Reproduction is one of the fundamental processes that characterize all living things. Every individual organism, whether it is a single-celled bacterium or a large vertebrate, has a finite life span. Reproduction serves to pass on the traits of individual members of the species to the next generation and thus ensures the survival of a species. Individual turtles have long life spans that factor into their reproductive strategy.

Turtles, like birds, are oviparous. This means that fertilization of eggs occurs internally but the eggs are deposited externally to complete their development. Turtles do not experience metamorphosis or pass through complex life cycles. Turtle hatchlings are miniaturized versions of the adults. Aside from growth, the only complex morphological changes they experience occur during sexual maturation.

Diamondback terrapins have developed a reproductive strategy that shows some subtle variations throughout the range of the species. Some of our knowledge about terrapin reproduction comes from early observations during the time period that terrapins were under cultivation, but there are increasing numbers of reports describing terrapin reproduction in wild populations. Predation of offspring will influence reproductive success. Therefore, most species face a trade-off: large numbers of offspring that are small and not well developed, or smaller numbers of larger, well-developed offspring, which may have a better chance of survival. In all types of turtles, the high rate of predation on eggs is an important factor that shapes the life history of the species. If high percentages of eggs are destroyed by predators, there is a better chance of passing on the genetic potential of the species if the clutch size is large.
However, a female turtle can only harbor a certain number of eggs. Each large female sea turtle may be able to produce more than a hundred eggs per clutch, but the smaller size of the diamondback terrapin will limit her clutch size to considerably fewer eggs. Although diamondback terrapins fall in the middle of the turtle reproductive spectrum in terms of clutch size, they are similar to other turtles in having well-developed but small and vulnerable offspring. Another factor that makes a positive contribution to reproductive success for some species is the investment of parental care. Turtles are deadbeat parents—their offspring are completely on their own once they emerge from their eggs.

The strategy for the survival of a species is next shaped by another trade-off: mature early, within a short time span while the animal is still small and at risk from predators, or spend the early years growing, mature later, and reproduce at a later age. The former strategy is geared to ensure a shot at reproduction before death by depredation; the latter strategy focuses on attaining a size that makes the animal immune from predation but delays reproduction. Turtles have chosen to delay reproduction until they are relatively predator proof. In the early years, their energy is devoted to growing their coat of armor so that when they reach maturity, they will live long and reproduce often.

**Sex Ratio**

Successful reproduction in turtles mandates that there are males and females in a population. However, not all diamondback terrapin populations have equal representation by the sexes. It is intriguing to speculate about the nature of the biased adult sex ratios for various clusters of diamondback terrapins. In some cases the number of mature males is approximately equal to the number of mature females, but in most locations sex ratios are biased toward females. A few sites appear to be exceptions, with males predominating. Before we try to understand the significance of the sex ratio for the sustainability of diamondback terrapin populations, it is reasonable to question the possible causes and the validity of published sex ratios.

The sex ratio in an adult turtle population may be partly explained by the sex ratio of hatchlings. The terrapin exhibits temperature-dependent sex determination (TSD). The sex of the terrapin hinges on the incubation temperature of the egg from which it emerges. Biased ratios of adults within a population may reflect disproportional sex ratios of hatchlings due to the nature and hence temperature of nesting sites in the various clusters. It is impossible to distin-
guish hatchling males from hatchling females with complete confidence based on external morphology. To determine the sex of hatchlings, investigators must use invasive techniques, such as laparoscopy, or sacrifice the turtles and perform histological examinations by looking at gonadal (sex organ) tissues under a microscope. Thus it is difficult to correlate incubation temperatures in natural nests with sex ratios in the emerging hatchlings.

Disproportionate numbers of males or females may also be attributed to differential mortality. There is no obvious reason why there should be differential mortality in wild hatchling or juvenile terrapins. For adult and subadult terrapins, other factors may affect life span. Depending on design of crab traps, males and females may suffer differential mortality rates as a result of accidental entrapment and subsequent drowning. Crab pots tend to trap more males than females (Roosenburg and Kelley, 1996) and can thus skew populations ratios toward a female bias. Because of their time spent on land, females are predictably more at risk of predation than males. In some regions, adult females are particularly vulnerable as victims of automobiles when they cross roadways to nest. With the expansion of the coastal road network to service resort and vacation areas, females in a population will be disproportionately killed as a result of road mortality. A study by Gibbs and Steen (2005) showed that sex ratios in turtle populations become more male biased where there are more roads and where females exhibit more terrestrial movement than males. For diamondback terrapins, the ratios in locations with high road mortality should show more sex bias toward males, which is not always the case. Since terrapins rarely roam, movement into clusters (immigration) or out of clusters (emigration) is unlikely to account for sex bias.

In some instances, reports of sex bias may be attributed to some degree to sampling bias. Female terrapins are easier to find because they spend more time on land. Small juvenile females can often be mistaken for mature males. Younger terrapins are almost impossible to find. And certainly, different seasonal sampling is expected to result in different sex sampling. This was reported by Seigel (1980a) in the Indian River, near the central Florida coast, when he showed that the winter sampling ratio of males to females was 10:1 while the late spring/summer ratio in the same location was 5:1. After mating, males may disperse and be more difficult to capture. Sampling in creeks adjacent to popular nesting areas may yield more females than males. Thus there are inherent problems when researchers attempt to ascertain the sex ratio of their terrapin colonies.

Lovich and Gibbons (1990) found a male biased population (average ratio
of males to females was 1.78:1) at Kiawah Island, South Carolina. They used a variety of collection techniques and equipment, including trammel nets and seine nets. Under conditions that produced almost the same probability of recapturing individuals from either sex (0.44 for males and 0.38 for females), males always outnumbered females despite some yearly variations in the ratio. Lovich and Gibbons attributed this male bias to the fact that terrapin males mature at a much faster rate than females. Males mature in about three years and females take twice as long to reach adulthood, so one would expect more males in the adult population. Although this seems like a very reasonable explanation for a male-biased sex ratio, researchers at other sites are not finding the same male bias. A recent population sampling at Kiawah Island may point to one potential cause of biased sex ratios. Long-term studies at the same Kiawah Island creeks indicate a change in the sex ratio. It seems that crab pots are responsible for increasing the mortality of males, and thus the population sex ratio has shifted to the point where there is now a slight female bias (Gibbons et al., 2004).

What would be the ideal sex ratio to sustain terrapin populations? In studies in the early 1900s of farm-raised terrapins, the highest fertility was observed when the ratio of females to males was 5:1 (Hildebrand, 1932). No one knows the answer to the question of optimal sex ratio for terrapins in the wild, but most researchers have the opinion that the loss of significant number of adult females can be the death knell for a population.

Sexual Size Dimorphism

As outlined in chapter 1, there is a dramatic size difference and age in reaching maturity between adult male and adult female terrapins (plate 1). Adult females are always much larger than males and require more time to mature. Secondary sexual characteristics also distinguish the sexes: Females have larger heads than males; males have thicker tails with the cloaca positioned outside the posterior margin of the plastron. Why should diamondback terrapin females be so much larger than males? The same phenomenon is true in some other turtle species, but in a few types of turtles, such as the snapping turtle, the male is larger than the female. If males display aggression and have to fight for mates, larger size would be an advantage. If females “select” mates, larger male size may be a factor in the selection process. Because male terrapins are so small, these factors are probably not significant in driving the size of males. It could be argued that it is an advantage to the species when adult
males and females achieve vastly different sizes because the sexes are not forced to compete for the same food resources.

Other size advantages are more obvious. If the male matures early, even though he achieves a smaller size, he increases the number of times he can mate throughout his lifetime, thus increasing his lifetime reproductive potential. If we assume that female body size has some correlation with clutch size; that is, the number of eggs that can develop and be laid at a given time, later maturation and attainment of a larger size would be an advantage by increasing the number of eggs per clutch and thus the female's lifetime reproductive output. The relationship between size of females and clutch size is explored later in this chapter.

Courtship and Mating

Although some aspects of terrapin mating have been elucidated from captive breeding programs, one of the missing areas in terrapin biology is observation of mating in the wild. In the North, we know that the more obvious sightings of mating horseshoe crabs signal the advent of terrapin mating season.

Mating has been observed in a few natural terrapin colonies. Chesapeake watermen told of great concentrations of terrapins in specific creeks in the early spring that may have represented mating aggregations. Siegel (1980c) reported mating aggregations at his study site at Merritt Island, Florida. On Cape Cod, an annual mating aggregation has been observed in one small cove in Wellfleet Harbor during late spring, although mating terrapins are sighted occasionally throughout their activity period. Aggregations make sense for mating in an aquatic species. It saves a lot of time for a seasonally active species in which males and females must search for a mate in a short time span. In Wellfleet, I have seen females being pursued by one or more males and it is often possible to scoop up a mating pair in a net from a kayak. In one scoop I once netted a three-year-old male and a precocious six-year-old female. Apparently, both turtles had recently reached maturity and were most likely mating for the first time or at least going through the motions. These ages are relatively young for terrapins to be mating on Cape Cod.

Even though terrapins have a distinct home range (the area they utilize for day-to-day and seasonal activities), males do not display territorial behavior. I have never observed males fighting for females. The whole scene is rather congenial, almost businesslike. It is still a mystery how and why the Wellfleet terrapins flock to this particular cove to mate. Other mysteries about
the mating process abound. How is this annual event initiated? Who attracts whom, and are chemical/olfactory attractants or pheromones involved? How common is mating at other times of the year and at other locations?

In one of the few studies of diamondback terrapin mating in the wild, attempts were made to observe mating behavior from behind natural blinds in water that offered only 1.0 meter visibility. Twelve matings were witnessed in canals near Merritt Island during spring aggregations where six to seventy-five individuals were observed. All mating occurred in daylight when air and water temperatures were similar. Water temperatures ranged from 24.8 to 27.0°C (76.6 to 80.6°F) and air temperatures ranged from 22.8 to 27.0°C (73 to 80.6°F). The turbidity of the water prevented the entire courtship and mating ritual from being witnessed. From snapshots of the ritual from different mating pairs, observers could put together a scenario in which a female first floats on the surface, with the male approaching from the rear and nudging the female’s cloacal region with his snout. Within a minute, the male mounts and copulation occurs immediately. Injection of sperm into the female is completed over the span of 1 to 2 minutes. If the female swims away before copulation, the male may follow in active pursuit (Seigel, 1980c). During May and June, in the clear waters of Chipman’s Cove on Cape Cod, terrapin mating plays out in a manner similar to that described by Seigel (1980c). The entire courtship and mating sequence appears to be fast and furtive (Brennessel and Lewis, personal observations). Only continued observations of mating in the wild will help to identify any courtship rituals or subtle behaviors that are important in the manner in which this event is orchestrated.

Female terrapins can store sperm for several years. Observation of females at terrapin farms revealed that some could produce eggs up to four years without contact with males. There was some indication that the ratio of fertile to infertile eggs may have decreased as a result of sperm storage (Coker, 1920). The obvious advantage for terrapins, as well as other species in which the phenomenon of sperm storage occurs, is that it is not imperative for a female to find a second or third mate if she will produce more than one clutch per year. Furthermore, a lack of males or low ratio of males to females in the population will not prevent the female from reproducing every year. The ability to store sperm would be an advantage in populations with female-biased ratios.

Because females can mate with more than one male each season and store sperm, there is the potential for each clutch of eggs to be fathered by more than one male. Several researchers are interested in whether a single clutch of eggs is the result of multiple paternity. With the possibility of multiple mat-
Reproduction

ings and the ability to store sperm, it is not clear how many males’ or which male’s sperm fertilize the eggs of each clutch. Molecular techniques are being used to address this question, and we will most likely find that some of the hatchlings in some clutches have different fathers. Using genetic tools, preliminary work in this area suggests that multiple paternity does occur but that it may not be as common in diamondback terrapins compared to other turtle species (Argyriou et al., 2004; Hauswaldt, 2004; Page and Brennessel, 2005). The possibility of sperm competition is related to the issue of multiple paternity. In many species with multiple matings, it has been shown that the sperm of one particular male may preferentially fertilize all or most of the eggs. When a female has mated with several males, the male whose sperm is utilized will have the best chance of passing on his genes to future generations. It may be a case of the survival of the fittest sperm!

Nesting

The nesting activities of female terrapins are inarguably the best understood aspect of the reproductive ecology of the species. There have been many studies and observations of terrapin nesting throughout their geographic range (table 3.1). However, these studies may not be as easy to conduct as one would think. A researcher must be in the right place at the right time to observe terrapin nesting. Not only are terrapins elusive in the water; they conduct their nesting activities in a most secretive manner. When a female is on a nesting run, we must remain quiet and hidden if we hope to observe her through the entire spectrum of her nesting activities. If we are lucky and are observing in a sandy area, we may find tracks that lead us from the creek or marsh to a nesting terrapin or a completed nest (fig. 3.1). In vegetated nesting areas, even Sherlock Holmes would find it difficult to remain on the trail of a nesting terrapin.

Female terrapins are known to lay eggs more than once each season. In captivity, where mating, nutrition, and growth were optimized, up to five clutches of eggs per female were recorded in one season (Hildebrand, 1932) although two to three clutches a year is more common. Clutches are separated by approximately 14 to 17 days, the length of time it takes for development of a set of eggs. Once she is ready to lay her eggs, the female must leave the relative safety of the water and trudge onto the land to dig a nest and deposit them. The trip has many perils. In some cases, the female may face vertebrate predators. Raccoons in Jamaica Bay Wildlife Refuge have been known to kill
Fig. 3.1. Tracks, disturbed sand, and the tracings of a tail indicate a terrapin nesting area.
and eat adult female terrapins when the females travel over land to nest (Feinberg and Burke, 2003). Long journeys on land also bring the danger of dehydration and overheating. Some females make the trip to nesting areas several times before conditions are right and they deposit their eggs. These forays are accompanied by sand sniffing, seemingly random digging with the snout and forelimbs, and even digging of a nesting chamber. But for reasons that are unclear, the female may abandon the nest before she deposits any eggs (fig. 3.2). It is fairly common, especially in the North, to observe aborted nesting attempts in which gravid females trudge onto land, scout around for a proper nesting site, and then about-face and return to the marsh. In some cases, this type of aborted nesting may be attributed to human activities: Noise, traffic, bicycles, pets, and other types of commotion may chase nesting terrapins back into the marsh. Peter Auger’s group (Auger and Giovannone, 1979) observed certain females attempting to lay eggs five to six times over a period of a week to ten days. Each attempt was accompanied by an anthropogenic disturbance that drove the female back into the water. This nesting delay may actually be detrimental to the population, because pushing nesting to later in the summer may delay hatchling into late fall, especially if a second clutch is involved. In the North, this poses problems for hatchlings when the temperature drops in October and November.

Human activity is not the only cause of aborted nesting. I have witnessed, time after time, a gravid female begin to prepare a nest and abandon it, only to move on and repeat her attempted nesting many times before she actually deposits eggs or calls it a day and heads back to the marsh . . . only to try again in a day or two. The area she digs up often looks like a battle zone, pocked with holes that are the remains of aborted nests. When I dig down into some of the abandoned nest cavities, I occasionally find a large rock or thick plant root that most likely signaled the female to try another spot. Sometimes there is no obvious reason why a nest has been abandoned. Perhaps the female is trying to confuse predators. Females also make nonnesting excursions. I have sometimes found nongravid females on land, rooting about as though they were preparing to deposit eggs but never getting down to business.

In some locations, human activities may actually shift the pattern of nesting from diurnal to nocturnal. Although nesting in daylight is the norm in most terrapin colonies, on Sandy Neck, Barnstable, Massachusetts, (Auger and Giovannone, 1979), Little Beach Island, New Jersey (Montevecchi and Burger, 1975), Patuxent River, Maryland (Roosenburg, 1994), Jamaica Bay,
New York (Feinberg and Burke, 2003), and in other locations, a significant amount of nesting activity may be occurring at night (table 3.1).

The weather is also a factor that determines nesting events. Air temperatures must be warm enough to power the movements of the nesting female but not so hot that she will overheat during a nesting foray. Feinberg and Burke (2003) have found the air temperature during optimal nesting times in Jamaica Bay to be $25.4 \pm 3.2^\circ C$ $(77.7 \pm 6^\circ F)$, while nesting did not occur when air temperatures exceeded $35^\circ C$ $(95^\circ F)$. Peak nesting was observed at 25 to 75 percent cloud cover. In central coastal Florida, Seigel (1980b) observed that most nesting occurred under clear skies with an air temperature maximum for nesting at $36^\circ C$ $(96.8^\circ F)$. Most investigators find that nesting does not usually occur under completely cloudy skies or during periods of rain. Perhaps female terrapins cue into the position of the sun to help with nest site selection. This

---

**Fig. 3.2.** Aborted nest. A female terrapin began to dig out a nest but then abandoned it. There were several aborted nests in the immediate vicinity, all dug by the same female.
visual cue may be important to position the nest in a location where the amount or angle of the sun will have a positive impact on nest temperatures throughout incubation.

Temperature restrictions may define the beginning and duration of the nesting season. The commencement of nesting for each colony varies predictably as a function of latitude as well as seasonal temperature variation. In the South, nesting may start as early as the beginning of May, while on Cape Cod, nesting rarely occurs before the middle of June and is sometimes delayed into the first weeks of July (table 3.1). In Florida, where the nesting season is longer, nesting and hatching may overlap; turtles in nests laid in May can be hatching while other nests are still being laid (Butler, 2000). With an extended nesting season, it is possible for southern terrapins to produce three clutches of eggs per year, while two is the maximum number of clutches for females in northern populations. Although double clutching has been observed on Cape Cod, it may not be the norm, especially when nesting does not begin until July.

In a study of reproductive ecology at Jamaica Bay Wildlife Refuge, Feinburg and Burke (2003) observed three distinct peaks of nesting activity separated by approximately two-week intervals. The first peak was characterized by large numbers of nesting females concentrated within a few days. During the following two nesting periods, fewer nesting terrapins were observed. In Wellfleet, three peaks of nesting activity are also observed but they occur over a more contracted time period. For example, in one year there were ten days between the first and second nesting peak and five days between the second and third. While it may be possible to speculate that the nesting time intervals observed at Jamaica Bay might correlate with multiple clutches from the same females, the intervals in Wellfleet nesting activity are not as easily related to multiple clutches and could possibly be related to variation in weather conditions.

There is a great variability in the distance females must travel on land in order to find suitable nesting habitat (table 3.1). On mangrove islands in Florida Bay, the nesting area is a few meters from the water. In Jamaica Bay, New York, nesting occurs about 180 meters (200 yards) or less from the water (Cook, 1989). When I visited Jamaica Bay during nesting season, I observed that the nesting areas were very close to the water. Female terrapins could be seen bobbing up and down within a few meters of the nesting beach, waiting for the opportune moment to come ashore. On Cape Cod, females sometimes make astounding trips across large expanses of marsh and sand dunes. Round
trips of up to 1600 meters (about a mile) are not unusual (Auger and Giovannone, 1979). In Barrington, Rhode Island, nesting areas are not visible from the marsh. It sometimes requires long treks through thickly wooded areas before a female finds suitable nesting substrate.

In areas in which upland nesting sites are a considerable distance from the water and in areas with a great deal of tidal height variation, tides may have a significant impact on nesting activity. In the Northeast, such as at Oyster Bay, Long Island (Bauer and Draud, 2004), Sandy Neck, Massachusetts (Auger and Giovannone, 1979), Jamaica Bay, New York (Feinberg and Burke, 2003), and Barnegat Bay, New Jersey (Burger and Montvecchi, 1975), nesting occurs at all tidal heights but is concentrated around the hours of high tide. In northeast Florida, tidal nesting preference was found to be several hours before to one hour after each high tide (Butler, 2000). High tides float females up into the marsh, where they are closer to potential nesting sites. Not only does this mean that they have less distance to travel to high, dry nesting areas, but it allows them to have a gauge for the high tide line and thus a location to dig a nest that will be safe from tidal inundation. In some areas, a significant increase in nesting activity is observed at the time of the full moon, when tides are at their highest (Bauer and Draud, 2004). Although high tide nesting is common, some females nest to the beat of a different drummer and are found on nesting runs at mid and low tides.

Nest Site Selection

It is not easy to decipher all of the instinctual factors that drive females to select the exact time and place for nesting. In most terrapin colonies, females display a preference for sandy, nonvegetated areas. Sandy soil of loose particle size may be optimal for gas diffusion and may be necessary for proper embryonic development. Sometimes sites are used in which the sand is relatively compact or strewn with gravel or shells. Large sandy areas offer less shading, and thus result in higher soil temperatures. Lack of nearby vegetation lessens the probability of destruction of eggs by plant roots. The disadvantages to open sandy areas include the higher probability of desiccation of eggs and chance of erosion due to wind. In contrast, nests in vegetated areas are more prone to root infiltration and are subject to cooler incubation temperatures. Terrapins are limited in terms of nesting sites and substrate type, depending on their location. On mangrove islands in Florida Bay, the nesting area is limited to a narrow margin of sandy marl between the hypersaline...
water and the swamp. In Chesapeake Bay, the sandy stretches around the bay are narrow, discontinuous, and interspersed with sections of salt marsh. In Cape May, New Jersey, females utilize the causeways to reach limited sandy areas that border the roads.

Terrapins may nest in clusters, with many females using the same small stretch of sandy terrain. This is especially true where nesting habitat is limited by natural topography or in areas populated by humans where much of the historical nesting habitat has been converted to home sites, driveways, and roadways. In many cases, sandy areas have been “hardened” with asphalt and other materials to allow the passage of automobiles. Seawalls and revetments may prevent females from utilizing some potential areas. Auger and Giovanone (1979) reported nest density in Sandy Neck, Barnstable, Massachusetts, to be fifty nests per 96 hectares (237 acres), which is relatively disperse compared to reported densities of 289 nests per hectare (2.4 acres) in northeast Florida (Butler, 2000), 220 nests per 1.4 hectares (3.5 acres) near Barnegat Bay, New Jersey (Montevecchi and Burger, 1975), and 446 nests per hectare (2.4 acres) in a nesting area within a Rhode Island wildlife refuge (Goodwin, 1994). Even within the same habitat, such as the Patuxent River, nest densities may vary from 60 nests per 0.25 hectares (0.6 acres) at one location to 225 nests per hectare (2.4 acres) at another location (Roosenburg, 1994). It is not clear whether nest densities in some localities are due to local colony size or to amount of suitable nesting habitat at each site.

Most nesting occurs above the high tide line, although there are some instances when a terrapin may not be savvy enough to anticipate the height of a spring tide and her nest may be flooded. If we happen to find a nest below the spring tide wrack line, we will relocate it to higher ground and hope the female’s progeny won’t repeat the mistake of their mother. Perhaps, it wasn’t a mistake at all. Erection of bulkheads and other attempts to stabilize shorelines have created obstacles that prevent terrapins from finding suitable nesting sites.

Observation of nesting females in some locations reveals a curious behavioral pattern. Before females select a nest site, they will often sniff, taste, or probe the substrate. We have no idea what they are looking for, but this behavior is persistent and pervasive for northern terrapins. It is very common to find a female on a nesting run who has sand all over her face.

We don’t know if a natal homing instinct plays a role in nest site selection. Do females return to the region where they emerged as hatchlings, or do they strike out for new nesting territory? Once females have located nesting areas,
they exhibit remarkable site fidelity, a phenomenon known as philopatry, returning to the same nesting area, clutch after clutch, year after year. There are always some exceptions. In Wellfleet, we discovered a single female terrapin who thumbs her nose at the convention; she has used alternate sides of a large creek for her first and second clutches. Another exception to the rule of philopatry involved a female who produced her first clutch on June 20 and came back to the same area on July 5 to try for a second nest. She was disturbed by tourists and returned to the water, only to be discovered on July 13 on a nesting run, 10 kilometers (6 miles) farther south.

Terrapins often select sandy roads to dig nests. In Jamaica Bay Wildlife Refuge, some nesting occurs on compacted trails covered with fine gravel. This certainly makes it more difficult for females to dig their nests but they are assured sunny, nonvegetated sites. On Merritt Island, Florida, when terrapin nesting was observed in the 1970s, terrapins opted for dike roads surrounding lagoons rather than sandy dunes (Seigel, 1980b). In Wellfleet, the sandy roads and driveways on Lieutenant Island and Indian Neck are certainly well utilized. Although these nests have the potential to survive an entire summer of traffic, they are jeopardized when hatchlings begin their emergence by digging and softening the substrate. Such softened nests easily succumb to the weight of automobiles or even bicycles as they roll over the nest. If “tire track” nests are found, we will often relocate the eggs to safer areas. Care must be taken so that the order and orientation of eggs is not altered when they are relocated (fig. 3.3). Very early in development, the turtle embryo attaches to the inside wall of the egg. There will be interference with development if eggs are turned to a new position after the first few hours following their deposition in the nest. In the event that natal homing is important in the reproduction of terrapins, hatchlings that result from these nest relocations are always released at the site of their original nest.

Aside from the openness of the sites, the predilection for roadways may have something to do with the slope of the location. Although we observe terrapins nesting on steeply sloped dunes, most nests are dug on flatter surfaces. This may be partially due to the fact that nests on slopes are more difficult to dig since sand is less stable and will tend to fill in recently excavated areas. Sometimes, the only area around a marsh is steeply sloped. Such is the case in one of the locations I study each summer. Determined females head up steep hills to look for nesting sites. On several occasions, I have found nesting females because they have tumbled backward down a steep hill and landed at my feet.
Once the urge to nest sets in, females are resolute and unwavering. I once witnessed a young female, about eleven years old, whose two front limbs and one rear limb were whittled down to stubs. It was not clear whether she suffered from a developmental defect or a serious accident. She was gravid and had thus obviously mated. When I spotted her, she had lumbered a long distance from the marsh and was attempting to climb a steep wooded embankment in hopes of finding a suitable nesting area.

In addition to being single-minded, females on nesting forays are extremely alert. The sense of movement, the cracking of dry saltmarsh hay underfoot, the click of a camera shutter or the flight of a gull overhead will cause her to remain motionless for long periods of time. Without moving, she will blend in with the marsh vegetation or sandy substrate and will be almost impossible to discern. I have watched immobilized females for up to thirty minutes before they ventured forth to complete their nesting run or retreated back into the marsh or water. If undiscovered, they could easily be mistaken for a rock. In some instances, the patient terrapin outlasts the researcher, who must move to swat a greenhead fly or scratch a mosquito bite.

**Digging the Nest**

Once a female has selected a nesting area and has made her final decision, the nesting process takes about thirty minutes. By observing twenty-eight females from the start of the process to completion, Feinberg and Burke (2003) found nesting times to vary from 13 to 47 minutes with a mean time of 24.8 ± 6.9
The female terrapin sculpts her nest in an invariable sequence of events. She first smooths and clears the area with her snout and front limbs (fig. 3.4). Then, as she alternates the scooping action of her rear limbs, she digs a small round hole approximately 4 centimeters (1.6 inches) in diameter. Still alternating her scooping, she expands the nest. Her nails help with the digging, her webbed toes with sand removal. The original hole never widens, but the nest chamber beneath slowly enlarges as she brings small loads of sand to the surface and deposits them around the nest for future use. She appears to rock from side to side as she repositions herself over the hole each time she alternates the action of her limbs. Gradually, the nest takes on a flask shape, narrow at the neck and wide at the bottom. The depth and overall measurements of nest cavities have been reported in a number of studies. Using composite data from several studies, the top of the main nest cavity varies from a mean depth of 6.0 to 10.65 centimeters (2.4 to 4.2 inches) from the surface to a mean depth of 14 to 16 centimeters (5.5 to 6.3 inches) to the bottom of the chamber (Montevecchi and Burger, 1975; Butler, 2000; Brennessel and Lewis, personal observation). Roosenburg (1994) reported the top of nests to be 5 to 17 cen-
timeters (2 to 6.7 inches) deep (mean = 12 centimeters (4.7 inches) and the bottom to be 10.5 to 24 centimeters (4.1 to 9.5 inches) (mean = 16.5 centimeters (6.5 inches)). The nest chamber itself measures an average of 4.67 centimeters (1.8 inches) deep and 7.29 centimeters (2.9 inches) wide (Montevecchi and Burger, 1975).

Nest depth not only has a great impact on incubation temperature, it also affects the overall success of hatchling development. Very shallow nests are prone to desiccation, erosion, and high temperature stress. Deep nests are jeopardized by low temperatures and perhaps also deficits of oxygen and moisture. Burger measured the absolute success rate of hatchling development as a function of nest depth. In twenty nests that averaged 18.2 ± 2 centimeters (7.2 ± 0.8 inches), all eggs developed. In shallow nests, with mean depth of 12.5 ± 1.83 centimeters (5 ± 0.72 inches), no eggs hatched, while 5 nests having a mean depth of 14.3 ± 1.27 centimeters (5.6 ± 0.5 inches), the top eggs did not hatch. In 11 nests of mean depth 18.7 ± 1.6 centimeters (7.4 ± 0.6 inches), bottom eggs did not develop. Excavation of nests and examination of eggs that did not hatch revealed incomplete development of embryos (Burger, 1976).

When the female terrapin is satisfied with the architecture of the egg chamber, she begins to deposit her eggs (fig. 3.5). She hunkers down so that her cloacal opening is at the top of the hole. Her front limbs support her in a semierect position. She is tilted at an angle and the bottom half of her carapace may be hidden from view. Once oxytocin-like hormones have kicked in, the eggs emerge, one at a time, and are dropped haphazardly into the nest cavity. The eggs are soft, pink-tinged, symmetric ovals and have a leathery casing (plate 9). The soft texture of the eggshells make them resilient and inhibits cracking as they tumble down on top of one another. The shells will
DIAMONDS IN THE MARSH

dry out somewhat over the next twenty-four hours but will never become brittle like bird eggs.

Once females begin to deposit eggs, they have rarely been observed to stop midstream. It is an atypical event that prevents her from depositing the full complement of eggs into her nest. Occasionally, a female on a nesting run will drop her eggs prematurely if she is handled. Females in captivity in tanks without nesting substrate will sometimes drop eggs into the water. Without optimal nesting habitat, some females in captivity may not lay a complete clutch within the usual time period. It was once noted that captive females deposited an average of two eggs in several discrete nesting attempts spread out over two to seven weeks (Burns and Williams, 1972).

After eggs are deposited the female backfills the moist sand that she excavated, into the nest between layers of eggs, and packs the sand down with rear limbs. Her motions are the reverse of those used in the digging process. She alternates her rear limbs, scooping behind her as she backfills sand into the nest cavity. The female is not quite done when the cavity is filled. She utilizes a push-up type motion to lift herself, then thumps the nest with her plastron as she descends. She performs this up-and-down motion several times to compact the sand on top of the nest. Some observers have noticed that the females may release fluid from their cloaca over the nest, perhaps to further compact the sand or to provide moisture. If she is not frightened or rushed, the female will tidy up around the nest and kick some sand over it during her departure so that it blends in with the surrounding substrate. Sometimes the nest can be found by searching for tracks and telltale marks, especially in very sandy areas. A rather smooth area, approximately 0.25 meters (about 10 inches) in diameter, may be outlined with terrapin footprints. The thin mark of her tail may be detected over the smooth area that forms the roof over her nest (fig. 3.1). More often than not, the nest becomes an integral part of the landscape as the female terrapin leaves her eggs and future progeny to their own fate. Although some reptiles, such as crocodiles, protect their nests and attempt to assure the success of their hatchlings, turtles are completely disinterested. Once the terrapin has laid her eggs, she still has a dangerous journey back to the marsh; her return to safety appears to be a strong driving instinct after nesting. Regarding the lack of parental care, Coker (1920) remarked, “Eggs are laid in a proper place and sometimes an improper place, and so far as we know, neither parent gives thought to the welfare of its offspring or even recognizes them when they meet in passing.”
Egg and Clutch Size

The egg and clutch sizes published for various studies of diamondback terrapin nesting ecology display geographic variation (table 3.1). The trend is for smaller clutches with larger eggs in the southern latitudes, and larger clutches, with smaller eggs in the North. The clutch sizes of \textit{M. t. tequesta} in central Florida (Seigel, 1980b) average 6.7 eggs, similar to those reported for \textit{M. t. centrata} in northeast Florida (Butler, 2000; Butler, et al., 2004). In Jamaica Bay, New York, the mean clutch size increases to 10.9, with a wide range of three to eighteen eggs per clutch (Feinberg and Burke, 2003). Wellfleet, Massachusetts, terrapins have average clutches of twelve eggs, with a range of four to twenty-two eggs per nest (Don Lewis, personal communication). The exception to this latitudinal correlation is the somewhat larger clutch and eggs size of Chesapeake Bay terrapins (average 12.29 eggs with a mass of 9.87 grams [0.35 ounces]) (Roosenburg and Dunham, 1997). In some studies conducted within local populations, clutch size appears to correlate with the size of the female. In general, the tendency is that the larger the female (as measured by mean plastron length), the larger is the clutch (Montevecchi and Burger, 1975; Seigel, 1980a; 1980b; Roosenburg and Dunham, 1997). Depending on climate, it may be more useful for terrapins to produce multiple small clutches, which occurs in the South, or fewer, larger clutches, which may be more typical of northern regions with a more contracted nesting season. Terrapins at the northern fringe for the species may be putting “all their eggs into one basket.”

One might be tempted to predict that within a terrapin colony, smaller clutches would contain larger eggs or that larger females would have larger eggs. But studies have shown that within a colony, there is no correlation between clutch size and egg size. There is also a lack of correlation between the size of a female and the size of her eggs (Montevecchi and Burger, 1975; Roosenburg and Dunham, 1997; Seigel, 1980b). In general, terrapins have a large variation in eggs size among clutches but little variation within clutches. In one study, egg size tended to decrease with clutches laid later in the season and no differences were found in the size of eggs deposited in different topographic regions of the nesting area (Montevecchi and Burger, 1975).

The trade-off between larger clutch size and larger egg size is not completely clear. Egg size may be an important contributor to hatching survivorship. Larger eggs, with more food reserves, usually produce larger hatchlings. This may be an important strategy in habitats in which hatchlings will be competing for resources. In contrast, production of more offspring may be a
<table>
<thead>
<tr>
<th>Study site</th>
<th>Nesting season</th>
<th>Time of day</th>
<th>Weather</th>
<th>Substrate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Louisiana</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Northeast Florida</td>
<td>Late April–late July, 78 days</td>
<td>Diurnal and nocturnal</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Central Florida, east coast</td>
<td>Late April– early July, 52–57 days</td>
<td>Diurnal</td>
<td>Clear skies</td>
<td>Dike roads, compacted sand</td>
</tr>
<tr>
<td>Beaufort, N.C., natural nests</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Beaufort, N.C., turtle farm</td>
<td>6 May–31 July, 80–90 days</td>
<td>—</td>
<td>—</td>
<td>Artificial sand pans</td>
</tr>
<tr>
<td>Patuxent River, Maryland</td>
<td>1 June–30 July, 60 days</td>
<td>Peaks at 1100 to 300 h; observed round the clock</td>
<td>Sunny</td>
<td>Narrow sandy beaches</td>
</tr>
<tr>
<td>Cape May, N.J.</td>
<td>Early June–mid July, 41 days</td>
<td>—</td>
<td>—</td>
<td>Road embankments</td>
</tr>
<tr>
<td>Brigantine National Wildlife Refuge, N.J. (now Edwin B. Forsythe National Wildlife Refuge)</td>
<td>34–44 days</td>
<td>Diurnal</td>
<td>25–75% cloud cover</td>
<td>—</td>
</tr>
<tr>
<td>Jamaica Bay Wildlife Refuge, N.Y.</td>
<td>3 June–13 July, 51 days in 1999; 9 June–4 August, 57 days in 2000</td>
<td>Diurnal 0930 to 2115 h</td>
<td>—</td>
<td>Partially vegetated sandy areas and gravel trails</td>
</tr>
<tr>
<td>Barrington, R.I.</td>
<td>10 June–13 July, 34 days</td>
<td>Diurnal mostly in morning</td>
<td>—</td>
<td>Non-vegetated, sandy areas</td>
</tr>
<tr>
<td>Barnstable, Mass.</td>
<td>—</td>
<td>45% nocturnal</td>
<td>—</td>
<td>50% on vegetated dunes; 50% on open dunes</td>
</tr>
<tr>
<td>Wellfleet, Mass.</td>
<td>Mid-June–late July, 23–42 days</td>
<td>Mostly diurnal; some nocturnal</td>
<td>Clear to partly cloudy skies</td>
<td>Sandy to partially vegetated dunes</td>
</tr>
<tr>
<td>Average nesting trek</td>
<td>Mean clutch size</td>
<td>Mean egg mass (grams)</td>
<td>Number of clutches/territory/year</td>
<td>Reference</td>
</tr>
<tr>
<td>----------------------</td>
<td>------------------</td>
<td>-----------------------</td>
<td>----------------------------------</td>
<td>-----------</td>
</tr>
<tr>
<td>—</td>
<td>8.5</td>
<td>—</td>
<td>1 observed</td>
<td>Burns and Williams (1972)</td>
</tr>
<tr>
<td>—</td>
<td>6.7 ± 1.4</td>
<td>—</td>
<td>Up to 3</td>
<td>Butler (2000); Butler et al. (2004)</td>
</tr>
<tr>
<td>Short; nesting areas near water</td>
<td>—</td>
<td>—</td>
<td>Up to 3</td>
<td>Seigel (1980b)</td>
</tr>
<tr>
<td>—</td>
<td>5.29</td>
<td>—</td>
<td>—</td>
<td>Coker (1906)</td>
</tr>
<tr>
<td>—</td>
<td>8</td>
<td>—</td>
<td>Up to 5</td>
<td>Hildebrand (1932)</td>
</tr>
<tr>
<td>&lt;10 m</td>
<td>12.29 ± 0.13</td>
<td>9.87 (0.35 oz)</td>
<td>Up to 3</td>
<td>Roosenburg (1994), Roosenburg and Dunham (1997)</td>
</tr>
<tr>
<td>—</td>
<td>8–12</td>
<td>—</td>
<td>—</td>
<td>Wood and Herlands (1995)</td>
</tr>
<tr>
<td>&lt;100 m</td>
<td>9.76 ± 2.61</td>
<td>7.7 (0.27 oz), range = 5–11 (0.18–0.39 oz)</td>
<td>—</td>
<td>Montevicchi and Burger (1975), Burger and Montevicchi (1975)</td>
</tr>
<tr>
<td>&lt;100 m*</td>
<td>10.9 ± 3.5</td>
<td>range = 3–18</td>
<td>Up to 3</td>
<td>Feinburg and Burke (2003)</td>
</tr>
<tr>
<td>Approx. 10–100 m, some up to 500 m.</td>
<td>11.9</td>
<td>—</td>
<td>Up to 2</td>
<td>Goodwin (1994)</td>
</tr>
<tr>
<td>Long treks up to 1600 m</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>Auger and Giovannone (1979)</td>
</tr>
<tr>
<td>Long treks are common</td>
<td>12 range = 4–22</td>
<td>7.75 (0.27 oz), range = 4.5–11 (0.16–0.39 oz)</td>
<td>Up to 2</td>
<td>Lewis (personal communication)</td>
</tr>
</tbody>
</table>

*personal observation
better strategy when hatchling mortality is high. Bitter winter temperatures that decrease soil temperatures below zero for prolonged periods may result in considerable hatchling mortality. Draud has shown a 50 percent temperature-induced mortality of terrapin hatchlings in Oyster Bay during the relatively cold winter of 2003 to 2004 (Draud, Zimnavoda, King, and Bossert, 2004). Mortality due to low temperatures would not have such a devastating impact in southern latitudes. Another possible reason for larger clutches in northern colonies may be the result of seasonal limitations in producing two clutches. Northern terrapins that produce a single clutch may be able to produce the same number of offspring per season as their Southern cousins who have the time to produce two clutches.

Roosenburg looked at the possible correlation between egg size and nesting location. Since egg mass correlates with hatchling size, he wondered where the larger eggs are laid. In the case of females, larger eggs that produce larger hatchlings would create a scenario in which females may mature several years before those that come from smaller eggs. (This relationship does not hold for males because both large and small male hatchlings reach maturation size in approximately the same time frame.) Roosenburg proposed a model for nesting in which placement of larger eggs under conditions that produce females would benefit the species. From this reasoning, Roosenburg hypothesized that larger eggs should be placed in open sites with warmer incubation temperatures than smaller eggs. Data collected at his field site in the Patuxent River, support this idea (Roosenburg, 1996).

**Incubation Temperature and Development**

For the entire range, the average incubation period for terrapin eggs is sixty to ninety days. Egg development is a function of environment, and hence soil temperature and moisture. The warmer the environment, the faster will development progress. The relatively shorter time required for development of a terrapin hatchling in Southern locations where spring temperatures are relatively warm allows multiple clutches per female per year, but cold spring and early summer temperatures may sometimes limit Northern females to one clutch per year. Auger and Giovannone (1979) reported an average incubation period of 108 days (range = 87 to 146) on Cape Cod. A more recent Cape Cod study (Lewis and Prescott, personal communication), conducted from 2000 to 2002, indicates a mean incubation period of 81 days (range 59 to 116 days), while Burger (1977) reported an average of 75 days (range 61 to 104) in New Jersey.
Temperature also has a profound effect on gonadogenesis (development of gonads) and thus on the resulting sex of each hatchling. As mentioned in chapter 1, diamondback terrapins lack X and Y or sex-determining chromosomes. Sex is strongly influenced by temperature. The phenomenon of temperature-dependent sex determination (TSD) is prevalent in most turtles. The benefit of TSD for a species is not completely clear. Nonetheless, the lack of sex chromosomes that guide the genotypic development of males and females has not hindered reptiles from producing progeny of both sexes.

In the laboratory, incubation temperatures below 28°C (82°F) produce male terrapins, while temperatures above 30°C (86°F) produce females. Intermediate temperatures result in a mixture of males and females (Jeyasuria and Place, 1997). In natural nests, temperature does not mimic controlled laboratory conditions. Furthermore, in some laboratory studies, the effect of temperature is not as nicely delineated. For example, in a laboratory setting with incubation temperatures set at 26, 28, 30, 32, and 34°C (79, 82, 86, 89, and 93°F) the percentage of male hatchlings was 100, 93.3, 61.1, 0.0, and 7.7, respectively (Giambanco, 2002). There is no clear explanation for the development of one male from thirteen eggs incubated at the highest temperature. For each turtle, TSD is believed to be an all-or-none phenomenon; hermaphrodites have rarely been observed.

In nature, incubation temperature is not constant; it fluctuates every day and also as a function of the time of year. In the early 1970s, Burger (1976) conducted studies of temperatures in four natural nests on Little Beach Island in New Jersey. Two of the nests were on south-facing slopes; the other two were on north-facing slopes. One of her first observations was a diel (daily) temperature variation of 2 to 12°C (approximately 4 to 22°F). In Maryland, a diel variation in soil temperature of as much as 10°C (19°F) was also observed (Jeyasuria et al., 1994; Roosenburg, 1996); while on Cape Cod we have recorded temperature variations of 2 to 7°C (about 4 to 13°F) per day (fig. 3.6). In the New Jersey study, daily low temperatures occurred at 0600 h and daily highs at 1500 h (Burger, 1976). In Maryland, the daily low temperature also occurred at 0600 h, while the daily high was measured between 1300 and 1400 h. On Cape Cod, daily lows also occur at about 0600 h and highs at 1600 to 1800 h (fig. 3.6). In New Jersey, the mean low temperature ranged from 19-24°C (66 to 75°F) and the high ranged from 23°C to 31°C (73 to 88°F). This compares to Cape Cod where we recorded mean nest temperatures from a low of 24°C (75°F) to a high of 31.5°C (88°F) in 2003. Nest high and low temperatures as well as diel temperature variations will be different from year to year.

97
 Depending on the weather conditions. Another parameter affecting incubation temperature is reflected in the difference between north- and south-facing slopes. North-facing slopes sustain slightly cooler nest temperatures, and hence slightly longer incubation periods (79 ± 3 days compared to 71 ± 3 days) (Burger, 1976). We have also found a significant mean temperature difference of 1 to 2°C (1.8 to 3.6°F) per day when north- and south-facing nests are compared throughout a nesting season (Brennessel and Lewis, personal observation).
One of the major differences in Burger’s study in the 1970s compared to our observations in 2003 is the detection of slightly higher temperatures in nests during the last three weeks of incubation compared to surrounding soil. Burger attributed this difference to metabolic heat. As yet, we have found no evidence for the production of metabolic heat in nests on Cape Cod.

Nest depth has an impact on temperature of incubation. Placement of an egg in the nest may also influence development time and sex of the resulting hatchling. Temperatures at the top of nests are several degrees higher than temperatures near the bottom of the nest. There is an approximately 2°C (3.6°F) difference in positional nest temperature when the top and bottom of a nest is compared. Furthermore, the top of the nest experiences a greater variation in diel temperature than the bottom, suggesting an insulating effect in the deeper regions of the nest (plate 10). Superimposed on the diel temperature variance in these shallow terrapin nests, seasonal and occasional temperature variations are also important to consider. During some seasons, a protracted period of cool or hot weather may have a role in determining the turtle phenotype. The time that the nest is laid within a season will also be a factor. Early spring clutches will be subjected to gradually increasing temperatures through June and July; late-season clutches may experience progressively decreasing temperatures at the end of summer or beginning of fall (Shine, 2004).

The temperature of nests throughout a season can be important in determining the time it takes for hatchlings to develop, but the sequence of temperature changes in the nest will be more important for TSD. In turtles, there is a narrow window of time during development, which usually occurs in the middle trimester, in which temperature must reach a critical threshold to trigger sexual differentiation. Prior to this critical time period, the embryonic turtle is neither male nor female; its gonads are said to be bipotential or indifferent (Porter, 1972; Pieau and Dorizzi, 2004).

The diel and seasonal variations in nest temperature depicted in fig. 3.6 and plate 10 were obtained by placing a temperature probe in a protected terrapin nest. The probe recorded temperature every thirty minutes during the entire course of the incubation period. The nest depicted in plate 10 was a late-season nest, laid on July 20, 2003, on a sunny, south-facing slope. Fifteen very small hatchlings (average weight 4.4 grams [0.16 ounces], range = 3.5 to 5 grams [0.12 to 0.18 ounces]) emerged from the nest, only eight of which survived. All surviving hatchlings were females. Even though the nest was laid late in the nesting season, in mid July, soil nest temperatures at the top of the
nest reached $30^\circ$C ($86^\circ$F) or higher (the temperature that produces females) on twenty days during the early incubation period. It is interesting to note that nest temperatures were maintained at high levels for short periods of time. On some days, high temperatures were reached for one to two hours; on other days, temperatures reached over $30^\circ$C ($86^\circ$F) for up to six hours. Throughout the incubation period, high temperatures were reached during the afternoon, from 1300h–1600h. This nest was coolest from about 0600h to 0800h. In this nest that produced females, the average temperature was $24^\circ$C ($75.2^\circ$F); the temperature varied from a low of $16^\circ$C ($60.8^\circ$F) to a high of $35^\circ$C ($95^\circ$F), with warmer temperatures during the first half of the summer, overlapping with the critical period for TSD.

In another study of the effects of temperature on terrapin sex determination, Giambanco (2002) correlated mean temperature, incubation period, and sex of hatchlings to solar exposure in three nests. One nest had no direct sunlight, one was shaded two to four hours each day, and one was in full sunlight. The nests hatched within eight days of each other. However, the nest without direct sun had slightly cooler mean, minimal, and maximal temperatures than the other two nests and produced over 90 percent males. The partially shaded nest produced a mixture of males and females. The nest in full sun had the highest minimal, maximal, and mean temperatures but produced only three viable hatchlings; all were female.

If sex of diamondback terrapins is influenced by temperature within a critical time period, we might ask how temperature dictates the outcome of sexual development. The explanation may hinge on biochemical events that affect the expression of specific genes. These events may be similar whether temperature or sex chromosomes provide the biochemical switch that initiates the sex-determining program. A likely candidate gene that may be key in sexual determination in vertebrates contains the code for an enzyme known as $P_{450}$ aromatase. This enzyme catalyzes the formation of “female” steroid hormones (estrogens) from androgens in two areas of the body: the brain and gonads. Estrogens such as estradiol are capable of switching the sex of turtles incubated at male-producing temperatures so that they develop as females. Thus, we can consider the early embryonic turtle gonad to be sexually “indifferent.” Estrogens will inhibit the formation of testes and stimulate the formation of ovaries. The $P_{450}$ aromatase enzyme is much higher in gonads of diamondback terrapins incubated at female-producing temperatures than at male-producing temperatures (Jeyasuria et al., 1994; Jeyasuria and Place, 1997; Place et al., 2001).
The explanation of TSD in diamondback terrapins is not as simple as a temperature-induced expression of P_{450} aromatase. Timing of embryonic estrogen synthesis in brain and gonads and amount of estrogen produced are other critical factors. Development of embryos occurs by a very specific sequence of events. The pattern in which these events unfold is key to TSD. Diamondback terrapin embryonic development has not been studied in detail, so most of our baseline knowledge of terrapin developmental stages comes from studies of snapping turtle (Chelydra serpentina) eggs, incubated in the laboratory at 20°C (68°F) (Porter, 1972). Under such conditions, investigators have categorized a presomite period (stages 0 to 3), in which the embryo is a tiny blob that can be described microscopically; a somite period (stages 4 to 10), in which differentiation of major body sections occurs—the head region can be distinguished from the tail region and placement of major organs can be discerned; and the limb period (stages 11 to 26), in which development is completed as the turtle shell is formed. The middle embryonic stages, 12 to 14, appear to be the critical times during development for TSD. The temperature-sensitive period coincides with the same developmental stages as the estrogen-sensitive period. This period is the developmental window in which administration of estrogen can shift development from male to female at male-producing temperatures. Chemical-induced inhibition of estrogen production or action during this period will produce males at female-producing temperatures. In snapping turtles, this stage occurs at approximately 30 to 42 days at 20°C (68°F), but proportionally faster at higher temperatures of incubation.

Place et al. (2001) used the basic staging hallmarks described for snapping turtles in their study of the expression of P_{450} aromatase in diamondback terrapin embryos incubated at male-determining temperature (26.5°C; 79.7°F) and female-determining temperature (30.5°C; 86.9°F). The temperature-sensitive period when sex is determined in diamondback terrapins incubated at 26.5°C (79.7°F) was between days 20 to 40, and for incubation at 30.5°C (86.0°F) it occurred between days 15 to 30. Place et al. found that differences in aromatase expression and production prior to embryonic stage 18 are the most critical for TSD. After this stage, it is very difficult to manipulate the sex of terrapin hatchlings by changing temperature or by using agents that inhibit aromatase activity. Place et al. (2001) believed that the interplay of aromatase activity between brain and gonads is critical for the sexual development in diamondback terrapins. In contrast, a review of TSD in other turtles suggests that the mechanism for TSD is confined to the gonads (Pieau and Dorizzi, 2004).
The expression of P₄₅₀ aromatase and the subsequent production of estrogens are clearly important in TSD, but there are missing links in our knowledge of the detailed temperature-dependent series of biochemical events that dictate sexual development. Much of the differentiation process involves a complex network of genetic regulatory mechanisms that play out at the molecular level and may be similar to those that control genotypic (determined by sex chromosomes) sex determination.

Given the effect of temperature on sex determination in terrapins, we can explore whether terrapins specifically manipulate the sex of offspring by selecting certain nesting sites over others. When Doody et al. (2003) asked this question about the pig-nosed turtle (*Carettochelys insculpta*), a turtle with TSD that inhabits rivers and billabongs in northern Australia and New Guinea, they found that the turtles did not favor beaches that were warmer or cooler than those that were generally available. The researchers concluded that Australian pig-nosed turtles do not manipulate sex of their offspring by beach selection.

Roosenburg (1996) asked the question about nest site selection in diamondback terrapins in a more complex scenario. He introduced the variable of egg size and concluded that terrapins select warmer nesting sites when their clutches contain larger eggs. This strategy would give a head start to larger female hatchlings that take longer to reach sexual maturity than male hatchlings.

Incubation temperature may also affect other aspects of reproductive success and hatchling development. In other reptiles, incubation temperature has been shown to have an impact on hatchling traits such as size and locomotor activity. To date, no one has reported on the effects of incubation temperature on hatchling size and other traits that may affect the ability of the hatchling to survive after emergence or reproduce in later years.

The global question about TSD is: Why does it occur at all? Even more perplexing is the fact that as common as TSD is in reptiles in general and turtles in particular, there are some emydid turtle species such as *Clemmys insculpta* and some *Apalone* (soft-shelled turtles) that have abandoned this strategy and rely on sex chromosomes to determine whether an egg develops as a male or female. The chromosomal strategy is referred to as genetic sex determination (GSD). There is no obvious reason why TSD, in contrast to GSD, would be an adaptive advantage for terrapins. With GSD, due to chance, there would be an equal probability of producing males or females. With TSD, however, the location, placement, and depth of the nest become
major factors in the resulting sex ratios of offspring. Instead of chromosomes, the nesting behavior of the mother turtle becomes the key component of sex determination.

It could be postulated that GSD may be an advantage for turtle species at the extremes of their range. For example, it would not be beneficial for Southern subspecies if all Southern nests produced primarily females. It will also not be beneficial if all Northern nests produced mostly males. GSD would eliminate the possibility of such skewed sex ratios. However, even with TSD, there is no evidence for such skewed ratios among hatchlings of any turtle species. Furthermore, there is no geographic variation in expression of TSD versus GSD among turtles.

From a study of the pattern of TSD utilization in twenty-two turtle species, a biological association between nest temperature and adult sexual dimorphism has been suggested (Ewert and Nelson, 2002). The sex that predominates at cooler temperatures is usually the smaller turtle. This is certainly the case for diamondback terrapins. Perhaps incubation temperatures produce physiological differences that optimize the fitness of adults. Appealing as this theory is, it does not account for those instances of TSD in turtle species in which males and females are approximately the same size.

Another possible adaptation that has been proposed to explain TSD is sib-avoidance. Since TSD often produces same-sex clutches, inbreeding can be prevented. GSD would produce approximately equal numbers of males and females that could potentially interbreed. This explanation doesn’t quite fit the diamondback terrapin reproductive strategy. Diamondback terrapins become sexually mature many years after they disperse from their nests. They do not form pair bonds, nor are they expected to find the same mates each season. The possibility of diamondback terrapins producing full siblings of the opposite sex from different clutches may be nonexistent. The lifetime yield of a mating between any individual female and a specific male may be only a single clutch.

Could TSD be a group structure adaptation (Ewert and Nelson, 2002)? Perhaps the strategy keeps the ratio of male to female diamondbacks optimal for the population. TSD has the potential to produce more females than males, which may offset the earlier maturation of males and hence the tendency of populations to exhibit a male bias. We don’t know the ratio of female to male hatchlings in most natural settings, but from the available studies it appears that many populations stabilize with an adult sex ratio that favors females.
Nesting Outcome

There are various estimates of the outcome of nesting efforts of female terrapins in the wild. Sometimes an egg or two in a clutch will fail to develop. Even if development occurs, some eggs will not produce viable hatchlings. The female terrapin never knows the outcome of her nesting foray. Should her nest be too shallow or too deep, hatchlings will not develop properly. Natural forces such as wind erosion and storm surges that inundate or wash away nests can certainly take their toll on eggs deposited in vulnerable locations. There is no way for the individual female terrapin to learn from her mistakes. Each year, we find a terrapin nest, most likely laid by the same clueless female, in an area that is subject to tidal inundation during spring tides. Perhaps the eggs can survive several floodings, but probably not a whole season of spring tides that wash over and may even erode the nest twice a month. We can relocate these eggs to drier ground and release them in their natal location after they hatch, but if natal homing is at work, the female’s hatchlings might return to the same vulnerable nesting area year after year when they are sexually mature.

Aside from environmental destruction, other factors may influence the outcome of successful hatching. Animal predators are the most important contributor to nest failure. In many locations throughout the range of the diamondback terrapin, the raccoon, Procyon lotor, is a major predator of terrapin eggs, especially within the first twenty-four hours after the eggs are laid. Most likely a transient indicator, such as scent, is used by raccoons to locate nests. Raccoons also display keen interest in areas that have been recently excavated. Nest depredation also occurs after more than twenty-four hours of egg deposition, but at a much lower frequency. In some instances, researchers mask the scent of a freshly laid nest by providing an alternate scent (e.g., human urine) until the nest survives its first twenty-four hours or until it can be effectively protected in another manner.

Raccoons are categorized as a subsidized predator because their numbers increase in areas where people live. They are attracted to locations where they have access to trash and other easy sources of food and they can prosper. These subsidized predators have greatly changed the outcome of nesting success for diamondback terrapins in Jamaica Bay Wildlife Refuge (JBWR). There were no signs of raccoons or nest depredation in the refuge in the late 1970s and early 1980s when Bob Cook first studied diamondback terrapins in this national park. Raccoons were introduced and/or found their way into the refuge in the mid 1980s and became fairly common in the 1990s. During a study conducted in 1998 and 1999, raccoons depredated 92.2 percent of terra-
pin nests on Ruler’s Bar Hassock, the prime terrapin nesting area in the refuge (Feinburg and Burke, 2003). During that time, raccoons were seen in the nesting area mostly at night. During my visit in 2004, several raccoons were spotted in the nesting area, bold as can be, in the middle of the day. One was eating terrapin eggs as the female deposited them in the nest (plate 7; described in chapter 2). Only the continued presence of researchers, volunteers, and refuge visitors prevented massive terrapin egg carnage during daylight hours.

Fig. 3.7. Field marks of raccoon predation.

105
Raccoons usually leave a pile of shell fragments close to the nest, and indeed, the presence of shells is often a field mark of a terrapin nest (fig. 3.7). At JBWR, raccoons also leave shells in conspicuous piles near the depredated nests. However, Feinburg and Burke (2003) found a curious behavior during the latter part of the nesting season: It appeared that raccoons sometimes consume entire eggs, including shells. No eggshells were found near certain marked nests that were depredated. Furthermore, raccoon scat (feces) contained large amounts of terrapin eggshells. The biological basis for the change in raccoon feeding behavior is not known and has not been extensively observed in other terrapin nesting areas. Butler (2000) and Burger (1977) reported some cases in which raccoons consumed entire eggs, but in general the shells are left behind. Shells may also be missing from nests if eggs are carried off by gulls to feed their chicks (Burger, 1977).

Raccoons are also the main terrapin nest predator at Sandy Neck, Massachusetts (Auger and Giovannone, 1979), and on Little Beach Island, New Jersey (Burger, 1976). At some sites, rates of predation vary from year to year for reasons that cannot be easily explained. On Little Beach Island, New Jersey, in 1973, at least some eggs developed in 84 percent of nests. Thirty-nine percent of all eggs produced hatchlings. In 1974, some eggs developed in only 25 percent of nests. Eighteen percent of all eggs produced hatchlings. These differences in hatching success rates were entirely due to predation.

In Wellfleet, Massachusetts, different nesting areas have suffered different degrees of nest depredation by raccoons. In more developed locations, where suitable nesting areas are at a premium, over 90 percent of nests are consistently lost to raccoons. In less populated nesting locations, such as those under conservation protection by the Massachusetts Audubon Society, predation occurs at a lower frequency. On Cape Cod, raccoon predation occurs mostly, if not exclusively, at night.

Other animal predators of diamondback terrapin eggs include red fox (Vulpes fulva), American crow (Corvus brachyrhynchos), laughing gulls (Larus atricilla) in New Jersey (Burger, 1977), fish crows (Corvus ossifragus) in northeast Florida (Butler, 2000), red fox and northern river otter (Lutra canadensis) in Maryland (Roosenburg, 1994), and ghost crabs (Ocypode quadrata) in Florida (Arndt, 1991, 1994; Butler et al., 2004). The avian predators may be more successful at the time of egg laying (Burger, 1977). Butler (2000) observed clever crows following gravid females to nesting areas and patiently observing researchers as they processed nests.

Relatively recent reports indicate that other animal predators may be
making an impact on diamondback terrapin reproduction: rats! On mangrove islands in Florida Bay, black rats have been a recent introduction and may be responsible for predation on nests (Mealy et al., 2004). A similar trend was seen during terrapin farming operations in the early twentieth century in North Carolina where rats ate both eggs and hatchlings (Hildebrand and Hatsel, 1926). In Oyster Bay, New York, the Norway rat apparently found a new food source in diamondback terrapin hatchlings (Draud, Bossert, and Zimnavoda, 2004). On nesting beaches, rats are a nocturnal predator. They have not been observed to disturb nests, but their teeth marks are evident on newly hatched terrapins (further described in chapter 4).

The site of the nest may affect the outcome of reproductive success. Burger (1977) found that nests near vegetation were more prone to predation by mammals, and those in open sandy areas to predation by birds. She also found that nests in high-density nesting areas, within 1 meter of each other, were preyed upon at a higher rate than nests that were dispersed. I have often observed a large number of predator digs in historically high-density nesting areas, sometimes up to two weeks before nesting begins. Predators such as raccoons have cued in to high-density nesting areas and may not need visual or olfactory cues to locate nest. Random digging in these areas will have a high probability of unearthing tasty terrapin eggs.

Although most incidents of nest predation by animal predators occur within the first day or two after eggs are laid, another spike of destruction affects nests as hatchlings are ready to emerge. On Little Beach Island in the 1970s, there was actually more nest depredation during hatching than nesting, occurring before all the hatchling turtles emerged from the nest (Burger, 1977). In Wellfleet, during September and October, we often locate nests that we have missed during the egg-laying season when we see signs of predation such as piles of broken shells and relatively large, predator-initiated digs in the sand. Perhaps the emergence of the first few hatchlings alerts predators to the nest location, either by scent or by visual cues.

Not only do the local fauna take a bite out of the future generations of terrapins, the flora can also destroy terrapin eggs. Roots from plants such as dune grass (Ammophila breviligulata) infiltrate eggs and halt their development (fig. 3.8). In some cases, the entire nest is invaded by roots even before development has progressed. Lazell and Auger (1981) were surprised and impressed by how rapidly roots can grow and infiltrate nests. The roots penetrate and pack the eggs. In other cases, development is almost complete before hatchlings become strangled by plant roots. We have observed dead, almost fully
developed hatchlings with ligature marks inflicted by plant roots.

It is sometimes easy to predict the location of nests that roots or rootlets will infiltrate. In vegetated areas, it is nearly impossible for diamondback terrapins to find a clear sandy patch to nest in. Therefore, terrapins will often nest in eroded areas, formed by humans and nonhuman animals as they trek through marsh uplands. If the eroded areas fill in with plants during the summer, terrapin eggs will be in jeopardy. In most cases, during the early part of the nesting season there are no clues for terrapins to predict the future location of luxuriant vegetation. By the end of August, a formerly bare dune may be covered in dense dune grass. I have protected nests from animal predators in late June only to have plants destroy the nests in July and August. Auger and Giovannone (1979) questioned whether the plant roots actively seek the moisture and nutrients contained in the egg, or whether root infiltration represents a chance event. No one has provided a satisfactory answer to this query.

Insects are an unlikely but significant predator of diamondback terrapin nests, affecting both eggs and hatchlings. On Sandy Neck, Barnstable, Mass-

Fig. 3.8. Eggs that have been infiltrated with plant roots.
achusetts, approximately 35 percent of nests laid in 1978 were victims of at least partial maggot infestation. The Systematic Entomology Department of Agriculture identified the maggots from Barnstable, Massachusetts, nests as larval forms of a member of the flesh fly family, *Sarcophagidae*. In Wellfleet, partial or entire nests full of small, fully formed terrapins suffer mortality when the hatchlings are infected by maggots. The route of entry in many cases appears to be via the yolk sac. Various species of ants have also been observed in nests, but in some cases it is not clear if they feeding on crushed eggs or dead hatchlings or if they are responsible for egg or hatchling fatality.

Predation on diamondback terrapin nests seems to be increasing at a dramatic rate. Whether this is the result of more comprehensive examination and reporting or actual decreases in reproductive success needs to be clearly defined.

Whether it is a raccoon, plant, or insect, predation on terrapin nests has been a facet in the course of this turtle’s natural history. As long as a sufficient number of hatchlings survive to reproductive age it is unlikely that the population will suffer. But the question becomes: What constitutes a sufficient percentage of hatchling survival to recruit turtles into the reproducing component of population? If there are additional stresses on the population, including those on juveniles and adults, it may not be possible for terrapins to sustain high nest depredation rates and still maintain stable populations.