George —
I thought this
had been sent to you
too. Sorry. Karla

DRAFT

does not include data collected after March 2003

A NUTRITIONAL ANALYSIS OF THE DIET AND FECAL PELLETS OF THE GREEN TURTLE, *CHELONIA MYDAS*, AT KALOKO-HONOKOHAU NATIONAL HISTORICAL PARK, HAWAII

Sara McCutcheon
University of Hawaii-Hilo

Mentor: Dr. Karla McDermid, Associate Professor
Department of Marine Science, University of Hawaii-Hilo

Abstract.

In 1978, the green turtle, *Chelonia mydas*, was listed as threatened in the United States under the Endangered Species Act. Any knowledge gained from an understanding of the diet and how it affects this species' ability to survive is crucial. Necropsies of turtles at Kaloko-Honokohau National Historical Park have revealed that some of the turtles have poor body condition, a problem that may be related to the nutrition of their diet. Turf algae, a primary component of the diet of *Chelonia mydas,* and turtle fecal pellets were collected from Kaloko-Honokohau National Historical Park at monthly intervals. The turf algae and fecal pellets were subjected to nutritional analyses for protein, carbohydrate, lipid, ash, and caloric content. The fecal pellets were higher in protein content than the turf algae, which may be related to fermentation carried out by bacteria in the turtle hindgut that makes the protein and other nutrients available for absorption. The turf algae had low caloric value; perhaps it is the pairing of low-energy and low-protein in the diet that has led to poor body conditions in the turtles at Kaloko-Honokohau National Historical Park.

Introduction.

The green turtle, *Chelonia mydas,* is listed as endangered worldwide by the

International Union for the Conservation of Nature and Natural Resources (National Marine Fisheries Service and U.S. Fish and Wildlife Service 1998). Green turtles were listed as threatened in 1978 under the U.S. Endangered Species Act of 1973, except for breeding populations in Florida and Mexico, where they are listed as endangered (National Marine Fisheries Service and U.S. Fish and Wildlife Service 1998). Their numbers in Hawaii have been increasing over the last 24 years, since the Act started protecting turtles from fisheries and harassment (Rillero 1999). Since the turtles are threatened, any work that could help benefit their populations in anyway is important to their survival.

Previously, the only studies on the dietary nutrition of green turtles used captive turtles fed artificial diets (Wood and Wood 1977, 1981), and turtles feeding on seagrass beds in Florida (Bjorndal 1980, 1985, Thayer et al. 1982, 1984). There have not been any studies on the nutritional composition of naturally occurring algae for sea turtles, nor has any work on the nutrition of the turtle's diet been conducted in Hawaii. The green turtle, *Chelonia mydas*, is herbivorous feeding on algae, and on seagrasses when available, but will readily eat animal matter in captivity (Mortimer 1995). Immature green turtles in the wild have very slow growth rates of only 1 cm/year straight carapace length (Zug et al. 2001), and once the turtles reach sexual maturity, their growth rate declines to 0.5 cm/year (NMFS and U.S. FWS 1998). Green turtles take anywhere from 20-50 years to reach sexual maturity in the wild (Bjorndal 1980, Limpus and Walter 1980, Mendonca 1981), whereas captive-raised green turtles can reach sexual maturity in 8-9 years (Wood and Wood 1980). Green turtles in captivity are fed high protein diets, in which protein is in an easily digestible form. Since turtles do not chew their food, the food is broken down in their cecums by gut microflora (Bjorndal 1985). This does not allow digestion of the protein until the latter half of the digestive tract; whereas, the protein in the artificial feed begins to be digested when it first enters the stomach (Bjorndal 1985). Balazs (1985) attributed differences in growth rates from different islands in the Hawaiian archipelago to dietary differences. Bjorndal (1980) concluded that growth rates of wild green turtles are determined nutritionally, not genetically.

Green turtles in Hawaii are thought to have fixed feeding areas and do not travel much, except for migrations to and from breeding grounds (Balazs NMFS, personal communication). If turtles feed in localized areas, the seaweed present in that area should be the major factor affecting their nutrition. Some green turtles studied at Kaloko-Honokohau National Historical Park (Kona Coast of Hawaii) have poor body condition although necropsies have revealed that they had sufficient amounts of food in their stomachs (Balazs NMFS, personal communication). The poor body condition of the turtles may be due to the turtles not getting enough nutrition from their diets.

Nutritional composition profiles of the turf algae, which the turtles graze, and fecal pellets at Kaloko-Honokohau National Historical Park were performed to analyze the nutritional quality of the turtles' diet. Assimilation efficiencies were going to be determined to assess how well the turtles incorporate the food they ingest. Assimilation efficiencies were not analyzed because a sufficient marker was lacking. The goal of the study is to gain an understanding of the nutritional aspects of the green turtles' diet, which will hopefully lead to a better comprehension of the turtles' biology and ecology.

Materials and Methods

The study site is Kaloko-Honokohau National Historical Park (NHP) located on the West coast of the island of Hawaii. Turf algae, a densely woven mat of algae approximately 1-3 cm tall, were collected where turtles were observed to feed at three locations within Kaloko-Honokohau NHP using a 15cm square ($0.0225m^2$) quadrat. The three zones within Kaloko-Honokohau NHP where turf algae were collected are zones lower B, upper B, and D (Figure 1). In order to determine how well the turtles are absorbing the nutrients in the seaweed they ingest, fecal pellets were collected along the entire stretch of beach at Kaloko-Honokohau NHP, and analyzed following the same methods as the seaweed analysis. Turf algae and fecal samples were collected monthly and then dried in a 60°C air oven, water content was calculated, and finally the samples were ground to a homogenous powder. The powder was used to analyze for crude lipid content, percent ash, percent protein, amino acid composition, percent soluble carbohydrate, and energy content. All analyses were performed in triplicate, except for ash determination, which requires five replicates. All results were recorded as a percent dry weight as the mean plus or minus the standard deviation.

Collection. Turf algae that the green turtle, *Chelonia mydas*, feeds on were collected from Kaloko-Honokohau NHP. The site was chosen because turtle-tagging trips to Kaloko-Honokohau NHP had revealed that many of the same turtles were being captured and recaptured in the same zones implying that the turtles have high site fidelity (Balazs NMFS, personal communication). The turtles have been observed feeding at the site on numerous occasions. Samples were usually collected at areas where a turtle was previously feeding and moved away.

Cleaning. Once the algal samples arrived at the laboratory, they were vigorously shaken to remove excess sand and silt. It was assumed that if an object or organism did not fall off after the shaking, the turtle would have ingested it. There were a few sponges, snails, worms that cemented themselves to the algae, and sand in the turf algae samples. The weight was then recorded to later determine biomass and water content.

Biomass. The biomass was determined by collecting all of the algae in a quadrat that was 15cm-length square (0.0225m^2), then the excess water was removed by blotting on a paper towel, and the wet weight was recorded in g/m^2.

Water Content. After cleaning, excess water was removed from the samples by blotting on a paper towel, and then they were dried at 60°C in air oven on aluminum foil until they reached a constant weight. The fecal pellets do not need to be blotted as they are very compact and do not retain much water; they are then weighed, dried, and then reweighed. Dried seaweed and pellets were ground to a homogenous powder following weighing and were then stored in airtight containers in a refrigerator. Water content was calculated by the difference of wet weight to dry weight of the samples, divided by the dry weight, and expressed as a percent.

Ash Determination. Percent ash was determined following methods outlined in Dawes (1998). A known amount of algae or fecal matter was weighed in a crucible, the crucible was placed in a muffle furnace at 500°C for four hours to allow all organic matter to burn away, and then percent ash was determined.

Total Protein Content. The Lowry method for protein analysis was followed (Lowry et. al. 1951). The samples were digested in 1N sodium hydroxide for 12h and then an alkaline copper citrate solution was added, followed by Folin-Ciocalteau phenol reagent. The sample was then measured colorimetrically using a Beckman Coulter DU 640 spectrophotometer at 660 nm wavelength light using Bovine Serum Albumin as a standard.

Amino Acid Analysis. Samples were sent to an independent laboratory (Texas A&M University) for amino acid analysis. Samples were examined for the all amino acids, including tryptophan.

Soluble Carbohydrate Analysis. The methods outlined in Dawes (1998) were followed in the colorimetric carbohydrate analysis. The samples were allowed to sit in 5% trichloroacetic acid overnight, were heated in an 80-90°C water bath, were allowed to react with 5% phenol, and then with concentrated sulfuric acid. The percent carbohydrate was then calculated by reading the absorbance at 490 nm wavelength light using a Beckman Coulter DU 640 spectrophotometer and compared against a glycogen standard.

Crude Lipid Content. A gravimetric analysis was performed as outlined in Dawes (1998). The crude lipid was extracted with a 2:1 (v/v) mixture of chloroform and methanol, was then purified with water, and was then evaporated under a constant stream of nitrogen gas. The weight of lipid residue was then recorded and percent lipid calculated.

Caloric Content. The algal samples were pressed into pellets that weighed between 0.1-0.2 g, and were combusted using a Parr 1425 Semimicro Calorimeter. Total energy (cal/g) was determined based on a benzoic acid standard. Total calories were recorded on an ash-free basis.

Results

Biomass. Biomass varied considerably with every monthly sample (Figure 2). Monthly variations range from 591.56 to 991.78 g/m^2 in zone lower B, from 683.11 to 1557.00 g/m^2 in zone upper B, and from 150.22 to 1038.78 g/m^2 in zone D. Overall, zone upper B had the highest biomass with a mean of 1182.71 g/m^2 and zones lower B and D had similar mean biomasses of 784.38 and 771.02 g/m^2, respectively. Biomass was not calculated for the months of September and October because sampling was done without a quadrat for those months.

Water Content. The water content ranged from 66-86% for all turf algae and fecal pellet samples (Table 1). The mean water content was higher for zone upper B (80.08%) than for zone lower B, D, and the fecal pellets (75.74%, 75.91%, and 76.25, respectively).

Ash. The percent of ash in the fecal pellets was lower than that of the turf algae for all months except for November, which is where the ash of zone D was at its lowest (Figure 3). The mean ash values for zones lower B, upper B, and D (67.29%, 67.31%, and 68.65%, respectively) were similar to one another and all were higher than the fecal pellet mean ash value (32.38%).

Protein. The percent protein in the fecal pellets was approximately twice as high as in the turf algae (Figure 4). The mean protein percent for zones lower B, upper B, and D (7.09%, 6.99%, and 4.38%, respectively) all were lower than that of the fecal pellets (12.95%). Zone D had the lowest protein content for all months, except November.

Amino Acid Analysis. The amount of amino acids necessary for normal growth and development for hatchling green turtles as determined by Wood and Wood (1977) were met by the turf algae for all amino acids, except for tryptophan (Table 2). The four amino acids that were in the greatest abundance, the four always present in the top five amino acids, were alanine, glycine, glutamate/glutamic acid, and aspartate/aspartic acid.

Soluble Carbohydrate. The percent soluble carbohydrate varied among months in the same zones and for fecal pellets (Figure 5). An average of all samples for each zone reveals that they are similar to one another (11.72% in zone lower B, 9.37% in zone upper B, and 9.77% in zone D) and to the fecal pellets (10.12%).

Crude Lipid. The turf algae have a much lower mean percent lipid (2.31% in zone lower B, 2.41% in zone upper B, and 1.95% in zone D) than the fecal pellets (14.80%). The turf algae samples range from as low as 0.35% to as high as 4.05% while the fecal pellets range from 12.50% to 17.02% (Figure 6).

Caloric Content. The amount of calories for the turf algae are rather low, ranging from 259.00 to 1501.96 calories per gram of dry sample matter (Figure 7). Zone D had higher caloric content (1501.96 cal/g) than zone lower B (259.00 to 665.70 cal/g).

Discussion

Biomass. Biomass varied between areas in the same zone and between months. This high variability may be due the heterogeneous nature of the habitat or to seasonal growth variations of different turf algae; some grow better at different months.

Water Content. The water content for the turf algae ranged from 66.40 to 86.95%, but the means for each zone were similar (zone lower B with 75.74%, zone upper B with 80.08%, and zone D with 75.91%). The fecal pellets were much less variable ranging from 71.90 to 82.04%, with a mean water content of 76.25%. The fecal pellets are compact and don't carry an excess amount of water, whereas the algae is composed of many species with varying levels of water retention.

Ash Determination. The mean ash values were higher for the turf algae (67.29% in zone lower B, 67.31% in zone upper B, and 68.65% in zone D) than they were for the fecal pellets (32.38%). It was expected that the ash in the fecal pellets would be equal to, or greater than, that of the turf algae the turtles feed on; however, this was not the case. This was expected because ash has a low digestibility, the turtles would absorb the organic nutrients and pass the ash through their system. However, some constituents of ash, mainly the carbonate portions, are hydrolyzed when they reach the high pH of vertebrate stomachs. Therefore, if there were a lot of calcareous algae in the sample, the ash would be higher in the algae than in the fecal pellets. Because some of the ash gets hydrolyzed, ash is an invalid marker for assimilation. To test how well ash worked as a marker for assimilation, assimilation values were calculated, however, it resulted in negative assimilation values. Negative assimilation should not be correct for any of the nutrients except for protein. Negative assimilation values indicate that the turtle is not absorbing any of the nutrients from its diet and is actually losing nutrients.

Protein Content. The percent of protein was higher in the fecal pellets (12.95%) of the green turtle than in the turf algae on which they feed (7.09% in lower B, 6.99% in upper B, and 4.38% in D), which may be what is decreasing the ash percent in the fecal pellets. The increase in protein observed here is not surprising. Bjorndal (1985) referred to green turtles having microbial fauna in their hindgut, which functions as a cecum. Since the green turtle does not chew its food, this functional cecum breaks down the plant matter through microbial fermentation. The breakdown that occurs in the hindgut is responsible for freeing the nutrients from the algal cells and making the nutrients available for absorption. The microbial organisms may be responsible for providing the turtle with the essential amino acids it needs for survival (Bjorndal 1985). These organisms may also be responsible for the increase of protein in the fecal pellets. In captivity, Wood and Wood (1977, 1981) fed their turtles are diets containing 25-36% crude protein. The low protein content of the turf algae at Kaloko-Honokohau, the primary food source of the green turtle, may be a large contributing factor as to why some of the turtles have poor body conditions.

Amino Acids. Four of the top five amino acids by percent for each sample are alanine, glycine, glutamate/glutamic acid, and aspartate/aspartic acid (Table 2). Wood and Wood (1977) performed a study to determine the amount of essential amino acids for hatchling green turtles necessary for normal growth and development and all of those requirements were met in the turf algae sampled, except that the turf appears to be lacking in tryptophan. Wood and Wood (1977) also found that with phenylalanine concentrations greater than 3%, the turtles started to experience decreased growth rates. Five of the six turf algae samples analyzed had phenylalanine concentrations in excess of 3%. This high level of phenylalanine may be a factor in the poor body conditions of the turtles at Kaloko-Honokohau NHP. However, green turtles undergo a dietary shift from hatchlings to immature turtles; while in the hatchling stage of their life, green turtles are pelagic and are more carnivorous (Wood and Wood 1981).

Soluble Carbohydrate. The percent soluble carbohydrate in the turf algae of all three zones was lowest during the months of December, January, and February (Figure 5). The decrease may be due to strong wave impacts and although the amount of soluble carbohydrates decreased, it's possible that the amount of insoluble, structural carbohydrates increased. Another possibility is that a shift in the species present occurred and the more wave tolerant species were persisting, however, this has yet to be determined.

Crude Lipid. The amount of lipids found in the turf algae is low, ranging from 0.35% to 4.05%. The turf algae were expected to be low in lipid content, as plants and algae tend to be low in fat. The fecal pellets, however, had higher amounts of lipids than the turf algae, with values ranging from 12.50% to 17.02%. The fecal pellet may be so much higher than the algae because some of the ash was hydrolyzed and did not show up in the fecal pellets at full concentrations, which would make all of the other nutrients appear to be larger than they really are. An alternative hypothesis is that when the bacteria performing the hindgut microbial fermentation die and are excreted, they are higher in lipid content than the turf algae.

Caloric Content. The calories were especially difficult to analyze because of an abnormally high rate of incomplete burns. The calories are especially low for seaweeds, other authors have found caloric values of single species of algae, that turtles are known to eat (Russell and Balazs 2000), ranging between 1670 to 4135 cal/g (Kennish and Williams 1997, Naidu et al 1992, Montgomery and Gerking 1980). These studies typically examine species of algae individually, not as a mixture, and their seaweeds were probably cleaner than the seaweed studied here. Perhaps sand or a chemical from a sponge, worm, or snail is retarding the ability of the seaweed to fully combust.

The necropsies of dead turtles at Kaloko-Honokohau NHP have revealed sufficient quantities of food in their stomachs but had a rather emaciated appearance.

There are typically a lot of turtles (14-96; Lisa Marrack, personal communication) inhabiting the beach. The turtles mainly stay in the lower B zone, and can only forage at the upper B zone during high tides because the area is high intertidal and exposed to air for a large part of the time. The low protein content of the food might be hindering their genetic potential of much higher growth rate and be another cause of the poor body conditions of the turtles at Kaloko-Honokohau NHP. In addition, the diet has phenylalanine percents in excess of 3% of the total amino acids, which may adversely affect growth. Based upon the energetic value of the turf algae, it appears that the turtles are faced with very low-energy foods. This may be why the turtles at Kaloko-Honokohau NHP are not as robust as they should be. The turtles must continually graze to meet their dietary needs, and, at times of high turtle density, the food may become limited.

Acknowledgements

I am grateful to the following people for their helpful suggestions and advice: Karla McDermid, Geroge Balazs, Karen Bjorndal, Sallie Beavers, and Lisa Marrack. The work at Kaloko-Honokohau NHP was funded by NMFS Turtle Program.

Literature Cited

AOAC. 1990. Official Methods of Analysis. Nutrient Components. 1105-1106.

Balazs, G.H. 1980. Synopsis of biological data on the green turtle in the Hawaiian Islands. NOAA technical memorandum NMFS.

Balazs, G.H. Growth rates of immature green turtles in the Hawaiian Archipelago, in

K.A. Bjorndal. 1995. Biology and Conservation of sea turtles. Smithsonian Institution Press; Washington and London.

Bjorndal, K.A. Foraging ecology and nutrition of sea turtles, in Lutz, P.L. and Musick, J.A. 1996. The biology of sea turtles. CRC Press; Florida.

Bjorndal, K.A. 1985. Nutritional ecology of sea turtles. Copeia 3: 736-751.

Bjorndal, K.A. 1980. Nutrition and grazing behavior of the green turtle *Chelonia mydas.* Marine Biology 56: 147-154.

Bjorndal, K.A., Bolten, A.B., and Chaloupka, M.Y. 2000. Green turtle somatic growth model: evidence for density dependence. Ecological Applications 10:269-282.

Chaloupka, M.Y. and Musick, J.A. Age, growth, and population dynamics, in Lutz, P.L. and Musick, J.A. 1996. The biology of sea turtles. CRC Press; Florida.

Kennish, R. and Williams, G.A. 1997. Feeding preferences of the herbivorous crab *Grapsus albolineatus*: the differential influence of algal nutrient content and morphology. Mar Ecol Prog Ser 147: 87-95.

Lowry O.H., Rosebrough N.J., Farr A.L., and Randall R.J. 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193: 265-275.

Montgomery W.L. and Gerking S.D. 1980. Marine macroalgae as food for fishes: an evaluation of potential food quality. Env. Biol. Fish. 5: 143-153.

Mortimer, J.A. Feeding ecology of sea turtles, in K.A. Bjorndal. 1995. Biology and Conservation of sea turtles. Smithsonian Institution Press; Washington and London.

Naidu, K.A., Tewari, A., Joshi, H.V., Viswanath, S., Ramesh, H.P., and Rao, S. V. 1992. Evaluation of nutritional quality and food safety of seaweeds of India. Journal of Food Safety 13: 77-90.

National Marine Fisheries Service and U.S. Fish and Wildlife Service. 1998. Recovery plan for U.S. Pacific populations of the green turtle (*Chelonia mydas*). National Marine Fisheries Service, Silver Spring, MD. 83pp.

Qasim R. 1991. Amino acid composition of some common seaweeds. Pak. J. Pharm. Sci. 449-454.

Russell, D.J. and Balazs, G.H. 2000. NOAA Technical Memorandum NMFS. Identification manual for the dietary vegetateion of the Hawaiian green turtle, *Chelonia mydas*. 49pp.

Russell, D.J. and Balazs, G.H. 1994. Colonization by the alien marine alga *Hypnea musciformis* (Wulfen) J. Ag. (Rhodophyta: Gigartinales) in the Hawaiian Islands and its utilization by the green turtle, *Chelonia mydas* L. Aquatic Botany 47: 53-60.

Thayer, G.W. and Engel, D.W. 1982. Evidence for short-circuiting of the detritus cycle of seagrass beds by the green turtle, *Chelonia mydas* L.

Thayer, G.W., K.A. Bjorndal, J.C. Ogden, S.L. Williams, and J.C. Zieman. 1984. Role of larger herbivores in seagrass communities. *Estuaries* 7: 351-376.

Wahbeh M.I. 1997. Amino acid and fatty acid profiles of four species of macroalgae from Aqaba and their suitability for use in fish diets. Aquaculture 159:101-109.

Wood, F.E. and Wood, J.R. 1977. Quantitative requirement of the hatchling green sea turtle, *Chelonia mydas*, for valine, leucine, isoleucine, and phenylalanine. J. Nutr. 107: 1502-1506.

Wood, J.R. and Wood, F.E. 1981. Growth and digestibility for the green turtle (*Chelonia mydas*) fed diets containing varying protein levels. Aquaculture 25: 269-274.

Zug, G.R., Balazs, G.H., Wetherall, J.A., Parker, D.M., and Murakawa, S.K.K. 2001.

Age and growth of Hawaiian green seaturtles (*Chelonia mydas*): an analysis based on skeletochronology. Fish. Bull. 100:117-127.

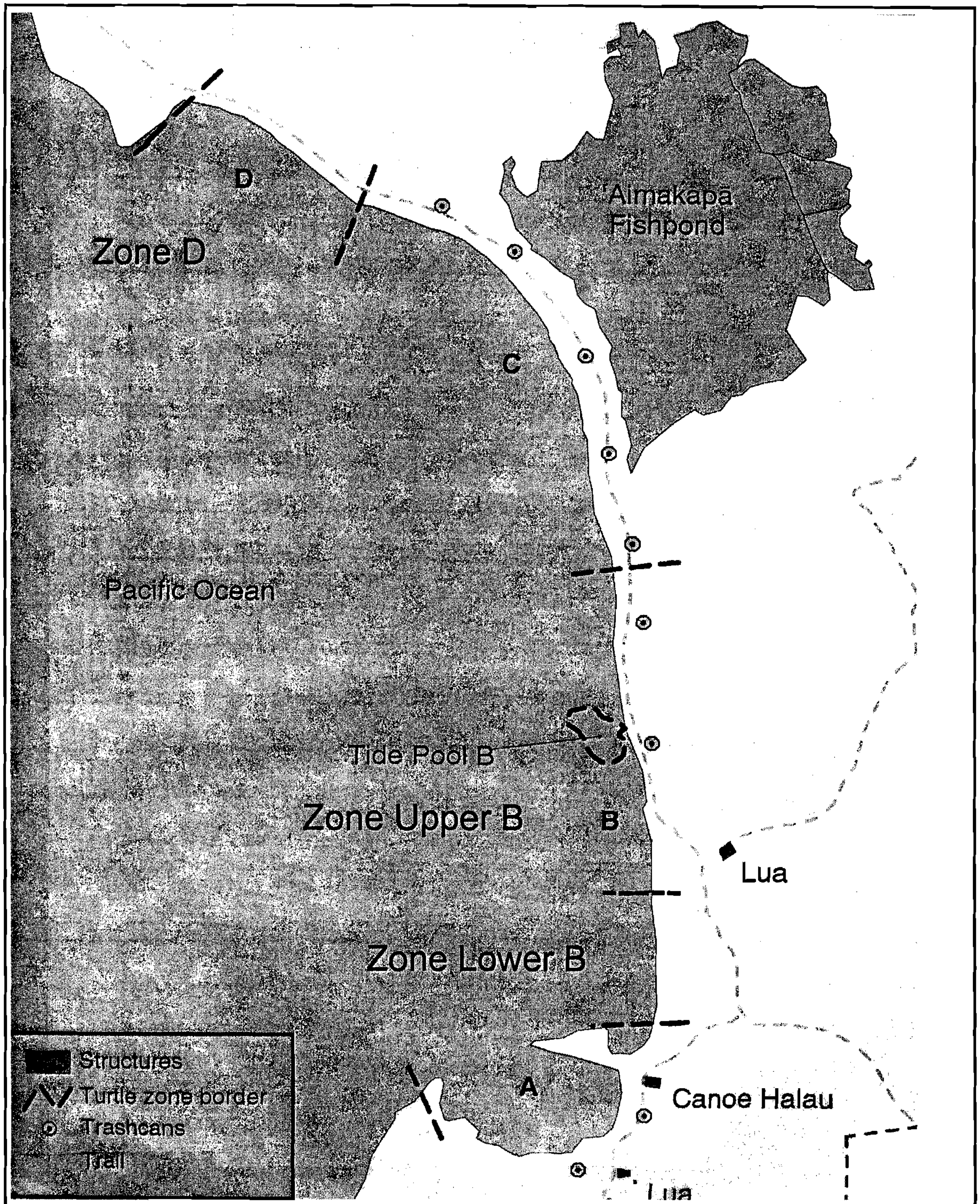

Fig. 1 The study site, Kaloko-Honokohau National Historical Park, is located on the west coast of Hawai'i. Turf algae were collected in zones Upper B, Lower B, and D. Fecal pellets were collected along the shore at high tide.

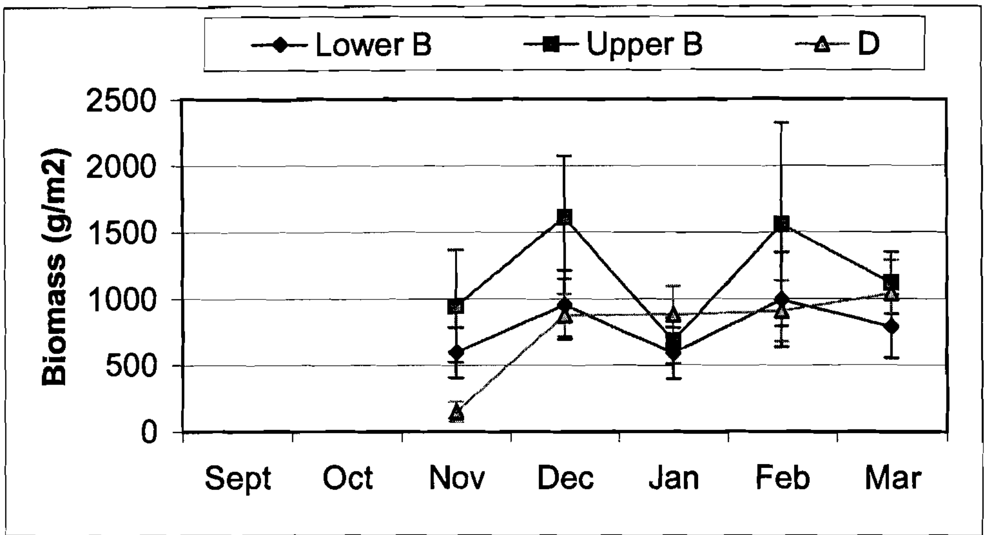

Figure 2. Biomass of the turf algae sampled at monthly intervals from Kaloko-Honokohau National Historical Park, Hawaii. Values are expressed as means and standard deviations.

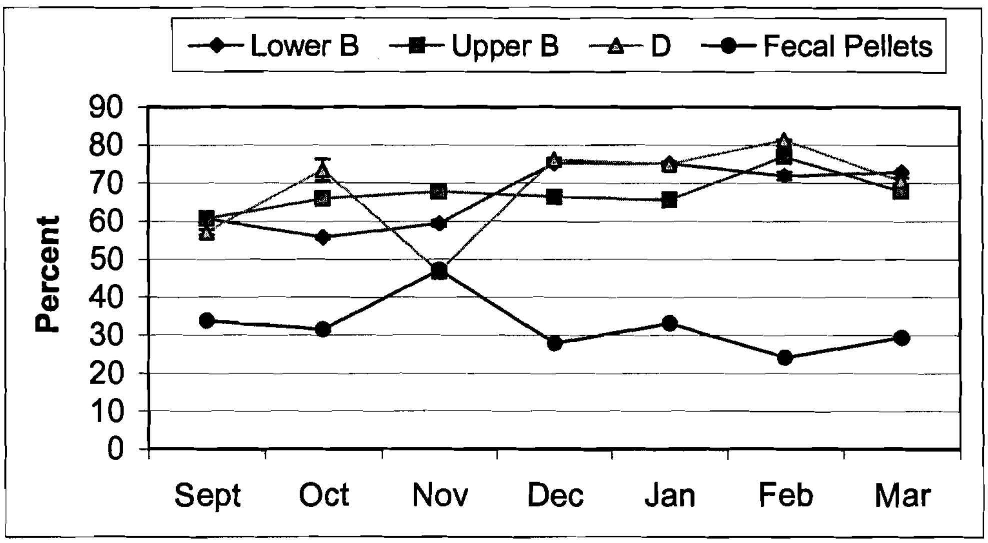

Figure 3. Monthly variation of the percent ash on a dry-weight basis for the turf algae sampled in zones D, upper and lower B, and turtle fecal pellets collected at Kaloko-Honokohau National Historical Park, Hawaii. Values are expressed as means and standard deviations.

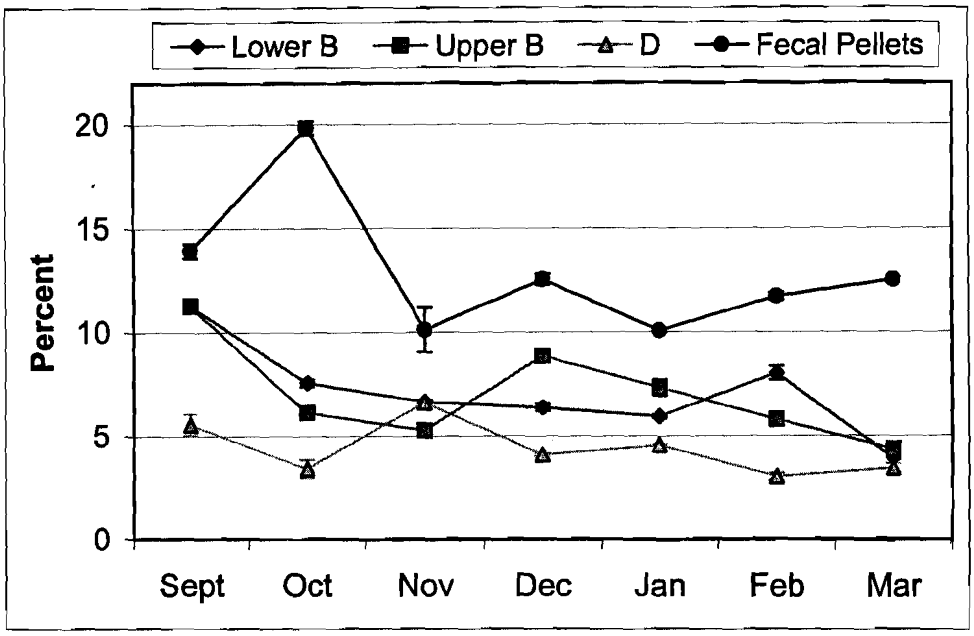

Figure 4. Percent total protein, on a dry-weight basis, in the fecal pellets of the green turtle and turf algae collected monthly from Kaloko-Honokohau National Historical Park from three zones, zones lower B, upper B, and D. Values are expressed as means and standard deviations.

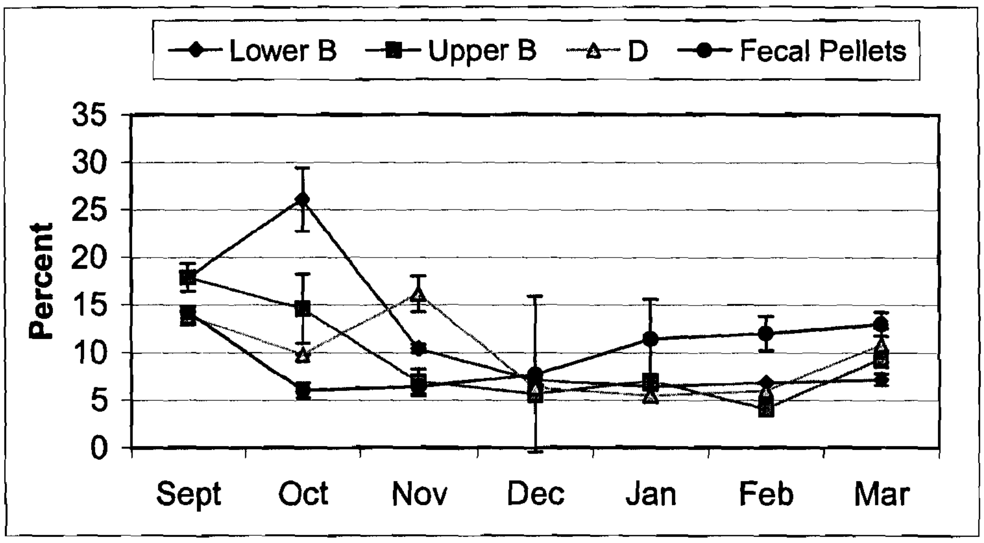

Figure 5. Mean and standard deviation of the soluble carbohydrate, as a percent dry weight, for turf algae collected from three zones and fecal pellets collected from Kaloko-Honokohau National Historical Park, Hawaii

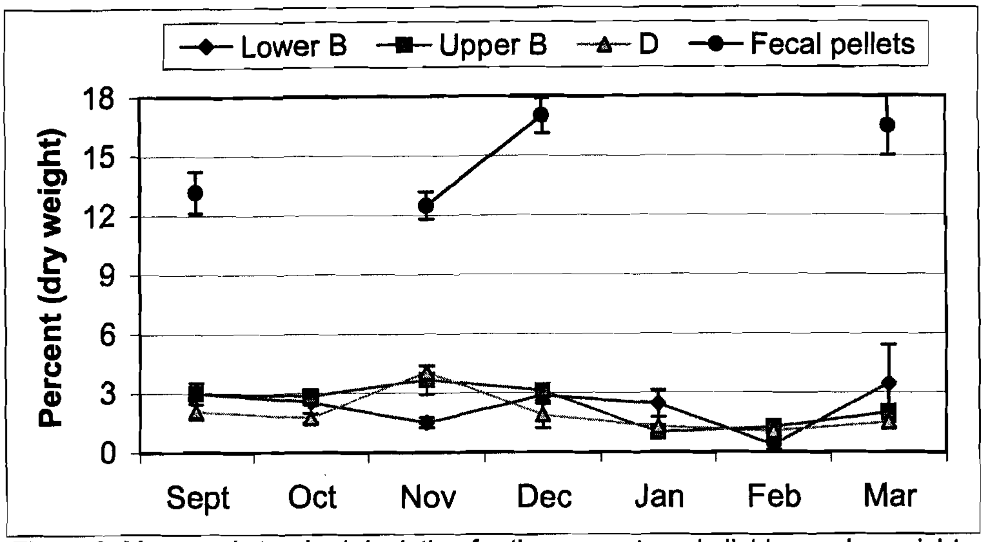

Figure 6. Mean and standard deviation for the percent crude lipid on a dry-weight basis for turf algae and fecal pellets collected montly at Kaloko-Honokohau National Historical Park.

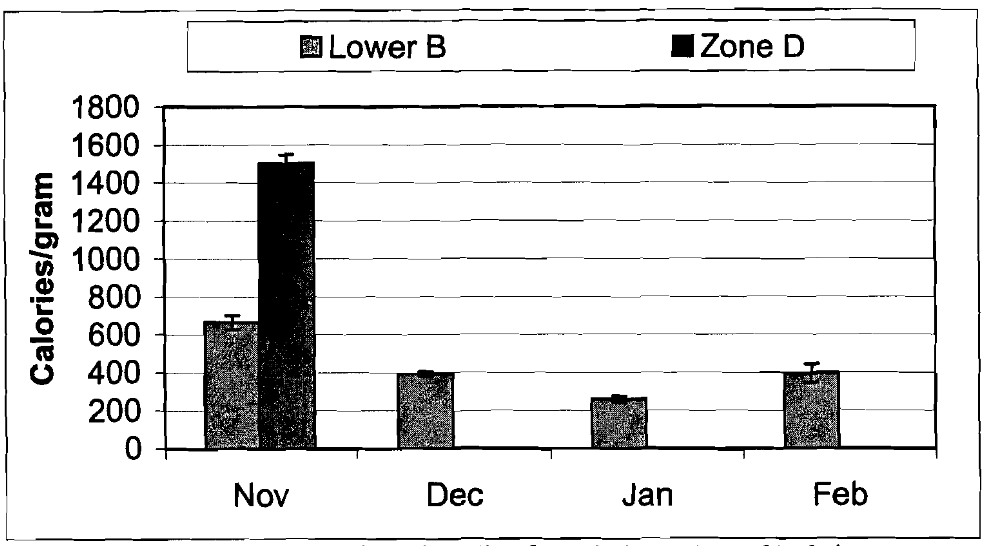

Figure 7. The mean and standard deviation for caloric content of turf algae collected in zones Lower B and D at Kaloko-Honokohau National Historical Park, Hawaii.

Table 1. Water content of fecal pellets and turf algae collected from zones D and lower and upper B at Kaloko-Honokohau National Historical Park along the Kona Coast of Hawaii

	Lower B	Upper B	D	Fecal pellets
Sept			86.95	72.98
Oct	85.26	84.68	85.53	74.98
Nov	81.90	81.90	79.06	71.90
Dec	73.40	81.12	73.16	74.84
Jan	70.97	82.30	68.88	80.83
Feb	71.38	74.11	66.40	76.20
Mar	71.51	76.34	71.38	82.04
Mean	**75.74**	**80.08**	**75.91**	**76.25**

Table 2. Amino acid profiles for turf algae collected from three zones within Kaloko-Honokohau National Historical Park, Hawaii. Amino acid content is expressed as percent of total amino acid per sample. The amino acids that Wood and Wood (1977) determined to be essential for hatchling green turtles are in boldface, along with the percent required for normal growth and development.

		November	December			January	February
amino acid	**Wood and Wood (1977)**	**Lower B**	**Lower B**	**Upper B**	**D**	**Lower B**	**Lower B**
LYS	**1.8%***	**5.53%**	**5.87%**	**5.40%**	**4.12%**	**5.53%**	**4.51%**
TRP	**0.22%***	**0.06%**	**0.08%**	**0.13%**	**0.06%**	**0.05%**	**0.08%**
MET	**0.5%***	**0.91%**	**1.92%**	**1.33%**	**0.75%**	**1.49%**	**0.99%**
VAL	**1.3%****	**6.73%**	**6.24%**	**6.43%**	**6.99%**	**6.35%**	**6.25%**
LEU	**1.6%****	**6.87%**	**6.63%**	**6.66%**	**7.41%**	**6.86%**	**6.86%**
ILE	**1.0%****	**4.83%**	**4.46%**	**4.49%**	**4.94%**	**4.63%**	**4.61%**
PHE	**1.0%****	**2.70%**	**3.49%**	**3.55%**	**3.41%**	**3.66%**	**3.39%**
HIS		**1.27%**	**1.36%**	**1.44%**	**1.33%**	**1.30%**	**1.54%**
THR		**5.66%**	**5.69%**	**5.86%**	**6.40%**	**5.68%**	**5.74%**
CYS		1.64%	2.34%	2.00%	2.35%	2.43%	2.22%
TYR		0.41%	1.57%	1.23%	0.89%	1.53%	1.32%
ARG		4.97%	5.85%	4.39%	4.87%	5.12%	5.03%
ASX		11.89%	14.30%	12.52%	13.40%	12.01%	11.67%
GLX		12.30%	8.87%	11.16%	9.27%	9.57%	10.98%
SER		7.19%	6.47%	6.70%	7.39%	6.71%	7.01%
GLY		9.88%	9.32%	9.94%	9.74%	9.25%	8.25%
ALA		11.42%	9.59%	10.37%	11.35%	10.33%	10.10%
PRO		5.80%	6.03%	6.56%	5.40%	7.55%	9.51%

* in the presence of 1.1% cysteine
** in the presence of 0.5% tyrosine

needs revision