

STUDIES OF THE PURITAN TIGER BEETLE (*CICINDELA PURITANA*)
AND ITS HABITAT: IMPLICATIONS FOR MANAGEMENT

Part 1. Habitat Parameters Determining the Distribution and Abundance of *C. puritana* in Maryland

Part 2. Annual Population Estimates of *C. puritana* at all Maryland Sites

Part 3. Laboratory Rearing, Oviposition Choice Experiment, and Field Translocations

Part 4. Laboratory Tests on the Effects of Herbicides on Tiger Beetles and Other Insects

FINAL REPORT

To: U. S. Fish and Wildlife Service, Chesapeake Bay Field Office
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Photo by Chris Wirth

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EXECUTIVE SUMMARY

Part 1. The Puritan Tiger Beetle (*Cicindela puritana*) (PTB) inhabits bluffs and beaches along the Upper Chesapeake Bay, and point bars within the Connecticut River in New England. This Federally Threatened species has shown a significant decline in numbers since the mid 1990's despite annual monitoring and preservation efforts. Adult PTBs actively prey on amphipods and other arthropods along the shoreline, but move to the upper adjacent bluffs to lay eggs and establish larval habitat. The purpose of this project was to determine the geologic and biologic controls on PTB populations in the remaining habitat sections in the Chesapeake Bay along the Calvert County and Sassafras River shores of Maryland.

Panoramic photography, Geographic Information Systems (GIS), and field and laboratory studies of the stratigraphic, sedimentologic, and biologic characteristics of high and low density PTB sites enabled an analysis of habitat parameters that influence the distribution and abundance of the PTB. For each panorama, a GIS photographic comparative analysis enabled a determination of potential (total exposed area and percent of bare bluff) and probable (exposed, non-vegetated bluff face that contains favorable geologic materials for burrowing) habitat. At each site, replicate samples were taken from representative locations within each of the major geologic units (formations) vertically from the base of the bluff to the top of the bluff (where accessible). Geologic parameters examined from the bluff faces included sediment compaction, temperature, moisture, grain size, conductivity, slope, and color. Beach parameters included width, slope, grain size, and percent and type of cover.

The first order GIS analysis revealed that, at regional scales, lithology (at the formation level) and vegetative cover (at a yet undefined threshold) control PTB distribution and abundance. The second order analysis using a Wilcoxon Signed Rank Test showed that no variable emerged as significantly different when comparing high and low beetle density sites by strata. However, when strata data were combined and the effect of site removed, the mean values of compaction (140.2 psi), mean grain size (0.3 mm) and sorting (0.24 mm; i.e., $\pm 1\sigma$) for all inhabited sites emerged as the statistically significant parameters (95% confidence level). These results corroborate those from other studies of the PTB along the Connecticut River and from other federally-threatened beach-dwelling tiger beetles that have shown that mean grain size, sorting, and compaction are biologically important habitat parameters. Consequently, multiple parameters affect the distribution and abundance of the PTBs. In particular, the bluff faces will exhibit characteristic exposures and vegetative cover in one of the stages of a well-documented bluff erosion cycle. This report presents a four stage multi-decadal cycle in which a fresh (well-exposed), unvegetated, steep (unstable) and geologically suitable bluff-face habitat [Stage 1] erodes through gradual slumping (translational slides) at the top of the bluff via groundwater seepage or by wave undercutting at the bluff toe. These processes reduce the bluff slope, stabilize the bluff, and encourage vegetative growth on the colluvium deposition and terraces produced by preferential groundwater erosion of strata [Stage 2]. Continued slumping of the top layers and/or slumping through wave cut activity of intact bluff material and oversteepening of the bluff face produce a stable colluvium fan at the bluff toe with slopes at angle of repose and that encourages further vegetative growth [Stage 3]. In the last stage [Stage 4], erosion and dispersal of colluvium fan results in the new, freshly exposed, steep bluff face of Stage 1.

The results from this study suggest that the year-to-year fluctuations (variations in spatial and temporal population trends) in PTB abundance relate to bluff face *quality* where both the antecedent geology of and the dynamics operating at the bluff face determine quality. While the PTB prefer the fresh bluff face portion of the cycle (created by bluff face erosion and ensuing removal of debris), other stages of less favorable habitat (e.g., stabilized bluffs with low slopes and colluvium fans and vegetative cover) can co-exist at any given time throughout the Bay. Both long-term, cumulative processes (spanning several decades) and shorter-term, episodic processes (individual storms) can result in colluvium removal and reactivation of the bluff face to create newly exposed, fresh surfaces for PTB burrowing and ovipositioning. The current bluff face conditions, beetle counts, the known two-year life cycle of *C. puritan* (i.e., no PTB recruitment would occur following the passage of a storm) and habitat parameter studies suggest that Hurricane (Tropical Storm) Isabel may have served as the trigger for the recent increase in beetle numbers observed beginning in 2006. Thus, the results from this study indicate that a three year lag exists between the impact of a large storm and the recovery of the beetle population.

Finally, this study provides the bluff quality (conditions) to consider for potential restoration sites through vegetation removal. Given that Ordinary Point has the second greatest amount of total bluff face area and both potential and probable habitat making this section a prime restoration site candidate. Along the Calvert Cliffs, the St. Leonard Member of the Choptank Formation (Fm.) appears to be the prime larval habitat along the Calvert Cliffs. This information along with the potential/probable habitat results indicates that Cliffs of Calvert, Little Cove Point, State Park, and Warrior's Rest have the most potential for restoration.

Part 2. Part 2 presents the results of several studies evaluating the accuracy of index counts for estimating populations of the PTB and results of the annual survey results of the PTB at all sites in Maryland. The study of survey methods demonstrated that index counts may vary significantly depending on the individual surveyor, seasonality of the beetles, and especially the time of day. For example, index counts were 2 or even 3 times lower when the beach was shaded compared to counts made when the shoreline was in full sun. It was also determined that index counts usually produced estimates that were half that of estimates from the removal method. Results of a comparison of index, removal and mark-recapture done with *C. dorsalis* also indicated a similar underestimation of index counts and confirmed that the removal method gave the most accurate results. In this same study, estimated from mark-recapture (Lincoln Index) were similar to the removal method if recaptures were done the same day, but recapture on the second or third day after marking produced overestimates of population size. Regardless, index counts may continue to be the preferred method because they are much less time consuming and suitable for relative comparisons of sites and annual trends.

The total number of adults of *C. puritana* counted at Calvert sites in 2008 was 5721, indicating a pattern of increase in the past 4 years (1101 in 2005, 3946 in 2006 and 2625 in 2007). In the prior years (2002-2005) counts were less than 2100 adults. The significant increase in 2008 is primarily a result of large increases in numbers at most major sites: Calvert Cliffs State Park (1609 in 2008, 292 in 2007), Little Cove Point (1116 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 minimal changes were recorded at the other sites. The results of surveys at all Sassafras sites produced a total count of 1764 in 2008, indicating a progressive and significant increase in the past 5 years, 398 in 2004, 408 in 2005, 1221 in 2006, and 1566 in 2007. Prior to

these years, the total counts declined significantly from 1996 (count of 1821) to 2002 (400). The lowest ever total numbers were recorded 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.

Part 3. This part of the report includes a short summary of the results of the rearing of adult *C. puritana* in the lab to produce offspring for laboratory, field translocations of these offspring, and a laboratory test of soil particle size preference by females for oviposition. Adult females readily oviposited in laboratory chambers with habitat soil. An estimate of over 400 eggs or first instars were produced from 30 females for an average of over 13 per female. Although this level of fecundity is much lower than the 40 or more eggs per female reported for several other tiger beetle species, it could probably be increased by appropriate adjustments such as increasing soil moisture. These studies further suggest that laboratory propagation may be a viable option for PTB recovery. A total of 88 first instars from the laboratory rearing were placed in plastic tubes and placed into the bluff at two field sites (Calvert Cliffs State Park, Grove Point) in association with over 250 native larvae. Both the translocated larvae and the native larvae experienced increasing mortality from early September to November 8, but most of those that survived developed to the second instar and some to the third instar. After the overwintering period at the April 5 survey, 20% of the native larvae at Calvert Cliffs State Park had survived and 24 % of those at Grove Point survived. None of the translocated larvae could be relocated, either as a result of loss through erosion or due to vandalism. There was evidence of significant erosion in the area of some of the native and translocated larvae.

The oviposition studies provided females with a choice of four different sand grain sizes in which to lay eggs. The results showed females preferred finer sand for oviposition: a mean of 7.4 per female in the fine sand (<.125mm) , 3.9 in medium sand (.250 mm), 1.0 in coarse sand (.50 mm) and one in very coarse sand (1.0 mm).

Part 4. This part includes tests of the effects of herbicides on tiger beetles and other insects. Tiger beetle habitats are increasingly being impacted by vegetation growth, especially invasive species, which eliminate bare, open patches needed by tiger beetle adults and larvae. Results indicated little or no mortality from direct or indirect applications of all formulations at or above highest recommended concentrations, including 5% Habitat with surfactant, 8 and 10% Roundup, and 10% Rodeo to the 6 species of tiger beetles or other insects (adult and larval flour beetles, adult and larval Tribolium, crickets, and soldier beetles) that were tested in the laboratory. Results of an earlier study with *Cicindela dorsalis* indicated no mortality from direct or indirect application of 1.75% Rodeo with 0.5% to larvae in lab tests and no apparent mortality in field trials. The results of these tests suggest that herbicides may be used to control vegetation in tiger beetle habitats without any impacts of tiger beetle adults or larvae or their prey

TABLE OF CONTENTS

Part 1. Habitat Parameters Determining the Distribution and Abundance of <i>C. puritana</i> in Maryland	
Introduction.....	1
Geology of Study Areas.....	3
Methods.....	14
Results and Discussion.....	18
Summary and Conclusions.....	25
Recommendations for <i>C. puritana</i> Management and Recovery in Maryland	30
Tables.....	32
Part 2. Annual Population Estimates of <i>C. puritana</i> at all Maryland Sites	
Introduction.....	1
Methods.....	1
Results and Discussion.....	2
Evaluation of the Index Method to Determine Population Size.....	2
Population Estimates at All Maryland Sites.....	5
Summary of <i>C. puritana</i> Trends at Sassafras Sites, 1988 to 2008...10	
Part 3. Laboratory Rearing, Oviposition Choice Experiment, and Field Translocations	
Introduction.....	1
Methods.....	1
Results and Discussion.....	2
Rearing.....	3
Oviposition Choice Trials.....	4
Part 4. Laboratory Tests of the Effects of Herbicides on Tiger Beetles and Other Insects	
Introduction.....	1
Methods.....	1
Results and Discussion.....	2
Literature Cited.....	7
Acknowledgments.....	8
APPENDICES	

Part 1

Part 1. HABITAT PARAMETERS DETERMINING THE DISTRIBUTION AND ABUNDANCE OF *C. PURITANA* IN MARYLAND

INTRODUCTION

The United States Fish and Wildlife Service (USFWS) listed the Puritan Tiger Beetle (PTB), *Cicindela puritana* as a Threatened Species in 1990 because of the loss of most populations along the Connecticut River and the lack of protection for most existing sites in Maryland (USFWS 1990). This species is also designated as “Endangered” by Maryland state law. Bluffs and beaches along the Upper Chesapeake Bay in Maryland, and point bars within the Connecticut River in New England contain the only known remaining habitat for the PTB (Figure 1). The New England populations have been small (totals of less than 1000 adults in most years) but relatively stable. Along the Chesapeake Bay, the species is found at two locations in Maryland separated by the Chesapeake Bay: the western shore along most of Calvert County (9 sites) and the eastern shore along or adjacent to the Sassafras River mouth in Cecil and Kent Counties (8 sites).

Results of annual counts for all populations since 1989 documented that the two Maryland metapopulations have declined dramatically from peak abundance in early to mid-1990’s to lowest abundance from 1999 to 2005. Numbers have increased significantly for both metapopulations in the past 3 years but are still well below peak abundance. A recent Population Viability Analysis (PVA) based on these annual monitoring data suggested that both metapopulations were at risk of extinction (Gowan and Knisley, 2005). The goal of this PVA is to inform future management and recovery efforts for this at risk species. Also important for these efforts is an analysis of habitat quality and how changes in various habitat parameters may be driving population dynamics. Despite this intensive survey work and additional biological studies, much about the habitat indicators and the specific causes of these year-to-year variations in abundance is unknown. Ashby (1986) and the Maryland Natural Heritage Program (2003) speculated that eroding shorelines, structural disturbances, increased coastal development, and increased vegetation growth on the bluffs all may be involved in habitat disruption and population declines. Indeed, development along the Chesapeake Bay shore has increased recently with waterfront housing, shoreline stabilization structures and recreational activities (Vogler et al. 1993).

Both larvae and adults of PTBs prey on a variety of arthropods and require habitats free of vegetation for their predatory activities. Larvae are sit-and-wait predators that live in burrows from which they capture prey organisms that pass near their burrow mouth. Adults of the PTB are visual hunters which actively prey on arthropods, amphipods, and occasionally scavenge on dead fish and crabs found along the shoreline or the bases of bluffs (Knisley and Schultz, 1997). The larvae occupy separate habitats from adults within permanent burrows on the vertical bluff faces adjacent to the narrow sandy beaches where adults are active. Earlier studies suggest larvae are largely restricted to patches of fine- to medium-grained sands, often in the upper strata of the bluffs (Vogler et al. 1993; Knisley and Schultz, 1997). These larval habitats are established by the selection of an oviposition site by the adult females which apparently move from their foraging areas along the beach to the adjacent bluffs, possibly at night. Studies in Maryland found that females oviposit their eggs primarily in sandy deposits on the vertical bluff faces, or sometimes at the base of the cliff in sediment from the cliff face (Hill and Knisley, 1993). The

first instars hatch during the late summer and dig a burrow at the site of oviposition in the cliffs. Development through the three larval instars continues for two years until the second spring when pupation occurs. Adults emerge from their pupal stages in mid-June and reach peak numbers in late June and early July (Hill and Knisley, 1993).

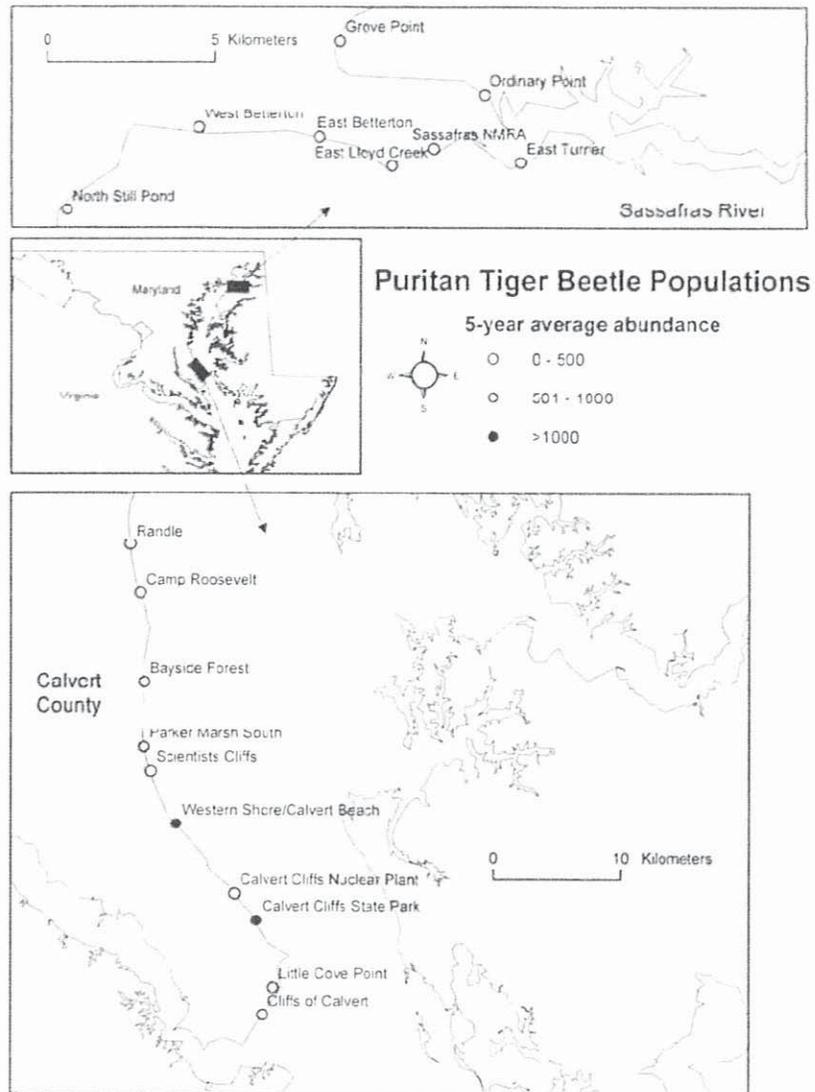


Figure 1. Map of the Sassafras River and Calvert County PTB sites within Maryland with five year average abundances shown for the study sites.

Although it is generally thought that habitat degradation negatively affects PTB abundance, the specific habitat parameters that control the presence/absence, distribution, and abundance of this declining species are not well known. Previous research on the PTB population along the Connecticut River analyzed the effects of moisture, vegetation cover, grain size and prey availability on the abundance of PTB populations (Omland 2002). Particle size emerged as the main density determining parameter. Omland (2002) found that sediment containing mostly medium and fine sand was positively correlated to areas of high larvae density. Also, the grain size on the surface of the burrows had higher correlation to larval density than the grain size of sediment at a depth of 30 cm into the burrows.

The purpose of this study was to determine which geological parameters are the best indicators of habitat quality for the PTB in Maryland where the habitat differs greatly from the New England habitat. The experimental design we used included a study of the geologic strata that comprise the bluffs, and comparisons of a series of beach and bluff parameters that included moisture content, conductivity, temperature, compaction, bluff slope, grain size and vegetation cover at high and low density adult patches at selected Calvert and Sassafras sites.

GEOLOGY OF THE STUDY AREAS

The two study areas containing PTB populations within the Chesapeake Bay are located approximately 110 km apart from each other, on opposite sides of the Chesapeake Bay, and their depositional environments were separated in geologic time by > 47.5 my. Both population sites contain unconsolidated to semi-consolidated Coastal Plain sediments that were deposited during multiple episodes of sea-level changes. The Calvert County study area contains Lower to Middle Miocene-aged units ranging in thickness from 25-35 m and in age from c. 6.3 my to 17.5 my (Kidwell, 1989), while the Sassafras River study area bluffs consist of Upper Cretaceous stratigraphic units which range in thickness from 4-21 m and age from 83 my to 65 my (Figure 2; Minard, 1974). While the lithology, thickness, and dip of the units vary as a function of broad, tectonic controls and more local variable depositional environments, the units generally dip to the south-southeast at <1° such that the older units descend below the surface and the younger formations crop out successively to the southeast. The separation of populations by geologic strata and geographic space raises intriguing questions about the geologic and geographic controls on the distribution and abundance of the PTB.

Calvert County. The distribution of the PTB in Calvert County, MD spans approximately 40 km along the western shore of the Chesapeake Bay and includes 9 study sites: Randle Cliffs, Bayside Forest, Warrior Rest, Scientist Cliffs, Western Shores, Power Plant, State Park, Little Cove Point and Cliffs of Calvert (Figure 3). The Calvert County bluffs that back the narrow beaches and provide habitat for larvae consist of three primary geologic formations. These three Tertiary (Miocene) -aged (c. 6.3-17.5 mya) Coastal Plain formations together comprise the Chesapeake Group of the Calvert County cliffs (Shattuck, 1902; 1904; Figure 4). Because of the southeastern dip (or tilt) to these formations (< 1°; approximately 11 ft/mi or 2 m/km), all formations decrease in exposed thickness to the south as they ultimately descend beneath the waters of the Chesapeake Bay. Consequently, in Calvert County, the youngest St. Mary's Formation outcrops along the southern part of the study area at the base of the bluffs from Drum Point to Calvert Cliffs State Park (Figures 5 and 6). The next oldest unit, the Choptank Fm., outcrops at the base of the Calvert Cliffs State Park, where it underlies the St. Mary's Fm., and

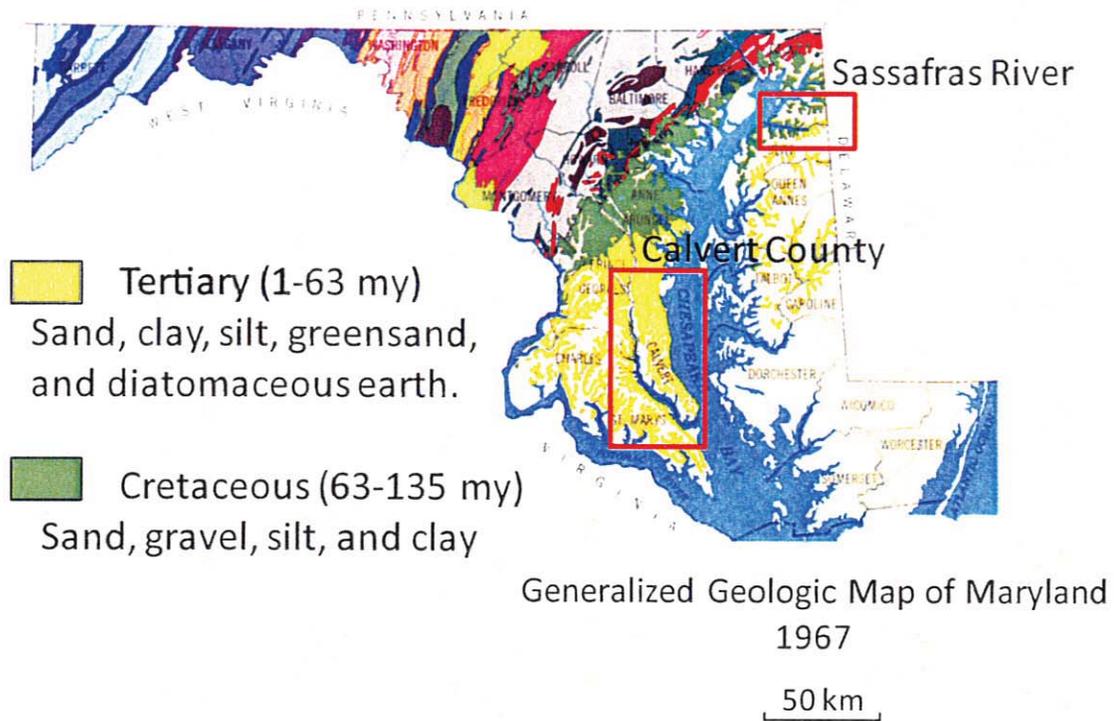


Figure 2. Generalized geologic map of Maryland showing differences in geology between the two PTB study sites (source: Maryland Geologic Survey, www.mgs.md.gov).

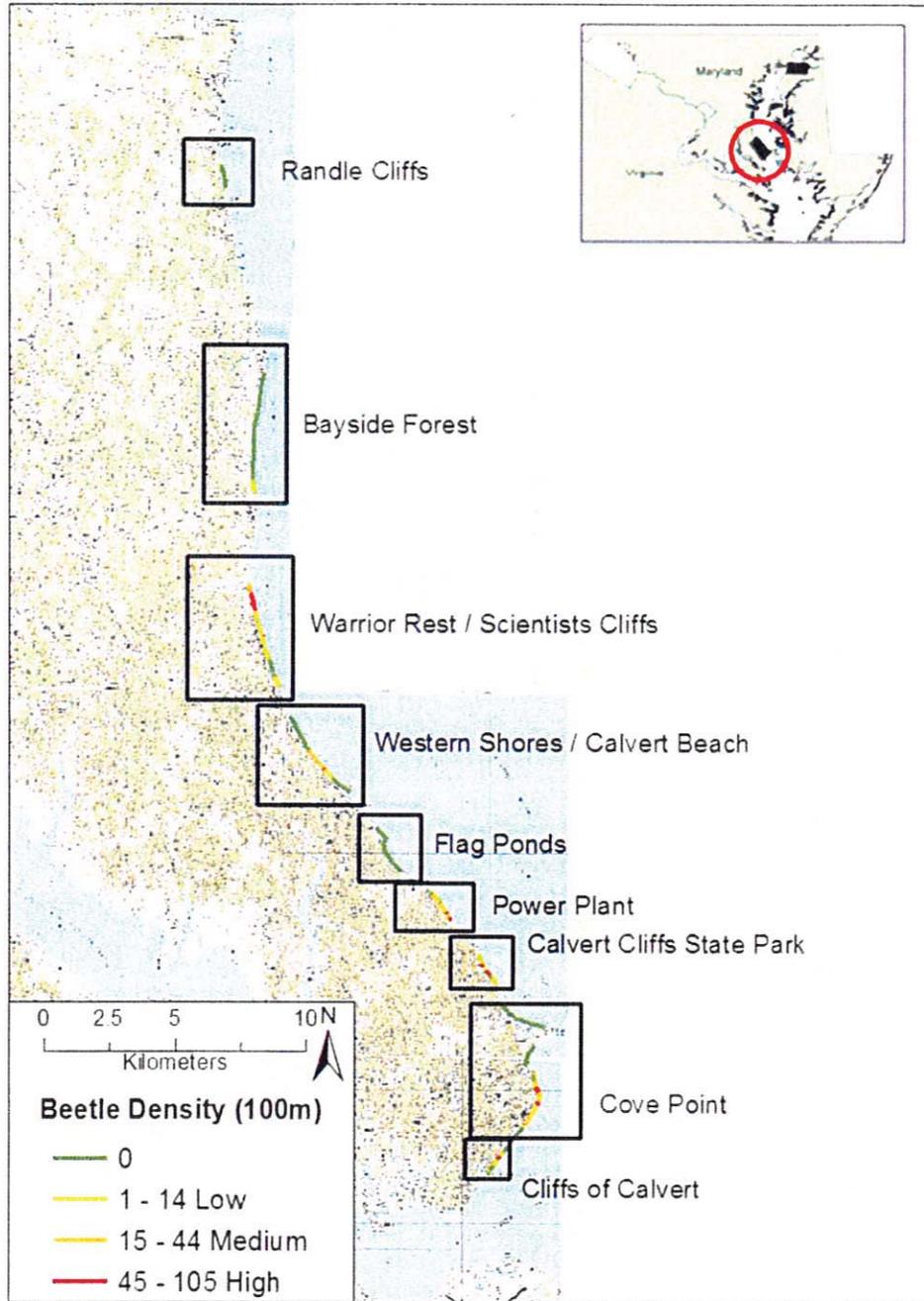


Figure 3. Map of the nine sampling sites within the Calvert County, MD study area.

EPOCH	FORMATION	MEMBER	DEPOSITIONAL		MOLLUSK		LITHOLOGY	
			BED	EVENT	ZONE			
PLIOCENE	Chowan River				XIX	4		
		Yorktown	Moore House			XVIII	5	
	Morgarts Beach				XVII			
	Rushmere				XVI	6		
	Sunken Meadow				XV	7		
	MIOCENE	Eastover	Claremont Manor			XIV	8	
				Windmill Point beds	24	XIII	9	
St. Marys		Little Cove Point beds		21-23	XII	10		
			Conoy	20	XI	11		
Choptank		Boston Cliffs		19	X			
			Drumcliff	18	IX	12		
			St. Leonard	17				
Calvert		Calvert	Plum Point Marl	Calvert Beach	14-16	VIII	13	
				12-13	VII			
				10-11	VI			
				4-9	V			
	Fairhaven				3b	IV		
		2-3a	III					
OLIGO-CENE	Old Church (VA) Belgrade (NC)					14		

Figure 4. Representative stratigraphic column for the Calvert County, MD study area (from Ward 1992).

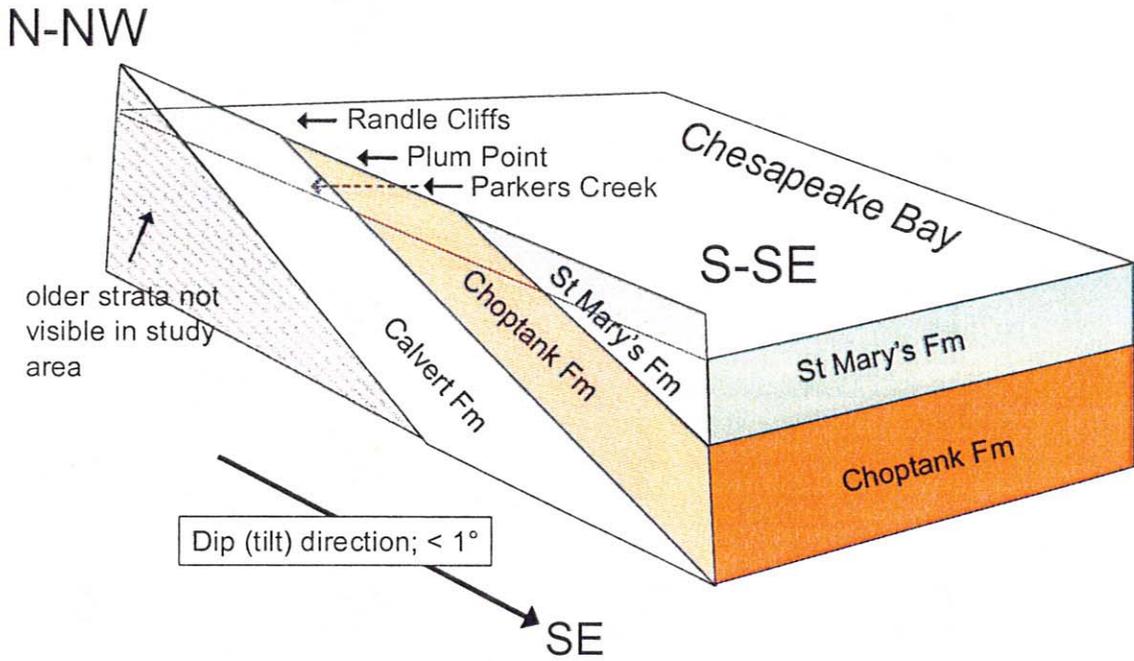


Figure 5. Generalized block diagram of Calvert County, Western Shore Chesapeake Bay (not to horizontal scale; greatly exaggerated vertical scale).

GEOLOGICAL PROFILE OF CALVERT CLIFFS ADAPTED FROM DIAGRAM BY PETER VOGT
 VERTICAL EXAGGERATION 20:1

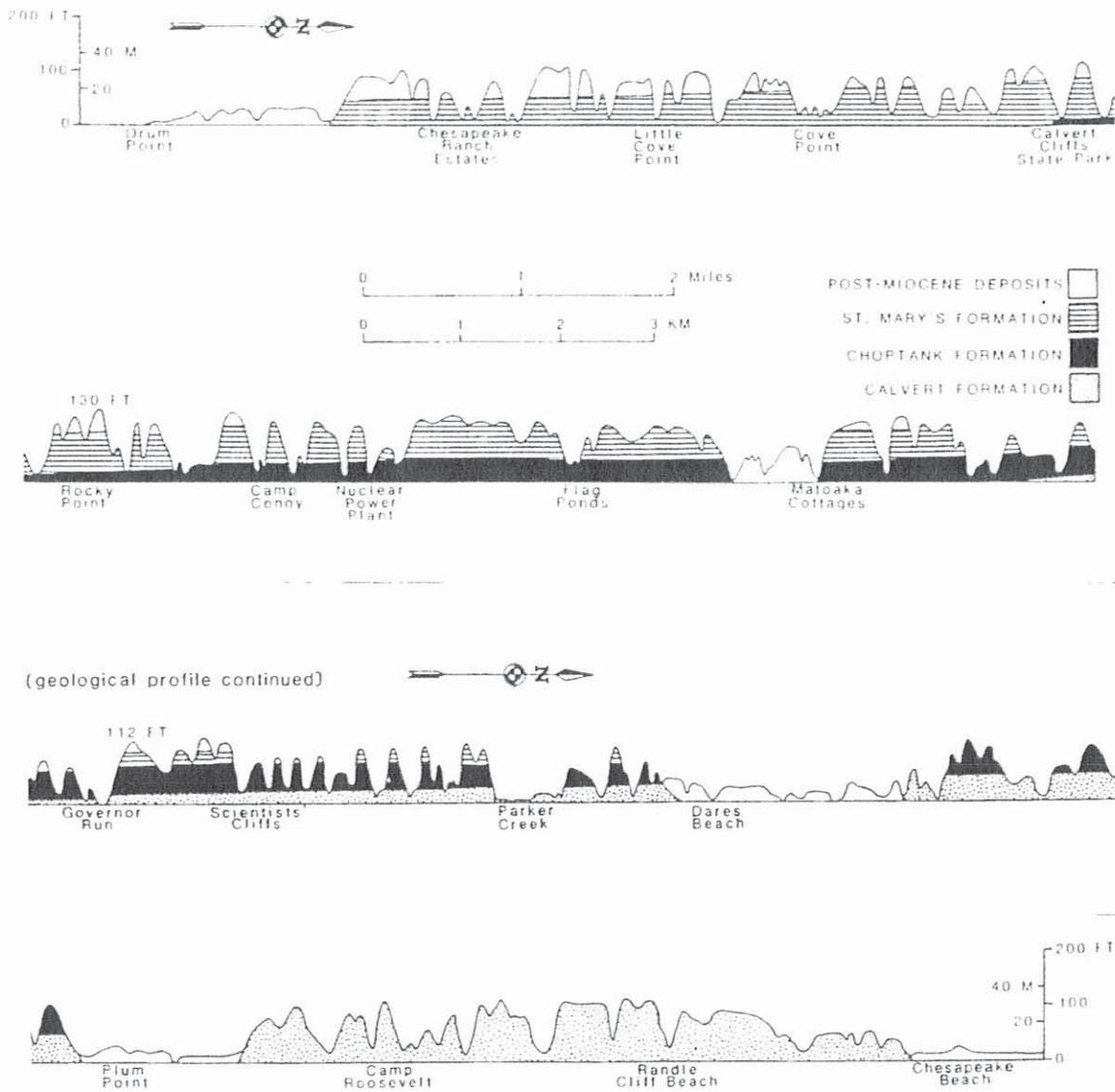


Figure 6. Generalized geological profile of Calvert Cliffs showing the regional southerly dip and exposures of geological formations at the various study sites. Note the predominance of the unfavorable (PTB habitat) Calvert Formation at the north end of the Calvert Cliffs (bottom), and the more favorable habitat Choptank Formation (black) to the south (middle two sections (from Ashby, 1995).

extends northward to Plum Point. South of Governor Run, the oldest unit of the study area, the Calvert Fm. occupies the base of the bluffs where it underlies the Choptank Fm. and increases in exposed thickness to the north. The Calvert Fm. completely dominates the geologic profile of the bluffs from Camp Roosevelt northward to Chesapeake Beach including the sites of Bayside Forest and Randle Cliffs (Figures 5 and 6).

The Calvert formations can contain substantial lateral and vertical facies changes. Each formation has been mapped as having three members, and as many as 24 stratigraphic units (zones) have been identified (Shattuck, 1904; Kidwell, 1989, 1997; Ward and Powers, 2004). While these zones do not meet the standards of the International or North American Codes of stratigraphic nomenclature, most authors of Calvert County bluff studies use the 24 “zones” or beds. This study sought to determine if the geologic variability (horizontal and vertical facies changes) within and among formations plays a role in the distribution and abundance of PTB.

A cursory qualitative comparison of the stratigraphy and geology of Warrior Rest and Randle Cliffs illustrates the first order geologic influence on tiger beetle populations (Figures 7 and 8). The apparent high quality bluff habitat at Warrior Rest consists of the Plum Point and Calvert Beach Members of the Calvert Fm. overlain by the Drumcliff, St. Leonard, and Boston Cliffs Members of the Choptank Fm (Figure 7). A thin layer of St. Mary’s Fm. caps this area. At Warrior Rest, the clayey, habitat-poor Plum Point Member of the Calvert Formation occupies the base of this bluff (≈ 4.6 m), but approximately 16 contiguous meters of five sandy Members (and eight beds/zones) overlie the Plum Point Member – including the apparent prime larval habitat of the St. Leonard Member of the Choptank Fm. By comparison, the Randle Cliff exposure at the northern limit of the Calvert PTB range contains fine-grained argillaceous (clay-rich) sand and sandy clay that seem largely unsuitable as PTB habitat (Figure 8).

Sassafras River. The Sassafras River location spans a length of approximately 14 km along the north and south shores of the Sassafras River in Kent and Cecil Counties and includes 8 study sites: Grove Point, Ordinary Point, West Turner, East Turner, East Lloyd, West Betterton, East Betterton and North Stillpond (Figure 9). The Sassafras site contains geologic units older than those found along the western shore and consist of 65 -100 mya Upper Cretaceous-aged Coastal Plain sediments of the Potomac Group that dip east-southeast (Owens et al., 1970). Several formations are well exposed along the south and north bank of the Sassafras River where PTB sites are found. These units include, from oldest (bottom) to youngest (top), the Merchantville Fm., the Englishtown Fm., the Marshalltown Fm., and the Mount Laurel Sand (Figure 10). Similar to the younger western Chesapeake Bay units, these units vary considerably laterally and vertically. The Merchantville Fm. consists of 6-12 m of thick bedded dark-gray to grayish-black clayey silt to fine and very fine sand. The Englishtown Fm. is characterized by fine to very fine quartz sand and prominent thinly-bedded cross stratification. The thickest outcrop of the Englishtown occurs east of the Betterton boat pier where 10 m of the formation is exposed. The Marshalltown Fm. (5-6 m thick) is usually thick bedded, mottled, fine- to medium glauconitic quartz sand. The Mt. Laurel Sand is a fine- to medium sized feldspathic quartz sand (with up to 15% glauconite) and is the thickest unit and the Sassafras region contains the best exposure of it in the North Atlantic Coastal Plain (Minard, 1974). The upper and lower parts can contain coarse sands and gravel and medium-greenish-gray to medium-dark-greenish-gray, fine to medium, silty, glauconitic quartz sand. The Mount Laurel is typically a thick layer (50 m in the northwest and 24 m thick at the western edge of the Betterton quadrangle) of glauconitic quartz sand above the Marshalltown Fm. (Minard 1974). The Mount Laurel Fm. is found primarily at

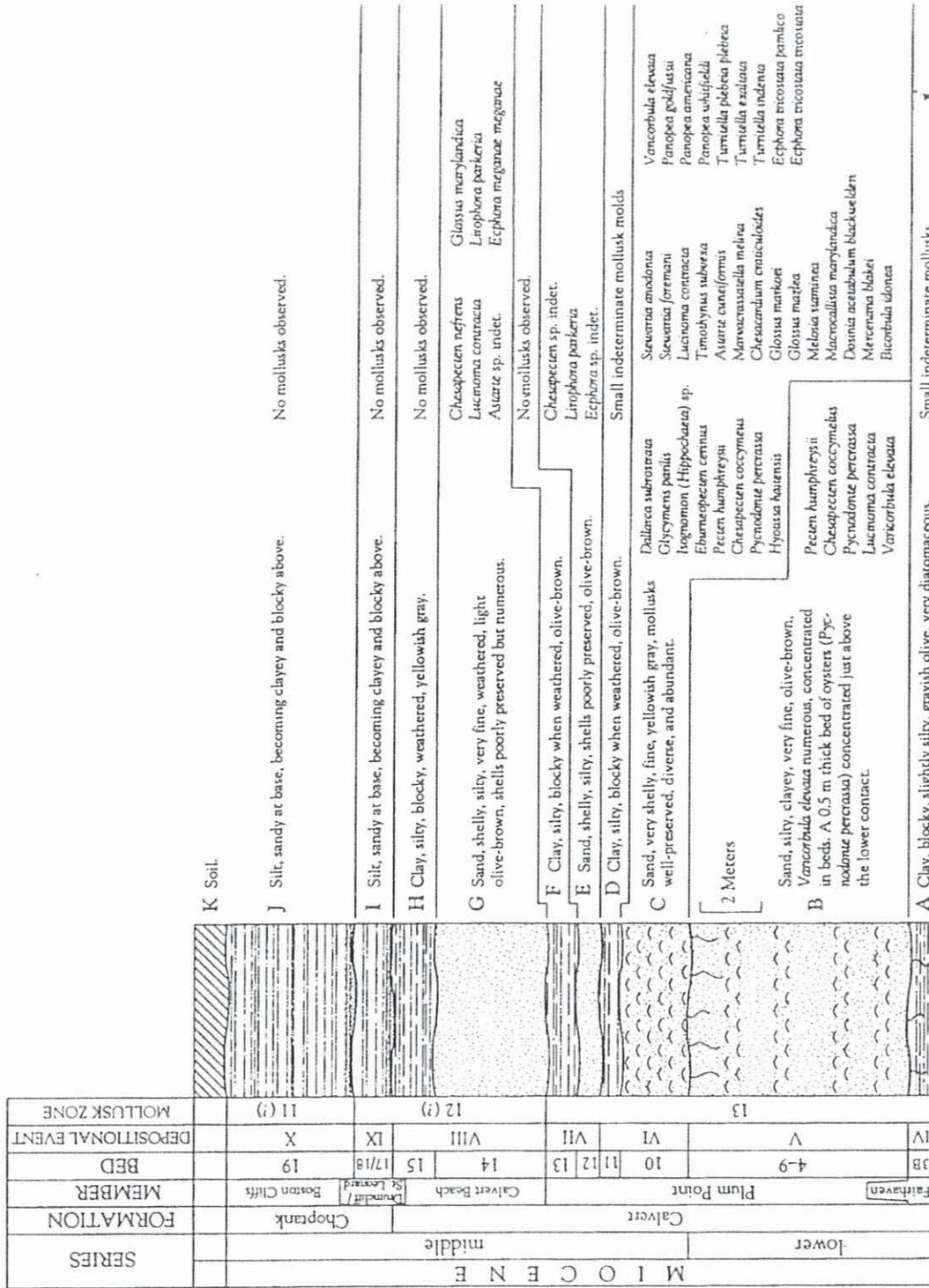


Figure 8. Randle Cliff stratigraphic column (from Ward, 1992).

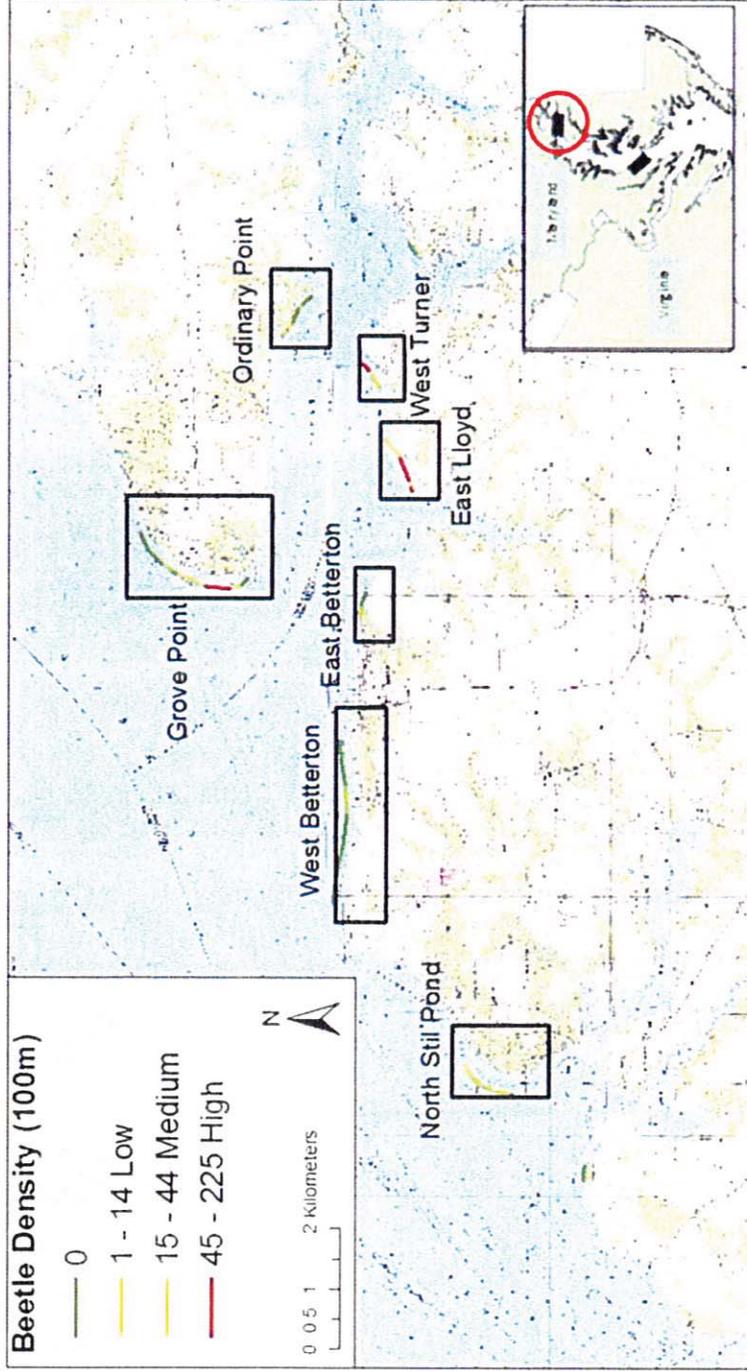


Figure 9. Map of the seven sampling sites within the Sassafras River study area.

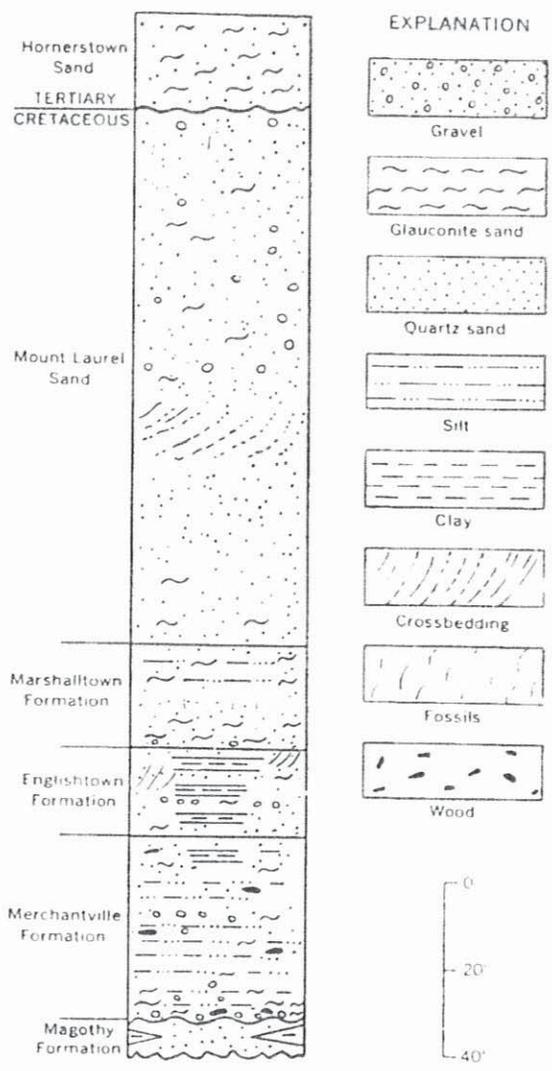


Figure 10. Representative stratigraphic column for the Sassafras River study area (from Owens et al. 1970)

the East Lloyd and West Turner locations along the southern coast of the Sassafras River. The Merchantville Fm. is well exposed as the lower formation at Grove Point and its fine- to very fine silts and clay make this formation an unfavorable habitat for the tiger beetles. However, the southerly regional dip of the units causes a decrease in the thickness of its exposure at Grove Point (and increases the more favorable Englishtown Fm.) from north to south.

METHODS

The methods used in this study consisted of field, laboratory, computer-based (Geographic Information Systems, GIS), and quantitative analyses. We used field studies to determine beetle numbers and assess habitat parameters. A GIS photographic analysis enabled a determination of potential and probable habitat (defined below). Both parametric and nonparametric statistics were used to identify parameters that control PTB densities.

Adult PTB Surveys. The methods used for adult beetle surveys are described in Part 2 of this report and not repeated here. We should note that in 2008, adult beetle counts were also recorded within much shorter sections of shoreline (about 10-20 meters depending on beetle numbers) to provide a more accurate measure of beetle abundance and density within each site. These new density data were used to select low and high density sites for habitat analysis. Topographic maps showing densities of beetles in both 2007 and 2008 were prepared using Terrain Navigator and are included in the Appendix of this report.

Larval PTB Surveys. We also conducted field surveys of larvae at selected sites by searching for the characteristic larval burrows in the bluffs, determining their identity (*C. puritana* or *C. repanda*), recording density (number/m²) and collecting sediment samples at some of these sites where larvae occurred. These surveys were conducted in mid-July when *C. repanda* larvae were most abundant and on several dates in late September and to early November when *C. puritana* larvae were active. Most survey locations were the more accessible lower strata of the bluffs (< 2-5 m), but other mid level strata (>5-10 m) were also searched. The survey method involved a visual search for larval burrows and when found, burrow stage and numbers within the surrounding 1 m² were counted. At most sites 2-5, 1 m² patches were sampled and results presented as the mean number of burrows. Species identity was based on burrow depth since earlier studies (Knisley and Hill, 1992) confirmed that burrows that were < 10 cm were *C. repanda* and those > 12-15 cm were *C. puritana*. Sediment samples were collected at representative sites with larvae by obtaining a sample from the surface down to 20 cm depth. Grain size was analyzed using the Ro-Tap methods described in more detail below.

Habitat Studies. A primary objective of this study was to determine the distribution, amount and quality of PTB larval habitat at both Calvert and Sassafras sites. Since larval habitat is selected by the adult female and is the site where their development occurs, the presence of larvae burrows is the best indicator of suitable habitat for tiger beetles. Using this factor to achieve our objective was difficult because larval burrows were found high on the bluff face at many sites (especially Calvert sites) and not accessible. An additional problem in surveying larvae is the co-occurrence of *Cicindela repanda* with *C. puritana*. Both have similar burrows, but can be distinguished by measuring burrow depth and generally by seasonality of occurrence.

PTB burrows are typically > 20 cm deep and most abundant during the fall while *C. repanda* burrows are usually < 15 cms and common during summer. Consequently, we used a combination of photographic analyses, geological analysis of the strata and adult densities to determine probable *C. puritana* habitat. Adult densities were the primary and *a priori* indicator of adult habitat and were verified by a comparative analysis of bluff and beach parameters. The approach enabled us to label sections as high or low density sites.

Photographic Vegetation Comparative Analysis. Vegetative cover, and stratigraphy were analyzed from photographs taken from offshore on 7-8 July 2008 showing the bluffs at all Calvert County and Sassafras River sites. All photographs were organized into panoramas to display complete sections of habitat for these two metapopulations (See Appendix B). Several problems were encountered in composing the panoramas and produced some errors in the subsequent analyses. Photos for the panoramas were taken at slightly different angles causing slight distortions in the view of the bluff faces. In some cases, vegetative cover obscured the outline of the bluffs. The total bluff face area for each site was determined from digital topographic maps (Terrain Navigator Pro) using the bluff face area between the shoreline or bluff toe, and the bluff “edge” at the top of the bluff. Common cultural features (e.g., houses, roads etc.) and natural features (e.g., creeks, depressions etc.) provided controls for the panorama photos and topographic maps. The error associated with this method was minimized by calculating the bluff face area at each site a minimum of 10 times until the standard error dropped below 10% of the mean bluff face area. Once the error fell below this acceptable limit, the replicated area calculations were averaged to obtain bluff area.

These panoramas were also used to delineate **potential** and **probable** habitat on the bluffs. Since earlier studies demonstrated that PTB larval habitat included only bare, unvegetated bluff faces, we categorized all areas of unvegetated bluff as **potential** habitat. To determine **potential** habitat, we used Geographic Information Systems software (GIS; Arc View version 9.3) to scale the photos using the area calculations from the maps, and to digitize the areas of the bare bluff faces and vegetative cover. The **potential** habitat (total exposed area and percent of bare bluff) was calculated by subtracting the area of the vegetative cover from the total bluff area at each site (See Appendix C).

However, earlier studies provided evidence that only unvegetated bluff strata with particular grain sizes – in the sand fraction size range – supported larvae. Thus, **probable** habitat consists of exposed bluff face (i.e., non-vegetated) that also contains favorable geologic materials for burrowing thereby restricting probable habitat to specific lithologies within the unvegetated sections. This prerequisite would exclude the Calvert Fm., for example, as probable habitat along the Calvert County coast. To calculate probable habitat, the area of each unsuitable Formation was determined and subtracted from the area of the potential habitat for each site. This analysis provided a first order examination of the geologic and biologic parameters that influence or control beetle density.

Difficulties arose when the contacts between Formations within the photos were not discernable. In some areas, slumping from higher formations or vegetation would cover the contacts. However, the lateral continuity and planar nature of the contacts enabled accurate interpolations in these cases. Given the potential for other variables to control preferred habitat, the calculated probable habitat area provided an overestimate of actual habitat.

Finally, an additional photo set taken in 2000 enabled us to determine if changes in bluff vegetation might explain changes in PTB numbers over time. To this end, we quantified the

percent (net) vegetation change that occurred between 2000 and 2007 at the Calvert County sites and compared those changes to changes in beetle numbers from the same time period.

Field Data Collection and Parameter Determination. A field test of **probable** habitat was conducted by analyzing a series of parameters measured at high and low density sections at selected PTB sites along the Calvert and Sassafras coasts. Sites selected for analysis along the Calvert coast included one high and one low density area at Calvert Cliffs State Park and two high and one low density sampling sites at Little Cove Point. Along the Sassafras shore, we sampled one high and one low density area at West Betterton, East Lloyd D, East Lloyd E and West Turner B sites; two high and two low density areas at Grove Point; and one high density area at West Turner A. Significant differences in parameters between high and low density areas were evidence that these parameters might explain the differences in abundance of PTBs at these low and high density areas.

Sampling at each site was carried out vertically on the bluff face within all accessible formations in order to capture the range of geologic conditions available to the beetles for burrowing. Where accessible, we used an extension ladder to access and sample a representative bed within each formation (and, in some cases, more than one bed) on the lower, middle, and upper bluff face. Three replicates were taken at each sample site on the bluff face (Figure 11). Data collected included moisture content (volumetric water content, VWC), temperature (°C) and conductivity (bulk dS/m) using a 5TE Decagon probe. Compaction was measured using an analog Spectrum Technologies 6100 penetrometer. Color of lithologies was determined using a Munsell color chart. The slope of the bluff face was measured with a clinometer. Sediment samples of the upper 5-10 cm of the bluff face were also taken in replicates of three at each of the vertical sample sites. The grain size analysis consisted of washing (in 100 ml of deionized water) and drying (at 120°C for 24 hr) each sample, splitting the dried sample to 20-40 g using an Ottoman-type sample splitter, and then sieving the subsample for 10 minutes using a Tyler (RoTap) mechanical sieve shaker. The mechanical shaker contained nested sieves at whole phi intervals (4 ϕ = 0.0625 mm; 3 ϕ = 0.125 mm; 2 ϕ = 0.25 mm; 1 ϕ = 0.5 mm; 0 ϕ = 1.0 mm; -1 ϕ = 2.0 mm). Gravel consisted of all material remaining on the 2.0 mm sieve and the pan fraction contained the mud (silt and clay). We did not remove the carbonates because of their relatively minor abundance and detrital nature. Grain size distributions were calculated using the logarithmic method of moments (Folk and Ward, 1957).

Other parameters such as percent slumping and percent vegetation coverage were measured using a box-transect approach. We also counted the number of larvae burrows at each site using this approach, but did not include these numbers in the statistical analysis as a habitat parameter.

Finally, we also measured the beach characteristics adjacent to each bluff face sample site. In particular, we measured beach slope near the high tide line and at the bluff toe; beach width; percent coverage by gravel, shells, woody debris, and heavy minerals; and shoreline orientation at each bluff sampling site.

Data Analysis. The block experimental design consisted of three treatment variables (factors) which included sample site, PTB density, and vertical elevation on the bluff face (strata)



Figure 11. Representative sampling site at Little Cove Point. Samples were taken (3 replicates – within yellow ovals and at yellow arrows) at three different levels, two within the St. Mary's Fm. and one within the Eastover Fm.

and the response variables as indicated above. To eliminate the effect of “site” on determining statistically significant differences between high and low beetle density sites for each of the strata, we tested the null hypothesis that no difference existed between high and low beetle density sites within a particular strata for each variable using the nonparametric paired Wilcoxon Signed Rank Test. The Wilcoxon test assesses the significance of the difference between population distributions of two samples consisting of matched pairs. We used a 95% confidence level ($\alpha = 0.05$) for a one-tailed test.

RESULTS AND DISCUSSION

PTB Abundance and Density at all Sites. The results of the 2007 and 2008 surveys – including those from earlier years – are included in more detail in Part 2 of this report. In short, abundance and densities at most sites increased in 2007 from previous years and continued with a significant increase in 2008.

Potential and Probable Habitat. The areas of total bluff, potential habitat and probable habitat for all PTB sites are shown in Figures 12 and 13 and Tables 1 and 2. Percentage differences of probable and potential habitat were also determined to account for the variation in site size (Figure 14). As indicated above, potential habitat included all unvegetated bluffs while probable habitat consisted only of those unvegetated bluffs with Formations having suitable larval habitat parameters. Examples of the end members of this analysis include West Turner, East Lloyd and Ordinary Point along the Sassafra River where the favorable Mt. Laurel Fm. dominates the composition of the bluff faces and, consequently, little change exists between potential and probable habitat. Given that West Turner, East Lloyd, and Ordinary Point have similar geologic strata (high potential and probable habitat), and that West Turner and East Lloyd have been converted to restoration sites, Ordinary Point may also serve as a suitable habitat restoration site. On the other end of the spectrum, the well exposed, but lithologically unfavorable Calvert Fm. at the Randle Cliffs of the Calvert coast causes a large difference between potential and probable habitat. However, for most areas, the amount (and percent) potential habitat substantially exceeded the amount (and percent) of probable habitat. In addition, the probable habitat was more localized than the potential habitat.

At the Calvert Sites, the Choptank and Eastover Fms. comprised the probable habitat because of the abundance of sand-sized particles within each Formation (See Appendix). The Calvert and St. Mary’s Fm., consisting mostly of clay and silt were considered unsuitable for the beetles. Consequently, because of the greatest exposures of the Calvert Fm. in the north and the regional southerly dip (Figures 5 and 6), the probable habitat increased to the south along Calvert with extreme values of probable habitat at both ends of the study area: No probable habitat was found to the north at Randle Cliffs and the greatest area of probable habitat occurred in the south at State Park, Little Cove Point, and Cliffs of Calvert.

As stated above, the northernmost sites in Calvert County (Randle Cliffs and Bayside Forest) had a relatively large area of potential habitat (i.e., unvegetated bluffs), but were dominated by the Calvert Fm. and therefore, had little to no probable habitat (Figures 12 and 14). Scientists Cliffs and Western Shores included long sections of shoreline. Consequently, these sites contained a large amount of total bluff area, but very little potential habitat because of heavy vegetative cover and little probable habitat because of unsuitable strata.

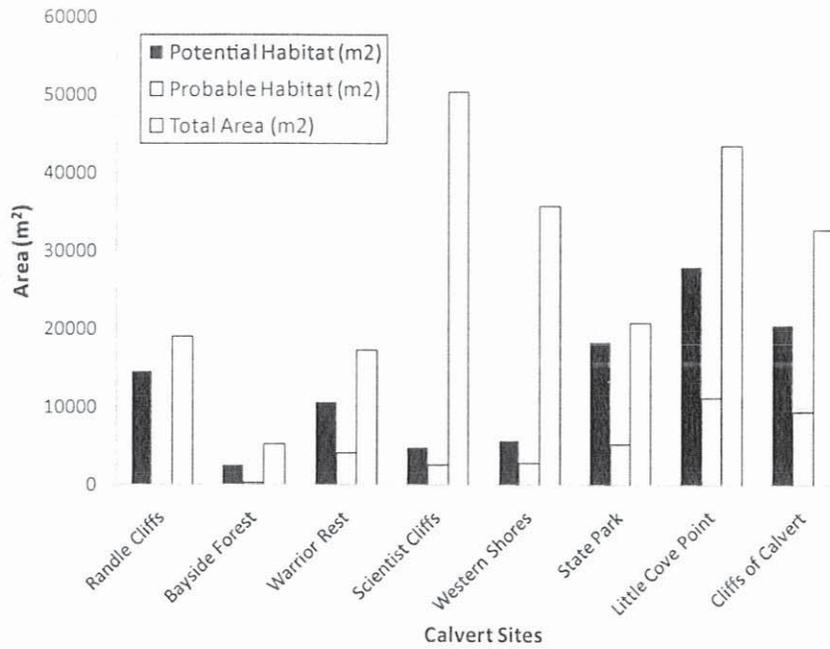


Figure 12. Areas of total bluff, potential habitat and probable habitat at all Calvert County sites.

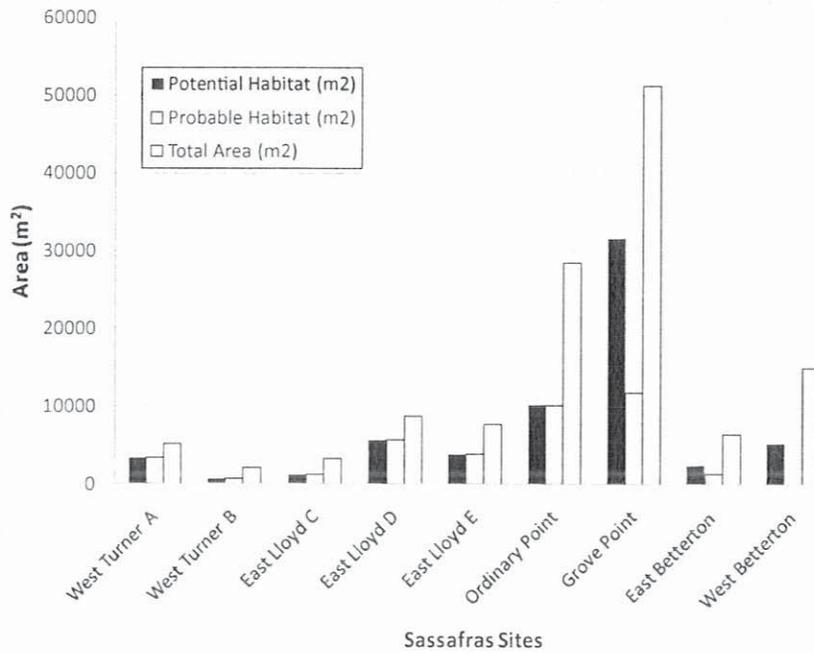


Figure 13. Areas of total bluff, potential habitat and probable habitat at all Sassafras River sites.

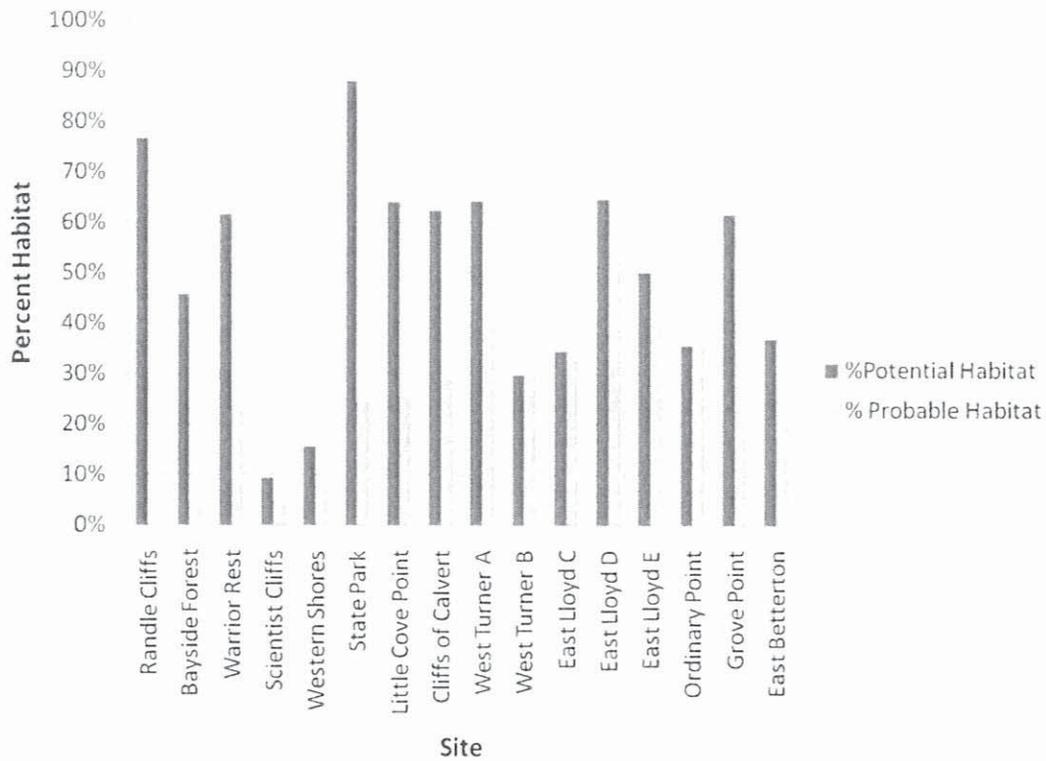


Figure 14. Comparison of potential and probable habitat area in percentage along the bluffs for both the Calvert and Sassafras sites. For all sites except the West Turner, East Lloyd and Ordinary Point sites, the amount of probable habitat is less than the amount of potential habitat. West Turner A and East Lloyd D emerge as having the largest percent probable habitat.

At Scientists Cliffs, a groin field has stabilized the shoreline and encouraged heavy vegetation growth on the bluffs thereby resulting in unsuitable habitat. Warrior Rest is a shorter reach than the adjacent Scientists Cliffs site, but the combination of favorable strata similar to Scientists Cliffs (i.e., the Choptank Formation, Figures 4 and 7), and less vegetation results in greater amounts by area (and percent) of potential and probable habitat. In fact, this site overall had the highest densities of adults despite having a very narrow beach and lithologically unfavorable lower strata (i.e., the Calvert Fm.). Although we could not survey the upper units along this high-bluffed section, the upper stratum (Choptank Fm.) of this site clearly supports a high density of larvae. The State Park site had nearly double the amount of probable habitat compared to any site to the north except for Warriors Rest, but half the amount of probable habitat compared to sites to the south. State Park contained relatively little vegetative cover, most likely due to persistent bluff face erosion, but had less probable habitat than sites to the south because, like Warrior Rest, both favorable habitat of the Choptank Fm. and unfavorable habitat of the St. Mary's Fm. was exposed at this site. Consequently, the State Park may serve as a transition "point" for probable habitat based on lithology and vegetative growth. Little Cove Point and Cliffs of Calvert included a long shoreline reach with abundant bluff area and potential habitat, as well as the largest area of probable habitat of any of the sites. These sites consisted of mostly the St. Mary's Fm. underlying the favorable Eastover Fm. (Figure 4).

As indicated above, the geology of the PTB habitats at the Sassafras sites differs from that of Calvert sites (in age and lithostratigraphy for the purposes of this study). The Sassafras sites – predominantly those to the east within the Sassafras River (e.g., East Lloyd, West Turner, and Ordinary Point)—contain the favorable, sandy Mount Laurel Fm. The Mt. Laurel Fm. overlies the less favorable, predominately fine-grained Marshalltown Fm. (Figure 10). The good exposure of the Mt. Laurel Fm. at these sites (minimal vegetative cover) results in nearly equal amounts of potential and probable habitat giving the East Lloyd site the second highest amount of probable habitat along the Sassafras River (Figure 13).

The Grove Point site contained the greatest amount of potential habitat of all sites studies along both the Calvert and Sassafras shores (Figure 13). Moreover, even though only approximately one-third of Grove Point contained probable habitat, the total area of this probable habitat exceeded every other site within the entire range of the tiger beetle.

The combined adjacent sections of West Turner A and B and East Lloyd C, D, and E included moderate lengths of shoreline and bluff area, but relatively little potential and probable habitat which was limited to several separate sections. Of these sections, West Turner A and East Lloyd D had high amounts of probable habitat, primarily due to a vegetation removal project here which eliminated much of the bluff vegetation in 2006. Two of the adjacent shoreline sections were control sites in the vegetation study (West Turner B and East Lloyd C), were heavily vegetated, and thus had little potential and probable habitat. The remaining site, East Lloyd E), also a control site was short in length but has very limited vegetation growth and favorable strata, thus a relatively large amount of probable habitat. All other sites, except West Betterton are much shorter in length than Grove Point and Ordinary Point, and thus have much less potential and probable habitat. West Betterton is heavily vegetated over much of its length and has very little probable habitat. From bottom (oldest) to top (youngest) this site and East Betterton consist of strata of Englishtown Fm., Marshalltown Fm. and the Mt. Laurel Sand. The Marshalltown Fm. is typically thick-bedded to massive, mottled fine to medium, silty, glauconitic quartz sand (Minard, 1974). The unit is 5-6 m thick in this area. The lower 2 m contains abundant coarse to very coarse gravels up to 2 cm in diameter. The Mount Laurel Fm. is

a thick unit of glauconitic quartz sand (Upper Cretaceous) and may provide the only probable habitat at these sites. During our field work we found considerable slumping of the Mt. Laurel Fm. which may have increased the amount of probable habitat. The Mount Laurel Fm. also dominates most of the bluff face at East Lloyd, West Turner, and Ordinary Point sites thus providing a large amount of probable habitat (Figure 14).

A bay-wide comparison of the Calvert and Sassafras potential and probable habitat indicates that Little Point Cove, Cliffs of Calvert, and Grove Point contain the greatest amounts (by area) of probable habitat. The 10,000 m² of probable habitat at these sites nearly doubles the probable habitat at any other site within the Bay (Figure 14). State Park in Calvert and E. Lloyd D on the Sassafras contain the next greatest amount of probable habitat, followed by E. Lloyd E, West Turner A, Warrior's Rest, Scientist's Cliffs, and Western Shores. As stated earlier, probable habitat is absent in the northern reach of Calvert (i.e., Randle Cliffs and Bayside Forest).

Adult PTB counts were then compared with the probable habitat found at Calvert and Sassafras sites (Figure 15). Sites were broken down into smaller sections for comparison to account for the probable habitat variability that may occur within larger sites. The low correlation ($R=0.0298$) suggests that additional factors can affect the distribution and abundance of PTBs at a site. For example, some sites with high amounts of probable habitat had relatively small PTB populations, while some areas with low amounts of probable habitat yielded large PTB populations. Vegetative cover and lithostratigraphic characteristics can control the distribution and abundance on regional scale (first order influences), but more localized habitat and microhabitat differences (second order influences) ultimately affect PTB abundance. It is also possible that specific beds within each formation yield more favorable habitats than others.

Bluff Parameters. The analysis of bluff parameters compared vertical sections (low, medium, high) of high and low density areas as response variables because of the variation in geological formations and beds vertically along the bluff face. The nonparametric paired Wilcoxon Signed Rank Test showed that no variable emerged as significantly different when comparing high and low beetle density sites by strata and removing the effect of site (Table 3). Thus, we could not reject the null hypothesis that no difference existed between high and low beetle density sites within a particular strata for each variable. However, when bluffs were compared by adding all strata into the paired analysis (i.e. no vertical delineation and eliminating the effect of site), compaction (140.2 psi; $p=0.010$), mean grain size (0.3 mm; $p=0.029$), and sorting (0.24 mm; $p=0.029$) emerged as the statistically significant (95% confidence level) mean values for all inhabited sites (Table 4). The mean grain size has been shown to be a biologically important habitat parameter to the PTB along the Connecticut River and to other federally-threatened beach-dwelling tiger beetles (Omland, 2002; Fenster and Knisley, 2006; Fenster et al., 2006).

The results of the preferred grain sizes are supported by two additional and independent studies conducted as part of this work. The samples obtained from the bluff face as part of the parameter study (and statistical analysis), the results from the laboratory oviposition experiment, and the field larval study (Table 5) all independently confirmed that beetles prefer fine- to medium-sized sand for burrowing and ovipositing. These results suggest that PTBs prefer first order geologic and biologic conditions and a suite of additional second order (more localized) variables including favorable grain size, sorting, and compaction.

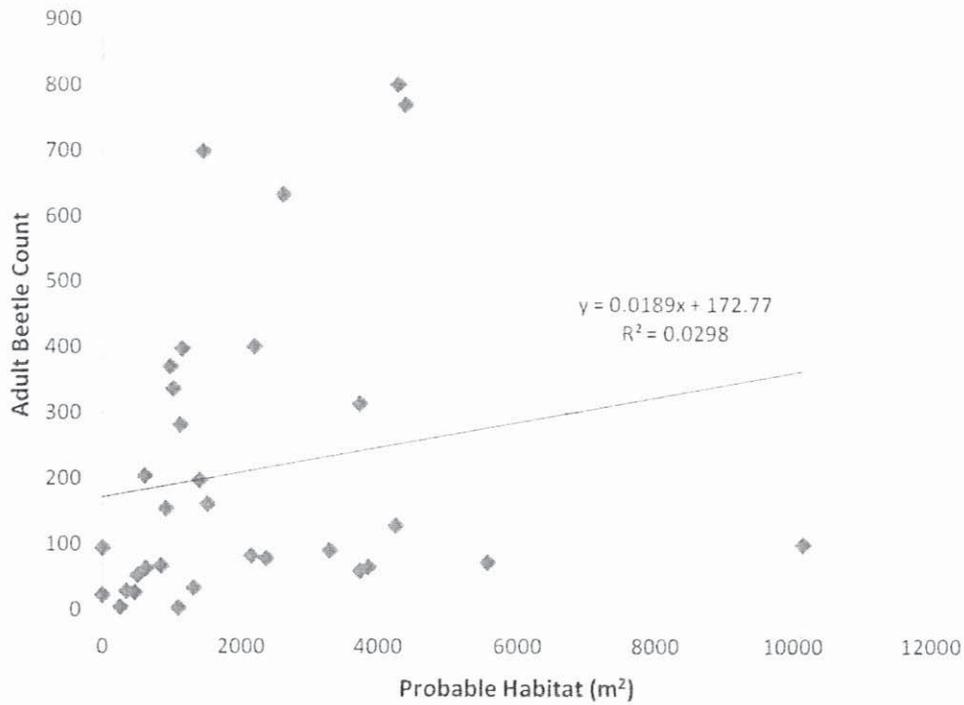


Figure 15. Analysis of the amount of available habitat (in m²) compared to the adult beetle count for individual bluffs within the Calvert and Sassafras sites. The low R value (0.0298) indicates a low correlation between these variables and suggests that other factors affect beetle populations at these sites.

Beach Parameters. Results of the statistical analysis of high versus low density sites (ANOVA, 1-tailed) indicated no significant differences for any parameters: width ($p=0.76$), % beach cover ($p=0.29$), beach cover type ($p=0.64$), %shell and gravel ($p=0.73$), % heavy mineral ($p=0.66$), shoreline orientation ($p=0.64$), slope at toe of bluff ($p=0.47$), slope at shoreline ($p=0.38$), mean grain size (mm) ($p=0.15$) and grain size sorting (mm) ($p=0.46$) (Table 6). The absence of significant differences suggests that beach characteristics do not account for patterns of abundance and distribution of PTBs. This finding is supported by the limited beach width available at the highest density Warrior Rest site. However, a minimal threshold beach set of parameters exists whereby it is likely that beach hardening structures or extensive debris and tree rubble on beach might affect the presence and abundance of adults foraging. Surveys indicate that adult PTBs need the beaches for hunting and therefore, could not exist in areas where a beach does not exist.

Larval Surveys. The results of these surveys indicated the presence of both *C. repanda* and *C. puritana* at West Turner A, B, D, E, Grove Point and at Calvert Cliffs State Park, but only *C. repanda* at West Betterton and West Turner C (Tables 5,7). These latter two sites had small adult numbers of *C. puritana* during the summer surveys apparently because of low quality larval habitat (dense vegetation and unsuitable geological parameters). Larvae of both species were common and at high densities along much of the W. Turner A site and East Lloyd E. Densities of *C. repanda* were as high as 20-30/m² during July in some patches at these two sites and as high as 18-22/m² for *C. puritana* in some patches in October. Both species were common but less dense at other sites with frequent co-occurrence in the same or nearby habitat patches. Despite a very large population of adult *C. puritana* at Calvert Cliffs State Park, very few larvae were found and none of the lower accessible strata seemed to be suitable as larval habitat. This situation was similar to that at Warrior Rest where adults were extremely dense but larval habitat was apparently restricted to the bluff top strata.

The results of grain size analysis indicated *C. puritana* were present in sediments with a higher percent of medium and fine sand and a more narrow range than *C. repanda* which was present in the same patches of sediment, but also in sediments that were much higher in clay and in coarse sand (Table 5). This may explain, in part, why *C. repanda*'s presence in a great variety of habitats over a wide geographic area while *C. puritana* is so limited in its distribution and abundance. Although these two species do overlap significantly in their larval distributions they do have different seasonal periods of activity and are at least partially temporally segregated. Adults of *C. repanda* emerge in late March to April and continue activity into June and July. First instar larvae first appear in April to May and progress to third instars which are at peak abundance in July when adults of *C. puritana* are most abundant and seeking oviposition sites. Consequently, females of *C. puritana* could be deterred or even preyed upon by these high density patches of third instar *C. repanda* as they move up the bluff face to find oviposition sites. Eggs of *C. puritana* that emerge as first instars in these same patches would compete likely use the same prey items as *C. repanda* during their early development, and because of their much smaller size would likely be at a significant competitive disadvantage. High densities of *C. puritana* larvae on these bluffs could also reduce food availability to adult *C. puritana* when they are on the bluffs to oviposit. These important and unexpected results of the larval surveys suggest that competition with *C. repanda* may be a significant limiting factor for populations of *C. puritana*, at least at some sites.

Vegetation Comparison, 2000 and 2007. The photographic analysis of sites for the years 2000 to 2007 show an increase in total potential habitat (= decrease in vegetation) for all sites except Western Shores, Scientists Cliffs, and Warrior Rest (Table 1). Although error exists in these determinations, the increase in vegetation at Western Shores and Scientists Cliffs is consistent with on ground surveys at these sites. The changes in vegetation that occurred over this time period corresponded with the bluff/colluvium erosion caused by Hurricane (Tropical Storm) Isabel in October 2003 and may explain changes in PTB numbers (discussed in more detail below). Observations indicated that many of the PTB sites experienced significant shoreline and bluff erosion and elimination of vegetation from the hurricane, so it is reasonable to expect that these impacts had a positive effect on PTB habitat and beetle numbers. Despite their aperiodic and episodic frequency, the cumulative effect of many storms and/or a large magnitude storm can create fresh (exposed) bluff habitat through wave-undercutting and slumping to expose elevated beds, or to remove colluvium covering potential and probable habitat behind a slump (discussed in more detail below; Leatherman, 1986; Wilcock et al. 1998; Clark et al., 2004).

Interpretations of possible effects would need to consider the two-year life cycle of the PTB and thus a several year delay in responding to habitat changes. Numbers at most Calvert sites were low around 2000 and remained low for several years before the trend increased from 2006 to 2008, especially at the State Park, Little Cove, and Cliffs of Calvert sites which experienced a significant increase in habitat during this period. (See Appendix D).

SUMMARY AND CONCLUSIONS

What Determines Habitat of the Puritan Tiger Beetle and How do we Account for Year-to-Year Fluctuations in Abundance? The results from this study suggest that the year-to-year fluctuations (variations in spatial and temporal population trends) in PTB abundance relate to bluff face *quality* where both the antecedent geology of and the dynamics operating at the bluff face determine quality. The potential/probable habitat analysis indicates that the PTB prefer fresh (i.e., newly exposed) and vegetation-free exposures of suitable geologic material (fine to medium, moderately-sorted, well-compacted sand) for burrowing and overpositing. The other geologic/biologic parameters tested, but not deemed statistically or biologically significant, included temperature, moisture, conductivity, slope, and color. Fresh bluff face surfaces result most often from cumulative and/or aperiodic processes acting over various time scales at both the bluff toe and higher on the bluff face. Within site variability of preferred habitat arises from vertical variations in geologic bedding along the bluff face. At some sites, the favorable beds occurred at the top of the bluff, while at other sites, favorable conditions existed lower on the bluff and closer to the beach. Additionally, none of the beach parameters tested, including beach width, slope, grain size, and percent and type of cover emerged as statistically significant. With respect to beaches, it appears that only the presence or absence of beaches influences PTB abundance.

Several studies have documented the processes responsible for bluff erosion in the Chesapeake Bay (Leatherman, 1986; Wilcock et al., 1998; Clark et al., 2004). These studies have shown that both oversteepening caused by wave activity at the bluff toe, and/or freeze-thaw action, and/or rotational slumping caused by groundwater infiltration and flow along the upper surface of an aquiclude (impermeable) layer can cause erosion of the bluff face (Figure 16). The

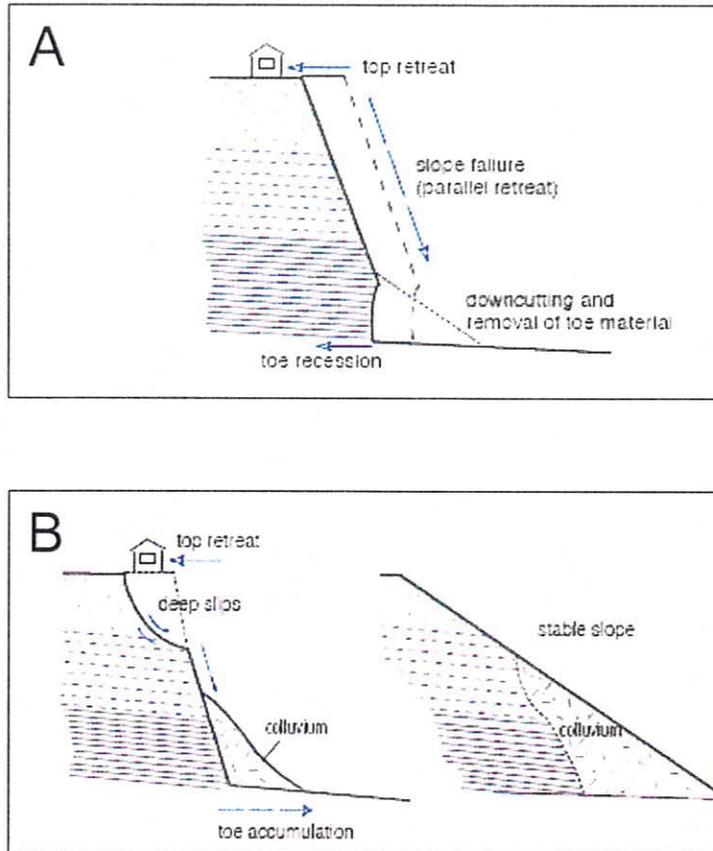


Figure 16. Bluff erosion processes and bluff stabilization through weathering, slumping, and consequent vegetative growth (from Clark et al., 2004). A. Wave action at the bluff toe erodes intact material on actively eroding bluffs. Slope failure occurs through top retreat, translational slides, and colluvial fan deposition. B. Continued slumping results in stable bluffs with slopes near the angle of repose.

ensuing erosion of the bluff face produces deposition on the beach and bayward of the bluff toe of loosely consolidated colluvium (a cone of detritus or colluvial fan). These deposits then become sites of vegetation growth and incursion. Given the (often) large volume of material contained in these deposits and the ensuing vegetative growth, the bluff stabilizes until that material is removed and redistributed to adjacent beaches and the nearshore. The main process responsible for removal of the colluvium is wave activity – especially large waves associated with tropical and extratropical storms (more specifically, the magnitude and frequency of storms). Clark et al. (2004) estimated that the timescale involved in slope stabilization is on the order of decades (i.e., 40 yr on average). This result indicates that the fresh bluff face to fresh bluff face erosion cycle (caused by bluff face erosion and ensuing removal of debris) is a long-term (decadal to possibly centennial scale) process (Figure 17).

Given sufficient time, bluff face erosion and colluvium deposition would reduce the pre-existing bluff face slope to the angle of repose ranging from 25° - 37° and averaging $\approx 31^{\circ}$ (Figure 16; Clark et al., 2004). Also, given that the PTB burrows are most often found on fresh bluff face surfaces with a slope of 65° , on average (range = 46° - 90°), bluff face erosion, colluvium deposition at the bluff toe, vegetation growth, and slope reduction decrease probable habitat.

Wilcock et al. (1998) showed that cumulative wave energy does not necessarily correlate with locations that experience the largest rates of slope recession. Instead, Wilcock et al. (1998) developed an index of relative wave strength (T/S), which is a function of both wave pressure (T) and the cohesive strength of the antecedent bluff material (S), to predict the wave strength required to erode intact material. A cumulative duration of ≥ 50 hr per year of a T/S index of 0.1 is a threshold for undercut and nonundercut slopes. Given that Calvert Cliffs shows a T/S of 0.05 – 0.1, for example, these bluffs would erode at durations less than 50 hr per year. While Wilcock et al. (1998) used this index to identify sites at risk to erosion, this parameter could also be used to predict beetle abundance (or probable habitat availability) and possibly, to identify potential restoration sites. Wilcock et al. (1998) address cumulative processes and the nature of the bluff material; however, they did not discuss the impact of short-term (episodic), large magnitude events. In particular, single, large magnitude tropical or extratropical storms may accelerate the bluff erosion cycle by relatively fast removal and dispersal of the sediment within a colluvium fan. Thus, creating fresh bluff exposures by removal of the colluvium fan can occur through longer-term, cumulative processes or shorter-term and episodic large magnitude events.

The recent passage and impact of Hurricane Isabel through Maryland in 2003 may demonstrate the impact of large magnitude events on bluff and habitat exposures, and of a process that results in rapid colluvium erosion and dispersion. While Hennessee and Halka (<http://www.mgs.md.gov/coastal/isabel/index.html>) discussed the irregular erosion that occurred throughout the Bay following Hurricane Isabel (a tropical storm when reaching Maryland; Figure 18), this large storm may explain the causes of post-Isabel PTB abundance increases (and reversal from a population decline from the late 1980s to 2003). In particular, the decline in PTB numbers during the late 1980s to 2003 may have resulted from limited storm activity (frequent and/or large magnitude storms), continued bluff erosion and slumping, and ensuing bluff stabilization (Stages 2 and 3 of the bluff erosion cycle; Figures 16 and 17). In contrast, the subsequent increase in PTB abundance at both metapopulation sites in the Chesapeake Bay beginning in 2006 may be directly linked to the creation of fresh bluff face exposures as a

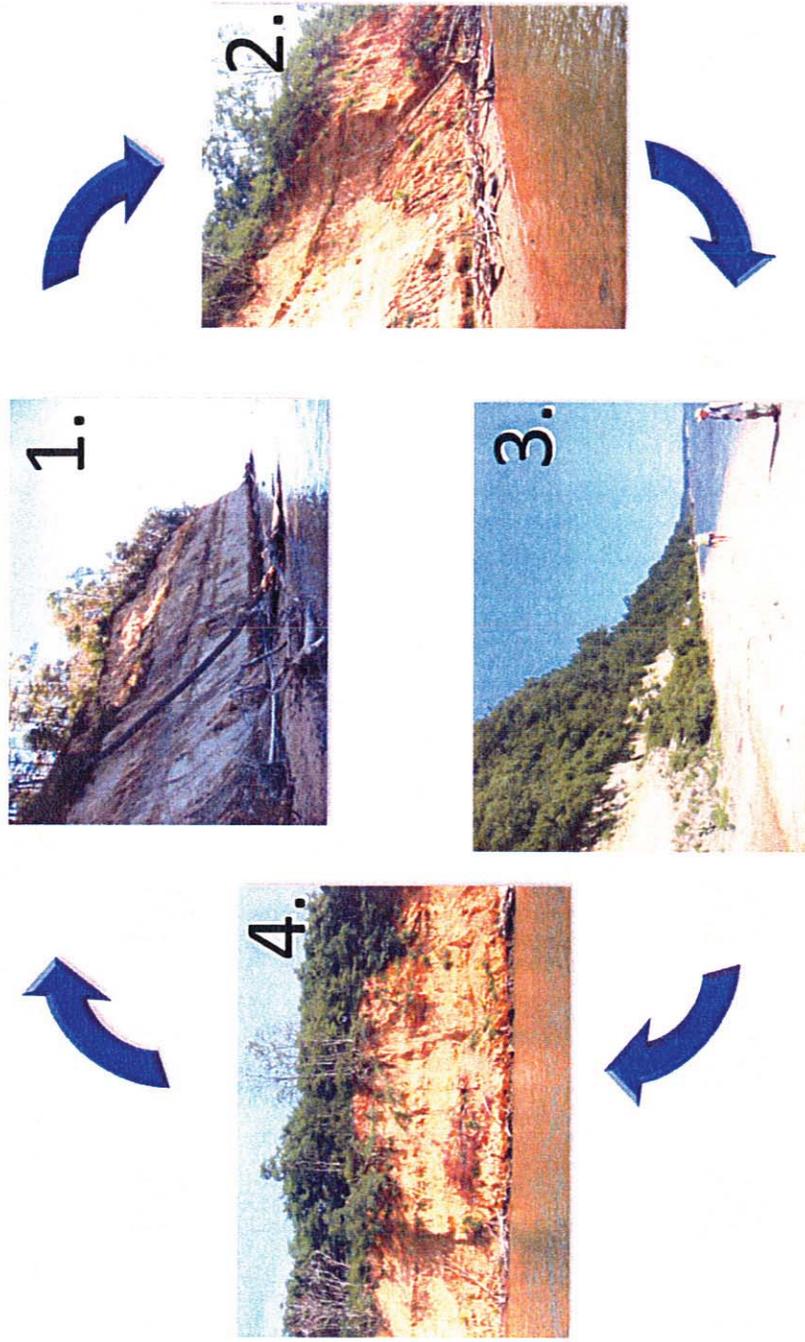


Figure 17. The bluff erosion cycle that shows bluffs in various stages ranging from steep, freshly-exposed bluff faces to low-angle, colluvium covered bluff faces. Stage 1. Fresh (unvegetated, unstable), steep, bluff face exposure; Stage 2. Gradual slumping (translational slides) at the top of the bluff through groundwater seepage, reduced bluff slope, and possible vegetative growth; Stage 3. Continued slumping of the top layers and/or slumping through wave cut activity of intact bluff material and oversteepening of the bluff face producing a stable colluvium fan at the bluff toe with slopes at angle of repose and that encourages vegetative growth; Stage 4. Erosion and dispersal of colluvium fan; Stage 5 = Stage 1 (back to beginning)

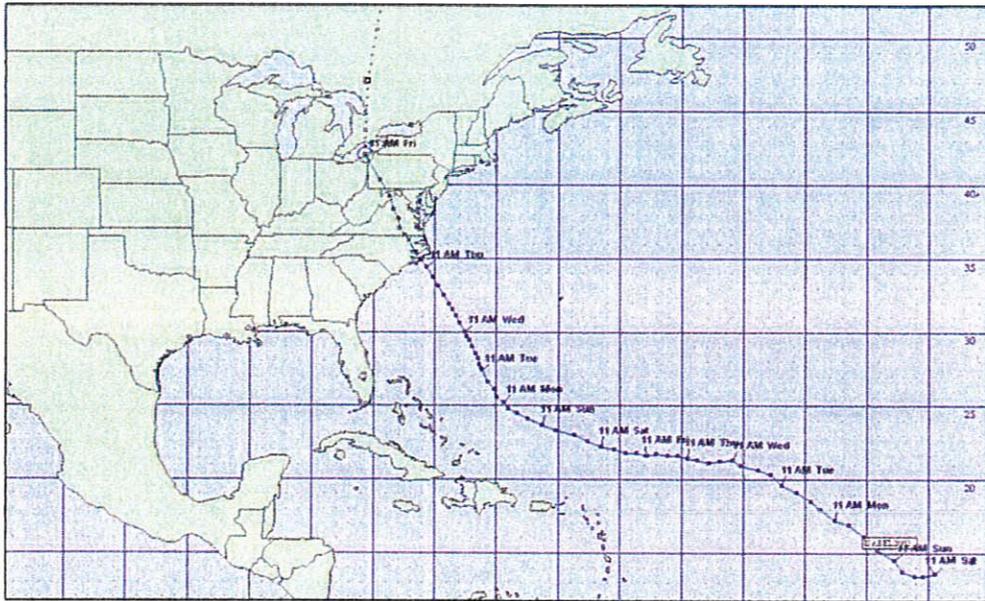


Figure 18. Storm track of Hurricane Isabel September 18-20, 2003. Note the eye passing to the west of the Chesapeake Bay through Virginia and Maryland (source: National Weather Service, Eastern Region Headquarters: http://www.erh.noaa.gov/er/akq/wx_events/hur/isabeltrack.jpg)

consequence of Isabel. Given that little or no PTB recruitment would have occurred during the spring and summer seasons immediately following the passage of Isabel, the creation of new bluff face exposures for the 2004 PTB adult population, and the two-year life PTB life cycle, we would expect to find the PTB numbers to increase beginning in 2006. The PTB count data support the hypothesis that a three year lag existed between the storm passage and a recovery of the population.

To summarize, multiple parameters affect the distribution and abundance of the PTBs. Locally, within each site, PTBs select the best available habitat consisting of fine- to medium-grained, moderately-sorted sand of moderate compaction. Given that these parameters can exist in beds of different formations, the PTBs have habitat available at two sites along Maryland's Chesapeake Bay coast but separated by approximately 120 km in distance and geologic environments that were deposited 45-85 million years apart. At both sites, the PTB prefer fresh, steep, unvegetated bluff exposures containing fine- to medium-, moderately-sorted, and well-compacted sand for burrowing and ovipositing. The natural processes that produce these exposures occur in a cyclical manner over a period of decades. At any given time, a bluff will be in one state (phase) of the bluff erosion cycle. The dynamics associated with this cycle –both cumulative and episodic – create Bay-wide differences in the states of bluffs around the Bay. The PTB apparently locates to bluffs where phases of this cycle and the antecedent geology are favorable to the survivability of this species.

Finally, Leatherman (1986) and Clark et al. (2004) suggest that planting vegetation will not stabilize slopes. While the presence of vegetation does correlate to bluff stability (Mickelson et al., 1977), vegetated slopes were not found to prevent slope failure along Calvert Cliffs and other bluffed areas that erode through groundwater seeping. This scenario suggests that a conservation strategy to remove vegetation will accelerate the process of fresh bluff face creation. Finally, Leatherman (1986) and Clark et al. (2004) claim that planting vegetation can exacerbate bluff erosion in areas where groundwater seeping is active.

Future studies should pursue a more accurate representation of beetle density by comparing habitat with larvae densities (as opposed to adults). However, the inaccessibility of many of the bluff faces at the Calvert and Sassafras sites makes a collection of such data difficult. Predator and prey abundance could also be a controlling factor of the PTB populations. More research is needed to understand the distribution of larvae within the bluff faces of Calvert County and Sassafras. In addition, little is known about the competition between the PTB and *C. repanda*. Finally, future studies should quantify the tropical and extratropical storm climate history of the upper Chesapeake Bay and relate those data to historical PTB distribution and abundance trends.

RECOMMENDATIONS FOR *C. PURITANA* MANAGEMENT AND RECOVERY IN MARYLAND

These recommendations are based in large part on this study with additional insights provided by other recent studies. They are not intended to replace strategies identified in the Recovery Plan, but rather update and supplement the recommendations in the Plan. Options for management and recovery are greatly limited because of the reduced range of the PTB and the private ownership of many sites, including some with the largest populations. Our recommendations are as follows:

1. Protect as many sites with existing populations as possible, either through purchase or landowner agreements.

2. Implement vegetation control at sites where there is evidence (much provided in this study) that vegetation encroachment is negatively affecting habitat and beetle population size. Notable sites in this category include the remaining bluff sections at West Turner and East Lloyd where vegetation control was not implemented, Ordinary Point, North Stillpond, Calvert Beach/Western Shores, and Scientist Cliffs. Several sites currently support large populations but also have vegetation encroachment in some of the most favorable strata and might support increased beetle populations. These sites are Warrior Rest, Little Cove Point, Cliffs of Calvert, and Grove Point.

3. Prevent or restrict the use of shoreline protection structures (groins, revetments, rip rap) or other modifications that result in increased growth of vegetation growth.

4. Investigate the role of competition by *C. repanda* on the *C. puritana*, and if appropriate develop an experimental study to reduce numbers of *C. repanda* at one or several sites.

5. Continue annual monitoring of beetle populations at all sites and conduct photographic studies at 2-3 year intervals to monitor bluff vegetation and erosion changes.

6. Quantify the tropical and extratropical storm climate history of the upper Chesapeake Bay and relate these data to historical *C. puritana* distribution and abundance trends.

Table 1. The change in vegetation and potential habitat for Calvert sites from 2000 to 2007.

Site	% Vegetation	%Potential Habitat	Vegetation (m ²)	Potential Habitat (m ²)
Cliffs of Calvert				
Section 3 2007	36%	64%	4766.2	8571.4
Section 3 2000	37%	63%	4974.0	8363.6
Little Cove Point				
Section 1 2007	30%	70%	4215.5	9687.5
Section 1 2000	33%	67%	4531.6	9371.4
Section 3 2007	80%	20%	4806.4	1191.4
Section 3 2000	87%	13%	5246.4	751.4
Section 5 2007	72%	28%	6644.4	2571.9
Section 5 2000	67%	33%	6207.0	3009.3
Western Shores				
Section 3 2007	90%	10%	11690.0	1357.0
Section 3 2000	83%	17%	10798.3	2248.8
State Park				
2007	12%	88%	2476.1	18293.6
2000	16%	84%	3260.0	17509.7
Scientist Cliffs				
2007	91%	9%	45670.8	4733.2
2000	85%	15%	42968.0	7436.0
Warrior Rest				
2007	38%	62%	6590.8	10602.8
2000	36%	64%	5750.7	11443.0

Table 2. Probable and potential habitat determined at all Calvert and Sassafras Sites (in % and m²). Total adult PTB counts also included for each site.

Site	% Vegetation	%Potential Habitat	% Probable Habitat	Vegetation (m ²)	Potential Habitat (m ²)	Probable Habitat (m ²)	Beetle Count
Randle Cliffs	23%	77%	0%	4416.9	14554.7	0.0	23
Bayside Forest	54%	46%	5%	2800.2	2377.0	255.5	5
Warrior Rest	38%	62%	24%	6590.8	10602.8	4043.2	1022
Scientist Cliffs	91%	9%	5%	45670.8	4733.2	2479.8	1225
Western Shores	84%	16%	8%	30173.5	5631.1	2789.4	685
State Park	12%	88%	25%	2476.1	18293.6	5146.1	1609
Little Cove Point	36%	64%	26%	15620.2	27934.5	11156.0	1116
Cliffs of Calvert	38%	62%	29%	12304.6	20412.1	9450.0	829
West Turner A	36%	64%	64%	1827.5	3292.6	3292.6	91
West Turner B	70%	30%	30%	1419.9	605.1	605.1	205
East Lloyd C	65%	35%	35%	2059.6	1090.1	1090.1	4
East Lloyd D	35%	65%	65%	3044.6	5575.2	5575.2	73
East Lloyd E	50%	50%	50%	3815.6	3855.2	3855.2	66
Ordinary Point	64%	36%	36%	18350.6	10148.5	10148.5	100
Grove Point	38%	62%	23%	19635.6	31653.4	11696.5	986
East Betterton	63%	37%	21%	4027.6	2368.8	1314.7	34
West Betterton	65%	35%	undetermined	9605.7	5277.8	undetermined	92

Table 3. Nonparametric paired Wilcoxon Signed Rank Test eliminating the effect of site on determining the significantly different variables between high and low beetle density sites for each strata. The terms “Low Strata,” “Medium Strata,” and “High Strata” in the table refer to vertical location (elevation) on the bluff face. A 95% confidence level ($\alpha = 0.05$) was used for a one-tailed test. No parameters emerged as statistically significant.

Strata	Variable	Significance (p-value)
Low Strata		
	Moisture	0.500
	Conductivity	0.250
	Temperature	0.125
	Compaction	0.406
	Slope	0.219
	Mean Grain Size	0.125
	Sorting	0.375
	Percent Sand/Mud	0.500
Medium Strata		
	Moisture	0.109
	Conductivity	0.188
	Temperature	0.344
	Compaction	0.055
	Slope	0.500
	Mean Grain Size	0.186
	Sorting	0.186
	Percent Sand/Mud	0.500
High Strata		
	Moisture	0.219
	Conductivity	0.500
	Temperature	0.281
	Compaction	0.078
	Slope	0.078
	Mean Grain Size	0.109
	Sorting	0.078
	Percent Sand/Mud	no data

Table 4. Nonparametric paired Wilcoxon Signed Rank Test eliminating the effect of site on determining statistically significant differences between high and low beetle densities (combined strata). A 95% confidence level ($\alpha = 0.05$) was used for a one-tailed test.

Variable	Significance (p-value)	High Density Mean	Low Density Mean
Moisture	0.058	16.2% VWC	16.6% VWC
Conductivity	0.183	0.3 μ S	0.2 μ S
Temperature	0.330	27.6°	25.6°
Compaction	0.010	140.2 psi	162.2 psi
Slope	0.351	64.1°	63.3°
Mean Grain Size	0.029	0.30 mm	0.50 mm
Sorting	0.029	0.24 mm	0.47 mm
Percent Sand/Mud	0.250	27.4%	23.2%

Table 5. Means and ranges of grain size for sediment samples where one or both *C. puritana* and *C. repanda* larvae were present. Grain sizes as follows: Coarse, > 0.50 mm; Medium, 0.250; Fine, 0.125; Clay, < 0.063 (numbers in percent).

	No. of Samples	Mean % Coarse	Range % Coarse	Mean % Medium	Range % Medium	Mean % Fine	Range % Fine	Mean % Clay	Range % Clay
<i>C. puritana</i>	17	22	4 - 64	18	11 - 66	36	7 - 61	5	0 - 15
<i>C. repanda</i>	22	26	4 - 86	26	6 - 54	26	6 - 62	22	1 - 71
Both species	9	23	4 - 64	39	14 - 48	35	7 - 62	3	0-15

Table 6. Means, standard deviations and p-values from high and low density beetle sites for beach parameters (1-tailed ANOVA at 95% C.I.)

Variable	Density	Mean	Standard Deviation	Significance (p-value)
Width	High	5.01	0.89	0.762
	Low	4.76	1.57	
Beach Cover (%)	High	25.22	12.66	0.294
	Low	36.88	22.19	
Beach Cover Type	High	2.63	1.60	0.646
	Low	3.13	1.96	
% Shell and Gravel	High	11.61	4.83	0.738
	Low	12.63	9.46	
% Heavy Mineral	High	1.28	1.44	0.667
	Low	1.63	2.20	
Shoreline Orientation	High	277.50	109.42	0.644
	Low	215.14	147.41	
Slope at Toe of Bluff	High	11.56	3.00	0.474
	Low	10.13	3.68	
Slope at Shoreline	High	7.56	2.13	0.384
	Low	8.63	2.92	
Mean Grain Size (mm)	High	0.44	0.21	0.153
	Low	0.60	0.45	
Sorting (mm)	High	0.31	0.23	0.466
	Low	0.41	0.32	

Table 7. Larval survey results for *C. puritana* and *C. repanda* at selected Calvert and Sassafras sites, including larval densities and percents of sediment grain sizes where sampled. Larval densities are means of 2-5 1 sq. m patches in the same locations.

Site	WyPt	Strata	Coordinates	C. pur.	Density	C. rep.	Density	coarse	medium	fine,vfi	clay
SASSAFRAS SITES											
W. Turner A	9	low face	18 S 413963 4358223	y	7	y	14				
W. Turner A	5	low slump	18 S 414006 4358266	y	15	y	26	5	36	56	3
W. Turner A	3	low face	18 S 414022 4358286	y	4	y	19	4	32	62	2
W. Turner A	1	mid face	18 S 414021 4358282	y	7	y	8	21	46	27	6
W. Turner A	3	low face	18 S 414022 4358286	y	3	y	1	30	48	21	1
W. Turner A	1	mid face	18 S 414021 4358282	y	1	y	14	64	14	22	0
W. Turner A	2	low face	18 S 414035 4358300	y	11	y	9	24	54	20	2
W. Turner A	13	mid slump	18 S 413932 4358191	y	36	y	44				
W. Turner A	15	low face	18 S 413914 4358177	y	7	y	18				
W. Turner A	17	upper slump	18 S 413896 4358162	y	20						
W. Turner B	23	mid face	18 S 413583 4358004	y	7	y	7	23	55	18	4
W. Turner B	26	low shelf,sandy	18 S 413535 4357967			y	4				
W. Turner B	28	low soft face	18 S 413526 4357960	y	6						
E. Turner C		low face	18 S 412821 4357867			y	4				
E. Lloyd D		low face	18 S 412698 4357830			y	3				
E. Lloyd D	443	low face	18 S 412675 4357794	y	5						
E. Lloyd D	450	low face	18 S 412518 4357692			y	3	20	52	27	1
E. Lloyd D	444	low face	18 S 412658 4357776	y	16			17	39	43	1
E. Lloyd D	202	low lens	18 S 412485 4357667			y	6				
E. Lloyd E	458	20' face	18 S 411971 4357429	y	17	y	5	4	33	61	2
E. Lloyd E	457	low face	18 S 411978 4357435	y	10						
Grove	59	mid face	18 S 410386 4360483								
Grove	417	mid slump	18 S 410341 4360796	y	8	y	11	29	55	16	0
Grove	450	mid face	18 S 412518 4357692					34	42	24	0
Grove	447	low sandy slump	18 S 412631 4357756	y	16						
Grove	454	low face, lens	18 S 410346 4360581	y	35	y	12	33	45	7	15
Grove	454	mid face	18 S 410346 4360581			y	2	45	47	8	0
W Betterton	440	mid slump	18 S 407448 4358537			y	3	72	22	6	0
W Betterton	441	low slump	18 S 407403 4358525					2	9	3	86
W Betterton	440	mid slump	18 S 407448 4358537			y	1	69	25	6	0
CALVERT SITES											
CC St. Park	207-1	low face	18 S 377335 4250862			y	2	8	8	16	71
CC St. Park	208	top strata	18 S 377231 4251147	y	6			26	66	8	0
CC St. Park	211	low face	18 S 377191 4251181			y	3	11	12	6	71
CC St. Park	215	low face	18 S 377155 4251245					13	9	8	70
CC St. Park	211	low face	18 S 377191 4251181			y	11	12	21	62	5
CC St. Park	207-2	low slump	18 S 377335 4250862			y	6	8	9	3	80
CC St. Park	222	mid face	18 S 376637 4252044			y	2	6	7	32	55
CC St. Park	264	mid face	18 S 376575 4252210	y	14						
Calv. Beach	225	mid slump	18 S 370734 4259272			y	1	4	11	27	58
Calv. Beach	238	low slump	18 S 370602 4259411			y	9	41	20	26	13
Calv. Beach	229-1	mid face	18 S 370560 4259425			y	2	86	6	8	0
Calv. Beach	79	mid-upper face	18 S 370552 4259487	y	8			18	44	28	10
Little Cove	2	mid face	18 S 378700 4247268			y	3	10	21	14	55
Little Cove	3	low slump	18 S 378659 4247475	y	6			37	30	24	9
Little Cove	6	mid slump	18 S 378657 4247499	y	11			15	15	60	10
Little Cove	6	mid slump	18 S 378657 4247499	y	2			15	15	60	10
Little Cove	7	mid face	18 S 378700 4247268					62	12	25	1
Little Cove	8	low face	18 S 378692 4247286			y	3	21	10	11	58
Little Cove	1	low face	18 S 378662 4247462			y	6	9	11	9	71
Little Cove	4	low slump	18 S 378659 4247475	y	9			22	11	61	6
Little Cove	5	low slump	18 S 378659 4247475	y	2			8	41	45	6

Part 2

PART 2: ANNUAL POPULATION ESTIMATES OF *C. PURITANA* AT ALL MARYLAND SITES

INTRODUCTION

Index counts have been consistently used as a quick, cost-effective method for estimating population size in tiger beetle populations. This method has been used for *C. puritana* and *C. d. dorsalis* at all survey sites since the mid-1980's. Although this method has been relatively consistently applied over the years and provides a good comparison among sites and years, it provides only a relative index of abundance and does not produce an accurate measure of actual population size. Index counts may also be affected by climatic conditions, time of day, surveyor, and seasonality of the beetle population (which varies from year-to-year). Studies with *C. dorsalis* and several other tiger beetle species have demonstrated that index counts may underestimate actual beetle numbers 2- to 3-fold. Consequently, it is important to obtain a better understanding of the causes of variations and how index count estimates compare with the true population size.

The 2008 PTB population estimates were conducted as a part of annual monitoring of Maryland *C. puritana* sites supported by MD DNR and the results below are taken from the 2008 report to Maryland DNR. Annual surveys for *C. puritana*, using similar methods, have been conducted at all Maryland sites since 1988, and are among the longest term monitoring of population size for any insect species. The 2008 surveys, as those in all previous years were to determine the distribution and abundance of the *C. puritana* at all sites for the two metapopulations. In all years, we have used the same methods by conducting surveys during peak season when daily weather conditions were ideal (sunny and/or warm). This consistent approach has allowed for valid comparisons of numbers over the years and among sites. Earlier studies showed that peak abundance is typically from the last week in June through mid-July.

METHODS

Evaluation of Index Counts. Results of three data sets from different studies were used to evaluate the accuracy of the index counts. The first data set was a comparison of multiple index counts at the West Turner and East Lloyd sites as a part of a vegetation removal study (2005-2008). The same visual index count method as describe below was used, but by different workers and during different times during the day. The second study was a comparison of estimates from index counts versus a removal method at the southern section of Scientists Cliffs in 2006. At this site we first conducted three separate index counts within a three hour period on the same date. This was followed by a removal method in which we moved along the same route as the index count and collected all adults seen. These were placed in individual plastic values and put into a cooler. A second and then a third pass were then made collecting all adults seen in each of these passes. The numbers from the three removal passes were used to calculate a populate estimate with Program Capture. The third study was a more comprehensive comparison of index counts, removal method and mark-recapture methods for populations of *Cicindela dorsalis* at several Virginia sites in 2008. Although this is a different species, we are confident that the results are at least generally applicable to *C. puritana*.

Annual Population Surveys. In 2008, all of the Calvert and Sassafras sites were surveyed between June 25 and July 14, on days that were sunny with temperatures in the mid 80's to low 90's. Surveys were done during low to mid-tide and when the sun was on the beach and cliff base, to provide a high level of activity. The survey method we used, as in previous years, involved one person walking slowly along the shoreline at the water edge and counting all adults that were seen on the ground surface. In areas where there was a narrow beach or cliffs near the water, the base of the cliffs was also examined and beetles there included in the count. In sections of wider beach the surveyor moved more slowly so the back portions of the beach could be surveyed. Since 2004 the counts have been made and reported within the same sections of shoreline and these verified using a GPS unit to reference these specific locations. These locations are shown as numbered waypoints on topo maps included with this report and the adult numbers within these sections shown in Tables 4 and 5 below. Shoreline characteristics were also recorded for each of these sections and included in the report tables. This year we also report adult numbers and resulting densities within more localized sections of the shorelines. These results were used in the habitat analysis (see Part 1). Topographic maps showing these densities are included in the Appendices of this report.

RESULTS AND DISCUSSION

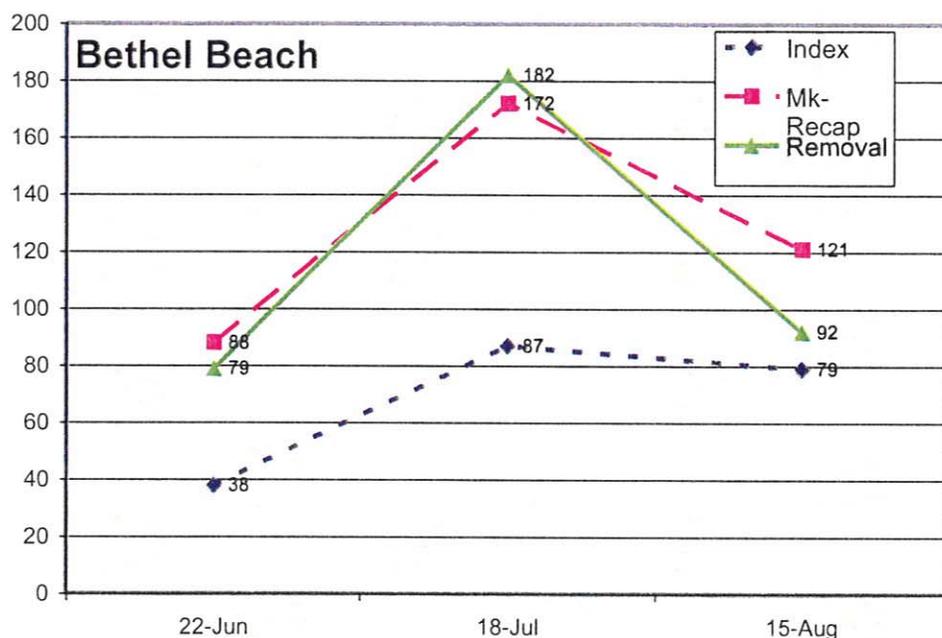
Evaluation of the Index Method to Determine Population Size

Variation in Index Counts at PTB sites. The multiple index counts at 5 separate cliff sections at West Turner and East Lloyd varied significantly in each of the 4 years that counts were compared (Table 1). Multiple counts varied greatly within a year at most sites and in all years. Two- fold differences in estimates were common in most years and differences of more than 3-fold were recorded in some cases. The range and standard deviation of these counts were correspondingly high. All surveyors were experienced in conducting these counts, our results in this study revealed minimal differences among the counts from the three surveyors. Differences in counts from one day versus the following day were also minimal. However, time of day had a very significant effect and accounted for most of the variation. Lowest counts were recorded during times when the beach was shaded; highest counts at a section were found when the beach and cliff base was in full sun. These differences are logically explained by tiger beetle biology. These insects are behavioral thermoregulators which move to sunny patches to obtain ideal body temperatures for their activity, and forage in the sunny water edge.

Table 1. Multiple index counts at 5 shoreline sections at West Turner and East Lloyd sites (5 bluff sections) in 4 years. Counts were made by three different workers at different times during the day.

Year	Survey date	Cliff section					
		A	B	C	D	E	
2005	7/12/2005	49	3	0	8	62	
		38	2	0	8	69	
		13	3	0	5	96	
	Max	49	3	0	8	96	
	Min	13	2	0	5	62	
	Mean	33.3	2.7	0.0	7.0	75.7	
	SD	18.4	0.6	0.0	1.7	18.0	
2006	7/11/2006	106	66	0	36	491	
		7/12/2006	61	35	1	47	446
		272	35	0	63	430	
		83	24	1	46	533	
		197	29		69	680	
		341	36		84	713	
	Max	341	66	1	84	713	
Min	61	24	0	36	430		
Mean	176.7	37.5	0.5	57.5	548.8		
SD	112.8	14.7	0.6	17.7	120.4		
n	6	6	4	6	6		
2007	7/9/2007	173	45	0	60	243	
		7/10/2007	187	29	0	54	330
		146	34	0	56	334	
		165	24	4	27	161	
		186	18	1	31	175	
		181	19	2	40	203	
	Max	187	45	4	60	334	
Min	146	18	0	27	161		
Mean	173.0	28.2	1.2	44.7	241.0		
SD	15.6	10.2	1.6	13.9	75.9		
n	6	6	6	6	6		
2008	7/9/2008	107	10	6	73	53	
		99	31	6	66	48	
		202	71	5	50	102	
		226	67	2	59	74	
		Max	226	71	6	73	102
		Min	99	10	2	50	48
	Mean	158.5	44.8	4.8	62.0	69.3	
SD	64.9	29.3	1.9	9.8	24.6		
n	4	4	4	4	4		

Figure 1. Comparative results of population estimates using 3 methods at one *C. dorsalis* site in Virginia, 2008.



Despite the problem of underestimation from index counts, this method is likely to remain the primary method for monitoring of *C. puritana* populations because available funding for surveys is often limited, and index counts are faster and thus less expensive. Even large sites can be surveyed by index counts in an hour or two while with the removal and mark-recapture methods, small to medium sites would typically require 2-6 hours with two-three people participating. If index counts are used for monitoring populations of the PTB, these counts will need to be adjusted to reflect the true population size, especially when this data is used to make decisions and policy about recovery and management.

Population Estimates at All Maryland Sites

Summary of Trends in Calvert C. puritana. The total number of adult *C. puritana* at all Calvert sites in 2008 was 5721 (Fig. 2, Table 2). This count represents a significant increase from 2625 in 2007 and importantly the highest count since 1998 when 9185 was counted and the other recent highest count of 3946 in 2006. Except for this high count in 2006, all other recent years (2002-2005) had counts of less than 2100 adults. The 2008 results suggest a general pattern of significant increase since 1999 and also confirm the pattern of alternate year abundance since 1996, with even years having consistently higher numbers than odd years. This is expected because of the two year life cycle of this species. The significant increase in 2008 is primarily a result of major increase in abundances at most major sites: Calvert Cliffs State Park (1609 in 2008, 292 in 2007), Little Cove Point (1116 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 minimal changes were recorded at the other sites. These year to year fluctuations over the years have been common, and reflect variations in recruitment, possibly tied to changes in habitat conditions or climatic factors.

Table 2. Total index counts for C. puritana at all Calvert County sites, 1986 to 2008.

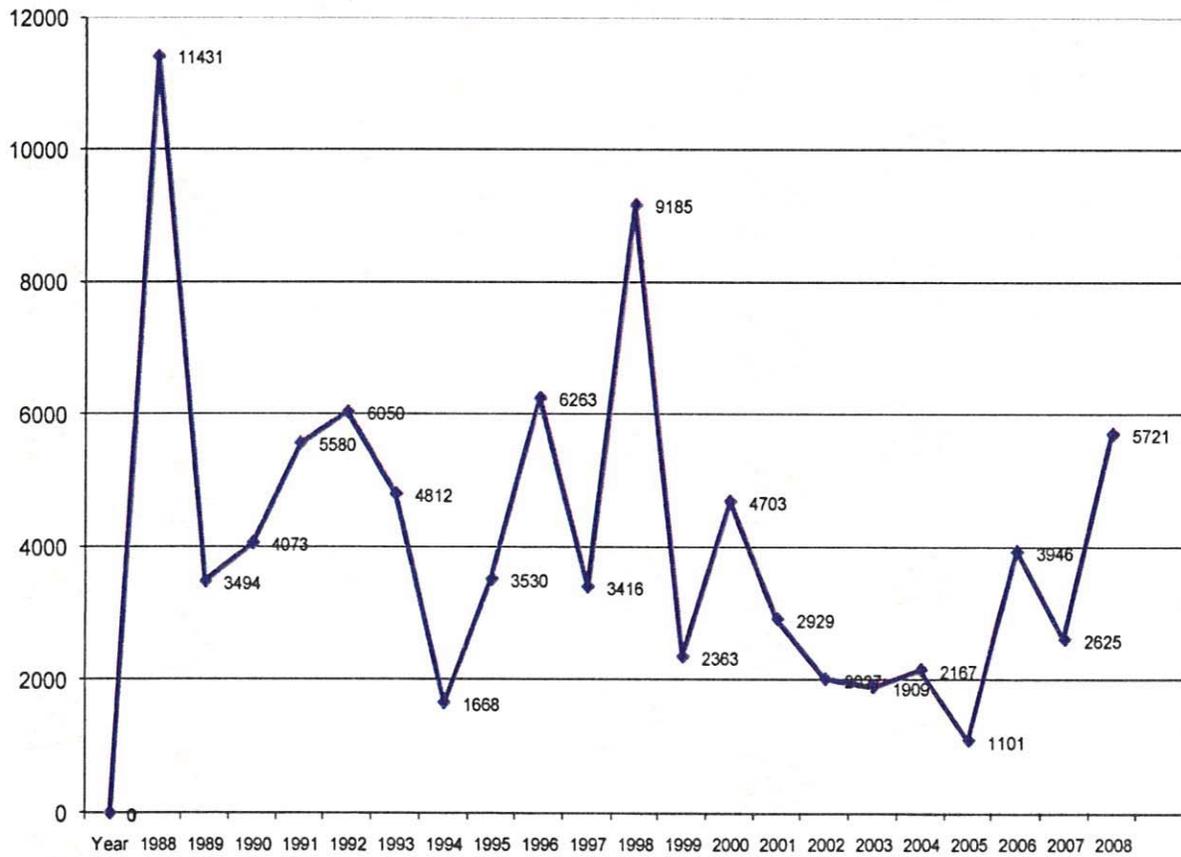
Year	Rand	CRsv	Bays	Wrest	ScCI	WS+CB	CCNP	CCSP	LCov	CofC	Total
1986	200	20	72		1000				250		1542
1988	93	73	22		3571	4891		2194	328	259	11431
1989	119	4	6		1491	1052		702	85	35	3494
1990	133		64		1342	1747		643	102	42	4073
1991	57	17	38		2057	1653		835	738	155	5550
1992	65	10	75		2029	767		2565	232	307	6050
1993	68	2	68		2007	731		1177	538	221	4812
1994	24		19		681	101		756	87		1668
1995	82	12	119		1146	1150		541	340	140	3530
1996	45	0	66		1904	1489		919	927	913	6263
1997	75	2	51		1091	851	119	507	525	195	3416
1998	83	1	44		3792	2597	616	984	566	502	9185
1999	29	0	41		408	1169	49		373	294	2363
2000	11	0	22		2317	1161	367		462	363	4703
2001	234	2	109		1375	502			352	355	2929
2002	52	0	28		691	621	80		397	158	2027
2003	31	0	149		256	577	226		586	84	1909
2004	27	0	0		447	1279	121		251	42	2167
2005	31	0	2	155	111	232		242	298	30	1101
2006	25	0	6	1366	218	1123	105	380	612	111	3946
2007	21	0	14	631	206	273	276	292	740	172	2625
2008	23	0	5	958	218	841	122	1609	1116	829	5721
Total	1528	143	1020	3110	28358	24807	2081	14346	9905	5207	

Counts for 1986 were incomplete.

Densities of PTBs within short sections of shoreline at all sites in 2008 are shown along with 2007 densities on topographic maps included in the Appendices of this report. Beetle density classes were determined using a regression analysis of the 2007 beetle densities per 100m. R-values for each class were maximized. Densities from 2007 were calculated between original waypoints and were placed in the zero, low (<15), medium (15-45) and high (>45) classes (Figure 10).

The northern most sites of Calvert County, Randle Cliffs and Bayside Forest had low numbers of adults and minimal densities (< 15 adults per 100m). Warrior Rest, Scientist Cliffs, Western Shores, the Power Plant, State Park, Little Cove Point and Calvert Cliffs all had a variety of high medium and low densities. The East and West Betterton sites and Ordinary Point site show low densities while Grove Point and the Lloyd and Turner sites had a variety of high, medium and low densities sections (See Appendices).

Fig. 2. Total index counts for C. puritana adults in Calvert County, 1988 to 2008.



Accounts for Individual Calvert Sites. Table 4 (below) gives the detailed results for all Calvert sites in 2008 as well as those from 2004-2007. Included are the adult numbers for *C. puritana* within each waypoint section, shoreline characteristics for each section of shoreline, and the coordinates for each waypoint. The locations of these waypoints are shown on the topographic maps included in the appendix of this report.

Randall Cliffs. This is the northernmost *C. puritana* site in Calvert County and has had consistently low numbers. Counts have typically been less than 100 since 1990, except for a count of 234 in 2001. The count of 23 in 2008 is similar to other recent low counts, ranging from 21 to 31 since 2004. It was unusual that this year 7 adults were on the beach section without cliffs at the north end. This site has not been suitable for supporting a large population, probably because of the limited suitable cliff substrate for larvae (seemingly too dry and with too little sand content). The shoreline is also very narrow with little adult foraging area. Typically, all adults have been found scattered in the few sections of wider beach. Also, there seems to be evidence of progressive and significant erosion throughout this site, especially the southern portion in the past 10 years, and this has eliminated some former habitat.

Camp Roosevelt. As in each survey since 2002, no adults were found at this site in 2008, further confirming the loss of this population. Even in earlier years counts at this site have been low, although 73 adults were found here in 1988. Records from collectors in the 1950's and 1960's suggest this site may have once supported a larger population. This site includes a long length of shoreline and cliffs, but the beach is narrow and/or the cliffs dry with little sand content, and little apparent suitable larval habitat for oviposition.

Bayside Forest. This site had only 5 adults in 2008 compared to 14 in 2007, 6 in 2006, 2 in 2005 and none in 2004. There were only 6 adults in 1989, but most other earlier years had 40 or more adults, and a peak number of 149 in 2003. All adults in 2008 were in waypoint section 31 which has supported most of the adults in recent years. Observations during the 2004 survey indicated this site experienced very severe erosion, apparently due to Hurricane Isabel. Most of the shoreline and especially the southern portion where beetles were always most common lost several meters or more of cliff face with extensive cliff breakdown and trees littering the beach and cliff base. There were also tracks and compaction from heavy equipment on the beach, apparently being used to clear the beach of downed trees. In 2005 there was no evidence of the downed trees and rubble or of heavy equipment on the beach. The beach was wide and cliffs relatively unvegetated. In 2007 and 2008 it appeared that the beach and cliffs have generally recovered from these earlier disturbances, but the small beetle numbers indicate the population has still not recovered and remains in serious danger of going extinct.

Warrior Rest and Scientists Cliffs. This very long section of shoreline is now separated into two sites because of differences in ownership and management. In previous years the beetle counts were combined and listed as Scientists Cliffs. The Warrior Rest count in 2008 was 958, compared to 633 last year and 1388 in 2006, further indicating the pattern of alternate year abundance. Conditions at the site did not appear significantly different than in any other recent years, so an explanation for these variations is unknown. The pattern of increase in these last 3 years is likely produced by the same thing that has affected increases in other sites in Calvert.

The distribution of adults at this site was as in previous years with adults present at high densities along most of the site and in most waypoint sections except the northernmost part.

The Scientists Cliffs population had 218 adults in 2008 compared to 206 in 2007, 213 in 2006 and 111 in 2005. As in past years most beetles were absent from much of the site, particularly the long northern section with groins. About a third of the adults counted in 2008 were in the section south of the public beach where cliffs provide some suitable habitat and the beach is wide. Groins added in this section about 5 years ago may be causing some deterioration of the habitat. Most of the other adults were at waypoints 44 and 45 where there were patches of open cliff habitat for larvae. Our observations in recent years suggest that much of the suitable habitat along the middle and northern section of Scientists Cliffs (the long section adjacent to Warrior Rest) has deteriorated due to increasing vegetation, apparently caused by the very extensive groin field along this portion of the shoreline. Some new groins have been added, but most are several decades old. However, the bulk of the population at these two sites has historically been the Warrior Rest section, and if this site continues to produce large numbers, the viability of this population and the whole Calvert metapopulation will be significantly improved. The Warriors Rest section has clearly experienced significant shoreline erosion in the past ten years and this may have contributed to even higher counts at this site 10-20 years ago. Regardless, the cliff habitat in the Warrior Rest section continues to be the best habitat for larvae in Calvert.

Western Shores/Calvert Beach. These two sites are now combined because they are adjacent shoreline sections and have comparable private ownership. This is also logical because they are part of the same section of shoreline and the same populations of *C. puritana* and *C. dorsalis*. This is the only site in Maryland with large populations of both species. The total number of *C. puritana* in 2008 was 841, compared to 273 in 2007, 886 in 2006, and 232 in 2005. These recent counts show the pattern of alternate year abundance. Odd year cohorts have been lower in most of the past 10 years at this site. As in previous years, the *C. puritana* were restricted to the southern end of the Western Shores part, adjacent to Calvert Beach where cliffs are very well developed and beaches wider than most other *C. puritana* sites. Although, Hurricane Isabel improved the habitat at this site by washing out some of the back beach vegetation and pushed sand onto the beach, this site continues to experience rapid back beach and low cliff vegetation growth. This vegetation now includes larger trees and dense shrubs which probably block the movement of adults of *C. puritana* up and down the cliffs as they switch from foraging on the beach to oviposition on the cliff face. At present, however, the population is viable although much lower than in earlier years. It will take a major storm to clear out this well developed vegetation and improve conditions.

Calvert Cliffs Nuclear Power Plant. This shoreline site has supported a moderate population of *C. puritana*, with numbers having been highly variable (high of 616 in 1998 and low of 49 in 1999), but less so in the past 4 surveys. The count was 122 in 2008 compared to 276 in 2007, 109 in 2006, and 121 in 2004. In most years adults have been concentrated near the middle and at the south end of the site where there is better cliff and beach habitat. However, in 2008 few were in the middle part and most were concentrated in the southern third of the site (waypoints 114-118). Much of the rest of the site has a very narrow and very rocky beach which is not suitable for adult foraging.

Calvert Cliffs State Park. The counts at this site showed a very great increase in 2008 when 1609 adults were found. This compares with consistently low counts in the previous 3 years: 292 in 2007, 338 in 2006, and 242 in 2005. In 2008, adults were found along nearly the whole length of the three cliff sections at the site and absent or sparsely present in the two marsh beach sections. The great increases in 2008 were seen in all of the waypoint sections (except 202) where adults were present, but were especially great in sections 207 (475 in 2008, 29 in 2007), 203 (318 in 2008, 23 in 2007) and 201 (228 in 2008, 3 in 2007). As in several other sites, there was no obvious shoreline or cliff changes apparent at this site in the past few years. No counts were made at this site from 1999 through 2004, but from 1988 to 1998, counts were over 600-700 in most years, with a peak of 2194 in 1988. The site has experienced significant shoreline and cliff erosion resulting in closure of the cliff sections to the public about 6 years ago. It is possible that this high and persistent erosion contributed to the lower counts in recent years, but ultimately to new habitat and increased recruitment to produce high numbers this year.

Little Cove Point. This long section of shoreline has extensive cliffs and mostly narrow to moderate width beach. It has consistently (except for a very few years) supported a medium to large population of *C. puritana*. The count of 1116 was the highest ever count and compares with a previous high count of 927 in 1996 and a second high count of 738 in 1991. The 2008 count along with the 740 in 2007 and 615 in 2006 represent a significant rebound in numbers at this site from the low counts of 298 in 2005 and 251 in 2004 (two of the lowest ever counts). The distribution of adults at the site has been comparable in recent years, with adults very widely distributed and consistently present along most of the site length, except for the north end and a section near the south end. The site remains as good cliff habitat and suitable beaches. A recent massive breakdown occurred at waypoints 157-159 in 2007 and several very large to smaller breakdowns at other locations in 2008. There have been some shoreline modifications (reef ball projects and revetment) in this and the adjacent Cliffs of Calvert shoreline in recent years, and the effect of these on beetle numbers is uncertain. We observed a significant cliff breakdown at one of these project sites during the conduct of our surveys this year.

Cliffs of Calvert. This site borders the above site and is a part of the same *C. puritana* population. The count was 829 in 2008 (the second highest count ever) and like the adjacent Little Cove experienced a very great increase in abundance; the 2007 count was only 172 and also low (111) in 2006. This count suggests an even greater pattern of increase than at Little Cove. It suggests the same factor was responsible for the increases. This site had a series of very low counts of less than 200 adults including lows of 30 and 42 from 2002 to 2007. The highest count was 913 adults in 1996, but numbers have fluctuated more at this site than most others. As in previous years, most of the adults in 2008 were in the middle section of the site, waypoints 179 to 185. The limiting factor at this site may be the narrow beaches over most of the site's length since there appears to be fairly extensive tall cliffs with suitable substrate over much of the site.

Summary of C. puritana Trends at Sassafras River Sites, 1989 to 2008. The results of annual surveys at all known sites produced a total count of 1764 in 2008, indicating a progressive and significant increase in the past 5 years, 398 in 2004, 408 in 2005, 1221 in 2006, and 1566 in 2007. Prior to these years, the total counts declined significantly from

1996 (count of 1821) to 2002 (400) (Fig. 3, Table 3). The lowest ever total numbers were recorded 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.

The causes of the progressive increase in numbers over the past few years are unknown as are those that caused the significant decline seen from 1996 to 2005. We have hypothesized that a progressive increase in bluff vegetation occurred during this period and reduced habitat quality, especially for recruitment and larval development. The cliff vegetation and especially that along the back beach and base of the cliffs will prevent or reduce the movement of adults from utilizing the foraging areas on the beach. Their movement to suitable oviposition sites on the cliff faces could also be impeded. It may also be that the composition of the vegetation on the cliffs is changing to more invasive species that are more resistant to erosion and/ or more effective in stabilizing the cliff faces. Shoreline and bluff erosion from Hurricane Isabel in 2003 could have countered this trend and reduced cliff face and base vegetation. Consequently, larval habitat improved, recruitment increased and populations of adults began to increase after this time. Because of the two year life cycle of *C. puritana* the improved conditions would take several years to be realized. Further discussion of causes of these trends was included in Part 1.

Table 3. Total index counts for all Sassafras sites, 1989-2008.

	1989	1991	1992	1993	1994	1995	1996	1997	1999	2002	2003	2004	2005	2006	2007	2008
Grove Point		1000+	1667	750	567	920	1230	452	150	78	195	254	156	273	843	986
Ordinary Point	650	12	215	88	110	208	78	45	120	0	9	40	28	30	53	100
North Stillpond.			217	190	87	133	138	92	44	220	119	42	26	143	66	120
W. Betterton		79	281	234	160	210	131	78	64	69	126	34	52	23	6	92
E. Betterton		0	20	19	40	44	21	28	7	11	16	6	12	6	12	34
East Lloyd		9	205	139	15	94	118	30	16	8	160	11	73	554	368	136
West Turner	150	0	51	12	47	88	80	19	10	12	3	3	26	172	218	293
East Turner	150	7	99	20	0	68	25	0	ns	2	2	8	35	20	0	3
Totals	950	1107+	2755	1452	1026	1765	1821	744	411	400	630	398	408	1221	1566	1764

Fig. 3. Total index counts for all Sassafras sites combined, 1989 to 2008.



Summary Results for Individual Sassafras Sites. Adult counts within the same standard waypoint sections for each of the Sassafras sites 2004-2008 are given in Table 5 and densities within more localized shoreline sections are shown on maps in the Appendix. Also included in Table 5 are shoreline characteristics for each section of shoreline, and the coordinates for the waypoints that separate the sites. The locations of these waypoints are shown on the topographic maps included with the report. The adult population at Grove Point continued the pattern of population increase seen in the past few years to a count of 1764 in 2008, an increase from 1556 in 2007, 843 in 2006 and 273 in 2006, and 408 in 2005. This site has consistently (except for 2002 and 2006) had the highest count of all Sassafras sites, usually with half or more of the metapopulation total. Numbers declined after 1996-1997 to a low of 78 in 2002 and remained at less than 300 adults until 2006. As in most recent years the main concentration of adults was from waypoints 61 to 64 where the best combination of beach and especially excellent cliff habitat was present. This section of shoreline was also the most densely populated in 2007 and 2008 which has accounted for most of the increase at this site. Few or no adults were found at the north and south ends of the site. We did not see any shoreline or cliff changes in the past few years which could account for this increase.

Ordinary Point had 100 adults in 2008, compared to 53 adults in 2007, 30 in 2006, 40 in 2004, and 29 in 2005. Most adults were concentrated near the north end of the site as in other recent years. Counts at this site were much higher in the mid-1990's, **peaking at 215** in 1992. This site includes a long section of shoreline but only limited sections of suitable cliff habitat.

The decline of the population at this site seems to be due to significant vegetation growth on the cliffs. Secondly, there is very little wide beach at this site which reduces overall habitat suitability.

North Still Pond had a count of 120 in 2008, relatively little change from 66 in 2007 and 143 in 2006. Adults were present along most of the length of the site and no apparent changes in shoreline or cliffs were noted. One negative feature of this site seems to be the orientation of the beach and cliffs which are shaded much of the day. Some sections of the cliffs have been quite heavily vegetated or rocky and seemingly unsuitable as larval habitat.

The numbers at East Betterton and West Betterton increased quite significantly in 2008, although numbers at both sites have been relatively low. The East Betterton count was 34 in 2008, up from 20 or less counts since 1996. This site has had consistently low numbers in all years (peak of 44 in 1995) and very little suitable habitat. The cliffs are very vegetated and the beach very narrow throughout. West Betterton has considerably more potential habitat and higher counts than East Betterton in all years (except 2007). Counts peaked at this site at near or over 200 in the 1990's. Vegetation on the cliffs at this site seems to have reduced habitat suitability in most recent years. The count of 92 in 2008 was the highest since 126 in 2003. This site is very long with extensive cliff habitat but most is very vegetated or with gravelly or clay soils. Adults have always been concentrated in the eastern end of the site where some sections of bare cliffs exist and absent from the long western section.

East Turner Creek had a count of 3 adults in 2008 but none in 2007 and only 20 in 2006 and 35 in 2005. The counts suggest this population may not be viable and could soon be extinct. The highest count at this site was 150 in 1989. Most of this site is very vegetated except a small patch at the west end.

Two adjacent sites on the south shoreline of the Sassafras that have experienced significant population changes in the past five years are East Lloyd and West Turner Creek. These sites are part of experimental study of the effect of vegetation removal on the *C. puritana* population. Details of this study will be included in another report. East Lloyd experienced a dramatic increase to 554 adults in 2006, from 96 in 2005, but declined to 368 in 2007 and 136 in 2008. This was a control site for the vegetation removal study and the cause of this decline, like the significant increase is unknown. It may be due, in part, of density dependent population factors of *C. puritana*. West Turner which included the vegetation removal section has increased very significantly in the past four years, from 3 in 2004, 18 in 2005, 172 in 2006, 218 in 2007, and 293 in 2008. Increases in 2006 were not a result of the vegetation removal experiment because that was not implemented until late summer 2006. Increases in the past two years, however could have been positively affected by increased habitat quality from the removal. More examination of this will be included in my FWS study now being finalized.

Table 4. Numbers of PTB counted, coordinates and characteristics within shoreline sections at all Calvert County sites, 2004 – 2008.

Map	2008	2007	2006	2005	2004	Shoreline Notes	LAT	LOX
Points	C.pur	pur	pur	C.pur.	C.pur.			
Randle Cliff								
1	7		0	0	0	public beach area, then cliffs begin; water level very high in 2005	4282206.00	366690.00
2	0		0	0	0	start main area of tall cliffs, very narrow, no beach	4282170.00	366700.00
3	0		0	0	0	same	4282169.00	366702.00
4	3	2	2	4	8	New breakdown area in 2005	4282063.00	366728.00
5	2	3	5	3	3	same, small sections of sandy beach	4281983.00	366763.00
6	0		0	18	0	same	4281923.00	366766.00
7	4	3	4	0	0	1-2 m wide, poor beach habitat; cliffs ok	4281870.00	366783.00
8	1	3	2	0	5	start breakdown with wider beach	4281857.00	366740.00
9	0	1	0	0	0	same but no beach; new sandy breakdown, mid height cliffs, dry	4281746.00	366806.00
10	1		1	2	8	wide, 3-4 m beach patch, then narrow; dry cliffs, small patch of beach	4281654.00	366832.00
11	1	4	1	2	3	narrow, no beach, even at mid-tide	4281501.00	366838.00
12	4	5	3	2	0	beach ends, no beach accessible to south, all v. narrow; breakdown at end	4281333.00	366857.00
	23	21	18	31	27			
Camp Roosevelt								
13	0		0	0	0	Start at north end at stream entry, no cliffs then wood area, then tall dry cliffs		
14	0		0	0	0	South end of site		
	0		0	0	0			
Bayside Forest								
15			0	0	0	N of BF, at Plum Point, at yellow house with lawn ornaments	4274428.23	368278.79
16			0	0	0	start series of white houses and no cliffs, some houses damaged by Isabel ?	4274050.32	368201.05
17			0	0	0	few small groins, cliffs fully vegetated, low, then wooded cliffs, trailer park	4273717.98	368134.07
18			0	0	0	creek, rock rip-rap, road bed to beach,	4273392.07	368107.36
19			0	0	0	start high cliffs, no suitable strata	4273246.13	368085.87
20			0	0	0	dry cliffs, most with vegetation	4272931.63	368026.85
21	0	0	0	0	0	cliffs end, marsh, creek entry, then woods, then low cliffs	4272475.73	367933.77
22	0	0	0	0	0	poor low, vegetated cliffs, breakdown; main survey area here to south+D61	4272131.05	367894.37
23	0	0	0	0	0	very tall cliffs, thin section of soft strata	4271673.71	367901.97
24	0	0	0	0	0	tall cliff section, narrow beach, many C. repanda	4271513.65	367901.33
25	0	see	0	0	0	pier posts in water, then no cliffs, then hard, mari cliffs	4271207.36	367883.21
26	0	to	0	0	0	cliffs dry, many fallen trees, then veg. cliffs	4270886.91	367855.87
27	0	right	0	0	0	same	4270884.91	367855.84
28	0	0	0	0	0	no cliffs, woods, then low cliffs; many C. hirticollis	4270696.80	367846.63
29	0	0	0	0	0	low cliffs, field behind, most cliffs bare, 20-25' high	4270437.65	367844.09
30	0	3	3*	0	0	start cliffs, lots of trees down, equipment and tracks; many C. hirticollis	4270322.93	367847.95
31	5	9	3*	2	0	road access, no cliff section	4270138.63	367883.34
32	0	1	0	0	0	low bare cliffs, very wide beach (due to 2004 erosion)	4269824.88	367927.75
33	0	1	0	0	0	Bayside Forest: south end access, low cliffs, evidence of severe erosion, cutback	4269741.77	367925.50
	5	14	6	2	0	*Note that these numbers were incorrectly placed further north in previous reports		
Warrior Rest								
N of 33a	0	0	3	0		Cliff section north of Parker Creek mouth	4266573	367708
33a	0	0	3	12		Far N end, start at beginning of cliffs, S edge of beach (no waypoint)	4266344	367687
33b	17	55	182	0			4266059	367729
34	96	48	34	13		Near N. end, no access for last 300 meters of cliffs habitat, severe erosion	4266119.45	367764.62
35	133	111	275	44		Good cliffs	4265995.33	367799.37
36	341	160	394	47		same; creek entry	4265795.36	367829.99
37	131	88	222	7		good cliffs, narrow beach	4265649.67	367873.71
38=39	240	171	275	31		continue tall cliffs; some vegetated cliff sections,	4265544.85	367896.40
	958	633	1388	154				
Scientists Cliffs								
40	22	25	0	6	26	at creek entry and cove; last groin, tall cliffs begin	4265331.95	367963.69
41	0	5	0	2	3	narrow, no cliff habitat	4265159.36	367995.99
42	0	4	0	2	0	same, no cliff habitat, beach narrows	4264839.36	368062.58
43	9	56	60	2	2	wider beach 6-8 m; old or broken groins, end at creek entry, driveway to beach	4264568.17	368130.93
44	25	18	8	4	16	section of rip rap; same low, veg. cliffs	4264327.43	368210.95
45	55	4	12	11	20	same; new gabion in 2005	4264219.09	368244.75
46	13	8	1	0	6	continue groins, low or vege cliffs	4263920.84	368332.12
47	11	10	9	0	0	same, groins	4263758.76	368380.74
Specific locales: '07: 2 at 8450, 2 at 8425, 6 at 8383, 3 at 8359, 1 at 8302, 4 at 8247, 18 at 8235(just S of stream channel); 56 from 8235 to 8200; 1 at 8119, 3 at 8036, 5 from 8018 to 8000, 25 from 7980 to 7964, 7964 to 7946, 12 from 7964 to 7946, 135 from 7946 to 7896, 88 from 7896 to 7873, 160 from 7883 to 7829, 111 from 7829 to 7800, 48 from 7800 to 7770; quit n								
48	0	0	1	8	20	6 groins, gabion, creek entry, low vegetated cliffs, ORV tracks	4263486.71	368474.83
49	0	0	0	0	0	groins end, beach narrows; ORV tracks	4263264.68	368571.49
50	0	0	0	0	0	SC North, S end, public beach rock groin, 10-12 m wide beach; heavy beach use	4263137.40	368636.56

51	0	0	0	2	0	Start Scientist Cliffs South, beginning of north end, groins	4263127.00	368621.00
52	1	2	4	0	5	open face cliffs, groins; 3rd, 4th gabions	4262981.00	368641.00
53	2	0	2	2	3	semi-vegetative cliffs, groins; most veg. cliffs	4262915.00	368665.00
54	3	3	19	9	8	semi-vegetative cliffs, end of groins; wide with breakdown bank	4262820.00	368708.00
55	7	16	18	16	12	open face cliffs(last groin at 8715)	4262758.00	368723.00
56	3	8	56	29	50	open face cliffs near waterline.	4262698.00	368749.00
57	34	34	18	12	12	same	4262638.00	368776.00
58	33	13	5	6	71	same, cliffs ending	4262564.00	368815.00
59	0				26	rock groin and residence, no open cliffs, far south end	4262435.00	368892.00
	218	206	213	111	280			
Western Shores/Calvert Beach								
60	0	0	0	0	0	north end of public beach	4261318.48	369396.39
61	0	0	0	0	0	narrow beach, ends at creek entry	4261219.24	369451.65
62	0	0	0	0	0	very narrow beach	4261067.20	369531.54
63	0	0	0	0	0	wider beach	4260966.85	369586.44
64	0	0	0	0	0	same, then beach narrows	4260854.48	369643.59
65	0	0	0	0	0	same	4260744.83	369690.67
66	0	0	0	0	0	same, but wider beach	4260660.75	369735.11
67	0	0	0	0	0	10-20 m wide beach, Phrag. On back, no cliffs	4260545.77	369802.17
68	0	0	10	0	0	vehicle tracks on beach, dense Phragmites on back, and at 2 creeks(2nd creek)	4260411.18	369867.78
69=70	3	0	2	10	0	cliffs low, recessed, Phragmites on beach, wide beach, end at first creek	4260233.24	369964.10
71=72	20	0	39	12	2	cliffs fully vegetated, trees on back beach	4260112.20	370042.84
73	44	25	28	31	30	same but cliffs become heavily vegetated and lower	4259999.63	370105.24
74	120	26	18	22	135	beach narrows then widens, tree rubble, cliffs tall, most bare	4259891.53	370186.91
75	61	41	66	42	65	vegetated cliffs, become lower, v. wide beach (10-15 m), then narrows	4259813.51	370255.01
76	84	25	63	23	115	very tall, excellent cliffs, thick habitat band of soft sand; heavy shrubs at base	4259710.25	370353.16
77	150	47	88	35	95	wide beach, heavy Phragmites cover (60+%), heavy shrub cover at base of cliffs	4259618.35	370421.75
						Subtotals for Western Shores		
78	143	35	101	28	122	N end Calvert: lower cliffs, good beach, 10-12 m wide, end at creek entry	4259516.99	370518.71
79	100	5	150	17	315	continue good cliff habitat, then lower but good, end at large fallen tree	4259432.09	370593.61
80	100	16	247	11	260	excellent high cliffs with wide habitat band, 3-5 m wide beach	4259378.77	370644.76
81	16	32	68	1	96	start bare cliffs, soft ideal upper strata	4259341.01	370680.54
82		16	6	0	25	upper cliffs vegetated and recessed, lower part is marl, 2-4 m wide beach	4259297.40	370721.02
83		3	0	0	19	North most groin, then bare cliffs, narrow beach	4259252.57	370782.25
84		2	0	0	0	groin, cliffs fully vine covered, 5-7 m wide beach, new sand bags?	4259032.11	370983.60
85			0	0	0	Calvert Beach and WSE: Start at access, creek, start cliffs	4258960.66	371022.77
86			0	0	0	Calvert Beach South, cliffs, residential, groins	4258899.00	371078.00
87			0	0	0	cliffs, end of groins	4258760.00	371228.00
88			0	0	0	residential, end of cliffs, starts flat back-beach	4258650.00	371340.00
89			0	0	0	far south end, residential, at jetty.	4258458.00	371687.00
				57	837	Subtotals for Calvert Beach		
	841	273	886	232	1279			
Calvert Cliffs Nuclear Power Plant								
100	0		0	no	0	North beach, wide beach, avg 20ft back-beach, no cliffs	4256074.00	373233.00
101	0		0	surv	0	same	4255928.00	373341.00
102	0		6		0	cliffs, avg 5ft back-beach	4255840.00	373426.00
103	0		0		0	cliffs, no back-beach	4255707.00	373559.00
104	0		3		0	same	4255538.00	373748.00
105	0		0		2	Start south of pier, main survey area, very rocky beach, no width, good cliffs	4254774.00	374636.00
106	0	6	6		0	same	4254697.00	374681.00
107	0	0	0		0	same	4254633.00	374786.00
108	8	2	7		6	same but wider beach	4254597.00	374818.00
109	10	43	13		44	wider sandy with shells beach (1-2 m)	4254527.00	374884.00
110	8	11	10		3	breakdown, rocky point, little habitat	4254412.00	374979.00
111	6	4	0		0	same, all very rock and no beach habitat	4254325.00	375085.00
112	4	0	0		0	same	4254288.00	375119.00
113	5	12	0		6	same but with sandy patches of beach: narrow some rocky, some sand	4254240.00	375146.00
114	11	36	6		18	arc beach, most sandy, 1-2 m	4254151.00	375163.00
115	24	23	36		28	same: rock, gravel, sand, medium width big breakdown just to south	4254053.00	375215.00
116	21	66	3		8	same	4253912.00	375341.00
117	4	28	0		0	point, rocky, no beach: very marginal, most beetles at S end	4253859.00	375397.00
118	21	45	19		6	start wide sandy beach, low cliffs	4253689.00	375475.00
119					0	end just to south, all hirticollis, no puntana habitat	4253617.00	375545.00
	122	276	109	ns	121			

Calvert Cliffs State Park								
201	228	3	20	0		Farthest N that can be accessed, rocky shoreline, no beach, even at low tide	4252322	376584
						N end of cliffs		6630
202	68	125	41	30		Here to north, very narrow, little beach habitat, but cliffs good	4251961	376687
						wider beach sections and good cliffs		6794
						start cliffs, no beach, inaccessible		6700
203	318	23	9	0		marsh and beach section, no habitat	4251855	376794
								6858
204	264	87	168	110		tall cliffs, tree rubble, narrow beach, then 4-5 m wide beach with most beetles	4251711	376896
								6906
						beach narrows, little or no width		6968
205	97	0	28	70		trail accessing beach, very narrow beach, dry cliffs, then good top cliff area	4251569	376987
206	46	5	8	0		marsh area and beach, no cliffs, no habitat	4251447	377087
207	475	29	30	11		very narrow beach, excellent cliffs	4251334	377139
208	67	7	8	10		arc beach, 0-1 m wide beach, then 2-3 m wide	4251150	377244
209	46	13	26	11		mid, tall cliffs, good, 2-4 m wide beach, end at Rocky Point	4250975	377300
210		0	0	0		south end of site, cliffs no beach		
	1609	292	338	242				
Little Cove Point- July 6								
140	32	0	0	0	8	cliffs, avg 20ft back-beach to cliffs, end open cliffs at this point	4247990.00	378548.00
141	47	14	21	8	14	cliffs, avg 4ft back-beach	4247932.00	378543.00
142=143	0	0	0	0	0	Cove Point Lake/Cove Lake, cliffs: beach end of cliffs	4247859.00	378548.00
144	0	0	0	0	0	start revetment	4247767.00	378578.00
145	60	25	0	0	0	end revetment	4247655.00	378624.00
146	99	29	22	0	11	Bannister point, arc beach	4247591.00	378668.00
147	161	13	40	0	3	same	4247522.00	378667.00
148	67	61	39	53	7	start point, then arc beach, low but good cliffs, breakdown area	4247387.00	378696.00
149	19	85	24	17	30	same, then high cliffs; good cliffs, wide beach; deck and stream entry	4247283.00	378706.00
150	17	3	2	4	0	no cliffs, then low cliffs; wood steps, terrace, rock revetment, set back	4247164.00	378755.00
151	8	43	11	5	0	point, then narrow arc beach, cliffs get lower	4247096.00	378822.00
152	73	52	0	0	0	another point section, then arc beach, no cliffs	4246977.00	378853.00
153	59	73	24	23	5	same, point; breakdown; 4th, N most pipe on back; breakdown; N end balls	4246798.00	378792.00
154	30	22	24	15	0	start point, no beach, good cliffs, break; drainage pipes on cliff sand bags	4246704.00	378742.00
155	3	15	26	4	16	same, medium cliffs, wide beach; stream channel and steps	4246584.00	378663.00
156	0	19	6	0	0	wider beach, low cliffs; hard cliffs	4246494.00	378601.00
157	77	27	11	6	2	same; massive breakdown	4246440.00	378572.00
158	21	5	90	9	4	same; breakdown, very high cliffs; massive breakdown	4246319.00	378518.00
159	46	23	33	22	6	mini-point, then recessed arc beach, narrow beach, good cliffs	4246233.00	378483.00
160	34	24	74	39	17	same, very high, good cliffs	4246147.00	378420.00
161	85	18	58	18	33	tree rubble but 1 m beach and good cliffs; breakdown	4246082.00	378376.00
162	8	29	36	15	22	same; N end of balls	4246018.00	378322.00
163	18	0	0	5	10	start good cliffs, 2 m beach, cliff terraced at top	4245951.00	378279.00
164	20	35	0	0	0	rock groin and rip rap (40 m long); S end of revetment	4245934.00	378254.00
165	6	0	0	4	8	start good cliff habitat, 1 m wide beach	4245870.00	378196.00
166	0	0	0	0	0	same	4245813.00	378153.00
167	0	0	0	0	0	wide beach, more groins; @ 12 total	4245723.00	378082.00
168	0	0	0	0	0	rock groins at south end of beach, then beach	4245646.00	378012.00
169	41	0	0	9	0	cliffs low, no habitat	4245561.00	377925.00
170	17	26	2	8	6	same, beach slightly wider	4245497.00	377878.00
171	53	14	40	14	13	eroded beach, tree rubble, but good cliffs; N end of balls	4245446.00	377834.00
172	15	85	32	20	36	S. end, start cliffs, 1-2 m beach, good cliff habitat	4245383.00	377774.00
	1116	740	615	298	251			
Cliffs of Calvert								
173	0	6	0	0	0	N end, rock pile, then small pond, no habitat	4245303.00	377719.00
174	0	0	0	0	0	no habitat; breakdown and minipoint	4245157.00	377615.00
175	0	0	0	0	0	same; jus N of N end of balls	4245058.00	377552.00
176	9	9	0	0	0	start creek and marsh with no beach, no habitat	4244953.00	377484.00
177	0	3	21	4	6	start good cliff habitat	4244878.00	377427.00
178	7	26	15	2	0	same	4244838.00	377392.00
179	52	51	0	0	0	lower cliffs, then vegetated cliffs; S end of balls	4244759.00	377325.00
180	183	0	16	6	6	same	4244700.00	377281.00
181	31	34	8	0	18	wider beach, good cliff habitat	4244638.00	377236.00
182	154	20	24	11	3	continue good cliff habitat	4244609.00	377217.00
183	59	7	4	1	2	same	4244538.00	377166.00
184	175	14	13	1	5	same	4244504.00	377146.00
185	144		10	2	2	same	4244409.00	377075.00
186	13	0	0	0	0	same; 0-1 m wide beach	4244312.00	377018.00
187	2	2	0	3	0	good cliffs with no beach	4244241.00	376974.00
188	0	0	0	0	0	end rip rap, start good cliff habitat, no beach	4244162.00	376920.00
189	0	0	0	0	0	S. end, start N of beach at large house with rip	4244057.00	376863.00
	829	172	111	30	42			

Table 5. Numbers of PTB counted, coordinates and characteristics within shoreline sections at all Calvert County sites, 2004 – 2008.

Waypoints	2008	2007	2006	2005	2004	Latitude	Longitude	Shoreline Characteristics
Grove Point								
57	0	0	0	0	0	4360277	410540	E end of site; dry cliffs, sand and some pebbles//very dry cliffs, 6-7 m beach, all bare
58	6	0	0	0	0	4360333	410498	very rocky, no beach, -1 m wide, cliffs vegetated//gets rocky, then trees;
59	8	7	3	5	4	4360415	410410	wider beach, good cliffs, 1 m, then tree rubble//wide beach, 6 m, ok cliffs
60	93	25	11	2	1	4360515	410374	narrow, -1 m wide, very good cliffs, then 1 m wide, end at camp/photo 6 looks N
61	169	143	39	7	15	4360577	410341	good cliffs, 1 m wide beach, earlier this was best prime section
62	113	111	92	14	0	4360639	410327	start pebble and stone beach, very narrow, cliffs veg., no habitat
63	353	166	81	61	146	4360695	410328	start good cliffs, 0-1 m wide, previously good, now beach narrower//
		78				60761	10334	
		139				60808	10339	
64	123	59	18	39	29	4360888	410361	good cliffs, same beach but then narrows// southern half very good sand beach and
65	55	49	11	7	18	4360950	410368	good cliffs but pebble, rocky beach, 1 m wide//still good cliffs
66	6	6	0	0	6	4361051	410387	very rocky, good cliffs end, then vegetated and non-habitat, road enters
67	6	12	0	0	12	4361138	410403	start N of road, narrow beach, 0-1 m, good upper cliffs
68	12	8	12	3	6	4361270	410472	same, some ok cliffs, 0 m beach
69	0	0	3	0	0	4361359	410507	low cliffs, no beach, -2 m wide
						4361384	410506	south end of new revetment; all logs and sticks behind revetment in 2007
70	42		0	18	8	4361401	410527	start bare cliffs, newly eroded, narrow beach/same
		38				4361427	410545	N end of revetment
71	0	0	0	0	0	4361506	410594	cliffs are ok habitat, beach ok, cliffs end, no habitat
72	0	0	0	0	3	4361552	410622	start low cliffs, ok habitat
73	0	2	0	0	0	4361586	410642	end, meet jim coming south
195	0	0	0	0	0	4362115	411315	Grove Neck, north end; gray clay, 12 m high then lower
196	0	0	0	0	0	4362060	411170	very rocky with many trees down/metal stakes; photo looking south, pier at top
197	0	0	0	0	1	4361936	410939	gray clay then red sand and rock; large sand stones on beach
198	0	3	0	0	5	4361720	410734	gray clay, 12 m high, then red sand top, very rocky beach
199	0	0	0	0	0	4361586	410647	end, meet bk
	986	843	273	156	254			
North Grove Point								
74				0	0	4362490	412209	rocky with sand, 1.5 m low dry cliffs, tree rubble
75				0	0	4362446	412101	red sand cliffs at top, bare, ok habitat
76				0	0	4362392	411990	all clay cliffs, no habitat
77				0	0	4362371	411945	end, meet jim, photo
200				0	45	4362317	411799	rocky beach, trees down; going N, new site, north of 200
201				0	0	4362368	411938	end site
				0	45			
Ordinary Point								
83	0	0	0	0	3	4359852	414418	Ordinary Pt. West, N end, dry stoney cliffs, 0-1 m wide
84	15	10	2	1	0	4359810	414442	good cliffs, no beach, then rocky cliffs, fully vegetated, tree rubble
85	33	0	5	15	22	4359753	414538	good cliffs, tree rubble, narrow beach, sandy
86	24	40	23	12	12	4359726	414570	breakdown, then ok cliffs, sandy 1 m wide
87	22	0	0	0	0	4359669	414655	end habitat
88	0	3	0	0	0	4359558	414922	continue Ordinary, N end, veg cliffs narrow to no beach, all tree rubble
89	6	0	0	0	3	4359508	414970	1 m beach and very veg. cliffs
90	0	0	0	0	0	4359464	415011	same
91	0	0	0	0	0	4359385	415062	same, end site
207	0	0	0	0	0	4359309	415117	variable beach width
208	0	0	0	0	0	4359384	415061	end of site
	100	53	30	28	40			
East Turner								
024	3	0	0	3	0	4357503	415759	East Turner Ck.; 0 m wide beach, tree rubble, good cliffs
025	0	0	0	1	0	4357525	415813	same
026	0	0	0	0	5	4357535	415831	same, good cliffs
027	0	0	17	30	0	4357556	415861	1 m beach width, cliffs low and very vegetated
028	0	0	3	1	3	4357587	415906	
029	0	0	0	0	0	4357627	415961	end at breakwater, shaded, photos 1, 2
	3	0	20	35	8			
West Turner B								
82	71	45	104	8	0	4357964	413531	West end of site; gravelly beach, many small trees down; Section B
West Turner A								
206	155	152	68	18	3	4358153	413887	many downed trees, many larvae in fall down (repana?); Section A
205	67	21	0	0	0	4358284	414014	east end of site
	222	173	68	18	3			
	293	218	172	26	3			

East Lloyd E												
78	57	35	347	44	8	4357434	411911	West Lloyd, photo: west end, good bare cliffs, no beach-	Section E, Control Site			
79	8	28	134	12	0	4357448	411987	narrow, -1 and rocky, but cliffs ok				
80	5	245	10	3	0	4357486	412089	sandy narrow beach				
81	3	10	0	3	0	4357490	412105	end site				
	73	318	491	62	8							
East Lloyd D												
202	25	10	0	0	0	4357668	412477	W. side of E. Turner Ck. Narrow beach, red sand, small stones;	Section D			
203	38	40	63	11	3	4357747	412613	no beach, many downed trees; all red sand bluff				
204			0			4357856	412706	trees down up river; end at end of bluff				
	63	60	63	11	3							
	136	368	554	73	11							
East Betterton												
30	0	0	0	0	0	4358265	410113	East Betterton, east end at pier, no cliffs				
31	0	0	0	2	2	4358264	410087	low cliffs, very vegetated, 1 m wide				
32	0	0	0	1	2	4358270	410046	heavy tree rubble, some bare cliffs				
33	1	0	0	0	0	4358301	409969	all very narrow beach, 0 m wide; bank swallows				
34	11	1	0	0	0	4358332	409866	start good cliffs but no beach, tree rubble				
35	22	3	3	3	0	4358328	409755	end of survey, meet jim from west				
180	0	8	3	6	2	4358366	409583	E. Betterton, start at W. end (bk east end)+G35				
181	0	0	0	0	0	4358367	409583					
182	0	0	0	0	0	4358330	409753	meet bk				
	34	12	6	12	6							
West Betterton												
36	2	0	0	2	6	4358536	407647	start east end, very good cliffs, 2 m wide				
37	17	0	0	12	10	4358535	407604	very good cliffs, 2 m wide				
38	25	0	3	10	6	4358540	407511	continue same, 1-2 m wide beach				
39	17	0	8	11	8	4358544	407433	point, tree and cliff breakdown, no beach, major cliff recession				
40	10	0	0	17	0	4358516	407367	beach wider but fully tree covered				
41	6	0	11	0	2	4358504	407279	bare cliffs and breakdown				
042=43	21	6	0	0	0	4358503	407265	end, meet Jim from west				
183	0	0	1	0	1	4358444	406876	West Betterton; heavily veg. west end, east end more open				
44	0	0	0	0	0	4358426	406418	W of West Betterton; very extensive riprap, east end, no cliffs, trees				
45	0	0	0	0	0	4358428	406330	tall mostly vegetated cliffs, non-habitat; 1 m beach				
46	0	0	0	0	0	4358447	406121	Tall, part bare cliffs, 1-2 m each, then continue poor habitat				
47	0	0	0	0	0	4358459	406039	cliffs low and most vegetated				
48	0	0	0	0	0	4358464	405987	start 50 m rip rap section				
49	0	0	0	0	0	4358475	405923	end rip rap but no habitat				
50	0	0	0	0	0	4358510	405748	same, no habitat				
51	0	0	0	0	0	4358555	405528	same, no habitat				
52	0	0	0	0	0	4358594	405289	end				
186	0	0	0	0	1	4358638	404891	Scout camp, go west to east, bk east to west; cliffs stabilized, trees				
187	0	0	0	0	0	4358616	405186	many trees on bluffs				
188	0	0	0	0	0	4358606	405286	end, meet bk				
	92	6	23	52	34							
North Still Pond												
189	0	0	0	0	0	4355728	402057	S end of Still Pond; rip rap area; then wider beach, many trees				
190	55	12	10	2	14	4355825	402055	gully, many hirticollis larvae; start eroded bluff, rocky				
191	25	4	28	7	14	4356068	402093	rocky, recent erosion on bluff, hardened sandstone				
192	34	24	50	17	11	4356301	402194	ending bluff, beach 3-5 m wide				
193	6	18	44	0	3	4356546	402428	end bluff, beach 3-5 m wide				
194	0	8	11			4356587	402565	end				
	120	66	143	26	42							
Totals	1764	1566	1221	408	398							

Part 3

PART 3. LABORATORY REARING, OVIPOSITION CHOICE EXPERIMENT, AND FIELD TRANSLOCATIONS

INTRODUCTION

Techniques for keeping and rearing tiger beetles in the laboratory are well- documented and have been successful for a variety of species (Knisley and Pearson 1984, Knisley and Schultz 1997, Pearson et al. 2005). Many of these studies have involved determinations of fecundity, effects of food, and rearing larvae for identification. Rearing tiger beetles through their complete life cycle has been more difficult and not often successful, especially for species that have a two-year life cycle in the field. The main difficulty is that these species enter an inactivity period during the second or third instar for prolonged periods and are susceptible to high mortality during this time (Knisley and Schultz 1997). Several workers (M.L. Brust, R. Gwiazdowski, pers. comm.; Knisley, unpublished studies) have taken individuals from egg to adult by excess feeding to get them through the life cycle rapidly, or by putting them through a winter/spring regime by altering temperature and photoperiod. The results of these studies are intended to provide a good basis for future success in rearing, translocation and recovery of rare tiger beetles, such as *C. puritana*.

The objective of this part of the study (conducted under permit #697823 issued by the USFWS Regional Office in Hadley, MA) was to develop rearing procedures and conduct additional studies of *C. puritana* that could potentially contribute to future translocation projects. We were especially interested in producing larvae from field collected adults and assessing fecundity in the lab. In addition, we used some of the resulting larvae to translocate to field sites and some of the field collected adults to test preferred grain size for oviposition in the laboratory.

METHODS

Rearing. The adults for this study were collected from two Calvert County, MD, sites, Calvert Beach and Little Cove Point. Thirty females and males of adult *C. puritana* were collected on June 27, 2007, placed in individual vials with moist paper towel and transferred to a cooler with ice. Soil from the habitat was also collected for use in laboratory rearing. These adults were transported the same day by Rodger Gwiazdowski and Joe Elkington to the University of Massachusetts insect rearing facility (Elkington Lab). The beetles were placed in separate plastic chambers (20 cm wide x 30 cm long by 18 cm high) with screen tops. The chambers were previously prepared by firmly packing an 8-10 cm layer of habitat soil into the chamber. At one end of the chambers a 10 cm thick vertical wall of packed soil was added to test if females might prefer a vertical substrate for oviposition. Single pairs of the field-collected adults were placed in each chamber and fed *ad libitum* with pinhead crickets. The soil was watered thoroughly each day with a misting sprayer. The chambers were checked each day to record the number of oviposition burrows and first instar larval burrows when they emerged. After about two weeks, the females were transferred to new chambers and offspring counted. Some of the emerging first instars from the MA rearings were fed occasionally with pinhead crickets or *Tribolium* larvae, and later those from some of the chambers were transferred to individual plastic tubes (2 cm diameter x 24 cm long) previously filled with a 50:50 mix of habitat soil and fine sand. Rearing of these larvae was continued into January 2008 to determine

development progress. Another group of chambers with an estimated 125 first instar larvae and some adults were taken to Knisley's lab at RMC and used in field translocations (see below).

Oviposition Trials. Adults brought from the Elkington lab were used to determine preferred sand grain size for oviposition. Ten females paired with males were placed in individual plastic chambers (30 x 15 x 12 cm) with a screened top and divided into four equal sections of sand representing four different particle sizes. Sand in the chambers was prepared by passing habitat soil (from MD sites) through standard USGS sieves to produce particle sizes of very coarse sand (1.0 mm, sieve # 16); coarse sand (0.50 mm, #35); medium sand (.250, #60), and fine (.125 mm, #120). A thin cardboard strip was used to separate the sections. Soil was placed to a depth of 10 cm and compacted. The beetles were fed *ad libitum* with adults and larvae of *Tribolium* and the chambers watered daily to provide moist soil. The test was run for 10 days after which adults were removed and the chambers watered daily for an additional 20 days until all eggs laid by females during the 10-day period had hatched and the first instars had emerged. All first instars in each chamber were counted along with any unhatched eggs found by searching through the soil in the chambers. Mean numbers of offspring per each section of chamber were determined and analyzed by ANOVA.

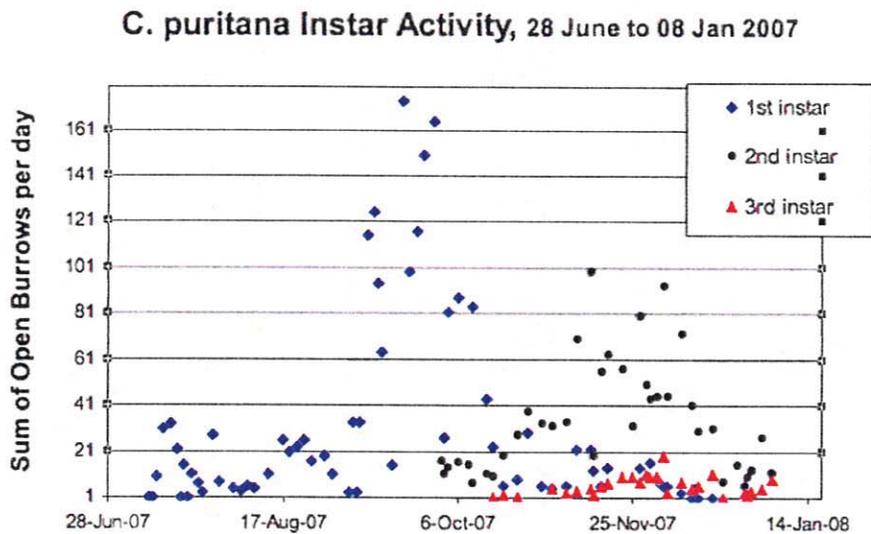
RESULTS AND DISCUSSION

Rearing. The rearing studies were very successful in getting adults to oviposit and yield a large number of first instar larvae. Adults set up in the lab June 28 were first observed ovipositing on July 2 and the first first instar was seen on July 10, 8 days after the first observed oviposition. The pattern of oviposition and early larval development was comparable to observations of field populations of *C. puritana*. The 30 captive females produced 218 first instar larvae that were accounted for but, a more probable total of about 400. The discrepancy between these two totals was a result of limited staff time to more closely monitor the chambers in the Elkington Lab. The multiple oviposition burrows and first instar larvae in each of the rearing chambers could not be individually tracked and within a few weeks, new first instars appeared while earlier ones were plugging their burrows in preparation for molting. Also some were possibly cannibalized or died from starvation. The resulting mean fecundity of about 13/female is much lower than reported for many tiger beetles (ranges of 40-50 to over 100 per female), but some females produced over 30 offspring. The mean per female would likely have been much higher if closer monitoring had occurred and rearing chambers were watered more frequently. It was found that during the first several weeks of the rearing, chambers were not kept moist enough, and oviposition increased significantly when watering was increased. Only a small percent of the burrows were found on the vertical wall of soil.

A total of 125 of the first instar larvae were taken by Knisley to his research lab at RMC for subsequent rearing and field translocation (see below) on August 8. The remaining first instars were transferred to individual rearing tubes in the Elkington Lab to follow their survival and developmental progress. Development progress of these lab-reared larvae was similar to what we have typically observed in the field in Maryland. Second instars were first seen on October 1 and more progressed to this stage through October and into November (Fig. 1). In the field overwintering as primarily second instars begins about mid to late October. The first lab reared third instars were seen in mid-October and continued to increase in numbers throughout

November and December. No further details on the rearing have been provided by Rodger Gwiazdowski except that these surviving larvae were to be subjected to overwintering conditions in an attempt to obtain emerge adults.

Fig. 1. Developmental progress of laboratory reared C. puritana larvae kept in individual rearing tubes. Results from Elkington Lab.



Larval Translocations and Monitoring. At Knisley’s RMC lab, the rearing chambers from the Elkington lab were examined for larvae on August 11. This was done by emptying the soil into a white tray and searching the soil for larvae. A total of 88 first instars (less than estimate of 125) were recovered, due either to overestimation or an inability to retrieve all larvae from the soil. These were transferred to individual rearing tubes (described above). Larvae readily established burrows in the rearing tubes and were fed *Tribolium* larvae several times per week until 15 September when they were transferred in the tubes to the Calvert Cliffs State Park (54 tubes) and Grove Point (34 tubes) for monitoring. An additional 250+ naturally occurring larvae of various instars were found in 8 separate patches at two sites, Calvert Cliffs State Park and Grove Point and marked with aluminum tags, as follows:

Calvert Cliffs State Park: Patch 1, 28 1st instars; 7 2nd instars; 0 3rd instars. Patch 2, 7-3-0; Patch 3, 11-2-0. Total of 46-12-0.

Grove Point: Patch 1: 5-15-1; patch 2, 0-55-2; patch 3, 9-11-2; patch 4, 2-58-3; patch 5, 4-36-0. Total of 20-175-8

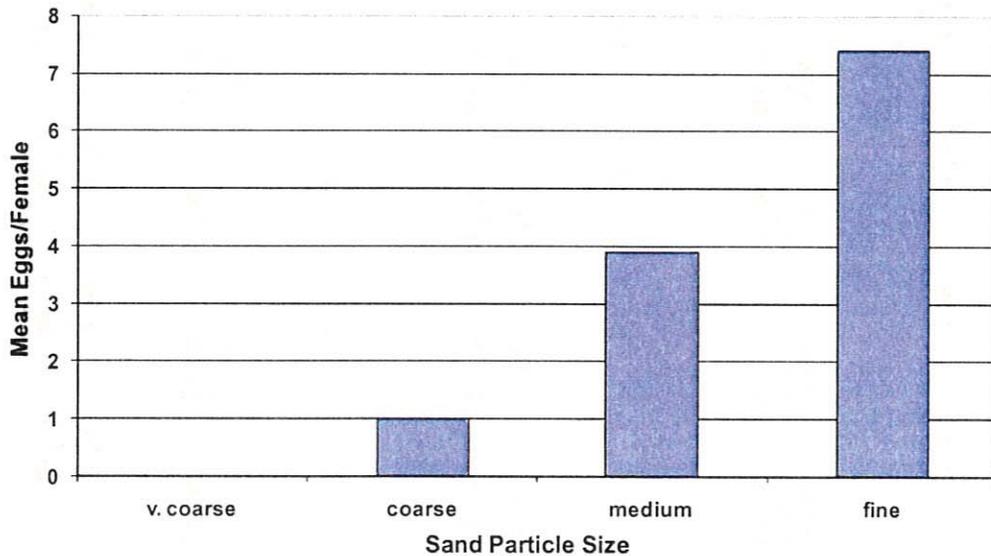
Maps were also made to indicate the location of larvae within each of these patches. The monitoring of the native larvae and those translocated at two field sites was conducted on 4 dates. The numbers of native and translocated larvae at both sites in the unknown category increased significantly throughout the monitoring period (Table 1). This category would include those larvae whose burrows were plugged, either because they were dead or in the premolting stage. Because most of these did not re-open, it was apparent that most were dead, so a percent survival (percent of initial total larvae that had open burrows on April 5) was calculated on April 5, 2009. Notable was that none of the tubes with translocated larvae could be found in this final check. It is uncertain if they were lost from erosion over the winter or had been vandalized. There was significant bluff erosion in and around some of the patches with native (some patches were completely lost) and translocated larvae so erosion was probably a significant cause of increasing numbers in the unknown group. Those larvae which survived, in both native and translocated groups at both field sites, progressed to the second and in some cases to the third instar (Table 1). Development appeared to be faster at the Grove Point site, but this was probably a result of marked larvae being mostly second instars when first marked. By October 5, most larvae were in the second instar with some remaining in the first instar and a few progressing to the third instar. By November 8, apparent mortality continued to increase and most surviving larvae were still second instars. By April 2009, accumulated mortality increased even more with relatively little development change since November. The developmental monitoring supports previous work indicating a two year life cycle for *C. puritana*. Surviving larvae will continue from spring through fall of 2009 and overwinter a second time before pupating and emerging in summer 2010. The observed larval mortality rate is typical of many other tiger beetles that have been studied (Knisley and Schultz 1997; Knisley, unpublished studies). Those studies documented survivorship of less than 5-8% from first instar to adult stage in a number of different species. The higher mortality of translocated larvae suggests vandalism may have been involved since none of the tubes were recovered. Future translocations should probably be done by placing first instars directly into the cliff face and avoid using rearing tubes which may attract attention by beach walkers. Both methods have been successful with other species.

Table 1. Results of development and survival of native and translocated larvae at two field sites.

DATE	No. Of NATIVE LARVAE Calvert Cliffs State Park				No of TRANSLOCATED LARVAE Calvert Cliffs State Park				No. Of NATIVE LARVAE Grove Point				No of TRANSLOCATED LARVAE Grove Point			
	1st	2nd	3rd	Unknown	1st	2nd	3rd	Unknown	1st	2nd	3rd	Unknown	1st	2nd	3rd	Unknown
20-Aug	46	12	0	0	54				34							
15-Sep	22	21	0	3	12	30		8	20	175	8	0	18	11	0	5
5-Oct	3	17	2	24	4	21		29	12	135	12	44	6	15	0	13
8-Nov	0	14	7	25	0	23	3	28	6	110	22	65	2	14	3	15
5-Apr	0	3	6	37	0	0	0	54	0	13	36	154	0	0	0	34
% Surviving	20				0				24				0			

Oviposition Choice Trials. Results of the laboratory oviposition test produced significantly different mean numbers of offspring in the 4 soil grain size sections (ANOVA, $p < .001$). Most offspring were in the two chambers with the finest sand, mean of 7.4 per female in fine sand (.125) and 3.9 in medium sand (.250 mm) (Fig. 2). The coarse sand had a mean of only 1 offspring and the very coarse sand no offspring. These results correspond with results from field collected larvae at Calvert and Sassafras sites suggesting the PTB prefers fine to very fine sand for oviposition, and consequently larvae are found in these same soil types (see Part 1 of this report). Additional studies with the co-occurring species, *C. repanda* would be important to determine its preferred oviposition substrate.

Fig. 2. Mean numbers of offspring (eggs and first instars) in each of 4 sections of rearing chambers after a 10-day oviposition period. Soil grain sizes were very coarse (1.0 mm, sieve # 16); coarse (0.50 mm, #35); medium (.250, #60), and fine (.125 mm, #120).



Part 4

PART 4. LABORATORY TESTS ON THE EFFECTS OF HERBICIDES ON TIGER BEETLES AND OTHER INSECTS

INTRODUCTION

Tiger beetles occur in a great variety of habitats, but most include open, bare soil patches where adults forage, thermoregulate and oviposit. Larvae also require these open patches for development through the three larval stages and subsequent adult emergence. Under normal conditions these open patches are maintained by the frequent disturbances which characterize the dynamic nature of tiger beetle habitats. Consequently, populations may decline or be lost when open patches are lost as a result of vegetation encroachment. Knisley and Hill (1992) presented two case histories of tiger beetle species that were extirpated by progressive vegetation growth in their habitat as a result of natural succession. Increasingly, invasive vegetation is becoming an additional serious threat to tiger beetle habitats. Several populations of the Ohlone tiger beetle have been lost and others have declined as a result of invasive vegetation eliminating open patches in their coastal grassland in California (Knisley and Arnold 2004). Invasive vegetation has also become a threat to *C. puritana* populations in New England (C. Davis, pers. comm.) and in Maryland (see Part 1). One option for controlling this detrimental vegetation in tiger beetle habitats is through the use of herbicides. However, their effect on tiger beetles or their prey is unknown. The objective of this study was to conduct a series of laboratory tests on the effects of several commonly used herbicides on tiger beetles and other insects.

METHODS

These tests included several of the most commonly used herbicides and formulations used to control vegetation and different methods of application,. All tests were run in my research lab at Randolph-Macon College under lab lighting conditions at temperatures of 22-25° C. All formulations were at or above the maximum recommended concentration indicated on the label. The following formulations were tested: 1. 5% Habitat with or without a 5% surfactant; 2. 10% mixture of Rodeo with surfactant; 3. 8 and 10% Round-Up, 4. commercial strength Triple Strike (used straight from container), and 5. 5% and 10% surfactant only. The recommended surfactant concentration with all of the herbicides is <1-3%. Insects tested included 8 species of tiger beetles (*C. repanda*, *C. t. tranquebarica*, *C. t. vibex*, *C. sexguttata*, *C. hirticollis*, *C. scutellaris*, *C. marginata*, and *C. punctulata*, adult and larvae of flour beetles (*Tribolium*) from laboratory cultures, *Tenebrio* larvae (mealworms) from lab cultures, house crickets (*Acheta domestica*) purchased locally, and field- collected soldier beetles.

The following methods of application were used :

1. Direct application to adults and larval tiger beetles and other insects kept in either 5-gallon terraria with moist soil or in finger bowls with damp filter paper. Test insects were placed into the test chambers and sprayed twice from a spray bottle with the herbicide to thoroughly cover them, then checked after one and 24 hours to determine survival.

2. Indirect application of the herbicide to soil in 5 gallon terraria. The soil was thoroughly saturated with 23-30 ml of the herbicide. Test insects were then introduced and exposed by contact with the soil for 24 hours, then checked to determine survival.

3. Indirect application to tiger beetle larvae in burrows. In this method 10 larvae were confined to the soil surface with a vial and allowed to dig burrows into the soil. After several hours when they completed digging their burrows, the vials were removed and the soil in the terraria was thoroughly saturated to a depth of 15-20 cm with the test herbicide. Exposure time was 24 h.

4. Indirect application of the herbicide to 10 cm diameter filter paper placed in a 10 cm diameter plastic petri dish. The filter paper was saturated with the herbicide and the test insects then placed in the closed petri dish for 24 hours.

5. Indirect application of herbicide to paper towels covering the bottom of a 5- gallon terraria. The paper towels were thoroughly saturated with the herbicide and insects then placed in the terraria for 24 hours and then checked for survival.

6. Controls were included with all of the above tests, using water applied in the same way as herbicides.

The results are presented as the percent survival for each of the individual tests. Since there was very minimal or no mortality in the control groups, these are not included in the results below. Also included here is a summary of an earlier field and laboratory study of the effects of the herbicide Rodeo on *Cicindela dorsalis* at Cove Point, MD (Knisley 1993). The study compared larval numbers in a series of shoreline transects before and after application of 1.75% Rodeo with 0.5% Li700 surfactant to the back beach area for the control of invasive Phragmites. In the laboratory study, there were 4 separate tests on tiger beetle larvae. In one test beetle larvae were sprayed directly as described above with the same Rodeo formulation and their response recorded. Two other tests were conducted in the same way except in one 1.75% Rodeo was used on 20 second and 20 third instars larvae and in the other only the 0.5% surfactant (15 second and 14 third instar larvae tested). The fourth test was designed to more accurately simulate field use of the herbicide. The same Rodeo and surfactant formulation was applied by a spray bottle to thoroughly saturate the soil around the mouth of larval burrows (15 second, 14 third instars) which had previously dug and became established. Larvae were monitored for a period of 3 days to determine potential effects.

RESULTS AND DISCUSSION

In general, the results of all tests produced very little mortality to tiger beetles and all other insects from the various herbicide formulations and methods of treatment. Somewhat higher mortality was seen with some concentrations of surfactant only. Among all species of tiger beetles tested (*C. repanda*, *C. t. tranquebarica*, *C. t. vibex*, *C. sexguttata*, *C. scutellaris*, *C. punctulata*, *C. hirticollis*, *C. marginata*), most individual tests resulted in 100% survival of adults and larvae, and only 4 of the total of 40 tests produced less than 80% survivorship (Table 1, Fig. 1). The results indicated no apparent difference among the different species of tiger beetles tested, no consistent difference between direct versus indirect application of the herbicides or among the types of herbicides, except that several of the surfactant only tests had higher mortality. All of the tests with tiger beetle larvae resulted in 100% survivorship.

Results with other insects tested produced similar results as those with tiger beetles. The majority of tests also produced 100% survival and most others had >80% survival. Survival of <70% occurred in several tests with Rodeo and very low survival was found in 6 of the tests using higher surfactant concentrations. In general, there was no apparent differences among the different species of insects tested, except that *Tenebrio* adults and especially *Tribolium* larvae had higher mortality in several tests. Results of the previous field tests with *C. dorsalis* indicated no changes in numbers of burrows before and after Rodeo treatment, and thus no apparent mortality. However, larvae numbers in pre- and post-treatment transects were very low so this study did not provide a reliable test of the herbicide effects. In the four laboratory tests with *C. dorsalis* there was no mortality recorded and no apparent effects to any larvae in any of the tests. All tested larvae were transferred to new terraria with habitat soil and readily dug new burrows, further suggesting no negative effects.

The results from these extensive tests with tiger beetles and other insects strongly suggest that there would be little or no mortality from field application of herbicides for control of vegetation in their habitats. In these tests mortality was very limited, even though concentrations used in the tests were at or above the maximum recommended levels and the mode of application more likely to produce mortality than field application methods. In the case of tiger beetles, application of the herbicides at a time when adults are not active would further insure that there would be no effect on them. Rodeo and Habitat are the two most commonly used herbicides to control vegetation in tiger beetle sites, and both caused little or no mortality in the laboratory tests. We could thus recommend the use of these herbicides for controlling invasive vegetation of *C. puritana* habitats. Application of herbicides during a time when adults are not active provide an additional safeguard to rare species of tiger beetles. Time of year restrictions for larvae are not as important since they are in burrows and even less susceptible to herbicide effects than adults. The herbicide Habitat was recently used to remove bluff vegetation in an experimental study of the effects of vegetation removal on population size of *C. puritana*.

Fig. 1. Results of laboratory tests of different herbicides on 7 species of tiger beetles.

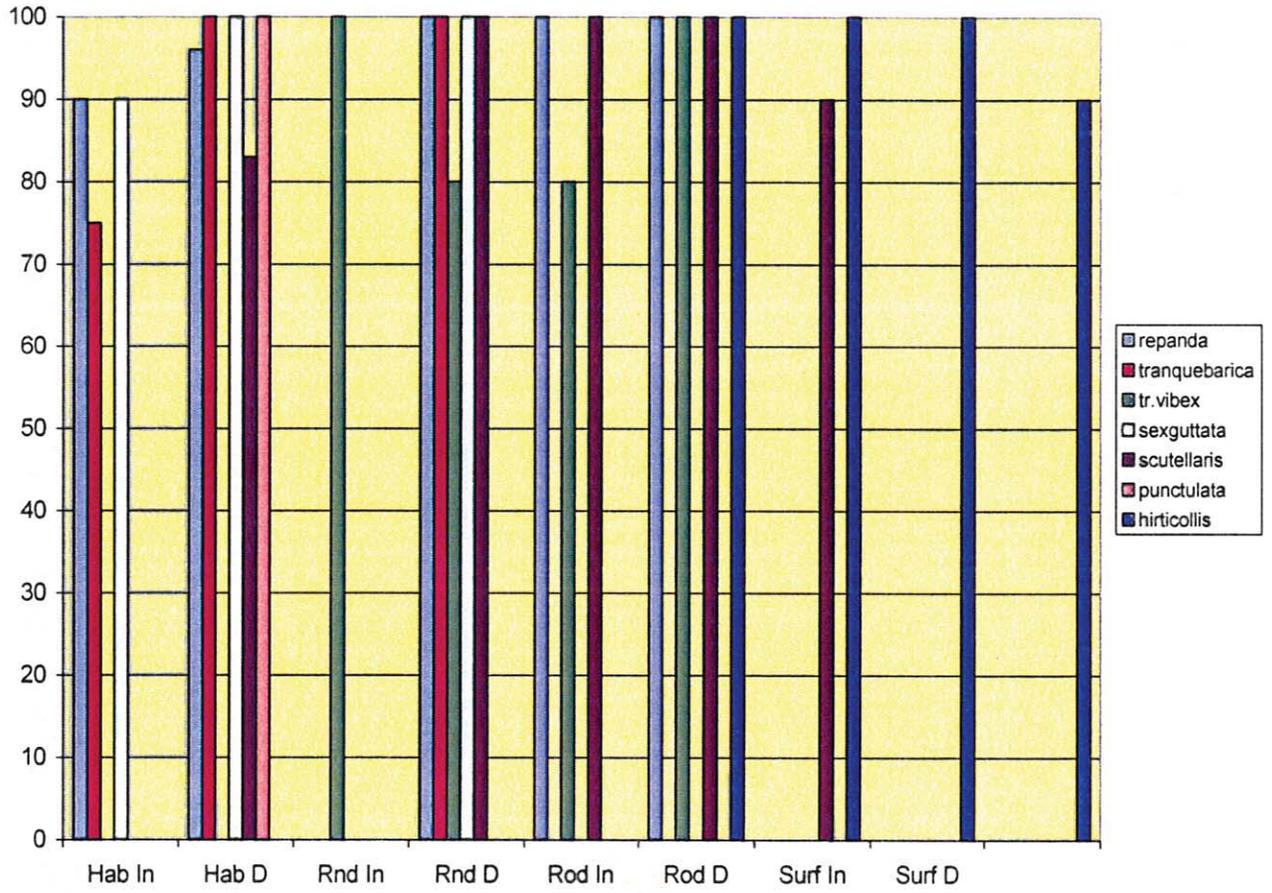


Table 1. All results of tests of different herbicides on tiger beetles and other insects.

Species	Stage	Date	Herbicide	Test Method	Type of Chamber	Percent Survival	Survivors/Total
C. repanda	A	16-Oct	8%Roundup	Soil	Terraria	95	19/20
C. repanda	A	29-Mar	5% Habitat	Soil	Terraria	90	9/10
C. repanda	A	10-Oct	5% Habitat	Direct	Terraria	92	12/13
C. repanda	A	10-Oct	5%Habitat	Direct	Terraria	100	15/15
C. repanda	A	10-Oct	5%Habitat	Direct	Terraria	100	15/15
C. repanda	A	29-Mar	10% Roundup	Direct	Terraria	100	15/15
C. repanda	A	15-Sep	10%Rodeo	Filter	Petri	100	10/10
C. repanda	A	15-Sep	10%Rodeo	Direct	Terraria	100	10/10
C. repanda	L	15-Sep	10%Rodeo	Direct	Terraria	100	12/12
C.t. tranquebarica	A	29-Mar	5% Habitat	Direct	Terraria	75	12/16
C.t. tranquebarica	A	29-Mar	10% Roundup	Direct	Terraria	100	15/15
C.t. tranquebarica	L	30-Mar	10%Roundup	Direct	Terraria	93	14/15
C.t. tranquebarica	A	11-Apr	5% Habitat+S	Direct	Terraria	100	10/10
C.t. tranquebarica	L	11-Apr	5% Habitat+S	Direct	Terraria	100	10/10
C.t.vibex	A	15-Mar	10%Rodeo	Filter	Dish	80	8/10
C.t.vibex	A	15-Mar	10%Rodeo	Direct	Dish	100	12/12
C.t.vibex	A	15-Mar	Roundup	Filter	Dish	100	8/8
C.t.vibex	A	15-Mar	Roundup	Direct	Dish	80	8/10
C.sexguttata	A	29-Mar	5% Habitat	Soil	Terraria	90	18/20
C.sexguttata	A	29-Mar	10% Roundup	Direct	Terraria	100	10/10
C.sexguttata	A	11-Apr	5% Habitat+S	Direct	Terraria	100	15/15
C.sexguttata	A	6-Jun	5% Habitat+S	Direct	Terraria	100	10/10
C.scutellaris	A	29-Mar	5% Habitat	Direct	Terraria	83	10/12
C.scutellaris	A	15-Oct	10%Rodeo	Filter	Dish	80	8/10
C.scutellaris	A	20-Oct	10%Rodeo	Direct	Dish	100	10/10
C.scutellaris	A	20-Oct	Surfactant 5%	Filter	Dish	100	10/10
C.scutellaris	A	20-Oct	Surfactant 5%	Direct	Dish	50	5/10
C.punctulata	A	11-Apr	5% Habitat+S	Direct	Terraria	100	15/15
C.punctulata	A	6-Jun	5% Habitat+S	Direct	Terraria	100	12/12
C.punctulata	A	29-Jun	Triple Strike	Direct	Terraria	88	7/8
C.hirticollis	L	19-Jun	5% Habitat	Direct	Terraria	100	21/21
C.hirticollis	A	29-Jun	Triple Strike	Direct	Terraria	88	7/8
C.hirticollis	A	15-Sep	10%Rodeo	Filter	Dish	100	10/10
C.hirticollis	L	15-Sep	10%Rodeo	Filter	Dish	100	15/15
C.hirticollis	L	15-Sep	10%Rodeo	Direct	Terraria	100	15/15
C.hirticollis	A	15-Sep	Surfactant 5%	Filter	Dish	90	9/10
C.hirticollis	A	15-Sep	Surfactant 10%	Filter	Dish	60	6/10
C.hirticollis	A	15-Sep	10%Rodeo	Direct	Dish	70	7/10
C. marginata	A	1-Jan	5% Habitat	Soil	Terraria	100	16/16
C. marginata	A	16-Oct	8%Roundup	Direct	Terraria	100	25/25

soldier beetles	A	10-Oct 5% Habitat	Direct	Terraria	100	12
soldier beetles	A	10-Oct 8%Roundup	Direct	Terraria	100	11
crickets	A	29-Mar 5% Habitat	Direct	Terraria	100	11/11
crickets	A	29-Mar 5% Habitat	Soil	Terraria	100	10/10
crickets	A	30-Oct 10%Rodeo	Direct	Terraria	92	46/50
crickets	A	30-Oct 10%Rodeo	Filter	Dish	88	44/50
crickets	A	30-Oct 10% Roundup	Direct	Terraria	87	26/30
crickets	A	30-Oct 10% Roundup	Filter	Dish	87	16/20
crickets	A	30-Oct Surfactant 5%	Direct	Terraria	96	48/50
crickets	A	30-Oct Surfactant 5%	Filter	Dish	95	38/40
Tenebrio	L	29-Mar 5% Habitat	Filter	Petri	95	52/55
Tenebrio	L	29-Mar 5% Habitat	Direct	Terraria	100	10/10
Tenebrio	L	20-Oct 10% Rodeo	Filter	Petri	100	50/50
Tenebrio	L	20-Oct 10% Rodeo	Direct	Terraria	100	50/50
Tenebrio	L	20-Oct 10% Roundup	Filter	Petri	100	50/50
Tenebrio	L	20-Oct 10% Roundup	Direct	Terraria	100	50/50
Tenebrio	L	20-Oct Surfactant 5%	Filter	Petri	88	44/50
Tenebrio	L	20-Oct Surfactant 5%	Indirect	Terraria	60	30/50
Tenebrio	L	20-Oct Surfactant 10%	Indirect	Petri	0	0/50
Tenebrio	L	20-Oct Surfactant 10%	Direct	Terraria	36	18/50
Tribolium	A	25-Oct 10%Rodeo	Indirect	Petri	100	50/50
Tribolium	A	25-Oct 10%Rodeo	Direct	Terraria	100	50/50
Tribolium	A	25-Oct 10%Roundup	Indirect	Petri	100	50/50
Tribolium	A	25-Oct 10%Roundup	Direct	Terraria	100	50/50
Tribolium	A	25-Oct Surfactant 5%	Indirect	Petri	100	50/50
Tribolium	A	25-Oct Surfactant 5%	Direct	Terraria	100	50/50
Tribolium	A	25-Oct Surfactant 10%	Indirect	Petri	16	8/50
Tribolium	A	25-Oct Surfactant 10%	Direct	Terraria	0	0/50
Tribolium	L	25-Oct 10%Rodeo	Indirect	Petri	60	30/50
Tribolium	L	25-Oct 10%Rodeo	Direct	Terraria	62	62/100
Tribolium	L	25-Oct 10%Roundup	Indirect	Petri	100	50/50
Tribolium	L	25-Oct 10%Roundup	Direct	Terraria	100	50/50
Tribolium	L	25-Oct Surfactant 20%	Indirect	Petri	40	20/50
Tribolium	L	25-Oct Surfactant 20%	Direct	Terraria	60	30/50

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APPENDICES

(all appendices also included on a CD with this report)

- I. USGS Topo maps with standard waypoints (used in annual reports since 2004) for adult PTB surveys at all Calvert and Sassafras Sites
- II. USGS Topo maps showing densities of adult PTB in 2007 and 2008 within standard waypoints (above) and also within short sections of all Calvert and Sassafras sites.
- III. Photographic panoramas of the shoreline and bluffs at all Calvert and Sassafras PTB sites in 2007 (North Stillpond not included).
- IV. Photographic panoramas of shoreline and bluffs at all Calvert and Sassafras sites from 2007 and from selected available sections in 2000 showing bluff habitat categories. Potential habitat is all of the bare unvegetated bluff face. Probable habitat is the area with suitable strata and shaded in light blue(North Stillpond not included).

Appendix I

I. USGS Topo maps with standard waypoints (used in annual reports since 2004) for adult PTB surveys at all Calvert and Sassafras Sites

Figure 1. Randle Cliff

Numbered points indicate GPS waypoints associated with beetle counts in Table 1.

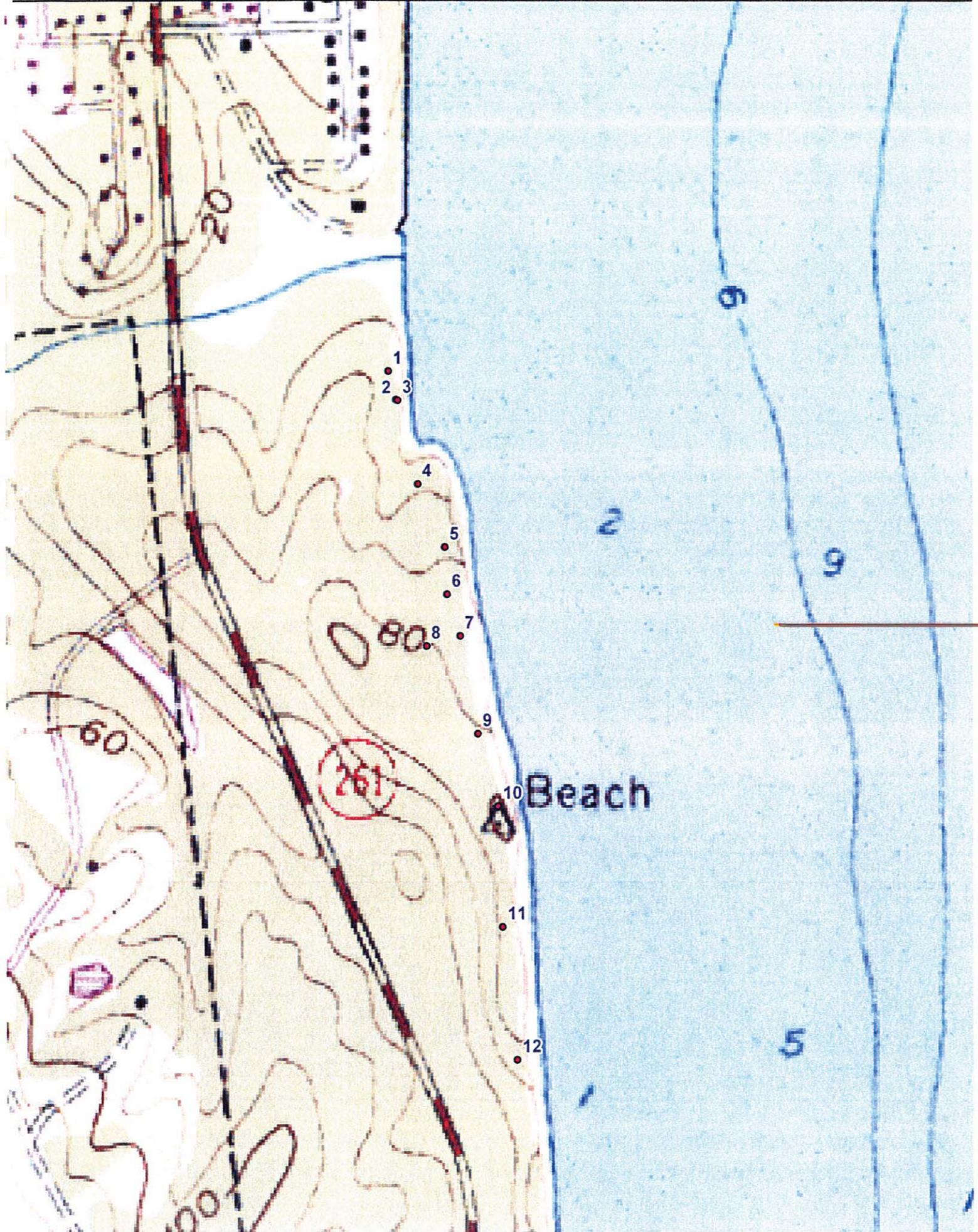
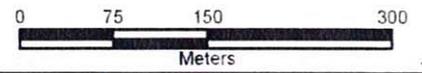


Figure 2. Camp Roosevelt

Numbered points indicate GPS waypoints associated with beetle counts in Table 1.

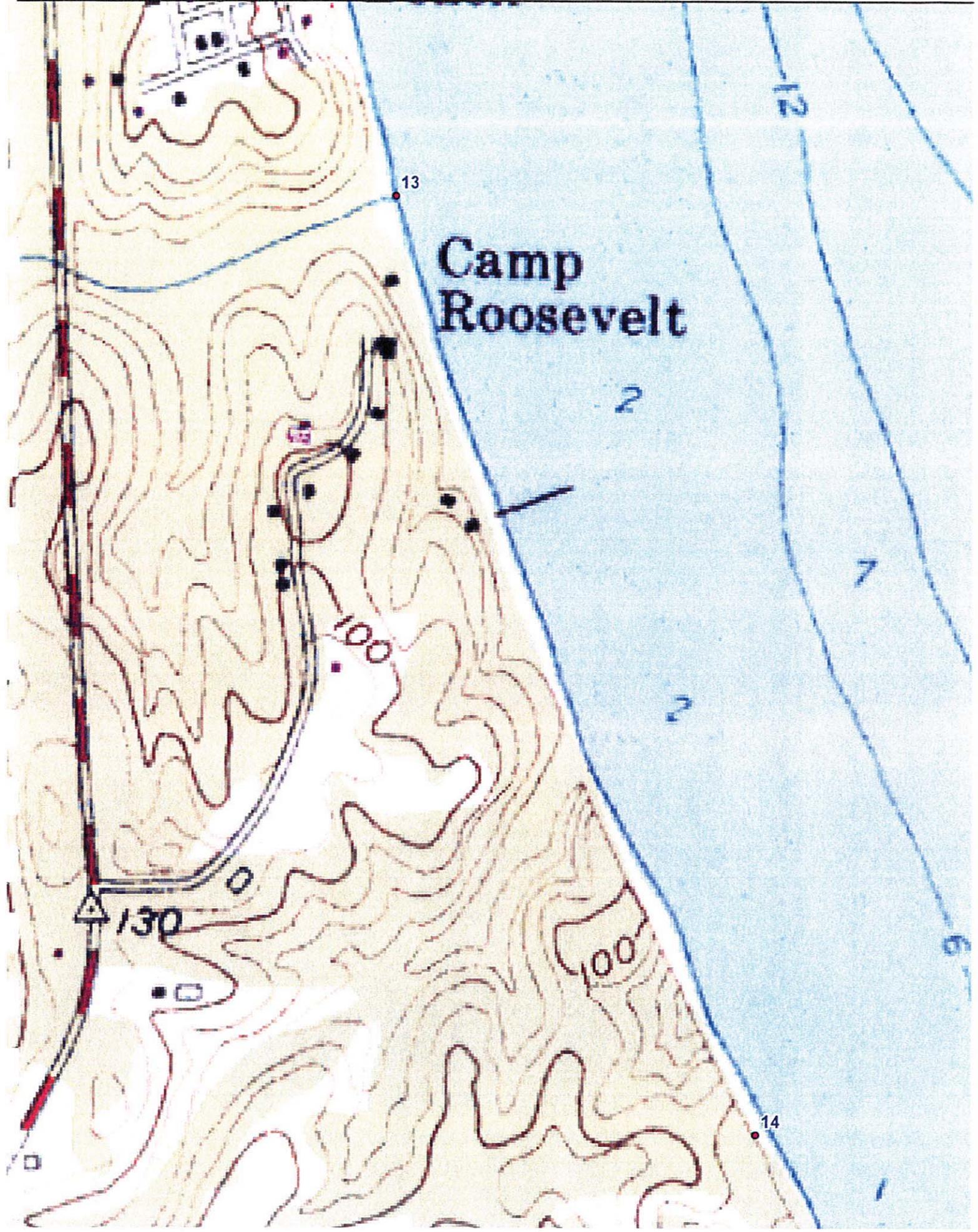
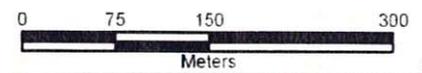


Figure 5. Western Shores Estates and Calvert Beach
Numbered points indicate GPS waypoints associated with beetle counts in Table 1.

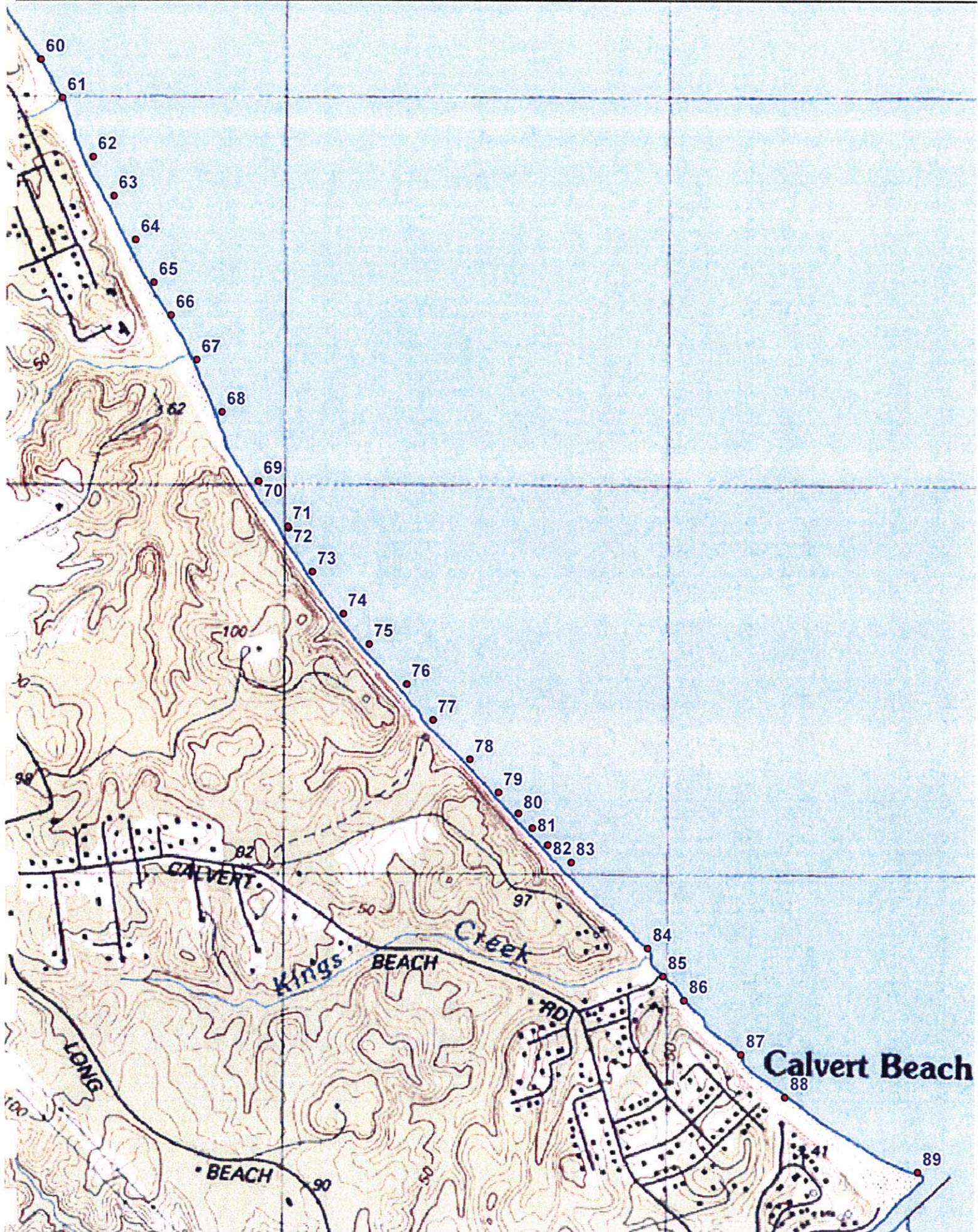
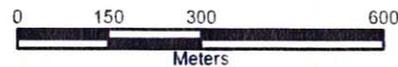


Figure 7a. Calvert Cliffs State Park

Numbered points indicate GPS waypoints associated with beetle counts in Table 1

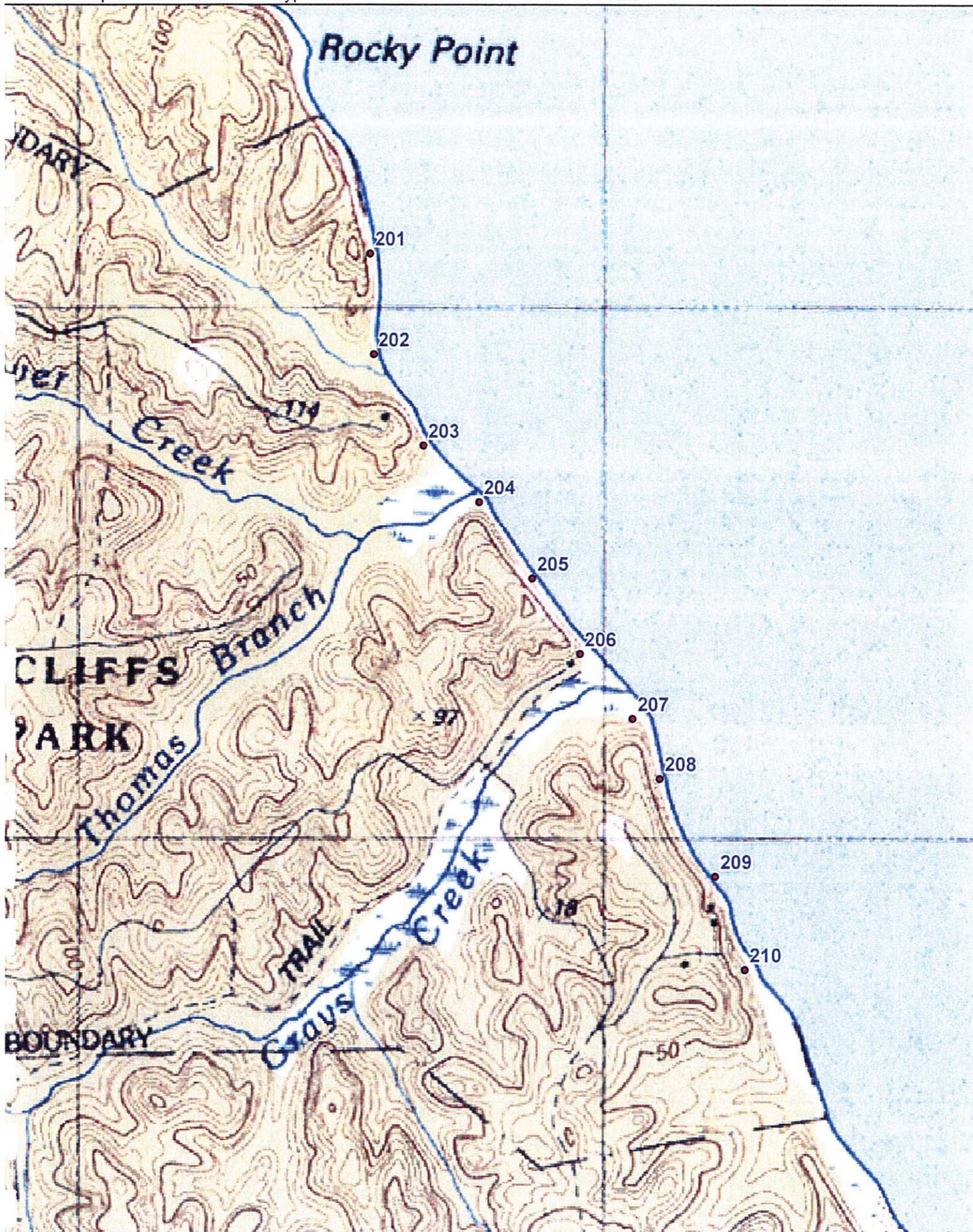


Figure 9. Little Cove Point

Numbered points indicate GPS waypoints associated with beetle counts in Table 1.

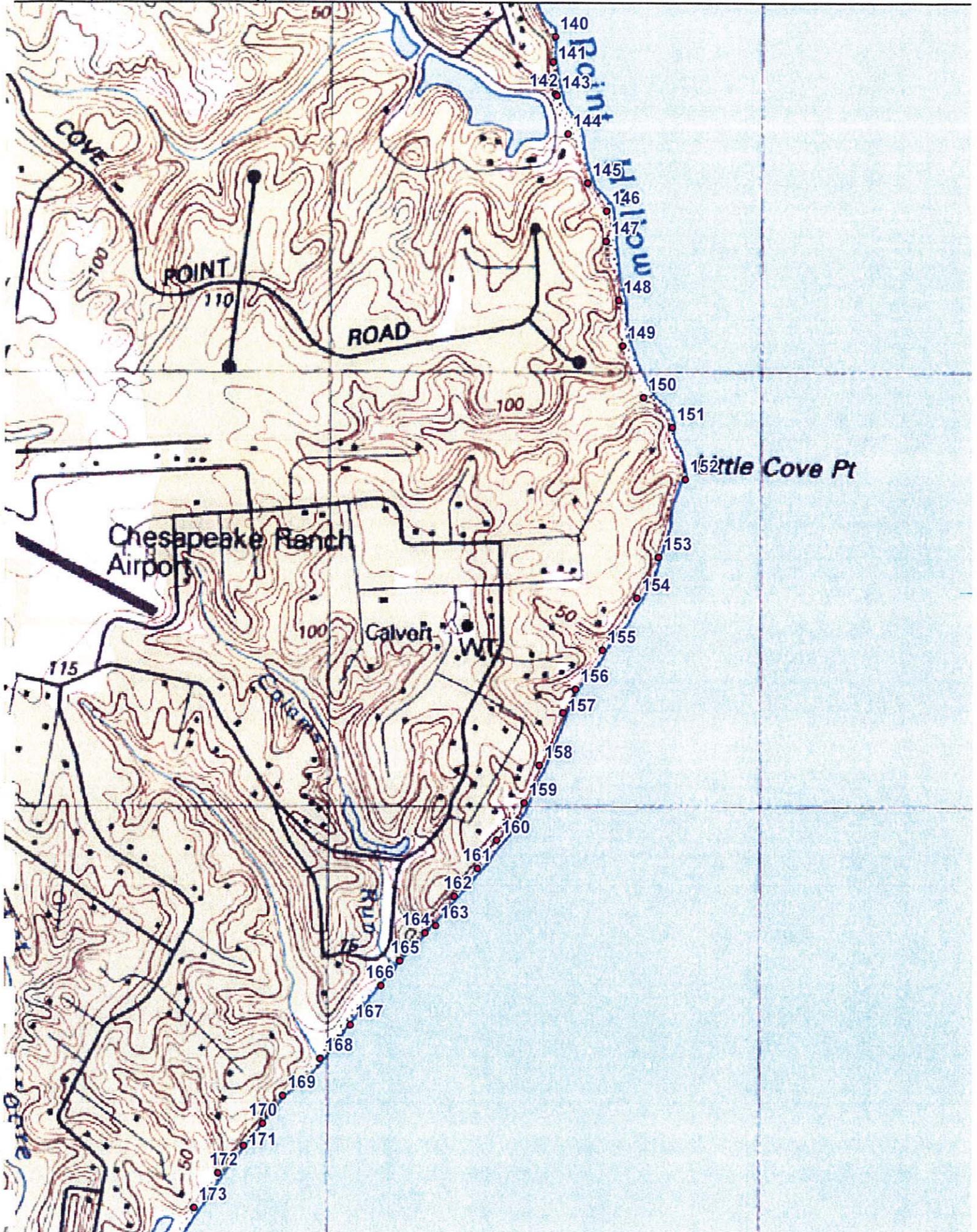
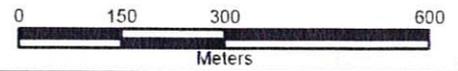
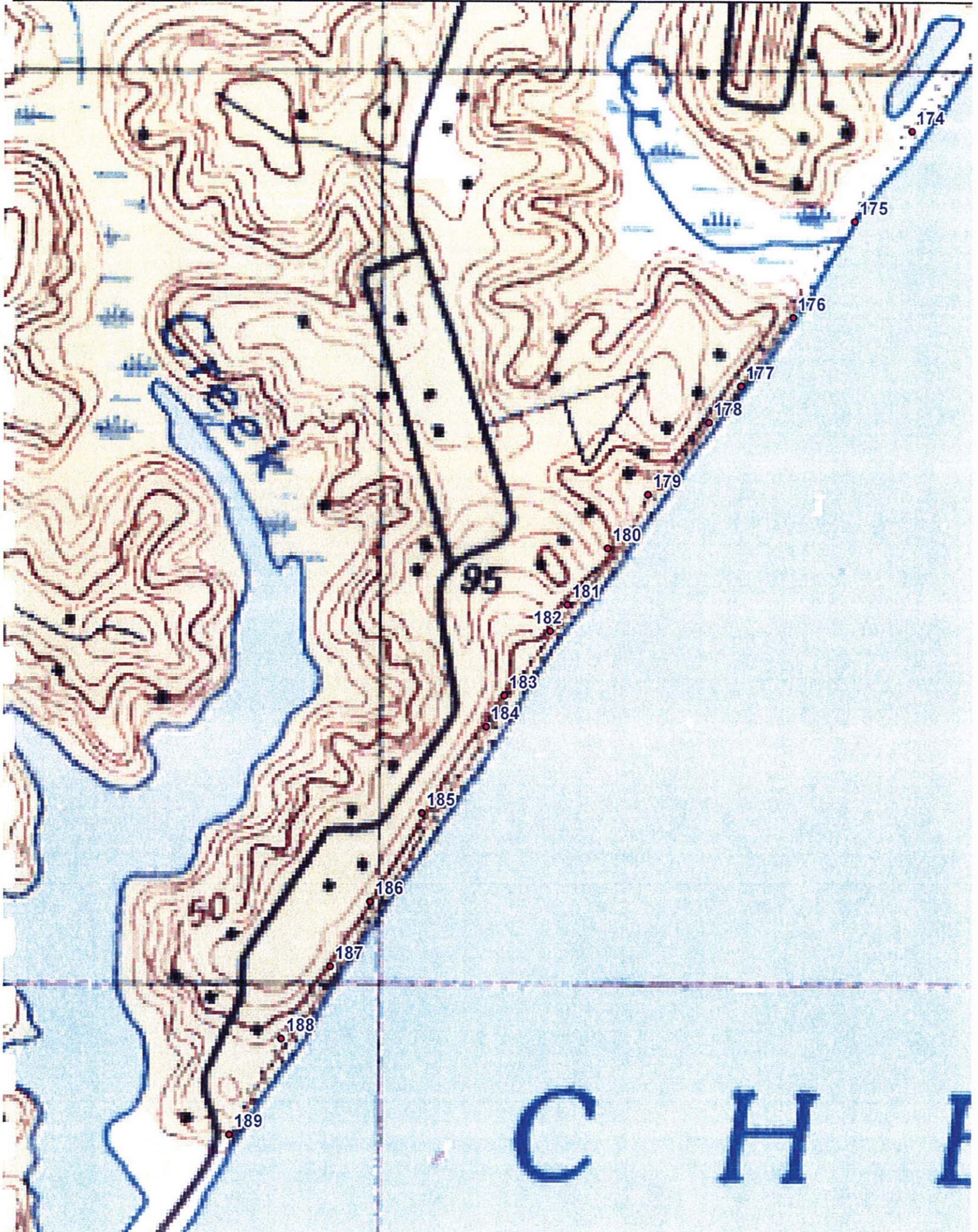
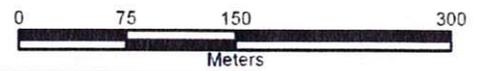
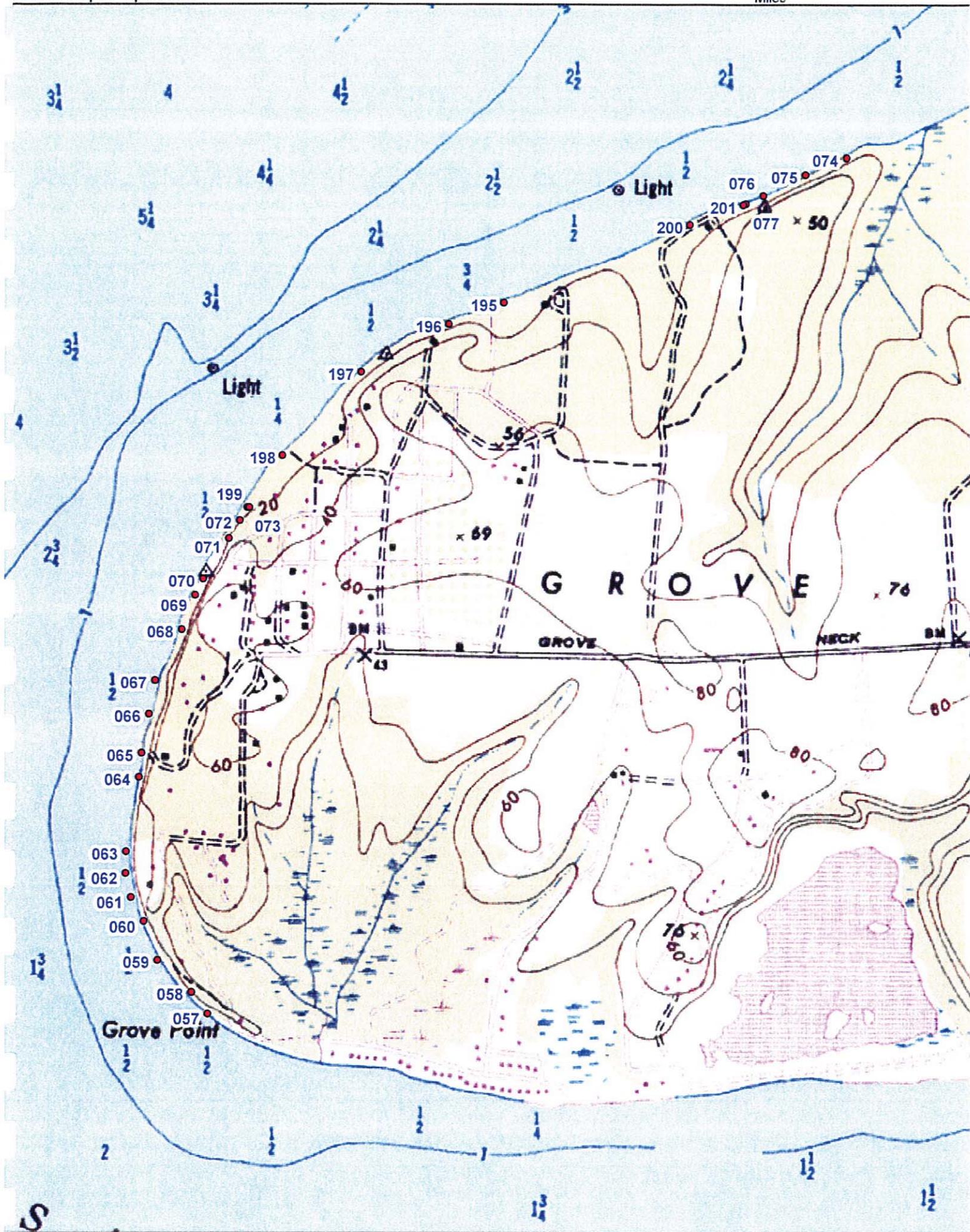
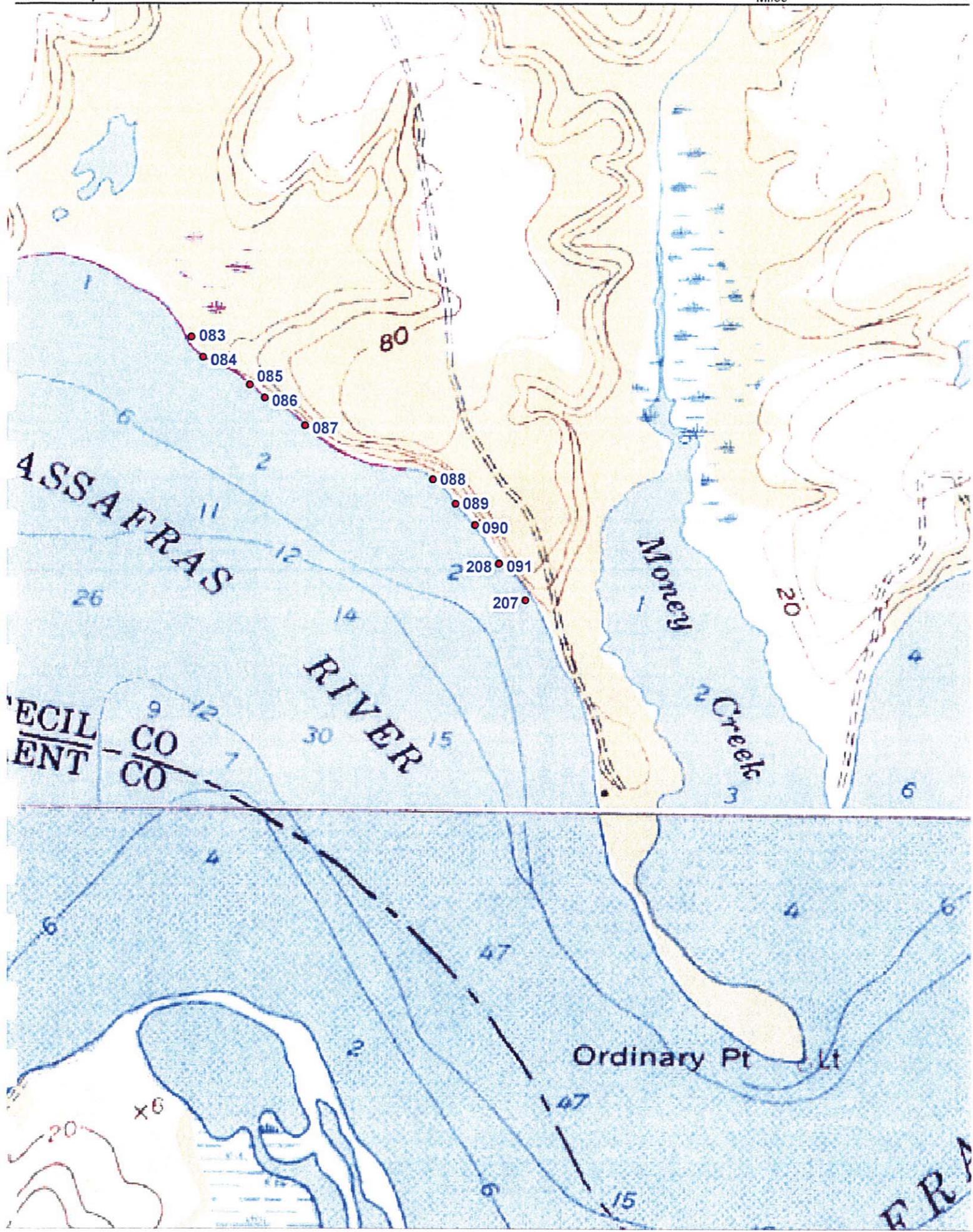


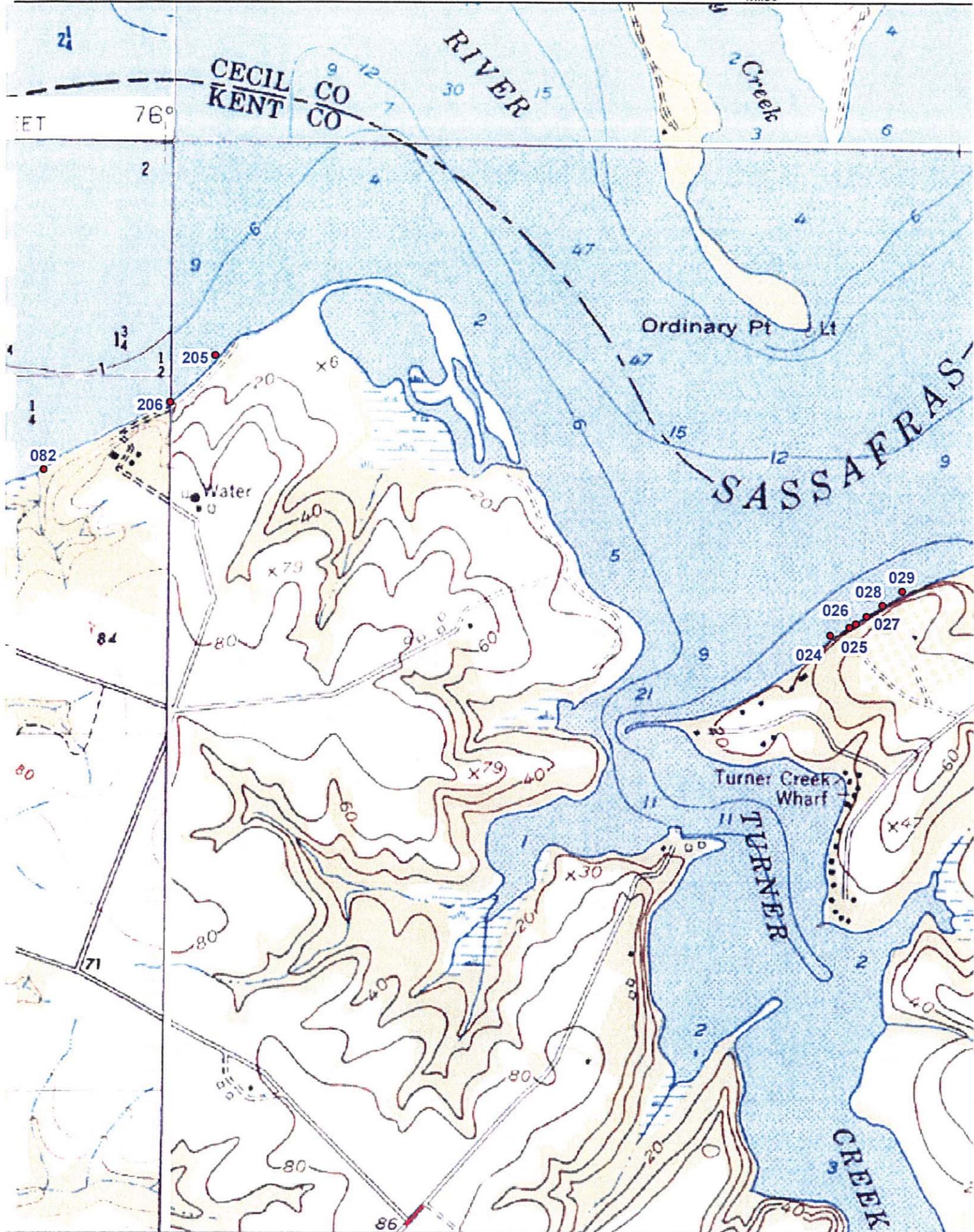
Figure 10. Cliffs of Calvert

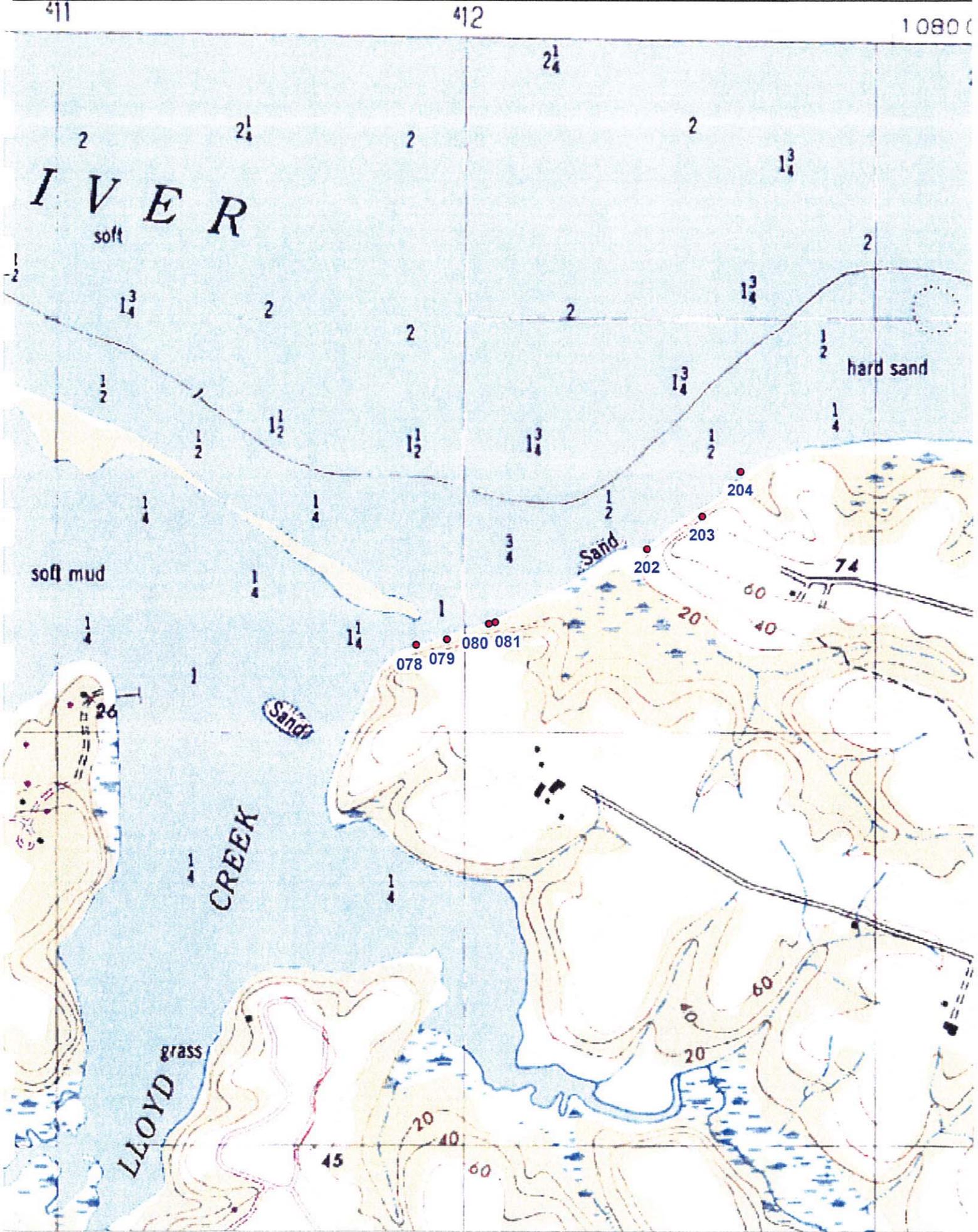
Numbered points indicate GPS waypoints associated with beetle counts in Table 1.

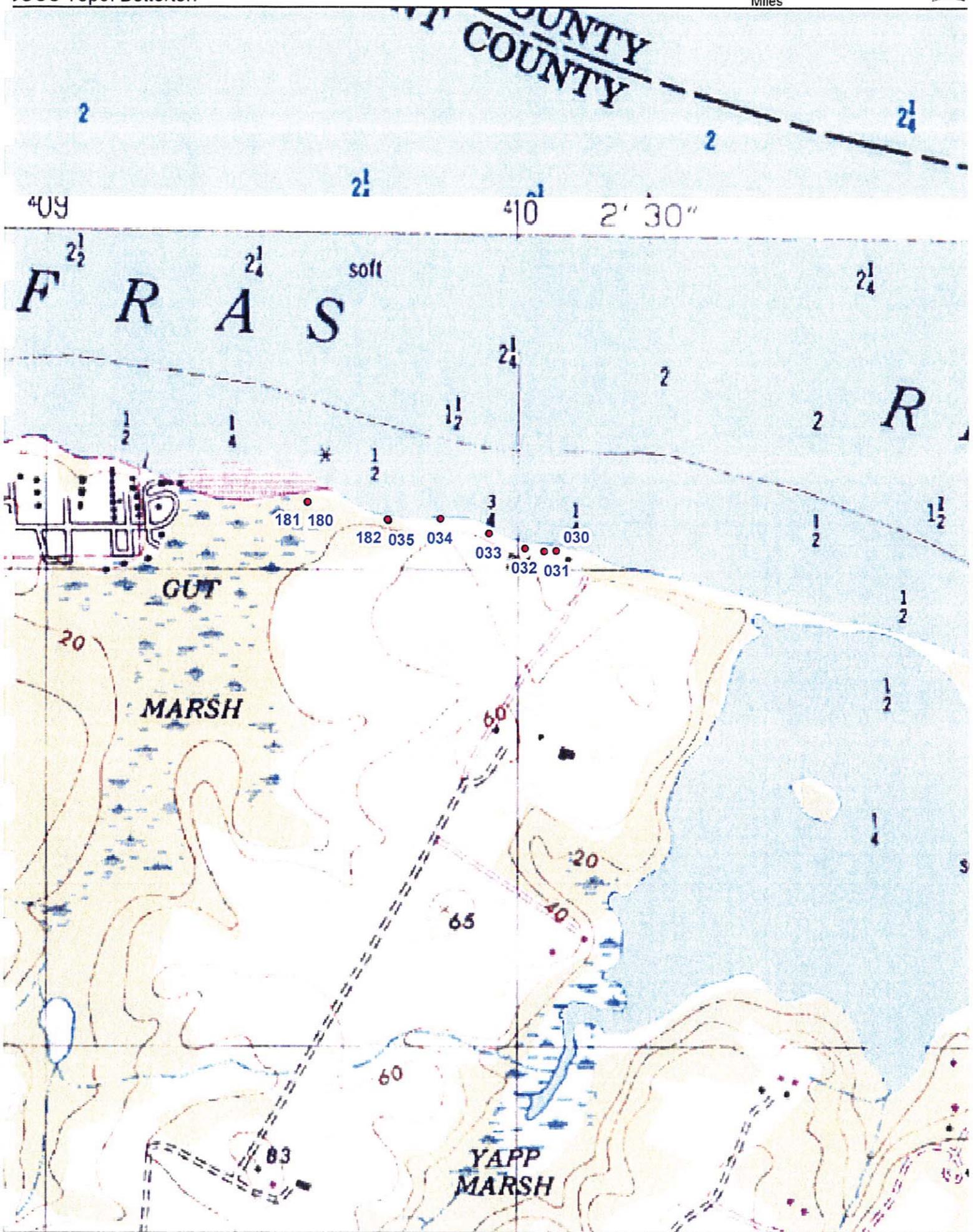


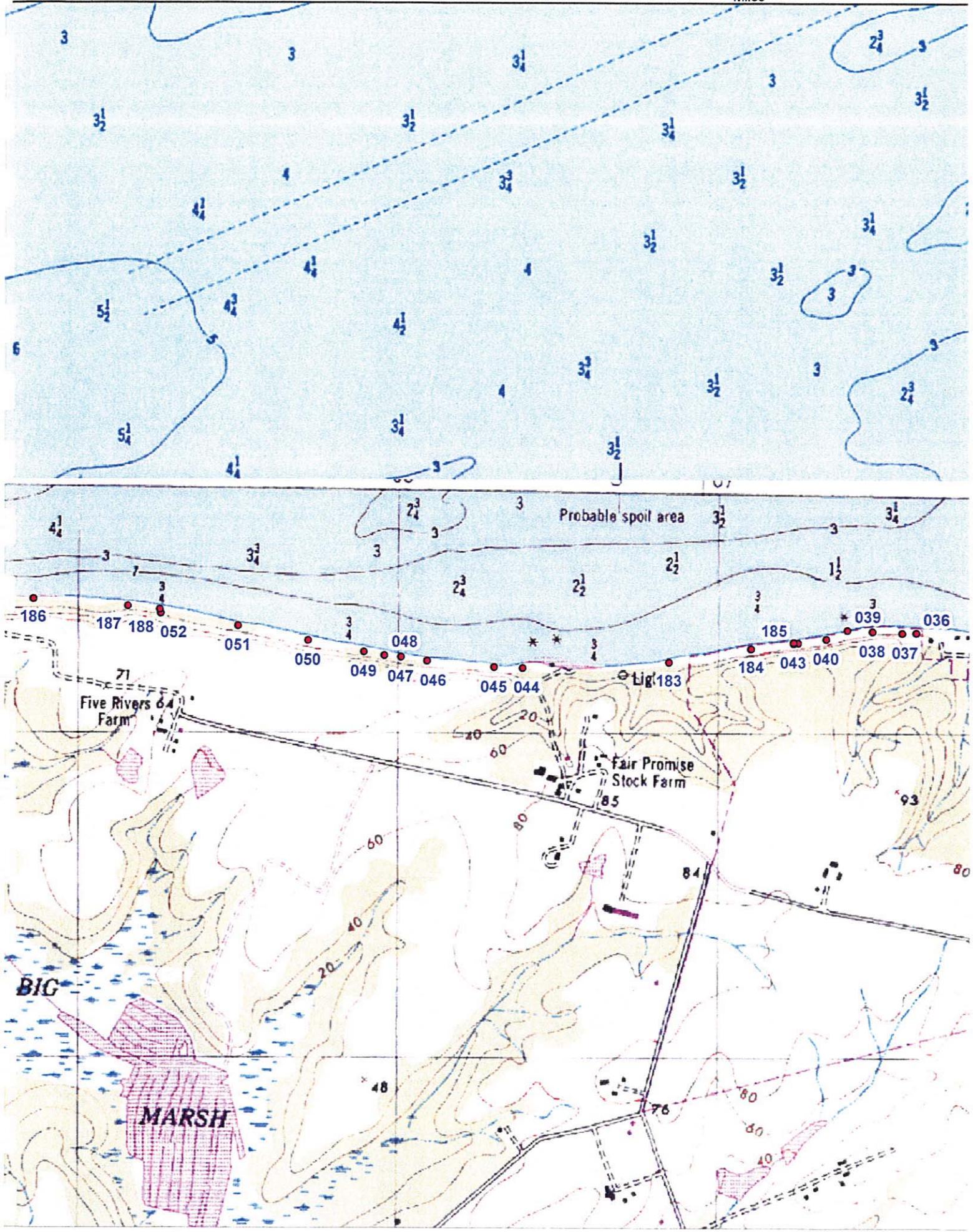






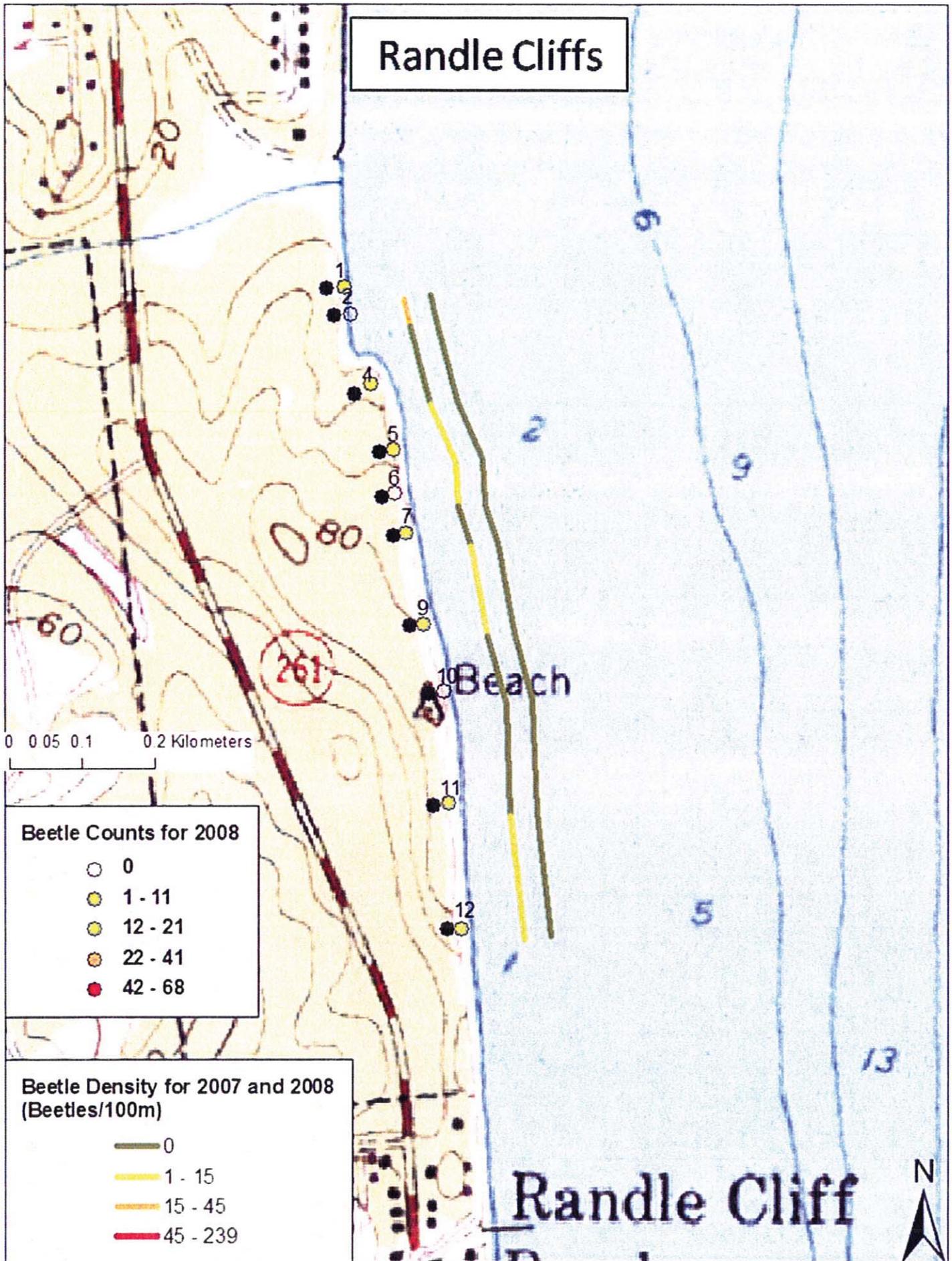








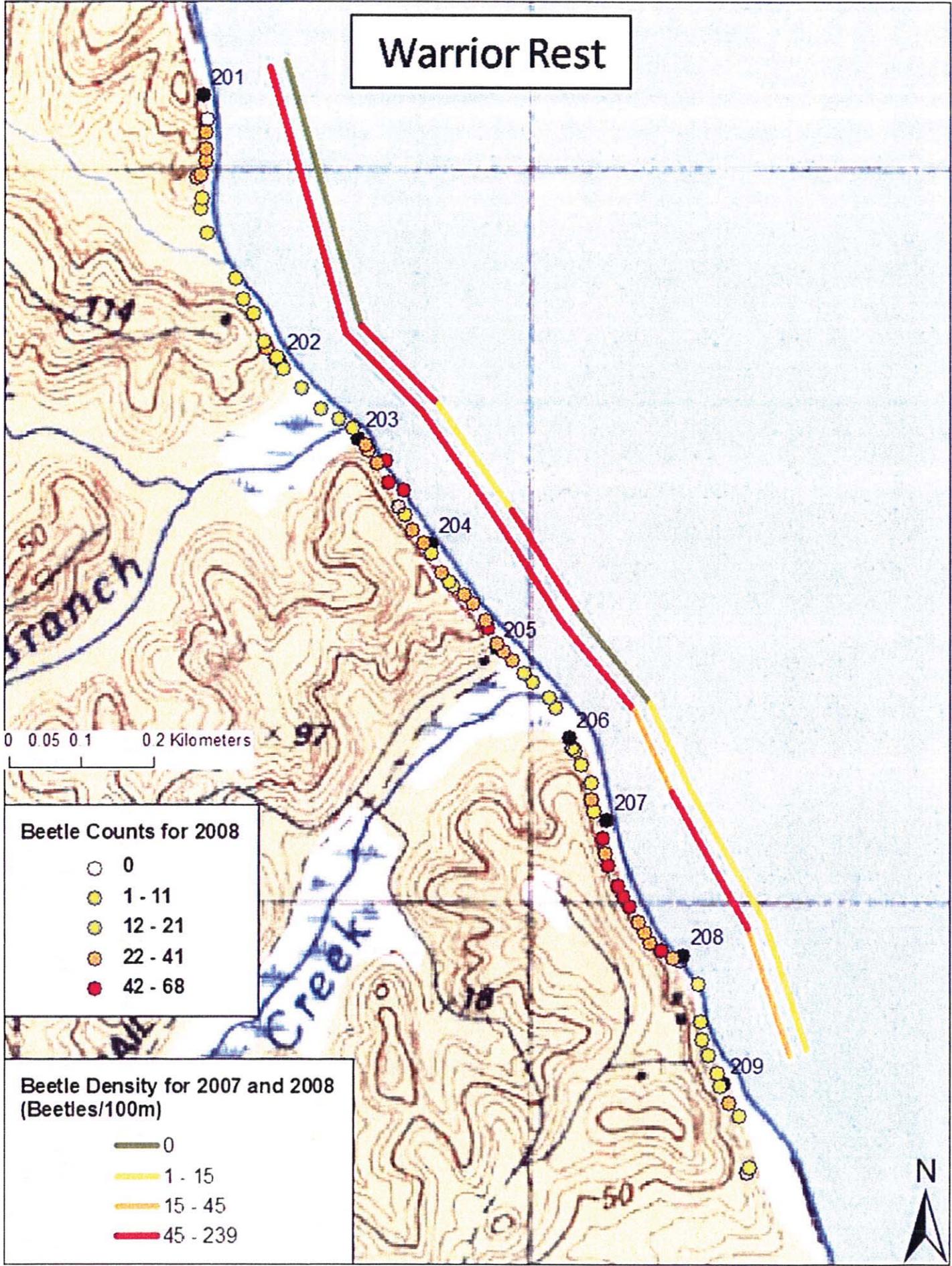
II. USGS Topo maps showing densities of adult PTB in 2007 and 2008 within standard waypoints (above) and also within short sections of all Calvert and Sassafras sites.



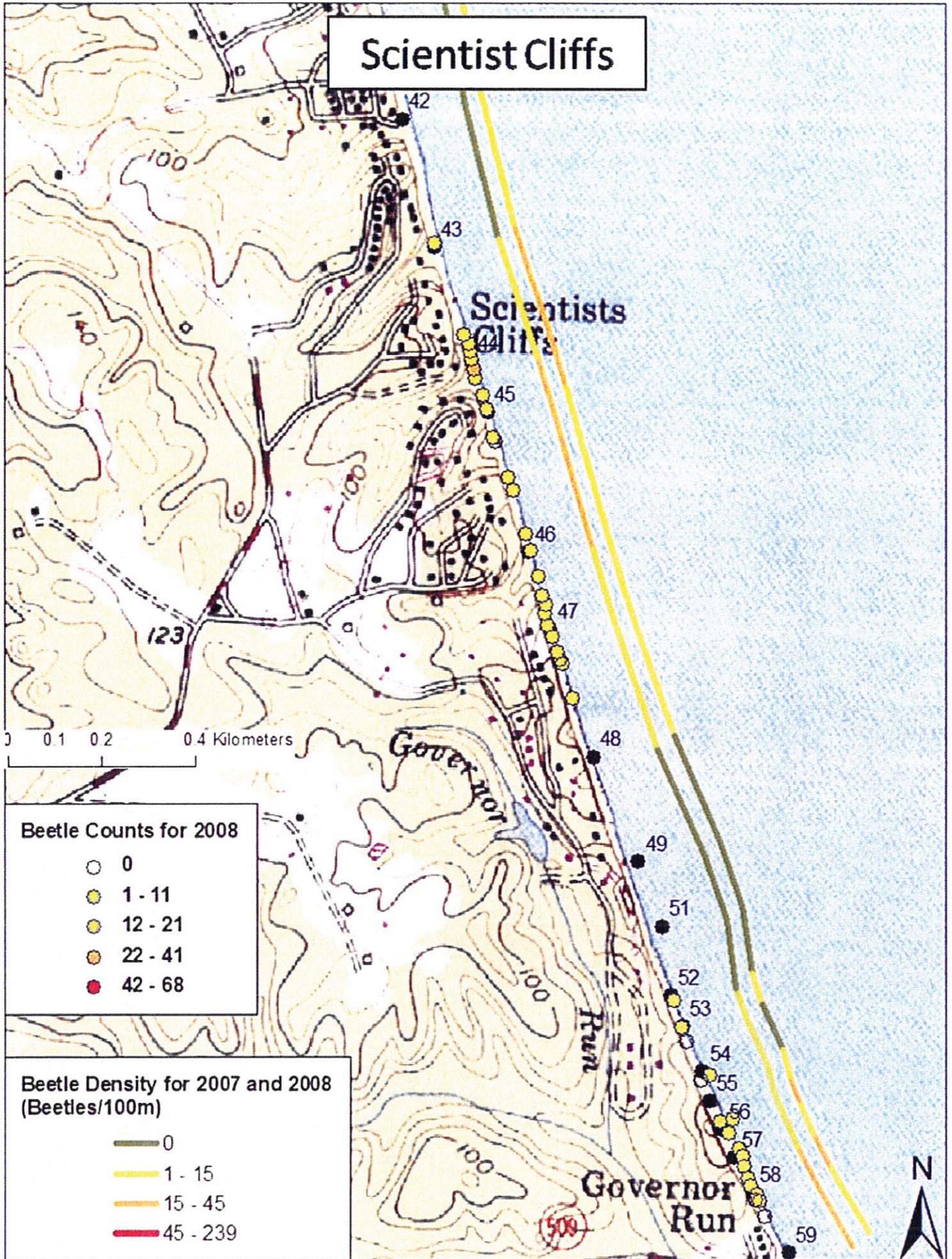
Bayside Forest



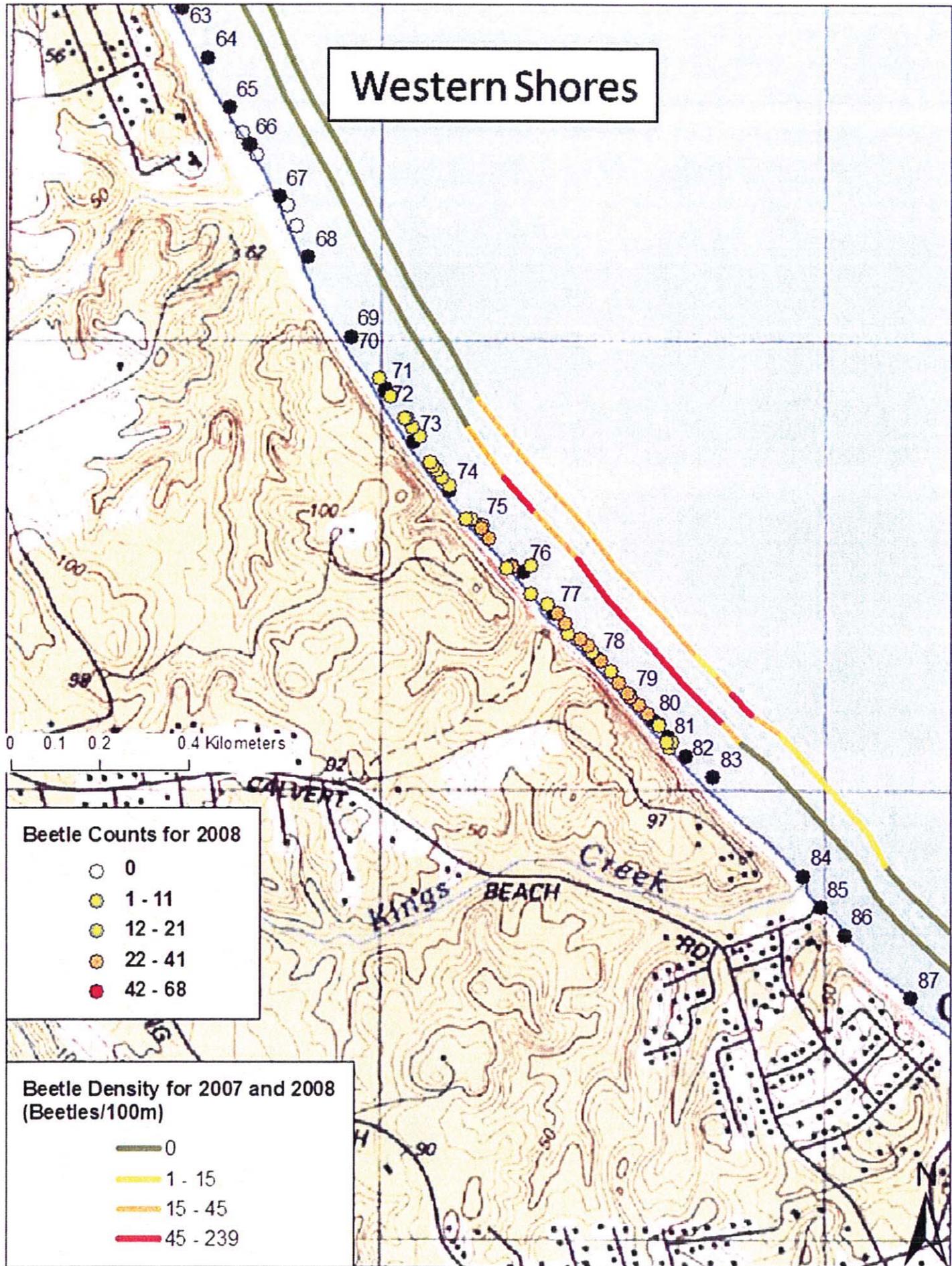
Warrior Rest



Scientist Cliffs

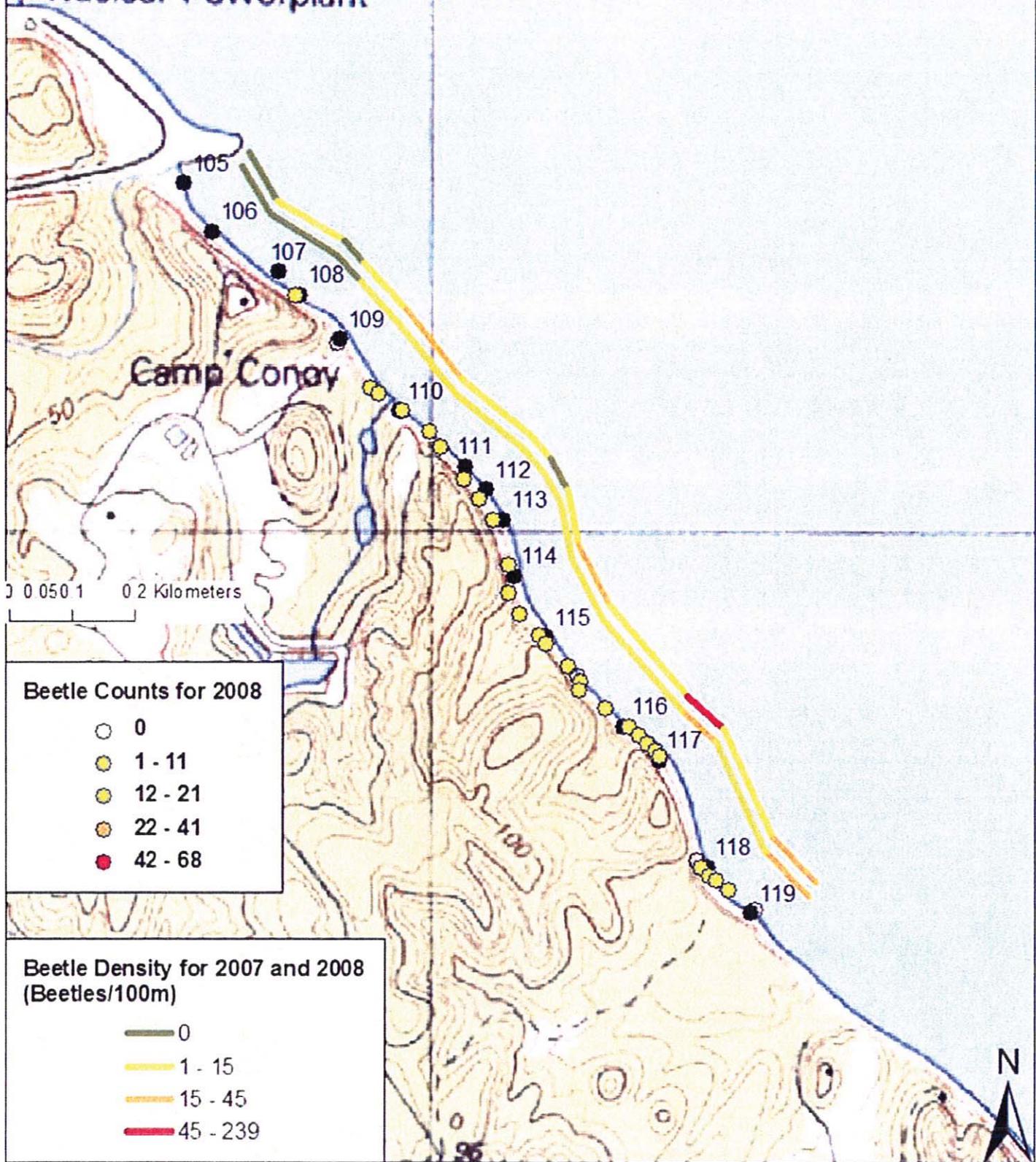


Western Shores



Power Plant

Calvert Cliffs
Nuclear Powerplant



Camp Conoy

0 0.05 0.1 0.2 Kilometers

Beetle Counts for 2008

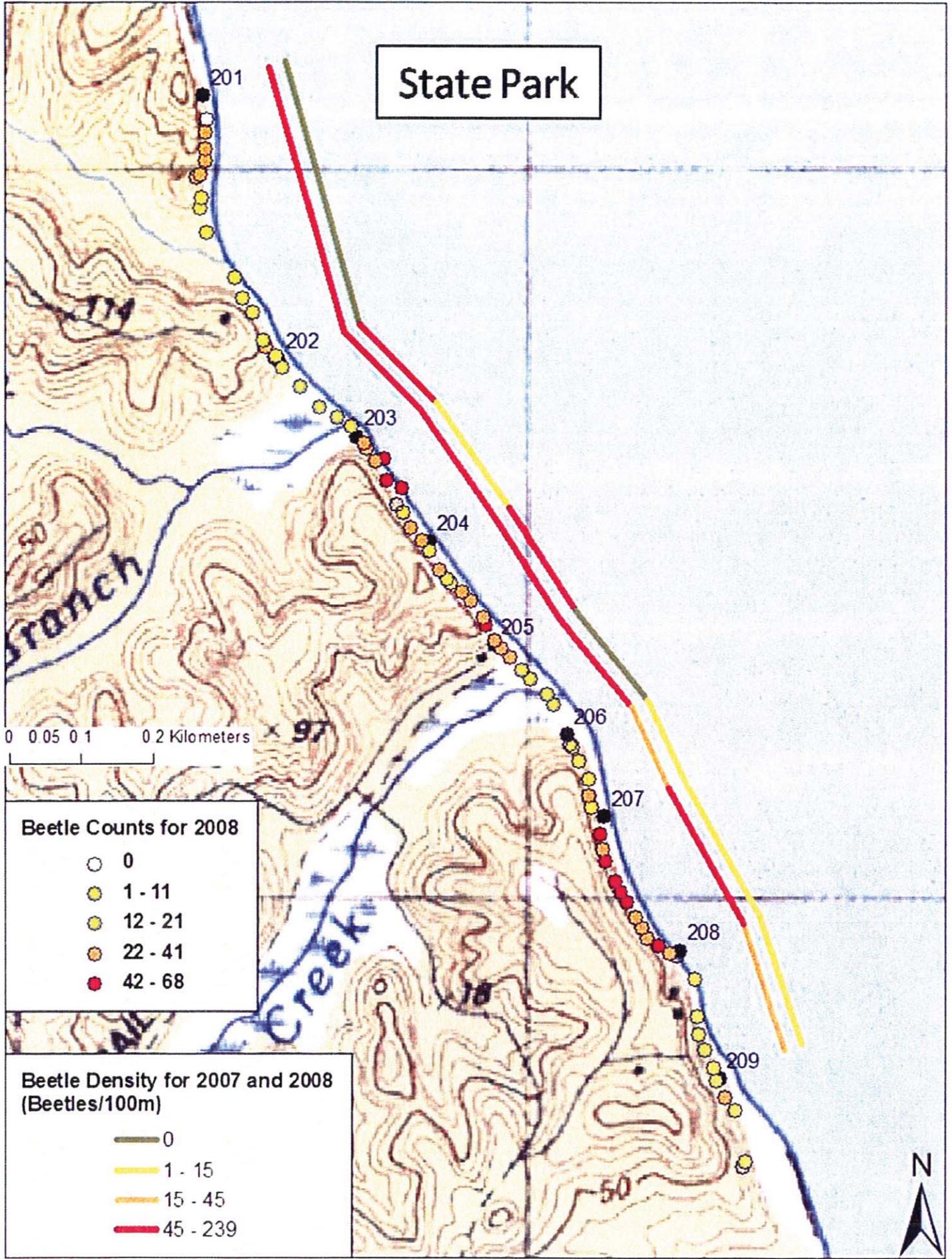
- 0
- 1 - 11
- 12 - 21
- 22 - 41
- 42 - 68

Beetle Density for 2007 and 2008 (Beetles/100m)

- 0
- 1 - 15
- 15 - 45
- 45 - 239



State Park



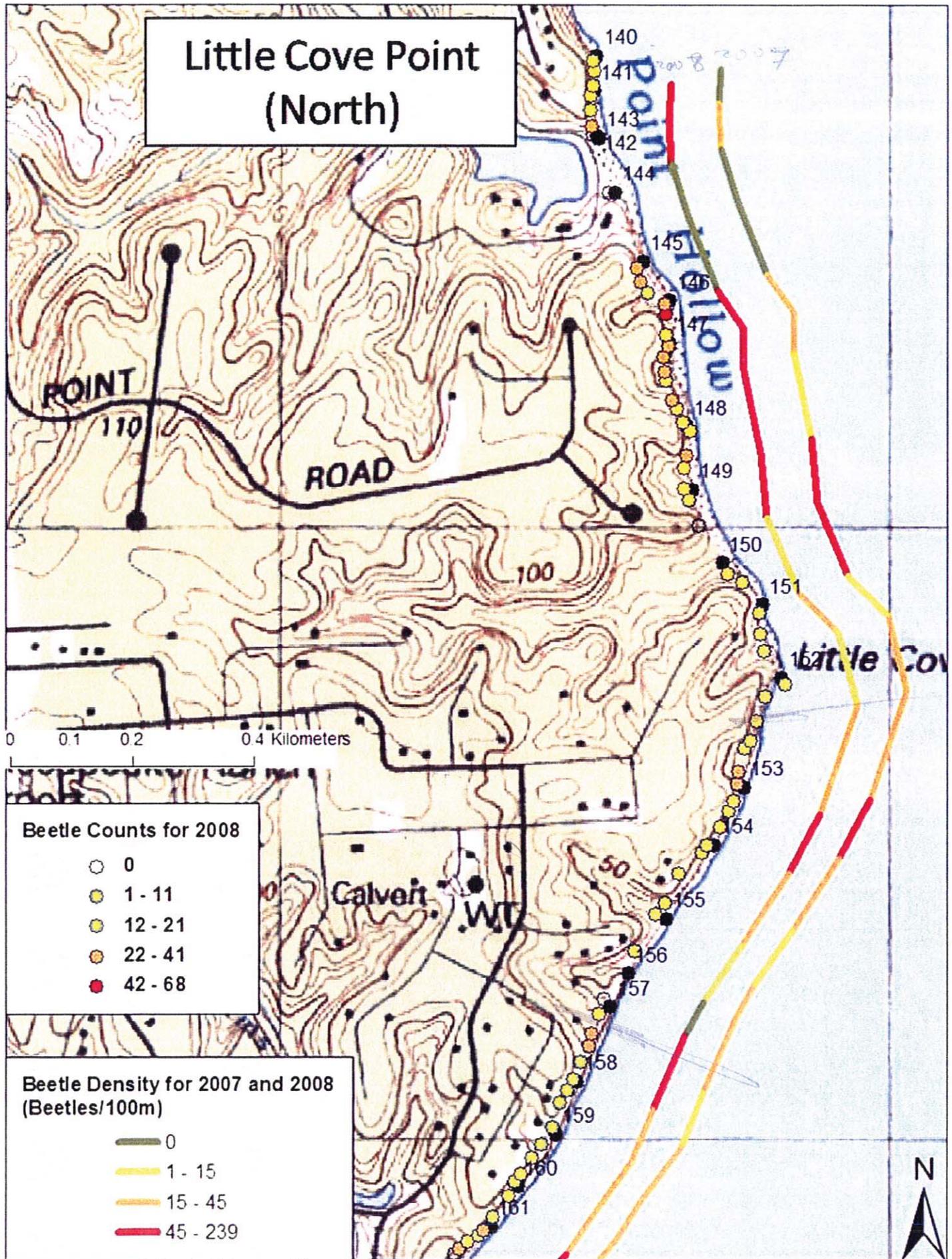
Beetle Counts for 2008

- 0
- 1 - 11
- 12 - 21
- 22 - 41
- 42 - 68

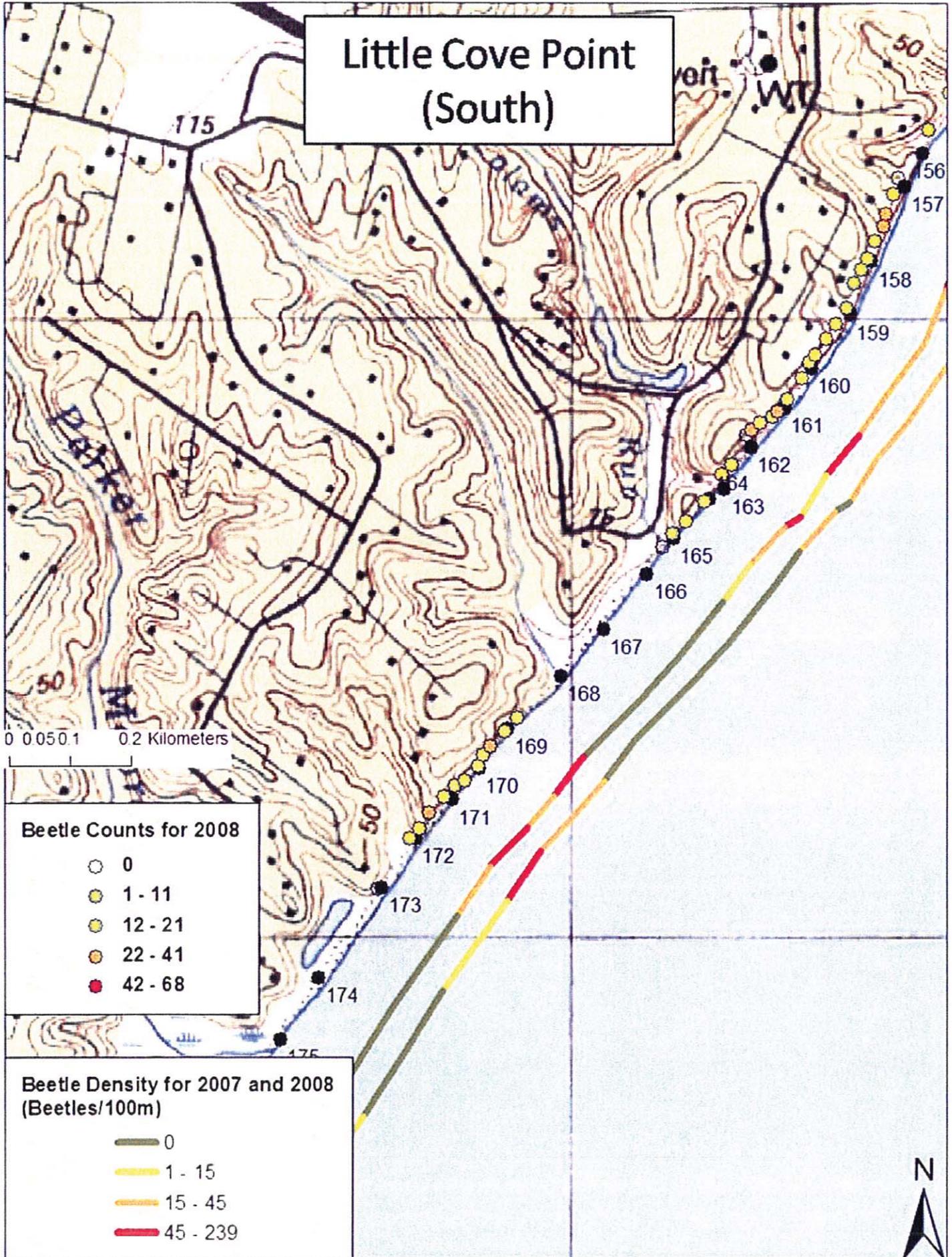
Beetle Density for 2007 and 2008 (Beetles/100m)

- 0
- 1 - 15
- 15 - 45
- 45 - 239

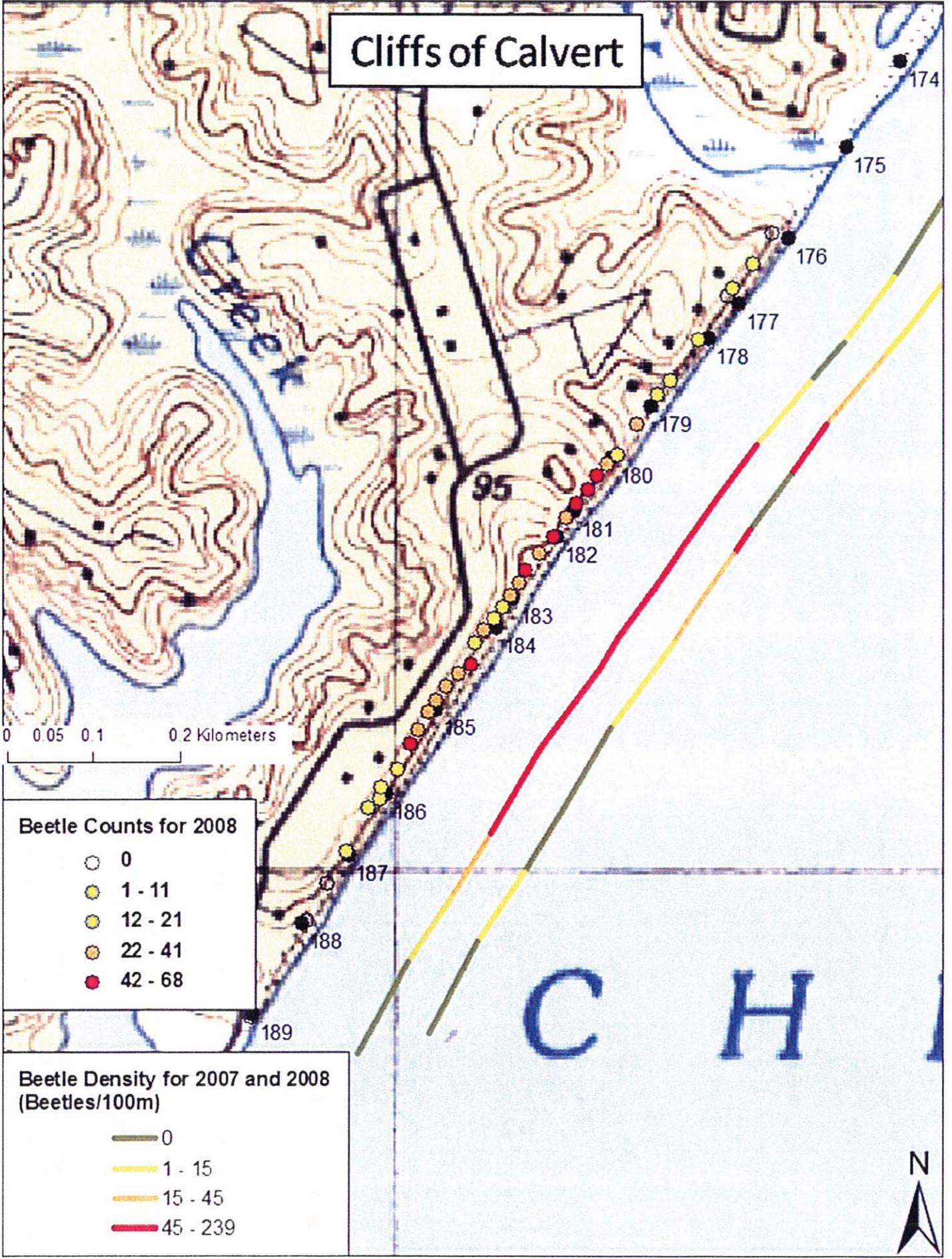
Little Cove Point (North)



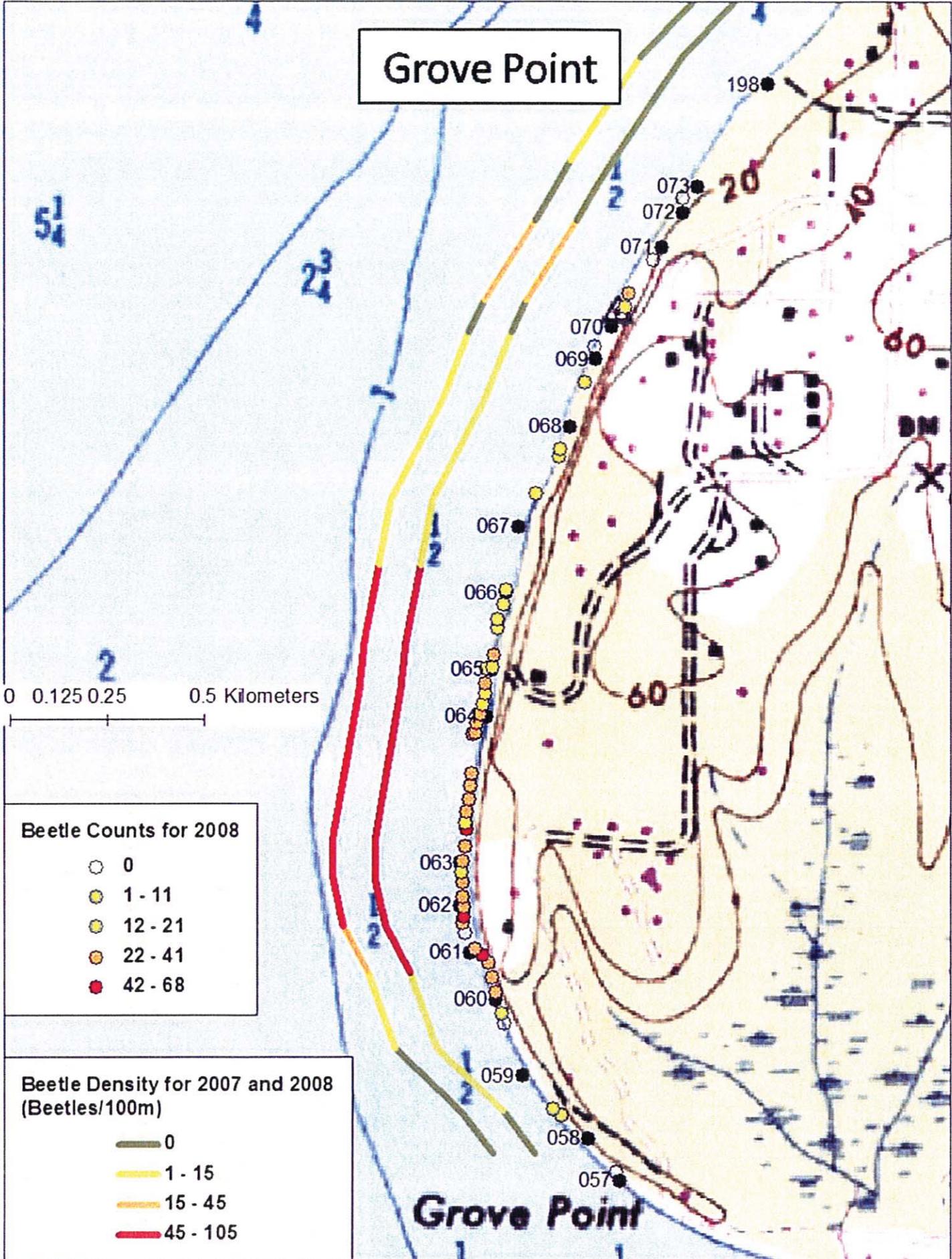
Little Cove Point (South)



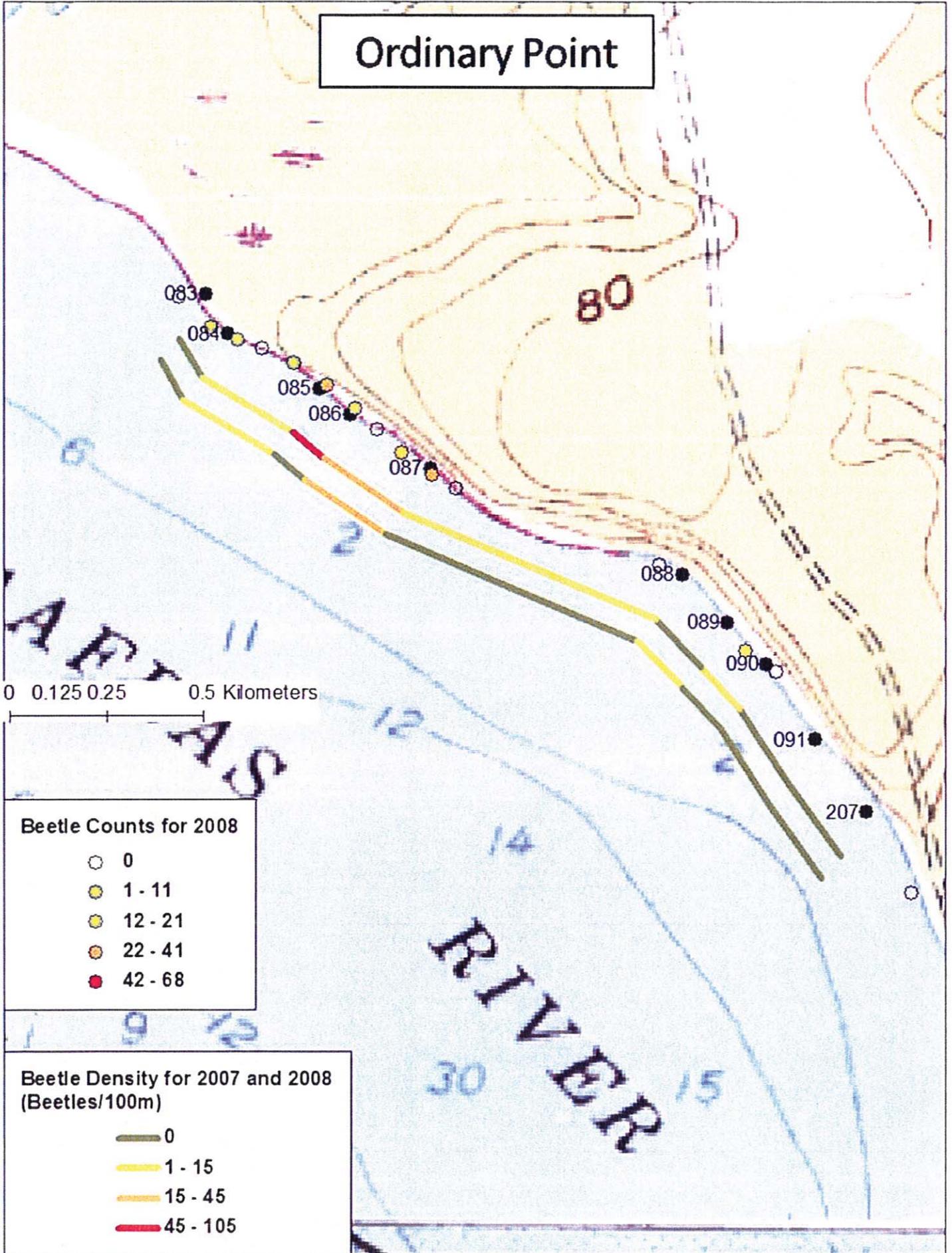
Cliffs of Calvert

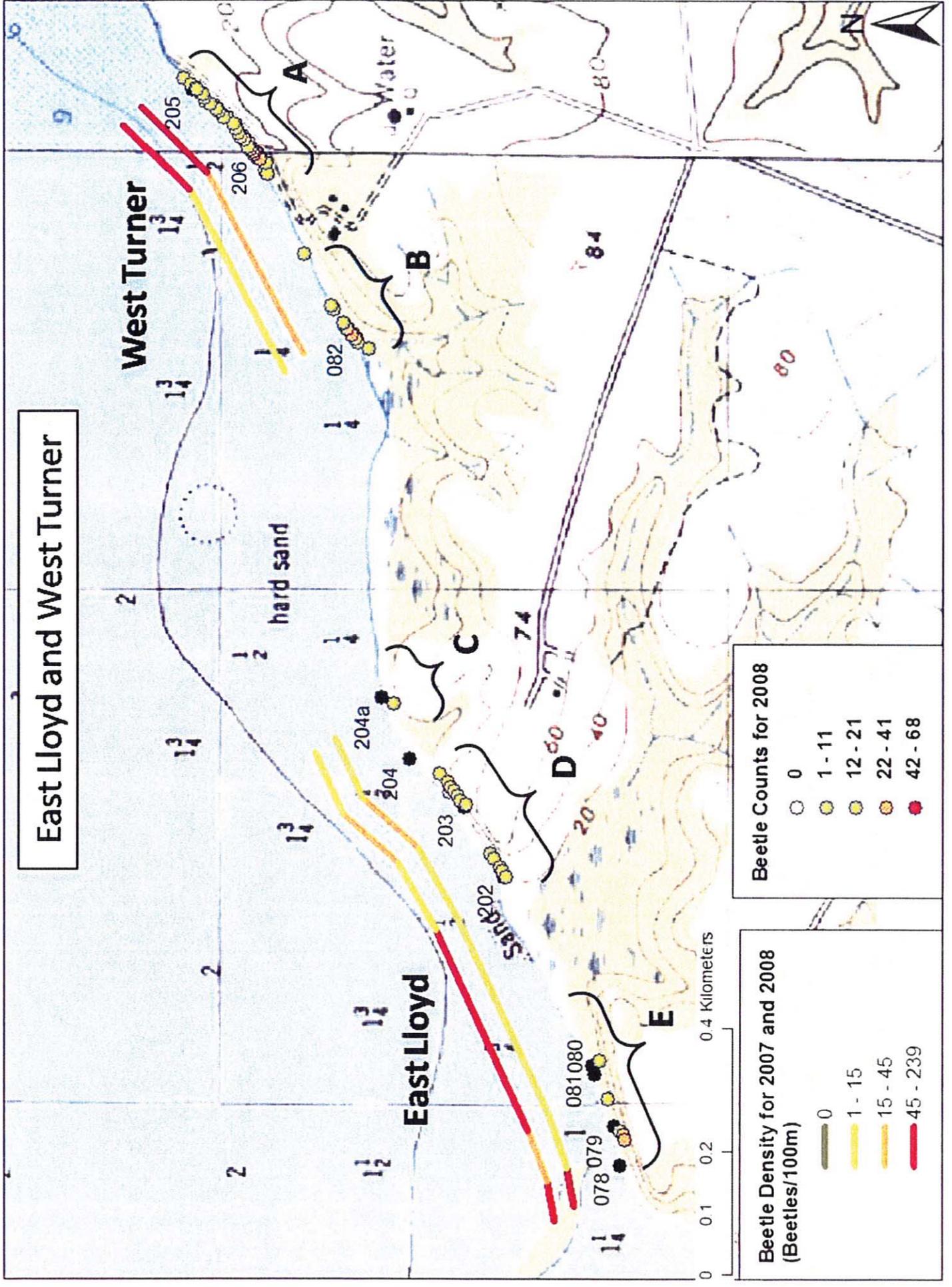


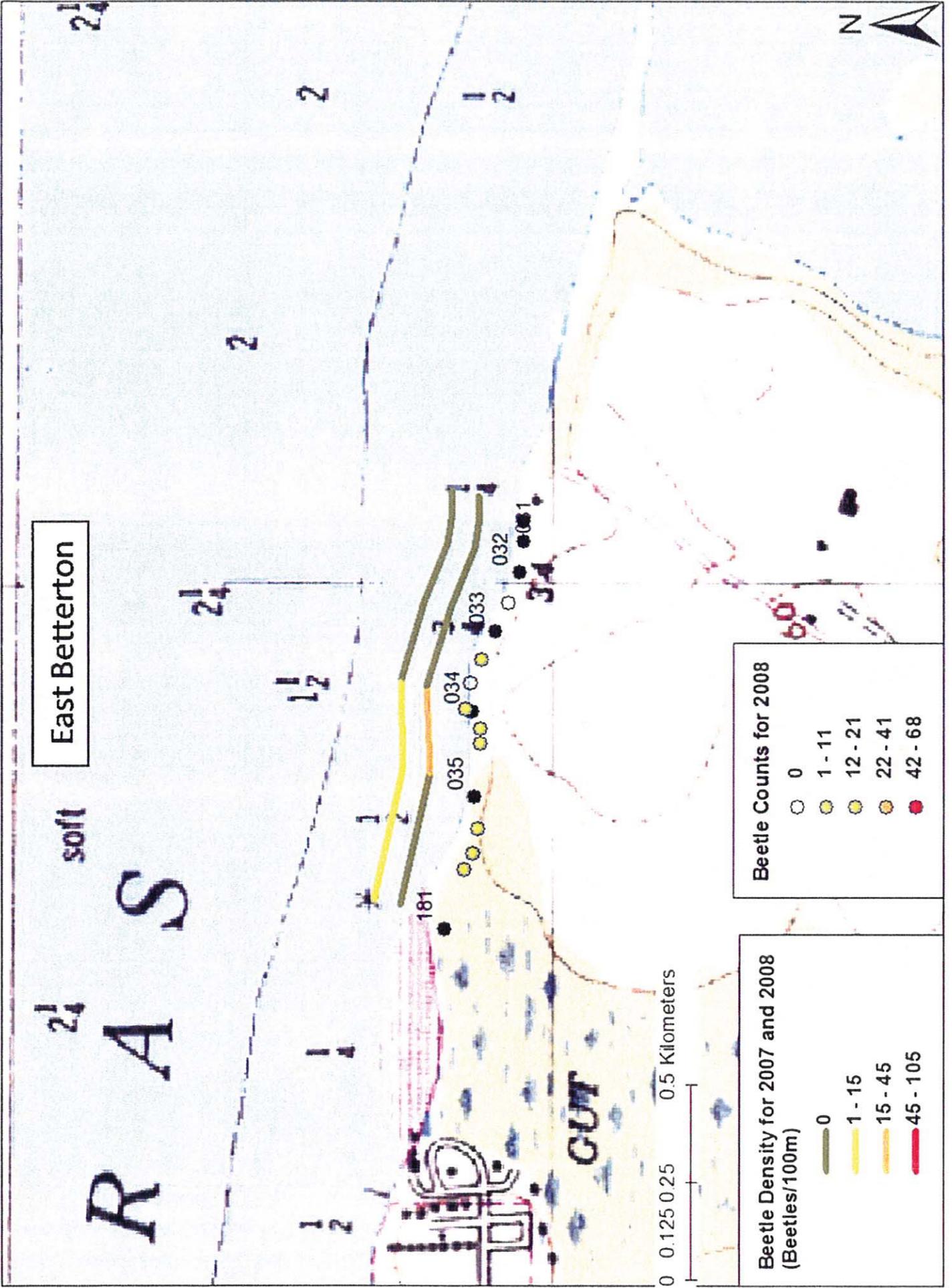
Grove Point



Ordinary Point







East Betterton

soft

RAS

GUT

0 0.125 0.25 0.5 Kilometers

Beetle Density for 2007 and 2008
(Beetles/100m)

- 0
- 1 - 15
- 15 - 45
- 45 - 105

Beetle Counts for 2008

- 0
- 1 - 11
- 12 - 21
- 22 - 41
- 42 - 68

24

2

2

1 2

24

1 2

181

035

034

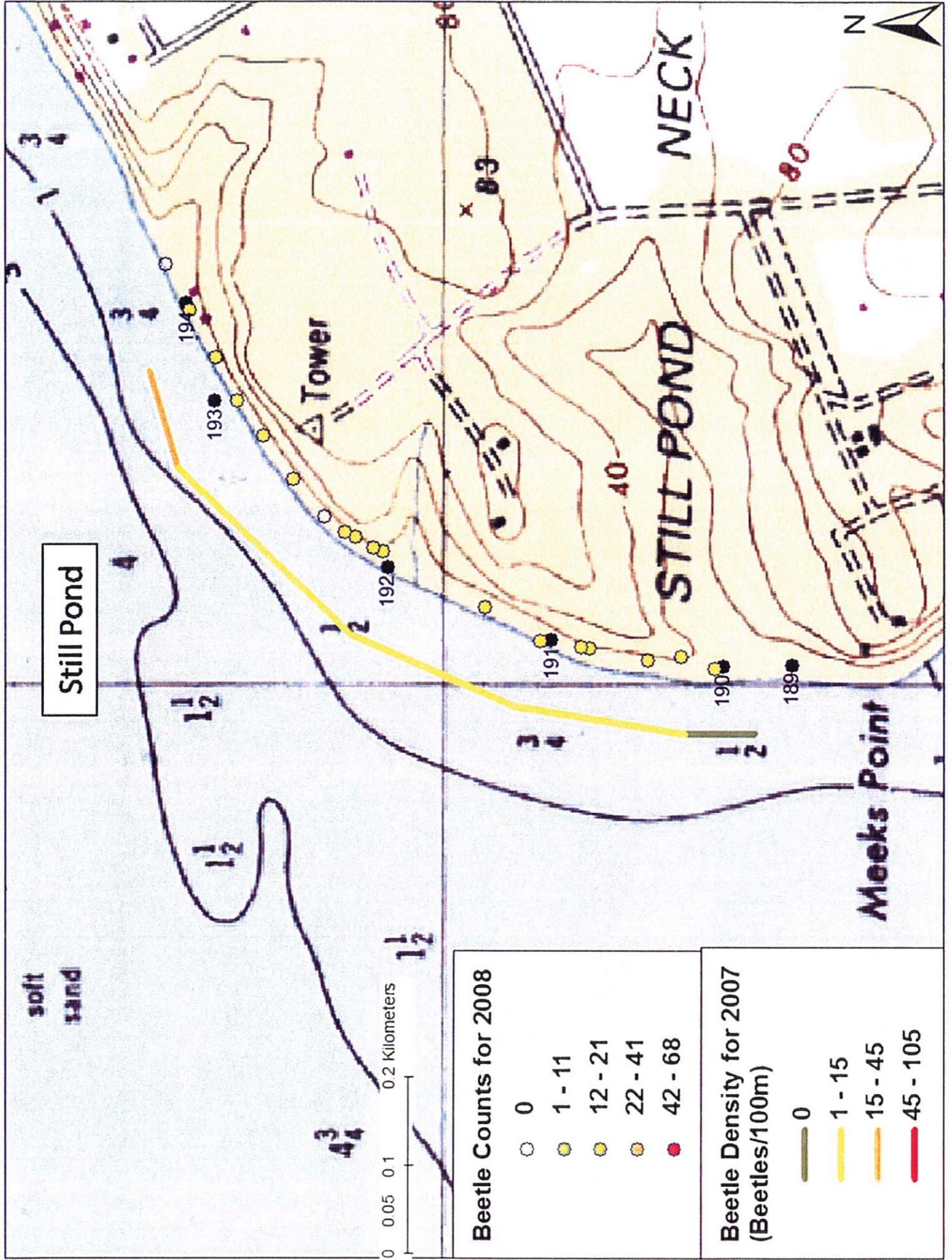
033

032

001

34





Appendix III

III. Photographic panoramas of the shoreline and bluffs at all Calvert and Sassafras PTB sites in 2007 (North Stillpond not included).

Calvert County Sites

2007

Randle Cliffs



Bayside Forest



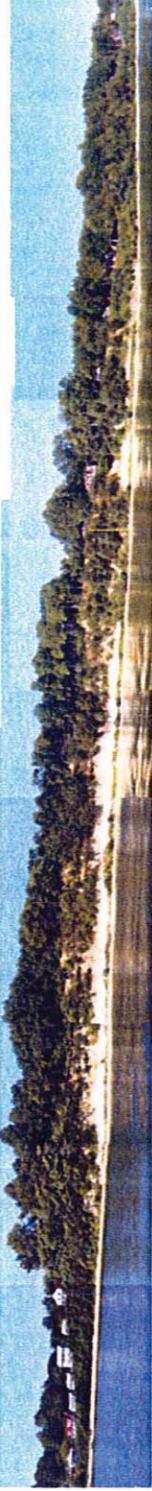
Warrior Rest

WR 1 WR 2 WR 3 WR 4



Scientist Cliffs

SC 1



SC 2



SC 3



SC 4



Western Shores

WS 1



WS

WS 2



WS 2

WS 3

WS 3

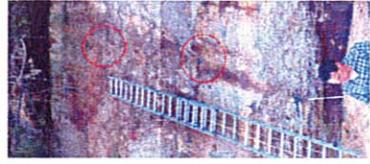
State Park

SP 1

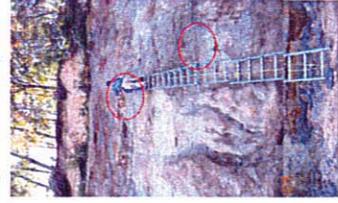


CD Start

Low Density



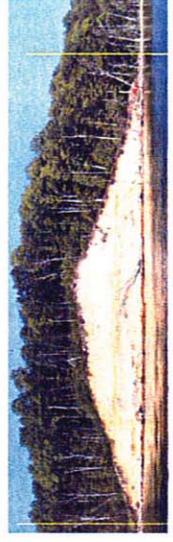
High Density



SP 2



SP 3



Little Cove Point

LCP 1



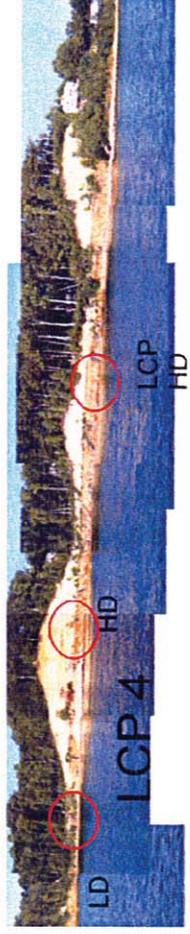
LCP 2



LCP 3



LCP 4



LCP 5



Cliffs of Calvert

CC 1



CC 2



CC 3



Low Density



High Density



High Density



Sassafras River Sites

2008

Grove Point

GP 1



GP 2



GP 3



High Density



High Density 2



Larvae



Grove Point Cont.

GP 4



Low Density



Low Density 2



GP 4 cont.



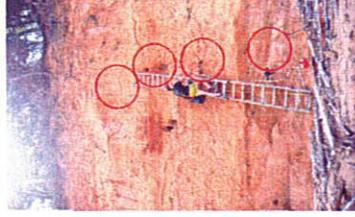
Ordinary Point



West Turner A



High Density



West Turner B



Low Density



High Density



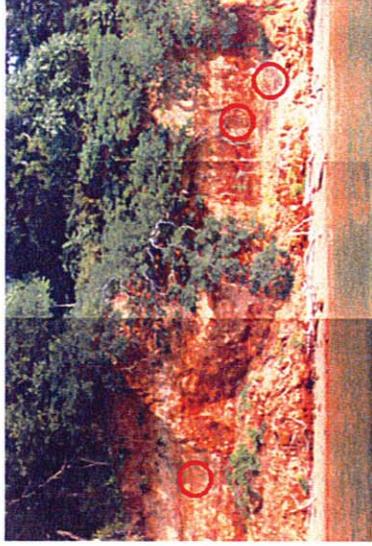
East Lloyd C



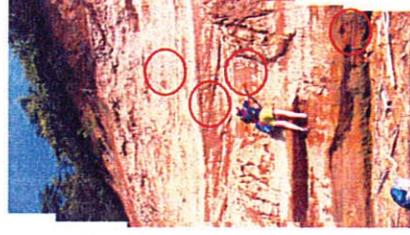
East Lloyd D



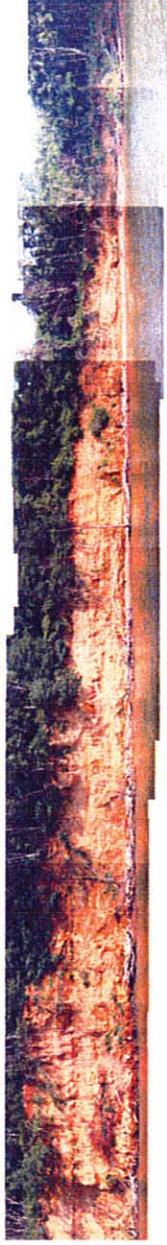
Low Density



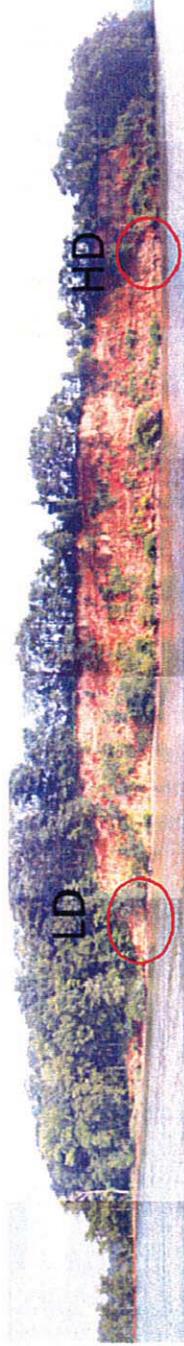
High Density



East Lloyd D cont.



East Lloyd E



Low Density



High Density



West Betterton



Low Density



High Density



East Betterton



Appendix IV

IV. Photographic panoramas of shoreline and bluffs at all Calvert and Sassafras sites from 2007 and from selected available sections in 2000 showing bluff habitat categories. Potential habitat is all of the bare unvegetated bluff face. Probable habitat is the area with suitable strata and shaded in light blue(North Stillpond not included).

Randle Cliffs 2007

Randle Cliffs South (top left) to North

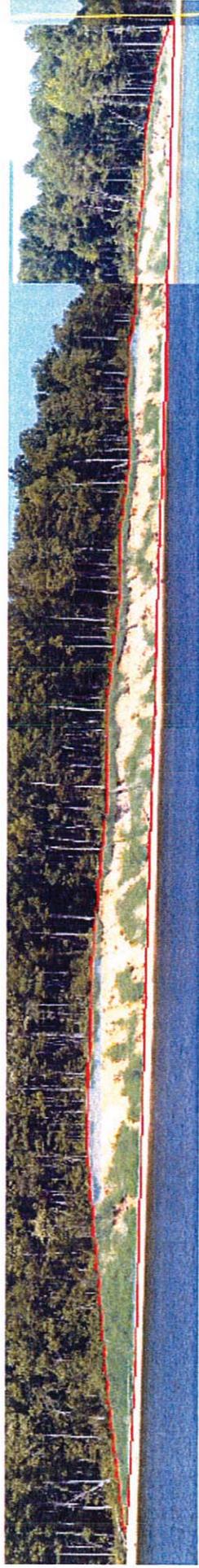
The bare region is the Calvert Formation which is unsuitable to PTBs and is therefore only characterized by potential habitat.



Bayside Forest 2007

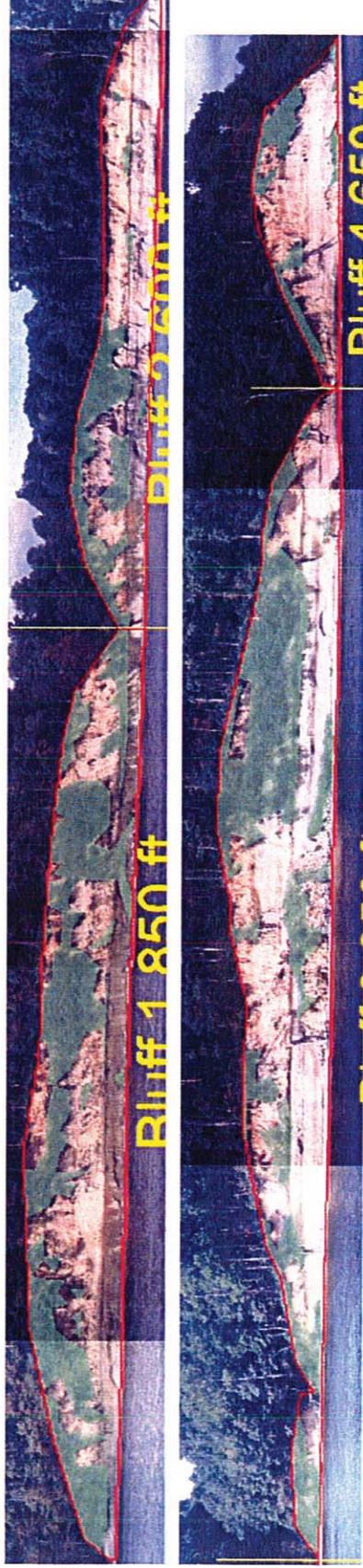
Bayside Forest South to North

The region indicated by the blue is Choptank Formation which is favorable to PTBs and is therefore probable habitat. The bare region is the Calvert Formation which is unsuitable to PTBs and is therefore only characterized as potential habitat.



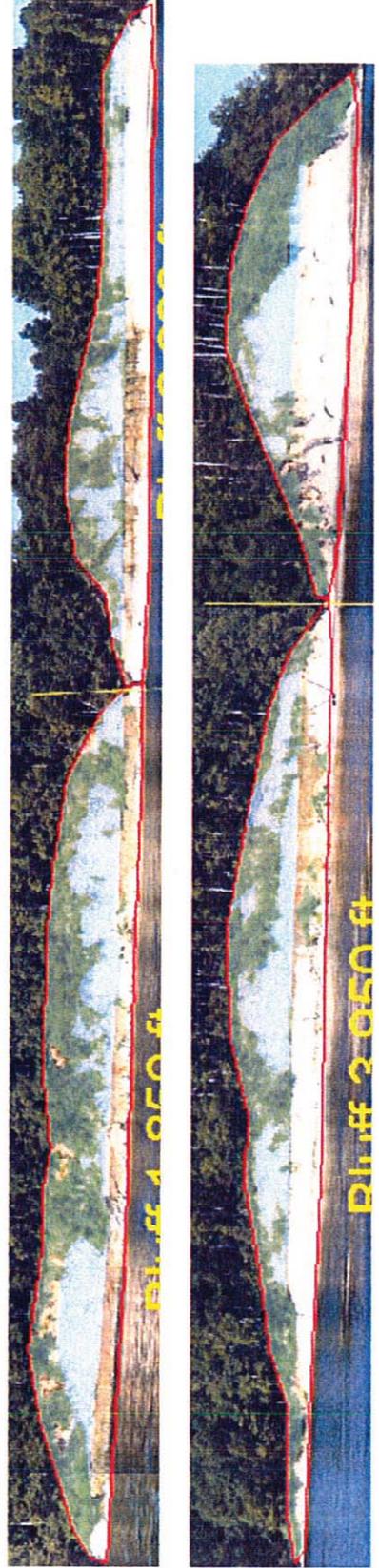
Warrior Rest

Warrior Rest 2000 South (left) to North
Yellow lines and numbers should be disregarded.



Warrior Rest 2007 South to North

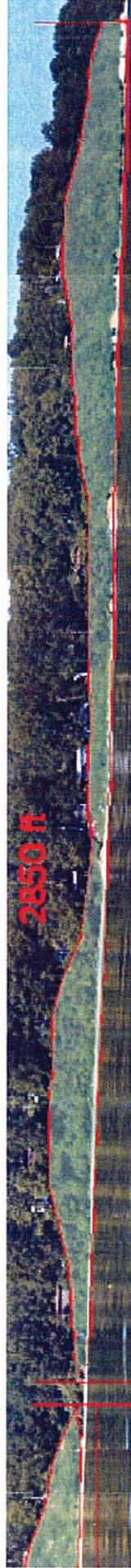
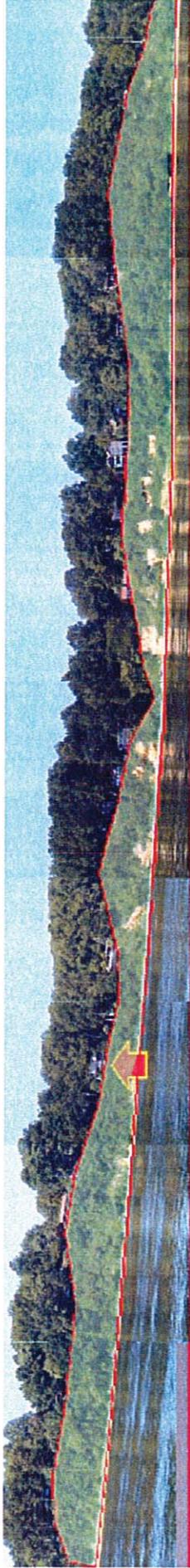
The region indicated by the blue is Choptank Formation which is favorable to PTBs and is therefore probable habitat. The bare region is the Calvert Formation which is unsuitable to PTBs and is therefore only characterized by potential habitat.



Scientist Cliffs 2007

Scientist Cliffs South (left) to North

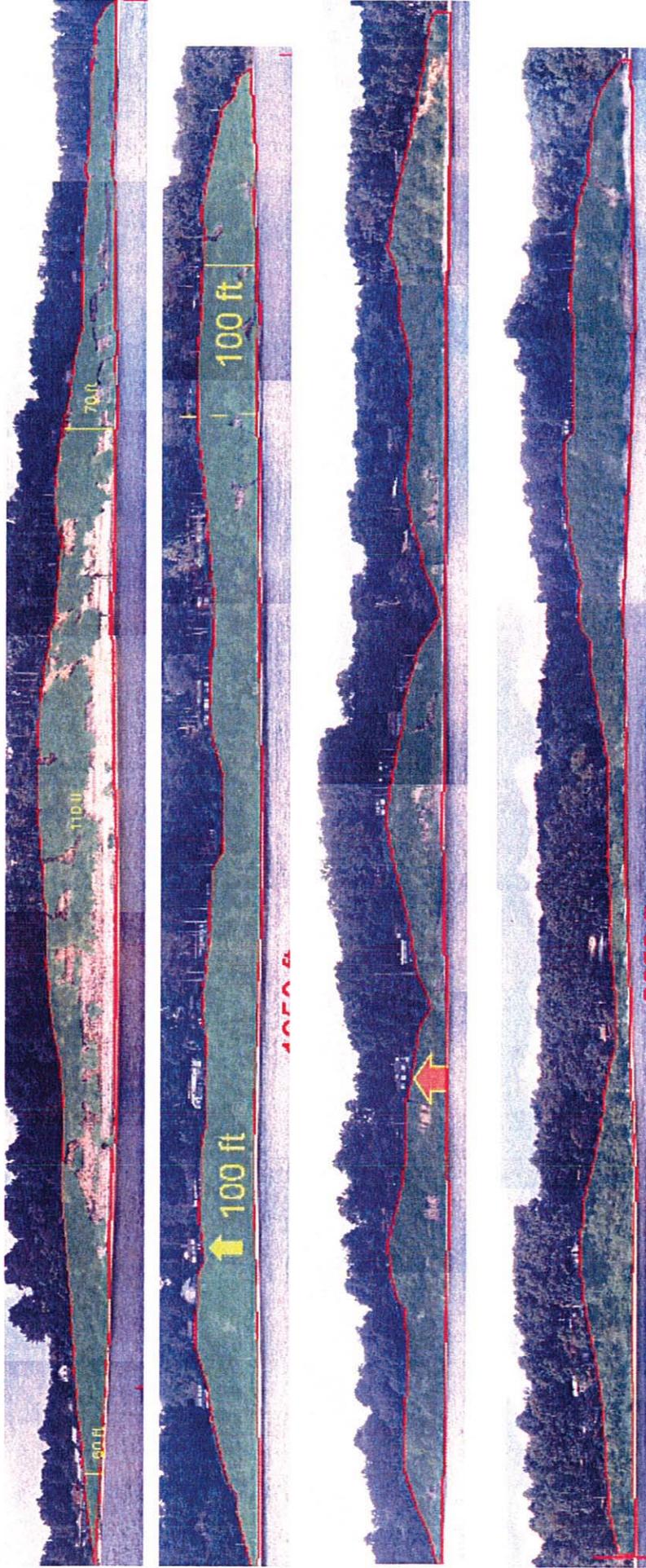
The region indicated by the blue is Choptank Formation which is favorable to PTBs and is therefore probable habitat. The bare region is the Calvert Formation which is unsuitable to PTBs and is therefore only characterized by potential habitat. Yellow lines and numbers should be disregarded.



Scientist Cliffs 2000

Scientist Cliffs South to North

Yellow lines and numbers should be disregarded.



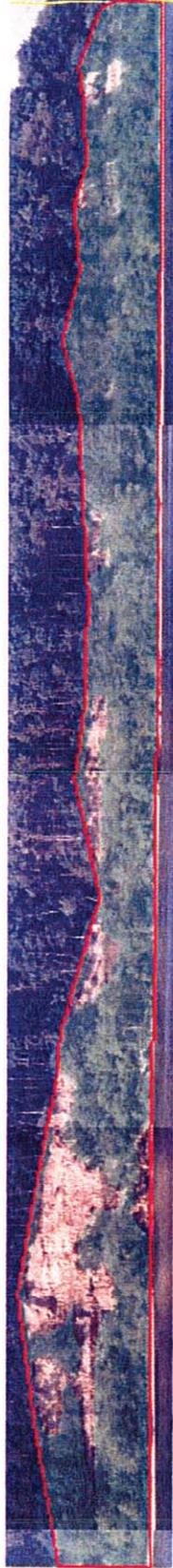
Western Shores

Western Shores 2007 South to North

The region indicated by the blue is Choptank Formation which is favorable to PTBs and probable habitat. The bare region is the Calvert Formation which is unsuitable to PTBs and is therefore potential habitat. Note that top panel includes shoreline groins.

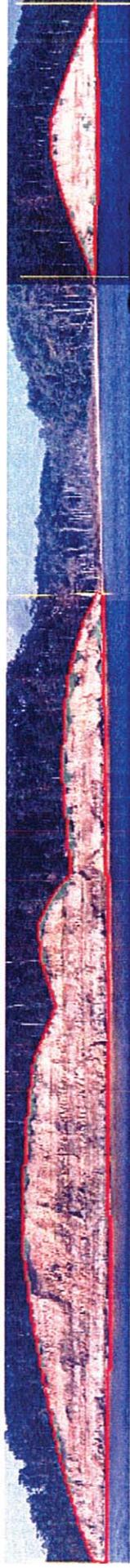
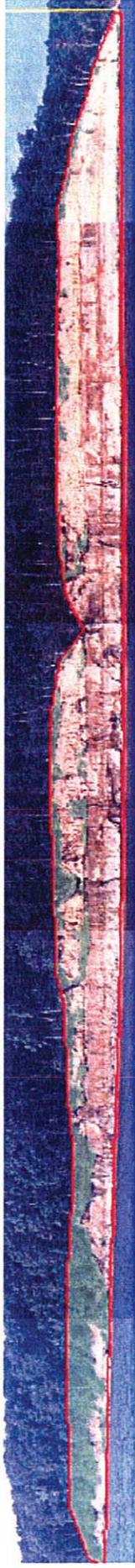


2000 photos



State Park

State Park 2000 South to North



State Park 2007 South to North

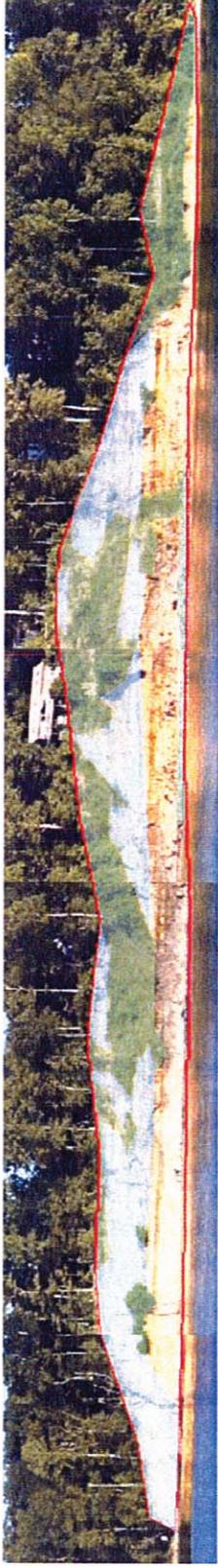
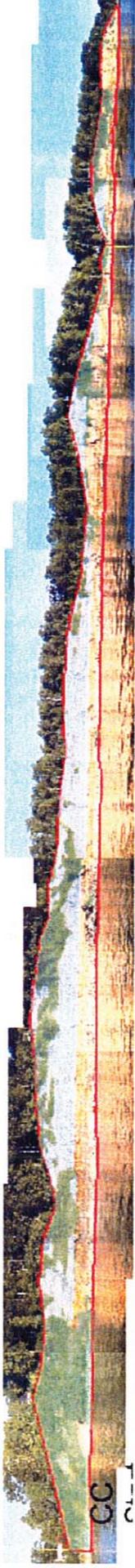
The region indicated by the blue is Eastover Formation which is favorable to PTBs and is therefore probable habitat. The bare region is the St. Mary's Formation which is unsuitable to PTBs and is therefore only characterized by potential habitat.



Cliffs of Calvert

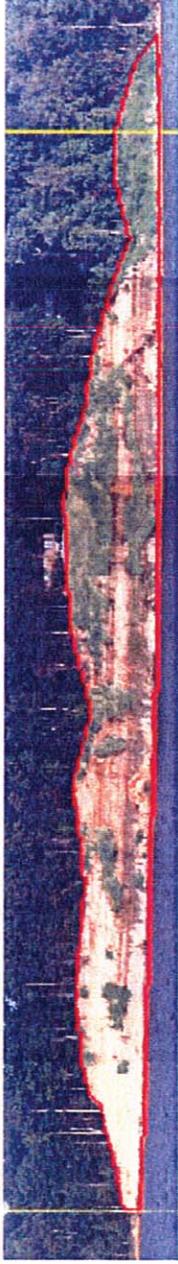
Cliffs of Calvert South to North 2007

The region indicated by the blue is Eastover Formation which is favorable to PTBs and is therefore probable habitat. The bare region is the St. Mary's Formation which is unsuitable to PTBs and is therefore only characterized by potential habitat.



Cliffs of Calvert South to North 2000

The bare region is the St. Mary's Formation which is unsuitable to PTBs and is therefore only characterized by potential habitat.



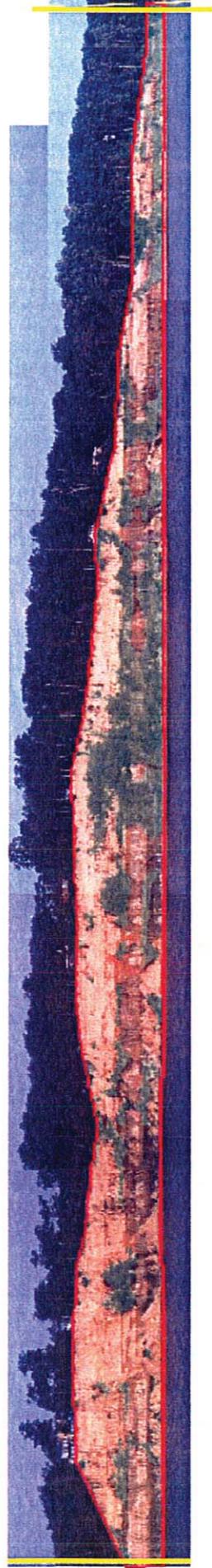
Little Cove Point 2007

Little Cove Point South to North

The region indicated by the blue is Eastover Formation which is favorable to PTBs and is therefore probable habitat. The bare region is the St. Mary's Formation which is unsuitable to PTBs and is therefore only characterized by potential habitat. The area between the yellow lines is the area used in the comparative analysis of vegetation coverage from 2000 to 2007



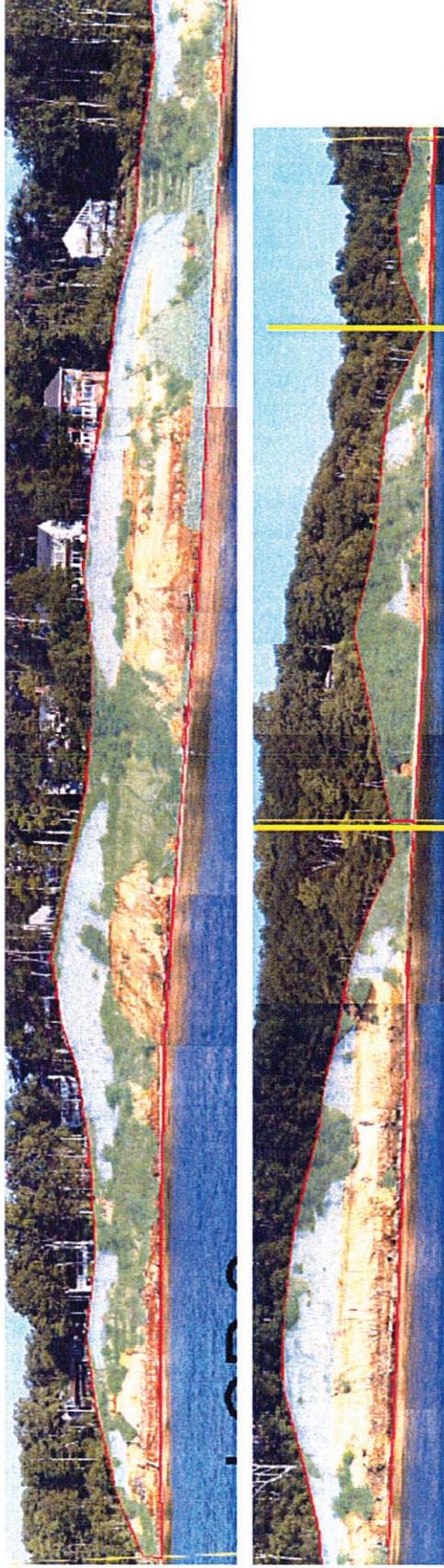
2000 Photos



Little Cove Point 2007 cont.

Little Cove Point South to North

The region indicated by the blue is Eastover Formation which is favorable to PTBs and is therefore probable habitat. The bare region is the St. Mary's Formation which is unsuitable to PTBs and is therefore only characterized by potential habitat. The area between the yellow lines is the area used in the comparative analysis of vegetation coverage from 2000 to 2007



2000 Photos



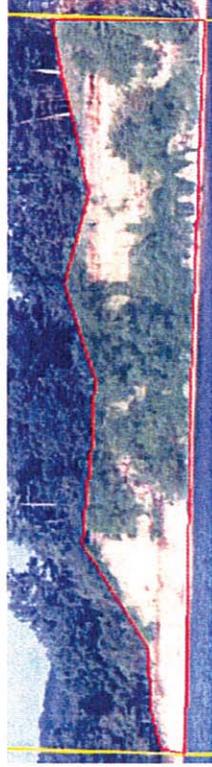
Little Cove Point 2007

Little Cove Point South to North

The region indicated by the blue is Eastover Formation which is favorable to PTBs and is therefore probable habitat. The bare region is the St. Mary's Formation which is unsuitable to PTBs and is therefore only characterized by potential habitat. The area between the yellow lines is the area used in the comparative analysis of vegetation coverage from 2000 to 2007



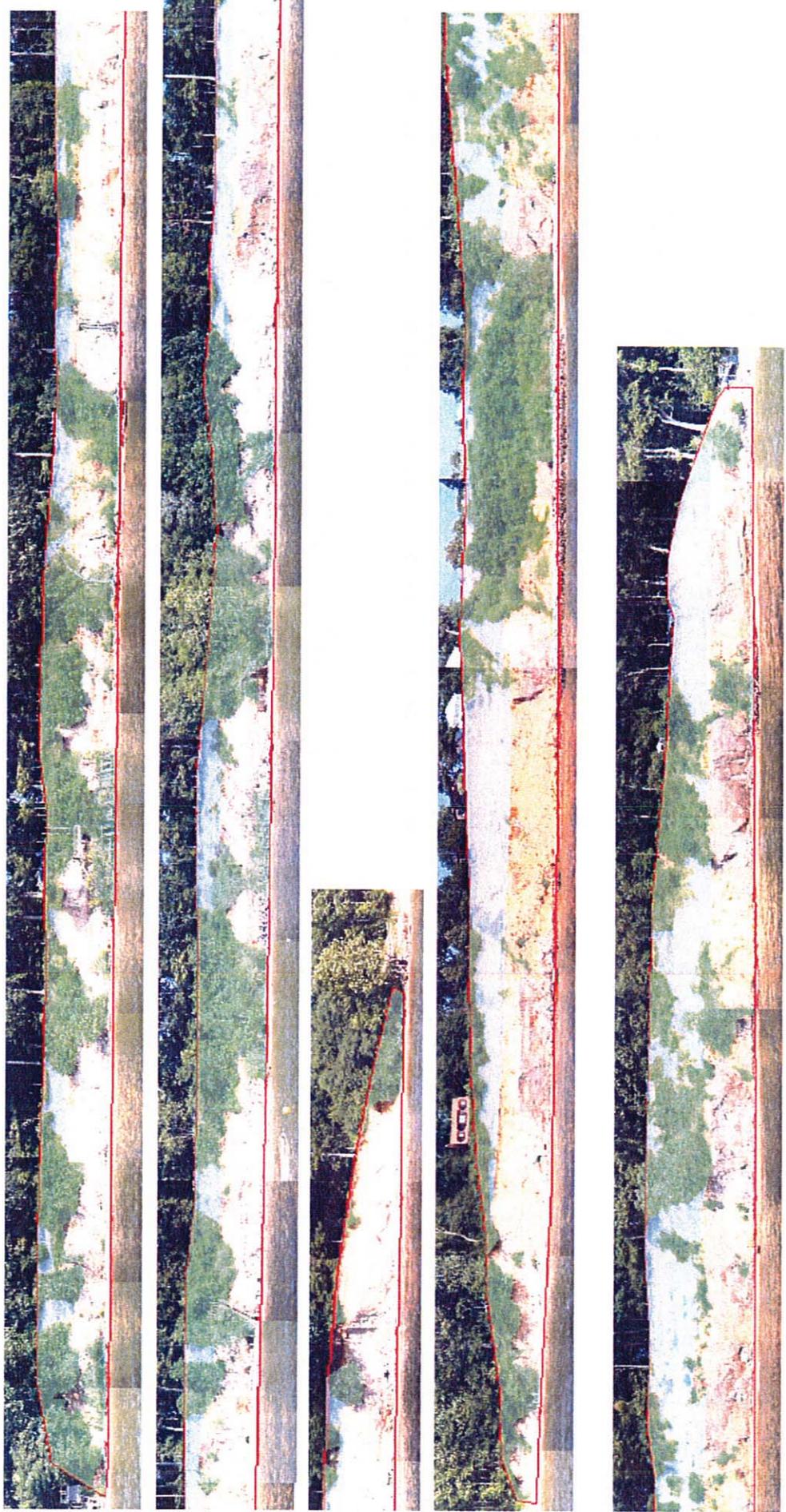
2000 photos



Grove Point 2007

Grove Point North (left) to South

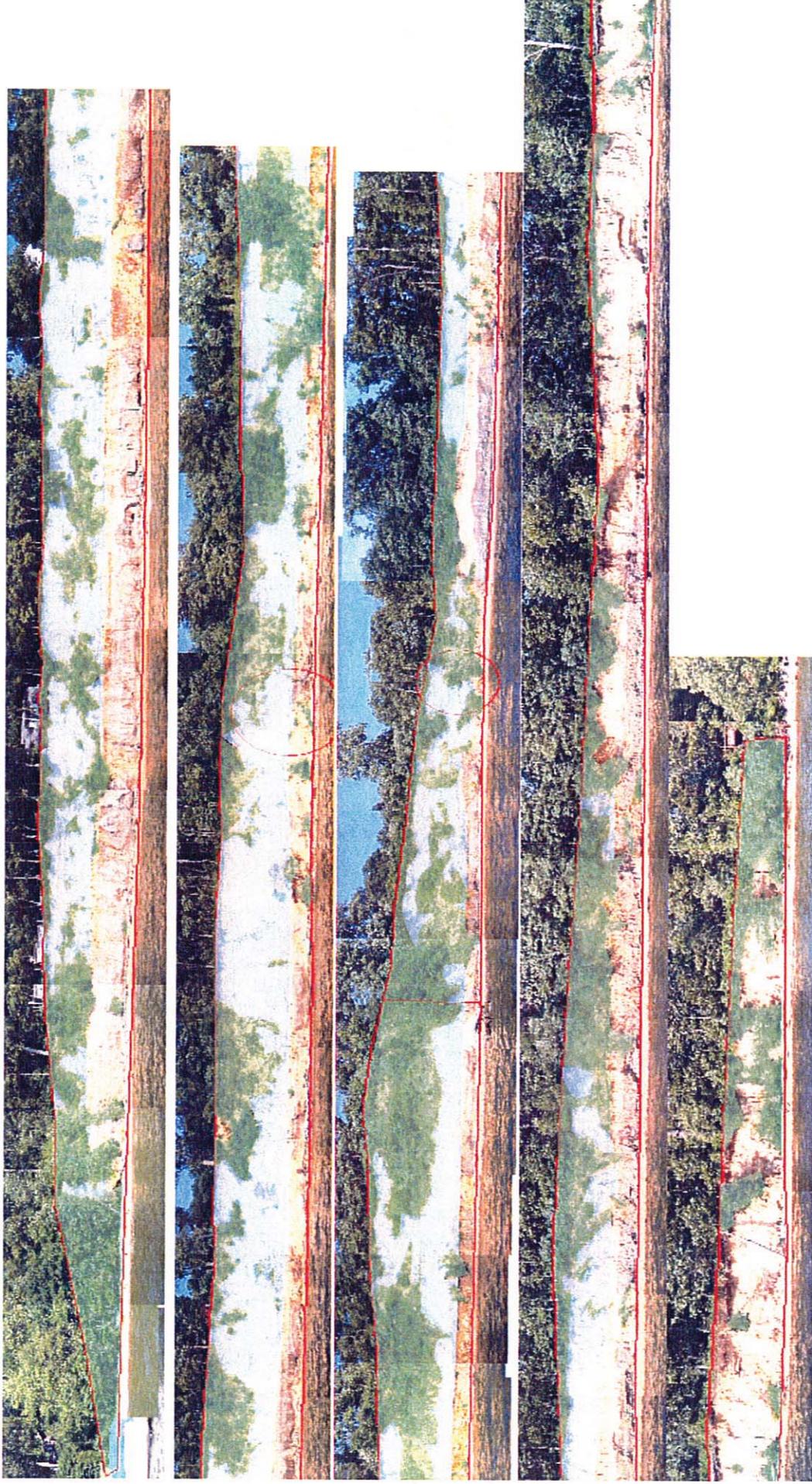
The region indicated by the blue is the Mount Laurel Formation which is favorable to PTBs and is therefore probable habitat. The bare region is the Merchantville Formation which is unsuitable to PTBs and is therefore only characterized by potential habitat.



Grove Point 2007 Cont.

Grove Point North to South

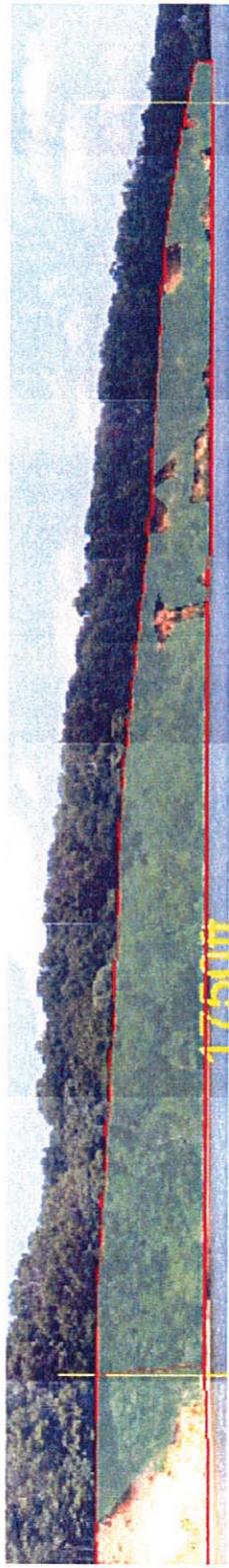
The region indicated by the blue is the Mount Laurel Formation which is favorable to PTBs and is therefore probable habitat. The bare region is the Merchantville Formation which is unsuitable to PTBs and is therefore only characterized by potential habitat.



Ordinary Point 2007

Ordinary Point West to East

IMPORTANT NOTE: All of the bare bluff area at Ordinary Point is probable habitat and should be colored blue like probable habitat in other panels



West Turner 2007

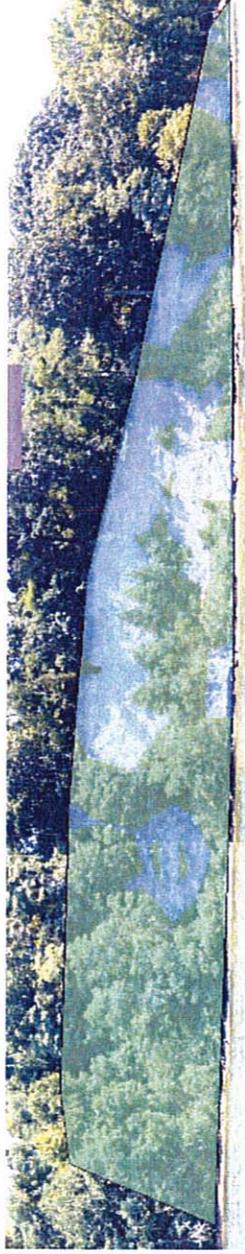
West Turner A

The region indicated by the blue is the Mount Laurel Formation which is favorable to PTBs and is therefore probable habitat.



West Turner B

The region indicated by the blue is the Mount Laurel Formation which is favorable to PTBs and is therefore probable habitat.



East Lloyd 2007

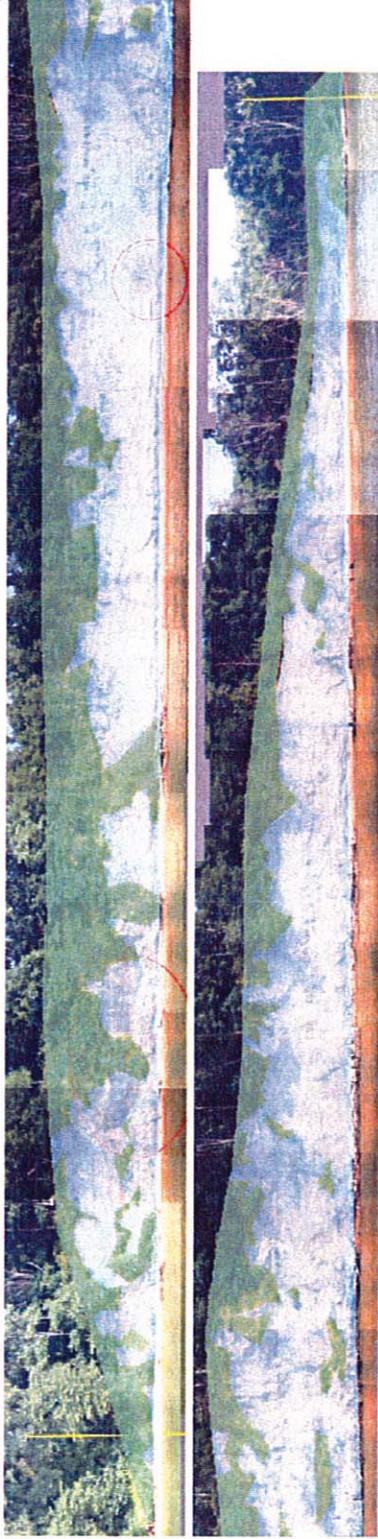
East Lloyd C

The region indicated by the light blue is the Mount Laurel Formation which is favorable to PTBs and is therefore probable habitat.



East Lloyd D East to West

The region indicated by the blue is the Mount Laurel Formation which is favorable to PTBs and is therefore probable habitat.



East Lloyd E

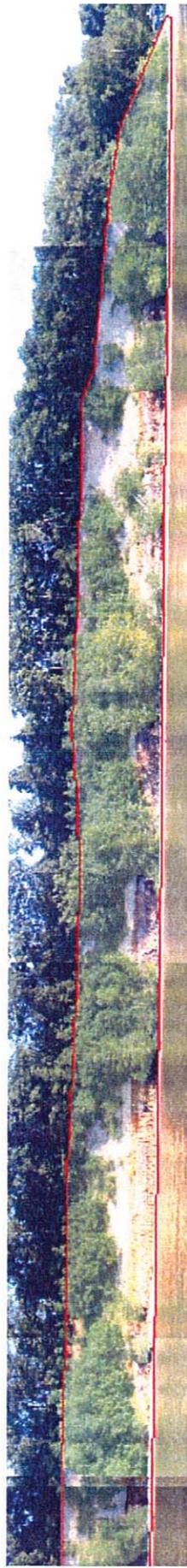
The region indicated by the blue is the Mount Laurel Formation which is favorable to PTBs and is therefore probable habitat.



East Betterton 2007

East Betterton North to South

The region indicated by the blue is the Mount Laurel Formation which is favorable to PTBs and is therefore probable habitat. The bare region is the Merchantville and Englishtown Formation which is unsuitable to PTBs and is therefore only characterized by potential habitat.



West Betterton 2007

West Betterton North to South

Non-vegetated areas were covered by slumping and formation contacts were not clear for photo analysis of probable habitat

