

Natural Diet of the Namaqualand Speckled Padloper (*Homopus signatus signatus*)

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NOTES AND FIELD REPORTS

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Status of Marine Turtles in the Gulf of Mannar, India

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ABSTRACT. – *Lepidochelys olivacea* was the most frequent sea turtle documented in the Gulf of Mannar during 2000–2001, whereas during the 1970s it was *Chelonia mydas*. Reduction in overall proportions of *C. mydas* and its size classes is attributed to exploitation of eggs, turtles, and incidental catch in fishing gear.

Five species of sea turtles occur in the Gulf of Mannar off the southeast tip of India, including the olive ridley (*Lepidochelys olivacea*), the green turtle (*Chelonia mydas*), the loggerhead (*Caretta caretta*), the hawksbill (*Eretmochelys imbricata*), and the leatherback (*Dermochelys coriacea*). Our study focuses on the status of marine turtles in Gulf of Mannar with particular emphasis on the changes in the population of green turtles, which occur there in considerable numbers but whose actual status is poorly documented. The Gulf of Mannar extends from Cape Comorin (lat 8°4'40"N, long 77°33'4"E) to Dhanushkodi (lat 9°9'9"N, long 79°26'46"E), and has about 250 km of coastline (Fig. 1). Extensive coral and

seagrass beds on the northern side and deep sea on the southern side make this area suitable for all 5 species of sea turtles found in this region. Over 20 islands are found in the Gulf of Mannar, including Krusadai and Rameswaram. Turtle fishing was practiced in this area for ages, and turtles were exported to Sri Lanka and other countries until 2 decades ago (Agastheesapillai and Thiagarajan 1979). Although the export of sea turtles was banned during the early 1980s by the Indian Wildlife Protection Act of 1972 (Anonymous 1991), the subsistence exploitation of turtles continues there even today.

Methods. — We surveyed the beaches south of Tuticorin (70 km) and Rameswaram Island (15 km) bimonthly, from November 2000 to April 2001. At least 10 km was covered during a day's survey, and the sampling was restricted to 0500–0800 hours. The number of tracks and shells of dead turtles found were recorded. We also monitored the fish market of Rameswaram Island and 6 others along the Tuticorin coast. On locating a turtle, we recorded the species and its curved carapace length (CCL). The shells of dead turtles were marked with paint to avoid repeat count.

Results and Discussion. — In this area, fishermen collect turtles using multifilament gill nets. These nets measured over 50 m in length and 6 m in width, and had mesh size from 15 to 22 cm. Entangled live turtles were taken for consumption, and the dead ones discarded in the sea. Considerable trawler fishing is also practiced throughout this range. Altogether, we observed 178 specimens, including 122 along the Tuticorin coast and 56 in Rameswaram Island (Table 1). In addition, 15 nests (14 on the Tuticorin beach and 1 on Rameswaram Island) were observed. Overall proportion of species showed that

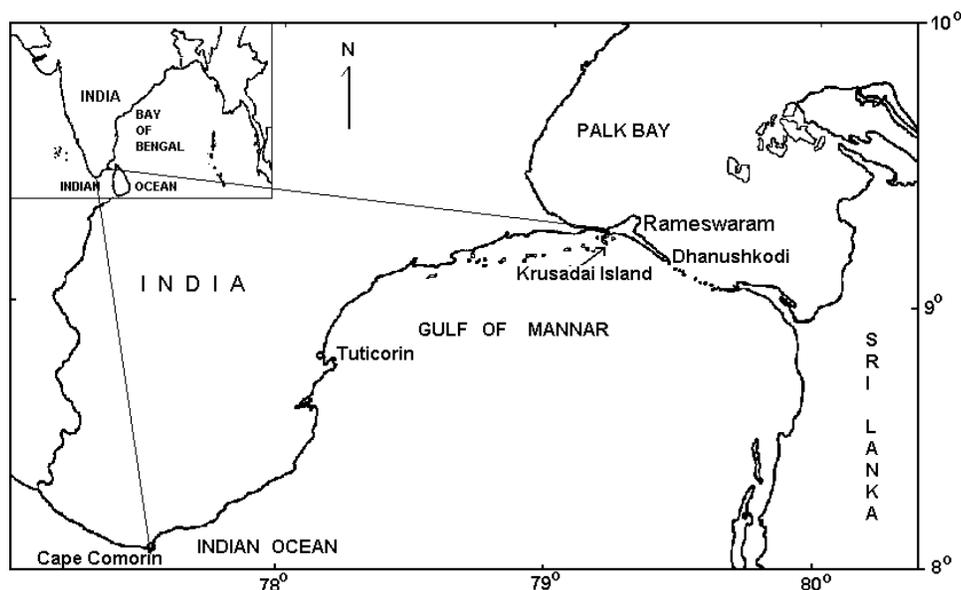


Figure 1. Map of southeast India showing the Gulf of Mannar.

Table 1. Species composition of sea turtles in the Gulf of Mannar, India. Numbers in parentheses are turtles or shells found in markets.

Species	Tuticorin Beach	Rameswaram Island	Number	%
<i>Lepidochelys olivacea</i>	64 (59)	36 (6)	100	56.2
<i>Chelonia mydas</i>	55 (52)	13 (3)	68	38.2
<i>Caretta caretta</i>	0	4 (2)	4	2.3
<i>Eretmochelys imbricata</i>	2 (2)	2 (0)	4	2.3
<i>Derموchelys coriacea</i>	1 (1)	1 (0)	2	1.1
Total	122 (114)	56 (11)	178	—

Table 2. Data on the size (CCL, curved carapace length, cm) of sea turtles in the Gulf of Mannar, India. SD = standard deviation.

Species	Mean CCL	SD	Range	n
<i>Lepidochelys olivacea</i>	65.3	6.5	46–74.5	99
<i>Chelonia mydas</i>	81.9	26.6	33–112	68
<i>Caretta caretta</i>	64.8	—	50–101	4
<i>Eretmochelys imbricata</i>	48.3	—	43–60	4
<i>Derموchelys coriacea</i>	145	—	140–150	2

L. olivacea was the most frequent (56.2%), followed by *C. mydas* (38.2%); other species together formed only 5.6%. Based on the turtle landing data prior to 1950 at Krusadai Island, Kuriyan (1950) reported that the majority of turtles caught in the area were *C. mydas*. This species formed about 89% of all turtles caught in the Gulf of Mannar and Palk Bay during 1971–1976 (Agastheesapillai and Thiagarajan 1979). Even though the method adapted for data collection in the earlier studies was different from that of the present one, available data indicate a reduction in the proportion of *C. mydas*.

Table 2 provides some aspects of morphometry of species found in the Gulf of Mannar. Length frequency of 99 *L. olivacea* specimens showed that about 15% of the turtles had CCL of less than 60 cm (Fig. 2). Dash and Kar

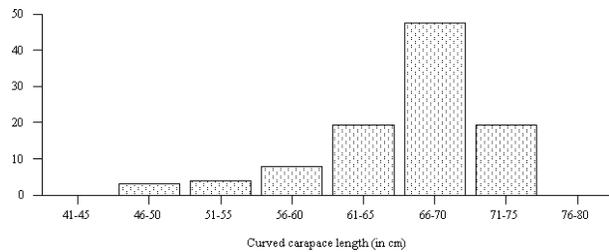


Figure 2. Size structure of *Lepidochelys olivacea* in the Gulf of Mannar, India, from November 2000 to April 2001 ($n=99$).

(1990) speculated that in the Bay of Bengal this species attains sexual maturity when it grows over 60 cm in shell length. Information on subadult or juvenile ridleys is scanty, and only 3 reports are available from the Bay of Bengal. Pandav et al. (1995) reported only 5 subadult ridleys during the last 20 years of research along Gahirmatha beach, which is one of the well-known mass nesting areas of *L. olivacea*. Along the Nagapattinam coast (southeastern coast of India) 2 subadults were recorded from November 2000 to May 2001 (Bhupathy and Karunakaran 2003). Deraniyagala (1953) reported 2 juveniles of this species from Morutuva in Sri Lanka. Our records of over 15 juveniles and subadult ridleys in the Gulf of Mannar during December–April, the breeding season for *L. olivacea*, partially fills the information gap on the developmental habitat for this species in this region.

Agastheesapillai and Thiagarajan (1979) predicted that in Gulf of Mannar, *C. mydas* attains sexual maturity when it grows over 81 cm in CCL. Of the 68 *C. mydas* observed during our study, about 70% of them had CCL below 80 cm (Fig. 3), indicating that the majority were immature. Prabavathy (1992) studied the biology of *C. mydas* from March 1991 to April 1992 along Tuticorin Bay. The study area (Tuticorin), and data collection methodology (i.e., based on stranded turtles along the beach and market surveys) were largely similar to the

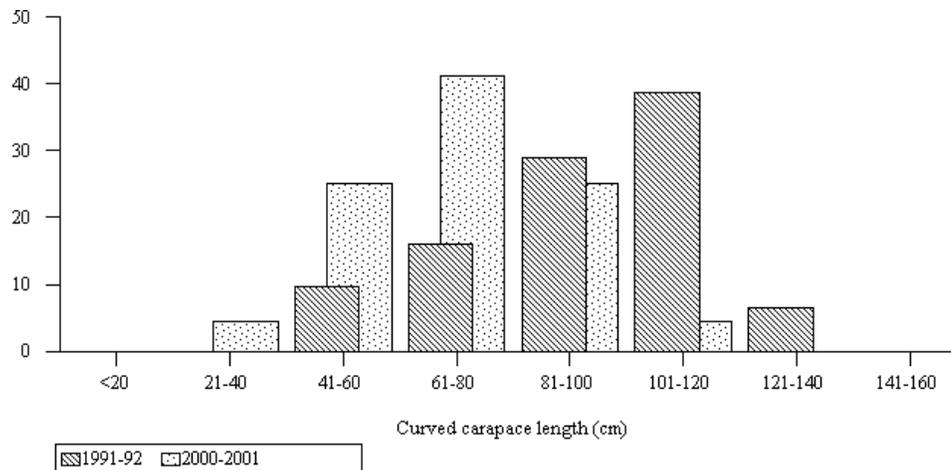


Figure 3. Comparison of size class distribution of *Chelonia mydas* in the Gulf of Mannar, from 1991–1992 ($n=31$) and 2000–2001 ($n=68$). Data for 1991–1992 were extracted from Prabavathi (1992). A significant difference was found between the size classes in the 2 sampling periods ($\chi^2=186.99$, $p < 0.001$).

present study. Of the 31 specimens observed from November 1991 to April 1992, only 25.8% had a CCL of less than 80 cm (Fig. 3). The size class distribution of turtles in both studies was significantly different ($\chi^2 = 186.99$, $p < 0.001$, $df = 4$). Reduction in the proportion of adult turtles could be due to exploitation over several decades, especially the take of nesting females.

Because both the overall proportions of *C. mydas* and the proportions of mature turtles have decreased, we conclude that, as elsewhere (Limpus 1995), the population of this species is declining in the Gulf of Mannar, India. All of the major detrimental factors, such as exploitation of eggs and turtles irrespective of size (Frazier 1980) and incidental death due to the careless operation of fishing gear, exist in this region.

The length frequencies of both *L. olivacea* and *C. mydas* (Fig. 3), show that the Gulf of Mannar is an important development and foraging habitat for turtles in the Bay of Bengal. However, no population estimates for these species are available for this region. Unless serious conservation measures such as exploitation control are undertaken, long-term survival for sea turtles in the Gulf of Mannar is bleak. An ecological study and sea turtle population monitoring program would provide the required data to propose conservation measures for sea turtles in this region.

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Diet of the Green Turtle (*Chelonia mydas*) at Ra's Al Hadd, Sultanate of Oman

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ABSTRACT. – Digestive tract contents of stranded green turtles (*Chelonia mydas*) from Ra's Al Hadd area, Oman, were examined. In decreasing order of importance, the food items (dry weight) comprised 19 taxa of algae (49%), 22 taxa of animal matter (26%), 2 seagrasses (15%), litter (7%), and substrate (3%).

The nesting and feeding grounds of the green turtle, *Chelonia mydas*, in Oman are of regional importance (Baldwin and Al-Kiyumi 1999). The present study focuses on the diet composition of stranded green turtles in the area of Ra's Al Hadd, a Nature Reserve internationally important as feeding and nesting grounds for this species.

The green turtle is the most commonly encountered marine reptile in Oman (Ross and Barwani 1982). Over 275 beaches along the 1700-km stretch of coastline are used for nesting, including those on offshore islands from the northern region of Musandam to the Oman-Yemen border in the south (Salm 1991). There are large concentrations of turtle nesting at Ra's Al Hadd (ca. 6000 females per year; Ross and Barwani 1982). Ra's Al Hadd is a protected area for nesting sea turtles, covering a stretch of coast of approximately 70 km from Khawr Al

Jaramah in the north to Ra's Al Ru'ays in the south, and an area of 120 km² including beaches, coastal lands, and offshore areas (Al-Kiyumi 1997).

The patterns of movements of green turtles between Oman nesting grounds and local or distant feeding grounds are unknown but appear to be rather complex. Extensive feeding grounds are in the Arabian Gulf, on the coasts of Oman and Yemen, and in the Red Sea (Ross and Barwani 1982). Many of Oman's green turtles are also known to feed in waters of other countries in the Arabian Gulf and Gulf of Aden (Salm and Salm 2001). Specifically in Oman, there are extensive and regionally important feeding grounds that occur along much of the coast, with particularly dense aggregations of feeding turtles to be found in the Barr al Hickman—Masirah Island region, along the Sahil al Jazir coastline and between Salalah and Hasik in Dhofar and scattered along the Batinah coast (Ross and Barwani 1982; Salm 1991). Although the feeding area along Arabian Sea coasts is extensive (around 800 km), development of dense beds of seagrasses is limited, especially during the summer season, due to the southwest monsoon known locally as *khareef*. The strong southwesterly winds drive currents parallel to Arabian Sea coasts from June to September with cold (21°C) and low salinity (35.5‰–35.7‰) water upwelled onto the continental shelf from depths of about 150 m. The high levels of nutrients in the sea combined with low temperatures account for the annual development of dense beds of macroalgae along Arabian Sea coasts. These conditions, however, are not well suited for the growth of seagrass leaves (Jupp et al. 1996).

The first dietary studies on *Chelonia mydas* in Oman were carried out by Ross (1985) who analyzed the stomach contents of 9 green turtles captured on the east side of the Masirah channel, and data showed that 1 turtle had eaten only algae, 3 others had eaten only seagrass, 2 had eaten seagrass and a trace of algae, while 3 others had eaten bulk amounts of both seagrass and algae. Quantitative data on food items were not obtained by species. The most important plant food items (forming > 40% by volume of stomach contents) included the seagrasses *Halodule uninervis* and *Halophila ovalis*, the green algae *Chaetomorpha aerea* and *Ulva lactuca*, and the brown macroalgae *Sargassum illicifolium*.

Green turtles in Oman are threatened by an assortment of human activity as well as by natural processes and events (Baldwin and Al-Kiyumi 1999). Of the human pressures, fishery-related threats are particularly severe, especially near feeding grounds or nesting beaches (Hare 1991). Most of the dead green turtles found in Ra's al Hadd have been incidentally caught in fishing gear. Other human activities, such as recreation and general coastal development, increasingly threaten turtle populations (Salm 1989). The first surveys of turtles in Oman were undertaken in 1977, focusing on Masirah Island and Ra's Al Hadd (the two Nature Reserves), starting with a turtle-tagging project. More than 23,000 turtles were tagged during the first 10 years of the project (1977–1986),

including a few on the feeding grounds of Masirah Island. Subsequently, additional turtles have been tagged, mostly at Ra's Al Hadd, increasing the total number of tagged individuals to more than 50,000 (Salm and Salm 2001).

Current management strategies in Oman are based on the protection of nesting beaches, largely from recreation-related threats and primarily within these two Nature Reserves. Protection of turtles at other stages of their life history, including feeding and migrating turtles, is vital for their survival (Salm and Salm 2001). Therefore, the knowledge of the diet and feeding grounds of these green turtle populations is essential for planning management actions for animals and their selected food resources.

Methods. — In July and August 2001, during the nesting season, 15 stranded green turtles (7 adult females, 7 adult males, 1 juvenile) were found on the beach in the Ra's Al Hadd area. Some of the females had shelled eggs, indicating active breeding. The probable cause of death was determined to be from artisanal fisheries activities (Ferreira 2002). The carapace length and width, the plastron length, the sex and site of recovery of all the turtles were recorded, and the digestive-tract contents were removed. From the 15 digestive tracts, only 11 contained food items (5 adult males, 5 adult females, 1 juvenile) that were used in this study. But it was not possible to quantify the diet contents of the juvenile sample due to the state of the food items, so only 10 samples were considered to quantify the green turtles diet composition.

The diet samples were collected and preserved in 4% buffered formalin/seawater solution in clear plastic vials stored in darkness to reduce color fading. After a week, the samples were transferred to 70% alcohol (Forbes 1999). Food items were identified to the lowest taxonomic level possible. The wet and dry weights of the items were obtained by drying them at 60°C until a constant mass was reached.

In the turtles with empty stomachs, the esophagus generally contained a bolus (also used for analyses), probably representing the last few bites taken before death. Some of the food found in the esophagus and stomach was in a remarkably fresh state, apparently unchanged by digestion, but in other cases the food was already digested.

Quantification of the diet composition allows the description of the food items found in the digestive tract contents, as well as their proportions. Two methods were applied to quantify the diet composition: the numeric method and the gravimetric method (Windell and Bowen 1978).

- 1) With the numeric method, the frequency of occurrence was determined. The frequency of occurrence (%FO) of a food item is given by the percentage of turtles that contain the same item in their digestive tracts:

$$\%FO = (n_i / N) \times 100$$

where n_i is the number of tracts that contain the item i , and N is the total number of tracts with prey (Windell and Bowen 1978). Following Hyslop

(1980), the turtles with an empty digestive tract were not considered for the calculation.

- 2) In the gravimetric method or weight contribution, the food items are separated according to their taxonomic categories and are weighed individually. The weight of each food item can be expressed as the percentage of the total weight of digestive tract contents found in all examined turtles (Hynes 1950):

$$\%W = (w_i / W_t) \times 100$$

where w_i is the total dry weight of a particular prey group or taxon, and W_t is the total weight of contents in all the digestive tracts.

The Index of Relative Importance (IRI) was adapted from Hyslop (1980) calculating each diet category i by the equation:

$$IRI = 100(\%W_i + \%FO_i) / \sum_{i=1}^n (\%FO_i \times \%W_i)$$

where FO is the frequency of occurrence, W the weight, and n is the number of diet categories.

The importance of diet items with a high ash content and therefore high relative weight (e.g., calcareous algae, sponges, spicules, and exoskeletons) will be overestimated in a gravimetric analysis whereas diet items with low ash content will be underestimated. The IRI provides a more reliable measure for ranking the relative importance of diet categories because frequency of occurrence and mass are integrated.

Results and Discussion. — The size of the adult turtles ranged between 98 and 111 cm CCL (curved carapace length) for the females and between 91.5 and 98 cm for the males; the juvenile had a CCL of 43 cm. Five adult males, 5 adult females, and the juvenile were considered for statistical analyses. It was possible to identify 42 different dietary items (Table 1); in decreasing order of importance (dry weight), these consisted of 19 taxa of algae (49%), 22 taxa of animal matter (26%), 2 seagrasses (15%), and substrate particles (3%; sand, pebbles, and shell fragments). Recovered items of litter (7%) included plastic bags, nylon chord, mesh bags, and tarp fragments. The digestive tract contents ranged from 3 to 16 g of dry weight. Weights of the taxa, litter, and substrate categories are presented, with FO and IRI data, in Table 2.

The digestive tract contents showed a strong tendency toward herbivory with indications of the turtles having foraged primarily on marine algae, although it was observed that they had also eaten seagrasses.

The brown algae *Nizamudinnia zanardinii* was the most commonly ingested food item, with 70% frequency of occurrence and an IRI of 24. The prevalence of *N. zanardinii* among the diet samples reflects its great abundance along Arabian Sea coasts. Every year thousands of tons of this seaweed appear along Arabian Sea coasts of Oman in the cold, nutrient-rich upwelled waters

Table 1. Taxonomic groups found in the digestive tracts of green turtles, *Chelonia mydas*, at Ra's Al Hadd, Oman.

Seagrasses
<i>Halodule uninervis</i>
<i>Halophila ovalis</i>
Green Algae
<i>Codium arabicum</i>
<i>Codium dwarkense</i>
<i>Cladophoropsis javanica</i>
<i>Enteromorpha flexuosa</i>
<i>Ulva rigida</i>
Red Algae
<i>Ahnfeltia plicata</i>
<i>Ceramium</i> sp.
<i>Polysiphonia</i> sp.
<i>Phyllophora</i> sp.
<i>Grateloupia</i> sp.
<i>Gracilaria canaliculata</i>
<i>Rhodymenia</i> sp.
Brown Algae
<i>Spatoglossum asperum</i>
<i>Stoechospermum marginatum</i>
<i>Padina tetrastratica</i>
<i>Hormophysa triquetra</i>
<i>Lobophora variegata</i>
<i>Nizamudinnia zanardinii</i>
Unidentified Algae
Bryozoa
Vesiculariidae
Walkeridae
Unidentified sp.
Hydrozoa
Aglaopheniidae
Campanuliniidae
Cephalopoda
Unidentified sp.
Bivalvia
Donacidae
Gastropoda
Cerithiidae
Trochidae sp. 1
Trochidae sp. 2
Turbinidae
Cancellariidae
Terebridae
Marginellidae
Nassariidae
Polichaeta
Sabellidae sp. 1
Sabellidae sp. 2
Chaetopteridae
Terebellidae
Aphroditidae
Owenidae
Eunice
Detritus

of the annual summer southwest monsoon. The Oman Seaweed Project (OSP 1999) carried out a comprehensive survey of seaweeds in Oman including data on taxonomy, the distribution, cover, and biomass of seaweeds found in various sites; maximum biomass data for *N. zanardinii* ranged from 0.60 to 2.33 kg DW/m².

Table 2. Quantitative description of the diet of green turtles (*Chelonia mydas*) at Ra's Al Hadd, Oman, and Index of Relative Importance (IRI).^a

Taxa		%FO	%W	IRI	
Seagrasses	<i>Halophila ovalis</i>	40	12.29	12.7	
	<i>Halodule uninervis</i>	50	2.42	3.1	
	<i>Nizamuddinina zanardinii</i>	70	13.03	23.5	
	<i>Cladophoropsis javanica</i>	50	10.61	13.7	
	<i>Hormophysa triquetra</i>	10	8.32	2.1	
	Unidentified Algae	20	4.98	2.6	
	<i>Stoechospermum marginatum</i>	10	4.49	1.2	
	<i>Ahnfeltia plicata</i>	10	2.10	1.54	
	<i>Gracilaria canaliculata</i>	20	2.04	1.05	
	Algae	<i>Rhodymenia</i> sp.	10	0.96	0.25
		<i>Codium dwarkense</i>	10	0.68	0.18
<i>Lobophora variegata</i>		10	0.53	0.14	
<i>Ulva rigida</i>		30	0.50	0.39	
<i>Spatoglossum asperum</i>		10	0.47	0.12	
<i>Grateloupia</i> sp.		20	0.44	0.23	
<i>Codium arabicum</i>		10	0.18	0.05	
<i>Padina tetrastromatica</i>		10	0.07	0.02	
Animals		Gastropoda	70	17.06	30.8
		Polichaeta	20	4.38	2.27
		Bivalvia	20	2.07	1.07
	Cephalopoda	70	2.02	3.6	
	Hydrozoa	50	0.40	0.5	
	Bryozoa	40	0.02	0.02	

^a %FO = frequency of occurrence; %W = dry weight percentage.

The green alga *Cladophoropsis javanica* was another common species in the diet samples. This species appears to be rare, and there are only two records of its existence: at the southern tip of Masirah Island (Wynne and Jupp 1998) and at Sur (B.P. Jupp, unpubl. data, 1999). However, dense beds of this species could have been overlooked in surveys to date.

Other commonly found species or that were a significant component by weight in the samples included *Ulva rigida*, *Gracilaria canaliculata*, *Hormophysa triquetra*, *Stoechospermum marginatum*, *Ahnfeltia plicata*, *Codium dwarkense*, and *Grateloupia* sp. These were also found in surveys in the Oman Seaweed Project (OSP 1999).

The red algae were only sparsely represented in these diet samples, in contrast to other studies where green turtles were found to forage primarily on Rhodophyta classes in Brazil and Mexico (Ferreira 1968; Seminoff et al. 1998).

The major components of the seagrass meadows in Oman are *Halophila* and *Halodule* species, which are hardy pioneer species well suited to the conditions along Arabian Sea coasts (Jupp et al. 1996). The seagrasses species *Halophila ovalis* and *Halodule uninervis* were abundant in the diet samples, both being preferred by the green turtles, similar to that reported in other green turtle diet studies (Oman: Ross 1985; India and Yemen: Bjorndal 1997). *Halodule uninervis* was found more commonly, occurring in 50% of the tracts but *Halophila ovalis* formed a higher weight percentage and had an IRI of 13. These species are also found along the north coast of Oman, limited to sandy/salty areas such as Ra's Suwadi, Bandar Jissah, and Sur Harbour (Jupp et al. 1996). Further

detailed studies on seagrasses in Oman, including biomass data, are given in Jupp et al. (1996).

Preference feeding tests with *C. mydas* in Oman (Al-Ajzoon 1993) showed a preference for macroalgae: *Nizamuddinina* > *Sargassum* > *Ulva*, and a preference for seagrasses: *Halophila* > *Syringodium* > *Halodule*. It is of interest that the same two preferred taxa, *Nizamuddinina* and *Halophila*, from these tests form the highest percentage by weight of plant components (13 and 12%, respectively, Table 2) in the digestive tract contents. This implies some selectivity on the part of the turtles for these macrophyte species. Ross (1985) attributed the differential abundance of algal species in the habitat and in the diet of green turtles in Oman to selective feeding. It is of interest that Ross (1985) reported a different suite of algae (*Chaetomorpha*, *Ulva*, and *Sargassum*) in the stomach contents he examined in the Masirah Channel, which may reflect different availabilities of algae in feeding grounds visited by the turtles. However, the number of samples from both studies is too small to conclude selective feeding. The extent to which the diet of green turtles is determined by selective feeding or by the relative abundance of different diet species has been addressed in several studies (Bjorndal 1997).

Analyzing males and females separately it was observed that the brown algae *Nizamuddinina zanardinii* was the most commonly ingested species by males, and the green algae *Cladophoropsis javanica* the most commonly ingested by females.

Data on any selective feeding and the distribution and biomass of both macroalgae and seagrasses are important in assessing the status of feeding grounds for *C. mydas*. To

better facilitate nutrient assimilation, green turtles appear to take small bites of algae during consumption. In the examined diet samples, algae were commonly fragmented to small size. The resulting small food particle size is functionally beneficial to nutrient assimilation providing opportunity for efficient digestion (Bjorndal 1997).

Green turtles in the Arabian Sea supplement their diet with animal matter. The possibility that such food resources make a major contribution of vitamins, trace minerals, or essential amino acids for herbivorous green turtles has been discussed (Bjorndal 1985) and may explain why, even with abundant algae and seagrass resources, green turtles feed on animal matter. Small invertebrates, including polychaete worms and tubes, small gastropod and bivalve shells, hydrozoans, and bryozoans, were found in almost all samples. The Gastropods and Cephalopods were the most abundant, with both occurring in 70% of the tracts, however, both show differences for IRI index, 41 and 4, respectively. This apparent higher use of nonalgal resources may reflect high prevalence of invertebrates in the shoreline habitats near Ra's Al Hadd. It is important to note that the animal matter species could also be associated with the algae and seagrasses. Little is known on the distribution of these fauna in feeding grounds.

The relative importance of animal matter, especially the gastropods, is believed to be exaggerated due to the use of dry weight as an index of measurement. Rigid invertebrate parts have overestimated weights compared with algal and seagrass specimens and soft-bodied invertebrates, like cephalopods, since they are completely digested and leave no identifiable remains behind. With the exception of the gastropods, the species richness and abundance of nonalgal species recovered in this study are likely to be underestimated.

The frequent occurrence of substrate particles (sand, pebbles, and shell fragments) in diet samples from all sites suggests that feeding turtles may be ingesting this material incidentally as they closely crop seagrasses and algae. Substrate may also be ingested as turtles feed on Sabellid worms and other benthic organisms. Green turtles also consumed plastic bags, nylon cord, mesh bags, and tarpaulin fragments. Again, the relative importance of these materials is exaggerated by the use of dry weight as an index of measurement, as with the animal matter. There is no evidence to suggest this as a cause of death, especially because they represent a small gravimetric percentage of the total diet. However, the amount of debris and other plastic materials ingested is usually small, and so it is important to put these apparently small percentages in perspective, even though mortality resulting from ingestion of debris is extremely difficult to estimate (National Research Council 1990).

The results from this study must be interpreted with care. Small sample sizes, possible misidentifications of prey species, the fact that data were obtained during the reproductive season, and analysis of dead turtles may contribute to erroneous conclusions.

Much research is needed to elucidate the relationships between the foraging ecology of sea turtles and their role in the marine ecosystems and between the nutrition of sea turtles and their productivity. Several gaps remain in our knowledge of feeding habits of green turtles in Oman, but understanding diet selection is critical for assessing the significance of feeding ground habitats, an undertaking that is ever more critical as wildlife managers are expected to make difficult decisions about which habitats to protect. Further quantitative studies are needed that address relative abundance of potential food items in the environment to the food items that are ingested.

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Calcium Depletion of Eggshell After Fungal Invasion of Sea Turtle Eggs

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ABSTRACT. – The calcium content of flatback (*Natator depressus*), loggerhead (*Caretta caretta*), hawksbill (*Eretmochelys imbricata*), and green (*Chelonia mydas*) sea turtle eggshells are not significantly different. Green turtle eggs with external fungal invasion by *Fusarium solani* had significantly reduced calcium content of the outer, inorganic portion of the eggshell.

The developmental failure of sea turtle eggs has often been related to fungal invasion (Solomon and Baird 1980; Wyneken et al. 1988; Eckert and Eckert 1990; Mo et al. 1990; Acuña-Mesén 1992; Peters et al. 1994; Phillott and Parmenter 2001), but the exact cause of embryo mortality is still unknown. Solomon and Baird (1980) suggested hyphal penetration of the eggshell and eggshell membranes may impair gaseous exchange, invade embryonic tissue, and/or impede normal embryonic development by depleting the amount of calcium in the eggshell. Phillott and Parmenter (2001) concluded that if hyphal impediment of gas exchange occurs, the severity of its influence would depend upon the size and location of the fungal growth, the sea turtle species and egg size. Fungi are capable of penetrating the eggshell and invading embryonic tissue (Phillott 2004). The potential for eggshell calcium depletion by fungi and the subsequent influence on embryogenesis have not been quantitatively established.

Solomon and Baird (1980) observed fungal hyphae between the soft shell membrane and crystalline shell layer in green sea turtle eggs. They concluded that the high calcium content of these hyphae, in conjunction with their proximity to the calcified eggshell, suggested fungi may be extracting calcium from the eggshell, thereby causing a deficiency in the embryo and impairing normal development.

Early in the growth phase during the second half of incubation, turtle embryos initially obtain calcium from the egg yolk. The yolk is quickly depleted of calcium, which must then be mobilized from the eggshell during the last trimester (Packard 1994; Sahoo et al. 1998). Calcium is the major inorganic constituent of sea turtle eggshell (20%–21%) (Solomon and Baird 1976; Sahoo et al. 1998), and the majority required for embryogenesis is derived from this source (60%—Sahoo et al. 1998; 62%—Bustard et al. 1969; 75%—Simkiss 1962). Consequently, calcium depletion could be expected to influence not only embryonic development (Solomon and Baird 1980) but also eggshell structure (Sahoo et al. 1996).

Sea turtle eggshell consists of 2 layers: an outer inorganic surface composed of organized, crystalline aggregates of calcium carbonate in the aragonite form (Solomon and Baird 1976) and an underlying organic component composed of the shell membrane or *membrana testacea*, consisting of dense fibrous organic matter (Packard and Packard 1979; Sahoo et al. 1996) with a

Table 1. Calcium content of hawksbill, loggerhead, flatback, and green sea turtle eggshell ($n=5$ for each species).

Species	Calcium content of eggshell (% by weight)			
	Organic component		Inorganic component	
	Mean	SD	Mean	SD
Hawksbill	9.02	9.57	43.06	10.94
Loggerhead	9.78	7.33	39.56	7.98
Flatback	9.01	9.73	43.37	8.04
Green	4.66	4.23	40.09	7.03

thin basement membrane (Sahoo et al. 1996). There is no cuticle on the egg exterior, and no distinct pore structure (Solomon and Baird 1976). The eggshell is the egg's first defense against microbial invasion, and, as such, its integrity is of great importance.

To determine the potential for fungal depletion of calcium from sea turtle eggshell, the calcium concentration of eggshell from oviposited eggs was compared with that of eggs that had been colonized by fungi but had not lost any of their calcium to the developing embryo.

Methods. — To estimate the calcium concentration of sea turtle eggshells, 1 egg was collected directly from a nesting female from each of 5 flatbacks (*Natator depressus*, Peak Island, 23°20.5'S, 150°56'E), loggerheads (*Caretta caretta*, Mon Repos Conservation Park, 24°48'S, 152°27'E), hawksbills (*Eretmochelys imbricata*, Milman Island, 11°10'S, 143°00'E), and greens (*Chelonia mydas*, Heron Island, 23°26'S, 152°27'E). Eggs were immediately frozen to -5°C for storage. The sample size was limited by permit regulations on the number of viable eggs allowed to be collected.

Prior to analysis, eggs were thawed to room temperature and their contents removed. The eggshells were air-dried and mounted on double-sided carbon tape attached to an aluminium stub and examined with a Jeol JSM-5300LV Scanning Microscope to determine calcium content. Energy dispersive X-ray analysis was conducted at an accelerating voltage of 15kV, with acquisition on an area of 10 × 10µm at ×1000 magnification, for 60 sec. Five fragments from each shell were analyzed to determine the percentage concentration of calcium. Statistical analysis of eggshell calcium content among species was carried out using a one-way nested ANOVA (with eggs as a nested factor within species).

To determine the calcium content of eggshells affected by fungus, 5 eggs from a single clutch of *C. mydas* (that had been collected for other research purposes) were analyzed for shell calcium. The clutch was collected at Heron Island and stored at 8°C for < 48 hrs at the Heron Island Research Centre before transport to Central Queensland University, following the procedures of low-temperature transport described by Harry and Limpus (1989). None of the eggs developed a white spot under incubation conditions of 28°C upon a natural sand

Table 2. Calcium content of nonaffected and fungal-affected green sea turtle eggshell ($n=5$ for each category).

Fungal Presence	Calcium content of eggshell (% by weight)			
	Organic component		Inorganic component	
	Mean	SD	Mean	SD
Yes	4.84	4.46	33.36	5.97
No	4.66	4.23	40.09	7.03

substrate, indicating failure of embryonic development beyond the gastrula present at oviposition (Decker 1967; Mahmoud et al. 1973; Ewert 1985). Fungus appeared on a single egg 7 days after being placed in the incubator and spread through the entire egg mass during the following 4 weeks. At this time 5 eggs with fungus visible on their exterior were randomly selected and 5 fragments per egg analyzed as described previously. Statistical analyses of eggshell calcium content among eggs with and without fungi was conducted using a one-way nested ANOVA (with eggs as a nested factor within fungal presence/absence) following arc-sine transformation to normalize these percentage data. Fungal hyphae were scraped from 5 different locations of each egg exterior with a sterile scalpel blade and mounted on double-sided carbon tape for calcium analysis. The fungus was identified following Booth (1971).

Results. — The calcium contents of unaffected eggs from each species are given in Table 1. There was no significant difference in calcium concentration of the eggshell inorganic or organic component among species (inorganic $F_{3,16} = 1.180$, $p > 0.05$; organic: $F_{3,16} = 1.384$, $p > 0.05$) or among the eggs nested within each species (inorganic component $F_{16,80} = 1.091$, $p > 0.05$; organic component $F_{16,80} = 1.692$, $p > 0.05$).

Fungal growth (by *Fusarium solani*) on the exterior of green sea turtle eggs (see Table 2) significantly reduced the calcium content of the outer, inorganic layer, but did not alter that of the inner, organic membrane (nested ANOVA: inorganic component $F_{1,8} = 11.223$, $p = 0.010$; organic component $F_{1,8} = 0.016$, $p > 0.05$). There was no difference among eggs nested within the two categories (inorganic component $F_{8,40} = 1.183$, $p > 0.05$; organic component $F_{8,40} = 1.263$, $p > 0.05$). SEM imagery of the fragments during calcium analysis indicated colonization by *F. solani* was superficial and did not penetrate the calcified layer into the shell membrane. The calcium concentrations of 5 samples of *F. solani* hyphae removed from the eggs are given in Table 3.

Pure calcium carbonate (Fisons Analytical Reagent, Code C/1120/53, Batch 9335052) was used to verify the precision of the Jeol microprobe calcium analysis. Calcium content was determined as 42.11% ± 1.96% (mean ± SD, $n = 5$) as compared to 40.08% actual.

Table 3. Calcium concentration of the fungus *F. solani* removed from the exterior of green sea turtle eggs.

Egg No.	Calcium content (% by weight)		n
	Average	SD	
1	2.16	0.48	5
2	3.32	0.83	5
3	5.56	3.53	5
4	2.18	0.55	5
5	2.76	0.91	5
Average	3.20	2.01	

Discussion. — This is the first report of calcium analyses for *E. imbricata*, *C. caretta*, and *N. depressus* sea turtle eggs. Solomon and Baird (1976) reported calcium to be 20% of *C. mydas* eggshell, but their analysis was performed on homogenized eggshell that included both the organic and inorganic components. Calculating the results of the present study in the same fashion gives an average of 19.15% calcium for *C. mydas* eggshell, which is almost identical to the results of Solomon and Baird (1976).

Although high variability in calcium content was detected in the organic and inorganic components of eggshell from all species (Table 1), there was no visual evidence of amorphous calcium deposits attributed to “calcium splash” (Chan and Solomon 1989). This eggshell phenomenon occurs in birds after delayed oviposition (often due to stress), resulting in irregularly distributed superficial and superfluous calcium. In sea turtles, delayed oviposition may occur after disturbance during nesting (by humans or other turtles), or through failure of nest construction due to unsuitable nesting substrate. However, this is unlikely to result in calcium splash because shell formation is already complete and the eggs are distal to the shelling region of the oviduct by this time. Calcium splash in sea turtles could only occur prior to nesting emergence, when severe disturbance may result in the cessation of oviductal motility during shelling and cause extra calcium to be deposited on the shell membrane or eggshell exterior. This would result in elevated calcium levels on isolated areas of a single egg.

Fusarium solani is 1 of 3 species of fungi regularly identified from failed eggs of turtles nesting in eastern Australia (Phillott et al. 2001, 2004) and has been implicated in embryo mortality (Phillott and Parmenter 2002). Its source was probably the natural sand substrate upon which the eggs were incubated (see Phillott and Parmenter 2002). Since there was no post-oviposition embryonic development, the calcium loss demonstrated by eggs affected by fungus can only be attributed to fungal presence, although ideally it would have been useful to compare calcium contents of eggs that had failed to develop but had not been subsequently colonized by fungi.

The similarity of eggshell calcium levels (this study) and structure (A.D. Phillott, unpub. data) of the 4 turtle species investigated suggests fungal depletion of calcium

is likely to be similar across all cheloniid species of sea turtle. Because the fungus was superficial on the egg and did not reach the shell membrane, it is not surprising that there was no calcium depletion of the latter. Analysis after a longer period of fungal presence would possibly show decreased levels of shell membrane calcium if the eggshell were eventually penetrated by fungus.

Two other species of fungi (*Fusarium oxysporum* and *Pseudallescheria boydii*) are also commonly isolated from failed sea turtle eggs in eastern Australia (Phillott et al. 2001, 2004). Their ability to cause calcium depletion of sea turtle eggshell requires further investigation.

Calcium requirements for fungal growth are poorly known. Available evidence is contradictory (Harold 1994) and regarded as both a macro- (Garraway and Evans 1984) and micro- (Jennings and Lysek 1996) nutrient. Uptake from the external medium may occur by facilitated diffusion, proton symport, or pinocytosis across the absorption zone of the hyphal tip (reviewed by Garraway and Evans 1984).

The effect of depleted eggshell calcium on the developing turtle embryo is poorly understood. Because the embryo does not extract calcium from the eggshell until the third trimester, it is assumed that embryonic development would proceed normally (with respect to calcium dynamics) until then, in the absence of other pathological factors. However, once calcium demand increases for osteogenesis, its insufficiency could possibly result in teratogenesis and/or embryo mortality.

Eggshell exfoliation of viable eggs is normally observed in the week prior to hatching (Miller 1982) and occurs due to calcium mobilization from the eggshell by the rapidly maturing embryo (Simkiss 1962). On inspection after hatching, the calcified layer of the eggshell appears disrupted (Schleich and Kästle 1988; Sahoo et al. 1996) as a result of calcium depletion (Sahoo et al. 1996). Premature loss of eggshell integrity, due to fungal depletion of calcium, would weaken its function as a barrier and allow easier hyphal penetration and subsequent access to the nutrient-rich yolk and embryonic material. Ultimately, both depletion of available calcium and/or destruction of the eggshell's integrity are likely to result in embryo mortality.

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Natural Diet of the Namaqualand Speckled Padloper (*Homopus signatus signatus*)

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ABSTRACT. – Feeding in the wild of the tortoise *Homopus signatus signatus* in South Africa was recorded and the diet compared to previously reported fecal analysis. Several new plant species were identified in the diet, and analyses differed by ca. 24% in terms of species present. Tortoises did not feed on all plant species or parts to the same extent, indicating selective feeding.

Homopus signatus signatus, the Namaqualand speckled padloper (or northern speckled cape tortoise), is the world's smallest tortoise species, occurring exclusively in rocky habitat in northwestern South Africa (Branch 1998; Boycott and Bourquin 2000). The biome in which it occurs is known as Succulent Karoo Shrubland (Branch

Table 1. Frequencies of plant species on which wild *Homopus signatus signatus* were observed feeding (present study), and frequencies of these species in tortoise fecal samples (from Loehr, 2002a).^a

Species	Frequency of feeding observations	Frequency of fecal observations
<i>Oxalis</i> spp.	22	31
<i>Leysera tenella</i>	16	20
<i>Crassula thunbergiana minutiflora</i>		
<i>Felicia merxmuelleri</i>	11	13
<i>Grielim humifusum</i>	6	1
<i>Diascia namaquensis</i>	4	13
<i>Heliophila variabilis</i>	3	7
<i>Felicia australis</i>	3	5
<i>Albuca</i> sp.	2	5
<i>Ballota africana</i>	2	3
<i>Cleretum papulosum</i>	1	0
<i>Cotula laxa</i>	1	0
<i>Crotalaria</i> sp.	1	3
<i>Erodium cicutarium</i>	1	0
<i>Hemimeris montana</i>	1	0
<i>Hermannia</i> sp.	1	6
<i>Lachenalia carnosus</i>	1	2
<i>Lebeckia sericea</i>	1	1
<i>Lotononis</i> sp.	1	0
<i>Moraea fugax</i>	1	0
<i>Massonia</i> sp.	1	0
<i>Microlooma sagittatum</i>	1	0
<i>Osteospermum amplectens</i>	1	0
<i>Wahlenbergia</i> sp.	1	0
<i>Wahlenbergia annularis</i>	1	0
<i>Diospyros ramulosa</i>	0	20
	$p < 0.05$	$p < 0.05$
	$p > 0.05$	

^a *Diospyros ramulosa* has been included in the fecal samples because it was subsequently recognized as an abundant species. Statistics represent frequency analyses by means of Kolmogorov-Smirnov tests (see text for details).

1998). Recently, available information on the ecology of *H. s. signatus* has increased considerably (Loehr 1999, 2002a, 2002b, 2004; Klerks 2002). One aspect studied is diet. In previous literature, the species has been suggested to have a specialized diet (Barzyk 1994), and to feed on succulents (Branch 1998). However, Loehr (1999) reported that captive specimens thrived on a diet that consisted of plantain, dandelion, clover, endive, chicory, and fruits. In a study of fecal material of wild *H. s. signatus*, the species appeared to feed on a variety of plants, with 4 (*Oxalis* spp., *Leysera tenella*, *Grielim humifusum*, *Crassula thunbergiana minutiflora*) making up an important proportion of the total (Loehr 2002a).

Loehr (2002a) was unable to identify more than about half of the collected fecal material. This was because much of the material was too digested for identification using available morphological plant features. In addition, 1 plant species that was frequently found in the feces could not be identified because it was not present in the reference collection. Although investigations of dietary preferences (Rall and Fairall 1993; Mason et al. 2000), nutrition (Ofstedal and Allen 1996), and digestive efficacy (Barboza

1995; Hailey 1997) will eventually be required to obtain a full understanding of the feeding ecology of *H. s. signatus*, the current incomplete information on its diet would also benefit from additional studies of the components. In this paper, I analyze focal observations of feeding in wild *H. s. signatus* accumulated during 5 study periods, and compare these with the previously published results of fecal analysis.

Method. — During 5–6 weeks in September and October 2001–2004, a 36,340-m² study site near Springbok, South Africa, was methodically inspected for *H. s. signatus*. In addition, an average of 15 tortoises were followed by means of thread-trailing (Loehr 2002b), or radiotelemetry. A detailed description of the study site can be found in Loehr (2002b), with the plant species listed in Loehr (2002a). When a tortoise was discovered, its behavior was recorded. For feeding specimens, the food items were identified in the field. Virtually all plant species in the study area had been identified previously by comparison with herbarium material (Loehr 2002a). When a nonfeeding tortoise had remains of plants in its beak, these were included as food items. Each plant species eaten by a tortoise represented 1 observation. The plant parts eaten (leaf, flower, stem, or fruit) were also recorded. To increase sample size, the results of the 2001–2004 study periods were combined with feeding observations made in August–September 2000 at the same site (Loehr 2002a).

Feeding observations were compared with the results of fecal analysis (Loehr 2002a). In the fecal analysis (samples collected in August–September 2000), each plant species present in the total produced fecal material during one tortoise encounter represented one observation. Plant parts were also distinguished and recorded. Plant species on which tortoises had been seen feeding were listed, and their frequencies were compared with the frequencies of these species in the feces by means of Kolmogorov-Smirnov tests (Sokal and Rohlf 1981). Similar analysis was performed for plant part frequencies. The results of the statistical tests are reported as D_{\max} statistic (D_m) and the critical (smallest) value of D_m for $p < 0.05$ ($D_{.05}$).

Results. — In total, 1580 observations of 244 tortoises were made, of which only 78 (4.9%) were observations of feeding on one or two plant species. Dietary items were recorded for 37 males, 38 females, and 9 juveniles, and the total number of food plant species identified was 25 (Table 1). Observations of feeding on *Oxalis* spp., *Leysera tenella*, and *Crassula thunbergiana minutiflora* were most frequent ($D_m = 0.502 > D_{.05} = 0.148$) (Table 1). A comparison of the frequencies of the 25 species consumed with their frequencies in the fecal samples revealed no significant difference ($D_m = 0.147 < D_{.05} = 0.148$) (Table 1). However, *Grielim humifusum* was strikingly absent from the observations made in 2001, 2002, and 2004 (1 and 3 observations in 2000 and 2003, respectively), although it was abundant in the fecal samples. The feeding observa-

tions in 2001–2004 revealed 11 new food plant species (five new families) for *H. s. signatus*: *Ballota africana* (Lamiaceae), *Cleretum papulosum* (Mesembryanthemaceae), *Erodium cicutarium* (Geraniaceae), *Hemimeris montana* (Scrophulariaceae), *Lotononis* sp. (Fabaceae), *Microloma sagittatum* (Asclepiadaceae), *Moraea fugax* (Iridaceae), *Massonia* sp. (Liliaceae), *Osteospermum amplexans* (Asteraceae), *Wahlenbergia annularis*, and *Wahlenbergia* sp. (Campanulaceae).

Feeding on leaves (44%) and flowers (35%) was more often observed than feeding on stems (20%) and fruits (2%) ($D_m = 0.289 > D_{.05} = 0.118$). Although fecal samples contained more flowers than leaves, no significant difference with the feeding observations could be distinguished ($D_m = 0.109 < D_{.05} = 0.118$). In September 2002 and 2004, observations were made of 2 males eating 8–10 flowers of *L. tenella* without eating any other readily available part of this plant species. A third observation of selective feeding was made in September 2002, when a female managed to eat a flower of *M. fugax* that was 15 cm above the surface (without showing any intention to eat other plant parts or species that were closer), by gradually pushing the flower stalk to the ground.

The unidentified food item that had been found in 41% of the fecal samples in 2000 (Tables 1 and 2 in Loehr 2002a) was found to be the flower of *Diospyros ramulosa*. These small flowers were present in relatively large quantities under *D. ramulosa* shrubs but had previously been overlooked.

Discussion. — Although Loehr (2002a) was able to identify only 50%–60% of the fecal material of *H. s. signatus*, the current analysis of focal feeding observations confirms that *Oxalis* spp., *L. tenella*, and *C. t. minutiflora* are among the most important food sources for this species in its studied habitat. Two plant species, *G. humifusum* and *D. ramulosa*, that occurred frequently in feces, were rarely or not at all recorded in feeding observations. The first species was common during most of the 2000 study period; in contrast, it was much scarcer in 2001–2004 and this may have been reflected in the feeding observations. *Diospyros ramulosa* flowers were eaten by tortoises when they had fallen off the shrubs and were scattered in dead foliage under them. Such locations were very hard to search for tortoises, and especially to make feeding observations. Tortoises usually stopped feeding immediately and moved away when they noticed an observer.

The new food plant species recorded in 2001–2004 add to the reported large variation in the diet of *H. s. signatus* (Loehr 2002a), and these species may have been part of the unidentifiable fecal material in 2000. The newly recorded species accounted for as much as 24% of the total number of species the tortoises were found to consume. Conversely, 37% of the plant species identified in the fecal matter were also noted as food items in this study. The research protocol did not allow temporal variation in dietary components to be determined (Nagy and Medica 1986; Moskovits and Bjorndal 1990; Rall and Fairall

1993), and both methods may underestimate the total number of food plants utilized.

Food items frequently eaten may have been preferred or may simply reflect abundance of the food item. The larger proportion of flowers in the feces, compared to feeding observations, may have been the result of two relatively dry study periods, 2003 and 2004, with fewer flowers (V.J.T. Loeh, *pers. obs.*, 2005). On the other hand, the observation of three tortoises specifically feeding on flowers of *L. tenella* and *M. fugax* suggests that preferences may exist. Captive *H. s. signatus* have also been reported to have a preference for feeding on flowers (Loehr 1999). Furthermore, since dietary preferences have been documented in other tortoises (Moskovits and Bjorndal 1990; Rall and Fairall 1993; Hailey et al. 1998; Mason et al. 1999, 2000; Jennings 2002; Oftedal et al. 2002), this aspect requires further investigation in *H. s. signatus*.

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Strandings of Juvenile Green Turtles at Samandağ, Turkey

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ABSTRACT. – Strandings of juvenile green turtles, *Chelonia mydas*, at Samandağ beach, southeastern

Turkey near Syria, suggest the area may represent a juvenile developmental habitat for the species.

Green turtles (*Chelonia mydas*) are one of two nesting sea turtle species in the Mediterranean. Their nesting activity has a distribution limited to the eastern part of the basin (Kasperek et al. 2001). The main green turtle nesting beaches are located in Turkey (Kazanlı, Akyatan, and Samandağ) and Cyprus. A few nests are also found elsewhere in Turkey (Dalyan, Kumluca, Belek, Side-Kızılot, Göksu Delta, and Ağyatan), in addition to Lebanon, Israel, and northern Sinai. Samandağ beach, located in southeastern Turkey near the Syrian border (Fig. 1), is considered among the most important areas for green turtles in the Mediterranean, hosting 7% of the entire population (Kasperek et al. 2001).

The importance of Samandağ beach for green turtle nesting activity has been confirmed by a monitoring program that recorded a nest density of 4.88, 22.4, and 22.4 nests per km in 2001, 2002, and 2003, respectively (Yalçın 2003; Yalçın-Özdilek and Sönmez 2003) and 126 nests in 2003 (Yalçın-Özdilek et al. 2004).

In the Mediterranean little is known about *C. mydas* population structure, distribution, and habitat utilization. In particular, the locations of juvenile developmental areas in the basin are still not clearly defined. The eastern part of the basin has been suggested to be a juvenile developmental area by different authors (Oruç et al. 1997; Laurent et al. 1996; Godley et al. 1998), whereas recent studies have reported two locations at more western areas: Lakonikos Bay, southern Peloponnese, Greece (Margaritoulis and Teneketzis 2001) and Fethiye beach, Turkey (Türkozan and Durmuş 2000).

Here, we examine stranding records of turtles found at Samandağ beach and discuss the possible location of a juvenile developmental area for the green turtle in the eastern Mediterranean (Fig. 1).

Methods. — The entire length of Samandağ beach (14.5 km) was monitored on foot every day from 28 June to 15 September 2002. Stranded sea turtles found dead on the beach were recorded and identified to species. Curved carapace length (CCL) and curved carapace width (CCW) were measured with a flexible tape measure according to Bolten (1999).

Results. — Twenty-eight stranded dead turtles were found along Samandağ beach during the 2002 nesting season (Table 1). Seventeen were collected at the beginning of the season, with 8 clearly old, probably stranded in previous years. An additional 11 turtles stranded during the season. Twenty-two (78.6%) were identified as *C. mydas*, with CCL ranging between 23.5 and 80 cm (mean = 40.6 ± 14.2 cm) (Fig. 2). One of them, with 35 cm CCL, had a stomach full of seagrass.

Discussion. — Green turtles are believed to be omnivorous with a strong tendency to carnivory during their pelagic stage, and then shift to an herbivorous diet

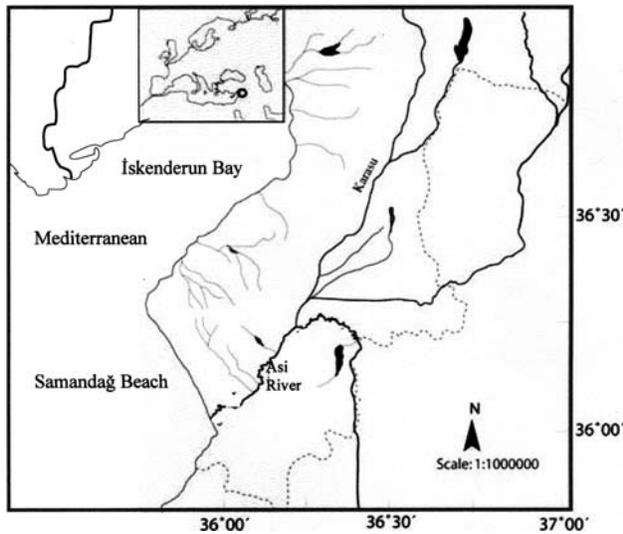


Figure 1. Map showing location of Samandağ beach, Turkey.

Table 1. Stranded sea turtles found on Samandağ beach, Turkey, in 2002.

Species	n	CCL (cm)	
		Mean	Range
<i>Chelonia mydas</i>	22	40.6	23.5–80
<i>Caretta caretta</i>	3	46.8	13.5–63.5
<i>Trionyx triunguis</i>	1	48.0	—
Unidentified	2	—	—

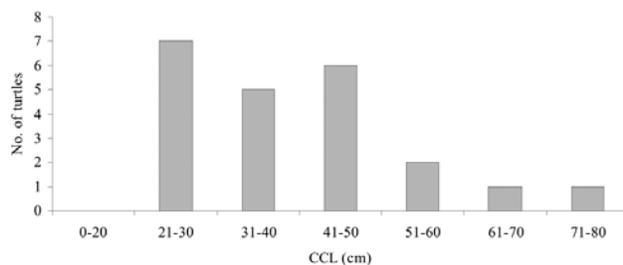


Figure 2. Curved carapace length (CCL) of stranded *C. mydas* ($n=22$) found on Samandağ beach, Turkey, in 2002.

(Bjorndal 1997). Being benthic herbivores (Bjorndal 1997) they can find very little food in the open ocean and they presumably stay in the neritic environment. At the size of ca. 30–40 cm CCL, green turtles generally recruit to neritic developmental habitats (Musick and Limpus 1997) where they are known to have strong feeding site fidelity and sometimes become resident until they approach maturity (Musick and Limpus 1997; Limpus and Walter 1980). Meylan and Meylan (1999) noted that juvenile green turtles of 20–30 cm in carapace length are commonly found in shallow-water neritic habitats.

In the Mediterranean, it is assumed that juvenile green turtles feed on seagrasses such as *Cymodocea nodosa*, *Zostera* spp., *Posidonia oceanica*, *Halophila stipulacea*, and algae. In Cyprus, 3 drowned green turtle juveniles had

been feeding exclusively on *C. nodosa* (Demetropoulos and Hadjichristophorou 1995).

The Turkish coastal area between Mersin and Yumurtalık is an area where juvenile green turtles can be suspected to forage. In fact, the Gulf of İskenderun was first cited by Gruvel (1933) (in Groombridge 1990) as frequented by turtles outside the nesting season, including adults, subadults, and smaller immature turtles. Successive observations from stranded turtles found during a survey along the Turkish coast (Baran and Kasperek 1989), suggested that green turtles reside in shallow water adjacent to their nesting beaches along the Çukurova region coast.

The frequent presence of juveniles in the Çukurova area has been confirmed by a recent study (Oruç et al. 1997), which showed that juveniles with CCL size ranging between 30 and 60 cm occur in the area and are mostly captured in winter. In addition, the physical characteristics of the coast in the Çukurova region (about 120 km long) and the sea characteristics within the 10-m isobath with an extension of 1.5–6 km offshore, support the hypothesis that this is a suitable area for green turtle foraging.

The size of stranded green turtles at Samandağ reported here suggests that juvenile *C. mydas* are probably foraging in the area. One was found with its stomach full of seagrass. Thus, *C. mydas* juvenile developmental habitat in the eastern Mediterranean may include areas around Samandağ beach. Research and conservation efforts should be focused on this area to help elucidate possible threats to juvenile sea turtles leading to strandings.

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Changes in Population Structure of Diamondback Terrapins (*Malaclemys terrapin terrapin*) in a Previously Surveyed Creek in Southern New Jersey

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ABSTRACT. – Population structure of *Malaclemys terrapin terrapin* in an unaltered creek in southern New Jersey was compared to data collected 12–13 years earlier. I found significantly lower average carapace size of terrapins and lower frequency of adult females compared to the earlier survey, and speculate that road mortality of nesting females may be responsible for these trends.

The diamondback terrapin (*Malaclemys terrapin*) is endemic to United States coastal salt marshes. Major threats to this species include habitat loss (coastal development and wetland destruction), mortality from drowning in crab traps, and road mortality (Roosenburg et al. 1997; Wood and Herlands 1997). A combination of these factors has resulted in concern for this species' status and its listing as a "Species of Special Concern" in several states (Wood and Herlands 1997).

In southern New Jersey, the nesting season for diamondback terrapins lasts from late May to mid-July, and occurs primarily in sand dune and salt marsh habitats (Wood and Herlands 1997). Unfortunately, thousands of female terrapins are killed by motor vehicles each summer as they traverse roads adjacent to their nesting habitats (Wood and Herlands 1997). Elasticity analyses on other turtle species indicate that changes in survival of adults and subadults have the greatest proportional effect on population growth rate (Heppell 1998). Because growth rates of diamondback terrapin populations are likely sensitive to the same demographic parameters, and road mortality is an unnatural threat to mature females, it is important to assess whether terrapin populations are responding to these losses.

The primary objective of my study was to investigate diamondback terrapin population structure in an unaltered subtidal creek in southern New Jersey, and compare results to those found by Rountree et al. (1992) 12–13 years

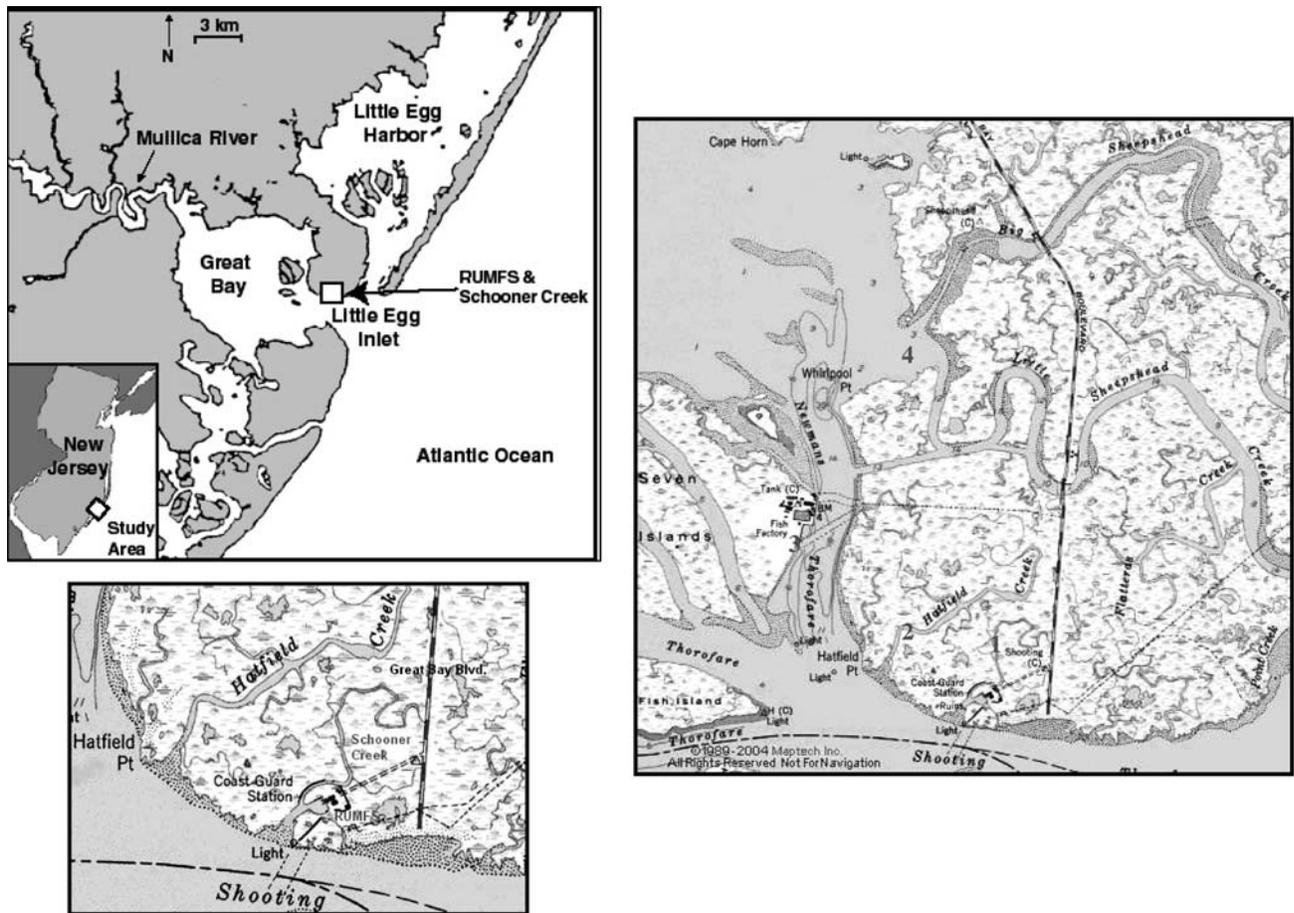


Figure 1. Trapping sites were located in Schooner Creek and surrounding areas in Great Bay–Little Egg Harbor estuarine complex in southern New Jersey. Study sites are labeled as (1) Schooner Creek, (2) Hatfield Creek, (3) Fish factory, and (4) Rand’s Marina cove. Detailed view of Schooner and Hatfield creeks are shown in bottom left map. Upper left map courtesy of S. Hagan, other maps from MapTech Inc.

earlier in the same creek system. During the years between my study and that by Rountree et al. (1992), Hoden and Able (2003) documented high road mortality of adult female terrapins along the main road adjacent to the creek. This mortality may have influenced terrapin population structure that occurred since Rountree et al.’s (1992) survey. Because body size is closely correlated to age in terrapins (Cagle 1952), changes in size structure relate to changes in a population’s age composition. I expected a lower mean body size of terrapins and fewer adult females compared to Rountree et al.’s (1992) survey results from when there was presumably less road mortality. The road adjacent to the creek was not paved until the early 1990s, so vehicle traffic was less intensive and less of a threat to terrapins before Rountree et al.’s (1992) survey in 1988–1989. However, there is no documented road mortality data prior to 1988 to confirm this (K. Able, *pers. comm.*, December 2001).

Although drowning in crab traps is a substantial threat to terrapins across their range (Roosenburg et al. 1997), it should not be a factor affecting this population because the creek and its surroundings are closed to crabbing. Because diamondback terrapins exhibit high site fidelity and limited

dispersal (Gibbons et al. 2001), this population is functionally “closed” and it is therefore appropriate to compare demographic information collected in this study to results of Rountree et al.’s (1992) survey.

Secondary objectives of this study were to determine terrapin sex ratio and population size. This information could serve as a baseline for comparison in future monitoring efforts. Aspects of non-nesting terrapin habitat use (movement and distribution) in an unaltered creek were also described.

Methods. — During August–September 2001, I sampled brackish creeks and inlets in the Great Bay–Little Egg Harbor estuarine complex of southern New Jersey, with an emphasis on Schooner Creek near Rutgers University Marine Field Station (RUMFS), Tuckerton (39.508881 N, 74.32586 W) (Fig. 1). Schooner Creek is an unaltered subtidal creek with one connection to Great Bay, located 1.3 km from Little Egg Harbor inlet. It is 1160 m in length with a creek bed area of 24,000 m² (Rountree et al. 1992). The dominant surrounding vegetation includes *Spartina alterniflora* and *S. patens*, and at its closest point to a road, Schooner Creek is < 100 m away from Great Bay Boulevard. Depths at high tide range from 0.9 to 3.7

m from upper creek to mouth. The entire length of the study creek was surveyed to determine terrapin movement and distribution with respect to distance from creek mouth. Catch per unit effort (CPUE) was compared to water temperature, salinity, and time of day.

Areas near Schooner Creek (Hatfield Creek, a cove near Rand's Marina, and the old fish factory in Great Bay) were also sampled to determine whether terrapins were moving between creeks and to experiment with different trapping gear, including crab traps and gill nets, set for up to 5 hours (Fig. 1). However, the terrapins captured in these areas were not considered in population structure comparisons between the two studies, to maintain consistency with Rountree et al.'s (1992) survey of Schooner Creek.

Twelve crab traps were set at various depths at 100-m intervals along the length of Schooner Creek, beginning at 50 m outside of the mouth (at the RUMFS boat basin). The crab traps were commercial-style 58-cm³ box wire frames with 4 oval openings and a biodegradable panel. The openings were stretched to a 15 cm diameter, which exceeds the maximum width of terrapins, to allow large individuals to enter the traps. Traps were deployed for 3 to 5 hours at flood tide and retrieved at high tide. Traps were set and pulled between the daylight hours of 0700 and 1900, 5 days a week from 16 August through 27 September 2001. Each trap was baited with Atlantic menhaden (*Brevoortia tyrannus*) or bluefish (*Pomatomus saltatrix*) at every setting. At each setting, I recorded the date, time of day, weather condition, surface water temperature, and salinity at mid-creek (500 m).

All captured terrapins were marked for individual identification by filing notches into unique combinations of their marginal scutes (Cagle 1939). Straight-line mid-carapace length (CL) was measured to the nearest mm with a caliper. The sex of each turtle was determined using head and tail characteristics: males have proportionally narrower heads and longer, thicker tails than females (Lovich and Gibbons 1990). Adult females are generally much larger than males, with males reaching maximum CL of 140 mm, while females can grow to 250 mm (Carr 1952). All terrapins were processed and released at capture location. Care was taken that terrapins did not remain in traps longer than 5 hours, and no terrapins drowned during this study.

Rountree et al. (1992) did not distinguish between males and females in their measurements of terrapins, so I could not back-calculate an accurate sex ratio for their population. To detect changes in sex ratio between the 2 data sets, I used frequency of adult females as an index for comparison. I classified any individual with a CL of 140 mm or greater as an adult female. That threshold value was chosen because it corresponds to the maximum size attained by males and concurs with estimates of female size at maturity (Carr 1952; Montevecchi and Burger 1975; Hurd et al. 1979). It is likely that many of the smaller terrapins (CL < 140 mm) in Rountree et al.'s

(1992) sample were also female, but they cannot be differentiated from the males by CL measurements alone.

To compare size structure data between the 2 studies, I pooled male and female size measurements and double-counted recaptures for consistency with Rountree et al.'s (1992) data, which did not discriminate between sexes or account for recaptured individuals. Only the August–September samples from Rountree et al.'s (1992) data were used for comparison to reduce potential biases due to seasonal changes.

The date and location of each recapture were recorded. The program CAPTURE was used to determine the probability of equal capture and to estimate population size (White et al. 1982). For this test, the 28 days of sampling were separated into 4 trapping "occasions" of 7 days each. Population size was also estimated using a modified Lincoln-Peterson model for comparison (Krebs 1998). The first 3 trapping/markings episodes were considered the initial trapping/markings sample (n_1), and the remaining trapping days were lumped as the second sample (n_2) because most of the marking occurred within the first 3 days of trapping.

Differences in mean CL between turtles captured in 1988–1989 (Rountree et al. 1992) and this study were compared using a two-sample *t*-test. A Chi-squared test was used to determine significance in frequency differences of adult females (CL \geq 140 mm) found in this study and Rountree et al.'s (1992). CPUE was calculated as the daily number of terrapins captured (including recaptures) divided by trap-hours (= number of traps multiplied by number of hours). The relationships between CPUE and time of day, water temperature, and salinity were analyzed for correlation. Statistical tests were computed using the 1996 version of PC-SAS.

Results. — Fifty-three diamondback terrapins were captured in Schooner Creek, and 9 were caught elsewhere (1 in Hatfield Creek, 2 by Rand's Marina in Great Bay, and 6 near the old fish factory) (Fig. 1). The latter were not included in population size and structure analyses, for consistency and comparability with Rountree et al.'s (1992) data. Eight of the 53 marked turtles were recaptured within Schooner Creek, some on multiple occasions, for a total of 11 recaptures and 64 (53 + 11) independent capture events.

Female terrapins in Schooner Creek ranged in size from 95.4 to 190.0 mm CL, and males ranged from 98.3 to 120.7 mm CL. The mean CL of terrapins I captured ($= 118.4 \pm 2.48$) was significantly lower than Rountree et al.'s (1992) mean terrapin size ($= 154.3 \pm 3.29$, $n = 89$) ($t = 19.3255$, $df = 1$, $p = 0.0001$) (Fig. 2). Of the 53 terrapins captured in crab pots in Schooner Creek, 36 (68%) were females (of all sizes) and 17 (32%) were males. Of these 36 females, 8 (22.2%) were considered large adults (CL \geq 140 mm). Given this proportion, it is likely that many of Rountree et al.'s (1992) smaller terrapins (CL < 140 mm) were also female, although actual ratios are unknown. The frequency of adult females was significantly lower in this study ($8/53 = 15.1\%$) than

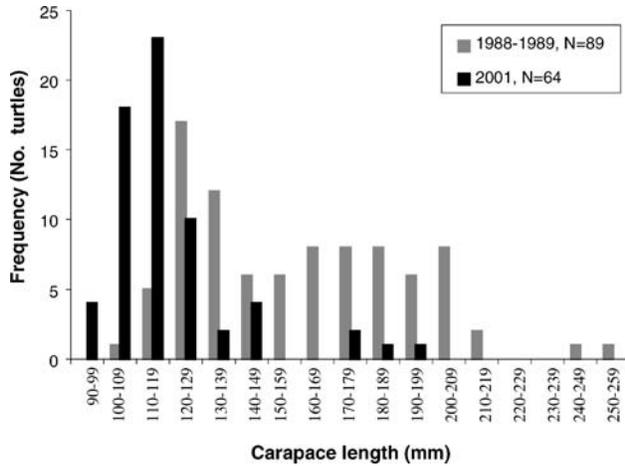


Figure 2. Size-frequency distributions of diamondback terrapins: comparison of CL data sets from 1988–1989 ($= 154.3 \pm 3.29$, $n = 89$; Rountree et al., 1992) and 2001 ($= 118.4 \pm 2.48$, $n = 64$; this study) ($p = 0.0001$).

in Rountree et al.’s (1992) survey ($55/89 = 61.8\%$) ($\chi^2 = 29.355$, $df = 1$, $p \leq 0.001$). Only 5 terrapins were caught in gill nets, 3 of which were female (60%) and 2 were males (40%).

The estimated population size in Schooner Creek was 119 ± 31.46 using the Lincoln-Peterson model. Program CAPTURE determined unequal capture probability of terrapins. Using the trap response model M_b (constant probability removal estimator), the program estimated a population size of 54 ± 1.65 individuals. With a 95% confidence interval, the population size in Schooner Creek was 57–181 (Lincoln-Peterson) to 54–62 (CAPTURE) individuals.

The 7 females that were recaptured ranged in CL from 104.4 to 129.7 mm, and the single recaptured male measured 116.7 mm. The number of days at liberty (days between captures and recaptures) was not correlated with distance traveled ($r^2 = 0.0033$). Days at liberty ranged from 1 to 25 days, and distance traveled ranged from 50 to 700 m (Fig. 3). No terrapin movement was detected

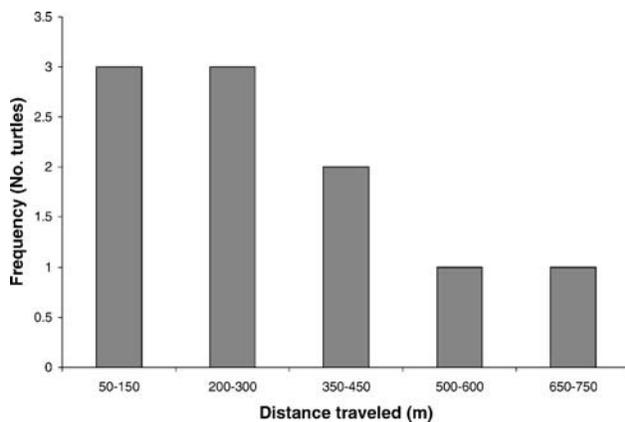


Figure 3. Frequency distribution of distance traveled between captures of diamondback terrapins in Schooner Creek, New Jersey, August–September 2001.

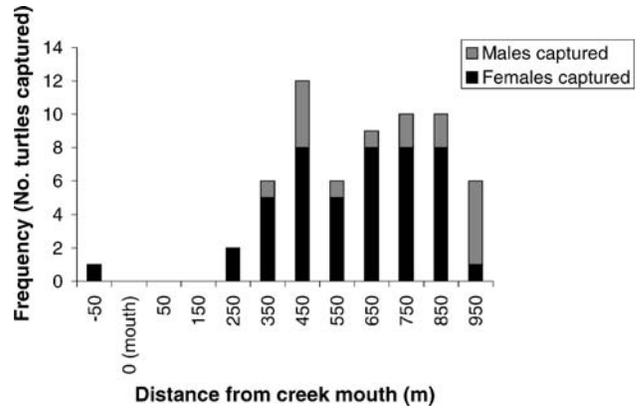


Figure 4. Diamondback terrapin capture frequency with respect to location in Schooner Creek, New Jersey, August–September 2001.

between Schooner Creek and the other sites. Only one terrapin was captured in a trap set within the lower 200 m of Schooner Creek, and all of the males were captured in the upper creek (Fig. 4).

Water temperatures during the trapping period ranged from 18° to 27°C, and salinity ranged from 27 to 30 ppt. The CPUE decreased over time (Fig. 5) and was significantly correlated with water temperature ($r^2 = 0.2507$, $df = 20$, $p = 0.0088$), but not with time of day ($r^2 = 0.0002$, $df = 25$, $p = 0.4708$) or salinity ($r^2 = 0.124$, $df = 21$, $p = 0.0964$).

Discussion. — Average CL was smaller in 2001 (118.4 ± 2.48 mm) than in 1988–1989 (154.3 ± 3.29 mm). The largest CL in this study was 190 mm, while Rountree et al. (1992) found CL up to 250 mm. They also found significantly more individuals in size classes greater than 140 mm, and fewer individuals in smaller-size categories (90–119 mm) than I found. The frequency of large individuals captured in my study was significantly less than it was in 1988–1989, which could be cause for concern. Because size and age are highly correlated in terrapins (Cagle 1952), the decrease in mean size may correspond to a loss of older females. The loss of adult females from the population due to road mortality could be responsible for this change in size composition.

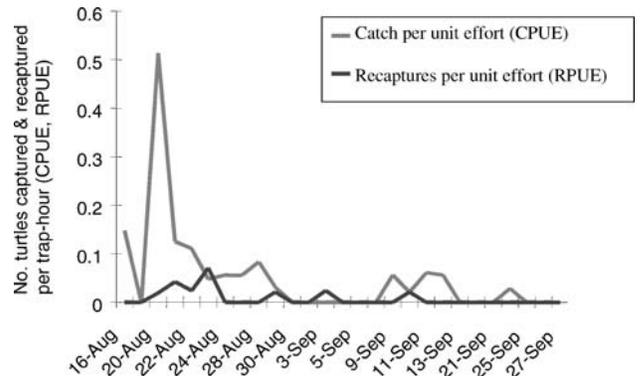


Figure 5. CPUE and recaptures per unit effort (RPUE) over time in Schooner Creek, New Jersey, August–September 2001.

Sampling bias may be partially responsible for the differences detected between the 2 studies. Rountree et al. (1992) pooled their captures from 3 subtidal creeks (including Schooner), sampled at the creek mouth only throughout ebb tides, and used active capture gear (weirs) rather than passive gear (crab traps). In this study, crab traps were set throughout Schooner Creek at flood tides and retrieved at high tide. Perhaps terrapins were rarely caught in the lower creek because they avoided this area at high tide. It may also be possible that smaller terrapins inhabit the upper creek while larger terrapins stay near the mouth, so Rountree et al.'s (1992) weir at the creek mouth yielded larger-sized captures. Furthermore, older (larger) individuals may have been more likely to avoid traps than younger individuals and thus could have been under-represented in this sample. If adults avoided crab traps and were distributed in the lower creek, it would explain why there were few captures in the lower creek, and few large individuals trapped in general.

However, it is doubtful that the gear-bias explanation fully accounts for the size-structure differences observed. I captured large individuals in the crab traps on several occasions, and other researchers have not found evidence for differential trap-avoidance by adults (M. Hoyle, *pers. comm.*, December 2001). I compared captures from crab traps and gill nets within Schooner Creek to determine whether there was a size bias due to collection gear. While there were too few captures in the nets ($n=4$) for significant conclusions, results indicated that terrapins caught in crab traps were similar in CL (100.0–118.7 mm) to those caught in gill nets (110.0–118.7 mm).

Although road mortality affects primarily female terrapins, the sex ratio could still be female-biased if the population has more females to start with (Hildebrand 1932; Hurd et al. 1979). Terrapin sex determination is temperature-dependent, with warmer incubation temperatures favoring production of females (Hildebrand 1932; Lovich and Gibbons 1990; Roosenburg 1996). There could be a naturally female-biased population near Schooner Creek if surrounding nest sites experienced warmer temperatures due to higher ambient temperatures, decreased shading from vegetation removal, or changes in characteristics of sediment deposits on the marsh. Because Schooner Creek is surrounded by abundant potential nesting habitat, there may also be a disproportionate number of females in the creek after the nesting season.

There are several assumptions associated with the Lincoln-Peterson model that, if not met, may cause biases in the population size estimate. The model assumes that the population is closed, meaning that no death, recruitment, or emigration/immigration occurred during the 2 sampling episodes n_1 and n_2 . It is unlikely that terrapins died or were recruited over the span of this sampling period, but movement in and out of Schooner Creek cannot be ruled out. However, none of the marked terrapins were captured at other sites, and terrapins recaptured in Schooner Creek generally did not move

far. Other studies confirm that terrapins exhibit high site fidelity, with little evidence for immigration or emigration (Hurd et al. 1979; Lovich and Gibbons 1990). These findings suggest that the closure assumption of this model can be accepted.

Program CAPTURE (White et al. 1982) concluded that there was a trap response and accounted for it in its population-size estimate by using the M_b model. If there actually was a behavioral response to traps by the terrapins (trap-avoidance), then program CAPTURE M_b is an appropriate population-size estimator. However, if unequal capture probability was due to emigration or reduced terrapin activity, the Lincoln-Peterson model is a more realistic estimator because it does not assume that marked terrapins have a lower capture probability than terrapins that were not previously captured.

There were too few recaptures to draw conclusions on terrapin movement patterns. However, it was noted that the terrapins were rarely captured in the lower 200 m of the creek, where Rountree et al. (1992) caught all of their terrapins with a weir. It is unclear why more terrapins were not captured in the lower creek, particularly males, as they were found exclusively in the upper creek (Fig. 4). Perhaps terrapins only passed through this area at certain times of day or tidal cycles (weir was set throughout ebb tides; traps set during flood tides). Disturbance, in the form of frequent boat activity or water intake by the field station, could also have influenced the terrapins' use of the lower creek. The abrupt drop in CPUE may have been due to a reduction in terrapin activity with the onset of cooler water temperatures, or perhaps a "trap-shy" behavioral response by the terrapins (Fig. 5). There was a trend for higher CPUE during sunny days than overcast days, but sunny days did not decrease in frequency over time.

Consistent long-term monitoring is necessary to detect temporal changes in the population size and structure. Regular surveys of roads and creeks, using comparable methods, should be conducted to detect population changes. It is also important to understand terrapins' habitat use after the nesting season and before the hibernation period, because environmental conditions and resources available to terrapins during this time may influence their future health and fecundity. Knowing when and how terrapins use marsh creeks could help identify critical habitat for protection.

Assuming that no gear or sampling bias occurred, the changes I detected in diamondback terrapin average body size and frequency of adult females over the 12–13 years are biologically and statistically significant. These time-related changes in population structure suggest selective mortality of adult females, a trend that terrapin populations cannot sustain, according to elasticity analyses. Because there is no crab-trap mortality in this area, road mortality of nesting females is a likely explanation for this age- and sex-specific loss, and if this is the case, conservation action will be required.

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Allometry and Sexual Dimorphism in the Snail-Eating Turtle *Malayemys macrocephala* from the Chao Phraya River Basin of Central Thailand

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ABSTRACT. – Allometric growth and sexual dimorphism of the shell is evident in *Malayemys macrocephala* from the Chao Phraya River Basin of central Thailand. Differences in allometric growth between males and females produce sexually dimorphic adults. Adult females exhibit larger sizes and have relatively wider and higher shells and longer plastra than males.

Brophy (2004) recently reviewed the systematics of the genus *Malayemys* (Testudines: Geoemydidae [Batacuridae]) and argued for the presence of two taxonomically distinct species. Analyses of head-stripe and shell characters revealed a clear pattern of geographic variation that was consistent with the topography of Southeast Asia and the poor dispersal abilities of these turtles. Turtles from the Mekong River Basin retained the name *M. subtrijuga* (Schlegel and Müller 1844), whereas those from the Chao

Phraya and Mae Klong river basins, coastal areas of southeastern Thailand, and the Malay Peninsula were assigned the name *M. macrocephala* (Gray 1859).

Malayemys macrocephala is a small geoemydid turtle reaching maximum sizes of 22 cm carapace length (Srinarumol 1995). This species has pronounced sexual dimorphism, with females exhibiting larger overall body sizes, proportionally wider carapaces, and shorter, narrower tails (Ernst and Barbour 1989; Srinarumol 1995; van Dijk and Thirakhupt, in press). Populations of *M. macrocephala* can be found in virtually all lowland areas of the Chao Phraya River Basin in central Thailand, where it is the most common turtle (van Dijk and Thirakhupt, in press).

Sexual dimorphism and allometry of the turtle shell have been studied extensively (reviews in Mosimann 1956; Berry and Shine 1980; Ernst and Lovich 1986; Gibbons and Lovich 1990). My research interest focused on geographic variation and the possibility of regional differentiation and speciation in *M. subtrijuga* (*sensu lato*). Studies of regional variation require the recognition and elimination of character variation due to factors such as sex, age, and ecology. Without such considerations, critical errors in taxonomic judgement are likely to occur.

Although *M. macrocephala* is a common turtle with high popularity in the pet trade, its biology is known only through an assortment of anecdotal reports. I discovered that despite the seeming abundance of *M. macrocephala* voucher specimens, few had precise locality data. I was able, however, to assemble a moderately large sample from the Chao Phraya River Basin. This sample permits the first published study to quantify allometry and sexual dimorphism in this species.

Methods. — I examined 97 museum specimens of *M. macrocephala* from the Chao Phraya River Basin of central Thailand. The geographic origin of each specimen was based on museum records, and the sample was divided by sex and life stage. Dial calipers (accurate to 0.1 mm) were used to take the following 29 straight-line measurements on the shell of each specimen: maximum carapace length (CL); carapace width at the level of the seam separating vertebral scutes 2 and 3 (CW); shell height at the level of the seam separating vertebral scutes 2 and 3 (SH); maximum plastron length (PL); maximum width (APLW and PPLW) and length (APLL and PPLL) of both plastral lobes; minimum bridge length (BrL); maximum width and length of vertebral scutes 1, 2, 3, and 5 (Vert1, 2, 3, 5W and L); maximum width and length of pleural scute 1 (Pleu1W and L); medial seam length of gular (GuL), humeral (HumL), pectoral (PecL), abdominal (AbdL), femoral (FemL), and anal (AnL) scutes; and maximum width of gular (GuW), humeral (HumW), femoral (FemW), and anal (AnW) scutes. One meristic character, RLatK, recorded the position (as a proportion) of the right lateral keel as it bisected pleural scute 2. Larger RLatK values corresponded to relatively greater distances

from the median keel. The condition of bilateral characters was recorded from the right side of the carapace and the left side of the plastron unless damaged.

Tail morphology was the primary characteristic used for sexual identification in this study. Sexual dimorphism of this character is pronounced in both subadults and adults, with males having much longer and thicker tails (Ernst and Barbour 1989; Srinarumol 1995; van Dijk and Thirakhupt, in press). When tail morphology was not available (shell and skeletal material; some dried specimens), information from museum records formed the basis of sexual identification. Assignment of specimens to appropriate life stages (juvenile, subadult, adult) was based primarily on Srinarumol (1995), who distinguished adults from subadults based on the complete development of testes and ovaries, and subadults from juveniles based on tail morphology.

To test for allometric variation, CL was used as the independent variable for regression analyses (least squares method) of other shell characters. Nontransformed data (mm) were utilized for all specimens that had a determinable sex (juveniles, subadults, adults), and males and females were analyzed separately. The slope and intercept of each regression equation were tested for differences from zero using Student *t*-tests. Intercepts that were significantly different from zero ($\alpha=0.05$) indicated differential growth (i.e., allometry) of the characters involved (Mosimann 1958; Stickel and Bunck 1989).

Sexual dimorphism of shell characters was examined using the regression analyses detailed above. The regression slopes of each bivariate relationship were compared for males and females using analysis of covariance (ANCOVA), with CL as covariate and sex as factor. Significantly different slopes ($\alpha=0.05$) indicated sexual dimorphism in the characters regressed against CL (Mosimann and Bider 1960; Mouton et al. 2000). In addition, sexual differences in CL were tested using Student *t*-test and expressed by the sexual dimorphism index (SDI) proposed by Gibbons and Lovich (1990) and modified by Lovich and Gibbons (1992). Kolmogorov-Smirnov and *F*-tests were used to verify normality and homogeneity of variances, respectively.

Sexual dimorphism of shell characters was also examined using multivariate techniques. Twenty-eight mensural shell characters were divided by CL, and the resulting ratios comprised most of the data set. RLatK was not divided by CL because it was standardized upon measurement (expressed as a proportion). Using all 29 shell variables, stepwise selection (PROC STEPDISC; SAS, 1989; significance level for entry and removal=0.30) was used to obtain a set of potential models that would classify turtles relative to their predetermined sex. Final selection of the best model was based on model size and classification accuracy. The best model gave the most accurate cross-validation results (PROC DISCRIM; SAS 1989) and had no more variables than the number of individuals in the smallest sample. This protocol was designed to select conservative models that had a low

number of variables and a high level of classification accuracy. Using the best model as defined above, the probability of correctly classifying each turtle relative to its predetermined sex was calculated using the cross-validation results of linear discriminant function analysis (PROC DISCRIM; SAS, 1989). To minimize the effects of allometric variation, only adult and larger subadult turtles of each sex (males ≥ 80 mm CL; females ≥ 100 mm CL) were compared.

Results and Discussion. — A frequency distribution of CL (Fig. 1) indicated that females were larger than males. Adult females averaged 148.60 ± 20.23 (mean ± 1 SD) mm CL (114.4–187.0 mm; $n=21$), whereas adult males were considerably smaller and averaged 117.21 ± 9.55 mm CL (100.3–130.7 mm; $n=15$). This difference in mean CL was statistically significant ($t=5.6$, $df=34$, $p < 0.0001$). Subadult females and males averaged 94.64 ± 9.56 (85.3–113.2 mm; $n=11$) and 85.74 ± 7.68 mm CL (69.7–95.4 mm; $n=24$), respectively. Juvenile females and juveniles of indeterminate sex averaged 75.75 ± 4.63 (68.1–83.4 mm; $n=18$) and 57.33 ± 9.33 mm CL (42.7–67.9 mm; $n=8$), respectively. Juvenile males could not be distinguished because all individuals < 68 mm CL lacked sexual dimorphism of tail morphology.

Srinarumol (1995) reported that adult females and males from his study area averaged 155.48 ± 27.91 mm CL (116.5–220.0 mm; $n=25$) and 112.20 ± 9.83 mm CL (100.8–133.0 mm; $n=14$), respectively. Srinarumol

(1995) also distinguished between subadults and juveniles and found that males could be identified at $CL \geq 80$ mm and females at $CL \geq 86$ mm.

Allometric growth of the shell was evident (Table 1). Among males, shell shape changed as CL increased proportionally more than shell width (CW, APLW, PPLW), shell height (SH), plastral length (PL and APLL), several scute widths (Pleu1W, Vert1W, Vert2W, Vert3W, HumW, FemW, and AnW), and a few scute lengths (Vert1L, BL, and AnL). For females, shell shape did not change as much because CL did not increase proportionally more than shell width or shell height but did increase proportionally more than plastral length (PL and PPLL) and a few scute widths (Vert1W, Vert3W, FemW, AnW) and lengths (BrL, AbdL, AnL).

Allometry of shell characters is a widespread phenomenon among turtles. Srinarumol (1995) performed regression analyses similar to those presented here, but he did not test for differential growth of shell characters. The allometric pattern that emerges for *M. macrocephala* is one where males grow proportionally longer than wider or higher, whereas females show proportional growth for most characters. This allometry yields adult males with relatively narrower, flatter shells and adult females with relatively wider and higher shells. It is critical to emphasize the interrelatedness of allometric growth and sexual dimorphism. The differences in allometric growth between male and female *M. macrocephala* produce the sexually dimorphic adults. Such a connection has been

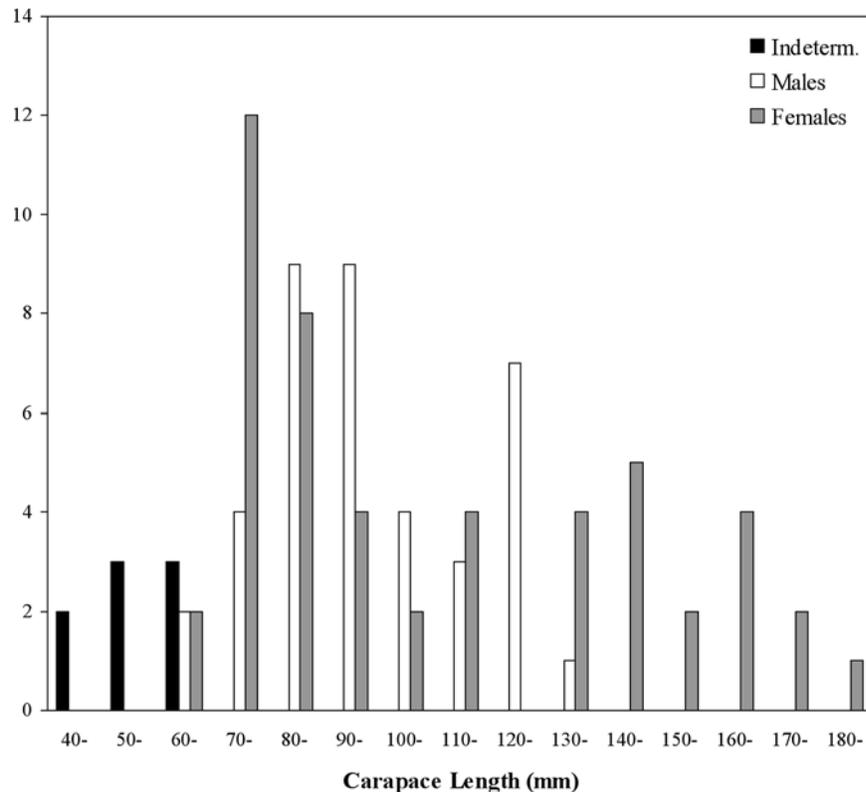


Figure 1. Frequency distribution of carapace length for *Malayemys macrocephala* from the Chao Phraya River Basin of central Thailand.

Table 1. Allometric relationships of shell characters to carapace length for *Malayemys macrocephala* from the Chao Phraya River Basin.

Character	Sex	n	Linear relation: y = a + bx (in mm)	R ²	Significance levels (p); intercept (a) (H ₀ : a = 0)
CW	F	48	CW = 2.43 + 0.75CL	0.98	ns
	M	38	CW = 14.77 + 0.58CL	0.94	<0.0001
SH	F	42	SH = 2.04 + 0.41CL	0.97	ns
	M	35	SH = 10.30 + 0.29CL	0.94	<0.0001
PL	F	43	PL = -4.43 + 0.92CL	0.99	0.0005
	M	36	PL = 4.89 + 0.80CL	0.99	0.0358
APLW	F	43	APLW = 0.02 + 0.45CL	0.99	ns
	M	36	APLW = 5.37 + 0.38CL	0.95	0.0015
APLL	F	43	APLL = -0.11 + 0.34CL	0.97	ns
	M	36	APLL = 3.97 + 0.29CL	0.92	0.0304
PPLW	F	43	PPLW = -0.67 + 0.45CL	0.98	ns
	M	36	PPLW = 7.21 + 0.35CL	0.94	0.0006
PPLL	F	43	PPLL = -6.71 + 0.61CL	0.99	<0.0001
	M	36	PPLL = 0.54 + 0.52CL	0.98	ns

^a All slopes are significantly different from zero ($p < 0.0001$). For significance levels, ns = $p > 0.05$. CW, carapace width; SH, shell height; PL, plastron length; APLW and PPLW, maximum plastral lobe widths; and APLL and PPLL, maximum plastral lobe lengths.

demonstrated by other authors working with a variety of turtle species (Mosimann 1956, 1958; Mosimann and Bider 1960; Stickel and Bunck 1989; Ernst et al. 1998).

Sexual dimorphism of the shell was also evident. ANCOVA indicated that the regression slopes of males and females differed significantly ($p < 0.05$) in 22 of the 28 characters examined (Table 2). Among these, differences in relative shell width, shell height, and plastral length were most significant ($p < 0.0001$). Females had relatively wider carapaces (CW, Vert1W, Vert2W, Vert3W), higher shells (SH), and wider (PPLW, FemW, AnW) and longer (PL, PPLL, BL, AnL) plastra (Fig. 2). Srinarumol (1995), using a similar method, found females to have relatively wider carapaces, longer plastra, and longer midline gular and pectoral lengths. The SDI for *M. macrocephala* from this study was calculated as +0.27. This is comparable to the SDI of +0.39 derived from Srinarumol's (1995) data.

One character of particular interest was anal scute length (AnL). The present data showed that males had relatively shorter AnL than females (Table 2). Van Dijk and Thirakhupt (in press) stated that males are distinguished from females by the shape of their anal notches. Males have deeper, V-shaped notches whereas females have shallower, round ones. A deeper anal notch would correspond to a shorter AnL. The V-shaped anal notch and relatively shorter AnL allow for a longer precloacal distance in males (Mosimann and Bider 1960). This is significant because the precloacal region of the tail accommodates the male's penis (Mosimann and Bider 1960).

Sexual dimorphism of the shell was also demonstrated by multivariate techniques. The best model to classify turtles according to predetermined sex contained 6 of the original 29 shell character ratios. These were AnL/CL, PPLL/CL, RLatK, Vert3W/CL, FemL/CL, and PecL/CL. Mean values for these 6 shell character ratios are presented in Table 3. Using the 6-variable model, cross-validation

Table 2. Comparison of regression slopes (ANCOVA) of shell characters vs. carapace length among male and female *Malayemys macrocephala* from the Chao Phraya River Basin.^a

Characters	Male vs. female slopes (b) (H ₀ : b _{males} = b _{females})		
	F	df	p
CW	26.26	1, 82	<0.0001
SH	24.33	1, 73	<0.0001
Pleu1W	12.38	1, 82	0.0007
Pleu1L	5.61	1, 82	0.0202
Vert1W	21.44	1, 81	<0.0001
Vert1L	5.95	1, 80	0.0169
Vert2W	32.40	1, 78	<0.0001
Vert2L	6.21	1, 79	0.0148
Vert3W	30.40	1, 81	<0.0001
Vert3L	3.58	1, 78	ns
Vert5W	0.19	1, 79	ns
Vert5L	8.02	1, 76	0.0059
PL	22.17	1, 75	<0.0001
APLW	14.16	1, 75	0.0003
APLL	6.87	1, 75	0.0106
PPLW	20.02	1, 75	<0.0001
PPLL	22.94	1, 75	<0.0001
BrL	51.65	1, 74	<0.0001
GulW	0.00	1, 76	ns
GulL	2.40	1, 76	ns
HumW	5.10	1, 76	0.0269
HumL	0.68	1, 76	ns
PecL	5.19	1, 75	0.0255
AbdL	9.65	1, 75	0.0027
FemW	21.56	1, 76	<0.0001
FemL	0.03	1, 76	ns
AnW	18.63	1, 76	<0.0001
AnL	32.57	1, 76	<0.0001

^a For significance levels, ns = $p > 0.05$. CW, carapace width; SH, shell height; Pleu1W, maximum pleural scute 1 width; Pleu1L, maximum pleural scute 1 length; Vert1W, Vert2W, Vert3W, and Vert5W, width of vertebral scutes 1, 2, 3, and 5, respectively; PL, plastron length; APLW and PPLW, plastral lobe widths; APLL and PPLL, plastral lobe lengths; BrL, bride length; GulW, HumW, FemW, and AnW, width of gular, humeral, femoral, and anal scutes, respectively; and GulL, HumL, PecL, AbdL, FemL, and AnL, seam length of gular, humeral, pectoral, abdominal, femoral, and anal scutes, respectively.

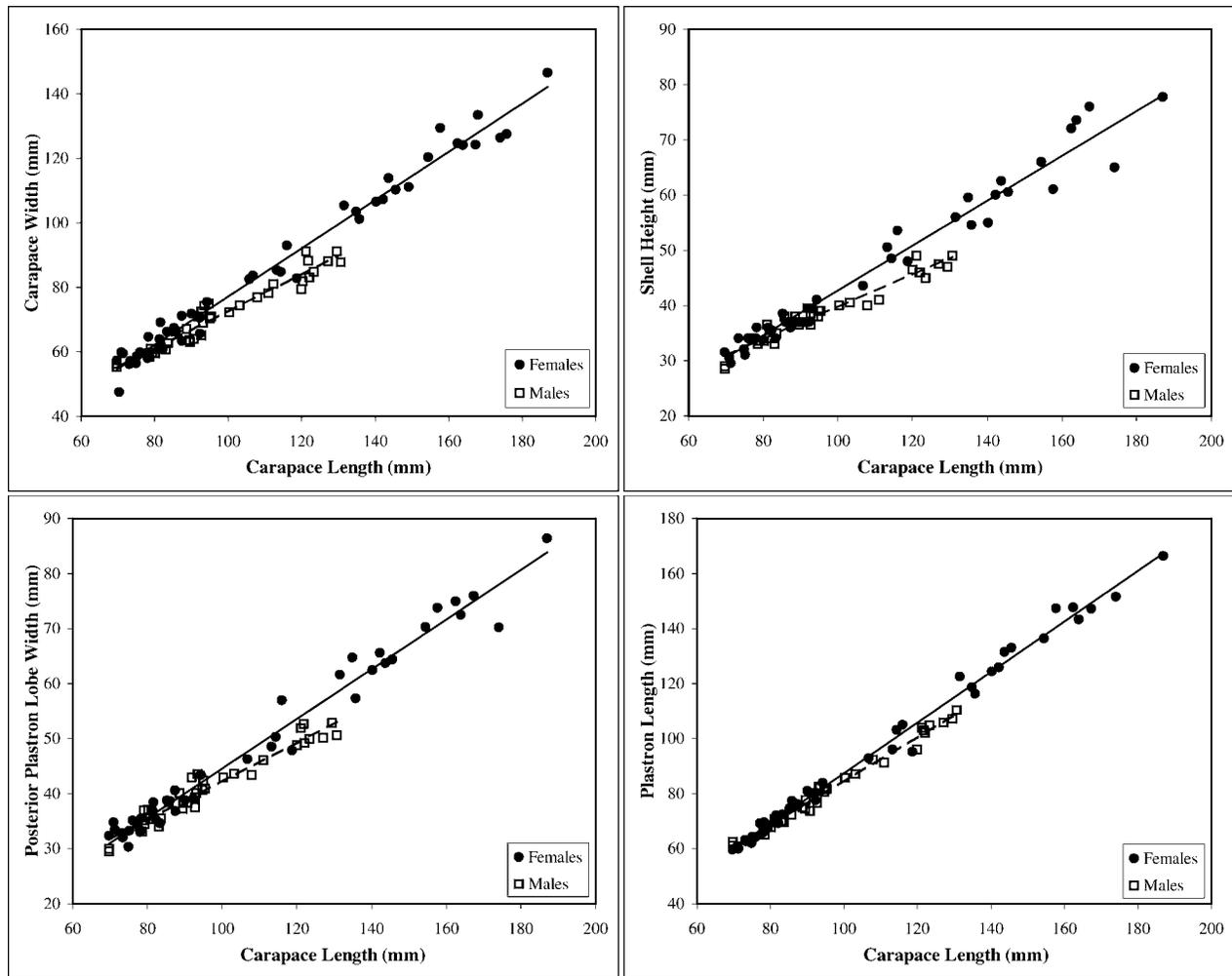


Figure 2. Allometry of carapace width, shell height, posterior plastron lobe width, and plastron length plotted as a function of carapace length and sex for *Malayemys macrocephala* from the Chao Phraya River Basin of central Thailand.

results of linear discriminant function analysis correctly classified 93.1% of males and 89.5% of females (Table 4).

Based on the preceding analyses, a clear pattern of sexual dimorphism emerges for *M. macrocephala*. Females attain larger sizes (Fig. 1) and have relatively wider and higher shells (carapace and plastron) and longer plastra than males (Fig. 2; Tables 2–4).

According to Gibbons and Lovich (1990), sexual size dimorphism (SSD) may be the result of a trade-off between the benefits of early maturity (increased matings leading to increased reproductive output) and the negative environmental consequences of small body size (increased risk of predation, desiccation, and thermal stress). Small body size may be favored in male *M. macrocephala* because the benefits of early maturity outweigh the risks of small body size.

Both Berry and Shine (1980) and Gibbons and Lovich (1990) recognized the importance of fecundity as a factor influencing body size in female turtles. Darwin's "fecundity advantage" hypothesis states that natural selection should favor large body size in females because this would

allow them to produce more offspring. For turtles in general, larger female size generally results in more or larger eggs (Gibbons et al. 1982). Such a relationship has also been suggested for *M. macrocephala* specifically (van Dijk and Thirakhupt, in press). Although fecundity selection could induce an increase in overall female size, it should primarily act on the relative size of the abdominal cavity (Mouton et al. 2000). This helps to explain the many relatively wider, higher, and longer shell characters exhibited by female *M. macrocephala*.

Some authors (review in Gibbons and Lovich 1990) have suggested that SSD is a result of ecological forces or natural selection. The most frequently cited ecological cause is probably competitive displacement (Brown and Wilson 1956; Dunham et al. 1979). In this model, the sexes evolve to exploit different resources in the environment, thereby reducing competition between them. Large females of *M. macrocephala* consume freshwater mussels, whereas males and other small individuals feed almost exclusively on aquatic snails (Srinarumol 1995; van Dijk and Thirakhupt, in press). The weakness of the displace-

Table 3. Shell character ratios—mean \pm 1 SE, (range), and [n]—used in discriminant function analysis to classify male and female *Malayemys macrocephala* from the Chao Phraya River Basin.

Character	Females	Males
AnL/CL	0.14 \pm 0.002 (0.12–0.16) [19]	0.12 \pm 0.002 (0.08–0.13) [30]
PPLL/CL	0.56 \pm 0.006 (0.49–0.60) [19]	0.52 \pm 0.003 (0.50–0.55) [30]
RLatK	0.22 \pm 0.007 (0.13–0.25) [23]	0.24 \pm 0.003 (0.20–0.25) [32]
Vert3W/CL	0.22 \pm 0.003 (0.19–0.24) [23]	0.20 \pm 0.002 (0.17–0.23) [31]
FemL/CL	0.14 \pm 0.003 (0.12–0.17) [19]	0.15 \pm 0.003 (0.12–0.18) [30]
PecL/CL	0.12 \pm 0.003 (0.09–0.15) [19]	0.10 \pm 0.003 (0.07–0.16) [30]

^a AnL, anal scute length; CL, carapace length; PPLL; plastral lobe length; RLatK, right lateral keel (as it bisects pleural scute 2); Vert3W, width of vertebral scute 3; FemL, seam length of femoral scute; and PecL, seam length of pectoral scute.

ment model in explaining this situation is that it cannot predict which sex should be larger (Gibbons and Lovich 1990). Rather than ecological factors being the cause of SSD in *M. macrocephala*, it may be that ecological differences between the sexes are simply consequences of sexually selected dimorphism (Shine 1986).

Malayemys macrocephala has SDI values ranging from +0.27 to +0.39 (Srinarumol 1995 and current study). SDI values for turtles range from –0.45 to +1.75 (Gibbons and Lovich 1990). When compared to other species that have females as the larger sex (mean SDI = +0.36; median SDI = +0.23), *M. macrocephala* displays average SDI values (Gibbons and Lovich 1990). In summary, the SSD pattern exhibited by *M. macrocephala* may be the result of a combination of selective pressures. Selection for increased fecundity may produce larger females (Berry and Shine 1980; Gibbons and Lovich 1990), whereas selection for early maturity may result in smaller males (Gibbons and Lovich 1990).

Table 4. Cross-validation results for male and female *Malayemys macrocephala* from the Chao Phraya River Basin based on linear discriminant function analysis of shell character ratios (percentages in parentheses).

Actual group	Group classification		
	Males	Females	Total
Males	27 (93.1)	2 (6.9)	29
Females	2 (10.5)	17 (89.5)	19
Total	29	19	48

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Reproductive Trends in Captive *Heosemys grandis* (Geomydidae)

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ABSTRACT. – A 20-year record of captive breeding of a female *Heosemys grandis* revealed a tradeoff between egg size and clutch size across the years when she produced 2 clutches per breeding season. First clutches had few large eggs and second clutches had a large number of smaller eggs. Four F1 progeny from this founder female began their reproductive years with much smaller eggs; however, their eggs increased in size over successive years until they were the same size as those of the long-term breeder.

Variation in egg size can be viewed as an adaptive form of bet-hedging (Kaplan and Cooper 1984). In this context, variation in egg size from a given female may be predictable within a short term, such as a year, but is at random in the long term. For instance, the growth of ovarian follicles to become eggs may occur during weather that is good or bad for ovarian growth, but the resultant hatchlings may face unrelated bad or good conditions for their type (e.g., large or small) because the weather has changed (Kaplan and Cooper 1984). Greater attention regarding turtles has focused on an energetic or space-limited reproductive tradeoff between egg size and clutch size (Elgar and Heaphy 1989). In natural populations, demonstration of a significant inverse correlation nearly always has required a statistical adjustment for female body size (Rowe 1994; Tucker et al. 1998), and even then, that correlation has not always been supported (Nieuwolt-Decaney 1997; Clark et al. 2001). Further, the statistical adjustment may confound interactions between female body size, age-related changes in the female reproductive system (Congdon et al. 2003), and a reversible, perhaps random tradeoff within fully mature females. Only one study has documented a significant egg size-clutch size tradeoff without adjustment for female body size and this tradeoff represented just one seasonal sample among three seasons (Roosenburg and Dunham 1997).

The world population of the Asian turtle *Heosemys grandis* (greater orange-headed earth turtle) is being

depleted through international trade. It is ranked by the IUCN as Vulnerable (www.iucn.org) and by CITES as Appendix II listing (www.cites.org). Rather little information is available on the natural history of the species in the wild. A female collected and held briefly captive in western Thailand (from 19 November to January) evidently nested because the holding pen yielded juveniles and eggshells by July (van Dijk 1998). Some adults presumably taken from the wild for international trade and “rescued” subsequently oviposited in captivity during the winter (Hudson and Buhlmann 2002). The species has temperature-dependent sex determination (Ewert et al. 1994).

The main focus of the present account concerns the 20-year record of reproduction of 1 captive founder female *Heosemys grandis* (“Founder or Mother”), 4 of her F1 progeny (daughters), and 1 sire for all (see Goode 1997, for a partial summary account). These data allowed us to examine the tradeoff between egg size and clutch size, age-related changes in reproduction, and some temporal aspects of reproduction particular to *H. grandis*, all in an artificially stable environment in captivity at the Columbus Zoo (CZ). Other accounts of reproduction in captivity include Foust (1989) and Rudolphi and Weser (2000).

Methods. — The parental pair and female young, upon reaching adulthood, were housed at CZ in a display area with 3.7×2.2 m of floor space. This space was divided between two-thirds land and one-third water (to 30 cm deep) parts. The land part included a sandpit (ca. 1 m^2 , > 25 cm deep) for nesting. Air temperature remained close to 27°C and water temperature was usually $23^\circ\text{--}25^\circ\text{C}$. The photo-period was set at 12 light:12 dark from mid-fall to mid spring and 15 L:9 D from late spring to early fall. Food was regularly available in excess of that consumed.

During the nesting season, female turtles were palpated to detect eggs, and in most cases left to nest on the display. On a few occasions, oviposition was induced with oxytocin. Although rare in occurrence, eggs laid after a main clutch could be associated with a given clutch because they were fully shelled, apparently overmature, and laid within 3–4 weeks of the main clutch, too soon for egg maturation from subsequent ovulation. Some of the eggs were incubated in containers of damp vermiculite (ca. -100 to -300 kPa) in warm or cooler parts of the room space ($26^\circ\text{--}30^\circ\text{C}$). Other eggs were incubated at closely controlled temperatures (25° , 28° , 30°C) in upright incubators at Indiana University.

Statistical applications included simple ANOVAs, linear correlation and regression (with residuals analyses where stated to adjust for female body mass), and Spearman’s rho or Wilcoxon’s signed rank tests in most cases involving binomial data. Most measurements include \pm standard deviation (SD).

RESULTS AND DISCUSSION

Founder. — The Founder Female (Mother) was an adult when obtained on 27 July 1967 and died on 2 March 1998.

Her mass averaged 5160 g (range 5075–5225 g, independent of age). Her carapace length (CL) remained at 295 mm throughout the study. The male (sire) measured 345 mm CL soon after arriving at CZ on 2 June 1977 and gradually grew to 368 mm (7060 g) by 1992.

During 20 years of observation, Founder produced 2 clutches seasonally during 15 years, 1 clutch seasonally during 2 years and did not reproduce during at least two of three other seasons (Fig. 1). Across all seasons, Founder’s clutch production occurred between 5 November (1986) and 11 March (1992). During two-clutch seasons, the first clutch was laid between 5 November and 11 January (range 67 days for 15 clutches, mean = 10 December). Second clutches were laid 21 December–11 March (range 81 days for 15 clutches, mean = 1 February). The average interval between clutches was 55 ± 9 days ($n=15$, range 46–79 days). The first and second oviposition dates were significantly correlated across years ($r = +0.91$, $p < 0.0001$). During seasons with a single clutch, Founder laid these on 2 and 3 December, i.e., ca. 1 week ahead of the average first clutch.

During the two-clutch seasons, the first clutch averaged significantly smaller than the second clutch by one egg (Fig. 1; first = 4.87 ± 1.06 eggs vs. second = 5.87 ± 1.457 eggs; $F_{1,14} = 9.54$, $p = 0.008$; $z = -2.51$, $p = 0.012$). Founder produced the largest clutches (9 and 10 eggs) during each of the two seasons when she produced just one clutch.

During the two-clutch seasons, average egg mass was significantly larger in the first clutch than in the second clutch (Fig. 2; first = 52.22 ± 2.75 g vs. second = $48.23 \pm$

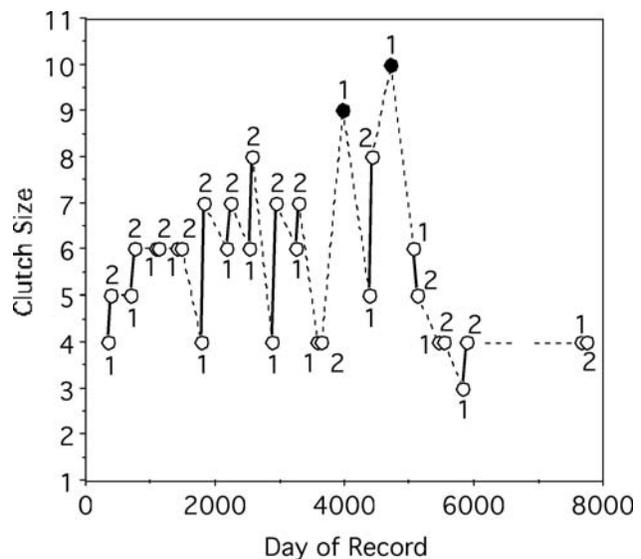


Figure 1. *Heosemys grandis*, seasonal records of clutch size during the life span of one individual, Founder Female (Mother). Open circles labeled 1 and 2 and connected with a solid line represent first and second clutches, respectively, within a given season. Solid circles labeled 1 represent seasons with a single clutch. The record follows continuously from 1 November 1977. Reproduction occurred every year into 1993, but did not occur during the subsequent three seasons.

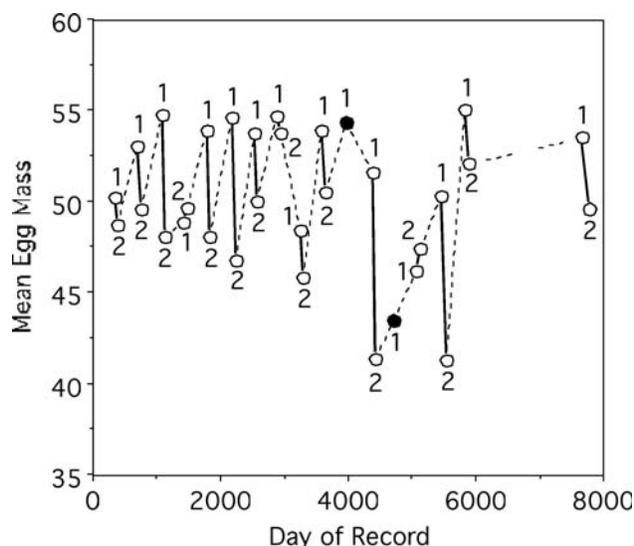


Figure 2. *Heosemys grandis*, seasonal records of clutch mean egg size during the life span of one individual, Founder Female (Mother). Symbols and other formats are as in Fig. 1.

3.42 g; $F_{1,14} = 21.1$, $p = 0.0004$). Mean egg size in the one-clutch seasons was not consistent relative to egg size in two-clutch seasons (Fig. 2).

The trend in egg size required estimating of egg mass for some clutches because data on eggs from early in the study (11 clutches) included only linear measurements. We used the apparent density of eggs with measured masses and linear measurements of later eggs (19 clutches) to estimate the masses of the early eggs. The mean egg mass of 87 weighed eggs was 48.8 ± 5.4 g (range 36.1–59.0). As estimated from the formula for a prolate ellipsoid, the densities of these eggs averaged 1.174 (range 0.994–1.395). The mean estimated mass of 75 unweighed eggs was 50.69 ± 3.5 g. The densities of 3 eggs directly measured through water displacement was 1.169–1.199 (mean = 1.180). Given the close correspondence between measured and estimated egg densities, the average egg shape of *H. grandis* (i.e., Founder) seems to conform closely to a prolate ellipsoid (see Iverson and Ewert 1991).

During Founder's two-clutch seasons, the fresh clutch mass of the first clutch (with 1 less egg on average) averaged slightly lighter than for the second clutch (260.1 g vs. 270.8 g, respectively; $F_{1,12} = 5.31$, $p < 0.04$), which relative to Founder's average mass (5160 g) gave relative clutch masses (RCM) of 0.050 and 0.052, respectively, and a seasonal RCM of 0.102. In the 2 years with single clutches, RCM was 0.095 and 0.085.

Mean clutch egg mass was inversely associated with clutch size across all of Founder's clutches ($r = -0.36$, $p = 0.043$; Spearman's $\rho = -0.375$, $p = 0.04$, $n = 32$). If one clutch (with just 1 unbroken egg for weighing) is removed the association is stronger ($r = -0.49$, $p = 0.0054$, Spearman's $\rho = -0.45$, $p = 0.014$, $n = 31$).

F1 Progeny. — Six of Founder's progeny were retained at CZ until they had matured and produced at

least one clutch. Four of these were retained for several additional years. The age at producing the first (primiparous) clutch in 5 of the 6 was 7.6–7.7 years; 1 was younger, 5.7 years. The size at the primiparous clutch was quite uniform (4496 ± 110 g, range = 4325–4650 g; 283 ± 6 mm CL, range 278–291 mm).

The oviposition dates of the progeny clutches (23 November–8 March) occurred within the range of Founder's dates. Three of the 4 retained progeny produced 1 clutch per season during their first 2–4 years of breeding; the fourth progeny started with 2 clutches. Each of these 4 progeny eventually had 2 two-clutch seasons. The mean interval between these clutches averaged 48 ± 10 days ($n = 8$, range 36–67 days), briefer than Founder's interval between clutches (55 days). Collectively, progeny first and second oviposition dates were significantly associated across years ($r = +0.85$, $p < 0.008$).

At CZ, the 7 females produced a total of 61 clutches during a 127-day period between early November and mid-March. At the Turtle Back Zoo (New Jersey), one female produced 7 clutches within the same period (Foust 1989). Thus, nesting in captivity was strongly seasonal, despite the artificial conditions. The range in Julian days includes that of one natural nesting in Thailand (van Dijk 1998) and represents the dry season in western central Thailand (Wernstedt 1959; van Dijk 1998), but includes the last 1–2 months of the wet season in the more southerly Malaysian range of *H. grandis*. Cues for reproduction at CZ may have been artificial reduction of the photoperiod, naturally drier winter air and accelerated evaporative cooling of the water in the enclosure.

During one-clutch seasons, the progeny produced the 4 largest clutches (1 with 11 eggs and 3 with 10 eggs). The largest clutches produced during two-clutch seasons were 9 eggs (1 season) and 8 eggs (3 seasons). In progeny two-clutch seasons, the first clutch did not differ significantly in size from the second. In two-clutch seasons, progeny egg mass in 7 of 8 first clutches was larger than in the second clutches from the same season. However, the trend was not statistically significant.

Upon achieving maturity, progeny egg size was small in their primiparous clutches (mean egg sizes of 29.4–39.4 g) and tended to remain small in the next few clutches (Fig. 3). In 3 of the 4 retained progeny, production of eggs more nearly as heavy as Founder's eggs (typically > 41 g) occurred only after three to four seasons. Thus, during this prolonged early period of reproduction, each progeny produced 3 to 5 clutches of small eggs. Progeny eggs weighing 50 g (common for Founder) first appeared after f4 seasons and in just 2 of the 4 retained progeny. Overall, the mean mass of progeny eggs was 38.6 ± 6.5 g (range 26.0–58.3 g, $n = 158$). The range of variation in progeny egg masses was much larger than for Founder (variances of 42.88 vs. 28.16, respectively). Estimated densities of progeny eggs averaged 1.187 ± 0.052 (range 1.014–

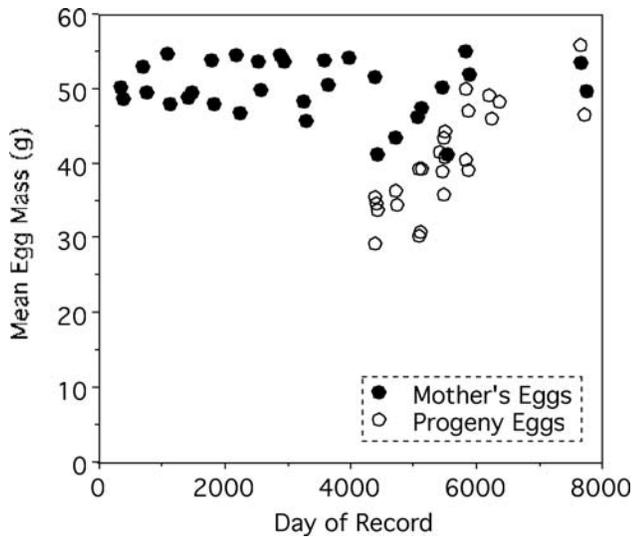


Figure 3. *Heosemys grandis*, continuous record of clutch mean egg size of Founder Female (Mother, solid circles) and F1 Progeny (open circles). The record follows continuously from 1 November 1977. The progeny hatched from eggs produced prior to day 1000 and began producing their own eggs when their own ages were 5.7 to 7.7 years.

1.288, $n=156$), which lies within the density range determined through water displacement.

Excluding the primiparous clutches from each progeny (clutches were atypically small), an inverse correlation was present in a contrast of mean clutch size with mean clutch egg mass (without adjustment for body size: Spearman's $\rho = -0.62$, $p = 0.0038$, $n = 23$). During this period, the variation across female masses was slight, deviating only up to ± 295 g from a mean of 4664 g. For the entire CZ series, the egg size-clutch size tradeoff with adjustment for female mass was strong ($r = -0.498$, $p < 0.0001$), and remained compelling without adjusting for female mass (Spearman's $\rho = -0.438$, $p = 0.0004$, $n = 60$ clutches). The association prevailed because the progeny produced relatively large clutches of small eggs (6.1 eggs; 41.6 g, respectively, $n = 29$ clutches) and Founder averaged smaller clutches of larger eggs (5.6 eggs; 50.5 g, respectively, $n = 32$ clutches).

Progeny RCMs averaged quite small for each of their primiparous clutches (mean 0.039, range 0.020–0.055, $n = 6$). Over the next two seasons, progeny seasonal RCMs increased to equal those of Founder (Fig. 4). Excluding primiparous clutches, progeny RCMs across one-clutch seasons averaged larger than RCMs of either clutch taken singly across two-clutch seasons (0.068 ± 0.011 , range 0.052–0.083, $n = 7$; vs. 0.054 ± 0.014 , range 0.032–0.071, $n = 14$), but below statistical significance (Mann-Whitney U, $p = 0.08$). Progeny seasonal RCMs in two-clutch seasons averaged 0.113 ± 0.019 , range 0.083–0.129). The RCM's of *H. grandis* are well within the range given for small samples of geoemydid turtles (Moll and Moll 1990) and for large southeastern USA emydid (Jackson 1988).

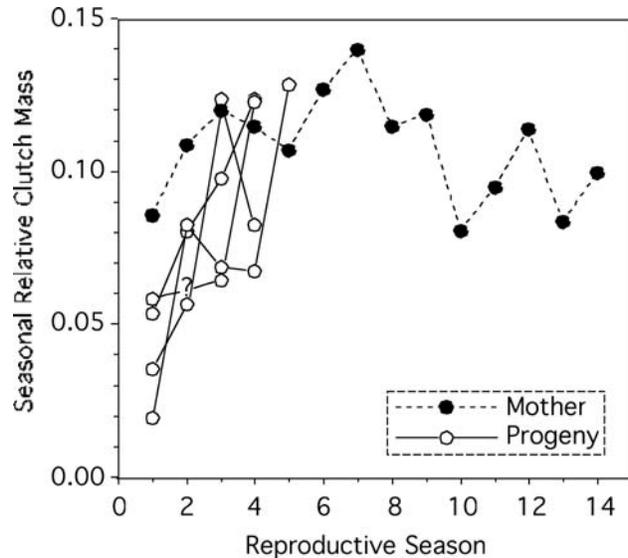


Figure 4. *Heosemys grandis*, continuous record of clutch mean egg size in Founder Female (Mother, solid circles) and F1 Progeny (open circles). The record commences with the first season in which an individual turtle produced a clutch and is continuous except for one season noted with a question mark. Note: Founder was >15 years old at her first recorded oviposition; the F1 progeny were <8 years old at their first recorded oviposition.

An Additional Female. — Another female (mass, 3599 g; 282 mm CL) maintained at IU, independently from the CZ females, produced only one clutch per season (2 eggs, 25 March 1976; 3 eggs, 7 August 1990; 3 eggs, 2 October 1991; 3 eggs, 30 January 1993). The first 2 clutches, while seemingly laid out-of-season, were also problematic (infertile or over-mature eggs). Egg size increased from 33.2–33.9 g in the first clutch to 52.9–61.0 g in the last 3 clutches. With this female and the CZ females included, egg mass across all females ranged from 26.0 g (47×30 mm, from a progeny) to 61.0 g (69×37 mm, from this non-CZ female).

Hatchlings. — Hatchlings were produced following incubation at 3 constant temperatures. The embryos developed directly (no post-ovipositional developmental arrest) and took the following times to pip: 25°C, 142.0 ± 11.3 days (range 133–158 days, $n = 4$); 28°C, 102.3 ± 4.5 days (range 94–106 days, $n = 6$); 30°C, 89.4 ± 4.6 days (range 85–98 days, $n = 6$). The live masses of 33 hatchlings (with internalized yolk sacs) from eggs of known weight averaged 31.5 ± 5.4 g (range 20.4–40.9 g) and were strongly isometric ($r = 0.92$) as a proportion (65.6%) of the fresh masses of their eggs. These hatchlings measured 52.7 ± 3.9 mm CL (range 45.2–60.2 mm) and 48.1 ± 3.6 mm PL (range 41–55 mm).

Across all females, the difference in egg size was 2.34-fold (2.27-fold for just Founder and her progeny; range 26–59 g). The range in masses of eggs that realized hatchlings was 28.2–61.0 g (2.2-fold). These ratios support the general observation that viable eggs within turtle populations tend to range 2- to 3-fold in mass (Ewert 1979).

Conclusions. — In considering variation in size as an adaptive feature of eggs, such as in bet-hedging (Kaplan and Cooper 1984), the egg mass variance for Founder and her progeny was 63.1 (cv = 18.0, $n = 339$ eggs). This value included a variance of 21.8 for Founder alone (cv = 9.4, $n = 169$ eggs), and 41.1 for the progeny alone (cv = 16.6, $n = 170$ eggs). Thus, although there was ample variation in egg size overall, the F1 progeny were responsible for most of it (Fig. 3). As shown above, much of the variation was nonrandom. The egg size-clutch size tradeoff was significant for just Founder, just her progeny, and overall. This tradeoff may be compatible with bet-hedging. However, the strong within-season trend for producing a few large eggs initially and several smaller eggs later (Fig. 2) contributed to this tradeoff, and may have little to do with bet-hedging. It is likely that this within-season decline in egg mass was a physiological consequence of the annual yolking of ovarian follicles for both clutches. The large change in progeny egg mass was age-related (Fig. 3) and thus non-random. This age-related increase may result from decreasing investment in somatic growth relative to investment in reproduction (Congdon et al. 2003), as expressed in seasonal RCM (Fig. 4). Residual variation in egg mass that remained unexplained and potentially attributable to bet-hedging could include the large decline in mean egg mass that occurred early in the second half of Founder's reproductive record (Fig. 2).

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