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## Evaluation Of The Factors Influencing Feed Intake And Satiety Of The Variegated Sea Urchin, *Lytechinus Variegatus*

Marlee Hayes  
*University of Alabama at Birmingham*

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EVALUATION OF THE FACTORS INFLUENCING FEED INTAKE AND SATIETY  
OF THE VARIEGATED SEA URCHIN, *LYTECHINUS VARIEGATUS*

by

MARLEE D. HAYES

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A THESIS

Submitted to the graduate faculty of The University of Alabama at Birmingham,  
in partial fulfillment of the requirements for the degree of  
Master of Science

BIRMINGHAM, ALABAMA

2018

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2017

EVUALATION OF THE FACTORS INFLUENCING FEED INTAKE AND SATIETY  
OF THE VAREIAGATED SEA URCHIN *LYTECHINUS VARIEGATUS*

MARLEE D. HAYES

BIOLOGY

ABSTRACT

Feed intake is the primary determinant of nutrient and energy acquisition for most metazoans. Satiety regulates feed intake and may have cascading effects on nutrient acquisition and health. Satiety of many higher organisms involves complex processes and a variety of signaling molecules that are responsive to many factors associated with nutrient intake. However, the ability to sate is observed in many simpler animals with less complex physiological processes. Sea urchins, with relatively simple digestive and nervous systems, provide an ideal model for the study of fundamental drivers of feed intake. Understanding the process of satiety in this primitive deuterostome can provide insight into satiety mechanisms in these and more complex taxa.

Evidence from laboratory and field observations indicate that sea urchins feed selectively, reducing feed intake even when food items remain available. In sea urchins, feed intake may be modified by volume satiation (fullness), nutrient satiation (consumption to a nutrient intake target), energy satiation (consumption to an energy target regardless of the source), or some combination thereof.

In the present study large *L. variegatus* were fed to excess formulated diets that varied in both food and nutrient density. Feed intake varied inversely with food density. Volume satiation was detected at the lowest levels of dry matter inclusion, during which

sea urchins consumed ca. 1/3 of their body wet weight/day. However, sea urchins primarily sated to the dry matter content of the feed, consuming ca. 1.4 to 2.1% of their live weight in dry matter at food densities ranging from 1 to 18%. There is evidence that sea urchins demonstrate a diffuse target for protein intake when offered a diet that is complex, indicating some level of protein leveraging. However, intake parameters cannot be fully explained by macronutrient intake. Evaluation of variable patterns of daily feed intake (amount consumed) indicated that daily intake variation occurs largely within an individual over time, and secondarily, between individuals within a population. Overall, variation in feed intake was highest when food density was lowest, and variation decreased with increasing food density. Macronutrients affected patterns of feed intake, and this complex interaction deserves further evaluation.

Keywords: sea urchin, nutrition, feed intake, satiety, deuterostome, aquaculture,

*Lytechinus variegatus*

## DEDICATION

To David Mims for his unfaltering love and support throughout my education. Thank you, David, for your monetary and moral support while I pursue my passions as a scientist and for the sacrifices you have made to enable me to do so. Thank you for the countless hours of assistance with my projects and for never giving up on me, even when I wanted to give up on myself. To my parents, David and Wanda Hayes, for both momentary and moral support throughout my education and my life. Also, to my mentor, Dr. Stephen A. Watts, without whom I would not be the scientist I am today. Dr. Watts, you have been my mentor, my advocate, my teacher, my collaborator, and my friend over the years and that influence has shaped who I am and who I aspire to be.

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## INTRODUCTION

Feed intake is an important component in understanding feeding strategies related to nutrient intake. An organism should exhibit a feed intake strategy that supports survival, maintenance, growth and reproduction, most likely shaped by natural selection. Across taxa, animals can adapt a variety of strategies for feed intake to meet their energetic and nutritional requirements. Generalists and specialists further direct feed intake as carnivores, omnivores, herbivores, and/or detritivores. Furthermore, feed intake is highly regulated by many internal and external factors.

For most animals, feeding is not continuous. Feed intake voluntarily ceases at a point indicative of satiation. The level at which an organism satiates is the point at which feed intake ceases. Satiety limits feed intake and the mechanisms leading to satiety may vary in complexity across taxa. Like other physiological processes, the mechanisms regulating satiety likely have evolved over time from less to more complex. In humans and other mammals, intricate signaling via hormones (gut and pancreatic) and vagal nerve inputs act to provide information to the brain about feeding and feed intake (Berthoud, 2008a; Berthoud 2008b; Sternson et al., 2013; Sclafani, 2014). Signals are continuously sent to the brain by the gut about both quality and amount of ingested nutrients (Berthoud, 2008b).

Despite the growing amount of research about satiety in higher vertebrates, a variety of questions related to the processes controlling feed intake and satiety in many animals remain (Berthoud and Morrison, 2008), especially in species with less complex

digestive, endocrine, and nervous systems. Sea urchins are some of the earliest deuterostomes to appear, in this group's lineage, dating back 460 million years (Smith and Savhill, 2001). Understanding factors that regulate feed intake and satiety in sea urchins may provide insight into the factors that control satiety across deuterostomes. Since their first appearance in the fossil record, sea urchins have diversified in ways that allow them to survive and colonize new areas (Smith and Kroh, 2013). Physical adaptations provided defenses against new predators (Smith and Kroh, 2013) and a variety of feeding strategies (Lawrence et al. 2013) may have aided their success. With 1,000 extant species (Kroh and Mooi, 2011), sea urchins are presently found in a variety of habitats, from the deep sea to polar seas and tropical reef flats, kelp forests, and seagrass beds (Lawrence, 2013). Sea urchins have varying life histories and these differences may be, in part, explained by the amount and quality of acquirable food sources (Lawrence, 2013). Sea urchins exhibit both carnivorous and herbivorous feeding modes, with omnivory a common strategy (Lawrence et al., 2013). Ecologically, sea urchins have a critical role in structuring marine ecosystems. They are crucial in regulating the abundance of foundation groups by acting as grazers in coral reefs, kelp forests and seagrass beds (Steneck, 2013), and are important prey items for a variety of large predatory fishes, spiny lobsters, sea otters and some crabs (Steneck, 2013). Considering their key trophic position, feed intake of sea urchins could have a cascading effect on complex marine food webs, which may affect how other higher-level consumers direct feed intake and allocate nutrients/energy.

Sea urchins feed selectively on a variety of plant and animal species but can reduce feed intake even when food items are readily available in stable environments

(Lawrence et al., 2013) implying sea urchins do sate on some component(s) of food. When offered choices, sea urchins have shown preferences among natural food types (Vadas, 1977; Ayling, 1978; Larson et al., 1980; McClintock et al., 1982; Lawrence et al., 2013), consuming some and avoiding others.

In the last century, sea urchins have become well known as a model for classic developmental and embryological research. Thus, culture conditions and standard husbandry procedures for several species are well documented. This previous use of sea urchins in a number of laboratory-based studies established a baseline of standard conditions that can be maintained and controlled. By coupling standardized conditions with strictly controlled formulated diets, specific aspects of feed intake and factors affecting feed intake, and thus ultimately satiety, can be observed in laboratory environments.

Sea urchins are regulating their feed intake, but the factors directing feed intake and satiety are not fully understood. Factors potentially affecting satiation range from mechanical fullness (gut volume satiation) to nutrient satiation (when an organism ceases consumption after consuming to a nutritional intake target) to energy satiation (the point at which enough energy, regardless of the source, is consumed and feed intake ceases), or some combination therein. At this time there is limited understanding about optimal daily dietary intake and daily intake patterns for sea urchins (Watts et al., 2013a).

The focus of the present study is to identify factors that promote satiety in the sea urchin *Lytechinus variegatus*. *Lytechinus variegatus* is an omnivorous urchin (Beddingfield and McClintock, 2000) and feeds on both plant and animal materials (Boone, 1928; Mortensen, 1943; Moore et al., 1963; Moore and McPherson, 1965; Kier



and Grant, 1965; Camp et al., 1973; Lowe, 1975; Greenway, 1977; Bach, 1979; Klinger, 1984a; McClintock et al., 1982; Klinger and Lawrence, 1984; Valentine and Heck, 1991; Montague et al., 1991; Valentine and Heck, 1993; Greenway, 1995; Beddingfield and McClintock, 1998; Peterson et al., 2002; Cobb and Lawrence, 2005). In shallow waters, *L. variegatus* graze a variety of seagrasses, consuming fresh blades and detrital blades (Watts et al. 2013b). Thus, *L. variegatus* has an important role in regulating seagrass growth and abundance (Camp et al. 1973; Valentine and Heck, 1991; Heck and Valentine, 1995; Valentine et al. 2000) and may have an important role in converting detritus into available energy in their food web (Watts et al, 2013b). Furthermore, overgrazing of seagrasses by *L. variegatus* may have detrimental and long-lasting impacts in their marine systems (Camp et al. 1973; Heck and Valentine, 1995; Rose et al. 1999; Peterson et al, 2002). Based on their varied ecological roles, *L. variegatus* is a key species in the food web, with their feeding activity having cascading impacts in seagrass ecosystems. It is paramount to understand what drives the feeding of *L. variegatus* in respect to their ecological role as a consumer. Additionally, understanding the factors that regulate feed intake and satiety in *L. variegatus* will not only aid in a greater understanding of the sea urchin's ecological role, but will provide insights into common themes of satiety across deuterostomes.

The present study will consider the following questions: What factors related to food content lead to satiety in *L. variegatus*? Does *L. variegatus* exhibit satiety upon achieving volume satiation (adequate volume of food consumed), macronutrient satiation (acquisition of specific nutrients), and/or energy satiation (adequate caloric intake), or does more than one of these factors, or other unknown factors, contribute to satiety? Are

there basic similarities or differences in the regulation of feed intake and satiety between simple (sea urchin) and more complex organisms? Do *L. variegatus* alter their daily feed intake patterns over time? Answering these and other questions will allow further understanding of the role of nutrition for *L. variegatus*, and by extrapolation, the fitness of sea urchins in general.

## METHODS

### Collection and Transport

Large *Lytechinus variegatus* (ca. 30mm diameter, 18-24 g) were obtained from shallow water (approximately 0.5 to 2 m depth) at Saint Joseph Bay, Florida (30°N, 85.5°) under a collection permit (Florida Fish and Wildlife Conservation Commission, Division of Marine Fisheries Management, SAL-16-0766-SR). Sea urchins were immediately transported to the University of Alabama at Birmingham. Upon arrival, sea urchins were exposed to laboratory conditions over a 24-hr period by providing water exchanges with 5 recirculating raceway containing synthetic seawater. Synthetic seawater was made with municipal water filtered by a Kent Marine Deluxe™ Hi-S Maxxima filter (50 Gallon/Day RO/DI System, Garden Grove CA, USA) with the addition of Instant Ocean® salt (manufactured by Aquarium Systems Inc; Marineland Company, Mentor, Ohio, USA) to a salinity of 32 ppt. After initial exposure to raceway water, sea urchins were distributed into several established 400L RAS raceways and fed a formulated maintenance feed, previously shown by Heflin et al (2016b) to support optimal growth in *L. variegatus*.

### Maintenance of Cultured Population

*Lytechinus variegatus* were held in culture in 5 aerated recirculating fiberglass raceways systems (235cm (L) x 53cm (W) x 31 cm (H) described by Taylor (2006) and

Heflin et al. (2016b). Raceways were equipped with a 160 cm (L) by 23cm (H) plastic baffle running longitudinally along the center to facilitate a recirculating current (Fig 1a). Circulation is accomplished by using an in-line utility pump that pulled raceway water from one side of the baffle, through the filtration unit (biological and mechanical), and then returned water to the opposite side of the baffle at a flow rate of approximately 9.7-12.6 cm s<sup>-1</sup>. (Supreme® Mag Drive Utility Pump, Danner™ Manufacturing, Inc., Islandia NY, USA, 700 gallons of water/hr). The water depth in each raceway was approximately 15cm (Heflin et al., 2016b).

Raceway water was maintained at a salinity of 32.0 ±5 ppt, a temperature of 22.0 ±1.0 °C and a pH of 8.2. Temperature and salinity were monitored daily using a YSI-30™ meter (YSI Inc./Xylem Inc., Yellow Springs, OH). Raceway salinity was adjusted by adding municipal water filter by Kent Marine Deluxe™ Hi-S Maxxima (50 Gallon/Day RO/DI System, Garden Grove CA, USA). Photoperiod was maintained in the room on a 12:12 light/dark cycle.

Raceways were cleaned by siphoning 2 times per week to remove solid waste. Water removed by siphoning was replaced with 32ppt synthetic seawater at a rate of ca. 25% water exchange per week. Alkalinity, pH, and concentrations of ammonia, nitrite, and nitrate were tested using Aqua Pharmaceuticals, LLC (Malvern, PA, USA) and La Motte Company (Chestertown, MD, USA) test kits once per week. Alkalinity and pH were adjusted by adding bicarbonate. If ammonia, nitrite, or nitrate increased above 0.25ppm, 0.25ppm, and 160 ppm, respectively, water exchanges were performed daily until concentrations return to normal.

## Experimental Conditions

Collected sea urchins were weighed and sorted according to size (wet weight), as determined by a top loading balance. Similar-sized ( $20.73 \pm 1.46\text{g}$ ) sea urchins were sorted randomly into one of 19 treatments with 10 urchins per treatment. Each sea urchin was housed individually in a plastic mesh cage (3mm mesh openings; 25cm high, 8.5cm diameter) as shown in figure 1c. A 2mm mesh circle was placed on the bottom side of each cage to allow feces to fall out of the cage. The cages were fitted into 8.7cm PVC coupling to maintain the balance of each individual cage, and to raise the bottom of the cage above the floor of the raceway. Three Tygon® spacers (0.5cm thick) were fitted on each PVC coupling to allow water flow underneath cages. Cages were haphazardly assigned to 1 of 5 raceways (Fig 1a). Within each raceway, cages were arranged such that water flow underneath and between cages was unrestricted. Individual cage positions were rotated within and between raceways bi-weekly to prevent any potential location effect.

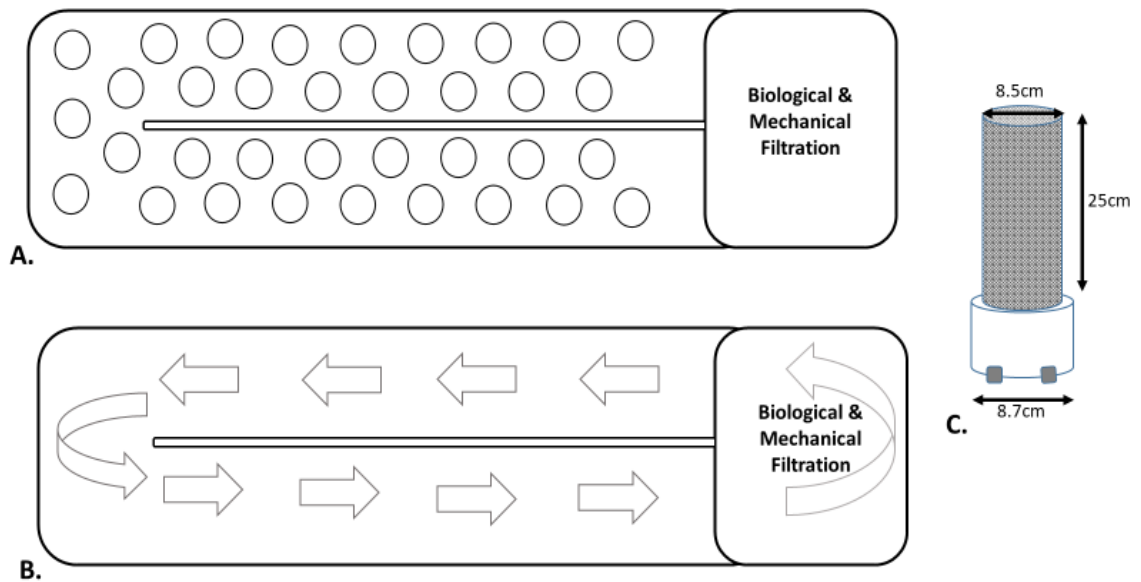


Figure 1. Schematic of recirculating raceway system and mesh cages. A) Aerial view of fiberglass raceway showing cage arrangement. B) Aerial view of fiberglass raceway with arrows indicating direction of water flow. C) Individual mesh cage used to house each individual.

## Experimental Protocol

Sea urchins were fed gel-based diets of uniform size and shape (rectangular) that varied in both food density and proximate composition. Individuals were offered one of three formulated diets (isocaloric within 0.1 kilocalories/kg dry weight) with varying ratios of protein (plant and animal sources) to carbohydrate (starch) content. All other components of the diet, including lipids and supplemented vitamins and minerals were held constant. These 3 diets included: a standard diet (described by Heflin et al., 2016b), with an equal protein to carbohydrate ratio (27% protein, 27% carbohydrate, as fed), a high protein low carbohydrate diet (38% protein, 13% carbohydrate, as fed), or a low protein high carbohydrate (18% protein, 40% carbohydrate, as fed). All animal and plant meals used to constitute the protein and carbohydrate in the formulated diets were varied proportionally. However, the caloric value of each of the diets was held relatively constant (within 0.1 kilocalories/kg dry weight; Table 1).

To prepare the formulated feed, dry ingredients were mixed with a PK twin shell® blender (Patterson-Kelley Co., East Stroudsburg, PA) for 10 mins. The ingredients were then placed in a commercial grade mixer (Hobart™ stand mixer, Model A-200, Hobart Corporation, Troy OH) and mixed for 40 mins. The liquid ingredients (fats and oils) were then added and mixed for 10 mins. The prepared feed was extruded as strands using a 4.8mm meat chopper attachment (Hobart™ Model A-200, Hobart Corporation, Troy, OH). Following extrusion, strands were separated by hand and placed on wire trays and dried at room temperature (22°C). Strands were dried for 48 hrs and then ground (twice) into a fine powder using a coffee grinder. The powder was held at 13°C until use to prepare gel-based diets.

<b>Diet (P:C)</b>	<b>% Protein</b>	<b>% Carbohydrate</b>	<b>% Fat</b>	<b>Energy (kcal/g)</b>
27:27	27.07	27.50	5.90	3.187
38:13	38.65	13.50	5.83	3.275
18:40	18.40	40.50	5.83	3.210

Table 1. Experimental diets. Macronutrient concentration (shown as percentages of diet, as fed) and energetic content (per gram dry weight) are given.

Each of the 3 diets was used to create gel-based food blocks with varying food densities % dry formulated feed of the total food cube) at 1, 3, 6, 10, 14 and 18% using non-nutritive agar as the binder. Food blocks of each diet were formulated at their respective food density, and with 1.5 % non-nutritive agar powder (Sigma A7002), and 32 ppt synthetic saltwater constituting the remaining percentage of the gel-based food cube. Additionally, a 0% food treatment was included, which consisted of a plain agar/seawater pellet containing no food. All 19 treatments and their components are shown in Table 2.

Synthetic saltwater was used to equate the density of the food cube and surrounding saltwater so that the food blocks would not be buoyant. Agar is an excellent binder and has been used to produce stable food blocks in previous sea urchin studies (Klinger 1982; Fabbroncini, 2012).

To create gel-based food blocks for 1 treatment, 32.2 ppt synthetic saltwater (amount dependent on treatment, table 2) and agar powder were placed in a flask, stirred with a magnetic stir bar, and heated until boiling on a stir hot plate. The agar was boiled for approximately 1–2 mins until the mixture became clear (indicating the agar powder has dissolved). The mixture was cooled at room temperature until it reached 38°C (a temperature unlikely to denature protein) and then the dry formulated ground feed (amount dependent on treatment, table 1) was added to the solution and stirred vigorously

with a metal whisk for 2 mins. The mixture was then poured into a 275 x 150 mm aluminum pan, which had been pre-cooled in an ice-water bath. The mixture solidified in approximately 5 mins. Once solid, the gel-based food was cut into rectangles of approximately 25x25x7 mm (length, width, height respectively). Previous research has illustrated square blocks with flat surfaces are an attractive food shape to *L. variegatus* (Klinger, 1982). Following production, the food blocks were stored under nitrogen gas and held in cold storage (appx. 4°C) to prevent/minimize bacterial or fungal growth. Fresh food blocks were prepared approximately every 4th day throughout the experimental period.



<b>Treatment</b>	<b>Protein (% as fed)</b>	<b>Carbohydrate (% as fed)</b>	<b>Food Density</b>	<b>Agar</b>	<b>32ppt Saltwater</b>
0%	0%	0%	0%	1.5% (7.5g)	492.5mL (19.7g salt)
27P:27C 1%	27%	27%	1% (5g)	1.5% (7.5g)	487.5mL (19.5g salt)
27P:27C 3%	27%	27%	3% (15g)	1.5% (7.5g)	477.5mL (19.1g salt)
27P:27C 6%	27%	27%	6% (30g)	1.5% (7.5g)	462.5mL (18.5g salt)
27P:27C 10%	27%	27%	10% (50g)	1.5% (7.5g)	442.5mL (17.7g salt)
27P:27C 14%	27%	27%	14% (70g)	1.5% (7.5g)	422.5mL (16.9g salt)
27P:27C 18%	27%	27%	18% (90g)	1.5% (7.5g)	402.5mL (16.1g salt)
38P:13c 1%	38%	13%	1% (5g)	1.5% (7.5g)	487.5mL (19.5g salt)
38P:13c 3%	38%	13%	3% (15g)	1.5% (7.5g)	477.5mL (19.1g salt)
38P:13c 6%	38%	13%	6% (30g)	1.5% (7.5g)	462.5mL (18.5g salt)
38P:13c 10%	38%	13%	10% (50g)	1.5% (7.5g)	442.5mL (17.7g salt)
38P:13c 14%	38%	13%	14% (70g)	1.5% (7.5g)	422.5mL (16.9g salt)
38P:13c 18%	38%	13%	18% (90g)	1.5% (7.5g)	402.5mL (16.1g salt)
18p:40C 1%	18%	40%	1% (5g)	1.5% (7.5g)	487.5mL (19.5g salt)
18p:40C 3%	18%	40%	3% (15g)	1.5% (7.5g)	477.5mL (19.1g salt)
18p:40C 6%	18%	40%	6% (30g)	1.5% (7.5g)	462.5mL (18.5g salt)
18p:40C 10%	18%	40%	10% (50g)	1.5% (7.5g)	442.5mL (17.7g salt)
18p:40C 14%	18%	40%	14% (70g)	1.5% (7.5g)	422.5mL (16.9g salt)
18p:40C 18%	18%	40%	18% (90g)	1.5% (7.5g)	402.5mL (16.1g salt)

Table 2. Experimental food treatments are shown. All components of the gel-based food blocks are listed. All percentages are based on a 500-mL total volume for a batch of gel-based food blocks. Diets included a standard reference diet (27% Protein, 27% Carbohydrate), a high protein diet (38% Protein, 13% Carbohydrate) or a high carbohydrate diet (18% Protein, 40% Carbohydrate).

Individually housed sea urchins (n=10 per treatment) were fed *ad libitum* a single pre-weighed food cube over a 24-hr period. After 24 hrs, the remaining food blocks were removed, rinsed with deionized water to remove surface salt, blotted dry, and weighed to measure daily consumption (wet weight) per animal. A new food cube was proffered each morning for an 11-day period. Wet feed intake measurements were used to calculate dry feed intake, dry matter intake, salt intake, energy intake, and macronutrient intake for sea urchins in each dietary treatment. Across dietary treatments, intake data were compiled and used to identify factors that contribute to satiety among sea urchins. Despite a relatively short trial, sea urchin wet weight gain (calculated as the difference between initial and final wet weight) was measured for each individual.

### Intake Calculations

Average daily feed intake (feed intake/individual/day) was calculated by the mean of daily intake for all individual sea urchins over the experimental period (11 days) (Equation 1). Autogenic controls were used for each treatment to standardize weight loss/gain of the food blocks due to leaching or absorption. Measured weight differences (percentage change of the food cube) in control blocks were used to adjust proffered weight before subtraction. Daily intake of formulated feed, salt, agar and total dry matter was determined (see equation 2, 3, 4, and 5, respectively). Salt content was empirically measured by drying salt water (32.2 ppt) samples at 50°C for 48 hrs in a convection drying oven. The remaining salt was weighed and salt content was calculated as an average of 0.04 g of salt per 1 g of saltwater. Total dry matter intake was calculated by the combined intake of dry formulated feed, agar, and salt. Protein and carbohydrate intake were determined by calculation based on dietary formulation (see equation 6 and

7, respectively). Energetic content of the three diets and agar was determined by bomb calorimetry. Energetic content was reported as 3277.4 Cal/g for the 38P:13C diet, 3173.0 Cal/g for the 27P:27C diet, 3215.5 Cal/g for the 18P:40C diet, and 3656.1 Cal/g for the agar powder. Daily energy intake was calculated as given in equation 8.

Equation 1

$$\text{Consumption}(g) = \text{Corrected proffered weight of food cube}(g) - \text{recovered weight of food cube}(g)$$

Equation 2

$$\text{Dry Feed Intake}(mg) = \text{Consumption}(g) * \text{Food Density}(\%) * 1000mg/1g$$

Equation 3

$$\text{Agar Intake}(mg) = \text{Consumption}(g) * \text{Agar Content}(\%) * 1000mg/1g$$

Equation 4

$$\begin{aligned} \text{Salt Intake}(mg) \\ = \text{Consumption}(g) * \text{Saltwater Intake}(\%) * \text{Salt}(g) * 1000mg/1g \end{aligned}$$

Equation 5

$$\begin{aligned} \text{Total Dry Matter Intake}(mg) = & \text{Dry formulated feed intake}(mg) \\ & + \text{salt intake}(mg) + \text{agar intake}(mg) \end{aligned}$$

Equation 6

$$\begin{aligned} \text{Protein Intake}(mg) = & \text{Dry formulated feed intake}(mg) \\ & * \text{protein content of diet}(\%) \end{aligned}$$

Equation 7

$$\begin{aligned} \text{Carbohydrate Intake}(mg) = & \text{Dry formulated feed intake}(mg) \\ & * \text{carbohydrate content of diet}(\%) \end{aligned}$$

Equation 8

$$\begin{aligned} \text{Energy Intake}(Cal) \\ = & \text{Dry Formulated Feed Intake}(mg) \\ & * \text{Energetic Content of Diet} \left( \frac{Cal}{g} \right) \end{aligned}$$

## Statistical Analysis

All statistical analyses were performed in R Studio. Test statistics were considered significant at a p-value  $< 0.05$ . Data for 0.5% food density of the 27P:27C diet treatment, which is included in some analyses in this thesis, were collected from a complementary experiment (see below). During the primary study described in this thesis, it became apparent the food densities utilized may not have been low enough to demonstrate a point of maximal feed intake. An aim of the present study was to identify the minimal amount of food (lowest density) that maximized sea urchin feed intake. Thus, a separate complementary study was designed to evaluate a lower food density, with 0.05% dry formulated feed. This experiment was conducted using the same methodology as described above for a period of 5 days with 5-10 sea urchins per treatment. Treatments of 0% and 1% food densities of the 27P:27C diet were fed in both experiments and consumption (wet weight intake) was used as a control between the two trials. A random effect linear model, using the R package *nlme* (Pinheiro et al., 2017), indicated no significant differences were present between these groups due to trial ( $p > 0.05$ ). In the model, both day and cohort were considered fixed effects and animal identification was considered a random effect. Thus, the data from 0.5% food density of the 27P:27C diet treatment from this separate trial were combined with data from the initial trial for further analyses.

For part I of the analysis, six random effects linear models were used to evaluate the differences of the 27P:27C dietary treatments (0-18%). Feed intake (wet weight), dry feed intake, agar intake, salt intake, total dry matter intake, and weight gain (wet weight) of individual sea urchins were each evaluated in separate models, using the R package

*nlme* (Pinheiro et al., 2017). In each analysis, food density was considered as a factor in the model, with individual animal identification as a random effect, so pairwise comparisons could be made. For each model, a Tukey's HSD *post-hoc* test was used to determine pairwise significance differences between food densities using the R package *multcomp* (Torsten et al., 2008). The 0% treatment was included in the feed intake (wet weight) analysis, but was excluded from other intake analyses, as intake was either not possible (e.g. no dry feed included in the block) or minimal. A broken line regression was also performed with the R packages *lm.br* and *Rcpp* (Eddelbuettel, 2013; Eddelbuettel and Francois 2011; Knowles et al., 1991) on individual weight gain data to indicate the food density of optimal weight gain. The 0.5% treatment was not included in the weight gain analysis since the experiment including the 0.05% treatment was performed in a separate trial.

For part II of the analysis, six random effects linear models were used to evaluate the differences of the 18 treatments in relation to both diet (27P:27C, 38P:13c, 18p:40C) and food density (1-18%), controlling for the effects of day, using the R package *nlme* (Pinheiro et al., 2017). AIC model selection indicated the effect of day was greatest when day was considered a continuous variable, thus day was considered as such in these models. In these analyses, food density was considered a factor in the model, with individual animal identification as a random effect, so pairwise comparisons could be made. For each model, a Tukey's HSD *post-hoc* test was used to determine pairwise significance differences, using the R package *multcomp* (Torsten et al., 2008). Tukey's HSD *post-hoc* test was used to determine pairwise significance differences within food densities across diets for feed intake (wet weight), dry feed intake, total dry matter intake,

protein intake, carbohydrate intake and energy intake. Tukey's HSD *post-hoc* test was used to determine pairwise significance differences within each diet across food densities for feed intake (wet weight), dry feed intake, total dry matter intake, protein intake, carbohydrate intake and energy intake. Agar contains both carbohydrate and energy sources and may or may not have digestible components for *L. variegatus*. Thus, carbohydrate and energy intake were evaluated both with and without including agar as a source of carbohydrate and energy.

For part III of the analysis, standard deviations for wet weight intake of total, between subjects, and within subject variation were extracted and plotted from the random effects linear model (mentioned above) for feed (wet weight) intake. Additionally, daily intake was plotted for individuals to evaluate patterns of variation over the experimental period. For the AIC analysis mentioned above, for each combination of diet and food density, two separate nested models were run. The first was a repeated measures regression in R (version 3.4.1) using the *lmer* function from the "lme4" library (version 1.1-13) with consumption as the dependent variable. The second model added a fixed day effect, where day was a continuous variable (1 df). The generic function AIC was used to calculate each models AIC value and the difference (model without day subtracted from the model with day). This difference, or delta AIC, should be interpreted as evidence in favor of one hypothesis compared to the other (Burnham and Anderson, 2002) and the small the value, the greater the day effect. The delta AI can be interpreted as an evidence ration, where  $\leq -6$  can be interpreted as evidence for a day effect (food consumption increasing or decreasing linearly over the course of the experiment).

## RESULTS

### Part I- Volume and Dry Matter Intake of a Reference Diet

Within the 27P:27C diet, sea urchins consumed from 1.5 to 7 grams per individual per day (Fig 2). Wet food intake was not significantly different between the 0.5% and 1% food density treatments, but both were significantly different from all other food densities (Tukey's HSD *post-hoc* test,  $p \leq 0.044$ ). When excluding pairwise comparisons of 14 and 18% and 14 and 10% food densities, all differences are highly significant ( $p < 0.001$ ).

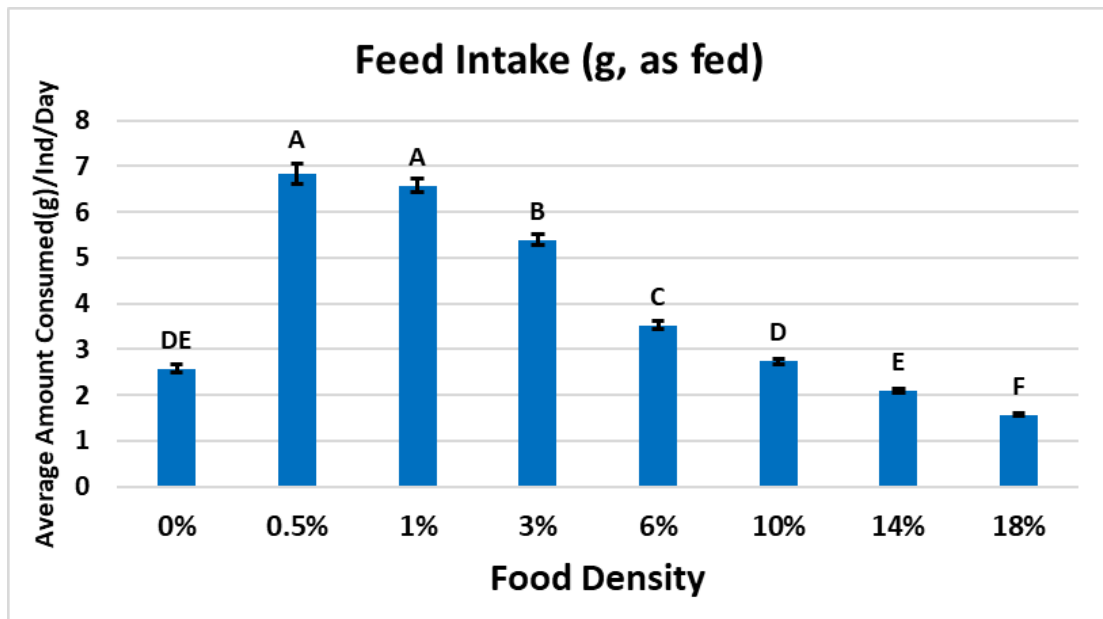


Figure 2. Average daily wet weight feed intake of gel-based food blocks. This includes total intake of the diet, which includes a dry powdered formulated feed, agar binder, and saltwater mass used to constitute the entire pellet of the 27P:27C diet (27% Protein + 27% Carbohydrate). The food density refers to the amount of the formulated feed as a percentage of the total mass of the constituted diet. Error bars represent SEM. Differing letters above columns illustrate significant differences determined by random effects linear model and a Tukey HSD test for *post-hoc* analysis ( $p \leq 0.044$ ;  $n=5-10$  replicates per treatment).

Wet food blocks are composed of several components, including dry ingredients containing specific nutrients (dry feed), salt, agar (as a binder), and water. Dry feed ingestion increased with increasing food density up to 10% and remained constant to 18% (Tukey's HSD *post-hoc* test,  $p \leq 0.001$ ).

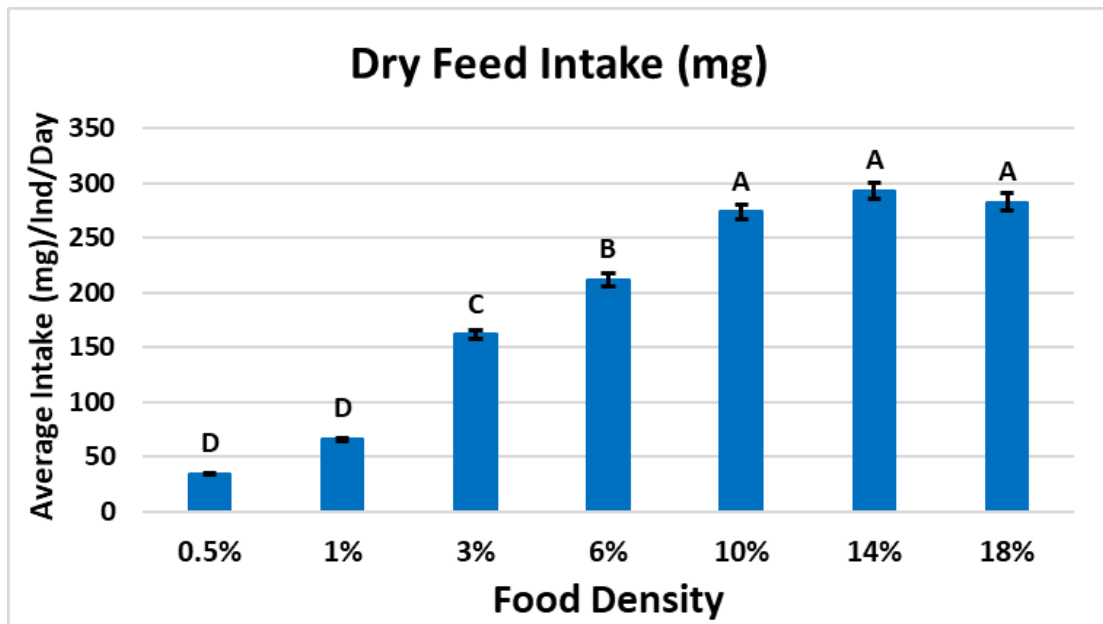


Figure 3. Average daily dry formulated feed intake of gel-based food blocks. The food density refers to the amount of the dry formulated feed as a percentage of the total mass of the constituted diet. Error bars represent SEM. Differing letters above columns illustrate significant differences determined by random effects linear model and a Tukey HSD test for *post-hoc* analysis ( $p < 0.001$ ;  $n=5-10$  individuals per treatment).

Due to compositional changes of the wet food blocks with increasing levels of dry feed, sea urchins consumed different amounts of agar across food densities (Fig 4). Total agar intake decreased significantly from 1 to 18% food densities (Tukey's HSD *post-hoc* test,  $p \leq 0.033$ ).



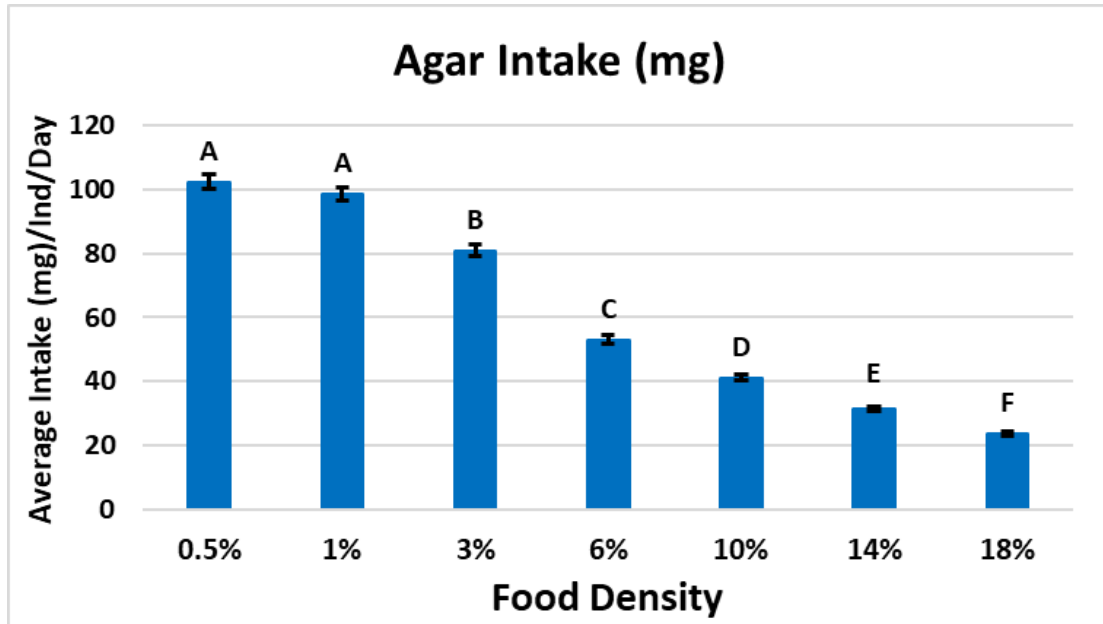


Figure 4. Average daily agar intake of gel-based food blocks. This includes total intake of agar, which is used as a binder to constitute the pellet of the 27P:27C diet (27% Protein + 27% Carbohydrate). The food density refers to the amount of the formulated feed as a percentage of the total mass of the constituted diet. Error bars represent SEM. Differing letters above columns illustrate significant differences determined by random effects linear model and a Tukey HSD test for *post-hoc* analysis ( $p \leq 0.033$ ;  $n=5-10$  replicates per treatment).

Sea urchins also consumed different amounts of sea salt across food densities (Fig 5). Salt intake decreased significantly from 1–18% food densities (Tukey’s HSD *post-hoc* test,  $p \leq 0.025$ ). Daily salt intake varied over 5-fold among the food densities tested.

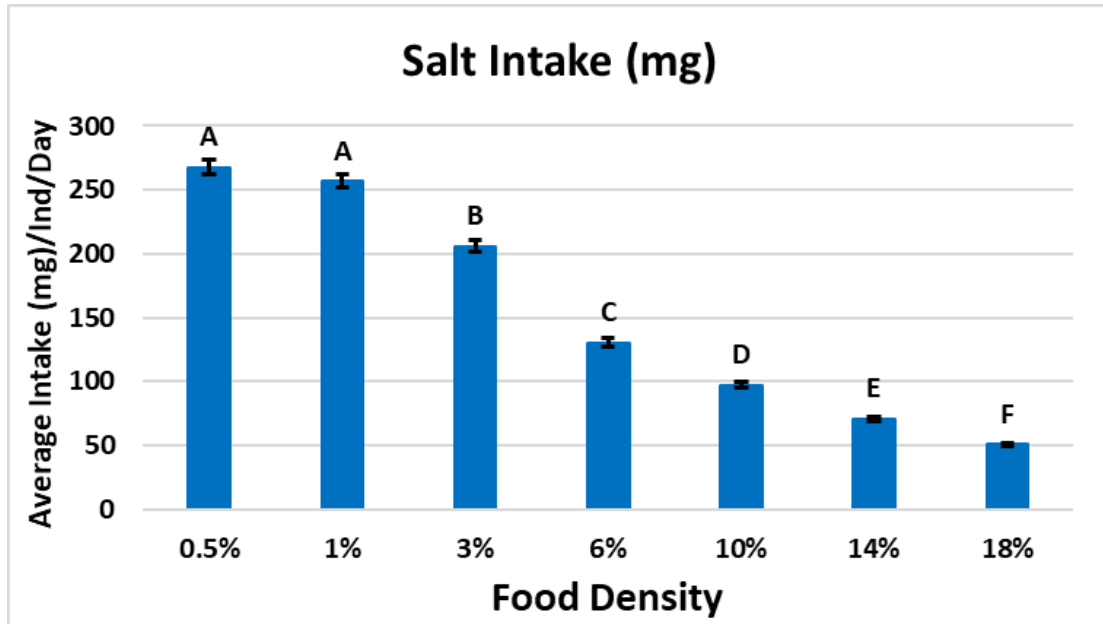


Figure 5. Average daily salt intake of gel-based food blocks. This includes total intake of salt which is used in part to constitute the 27P:27C diet (27% Protein + 27% Carbohydrate). The food density refers to the amount of the formulated feed as a percentage of the total mass of the constituted diet. Error bars represent SEM. Differing letters above columns illustrate significant differences determined by random effects linear model and a Tukey HSD test for *post-hoc* analysis ( $p \leq 0.025$ ;  $n=5-10$  replicates per treatment).

For all food densities (0.5%-18%), sea urchins consumed between 350–450 mg of total dry matter (combined dry feed, salt and agar) per individual per day (Fig 6).

Significant differences were found between some food densities, however not all groups are significantly different from each other (random effects linear model,  $p = 0.042$ ).

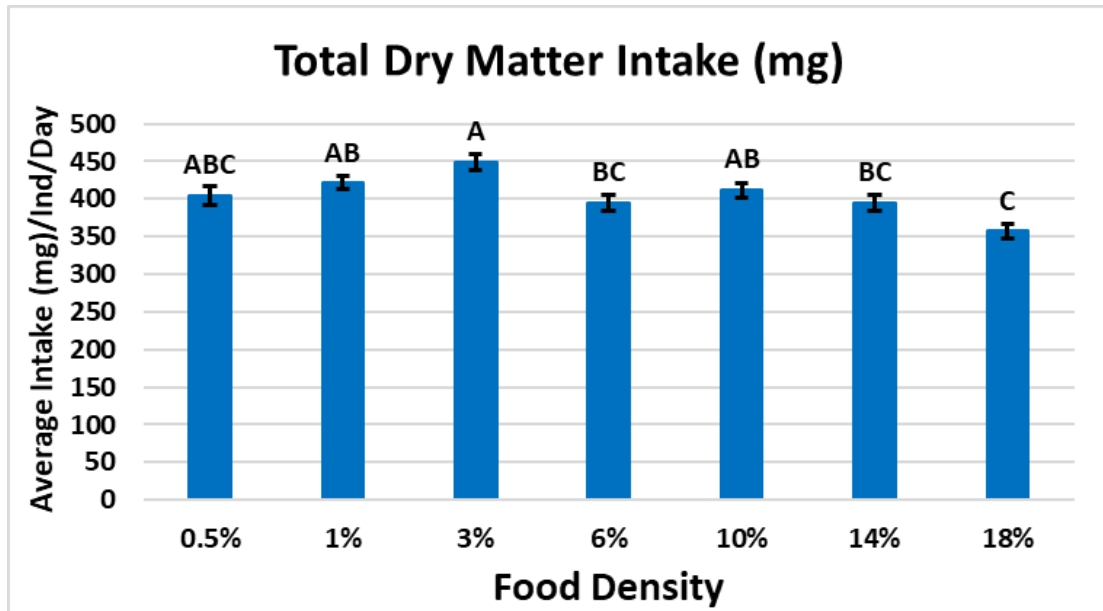


Figure 6. Average daily total dry matter intake of gel-based food blocks. This includes total intake of the dry feed, agar binder, and salt used to constitute the 27P:27C diet (27% Protein + 27% Carbohydrate). The food density refers to the amount of the formulated feed as a percentage of the total mass of the constituted diet. Error bars represent SEM. Differing letters above columns illustrate significant differences determined by random effects linear model and a Tukey HSD test for *post-hoc* analysis ( $p \leq 0.042$ ;  $n=5-10$  replicates per treatment).

Total wet weight gain for all individual sea urchins was maximized at 3% and greater food density (Fig 7). All sea urchins treatments (0 to 18% food density) gained wet weight over the 11-day period, with an average of 5.08 g gained per individual overall. Significant differences were determined among food densities (random effect liner model,  $p < 0.001$ ). Individual sea urchin wet weight gain at 3–18% food densities were not significantly different from each other but were significantly different from the 0% and 1% food densities (Tukey test,  $p < 0.0001$ ). A broken line regression indicated an inflection point at 2.104% food density, where increasing food density no longer has an increasing effect on weight gain.

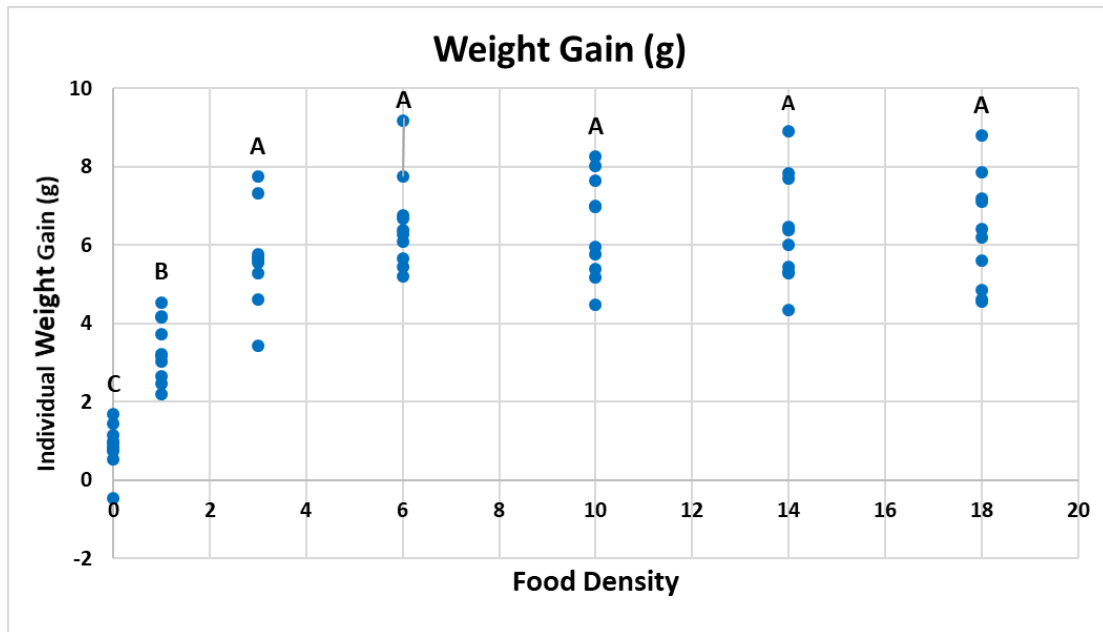


Figure 7. Total wet weight gain of sea urchins for each food density. Each dot represents one individual. The food density refers to the amount of the formulated feed as a percentage of the total mass of the constituted diet. Differing letters above columns illustrate significant differences determined by a random effects linear model and a Tukey HSD test for *post-hoc* analysis ( $p < 0.001$ ;  $n = 10$  replicates per treatment). The inflection point across food density was 2.104 (broken line regression). The slope before of the line before the inflection point was 6.055 and the slope of the line after the inflection point was 0.027.

## Part II- Macronutrient Intake of a Reference Diet

In this second comparison, the three diets varying in protein and carbohydrate content were evaluated. Sea urchins consumed approximately 1.5 to 7 grams per individual per day over the range of food densities tested (Fig 8). There was a significant effect of food density and a significant effect of diet on wet food intake) (random effects linear model,  $p \leq 0.005$ ); sea urchins consumed less wet food intake as food density increased for all three diets (27P:27C, 18p:40C, 38P:13c). There was a significant diet by food density interaction in which the 18p:40C diet decreased at a different rate than did the 27P:27C diet across food densities (random effects linear model,  $p = 0.004$ ). There was a significant effect of day on consumption (random effect linear model,  $p < 0.0001$ ). The effect of day and time will be further expanded in part III.

Within the 18P:40C diet, significant differences occurred across food densities, but not all food densities were significantly different (Tukey's HSD *post-hoc* test,  $p \leq 0.001$ ). In pairwise comparisons, 6 and 10%, 10 and 14%, and 14 and 18% food densities of the 18P:40C were not different. Within the 38P:13C diet, significant differences were present across food densities, with the exception of the 10 and 14% food densities (Tukey's HSD *post-hoc* test,  $p \leq 0.001$ ).

Despite significant differences among diets within a food density, sea urchins still consumed relatively similar amounts of food. At lower food densities (1 and 3%) greater amounts of the diet with a balanced ratio of protein: carbohydrate (27P:27C) was consumed than the two diets with higher variation in protein: carbohydrate ratios (18p:40C and 38P:13c). At 1% food density, sea urchins consumed significantly less of the low protein: high carbohydrate diet (18p:40C) when compared with the balanced diet (27P:27C). At 3% food density, significantly less of both extreme diets (38P:13c and 18p:40C) was consumed in comparison to the balanced diet (27P:27C) (Tukey's HSD *post-hoc* test,  $p \leq 0.0032$ ). Within the 6, 14, and 18% food densities, no significant differences in wet weight intake were illustrated across diets. At 10% food density, significantly less of the high protein diet (38P:13C) was consumed when compared to the balanced diet (27P:27C) (Tukey's HSD *post-hoc* test,  $p \leq 0.049$ ). An interesting trend to note was the slight increase in consumption of the high protein diet (38P:13c) when compared with the low protein diet (18p:40C) at lower food densities (1–6%). However, at higher food densities (10–18%) a trend of slightly less consumption of the high protein diet (38P:13c) occurred when compared with the low protein diet (18p:40C).

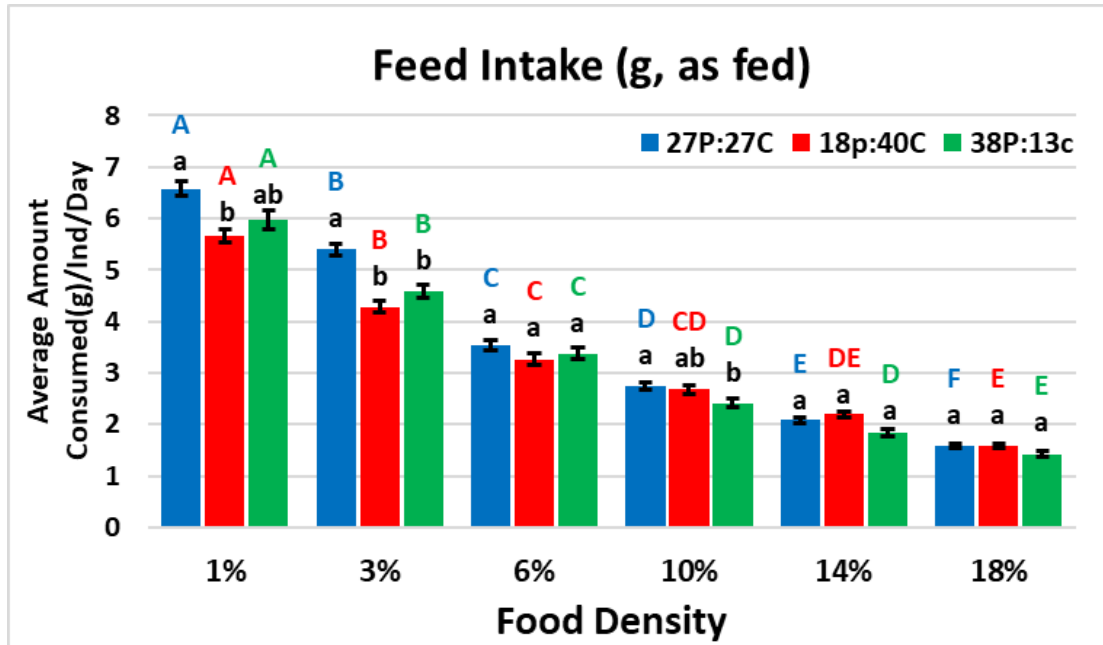


Figure 8. Average daily wet weight feed intake of gel-based food blocks for the 3 diets. This includes total intake of the diet, which includes a dry powdered formulated feed, agar binder, and saltwater mass used to constitute the entire pellet of the respective diet. The food density refers to the amount of the formulated feed as a percentage of the total mass of the constituted diet. Error bars represent SEM. Differing colored upper-case letters above columns illustrate significant differences within a diet across food densities determined by random effects linear model and a Tukey HSD test for *post-hoc* analysis ( $p \leq 0.024$ ;  $n=10$  replicates per treatment). Differing black lower-case letters above columns illustrate significant differences within a food density across the three diets determined by random effects linear model and a Tukey HSD test for *post-hoc* analysis ( $p \leq 0.049$ ;  $n=10$  replicates per treatment).

When comparing all three diets, sea urchins consumed ranges of dry feed from approximately 50 to 300 mg per individual per day (Fig 9). Sea urchins consumed more dry feed as food density increased, despite consuming less wet food weight for all three diets. There is a significant effect of food density on dry formulated feed intake (random effects linear model,  $p \leq 0.0001$ ). There was not a significant effect of diet on dry feed intake. Within the 27P:27C diet and the 38P:13c diet, a significant increase in feed intake across food densities occurred from 1–10%, with a maximum at 10% or higher food density (Tukey's HSD *post-hoc* test,  $p \leq 0.001$ ;  $p \leq 0.037$ ). Within the 18p:40C diet, a significant increase in intake across food densities occurred from 1 to 14% (Tukey's HSD

*post-hoc* test,  $p \leq 0.021$ ). In pairwise comparison of the 18p:40C diet, feed intake of 10 and 18%, and 14 and 18% are not different.

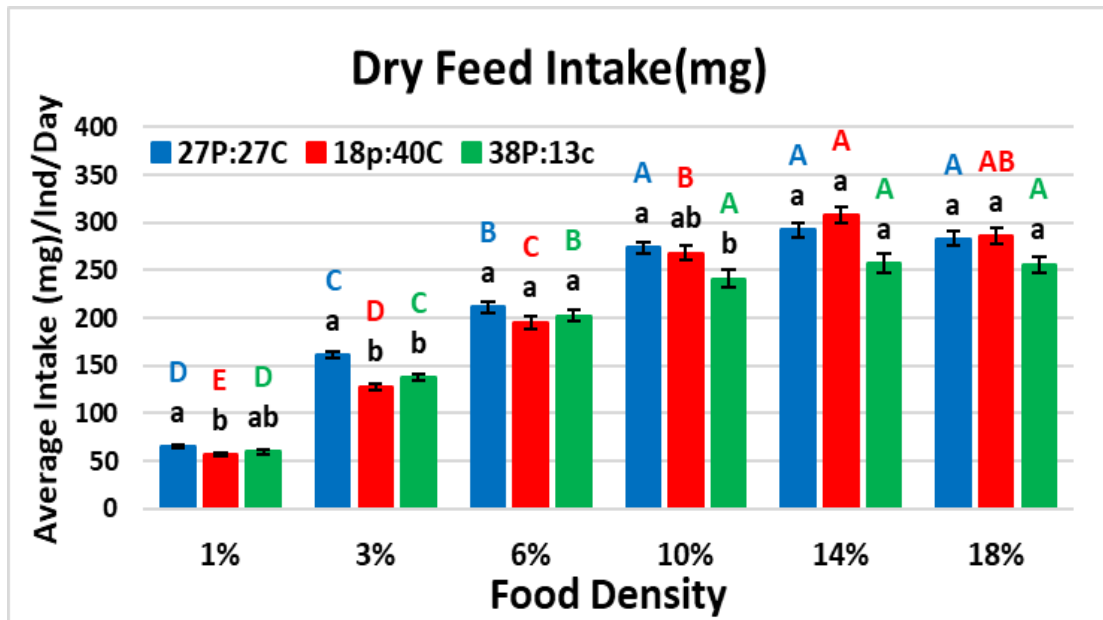


Figure 9. Average daily dry formulated feed intake of gel-based food blocks for all diets. The food density refers to the amount of the formulated feed as a percentage of the total mass of the constituted diet. Error bars represent SEM. Differing colored upper-case letters above columns illustrate significant differences within a diet across food densities determined by random effects linear model and a Tukey HSD test for *post-hoc* analysis ( $p \leq 0.037$ ;  $n=10$  replicates per treatment). Differing black lower-case letters above columns illustrate significant differences within a food density across the three diets determined by random effects linear model and a Tukey HSD test for *post-hoc* analysis ( $p \leq 0.049$ ;  $n=10$  replicates per treatment).

When all three diets are compared, total dry matter intake ranges from 325–450 mg per individual sea urchin per day across all food densities (Fig 10). Diet and food density had significant effects on total dry matter intake (random effects linear model,  $p \leq 0.035$ ). There was a significant diet by food density interaction in which the 18p:40C diet decreases at a different rate than 27P:27C diet (random effects liner model,  $p < 0.001$ ).

Sea urchin consumed relatively similar amounts of total dry matter across all diets and food densities. Significant differences occurred across food densities within the 27P:27C diet and the 38P:13c diet (Tukey's HSD *post-hoc* test,  $p \leq 0.037$ ) with

differences mainly occurring between the high and low food densities (1% and 3% when compared to 18%). No significant differences in total dry matter intake were detected for the 18p:40C diet across food densities.

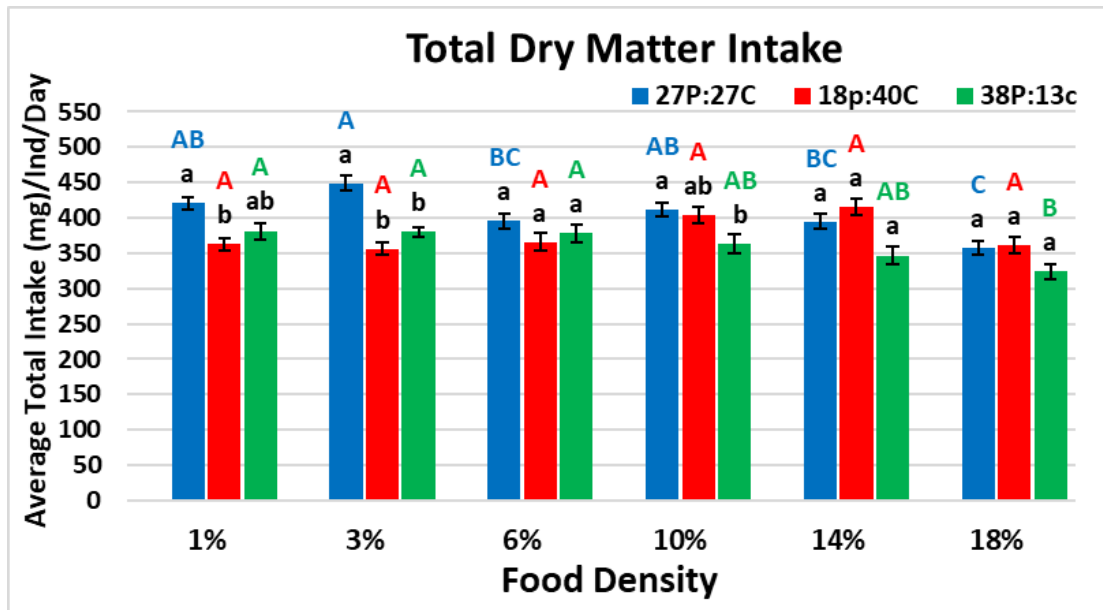


Figure 10. Average daily total dry matter intake of gel-based food blocks for all diets. This represents total dry matter intake of the diet, which includes a dry powdered formulated feed, agar binder, and salt (of the saltwater matrix) used to constitute the entire pellet of each diet. The food density refers to the amount of the formulated feed as a percentage of the total mass of the constituted diet. Error bars represent SEM. Differing colored upper-case letters above columns illustrate significant differences within a diet across food densities determined by random effects linear model and a Tukey HSD test for *post-hoc* analysis ( $p \leq 0.037$ ;  $n=10$  replicates per treatment). Differing black lower-case letters above columns illustrate significant differences within a food density across the three diets determined by random effects linear model and a Tukey HSD test for *post-hoc* analysis ( $p \leq 0.049$ ;  $n=10$  replicates per treatment).

When all three diets are compared, sea urchins consumed variable amounts of protein per individual per day (Fig 11). Diet and food density had significant effects on protein intake (random effects linear model,  $p \leq 0.007$ ). Within each diet, a maxima of protein intake occurred at 10% and greater food densities. However, the amount of protein intake at which the maxima occurs is different for each diet. Within both the 27P:27C and the 38P:13c diets, significant increases in protein intake are detected from food densities of 1-10% for each diet (Tukey's HSD *post-hoc* test,  $p \leq 0.035$ ). Within both



the 27P:27C and the 38P:13c diets significant differences in protein intake are not detected at 10–18% food densities for each diet. Within the 18p:40C diet, significant differences in protein intake are detected from 1–14% (Tukey's HSD *post-hoc* test,  $p \leq 0.021$ ).

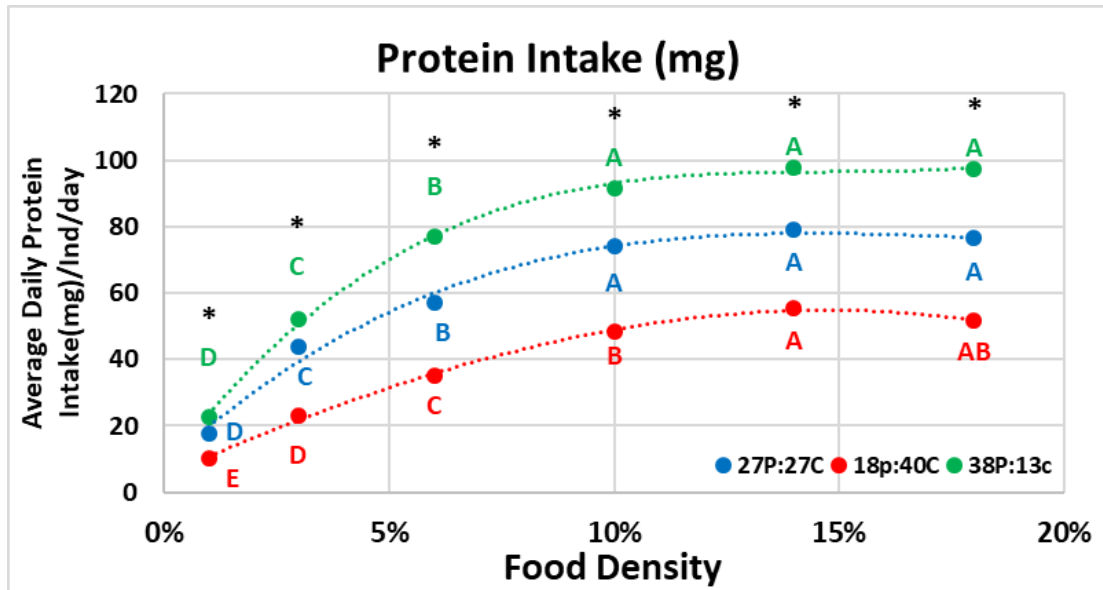


Figure 11. Average daily protein intake of gel-based food blocks for each diet. The food density refers to the amount of the formulated feed as a percentage of the total mass of the constituted diet. Differing colored upper-case letters above or below points illustrate significant differences within a diet across food densities determined by random effects linear model and a Tukey HSD test for *post-hoc* analysis ( $p \leq 0.035$ ;  $n=10$  replicates per treatment). Black asterisks above points illustrate significant differences within a food density across the three diets determined by random effects linear model and a Tukey HSD test for *post-hoc* analysis ( $p \leq 0.018$ ;  $n=10$  replicates per treatment).

When comparing all three diets, sea urchins consumed variable amounts of carbohydrate per individual per day. When all three diets are compared, sea urchins consumed variable amounts of carbohydrate (Fig 11). When carbohydrate intake derived from the dry feed was evaluated, diet and food density had significant effects on carbohydrate intake (random effects linear model,  $p \leq 0.002$ ). Within each diet, an increase of carbohydrate intake is seen across food densities, with a decreasing rate of

intake at higher food densities. Within both the 27P:27C and the 38P:13c diets, significant increases in carbohydrate intake are detected from food densities 1–10% for each diet (Tukey's HSD *post-hoc* test,  $p \leq 0.027$ ). Within both the 27P:27C and the 38P:13c diets significant differences are not detected at 10–18% food densities for each diet. Within the 18p:40C diet, significant differences in carbohydrate intake are detected from 1–14% (Tukey's HSD *post-hoc* test,  $p \leq 0.02$ ). When comparing within food densities across diets, highly significant differences were detected for carbohydrate intake across diets for all food densities (Tukey's HSD *post-hoc* test,  $p \leq 0.0001$ ).

When carbohydrate intake derived from both the dry feed and agar (100% carbohydrate) was evaluated, diet and food density had significant effects on carbohydrate intake (random effects linear model,  $p \leq 0.002$ ). There was a significant diet by food density interactions in which the carbohydrate intake of 18p:40C diet and the 38P:13c diets change at different rates than 27P:27C diet (random effects liner model,  $p \leq 0.001$ ). Within all three diets, sea urchins consumed significantly different amounts of total carbohydrate when comparing low (1-3%) and high food densities (18%). Within both the 27P:27C and the 38P:13c diets, significant decreases in total carbohydrate intake are detected from food densities 1-18% (Tukey's HSD *post-hoc* test,  $p \leq 0.049$ ). Within 18p:40C diet, significant increases in total carbohydrate intake is detected from 1–18% food densities (Tukey's HSD *post-hoc* test,  $p \leq 0.038$ ).

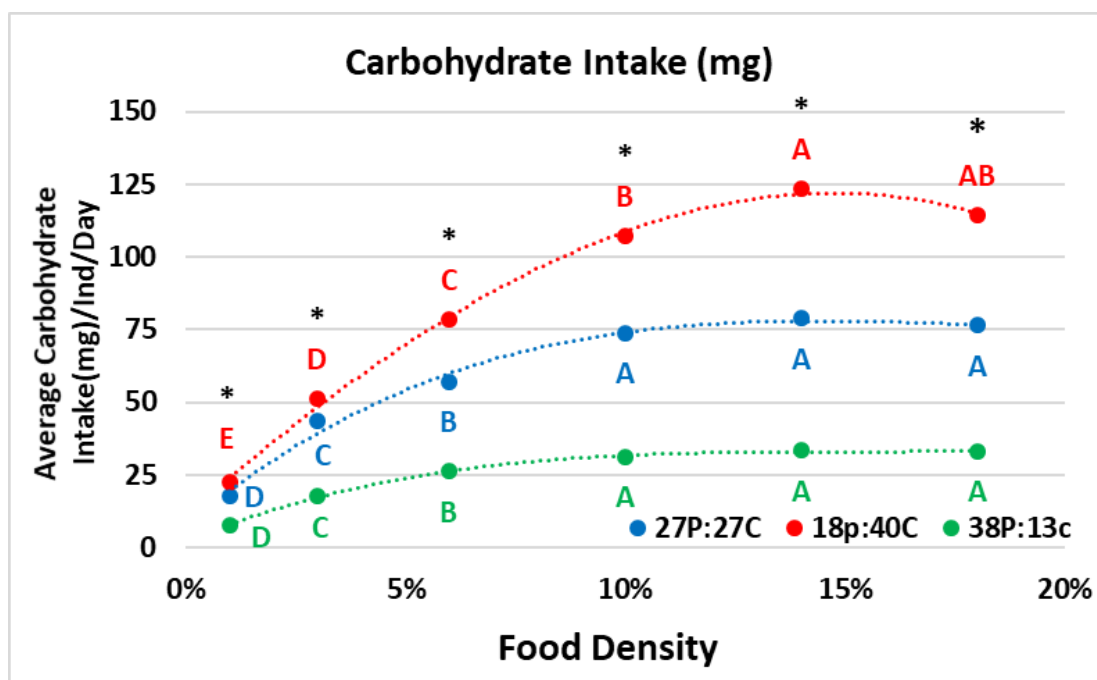


Figure 12. Average daily carbohydrate intake, provided by dry formulated feed, of gel-based food blocks. The food density refers to the amount of the formulated feed as a percentage of the total mass of the constituted diet. Differing colored upper-case letters above or below points illustrate significant differences within a diet across food densities determined by random effects linear model and a Tukey HSD test for *post-hoc* analysis ( $p \leq 0.027$ ;  $n=10$  replicates per treatment). Black asterisks above points illustrate significant differences within a food density across the three diets determined by random effects linear model and a Tukey HSD test for *post-hoc* analysis ( $p \leq 0.001$ ;  $n=10$  replicates per treatment).

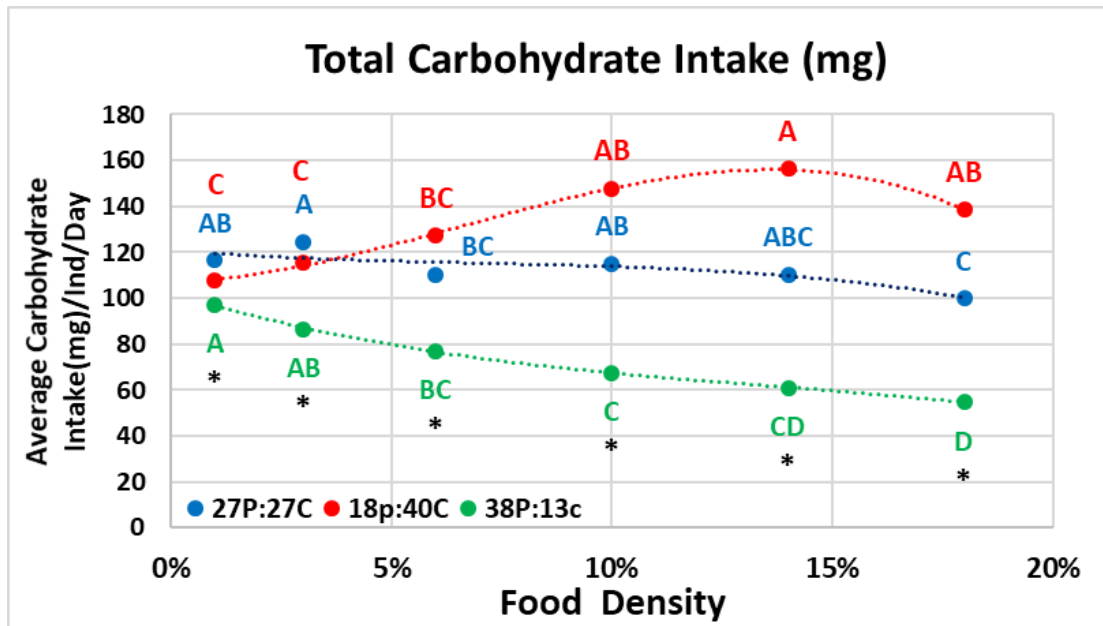


Figure 13. Average daily carbohydrate intake, provided by dry formulated feed and agar binder, of gel-based food blocks. The food density refers to the amount of the formulated feed as a percentage of the total mass of the constituted diet. Differing colored upper-case letters above or below points illustrate significant differences within a diet across food densities determined by random effects linear model and a Tukey HSD test for *post-hoc* analysis ( $p \leq 0.049$ ;  $n=10$  replicates per treatment). Black asterisks below points illustrate significant differences within a food density across the three diets determined by random effects linear model and a Tukey HSD test for *post-hoc* analysis ( $p \leq 0.016$ ;  $n=10$  replicates per treatment).

When comparing all three diets, sea urchins consumed varying amounts of energy per individual per day. The dry feed contains primarily soluble, digestible carbohydrates. When considering only the energy provided from the dry feed, there was a significant effect of food density on energy intake (random effects linear model,  $p \leq 0.0001$ ), but there was not a significant effect of diet on energy intake. When considering energetic content provided from both the dry feed and agar, there were significant effects of both food density and diet on energy intake, with the 18p:40C and 27P:27C diets differing significantly (random effects linear model,  $p \leq 0.005$ ).

When considering only the energy provided by dry feed, significant differences in energy intake were detected both within diets across food densities and within food densities across diet. Within diets across food density of 1-10%, significant increases in

energy intake were detected for all three diets (Tukey's HSD *post-hoc* test,  $p \leq 0.027$ ). At food densities of 10–18% significant differences in energy intake were only detected for the 18p:40C diet, with the 10 and 14% food densities differing significantly (Tukey's HSD *post-hoc* test,  $p \leq 0.02$ ). When comparing within each food density across diets, significant differences were only detected at 3% food density, with the 27P:27C diet differing from both the 18p:40C and 38P:13c diets (Tukey's HSD *post-hoc* test,  $p \leq 0.0346$ ).

When considering the energy provide by dry formulated feed and agar powder, significant differences in energy intake are detected both within diets across food densities and within food densities across diet. When comparing within the 27P:27C and 18p:40 diets across food densities, significant differences in energy intake were detected at food densities of 1–10% within each diet (Tukey's HSD *post-hoc* test,  $p \leq 0.0254$ ). At 10–18% food densities, no significant differences were detected within each diet (27P:27C and 18p:40). When comparing within the 38P:13c diet across food densities, significant differences were detected between food densities of 1-6% (Tukey's HSD *post-hoc* test,  $p \leq 0.048$ ). At food densities of 6-18%, no significant differences were detected within the 38P:13c diet. When comparing within each food density across diets, significant difference were only detected at 1 and 3% food densities (Tukey's HSD *post-hoc* test,  $p \leq 0.041$ ).

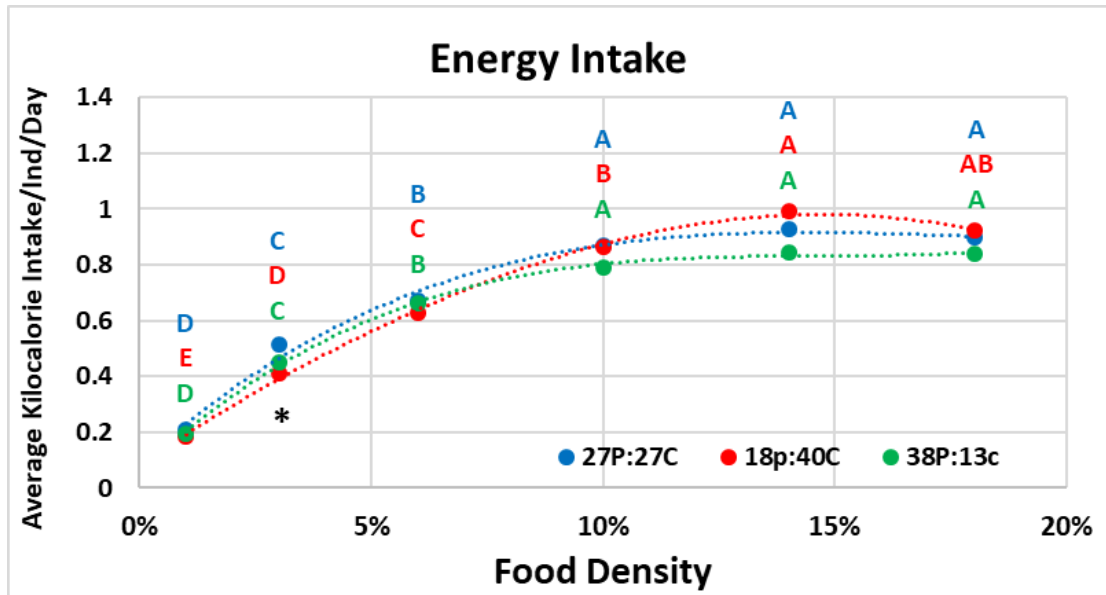


Figure 14. Average daily energy (caloric) intake, provided by dry formulated feed only, of gel-based food blocks. Bomb calorimetry was used to evaluate the total energetic value of the diets. The food density refers to the amount of the formulated feed as a percentage of the total mass of the constituted diet. Error bars represent SEM. Differing colored upper-case letters above points illustrate significant differences within a diet across food densities determined by random effects linear model and a Tukey HSD test for *post-hoc* analysis ( $p \leq 0.027$ ;  $n=10$  replicates per treatment). Black asterisks below points illustrate significant differences within a food density across the three diets determined by random effects linear model and a Tukey HSD test for *post-hoc* analysis ( $p \leq 0.0364$ ;  $n=10$  replicates per treatment).

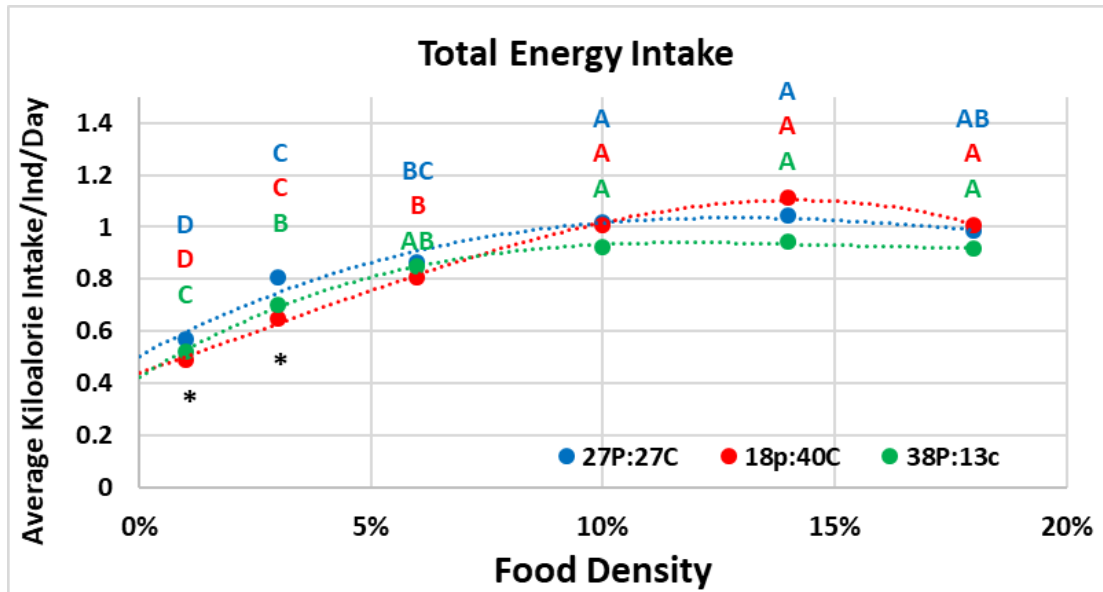


Figure 15. Average daily energy (caloric) intake, provided by dry formulated feed and agar binder, of gel-based food blocks. Bomb calorimetry was used to evaluate the total energetic value of the diets and agar binder. The food density refers to the amount of the formulated feed as a percentage of the total mass of the constituted diet. Error bars represent SEM. Differing colored upper-case letters above points illustrate significant differences within a diet across food densities determined by random effects linear model and a Tukey HSD test for *post-hoc* analysis ( $p \leq 0.0254$ ;  $n=10$  replicates per treatment). Black asterisks below points illustrate significant differences within a food density across the three diets determined by random effects linear model and a Tukey HSD test for *post-hoc* analysis ( $p \leq 0.041$ ;  $n=10$  replicates per treatment).

### Part III-Variation in Food Intake and Patterns of Daily Food Intake

Feed intake varied each day during the experimental period. Variance was calculated as total variance (including within and between individuals), and also calculated to both variance within an individual over time and between individuals over time. When comparing standard deviation in feed intake (wet weight) across the experimental period for all three diets, total standard deviation for feeding was highest at 1% food density for all three diets (Fig 16). Total standard deviation generally decreased as food density increased for all diets.

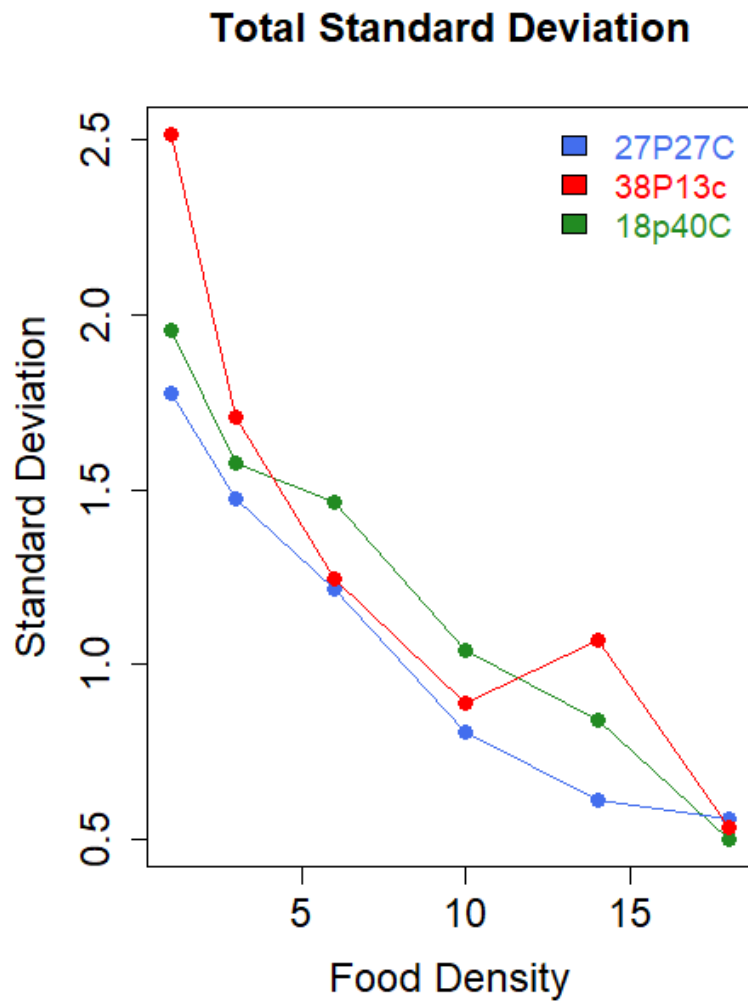


Figure 16. Total standard deviation in feed intake (wet weight intake) for the three diets. The food density refers to the amount of the formulated feed as a percentage of the total mass of the constituted diet.

When total standard deviation is separated into those attributes contributing to variation, principally within individual and between individual variance, the within individual variance accounts for a greater portion of the total variance than the between individual variance. When comparing standard deviation within individuals in feed intake (wet weight) across the experimental period for all three diets, between individual standard deviation for feeding was still highest at 1% food density for all three diets (Fig



18). Within individual standard deviation generally decreased as food density increased for all diets.

When comparing standard deviation between individuals in feed intake (wet weight) across the experimental period for all three diets, between individual standard deviation was still highest at 1% food density for all three diets (Fig 17). Between individual standard deviation generally decreased as food density increased for all diets, with an exception of the 14% food density of the 38P:13c diet.

## Standard Deviation Within Individuals

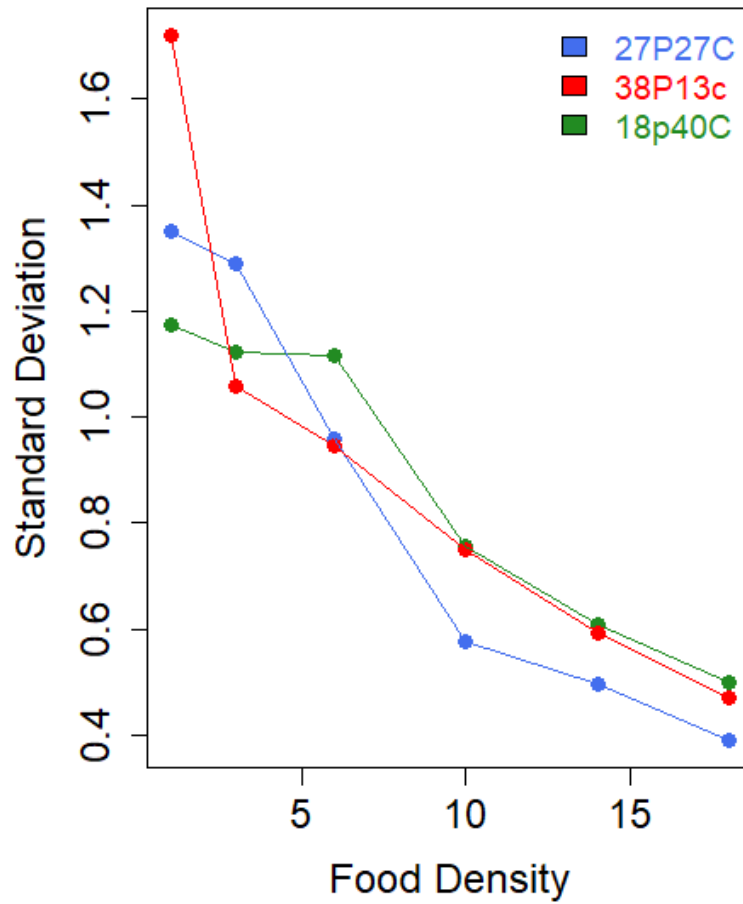


Figure 17. Standard deviation within individuals in feed intake (wet weight intake) for the three diets. The food density refers to the amount of the formulated feed as a percentage of the total mass of the constituted diet.

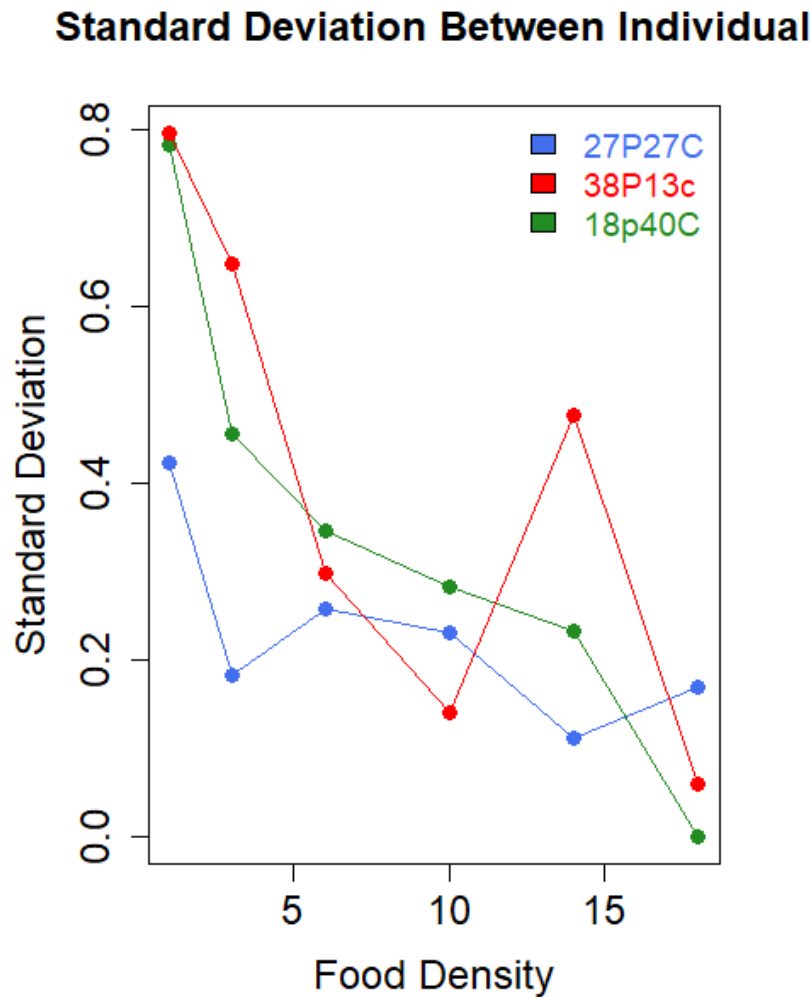


Figure 18. Standard deviation between individuals in feed intake (wet weight intake) for the three diets. The food density refers to the amount of the formulated feed as a percentage of the total mass of the constituted diet.

Based on an AIC model, day was treated as a continuous variable for the analysis in the present study. Figure 19 depicts whether consumption (wet weight intake) increased decreased or remained the same over the experimental period for each combination of diet and food density. All delta AIC values less than or equal to  $-6$  should be interpreted as strong evidence of a linear day effect (at least a 20:1 evidence ratio), while values greater than  $-6$  should be imply that no linear day effect was detected

(Burnham and Anderson, 2002). For all 5 diet and food density combinations with high evidence of a linear trend in food consumption (38P:13c 3, 6 and 10% and 27P:27C 10 and 14%), the trend was positive and consumption increased throughout the experiment.

When comparing feed intake (wet weight intake) across diets and food densities, day (time) had a significant effect on consumption overall (random effects linear model,  $p < 0.0001$ ). However, the effect of time varied when compared within treatments. Daily intake for individual sea urchins for each diet and food density combinations are shown in figures 20-37. With the 27P:27C 1, 3, and 6% food densities, there was no significant effect of day(time) on consumption. Within the 27P:27C 10, 14 and 18% food densities, there was a significant effect of time (random effects linear model,  $p < 0.004$ ). When the 27P:27C food densities were tracked daily across individuals, the combined slope over time remained minimal (Figs 20–25). Within the 18p:40C diet, only the 10% food density was significantly affected by day (random effects linear model,  $p < 0.001$ ). Similar to the 27P:27C groups, when the 18p:40C groups are tracked daily by individual, the slope over time remained minimal. Within the 38P:13c diet, consumption at all food densities is significantly affected by day (random effects linear model,  $p < 0.003$ ). Within the 38P:13c diet, when intake is tracked daily by individual, the combined trend in consumption seems to increase over time for most food densities, with the most exaggerated increases at the 3, 6, and 10%. This was predicted by the AIC model which suggested the strongest effect of day occurred at these food densities within the high protein diet.

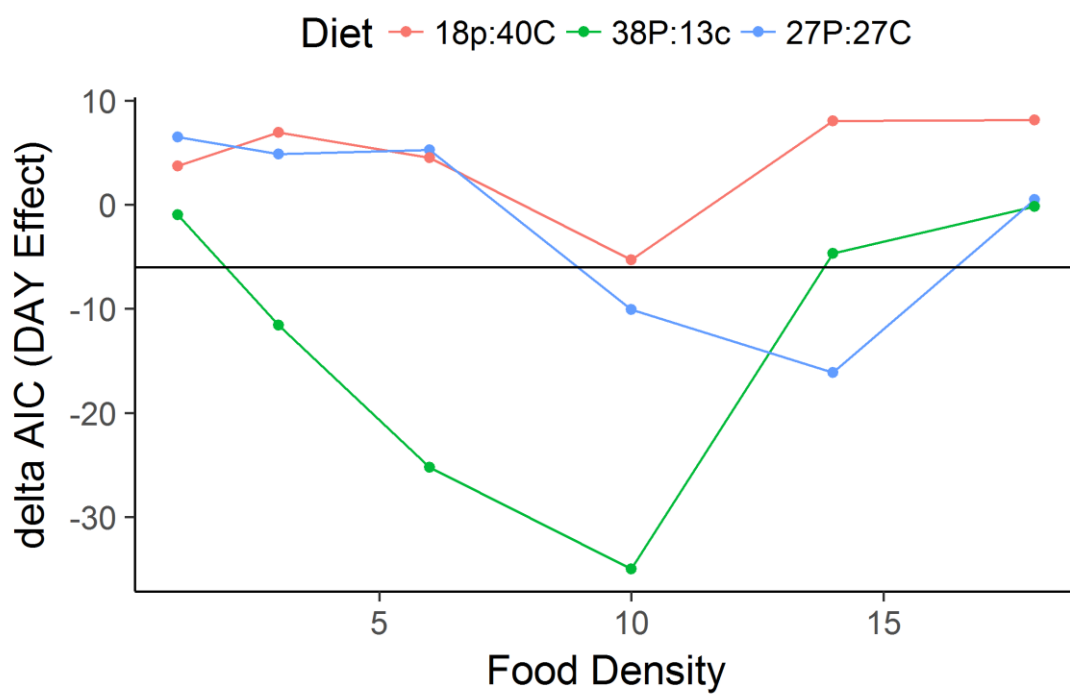


Figure 19. Dot and line plot depicting the importance of the continuous variable DAY, for each combination of diet and food density. Importance is quantified using the difference in AIC scores between models with and without a DAY effect. Values less than  $-6$  (those falling below the horizontal solid black line) have an evidence ration of at least 20:1 in favor of a DAY effect.

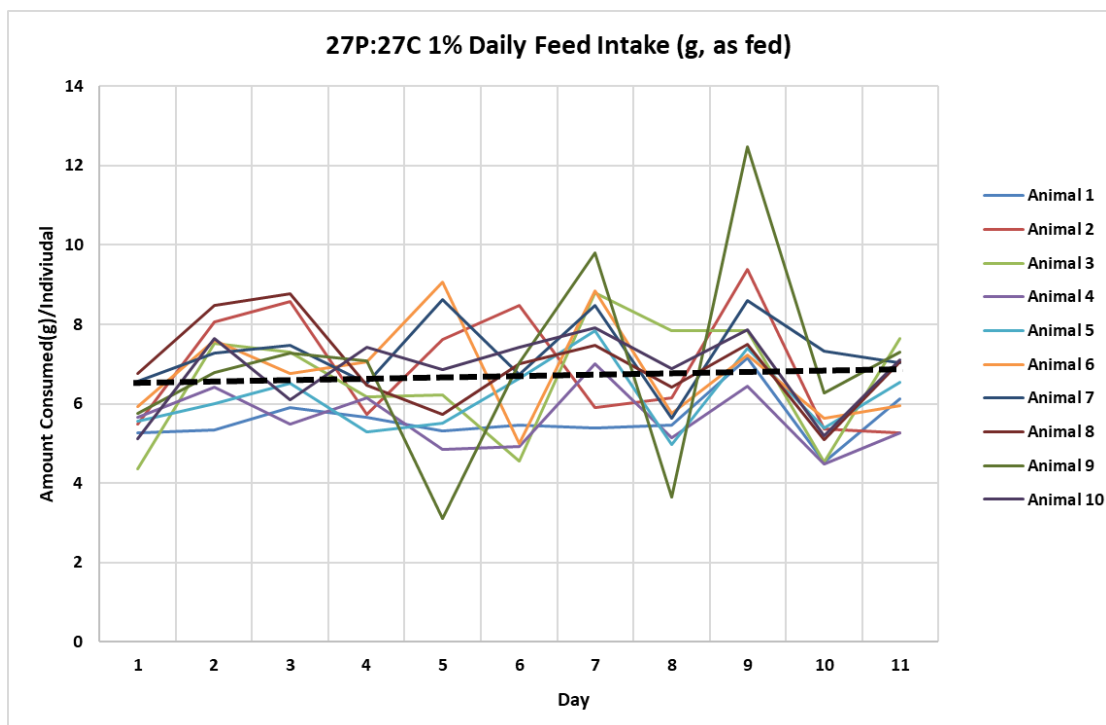


Figure 20. Daily feed intake (wet weight) for individuals fed the 27P:27C diet at 1% food density. Each colored line represents intake for an individual (1-10). The dashed black line illustrates the trend over the 11-day period for all individuals.

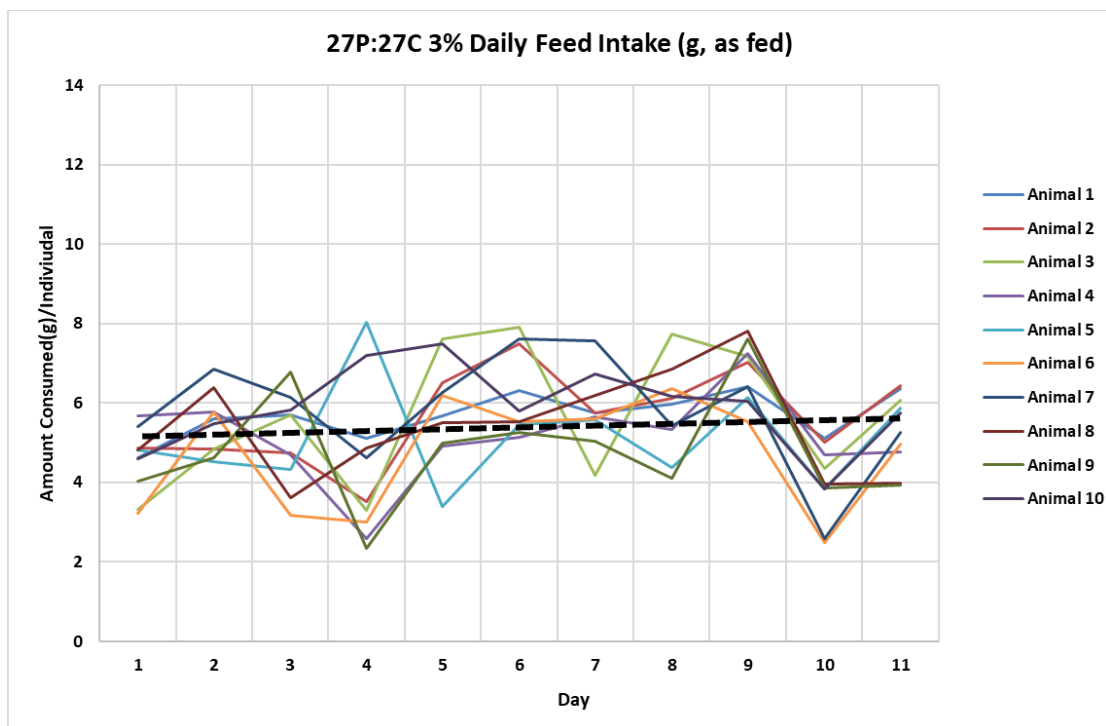


Figure 21. Daily feed intake (wet weight) for individuals fed the 27P:27C diet at 3% food density. Each colored line represents intake for an individual (1-10). The dashed black line illustrates the trend over the 11-day period for all individuals.

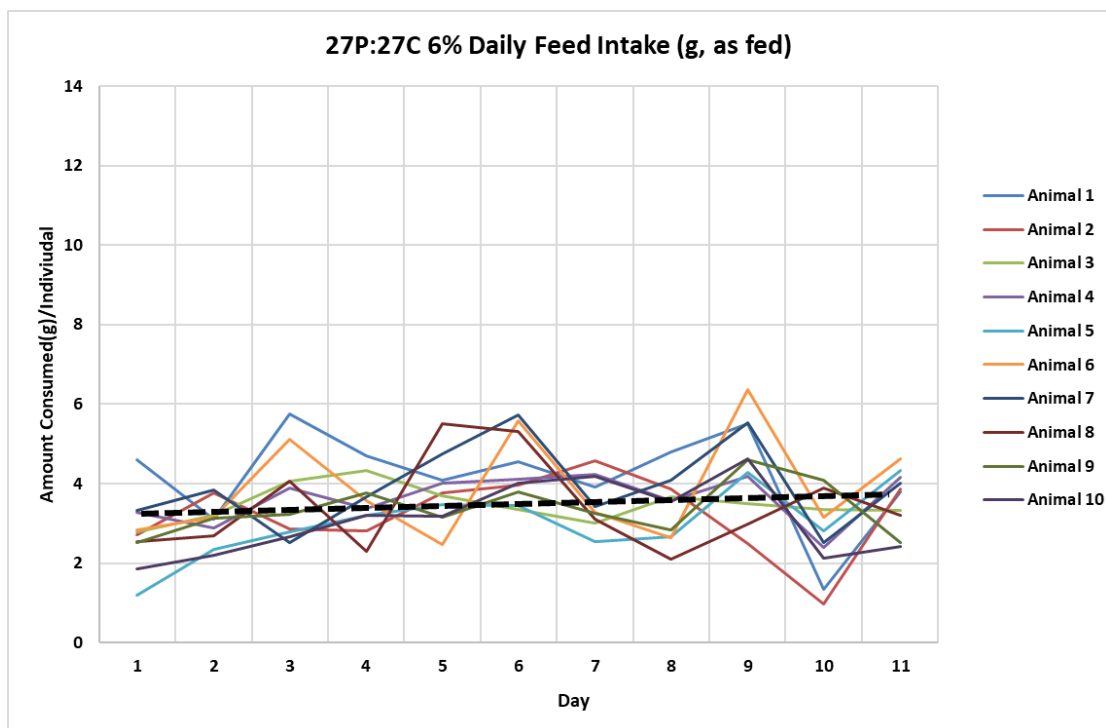


Figure 22. Daily feed intake (wet weight) for individuals fed the 27P:27C diet at 6% food density. Each colored line represents intake for an individual (1-10). The dashed black line illustrates the trend over the 11-day period for all individuals.

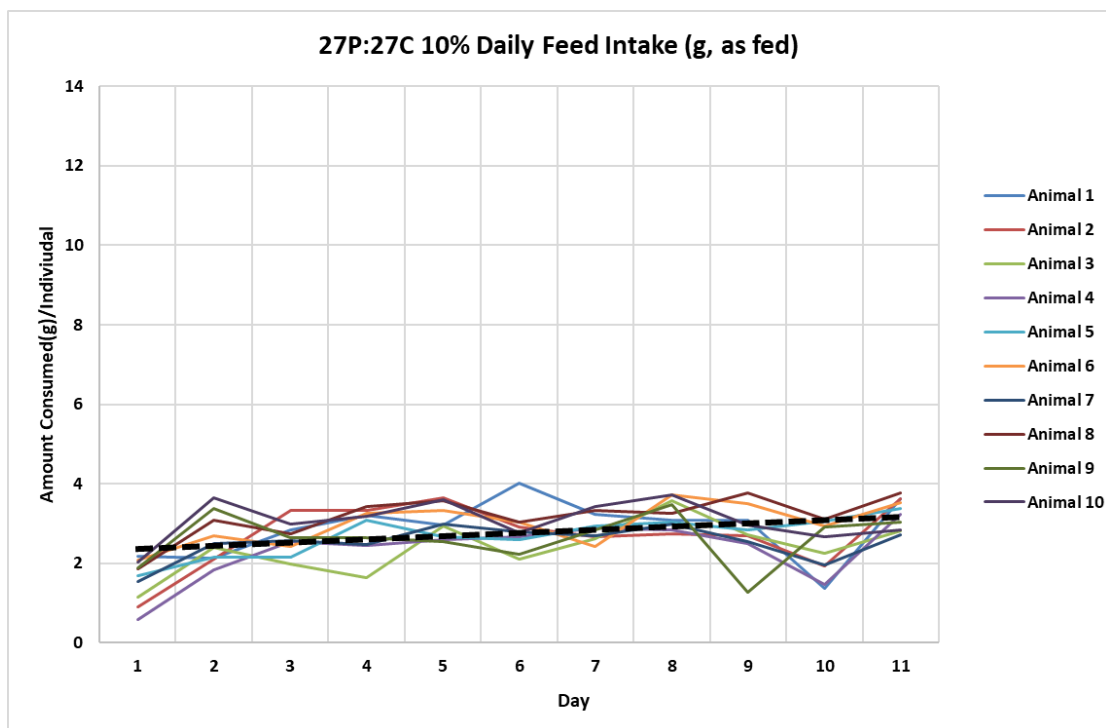


Figure 23. Daily feed intake (wet weight) for individuals fed the 27P:27C diet at 10% food density. Each colored line represents intake for an individual (1-10). The dashed black line illustrates the trend over the 11-day period for all individuals.

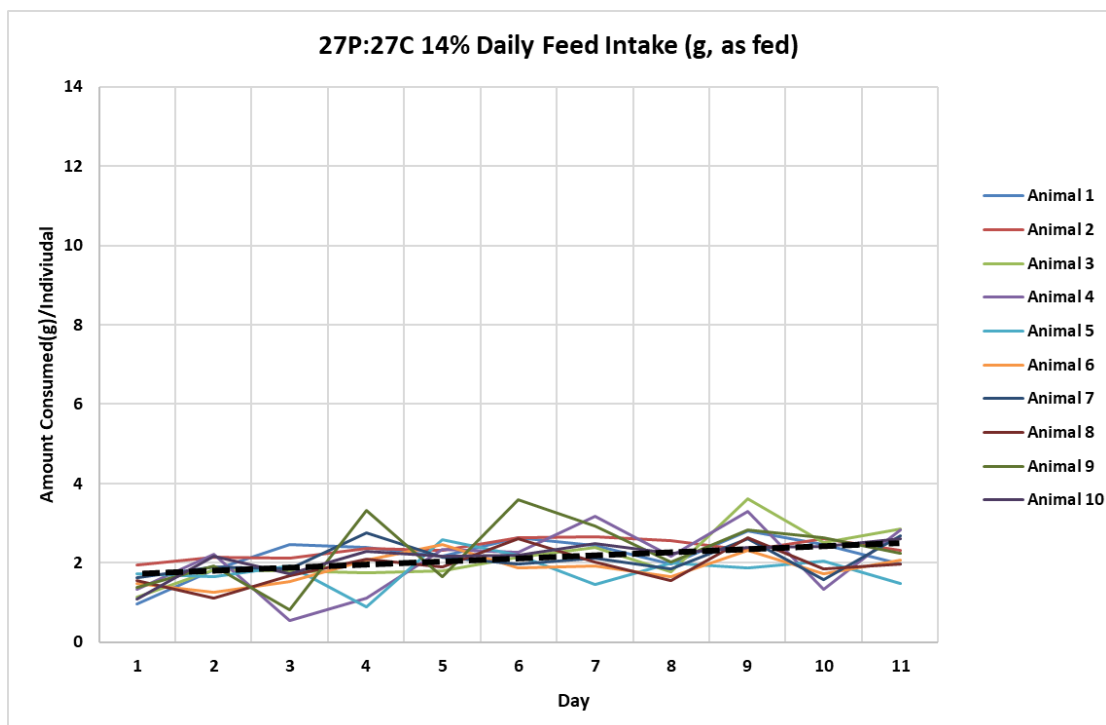


Figure 24. Daily feed intake (wet weight) for individuals fed the 27P:27C diet at 14% food density. Each colored line represents intake for an individual (1-10). The dashed black line illustrates the trend over the 11-day period for all individuals.

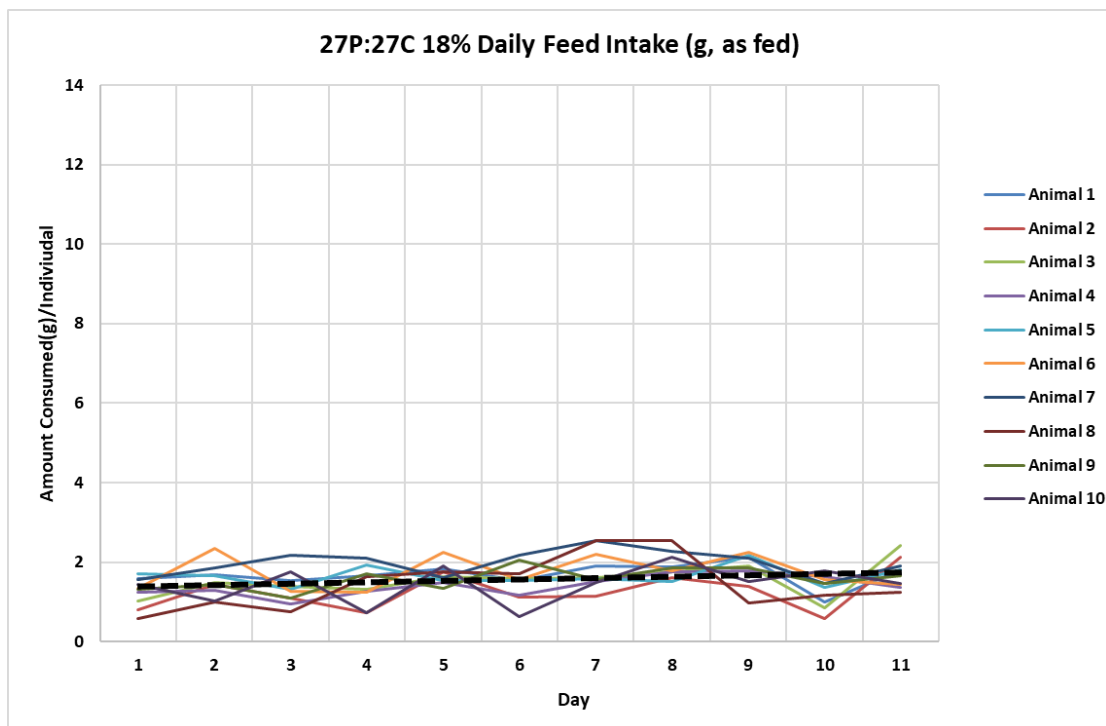


Figure 25. Daily feed intake (wet weight) for individuals fed the 27P:27C diet at 18% food density. Each colored line represents intake for an individual (1-10). The dashed black line illustrates the trend over the 11-day period for all individuals.



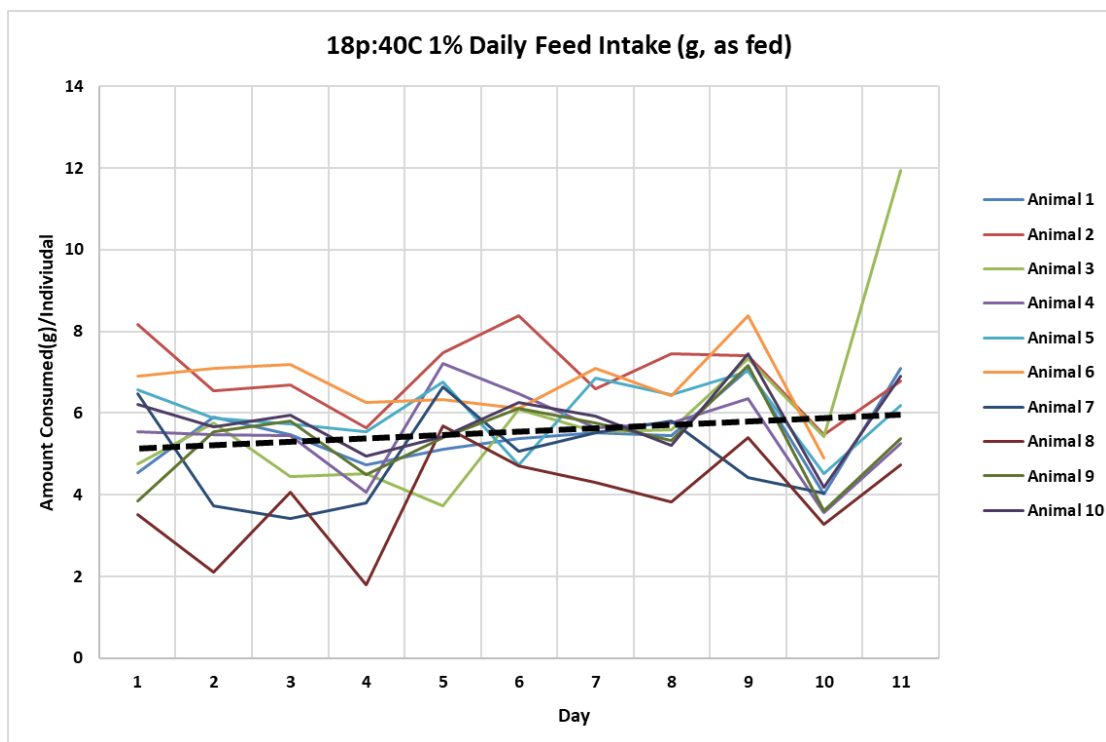


Figure 26. Daily feed intake (wet weight) for individuals fed the 18p:40C diet at 1% food density. Each colored line represents intake for an individual (1-10). The dashed black line illustrates the trend over the 11-day period for all individuals.

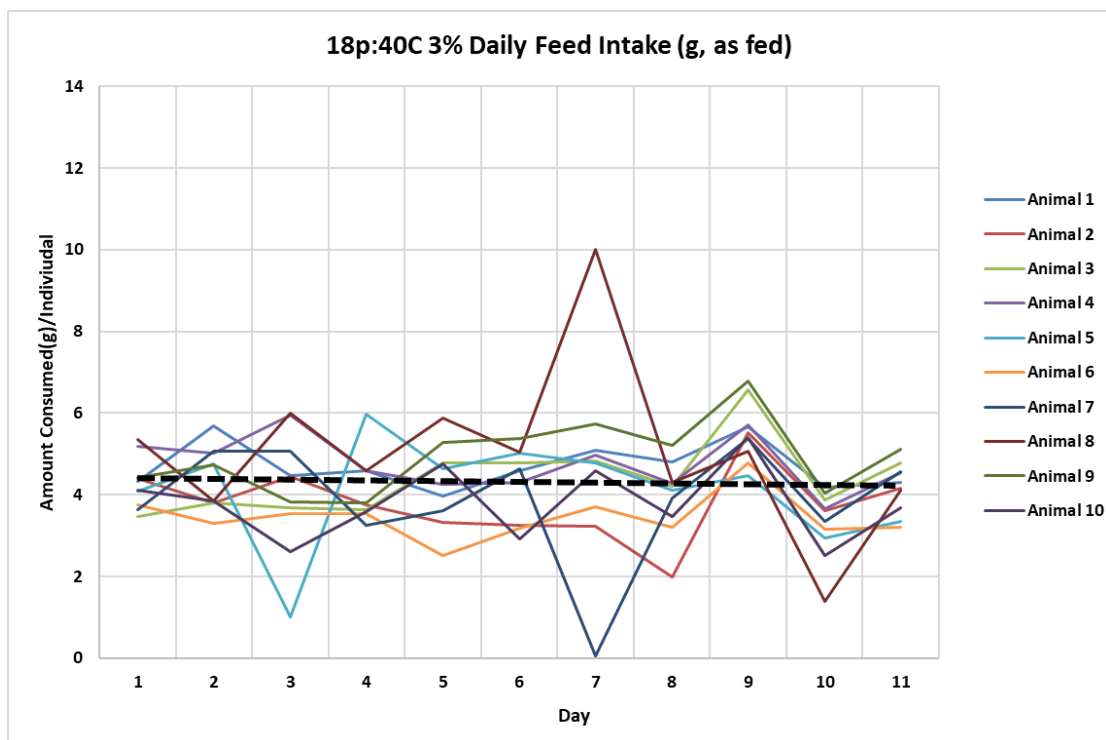


Figure 27. Daily feed intake (wet weight) for individuals fed the 18p:40C diet at 3% food density. Each colored line represents intake for an individual (1-10). The dashed black line illustrates the trend over the 11-day period for all individuals.

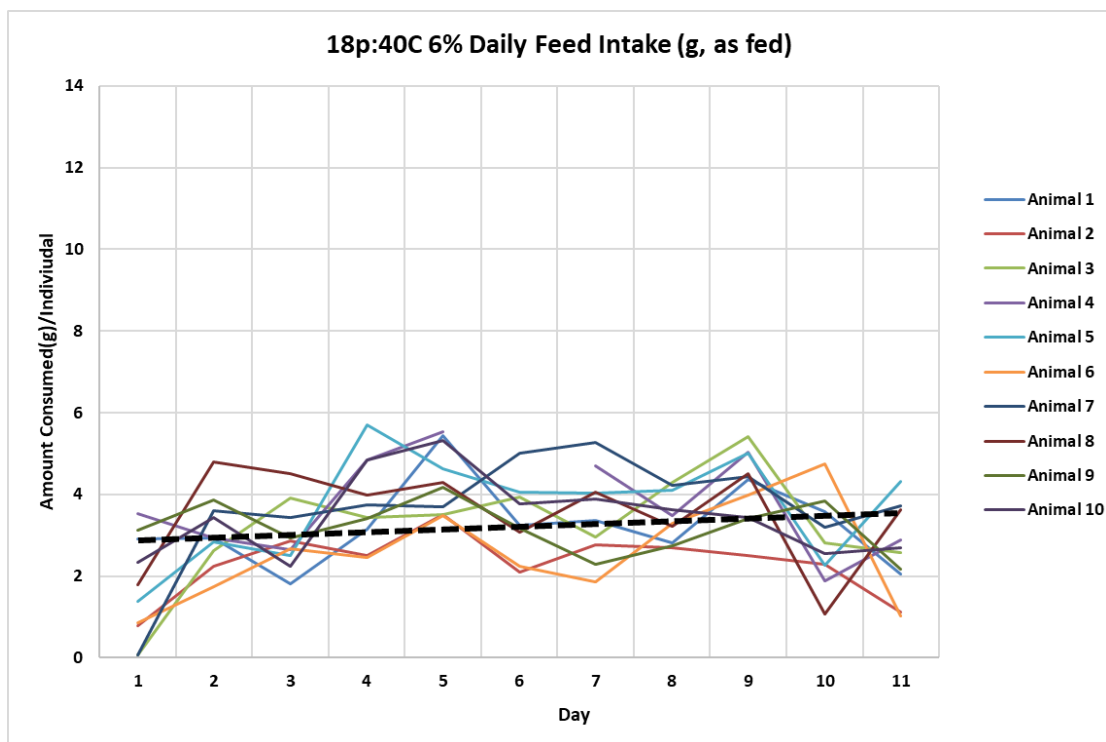


Figure 28. Daily feed intake (wet weight) for individuals fed the 18p:40C diet at 6% food density. Each colored line represents intake for an individual (1-10). The dashed black line illustrates the trend over the 11-day period for all individuals.

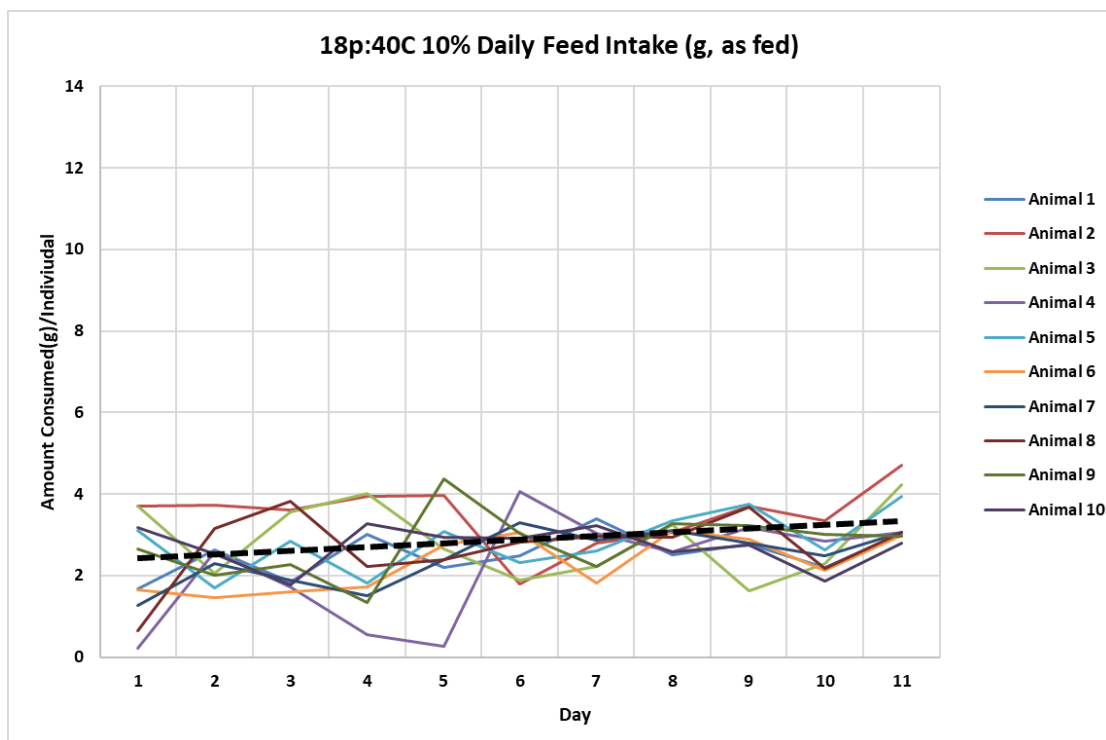


Figure 29. Daily feed intake (wet weight) for individuals fed the 18p:40C diet at 10% food density. Each colored line represents intake for an individual (1-10). The dashed black line illustrates the trend over the 11-day period for all individuals.

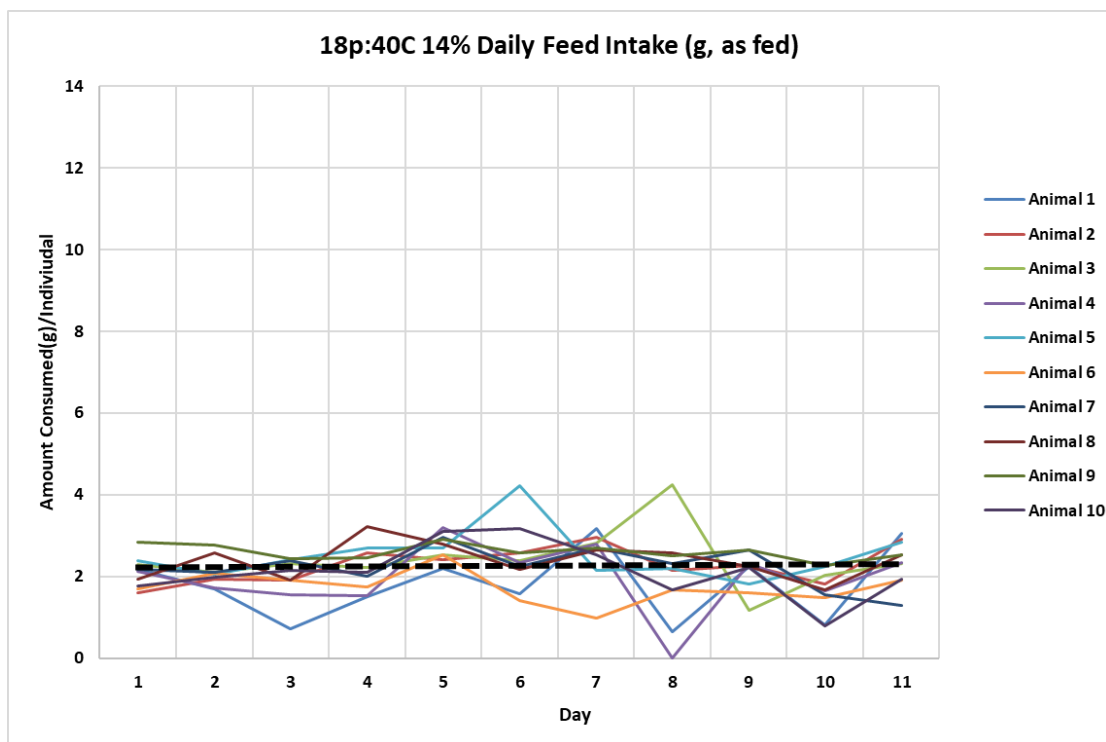


Figure 30. Daily feed intake (wet weight) for individuals fed the 18p:40C diet at 14% food density. Each colored line represents intake for an individual (1-10). The dashed black line illustrates the trend over the 11-day period for all individuals.

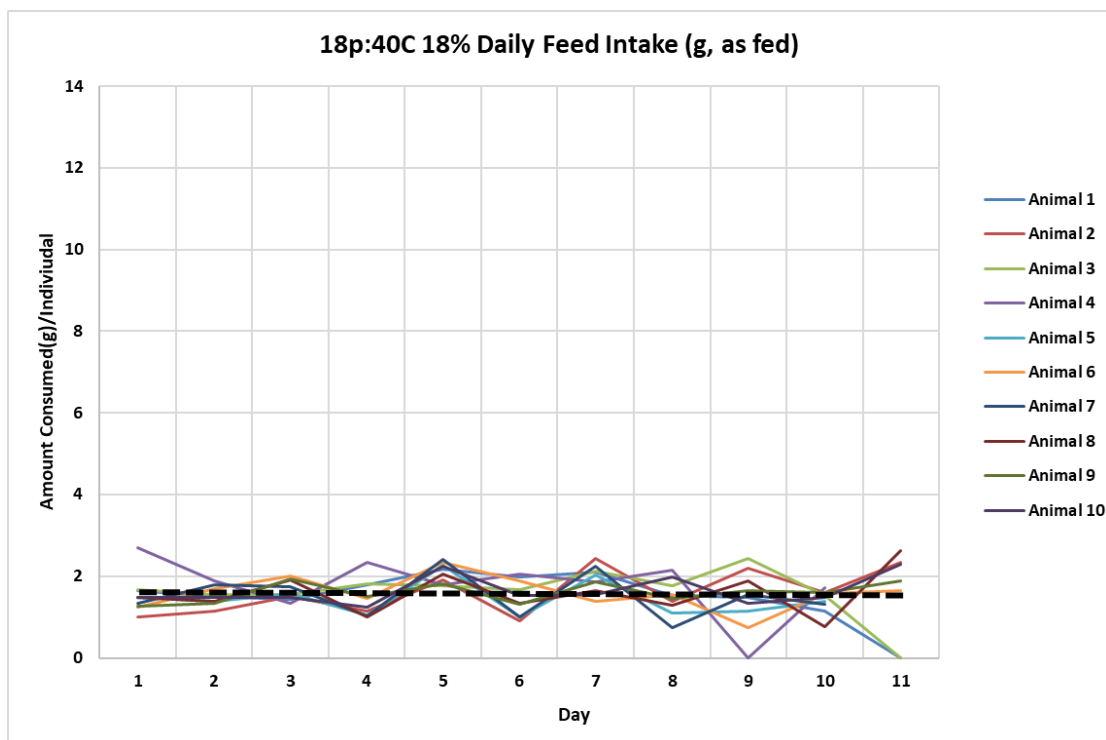


Figure 31. Daily feed intake (wet weight) for individuals fed the 18p:40C diet at 18% food density. Each colored line represents intake for an individual (1-10). The dashed black line illustrates the trend over the 11-day period for all individuals.

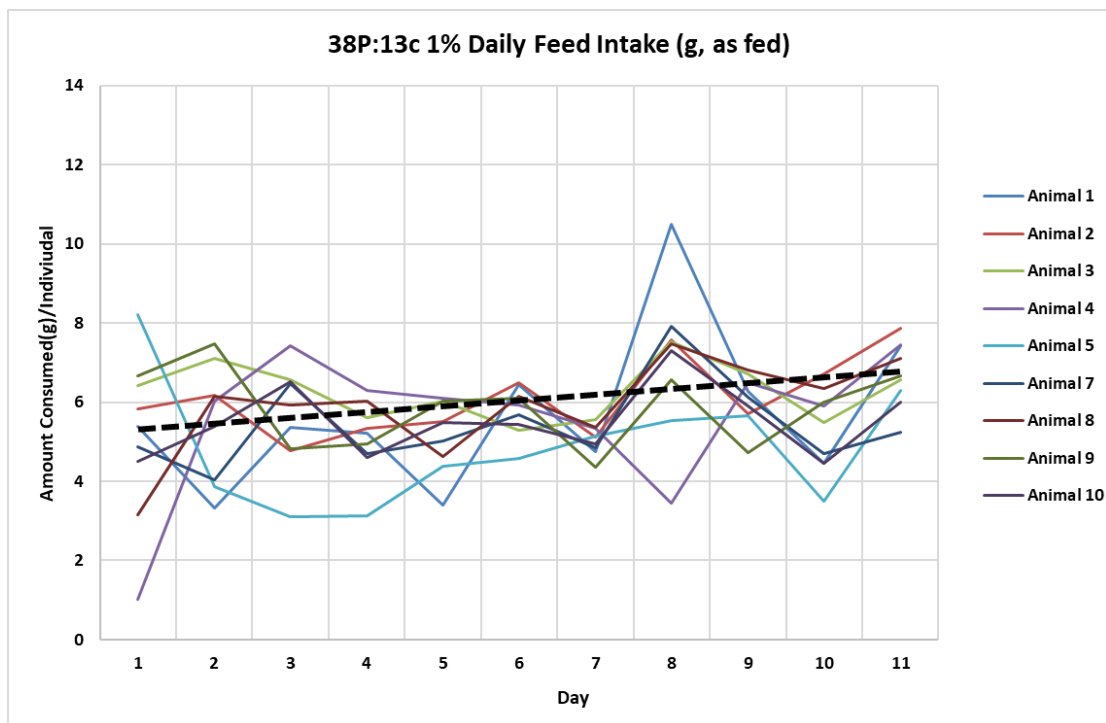


Figure 32. Daily feed intake (wet weight) for individuals fed the 38P:13c diet at 1% food density. Each colored line represents intake for an individual (1-10). The dashed black line illustrates the trend over the 11-day period for all individuals.

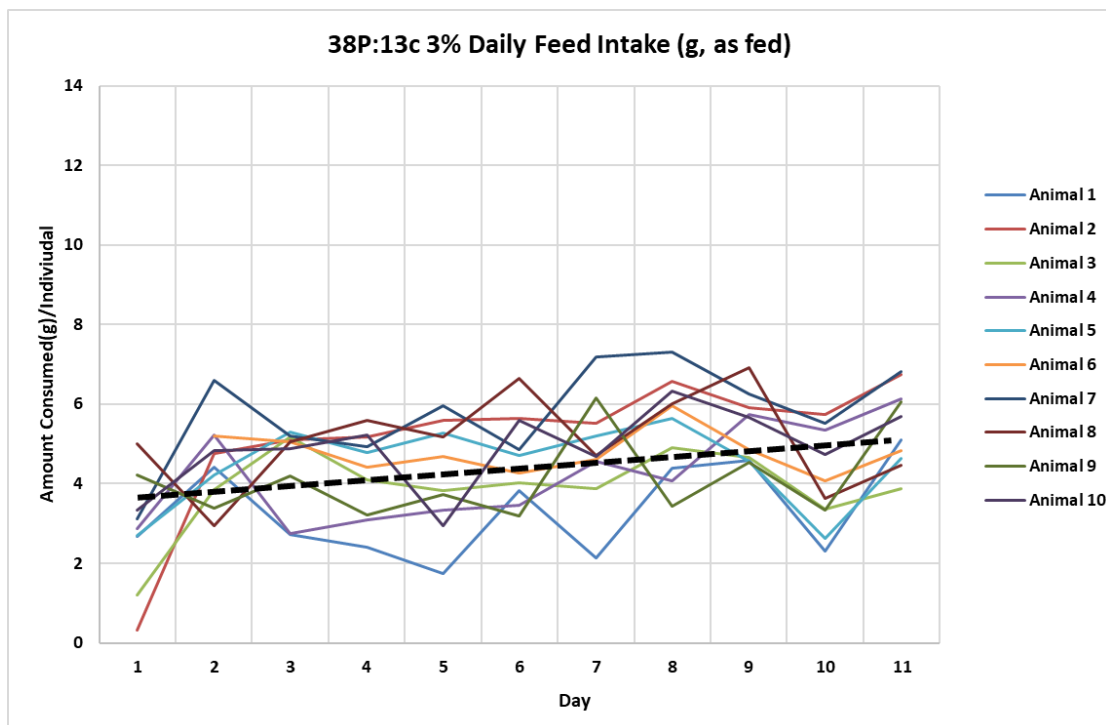


Figure 33. Daily feed intake (wet weight) for individuals fed the 38P:13c diet at 3% food density. Each colored line represents intake for an individual (1-10). The dashed black line illustrates the trend over the 11-day period for all individuals.

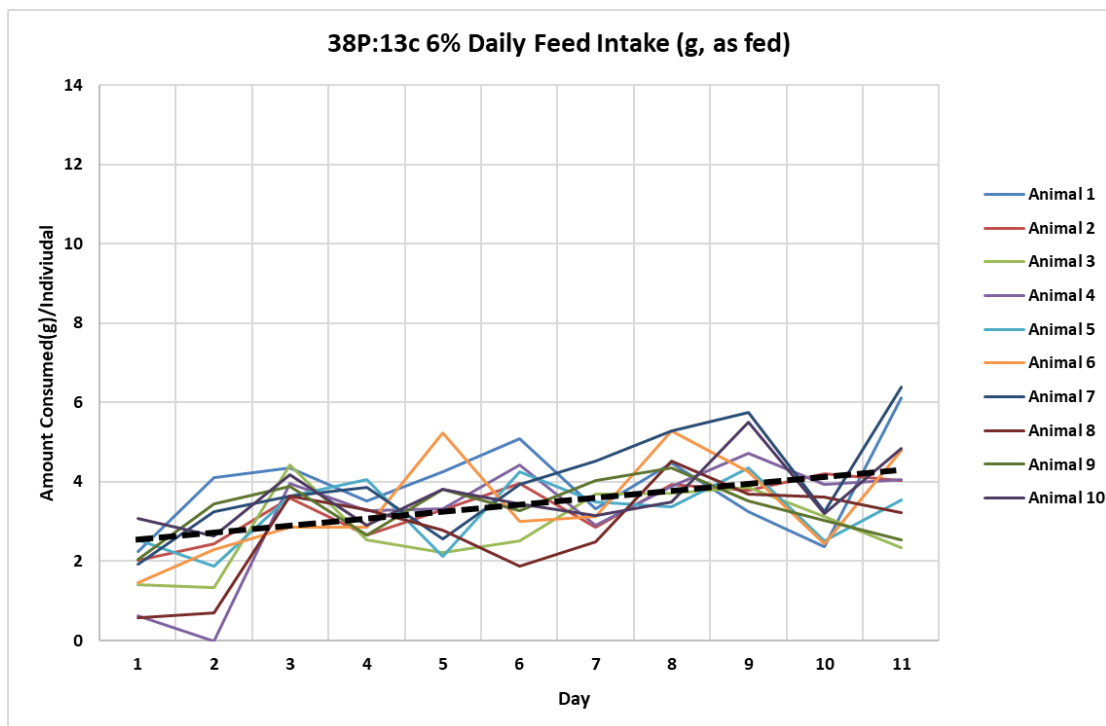


Figure 34. Daily feed intake (wet weight) for individuals fed the 38P:13c diet at 6% food density. Each colored line represents intake for an individual (1-10). The dashed black line illustrates the trend over the 11-day period for all individuals.

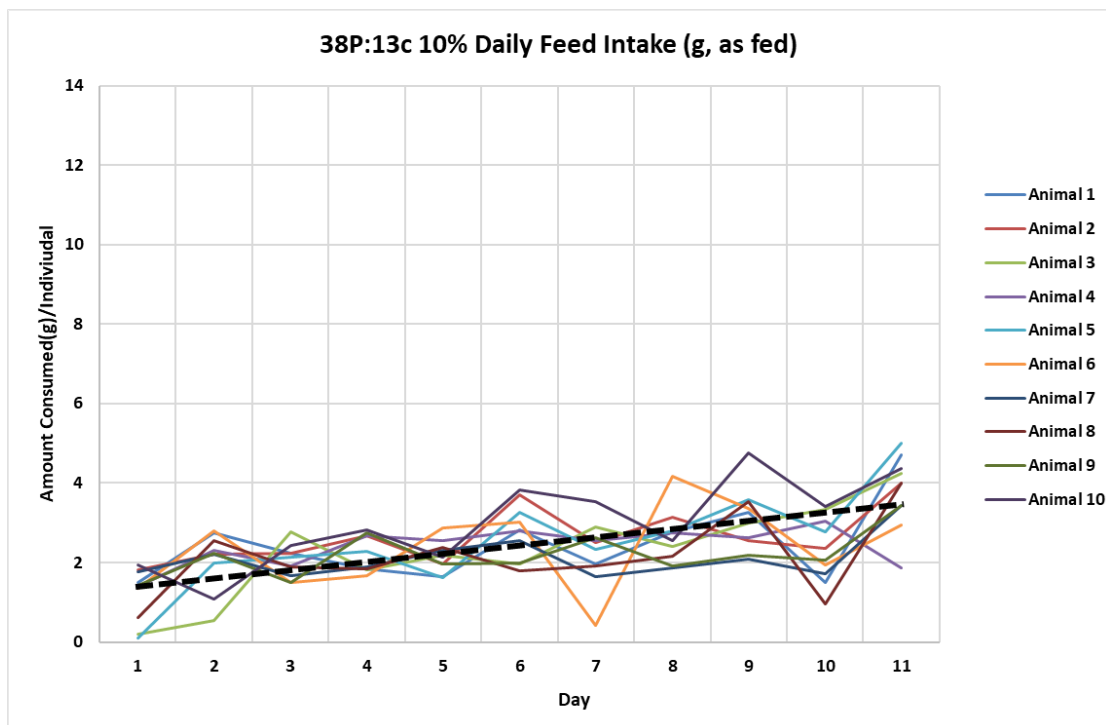


Figure 35. Daily feed intake (wet weight) for individuals fed the 38P:13c diet at 10% food density. Each colored line represents intake for an individual (1-10). The dashed black line illustrates the trend over the 11-day period for all individuals.

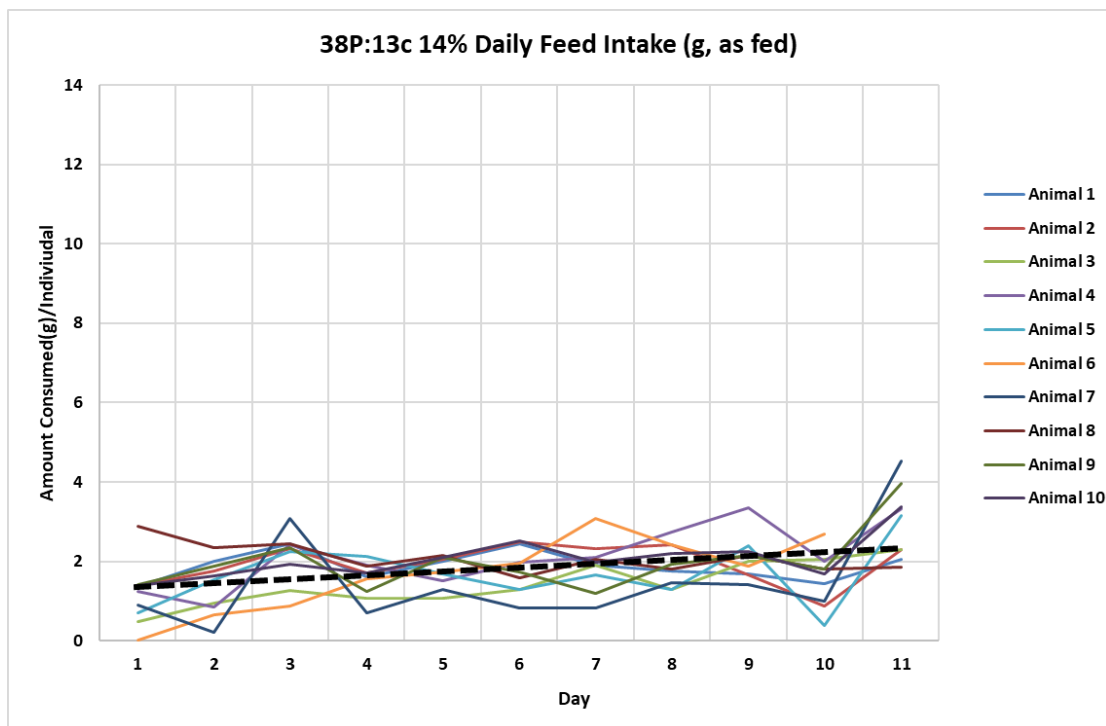


Figure 36. Daily feed intake (wet weight) for individuals fed the 38P:13c diet at 14% food density. Each colored line represents intake for an individual (1-10). The dashed black line illustrates the trend over the 11-day period for all individuals.

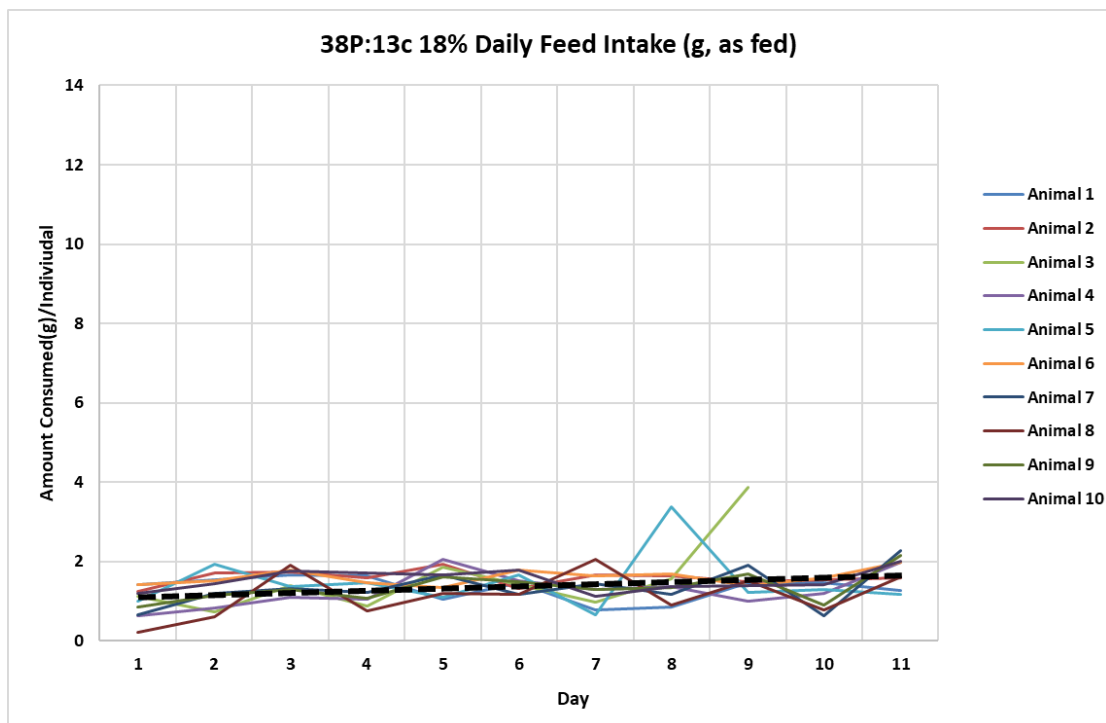


Figure 37. Daily feed intake (wet weight) for individuals fed the 38P:13c diet at 18% food density. Each colored line represents intake for an individual (1-10). The dashed black line illustrates the trend over the 11-day period for all individuals.

## DISCUSSION

### Part I- Satiety is Induced by Both Volume and Dry Matter

In the natural environment, feeding in *Lytechinus variegatus* is in part based on food availability and rate of encounter, due to the low phagostimulatory responses (Klinger and Lawrence, 1985). *Lytechinus variegatus* often initially demonstrates little preference when offered multiple food sources; however, consumption rates over a longer period of time are variable, suggesting consumption is necessary to determine preference and intake (Klinger and Lawrence, 1984; Klinger and Lawrence, 1985; Beddingfield and McClintock, 1998). After initial consumption, or testing, of a given food item, *L. variegatus* directs feed intake by determining whether or not to continue consumption (Klinger and Lawrence, 1984; Beddingfield and McClintock, 1998). The results of the present study are consistent with the previous suggestion that sea urchins taste test potential foods. In the present study, at 0% food density (no food inclusion), sea urchins consumed approximately 2.5 g per individual per day. This pattern of intake is relatively constant across the experimental period, indicating sea urchins continue to consume some of the gel-based food blocks each day, even when nutrients are limited. Sea urchins fed the 0% food density diet may deem the gel-based food blocks a not worthwhile food items and cease consumption after initial ingestion on a daily basis. Daily intake patterns and individual variability will be further expanded later in the discussion. It remains unclear why sea urchins consumed over 2 grams of these food blocks each day before ceasing consumption. One possibility may be that if sea urchins are feeding at a

maximum rate, then around 2 grams of total wet weight intake corresponds to a point metabolically where processing a non-nutritive food item becomes costlier than its value leading to cessation of feeding.

Sea urchins consumed less wet weight of gel-based food blocks as food density increased from 1 to 18% for all diets, indicating food density affects consumption. Klinger et al. (1994) reported *L. variegatus* consumed wet weight that ranged from less than 1 to 7 g of food (wet weight) per individual per day<sup>-1</sup>. In their review, Ridder and Lawrence (1982) reported studies in which *L. variegatus* consumed from 0.9 to 1.4 g food per urchin per day. Ridder and Lawrence (1982) reported *L. variegatus* consumed up to 7.15 g dry sea grass per urchin per day. These values for wet and dry weight intake are consistent with values observed in the present study.

Klinger and Lawrence (1984) detected that varying the level of plant tissue inclusions in agarose foods altered feed feeding rate in *L. variegatus*. Lares and McClintock (1991) detected that *Eucidaris tribuloides* had higher feeding rates and higher nutrient absorption efficiencies when fed agar blocks containing low-quality food (1% fish meal) in comparison to high-quality food (10% fish meal). These data combined with findings of the present study suggest that sea urchins regulate feed intake based on food density and/or food quality.

In the present study, *L. variegatus* consumed around 6-7 grams of wet weight intake per individual per day at the lowest food densities of 0.5% and 1%. This level of consumption is approximately 1/3 of these sea urchins' total wet body weights, suggesting a threshold of volumetric satiety for adult *L. variegatus* (at least in this size class). Suskiewicz and Johnson (2017) reported sea urchins consumed highly variable



percentages of their body weight per day in wet weight intake, with ranges of intake from less than 1 to 18% of the sea urchin's body weight. In the present study, sea urchins fed low food densities consumed, on average, approximately 33% of their body weight per day in wet weight intake. To our knowledge, no study to date has demonstrated intake at such a high percentage of body weight in *L. variegatus*. Suskiewicz and Johnson (2017) also noted that consuming greater than 10% of body weight per day was rare and may indicate short periods of time in which *Strongylocentrotus droebachiensis* feeds indiscriminately. Thus, these higher volumes may indicate maximal gut capacity for *S. droebachiensis* (Suskiewicz and Johnson 2017). Similarly, lower food densities in the present study may indicate a maximal gut capacity for *L. variegatus*. However, at higher food densities (10-18%), *L. variegatus* consumed between 7-12% of body weight per day in wet weight intake (1.5-2.5 grams). Suskiewicz and Johnson (2017) reported *S. droebachiensis* generally consumed 2-4% of body weight in weight wet intake daily (1-2 grams of intake). When *L. variegatus* feeds below the point of volumetric satiety (ca 1/3 of body weight), it is apparent some factor(s) or process(es) direct feed intake and regulate satiety, as these urchins do not feed indiscriminately.

When comparing intake across the 3 diets within each food density, it is interesting that significant differences in wet weight consumption occurred between diets at several discrete food densities. At lower food densities (1 and 3%) sea urchins consumed more of the balanced diet, defined by Heflin 2016b, (27P:27C) than the two unbalanced diets (38P:13c and 18P:40C). Differential consumption of diets that vary in quality at lower food densities may reflect an opportunistic feeding strategy indicative of some level of discrimination Sea urchins may favor consuming more of the balanced

27P:27C diet, especially when specific nutrients may be limited in the unbalanced diets in respect to both reduced availability of food (low density food) and options (no choices of different food items).

At 10% food density there were only marginal although significant differences among diets. These intake patterns suggest that food quality (diet option in this study) has a greater impact when specific nutrients are limited. Larson (1980) demonstrated that *S. droebachiensis* may adopt a facultative specialist strategy when choices of food are available. Studies in which multiple food choices are provided to individuals both at limited volumes and unlimited volumes may help clarify which strategies *L. variegatus* and other sea urchin's species might adopt when multiple food items are available under different conditions.

As food density increased, despite consuming less wet biomass, sea urchins consumed more dry formulated feed. This suggests an intake target for dry feed (or inclusive nutrients) at higher food densities in *L. variegatus*. When considering other dry components of the gel-based food blocks (agar and salt of the saltwater matrix), intake decreased as food density increased. Agar powder was held constant, included at 1.5% for all food densities and diets. Salt content was provided from the 32ppt saltwater that constituted, in part, the matrix of the gel-based food blocks. As wet food intake decreased across food densities, both agar and salt intake decreased. When dry formulated feed, salt, and agar are considered together, these components constituted the total dry matter of the gel-based blocks. Sea urchins consumed 325 – 450 mg of total dry matter per individual per day for all three diets. These data suggest *L. variegatus* sates based on total dry matter of food items. Data from Beddingfield and McClintock (1998) demonstrated

when fed 6 diets of natural food sources in agarose food, *L. variegatus* consumed similar amounts of total dry matter in 5 of the 6 diets, despite consuming varying amounts of wet weight. Consuming to satiety of total dry matter intake may be an adaptive strategy for a marine omnivore with herbivorous tendencies. *L. variegatus* may encounter food items with high water content in the wild, including seagrass. Dawes and Lawrence (1980) report that the sea grass *Thalassia testudinum* in Florida has water content of  $80 \pm 2.8$  to  $85 \pm 1.0$  % in its blades and  $87 \pm 2.8$  to  $90 \pm 1.6$  % in its short shoots, which vary seasonally. Thus, consuming to a total dry matter intake target may be an effective way to insure adequate nutrient intake when consuming complex (or mixed) diets such as sea grasses and their associated epibionts, mussels, and/or detritus.

Despite *L. variegatus* consuming similar ranges of total dry matter intake, significant differences in intake were detected both within diets across food densities and within food densities across diets. It is likely that the control of food intake and satiety in this species and other sea urchins is complex, with many levels of regulation. It is possible that an intake target of total dry matter exists as a primary target, and that once consumption approaches a target value, other factors (specific nutrients, attractants, deterrents) further direct feed intake, and ultimately, satiation. It may also be possible that total dry matter intake is a secondary or alternative target for regulation if other feed characteristics override feed intake regulation. Of course, these levels of regulation are even more complicated if sea urchins are not able to reach intake targets within the volumetric constraints of consumption.

## Part II- Macronutrients and Energy

Although volume and dry matter content are factors influencing satiety, *Lytechinus variegatus* and other species of sea urchin may have dietary intake requirements for specific nutrients (both quality and quantity); yet, such requirements are not well understood (Watts et al., 2013a). Varying amounts of macronutrients and/or nutrient ratios may influence feed intake. Heflin et al. (2016a) concluded that *L. variegatus* will consume to an intake target for protein, and either over or under consume carbohydrate in doing so. In their study, Heflin et al. (2016a) offered sea urchins a diet containing either protein or carbohydrate obtained from a single source. Sea urchins were offered a choice of the two diets. It is important to note that the diets used in the present study varied in protein and carbohydrate content (both in quantity and quality) and inclusive of other macro- and micronutrients. The complexity of the diets used may affect perception of intake targets compared to a less complex (single ingredient) food items. Another consideration is that in the present study sea urchins were fed each diet singularly with no choice. Here, sea urchins were constrained to eating more or less of the same diet (with fixed macronutrient ratios).

Protein is an essential component for proper physiological functions in sea urchins (Marsh et al., 2013). Increasing dietary protein levels are inversely related to feed intake, and higher protein levels lead to more rapid satiation (ceasing of feeding) (Frantzis and Grémare, 1992; Fernandez and Bourdouresque, 1998; McBride et al., 1998; Meidel and Scheilbling, 1999; Agatsuma, 2000; Fernandez and Bourdouresque, 2000; Hammer et al., 2004; Daggett et al., 2005; H.S. Hammer et al 2006). Protein content in

the diet positively correlates with growth in many sea urchin species (Watts et al., 2013a). An optimal protein content of 20% dry feed inclusion has been reported (Hammer et al., 2006) in adult *L. variegatus*. Heflin et al (2016a) suggested protein levels of 25 to 30% may be optimal for juvenile *L. variegatus* raised in culture. A number of studies have suggested, though, that there is an upper limit for protein intake at which growth ceases (McBride et al., 1998; Kennedy et al., 2005; Senaratna et al., 2005; H.S. Hammer et al., 2006; Marsh et al., 2013; Heflin et al. 2016b) or even begins to decrease (Eddy et al., 2012).

In the present study, sea urchins exhibited a wide range of protein intake (approximately 10-100 mg) per individual per day. Within each diet, significant increases in protein intake occurred as food densities increased from 1 to 10%. At higher food densities, the amount of protein intake at which maxima intake occurred varied for each diet, suggesting that nutrient ratios could affect intake of a single nutrient.

Recently, Heflin et al (2016a) demonstrated a narrow protein target (0.047-0.061 g/day) for *L. variegatus* fed paired dietary combinations of protein and carbohydrate at varying dry feed levels. In contrast to some previous studies, the present study suggests that *L. variegatus* prioritizes protein even at the expense of over or under consumption of carbohydrate, regardless of pairwise macronutrient combinations and ratios. Heflin et al (2016a) further suggest that sea urchins will primarily regulate protein (protein leverage) and opportunistically store excess carbohydrate, if available. The ranges for protein intake demonstrated by Heflin (2016a) are similar to those measured in the present study. However, protein leveraging is not demonstrated across diets, and the range of intake was broader in the present study. At 3% and greater food densities, sea urchins had the

capacity to consume more wet weight intake, based on the previously demonstrated volumetric threshold, to increase intake of any given macronutrient. Sea urchins fed the lower protein diet (18p:40C) and the balanced diet (27P:27C) could have consumed more wet weight to increase their protein intake to reach an intake target. Alternatively, sea urchins fed the high protein diet (38P:13c) or the balanced diet (27P:27C) could have decreased wet weight intake to lower protein intake if a target existed at lower intake levels. Sea urchins fed each diet consumed significantly different amounts of protein intake across diet and food density. Thus, satiety based solely on protein intake was not demonstrated in the present study.

The sources of protein also warrant consideration for directing feed intake of sea urchins. Valentine and Heck (2001) demonstrated that *L. variegatus* will consume more non-nitrogen enriched, than nitrogen enriched seagrass, suggesting they are compensating for low nutritional quality by consuming more. It is possible that sea urchins may be targeting nitrogen content or specific amino acid content, with respect to protein intake, but this remains unclear.

Sea urchins use carbohydrates as a primary energy source (Marsh et al., 2013) and may be able to process and utilize carbohydrates as an energy source more efficiently than proteins or lipids (Marsh et al., 2013; Watts et al., 2013a). Nonetheless, many sea urchin species poorly digest/assimilate structural/insoluble carbohydrates (Lawrence et al., 2013; Klinger, 1984b). Several studies have suggested *L. variegatus* may consume food to an energetic target relative to carbohydrate intake (Taylor, 2006; Hammer et al., 2012). In the present study, sea urchins consumed variable amounts of carbohydrate per individual per day. Carbohydrate intake was analyzed both by accounting solely for the

carbohydrate provided from the formulated feed and for both formulated feed and agar. It remains unresolved if the agar powder used in the present study provides a carbohydrate source that *L. variegatus* can digest and assimilate. Thus, the effect of carbohydrate on intake and satiety were evaluated both with and without agar as a carbohydrate source.

When carbohydrate intake from formulated feed intake only is evaluated, very similar patterns to protein intake are observed. Sea urchins consumed carbohydrate from approximately 10 to 125 mg per individual per day. Intake varied with diet and food density. A maxima for carbohydrate intake was established at 10% or greater food densities within each diet. Nonetheless, the amount of carbohydrate intake at which the maxima occurred varied for each diet. The high carbohydrate diet (18P:40C) had the highest amount of carbohydrate intake and the low carbohydrate diet (38P:13C) the lowest. When both carbohydrate from formulated feed and agar powder were considered, total carbohydrate intake decreased with increased food densities for the 27P:27C diet and the 38P:13c diets but increased with increased food densities of the 18p:40C diet. Agar was held constant at 1.5% inclusion across diets and densities. Thus, at lower densities, when larger amounts of wet weight of gel-based food blocks were consumed, agar contributed a greater impact to total carbohydrate intake. At higher food densities, when wet food weight intake was reduced, agar had a minimal impact on total carbohydrate intake.

Whether carbohydrate is evaluated with or without agar as a carbohydrate source, differences in intake are detected across diets and food densities. Again, similar to protein, sea urchins were able to consume more or less wet weight of the gel-based food blocks to increase or decrease their carbohydrate intake if they were attempting to

consume to a specific carbohydrate target. Two studies have suggested *L. variegatus* may consume to a carbohydrate-based energy target at the expense of under or over consumption of protein (Taylor, 2006; Hammer et al., 2012). However, *L. variegatus* did not demonstrate intake patterns that would suggest a carbohydrate intake target, point of satiety, or a prioritization of carbohydrate intake in the present study.

For many herbivores, achieving separate individual nutritional targets may not be feasible based on the complexity of diets they consume (Simpson et al., 2004). Thus, trade-offs between over and under eating various macro- and micro-nutrients will occur (Simpson et al., 2004). *L. variegatus* has been observed to consume a variety of food items in the field based on both their palatability or availability, indicative of a generalist feeding strategy (Beddingfield and McClintock, 1999). Furthermore, *L. variegatus* has been observed feeding in the field preferentially on detritus over living seagrasses, perhaps a strategy to consume variable matter with a reduced structural carbohydrate content (Lowe and Lawrence, 1976).

Trade-offs between macronutrient intake, particularly of protein and carbohydrate, have been suggested for *L. variegatus* in the laboratory (Heflin, 2016a; Taylor, 2006; Hammer et al., 2012; Fernandez and Boudouresque, 2000). *L. variegatus* has been reported to preferentially consume animal or plant material (McClintock et al., 1982). Moreover, they prefer consuming associated epibionts on seagrasses rather than the blades themselves (Greenway, 1995), and have been shown to prefer detrital seagrass to fresh seagrass (Montegue et al., 1991). Collectively, these studies are indicative of a preference in nature for protein.



Nutritional trade-offs of macronutrients, such as those illustrated above, may have consequences, particularly when it comes to over or under consumption of a given nutrient when targeting another (Simpson et al., 2004). Some nutrients may be essential to physiological processes (Watts et al. 2013a), others when, consumed in high levels, may be costly to process or even toxic (Watts et al, 2013a). Feeding preferences demonstrated by *L. variegatus* may reflect consumption to a nutrient target or may reflect an attempt to balance a generalist diet over time. It is also likely such nutritional targets are not static in sea urchins but vary based on many internal and external factors, including life stage and environmental conditions. Patterns of macronutrient intake in sea urchins still warrants further investigation as nutritional research continues to unravel the complexity of feeding and satiety.

The energetic content of foods is also an important consideration in an animal's diet. Paine and Vadas (1969) suggested that food items with low to intermediate caloric values, (ranging from 4.32 to 4.67 kcals/g dry) seem to be consumed most readily by marine herbivores. This would suggest energetic value (calories) are not the primary driver of feed intake. Larson et al (1980) demonstrated food preference was not correlated with caloric content in the sea urchin *S. droebachiensis*. An inverse correlation in caloric intake and feeding preference for algae was reported in the sea urchins *S. droebachiensis* and *Mesocentrotus* (as *Strongylocentrotus*) *franciscanus* (Vadas, 1980). Beddingfield and McClintock (1998) found no relationship between prey nutritional quality and feeding preference in *L. variegatus*.

Similar to the analysis of carbohydrate intake, energy intake was considered both with and without the contribution of the agar powder. The impact of agar on energy

intake had little impact on the pattern of intake across diets and food densities. The contribution of agar as an energy source only altered the scale on which energy intake was measured. When only considering energy provided from consumption of formulated feed, energy intake per individual per day ranged from 0.2-1 kilocalories. When considering energy from both formulated feed and agar powder, energy intake per individual per day ranged from 0.4 to 1.1 kilocalories. Sea urchins consumed similar amount of energy within each diet at 10-18% food densities. At 1 to 3% food densities, significant differences in energy intake were detected across diets. At 6% and higher food densities, no significant differences in energy intake across diets at each food density were detected.

Results of the present study may suggest an energetic target for *L. variegatus*; however, by design these diets were formulated to be isocaloric (within 0.1 kilocalorie). Thus, if sea urchins were consuming to another intake target or point of satiety (such as total dry matter or a balance of macronutrients) they would, by default, consume similar amounts of total energy. McClintock (1986) suggested that energy provided by digestible organic components of food may be more important in directing feed intake than total energetic content (calories of digestible and indigestible components). A high protein to energy ratio in foods consumed by *L. variegatus* has also been suggested as optimal (Taylor, 2006; Hammer et al., 2012; Heflin et al., 2012). This may be due to a low requirement for energy in *L. variegatus* (Watt et al., 2013a). It is also possible that macronutrient content in relation to energy is an intake target for *L. variegatus*, and that caloric value alone has little influence on feed intake. Further investigations of energetic targets of feed intake for sea urchins are needed.

The ability to gain nutrients and energy in the appropriate amounts will have important physiological consequences for processes such as growth. Sea urchins will invest resources in both somatic and gonadal growth. In the present study, wet weight gains of individual sea urchins were analyzed for all food densities for the 27P:27C diet. All treatment groups, on average, gained wet weight over the 11-day period. Wet weight gain significantly increased with increases in food densities from 0 to 3%. At 3% and higher food densities, *L. variegatus* did not gain significantly more weight. This suggests a point of maximal wet weight gain at the 3% food density. It is interesting that sea urchins offered food densities of 3-18% did not consume the same wet weight of food blocks, dry formulated feed, or even the same macronutrient amounts, yet gained similar wet weight. Dissections and body composition were not integrated in the present study; thus, no inferences about nutrient allocation to specific tissues can be made from this work. In addition, the period for growth was only 11 days, which is a limited time. The relationship between food density and growth in sea urchins warrants further investigation. If sea urchins fed lower food densities were shown to gain not only body mass, but also develop gonads of high quality, this would have important implications for cost effective feed production in sea urchin aquaculture.

### Part III- Variability in Feed Intake and Patterns of Feed Intake Over Time

Substantial amounts of food are consumed by *L. variegatus* in a single day, and food intake is easily quantifiable. Most studies that measure food intake in sea urchins have done so over a defined period of time, and these data are then presented as average or total consumption for the defined time period. Therefore, little is known about the day

to day variability of feed intake in sea urchins. In the present study, total standard deviations of feed intake, representing the combined day to day variability observed both within individuals and between individuals, were highest for individuals fed low food densities and decreased significantly as food density increased. This high variability at low food densities may be the result of the limited availability of one or more nutrients to elicit physiological or cellular processes as related to chemical nutrient signaling. Simply put, additional nutrient(s) are required for determinative “taste testing” of foods, a necessity when food is less dense or when available food is not of high quality.

If the metric representing total variation is parsed into its component parts, most of the variation is attributed to that observed within an individual over time. About a third of the total variation observed can also be attributed to variation observed among (between) individuals. In other words, the variability observed within a population is less than that observed within an individual. Many studies to date have focused on intake averages, but few have evaluated the patterns of feed intake and changes over time. Watts et al. (2011) reported feed intake varied daily in *Lytechinus variegatus* (Watts et al., 2011). In their study, feed intake was highly dependent on environmental temperature, and changes in feed intake likely reflected patterns caused by acclimation. Similarly, the present study illustrated high variability in day to day feed intake for *L. variegatus*. Large differences in daily intake (between 2-5 g) were not uncommon for individuals fed lower food densities. However, as food density increased, day to day variation in individual feed intakes decreased.

Interestingly, the greatest impact of day (as a model factor) was on feed intake measured for the 38P:13c diet, with extremely strong evidence for changes in feed intake

over time at the 3, 6 and 10% food densities. When individuals in these groups (38P:13c 3, 6 and 10%) were tracked over the experimental period by day, a significant increase in wet feed intake was observed over time. This overall increase in feed intake was not observed in other treatments when tracked individually over time. The implications of these findings are unclear. These results may suggest that increasing feed intake over time for the high protein diet represented either a) an increasing requirement for a diet high in dietary protein, b) an increasing requirement for a diet with a high protein: energy ratio, c) recognition (habituation) of the individuals to the attractable components of this diet, or d) unknown variables leading to increased consumption of this diet. The food densities most responsive, including those with 3, 6, or 12% dry feed, are likely within the range of nutrients found in natural food items (a high water content relative to dry matter and nutrients). The variation in wet feed intake is affected by many factors and day/time is only a portion of that variation. However, the effect of day on the 38P:13c groups mentioned above is a strong effect based on the AIC model and should not be discounted without further evaluation. Lawrence et al. (2003) suggested *L. variegatus* alters feeding rates over time with differences in feeding apparent after 12 days. The sea urchins in the present study were fed for a 14-day period and analysis limited to the last 11 days (the first 3 days were provided as standardization time). Studies over longer periods may be necessary to fully evaluate the patterns of intake over time and to evaluate daily variations in consumption for sea urchins.

## Summary

In the present study, the focus has been on the fundamental factors that control feed intake and satiety in a deuterostome with a relatively simple physiology. Many components of satiety signaling in vertebrates have not been identified in less complex deuterostomes, including the Echinodermata. Echinoderms lack a complex localized brain, thus signaling through a brain-gut axis cannot occur via typical hormonal or vagal inputs. However, it is interesting that echinoderms do possess a distinct arrangement of the nervous system when compared with other deuterostomes, where most all body organs, tube feet, spine, and viscera are innervated with neurons (Burke et al., 2006). Burke and co-authors (2006) suggest that among metazoan nervous systems, the echinoderms may be the least well studied in terms of signaling, despite the unique insights such studies would provide to understanding neuronal evolutionary patterns among deuterostomes.

Evolutionarily conserved pathways of nutrient-sensing allow molecules such as leptin and ghrelin to act on and alter neural functions in mammals (Berthoud and Morrison, 2008). The ghrelin gene is evolutionarily conserved in many vertebrates, but functions of ghrelin may be both conserved, and have species-specific impacts among animal taxa (Kaiya et al., 2008). The identification of genes in echinoderms, like that of the recent sequencing of the sea urchin genome, illustrated the need for a greater understanding of the nervous systems of these animals (Burke et al., 2006). Recent work has demonstrated the sea urchin *Strongylocentrotus purpuratus*, expresses receptors in the NPY receptor family. These receptors are related to a variety of physiological functions including gustatory functions (Semmens and Elphick, 2017). Since the NPY

genes/receptors occur in sea urchins, and the presence of related peptides (which bind NPY receptors) has been hypothesized; however, such peptides have yet to be documented in sea urchins (Semmens and Elphick, 2017). The implications of the presence of this gene family have not been unraveled, but it may illustrate a tightly conserved relationship among deuterostome neuropeptide evolution that may ultimately shed light on the evolution of satiety at the molecular level.

Using a model such as the sea urchin, which likely lacks many of the complexities responsible for modulation the satiation processes in higher vertebrates, provides a means to better understand the fundamental factors that direct satiety including food volume and food macronutrients and energetic content. These factors are likely to direct signaling via taxa-dependent mechanisms and provide novel information about baseline factors that direct feed intake and satiety. Such baseline factors may be universal across taxa and may provide a framework to understanding how more complex processes of satiety signaling evolved. It is apparent the mechanism(s) controlling feed intake and satiation in sea urchins are multi-faceted and requires further research and many unresolved questions remain about their regulation (Suskiewicz and Johnson, 2017).

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## APPENDIX

### INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE APPROVAL



THE UNIVERSITY OF ALABAMA AT BIRMINGHAM  
*Institutional Animal Care and Use Committee (IACUC)*

MEMORANDUM

DATE: 27-Jan-2017  
TO: Watts, Stephen A  
FROM:   
Robert A. Kesterson, Ph.D., Chair  
Institutional Animal Care and Use Committee (IACUC)  
SUBJECT: NOTICE OF APPROVAL

The following application was approved by the University of Alabama at Birmingham Institutional Animal Care and Use Committee (IACUC) on 27-Jan-2017.

Protocol PI: Watts, Stephen A  
Title: Evaluation of a Stacked Polyculture System Involving the Sea Urchin *Lytechinus Variegatus* and the Pacific Whiteleg Shrimp *Litopenaeus Vannamei*  
Animal Project Number (APN): IACUC-10043

This institution has an Animal Welfare Assurance on file with the Office of Laboratory Animal Welfare (OLAW), is registered as a Research Facility with the USDA, and is accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC).

Institutional Animal Care and Use Committee (IACUC)		Mailing Address:
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933 19th Street South		1530 3rd Ave S
(205) 934-7692		Birmingham, AL 35294-0019
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