

GRANT AGREEMENT PERFORMANCE REPORT

TO: U.S. FISH AND WILDLIFE SERVICE
WILDLIFE AND SPORT FISH AND RESTORATION
HADLEY, MA

FROM: MARYLAND DEPARTMENT OF NATURAL RESOURCES
ANNAPOLIS, MD

GRANT AGREEMENT: E-4-20

GRANT TITLE: ENDANGERED SPECIES CONSERVATION

TOTAL COSTS: \$42,918

JOB PERFORMANCE REPORT

State: Maryland

Project Title: Endangered Species Conservation

Job No.: 657

Job Title: Restore and Manage Sandplain Gerardia Habitat

Principal Investigator: Tyndall

Job Objective:

Restore and maintain more than 1,000 acres of sandplain gerardia (*Agalinis acuta*) habitat.

Activities/Findings:

During the period 1 July 2008 - 30 June 2009, we were unable to clear additional potential habitat due to chipper malfunctions; after 2 expensive and unsuccessful repair attempts, we postponed reuse of the chipper until SFY 2010. Instead, we killed reinvading seedlings of Virginia pine and Red cedar in about 100 acres of previously cleared habitat. With the assistance of Blackwater National Wildlife Refuge, we successfully burned a 26-acre unit on 11 November 2008

We searched and managed all previously cleared habitat for non-native invasive species; of special concern is the recent invasion of the highly invasive grass, *Miscanthus*.

We completed the annual monitoring survey; the sandplain gerardia population was 5% of the average for the period 1990-2007, probably due to another summer of low rainfall and deer herbivory. A preliminary survey estimated 24-33% of plants grazed by deer.

Reasons for deviations (if any):

None.

Recommendations:

Funding needs continue for (1) chipper repair and use, (2) prescribed burning of recovered habitat, (3) search and eradication of incipient populations of highly invasive non-native plant species such as Tree-of-heaven, Mile-a-minute, and *Miscanthus*, and (4) annual population surveys to understand annual size fluctuations in relation to weather and deer herbivory.

JOB PERFORMANCE REPORT

State: Maryland

Project Title: Endangered Species Conservation

Job No.: 659

Job Title: Seabeach Amaranth Reintroduction

Principal Investigator: Tyndall

Job Objective:

To reestablish a viable seabeach amaranth (*Amaranthus pumilus*) population on Assateague Island National Seashore.

Activities/Findings:

During the period 1 July 2008 - 30 June 2009, we documented 1,048 plants on the Maryland portion of Assateague Island, with 7 plants found in Virginia. The highest count on record was in August 2006 with 1,552 plants in Maryland and 9 in Virginia. Excessive deer and horse herbivory continue to significantly limit reproductive output and expansion of the population. We did not relocate plants on Tom's Hook in Virginia.

Reasons for deviations (if any):

None.

Recommendations for the continuance of job:

We recommend continuing this job to determine reintroduction success or failure.

JOB PERFORMANCE REPORT

State: Maryland

Project Title: Endangered Species Conservation

Job No.: 661

Job Title: Survey and Monitor Sensitive Joint-vetch Populations

Principal Investigator: Knapp

Job Objective:

To determine the status of sensitive joint-vetch (*Aeschynomene virginica*) in Maryland and to identify critical elements to be included in a conservation/recovery strategy, to provide for the long-term sustainability of the species in Maryland.

Activities/Findings:

During the period 1 July 2008 - 30 June 2009, we conducted surveys for the Eastern Shore populations. The Taylor Branch subpopulation consisted of only 88 plants compared to its annual average of 440 plants. However, the Upper Manokin subpopulation was 3,441 plants, substantially higher than its annual average of 1,666 plants. The difference in subpopulation size was unexpected and not understood. We present the fluctuations in the sensitive joint-vetch population from 1994 to present in Figure 1.

In the previous performance report (E-4-19) for this job, we erroneously reported the results from the 2008 survey period (as provided above) instead of the results for the 2007 surveys. In August 2007, the Taylor Branch subpopulation count was 88 plants and the Upper Manokin population was 3,774 plants.

Reasons for deviations (if any):

None.

Recommendations for the continuation of the job:

WHS should monitor existing subpopulations annually to observe invasive species populations and shoreline development projects. Likewise, we will understand natural fluctuations in population size as more population data is collected.

Number of Plants

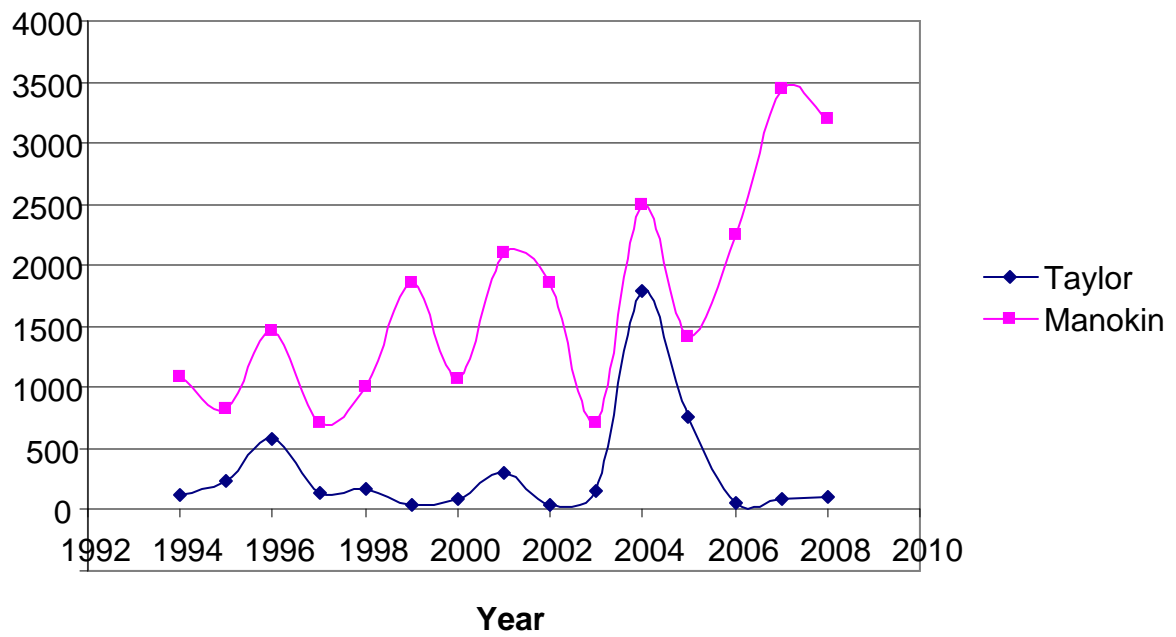


Figure 1. Sensitive joint-vetch population fluctuations in Maryland since 1994 by subpopulation.

JOB PERFORMANCE REPORT

State: Maryland

Project Title: Endangered Species Conservation

Job No.: 687

Job Title: Monitor Puritan and Northeastern Beach Tiger Beetles

Principal Investigator: Therres

Job Objective:

To monitor the highly sensitive populations of Puritan tiger beetle and northeastern beach tiger beetle occurring in Calvert County and on the Eastern Shore of Maryland and identify potential threats and direct habitat/land use conflicts as shoreline development and stabilization continues along the shores of the Chesapeake Bay.

Activities/Findings:

WHS contracted with Dr. Barry Knisley, Randolph-Macon College to conduct tiger beetle research in Maryland. Dr. Knisley and staff conducted surveys for adult northeastern beach tiger beetles (*Cicindela dorsalis dorsalis*) and Puritan tiger beetles (*C. puritana*) at Calvert County (Table 1) and Sassafras River (Table 2) sites in 2008. In previous surveys in Calvert County, we reported Western Shores and Calvert Beach as separate areas but we combined them as one unit in 2006. In addition, we reported the Warrior's Rest section of Scientists Cliffs as a separate unit in Calvert County beginning in 2006.

The total number of adults of *C. puritana* counted at Calvert sites in 2008 was 5,721, showing a pattern of increase in the past 4 years (1,101 in 2005, 3,946 in 2006 and 2,625 in 2007). From 2002-2005 counts were less than 2,100 adults. The significant increase in 2008 is primarily a result of large increases in numbers at most major sites: Calvert Cliffs State Park (1,609 in 2008, 292 in 2007), Little Cove Point (1,116 in 2008, 740 in 2007), Cliffs of Calvert (829 in 2008, 172 in 2007), and Western Shores/Calvert Beach (841 in 2008, 272 in 2007). There was also a significant increase at Warrior Rest (958 in 2008 compared to 631 in 2007), but we noticed minimal changes at the other sites. The total count for adult *C. d. dorsalis* at all sites in 2008 was 190, dramatically lower than any other years, and a serious threat to the survival of the Calvert metapopulation of this species. Adult numbers in Calvert have declined significantly since the counts of 3,000-10,000 adults in the early 1990s to a consistent range of 674-767 from 2004 to 2007. Counts were 188 at Western Shores and only 2 at Flag Ponds. There were no apparent shoreline changes at any of these sites since last year, and the cause of the decline is unknown.

The results of surveys at all Sassafras sites produced a total count of 1,764 *C. puritana* in 2008, indicating a progressive and significant increase in the past 5 years, 398 in 2004, 408 in 2005, 1,221 in 2006, and 1,566 in 2007. Prior to these years, the total counts declined significantly from 1996 (count of 1,821) to 2002 (400). We recorded the lowest total numbers from 1999 to 2004. Most of the increase in 2008 and nearly all of the increase in 2007 was due to the increase at the Grove Point site (273 in 2006, 843 in 2007 and 986 in 2008). Most other sites experienced moderate increases, but interestingly East Lloyd showed a decline in the past 3 years, from 554 in 2006 to 136 in 2008.

These year to year fluctuations of *C. puritana* at both Calvert and Sassafras sites have been the norm, and reflect variations in recruitment, possibly tied to changes in habitat conditions or climatic factors. However, we have been unable to effectively determine the specific factors and the nature of their impacts. We hypothesized that Hurricane Isabel in 2003 may have had a positive effect by reducing vegetation and creating more favorable habitat, possibly for both *C. puritana* and *C. d. dorsalis*. This could have resulted in a pattern of gradual increase that we have seen in these recent years. Hurricanes and other erosional events can clear out cliff base or cliff face vegetation, making these areas more favorable for adult oviposition and recruitment. Storms and erosion can also bring new sand onto beaches and improve the habitat for *C. d. dorsalis*, which seems to do best in dynamic, accretion shorelines. Both species are also affected by other aspects of weather, such as increased rainfall that could increase the prey resource base and thus increase survival and recruitment. It is also certain that aspects of the beetle biology (density dependent population dynamics, competition, parasitism, etc.) are key factors in driving changing levels of abundance, but these have not been identified.

Reasons for deviations (if any):

None.

Recommendations for the continuance of job:

Continue this job at least until such time as the tiger beetle population has fully recovered. Annual monitoring of all tiger beetle populations within Maryland is needed to assess changes or threats to the tiger beetle populations.

Table 1. Population census counts of adult *Cicindela dorsalis dorsalis* and *C. puritana* at all Calvert County survey sites during 2008 surveys.

Site	Number of adults	
	<i>C. d. dorsalis</i>	<i>C. puritana</i>
Randle Cliff	0	23
Camp Roosevelt	0	0
Bayside Forest	0	5
Warrior's Rest	0	958
Scientists Cliffs	0	218
Western Shores & Calvert Beach	188	841
Flag Ponds	2	0
Calvert Cliffs State Park	0	1609
Nuclear Power Plant	0	122
Cove Point	0	0
Little Cove Point	0	1116
Cliffs of Calvert	0	829
	190	5,721

Table 2. Population census counts of adult *C. puritana* at Sassafras River survey sites during 2008 surveys.

Site	Number of adults
Grove Point	986
Ordinary Point	100
North Stillpond	120
West Betterton	92
East Betterton	34
East Lloyd	136
West Turner	293
East Turner	3
	<hr/>
	1,764

JOB PERFORMANCE REPORT

State: Maryland

Project Title: Endangered Species Conservation

Job No.: 695

Job Title: Monitor Dwarf Wedge Mussel Populations

Principal Investigator: McCann

Job Objective:

To provide more detailed information on the distribution and relative abundance of the dwarf wedge mussel (*Alasmidonta heterodon*) in the Three Bridges Branch watershed, and continue population monitoring at other streams.

Activities/Findings:

Herring Run Watershed Inventory

In 2007, during routine stream monitoring by DNR's Biological Stream Survey (MBSS) staff, a single fresh dead dwarf wedge mussel shell was discovered in Herring Run, a small tributary of the upper Choptank River in Caroline County, approximately 3 km southeast of Denton. Although the species was documented in 2 other Choptank tributaries (Norwich Creek, Long Marsh Ditch-Mason Branch), this discovery likely represents the only extant population remaining in the Choptank watershed. The nearest other extant population occurs about 30 km to the northwest in Three Bridges Branch, Queen Anne's County, within the Corsica River watershed.

During August 2008, we conducted intensive surveys throughout the Herring Run watershed to determine the extent, relative abundance and age/size distribution of this newly discovered population. We surveyed 13 100m long stream sections spaced at approximately 500m intervals throughout Herring Run, from its confluence with Watts Creek upstream to its headwaters, as well as 2 perennial tributaries. We used a combination of snorkeling, bucketing and shoreline-midden searches during which we recorded the number of all live and dead freshwater mussels found by species. For rare, threatened and endangered mussel species, we tagged, aged (based on external shell annuli counts), and recorded the length, width and height of each live individual. We also recorded macro and microhabitat information (e.g., stream width, depth, gradient, substrate, etc.) for each capture location.

We surveyed a total of 1.3 km of stream length along Herring Run, which represents about 25% of the nontidal, perennial extent (~5 km total length) of this stream. In addition, we spot-checked numerous areas in between each survey station. With a total survey effort of 25 person-hours, we found 2,082 live native unionids along with 265 dead shells, which together comprised 4 species (Table 1). The eastern elliptio (*Elliptio complanata*) was, by far, the most common and widespread species which represented over 95% of all live individuals. The northern lance (*Elliptio fisheriana*) was the second most common species (4.8% of live mussels) followed by the eastern floater (*Pyganodon cataracta*) and dwarf wedge mussel. We found no live or dead mussels in the 2 perennial unnamed tributaries and field reconnaissance indicated that other tributaries are probably too intermittent and seasonally dry to support any unionids.

Table 1. Summary of freshwater mussels observed during surveys of 13 100-m long stream sections in the Herring Run watershed (Caroline Co., Maryland) during August 2008.

	# live	# dead	% live total	# sites ¹	% sites
<i>Alasmidonta heterodon</i>	1	1	0.1	2	15.4
<i>Elliptio complanata</i>	1,981	204	95.2	10	76.9
<i>Elliptio fisheriana</i>	99	52	4.8	7	53.9
<i>Pyganodon cataracta</i>	1	8	0.1	2	15.4
TOTAL	2,082	265			

¹No sites where either live or dead shells were found.

We found only a single live individual of dwarf wedge mussel along with 1 old dead shell. The live individual was relatively large and old with an estimated age of 9 years. It measured 45.8 mm in length, 19.4 mm in width and 25.4 mm in height. The dead shell was too worn and fragmented to estimate age or obtain precise size measurements but it too was clearly a large, old individual. We found the dead shell within 50 m of the same location where we discovered the species in 2007. This area occurred just downstream from a road crossing in a section that was obviously scoured and periodically blown out by large flows occurring through the road culvert. The live individual occurred about 500 m downstream from this location in a section that supported, by far, the highest densities of mussels found anywhere in the Herring Run watershed. Seven hundred and sixty-seven live mussels comprising 3 species occurred in this section, which represented over a third of the mussels found in the entire watershed. Characteristics of the survey station were mostly gently flowing long, shallow pools with a few small slow riffles and runs and a substrate dominated by small, dark gravel and coarse sand. Mean stream width here was 2.4 m and stream depth was about 0.2 m. A ~300 m wide, mature deciduous floodplain forest bordered the stream. Large agricultural fields planted in corn or soybean surrounded the floodplain forest. The actual capture location of the Dwarf Wedge Mussel was a small, shallow pool near the bank in a silt-muck dominated substrate in 20 cm deep water. Eastern elliptio densities were high near the capture site.

Our findings indicate that an extremely small, isolated and perhaps senescent population with very low viability occurs in Herring Run. Despite intensive surveys throughout the watershed, we were able to only find a single, old individual and 1 old dead shell. While it is highly unlikely that our surveys detected all individuals (our surveys covered about 25% of the total stream length and did not involve subsurface sampling where over 50% of a population may reside), the rate of detection was extremely low compared to other populations in Maryland. Moreover, we found that stream conditions and water quality in Herring Run were somewhat degraded. MBSS data supports these observations. In addition, the exotic Asiatic clam (*Corbicula fluminea*) occurred throughout most of the watershed. This nonnative bivalve may displace native mussels via competition for food and space. Its presence and abundance appears to be inversely related to dwarf wedge mussel presence-abundance in other Maryland streams and, in most cases, is completely absent in streams supporting *A. heterodon*. Finally, in many sections of Herring Run both up and downstream from the section where we

found *A. heterodon*, there was no continuous surface flow stream and numerous isolated standing pools of water. Although Maryland's Eastern Shore was experiencing below-average rainfall at the time of the surveys, the rain deficit had clearly not approached drought levels. Such low flow conditions may have been due to groundwater withdrawal from pivot irrigation systems in the surrounding watershed. Several ponds along and in the floodplain may also be contributing to low flows. While forest stream buffers appeared adequate in most of the upper half of the watershed, which is dominated by agricultural fields, the lack of sufficient buffers and the amount of impervious surface is a concern in the lower half of the watershed. Development is more predominant in this area in the form of several small subdivisions with some backyards occurring adjacent to or in the floodplain.

Population monitoring at other Dwarf Wedge Mussel streams

During August-September 2008, we monitored dwarf wedge mussel populations in Nanjemoy Creek (Charles Co.) and McIntosh Run (St. Mary's Co.). We used the same survey methods as described above. However, as in previous years, instead of searching continuous 100-m sections, we confined surveys to 2 established 200-m long stations per stream. At each station, we surveyed 5 10-m long sections with each section spaced systematically at 40 m intervals. Biologists from U.S. Fish and Wildlife Service-Chesapeake Field Office, DNR-MBSS and Skelly and Loy, Inc. Engineering assisted with monitoring (Table 2).

At Nanjemoy Creek, we found 2,306 live native unionids comprising 3 species with an overall detection rate of 114.2 live mussels per observer-hour (Table 2). As in previous years, exceptionally high densities of eastern elliptio were present along with one of the state's highest densities of the state-listed Atlantic spike (*E. producta*). We found 13 live and 1 dead Dwarf Wedge Mussel including 1 live recapture of an individual tagged in 2006. The live detection rate was 0.64 mussels per observer hour. Age-size data show an approximately normal distribution of age and size classes and evidence of recent reproduction. Age ranged from 3-8+ years (some older, worn shells could not be aged accurately beyond 8 years) and shell length ranged from 14.6-41.8 mm. These findings indicate that Nanjemoy Creek continues to support what is probably the state's largest, most viable population. Data also indicate the presence of a relatively stable and recovering population since 2003, when it experienced a major decline due to the combined effects of a severe drought followed by extreme flooding from Hurricane Isabel.

At McIntosh Run, we recorded 722 live native unionids represented by just 2 species, eastern elliptio and dwarf wedge mussel, with an overall detection rate of 69.4 mussels per observer-hour (Table 2). Eastern elliptio abundance continued to be moderately high. We found 4 live *A. heterodon* including 1 live recapture of an individual tagged in 2004. The *A. heterodon* detection rate was 0.39, which like Nanjemoy Creek, indicates a relatively stable and recovering population since 2003. *A. heterodon* age classes ranged from 6 - 10 years old and mussel length ranged from 32.2-44.5 mm. The lack of smaller and younger individuals may have been due to under sampling rather than an actual absence of these age-size classes. It remains to be seen through future monitoring whether this metric indicates a lack of reproduction or sampling error.

Table 2. Summary of freshwater mussels observed during Dwarf Wedge Mussel monitoring in McIntosh Run (St. Mary's Co.) and Nanjemoy Creek (Charles Co.) during August-September 2008.

Stream		Species ¹			Total
		<i>Ah</i>	<i>Ec</i>	<i>Ep</i>	
McIntosh Run	Live	4	718	0	722
	Dead	0	24	0	24
	Tagged	3	0	0	3
	Recaptures	1	0	0	1
	Live Detection Rate ²	0.39	69.0	0.0	69.4
Nanjemoy Creek	Live	13	2242	51	2,306
	Dead	1	56	3	60
	Tagged	12	0	13	25
	Recaptures	1	0	2	3
	Live Detection Rate ²	0.64	111.0	2.5	114.2

¹ *Ah* - *Alasmidonta heterodon*, *Ec* = *Elliptio complanata*, *Ep* = *Elliptio producta*.

² Live detection rate = no. live individuals observed per observer hour, where observer hour = no. observers x survey hours.

Reasons for deviations (if any):

None.

Recommendations for the continuation of the job:

Continue population monitoring. Conduct additional denovo inventory work in other streams with potential *Alasmidonta heterodon* habitat. These efforts provide important information on the status, distribution, population trends and viability of the mussel in each stream, and yield valuable insights into potential threats and conservation needs.

JOB PERFORMANCE REPORT

State: Maryland

Project Title: Endangered Species Conservation

Job No.: 697

Job Title: Puritan Tiger Beetle Habitat Restoration

Principal Investigator: McCann

Job Objective:

To restore suitable cliff habitat for puritan tiger beetles on Sassafras River Natural Resources Management Area by controlling encroaching vegetation.

Activities/Findings:

During 2006, habitat restoration in the form of vegetation control (using herbicides and mechanical control) occurred at 2 puritan tiger beetle cliff sites along the Chesapeake Bay. We believe that vegetation encroachment on these cliffs has significantly reduced habitat suitability for the beetle. These habitat changes also may have contributed to a major decline in the overall Sassafras River metapopulation, which represents approximately 20% of the world's remaining populations. This first-ever attempt at habitat restoration for the species in the Chesapeake Bay region promises to help bolster the long-term viability of the Sassafras River metapopulation and species as a whole.

To determine the effectiveness of the herbicide treatment and its effects on puritan tiger beetle populations, we collected baseline cliff vegetation and beetle population data during 2005-2006. We followed this by annual post-treatment data collection in 2007-2009. Population monitoring involved multiple adult beetle index counts (3-6) at the 2 treatment sites as well as 3 adjacent cliff sites where no vegetation control occurred (i.e., control sites). The counts occurred on 1-2 days in mid-July when adult numbers peak. These counts took place on the same day or within several days of annual monitoring at other sites in the Sassafras River area; this monitoring involved single counts.

We evaluated cliff vegetation using a combination of photo-monitoring and plot-based sampling. We took photos annually from a boat during late August-September 2006-2008 at a series of 31 GPS-referenced, offshore locations and 38 on-shore/near-shore locations. The primary purpose of photo-monitoring was to evaluate changes in vegetation cover and distribution along each cliff face. We used vegetation plot data primarily to evaluate changes in species composition, particularly with regard to invasive and non-native species, the spread of which is a concern following herbicide treatment. We collected plot data during late August-September 2006 and 2008 at 13 10-m wide plots that extended from the cliff base to cliff top. At each plot, we estimated the percent cover of each vascular plant species as a vertical projection onto the plot area and assigned to 1 of 9 numerical cover classes.

We detailed monitoring results, as of late July 2008, in a report submitted in August 2008 to the U.S. Fish and Wildlife Service, Chesapeake Field Office entitled "Restoration of Puritan Tiger Beetle (*Cicindela puritana*) habitat at Sassafras Natural Resources Management Area, Kent County, Maryland" by J. M. McCann et al. We summarize the report below.

Herbicide treatment at the 2 cliff sites during 2006 was very successful in terms of eliminating most cliff vegetation and significantly improving habitat conditions for puritan tiger beetle. Two years after the herbicide treatment (2008), vegetative cover remained quite low. We also observed no significant increases in the presence of invasive plant species.

Although we need additional monitoring to fully evaluate beetle response to habitat changes, beetle abundance has so far showed a moderately positive response. Following remarkably high numbers in 2006 (probably due to the latent effects of Hurricane Isabel which struck the Chesapeake Bay in 2003), beetle abundance declined in 2007 at both control and treatment sites (Figure 1). Similar declines occurred at several other sites in the Sassafras area. However, the decline was much less severe at the treatment sites than at the control sites. Beetle numbers were also more stable at treatment sites and even showed a small increase from 2007 to 2008 while numbers at control sites continued to decline in 2008.

The effects of cliff vegetation control on beetle abundance should be better known in several years. Still, monitoring results thus far indicate it has helped dampen the severity of a short-term decline and stabilize beetle numbers. Continued annual beetle monitoring is essential for evaluating this species' response to vegetation control, and should continue through at least 2011. Likewise, cliff vegetation plot sampling and photo-monitoring should continue every 1-2 years through at least 2011. Vegetation monitoring will help us evaluate beetle response to vegetation conditions as well as the frequency of vegetation control, the techniques used, and the overall benefits of doing so. It will also help us assess habitat restoration needs at other cliff sites.

Reasons for deviations (if any):

None

Recommendations for the continuation of the job:

Annual monitoring of beetle populations and monitoring of cliff vegetation every 1-2 years should continue through at least 2011 so that we can adequately evaluate restoration efforts.

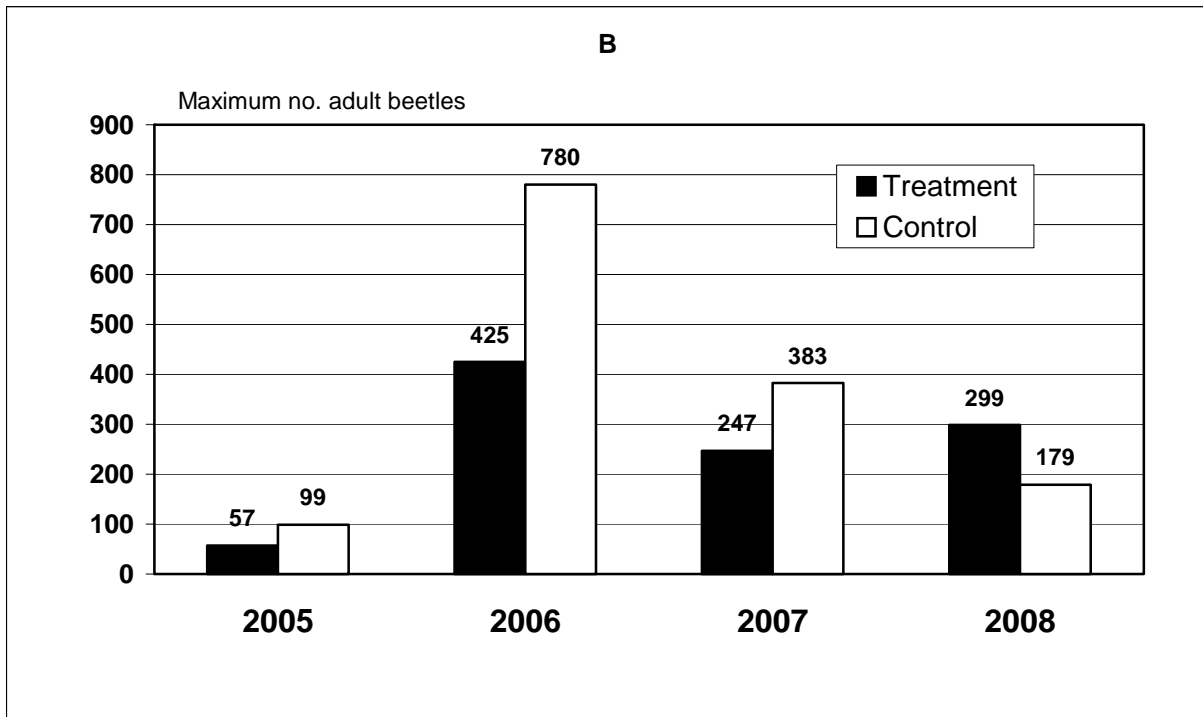
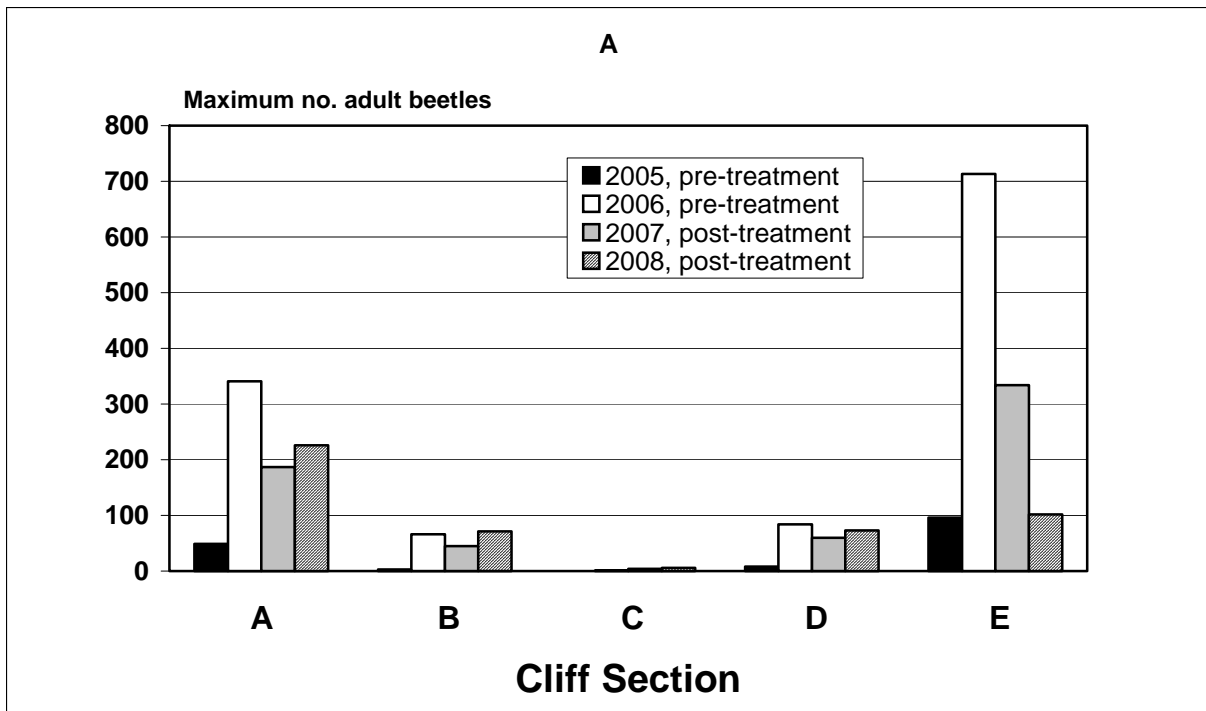


Figure 1. Puritan tiger beetle (*C. puritana*) abundance for cliffs A-E at Sassafras Natural Resource Management Area (Kent Co., MD) during 2005-2008 by (a) cliff section and (b) by year with pooled data for control and treatment sites, based on the maximum number of adult beetles detected during multiple index counts.

JOB PERFORMANCE REPORT

State: Maryland

Project Title: Endangered Species Conservation

Job No.: 698

Job Title: Harperella Monitoring and Mapping

Principal Investigator: Frye

Job Objective:

To complete a systematic stream-level inventory of Sideling Hill and Fifteen Mile Creek and to collect leaf tissue for an analysis of patterns of gene flow using molecular markers.

Activities/Findings:

We collected tissue samples from Sideling Hill Creek from each of the 45 previously established transects with depth sampling of one extensive patch in 0.25 m² quadrats (4 corners method) up to a depth of 1 m². We air dried and extracted tissue using standard methods according to manufacturer's instructions (Qiagen Kit). In a preliminary sample, total DNA content was relatively low but of high molecular weight. We performed additional extractions using a nanodrop procedure in order to produce statistics for nanograms of DNA for each milligram of dried tissue. Initial PCR's using known chloroplast primers were successful but the sequences obtained showed no variation. Initial PCR's using common primers for nuclear markers were successful but we were unable to resolve the sequences due to large indels.

Currently, we are using ISSR (intersimple sequence repeats) to determine whether there is detectable genetic variation in the Sideling Hill population. ISSR constitutes a whole-genome approach using anonymous DNA segments. If these results are promising then we will continue with an analysis of many more individuals to produce a population map that we can compare with geographic distance. The attached table provides the primer combinations and whether they were successful (positive) or not (negative).

Table 1. ISSR primers used on *Ptilimnium nodosum* to identify polymorphic primers for ISSR analysis.

Primer	Sequence	Results
807	AGA GAG AGA GAG AGA GT	+
810	GAG AGA GAG AGA GAG AT	+
811	GAG AGA GAG AGA GAG AC	-
812	GAG AGA GAG AGA GAG AA	+
825	ACA CAC ACA CAC ACA CT	+
836	AGA GAG AGA GAG	+

	AGA GYA	
839	TAT ATA TAT ATA TAT ARG	-
840	GAG AGA GAG AGA GAG AYT	+
841	GAG AGA GAG AGA GAG AYC	+
846	CAC ACA CAC ACA CAC ART	-
855	ACA CAC ACA CAC ACA CYT	+
856	ACA CAC ACA CAC ACA CYG	-
864	ATG ATG ATG ATG ATG ATG	-
868	GAA GAA GAA GAA GAA GAA	+
878	GGA TGG ATG GAT GGA T	+

Reasons for deviations (if any):

Initial results from chloroplast markers showed no variation and results from nuclear markers showed variation but only in indel structure. We are currently using ISSR methods to determine population-level genetic variation. We are using gradient PCR's to determine optimal primer combinations and conditions.

Recommendations:

We recommend continuing this work to resolve best methods.