EFFECTS OF DIETARY CARBOHYDRATE ON WEIGHT GAIN AND GONAD PRODUCTION IN JUVENILE SEA URCHINS, *LYTECHINUS VARIEGATUS*

by

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ABSTRACT

Formulated feeds that support weight gain and survival in juvenile sea urchins are necessary for successful sea urchin aquaculture. In Chapter 1, juvenile _Lytechinus variegatus_ were fed _ad libitum_ one of four experimental feeds: 1) mixed-taxa algal biofilm (MTAB), 2) freeze-dried MTAB, 3) a commercial, small mammal feed (Friskies® cat treats), or 4) a commercial, formulated feed for sea urchins. The MTAB and sea urchin feed supported weight gain and survival, suggesting that juveniles as small as 3 to 4 mm can utilize formulated feeds for growth.

In Chapter 2, juvenile _Lytechinus variegatus_ were fed _ad libitum_ one of five feeds: three semi-purified, formulated feeds with constant protein (31% dry weight) and varying levels of dietary carbohydrate, low, medium, and high (19, 26, and 38% dry weight, respectively); one semi-purified, formulated feed with high protein (45% dry weight) and medium carbohydrate (24% dry weight); and a live, mixed-taxa algal biofilm (MTAB). Sea urchins fed the 19% carbohydrate and 24% carbohydrate, 45% protein feed gained significantly more weight over the 8-wk study. For those sea urchins fed feeds that varied only in carbohydrate level, we hypothesize that weight gain, while inversely proportional to carbohydrate level, was directly related to consumption. Sea urchins fed the 19% feed consumed the same amount of protein as sea urchins fed the 24% carbohydrate, 45% protein feed, and all treatments consumed the same amount of energy. In consuming feeds to meet a possible energy requirement, the sea urchins that consumed more feed also consumed more protein, as well as other nutrients, which resulted in
higher weight gain. Consequently, protein: energy ratio may be important in determining feed utilization and growth. Sea urchins fed the formulated feeds had measurable gonads at a minimum diameter of at least 13 mm. Finally, we suggest that more information is needed to determine the effects of leaching in nutrition studies.
DEDICATION

To my family and husband for their unconditional love and support.
ACKNOWLEDGEMENTS

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I am very grateful for the help of Dr. Anthony Siccardi III, who greatly contributed to this work technically and intellectually. Also, I would like to thank the committee chairman, Dr. Stephen Watts, for all of the quality time and effort that he has dedicated to this work and helping me attain my goals; Dr. John M. Lawrence, for contributing to many aspects of this work; Dr. Mickie Powell, for her technical assistance and review comments; and lastly, Dr. Addison Lawrence for contributing a wealth of knowledge and many helpful comments.

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FORMULATED FEED SUPPORTS WEIGHT GAIN AND SURVIVORSHIP IN JUVENILE SEA URCHINS, LYTECHINUS VARIEGATUS

by

ANNA M. TAYLOR, MICKIE L. POWELL, ADDISON L. LAWRENCE, STEPHEN A. WATTS

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CHAPTER 1

FORMULATED FEED SUPPORTS WEIGHT GAIN AND SURVIVORSHIP IN JUVENILE SEA URCHINS, LYTECHINUS VARIEGATUS

Abstract

In adult sea urchins, formulated feeds support weight gain and gonad production; however, studies demonstrating the effects of formulated feeds on juvenile sea urchin growth are limited. In this study, juvenile sea urchins (3.20-7.33 mm, N = 12/treatment) were reared individually in artificial seawater and fed one of four experimental feeds: 1) mixed-taxa algal biofilm (MTAB), 2) freeze-dried MTAB, 3) a commercial, small mammal feed (Friskies® cat treats), or 4) a semi-purified feed formulated for sea urchins. The MTAB and sea urchin feed supported weight gain and survival throughout the study; however, those fed the sea urchin feed exhibited a short lag period at the onset of feeding. This short lag period may be, in part, due to the initial attraction of the urchins to the formulated feed. Furthermore, we hypothesize that gut physiology or gut flora must acclimatize to a new diet (all sea urchins were reared initially on the MTAB). The freeze-dried MTAB and mammal feed did not support substantial weight gain. This study suggests that juveniles as small as 3 to 4 mm can utilize formulated feeds for growth.
Sea urchin gonads (roe or uni) are regarded as a delicacy around the world, particularly in Asia and Europe. The high demand for roe has resulted in overfishing of wild populations (Keesing and Hall 1999; Andrews et al. 2002), promoting the development of sea urchin aquaculture (Klinger et al. 1997; McBride et al. 1997; Watts et al. 1998). For a successful sea urchin aquaculture industry, economically-viable, complete life cycle culture must be developed. A nutritionally-complete formulated feed is an absolute requirement for a commercial sea urchin aquaculture industry (Lawrence and Lawrence 2004). Although studies using formulated feeds have reported enhanced gonad production in adults (Lawrence and Lawrence 2004), few studies have evaluated formulated feeds in juvenile and small sea urchins (Fernandez and Boudouresque 1998; Williams and Harris 1998; Kennedy et al. 1999; Akiyama et al. 2001; Hammer et al. 2004; Pearce et al. 2004; Daggett et al. 2005). Natural diets support reasonable growth in juveniles of many sea urchin species; however, feeding sea urchins a diet consisting of live, natural biota is infeasible due to the large quantities needed and the high costs of collection and storage. Therefore, the production of formulated feeds will be a priority for developing a sea urchin aquaculture industry, particularly in the early life-history stages.

This study compares the effects of a formulated, semi-purified sea urchin feed, a commercial feline mammal feed (Friskies®) of similar proximate composition, a live, mixed-taxa algal biofilm, and a freeze-dried form of biofilm on growth parameters of juvenile sea urchins, *Lytechinus variegatus*. 
Materials and Methods

Adult *Lytechinus variegatus* were collected from Saint Joseph’s Bay (30°N, 85.5°W), Florida in May 2004. Adult urchins were spawned by injecting 1 mL of 0.1 M acetylcholine into the coelomic cavity. Spawned gametes were collected, ova were fertilized, and developing larvae cultured on a mixed unicellular algal diet containing *Isochrysis galbana*, *Rhodomonas salina*, and *Dunaliella tertiolecta* in 100% synthetic seawater (Instant Ocean Sea Salt, 32 ppt salinity). After metamorphosis, juvenile sea urchins were reared on cultures of the benthic diatom *Amphora heleninsis* until ca. 1-1.5 mm diameter. Juveniles were then fed live, mixed-taxon green algal biofilm reported previously to support growth and survivorship in juveniles (Powell et al. 2005). Juveniles were selected at random and assigned to one of four experimental feed treatments (N = 12 for each treatment, diameters ranged from 3.20-7.33 mm, wet weights ranged from 0.03-0.35g). Individuals were held at constant photoperiod (12 hr: 12 hr, light:dark) and water temperature maintained at 27 ± 1 C. Initial wet weights did not vary significantly among treatments (ANOVA, P > 0.05).

Individuals were fed one of four diets ad libitum for a maximum of eight weeks. These diets include (1) a feed formulated for sea urchins (provided by Wenger International) (described by Hofer 2002), (2) a commercial, small-mammal feed of similar proximate composition to the sea urchin feed (Friskies® Cat Treats, Crunchy Tuna and Salmon Flavor Treats; Purina®), (3) a live, mixed-taxon algal biofilm (MTAB) containing green filamentous algae, red algae, diatoms, and an associated mucilage, and
Table 1. Composition (% dry weight basis) and moisture content of the sea urchin feed, Friskies® feed, mixed-taxon algal biofilm (MTAB), and freeze-dried MTAB.

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<th>Sea urchin feed&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Friskies® feed&lt;sup&gt;b&lt;/sup&gt;</th>
<th>MTAB&lt;sup&gt;c&lt;/sup&gt;</th>
<th>Freeze-dried MTAB&lt;sup&gt;c&lt;/sup&gt;</th>
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<tr>
<td>Crude protein</td>
<td>32.09</td>
<td>29.0</td>
<td>42.2</td>
<td>42.2</td>
</tr>
<tr>
<td>Crude fat</td>
<td>9.82</td>
<td>10.0</td>
<td>4.3</td>
<td>4.3</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>37.50</td>
<td>-</td>
<td>21.7</td>
<td>21.7</td>
</tr>
<tr>
<td>Crude fiber</td>
<td>6.66</td>
<td>4.0</td>
<td>1.6</td>
<td>1.6</td>
</tr>
<tr>
<td>Total ash</td>
<td>8.66</td>
<td>-</td>
<td>30.3</td>
<td>30.3</td>
</tr>
<tr>
<td>Moisture of constituted diet</td>
<td>78.0</td>
<td>78.0</td>
<td>93.0</td>
<td>78.0</td>
</tr>
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<sup>a</sup> Calculated composition provided by Dr. Addison Lawrence (TAMUS).

<sup>b</sup> Analysis provided by Purina®.

<sup>c</sup> Analysis provided by Dr. Louis D’Abramo and the Food Science Department at Mississippi State University.
(4) a freeze-dried MTAB. Proximate compositions of the diets are shown in Table 1. The sea urchin feed, Friskies® feed, and freeze-dried MTAB were ground using a Wiley mill (#40 mesh), and the dry feed was constituted into a soft pellet by adding 20 g of dry feed to a solution of heated synthetic seawater (78 mL, 60-70°C) containing 2 g of agar binder. The wet slurry was allowed to cool and solidify and was cut into pellets of ca. 1 cm³.

Fresh feed was prepared every four days and stored in a refrigerator at 4°C. The MTAB was fed to the sea urchins as a thin, filamentous sheet harvested from the sides of glass culture vessels. For the first four weeks, juvenile sea urchins were held individually in small 500 mL polyethylene containers (static-renewal). Uneaten food and feces was removed every day (only one day on the weekend) and new food was provided (consumption rates were not determined). Seawater was exchanged (100%) every two or three days to maintain water quality.

At week four, the Friskies® feed was terminated since sea urchins were consuming minimal feed quantities. All sea urchins in the remaining treatments were transferred individually to 1L polyethylene containers placed in 80 L aquaria (N=8 sea urchins/aquaria) with recirculating synthetic seawater (Hofer 2002). Water temperature was maintained at 24 ± 1°C. Water quality was maintained within optimal parameters (pH 8.2, ammonia and nitrite < 0.5 ppm, nitrate < 10 ppm, as determined using Lamotte reference assays).

Every two weeks, each sea urchin was blotted on a paper towel and weighed. To determine test diameter, individual sea urchins were photographed weekly with a Nikon digital camera using a submerged ruler as calibration and analyzed using the Optimus
6.51 imaging software (Media Cybernetics, L.P.). Individual specific growth rates (% body weight gain/ day, SGR) were calculated using the formula:

$$SGR = \frac{\ln (\text{final weight}) - \ln (\text{initial weight})}{\text{number of days}}$$

An ANOVA with a Tukey’s adjustment for multiple comparisons was used to determine significant differences of diameters, wet weights, and SGR. To analyze the scatter plot of diameter versus wet weight data for the sea urchin feed and MTAB, a log transform was applied to linearize the data from weeks four, six, and eight. An Analysis of Covariance (ANCOVA), with diameter and wet weight as the covariates and a Tukey’s adjustment for multiple comparisons, was performed on the regression lines to determine significant difference and interaction between the variables. All data were analyzed using SAS version 9.1 (SAS Institute, Inc., Cary, North Carolina, USA) and considered significant when P < 0.05.
Results

The MTAB supported the highest weight gain over the eight week period (ca. 3.20 ± 0.17 g/individual) (Fig. 1). Individuals fed the freeze-dried MTAB gained the least (0.39 ± 0.17 g/individual). Significant differences in weight gain were observed by week two; individuals fed the MTAB had gained significantly more weight than all other diets. By week six, the average wet weights of all treatments were significantly different (P < 0.0001). Relative increases in diameters exhibited trends similar to weight gain (Fig. 2).

Sea urchins fed the MTAB had the maximum SGR at week two (9.3% body weight gain/day), which decreased as individuals increased in weight (Fig. 3). Sea urchins fed the sea urchin feed exhibited an increase in SGR from week two to week four, reaching their highest SGR at week four. Growth rates of these individuals were high throughout the remainder of the study period.

Survivorship was high (> 83%) in sea urchins fed the sea urchin feed, the Friskies® feed, and MTAB (Fig. 4). Survivorship was reduced in those fed the freeze-dried MTAB (five of twelve individuals died within four weeks).

For all diets combined, the relation of wet weight to diameter could be described by the equation $y = 0.012x^2 - 0.094x + 0.26$, $R^2 = 0.98$ (Fig. 5). Regression analyses of log transformations of wet weights and diameters in sea urchins fed the sea urchin feed or MTAB (from week 4 to week 8) indicated a strong relation between test diameter and wet weight ($R^2 = 0.98$ and $R^2 = 0.95$, respectively) (Fig. 6). ANCOVA results from the transformed data indicated a significant difference between treatments, although there
was no significant interaction or difference in the slopes of the two regression lines (Fig. 6). Individuals fed the sea urchin feed weighed significantly more than individuals of the same diameter that were fed the MTAB.
Figure 1. Wet weight (g) of juvenile sea urchins, *Lytechinus variegatus*, fed one of four diets: commercial sea urchin feed, Friskies® feed, mixed-taxa algal biofilm (MTAB), and freeze-dried MTAB. Values represent means ± SE (N = 12 sea urchins/treatment).
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Figure 3. Specific growth rate (% body weight gain/day) calculated in two week intervals of juvenile sea urchins, *Lytechinus variegatus*, fed one of four diets: commercial sea urchin feed, Friskies® feed, mixed-taxa algal biofilm (MTAB), and freeze-dried MTAB. Values represent means ± SE (N = 12 sea urchins/treatment).
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Discussion

Comparatively high weight gain in individuals fed the MTAB may be attributed to nutritional history of the individual sea urchins and/or the nutritional value of the MTAB diet. All juveniles were reared initially on the MTAB prior to the study. The digestive physiology (enzyme activities, establishment of an appropriate bacterial flora in the gut, etc.) of the sea urchins may have acclimatized to the biofilm, and these sea urchins may have been able to digest and assimilate a biofilm more efficiently (Prim and Lawrence 1975; De Ridder and Foret 2001).

Natural diets consisting of live algae or kelp support growth in many juvenile sea urchins, including *Loxechinus albus* (Gonzalez et al. 1993), *Strongylocentrotus droebachiensis* (Kennedy et al. 1999; Nishizaki and Ackerman 2004; Daggett et al. 2005) and *Strongylocentrotus franciscanus* (Nishizaki and Ackerman 2004). In general, kelp diets contain low protein levels, 12-17% crude dry protein (Chapman and Craigie 1977). The MTAB used in this study had a higher protein level (42% crude dry protein). Nestler and Harris (1994), Williams and Harris (1998), and Meidal and Scheibling (1999) suggested that a diet of live kelp, *Laminaria* species, and an additional source of protein (the bryozoan, *Membranipora membranacea* or clam flesh, *Mytilus* species) supported growth better than a diet consisting of kelp alone, suggesting protein concentration is important for juvenile sea urchin growth. Regardless of its inherent value as a feed, a diet consisting of live, natural biota is infeasible due to the large quantities needed and the high costs of collection and storage.
The sea urchin feed supported significant weight gain and these individuals exhibited interesting growth trends. The rate of weight gain (SGR) of sea urchins fed the sea urchin feed increased significantly after sea urchins were fed the sea urchin feed for two weeks. These data suggest that the nutrient content of the sea urchin feed was sufficient to promote weight gain, but that a lag period was required. This lag period may be, in part, due to the initial attraction of the urchins to the formulated feed. We have previously observed a lag of about one week when small adults initially fed natural diets were switched to formulated feeds (unpub. data). Furthermore, we hypothesize that gut physiology or gut flora must acclimatize to a new diet (these sea urchins were reared previously on the MTAB). Alternatively, macro- or micronutrients in the sea urchin feed may have been limiting during the early period of juvenile growth. Sea urchins fed the sea urchin feed exhibited weight gain comparable to those fed the MTAB during the last four weeks of the study. Formulated feeds have also been shown to support growth in small *Paracentrotus lividus* (Fernandez and Boudouresque 1998), *Strongylocentrotus droebachiensis* (Williams and Harris 1998; Kennedy et al. 1999; Pearce et al. 2004; Daggett et al. 2005), *Pseudocentrotus depressus* (Akiyama et al. 2001), and *Lytechinus variegatus* (Hammer et al. 2004).

The Friskies® feed is a commercial feed adequate for supporting growth and maintenance of feline mammals. The Friskies® feed is similar in proximate composition to the formulated sea urchin feed; however, the Friskies® feed did not support weight gain in juvenile sea urchins. These data suggest that there are differences in the nutritional requirements of sea urchins compared to mammals. The similarities in proximate composition of these two feeds would suggest that differences in weight gain
were due to qualitative differences in macronutrients (e.g. essential amino acids) or to micro-nutrient composition (e.g. vitamins, minerals).

The freeze-dried MTAB had the same proximate composition as the MTAB, but it did not support weight gain and survivorship decreased. Obviously, one or more essential components (e.g. vitamins, growth factors, etc.) within the algae or the associated mucilage (bacteria) were destroyed or made unavailable when MTAB was dehydrated.

Regression analysis of log transformed wet weights and diameters of sea urchins fed the sea urchin feed and MTAB indicated that individuals fed the formulated sea urchin feed had significantly greater mass than individuals of the same diameter fed the MTAB. We hypothesize that weight differences between treatments were attributed to differences in the apparent energy content of the diets (sea urchin feed > MTAB), which resulted in differences in relative skeletal mass or soft tissue production (gut and/or gonad). Since no dissections were performed, the specific difference could not be determined. Meidal and Scheibling (1999) suggested that diets of high food quality and quantity accelerated reproductive maturation, growth rate, and enhanced gonad production in juvenile and young adult *Strongylocentrotus droebachiensis*.

We conclude that the formulated sea urchin feed and the live MTAB provided sufficient nutrients to promote weight gain in juvenile *L. variegatus*. However, the use of live diets may not be practical in large-scale culture operations, and the freeze-dried MTAB did not support weight gain or survivorship. Comparison of a feed formulated for sea urchins and a feed formulated for mammals indicate substantial differences in nutritional requirements of these organisms despite similar proximate compositions. This
study suggests that juveniles as small as 3 to 4 mm can utilize formulated feeds for
growth. Formulated feeds for juvenile sea urchins are feasible and more practical than
live, natural diets for supporting and maintaining growth in culture.
Literature Cited


EFFECTS OF DIETARY CARBOHYDRATE ON WEIGHT GAIN AND GONAD PRODUCTION IN JUVENILE SEA URCHINS, LYTECHINUS VARIEGATUS

by

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CHAPTER 2
EFFECT OF DIETARY CARBOHYDRATE ON WEIGHT GAIN AND GONAD PRODUCTION IN JUVENILE SEA URCHINS, LYTECHINUS VARIEGATUS

Abstract

Cultured, juvenile sea urchins (N = 80, 0.088 ± 0.001 g wet weight and 5.72 ± 0.04 mm diameter) were held individually and randomly assigned to one of five feeds (N = 16 individuals/treatment). The feeds included three semi-purified, formulated feeds with constant protein (31% dry weight) with varying levels of dietary carbohydrate, low (19% dry weight), medium (26% dry weight), and high (38% dry weight); one semi-purified, formulated feed with high protein (45% dry weight) and medium carbohydrate (24% dry weight); and a live, mixed taxa algal biofilm (MTAB) fed *ad libitum*. Wet weights and diameters were measured every 2 weeks, and sea urchins were dissected and organs removed at the end of the 8-week study period.

At weeks 6 and 8, sea urchins fed the 19% carbohydrate feed had gained significantly more wet weight than sea urchins fed the 38% carbohydrate feed. There were no significant differences between wet or dry weights of the test, lantern, or gut, and sea urchins fed the MTAB had wet and dry gonad weights that were slightly smaller than the gonads of sea urchins fed formulated feeds (P= 0.08 and P=0.09, respectively). Gonads were measurable in sea urchins having a minimum test diameter of at least
13mm. Gonads increased appreciably for sea urchins having a test diameter of at least 18mm.

A consumption study was conducted to determine the apparent consumption of the four formulated feeds. Sea urchins (N = 10 individuals/treatment) were fed a pre-weighed amount of one of four formulated feeds ad libitum each day for 12 days. Uneaten feed and feces were removed each day and collected over the 12-day study period. The amount of dry matter leached in 24 hours was determined (26.7 to 37.7% loss) and used to estimate the amount of feed available for sea urchins after the food was exposed to water for 24 hrs. Sea urchins fed the 19% carbohydrate feed consumed significantly more dry feed than sea urchins fed other feeds. Thus, sea urchins fed the 19% carbohydrate feed consumed more protein than sea urchins fed the 26 and 38% carbohydrate feeds since all three feeds contain the same protein level.

Weight gain was inversely proportional to the carbohydrate level when protein in the feed was constant. For sea urchins fed feeds that varied in carbohydrate level, we hypothesize that weight gain, while inversely proportion to carbohydrate level, was directly related to consumption. In consuming feeds to meet energy requirements, sea urchins that consumed higher levels of protein and other dietary nutrients as well, had the highest weight gain. Consequently, the protein: energy ratio may be important in determining feed utilization and growth. Finally, we suggest that more information is needed to determine the effects of leaching for future consideration for some nutrition studies.
Sea urchin gonads (roe or uni) are regarded as an important seafood product in many parts of the world, particularly Asia and Europe. Historically, sea urchins have been harvested from natural habitats for their roe. The high demand for roe, the ease of capture and long recruitment time has resulted in overfishing of wild populations (Keesing and Hall 1999; Andrews et al. 2002). Depletion of wild populations could be ecologically detrimental; therefore, promoting the development of sea urchin aquaculture is desirable (Klinger et al. 1997; McBride et al. 1997; Watts et al. 1998). For the development of a successful commercial sea urchin aquaculture industry, a nutritionally-complete formulated feed will be required (Lawrence and Lawrence 2004).

Lawrence and Lawrence (2004) suggested that a viable formulated feed must have the appropriate nutrient quantity and quality. Therefore, feed ingredients should be assessed for quality, and the nutritional requirements of the organism must be determined to produce a cost-effective and nutritionally-complete feed. In addition, formulated feeds should support growth throughout most, if not all, life-history stages. Although growth is desirable, a formulated feed should support rapid somatic growth in juveniles while limiting early gonad production (Lawrence 2000) and the feed should promote gonad growth and maintenance in adult urchins of marketable size. Consequently, comparison of natural diets and formulated feeds at various life-history stages is one experimental approach which may provide information for the development of a commercial, formulated feed that is appropriate for adult and juvenile sea urchins.
In wild populations, adult sea urchins are found feeding mainly on attached or drifting plant material, encrusting animals or algae, or bottom material (Lawrence 1975). Other studies have shown that diet can change with age. Elmhirst (1922) reported that young *Echinus esculentes* consumed calcareous foods while Kawamura (1974) and Fuji (1967) noted that juvenile *Strongylocentrotus intermedius* consumed only detritus. Leighton (1968) suggested that decaying organic material may be more beneficial to young urchins than adults. Fenchel (1970) suggested that when young urchins consume decaying organic material, they are also consuming the associated microflora which could contribute to urchin nutrition. Jenson (1969), Nestler and Harris (1994), Williams and Harris (1998), and Meidal and Scheibling (1999) suggested that a diet of live kelp, *Laminaria* spp., and an additional source of protein (the bryozoan, *Membranipora membranacea* or clam flesh, *Mytilus* spp.) supported growth better than a diet consisting of kelp alone.

In addition to differences in types of food consumed by juvenile and adult sea urchins, feeding parameters and growth trends also differ. Small sea urchins exhibit higher specific growth rates compared to large urchins (Kennedy et al. 1999; Hammer et al. 2004). Small urchins can also exhibit an increase in feeding rates (Lawrence 1975; Barker et al. 1998), and since there is little or no gonad development in juvenile sea urchins, more energy can be allocated to survival and/or somatic growth (Lawrence and Bazhin 1998).

Although there are apparent differences in the diets of juvenile and adult sea urchins, several studies have demonstrated that natural diets can support reasonable growth in juvenile *Evechinus choloticus* (Barker et al. 1998), *Strongylocentrotus*...
droebachiensis (Nestler and Harris 1994; Williams and Harris 1998; Kennedy et al. 1999; Medial and Scheibling 1999; Nishizaki and Ackerman 2004; Pearce et al. 2004; Daggett et al. 2005), Strongylocentrotus franciscanus (Nishizaki and Ackerman 2004), and Loxechinus albus (Gonzalez et al. 1993). However, feeding sea urchins a diet consisting of live, natural biota in commercial aquaculture is infeasible due to the large quantities of feed needed and the high costs of collection and storage. These difficulties emphasize the need for the future development of formulated feeds (Lawrence and Lawrence 2004).

Several studies have shown that formulated feeds can support growth in small sea urchins including Paracentrotus lividus (Fernandez and Boudouresque 1998), Strongylocentrotus droebachiensis (Williams and Harris 1998; Kennedy et al. 1999; Pearce et al. 2004; Daggett et al. 2005), Pseudocentrotus depressus (Akiyama et al. 2001), and Lytechinus variegatus (Hammer et al. 2004; Taylor Chapter 1). These studies suggest that growth of juvenile sea urchins can be supported by formulated feeds. However, nothing is known in respect to the specific dietary nutrient and energy requirements of juvenile sea urchins.

Organisms require diets that provide sufficient energy for maintenance, growth, and reproduction. Dietary macro-nutrients (protein, lipid, and carbohydrates) all contribute to metabolic energy. However, sea urchins will probably utilize energy from certain classes or sources of macro-nutrients more efficiently than others. Lipids are the most energy dense macro-nutrient, provide important structural components of cellular membranes (Marsh and Watts 2007), and can function as vitamins or hormones. However, lipids are digested with lipases, and there are limited numbers of lipases found in the gut of sea urchins (Lawrence 1975; Lawrence et al. 2007). Also, lipids require the
availability of oxygen for β-oxidation. Sea urchins are believed to have limited oxygen availability in internal tissues; therefore, lipids are not believed to be a significant contributor to the metabolic energy of adult sea urchins (Marsh and Watts 2007).

Protein is an important macro-nutrient that provides essential amino acids for growth, maintenance, and reproduction (Morris 1991). Since many physiological processes are dependent upon protein, utilization of protein as an energy source when protein is limiting in the diet could reduce the amount allocated to these processes, and could ultimately reduce growth and/or reproduction. In adult sea urchins, the amount of protein in the feed affects gonad production (McBride et al. 1998; Akiyama et al. 2001; Pearce et al. 2002; Hammer et al. 2004; Hammer et al. 2006). Schlosser (2005) suggested Paracentrotus lividus fed a natural algae diet used protein as an energy source, which resulted in decreased gonad production. Similarly, Hammer et al. (2006) reported that protein efficiency ratios decreased in urchins fed high levels of dietary protein, further suggesting that protein could be used as an energy source. The known effects of dietary protein is limiting in juvenile sea urchins (Hammer et al. 2004). However, we hypothesize that the level of dietary protein in the feed is very important since juveniles typically exhibit high rates of growth. Because of the potential limitation of protein in natural diets (Lowe and Lawrence 1976; Chapman and Craigie 1977), the use of protein as a metabolic energy source for sea urchins may not be desirable.

Carbohydrate is the macro-nutrient that is most commonly oxidized and utilized as an energy source by herbivores and many omnivores (Morris 1991). Lawrence et al. (2007) noted that there are many carbohydrases in the sea urchin gut that oxidize complex polysaccharides into functional monosaccharides. Carbohydrates may also be
stored by the nutritive phagocytes in the gonad (Pearse and Cameron 1991; Marsh and Watts 2007; Hammer et al. 2006).Stored carbohydrates may affect gonad color and taste (Pearce et al. 2002; Unuma 2002) and/or provide energy for gametogenesis (Pearse and Cameron 1991; Marsh and Watts 2007). Knowledge of the carbohydrate requirements for juvenile sea urchins is not available.

Since carbohydrates and proteins may be the primary macro-nutrients utilized for metabolic energy and growth, respectively, it is very important that the dietary requirements for these nutrients be evaluated. Hammer (2006) recently evaluated feeds that varied in dry protein content, 21 to 31%, and dry carbohydrate, 44 to 33%, in adult *Lytechinus variegatus*. He found that individuals had the highest growth rates when fed high protein, low carbohydrate feeds. These feeds also had the highest protein: energy ratios, suggesting that adequate protein levels relative to energy were essential to promote optimal growth. The contribution of carbohydrate needed to satisfy energy requirements has not been determined. Sufficient carbohydrate in the feed could allow available protein to be assimilated into tissue and, as such, protein would not be metabolized as an energy source. For juvenile urchins, the carbohydrate content of a formulated feed should be sufficient to provide the urchin with enough metabolic energy to promote somatic growth, but it should not be in excess as to promote premature gonad growth. According to the energy budget constructed by Lawrence and Bahzin (1998), if metabolic energy is assimilated into somatic growth then gonadal growth should be minimal. For juvenile sea urchins, it would be desirable to have rapid growth to marketable size and no resulting conflict in energy allocation. Therefore, knowledge of the appropriate protein: energy
ratio and appropriate energy sources will provide the basis for the formulation of a feed that will promote rapid somatic growth and limit gonadal growth in juvenile sea urchins.

Although, the level of dietary carbohydrate may influence the growth of juvenile *L. variegatus*, consumption rates may also affect growth rates. Hammer (2006) suggested that the amount of protein, and potentially carbohydrate, in the feed affected consumption and growth parameters (test diameter and wet gain) in adult *L. variegatus* fed feeds with different levels of both protein and carbohydrate. This suggests that the dietary carbohydrate value of a feed may also influence consumption and possibly growth of juvenile *L. variegatus*.

In this study, sea urchins were fed four different semi-purified, cold-extruded formulated feeds. Three experimental feeds contained low, medium and high carbohydrate levels (19, 26, and 38% dry weight, respectively) at a protein level of 31% crude protein, and one contained medium carbohydrate (24% dry weight) and high protein (45% dry weight). Experimental feeds will be compared to a reference diet consisting of a live, mixed-taxa algal biofilm (MTAB). A separate leaching and consumption study will also be conducted to determine the effect of leaching on the feeds, along with the effect of the amount of dietary carbohydrate on consumption of juvenile sea urchins, *L. variegatus*. 
Materials and Methods

Adult Lytechinus variegatus were collected from Saint Joseph Bay, Florida (30° N, 85.5°W) in May 2005. Adult urchins were spawned by injecting 1 mL of 0.1M acetylcholine through the peristomial membrane into the coelomic cavity. Spawned gametes were collected, ova were fertilized, and developing larvae cultured on a mixed, unicellular algal diet containing Isochrysis galbana, Rhodomonas salina, and Dunaliella tertiolecta in 100% artificial saltwater (Instant Ocean Sea Salt, 32 ppt salinity). After metamorphosis, juvenile urchins were reared on cultures of the benthic diatom Amphora helenensis until ca. 1-1.5 mm diameter. Juveniles were then fed a live, mixed-taxa green algal biofilm (MTAB) reported previously to support growth and survivorship in juveniles (Powell et al. 2005). Individuals were maintained on a constant photoperiod (12 hr:12 hr, light:dark) and water temperature maintained at 24 ± 1 C.

Over five weeks, juvenile sea urchins were selected at random from large culture tanks. Initial juvenile wet weights ranged 0.07 to 0.123 g and diameters ranged from 4.9 to 6.8 mm; individuals that died within one week were replaced. Individuals were randomly assigned and fed ad libitum one of five feeds for eight weeks (N=16 sea urchins/treatment). These feeds included a live, mixed-taxa algal biofilm (MTAB) containing green filamentous algae, red algae, diatoms, and an associated mucilage, three semi-purified, formulated feeds with constant protein (31% dry protein) and varying carbohydrate content (19%, 26%, and 38% dry carbohydrate), and one formulated feed which has similar proximate nutrient values of the MTAB (24% dry carbohydrate and
45% dry protein). Table 1 contains the calculated values for the formulated feeds. Table 2 shows the proximate analyses of the feeds that were performed by Eurofins, Memphis, TN. Percent crude protein was determined by AOAC Method 990.3; FP-528 Nitrogen/Protein Determination; Leco Corporation, St. Joseph, MI. Fat content was determined by acid hydrolysis, carbohydrate content was determined by subtraction, and energy content was determined by micro-bomb calorimetry (Parr Instrument Company, Moline, Illinois).

All formulated feeds were prepared at the Texas A & M Experimental Station, Port Aransas, Texas. The various levels of carbohydrate were produced by substituting purified wheat starch for acid washed diatomaceous earth (Tables 1, 2). Dry ingredients were thoroughly mixed using a V-mixer for 10 minutes and the mixed-dry and liquid ingredients were combined for 40 minutes using a Hobart mixer. A moist feed was extruded, cold, through a Hobart A-200 extruder (Hobart, Troy, OH). Formulated feeds were proffered as a dry (9 to 11% moisture, as fed), cold-extruded pellet 1.5-4.5 cm in length depending on sea urchin size and the MTAB was offered as a thin, filamentous sheet (93% moisture content).

Sea urchins were reared in two linked rectangular fiberglass raceways with recirculating artificial saltwater (235 cm x 53 cm x 31 cm, L x W x H, N = 56 sea urchins/raceway, 22 ± 1 C, 32 ppt salinity) (Fig. 1). A 160 x 23 cm (L x H) center baffle was constructed to partially separate the raceway, allowing for recirculating water flow. Saltwater was circulated by an in-line pump (Supreme® Mag Drive Utility Pump, 500 gallons of water/hr). Saltwater was removed from the raceway by the utility pump,
passed through a mechanical and biological filter and was returned to the raceway downstream, creating a current with a flow rate of approximately (9.7-12.6 cm/s) (Fig. 1).
Table 1: Calculated nutrient values of formulated feeds and the mixed-taxa algal biofilm (MTAB). All values are “as fed” unless noted (* units are mg/kg or ppm, ** units are IU/kg).

<table>
<thead>
<tr>
<th>Nutrients</th>
<th>19%</th>
<th>26%</th>
<th>38%</th>
<th>24%</th>
<th>45%</th>
<th>MTAB</th>
</tr>
</thead>
<tbody>
<tr>
<td>06-Crude Protein</td>
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<td>28.7</td>
<td>28.7</td>
<td>41.3</td>
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<td>08-Carbohydrate</td>
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<td>36</td>
<td>22.3</td>
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<tr>
<td>09-Crude Fiber</td>
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<tr>
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<tr>
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<td>25-Calcium</td>
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<td>79.6</td>
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<td>34-Copper*</td>
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<td>0.837</td>
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<td>52-Threonine</td>
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<td>1.22</td>
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<td>60-Vitamin D**</td>
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<tr>
<td>63-Vitamin C*</td>
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<tr>
<td>64-Thiamine*</td>
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<td>65-Riboflavin*</td>
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<tr>
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<td>68-Pantothenic Acid*</td>
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<tr>
<td>69-Biotin*</td>
<td>0.962</td>
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<tr>
<td>70-Inositol*</td>
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<tr>
<td>71-Choline*</td>
<td>-</td>
<td>-</td>
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<tr>
<td>72-Folic Acid*</td>
<td>24</td>
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<tr>
<td>73-Cyanocobalimine*</td>
<td>0.182</td>
<td>0.182</td>
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</tr>
</tbody>
</table>
Table 2. Determined proximate nutrient composition (%) of five feeds including four semi-purified, formulated feeds (19, 26, or 38% carbohydrate and 31% protein; 24% carbohydrate and 45% protein) and a mixed-taxa algal biofilm (MTAB). Values are expressed as dry weight. Carbohydrate values were determined by subtraction.

<table>
<thead>
<tr>
<th>Feed</th>
<th>19%</th>
<th>26%</th>
<th>38%</th>
<th>24% 45%</th>
<th>MTAB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude Protein</td>
<td>31.59</td>
<td>31.29</td>
<td>31.71</td>
<td>44.96</td>
<td>42.20</td>
</tr>
<tr>
<td>Crude Fat</td>
<td>6.47</td>
<td>6.00</td>
<td>6.77</td>
<td>7.14</td>
<td>4.20</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>18.89</td>
<td>26.15</td>
<td>38.47</td>
<td>24.48</td>
<td>21.90</td>
</tr>
<tr>
<td>Crude Fiber</td>
<td>3.38</td>
<td>3.20</td>
<td>1.67</td>
<td>1.67</td>
<td>1.40</td>
</tr>
<tr>
<td>Moisture</td>
<td>9.17</td>
<td>10.21</td>
<td>11.15</td>
<td>11.01</td>
<td>93.00</td>
</tr>
<tr>
<td>Pre-leaching Caloric Value</td>
<td>3.07</td>
<td>3.28</td>
<td>3.82</td>
<td>3.98</td>
<td>3.50</td>
</tr>
<tr>
<td>Post-leaching Caloric Value</td>
<td>2.71</td>
<td>3.06</td>
<td>3.67</td>
<td>3.75</td>
<td>-</td>
</tr>
<tr>
<td>Pre-leaching Protein: Energy</td>
<td>104</td>
<td>96</td>
<td>82</td>
<td>112</td>
<td>121</td>
</tr>
</tbody>
</table>

...
Sea urchins were placed in individual cylindrical cages made of 3 mm polypropylene mesh to allow water flow-through. The cages were 25 cm tall and had a diameter of approximately 8.5 cm. A circular bottom, also made of 3 mm polypropylene mesh, enclosed the bottom of the cages and was attached to the cylindrical portion of the cages by polypropylene cable ties. Each of the cylindrical cages was placed in a polyvinyl chloride (PVC) coupling with an internal diameter of 8.7 cm. The PVC coupling was elevated from the floor of the raceway, by approximately 1-2 mm, by attaching three small pieces of polypropylene tubing to the base of the coupling. This elevation helped ensure that there was continuous flow under the cages and couplings, and it also helped restrict the movement of feces and uneaten food.

The depth of the saltwater in the raceways was maintained from 10 to 15 cm throughout the study, increasing in depth as sea urchins increased in size. Salinities were checked and adjusted daily by adding filtered freshwater. Every two weeks, sea urchins were randomly redistributed and relocated throughout the raceways to eliminate bias due to cage positioning or current, and the cages were scrubbed weekly to ensure that there was minimal bacterial growth on the cages that could contribute to the nutrition of the sea urchins.

Fresh food was provided daily, the MTAB was replenished every one or two days, as needed. Uneaten feed and feces were removed by siphon every two days. Water quality parameters were tested weekly using a LaMotte’s saltwater test kit and maintained within optimal range (pH= 8.2, ammonia and nitrites < 0.5 ppm, nitrates < 10 ppm, alkalinity > 150 ppt).
Growth

The wet weight and test diameter of each individual sea urchin was measured at the beginning of the study and every 2 weeks thereafter. For wet weights, individuals were blotted dry on a paper towel to remove excess water and weighed. To acquire test diameters (mm), individuals were photographed out of water with a reference ruler. Sea urchins were photographed with a Nikon® digital camera, and the images were analyzed using Optimus 6.51 imaging software (Media Cybernetics, L.P.). Individual specific growth rates (% body weight gain/day, SGR) were calculated using the equation:

\[
\frac{\ln (\text{final weight}) - \ln (\text{initial weight})}{\text{Number of days}} \times 100
\]

A repeated measures analysis using The Mixed Procedure (PROC MIXED) was used to determine significance of the dependent variables, wet weights and diameters. An ANOVA with a Tukey’s adjustment for multiple comparisons was used to determine significance of SGR. To analyze the scatter plot of diameter versus wet weight data for the feeds, a log transform was applied to linearize the data. An ANCOVA, with diameter and wet weight as the covariates, was performed and a comparison of estimated marginal means separated by a Tukey’s test at the mean diameter or wet weight of all treatments (19.0 mm and 2.67 g, respectively) was used to determine significant differences and interaction between the variables. All growth data were analyzed using the statistic software SAS version 9.1 (SAS Institute, Inc., Cary, North Carolina, USA). All values were considered significant when \( P < 0.05 \).
Figure 1: Schematic of recirculating system. A) Side view of one fiberglass raceway (235 cm x 53 cm x 31 cm, L x W x H) with a 160 x 23 cm (L x H) center baffle and 56 individual flow-through cages. B) End view of one fiberglass raceway (schematic is not drawn to scale).
Organ Analyses

Sea urchins were dissected at the end of 8 weeks. The gonad, lantern, and test were blotted on a paper towel and weighed. The gut (esophagus, stomach, and intestine) was removed, rinsed to remove any food or feces, blotted on a paper towel, and weighed. Moisture content of each organ was calculated as:

\[
\frac{\text{Organ wet weight} - \text{Organ dry weight}}{\text{Organ wet weight}} \times 100
\]

To determine dry organ weights, organs were removed and dried in a convection oven at 60°C, for 48 hrs or until constant dry weight. Wet and dry skeletal components, test and lantern, were analyzed using ANCOVA using sea urchin test diameter as the covariate. Treatments were compared at the average test diameter of all sea urchins (19.0 mm). Gut wet and dry weights were analyzed by ANCOVA using the sea urchin final wet and total dry weights as the covariates, respectively. Treatments were compared at the average final wet and dry weights of all sea urchins (2.67 g and 0.60 g, respectively). However, the gonad wet and dry data had unequal variance and was not normal; therefore, a non-parametric Krusal-Wallis Mean Rank Test was performed. To determine differences for moisture content an ANOVA was performed were separated by a Tukey’s HSD adjustment. All dissection data were analyzed using the statistic software SAS version 9.1 (SAS Institute, Inc., Cary, North Carolina, USA). All values were considered significant when P < 0.05.
Leaching

The stability of each of the formulated feeds over a 24-hr period was determined by placing a pre-weighed piece of each of the four formulated feeds (approximately 0.15g) into individual cages (N = 10/treatment) and was repeated for 3 days. The feed remained in the individual cage undisturbed for 24 hrs after which it was removed by siphon, rinsed with deionized water, and dried in a convection oven at 60 C for 48 hrs, or until constant dry weight. After the samples were dried, the remaining food was weighed. The leaching and stability over the 24-hr period was calculated using the formula:

\[
\frac{\text{Dry weight feed in (g)} - \text{Dry weight feed removed (g)}}{\text{Dry weight feed in (g)}} \times 100
\]

Pre-leaching (content of feed prior to introduction to the water) and post-leaching (content of feed after 24 hr in the water) values were calculated for each of the four formulated feeds, and these values will be considered when analyzing the consumption and digestibility data to account for the amount of feed that may be lost due to leaching. The energy value of the feed after the 24-hr period was also analyzed by micro-bomb calorimetry (Parr Instrument Company, Moline, Illinois) to determine the energy value of the post-leached feed. The leaching data were analyzed using ANOVA (SAS Institute, Inc., Cary, North Carolina, USA) and significant differences (P < 0.05) were separated using a Student-Newman-Keul’s adjustment.

Apparent Consumption and Digestibility

To determine apparent consumption (AC), cultured sea urchins (wet weight = 5.60 ± 0.48g, N= 40) were held in individual containers in the linked raceway system.
All sea urchins were fed individually a reference feed (approximately 0.15 g/day) for 7 days, and on day 8, sea urchins were randomly assigned to be fed one of the four experimental feeds (19, 26, 38% carbohydrate or 24% carbohydrate, 45% protein; N = 10/treatment). Sea urchins were fed their respective experimental feed (approximately 0.15 g/day) for an additional 7 days with uneaten food and feces being removed every 2 days. On day 15, sea urchins were weighed (there were no significant differences in wet weights among treatments) and fed ad libitum a pre-weighed amount of the respective, assigned formulated feed. Uneaten food and fecal material were collected daily for 12 days. On day 27, the last day of the consumption study, sea urchins were weighed. At the end of the study, the uneaten food and feces were dried in a convection oven at 60 C for 48 hrs, or until constant dry weight. All of the consumption data were derived two ways: without considering the amount of feed or energy lost due to leaching (pre-leaching) and with consideration for the amount of feed and energy lost over the 24-hr period (post-leaching). Therefore, the equations for the data calculated pre-leaching consideration will be describe first.

The amount of dry feed consumed/day (mg/day) will be calculated using the formula:

\[
\text{Dry feed fed (mg) – Dry feed removed (mg)} \div \text{# of days}
\]

The amount of energy consumed/day (cal/day) was determined by the formula:

Pre-leached caloric value (cal/mg) \times \text{Dry feed consumed/ day (mg/day)}
The % digestible energy of the feeds was determined using the formula:

\[
\frac{\text{Amount of energy consumed (cal)} - \text{Amount of energy of feces (cal)}}{\text{Amount of energy consumed (cal)}} \times 100
\]

The amount of digestible energy consumed/ day was determined using the formula:

\[
\text{Pre-leaching caloric value of feed consumed (cal/mg)} \times \% \text{ digestible energy}
\]

The apparent dry matter digestibility (ADMD) of the four formulated feeds was determined by gravimetric collection. The collected feces were used to determine ADMD of each of the feeds. The ADMD of the formulated feeds were calculated using the formula:

\[
\frac{\text{Dry feed consumed (g)} - \text{Dry fecal material removed (g)}}{\text{Dry feed consumed (g)}} \times 100
\]

Secondarily, to account for the leaching the following formulas were used. The dry amount of feed available for consumption by the sea urchin was calculated by the formula:

\[
\text{Dry feed fed (mg)} - \% \text{ loss due to leaching}
\]

The amount dry feed consumed (mg) was then determined using the formula:

\[
\text{Dry feed available (mg)} - \text{Dry feed remaining (mg)}
\]

The % digestible energy was determined using the formula:

\[
\frac{\text{Energy consumed (cal)} - \text{Energy in feces (cal)}}{\text{Energy consumed (cal)}} \times 100
\]

The amount of digestible energy consumed (kcal) was determined by:

\[
\text{Post-leaching caloric value of feed consumed (cal/mg)} \times \% \text{ digestible energy}
\]
ADMD were calculated using the formula:

\[
\text{Dry food consumed (g) – Dry fecal material removed (g) \times 100}
\]

\[
\text{Dry food consumed (g)}
\]

All consumption and digestibility data were analyzed using an ANOVA with a Student-Newman-Keul’s test for multiple comparisons. One sea urchin fed the 19% carbohydrate feed was observed not consuming feed and did not gain a significant amount of weight over the consumption study, and the data collected was analyzed using Cook’s Distances and Leverage Values. The data from this sea urchin was not included in the study values due to observational and statistical evidence. All data were analyzed using the statistic software SAS version 9.1 (SAS Institute, Inc., Cary, North Carolina, USA). All values were considered significant when \( P < 0.05 \).
Results

Survival

Survival was 100% in sea urchins fed the experimental formulated feeds. Sea urchins fed the MTAB had 93% survival with only one sea urchin dying over the eight-week study period. The observed values for ammonia, nitrite, nitrate, temperature, salinity and pH remained within acceptable levels throughout the study.

Growth

Sea urchins fed formulated feeds and the live, MTAB exhibited similar trends in weight gain over the eight-week study (Fig. 2). At week eight, sea urchins fed the 19% carbohydrate and the 24% carbohydrate, 45% protein feeds weighed significantly more than sea urchins fed the 38% carbohydrate feed (P = 0.03, Fig. 2). There were no other significant differences detected among any other treatments.

Sea urchins fed the 19% carbohydrate feed had significantly larger test diameters than sea urchins fed the 38% carbohydrate feed at weeks six and eight (P < 0.02, Fig. 3). Sea urchins fed the 24% carbohydrate, 45% protein feed had a significantly larger test diameter than those fed the 38% carbohydrate feed at weeks four, six, and eight (P < 0.04, Fig. 3). Sea urchins fed the 26% carbohydrate feed and the MTAB were not significantly different from any treatment during the 8-wk study.

Specific growth rates (SGR) ranged from 6.7 to 9.1 for all treatments during the first 2 weeks of the study (Fig. 4). The maximum SGR for sea urchins fed MTAB
occurred from week two to four and then decreased throughout the remainder of the study. SGR decreased in all other treatments with time, and there were no significant differences among feeds over the study period.

For all treatments combined, the relationship between diameter (mm) and wet weight (g) was best described by the equation $y = 0.0006x^{2.86}, R^2 = 0.99$ (Fig. 5). Linear regression of the log transformed values for all treatments over the 8-wk study period indicates a strong relationship between diameter and wet weight described by the equation $y = 2.84x - 3.21, R^2 = 0.99$ (Fig. 6). No significant differences were observed among the treatments.

**Organ Analyses**

Test wet and dry weight did not vary with treatment ($P = 0.686$ and $P = 0.864$, respectively, Table 3). Lantern wet and dry weights were not significantly different between treatments when covaried with test diameter ($P = 0.08$ and $P = 0.87$, respectively).

Gut wet or dry weights did not vary significantly among treatments ($P = 0.93$ and $P = 0.41$, respectively; Table 3). There were no significant differences among the wet or dry gonad weight of sea urchins fed the formulated feed; however, sea urchins fed the live, MTAB diet had gonads that were smaller in wet and dry weights ($P = 0.09$ and $P = 0.08$ level, respectively; Table 4).

Measurable gonads were first apparent in sea urchins of approximately 13 mm diameter or 0.9 g wet weight (Fig. 7). However, gonad mass showed appreciable
increases after sea urchins reached a size of approximately 18.5 mm diameter or 2.7 g wet weight.
Figure 2. Total wet weight (g) of juvenile sea urchins, *Lytechinus variegatus*, fed one of five feeds including four semi-purified, formulated feeds (19, 26, or 38% carbohydrate and 31% protein; 24% carbohydrate and 45% protein) and a mixed-taxa algal biofilm (MTAB). Values represent means ± SE (N = 16 sea urchins/treatment).
Figure 3. Test diameter (mm) of juvenile sea urchins, *Lytechinus variegatus*, fed one of five feeds including four semi-purified, formulated feeds (19, 26, or 38% carbohydrate and 31% protein; 24% carbohydrate and 45% protein) and a mixed-taxa algal biofilm (MTAB). Values represent means ± SE (N = 16 sea urchins/treatment).
Figure 4. Specific growth rates (% body weight gain/day) calculated in 2-wk intervals of juvenile sea urchins, *Lytechinus variegatus*, fed one of five feeds including four semi-purified, formulated feeds (19, 26, or 38% carbohydrate and 31% protein; 24% carbohydrate and 45% protein) and a mixed-taxa algal biofilm (MTAB). Values represent means ± SE (N = 16 sea urchins/treatment).
Figure 5. Relationship of diameter (mm) to total wet weight (g) of juvenile sea urchins, *Lytechinus variegatus*, fed one of five feeds including four semi-purified, formulated feeds (19, 26, or 38% carbohydrate and 31% protein; 24% carbohydrate and 45% protein) and a mixed-taxa algal biofilm (MTAB). Values represent means ± SE (N = 16 sea urchins/treatment).
Figure 6. Linear regression analysis of diameters and wet weights (log transformation) of juvenile sea urchins, *Lytechinus variegatus*, fed one of five feeds including four semi-purified, formulated feeds (19, 26, or 38% carbohydrate and 31% protein; 24% carbohydrate and 45% protein) and a mixed-taxa algal biofilm (MTAB). Values represent means ± SE (N = 16 sea urchins/treatment).
Figure 7. Relation of wet gonad weight to (A) diameter and (B) wet weight of individual juvenile sea urchins, *Lytechinus variegatus*, fed one of five feeds including four semi-purified, formulated feeds (19, 26, or 38% carbohydrate and 31% protein; 24% carbohydrate and 45% protein) and a mixed-taxa algal biofilm (MTAB).
Leaching

The 38% carbohydrate had the highest percent loss due to leaching (38% loss in 24 hr) (Table 6). Leaching decreased in the 19 and 26% carbohydrate feeds (26.4 and 27.1% average loss in 24 hr, respectively). After the 24-hr period, the determined energy values were significantly different from the determined energy values of each formulated feed pre-leaching (P < 0.001).

Apparent Consumption and Digestibility

Although absolute values of consumption and digestibility varied considerably whether evaluated pre- or post-leaching, general trends among the feeds remained evident (Tables 5, 6). Sea urchins fed the 19% carbohydrate feed apparently consumed significantly more dry feed per day than sea urchins fed the 26% carbohydrate and 24% carbohydrate, 45% protein feeds (P = 0.003, Table 5) and the 38% carbohydrate feed (post-leaching; Table 6). Based on these apparent consumption values, sea urchins fed the 19% carbohydrate consumed significantly more protein than sea urchins fed the 26 and 38% carbohydrate feed (post-leaching; P = 0.046; Table 6). Based on post-leaching analyses, sea urchins consumed similar amounts of digestible energy regardless of the feed (P = 0.40). ADMD values varied greatly depending on whether the calculation considered pre- or post-leaching feed; however, sea urchins fed the 24% carbohydrate, 45% protein feeds generally had higher dry matter absorption that those fed the 19 or 26% carbohydrate feed. Digestible energy values were similar (post-leaching) among all feeds (Table 6). Consequently, all consumption and digestibility values must be considered in terms of the leaching characteristics of the feed.
Table 3. Test and lantern wet and dry weights and moisture content of juvenile sea urchins, *Lytechinus variegatus*, fed one of five feeds including four semi-purified, formulated feeds (19, 26, or 38% carbohydrate and 31% protein; 24% carbohydrate and 45% protein) and a mixed-taxa algal biofilm (MTAB). Values represent means ± SE (N = 16 sea urchins/treatment). Means with similar superscripts are not significantly different (P > 0.05).

<table>
<thead>
<tr>
<th>Feed</th>
<th>19%</th>
<th>26%</th>
<th>38%</th>
<th>24%</th>
<th>45%</th>
<th>MTAB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test Wet Weight (g)</td>
<td>1.33 ± 0.14 A</td>
<td>1.16 ± 0.14 A</td>
<td>1.05 ± 0.15 A</td>
<td>1.29 ± 0.12 A</td>
<td>1.11 ± 0.16 A</td>
<td></td>
</tr>
<tr>
<td>Test Dry Weight (g)</td>
<td>0.58 ± 0.06 A</td>
<td>0.50 ± 0.06 A</td>
<td>0.48 ± 0.06 A</td>
<td>0.58 ± 0.05 A</td>
<td>0.52 ± 0.07 A</td>
<td></td>
</tr>
<tr>
<td>Test Moisture Content (%)</td>
<td>56.5 A</td>
<td>55.9 A</td>
<td>53.3 AB</td>
<td>54.6 AB</td>
<td>52.4 B</td>
<td></td>
</tr>
<tr>
<td>Lantern Wet Weight (mg)</td>
<td>81.2 ± 10.9 A</td>
<td>66.7 ± 9.22 A</td>
<td>72.7 ± 10.0 A</td>
<td>81.2 ± 8.50 A</td>
<td>82.8 ± 13.0 A</td>
<td></td>
</tr>
<tr>
<td>Lantern Dry Weight (mg)</td>
<td>43.4 ± 5.32 A</td>
<td>38.6 ± 5.13 A</td>
<td>40.8 ± 5.56 A</td>
<td>45.3 ± 4.50 A</td>
<td>46.0 ± 6.42 A</td>
<td></td>
</tr>
<tr>
<td>Lantern Moisture Content (%)</td>
<td>45.5 A</td>
<td>41.1 A</td>
<td>42.6 A</td>
<td>43.7 A</td>
<td>41.4 A</td>
<td></td>
</tr>
</tbody>
</table>
Table 4. Gut and gonad wet and dry weights and moisture content of juvenile sea urchins, *Lytechinus variegatus*, fed one of five feeds including four semi-purified, formulated feeds (19, 26, or 38% carbohydrate and 31% protein; 24% carbohydrate and 45% protein) and a mixed-taxon algal biofilm (MTAB). Values represent means ± SE (N = 16 sea urchins/treatment). Means with similar superscripts are not significantly different (P > 0.05).

<table>
<thead>
<tr>
<th>Feed</th>
<th>19%</th>
<th>26%</th>
<th>38%</th>
<th>24%</th>
<th>45%</th>
<th>MTAB&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gut Wet Weight (mg)</td>
<td>101 ± 11.4&lt;sup&gt;A&lt;/sup&gt;</td>
<td>89.2 ± 11.0&lt;sup&gt;A&lt;/sup&gt;</td>
<td>79.9 ± 13.6&lt;sup&gt;A&lt;/sup&gt;</td>
<td>106 ± 9.83&lt;sup&gt;A&lt;/sup&gt;</td>
<td>88.8 ± 13.9&lt;sup&gt;A&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Gut Dry Weight (mg)</td>
<td>21.9 ± 2.44&lt;sup&gt;A&lt;/sup&gt;</td>
<td>19.0 ± 2.36&lt;sup&gt;A&lt;/sup&gt;</td>
<td>16.7 ± 2.85&lt;sup&gt;A&lt;/sup&gt;</td>
<td>21.9 ± 1.97&lt;sup&gt;A&lt;/sup&gt;</td>
<td>16.9 ± 3.06&lt;sup&gt;A&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Gut Moisture Content (%)</td>
<td>78.3&lt;sup&gt;A&lt;/sup&gt;</td>
<td>78.7&lt;sup&gt;A&lt;/sup&gt;</td>
<td>78.9&lt;sup&gt;A&lt;/sup&gt;</td>
<td>79.4&lt;sup&gt;A&lt;/sup&gt;</td>
<td>80.7&lt;sup&gt;A&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Gonad Wet Weight (mg)</td>
<td>55.1 ± 25.4</td>
<td>43.4 ± 20.3</td>
<td>25.9 ± 11.5</td>
<td>44.2 ± 15.8</td>
<td>7.49 ± 3.70&lt;sup&gt;●&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Gonad Dry Weight (mg)</td>
<td>13.0 ± 6.96</td>
<td>11.0 ± 5.85</td>
<td>5.37 ± 2.47</td>
<td>10.5 ± 4.24</td>
<td>1.37 ± 0.67&lt;sup&gt;●&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Gonad Moisture Content (%)</td>
<td>80.9&lt;sup&gt;A&lt;/sup&gt;</td>
<td>78.1&lt;sup&gt;A&lt;/sup&gt;</td>
<td>79.5&lt;sup&gt;A&lt;/sup&gt;</td>
<td>78.5&lt;sup&gt;A&lt;/sup&gt;</td>
<td>75.9&lt;sup&gt;A&lt;/sup&gt;</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> MTAB significantly different at *P = 0.076, *P = 0.085 (Kruskal-Wallis test).
Table 5: Apparent dry matter digestibility (ADMD), apparent consumption, and feeding rates of individual juvenile sea urchins, *Lytechinus variegatus*, fed one of four semi-purified, formulated feeds (19, 26, or 38% carbohydrate and 31% protein; 24% carbohydrate and 45% protein) over the 12-day study period (N = 10 individuals per treatment). All consumption parameters are expressed on a daily basis (consumption data considering no feed was lost over the 24-hr period and using determined pre-leaching energy values). Means with similar superscripts are not significantly different (P > 0.05).

<table>
<thead>
<tr>
<th>Feed</th>
<th>19%</th>
<th>26%</th>
<th>38%</th>
<th>24% 45%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amount feed consumed (mg)</td>
<td>162.2 ± 10.1^A</td>
<td>120.0 ± 8.43^B</td>
<td>141.0 ± 12.0^AB</td>
<td>110.0 ± 4.47^B</td>
</tr>
<tr>
<td>Amount protein consumed (mg)</td>
<td>51.1 ± 2.61^A</td>
<td>38.0 ± 3.27^B</td>
<td>45.0 ± 3.73^AB</td>
<td>49.0 ± 1.90^A</td>
</tr>
<tr>
<td>Amount digestible energy consumed (cal)</td>
<td>405.6 ± 20.4^AB</td>
<td>328.0 ± 20.8^B</td>
<td>447.0 ± 35.7^A</td>
<td>388.0 ± 10.3^AB</td>
</tr>
<tr>
<td>ADMD (%)</td>
<td>74.3 ± 1.56^B</td>
<td>73.3 ± 2.48^B</td>
<td>81.2 ± 1.31^A</td>
<td>82.5 ± 1.31^A</td>
</tr>
<tr>
<td>Digestible energy (%)</td>
<td>82.2 ± 1.07^B</td>
<td>83.4 ± 0.84^B</td>
<td>85.2 ± 1.23^AB</td>
<td>87.4 ± 1.10^A</td>
</tr>
<tr>
<td>Feeding rate (% body weight consumed/day, as fed)</td>
<td>3.35 ± 0.33^A</td>
<td>2.87 ± 0.34^A</td>
<td>3.37 ± 0.44^A</td>
<td>2.89 ± 0.40^A</td>
</tr>
</tbody>
</table>
Table 6: Feed stability, apparent dry matter digestibility (ADMD), and apparent consumption of individual juvenile sea urchins, *Lytechinus variegatus*, fed one of four semi-purified, formulated feeds (19, 26, or 38% carbohydrate and 31% protein; 24% carbohydrate and 45% protein) over the 12-day study period (N = 10 individuals/treatment). All consumption parameters are expressed on a daily basis (consumption data considering the amount of feed lost due to leaching over the 24-hr period, and using the post-leaching energy value). Means with similar superscripts are not significantly different (P > 0.05).

<table>
<thead>
<tr>
<th>Feed</th>
<th>19%</th>
<th>26%</th>
<th>38%</th>
<th>24% 45%</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Leaching (% loss)</strong></td>
<td>26.7 ± 0.32&lt;sup&gt;C&lt;/sup&gt;</td>
<td>27.1 ± 0.32&lt;sup&gt;C&lt;/sup&gt;</td>
<td>37.7 ± 0.53&lt;sup&gt;A&lt;/sup&gt;</td>
<td>31.3 ± 1.79&lt;sup&gt;B&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Amount feed consumed (mg)</strong></td>
<td>83.3 ± 9.43&lt;sup&gt;A&lt;/sup&gt;</td>
<td>53.0 ± 7.31&lt;sup&gt;B&lt;/sup&gt;</td>
<td>56.0 ± 8.72&lt;sup&gt;B&lt;/sup&gt;</td>
<td>43.0 ± 5.18&lt;sup&gt;B&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Amount protein consumed (mg)</strong></td>
<td>27.8 ± 3.24&lt;sup&gt;A&lt;/sup&gt;</td>
<td>16.0 ± 2.67&lt;sup&gt;B&lt;/sup&gt;</td>
<td>19.0 ± 2.33&lt;sup&gt;B&lt;/sup&gt;</td>
<td>20.0 ± 2.11&lt;sup&gt;AB&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Amount digestible energy consumed (cal)</strong></td>
<td>137.8 ± 15.4&lt;sup&gt;A&lt;/sup&gt;</td>
<td>97.0 ± 16.7&lt;sup&gt;A&lt;/sup&gt;</td>
<td>118.0 ± 18.7&lt;sup&gt;A&lt;/sup&gt;</td>
<td>106.0 ± 16.7&lt;sup&gt;A&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>ADMD (%)</strong></td>
<td>40.8 ± 1.76&lt;sup&gt;B&lt;/sup&gt;</td>
<td>39.3 ± 2.18&lt;sup&gt;B&lt;/sup&gt;</td>
<td>48.1 ± 2.49&lt;sup&gt;AB&lt;/sup&gt;</td>
<td>52.3 ± 3.97&lt;sup&gt;A&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Digestible energy (%)</strong></td>
<td>60.6 ± 1.06&lt;sup&gt;A&lt;/sup&gt;</td>
<td>57.8 ± 1.70&lt;sup&gt;A&lt;/sup&gt;</td>
<td>57.9 ± 2.08&lt;sup&gt;A&lt;/sup&gt;</td>
<td>63.7 ± 3.22&lt;sup&gt;A&lt;/sup&gt;</td>
</tr>
</tbody>
</table>
Discussion

Survival

Formulated feeds used in this study promoted 100% survival, indicating juvenile *L. variegatus* are capable of utilizing nutrients in these feeds and that adequate water quality was maintained during the experiment. Survival rates were equal or higher to those for the live MTAB. Taylor (Chapter 1) had demonstrated previously that formulated feeds can support survival and growth. Successful aquaculture will require a formulated feed that can support juvenile growth (Lawrence and Lawrence 2004).

Growth

In this study, carbohydrate levels were adjusted by co-varying the levels of diatomaceous earth. At these levels of diatomaceous earth, no effects on growth have been found (unpublished data); thus, the results of this study are attributed to changes in dietary carbohydrate or protein. Under the conditions of this study, dietary carbohydrate level significantly affected weight gain; however, the rate of weight gain was not directly related to the energy of the feed as suggested by Taylor (Chapter 1). Taylor (Chapter 1) suggested that sea urchins fed a semi-purified, formulated feed gained more weight per unit diameter than sea urchins fed a natural diet because of the increased caloric value of the formulated feed. However, results from this study suggest that the caloric value of the feed is not the only determining factor of sea urchin growth. Instead, we suggest the
difference in weight gain may be attributed to the protein: energy ratio or consumption rate of the feeds.

The protein: energy ratio of the feeds used in this study ranged from 82 to 112 mg protein/kcal. The two formulated feeds that supported the most weight gain, 19% carbohydrate and 24% carbohydrate, 45% protein, had the highest protein: energy ratios of 104 and 112 mg protein/kcal, respectively. The feed with the highest carbohydrate content, and consequently the highest energy content, supported the lowest growth rate. Hammer (2006) found that adult sea urchins (29.5 ± 0.11 mm test diameter) fed a formulated feed with the highest protein:energy ratio (80.7 mg protein/ kcal) had the highest weight gain throughout the 12-week study. We suggest that higher protein: energy ratios are needed to promote high rates of growth of juvenile sea urchins. Cuzon and Guillaume (1997) suggested that feeds with protein: energy ratios ranging from 90-160 mg protein/kcal are optimal for many commercial shrimp species.

Although the MTAB also had high protein: energy ratio, the MTAB was 93% moisture; therefore, sea urchins must consume large quantities of the MTAB to consume the dry weight equivalent of the formulated feeds. Consumption of MTAB could not be determined in this stud; however, these data suggest that formulated feeds supported weight gain as well as the live MTAB.

The initial specific growth rates (% body weight gain/day, SGR) of sea urchins fed the formulated feeds in this study were higher (8.3 to 9.2 % body weight gain/day) than the reported SGR of *L. variegatus* observed in the field (Moore et al. 1963, Beddingfield and McClintock 2000). These data were also higher that the SGR for juvenile *L. variegatus* (5.5% body weight gain/day) in the study reported by Taylor
(Chapter 1) in which juvenile sea urchins were fed a commercial feed formulated for sea urchins (32% protein, 37% carbohydrate, dry weight). Wallace (2001) reported SGRs of approximately 5% body weight gain/day in small *L. variegatus* (14.6 ± 0.15 mm, 1.1 ± 0.35 g) fed formulated feeds. These data suggest that the quality of the formulated feeds used in this study exceeded the quality of the feeds used in these previous studies.

**Organ Analyses**

Organ growth (test, lantern, and gut) was generally isometric with whole sea urchin growth. Although gonad growth did not vary among the formulated feeds, the reduced gonad growth in those fed the MTAB suggests that other nutrients/signals are important in stimulating gonad growth and maturation. Gonad tissues were often observed in small urchins (ca. 13 mm diameter) regardless of the diet, but precocious growth was observed in *L. variegatus* as small as ca. 18 mm fed formulated feeds or MTAB (although precocious gonad growth was less obvious in the MTAB treatment). Some of these gonads were observed to be in advanced stages of gametogenesis, because mature sperm and eggs (fertilizable) were found. This suggests that with the proper nutrition sea urchins could become sexually mature at a much smaller size than reported in natural conditions (Lawrence 2001). In the wild, *L. variegatus* develop large gonads only after reaching a size of ca. 40 mm diameter (Moore et al. 1963). Assuming essential nutrients are not limiting, these data suggest that other biotic or abiotic factors are involved in stimulating gonad growth and maturation in wild populations.

Additional studies are needed to determine a more precise timeline for gonad development in juvenile urchins. A gonad development timeline (nutritive phagocytes vs
gametes) would provide information concerning the nutritional requirements of nutrient storage and gametogenesis. Once gonads have begun to develop, the metabolism and nutrient requirements of the sea urchin may change. This could alter animal husbandry techniques, along with the nutrient content of required feeds. Lawrence (2001) suggested that early gonad development was unfavorable since it may restrict energy allocation to somatic growth. Lawrence (2001) also suggested that during juvenile growth more energy should be allocated to somatic growth than to gonad growth.

**Leaching**

The amount of dry matter leaching in this study was directly related to the amount of carbohydrate and protein ingredients in the feed. In many dry feeds prepared for aquatic organisms, feed ingredients and the feed production methods can result in a feed that loses nutrients quickly when exposed to water. Since the leaching rate was related to the content of specific ingredients (primarily starch), we suggest that more research is needed to determine the exact rate of leaching for various nutrients. Pearce et al. (2002) investigated the effects of binder type and concentration on feed stability, and reported that the binder type did have a significant effect on stability; however, binder concentration did not influence stability. Pearce et al. (2002) reported feed loss values ranging from 9.1 to 13% dry matter. These values are significantly lower than the values reported for the formulated feeds used in this study. In the laboratory, we have observed that as much as 5% of the dry feed may be leached by rinsing the feed with deionized water, and that up to 15% may be lost within the first ten hours of exposure to water (unpublished data). In fact, energy values of the formulated feeds decreased from 4 to
12% within 24 hrs. Leaching could also be due in part to the different extrusion processes, experimental design, and/or feed types. These studies show the importance of considering feed stability and leaching when conducting nutrition studies.

Many studies with other organisms, such as shrimp or fish, have not considered the leaching of feeds since the feeds are consumed within several minutes of exposure of the feed to water. However, sea urchins are slow feeders, and the feeding pattern of sea urchins is unpredictable and irregular. Typically, sea urchins are exposed to feeds for a few hours or a few days. These data clearly show that the calculated nutrient values of the feeds may become compromised and inaccurate after exposure to water. Leaching will influence the amount of feed and nutrients available for consumption by the sea urchin. More knowledge as to the characteristics of feed leaching would be useful in the development of feeds that are more stable, which may help reduce aquaculture pollution and aid in the production of cost-effective, commercial feeds.

**Apparent Consumption and Digestibility**

Values of consumption and digestibility were dependent on whether the consumption data was analyzed pre- or post-leaching. Consumption, if calculated based on the nutrient composition as fed or dry weight (pre-leaching), represents maximum consumption values. These values do not account for any feed lost due to instability and leaching. Consumption, if calculated based on nutrient composition post-leaching for 24 hours, represents a minimum value (most sea urchins consumed their feed in less than 24 hours). We suggest that true consumption values are between the pre- and post-leaching
values; however, the individual variability in feeding and feeding rates makes absolute determination of consumption difficult.

For those fed the formulated feeds, weight gain and test diameter were inversely related to the caloric value of the feed. We suggest that this difference can be attributed to the amount of feed consumed by each treatment. Since sea urchins fed the 19% carbohydrate feed consumed significantly more dry feed, they also consumed more protein than the 26 and 38% carbohydrate feeds (post-leaching), even though the amount of energy consumed was similar. Those fed 19% carbohydrate or 24% carbohydrate, 45% protein consumed different amounts of feed, but similar amounts of protein and energy, resulting in similar weight gain.

Hammer (2006) found that adult *L. variegatus* fed feeds with high levels of protein (31% dry weight) had significantly more wet weight gain, larger test diameters, and higher specific growth rates than sea urchins fed low levels of protein (17% dry weight). Sea urchins fed the 31% protein feed also consumed less feed and energy; therefore, Hammer (2006) suggested that the amount of protein consumed regulated feed intake in *L. variegatus*. Miller and Mann (1973), Lowe and Lawrence (1976), Fernandez and Boudouresque (1998), and Daggett et al. (2005) have also suggested that sea urchins may consume high quantities of low nitrogen diets. Kureshy and Davis (2002) also suggested that the protein consumed regulated feed intake in shrimp.

In contrast to Hammer (2006), sea urchins in this study consumed the same amount of digestible energy over the 12-day study period (post-leaching). These data suggest that sea urchins are consuming feed to meet an energy requirement. It has been suggested that the amount of digestible energy is a major factor controlling feed intake in

Although, sea urchins may have consumed feed to meet an energy requirement, the calories required to sustain a sea urchin are relatively low. These data are supported by Lawrence and Lane (1982) and Hill and Lawrence (2006) who reported low respiratory rates for *L. variegatus*. Compared to other organisms, *L. variegatus* have a low daily caloric requirement. Cuzon et al. (2004) suggested that 33.5 kcal/day for a 100g shrimp, *Litopenaeus vannamei* (335 kcal/kg body weight/day), and Siccardi (2006) suggested that 1.02 kcal/day was sufficient for *Litopenaeus vannamei* weighing 7.68-13.08g (96 kcal/kg body weight/day). Gatlin (1986) suggested that 99.7 kcal/kg body weight/day for channel catfish, and McGoogan and Gatlin (1998) suggested that 185 kcal/kg body weight/day was ideal for carnivorous red drum. In this study, the average digestible energy consumption was between 70 kcal/kg body weight/day (pre-leaching) and 21 kcal/kg body weight/day (post-leaching).

The ADMD values were inversely proportional to the amount of ash in the feeds. In this study, ash was substituted with purified starch to produce various carbohydrate levels. Consequently, the feeds with less carbohydrate (19 and 26% carbohydrate) had a higher ash content, which could account for the overall differences detected among ADMD values in the various treatments. These pre-leaching ADMD values are similar to those reported by Hammer (2006) and were calculated similarly to those in other studies of aquatic organisms. Because of low and irregular consumption rates in sea urchins and
recognizing that dry matter is leached when feeds are placed in the seawater, post-leaching ADMD values were significantly lower, suggesting that the feeds were less digestible than previously considered. We suggest that soluble components such as carbohydrate and protein are the main ingredients leached; therefore, loss of these ingredients would lower the ADMD.

The feeding rate (% body weight consumed/day) of juvenile sea urchins in this study was comparable with feeding rates of adult Strongylocentrotus intermedius fed various algae, but lower than sub-adult S. intermedius (average test diameter = 24.6) fed Laminaria japonica (Fuji 1967). Feeding rates were also similar to S. droebachiensis fed formulated feeds during the summer months (Daggett 2005), and slightly higher than the feeding rates reported by Barker et al. (1998) for small Evechinus chloroticus fed formulated feeds.

In summary, weight gain was generally inversely proportional to the carbohydrate level in the feed. For those sea urchins fed feeds that varied only in carbohydrate level, we hypothesize that weight gain, while inversely proportion to carbohydrate level, was directly related to consumption. In consuming feeds to meet energy requirements, sea urchins that consumed more feed also consumed higher levels of protein and had the highest weight gain. Consequently, protein: energy ratio may be important in determining feed utilization and growth. Furthermore, we suggest that studies involving the use of formulated feeds should consider feed leaching. The leaching of ingredients can significantly influence the amount of energy and nutrients available to the sea urchin, and consequentially, influence the analysis and conclusions of nutrition studies. Leaching
should also be considered to help develop feeds that are economical and environmentally friendly.
Literature Cited


Boonyaratpalin, M. 1978. Effect of dietary levels of energy and protein on voluntary food consumption, growth, and body and serum composition of channel catfish. Ph.D. Dissertation, Auburn University, Auburn, Alabama, USA.


APPENDIX A

IACUC APPROVAL FORM

NOTICE OF APPROVAL

DATE: April 8, 2005

TO: 
Stephen A. Watts, M.S., Ph.D. 
CH-375 1170 
FAX: 975-6097

FROM: Suzanne M. Michalek, Ph.D., Chair 
Institutional Animal Care and Use Committee

SUBJECT: Title: Assessment of the Sea Urchin Tripneustes Ventricosus as a Candidate for 
Aquaculture in the Gulf of Mexico Region 
Sponsor: Mississippi-Alabama Sea Grant Consortium 
Animal Project Number: 050406856

On April 8, 2005, the University of Alabama at Birmingham Institutional Animal Care and Use Committee (IACUC) reviewed the animal use proposed in the above referenced application. It approved the use of the following species and numbers of animals:

<table>
<thead>
<tr>
<th>Species</th>
<th>Use Category</th>
<th>Number in Category</th>
</tr>
</thead>
<tbody>
<tr>
<td>Invertebrates</td>
<td>A</td>
<td>200</td>
</tr>
</tbody>
</table>

Animal use is scheduled for review one year from April 2005. Approval from the IACUC must be obtained before implementing any changes or modifications in the approved animal use.

Please keep this record for your files, and forward the attached letter to the appropriate granting agency.

Refer to Animal Protocol Number (APN) 050406856 when ordering animals or in any correspondence with the IACUC or Animal Resource Program (ARP) offices regarding this study. If you have concerns or questions regarding this notice, please call the IACUC office at 934-7602.

Institutional Animal Care and Use Committee 
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1717 7th Avenue South 
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