproductive behavior, etc., also may have influenced movement. In the natural habitat, niche division (intra- or interspecific competition) and predator avoidance may also largely determine habitat selection. Very little is known about this insect and more research is necessary to discern how it would behave under different flow conditions in its natural habitat.

1.0 INTRODUCTION

The Comal Springs riffle beetle (*Heterelmis comalensis*) was first collected by Linda Bosse in 1976 and later described from specimens found in the headwaters of the Comal River, New Braunfels, Texas (Bosse et al., 1988). All specimens (38 adults and later 30 adults and 31 larvae collected by Harley Brown in 1977) were found in spring run 2 (Figure 1) where water depth ranged from 2 to 10 cm, flowing over a gravel substrate into Landa Lake (Bosse, 1979 unpublished thesis, Bosse et. al. 1988). Adult Comal Springs riffle beetles have reduced hind wings, rendering them flightless, and are approximately 2 mm in length; females are slightly larger than males. This population is believed to reach its greatest density from February to April (Bosse et. al., 1988). Larvae were collected with adults in the gravel substrate and not on submerged wood as is typical of most *Heterelmis* (Brown and Barr, 1988).

The nearest relative to *H. comalensis* is *H. glabra* found in west Texas, Arizona, Mexico, and Central America (Brown and Barr, 1988). *Heterelmis vulnerata* occurs in the nearby San Marcos River and is widely distributed in Texas and Oklahoma (Brown, 1972). Until recently, the Comal Springs riffle beetle had only been collected in two other spring runs (1 and 3) in Landa Park (a single specimen also was reported from the San Marcos River, Hays County, 32 km northeast of Comal Springs, but no other observations have been made there). With limited information on its distribution, the U.S. Fish and Wildlife Service listed *H. comalensis* as an endangered species in 1998 with the primary threat being reduction of water quantity and quality due to human activities (USFWS, 1997). BIO-WEST, Inc. (2002) has since documented an expansion of the known range of this species to include areas along the western shore of Landa Lake and around upwellings in the lake itself; however the population is still confined to a relatively small area. Despite its limited distribution, the Comal Springs riffle beetle has survived a drought resulting in cessation of flow from Comal Springs between 13 June and 3 November 1956 (Brune, 1981).

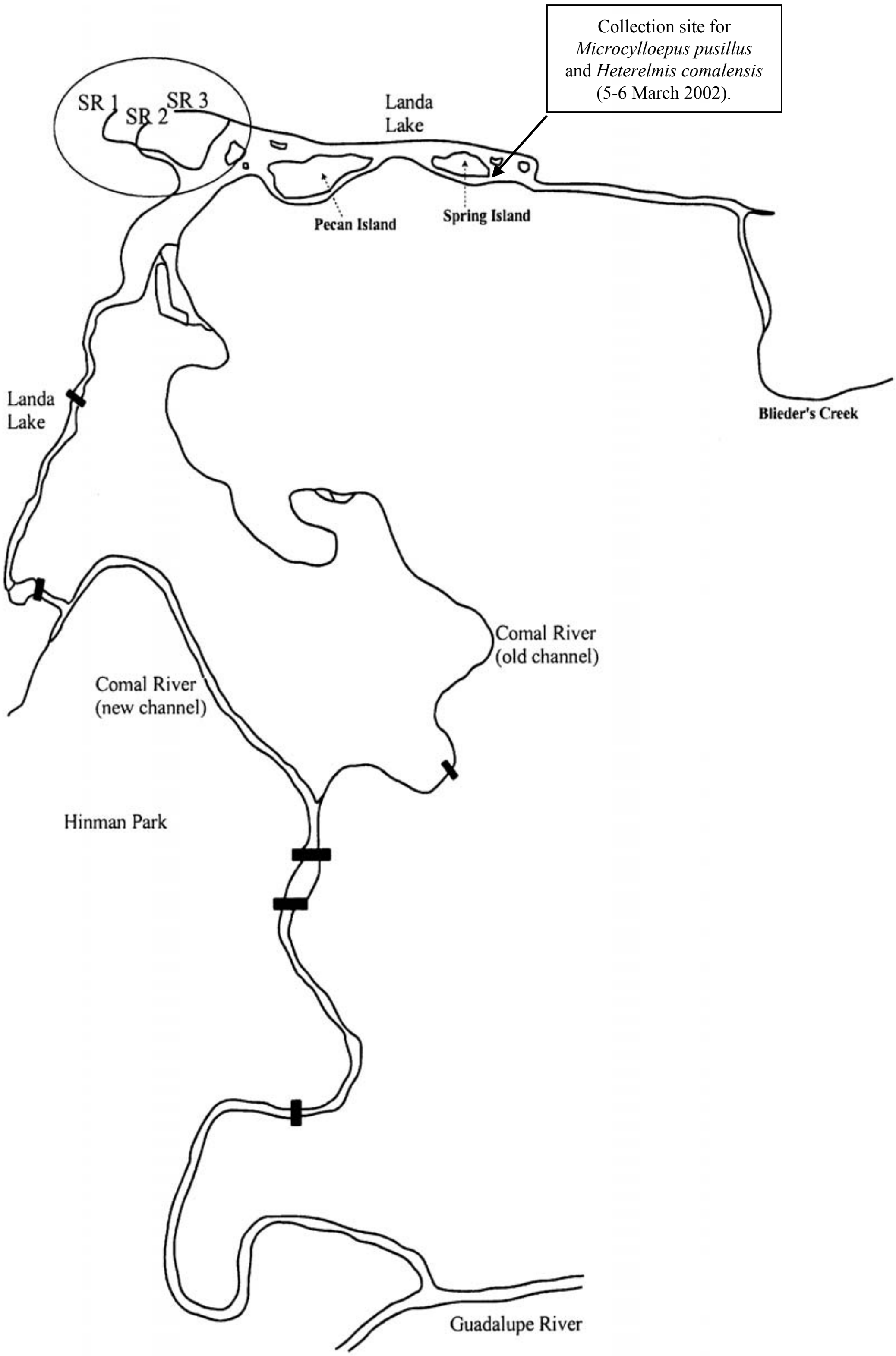


Figure 1. Comal River, New Braunfels, Texas: Spring runs 1, 2 and 3 shown in oval.

How the species survived the drought of 1956 remains unknown. Because the species is closely associated with areas of spring upwelling, one hypothesis is that the Comal Springs riffle beetle "follows" the flow stimulus and retreats into springheads during periods of low flow. This hypothesis also raises the possibility that a portion of the population may regularly occupy hyporheos regions (area under a streambed where interstitial water moves by percolation); only the uppermost layer of gravel and rock has been sampled to date. The current study explored this possibility with laboratory experimentation to determine vertical (depth) preference within the substrate and orientation to current (rheotaxis) in normal and reduced flow conditions.

2.0 METHODS

Beetle collections:

Preliminary trials were run with a surrogate species, *Microcylloepus pusillus*, during development of the experimental systems. On 23 January 2002, fifty *M. pusillus* were collected from rocks surrounding an upwelling spring on the bottom of Landa Lake just upstream of Spring Island (Figure 1). Ten adult and 2 larval *H. comalensis* also were observed at this site during the collection. The *M. pusillus* were taken to the National Fish Hatchery and Technology Center, San Marcos, Texas (NFHTC) and stored in a holding tank. After the preliminary testing, these beetles were removed and preserved in 70% isopropyl alcohol on 5 March 2002.

On 5-6 March 2002, fifty-four *H. comalensis* were collected and transported to the NHFTC and stored in the existing refugium. The sites of collection are indicated in Figure 2: 11 beetles were collected from spring run 1; three beetles were located in spring run 2 but returned to the site; and 48 beetles were retrieved from spring run 3 with 5 returned to the site. Most of the beetles were found on rocks, gravel, or leaves in the shallow marginal waters of side springs flowing into the spring runs. Beetles were carefully removed by hand or soft forceps and placed in a small cooler containing spring water and a few leaves. On 19 March 2002, identification of the beetles was confirmed in the laboratory with the aid of a dissecting microscope. Almost all the beetles had attached protozoans primarily near the head region, which is a common circumstance for elmids (Brown, 1987). Fifty-two of the beetles were then moved to the holding tank on the experimental system. One beetle with a missing leg was returned to the refugium, and one mortality was preserved in 70% isopropyl alcohol. Seven additional mortalities occurred during the experiment (Table 1). Water quality measurements from the collection sites on 22 March 2002 are presented in Table 2.

System Design:

The experimental setup is represented in Figure 3. Beetles were stored in a 55-L glass aquarium (holding tank) sitting on top of a 950-L insulated fiberglass tank that served as the shared reservoir, (Living Stream model MT-1024, Frigid Unit, Toledo, Ohio). A 1-hp chiller/1KW heater unit (Universal Marine Industries, Inc., San Leandro, California) maintained water temperature between 21-23° C (range recorded in natural habitat, Brune 1981), and a 0.5-hp Hayward pump (model SP125J; Hayward Pool Products, Inc., Elizabeth, New Jersey) circulated water throughout the

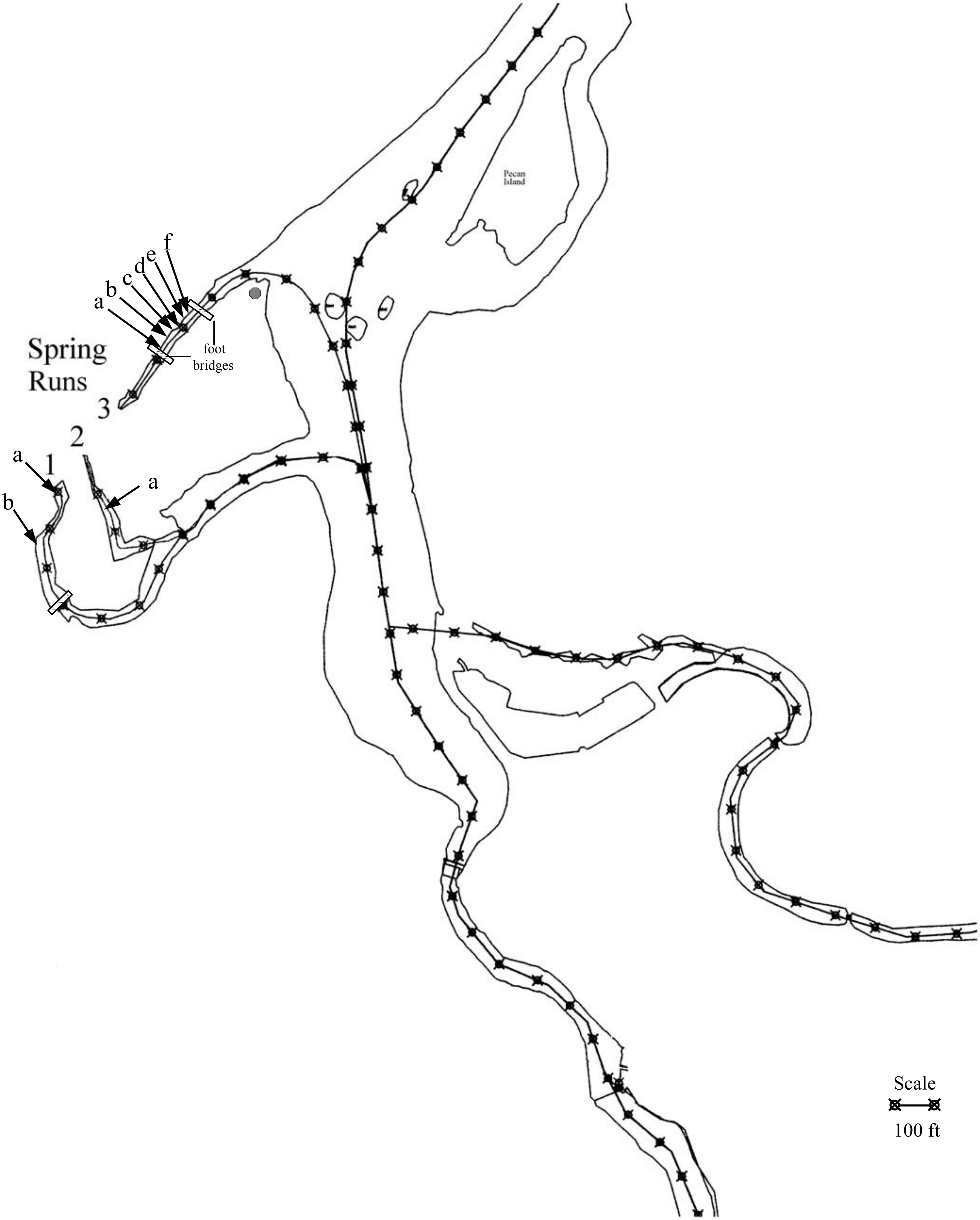


Figure 2. Collection sites of *Heterelmis comalensis* from the spring runs of Landa Lake, March 2002. Five to six beetles were collected in each side spring indicated by arrows a-f.

Table 1. Mortalities of wild caught *Heterelmis comalensis*.

Date	Note	Number of Surviving Beetles	
		Experimental Tank	Refugium
5 March	11 beetles collected from spring run 1		11
6 March	43 beetles collected from spring run 3		54
19 March	mortality in refugium one missing leg, rest moved to holding tank	52	1
27 March	mortality (recent) in experimental block mortality (few pieces found) in holding tank	50	1
26 April	mortality (in pieces) in experimental block mortality (exoskeleton) in holding tank	48	
30 April	mortality (recent) in holding tank mortality (headless exoskeleton) in holding tank	46	1
17 May	mortality (headless exoskeleton) in holding tank	45	1
19 June	no mortalities, holding tank serves as refugium	45	1
	8 total mortalities 85 % survival Average death rate = 2 mortalities/month		

Table 2. Water quality measurements from Comal Springs, New Braunfels, Texas, 22 March 2002.

	Spring Run 1	**Spring Run 2**	**Spring Run 3**
Temperature ºC	23.5	23.0	23.1
Conductivity, µS/cm	0.575	0.577	0.575
pH	7.92	7.92	7.92
Dissolved oxygen, mg/L	5.1	5.1	4.6
TDG (% saturation)	-	-	110.5

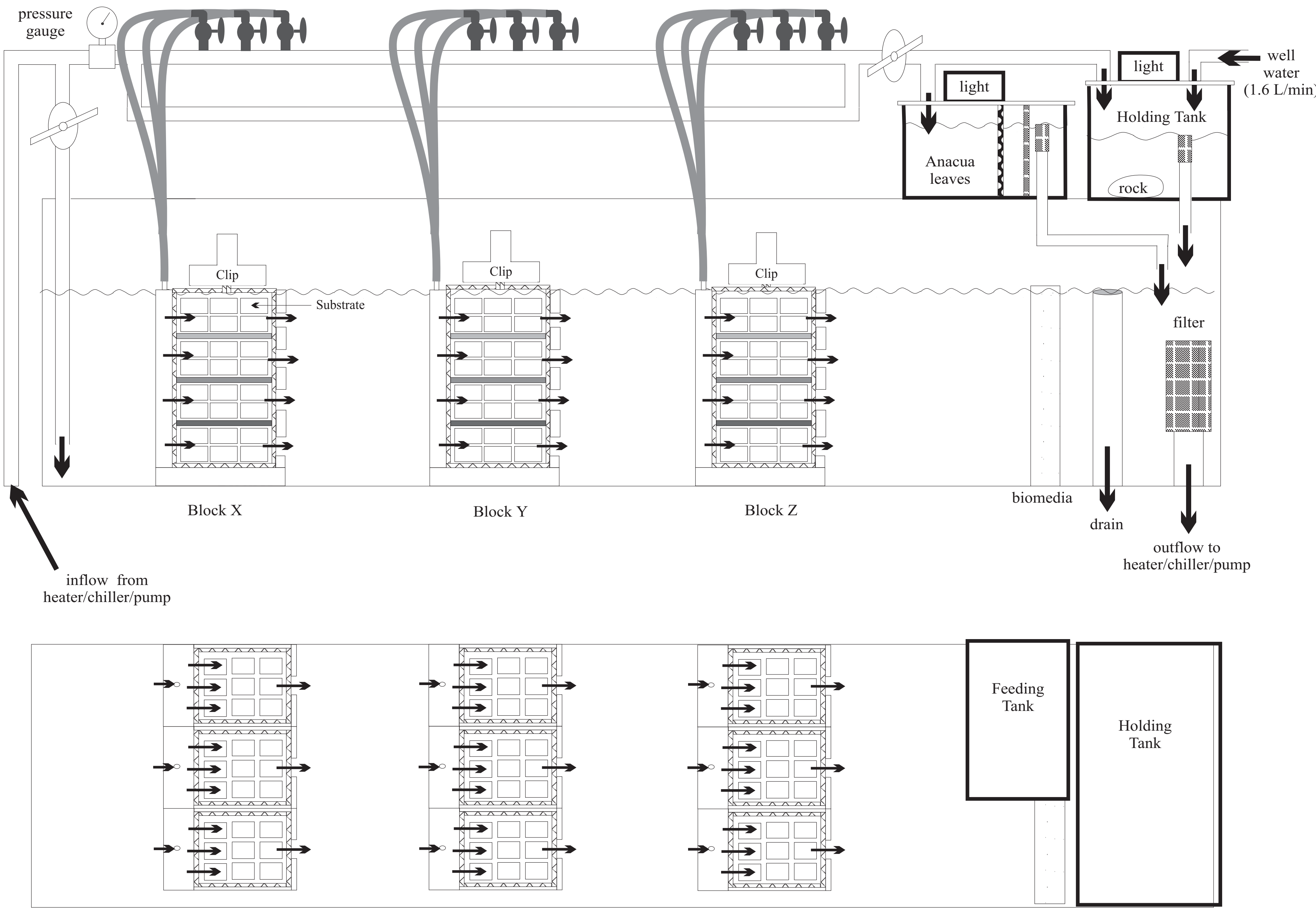


Figure 3. Profile and overhead view of system layout.

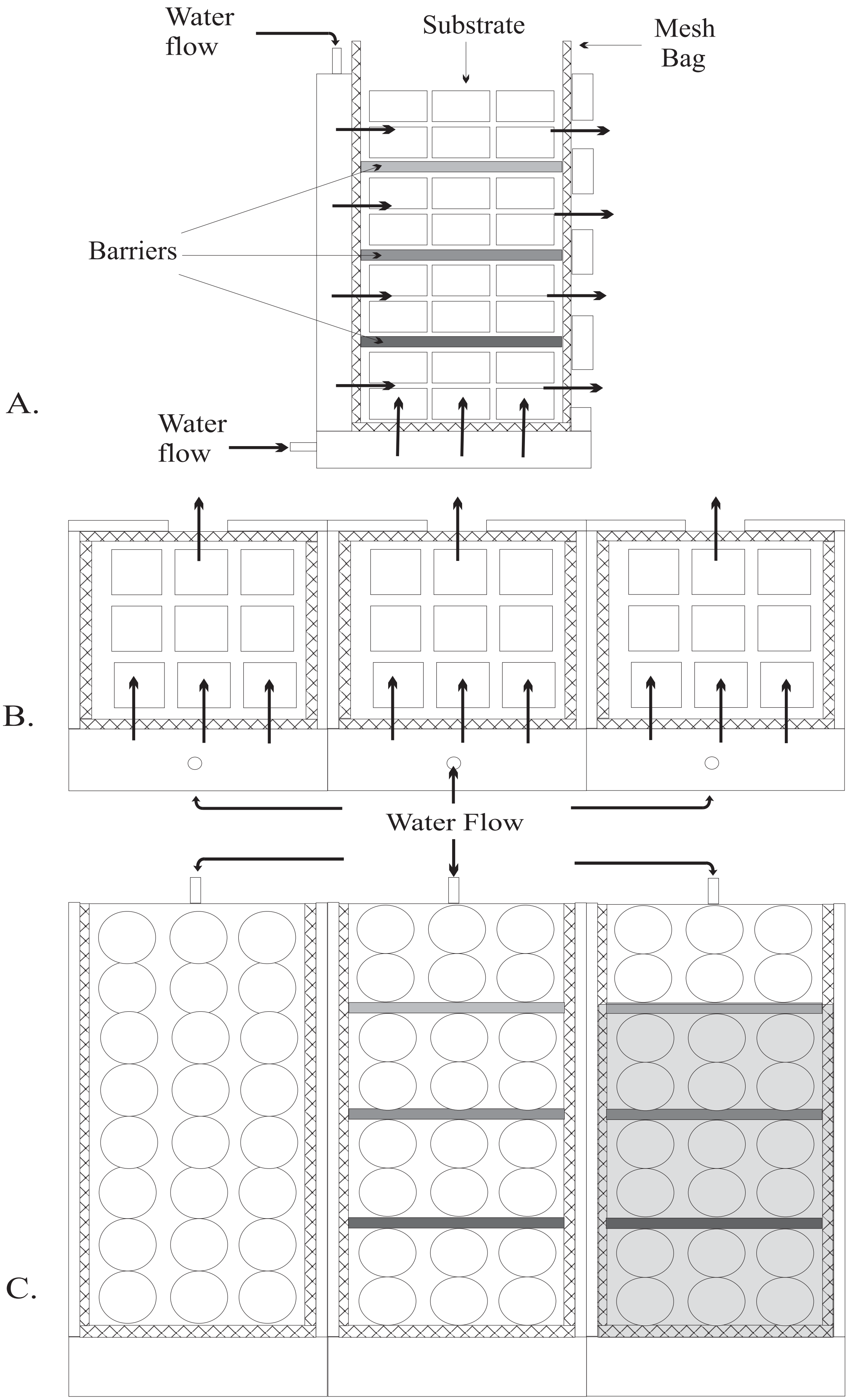


Figure 4. Diagram of a three-chambered block filled with substrate. A) side view with barriers and combination flow shown, B) top view, and C) front view with no barriers in the left section, barriers in the center section, and horizontal top flow only in the right section.

system. Untreated water pumped directly from the Edwards Aquifer was added continuously (1.6 L/min) to the holding tank along with recirculation water and both drained back into the shared reservoir. Anacua (*Ehretia anacua*) leaves collected from the terrestrial areas surrounding the Comal River were dried and introduced into a 16.5-L aquarium to serve as a source of bacterial and fungal growth and evenly inoculate other areas of the tank with epiphyton on which the beetles might feed. Water was circulated through these leaves and filtered back into the shared reservoir. Standard aquarium light sources with fluorescent bulbs were placed on top of the food and holding tanks.

Three identical blocks were constructed with black acrylic plexiglass (Figure 4). Each block was divided into three smaller identical compartment, or "sections" (14" x 5" x 5") with the bottom and one side having the ability for water to flow in and the opposite side with open areas for water to pass out. During most trials, including all with *H. comalensis*, each compartment was filled with Bio Barrel polypropylene media (Part No. BF44A, Aquatic Eco-Systems, Inc., Apopka, Florida). Each piece was cylindrical and measured 1.5" x 1.5" and was designed to maximize interstitial surface area while allowing flow vertically, horizontally, or in both directions. These pieces were glued together into 3 x 3 grids (4.5" x 4.5") to form a single layer of substrate. Eight of these layers were stacked inside bags constructed from 300-micron and 325-micron nylon mesh and inserted into each section of the blocks. Three trials conducted with *M. pusillus* contained differing substrate: two had 7 limestone rocks for layers and a third had Bio Barrels with small bricks. Each of the three blocks had recirculation water pumped through hoses (one for vertical flow and one for horizontal flow for each of the three compartments). Faucets (Figure 3) were turned on or off to choose a vertical, horizontal, or combined flow direction, and flow regulators were placed inside the faucets to evenly distribute the water flow throughout all chambers. Current velocities (Table 3) were adjusted by pressure (PSI guage) and flow regulators and measured using a USGS Price pygmy current meter, modified to fit within compartments, and current meter digitizer (CMD 2.0).

<u>Data Collection</u>

For each trial, three treatment levels (Tables 4 and 5) were assigned to sections within a block using a random number generator and replicated across the three blocks (X, Y, Z). Treatments were not consistent across trials as some changes were necessary (e.g., the addition of barriers). To produce the desired flow levels for each treatment, faucets were toggled and the valve on the exhaust pipe was adjusted to reach the water pressure needed, as indicated by the PSI reading on the pressure gauge. Mesh bags were filled with substrate and inserted into each section. The water level was adjusted to cover the top layer of substrate by maneuvering the drain standpipe of the shared reservoir tank. Beetles were carefully removed from the holding tank with soft forceps or disposable plastic transfer pipettes and placed in 50-ml plastic beakers (five/beaker) partially filled with water. Water and beetles were poured over the center of the top layer of substrate. Five beetles were introduced to each section (3 sections x 3 blocks x 5 beetles = 45 beetles per trial). Clips were used to secure the tops of the mesh bags and prevent escape from the experimental setup. After 2 days or 1 week for a given experimental trial, the sections were checked. Each mesh bag was carefully removed, clipped on a hanger, and an initial observation was made of the mesh; many beetles were observed clinging on the mesh and localities were easily noticed with this inspection. Subsequently, bags were opened and each layer was carefully removed and

Table 3. Velocity measurements (m/s) for each section with 3-gal/min flow regulators on the horizontal hoses and 1-gal/min regulators on the vertical hoses of the riffle beetle experimental setup.

Block	Section	Horizontal (10 psi) higher flow	Vertical (10 psi) higher flow	Horizontal (5 psi) lower flow
X	1	0.33	0.33	0.17
X	2	0.45	0.37	0.20
X	3	0.40	0.33	0.18
Y	1	0.35	0.43	0.18
Y	2	0.34	0.43	0.17
Y	3	0.34	0.42	0.15
Z	1	0.31	0.41	0.14
Z	2	0.32	0.34	0.15
Z	3	0.29	0.40	0.20

Table 4. Treatments conducted in preliminary trials with *Microcylloepus pusillus* (30 Jan – 1 Mar 2002). Treatments included moderate (≅ 0.4 m/s horizontal, ≅ 0.6 m/s vertical) and high (≅ 0.8 m/s horizontal, ≅ 1.3 m/s vertical) flow levels and three different substrate types (biomedia only, biomedia + bricks, and limestone rocks). In each block, one section had horizontal flow, one had vertical flow, and one had both.

Trial	**Time Period**	**Blocks**	**Flow Level**	**Substrate**
P1	30 Jan – 1 Feb (2 days)	Y	moderate	8 layers biomedia
P2	30 Jan – 5 Feb (1 week)	X	moderate	8 layers biomedia
P3	5-12 Feb (1 week)	X, Y, Z	moderate	8 layers biomedia
P4	13-20 Feb (1 week)	X	moderate	7 limestone rocks
		Y	moderate	6 layers biomedia with bricks between
		Z	none	8 layers biomedia
P5	20 Feb – 1 Mar (1 week)	X	high	7 limestone rocks
		Y, Z	high	8 layers biomedia

Table 5. Treatments conducted in weekly trials with *Heterelmis comalensis* (20 March – 24 May 2002). All treatments contained 8 layers of plastic biomedia substrate and 15 beetles (3 replications x 5 beetles). High flow was ≅ 0.37 m/s and low flow ≅ 0.17 m/s; in trial 8, flow was lowered in daily increments until flow was ceased one day prior to sampling.

Trial	**Time Period**	**Flow Level**	**Treatment**
R1	20 – 27 Mar	high	A – full horizontal flow B – vertical flow C – combination
R2	27 Mar – 3 Apr	low	A – full horizontal flow B – vertical flow C – combination
R3	4 – 12 Apr	high	A – full horizontal flow B – full horizontal with barriers C – top horizontal flow with barriers
R4	12 – 19 Apr	low	A – top horizontal flow with extensive barriers B – full horizontal with barriers C – top horizontal flow with barriers
R5	19 – 26 Apr	high	A – top horizontal flow with barriers B – full horizontal with extensive barriers C – top horizontal flow with extensive barriers
R6	30 Apr – 7 May	low	A – vertical flow with extensive barriers B – full horizontal with extensive barriers C – top horizontal flow with extensive barriers
R7	7 – 14 May	high	A – vertical flow with extensive barriers B – full horizontal with extensive barriers C – top horizontal flow with extensive barriers
R8	17 – 24 May	low to none	A – vertical flow with extensive barriers B – full horizontal with extensive barriers C – top horizontal flow with extensive barriers

Table 6. Water quality data from the shared reservoir of the Comal Springs riffle beetle experiment (18 March – 28 May, 2002). All weekly ammonia levels were ≤ 0.05 mg/L.

Date	Temperature ºC	Conductivity µS/cm	pH	Dissolved Oxygen, mg/L	TDG (% Saturation)
18 March	22.06	0.633	7.75	5.04	-
21 March	20.89	0.633	-	4.54	102.7
25 March	22.70	0.630	7.62	4.46	101.3
28 March	21.04	0.633	7.66	5.25	100.2
1 April	20.84	0.634	7.51	5.04	100.1
3 April	20.70	0.611	7.47	5.19	101.0
8 April	21.99	0.631	-	5.46	100.3
10 April	21.96	0.658	7.82	5.21	101.5
16 April	22.19	0.654	7.74	5.72	100.2
18 April	22.34	0.633	7.84	6.08	101.5
22 April	22.14	0.665	7.19	5.50	97.7
25 April	22.14	0.630	8.19	5.05	104.5
30 April	22.18	0.655	7.73	5.70	103.2
2 May	22.47	0.630	7.60	5.43	104.4
6 May	22.75	0.628	7.70	5.43	104.3
9 May	22.30	0.631	7.69	3.94	104.2
13 May	21.05	0.632	7.76	4.64	105.1
16 May	22.52	0.621	8.18	5.88	104.8
20 May	20.61	0.632	7.73	4.42	104.8
24 May	22.52	0.621	7.81	5.74	102.0
28 May	22.74	0.623	7.94	5.51	103.8
Average	**21.82**	**0.638**	**7.56**	**5.14**	**102.0**

examined. Vertical and horizontal location was recorded for each beetle within a section and beetles were returned to the holding tank until the next trial.

Dissolved oxygen, conductivity, water temperature, and pH were recorded twice weekly using a Hydrolab multi-probe and data sonde (model 2; Hydrolab Corporation, Austin, Texas). Total gas saturation was measured weekly using a gas saturometer (model DS-1B; Sweeney Aquametrics, Stony Creek, Connecticut). Ammonia levels were measured at the A. E. Wood State Fish Hatchery laboratory weekly (Table 6).

3.0 RESULTS AND DISCUSSION

Preliminary trials with *Microcylloepus pusillus*

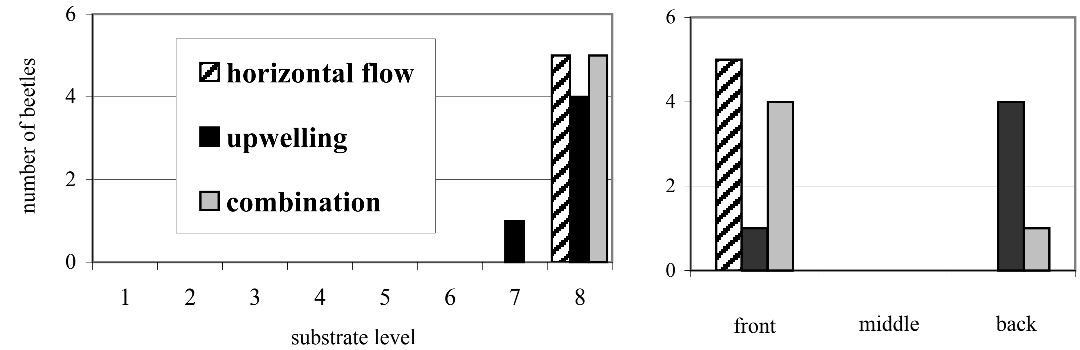
Microcylloepus pusillus was used in five preliminary trials with a variety of flow levels and directions, substrate types, and time periods as outlined in Table 4. With the exception of Block Z on trial P4, which had no flow, all blocks had one section with horizontal flow only (treatment A); one section with vertical flow only (treatment B); and one section with flow in both directions combined (treatment C, Figure 4a). Beetles were found mainly in the bottom layers in all treatments and variable conditions tested, including the three different substrates (Figures 5, 6 and 7). Because results remained similar using the different substrate types, the plastic biomedia substrate layers were used in all trials with *H. comalensis* for ease of location of beetles and reduced danger in crushing or loss of individuals.

Trials with *Heterelmis comalensis*

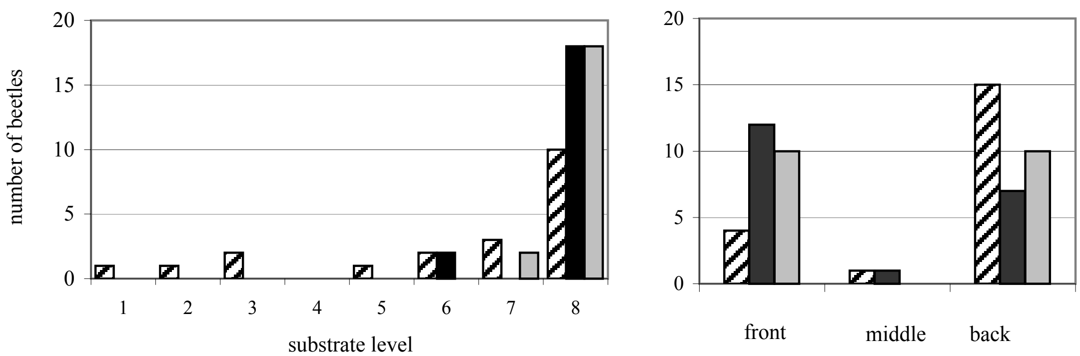
Eight trials were completed using *H. comalensis* with plastic biomedia substrate and differing flow levels and treatments (outlined in Table 5). The first two trials (R1 and R2), which had the same treatments (horizontal, vertical, and combined flow) as the preliminary trials, showed results similar to that of *M. pusillus* with minimal difference between treatments (Figure 8).

Results to this point showed a strong preference for the bottom layers of substrate by both *M. pusillus* and *H. comalensis,* regardless of flow. If extrapolated to populations in the wild, it would appear that most of the *H. comalensis* should be subterranean and found at least a foot below the surface. However, some questions remained regarding the representation of natural conditions in the experimental setup. To discount the possibility that the beetles were using the lower layers of substrate to avoid light entering from overhead, black plexiglass covers were placed above each block on all subsequent trials. In addition, it was hypothesized that the interstitial spaces of the artificial media were too large and did not adequately represent the increasingly small spaces likely to be encountered when moving down from the cobble at the surface into finer substrates in the wild. To overcome this concern, three increasingly difficult-to-cross barriers were placed between every other substrate layer to hinder downward movement (Figure 4a and 4c). These barriers were constructed from plastic pond liner with different numbers of 6-mm diameter holes punched evenly throughout. The surface of the first barrier placed between layers 2 and 3 was 29% passable (percent of surface area composed of holes); the second barrier between the layers 4 and 5 was 19% passable; and the third barrier between the layer 7 and 8 was 11% passable.

P1 - Two-day run at moderate flow (5 individuals/treatment)



P2 and P3 - One-week run at moderate flow (20 individuals/treatment)



P5, block Y and Z - One-week run at high flow (10 individuals/treatment)

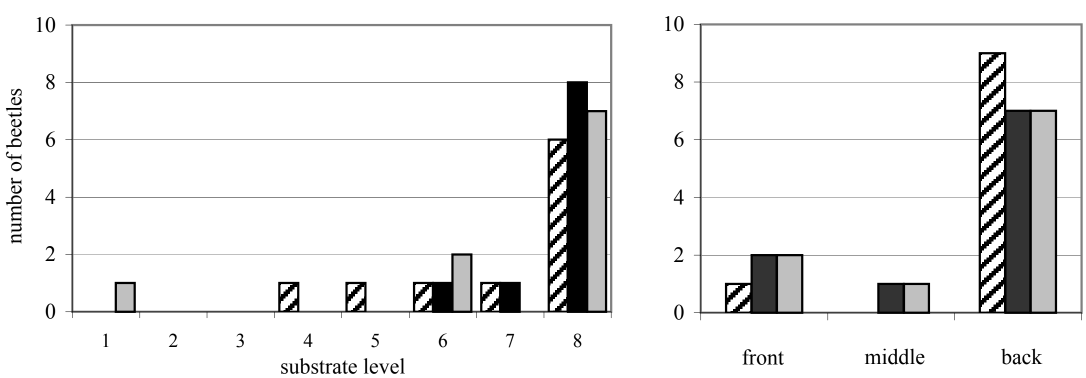
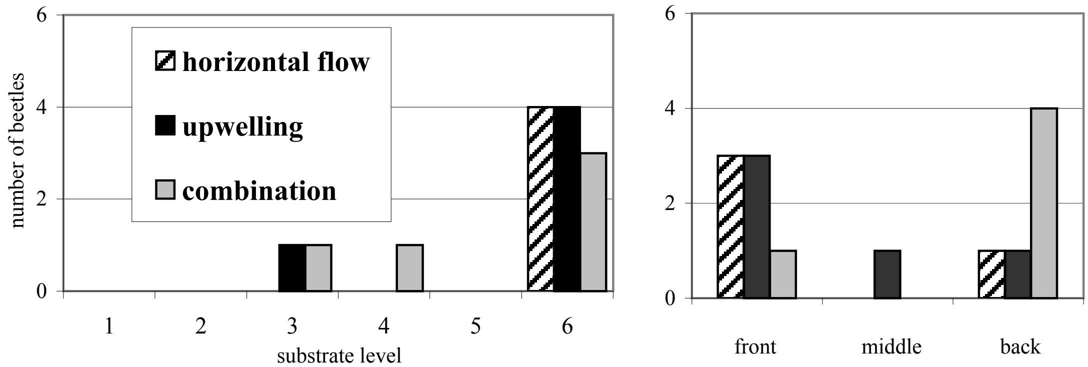
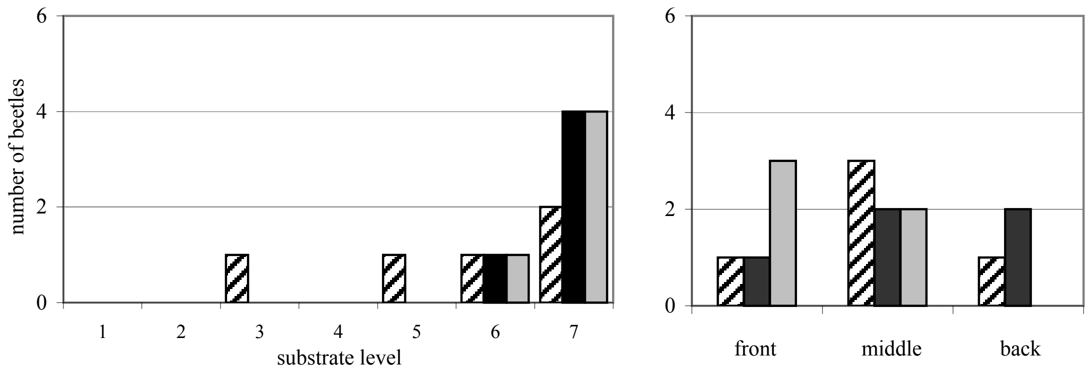


Figure 5. Preliminary runs with *Microcylloepus pusillus* and Bio Barrel substrate; depth (1= top layer; 8 = bottom layer) and orientation to flow (front = towards origin of horizontal flow; back = away from origin of horizontal flow) are represented.

P4, block Y - Brick/biomedia substrate at moderate flow (5 individuals/treatment)



P4, block X - Limstone rock substrate at moderate flow (5 individuals/treatment)



P5, block X - Limestone rock substrate at high flow (5 individuals/treatment)

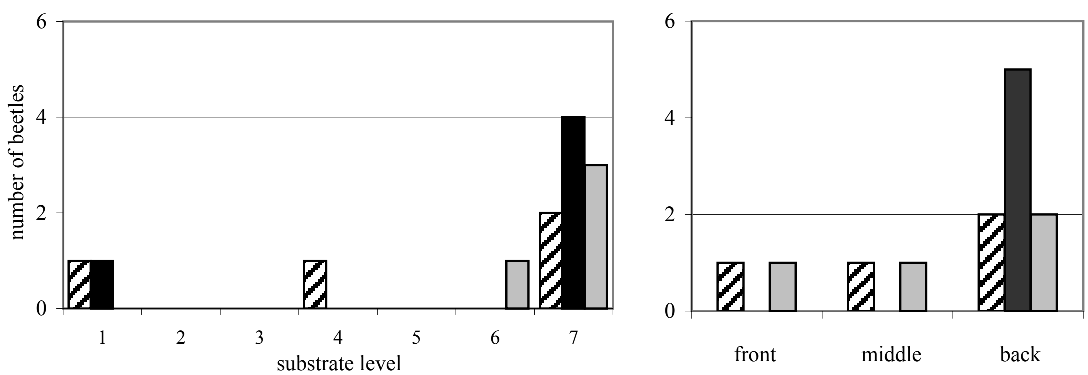
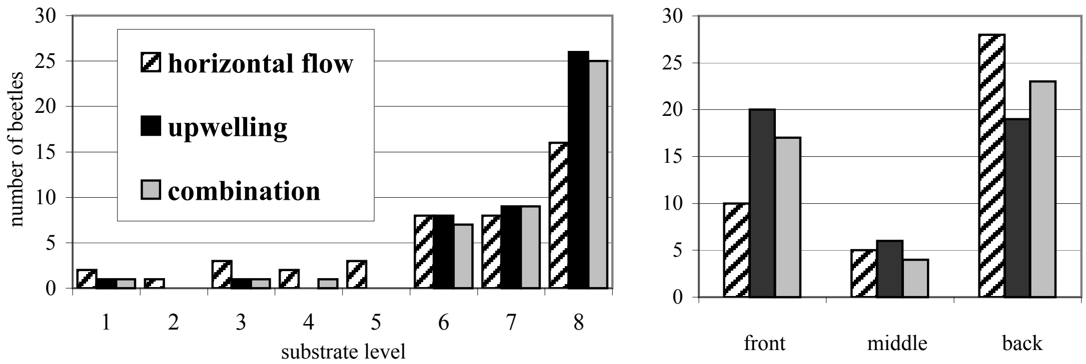


Figure 6. Preliminary runs with *Microcylloepus pusillus* using alternate substrates; depth (1= top layer; 8 = bottom layer) and orientation to flow (front = towards origin of horizontal flow; back = away from origin of horizontal flow) are represented.

All week-long preliminary runs with flow (45 individuals/treatment)



P4, block Z - Biomedia substrate with no flow (5 individuals/treatment)

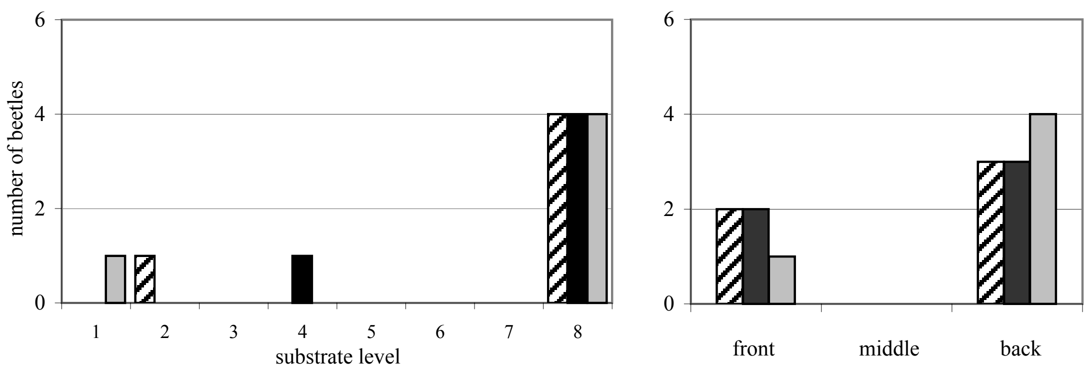
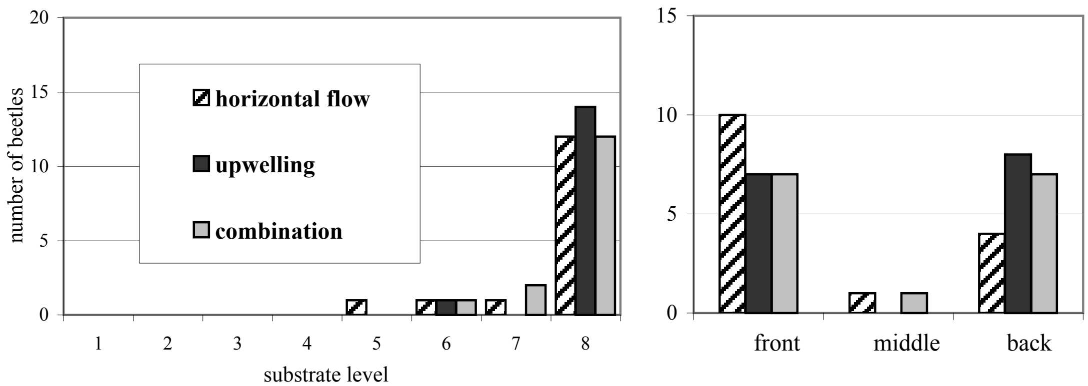


Figure 7. Combined data on preliminary runs with flow and results of one trial without flow; depth (1= top layer; 8 = bottom layer) and orientation to flow (front = towards origin of horizontal flow; back = away from origin of horizontal flow) are represented.

R1 - Trial with *H. comalensis* at higher flow (0.37 m/s)



R2 - Trial with *H. comalensis* at lower flow (0.17 m/s)

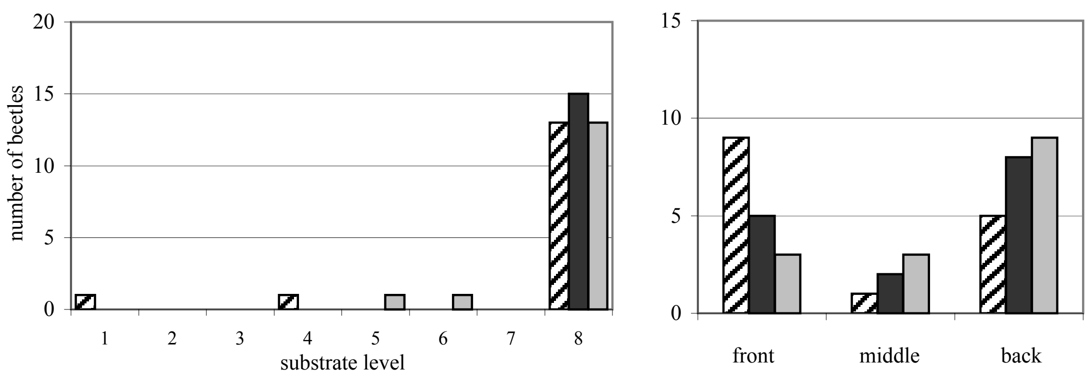


Figure 8. Experimental runs with *Heterelmis comalensis* and no barriers; depth (1= top layer; 8 = bottom layer) and orientation to flow (front = towards origin of horizontal flow; back = away from origin of horizontal flow) are represented. All runs had 15 beetles per treatment.

Also in subsequent trials, the upwelling treatment was replaced by one with horizontal water flow only through the upper two layers of substrate, above the first barrier (Figure 4c). The intent was to evaluate whether the addition of barriers would allow them to respond more directly to flow, rather than moving downward regardless of conditions. The expectation was that beetles would not be found in lower layers where flow would be absent. This design was accomplished by placing a square bottom plastic bag cut to the desired height around the mesh bag filled with substrate, consequently blocking all flow through the section except for the top two layers.

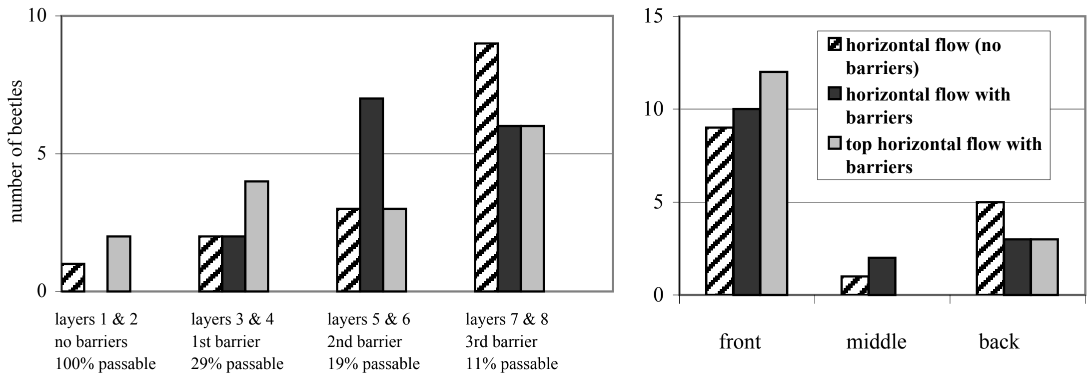
In the third trial (R3), beetles in treatments with the barriers were not as concentrated toward the bottom layers compared to treatments without barriers, and most beetles were located in the front of the sections, towards the current (Figure 9). In addition to simulating increasingly small pore size between substrates, the barriers may have improved horizontal flow across the blocks, making current direction more detectable by the beetles. However, despite barriers and adjusted treatments, most of the beetles were still found in the bottom half of the blocks.

For the fourth trial (R4), more restrictive barriers were constructed and placed in the same locations. These were designed from the same material and had fewer of the same-sized holes punched in a symmetrical pattern around the center of the barriers. The first barrier was 5% passable; the second was 3% passable; and the third was 1% passable. These more restrictive layers were added to one section with horizontal flow only across the upper layers and the majority of beetles were found in the top half of the block. The treatments with the less restrictive barriers had similar results to the previous trial (Figure 9). Those results could have been either an increased response to flow, or a decreased ability to pass through the first barrier. Thus, a fifth trial (R5) included the more restrictive barriers on treatments with horizontal flow only across the upper layers ("top-flow" treatment) and full horizontal flow. This trial resulted in a relatively even vertical distribution of beetles in the full horizontal treatment and beetles concentrated near the top in the top flow treatment (Figure 9). This suggests that the more restrictive barriers did not prevent movement to the bottom layers, yet beetles remained in the upper layers when that was the only area with flow. The more restrictive barriers were used exclusively in the last three trials (R6-8) with similar results.

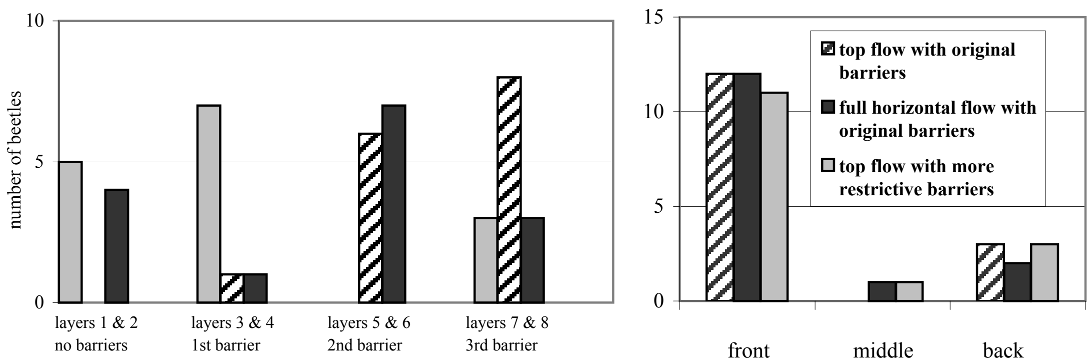
Also added to trials 6-8 was an upwelling (only) treatment to evaluate whether the beetles would congregate in the bottom despite increased difficulty (restrictive barriers) and the lack of stimuli (upwelling flow would not have been detected in the upper layers because of intervening barriers). Results indicate that the beetles somehow found the source of upwelling and congregated there (Figure 10).

The final trial (R8) was set up identical to the previous two, but flow levels were slowly reduced over time. The trial began at the lowered flow rate $\cong$ 0.17m/s; two days later the flow was reduced to $\cong$ 0.1 m/s; after two additional days, the flow was further reduced to $\cong$ 0.07 m/s. After a total of 6 days, the hoses leading to the blocks were removed from the faucets and flow = 0 m/s; beetles were checked the following day. Compared with the previous two runs, results were similar but with beetles in the upwelling and top horizontal flow treatments shifted slightly away from flow, and beetles in the full horizontal flow treatment shifted slightly toward the bottom layers. Also beetles in all treatments were shifted slightly away from the front end of the blocks.

R3 - *H. comalensis* with barriers at higher flow (0.37 m/s)



R4 - *H. comalensis* with barriers at lower flow (0.17 m/s)



R5 - *H. comalensis* with barriers at higher flow (0.37 m/s)

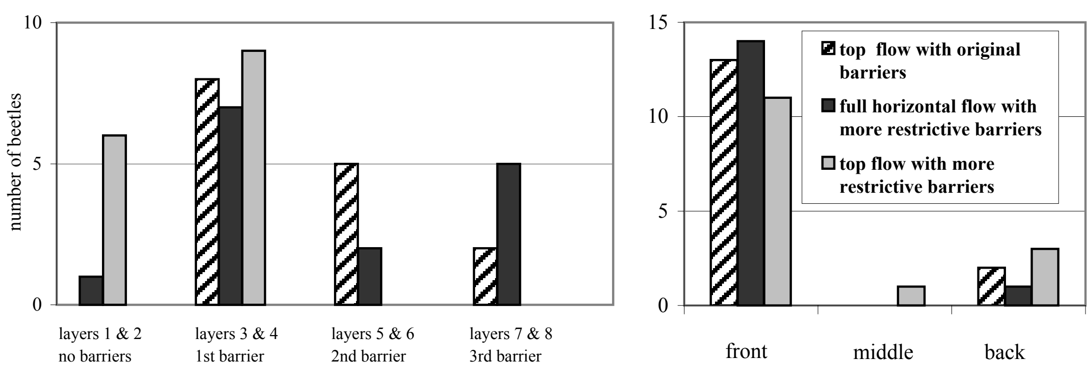
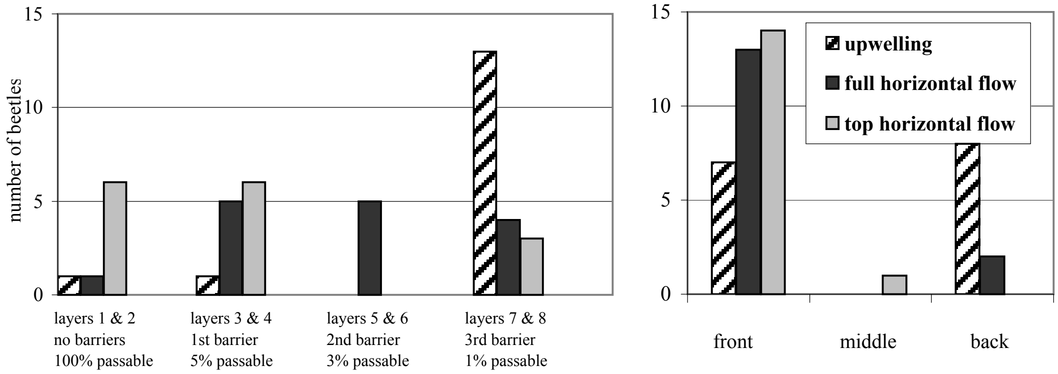
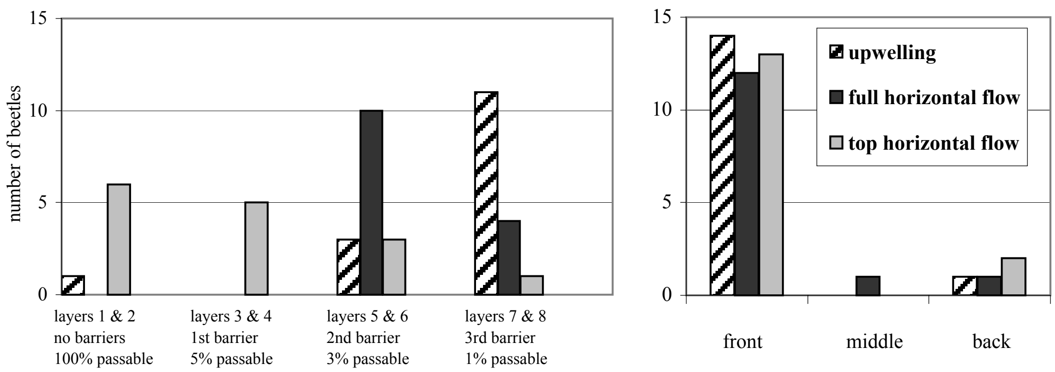


Figure 9. Experimental runs with *Heterelmis comalensis* and barriers added; depth and orientation to flow (front = towards origin of horizontal flow; back = away from origin of horizontal flow) are represented. All runs had 15 beetles per treatment. More restrictive barriers were as follows; 1st = 5% open, 2nd = 3% open, 3rd = 1% open.

R6 - *H. comalensis* with barriers at lower flow (0.17 m/s)



R7 - *H. comalensis* with barriers at higher flow (0.37 m/s)



R8 - *H. comalensis* with barriers and decreasing flow (0.17 - 0 m/s)

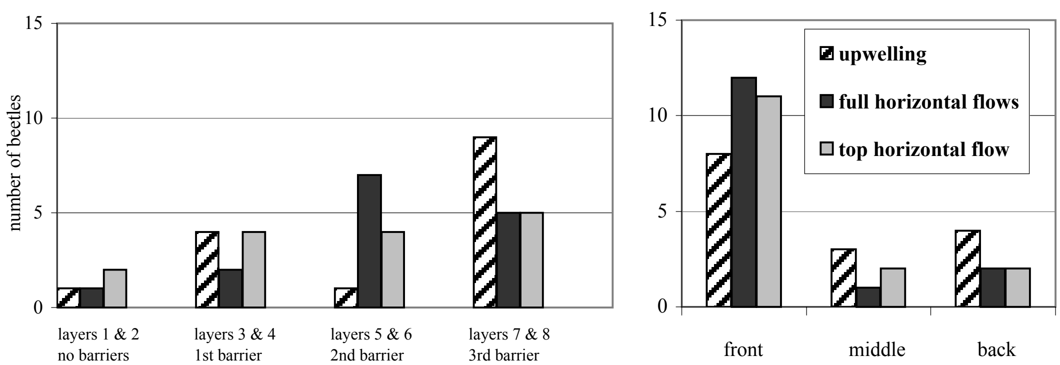


Figure 10. Experimental runs with*Heterelmis comalensis* and more restrictive barriers added; depth and orientation to flow (front = towards origin of horizontal flow; back = away from origin of horizontal flo are represented. All runs had 15 beetles per treatment.

The graphs presented in Figures 11 through 13 display aggregate data from all trials. Identical treatments, run at the same flow level, are combined; the overall number of beetles in each treatment often differs because of the variability of treatments included across trials. In order to compare treatments, each beetle's location (x-axis) is represented as a percentage. Trials using *H. comalensis* without barriers at higher flow did not differ much from lower-flow trials (Figure 11). These beetles, like *M. pusillus*, tended to migrate to the bottom layer, but a few occupied the upper levels in the full horizontal flow treatment. Although a slightly larger percentage of beetles in the full horizontal flow treatment were found closer to the front, no strong preference toward or away from current was displayed.

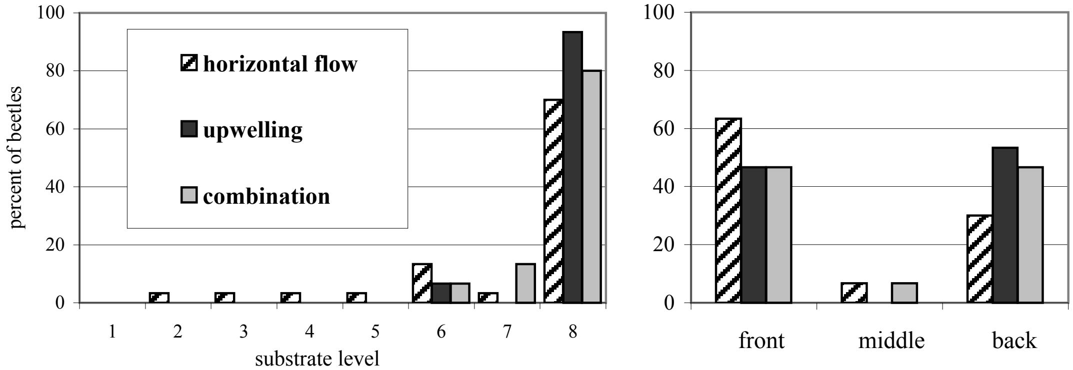
Trials with the first set of barriers (less restrictive) did not differ much between lower flow and higher flow (Figure 12). A higher percentage of beetles occupied layers above the bottom-most one in the full horizontal flow treatment with barriers compared to the same treatment without barriers. This is even more noticeable in the top-flow treatment with barriers. Also, a distinct shift towards the origin of flow is evident and persists with both sets of barriers (Figures 12 and 13).

All treatments run with more restrictive barriers had minimal differences between higher and lower flow rates (Figure 13). Beetles were found to occupy the layers where flow was strongest. The beetles in the top-flow treatment were found primarily in the upper half of the blocks. The beetles in the full horizontal flow treatment were found more scattered throughout the blocks, and the beetles in the upwelling treatment were found mostly in the bottom layers below the 1% passable barrier.

Due to time constraints, unavailability of large numbers of beetles, and preliminary nature of work, trials were not replicated sufficiently to produce statistically testable data. However, in this laboratory study, captive *H. comalensis* displayed a tendency for downward movement through the substrate and a preference to be in, and move toward, current (positive rheotaxis). The different flow intensities used in the study did not have a substantial affect on behavior. If this behavior is occurring in their natural habitat, then it is likely that these beetles would respond to the decreased flows associated with drought by moving downward in the substrate in search of a flow stimulus. It also appears feasible that these beetles regularly inhabit areas deeper in the gravel and sediment around the many spring orifices than have been previously sampled.

Despite these interesting observations, knowledge of this insect remains limited. Although the results of this study indicate movement of *H. comalensis* in response to flow, movement within their natural habitat may also be influenced by dietary needs (the search and preference for particular foods), reproductive behavior (location and competition for mates, finding appropriate places for laying eggs), niche division (avoiding competition with same and other species including own larvae), and predator avoidance. None of these aspects have been previously studied and therefore should be investigated to understand the behavior and requirements for survival in order to appropriately protect this endangered species.

Runs with *H. comalensis* and no barriers at higher flow (0.37 m/s)



Run with *H. comalensis* and no barriers at lower flow (0.17 m/s)

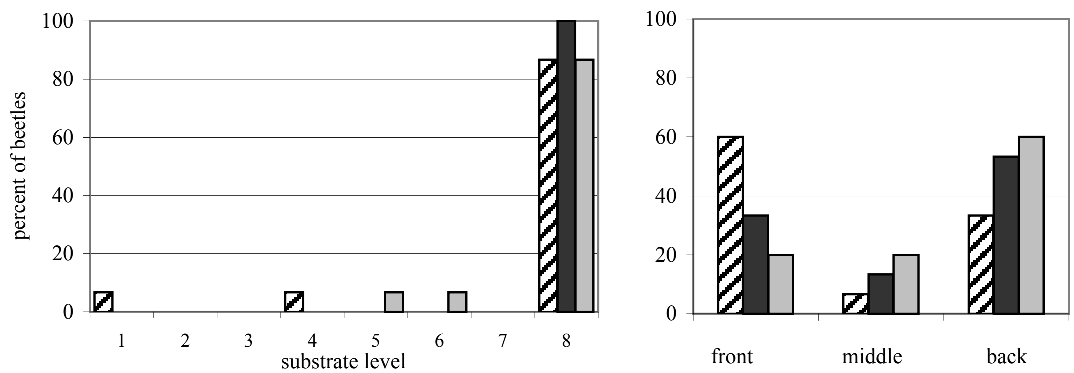
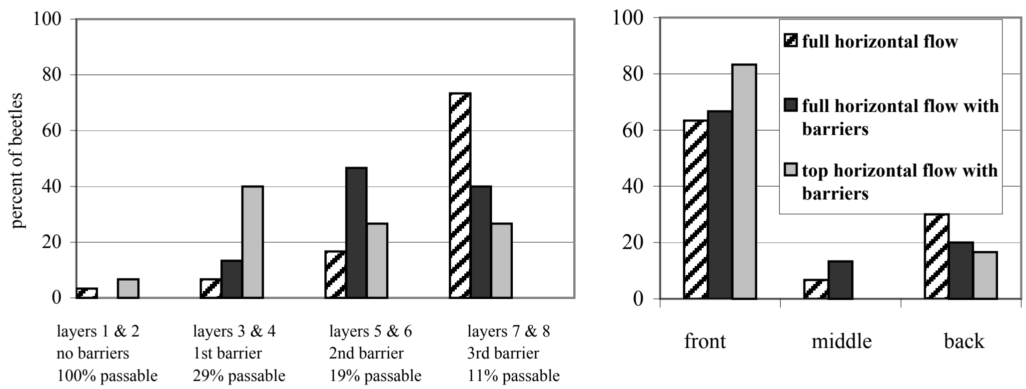


Figure 11. Combined data for all trials with Heterelmis comalensis and no barriers at higher and lower flows; depth (1= top layer; 8 = bottom layer) and orientation to flow (front = towards origin of horizontal flow; back = away from origin of horizontal flow) are represented.

Runs with *H. comalensis* and barriers at higher flow (0.3 m/s)



Runs with *H. comalensis* and barriers at lower flow (0.17 m/s)

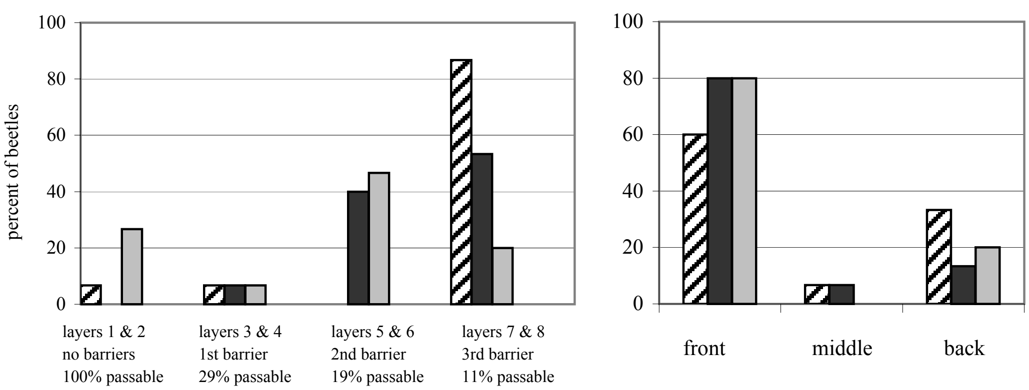
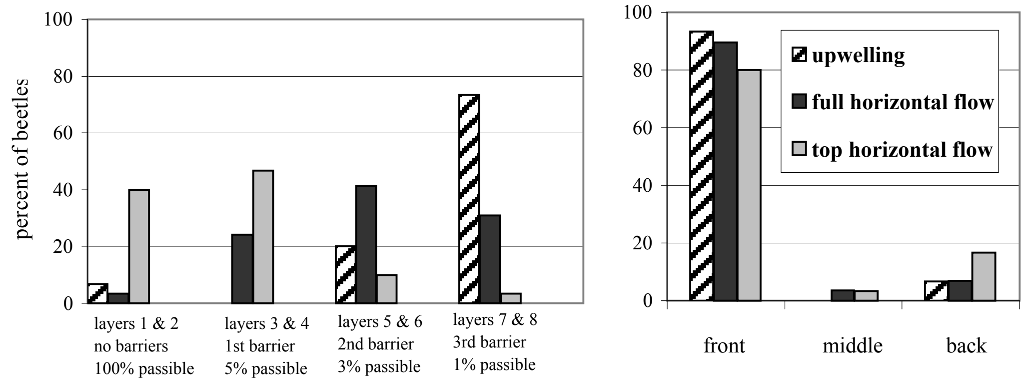
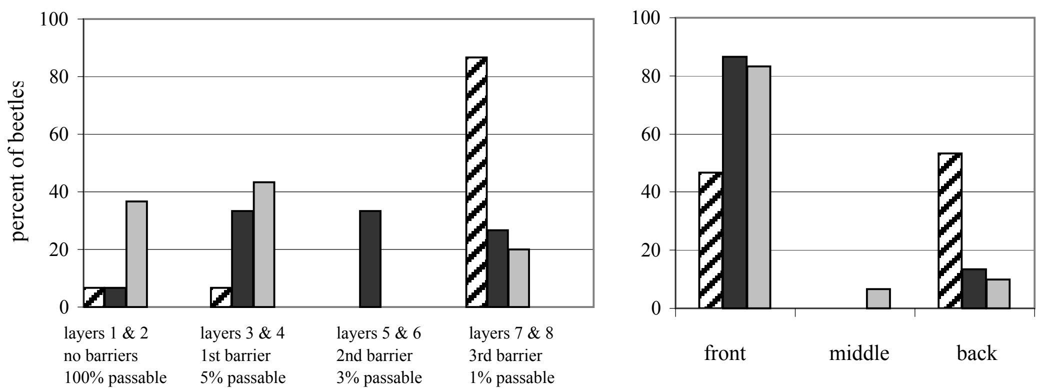


Figure 12. Combined data for all trials with *Heterelmis comalensis* and less restrictive barriers; depth and orientation to flow (front = towards origin of horizontal flow; back = away from origin of horizontal flow) are represented.

Runs with *H. comalensis* and more restrictive barriers at higher flow (0.37 m/s)



Runs with *H. comalensis* and more restrictive barriers at lower flow (0.17 m/s)



Runs with *H. comalensis,* more restrictive barriers and decreasing flow (0.17 - 0 m/s

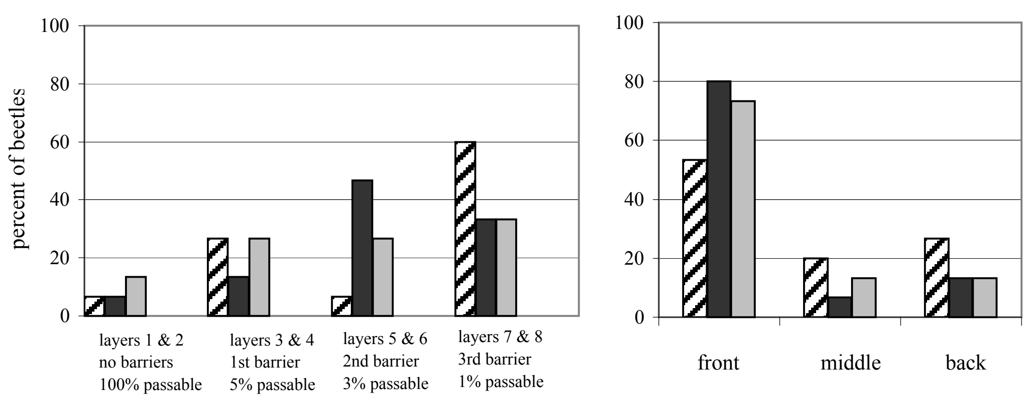


Figure 13. Combined data for all trials with *Heterelmis comalensis* and more restrictive barriers at higher and lower flows; depth and orientation to flow (front = towards origin of horizontal flow; back = away from origin of horizontal flow) are represented.

4.0 REFERENCES

BIO-WEST. 2002. Riffle beetle habitat and population evaluation. Final report. Edwards Aquifer Authority, San Antonio, Texas. 11p.

Bowles, D. E., R. Stanford, and C. B. Barr. 2001. Preliminary habitat characterization and phenology of the endangered riffle beetle *Heterelmis comalensis* and a coexisting species, *Microcylloepus pusillus*, (Coleoptera:Elmidae) at Comal Springs, Texas, USA. Draft Report submitted to Journal of Insect Conservation.

Bosse, L. S. 1979. A survey of adult Dryopoids (Coleoptera) in the San Marcos and Comal Rivers in central Texas. Unpublished thesis, Southwest Texas State University.

Bosse, L. S., D.W. Tuff, H. P. Brown. 1988. A new species of *Heterelmis* from Texas (Coleoptera: Elmidae). The Southwestern Naturalist 33(2):199-203.

Brown, H. P. 1972. Synopsis of the Genus *Heterelmis* Sharp in the United States, with descriptions of a new species from Arizona (Coleoptera, Dryopoidea, Elmidae). Entomological News 83:229-238.

Brown, H. P. 1987. Biology of Riffle Beetles. Annual Review of Entomology 32:253-273.

Brown, H. P. and C. B. Barr. 1988. First report of stygobiontic (subterranean) riffle beetles in North America. Program abstract for April 22, 1988, meeting of Southwestern Association of Naturalists. 5 pp.

Brune, G. 1981. Springs of Texas, Volume 1. Branch-Smith Inc., Fort Worth, Texas.

[USFWS] U.S. Fish and Wildlife Service. 1997. Endangered and threatened wildlife and plants; final rule to list three aquatic invertebrates in Comal and Hays Counties, TX, as endangered. Federal Register Vol. 62, No. 243, pp 66290-66303.