

## **Supplemental Methods:**

### *Shark Capture*

A gill net (450' x 10' with 4 5/8" stretched mesh) was positioned perpendicular to the shoreline and the surrounding water was exposed to menhaden chum. Every 15mins, the length of the gill net was checked by a snorkeler to see if a bonnethead shark had been caught. If a shark was captured, it was brought back to a boat anchored nearby and placed into a circular live-well with fresh seawater. A Rio 2100 aquarium pump circulated the water while a micro-bubble diffuser was used to bubble oxygen into the water until the shark could be transported back to the FIU outdoor mesocosm facility.

### *Food Preparation, Feeding, and Fecal Collections*

The food was prepared by weighing out the appropriate amount of seagrass, thoroughly rinsing the seagrass with deionized water, and folding it inside a thin layer of the appropriate amount of squid mantle (frozen squid was obtained from West Marine). The prepared food was mounted to a piece of PVC piping and dipped under the surface of the water for the shark to retrieve (<https://goo.gl/xUUuK6>).

Feedings were divided into three feeding events per day. Sharks were moved into nearby individual 946 L circular, closed-system, tanks during the day for feedings and fecal collections in order to ensure that all sharks received the appropriate amount of food and to ensure the accuracy of identifying which shark produced which fecal material. Since the smaller 946 L tanks were closed systems, the sharks were moved back into the larger (40,337 L) tank in the evening and overnight so that they could be exposed to fresh, flowing seawater and oxygen. The smaller 946 L tanks were drained and cleaned at

the conclusion of each day and filled with fresh seawater the following morning to repeat the feeding and fecal material collection process.

### *Tissue Preparation*

The length and mass of each individual gut region was measured. The gut contents were removed from each section by pushing along the intestine with the edge of a glass microscope slide, placed into a 1.5 mL microcentrifuge tube, and frozen on dry ice before storage at  $-80^{\circ}\text{C}$ . The remaining tissue from each gut region was weighed and then cut open. The mucosal layer was scraped from the internal tissue of each intestine region using the edge of a glass microscope slide, placed into a 1.5 mL microcentrifuge tube, and frozen on dry ice before storage at  $-80^{\circ}\text{C}$ . The remaining intestinal tissue was also frozen on dry ice, placed into separately labeled microcentrifuge tubes, and stored at  $-80^{\circ}\text{C}$ . A piece of the liver from each shark, as well as a piece of muscle tissue from the body of each shark, was removed, frozen on dry ice and stored at  $-80^{\circ}\text{C}$ .

### *Nutrient Content Determination*

To determine total organic matter content, samples were dried at  $105^{\circ}\text{C}$  to a constant mass. Samples were then combusted at  $550^{\circ}\text{C}$  for 3 hrs. The remaining content was ash. The mass of the ash subtracted from the initial dry mass determined total organic matter. The final mass was multiplied by 100 to calculate the percent organic matter in the sample. Since the sharks were not able to be kept in their individual tanks for 24 hrs at a time (due to the lack of water flow), total collection of fecal material was not possible, hence the reason for adjusting the digestibility by ash (as noted in the main manuscript) to ensure that we are comparing the appropriate amount of food consumed to the feces that were collected.

A Pierce© BCA protein assay kit was used to determine protein content. The incubation occurred for 30 mins at 37°C. A standard curve with bovine serum albumin (BSA; pH 7.5) was used to calculate the protein concentration. A BioTek Synergy H1 Hybrid spectrophotometer/fluorometer equipped with a monochromator (BioTek, Winooski, VT) read samples at a wavelength of 562 nm.

Soluble carbohydrate content was determined using the method developed by Dubois et al.<sup>67</sup>. 5% phenol and concentrated 18 M sulfuric acid were used as solvents and a standard curve with 12 mM glucose was used to determine carbohydrate concentration. The microplate absorbance was 490 nm.

The charring method using 36 M sulfuric acid for the determination of total lipid content was developed by Marsh and Weinstein<sup>68</sup> and the lipid extraction method was developed by Bligh and Dyer<sup>69</sup>. A 2:1 chloroform: methanol reagent was used as the solvent and a standard curve using stearic acid prepared in 100% chloroform (4000 ug/mL) was used to determine lipid content. The microplate absorbance was 375 nm.

For fiber digestibility, samples (lab-fed shark fecal material and the 90% seagrass/10% squid diet) were dried at 60°C overnight. Samples were then briefly exposed to liquid nitrogen and immediately ground with a mortar and pestle to pass through a 1 mm screen and then dried overnight again at 60°C. Approximately 1 g per sample was placed into individual ANKOM filter bags. The samples then underwent the NDF procedure<sup>33</sup>, were dried overnight at 105°C, and weighed. Samples then underwent the ADF procedure<sup>33</sup>, were dried overnight at 105°C, and weighed. Lignin was subtracted from both the NDF and ADF digestibility calculations based on Bjorndal's<sup>18</sup> determination of the lignin components of seagrass.

### *Metabolic Rate Calculations*

We determined the amount (g) of protein, soluble carbohydrates, lipids, and fiber consumed daily by each shark using the methods detailed in the previous paragraphs. Assuming 4 Cal/g of protein and carbohydrates and 9 Cal/g of lipid, we determined the amount of Calories of protein, soluble carbohydrates, lipids, and fiber consumed daily by each shark. Given the digestibility (%) of each of these nutrient types determined for each shark using the procedures outlined in previous paragraphs, we determined the total number of Calories digested per day by each shark (Table S1).

### *Enzyme Assays*

All pH values listed for buffers were measured at room temperature (22°C), and all reagents were purchased from Sigma-Aldrich Chemical (St. Louis). All reactions were run at saturating substrate concentrations as determined for each enzyme with gut tissues from bonnethead sharks. Each enzyme activity was measured in each gut region of each individual shark, and blanks consisting of substrate only and homogenate only (in buffer) were conducted simultaneously to account for endogenous substrate and/or product in the tissue homogenates and substrate solutions.

$\alpha$ -Amylase activity was measured using 1% potato starch dissolved in 25 mM Tris-HCl containing 1 mM CaCl<sub>2</sub>. Previous work had shown that low concentrations of Tris are suitable for the measurement of amylase and maltase<sup>53</sup>. The  $\alpha$ -amylase activity was determined from a glucose standard curve and expressed in U ( $\mu$ mol glucose liberated per minute) per gram wet weight of gut tissue.

Maltase activities were measured as described by German and Bittong<sup>70</sup>. We used 112 mM maltose dissolved in 200 mM phosphate buffer, pH 7.5. The maltase activity

was determined from a glucose standard curve and expressed in U ( $\mu\text{mol}$  glucose liberated per minute) per gram wet weight of gut tissue.

Trypsin activity was assayed using a modified version of the method designed by Erlanger et al.<sup>71</sup>. The substrate, 2 mM N $\alpha$ -benzoyl-L-arginine-p-nitroanilide hydrochloride (BAPNA), was dissolved in 100 mM Tris-HCl buffer (pH 7.5). Trypsin activity was determined with a p-nitroaniline standard curve, and expressed in U ( $\mu\text{mol}$  p-nitroaniline liberated per minute) per gram wet weight of gut tissue.

Aminoamidase activity was measured using 2.04 mM L-alanine-p-nitroanilide HCl dissolved in 200 mM sodium phosphate buffer (pH 7.5). Aminoamidase activity was determined with a p-nitroaniline standard curve, and activity was expressed in U ( $\mu\text{mol}$  p-nitroaniline liberated per minute) per gram wet weight of gut tissue.

Lipase (non-specific bile-salt activated) activity was assayed using 0.55 mM p-nitrophenyl myristate (in ethanol) in the presence of 5.2 mM sodium cholate dissolved in 25 mM Tris-HCl (pH 7.5). Lipase activity was determined with a p-nitrophenol standard curve, and expressed in U ( $\mu\text{mol}$  p-nitrophenol liberated per minute) per gram wet weight of gut tissue.

$\beta$ -Glucosidase and N-acetyl- $\beta$ -D-glucosaminidase activities were measured following German et al.<sup>13</sup> using 200  $\mu\text{M}$  solutions of the substrates 4-methylumbelliferyl- $\beta$ -D-glucoside and 4-methylumbelliferyl-N-acetyl- $\beta$ -D-glucosaminide, respectively, dissolved in 25 mM Tris-HCl (pH 7.5; sodium acetate pH 5.5 for the colon tissue and contents). Briefly, 90  $\mu\text{L}$  of substrate was combined with 10  $\mu\text{L}$  of homogenate in a black microplate and incubated for 30 min. Following incubation, 2.5  $\mu\text{L}$  of 1 M NaOH was added to each microplate well, and the fluorescence read immediately at 365 nm

excitation and 450 nm emission. Each plate included a standard curve of the product (4-methylumbelliferone), substrate controls, and homogenate controls, and enzymatic activity ( $\mu\text{mol}$  product released per minute per gram wet weight tissue) was calculated from the MUB standard curve<sup>72</sup>.

#### *Compound Specific Stable Isotope Preparation*

Lipids were extracted prior to analysis using a soxhlet<sup>68</sup>. Liver samples (7 mg per sample) and seagrass samples (40 mg per sample) were ground, dried at 60°C overnight, weighed, placed in glass vials, and were sent to the University of California, Davis Stable Isotope Facility for compound specific stable isotope analyses using a Trace Ultra GC gas chromatograph coupled to Thermo Delta V Plus through a GC IsoLink.

#### **Supplemental References:**

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