Comparison of northern diamondback terrapin (*Malaclemys terrapin terrapin*) hatching success among variably oiled nesting sites along the Patuxent River following the Chalk Point Oil Spill of April 7, 2000

By
Roger C. Wood, Ph.D.
and
L. Stanton Hales, Jr., Ph.D.

Final Report

December 7, 2001

INTRODUCTION

On April 7, 2000, approximately 126,000 gallons of a mixture of #2 and #6 fuel oil were released from a break in a pipeline providing fuel to the Chalk Point generating station. At the time of the oil spill the pipeline was owned by the Potomac Electric Power Company (PEPCO) and operated by Support Terminal Services Operating Partnership, LLP (ST Services). The spill initially leaked into Swanson Creek and the surrounding tidal wetlands. On April 8, high winds, rain and tides spread the oil downstream approximately 27 linear kilometers along the Patuxent River and into several tributaries, including Indian Creek, Trent Hall Creek, Washington Creek, and Cremona Creek, fouling many more kilometers of shoreline.

One of many resident species potentially affected by the oil spill in the Patuxent River and its tributary creeks is the northern diamondback terrapin, *Malaclemys terrapin terrrapin*.
Female terrapins are known to nest on sandy beaches along the shores of the spill zone. Thus, not only were adult terrapins potentially exposed to and affected by the oil spill, but also eggs laid under the surface of the nesting beaches were potentially exposed and affected.

Therefore, we were asked to assess the impact of the oil spill upon terrapin reproductive success along the Patuxent River downstream from Swanson Creek. We were asked to undertake our research at the beginning of September, nearly five months after the April 7th spill. Thus we first became actively involved with this project during the second month of the hatching season (well after the nesting season had ended).

Since 1987, various aspects of the life history, ecology, and behavior of diamondback terrapins in the Patuxent River have been the subject of long-term studies (see below) by Dr. Willem Roosenburg and his many colleagues. Some of the shoreline that was oiled included the primary study site for much of this research, an eight-kilometer stretch along the western bank of the river from Long Point, just downstream from Benedict, to Marsh Point. Oiling of the shoreline within this area ranged from heavy to light. Thus, some of those studies (see below) were helpful in our developing a plan of investigation.

Some characteristics of the Patuxent terrapin population reported in the scientific literature are summarized in Table 1. Egg incubation time varies between 56 and 110 days, with hatching typically commencing in early August and continuing into November, depending on the weather (Roosenburg, 1992). Roosenburg (1994) has reported nesting densities ranging from 240 to 1125 nests ha^{-1}. Nest predation rates were highest (up to 95 % of all nests) at sites with the highest nesting density; thus, beaches with low nest densities may produce a larger number of hatchlings than beaches with high nest densities (Roosenburg, 1994).

OBJECTIVE

The objective of our investigation is to determine the hatching success of terrapins at nesting beaches that were variously oiled (ranging from heavily to lightly) compared to beaches that were not oiled. Data from this study are intended to provide a basis for assessing the magnitude and extent of injury to the year 2000 hatchling cohort within the spill zone. Results will be used to assist in determining the level of compensation necessary to replace terrapin losses resulting from the spill.

METHODS

We designed a methodology to compensate for the start of our investigation in September, after the entire nesting season and a portion of the hatching season had passed. The methodology consisted of the following components: (1) a preliminary survey to look for evidence of nesting activity in Swanson Creek, and along creeks and both banks of the Patuxent River downstream from the mouth of Swanson Creek; (2) selection of suitable sites for monitoring of continuing hatching activity, and the construction of standardized exclosures at each of these sites; (3) monitoring of those sites throughout the fall to collect terrapins, and obtain other size and developmental information from collected hatchlings; and (4) excavation of selected exclosures at the end of the fall hatching season to look for hatchlings or nests overwintering underground, as well as to identify the locations of nests from which hatchlings had already emerged. Although not equivalent to being present at the start of the nesting season, all of this information gives us a reasonably accurate picture of nesting at these sites.

Though terrapins lay their eggs in nests in the supratidal zone (above mean high water), there are several potential mechanisms by which nesting success at oiled beaches might be affected by the oil spill. First, females nesting at oiled sites presumably lived and fed in areas impacted by the spill. Thus, any reduction in the available prey base in their feeding areas could possibly reduce the number or quality of eggs that females produce. Even without any prey-base effect, oil is a potential stressor of their physiology and might affect reproduction. Finally, though nests were above the high tide mark, high spring and storm tides inundate most nests in the study area (Roosenburg, 1992). Thus, nests in the supratidal zone at those sites could also potentially experience effects of any residual oil.

Site Selection and Designation of Oil Category

The initial three days (beginning Sunday, September 3, 2000) of field work were dedicated to surveying potential nesting sites along the banks of Swanson Creek and downstream from its mouth along the shoreline of the Patuxent River for several kilometers. Some 22 nesting sites had previously been identified by Mountford (2000) within the area affected by the oil spill. We visited the majority of these, including two beaches (Burton's Beach and Marsh Point) upon which Roosenburg (1992) had focused much of his previous field research.

At each beach we visited we looked for the remains of terrapin egg shells on the surface. This shell material is evidence of nest predation, which in turn means that one or more female terrapins had previously dug a nest and deposited eggs at that site. Samples of egg shells were collected at many of these localities.

Based on this preliminary survey, and with the aid of a map provided by ENTRIX documenting the severity of shoreline oiling, we selected nine study sites (Table 2 and Figure 1). Three of these were characterized on our map as heavily-oiled nesting beaches; three of these represented moderately- to lightly-oiled nesting beaches; and finally, three were unoiled nesting beaches. We divided these nine beaches into three categories: 1) heavily-oiled sites, 2) moderately- to lightly-oiled sites, and 3) unoiled, control sites.

Several months after our field work was initiated, the oiling assessment of one site originally designated as heavily oiled (Playground) was changed to very lightly oiled (ENTRIX, 2000). To keep equal sample sizes within oiling categories for nested analyses of variance (Zar, 1984), we subsequently regrouped the sites into new categories: 1) heavily- to moderately-oiled sites, 2) lightly- to very-lightly oiled, and 3) unoiled, control sites (see Table 2). This regrouping essentially enabled us to maintain our original study goals and experimental design.

It was our original expectation that two nesting localities included in our study would be Burrton's Beach and Marsh Point, where terrapin nesting was previously studied (Roosenburg, 1992). After visits to these sites, it was apparent that neither was suitable for inclusion in this study. Dense vegetation present at Burton's Beach made it an unsuitable site for exclosures. Successional changes and other observations (*e. g*., frequent tidal inundation, dense vegetation, lack of nest shell material compared to other sites) at Marsh Point suggested that this beach was no longer the highly productive nesting site that it once was.

Exclosure Design and Construction

At each of the nine selected nesting beaches we constructed two approximately 50-square meter exclosures that would enable us to detect any terrapin hatching activity within them. An exclosure is designed to retain hatchlings within it until they can be censused, while at the same time keeping out potential hatchling predators, such as raccoons, foxes and birds. The number, size and shape of these exclosures varied slightly from one study site to the next because of varying terrain and vegetation cover, but the total area enclosed at each of our study sites was as close to 100 m^2 as beach topography would allow (Table 2).

The total area of the exclosures at each site was based on reported nest densities ranging from 240 to 1125 nests ha^{-1} (Roosenburg, 1994). Thus, at the low density (240 nests ha^{-1}= 0.024 nests m^{-2}), our 50-m^2 exclosures should be sufficiently large to yield one nest. At the high nest density (=0.113 nests m^{-2}), the exclosures would be predicted to produce about six nests. Thus, these exclosures were considered suitable to recognize significant impacts to terrapin hatchlings.

A typical exclosure is shown in Figure 2. The perimeter fencing (1.5 cm-square plastic mesh) was buried 10 to 15 cm into the substrate. This was intended to discourage predators outside the exclosure from burrowing underneath to get in, and it also prevented hatchlings that have emerged from their nests from escaping. (During the course of our field work, we did not find any evidence of attempts to burrow underneath the buried fencing.) Plastic fencing (2.5 cm-square mesh) was stretched across the top of the exclosure to deter predators from above.

Construction of the last of our exclosures was completed on September 21st. Thereafter, we monitored the exclosures regularly (almost daily) through early November. We concluded our exclosure monitoring activities on November 10th, one week after no new hatchlings were found.

Exclosure Monitoring, Data Collection and Statistical Comparison

Within each exclosure, the vegetation was identified using two regional keys (Duncan and Duncan 1987, Silberhorn 1999). Vegetation was also mapped (using tape measures) onto graph paper to determine the extent to which the exclosures were vegetated. We recorded ground temperatures inside and outside the exclosures. The purpose of this was to determine whether the plastic mesh cloth stretched over the exclosures was either 1) warming the exclosure by absorbing solar radiation or 2) shading the ground inside the fencing enough to lower its temperature significantly compared to the ground temperatures outside the fencing. This was important to determine, because 1) we did not wish to alter the rate of development of hatchlings compared to other, non-exclosed areas and 2) terrapins are characterized by temperature-dependent sex determination. Eggs deposited in nests that are dug in sunny areas develop at relatively warm temperatures, resulting in relatively short incubation times and female hatchlings. Eggs deposited in nests that are dug in shaded areas develop at relatively low temperatures and produce male hatchlings.

Not surprisingly, ground temperatures varied from day to day over the time that we monitored our sites (Appendix 1). However, our measurements of ground temperatures inside (mean = 13.1°C, standard error = 0.37°C, N = 138) and outside (mean =13.2°C, standard error = 0.38°C, N =138) exclosures did not significantly differ ($t = 0.08$, $p > 0.50$). This lack of a significant difference suggests that the exclosures should not have any effect on the development of any nests located within them due to temperature effects.

Except for the first seven hatchlings that were recovered prior to availability of needed equipment, every hatchling that we discovered was examined in a standardized manner (Herlands *et al*. 1993). For those first seven hatchlings, only carapace and plastron lengths were obtained. For all other terrapins, four shell dimensions were measured to the nearest 0.1 mm with a pair of calipers: carapace length = midline length of the carapace (top part of the shell); carapace width = maximum width of the carapace; plastron length = midline length of the plastron (bottom part of the shell); and maximum depth = depth of the shell from the most dorsal margin of the carapace to the most ventral margin of the plastron). Weight was determined to the nearest 0.1 g on an Ohaus portable scale. In addition, scute anomalies were recorded (scutes are the enlarged scales that cover terrapin shells).

All of these parameters were of interest as potential indicators of the effects of oil as a developmental stressor. For example, if hatchlings from heavily-oiled beaches had low weights and/or a high frequency of scute abnormalities compared to hatchlings from unoiled beaches, then one might reasonably suspect that these differences were attributable to detrimental effects of oiled substrate on embryonic development.

Because terrapins were not obtained from exclosures at all sites in each oiling category (and thus, there were no measurements to include in any statistical comparison), one-way analysis of variance was used to compare size measurements among oiling categories and sites (Zar, 1984). Contingency table analyses employing log-likelihood ratios were used to compare the frequency of anomalies among oiling categories (Zar, 1984).

Excavation of Selected Exclosures

In typical terrapin populations, not all hatchlings emerge in the fall. Some hatchlings remain underground throughout the winter and do not dig their way to the surface until the following spring. Anything that slows development of hatchlings, such as the relatively cool weather that characterized the summer of 2000 or potential effects of the oil spill, might 1) increase the percentage of spring emergers (hatchlings that overwinter in the ground and emerge the following spring) or 2) sufficiently slow or interrupt development to (directly or indirectly) cause increased mortality. In southern New Jersey, the only place where attempts to quantify the spring emergence of terrapin hatchlings have been made, approximately 25% of the hatchlings remain underground throughout the winter and emerge the following spring (R. C. Wood, unpublished data).

In order to supplement the data from our previous field work, we undertook a second type of investigation in December, 2000. This involved excavation of exclosures at both oiled and unoiled sites to document the number of overwintering hatchlings, unhatched eggs, and opened eggshells (representing successfully hatched terrapins) beneath the surface to a depth of approximately 15 to 20 cm below the ground. Because the upper part of a typical terrapin nest chamber is no more than eight to ten cm below the surface, this enabled us to locate the positions of all nest chambers within each exclosure that we excavated.

Each excavated exclosure had the potential to provide the following information:

(1) the density of nests as indicated by concentrations of eggshell (from successfully hatched nests) found underground, or clusters of hatchlings that have liberated themselves from their eggs but remained underground, or nests of unhatched eggs, either viable or not;

(2) the number of eggs in each nest (here defined as nest size; however, nest size in this study is not being determined at the time of nesting as in previous studies in this area [Roosenburg, 1992]); and

(3) the condition of eggs in each nest. Viable eggs are chalk-white in color, non-viable eggs are buff-colored, and open-shell remnants underground represent successfully hatched terrapins. Inviable eggs were taken to our laboratory and dissected to determine the developmental stage at which death occurred. Eggs believed to be viable were given to personnel of the Maryland Department of Natural Resources for incubation and possible rearing.

This information, in effect, would be <u>nearly</u> equivalent to our having been present during the nesting season and marking every nest within each exclosure. Combining this information with other data previously accumulated should afford a fairly complete evaluation of nesting success at representative sites during the summer of 2000 along the banks of the Patuxent River downstream from the power plant.

This phase of our field work commenced on December 7th and continued through December 22nd. Snow, frozen ground, and bitter cold weather prevented us from excavating nests from all exclosures; nonetheless, all remaining exclosures were removed from all sites by December 22nd. This was reportedly one of the coldest Decembers on record according to the National Weather Service (Associated Press article in <u>Atlantic City Press</u>, January 6, 2001).

One-way analysis of variance (Zar, 1984) was used to compare nest sizes among oiling categories and sites, and to compare nest sizes from this study with nest sizes reported by Roosenburg (1992) for five previous years. Scheffe's multiple contrast was used to compare data from this study with previous studies (Zar, 1984). Contingency table analyses employing log-likelihood ratios were used to compare the different condition of embryos (hatched, viable, and dead) among oiling categories (Zar, 1984). Inter- and intra-nest variability were not considered in those contingency table analyses.

RESULTS

Hatching Study

Over the course of nearly 9 weeks (from September 10th to November 9th, 2000), we recovered 63 terrapin hatchlings from seven of the nine sites that we monitored (Table 3). No terrapins were recovered from the Trent Hall beach (a lightly-oiled site) or Prison Point (a control site).

It should be emphasized that the absence of hatchlings at certain sites does not mean that there was no nesting activity at these places. To the contrary, our exclosure sites were selected in part because we had found evidence of recent nesting activity (*i. e*., moderate to considerable amounts of fresh egg shell remains littering the ground) at all of them during our preliminary survey. Based on our knowledge of terrapin nesting habits both along the banks of the Patuxent River and elsewhere, we suspect that the relatively large numbers of hatchlings at Caney Creek and Jack Bay indicate that these were sites where 1) adult female terrapins double-clutched (*i. e*., laid a second set of eggs) later in the nesting season or 2) predation was lower. Such eggs would have been likely to produce hatchlings later than eggs laid earlier in the nesting season. Presumably, hatching at sites where we did not find terrapin hatchlings was completed prior to the initiation of our study; alternatively, nests at those sites may have experienced high predation rates.

In terms of numbers of hatchlings found, there was considerable variability among sites irrespective of oiling category. The majority of hatchlings came from only two sites, one heavily oiled (Caney Creek; N=26) and one unoiled, control (Jack Bay; N=19). Nested (or hierarchical) analysis of variance showed no significant difference in numbers of hatchlings (adjusted for minor differences in areas of the exclosures) at heavily oiled, moderately to lightly oiled, and control sites ($F_{[2, 6]}$=1.13, P>0.05). In addition, there were significant differences in the numbers of hatchlings among sites within oiling categories ($F_{[6, 9]}$=1.59, P>0.05).

Analysis of variance indicated that there were no significant differences in any size measurements of hatchlings among oiling categories. Carapace length ($F_{[2, 61]}$= 2.98, P=0.06), plastron length ($F_{[2, 61]}$= 2.68, P=0.08), maximum width ($F_{[2,56]}$=1.02, P=0.40), maximum height ($F_{[2, 54]}$=0.69, P=0.51), and weight ($F_{[2, 54]}$=0.28, P=0.76) did not differ among oiling categories (Table 5). Carapace length and plastron length, hatchling measurements that exhibited marginal levels of significance (P=0.06 and P=0.08), exhibited an unexpected trend: average lengths of hatchlings at lightly oiled sites (CL= 3.09 cm, PL=2.68 cm) > average lengths at heavily oiled sites (CL=3.02 cm, PL=2.64 cm) > average lengths at unoiled, control sites (CL=2.96 cm, PL=2.55 cm). Trends in those data may have been affected by site-specific factors on development. Size measurements varied considerably across sites, but only carapace length and maximum height differed significantly between sites within the same oiling categories (see Table 5).

Significant differences in some developmental features among oiling categories were infrequent and generally inexplicable, and potentially due to a number of site-specific environmental variables (*e. g*., temperature). The occurrence of an egg tooth on hatchlings did not differ among oiling categories ($G_{[2]}$=3.54, P=0.19), whereas the occurrence of a yolk sac differed inexplicably among heavily oiled (42%), lightly oiled (11%), and unoiled, control categories (69%; $G_{[2]}$=8.67, P=0.02*). The number of axial scutes ($G_{[2]}$=2.92, P=0.24) and inguinal scutes ($G_{[2]}$=3.40, P=0.20) did not differ among oiling categories (see Table 6). The only scute anomalies observed to differ among oiling categories were split cervical scutes ($G_{[2]}$=6.84, P=0.03*), which occurred in 32% of hatchlings at heavily oiled sites, 11% of hatchlings at lightly oiled sites, and 5% of hatchlings at control sites. Split cervical scutes occur occasionally in terrapins in southern New Jersey that we have had under observation for many years (R. C. Wood, unpublished data). No grotesquely deformed specimens were encountered throughout the course of our study.

Nest Excavation Study

Two heavily oiled sites (Caney Creek and Long Point) and two control sites (Jack Bay and Sheridan Point) were excavated. Inclement weather prohibited excavation of any of the other sites. A total of 68 different “nests” containing more than 539 eggs and/or hatchlings in different stages of viability and development were excavated (Table 7, Appendix 2). In some cases these groups undoubtedly represent complete nests; in other cases, it seemed clear that the group was not a complete nest (*i. e.*, a "partial" nest, such as finding a single hatchling). For these reasons, information from nests containing one or two eggs and/or hatchlings were excluded from some analyses or the analyses were performed on data sets that included and excluded those "partial" nests.

Including all data, nest densities ranged from one per 100 m^2 at Sheridan Point to 26 per 100 m^2 at Jack Bay. Both of these were non-oiled control sites. Nest densities were relatively high at the two heavily oiled sites (approximately 22 per 100 m^2 at Caney Creek and 23 per 100 m^2 at Long Point). If data from "partial" nests are excluded, nest densities ranged from one per 100 m^2 at Sheridan Point to 23 per 100 m^2 at Jack Bay; nest densities at the two heavily oiled sites ranged from 16 to 17 per 100 m^2 at Long Point and Caney Creek respectively. Irrespective of the data used, nest density at three of the four sites that we excavated far exceeded the maximum nest density (approximately six nests per 100 m^2) previously reported for terrapins in the Patuxent River (Roosenburg, 1994).

The oil contamination and subsequent cleanup did not appear to result in localized effects on terrapin nest density within the spill area. Irrespective of the inclusion or exclusion of data from partial nests, nest density did not differ between oiled (mean=22.5 vs. 16.5 nests per 100 m^2) and unoiled (mean=13.5 vs. 12 nests per 100 m^2) exclosures (ANOVA, $F_{[1, 2]}$=0.52, P>0.50 for all data; $F_{[1, 2]}$=0.16, P>0.50 without partial nest data. Similarly, nest density did not differ among sites within oiling levels (ANOVA, $F_{[2, 4]}$=0.53, P>0.50 for all data; $F_{[1, 2]}$=0.63, P>0.50 without partial nest data).

As with nest density, the oil contamination and subsequent cleanup did not appear to result in localized effects on terrapin nest size within the spill area. Nest sizes in oiled (mean= 9.2 eggs $nest^{-1}$, SE=0.74) and unoiled, control exclosures (mean = 8.6 eggs $nest^{-1}$, SE=0.83 eggs) did not differ (ANOVA, $F_{(1,59)}$=0.22, P=0.64). Similarly, nest size did not differ among the four excavated sites (ANOVA, $F_{(4,59)}$=0.66, P=0.62), where average nest sizes ranged from a low of 6 eggs $nest^{-1}$ at Sheridan Point to a high of 10.1 eggs $nest^{-1}$ at Caney Creek.

However, even with data from partial nests <u>not</u> included in the analyses, nest sizes from this stretch of the Patuxent River in 2000 (overall mean=8.92, S.E.=0.55, N=60) are significantly smaller than the nest sizes reported by Roosenburg (1992) for terrapins in the Patuxent River from 1987 to 1991 (overall mean= 12.90, S.E. 0.13, N=504; ANOVA, F [5,559]=19.65, P<0.01; Scheffe's S for 2001 vs. all other years =9.62, P<0.01). Our nest size estimates for 2000 did not use all observations from our excavations, that is, presumably partial nests consisting of only one or two terrapins and/or eggs were not included in these comparisons.

It is unknown whether nests included in this analysis were complete (because nest size was not determined at nesting, but several months later) and whether the low nest size is directly attributable to effects of the oil contamination; however, these low nest sizes concern us for several reasons. First, selective removal of very small nests (nest size < 3) from our analyses of nest size would not change the significance of the differences we observed. Second, we do not believe that the low nest sizes in all exclosures are due to partial predation of nests. Previous studies reported considerable variability in predation (Roosenburg, 1992) but average nest sizes were low at all excavated sites. We also do not believe that the low nest sizes were due to nests being partially washed away. With the exception of one nest at Caney Creek (embryo and egg N=8) excavated only 4 to 5 cm below the substrate surface, all nests at the four sites were excavated at typical depths (at least 10 to 12 cm) and did not exhibit any signs of having been partially washed away (*e. g.*, some eggs visible at the substrate surface). Finally, if oil contamination affected adult female terrapins prior to egg-laying, then one would not necessarily expect diminished nest size to occur only at nesting sites that were oiled.

Unfortunately no studies have ever been done on the effects of oil on terrapins (Rattner *et al*., 2000). Very little information, in fact, is available about the effects of oil on any kind of turtles. Studies of this nature to date have focused on marine turtles (Alam and Brim, 2000; Fritts and McGehee, 1982; Geraci and St. Aubin, 1987), which are not closely related to diamondback terrapins (and are therefore not necessarily good models for the effects of oil on terrapins). However, those studies indicate that oiling of nests can result in embryonic abnormalities and mortality.

Significant differences in the condition of eggs excavated from nests in exclosures at oiled and unoiled (control) sites could be a consequence of oil contamination. Consistent with our previous comparison of hatchlings collected in the different exclosures, the frequency of eggs that had apparently hatched did not differ between oiled (177 of 311 eggs) and unoiled, control areas (132 of 228 eggs,

$G_{[1]}$=0.05, P>0.50.). However, the frequency of potential spring emergers (viable eggs that would presumably result in spring emergers) differed significantly between oiled (50 of 311 eggs) and unoiled sites (63 of 228 eggs, $G_{[1]}$=10.51, P<0.001). This significant difference in the number of spring emergers was consistent with the significantly higher numbers of dead embryos at the oiled sites (84 of 311 eggs) compared to unoiled, control sites (33 of 228, $G_{[1]}$= 10.51, P<0.001).

At least 18 inviable eggs from several different nests at one Caney Creek exclosure (#2) are of particular interest. These nests contained the skeletons of late-stage embryos inside well-formed eggs that did not hatch. Based upon our extensive experience of rearing thousands of embryos in our laboratory hatching program over the past 12 years, mortality of late-stage embryos is rare (R. C. Wood, unpublished data).

The cause of mortality of these embryos is unknown and may be due to many factors other than the effects of the embryos' exposure to residual oil present in sediment after the cleanup. It is possible that the embryos might have died as a result of being inundated too frequently by tidal waters. Or, conceivably, the embryos might have been killed by excessive thermal stress if their nest chamber overheated. This could happen, for example, if wind erosion removed several inches of sand from above the nest chambers within which the eggs were laid. However, the summer of 2000 was cool, and there was no evidence of erosion at those exclosures.

Finally, as part of the effort to assess the impact of the oil spill on terrapin reproductive success in the year 2000, it is helpful to keep in mind some of the important population characteristics of these turtles. They have long adult lives during which females nest every year, sometimes multiple times, for periods of time that may exceed two decades. There is naturally high predation upon their nests and undoubtedly upon hatchlings too, although this latter probability has not been sufficiently documented. Thus, a typical terrapin population consists of a high proportion of adults and a relatively small number of juveniles. There is a very low probability that hatchlings from any annual cohort, even under the best of circumstances, will survive to become adults.

SUMMARY

Oiling of terrapin nesting sites potentially has a number of impacts upon terrapins. Based on comparisons of hatching and hatchlings between variously oiled and unoiled sites, and comparison of egg and nest information from the excavation of terrapin nests at heavily oiled and unoiled sites, we conclude the following about the impacts of the oil spill upon the diamondback terrapin population(s) in the Patuxent River.

(1) Terrapin nest density on oiled and unoiled beaches downriver from the Chalk Point generating station did not differ during the 2000 nesting season.

(2) The hatching of terrapins in fall (as reflected by number of hatchlings recovered over a two-month period at all study sites and our assessment of fall hatching from excavations at selected sites) did not differ between oiled and unoiled nesting beaches downriver from the Chalk Point generating station.

(3) Fall hatchlings recovered from variously oiled and unoiled sites were comparable in size and weight. Although some minor differences in developmental features of hatchlings at oiled and unoiled sites were observed, no consistent or noteworthy developmental abnormalities were observed.

(4) Our best estimates of nest sizes across oiled and unoiled sites did not differ. On average, however, nest sizes in 2000 were significantly lower than the average nest reported by Roosenburg (1994) for the years 1987-1991 in that same stretch of the Patuxent River. If real, those differences probably do not indicate an effect of oil contamination <u>at nest sites</u>, but more likely represent some widespread factor affecting adult female terrapins that may or may not be related to oil contamination.

(5) Comparison of the condition of eggs recovered at selected oiled and unoiled sites revealed a significantly higher frequency of dead embryos and a lower frequency of presumed spring-emergers at oiled sites compared to unoiled sites. The cause of death of those embryos is not known and may not necessarily be attributed to the oil spill, but the observed mortality is of concern for two reasons. (A) Spring emergers would experience the potential effects of any residual oil contamination for a protracted

period compared to fall hatchlings, and (B) based upon our extensive observations of mortality in laboratory rearing studies, the high observed mortality of terrapin embryos in late stages of development at oiled sites is unusual.

In summary, results of our study suggest that the oil spill may have contributed to a reduction in nest size throughout the area of study and may have increased the mortality of presumed spring emergers at oiled sites. Based on the life history characteristics of this species, we believe these potential impacts to terrapin reproduction during 2000 are probably minor. We found no evidence of a major, catastrophic impact of the oil spill on terrapin reproduction in the Patuxent River.

REFERENCES CITED

Alam, S. K., M. S Brim. 2000. Organochlorine. PCB, PAH and metal concentrations in eggs of loggerhead sea turtles (*Caretta caretta*) from Northwest Florida, USA. Journal of Environmental Science and Health: Part B, Pesticides, Food Contaminants and Agricultural Waste, 35(6), pp. 705-724.

Duncan,W. and M. 1987. Smithsonian Guide to Seaside Plants of the Gulf and Atlantic Coasts. Smithsonian Press, Washington, D. C. 409 pp.

ENTRIX Inc. 2000. Swanson Creek incident summary of SCAT activities and data management.

Fritts, T. H. and M. A. McGehee. 1982. Effects of petroleum on the development and survival of marine turtle embryos. U. S. Fish and Wildlife Service, Biological Services Program, FWS/OBS-82/37: 41 pp.

Geraci, J. R., and D. J. St.Aubin. 1987. Effects of offshore oil and gas development on marine mammals and turtles. In Long Term Environmental Effects of Oil and Gas Development. Boesch, D. F. and, N. N. Rabalais (eds.). London, Elsevier Applied Science, pp. 587-617.

Herlands, R. L., S. Schnackenberg, and R. C. Wood. 1993. Life after death: conservation of northern diamondback terrapins in New Jersey. Proceedings, 49th Annual Northeast Fish and Wildlife Conference, pg. 135.

Jeyasuria, P., W. M. Roosenburg and, A. R. Place. 1994. Role of P-450 aromatase in sex determination of the diamondback terrapin, *Malaclemys terrapin.* The Journal of Experimental Zoology 270, pp. 95-111.

Mountford, K. 2000. Diamondback terrapin survey note. Unpublished. US Environmental Protection Agency, Chesapeake Bay Program, Note and map.

Rattner, B.A., N. H. Golden, J. L. Pearson, J. B. Cohen, L. J. Garrett, M. A. Ottinger and, J. B. Erwin. 2000. Biological and ecotoxicological characteristics of terrestrial vertebrate species residing in estuaries: diamondback terrapin. U. S. Geological Survey. www.pwrc.usgs.gov/bioeco/terrapin.httm. 3 pp.

Roosenburg, W. M. 1990. Chesapeake diamondback terrapin investigations. Chesapeake Research Consortium, Publication Number 133.

Roosenburg, W. M. 1991. The diamondback terrapin: population dynamics, habitat requirements, and opportunities for conservation. Chesapeake Research Consortium, Publication Number 137, pp. 227-234.

Roosenburg, W. M. 1992. Life history consequences of nest site choice by the daiamondback terrapin, *Malaclemys terrapin*. Doctoral dissertation, University of Pennsylvania, 206 pp.

Roosenburg, W. M. 1994. Nesting habitat requirements of the diamondback terrapin: a geographic comparison. Wetland Journal 6(2), pp. 8-11.

Roosenburg, W. M. 1996. Maternal condition and nest site choice: an alternative for the maintenance of environmental sex determination? American Zoologist 36, pp. 157-168.

Roosenburg, W. M. and, A. E. Dunham. 1997. Allocation of reproductive output: egg- and clutch-size variation in the diamondback terrapin. Copeia 1997(2), pp. 290-297.

Roosenburg, W. M. and, K. C. Kelley. 1996. The effect of egg size and incubation temperature on growth in the turtle, *Malaclemys terrapin.* Journal of Herpetology, 30(2), pp. 198- 204.

Roosenburg, W. M. and, A. R Place. 1994. Nest predation and hatchling sex ratio in the diamondback terrapin: implications for management and conservation. Chesapeake Research Consortium, Publication Number 149. pp. 65-70.

Silberhorn, G. 1999. Common plants of the Mid-Atlantic Coast: A Field Guide, Revised Ed., JHU Press, 294 p

Zar, J. H. 1984. Biostatistical analysis, 2nd ed. Prentice Hall, Englewood Cliffs, New Jersey, 716 pp.

TABLES, FIGURES AND APPENDICES

Table 1. Summary of published information about the terrapin population in the Patuxent River. All information is taken from Roosenburg, 1990, 1991, 1992, 1994, 1996; Roosenburg and Place 1994; and Jeyasuria *et al.* 1994.

Estimated size of population within study area: between 1700 and 2900 adults

Nesting season: roughly from the beginning of June through the end of July

Number of eggs per nest (a nest = all the eggs laid in a single nest):
7 to 22 (mean = 13)

Number of nestes per female per nesting season: 1 to 3

Length of egg incubation: minimum number of days = 56; shaded nests take longer (80-110 days); some hatchlings are reported to overwinter in their nests

Number of hatchlings produced per successful nest: 3.4 to 10.75

Known nest predators:
- raccoons (responsible for 59 to 70 % of all nest predation);
- foxes (5 to 9 %);
- other mammal species (1 to 3 %);
- unknown: (20 % or more)

Effect of natural predation: 55 to 95 % of all nests destroyed

Table 2. Dimension (m x m), area (m^2), and vegetated area (area in m^2) and % vegetation (%Veg) of the terrapin exclosures at each site for each oiling category. NR indicates non-rectangular shape. [1] indicates that assignment of the site to this oiling category was changed due to modification of the oiling category of another site. [2] indicates the original oiling level was changed from heavy to very light, which changed the grouping of sites in the oiled categories.

Oiling category & site	Dimension	Area	Vegetated Area	% Veg
Heavily (h) to moderately (m) oiled sites				
Caney Creek 1(h)	2.5 X 20.0	50.0	1.6	3.2
Caney Creek 2(h)	NR	37.8	1.9	5.0
Long Point 1 (h)	NR	75.0	18.8	25.0
Long Point 2 (h)	5.0 X 10.0	50.0	37.5	75.0
Hoyer Farm 1[1] (m)	5.0 X 10.0	50.0	34.9	69.8
Hoyer Farm 2[1] (m)	2.5 X 20.0	50.0	37.5	75.0
Lightly (l) to very lightly (vl) oiled sites				
Grandfather's Pond 1 (lt)	5.0 X 10.0	50.0	37.5	75.0
Grandfather's Pond 2 (lt)	5.0 X 10.0	50.0	37.5	75.0
Trent Hall 1 (lt)	2.5 X 20.0	50.0	37.5	75.0
Trent Hall 2 (lt)	2.5 X 20.0	50.0	37.5	75.0
Playground 1[2] (vl)	5.0 X 10.0	50.0	35.6	71.3
Playground 2[2] (vl)	5.0 X 10.0	50.0	32.8	65.6
Non-oiled, control (c) sites				
Prison Point 1 (c)	7.5 X 7.5	56.3	28.1	50.0
Prison Point 2 (c)	7.5 X 6.0	45.0	22.5	50.0
Sheridan Point 1 (c)	5.0 X 10.0	50.0	26.8	53.6
Sheridan Point 2 (c)	5.0 X 10.0	50.0	36.8	73.5
Jack Bay 1 (c)	5.0 X10.0	50.0	21.2	42.3
Jack Bay 2 (c)	NR	28.8	8.6	29.9

Table 3. Numbers of hatchlings of diamondback terrapins (n) collected from each exclosure during the nesting study at each site for each oiling category. Summary statistics (mean ± 2 standard errors, and the total size [N] of the catch) for each oiling category are also provided. Other abbreviations as in Table 2.

	n
Heavily (h) to moderately (m) oiled sites (5.5± 5.7, N=33)	
Caney Creek 1 (h)	7
Caney Creek 2 (h)	19
Long Point 1 (h)	0
Long Point 2 (h)	2
Hoyer Farm 1[1] (m)	2
Hoyer Farm 2[1] (m)	3
Lightly (lt) to very lightly (vl) oiled sites (1.5± 1.8, N=9)	
Grandfather's Pond 1 (lt)	0
Grandfather's Pond 2 (lt)	2
Trent Hall 1 (lt)	0
Trent Hall 2 (lt)	0
Playground 1[2] (vl)	2
Playground 2[2] (vl)	5
Non-oiled, control (c) sites (3.5±6.2, N=21)	
Prison Point 1 (c)	0
Prison Point 2 (c)	0
Sheridan Point 1 (c)	1
Sheridan Point 2 (c)	1
Jack Bay 1 (c)	19
Jack Bay 2 (c)	0

Table 4. Collection data (terrapin ID #, date, oil category [Oil Cat], Site, exclosure number [Excl]) and measurements (carapace length [CL], plastron length [PL], maximum width [Wth], maximum height [Ht], and weight [Wt]) of terrapins collected in each exclosure. Measurements in cm unless noted.

#	Date	Oil Cat	Site	Excl	CL	PL	Wth	Ht	Wt (g)
1	9/10/00	Control	Jack Bay	1	3.00	2.56			
2	9/10/00	Control	Sheridan Point	2	3.14	2.73			
3	9/12/00	Control	Jack Bay	1	2.89	2.40			
4	9/13/00	Heavy	Caney Creek	1	3.00	2.80			
5	9/13/00	Heavy	Caney Creek	1	3.00	2.80			
6	9/16/00	Control	Jack Bay	1	3.26				
7	9/19/00	Control	Jack Bay	1	2.97	2.61	2.71		
8	9/21/00	Heavy	Hoyer Farm	1	2.95	2.44	2.39	1.40	5.90
9	9/21/00	Heavy	Hoyer Farm	2	3.12	2.69	2.83	1.63	7.40
10	9/22/00	Control	Jack Bay	1	2.79	2.35	2.43	1.51	5.40
11	9/22/00	Heavy	Hoyer Farm	2	2.97	2.52	2.57	1.45	7.50
12	9/22/00	Heavy	Hoyer Farm	2	3.08	2.79	2.59	1.54	7.60
13	9/22/00	Heavy	Long Point	2	2.52	2.21	2.30	1.30	4.00
14	9/22/00	Heavy	Caney Creek	2	2.97	2.71	2.64	1.55	7.50
15	9/22/00	Heavy	Caney Creek	2	3.08	2.73	2.60	1.59	7.60
16	9/22/00	Heavy	Caney Creek	2	2.95	2.35	2.66	1.63	6.10
17	9/22/00	Heavy	Caney Creek	2	2.91	2.62	2.47	1.63	6.20
18	9/22/00	Heavy	Caney Creek	2	3.04	2.64	2.58	1.64	7.60
19	9/22/00	Heavy	Caney Creek	2	3.02	2.60	2.49	1.78	7.60
20	9/22/00	Heavy	Caney Creek	2	2.97	2.65	2.73	1.74	7.80
21	9/25/00	Heavy	Caney Creek	2	3.06	2.69	2.67	1.61	7.80
22	9/25/00	Heavy	Caney Creek	2	3.21	2.73	2.58	1.66	8.50
23	9/25/00	Heavy	Caney Creek	2	3.08	2.58	2.51	1.58	7.80
24	9/25/00	Heavy	Caney Creek	2	2.99	2.59	2.42	1.56	7.00
25	9/25/00	Heavy	Caney Creek	2	3.16	2.85	2.65	1.67	8.60
26	9/25/00	Heavy	Caney Creek	2	3.12	2.81	2.49	1.66	8.00
27	9/25/00	Heavy	Caney Creek	2	3.23	2.94	2.69	1.68	9.30
28	9/25/00	Light	Grndfthr Pnd	2	3.31	2.86	2.40	1.66	7.80
29	9/28/00	Light	Playground	1	2.83	2.48	2.60	1.49	5.90
30	9/28/00	Light	Playground	2	2.86	2.49	2.37	1.48	5.90
31	9/29/00	Control	Jack Bay	1	2.94	2.42	2.63	1.56	7.00
32	9/30/00	Heavy	Caney Creek	2	2.99	2.62	2.64	1.51	4.70
33	9/30/00	Light	Grndfthr Pnd	2	3.24	2.75	2.81	1.58	7.40
34	10/5/00	Control	Jack Bay	1	2.89	2.53	2.53	1.69	6.80
35	10/6/00	Control	Jack Bay	1	2.97	2.66	2.60	1.79	7.80
36	10/12/00	Heavy	Long Point	2	2.93	2.50	2.50	1.51	5.70
37	10/13/00	Control	Jack Bay	1	2.95	2.48	2.48	1.68	6.40
38	10/18/00	Control	Sheridan Point	1	3.04	2.56	2.68	1.49	6.30
39	10/19/00	Light	Playground	2	3.08	2.63	2.68	1.54	7.10
40	10/19/00	Light	Playground	2	2.98	2.63	2.46	1.58	7.30
41	10/19/00	Heavy	Caney Creek	2	2.91	2.30	2.30	1.42	5.90

Table 4 continued.

#	Date	Oil Cat	Site	Excl	CL	PL	Wth	Ht	Wt (g)
42	10/20/00	Heavy	Caney Creek	2	2.97	2.54	2.49	1.46	7.00
43	10/24/00	Control	Jack Bay	1	2.88	2.50	2.61	1.54	7.20
44	10/24/00	Control	Jack Bay	1	2.91	2.50	2.45	1.53	6.90
45	10/24/00	Control	Jack Bay	1	2.68	2.23	2.16	1.41	4.50
46	10/24/00	Control	Jack Bay	1	3.03	2.71	2.49	1.60	7.40
47	10/24/00	Control	Jack Bay	1	3.00	2.63	2.47	1.64	7.50
48	10/24/00	Control	Jack Bay	1	3.04	2.81	2.50	1.73	7.90
49	10/24/00	Heavy	Caney Creek	2	3.14	2.72	2.60	1.61	7.30
50	10/24/00	Light	Playground	1	3.11	2.74	2.63	1.45	6.30
51	10/25/00	Control	Jack Bay	1	2.78	2.35	2.34	1.52	6.30
52	10/25/00	Heavy	Caney Creek	1	2.94	2.68	2.57	1.45	5.90
53	10/25/00	Heavy	Caney Creek	1	3.03	2.66	2.62	1.71	7.60
54	10/25/00	Heavy	Caney Creek	1	2.93	2.53	2.57	1.53	5.50
55	10/25/00	Heavy	Caney Creek	1	3.00	2.61	2.49	1.64	6.00
56	10/25/00	Heavy	Caney Creek	1	3.17	2.80	2.76	1.67	8.20
57	10/27/00	Control	Jack Bay	1	3.05	2.65	2.74	1.69	7.60
58	10/29/00	Light	Playground	2	3.15	2.65	2.80	1.57	6.50
59	10/30/00	Heavy	Hoyer Farm	1	3.10	2.62	2.72	1.57	8.40
60	10/30/00	Light	Playground	2	3.21	2.89	2.67	1.57	9.50
61	11/1/00	Heavy	Caney Creek	2	3.02	2.64	2.59	1.62	6.90
62	11/3/00	Control	Jack Bay	1	2.98	2.62	2.45	1.56	7.20

Table 5. Summary statistics (mean ± 2 SE, sample size) of all terrapin measurements (carapace length [CL], plastron length [PL], maximum width [MW], maximum height [MH], and weight [Wt]) by oiling category and by exclosure. Some measurements were not made on a limited number of terrapins early in the experiment. All length measurements are given in centimeters and were made to the nearest mm; weight was measured to the nearest 0.1 g. (- indicates that no terrapins were collected in that exclosure)

Oiling category & site	CL	PL	MW	MH	Wt
Heavy (h) to moderate (m)	3.02 ± 0.044, 33	2.64 ± 0.054, 33	2.57 ± 0.044, 31	1.58 ± 0.38, 31	7.06 ± 0.43, 31
Caney Creek (h)	3.01 ± 0.060, 7	2.70 ± 0.082, 7	2.60 ± 0.090, 5	1.60 ± 0.096, 5	6.64 ± 1.06, 5
Caney Creek (h)	3.04 ± 0.044, 19	2.65 ± 0.070, 19	2.57 ± 0.048, 19	1.61 ± 0.040, 19	7.33 ± 0.49, 19
Long Point (h)	-	-	-	-	-
Long Point (h)	2.73 ± 0.41, 2	2.36 ± 0.29, 2	2.40 ± 0.20, 2	1.41 ± 0.21, 2	4.85 ± 1.70, 2
Hoyer Farm (m)	3.03 ± 0.15, 2	2.53 ± 0.18, 2	2.56 ± 0.33, 2	1.49 ± 0.17, 2	7.15 ± 2.50, 2
Hoyer Farm (m)	3.06 ± 0.09, 3	2.67 ± 0.16, 3	2.66 ± 0.17, 3	1.54 ± 0.10, 3	7.50 ± 0.12, 3
Light (l) to very light (vl)	3.09 ± 0.11, 9	2.68 ± 0.11, 9	2.60 ± 0.011, 9	1.55 ± 0.042, 9	7.08 ± 0.75, 9
Grandfather's Pond (l)	-	-	-	-	-
Grandfather's Pond (l)	3.28 ± 0.07, 2	2.81 ± 0.11, 2	2.61 ± 0.41, 2	1.62 ± 0.080, 2	7.60 ± 0.40, 2
Trent Hall (l)	-	-	-	-	-
Trent Hall (l)	-	-	-	-	-
Playground (vl)	2.97 ± 0.28, 2	2.61 ± 0.26, 2	2.62 ± 0.030, 2	1.47 ± 0.040, 2	6.10 ± 0.40, 2
Playground (vl)	3.06 ± 0.12, 5	2.66 ± 0.13, 5	2.60 ± 0.16, 5	1.55 ± 0.036, 5	7.26 ± 1.22, 5
Non-oiled, control (c)	2.96 ± 0.058, 20	2.55 ± 0.068, 20	2.53 ± 0.072, 17	1.60 ± 0.054,15	6.81 ± 0.48,15
Prison Point (c)	-	-	-	-	-
Prison Point (c)	-	-	-	-	-
Sheridan (c)	3.04, 1	2.56, 1	2.68, 1	1.49, 1	6.30, 1
Sheridan (c)	3.14, 1	2.73, 1	-	-	-
Jack Bay (c)	2.95 ± 0.060,18	2.54 ± 0.074, 18	2.52 ± 0.074, 16	1.60 ± 0.056, 14	6.85 ± 0.51,14
Jack Bay (c)	-	-	-	-	-

Table 6. Frequency of occurrence (first number) and number of terrapins (second number) examined for various developmental features (egg tooth [ET], yolk sac [YS], occurrence of axial scutes [AS], occurrence of inguinal scutes [IS], and split cervical scutes [CS]) of terrapins by oiling category and by exclosure. The mean number ([AS(#)] ± 2 SE) of axial scutes is also indicated. (- indicates that no terrapins were collected in that exclosure; other abbreviations as in Table 2.)

Oiling category & site	ET (%)	YS (%)	AS (%)	AS (#)	IS (%)	CS (%)
Heavily & moderately oiled	74, 31	42, 31	84, 31	1.55±0.28	10, 31	32, 31
Caney Creek 1 (h)	100, 5	20, 5	100, 5	1.80±0.40	0, 5	40, 5
Caney Creek 2 (h)	84, 19	63, 19	95, 19	1.74±0.26	11, 19	42, 19
Long Point 1 (h)	-	-	-	-	-	-
Long Point 2 (h)	50, 2	0, 2	50, 2	1.00±1.00	50, 2	0, 2
Hoyer Farm 1 (m)	50, 2	0, 2	50, 2	1.00±1.00	0, 2	0, 2
Hoyer Farm 2 (m)	0, 3	0, 3	33, 3	0.67±1.33	0, 3	0, 3
Lightly to very lightly oiled	44, 9	11, 9	89, 9	1.78±0.44	11, 9	11, 9
Grndfthr's Pond 1 (lt)	-	-	-	-	-	-
Grndfthr's Pond 2 (lt)	50, 2	0, 2	100, 2	2.00	0, 2	0, 2
Trent Hall 1 (lt)	-	-	-	-	-	-
Trent Hall 2 (lt)	-	-	-	-	-	-
Playground 1 (vl)	50, 2	50, 2	100, 2	2.00	0, 2	50, 2
Playground 2 (vl)	40, 5	0, 5	80, 5	1.60±0.80	20, 5	0, 5
Non-oiled, control (c)	80, 15	69, 16	65, 17	1.29±0.48	0, 20	5, 20
Prison Point 1 (c)	-	-	-	-	-	-
Prison Point 2 (c)	-	-	-	-	-	-
Sheridan Point 1 (c)	0, 1	0, 1	100, 1	2.00	0, 1	0, 1
Sheridan Point 2 (c)	-	-	-	-	0, 1	0, 1
Jack Bay 1 (c)	85, 14	73, 15	63, 16	1.25±0.50	0, 18	6, 18
Jack Bay 2 (c)	-	-	-	-	-	-

Table 7. Number of excavated nests (# Nests), number of recovered eggs (# Eggs, with mean nest size ± 2 SE in parentheses), and the number of all recovered eggs and/or egg remains in different conditions (hatched [H], viable [V], and inviable [dead, D) recovered from those excavations at two non-oiled, control sites and two heavily oiled sites. The number of eggs equals the sum of the number hatched, the number viable, and the number inviable (# Eggs= H + V + D). * indicates that nest size could not be determined from three nests; thus data from only 15 nests were used to compute averages.

Heavily oiled sites	**# Nests**	**# Eggs**	**# Hatched**	**# Viable**	**# Dead**
Caney Creek 1	0	0	0	0	0
Caney Creek 2	18*	135 (9.0 ± 2.77)	69	48	18
Long Point 1	9	55 (6.11 ± 2.72)	43	2	10
Long Point 2	14	121 (8.64 ± 2.22)	65	0	56
Non-oiled, control sites					
Sheridan Point 1	1	6	6	0	0
Sheridan Point 2	0	0	0	0	0
Jack Bay 1	26	227 (8.73 ± 1.71)	126	63	33
Jack Bay 2	0	0	0	0	0

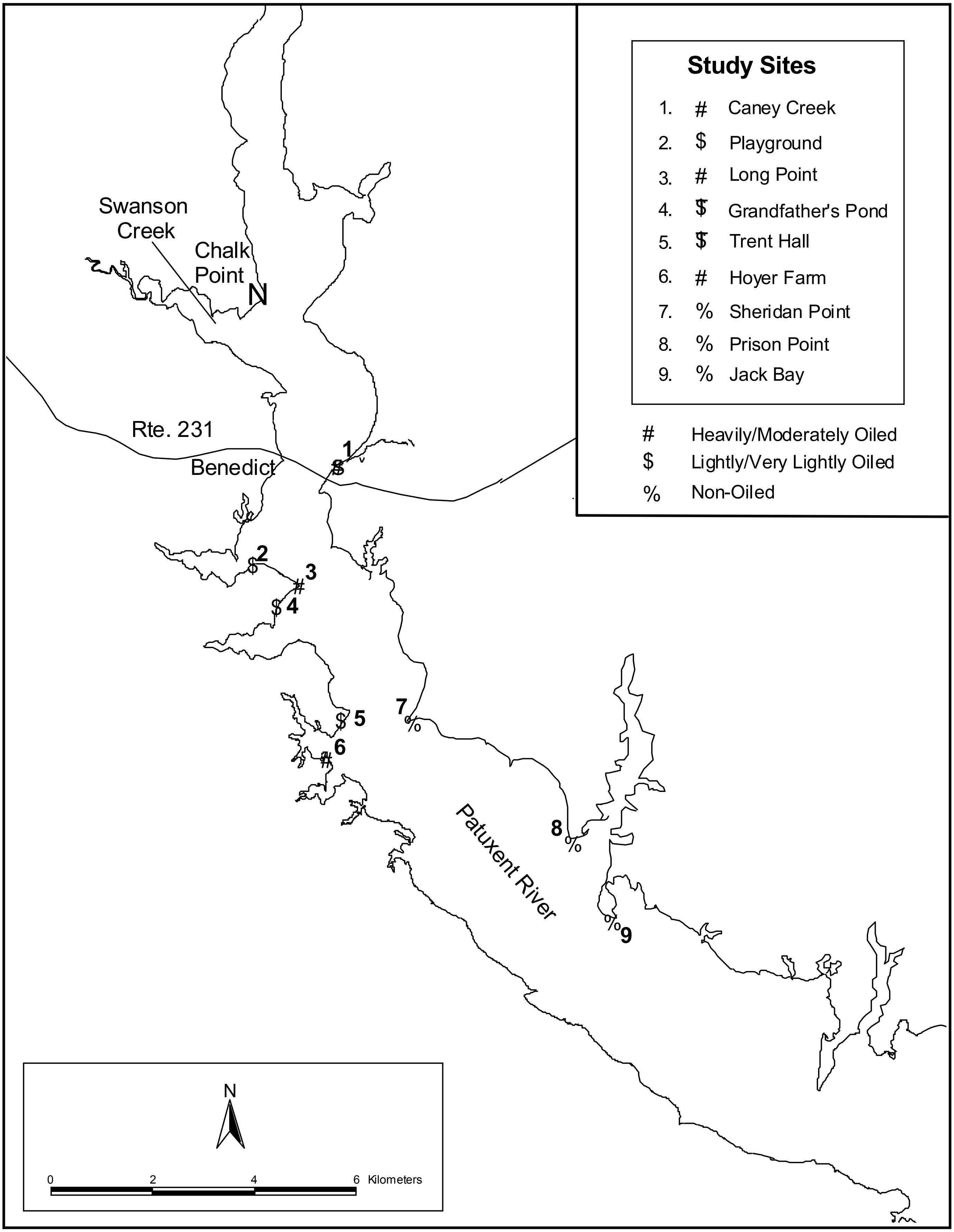

Figure 1: Location of nine study sites to assess the hatching success of northern diamondback terrapins along the Patuxent River.

Figure 2. Construction of terrapin enclosures, September, 2000. (a) Installation of a wooden frame at the Hoyer Farm site for the 50m² exclosure. (b) Attaching vertical panel of 1.5 cm² mesh plastic fencing at the corner of the wooden frame. The plastic fencing is attached with plastic cable ties and metal staples, and is buried 15 to 20 cm into the ground to discourage potential predators. (c) Close-up view showing the border of an exclosure with 2.5 cm² mesh plastic deer cloth attached. (d) A completed exclosure at the Playground site.

Figure 3. Excavation of exclosures, December, 2000. (a) Edge of a terrapin nest at Jack Bay with one egg exposed. In the months subsequent to the deposition of eggs into this nest, shifting sands have roughly doubled the thickness of sand overlying the nest. (b) Four terrapin eggs removed from a nest at Jack Bay.

Appendix 1. Soil temperature (degrees Celsius) collected inside (temp in) and outside (temp out) exclosures

Date	Time	Location	Temp in (C)	Temp out (C)
9/28/00	1310	Caney Creek 1	22	22
9/27/00	1120	Hoyer Farm 1	17	17
9/27/00	1120	Hoyer Farm 2	19	19
9/27/00	900	Jack Bay 1	14	14
9/27/00	900	Jack Bay 2	14	14
9/27/00	945	Prison Point 1	15.5	15.5
9/27/00	945	Prison Point 2	15.5	15.5
9/27/00	1030	Sheridan point 1	16.5	16
9/27/00	1030	Sheridan point 2	16	15
9/27/00	1145	Trent Hall 1	20	21
9/27/00	1145	Trent Hall 2	20	21
9/28/00	1310	Caney Creek 2	22	22
9/28/00	1235	Grandfather's pond 1	24	24
9/28/00	1235	Grandfather's pond 2	24	24
9/28/00	1210	Long Point 1	22	22
9/28/00	1210	Long Point 2	24	25
9/28/00	1145	Playground 1	23	22
9/28/00	1145	Playground 2	23	22
9/30/00	1010	Caney Creek 2	15	16
9/30/00	945	Grandfather's pond 2	16	17
10/2/00	930	Caney Creek 1	12	12
10/2/00	930	Caney Creek 2	12	12
10/2/00	1230	Jack Bay 1	17	17
10/2/00	1230	Jack Bay 2	20	20
10/2/00	1150	Prison Point 1	22.5	23
10/2/00	1150	Prison Point 2	22.5	23
10/2/00	1130	Sheridan point 1	21	22
10/2/00	1130	Sheridan point 2	20	20
10/2/00	1100	Trent Hall 1	21	21
10/2/00	1100	Trent Hall 2	21	21
10/3/00	1030	Grandfather's pond 1	15.5	16
10/3/00	1030	Grandfather's pond 2	15.5	16
10/3/00	1015	Long Point 1	15	14
10/3/00	1015	Long Point 2	14.5	15
10/3/00	1000	Playground 1	14	14
10/3/00	1000	Playground 1	14	14
10/6/00	1035	Jack Bay 1	15.5	15.5
10/9/00	1015	Caney Creek 1	8	7.5
10/9/00	1200	Grandfather's pond 1	12	12
10/9/00	1200	Grandfather's pond 2	12	12
10/9/00	1230	Hoyer Farm 1	12	12
10/9/00	1230	Hoyer Farm 2	11	10
10/9/00	1200	Jack Bay 1	7	7
10/9/00	1130	Long Point 1	9.5	8.5
10/9/00	1130	Long Point 2	10	10

Appendix 1 continued.

Date	Time	Location	Temp in (C)	Temp out (C)

10/9/00	1330	Sheridan point 1	12	12
10/9/00	1330	Sheridan point 2	12	12
10/9/00	1040	Playground 1	9	9
10/9/00	1040	Playground 2	9	9
10/9/00	1300	Trent Hall 1	14	15
10/9/00	1300	Trent Hall 2	14	15
10/10/00	1025	Jack Bay 1	7	7
10/10/00	1110	Jack Bay 2	8	7.5
10/10/00	1110	Jack Bay 2b	7	7
10/10/00	1300	Prison Point 1	11	11
10/10/00	1300	Prison Point 2	11	11
10/12/00	1415	Long Point 2	17	18
10/13/00	1245	Jack Bay 1	12	11
10/16/00	1450	Caney Creek 1	15	14
10/16/00	1450	Caney Creek 2	15	14
10/16/00	1335	Grandfather's pond 1	18	17
10/16/00	1335	Grandfather's pond 2	18	17
10/16/00	1400	Hoyer Farm 1	14	14
10/16/00	1410	Hoyer Farm 2	16.5	15
10/16/00	1030	Jack Bay 1	11	11.5
10/16/00	1030	Jack Bay 2	11	11
10/16/00	1030	Jack Bay 2b	11	11
10/16/00	1510	Long Point 1	18.5	16
10/16/00	1450	Long Point 2	15.5	17
10/16/00	1135	Prison Point 1	13.5	13
10/16/00	1135	Prison Point 2	13.5	13
10/16/00	1220	Sheridan point 1	14	13
10/16/00	1220	Sheridan point 2	13.5	13.5
10/16/00	1525	Playground 1	16	16
10/16/00	1525	Playground 2	16	16
10/16/00	1335	Trent Hall 1	15	15
10/16/00	1335	Trent Hall 2	15	15
10/18/00	1320	Sheridan point 1	14.5	14.5
10/19/00	1240	Caney Creek 2	15	15
10/19/00	1140	Playground 2	13	13
10/20/00	1430	Caney Creek 2	10	10
10/24/00	1305	Caney Creek 1	11	11
10/24/00	1305	Caney Creek 2	11	11
10/24/00	1435	Grandfather's pond 1	15	13
10/24/00	1435	Grandfather's pond 2	15	13
10/24/00	1230	Hoyer Farm 1	10	10
10/24/00	1230	Hoyer Farm 2	11.5	11.5
10/24/00	1130	Jack Bay 1	9.5	10
10/24/00	1110	Jack Bay 2	10	10
10/24/00	1110	Jack Bay 2b	10	10
10/24/00	1455	Long Point 1	15.5	13.5

Appendix 1 continued.

Date	Time	Location	Temp in (C)	Temp out (C)
10/24/00	1445	Long Point 2	15	15

10/24/00	1100	Prison Point 1	10	10
10/24/00	1100	Prison Point 2	10	10
10/24/00	1040	Sheridan point 1	9.5	9.5
10/24/00	1040	Sheridan point 2	10.5	10.5
10/24/00	1515	Playground 1	15	14
10/24/00	1515	Playground 2	15	14
10/24/00	1245	Trent Hall 1	15	15
10/24/00	1245	Trent Hall 2	15	16
10/25/00	1330	Caney Creek 1	12	12
10/25/00	1140	Jack Bay 1	10	10
10/27/00	1300	Jack Bay 1	12	12
10/29/00	1110	Playground 2	9	6
10/30/00	1415	Grandfather's pond 1	12	13
10/30/00	1415	Grandfather's pond 2	12	13
10/30/00	1300	Hoyer Farm 1	11	10
10/30/00	1300	Hoyer Farm 2	11	10
10/30/00	1205	Jack Bay 1	7	6.5
10/30/00	1145	Jack Bay 2	8	7
10/30/00	1430	Long Point 1	6.5	7
10/30/00	1440	Long Point 2	11	12
10/30/00	1100	Prison Point 1	8.5	6.5
10/30/00	1100	Prison Point 2	8.5	6.5
10/30/00	1015	Sheridan point 1	9	8
10/30/00	1015	Sheridan point 2	7	6
10/30/00	1355	Playground 1	10	9
10/30/00	1355	Playground 1	10	9
10/30/00	1320	Trent Hall 1	11.5	13.5
10/30/00	1320	Trent Hall 2	11.5	13.5
11/1/00	1345	Caney Creek 2	8	7
11/3/00	1210	Jack Bay 1	10	10
11/8/00	1345	Caney Creek 1	8.5	8
11/8/00	1345	Caney Creek 2	8.5	8
11/8/00	1100	Grandfather's pond 1	11	11
11/8/00	1100	Grandfather's pond 2	11	11
11/8/00	1130	Hoyer Farm 1	8.5	8.5
11/8/00	1130	Hoyer Farm 2	8.5	8.5
11/8/00	1245	Jack Bay 1	8.5	8.5
11/8/00	1245	Jack Bay 2	7.5	8.5
11/8/00	1320	Long Point 1	9	8
11/8/00	1320	Long Point 2	11	11.5
11/8/00	1220	Prison Point 1	7	7.5
11/8/00	1220	Prison Point 2	7	7.5
11/8/00	1200	Sheridan point 1	9.5	9.5
11/8/00	1200	Sheridan point 2	9.5	9.5
11/8/00	1115	Trent Hall 1	9	9
11/8/00	1115	Trent Hall 2	9	9

Appendix 2. Information collected from excavated exclosures: date, site, identification of individual nests, numbers of eggs in each nest (Egg N), numbers of terrapins that hatched (H), numbers of viable embryos (V/L), and numbers of inviable embryos and/or remains of dead terrapins (I/D). P* indicates that the number of eggs could not be determined and could not be included in determination of average clutch size.

Index #	Date	Location	Nest #	Egg N	H	V/L	I/D
1	12/7/00	Caney Creek 2	1	P*			
2	12/7/00	Caney Creek 2	2	P*			
3	12/7/00	Caney Creek 2	3	16	0	14	2
4	12/7/00	Caney Creek 2	4	4	4	0	0
5	12/7/00	Caney Creek 2	5	1	0	0	1
6	12/7/00	Caney Creek 2	6	P*			
7	12/7/00	Caney Creek 2	7	9	9	0	0
8	12/7/00	Caney Creek 2	8	5	3	0	2
9	12/7/00	Caney Creek 2	9	2	2	0	0
10	12/7/00	Caney Creek 2	10	16	0	16	0
11	12/7/00	Caney Creek 2	11	12	10	0	2
12	12/7/00	Caney Creek 2	12	14	8	0	6
13	12/7/00	Caney Creek 2	13	8	0	4	4
14	12/8/00	Caney Creek 2	1	12	12	0	0
15	12/8/00	Caney Creek 2	2	4	4	0	0
16	12/8/00	Caney Creek 2	3	16	1	14	1
17	12/8/00	Caney Creek 2	4	4	4	0	0
18	12/8/00	Caney Creek 2	5	12	12	0	0
19	12/9/00	Long Point 1	1	12	12	0	0
20	12/9/00	Long Point 1	2	9	9	0	0
21	12/9/00	Long Point 1	3	5	5	0	0
22	12/9/00	Long Point 1	4	2	2	0	0
23	12/9/00	Long Point 1	5	12	0	2	10
24	12/9/00	Long Point 1	6	2	2	0	0
25	12/9/00	Long Point 1	7	3	3	0	0
26	12/10/00	Long Point 1	1	3	3	0	0
27	12/10/00	Long Point 1	2	7	7	0	0
28	12/11/00	Long Point 2	1	6	4	0	2
29	12/11/00	Long Point 2	2	15	2	0	13
30	12/11/00	Long Point 2	3	8	0	0	8
31	12/11/00	Long Point 2	4	5	4	0	1
32	12/11/00	Long Point 2	5	10	9	0	1
33	12/11/00	Long Point 2	6	1	0	0	1
34	12/11/00	Long Point 2	7	6	6	0	0
35	12/11/00	Long Point 2	8	11	0	0	11
36	12/12/00	Long Point 2	1	17	15	0	2
37	12/12/00	Long Point 2	2	10	3	0	7
38	12/12/00	Long Point 2	3	5	0	0	5
39	12/12/00	Long Point 2	4	8	4	0	4
40	12/12/00	Long Point 2	5	11	10	0	1
41	12/12/00	Long Point 2	6	8	8	0	0
42	12/14/00	Sheridan Point	1	6	6	0	0
43	12/18/00	Jack Bay 1	1	4	0	2	2

Index #	Date	Location	Nest#	Egg N	H	V/L	I/D
44	12/18/00	Jack Bay 1	2	5	5	0	0
45	12/18/00	Jack Bay 1	3	14	2	9	0
46	12/18/00	Jack Bay 1	4	6	6	0	0
47	12/18/00	Jack Bay 1	5	3	3	0	0

48	12/18/00	Jack Bay 1	6	10	10	0	0
49	12/18/00	Jack Bay 1	7	13	13	0	0
50	12/18/00	Jack Bay 1	8	12	7	5	0
51	12/18/00	Jack Bay 1	9	10	2	8	0
52	12/18/00	Jack Bay 1	10	20	20	0	0
53	12/18/00	Jack Bay 1	11	12	2	9	1
54	12/18/00	Jack Bay 1	12	3	3	0	0
55	12/18/00	Jack Bay 1	13	12	12	0	0
56	12/18/00	Jack Bay 1	14	9	1	7	1
57	12/18/00	Jack Bay 1	15	11	2	7	2
58	12/18/00	Jack Bay 1	16	4	4	0	0
59	12/18/00	Jack Bay 1	17	10	1	4	5
60	12/18/00	Jack Bay 1	18	13	0	12	1
61	12/18/00	Jack Bay 1	19	11	11	0	0
62	12/19/00	Jack Bay 1	1	12	4	0	8
63	12/19/00	Jack Bay 1	2	4	4	0	0
64	12/19/00	Jack Bay 1	3	6	6	0	0
65	12/19/00	Jack Bay 1	4	3	0	0	3
66	12/19/00	Jack Bay 1	5	5	5	0	0
67	12/19/00	Jack Bay 1	6	5	5	0	0
68	12/19/00	Jack Bay 1	7	10	0	0	10

September 7, 2001

Norman Meade
NOAA Damage Assessment Center (N/ORR3)
1305 East-West Highway
Room 10357
Silver Spring, MD 20910

Dear Norman:

The following are my comments on "Comparison of northern diamondback terrapin (*Malaclemys terrapin terrapin*) hatching success among variably oiled nesting sites along the Patuxent River following the Chalk Point Oil Spill of April 7, 2000" by authors anonymous to me.

I would also like to take this opportunity to comment on the highly professional manner in which the review process itself was handled by you and others involved in the transfer of materials and the giving of instructions for the review. Objectivity and impartiality were maintained at all levels. If any of the parties involved wanted a supportive review or a dissenting one, it was not revealed to me. Hence, my comments are as objective as I can be based only on the written report that I received.

My review of the report ("Comparison of northern diamondback terrapin (*Malaclemys terrapin terrapin*) hatching success among variably oiled nesting sites along the Patuxent River following the Chalk Point Oil Spill of April 7, 2000") addresses the following queries:

1. Are the methods used appropriate for undertaking the stated goals of the study?
2. Were the methods employed properly implemented?
3. Are the conclusions adequately supported by the data and the statistical analyses?

In addition, comments are given regarding specific statements in the report that could be made more succinctly, might be confusing, or that would benefit from editorial modification.

General Statement Regarding Study and Report:

Overall, the study appears to have used a satisfactory approach and methodologies for determining "the hatching success of terrapins at nesting beaches that were variously oiled compared to beaches that were not oiled . . . for assessing the magnitude and extent of injury to the year 2000 hatchling cohort" in consideration of the constraints on the timing of when the study was initiated. The report gives an understandable presentation of the approach and most of the findings, interpretations, and conclusions seem reasonable.

Answers to Specific Questions:

1. Are the methods used appropriate for undertaking the stated goals of the study?

In consideration of the timing of the study (approximately five months after the oil spill), using exclosures to locate hatchings in the early fall and excavating enclosures during late fall were reasonable approaches for assessing the impact of the oil spill on reproductive success of nests during the first year. The Objective section of the report states that the emphasis of the survey was on the 2000 hatchling cohort, so assessing hatchling success was appropriate since there was no opportunity to observe the behavioral responses of females to nesting beaches within the spill zone or to determine comparatively whether females in the spill zone showed different clutch, nesting, or other reproductive patterns than females from non-spill areas. The plan to search for previously hatched terrapins and for overwintering nests by excavating enclosures was a reasonable extension of the exclosure comparisons, in order to obtain a more accurate estimate of reproductive output by female terrapins the previous nesting season.

2. Were the methods employed properly implemented?

Based on the seasonal timing (late summer/autumn after many hatchlings would have departed nests), the selection of nesting beaches and the construction of exclosures were apparently completed in less than a month's time. Continuing the surveys for hatchlings until November 10 following a week with no hatchlings being found was rational. Thus, the study plan of using exclosures appears to have been implemented in a reasonably efficient manner. The recording of hatchling emergences and the body measurements taken also appear to have been done properly. The plan to excavate at each of the nine study sites was thwarted by cold weather, but the alternate plan at least provided a comparison between two sites receiving heavy oil and two control sites without oil. The comparison, therefore, provided a complement to the exclosure studies for 4 of the 9 sites and permitted a comparison between extreme sites in regard to the oil spill.

3. Are the conclusions adequately supported by the data and the statistical analyses?

Interpretation of nest densities at sites:

The conclusion that "terrapin nest density on oiled and unoiled beaches downriver from the Chalk Point generating station did not differ during the 2000 nesting season" is supported by the data. Based on hatchlings captured in exclosures, on hatchlings, hatched eggs, and eggs present in the excavated enclosures, and on the combination of samples from the two approaches the heavily oiled beaches did not differ significantly from those of control beaches in numbers of nests per area.

Interpretation of hatchling emergence in the fall:

The conclusion that "the hatching of terrapins in fall did not differ between oiled and unoiled nesting beaches downriver from the Chalk Point generating station" is supported by the data available. The finding that a total of 33 live hatchlings were encountered in 5 of the 6 exclosures in the heavily oiled sections compared to only 21 from 3 of the 6 in the control sites can be interpreted as being insufficient evidence that the oil spill hindered hatchling success the year of the spill. The statistical tests used (nested analysis of variance) are adequate for the particular purpose of comparing whether difference in hatchling numbers occurred among or within the three oil categories. A conclusion that the findings were not significant is warranted, and the fact that the highest absolute numbers came from the heavily oiled sites further reduces cause for question.

Interpretation of hatchling developmental anomalies and size measurements:

The statements that 1) "no significant differences in any size measurements of hatchlings" occurred among the two oil sites and the control sites and that 2) "fall hatchlings recovered from variously oiled and unoiled sites were comparable in size and weight" are supported by the analysis of variance and Table 5. The sample sizes are adequate to be convincing that no appreciable differences exist.

Interpretation of nest size comparisons:

The statement that "best estimates of nest size at oiled and unoiled sites did not differ" is supported by the data and by the interpretation of the partial nest phenomenon within the report.

Interpretation of egg conditions from selected sites:

The conclusion that "the condition of eggs recovered at selected sites revealed a significantly higher frequency of dead embryos and a lower frequency of presumably spring-emergers at oiled sites compared to unoiled sites" seems equivocal. The report itself even makes the statement on page 14 that “the cause of mortality of these embryos is unknown and may be due to many factors other than the effects of the embryos’ exposure to residual oil present in sediments after cleanup.” This does not provide convincing evidence of an oil-based impact. See additional comments (#4) below.

The following points should probably be clarified in the final report:

1. The statement on page 4 that the designation of the Playground site from “heavily oiled” to “moderately to lightly oiled” altered the “original grouping of beaches in the experimental design” is confusing based on the subsequent presentation of three beaches in each “oiling” category in tables 2, 3, 5, 6, etc. That is, if Playground was originally designated in the “heavily oiled” category, what beach did it replace in the “lightly oiled” category and which beach replaced it in the “heavily oiled” category. The statements on page 4 that address this point are not particularly lucid.

2. The report states on page 10 (lines 14-15) that "Analysis of variance indicated that there were no significant differences in any size measurements of hatchlings among oiling categories." Carapace length and maximum height are included among the measurements. On lines 23-24, the report states "carapace length and maximum height also differed significantly among sites (see Table 5)." This seems contradictory. The latter statement should probably be either dropped or reworded to indicate that "sites" include both oil categories and controls and that the statement has no bearing on a comparison of the impact of oil sites versus control sites (other than to show the high variability in the characters measured). Including the comparison obfuscates the issue.

3. The suggestion made in the summary (page 16) that the oil spill may have contributed to a reduction in nest size seems totally unsupported by the evidence presented. Numerous publications in turtle ecology have demonstrated that reproductive output, including both clutch size and frequency, can vary among years as a consequence of environmental factors unrelated to oil pollution. Also, the interpretation that nest sizes were lower in 2000 appears to be based on an unsupported assumption that clutch sizes determined for females by x-radiography in previous years are applicable to nest sizes in another year. And finally, if the presumed reduction in nest size were a function of oil pollution, why would it not be more apparent on the "oiled" beaches compared to the controls?

4. Table 7 is troublesome for several reasons that collectively cause the results and interpretations presented on this aspect of the study to be questionable and confusing. Listing the most salient points is probably the most efficient way to address the problems associated with this part of the study.

a. At least two or three of the mean number of eggs per nest seem miscalculated in Table 7. For example, under Jack Bay 1, 222 eggs divided by 26 nests yields 8.54 eggs/nest. The table has the mean as 8.73. However, in Appendix 2, the total number of eggs is 227, which would give a mean of 8.73. Which is correct?

b. The intra-nest variability is presented in Appendix 2 but it is not clear how or if it is considered in the analysis that compares the heavily oiled sites with the control sites.

c. In the G-tests on pages 13-14 an implicit assumption is made that the #Dead category represents animals that are comparable to the #Viable category (i.e., that they would have overwintered if they had lived). In reality, a dead embryo could have been comparable to one in the #Hatched category (i.e., it could have emerged in the fall if it had not died). The analysis is based on an assumption that all dead embryos were in the overwintering category. Although there would be no way to know which category a dead embryo should be assigned to, the analysis becomes somewhat confounded, and this point needs to be considered.

d. An inconsistency appears to be present between the statement on page 14 that "At least 28 inviable eggs from several different nests at one Caney Creek exclosure (#2) are of particular interest" and the data on inviable embryos presented in Appendix 2. I only count 18 inviable embryos in Table 7 and Appendix 2.

e. The clutch size of terrapins ranges from 4-18 according to Ernst, Lovich, and Barbour. Appendix 2 indicates several nests with fewer than 4 eggs (2 with only 1) and one nest with 20. The report refers to having concerns about whether those with 1 or 2 eggs represented a complete clutch, and they were thus eliminated from the analyses. Should there also have been concerns about the 5 nests with only 3 eggs each? Should they have been eliminated also? Should there also be concerns about whether the nest with 20 eggs represented a clutch laid by a single female? If other reports exist of clutch sizes/nest sizes outside the range reported by Ernst et al., these should probably be mentioned. This would seem important in an analysis that is based on comparing intra- and inter-nest size among sites, and the justification for using some eggs and not others bears careful consideration.

5. The report should explain how "oiled" beaches would be expected to result in a higher mortality of eggs than control beaches in the same area. The oil presumably would not be on the beach itself above the high tide mark, yet all eggs would be laid in nests above the high tide mark. Therefore, the eggs would not be exposed to oil during incubation. As female terrapins in some areas are known to move more than a mile to nest in some instances, the report should make it clear that a female that nested on an "oiled" beach presumably lived and fed in the affected area, which is extensive, and that the control beaches are sufficiently distant from the oil-impacted areas to have assurance that nesting females did not live and feed in oil-impacted habitat. These considerations should probably be mentioned in the report.

6. The meaning and purpose of a couple of sentences in the report were not clear to me in the context they were presented, as follows:

a. page 12, line 2 from bottom – "It is unknown if data from any nests included in this analysis were complete . . . ." Not sure exactly what this would mean.

b. page 15, lines 1-2 from bottom – "Spring emergers would experience the potential effects of any residual oil contamination for a protracted period compared to fall hatchlings." I assume this means because they would be in the soil for a longer period, but this would be based on a premise that the sand itself was contaminated above the high tide mark. How could that happen?

The following points are for consideration only and not necessarily required as part of the report on hatching success among variably oiled nesting sites in 2000.

1. Open-literature references are minimal in the report, although citations may not be an important aspect. A variety of references could be cited about terrapins and about reproductive traits in emydid turtles that would be pertinent for the topics addressed.

2. Could mammalian predators have been deterred from entering some of the nesting areas (due to olfactory aversion), resulting in a reduction in predation and therefore a nest density that would be higher in the fall? This could perhaps explain the statement on page 12 that "three of the four sites we excavated far exceeded the maximum nest density . . . previously reported." An examination of predator density based on tracks or using more sophisticated approaches with break-beam photography could address this question, but was not possible during the nesting season of 2000 due to the seasonal timing of the study. However, the point should perhaps be mentioned in explaining the higher nest densities.

3. Will the most dramatic impact on reproductive output of terrapins result from a decrease in prey base productivity (e.g., crustaceans and mollusks) in the heavily oiled areas which in turn leads to reduced growth rates of individuals and smaller (or less frequent) clutch sizes of

females? If this were true, the evidence would not become apparent until the nesting seasons of 2001 or later.

Sincerely,

J. Whitfield Gibbons
Professor of Ecology

Office 803 725-5852
Email gibbons@srel.edu