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EFFECT OF DIET QUALITY ON NUTRIENT ALLOCATION TO THE TEST AND ARISTOTLE'S LANTERN IN THE SEA URCHIN *LYTECHINUS VARIEGATUS* (LAMARCK, 1816)

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Abstract

Small adult (19.50 ± 2.01 g wet weight) *Lytechinus variegatus* were fed eight formulated diets with different protein (12 to 36% dry weight as fed) and carbohydrate (21 to 39 % dry weight) levels. Each sea urchin ($n = 8$ per treatment) was fed a daily ration of 1.5% of the average body weight of all individuals for 9 weeks. Akaike information criterion scores were used to compare six different dietary composition hypotheses for eight growth measurements. For each physical growth response, different mathematical models representing a priori hypotheses were compared using the Akaike Information Criterion (AIC) score. The AIC is one of many information-theoretic approaches that allows for direct comparison of non-nested models with varying number of parameters. Dietary protein level and protein: energy ratio were the best models for prediction of test diameter increase. Dietary protein level was the best model of test with spines wet weight gain and test with spines dry matter production. When the Aristotle's lantern was corrected for size of the test, there was an inverse relationship with dietary protein level. Log transformed lantern to test with spines index was also best associated with the dietary protein model. Dietary carbohydrate level was a poor predictor for growth parameters. However, the protein $\times$ carbohydrate interaction model was the best model of organic content (% dry weight) of the test without spines. These data suggest that there is a differential allocation of resources when dietary protein is limiting and the test with spines, but not the Aristotle's lantern, is affected by availability of dietary nutrients.

Keywords

Aristotle's lantern; sea urchin; *Lytechinus variegatus*; plasticity

INTRODUCTION

Natural environments are often highly variable. To maximize fitness, organisms need the ability to adjust not only from generation to generation, but also at the individual level (plasticity) with changes occurring within a lifetime (Newman, 1992; Scheiner, 1993; DeWitt, 1998). The energetic cost associated with maintaining plasticity of traits is higher than that of maintaining a fixed trait (DeWitt, 1998; Lau et al., 2009; Newman, 1992;

Scheiner, 1993; Scheiner and Berrigan, 1998). However, the additional energetic investment may be worthwhile if survival of the individual is increased. Phenotypic plasticity is important in plants (Schlichting, 1986) and in animals that are either sessile or unable to travel quickly to a habitat with more suitable conditions (Ebert, 1996; Russell, 1998). Given the sedentary nature of most adult echinoderms, a high degree of plasticity would be predicted.

Phenotypic plasticity is an adaptive response to an environmental change or stress (Ebert, 1980; Miner, 2005; Newman, 1992; Russell, 1997). Plasticity may be interpreted as normal progression and variation in the pattern of iso- or allometric organ growth, defined herein as modeling. Alternatively, plasticity could be interpreted as a regression, including resorption (or shrinking) or shifting (movement), of organs in response to a biotic or abiotic stressor. This regression leading to an altered progression of growth would be defined as remodeling.

Low food quality and low food availability are both stressors that are suggested to lead to differential phenotypic expression in adult echinoderms (Ebert, 1996; Fernandez and Boudouresque, 1997; Guillou, 2000). Plasticity (relative increase or decrease in size) of the feeding apparatus (Aristotle's lantern) has been reported in several echinoderm species (Black et al., 1982; Black et al., 1984; Constable, 1993; Ebert, 1968, 1980; Fansler, 2004; Fernandez and Boudouresque, 1997; Guillou, 2000; Hagen, 2008; Lau et al., 2009; Levitan, 1991; McShane and Anderson, 1997). In contrast, both Russell (1998) and Lawrence et al. (1996) observed no significant differences in relative lantern size among *Strongylocentrotus droebachiensis* and *Tetrapyrgus niger* fed varying feeds or varying feed rations.

The functional significance of a comparatively large jaw apparatus is not entirely understood. It has been suggested that the increased size may, in part, be an effect of enlarging the muscles associated with the jaws due to increased scraping effort (Ebert, 1980). Another explanation is that a large lantern may help an individual obtain more food because it allows the urchin to graze upon a larger surface area (Black, 1984; Ebert, 1980, 1996; Lau et al., 2009; Lawrence et al., 1995; Minor and Scheibling 1997).

Using a formulated dry feed, the effect of specific nutrients has only been investigated in one study. Jones et al. (2010) suggested that dietary manipulation of the mineral selenium affected the ratio of dry lantern weight to the dry weight of the test with spines, indicating a specific nutrient can affect phenotypic variation in lantern and/or test size. Comparatively, those nutrients which contribute to energy production or utilization may ultimately affect body organ allometry. In sea urchins, protein and carbohydrate are the two primary energy sources used for maintenance and growth (Marsh and Watts, 2007). Protein, carbohydrate, and their value as anabolic precursors and energy sources affected total body weight gain in *L. variegatus* (Heflin, 2010). Dietary changes in their profiles could potentially affect organ growth of the test, lantern and spines. The purpose of this study is to examine the effect of variations in dietary protein and carbohydrate level, protein: energy ratio, protein: carbohydrate ratio, and total dietary energy level on the relative size and composition of the test, spines, and Aristotle's lantern using a defined dry formulated diet in growing *Lytechinus variegatus*.

MATERIALS AND METHODS

Collection and Initial Measurements

Small adult *Lytechinus variegatus* (ca. 19.50 ± 2.01 g initial wet weight) were collected from St. Joseph Bay (30°N, 85.5°W), FL and transported to Texas AgriLIFE Mariculture Research Laboratory in Port Aransas, TX. Nineteen individuals were randomly selected for initial evaluation. Individuals were weighed (to the nearest mg) and photographed for diameter measurements (to the nearest 0.01 mm) using ImageJ® software. We suggest that this method is more accurate and causes less stress than use of calipers. Urchins were dissected by making a circular incision around the peristomial membrane. The gut, gonads and Aristotle's lantern (including muscles and pharynx) were removed. The interior surface of the test was scraped with a spatula to remove any soft tissue. The test and lantern were blotted with paper towels to remove excess moisture. The test with spines (including peristomial membrane) and Aristotle's lantern were weighed to the nearest mg. The tests with spines and the lanterns were dried at 60°C for 48 hours to a constant weight. Mean dry lantern and test with spines weights were calculated for the initial sample and used as an estimate for initial dry lantern and dry test with spines weights for urchins (n = 64) used in the study. Initial test diameters were measured for these urchins, and individuals were assigned randomly to one of eight dietary treatments (n=8 per diet). Initial test diameters did not differ significantly among dietary treatments.

Culture Conditions

Sea urchins were held in a semi-recirculating system with both mechanical and biological filtration and UV sterilization of the seawater. The culture system (2400 L) consisted of 16 interconnected 20 L fiberglass tanks containing water distributed from a central sump. Each tank held four cylindrical plastic mesh cages (12 cm dia., 30 cm height, and a 4 mm open mesh). Each plastic cage was inserted into a PVC coupling (11.5 cm I.D.) and elevated with PVC spacers to allow unimpeded seawater circulation throughout the cage. Each cage housed one individual. Cages were identical to eliminate the potential effects of vessel shape on test phenotype (Hernandez and Russell, 2010).

Water volume in each tank was maintained by a central standpipe, and natural seawater was supplied to each mesh enclosure at a ca. rate of 25 L hr^{-1} (water exchange rate of 3000% per day). Fresh seawater was passed through a stratified sand filter and a Diamond water filter (Diamond Water Conditioning, Horton, WI). Seawater in the entire culture system was exchanged at a rate of 10% per day. Water quality parameters measured included total ammonia nitrogen, nitrate nitrogen, nitrite nitrogen, pH (Brinkman Metrohm® pH meter), temperature, and salinity (Yellow Springs International Inc., Yellow Springs, OH) based on method modified from those of Spotte (1979a,b), Solarzano (1969), Mullen and Riley (1955), and Strickland and Parsons (1972).

Diet and Diet Preparation

Eight semi-purified diets were formulated and produced using both purified and practical ingredients. Levels of dietary protein and carbohydrate (Table 1, Table 2) ranged from 12 to 36 % protein (using purified plant and animal sources) and 21 to 39% carbohydrate (using a

purified starch source). Levels of protein and carbohydrate were adjusted with acid-washed diatomaceous earth, which has no effect on sea urchins at these levels (unpublished data). All other nutrients were constant among treatments. The proximate components are shown in Table 2.

Dry ingredients were mixed with a PK twin shell® blender (Patterson-Kelley Co., East Stroudsburg, PA) for 10 minutes. Dry ingredients were then transferred to a Hobart stand mixer (Model A-200, Hobart Corporation, Troy, OH) and blended for 40 minutes. Liquid ingredients were added, and the mixture was blended for an additional 10 minutes to a mash-like consistency. The diets were extruded using a meat chopper attachment (Model A-200, Hobart Corporation, Troy, OH) fitted with a 4.8 mm die. Feed strands were separated and dried on wire trays in a forced air oven (35°C) for 48 hours. Final moisture content of all feed treatments was 8–10%. Feed was stored in air-tight storage bags at 4°C until used.

Feeding Rate

Each sea urchin was proffered a limiting daily ration (sub-satiation) equal to 1.5% of the initial average wet body weight of all individuals. Feeding at sub-satiation ensured that urchins consumed all food proffered within a 24-hour period and allowed for direct measure of feed intake. A sub-satiation feeding regime also prevented individuals from compensating for a dietary deficiency by increasing consumption (Taylor, 2006). Individuals were photographed for diameter measurements every three weeks and feed rations were adjusted to be equivalent to 1.5% of the average body weight at that time (Table 3). Consumption of all food proffered was confirmed by direct observation. Feces were removed by siphon immediately prior to feeding each day.

Daily feeding rate was calculated as:

$$(1) \quad \text{Average wet weight of individuals in the study (g)} \times 0.015$$

Protein: energy ratio of each feed was calculated as:

$$(2) \quad \text{Protein (mg)/energy content (Kcal)}$$

Protein: Carbohydrate ratio of each feed was calculated as:

$$(3) \quad \text{mg protein mg carbohydrate}$$

Total energy content of each feed was calculated with the caloric equivalents of Phillips (1972):

$$*(4) \quad \% \text{ protein}/100 \times 5650 \text{ (cal g}^{-1}\text{)} + \% \text{ carbohydrate}/100 \times 4000 \text{ (cal g}^{-1}\text{)} + \% \text{ lipid}/100 \times 9450 \text{ (cal g}^{-1}\text{)}$$

After nine weeks, urchins were dissected as previously described.

Growth

To measure test diameters, urchins were photographed from above and diameters were measured (0.01 mm) using ImageJ® software. For each individual, two diameters were

recorded across the ambitus (each perpendicular to the other) and the average calculated. Test diameter was measured every three weeks. Diameter increase was calculated as:

$$(5) \quad \text{Final diameter (mm)} - \text{initial diameter (mm)}$$

Estimated Aristotle's lantern and test with spines dry matter production were calculated for each individual as:

$$(6) \quad \text{Final dry weight of lantern (or test with spines and peristomial membrane (g))} - \text{initial average dry weight of lantern (or test with spines and peristomial membrane (g))}$$

Final dry Aristotle's lantern or test with spines index of an individual was calculated as:

$$(7) \quad \text{Dry weight of Aristotle's lantern (or test with spines and peristomial membrane (g))} / \text{dry weight of individual (g)} \times 100$$

Final dry Aristotle's lantern to final dry test with spines index was calculated as:

$$(8) \quad \text{Dry weight of Aristotle's lantern (g)} / \text{dry weight of test with spines and peristomial membrane (g)} \times 100$$

Percent Organic Matter of Test and Spines

For each individual, a ¼ section of the dry test was analyzed, with oral and aboral plates and spines removed from a lateral quadrant (not including the peristomial membrane). Test plates and spines were placed separately in pre-weighed crucibles. The combined crucible and tissue weight was recorded, and tissues were ashed in a muffle furnace at 500°C for 4 hours. After cooling, the combined weight of the crucible and tissue was recorded, and the percent organic matter was calculated as:

$$(9) \quad (\text{dry tissue weight} - \text{weight of inorganic matter (g)}) / \text{dry weight of tissue (g)} \times 100$$

Statistics

To determine the relationship between carbohydrate and protein level on lantern and test growth measurements, multiple linear regressions were conducted in R 2.11.1 (www.r-project.org). For each physical growth response, different mathematical models representing *a priori* hypotheses were compared using the Akaike Information Criterion (AIC) score (Burnham and Anderson, 2002). The AIC is one of many information-theoretic approaches that allows for direct comparison of non-nested models with varying number of parameters. The *a priori* hypotheses compared were that lantern and test size (see Table 3 for the complete list of response variables examined) are dependent upon: 1) protein level, carbohydrate level, and their interaction, 2) total energy, 3) protein: energy ratio, and 4) protein:carbohydrate ratio (descriptions of equations used to derive values provided above). Because initial analyses showed that, at the dietary levels used, the interaction between protein and carbohydrate levels as well as carbohydrate levels themselves were often statistically unimportant, two parameter-reduced models were considered. These included models with protein and carbohydrate level and only the protein level. Model fit was checked by examining the residuals for normality and homoscedasticity visually. For the

lantern/total size index, lantern was included as the response and total size was a covariate. One response variable, the lantern/test index, was log transformed to better satisfy linearity.

RESULTS

Water Quality

Water conditions were maintained as follows: 32 ± 0.5 ppt salinity, $22 \pm 2^\circ\text{C}$, D.O. 7 ± 2 ppm., ammonia <0.1 ppm, nitrite <0.1 ppm, nitrate <0.1 ppm, and pH 8.2. A 12:12 light: dark photoperiod was maintained.

Test and Spines Analyses

The best models of test diameter increase included dietary protein level and protein: energy ratio (Table 3). Parameter estimates indicated test diameter of individuals increased by 0.19 mm for every one percent increase in dietary protein level, (Table 4, Fig. 1) and by 0.08 mm for every one mg P kcal⁻¹ increase in protein: energy ratio (Table 4).

The dietary protein level model was also the best predictor of test with spines wet weight gain and dry matter production (Table 3). Test with spines wet weight gain and dry matter production were directly proportional to protein consumption (Table 5, Fig. 2, Fig. 3). Parameter estimates showed that test with spines wet weight of individuals increased by 0.201 g and dry matter production increased by 0.085 g for every one percent increase in dietary protein level (Table 4). Dietary carbohydrate level was a poor model of test with spines growth in terms of diameter increase, wet weight gain, and dry matter production.

Organic content (%) of the test (without spines) varied among dietary treatments (Table 6). The protein: carbohydrate interaction effect model was best associated with variations in organic content (Table 3, Fig. 4). When the carbohydrate level was held constant, organic content of the test varied directly with dietary protein at levels below 19%. However, at dietary protein levels above 19%, there was an inverse correlation between dietary protein level and test organic content. Test organic content was inversely correlated with dietary carbohydrate levels used in this study.

Organic content of the spines varied among dietary treatments (Table 6). The percent organic content of the spines was best associated with the protein: energy ratio model (Table 4). Parameter estimates showed that organic content increased 0.015 percent for every mg P kcal⁻¹ increase in protein: energy ratio (Table 4).

Lantern Analysis

Lantern wet weight gain corrected for size of the individual was best associated and inversely correlated with dietary protein level (Fig.7).

Lantern to Test with Spines Index

The dietary protein level model was the best predictor of the log transformed dry lantern to dry test with spines index (Table 3). Parameter estimates showed that for every one percent

increase in dietary protein, the log of the lantern to test with spines index decreased by 0.011 ($p < 0.001$, Table 4).

DISCUSSION

Water quality parameters maintained in this study were within acceptable ranges for sea urchins (Basuyaux and Mathieu, 1998). This is further supported by the high survivorship and high growth rates exhibited by all treatments.

Like most organisms, the growth rate of sea urchins is greatly influenced by the quality and quantity of food available (Lawrence and Lane, 1982). Urchins increase growth rate with an increase in intake of nutrients. As feed rations in this study were below satiation, any variations in the size of the test with spines and the relative size of the Aristotle's lantern can be directly attributed to variations in the nutrient and/or energy content of the diets instead of the quantity of dry matter consumed. Additionally, feeding at defined levels below satiation removed the potential effect of compensatory feed intake.

During severe food limitation, sea urchins may exhibit plasticity of the test, remodeling the body wall to use stored nutrients for metabolism which results in a decrease in test size (Ebert, 1968, 1980; Guillou, 2000; Levitan, 1991). This process may be slow to occur (Lares, 1998) and, while it may be beneficial to the individual (since a smaller body size requires fewer resources to maintain (Ebert, 1996), test remodeling may be energetically costly and probably only occurs under conditions of extreme food restriction. Lares (1998) starved *L. variegatus* for 2.5 months and did not observe changes in test diameter; however, nutrient stores in the test of starved individuals decreased, suggesting that the test diameter of *L. variegatus* may shrink under extended conditions of extreme food restriction. Individuals in the present study were not starved or subjected to significant food restriction and all tests increased in diameter and weight.

Dietary protein levels directly influence test growth in sea urchins (reviewed by McBride et al., 1998). Hammer (2006) reported larger diameters and higher wet and dry test weights in *L. variegatus* fed 20% protein as compared to individuals fed 9% protein. McBride et al (1998) found no differences in test diameter among *Strongylocentrotus franciscanus* fed prepared diets with protein levels ranging from 30–50%. Although diets *used in these studies were not isocaloric, the observed growth differences in the present study were not the result of differences in dietary energy, but seemingly were affected primarily by dietary protein content. The 12% protein diets, while adequate for maintenance and survival, do not provide enough protein for maximal test with spines growth.

Dietary protein and carbohydrate levels both correlated with nutrient storage in the body wall. The concentration (% dry weight) of organic matter in the tests of urchins fed diets with 12% protein was lower than that of urchins fed diets with 19% protein. However, at the 19 and 12% levels of dietary protein, we observed an inverse relationship between dietary carbohydrate level and the concentration of organic matter in the test, with the largest effect observed at the 19% protein level. We hypothesize that, under the conditions of this study, high levels of dietary carbohydrate in some way inhibited the processing and/or assimilation

of protein. Because protein is the major component of the sea urchin test (Lawrence and Lane, 1982), we believe that the difference in organic matter concentration in the test most likely indicates that minimal protein was allocated to storage in the test of the individuals fed 12% protein, suggesting this level is not adequate for optimal test growth and nutrient storage. Above 19%, the decrease in percent organic matter suggests that, in addition to dietary protein and carbohydrate levels, another factor also influenced the storage of organic matter in the tests of these individuals. We hypothesize that the high metabolic cost of absorbing and assimilating high levels of dietary protein may decrease the deposition of organic matter to the test. A similar trend was observed by Hammer et al. (2006) in *L. variegatus* fed at 9%, 20%, or 31% protein (decrease observed at 31% protein).

Percent organic matter of the spines was lower than that of the test in all treatments, most likely reflecting structural and functional differences in these tissues. Percent organic matter of the spines varied among dietary treatments, but the biological significance of these differences is not apparent.

Final wet weight and dry matter production of the Aristotle's lantern did not vary among treatments, indicating little or no variation in the growth rate of the lantern in response to large differences in dietary protein and carbohydrate content during the 9- week study period. Although the size of the lantern did not vary among treatments, the relative size of the lantern (indexed to the size of the test with spines) did vary significantly with diet, due primarily to differences in test growth. These data indicate that growth rates of the test with spines increased directly with dietary protein, but the growth rates of the lantern did not, under the conditions of this study. Consequently, resource allocation to the test with spines varied with the quality of the diet (particularly protein), but resource allocation to the Aristotle's lantern remained constant, regardless of dietary quality.

In this study, specific nutrients affected resource allocation to the test and lantern in *L. variegatus*, inducing allometry in organ growth. Thus, organ allometry in sea urchins appears to be influenced not only by the quantity of food available, but also by availability of specific nutrients. Future studies can use formulated diets with varying levels of specific nutrients proffered at varying rations to evaluate the effects of both food quality and quantity (including starvation) on modeling and remodeling of the calcareous tissues (test and lantern).

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Highlights

Nutrient levels affect resource allocation to the Aristotle's lantern of *Lytechinus variegatus*

Nutrient levels affect resource allocation to the test of *Lytechinus variegatus*

Figure 1. Relationship between diameter increase (mm) and dietary protein level (%) of individual *L. variegatus* fed semipurified diets varying in protein level for 9 weeks.

Figure 2. Relationship between test with spines wet weight gain (g) and dietary protein level (%) of individual *L. variegatus* fed semipurified diets varying in protein level for 9 weeks.

Figure 3. Relationship between test with spines dry matter production (g) and dietary protein level (%) of individual *L. variegatus* fed semipurified diets varying in protein level for 9 weeks.

Figure 4. Surface plot of test with spines organic matter (%) as compared to dietary protein and carbohydrate in individual *L. variegatus* fed semipurified diets varying in protein level for 9 weeks.

Figure 5. Relationship between lantern wet weight (g) and total dietary energy level (cal g^{-1}) of individual *L. variegatus* fed semipurified diets varying in protein level for 9 weeks.

Figure 6. Relationship between lantern dry matter production (g) and dietary protein level (%) of individual *L. variegatus* fed semipurified diets varying in protein level for 9 weeks.

Figure 7.
Relationship between size-corrected lantern and dietary protein level (%) of individual *L. variegatus* fed semipurified diets varying in protein level for 9 weeks.

Figure 8.
Relationship between log (lantern/test with spines index) and dietary protein level (%) of individual *L. variegatus* fed semipurified diets varying in protein level for 9 weeks.

Table 1

Calculated protein and carbohydrate levels, total energy, protein: energy, and protein: carbohydrate ratios in each of the eight diets.

Protein (% dry weight)	Carbohydrate (% dry weight)	Total Energy (cal g ⁻¹)	Protein: Energy (mg P:kcal ⁻¹)	Protein: Carbohydrate (mg P : mg C ⁻¹)
36	21	3749	95	1.7
28	30	3299	76	0.93
19	21	2783	68	0.90
19	30	3130	60	0.63
19	39	3478	54	0.49
12	21	2380	50	0.57
12	30	2727	44	0.40
12	39	3075	39	0.31

Table 2

Proximate composition of the formulations used to produce diets varying in protein and carbohydrate levels.

	12% P: 21% C	12% P: 30% C	12% P: 39% C	19% P: 21% C	19% P: 30% C	19% P: 39% C	28% P: 30% C	36% P: 21% C
Crude protein (%)	12	12	12	19	19	19	28	36
Carbohydrate (%)	21	30	39	21	30	39	30	21
Fiber (%)	4.5	4.5	4.5	4.5	4.5	4.5	4.6	4.6
Diatomaceous Earth (DE, %)	27	18	9	19	10	1	0	0
Non-DE Ash (%)	24	24	24	24	24	24	24	25
Crude fat (%)	7	7	7	7	7	7	7	7

All values are calculated, and on an "as fed" basis. All diets contain up to 28% marine ingredients, 28.7% plant ingredients, 1.1% carotenoids, 0.7% vitamin premix, 24 % mineral mix, 7.2% binder and anti-fungal-antioxidant agents.

Table 3

AIC scores for each growth model. Rows are the response variable and columns are the variables in the model. Scores are only comparable for models with the same response variable. All models within one information unit of the best model are in **bold**, while the best model is in **bold and underlined**. $P + C + (P \times C) = \text{Protein} + \text{Carbohydrate} + (\text{Protein} \times \text{Carbohydrate})$. TE = Total Dietary Energy. P: E = Protein: Energy Ratio. PC = Protein: Carbohydrate Ratio.

Response Variable	P + C + (P×C)	Protein + Carbohydrate	Protein (%dry weight)	Total Energy (cal g ⁻¹ dry weight ¹)	Protein: Energy (mg P: kcal ⁻¹)	P: C (mg P: mg C ⁻¹)
Test with Spines Wet Weight Gain (g)	243.02	241.09	<u>239.14</u>	250.10	241.35	245.26
Test with Spines Dry Matter Production (g)	150.94	148.94	<u>146.97</u>	157.18	149.44	151.99
Lantern Wet Weight Gain (g)	-35.48	-35.24	-37.17	-37.20	-37.16	-37.19
Lantern Dry Matter Production (g)	-111.36	-112.18	<u>-114.18</u>	-114.12	-114.10	-114.09
Log (Lantern/Test with Spines Index)	-70.58	-70.93	<u>-72.91</u>	-64.25	-69.92	-67.52
Test Diameter Increase (mm)	298.36	287.40	<u>285.40</u>	290.99	285.74	288.69
Test Percent Organic Matter ^a	<u>162.8</u>	167.5	165.5	177.2	173.65	182.14
Spines Percent Organic Matter	<u>172.05</u>	<u>171.06</u>	174.39	176.55	171.78	172.6

^aFor this response, all models included a quadratic term (protein in the multiple regression)

Table 4

Parameter estimates and tests of significance for various measures of *Lytechinus variegatus* growth models. Only statistically significant terms ($P < 0.05$) are included (if an interaction was found to be significant, main effects were included regardless of associated p-values). Associated p-values for parameter estimates being significantly different than 0 are included as * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$.

Response Variable	Separate effects		Combined effects			
	Protein (% dry weight)	Carbohydrate (%dry weight)	Protein × Carbohydrate	Total energy (cal g dry weight ⁻¹)	Protein: Energy (mg P: kcal ⁻¹)	Protein: Carbohydrate (mg P mg C ⁻¹)
Test with Spines Wet Weight Gain (g)	0.201***	-	-	0.0031***	0.089***	4.498***
Test with Spines Dry Matter Production (g)	0.085***	-	-	0.0013***	0.037***	1.912***
Lantern Wet Weight Gain (g)	-	-	-	-	-	-
Lantern Dry Matter Production (g)	-	-	-	-	-	-
Log (Lantern/Test with Spines Index) ^a	-0.011***	-	-	-0.00017***	-0.0049***	-0.248***
Lantern/Total Index	-0.005**	-	-	-0.00007*	-0.0019*	-0.092*
Diameter Increase (mm)	0.19***	-	-	-	0.09***	3.29***
Test Percent Organic Matter (quadratic term)	.989*** (-0.015***)	0.162*	-0.01*	0.013** (-0.000002*)	0.207*** (-0.0014**)	4.08* (-1.73*)
Spines Percent Organic Matter	-	-	-	-	0.015*	-

^a parameters shown for this response are given in terms of the log scale of the lantern to test with spines index but can be converted for any dietary protein level (%) by using $e^{-0.011}$

* protein level.

Table 5

Mean values ($\pm$ SEM) at the end of the experiment for test with spines wet weight, test with spines wet weight gain, dry test with spines index, test with spines production and test diameter increase of *Lytechinus variegatus* fed diets with varying protein and carbohydrate levels, protein: energy ratios (P: E), total energy (TE), and protein: carbohydrate ratios (P: C). P: E represents mg of protein per kilocalorie. TE represents total dietary energy in calories per gram. P: C represents protein: carbohydrate ratio in mg per mg.

Protein (%)	Carbohydrate (%)	P:E (mg P kcal ⁻¹)	TE (cal g ⁻¹)	P:C (mg mg ⁻¹)	Final Wet Test with Spines Weight (g)	Wet Test with Spines Weight Gain (g)	Final Dry Test with Spines Weight (g)	Dry Test with Spines Production (g)	Final Test Diameter (mm)	Test Diameter Increase (mm)
12	21	50.8	2380	0.57	13.72 $\pm$ 0.69	6.87 $\pm$ 0.69	7.19 $\pm$ 0.33	3.50 $\pm$ 0.33	49.94 $\pm$ 0.97	11.19 $\pm$ 0.91
12	30	44.3	2728	0.40	14.65 $\pm$ 0.57	7.80 $\pm$ 0.57	7.46 $\pm$ 0.24	3.77 $\pm$ 0.24	49.96 $\pm$ 0.97	11.74 $\pm$ 0.82
12	39	39.3	3075	0.31	14.57 $\pm$ 0.65	7.72 $\pm$ 0.65	7.58 $\pm$ 0.33	3.89 $\pm$ 0.33	50.38 $\pm$ 0.78	12.23 $\pm$ 0.69
19	21	68.6	2783	0.90	16.60 $\pm$ 0.68	9.75 $\pm$ 0.68	8.26 $\pm$ 0.29	4.56 $\pm$ 0.29	52.47 $\pm$ 0.84	14.39 $\pm$ 0.76
19	30	60.9	3131	0.63	16.39 $\pm$ 0.90	9.54 $\pm$ 0.90	8.14 $\pm$ 0.36	4.44 $\pm$ 0.36	50.89 $\pm$ 0.67	12.80 $\pm$ 0.88
19	39	54.9	3478	0.49	15.31 $\pm$ 0.41	8.46 $\pm$ 0.41	7.60 $\pm$ 0.25	3.91 $\pm$ 0.25	51.02 $\pm$ 1.78	12.63 $\pm$ 1.43
28	30	76.9	3647	0.93	18.22 $\pm$ 0.97	11.37 $\pm$ 0.97	9.08 $\pm$ 0.42	5.39 $\pm$ 0.42	53.47 $\pm$ 0.68	15.47 $\pm$ 1.17
36	21	95.6	3749	1.71	18.84 $\pm$ 0.57	11.99 $\pm$ 0.57	9.31 $\pm$ 0.25	5.61 $\pm$ 0.25	55.37 $\pm$ 0.82	16.30 $\pm$ 1.22

Initial average wet test with spines weight was 6.85 $\pm$ 0.17 g

Initial average dry test with spines weight was 3.65 $\pm$ 0.33 g

Table 6

Mean ($\pm$ SEM) test and spine organic matter (%) of *Lytechinus variegatus* fed diets with varying protein and carbohydrate levels, protein: energy ratios (P: E), total energy (TE), and protein: carbohydrate ratios (P: C). P: E represents mg of protein per kilocalorie. TE represents total dietary energy in calories per gram. P: C represents protein: carbohydrate ratio in mg per mg.

Protein (%)	Carbohydrate (%)	P: E (mg P kcal ⁻¹)	TE (cal g ⁻¹)	P: C (mg mg ⁻¹)	Test Organic Matter (% dry weight)	Spines Organic Matter (% dry weight)
12	39	39.3	3075	0.31	12.36 $\pm$ 0.34	10.04 $\pm$ 0.29
12	30	44.3	2728	0.40	12.78 $\pm$ 0.19	10.02 $\pm$ 0.22
12	21	50.8	2380	0.57	12.16 $\pm$ 0.28	10.92 $\pm$ 0.32
19	39	54.9	3478	0.49	14.26 $\pm$ 2.03	10.65 $\pm$ 0.42
19	30	60.9	3131	0.63	14.54 $\pm$ 0.42	10.97 $\pm$ 0.25
19	21	68.6	2783	0.90	14.01 $\pm$ 0.22	11.43 $\pm$ 0.34
28	30	76.9	3647	0.93	13.23 $\pm$ 0.24	10.28 $\pm$ 0.42
36	21	95.6	3749	1.71	13.51 $\pm$ 0.16	11.40 $\pm$ 0.38

Table 7

Mean ($\pm$ SEM) final lantern wet weight, lantern wet weight gain, lantern production, dry lantern/dry test with spines index of *Lytechinus variegatus* fed diets with varying protein and carbohydrate levels, protein: energy ratios (P: E), total energy (TE), and protein: carbohydrate ratios (P: C). P: E represents mg of protein per kilocalorie. TE represents total dietary energy in calories per gram. P: C represents protein: carbohydrate ratio in mg per mg.

Protein (%)	Carbohydrate (%)	P: E (mg P/ kcal)	TE (cal g ⁻¹)	P: C (mg mg ⁻¹)	Final Wet Lantern Weight (g)	Wet Lantern Weight Gain (g)	Final Dry Lantern Weight (g)	Lantern Dry Matter Production (g)	Lantern Index (%)
12	21	50.8	2380	0.57	1.07 $\pm$ 0.09	0.34 $\pm$ 0.09	0.64 $\pm$ 0.05	0.22 $\pm$ 0.05	8.93 $\pm$ 0.60
12	30	44.3	2728	0.40	1.19 $\pm$ 0.05	0.45 $\pm$ 0.05	0.67 $\pm$ 0.01	0.25 $\pm$ 0.01	9.04 $\pm$ 0.22
12	39	39.3	3075	0.31	1.17 $\pm$ 0.05	0.43 $\pm$ 0.05	0.69 $\pm$ 0.02	0.27 $\pm$ 0.02	9.20 $\pm$ 0.30
19	21	68.6	2783	0.90	1.17 $\pm$ 0.05	0.43 $\pm$ 0.05	0.69 $\pm$ 0.02	0.27 $\pm$ 0.02	8.43 $\pm$ 0.47
19	30	60.9	3131	0.63	1.19 $\pm$ 0.07	0.45 $\pm$ 0.07	0.71 $\pm$ 0.03	0.29 $\pm$ 0.03	8.72 $\pm$ 0.27
19	39	54.9	3478	0.49	1.04 $\pm$ 0.08	0.30 $\pm$ 0.07	0.63 $\pm$ 0.05	0.21 $\pm$ 0.05	8.33 $\pm$ 0.67
28	30	76.9	3647	0.93	1.09 $\pm$ 0.05	0.35 $\pm$ 0.05	0.64 $\pm$ 0.04	0.22 $\pm$ 0.04	7.08 $\pm$ 0.40
36	21	95.6	3749	1.71	1.16 $\pm$ 0.05	0.43 $\pm$ 0.05	0.66 $\pm$ 0.32	0.24 $\pm$ -0.32	7.12 $\pm$ 0.31

Initial average lantern wet weight was 0.74 $\pm$ 0.04 g

Initial average lantern dry weight was 0.64 $\pm$ 0.05 g