The toxic cyanobacteria, *Lyngbya majuscula*, as a component of green turtle (*Chelonia mydas*) diet in the Hawaiian Islands January 2003

Preliminary Findings for
George Balazs
Leader Marine Turtle Research

by

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Good!

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Karen Arthur

Preliminary Results from January 2003 Field Trip for George Balazs (and anyone else interested)

Summary

- *Lyngbya majuscula* was present at five of the sites visited, however, it was only present in sparse amounts. No blooms were observed during January 2003.
- The toxicity of *L. majuscula* was highly variable and ranged between below the detection limit (0.01mg/kg dry weight) and 168.5mg/kg and 540.0mg/kg for Lyngbyatoxin A and Debromoaplysiatoxin. It was found to be highest in samples from Kaneohe Bay, Oahu and Palaau, Molokai.
- *Lyngbya majuscula* was present in 27% of diet samples examined, however, it contributed a negligible amount (<1%) of green turtle diets in the Hawaiian Islands during January 2003.
- Green turtle diet in the Hawaiian Islands is predominantly algal based, although seagrass and animal material are also present in diet samples.

Introduction

Lyngbya majuscula is a bloom-forming filamentous cyanobacterium with a pan-tropic distribution (Osborne, Webb et al. 2001). It is a prolific producer of biologically active compounds including Lyngbyatoxin A and Debromoaplysiatoxin, which are potent tumour promoters (Fujiki, Sugimura et al. 1983; Fujiki, Suganuma et al. 1984). *L. majuscula* forms dense benthic mats covering seagrass and macroalgae. These marine plants also form the basis of the diet of the herbivorous green turtle (*Chelonia mydas*). *L. majuscula* has been documented to grow prolifically in the Hawaiian Islands (Banner 1959; Moikeha 1968; Izumi and Moore 1987), an area that provides an important feeding ground for green turtles (Balazs, Forsyth et al. 1987). *L. majuscula* has been observed in diet samples from green turtles in Hawaii (Russell and Balazs 2000) and captive turtles have been observed consuming *L. majuscula* (McMaster 2002), however, the amount of *L. majuscula* consumed by green turtles has never been quantified, nor has the amount of toxin to which the turtles are potentially exposed.

Tumour promoting toxins from marine organisms have been implicated in the debilitating marine turtle disease fibropapillomatosis (FP) (Landsberg, Balazs et al. 1999), a disease that has been observed in turtles in areas where *L. majuscula* is in bloom. FP occurs world wide in epizootic levels, but is in greatest prevalence in inshore regions of high human impact (Herbst 1994). Although the cause of the disease has not been conclusively isolated, it is believed to be associated with a herpes virus (Jacobson, Buergelt et al. 1991; Quackenbush, Limpus et al. 1999; Quackenbush, Cassey et al. 2001) and tumour promoting toxins from marine organisms have also been implicated as potential environmental co-factors (Landsberg, Balazs et al. 1999). In Hawaii, the region with the highest recorded prevalence of FP (up to 92% of animals (Balazs 1991)), *L. majuscula* is commonly found in the gut contents of green turtles (Russell and Balazs 2000) and as *L. majuscula* contains tumour promoting toxins it is possible that the disease is related to assimilation of tumour promoting toxins. Understanding the amount of *L. majuscula* that turtles consume in the wild and the amount of toxin to which the animals are potentially exposed may provide an insight into the aetiology of FP.

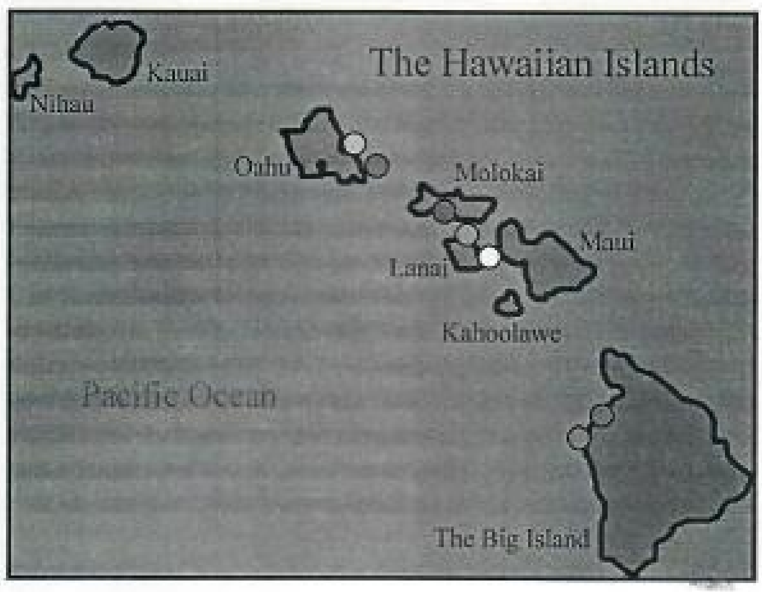
Objectives

This study of wild green turtles in the Hawaiian Islands, USA, aims to examine whether turtles are exposed to *L. majuscula* and its toxins. To do this is it necessary to understand the availability of *L. majuscula*, the concentration of Lyngbyatoxin A and Debromoaplysiatoxin and also whether the turtles consume the cyanobacteria and if so, in what quantities. This study looks at the effects of *L. majuscula* on green turtles in the Hawaiian Islands in terms of diet, FP prevalence and the potential for turtles to be exposed to LA and DAT.

Methods

Study Sites

Seven sites on four islands were selected for this study. Kaneohe Bay and Kawainui Bay on Oahu; a local fishing area, Palaau, on Molokai; White Rock and Federation Camp on Lanai; and Kiholo Bay and Kaloko Beach Park on the Big Island of Hawaii (Map 1). These sites were selected as they are all part of a long-term tag and recapture program and hence population demographic parameters such as FP rate are already known.



Map 1
The Hawaiian Islands.
Study sites January 2003:

Kaneohe Bay, Oahu
Kawainui, Oahu
Palaau, Molokai
White Rock, Lanai
Federation Camp, Lanai
Kiholo, Hawaii
Kaloko, Hawaii

Availability and toxicolgy of *Lyngbya majuscula*

A careful search for *L. majuscula* was made at each site. Where observed, its coverage was estimated and where possible up to four samples were collected for toxin analysis. These were frozen and returned to the University of Queensland, Australia for analysis. The samples were freeze-dried, ground and toxins extracted using acetone. The quantity of LA and DAT was determined with the use of High Performance Liquid Chromatography (HPLC).

Diet analysis

Green turtles (*Chelonia mydas*) were captured by hand on snorkel, and in bullpen nets. They were tagged, measured and weighed and a crop sample was obtained using the stomach flush technique (Balazs 1980; Forbes and Limpus 1993). At Federation Rock, Lanai, 15 fecal samples were collected along the beach adjacent to the area where turtles were captured. It is assumed that the fecal material was from the same population of turtles that were sampled in the area.

The relative volume of each food type identified from crop samples was determined using the principles of microstereology (Schaefer 1970) after the modified methods of Forbes (1995) and Read (1991). The sample was placed in a petri dish and mixed until visually homogenous and viewed under dissection microscope with a 19mm field of view. All food items present were identified to the lowest possible taxa (seagrass were identified to species and algae were generally identified to genus). To quantify the crop samples, a graticule with 33 marked positions was used to determine the volumetric proportion of each food item by counting the number points covering each food type. Ten non-overlapping fields of view were observed and a total of 330 points used for each sample. The relative proportion of each food type present was then determined using the following equation:

$$Pt = Nt / Tp * 100$$

Where: Pt = Proportion each food type

Nt = number of points coving that food type

Tp = total number of points observed

Algae samples were identified with the use of Abbott (1999), Cribb (1983), Magruder & Hunt (1979), Russell & Balazs (2000), Wormersley (1984) and Wormsley (1987), and with the assistance and advice of Dr. Dennis Russel. Simon Albert (Marine Botany Group, Centre for Marine Studies, University of Queensland) confirmed the identification of *L. majuscula*. Classification of the algae into functional groups was based on Steneck & Dethier (1994). This classification can be useful in understanding the algae from a morphological basis. The advantage of classifying algae according to their form and function is that it provides a basis for functional comparisons. For example, understanding which algae *L. majuscula* is most likely to grow on or become tangled in and therefore incidentally eaten by turtles.

Results

Between 21st January and 1st February 2003, 103 turtles were sampled from the seven sites. One turtle was sampled on consecutive days and, except where specified, the second sample has not been included in the following analysis as it was not considered to be independent of the first sampling. A summary of the number of turtles sampled at each site can be found in table 1.

Table 1: The number of turtles sampled at each site with previously observed FP rates for each population (pers. comm. George Balazs).

Island	Site	Number turtles sampled	FP rate
Oahu	Kawainui Bay	16	Moderate
	Kaneohe Bay	15 + 1 repeat	High
	Kailua Beach Park	0	-
Molokai	Palaau	15	High
Lanai	Federation Camp	3	None
	White Rock	7	None
The Island of Hawaii	Kiholo Bay	25	None
	Kaloko Beach Park	22	None
Total		103 + 1 repeat	

LYNGBYA MAJUSCULA ABUNDANCE AND TOXICITY

L. majuscula was not observed to be in bloom at any of the sites visited in January 2003. However, it was observed in very small amounts at most sites (table 2).

As previously noted, the toxicity of *L. majuscula* is highly variable (Osborne, Webb et al. 2001). As can be observed in table 2 and figure 1, the toxicity of *L. majuscula* in the Hawaiian Islands is highly variable both on a temporal and geographic scale. Toxicity ranged between below the detection limit (0.01mg/kg dry weight) and 168.5mg/kg and 540.0mg/kg for Lyngbyatoxin A and Debromoaplysiatoxin respectively. Toxin content was found to be highest in samples from Kaneohe Bay and Honokawai (August 2002) and Palaau (January 2003) (Figure 1).

Table 2: The abundance and toxicity of Lyngbya majuscula at each site examined in the Hawaiian Islands, January 2003, plus result for Kaneohe Bay, Oahu and Honokawai, Maui from August 2002. B.D.L = below detection limit (0.01mg/kg) and ND= standard deviation not calculated as all but one result below detection limit.

Site	Lyngbya presence and abundance	Toxicity Mean (Standard Deviation)	
		Lyngbyatoxin A (mg/kg)	Debromoaplysiatoxin (mg/kg)
Kawainui Bay, Oahu	None observed	-	-
Kaneohe Bay, Oahu	Yes - sparse	7.7 (7.5)	1.2 (0.8)
Kailua Beach Park, Oahu	Yes – one patch	B.D.L.	B.D.L.
Palaau, Molokai	Yes - sparse	17.5 (9.0)	115.8 (200.6)
Federation Camp, Lanai	Yes - sparse	B.D.L.	B.D.L.
White Rock, Lanai	Yes - sparse	B.D.L.	B.D.L.
Kiholo Bay, Hawaii	None observed	-	-
Kaloko Beach Park, Hawaii	None observed	-	-
Kaneohe Bay (Aug, 2002)	Yes – sparse	160.5 (10.6)	13.8 (ND)
Honokowai, Maui (Aug, 2002)	Yes – abundant	6.5 (7.2)	73.3 (155.4)

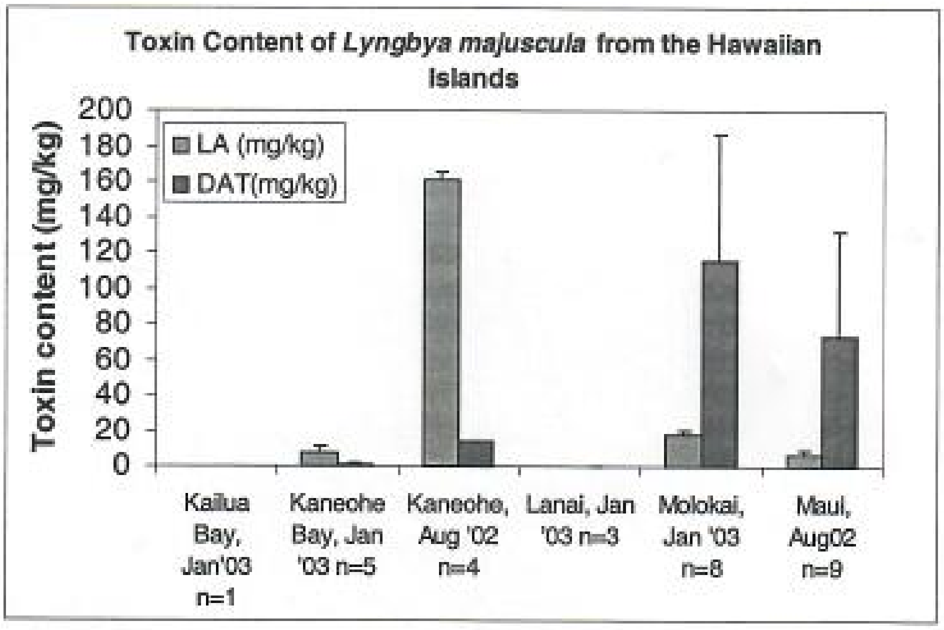


Figure 1: The Lyngbyatoxin A (LA) and Debromoaplysiatoxin (DAT) content of *Lyngbya majuscula* from each site where *L. majuscula* was observed in August 2002 and January 2003. Error bars represent standard error.

LYNGBYA MAJUSCULA AS A COMPONENT OF GREEN TURTLE DIET

Although *L. majuscula* was observed in 27% of all samples analysed, it only contributed a small amount to the overall diet of green turtles in the Hawaiian Islands. It was most commonly observed in samples from White Rock, Palauu, Kaneohe Bay and Kawainui Bay where approximately half the samples contained *L. majuscula* (Figure 2). However, the mean relative volume of *L. majuscula* in turtle diet was less than 1% (Figure 3) and did not contribute significantly to green turtle diet.

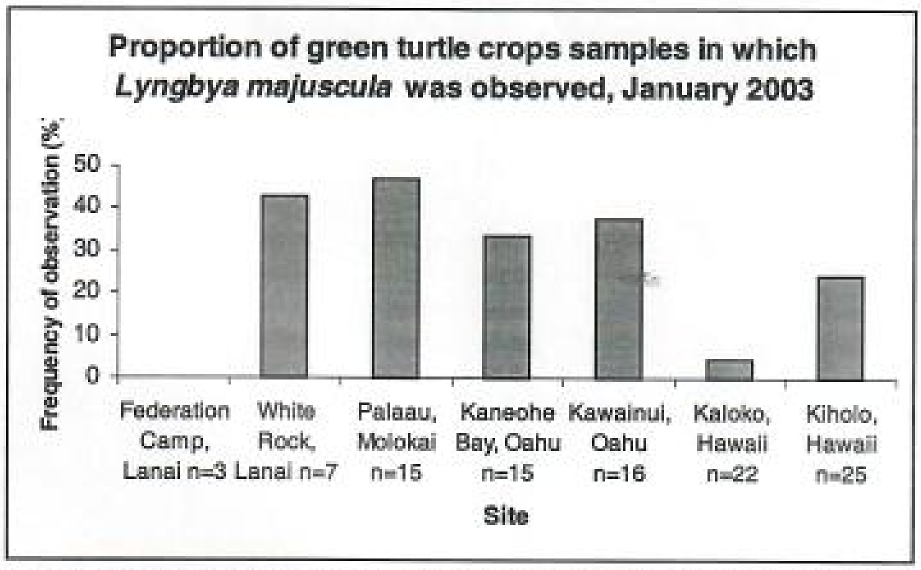


Figure 2: The frequency of green turtle samples from the Hawaiian Islands (January 2003) that contained *Lyngbya majuscula*.

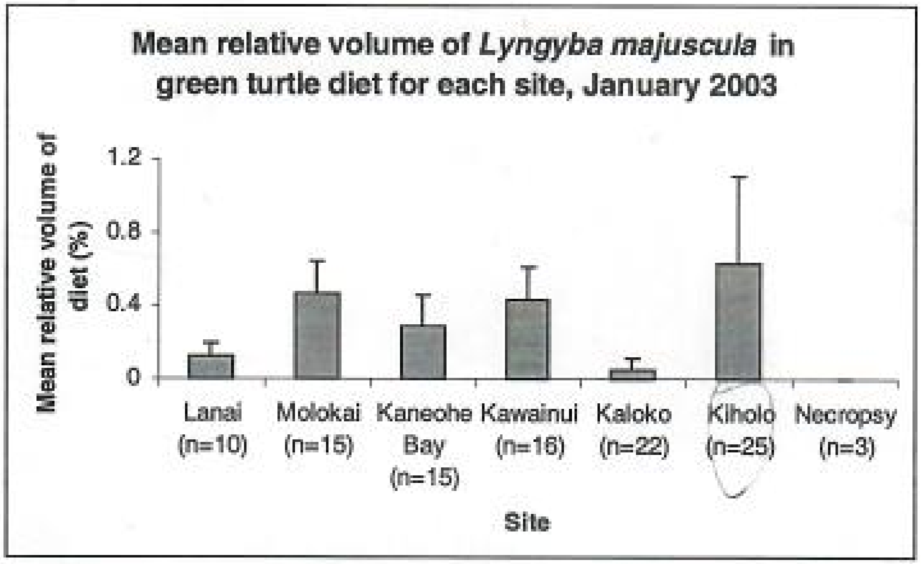


Figure 3: The mean relative volume for each site of *Lyngbya majuscula* in green turtle crop samples from the Hawaiian Islands, January 2003. Error bars represent standard error

Green turtle diet consisted predominantly of algae (table 3). Seagrass was only observed in samples from Kaneohe Bay, Oahu and the only seagrasses identified from green turtle crop samples were *Halophila decipiens* and *Halophila hawaiiana*. When *Halophila decipiens* was present it generally contributed the large majority of the sample, however, when *Halophila hawaiiana* was present it was often mixed with *Hypnea* sp. or *Gracilaria* sp..

Table 3: Food types found in the crop samples collected from green turtles in the Hawaiian Islands during January 2003.

Food type	Dietary item	Functional Group
Seagrass	*Halophila decipiens*	Seagrass
	Halophila hawaiiana	Seagrass
Rhodophyta	*Acanthophora spicifera*	Corticated macrophyte
	Amphiroa sp.	Articulated calcareous
	Cerium sp.	algae
	Centroceras clavulatum	Filamentous algae
	Centroceras sp.	Filamentous algae
	Gelidiella sp.	Filamentous algae
	Gracilaria salicornia	Corticated macrophyte
	Gracilaria sp.	Corticated macrophyte
	Hypnea spinella	Corticated macrophyte
	Hypnea sp.	Corticated macrophyte
	Laurencia sp.	Corticated macrophyte
	Melanamansia glomerate	Corticated macrophyte
	Polysiphonia sp.	Corticated foliose algae
	Pterocladiella capillacea	Filamentous algae
	Pterocladiella sp.	Corticated foliose algae
	Tolypiocladia glomerulata	Corticated foliose algae
		Filamentous algae
Phaeophyta	*Dictyota sp.*	Corticated foliose algae
	Lobophora variegata	Foliose algae
	Sargassum sp.	Leathery macrophyte

	Turbinaria sp.	Leathery macrophyte
Chlorophyta	*Bryopsis sp.*	Filamentous algae
	Cladophora seriacea	Filamentous algae
	Cladophora sp.	Filamentous algae
	Codium arabicum	Mass forming
	Codium edule	Mass forming
	Codium sp.	Mass forming
	Dictyosphaeria cavernosa	Foliose algae
	Dictyosphaeria versluysii	Foliose algae
	Halimeda opuntia	Articulated calcareous algae
	Rhizoclonium grande	Filamentous
	Ulva fasciata	Foliose algae
	Ulva sp.	Foliose algae
Cyanobacteria	*Lyngbya majuscula*	Filamentous algae
	Lyngbya sempiplena	Filamentous algae
	Lyngbya porphyrosiphonis	Filamentous algae
	Schizothrix sp.	Filamentous algae
Animal material	Unidentified shell material	Animal material
	Unidentified crustacean	Animal material
	Unidentified jellyfish	Animal material
Sediment	Black, volcanic	
	Calcareous	
	Sand	
Anthropogenic material	Green plastic	
Unidentified material	Unidentified terrestrial plant material	
	Unidentified sponge	
	Various algae	

L. majuscula was most often observed in samples that contained other filamentous algae, however, no correlation was found between the presence of *L. majuscula* and the presence of algae from the filamentous algae functional group. Corticated macrophytes such as *Gracilaria* sp., *Hypnea* sp. and *Pterocladia* made up the majority of green turtle diets in the Hawaiian Islands (Figure 4). These branching algae can entangle *L. majuscula* filaments and cause the turtles to ingest *L. majuscula* incidentally. This was observed to be the cause of human poisonings in Hawaii during 1994 when toxins from an epiphytic blue green algae (probably *L. majuscula*) growing on the edible *Graclaria cornopifolia* caused vomiting and diarrhea and burning around the mouth (Nagai, Yasumoto et al. 1996).

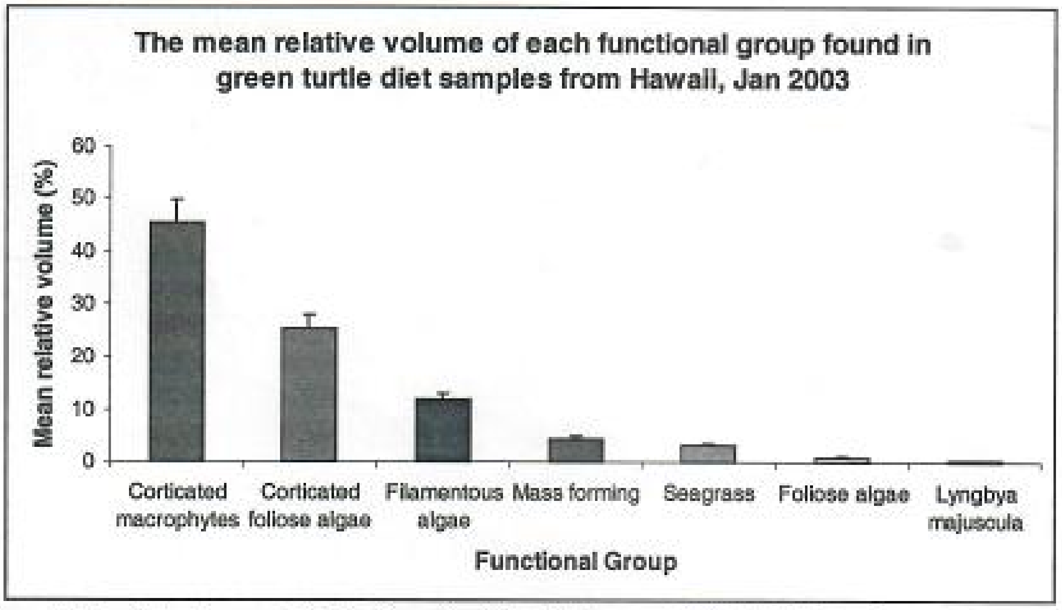


Figure 4: The diet of green turtles in Hawaiian Island in January 2003 with dietary items characterised by a morphological and functional grouping. Error bars represent standard error.

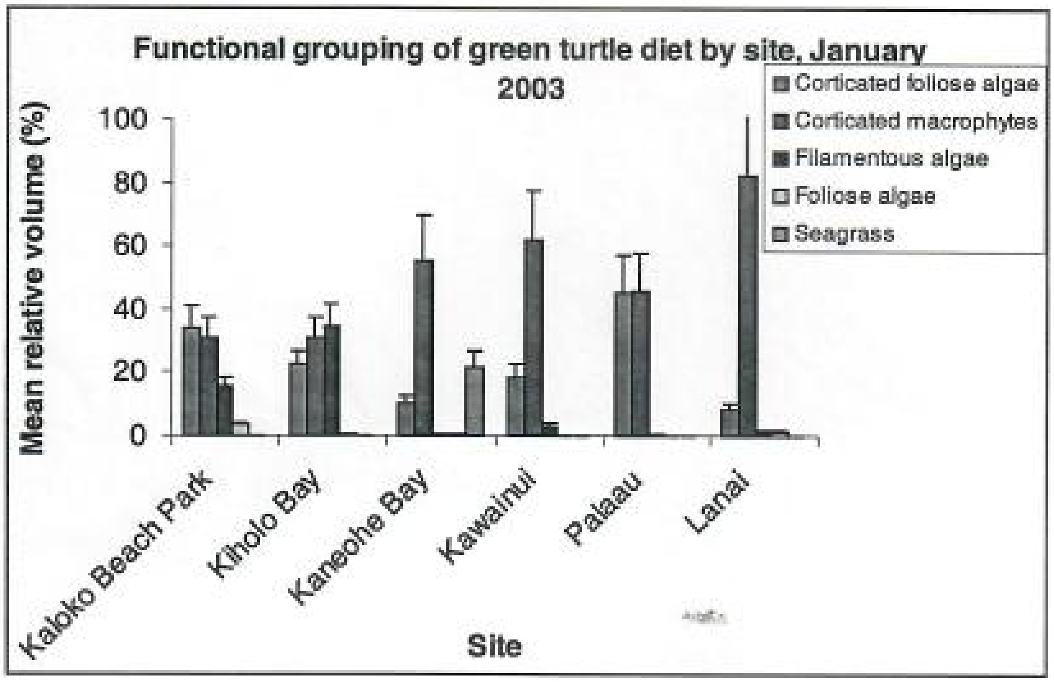


Figure 5: Green turtle diet analysis by functional group for each site, January 2003. Error bars represent standard error.

FECAL SAMPLES

L. majuscula was observed to be identifiable in faeces collected from White Rock, Lanai, as was *Melanamansia glomerata*. However, it has not yet been determined how to quantify faecal pellets as there is a large amount on unidentifiable material present. Any suggestions are welcomed.

POTENTIAL TOXIN EXPOSURE

Based on the daily food intake calculated by Bjorndal (1997), a 30kg green turtle in Kaneohe Bay consuming 0.3% of its diet as *L. majuscula* would have be exposed to 63$\mu g.kg_{(turtle)}^{-1}.day^{-1}$ Lyngbyatoxin A and 10$\mu g.kg_{(turtle)}^{-1}.day^{-1}$ Debromoaplysiatoxin during January 2003. The only study to look at the effects of ingesting Lyngbyatoxin A demonstrated that a 13g mouse that was fed 1000µg of Lyngbyatoxin A in a single dose suffered erosion of the stomach and small intestine (Ito, Satake et al. 2002). It did not lead to death and the mice recovered over the following days. However, this was a single dose study and there is currently no data on long term exposure to LA or DAT.

Acknowledgements

This project would not be possible without the many hands that help to catch, to hold, to process and to write. Great appreciation is extended to George and his team of eager volunteers and associates in Hawaii. These include Marc Rice and all the students at the Hawaiian Prepatory Academy; Sally Beavers and Lisa Marack and the team from State Parks Service; Dr. Thierry Work and Dr. Bob Morris the turtle vets; Ed, Dart, Julie, Terry and Annie the Molokai fishing team; and Mrs Sherman for the use of her front yard. I also want to acknowledge the assistance of Glen Shaw, Ange Capper and Geoff Eaglesham for their assistance in the toxicology for this project

NOAA provided funding for this project and The University of Queensland provided facilities in Australia for analysis.

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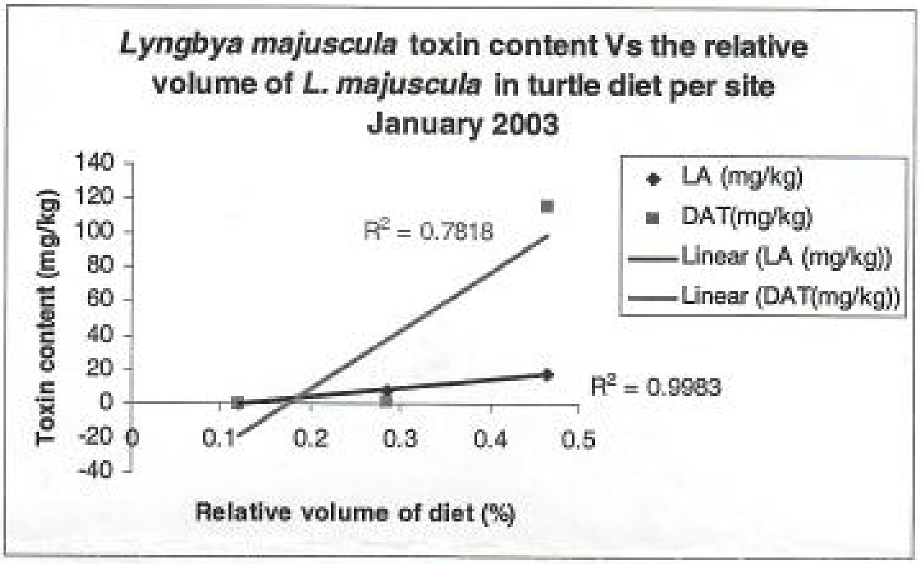
Addition to report submitted 24th July 2003

Toxin content of Lyngbya majuscula at three sites Vs the mean relative volume of L. majuscula consumed by turtles at the same site:



This shows the relationship between toxin content of *L. majuscula* at three sites: Lanai, Kaneohe Bay and Molokai Vs the amount that *L majuscula* contributes to diet at each site. Although the R^2 values are strong, I don't believe this is a good relationship as we are 1. talking about different Lygnbya (ie what is found growing and what is inside the animal) and as toxicity is highly variable I don't necessarily believe there would be distinct site differences and 2. we are only looking at three sites.

Besides, the turtles should be eating LESS the more toxic the Lygnbya.

...but I thought I would run it by you anyway.

Karen