“eDNA metabarcoding for diet analyses of green sea turtles (*Chelonia mydas*)”

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Table S1. Summary of advantages and limitations of different approaches that have been used throughout the years to study sea turtle diet.

Technique	Advantage	Limitation	References
Stomach flushing (gastric lavage)	If a good sample is retrieved, it can easily be seen what the turtle was eating	Turtle could be on an empty stomach Very invasive, and difficult method Limited temporal window into diet - can result in incomplete description of diet	Legler 1977; Seminoff et al. 2002; Burkholder et al. 2011
Esophagus lavage	Allows rapid retrieval of large volumes of food from the esophagus and anterior stomach regions Quick, safe and inexpensive method to obtain stomach samples in the field without injury to the animal	Limited temporal window into diet - can result in incomplete description of diet Care needs to be taken when interpreting the significance of the sample retrieved	Forbes and Limpus, 1993
Animal- borne video camera	Not invasive Allows direct assesment of items the animal feeds on	Need to retrieve the camera which can be very difficult and expensive	Heithaus et al. 2002; Fukuoka et al. 2016
Stable Isotope Analysis (SIA)	Reflects a time-integrated diet, with the timescale determined by its metabolic activity Gives information on prey effectively assimilated and not only on those consumed	Does not allow for direct species identification and quantification of prey, and information on the isotopic composition of putative prey is needed to interpret results	Peterson and Fry 1987; Gannes et al. 1998; Post 2002; Burkholder et al. 2011
DNA metabarcoding	Time-efficient fieldwork Less intrusive than other techniques Easy to sample High resolution If baseline of putative diet items is available, superior taxonomic resolution	Taxonomic assignment of the diet items is really dependent of the database used Only provides a snapshot of what the animals have eaten in recent feeding events Detection bias	This study

Table S2. Summary information of the green turtles captured for this study. CCL – curved carapace length; CCW – curved carapace width. Only data for the juvenile (animal code: J stands for "Juvenile") individuals is included.

Date of capture	Time of capture	Site of capture	Lat (N)	Long (W)	Animal code	Life-stage	PIT tag	CCL (cm)	CCW (cm)
27/10/2019	13:00 - 14:00	Ancante	11.3121	-16.4007	J1	Juvenile	977200009430768	39.4	37.6
27/10/2019	13:00 - 14:00	Ancante	11.3121	-16.4007	J2	Juvenile	977200009429926	36.5	32.5
27/10/2019	13:00 - 14:00	Ancante	11.3121	-16.4007	J3	Juvenile	977200009427885	38.3	34.3
27/10/2019	13:00 - 14:00	Ancante	11.3121	-16.4007	J4	Juvenile	977200009427231	48.1	41.5
27/10/2019	13:00 - 14:00	Ancante	11.3121	-16.4007	J5	Juvenile	977200009427097	39.5	35.7
27/10/2019	13:00 - 14:00	Ancante	11.3121	-16.4007	J6	Juvenile	977200009430232	40.6	36.2

Table S3. Animal code (J "juveniles", H "hatchling") and corresponding information regarding sample type. Three different sample types were collected: cloaca and esophagus from juveniles, and intestines from dead hatchlings. Different sample types were collected from each animal (for instance, there are two samples of animal J5, one for cloaca and one for esophagus). Number of reads per sample before and after sequencing filtering with DADA2 package in QIMME2. Input column corresponds to the initial number of reads in each sample and non-chimeric column corresponds to the final number of reads obtained after the sequencing filtering and the number of reads per sample used in the rest of the analyses.

Animal code	Sample type	Input	Filtered	Denoised	Merged	Non-chimeric
J3	cloaca	126550	72326	72324	72207	67487
H1	intestines	115133	65358	65307	65028	61202
J3	esophagus	113989	61512	61438	60468	56596
J1	esophagus	72815	36919	36791	35262	30878
J1	cloaca	128664	71905	71862	71418	66943
J5	cloaca	115534	63114	63068	62659	58919
J5	esophagus	104737	52470	52445	51496	45270
J6	esophagus	74419	31021	30877	29924	28578
J6	cloaca	109286	55864	55826	55583	55510
J2	esophagus	82119	39668	39539	37390	31173
J4	esophagus	97467	50274	50099	47888	42374
J4	cloaca	120692	65411	65340	64769	60932
H2	intestines	104280	55659	55652	55571	55571
H3	intestines	113478	59928	59926	59485	56221
J2	cloaca	96543	51802	51752	51394	49260

Table S4. Frequency of occurrence of main macroalgae and seagrass species found in the sampling area, listed taxonomically. Data obtained by 30 random 50x50cm quadrats per session (two sessions). FO represents the percentage of quadrats for which the taxon was found to be present.

Division	Class	Order	Family	Species	Presence	(F.O.)
Tracheophyta	Magnoliopsida	Alismatales	Cymodoceaceae	*Halodule wrightii*	19/30	63.33%
Ochrophyta	Phaeophyceae	Dictyotales	Dictyotaceae	Tribe: Dictyoteae	21/30	70.00%
				Padina sp.	17/30	56.67%
		Fucales	Sargassaceae	*Sargassum sp.*	17/30	56.67%
Rhodophyta	Florideophyceae (Corallinophycidae)	Corallinales	Corallinaceae	*Jania sp.*	15/30	50.00%
	Florideophyceae (Rhodymeniophycidae)	Ceramiales	Rhodomelaceae	*Laurencia dendroidea*	4/30	13.33%
			Spyridaceae	*Spyridia filamentosa*	3/30	10.00%
	Florideophyceae (Nemaliophycidae)	Nemaliales	Liagoraceae	*Liagora sp.*	1/30	3.33%
Chlorophyta	Ulvophyceae	Ulvales	Ulvaceae	*Ulva sp.*	6/30	20.00%
		Cladophorales	Siphonocladaceae	*Ernodesmis verticilata*	1/30	3.33%
		Bryopsidales	Caulerpaceae	*Caulerpa sertularioides*	1/30	3.33%

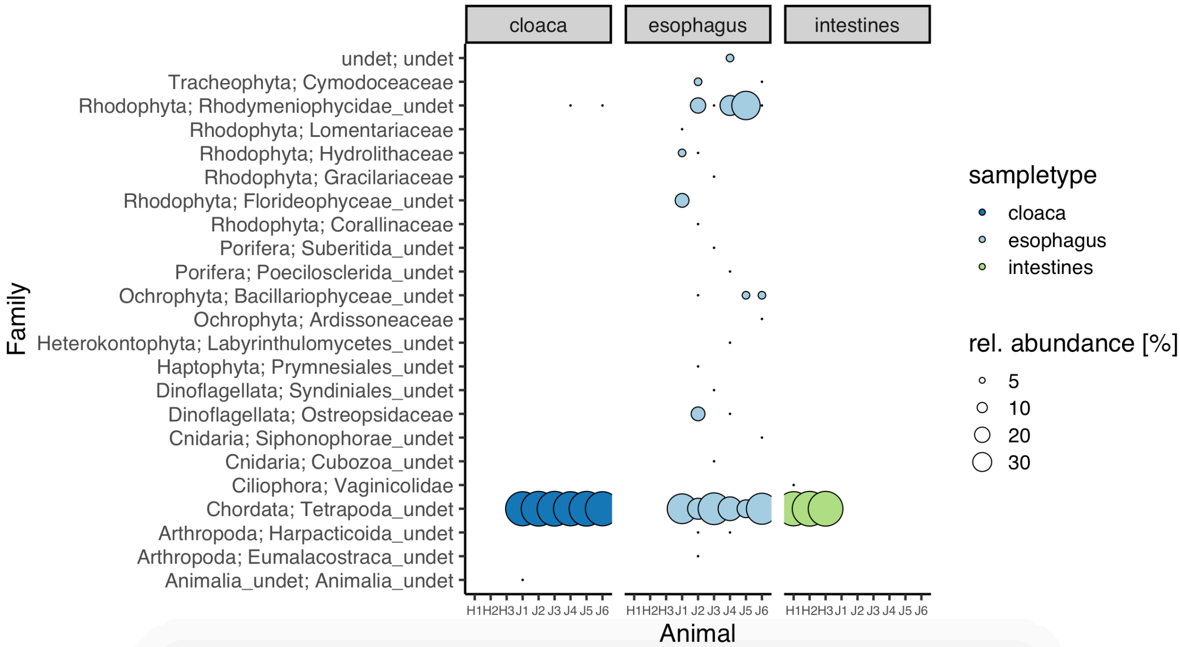

Figure S1. Relative abundance of the green turtle diet community composition for all three sample types of individual turtles included in the study (i.e., Animal). Diet items given at the taxonomic level of Phylum; Family. Tetrapoda_undet was the most abundant taxon identified for all three sample types, corresponding to turtle 18S rRNA gene reads. When Family affiliation was not possible to determine, the lowest identified taxonomic rank is shown (followed by "undet", i.e., undetermined).

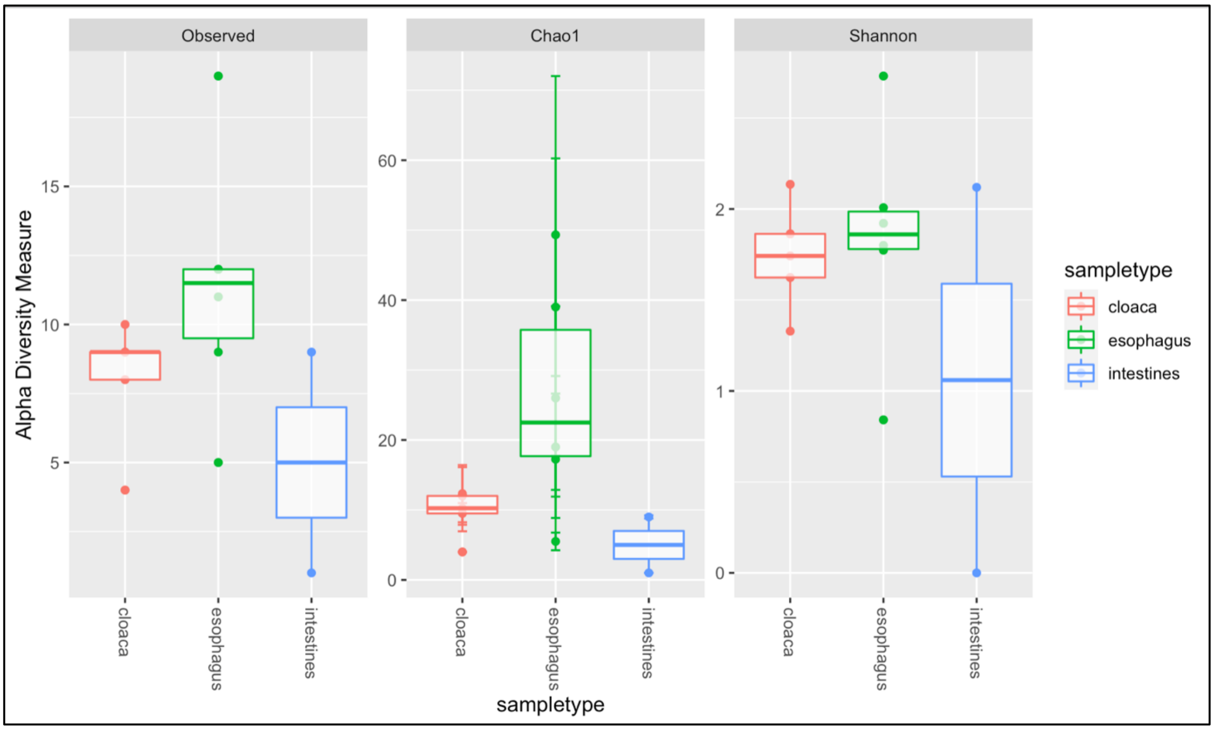

Figure S2. Alpha-diversity plots for intestine (from hatchlings), cloaca and esophagus (from juveniles) sample types. Observed species (count of unique ASVs) in each sample type (turtle DNA removed) are shown on the left panel. Middle panel represents the Chao1 index. Shannon diversity index is shown on the right panel. Overall, alpha diversity differed among the sample types analyzed. Esophagus samples exhibited a higher richness than cloacal ones and intestine samples yielded the lowest diversity.

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