

# Spongivory in Hawksbill Turtles: A Diet of Glass

ANNE MEYLAN\*

The hawksbill (*Eretmochelys imbricata*), an endangered marine turtle associated with coral reefs throughout the tropics, feeds almost exclusively on sponges in the Caribbean, and possibly throughout its range. It is one of fewer than a dozen vertebrates that are known to specialize on this widely distributed but well-defended food resource. The diet is taxonomically narrow and highly uniform geographically, includes sponges that are toxic to other vertebrates, and contains more silica than that of other vertebrates. By affecting space competition, spongivory by hawksbills may influence succession and diversity of reef communities.

S PONGES ARE AN IMPORTANT COMPONENT of hard-substrate marine communities. On Caribbean coral reefs, they frequently rival, or even surpass, reef-building corals in terms of biomass contribution (1). They are aggressive competitors for space, particularly in deep, open-water habitats and in cryptic environments such as the undersurfaces of foliaceous corals (2). With rare exception (3), biotic factors that regulate sponge populations remain poorly known. Predation is considered a minimal influence, and is presumed to be limited by mechanical defenses, such as siliceous spicules and tough organic fibers, and by chemical defenses, for which the phylum Porifera is noted (4). The only vertebrates known to feed exclusively or nearly exclusively on sponges (spongivores) are a small number of highly evolved teleost fishes (5).

Results of a Caribbean-wide study of the feeding habits and ecology of the hawksbill turtle (*Eretmochelys imbricata*) suggest that this species is also a dedicated spongivore (6). Sponges contributed 95.3% of the total dry mass of all food items in digestive tract samples from 61 animals from seven Caribbean countries (7). Sponges were identified from all but one turtle, and were the predominant food item in 51.

When the sample was limited to 34 turtles of known size for which complete digestive tract contents were recovered, a high (72.3 to 99.9) percentage contribution of sponges was observed for turtles of all sizes with the exception of one 23-cm juvenile and three gravid females. Twenty-three- to 25-cm carapace length appears to be the size at which hawksbills end a pelagic life history phase (with epipelagic feeding habits) and begin benthic feeding in coastal habitats (8). The predominant organic food item in seven of nine gravid females was also sponges, but these animals also contained substantial amounts of calcareous substrate material

that appears to have been ingested purposefully. When gravid females were excluded from the sample, the average percentage dry weight contribution of sponges in the samples was 94.2 (SD = 12.0,  $n = 28$ ), with no statistically significant differences between samples of males and females (9).

Sponges accounted for a high percentage of the digestive tract samples throughout the Caribbean. No statistically significant differences were observed in average contribution of sponges in the three major regions included in the study: Panama, the Dominican Republic, and the Lesser Antilles (10).

The high percentage of sponges in digestive tract samples and the high degree of homogeneity in turtles of different sexes, sizes, and geographic origins suggest that the hawksbill is a sponge specialist. In a previous study of the feeding habits of *Eretmochelys*, both sponges and tunicates were found to be important in the diet (11). Use of frequency of occurrence as the only quantitative measure, however, led the authors to conclude that the hawksbill is a "relatively indiscriminant feeder whose food consists mainly of benthic invertebrates." The study was conducted at a single locality, primarily during the nesting season, but this description of the hawksbill has been used worldwide to characterize the feeding habits of the species (12). Evidence presented here refutes that diagnosis. Sponges have been recorded in the diet of two other marine turtles (*Chelonia mydas* and *Caretta caretta*) and in four freshwater turtles, but with the exception of *Graptemys nigrinoda delticola*, they appear to be of little importance (13). *Eretmochelys imbricata* is the only known sponge specialist among the Reptilia.

My study focused on the Caribbean, but there is evidence to suggest that spongivory is a worldwide habit for *Eretmochelys*. Many of the reports are anecdotal, but samples in which sponges were the predominant or exclusive food item have been recorded in Oman, the Seychelles, Hawaii, and the Bahamas (14). I also found sponges to be the predominant food item in two samples from South Africa, one from Pacific Panama, one

from Veracruz, Mexico, and one from Texas.

Samples from Caribbean hawksbills were taxonomically narrow with respect to sponge composition: 98.9% of the dry mass of all identified sponge could be assigned to 3 of the 13 orders of demosponges (Astrophorida, Spirophorida, and Hadromerida) (15). The ten most highly ranked prey species (Table 1) accounted for 79.1% of the dry mass of all identified sponges (16). Several hundred species occur within the geographic range and habitats encompassed by the samples. The degree to which this narrow taxonomic pattern is upheld worldwide is not known, but preliminary information indicates that many of the same genera of sponges are eaten in other regions.

Members of the phylum Porifera produce a diverse array of secondary compounds (alkaloids, terpenes, and brominated compounds) that are known to have noxious or toxic properties (17). Several sponge species consumed by *Eretmochelys* that have been shown to be toxic to fish include *Chondrilla nucula*, *Tethya actinia*, and *Spheciospongia vesparium* (18). Extracts of *Suberites domuncula* (*Suberites* sp. was the fifth most highly ranked species in the study) cause hemorrhaging and death in a wide variety of lab animals including turtles (species not identified) (19). Suberitine, a neurotoxic protein isolated from this sponge exhibits strong adenosinetriphosphatase and hemolytic properties (20). *Aaptos aaptos* (*Aaptos* sp. was the ninth-ranked sponge) possesses adrenoreceptor-blocking activity (21). The possible link between these potentially toxic sponge metabolites and the sporadic mass fatalities of humans caused by consumption of hawksbill meat (22) deserves further study.

**Table 1.** The ten highest ranked prey sponges of hawksbill turtles (*Eretmochelys imbricata*). Ranks are based on product of average percentage dry mass contribution in stomach contents and frequency of occurrence ( $n = 54$  turtles).

| Sponge                           | Rank |
|----------------------------------|------|
| Order Astrophorida               |      |
| Family Geodiidae                 |      |
| <i>Geodia</i> sp.                | 3    |
| Family Stellettidae              |      |
| <i>Ancorina</i> sp.              | 2    |
| <i>Ecionemia</i> sp.             | 7    |
| <i>Myriastrra</i> sp.            | 6    |
| Incertae sedis                   |      |
| Family Chondrosiidae             |      |
| <i>Chondrosia</i> sp.            | 8    |
| <i>Chondrilla nucula</i>         | 1    |
| Order Hadromerida                |      |
| Family Tethyidae                 |      |
| <i>Tethya</i> cf. <i>actinia</i> | 10   |
| <i>Aaptos</i> sp.                | 9    |
| Family Suberitidae               |      |
| <i>Suberites</i> sp.             | 5    |
| Family Placospongiidae           |      |
| <i>Placospongia</i> sp.          | 4    |

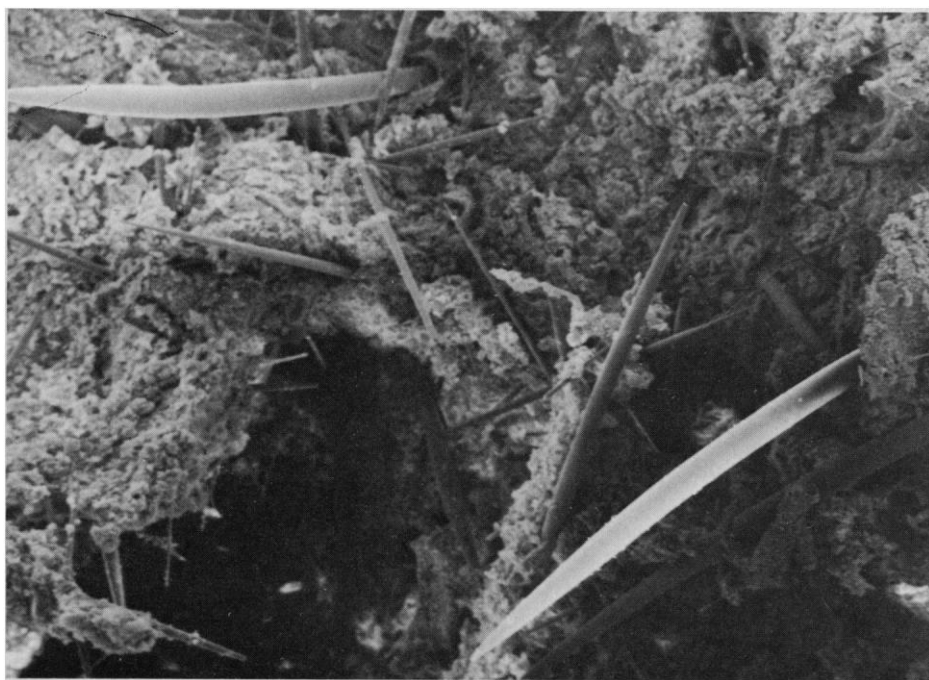
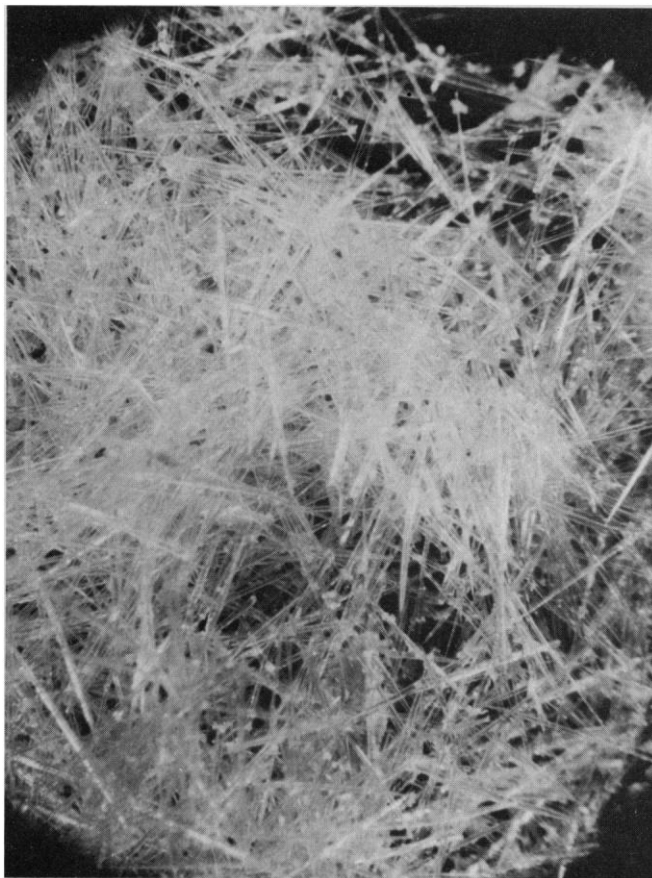
Department of Herpetology and Ichthyology, American Museum of Natural History, New York, NY 10024.

\*Present address: Bureau of Marine Research, Department of Natural Resources, St. Petersburg, FL 33701-5095.

Prey sponges such as *Geodia*, *Ancorina*, *Ecionemia*, and *Placospongia* are among the most highly silicified demosponges; siliceous spicules constitute up to 67.1% of the dry mass of *Geodia neptuni* (23). The silica in

spicules is present in an amorphous, hydrated form that is similar to opal, a type of glass. Intestinal contents of hawksbills typically consisted of masses of dissociated spicules (as long as 5 mm) (Fig. 1 and cover).

**Fig. 1.** Dried intestinal contents from a hawksbill turtle. Glass-like needles are siliceous sponge spicules. Some spicules are up to 5 mm long.



**Fig. 2.** Scanning electron micrograph of intestinal epithelium of a hawksbill turtle, showing siliceous sponge spicules (magnification,  $\times 75$ ).

Three randomly selected samples of intestinal contents consisted of 92.0, 76.6, and 74.3% ash (dry mass basis), virtually all of which was silica. Assuming that digestive tract contents average 50% silica on a dry mass basis, an actively feeding adult turtle could contain over 500 g of silica in the digestive tract.

No vertebrate diet comparable in silica content has been described, although diets of exclusively spongivorous fish can be predicted to be similar. Silica is a prominent structural component in a few groups of algae, protozoans, and plants, but in few, if any, of these groups does the silica content approach that of the highly siliceous sponges in the hawksbill's diet. Scouring rushes and rice, which are considered to be the most heavily silicified plants, contain only 20% silica by dry mass (24).

Dissociated siliceous spicules were found embedded in the intestinal epithelia of hawksbills (Fig. 2). No morphological adaptations were identified that might facilitate the handling of spicules. In other spongivores, spicule-compacting organs, modifications of masticatory structures, and copious mucus production are thought to serve this purpose.

Predation by a large (up to 127 kg), mobile predator such as the hawksbill can be expected to have significant ecological consequences for prey sponges, some of which are known to be slow-growing, long-lived species (25). From a community perspective, the removal by hawksbills of large amounts of sponge from the reef and the consequent opening up of free space could be expected to influence reef succession and diversity. Current population levels of hawksbills are low throughout the range of the species, but repeated foraging by individuals within their limited home ranges may result in localized impact. By virtue of their powerful jaws, hawksbills may also facilitate sponge feeding by fish, thus augmenting their own impact. Divers report that both spongivorous and nonspongivorous fish feed voraciously on soft choanosome and endosymbionts exposed by foraging hawksbills.

The highly specific diet of the hawksbill and the dependence of this turtle on filter-feeding, hard-bottom communities make it vulnerable to deteriorating conditions on the world's reefs. Populations throughout the circumtropical range of the species are already highly endangered by international trade in tortoiseshell.

#### REFERENCES AND NOTES

1. K. Ruetzler, in *Coral Reefs: Research Methods*, D. Stoddart and R. Johannes, Eds. (Unesco, Paris, 1978), pp. 299–313.

2. J. B. C. Jackson and L. Buss, *Proc. Natl. Acad. Sci. U.S.A.* **72**, 5160 (1975); T. Suchanek, R. Carpenter, J. Witman, D. Harvell, *NOAA Symp. Ser. Undersea Res.* **3**, 9 (1985).
3. P. Dayton *et al.*, *Ecol. Monogr.* **44**, 105 (1974).
4. J. Randall and W. Hartman, *Mar. Biol.* **1**, 216 (1968); M. Sara and J. Vacelet, in *Traité de Zoologie*, P.-P. Grasse, Ed. (Masson, Paris, 1973), vol. 3, pp. 463–576; G. J. Bakus, *Science* **211**, 497 (1981).
5. J. Randall and W. Hartman, *Mar. Biol.* **1**, 216 (1968); E. Hobsen, *Fish. Bull.* **72**, 915 (1974); M. Sano, M. Shimizu, Y. Nose, *Bull. Univ. Mus. Univ. Tokyo* **25** (1984).
6. A. Meylan, thesis, University of Florida, Gainesville, FL (1984); in *Biology of Invertebrate and Lower Vertebrate Collagens*, A. Bairati and R. Garrone, Eds. (Plenum, New York, 1985), pp. 191–196.
7. Sample consisted of turtles (23.0- to 87.7-cm straight carapace length) captured by fishermen with nets or harpoons, or on nesting beaches at 19 localities in the Lesser Antilles, the Dominican Republic, and Caribbean Panama. Collection dates included all months except December, January, and August. Complete digestive tract contents (total 12.4 kg dry mass) were available for 37 hawksbills, partial samples for 24.
8. A. Meylan, unpublished data.
9. Females:  $\bar{x}$  = 95.2%, SD = 7.5, range 78.3 to 99.2,  $n$  = 7; males:  $\bar{x}$  = 96.4%, SD = 7.7, range 72.3 to 99.9,  $n$  = 12, Mann-Whitney  $U$  test,  $P$  = 0.2067.
10. Panama:  $\bar{x}$  = 96.3%, SD = 8.0, range 72.3 to 99.9,  $n$  = 11; Dominican Republic:  $\bar{x}$  = 95.8%, SD = 2.2, range 93.4 to 97.9,  $n$  = 4; Lesser Antilles:  $\bar{x}$  = 96.2%, SD = 5.8, range 78.3 to 99.6,  $n$  = 12; Kruskal-Wallis test,  $P$  = 0.1089. Sample includes nongravid turtles greater than 25-cm carapace length with complete digestive tract contents.
11. A. Carr and S. Stancyk, *Biol. Conserv.* **8**, 161 (1975).
12. W. Witzell, *FAO Fish. Synop.* **137** (1983); K. Bjørndal, *Copeia* **1985**, 736 (1985).
13. P. Lahanas, thesis, Auburn University, Auburn, AL (1982).
14. See references in Meylan, thesis (6).
15. Identifications were based on microscopic examination of siliceous spicules of sponges in the stomach only. An average of 90.1% (SD = 15.62, dry mass basis) of the sponge in individual samples was identifiable.
16. Rank was calculated as the average percentage dry mass contribution times the percentage of occurrence.
17. D. Faulkner, *Natl. Prod. Rep.* **1**, 551 (1984).
18. G. Green, *Mar. Biol.* **40**, 207 (1977); G. Bakus and M. Thun, *Colloq. Int. C.N.R.S.* **291**, 417 (1979).
19. C. Richet, *C. R. Soc. Biol.* **61**, 686 (1906).
20. L. Cariello and L. Zanetti, *Comp. Biochem. Physiol.* **64C**, 15 (1979).
21. H. Nakamura *et al.*, *Tetrahedron Lett.* **23**, 5555 (1982).
22. E. Silas and B. Fernando, *Gen. Mar. Fish. Res. Inst. Bull. (India)* **35**, 62 (1984).
23. K. Ruetzler and I. Macintyre, *Mar. Biol.* **49**, 147 (1978).
24. P. Kaufman *et al.*, in *Silicon and Siliceous Structures in Biological Systems*, T. Simpson and B. Volcani, Eds. (Springer-Verlag, New York, 1981), pp. 409–449.
25. P. Bergquist, *Sponges* (Univ. of California Press, Berkeley, CA, 1978).
26. A. Carr helped me realize the value of feeding studies in understanding the natural history of sea turtles. I thank K. Ruetzler for assistance with sponge identifications. Funding was provided by World Wildlife Fund/International, Caribbean Conservation Corporation, and the National Marine Fisheries Service. I thank K. Bjørndal, S. Pomponi, P. Meylan, and J. Winston for editorial comments.

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## Hormone Conjugated with Antibody to CD3 Mediates Cytotoxic T Cell Lysis of Human Melanoma Cells

MARGARET ANN LIU, SAMUEL R. NUSSBAUM, HERMAN N. EISEN

Cytotoxic T lymphocytes can be activated by antibodies to their antigen-specific receptor complex (TCR-CD3) to destroy target cells, regardless of the specificity of the cytotoxic T cells. A novel hormone-antibody conjugate, consisting of an analog of melanocyte-stimulating hormone chemically coupled to a monoclonal antibody to CD3, the invariant component of the T cell receptor complex, was used to target human melanoma cells for destruction by human cytotoxic T lymphocytes that bear no specificity for the tumor cells. As targeting components of such anti-CD3 conjugates, hormones or growth factors are expected to prove more effective than antibodies to tumor-associated antigens in focusing the destructive activity of cytotoxic T cells on tumor target cells.

IT IS WIDELY RECOGNIZED THAT THE specificity of antibodies for cell-surface ligands and of hormones for receptors on cells offers possibilities for delivering therapeutic agents to selected target cells. Thus, chemotherapeutic drugs and toxins have been attached to antibodies that are specific for tumor-associated antigens [reviewed in (1)], and hormones have been used as vectors for directing chemotherapeutic agents to tumor cells (2). In alternative approaches, antibody conjugates, referred to as heterobifunctional antibody duplexes, have been used to target tumor cells for destruction by killer T cells (3). In the

duplexes, one antibody (the "targeting" antibody) is specific for a tumor antigen and the other (the "effector" antibody) binds specifically to the antigen receptor complexes on T cells, activating their effector functions regardless of the antigens they normally recognize. Activated cytotoxic T lymphocytes (CTLs) lyse the cells to which they are adherent, and the antibody duplexes can thus induce CTLs to kill tumor cells for which they bear no specificity. However, tumor cell variants that lack tumor-associated antigens are often found (4). In contrast, the growth of many tumors is dependent on their cell-surface receptors for hormones or autocrine growth factors (5). Hence, hormones or autocrine growth factors might offer advantages over antibodies to tumor-associated antigens as targeting reagents.

We describe a novel conjugate in which a hormone, substituting for the targeting antibody, is covalently joined to an antibody to the CD3 component of the T cell receptor (TCR)-CD3 complex. The hormone,

termed MSHa, is an analog of  $\alpha$ -melanocyte-stimulating hormone (MSH); it is specific for the MSH receptor on melanoma cells (6). We show that, in the presence of this conjugate, CTLs destroy melanoma cells. The conjugate may be regarded as a useful model for exploring the more general use of growth factor-antibody conjugates to mediate destruction of tumor cells and other factor-dependent cells by cytotoxic cells.

The MSHa-anti-CD3 conjugate (7) mediated specific lysis of the human melanoma line B16F10 (8) by the human CD8<sup>+</sup> cloned CTL cell line 6B7 (9) (Fig. 1). That these CTLs were not specific for the melanoma cells they killed was evident from their failure to lyse these cells in the absence of the conjugate. Individual components of the conjugates—that is, the free MSHa alone (6) and anti-CD3 alone (10)—did not mediate CTL lysis of the melanoma cells. Indeed, each of them, and also antibodies to MSH (11), blocked the effect of the conjugate (Fig. 1B) in a dose-dependent manner (Fig. 2, A, B, and C). These results indicate that conjugate-mediated lysis of the melanoma cells depends on the binding of the conjugate's anti-CD3 moiety to the CTL and the binding of its MSHa moiety to the MSH receptor on the B16F10 cells; unconjugated anti-CD3 blocked the former interaction and unconjugated MSHa and anti-MSH blocked the latter interaction.

As in conventional antigen-dependent lysis of target cells by CTLs, the conjugate-mediated lysis of melanoma cells also increased as the ratio of CTLs to melanoma cells increased. Thus, at ratios of 0.5, 1, 5, 10, and 20 CTLs to one melanoma cell, the amount of specific lysis was 9, 14, 30, 34, and 42 percent, respectively, in the presence of conjugate ( $\sim 2 \times 10^{-8} M$ ). When the ra-

M. A. Liu, Center for Cancer Research, Massachusetts Institute of Technology, Cambridge, MA 02139, and Endocrine Unit, Medical Services, Massachusetts General Hospital and Harvard Medical School, Boston, MA 02114.

S. R. Nussbaum, Endocrine Unit, Medical Services, Massachusetts General Hospital and Harvard Medical School, Boston, MA 02114.

H. N. Eisen, Center for Cancer Research and Department of Biology, Massachusetts Institute of Technology, Cambridge, MA 02139.