Statewide survey for *Hygrotus diversipes* in Wyoming

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Introduction

Hygrotus diversipes, the narrow-foot predaceous diving beetle, is an endemic aquatic beetle only known from 11 sites in eastern Wyoming (Miller 2002). *H. diversipes* was a category 2 candidate species under the endangered species act (ESA) in 1989 and removed as a candidate in 1996. In 2007, *H. diversipes* was petitioned for listing again under ESA by Forest Guardians. Another petition by WildEarth Guardians in 2008 proposed emergency listing of *H. diversipes*. The US Fish and Wildlife Service issued a negative 90-day finding in February 2009 over which WildEarth Guardians are currently suing the US Fish and Wildlife Service.

H. diversipes was first collected and described in 1964 by Hugh Leech (Leech 1966), but relatively little has been learned about this beetle since that time. Leech (1966) discovered *H. diversipes* in Dugout Creek, northwest of Midwest, Wyoming. Since that time, Bureau of Land Management (1985, 1988, 1992), Professional Entomological Services Technology, Inc. (1992 and 1993), and Kelly Miller (1996 and 2002) surveyed for *H. diversipes* (Miller 2002). Additionally, Anderson (1983) and Larson et al. (2000) published identification keys. Currently, *H. diversipes* is known from the Powder River Basin, and from one site within the Wind River Basin, Wyoming (Miller 2002).

To estimate the current distribution of *H. diversipes* in Wyoming, we made a predictive distribution map and surveyed for the beetle throughout Wyoming. Predictive distribution models were created for *Hygrotus diversipes* based on prior survey data (Miller 2002), using both Maximum Entropy (Phillips et al. 2006; hereafter Maxent) and Random Forest (Cutler et al. 2007) algorithms. Maxent is a presence-only method that performs well with limited sample sizes (Hernandez et al. 2006). Random Forest also has performed well in past modeling efforts and has the added advantage of using absence data in conjunction with presence data to predict presence (Cutler et al. 2007). Models created using these two algorithms were combined to identify stream segments estimated to have the highest likelihood of presence of *H. diversipes* based on both algorithms. Following the initial surveys in selected basins guided by these models in early-summer of 2011, a revised Random Forest model was created by incorporating the new negative (absence) data. Subsequent surveys were informed by both the initial stream segment prioritization and a prioritization based on the revised Random Forest model. Our objectives were to 1.) identify stream segments in Wyoming predicted to be suitable for *H. diversipes*, 2.) collect aquatic beetles from suitable stream segment across the state, and 3.) estimate the current distribution of *H. diversipes* in Wyoming.

The *H. diversipes* survey was a cooperative venture among the 3 authors. Lusha Tronstad was responsible for organizing and logistics for the 2011 surveys. Lusha also surveyed a portion of the sites in 2011. Mark Andersen made the predictive distribution models. Kelsey Swanson surveyed for *H. diversipes* in 2010 and a portion of the sites in 2011. Kelsey also identified all the beetles collected during this study.

Methods

Predictive distribution modeling

To predict what streams in Wyoming had habitat suitable for *H. diversipes*, we made predictive distribution models. Our sampling unit was stream segments from the Enhanced 100k Digital Line Graph (DLG) stream data generated for the Wyoming GAP project (Merrill et al. 1996). Previously surveyed sites where *H. diversipes* was found to be present or absent were matched to the appropriate stream segments from the DLG dataset using ArcGIS. A presence/absence attribute was also added to each of the associated segments and used as the response variable. Eleven presence sites and twenty-seven absence sites were available for generating the initial models (Miller 2002).

The DLG dataset included attributes for both Stahler stream order (Strahler 1952) and stream flow (i.e., perennial, intermittent, ephemeral). We attributed each of the stream segments with 55 additional potential predictors representing various aspects of climate, topography, substrate, and vegetation (Appendix 1). Stream slope was calculated as the maximum segment elevation minus the minimum segment elevation divided by the segment length. Depth to water table, electrical conductivity, soil organic matter, and soil pH were calculated using the Soil Data Viewer Tool 5.2 (USDA-NRCS 2008) with STATSGO data (Soil Survey Staff n.d.). All other variables were created from the predictor set generated by Keinath et al. (2010). Except for predictors pertaining to a particular stream segment (e.g., stream slope, stream order), predictors were summarized using the Line Raster Intersection Statistics with the "Length-Weighted Mean" option within Hawth's Tools (Beyer 2004).

A Maxent model was generated using ten-fold cross-validation with all 57 of the potential predictors to identify the most powerful predictors. The top twelve predictors were identified by summarizing the scores for overall contribution and jackknife contributions, and eliminating variables too highly correlated ($R^2 > 0.8$, Menard 2002) with other, higher-ranked variables. A final Maxent model was then generated using only these twelve predictors (depth to water table, stream flow, hottest month mean maximum temperature, stream order, herbaceous cover, sagebrush cover, cottonwood cover, deciduous cover, pinion-juniper cover, variation of monthly precipitation, slope, and conifer cover). A binary prediction was created by applying the Minimum Training Presence threshold (i.e., the lowest logistic prediction assigned to a point of known presence) to the logistic prediction output for all stream segments. Logistic and binary predictions for both the presence training locations and the background locations (i.e., all other segments) based on this model were then added as attribute fields to the DLG layer.

An initial Random Forest model was generated using the RATTLE package (Williams 2009) in R (Venables et al. 2010) with the existing presence/absence data, using all 57 potential predictors. Seven predictors were evaluated at each split, with a total of 500 trees built. As Random Forest is particularly robust to large numbers of predictors (Cutler et al. 2007), no subsequent variable reduction was done. As with the Maxent predictions, Random Forest predictions were written to each stream segment for both logistic and binary output (Minimum Training Presence).

Initial sampling sites for major drainage basins in Wyoming were determined by ranking stream segments in each basin according to a score determined by the following formula:

$$Score = (Bin_{RF} + Bin_{ME} + Log_{RF} + Log_{ME}) * Road * Public$$

where Bin_{RF} and Log_{RF} were the binary and logistic predictions, respectively, of the initial Random Forest model; Bin_{ME} and Log_{ME} were the binary and logistic predictions, respectively, of the Maxent model; *Road* was a binary value indicating whether a stream segment did (1) or did not (0) intersect a public road; and *Public* was assigned a value of 1.0 when the road/stream intersection occurred on public land or a value of 0.5 when the road/stream intersection did not occur on public land. This scoring was intended to prioritize stream segments on public land accessible by public roads that were predicted as present by both models and had the highest probability according to both models. The top twenty stream segments in each basin according to this score were selected as priority sampling sites for the initial surveys in early-summer of 2011.

After the initial surveys conducted by WYNDD in May and June of 2011, a revised Random Forest model was generated by incorporating the twenty-three additional negative locations from these surveys, using the same settings as the initial model. A new score field was generated using the following formula:

$$Score2 = Log_{RF2} * Road * Public$$

where Log_{RF2} was the logistic score for the revised Random Forest model, and the *Road* and *Public* terms were as defined above. Because we only used one model, we used the logistic prediction to rank 20 stream segments with the highest probability of predicting *H. diversipes* presence. For basins not surveyed during the initial survey period, sampling sites were prioritized based on a review of the original and revised scores by basin.

Field sampling

To estimate the distribution of *H. diversipes* in Wyoming, we collected aquatic beetles in 2010 and 2011. In 2010, we surveyed 3 streams that *H. diversipes* was known from: Dugout Creek, Dead Horse Creek and 2 locations in Cloud Creek. Additionally, we did not sample Hay Draw for *H. diversipes*, because the site is located on private land and we were unable to obtain permission. We surveyed in the Powder River Basin to assess the presence of *H. diversipes* near the type location. For each of the sites, we collected beetles 1 mile up and downstream or until the stream dried. At the time, we were unaware of the 6 new locations discovered by Miller (2002). We recorded site location with a GPS unit, described conditions, and took photos at all sites. Aquatic beetles were collected using a D-frame dip net.

To estimate the distribution of *H. diversipes* across Wyoming, we visited stream reaches predicted to have suitable habitat for the beetle in 2011. To survey for *H. diversipes*, we attempted to visit 20 to 40 stream reaches with the highest predicted probability of containing *H. diversipes* in each river basin. At sites with water, we recorded location using a GPS unit, site description, and photos. Dissolved oxygen, pH, specific conductivity, salinity, water temperature, and oxidation-reduction potential were measured using a YSI Profession Plus that was calibrated daily. We collected aquatic invertebrates using either a D-frame dip net or bottle traps (Aiken and Roughley 1985) left in the stream overnight. Aquatic invertebrates were preserved in 75% ethanol and beetles were identified using Larson et al. (2000).

Results

Predictive distribution models

The Maxent model (Figure 1) had a training AUC of 0.985, indicating a good fit of the model to the training data. This model indicated that *H. diversipes* distribution is most closely associated with various aspects of water regimes, climate, and vegetative cover. Specifically, the species was predicted to be more likely to occur in intermittent streams with a Strahler order of 3-4, a gentle stream gradient, and in areas with a shallow water table. Maxent also indicated that the species was associated with sparse forest and herbaceous cover, moderate sagebrush cover, in warm areas of the state with highly variable seasonal precipitation patterns.

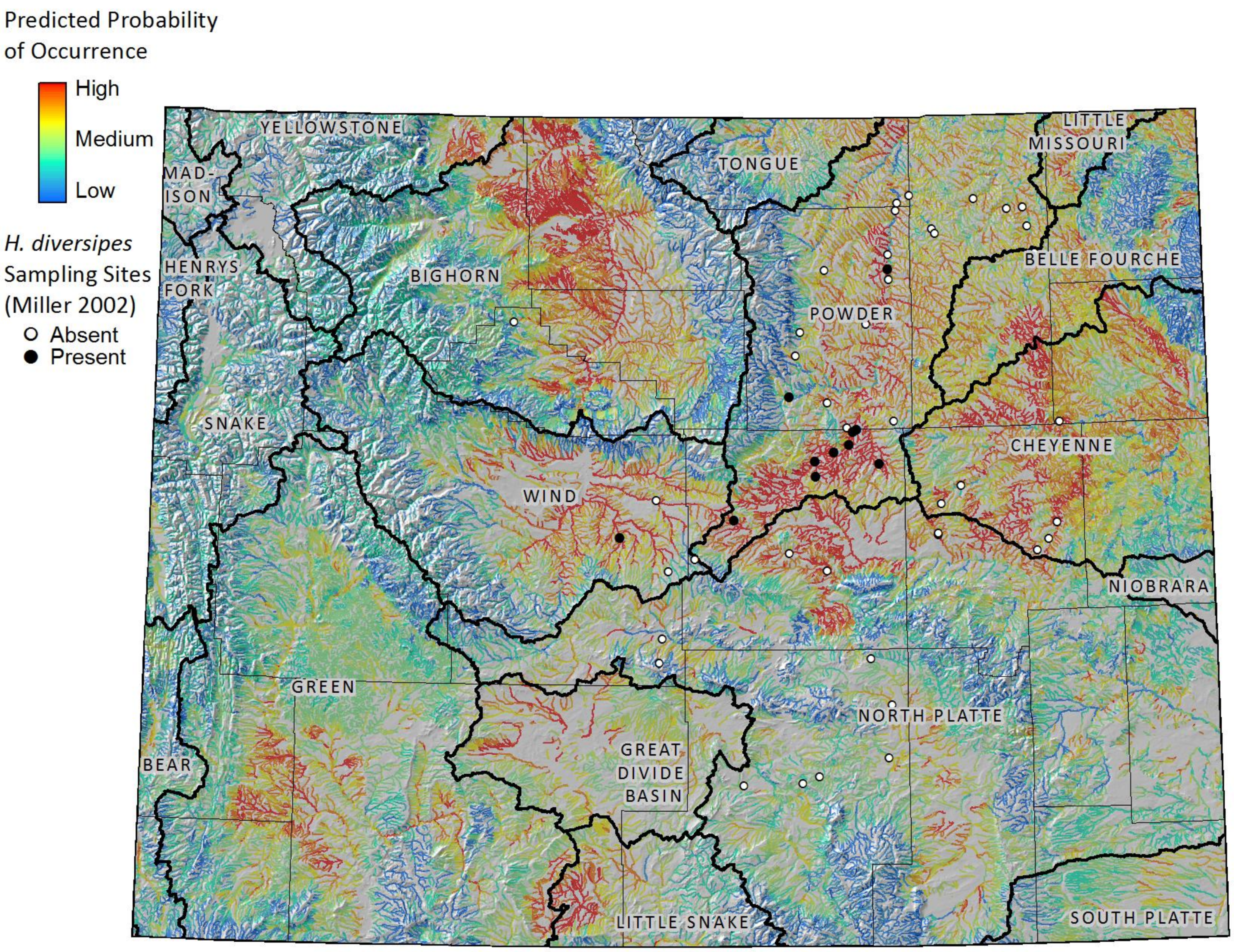

Figure 1. Logistic predictions for all streams in Wyoming based on the Maxent model.

The initial Random Forest model (Figure 2) had an out-of-bag error estimate (Cutler et al. 2007) of 13.16%, though the sensitivity based on out-of-bag samples was just 63.6%, indicating relatively poor identification of presence locations. The predictors identified as most important to the Random Forest model were similar to those selected for the Maxent model, with the addition of several soil chemistry and texture predictors. In particular, electrical conductivity (a measure of soil salinity), texture, and water-holding capacity were selected as the most important variables to model accuracy. Electrical conductivity was much higher at training presence points than at training absence points.

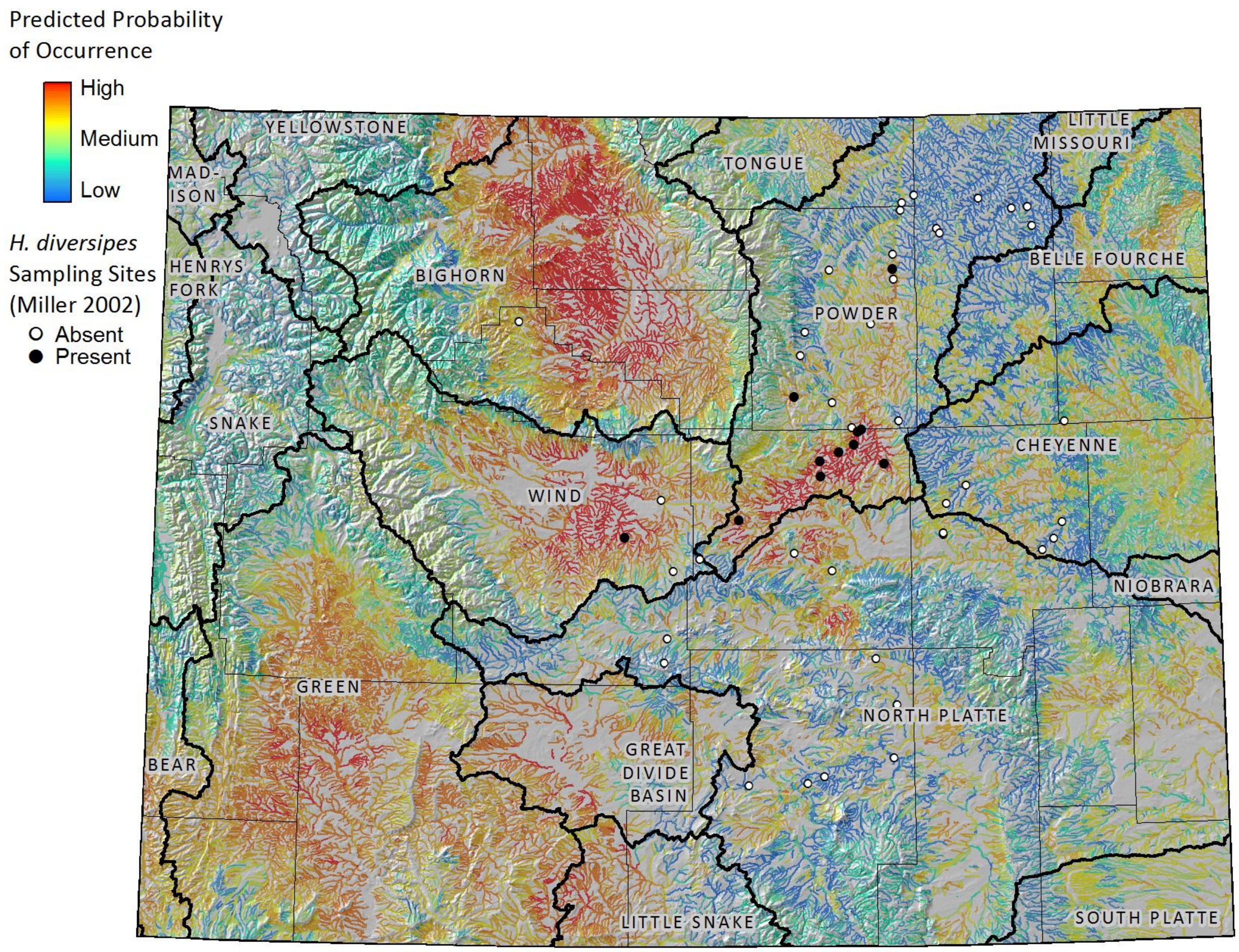

Figure 2. Logistic predictions for all streams in Wyoming based on the initial Random Forest model.

The revised Random Forest model (Figure 3) had a better out-of-bag error estimate of 11.48%, but a worse sensitivity score of 45.5%. The most important variable in the revised model was soil percent clay, with stream segments at training presence sites having a higher mean clay percentage (28.0%, SD 8.8%) than stream segments at training absence sites (19.9%, SD 5.4%). As with the Maxent model, hottest month mean maximum temperature was identified as important in the revised Random Forest model; this variable was relatively unimportant to the initial Random Forest model. Both the Maxent model and the revised Random Forest model suggest that *H. diversipes* are more likely to occur in the areas with the highest hottest month mean temperatures.

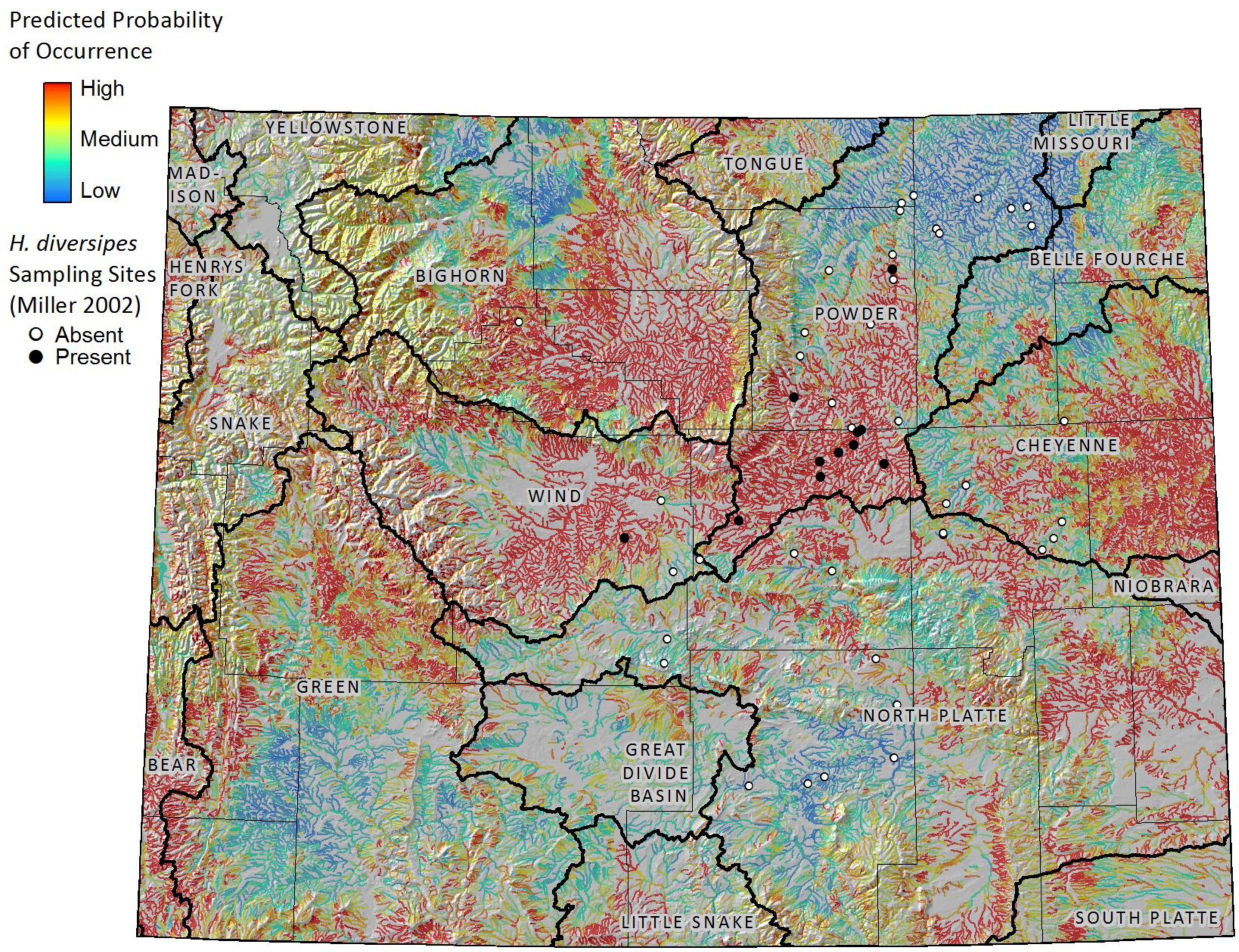

Figure 3. Logistic predictions for all streams in Wyoming based on the revised Random Forest model.

Field survey

In 2010, we visited 37 sites with water in Natrona County between June and August (Figure 4). We identified 3 families, 8 genera, and 14 different species of aquatic beetles (Appendix 2). Of the 37 sites, we only collected *H. diversipes* at 2 sites (Dead Horse Creek and Cloud Creek at Wild Horse Road), collecting a total of 24 *H. diversipes* specimens. We did not collect *H. diversipes* at Dugout Creek, the original site where this beetle was discovered. Elevations ranged between 1451 to 1737 m (4760 to 5700 ft).

In 2011, we surveyed for *H. diversipes* in the Green, Wind, Yellowstone, Bighorn, Great Divide, Little Snake, North Platte, Powder, Little Missouri, Belle Fourche, and Cheyenne River basins because the models suggested that these areas contained the best habitat for *H. diversipes*. We attempted to visit 305 sites from May through August 2011 to survey for *H. diversipes*. Of these, 134 sites were dry, 131 sites were inaccessible, and 40 sites contained water (Figure 4). We identified 8 families, 17 genera, and 26 species of aquatic beetles (Appendix 3). From the 40 sites with water, we only collected 2 specimens of *H. diversipes* at one location, Dead Horse Creek. We did not collect *H. diversipes* at Dugout Creek, the type location, despite visits in May, July, and September. The elevation of sites with water ranged between 1084 to 2718 m (3557 to 8916 ft) with a mean of 1646 m (5400 ft).

Table 1. Minimum, median, mean, and maximum water temperature, dissolved oxygen (DO), specific conductivity (SPC), salinity, pH, and oxidation-reduction potential (ORP) at the 40 sites we sampled for H. diversipes, plus Dead Horse Creek where we collected H. diversipes.

	Water temperature (°C)	DO (%)	DO (mg/L)	SPC (µS/cm)	Salinity (mg/L)	pH	ORP (mV)
Minimum	9	48	4.6	179	80	7.09	-84
Median	15	100	8.0	669	330	8.65	184
Mean	16	99	7.9	2825	3470	8.54	234
Maximum	25	136	10.1	25,040	76,000	9.85	2016
Dead Horse Ck	12	98	8.5	5297	2870	8.44	169.2

Basic water quality from the sampled sites varied (Table 1). In general, most aquatic ecosystems had ample dissolved oxygen for aquatic invertebrates. The salinity of most sites we visited was above the average salinity for rivers worldwide (120 mg/L, Wetzel 2001). pH was basic (>7), as is commonly found around Wyoming. The aquatic ecosystems we sampled varied between oxidizing (e.g., >200 mV) and reducing (e.g., <200 mV) environments.

The majority of sites with water were rangeland (85%), while fewer had energy development (32.5%). The dominant vegetation on the landscape was sagebrush (*Artemisia tridentata*; 90% of sites), greasewood (*Sarcobatus* sp.; 65% of sites), shortgrass prairie (35% of sites), rabbitbrush (*Chrysothamnus* and *Ericameria* sp.; 20% of sites), saltbush (*Atriplex* spp.; 15% of sites), and willows (*Salix* spp.; 7.5% of sites). Estimated stream width varied between 0.3 and 46 m (1 and 150 ft) with a mean width of 3.3 m (11 ft). Estimated stream depth varied between 0.06 and 1.2 m (0.2 and 4 ft) with a mean depth of 0.3 m (1 ft). The substrate at all sites was fine with the exception of one site that was sandy. Dead Horse Creek was surrounded by rangeland, and the dominant vegetation was sagebrush, greasewood, rabbitbrush, and grass. When we sampled Dead Horse Creek, the small stream was a series of disconnected pools with fine substrate.

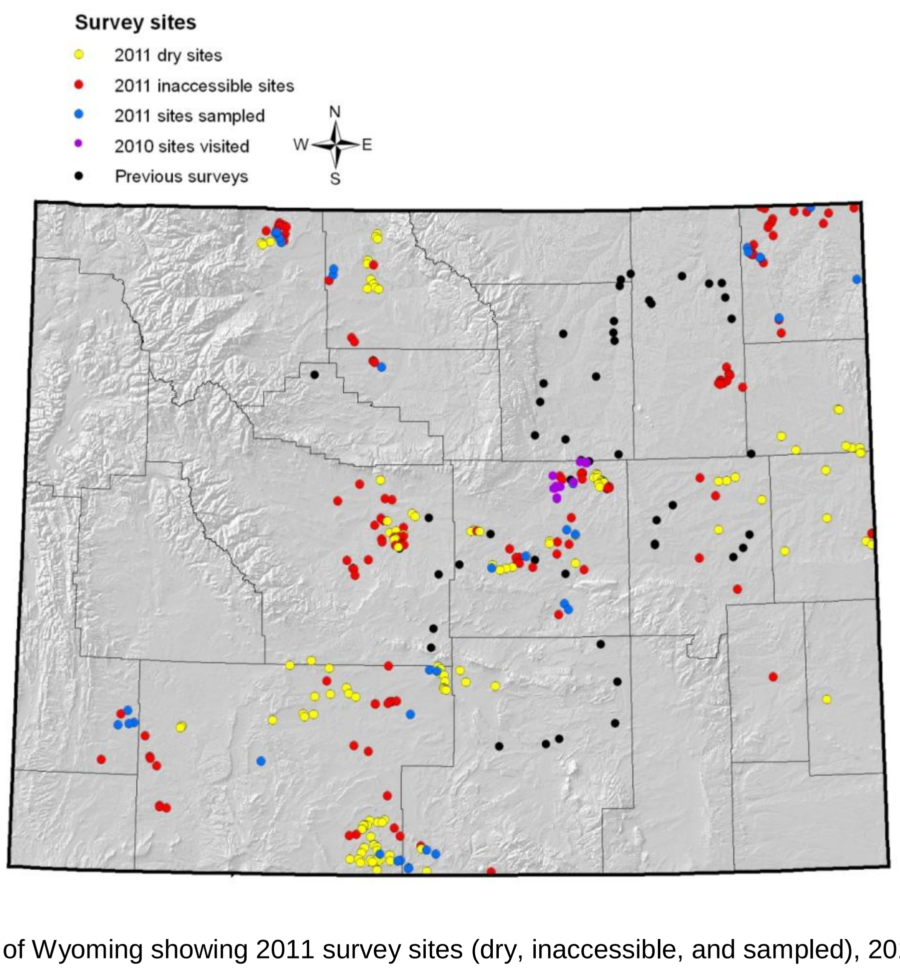

Figure 4. Map of Wyoming showing 2011 survey sites (dry, inaccessible, and sampled), 2010 sites sampled, and past sites surveyed for Hygrotus diversipes.

Discussion

Relatively little is known about the life history of *H. diversipes*. Beetles have 3 life stages: larvae, pupae, and adult. The larvae of predaceous diving beetles hatch from eggs and are aquatic; however, these insects pupate on land. The adults return to water and breathe atmospheric air. Larvae are typically restricted to the habitat in which eggs were laid, but the adults can disperse among water bodies because they have wings. In general, *Hygrotus* species are thought to overwinter as adults, and to breed in spring and early summer. To learn more about the life history of *H. diversipes*, we must first identify and describe the larvae. After we can identify larval *H. diversipes*, we can learn more about where they live, breed, and overwinter.

As adults, *H. diversipes* are probably good fliers and can likely move among aquatic ecosystems. Other studies have found that predaceous diving beetles are excellent dispersers (e.g., Tronstad et al. 2007). Therefore, *H. diversipes* adults should be capable of moving to a new habitat as a stream dries. *H. diversipes* were collected in streams (Miller 2002) and our models predicted the best habitat as intermittent streams (e.g., seasonally wet streams). We suspect that suitable habitat for *H. diversipes* is quite dynamic in both space and time; suitable habitat may shift position across days, months and years, with adult beetles likely changing their positions in response on similar time scales. For example, less habitat is probably available during drought years, when intermittent streams contain little water. Conversely, *H. diversipes* may find abundant habitat during wet years when more intermittent streams likely contain surface water. However, we do not know what refuge or survival strategy *H. diversipes* uses during drought.

Most members of the genus *Hygrotus* have very specific habitat requirements and are typically found in lentic habitats (ponds and lakes; Larson et al. 2000). However, *H. diversipes* has only been collected from streams (Miller 2002). We collected most samples from intermittent streams, but we also collected beetles from ponds, similar to Miller's (2002) survey. Based on available data, *H. diversipes* lives in intermittent streams that are often a series of disconnected pools. Miller (2002) observed that streams with *H. diversipes* often contained organic matter, fine substrates (clay), sparse gravel, and sedges at the water's edge. These types of prairie streams depend on precipitation and may heavily rely on moisture from spring snow storms. Thus, the suitability of intermittent streams as habitat for *H. diversipes* may vary from day to day, depending on local precipitation patterns, leading to complex and unpredictable patterns of occupation by the beetle.

H. diversipes may have a competitive advantage in certain aquatic ecosystems by being tolerant of salinity. Other *Hygrotus* species are able to live in or are restricted to saline waters because they possess special osmoregulatory capabilities. For example, *H. salinarius* lives in a Canadian lake with higher salinity than ocean water (Tones 1978). This species survives as an adult by having hyposmotic haemolymph and hyperosmotic urine relative to the water. Additionally, we collected *H. masculinus* in a highly saline lake (>25,000 µS/cm) in the Great Divide Basin. Miller (2002) stated that he collected *H. diversipes* in "... small, highly mineralized pools in gulches where there is often white crusts of salts along the margins of the water". PEST (1995) collected *H. diversipes* in similarly described habitats. Additionally, our models estimated that soil salinity was higher at sites predicted to contain *H. diversipes*. Based on our sampling, *H. diversipes* can live in saline waters such as Dead Horse Creek (Table 1); however, we do not know the maximum concentration of salinity that *H. diversipes* can survive.

H. diversipes has been collected from 10 sites in the Powder River Basin and 1 site in the Wind River Basin (Miller 2002); however, our predictive distribution modeling suggests that this beetle may be more widely distributed. Based on our survey, *H. diversipes* was not distributed throughout the state. However, suitable *H. diversipes* habitat may have been scarce in 2011, because the lowlands were dry throughout much of Wyoming. Although the mountains held record snow packs, the lowlands did not receive a spring snow storm that provides the essential moisture that penetrates the soil and recharges groundwater. Distance to ground water was an important predictor in our models and lower ground water levels would result in fewer intermittent streams with water. In fact, many stream segments were dry, even when we surveyed in May (Figure 4).

Surveying for *H. diversipes* during a wetter year may reveal that the beetle is more widespread. First, our models predicted that suitable habitat may be located in several river basins, including the Bighorn, Green, and North Platte River Basins (Figures 1, 2, and 3). Second, adults of *H. diversipes* are probably good fliers suggesting that they may be capable of widely dispersing among suitable aquatic habitats. Third, *H. diversipes* may have a competitive advantage in intermittent streams with saline water. Other studies observed that *H. diversipes* was sometimes the dominant beetle in habitats where they were collected (Miller 2002). Given the dynamic nature of *H. diversipes* habitat, and the number of sites that did not contain water or that we could not access in 2011, *H. diversipes* may be collected from more sites in a larger area of Wyoming in a wetter year.

Conclusions

H. diversipes has been collected from 11 sites in eastern Wyoming (Miller 2002). However, we only collected *H. diversipes* from 2 sites in 2010 and 1 site in 2011, despite a state-wide survey in 2011. Suitable habitat for *H. diversipes* is likely dynamic and the beetle probably responds quickly to local conditions. Many predicted sites with suitable habitat for *H. diversipes* were dry, which may have limited the distribution of this beetle in 2011. Overall, little basic information is known about *H. diversipes*, such as life history information, overwinter strategies, and drought refuges. In order to learn more about this beetle, we must first describe larval *H. diversipes*. Currently, *H. diversipes* appears to be restricted to saline, intermittent streams with disconnected pools in eastern Wyoming. Although *H. diversipes* does not appear to be abundant across all of Wyoming, additional surveys are needed to assess the distribution of this beetle, especially in wet years when *H. diversipes* may be more widely collected.

Acknowledgements

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Appendix 1. Potential predictor layers used in building H. diversipes predictive distribution models.

Variable	Name	Resulting Units	Used in Maxent Model
Climate			
Annual mean precipitation	"p1"	0. 1 cm	
Precipitation of the wettest month	"p2"	0. 1 cm	
Precipitation of the driest month	"p3"	0. 1 cm	
Annual precipitation range (p3 – p2)	"p4"	0. 1 cm	
Precipitation of the wettest quarter	"p5"	0. 1 cm	
Precipitation of the driest quarter	"p6"	0. 1 cm	
Precipitation of the warmest quarter	"p7"	0. 1 cm	
Precipitation of the coldest quarter	"p8"	0. 1 cm	
Variation of monthly precipitation	"p9"	0. 1 cm	x
Annual mean relative humidity	"h1"	0.01%	
Relative Humidity of the most humid month	"h2"	0.01%	
Relative Humidity of the least humid month	"h3"	0.01%	
Annual RH range	"h4"	0.01%	
Variation of monthly RH	"h5"	0.01%	
Annual total radiation	"r1"	0.01 MJ/m²/day	
Radiation of the lightest month	"r2"	0.01 MJ/m²/day	
Radiation of the darkest month	"r3"	0.01 MJ/m²/day	
Annual radiation range	"r4"	0.01 MJ/m²/day	
Variation of monthly radiation	"r5"	0.01 MJ/m²/day	
Annual mean temperature	"t1"	0.1 °C	
Mean diurnal temperature range	"t2"	0.1 °C	
Hottest month mean maximum temperature	"t3"	0.1 °C	x
Coldest month mean minimum temperature	"t4"	0.1 °C	
Annual temperature range (T3 – T4)	"t5"	0.1 °C	
Isothermality (T2/T5)	"t6"	0.1 °C	
Standard deviation of monthly temperature	"t7"	0.1 °C	
Wettest quarter mean temperature	"t8"	0.1 °C	
Driest quarter mean temperature	"t9"	0.1 °C	
Warmest quarter mean temperature	"t10"	0.1 °C	
Coldest quarter mean temperature	"t11"	0.1 °C	
Annual number of frost days	"tf_a"	0.1 Days	
Interannual variation in annual number of frost days	"tf_s"	0.1 Days	

Appendix 1 (continued).

Variable	Name	Resulting Units	Used in Maxent Model
Hydrology			
Stream Flow	"flow"	Categorical	x
Stream Order	"order"	Ordinal	x
Stream Slope	"slope"	Percent	x
Depth to Water Table	"d2wtab_LWM"	cm	x
Substrate			
Depth to Shallowest Restrictive Layer	"d2srl_LWM"	cm	
Electrical Conductivity	"elcond_LWM"	millimhos/cm	
Soil Organic Matter	"orgmat_LWM"	Percent, by weight	
Soil Percent Clay	"pclay_LWM"	Percent	
Soil Percent Sand	"psand_LWM"	Percent	
Soil Percent Silt	"psilt_LWM"	Percent	
Soil pH	"soilph_LWM"	pH	
Topography			
Compound Topographic Index	"cti_LWM"	Unitless; greater values indicate greater moisture	
Elevation	"elev_LWM"	Meters	
Radiation Load	"radld_LWM"	Unitless; greater values indicate greater incident radiation	

Appendix 1 (continued).

Variable	Name	Resulting Units	Used in Maxent Model
Vegetation			
Bare Ground Index	“bare_LWM”	See Keinath et al. 2010	
Conifer Index	"confr_LWM"	See Keinath et al. 2010	x
Cottonwood Index	"pode_LWM"	See Keinath et al. 2010	x
Deciduous Forest Index	"decid_LWM"	See Keinath et al. 2010	x
Forest Cover	“frstc_LWM”	See Keinath et al. 2010	
Herbaceous Cover Index	"herb_LWM"	See Keinath et al. 2010	x
Percent Cover of Sagebrush	“usage_LWM”	See Keinath et al. 2010	
Pinion-Juniper Index	“pj_LWM”	See Keinath et al. 2010	x
Ponderosa Pine Index	"pipoc_LWM"	See Keinath et al. 2010	
Sagebrush Index	"sage_LWM"	See Keinath et al. 2010	x
Shrub Cover Index	"shrub_LWM"	See Keinath et al. 2010	

Appendix 2. Sampling locations (stream name and coordinates in NAD83), aquatic beetles collected (identified according to Larson et al. (2000)), and sampling dates in 2010.

Stream	Zone	Latitude	Longitude	Family	Genus	Species	Date
Puddle by Lone Bear Rd	13T	363473N	4806944E	Dytiscidae	Hygrotus	patruelis	6/18/2010
				Hydrophilidae			
				Hydrophilidae	Helophorus	sp.	
Dugout Creek	13T	386186N	4815189E	Hydrophilidae	Helophorus	sp.	7/9/2010
				Hydrophilidae	Berosus	stylifer	
	13T	386186N	4815205E	Hydrophilidae	Berosus	stylifer	7/9/2010
	13T	386211N	485260E				7/9/2010
	13T	386166N	4815260E	Hydrophilidae	Helophorus	sp.	7/9/2010
				Hydrophilidae	Berosus	stylifer	
	13T	386083N	4815154E	Hydrophilidae	Berosus	stylifer	7/9/2010
	13T	385979N	481081E				7/9/2010
	13T	385881N	4814946E	Dytiscidae	Laccophilus	maculosus decipiens	7/9/2010
	13T	385855N	4814880E	Haliplidae	Haliplus	immaculicollis	7/9/2010
				Hydrophilidae	Berosus	stylifer	
				Hydrophilidae	Helophorus	sp.	
	13T	385771N	4814813E	Hydrophilidae	Berosus	stylifer	7/9/2010
North Fork Dead Horse Creek	13T	377165N	4802489E	Dytiscidae	Hygrotus	diversipes	7/9/2010
				Dytiscidae	Hydroporus	signatus	
				Dytiscidae	Hygrotus	semivittatus	
				Hydrophilidae	Helophorus	sp.	
	13T	377117N	4802489E	Hydrophilidae	Helophorus	sp.	7/9/2010
Cloud Creek at Wild Horse Rd	13T	365344N	4799496E	Hydrophilidae	Helophorus	sp.	6/18/2010
	13T	365334N	4799456E	Dytiscidae	Stictotarsus	griseostriatus	7/9/2010
				Haliplidae	Haliplus	immaculicollis	
				Hydrophilidae	Helophorus	sp.	
	13T	365330N	4799448E	Dytiscidae	Hygrotus	diversipes	7/9/2010
				Dytiscidae	Stictotarsus	griseostriatus	
				Hydrophilidae	Berosus	sp.	
				Hydrophilidae	Helophorus	sp.	
	13T	365324N	4799432E	Hydrophilidae	Helophorus	sp.	7/9/2010
	13T	365294N	4799324E	Dytiscidae	Hygrotus	diversipes	7/9/2010
				Dytiscidae	Stictotarsus	griseostriatus	
	13T	365313N	4799269E	Dytiscidae	Hygrotus	diversipes	7/9/2010
	13T	365446N	4799224E	Dytiscidae	Hygrotus	diversipes	7/9/2010
				Dytiscidae	Hygrotus	patruelis	
				Hydrophilidae	Helophorus	sp.	
	13T	365546N	4799033E	Dytiscidae	Hygrotus	diversipes	7/9/2010
	13T	365452N	4799863E	Dytiscidae	Hygrotus	diversipes	7/9/2010
	13T	365464N	4799830E	Dytiscidae	Hygrotus	diversipes	7/9/2010
				Dytiscidae	Liodessus	obscurellus	
	13T	365495N	4799717E	Dytiscidae	Hygrotus	diversipes	7/9/2010
	13T	365355N	4799767E	Dytiscidae	Hygrotus	diversipes	7/9/2010
	13T	365332N	4799678E	Dytiscidae	Stictotarsus	striatellus	7/9/2010
	13T	365366N	4799512E	Dytiscidae	Hygrotus	diversipes	7/9/2010
	13T	365366N	4799512E	Dytiscidae	Hygrotus	nubilus	7/9/2010
				Hydrophilidae	Helophorus	sp.	
Cloud Creek at 33 Mile Rd	13T	365541N	4792206E	Dytiscidae	Hygrotus	impressopuncatatus	7/9/2010
				Dytiscidae	Hygrotus	suturalis	
				Hydrophilidae	Helophorus	sp.	
	13T	365483N	4792078E	Dytiscidae	Hygrotus	patruelis	7/9/2010
Pond by Wild Horse Rd	13T	368165N	4799434E	Dytiscidae	Hygrotus	nubilus	8/13/2010
				Dytiscidae	Hygrotus	sellatus	
				Dytiscidae	Hygrotus	suturalis	

Appendix 3. Sampling locations (stream name and coordinates in NAD83), aquatic beetles collected (identified according to Larson et al. (2000)), and sampling dates in 2011.

Stream	Zone	Latitude	Longitude	Family	Genus	Species	Date
Trib #2 of Slate Creek	12T	0572483N	4637183E	Dytiscidae	Larvae		6/1/2011
				Hydrophilidae	Helophorus	sp.	
				Gyrinidae	Gyrinus	sp.	
				Dytiscidae	Dytiscus	dauricus	
				Dytiscidae	Hygrotus	infuscatus	
Dugout Creek	13T	0385948N	4815029E	Dytiscidae	Larvae		5/10/2011
				Haliplidae	Larvae		
				Gyrinidae	Gyrinus	sp.	
				Hydrophilidae	Tropisternus	sp.	
				Hydrophilidae	Berosus	sp.	
				Hydrophilidae	sp.		
				Haliplidae	Peltodytes	callosus	
				Dytiscidae	Laccophilus	maculosus decipiens	
				Dytiscidae	Stictotarsus	striatellus	
				Dytiscidae	Dytiscus	hybridus	
Dead Horse Creek	13T	0376909N	4801155E	Hydrophilidae	Helophorus	sp.	5/10/2011
				Dytiscidae	Hygrotus	patruelis	
				Dytiscidae	Hygrotus	patruelis	
				Dytiscidae	Stictotarsus	striatellus	
				Dytiscidae	Hygrotus	diversipes	
Trib to L. Missouri	13T	0541143N	4981034E	Dytiscidae	Larvae		5/25/2011
				Hydrophilidae	Berosus	sp.	
				Hydrophilidae	Helophorus	sp.	
				Curculionidae			
				Dytiscidae	Coptotomus	longulus longulus	
				Dytiscidae	Hygrotus	patruelis	
Big Sand Coulee	12T	0660886N	4962752E	Hydrophilidae	Tropisternus	sp.	5/18/2011
				Dytiscidae	Laccophilus	maculosus decipiens	
				Dytiscidae	Hygrotus	punctilineatus	
Coon Creek	12T	0696680N	4942698E	Chrysomelidae			5/17/2011
				Hydrophilidae	Helophorus	sp.	
				Dytiscidae	Laccophilus	maculosus decipiens	
				Dytiscidae	Agabus	seriatus	
N. Fork Casper Creek	13T	0371446N	4770671E	Dytiscidae	Hygrotus	patruelis	5/11/2011
				Dytiscidae	Larvae		
				Hydrophilidae	Tropisternus	sp.	
				Dytiscidae	Laccophilus	maculosus decipiens	
				Dytiscidae	Rhantus	binotatus	
				Dytiscidae	Colymbetes	incognitus	
				Hydrophilidae	Berosus	sp.	
				Hydrophilidae	Helophorus	sp.	
				Dytiscidae	Rhantus	gutticollis	
				Dytiscidae	Hygrotus	marklini	
				Dytiscidae	Larvae		5/26/2011
				Hydrophilidae	Berosus	sp.	
				Hydrophilidae	Helophorus	sp.	
				Dytiscidae	Liodessus	obscurellus	
				Dytiscidae	Hygrotus	patruelis	
				Dytiscidae	Hygrotus	unguicularis	
				Dytiscidae	Hygrotus	marklini	

Appendix 3 (continued).

Stream	Zone	Latitude	Longitude	Family	Genus	Species	Date
Mule Creek	13T	0517852N	4907769E	Gyrinidae	Gyrinus	sp.	5/25/2011
				Heteroceridae			
				Haliplidae	Peltodytes	edentulus	
15 Mile Creek	12T	0731526N	4882393E	Hydrophilidae	Berosus	sp.	5/16/2011
				Dytiscidae	Laccophilus	maculosus decipiens	
Trib #2 Big Sand Coulee	12T	0657521N	4969525E	Hydrophilidae	Helophorus	sp.	5/19/2011
				Hydrophilidae	Tropisternus	sp.	
				Dytiscidae	Rhantus	gutticollis	
Trib to Big Sand Coulee	12T	0656682N	4968830E	Hydrophilidae	Helophorus	sp.	5/18/2011
				Dytiscidae	Hygrotus	patruelis	
Shute Creek	12T	0576215N	4638148E	Hydrophilidae	Helophorus	sp.	6/1/2011
Cracker Creek	13T	0498001N	4955631E	Dytiscidae	Larvae		5/26/2011
				Hydrophilidae	Berosus	sp.	
				Curculionidae			
				Dytiscidae	Rhantus	gutticollis	
				Dytiscidae	Agabus	seriatus	
Prairie Creek	13T	0498669N	4952483E	Hydrophilidae	Berosus	sp.	5/26/2011
				Dytiscidae	Agabus	seriatus	
				Dytiscidae	Rhantus	gutticollis	
				Dytiscidae	Laccophilus	maculosus maculosus	
				Dytiscidae	Laccophilus	maculosus decipiens	
				Haliplidae	Peltodytes	callosus	
				Haliplidae	Haliplus	immaculicollis	
				Dytiscidae	Neoporus	superioris	
Alkalii Creek	13T	0506130N	4948584E	Dytiscidae	Larvae		5/26/2011
				Hydrophilidae	Berosus	sp.	
				Dytiscidae	Agabus	seriatus	
				Dytiscidae	Laccophilus	maculosus maculosus	
				Dytiscidae	Laccophilus	maculosus decipiens	
				Haliplidae	Peltodytes	callosus	
				Haliplidae	Peltodytes	edentulus	
W. Fork of Big Sand Coulee	12T	0659665N	4965821E	Dytiscidae	Rhantus	binotatus	5/18/2011
				Hydrophilidae	Helophorus	sp.	
				Hydrophilidae	Tropisternus	sp.	
				Chrysomelidae			
				Curculionidae			
				Dytiscidae	Agabus	seriatus	
				Dytiscidae	Laccophilus	maculosus decipiens	
S. Red Water Creek	13T	0570483N	4931979E	Hydrophilidae	Helophorus	sp.	5/25/2011
20 Mile Creek	13T	0377333N	4767086E	Hydrophilidae	Larvae		5/11/2011
Bear Creek	13T	0371145N	4717092E	Hydrophilidae	Berosus	sp.	5/24/2011
Trib #2 Shute Creek	12T	0572483N	4637183E	Hydrophilidae	Berosus	sp.	6/1/2011
Trib Slate Creek	12T	0571311N	4646081E	Hydrophilidae	Helophorus	sp.	6/2/2011
Big Sand Coulee	12T	0660886N	4962752E	Chrysomelidae			5/18/2011
				Hydrophilidae	Berosus	sp.	
				Gyrinidae	Gyrinus	sp.	
				Dytiscidae	Laccophilus	maculosus decipiens	
				Dytiscidae	Hygrotus	nubilus	
				Dytiscidae	Hygrotus	semivittatus	

Appendix 3 (continued).

Stream	Zone	Latitude	Longitude	Family	Genus	Species	Date
Coon Creek (N. Branch)	12T	0696696N	4046568E	Chrysomelidae			5/17/2011
				Curculionidae			
				Hydrophilidae	Helophorus	sp.	
				Dytiscidae	Agabus	seriatus	
Unnamed Pond	13T	4753744N	343685E	Hydrophilidae	Tropisternus	sp.	7/20/2011
				Dytiscidae	Hygrotus	punctilineatus	
				Dytiscidae	Hygrotus	patruelis	
Cloud Creek	13T	4799441N	365328E	Hydrophilidae	Helophorus	sp.	7/20/2011
				Dytiscidae	Hygrotus	patruelis	
Hangout Wash	13T	254044N	4552717E	Hydrophilidae	Helophorus	sp.	8/9/2011
				Dytiscidae	Larvae		
				Dytiscidae	Laccophilus	maculosus decipiens	
				Dytiscidae	Liodessus	obscurellus	
				Elmidae	Heterlimnius	corpulentus	
Chain Lake	13T	263442N	4650070E	Hydrophilidae	Helophorus	sp.	8/11/2011
				Dytiscidae	Hygrotus	masculinus	
Cottonwood Creek	13T	277533N	4556468E	Hydrophilidae	Helophorus	sp.	8/10/2011
				Gyrinidae	Gyrinus	sp.	
				Haliplidae	Haliplus	immaculicollis	
				Dytiscidae	Laccophilus	maculosus decipiens	
				Dytiscidae	Liodessus	obscurellus	
				Dytiscidae	Rhantus	binotatus	
Pond 2	13T	252250N	4552303E	Gyrinidae	Gyrinus	sp.	8/9/2011
				Hydrophilidae	Berosus	sp.	
Lost Soldier Creek	13T	281847N	4678742E	Haliplidae	Haliplus	immaculicollis	8/11/2011
				Gyrinidae	Gyrinus	sp.	
				Dytiscidae	Hygrotus	marklini	
				Dytiscidae	Rhantus	binotatus	
Sand Creek &Red Creek	13T	258824N	4547281E	Dytiscidae	Larvae		8/9/2011
				Hydrophilidae	Helophorus	sp.	
				Hydrophilidae	Tropisternus	sp.	
				Hydrophilidae	Tropisternus	sp.	
				Gyrinidae	Gyrinus	sp.	
				Dytiscidae	Laccophilus	maculosus decipiens	
				Dytiscidae	Stictotarsus	griseostriatus	
				Dytiscidae	Hygrotus	nubilus	
A & M Reservoir	13T	276875N	4679408E	Haliplidae	Haliplus	immaculicollis	8/11/2011
				Hydrophilidae	Tropisternus	sp.	
N. Fork of Cottonwood Creek	13T	271383N	4559298E	Hydrophilidae	Helophorus	sp.	8/10/2011
				Hydrophilidae	Tropisternus	sp.	
				Dytiscidae	Agabus	seriatus	
				Dytiscidae	Colymbetes	incognitus	