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Determination of dietary protein, carbohydrate, and lipid requirements for the sea urchin Lytechinus variegatus fed semi-purified feeds.

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DETERMINATION OF DIETARY PROTEIN, CARBOHYDRATE, AND LIPID REQUIREMENTS FOR THE SEA URCHIN *LYTECHINUS VARIEGATUS* FED SEMI-PURIFIED FEEDS

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DETERMINATION OF DIETARY PROTEIN, CARBOHYDRATE, AND LIPID REQUIREMENTS FOR THE SEA URCHIN *LYTECHINUS VARIEGATUS* FED SEMI-PURIFIED FEEDS

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Understanding the effects of dietary protein, carbohydrate, and lipid in *Lytechinus variegatus* would contribute to the development of commercial feeds for sea urchin aquaculture. Adult sea urchins were fed formulated feeds with different protein levels, protein: carbohydrate levels and lipid sources and levels to observe effects on sea urchin growth and quality. Sea urchins fed the 20% protein feed had moderate consumption, high survival, weight gain, production efficiency and protein efficiency suggesting this feed was utilized most efficiently. The protein and carbohydrate composition of the gonad varied directly with the level of these macronutrients consumed.

Image analysis indicated that the volume of nutritive phagocytes varied indirectly with dietary protein levels and the volume of the germinal epithelium and gamete numbers varied directly with dietary protein levels in the gonad. Data indicate macronutrient storage and gametogenic development are manipulated by diet. The effects of dietary protein were further evaluated with an improved feed containing protein: carbohydrate levels that bracketed the 20% protein feed. Improvements were made in feed formulation, feed physical form, experimental systems, and experimental methods. Sea urchins fed the 31:33% protein: carbohydrate feed had lower feed and energy consumption yet higher weight gain, production, production efficiency, gonad production, and gonad production efficiency.

Similar protein efficiency ratios among the feed treatments suggest protein sparing. Increases in supplemented menhaden oil levels had no effect on growth but reduced

production efficiency above and below the 1% level, suggesting that a source of marine-derived fatty acids are necessary for optimal production efficiency. Increases in supplemented soy oil levels had negative effects on growth, production and production efficiency. The digestibility of dry matter, crude protein, and carbohydrate were reduced when menhaden or soy oil was supplemented at the 4% level suggesting that dietary lipid levels affect the digestibility of macronutrients. These data indicate that (1) protein is an important component of sea urchin feeds, (2) dietary protein and carbohydrate directly affect the biochemical and cellular composition of the gonad, (3) the protein:energy ratio may be important for optimizing sea urchin growth, and (4) the source and level of neutral lipid affects sea urchin growth.

DEDICATION

First and foremost, this work is dedicated to my wonderful wife Brenda for believing in me when I couldn't believe in myself. We finally made it. She put her life and career on hold; sacrificing more than any person should to make this work reality. It is only through her powerful persuasion, unconditional love, limitless patience, and our stubborn efforts together that much of this work was completed. This work is also dedicated to my family, Harold, Helen, and Heather, who always believed I could and sacrificed tremendously to make it happen. Finally, I dedicate this work to my many mentors, Drs. Ken Stuck, Dan Bishop, Paula Nemeth, Bill Falls, and Stephen Watts, for taking the time and effort to create a teacher and researcher.

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INTRODUCTION

Sea urchins are an important grazing species in many marine ecosystems including kelp forests, coral reefs, rocky shores and near-shore sea-grass beds (Camp et al., 1973; Lawrence, 1975; Lawrence and Sammarco, 1982; Tegner et al., 1995; Valentine and Heck, 1999; Haley and Solandt, 2001). These echinoids frequently influence marine plant distribution and abundance (Lawrence, 1975; Valentine and Heck, 1999; Valentine et al., 2000). Large numbers of sea urchins have been associated with the occurrence of barren grounds in regions previously known to have extensive sea-grass beds (reviewed in Valentine and Heck, 1999). Furthermore, removal of large numbers of sea urchins from marine communities by disease or over-fishing causes major changes in community structure to occur (reviewed in Lawrence and Sammarco, 1982; Lessios et al., 1984; Scheibling, 1984; Lawrence, 2001; Tajima and Lawrence, 2001).

In addition to the ecological impacts that sea urchins can exert in marine communities, an economic interest in the development and enhancement of sea urchin roe production for food has arisen. Sea urchin roe is a popular seafoood in Japan, France, Chile and Barbados (Sloan, 1985; Hagen, 1996; Keesing and Hall, 1998; reviewed in Lawrence, 2001). Annual world-wide fisheries of sea urchins has been estimated to be approximately 117,000 metric tons (whole weight of sea urchin) with the world's major producers being Chile, Japan and the United States (Keesing and Hall, 1998; Andrews et al., 2002). Japanese imports alone are valued in excess of $260 million dollars U.S. annually (Keesing and Hall, 1998, Andrews et al., 2002). Sea urchin roe production has

decreased in recent years due to over-fishing of sea urchin stocks world-wide (Sloan, 1985; Keesing and Hall, 1998). Aquaculture of sea urchins is in its infancy (Schlosser, 2005), but future demand, price incentives and breakthroughs in research may make closed-cycle aquaculture feasible (Hagen, 1996). Aquaculture of sea urchins for wild seed-stock fisheries enhancement and roe enhancement has been practiced for several years in Japan and Chile (Hagen, 1996; Lawrence, 2001).

The life cycle of sea urchins begins with adults releasing large numbers of gametes into seawater (McEdward and Miner, 2001). Fertilization is internal or external and the basic biology of the early developmental stages (cleavage through larval morphogenesis) has been intensely studied by embryologists. A first feeding larva is called a echinopleuteus and feeds primarily on small particles suspended in the plankton. In the lab, larvae can be successfully maintained and supported on a wide variety of marine algae and diatom species. Larvae grow and develop in the plankton, but development is arrested when the larvae become competent to undergo metamorphosis. Metamorphosis is induced when a suitable substrate and the proper environmental cues are present. The post metamorphic sea urchin resembles a miniature adult and continuous or seasonal growth occurs (McEdward and Miner, 2001).

The feeding preferences of adult sea urchins in the field have been documented for most of the commercially important species (Lawrence, 1975; DeRidder and Lawrence, 1982; Lawrence, 2001). Sea urchins are largely regarded as being generalist omnivores despite their ecological importance as marine herbivores (reviewed in Lawrence, 1975; DeRidder and Lawrence, 1982; Briscoe and Sebens, 1988; Nestler and Harris, 1994; Beddingfield and McClintock, 1998, 1999; reviewed in Lawrence, 2001). The

foods most frequently documented from the guts of sea urchins in the field include plant material, encrusting algae or animals, sessile animals, detritus, substratum, carrion, and fecal material (Lawrence, 1975). Food preferences vary widely among species and sea urchins seem to be largely opportunistic, eating whatever is readily available in a particular habitat and during a particular season (Lawrence, 1975; DeRidder and Lawrence, 1982; Beddingfield and McClintock, 1999).

The nutrients that sea urchins obtain through feeding are used for maintenance, somatic growth and reproduction (Lawrence and Lane, 1982). These nutrients can be stored in the cells of the gut, test, and gonad (Lawrence and Lane, 1982). The nutritional value of natural or formulated feeds is usually evaluated by measuring changes in survivorship, weight gain, feed efficiency, and the relative size and composition of tissues in urchins fed a particular feed (Lawrence, 1975; Lawrence and Lane, 1982; Klinger et al., 1988; Bishop and Watts, 1992; Klinger et al., 1994; Fernandez et al., 1995; Klinger et al., 1996; Klinger et al., 1997;Lawrence et al., 1997; Beddingfield and McClintock, 1998; Fernandez and Bouderesque, 1998; Olave et al., 2001; Hofer, 2002; Pearce et al., 2002; Hammer et al., 2004). Formulated feeds have been shown to support sea urchin tissue growth better than natural diets (Fernandez et al., 1995; Levin and Naidenko, 1987; Lawrence et al., 1997; reviewed in Klinger et al., 1996; Olave et al., 2001). Though many of the early formulated feeds provided valuable insights into nutritional requirements and clearly supported weight gain in adult urchins, the physical characteristics of the feeds were not feasible for use in commercial aquaculture.

Many of the previous studies with sea urchins have utilized natural, practical or semi-purified feeds that would not be feasible for use in production aquaculture (Law-

rence, 1989; Fernandez et al., 1995; Akiyama et al., 1997; McBride et al., 1998; Pantazis et al., 2000; Otero-Villanueva et al., 2004; Hofer, 2002; Hammer et al., 2004). Natural feeds can be used to determine nutrient requirements, but are not feasible for commercial production because they cannot be conveniently stored, may spoil rapidly, can be difficult to proffer, may be inconsistent in quality, would require significant costs and labor for collection and, consequently, would not be cost effective (Pearce et al., 2002; Lawrence and Lawrence, 2003). Moist practical or semi-purified feeds have the advantages of being consistent in quality and nutrient content but are problematic because they cannot be easily or conveniently stored and tend to spoil quickly. Commercial aquaculture feeds are usually extruded or pelleted. They are cost-effective to produce and store, and relatively stable from spoilage due to low moisture content (Pearce et al., 2002; Lawrence and Lawrence, 2003). Recent nutritional studies have utilized feeds that meet many of these requirements (Pearce et al., 2002; Castell et al., 2004; Schlosser et al., 2005).

Commercial feed production is a requirement before the aquaculture of sea urchins can become feasible and sustainable. These feeds must be nutritionally optimized for each species and the life history stage of that species. They must also be in a physical form that is easily available for consumption, and feed management strategies need to be developed (Lawrence and Lawrence, 2003). First and foremost, development of high quality sea urchin feeds requires knowledge of specific macro-nutrient sources, levels, and the effects of these macro-nutrients on growth and development.

Protein is a primary component of all multi-cellular animals (Morris, 1991). Dietary proteins supply essential and nonessential amino acids and serve as a source of nitrogen for other compounds. For the most part, animal proteins are continually being recy-

cled and adult animals require a regular supply of amino acids to replace those not recovered from degraded tissue proteins. In growing animals, amino acids, in addition to those required for protein turnover, are needed for new tissue growth (Morris, 1991). In fish, inadequate dietary protein results in a decrease of growth, the loss of body weight and the removal of proteins from less vital tissues for use in more vital tissues (Wilson, 2002). In contrast, an overabundance of dietary protein results in excess protein being used for energy (Wilson, 2002).

Lowe and Lawrence (1976) suggested that the growth and reproductive rates of sea urchins may be more dependent on the amount of protein than on the amount of energy acquired through feeding. Miller and Mann (1973) and Field (pers. comm. to Ebert, 1975) have suggested that sea urchins in nature must ingest and process large quantities of protein-poor foods to meet nutritional requirements for protein. In the laboratory, Hammer et al. (2004) found that small urchins ingested greater quantities of protein-deficient formulated feeds than urchins fed protein-rich feeds. The protein requirements (using formulated feeds) of some urchin species have been evaluated for adults (de Jong-Westman, 1995; Fernandez et al., 1995; Fernandez and Bouderesque, 1998; Pearce et al., 2002) and for small urchins (McBride et al., 1998; Akiyama et al., 2001; Hammer et al., 2004). All of these studies have suggested that moderate protein levels (between 20 and 30 % dry weight of feed) support growth and development of sea urchin organs (test, lantern, gut, and gonad). In addition, several studies suggest that moderate protein levels are used most efficiently (McBride et al., 1998; Akiyama et al., 2001; Hammer et al., 2004). As protein is the most expensive component of aquaculture feeds, it is essential to determine the levels of protein required to optimize growth and use protein most efficiently.

The oxidation of dietary carbohydrates is the primary source of energy for all herbivorous and many omnivorous animals (Morris, 1991). Carbohydrates provide the energy that is required for cellular metabolic processes and for tissue synthesis (Morris, 1991). Feeds low in energy result in poor growth and feed conversion as proteins are catabolized for energy (Cuzon and Guillaume, 1997). Feeds of high-energy content (low protein content) result in reduced protein intake and restricted growth in fish and shrimps (Cuzon and Guillaume, 1997; Bureau et al., 2002). Information on carbohydrate utilization is essential in developing cost-effective feeds because energy must be supplied in sufficient amounts so that protein is almost exclusively used for tissue synthesis (Cuzon and Guillaume, 1997).

Carbohydrates are thought to be the primary source of energy in sea urchins (Marsh and Watts, in press). Sea urchins lack an efficient circulatory system to transport oxygen to internal tissues and may therefore rely on primarily anaerobic mechanisms to produce energy within internal organs (Marsh and Watts, in press). Large quantities of oxygen are required to oxidize lipids for energy production and it appears unlikely that sea urchins could provide oxygen in the quantities required for beta-oxidation (Marsh and Watts, in press). Therefore it becomes even more important to understand the role of dietary carbohydrates in energy production, growth, and development.

Lipids have several very important functions in animals including: energy storage, energy production, cell membrane production, and as precursors in the synthesis of many regulatory molecules (Morris, 1991). Despite the importance of lipids, we know less about the lipid requirements for fish, crustaceans and other animals than about other nutrients (Sargent et al., 2002). The determination of lipid requirements is difficult and

entails consideration of relative and absolute amounts of individual fatty acids combined with abilities to metabolize and biosynthesize fatty acids (Sargent et al., 2002).

The effect of dietary lipid source and level on somatic growth has received little attention in sea urchins. Pantazis et al. (2000) varied the lipid concentration of feeds in *Psammechinus miliaris* and found no significant differences in somatic growth among the feeds examined. They were the first to suggest that commonly presumed essential fatty acids were not required by sea urchins. Castell et al. (2004) studied the effects of dietary fat source on fatty acid composition and metabolism in *Strongylocentrotus droebachiensis*. They also found no significant differences in weight gain or test diameter among feeds that varied in qualitative and quantitative fatty acid composition and confirmed the findings of Pantazis et al. (2000) about essential fatty acid requirements. However, Gibbs et al. (2006) recently observed that an increase in levels of dietary phospholipid increased weight gain and gonad production in *L. variegatus*. Knowledge of lipid source, level, and form is important because provision of this nutrient (often fish oils) can be expensive in the formulation of aquaculture feeds. Lipids have been shown previously to be necessary for optimal weight gain in aquacultured crustaceans and fish (reviewed in D'Abramo, 1997; reviewed in Sargent et al., 2002).

Formulating diets with the proper sources and levels of macro-nutrients is important; however, the organism must be able to digest and absorb those nutrients if the diet is to be efficiently utilized. Formulated feeds can be nutritionally balanced and still fail to produce favorable growth if the nutrients are not biologically available for use by the organism (Lee and Lawrence, 1997). Biological availability is usually assessed by evaluating digestibility (absorption). The digestibility of a feed depends not only on the animal's

digestive anatomy and physiology but also on the feed's physical and nutritional characteristics (Lee and Lawrence, 1997). Sea urchin digestibilities of natural and formulated feeds have been examined (reviewed in Lawrence et al., in press). With each new feed that is evaluated, detailed studies of feeding and growth parameters, including digestibility, are required.

The sea urchin *Lytechinus variegatus* is frequently found in sea-grass ecosystems from North Carolina to Brazil (Moore et al. 1963; Beddingfield and McClintock 1999; reviewed in Watts et al., in press). *Lytechinus variegatus* has been described as a ruderal species (Lawrence and Bazhin, 1998) and, as such, has a rapid growth rate. Individuals in the field have been reported to grow to a diameter of 35 mm in a single year in the northern Gulf of Mexico (Beddingfield and McClintock, 2000) and from 10 mm to over 50 mm in Key Biscayne, Florida (Moore et al., 1963). This species can grow to greater than 90 mm in test diameter (Moore et al. 1963).

Lytechinus variegatus is an omnivore that feeds on a wide variety of foods including but not limited to *Thalassia testudinum*, *Syringodium filiforme*, *Cymodocea manatorum*, *Haladule wrightii*, filamentous algae, sea grass epibionts, drift material, sand, shells, the mussel *Modiolus americanus*, and gastropods (reviewed in Watts et al. in press). Such a diverse diet is beneficial to the growth of *L. variegatus* because the urchin is provided with a wide variety of nutrients (Lowe and Lawrence, 1976; Klinger et al., 1994; Beddingfield and McClintock, 1998).

The sea urchin *Lytechinus variegatus* has been an important model for the study of sea urchin nutrition due to rapid growth rates and the ease of culture in the laboratory (Lowe and Lawrence, 1976; Klinger, 1982; Klinger et al, 1986; Klinger et al., 1988;

Klinger et al., 1994; Lares, 1999; Bishop and Watts, 1992; Bishop and Watts, 1994; Watts et al., 1998; Hammer et al., 2004). In the laboratory, high survivorship and weight gain of *L. variegatus* is supported by formulated feeds (Klinger, 1982; Klinger et al., 1988; Bishop and Watts, 1992; Beddingfield and McClintock, 1998; Hofer, 2002; Hammer et al., 2004). Formulated feed research has focused mainly on tissue growth, absorption efficiency and food preferences while very little research has been completed regarding the effect of specific macro-nutrients on the overall growth, tissue growth and tissue composition. A recent study by Hammer et al. (2004) found small *L. variegatus* grew rapidly on formulated feeds of greater than 15% dry protein but survivorship was greatly reduced in feeds with a dry protein content of less than 20%. Sea urchins proffered a low protein feed (9%) attempted to compensate by consuming more feed per gram wet weight, but production efficiency was greatly reduced. Sea urchins proffered formulated feeds $\geq$15% protein had good gonad growth. Protein (amino acid) requirements for growth and development are not known for adult *L. variegatus*, and are not fully understood for any sea urchin species. Carbohydrate and lipid requirements have not been investigated.

This dissertation represents a step-wise research program evaluating the daily dietary nutritional requirements and the nutritional value of specific ingredients for feed development in sea urchins. Manuscript 1 represents the initial approach in evaluating protein requirements for adults of this species through the assessment of survival, consumption, growth and efficiency parameters. Three semi-purified moist feeds with different levels of protein (and consequently carbohydrate) level were prepared. The feeds were based on nutritional information available from research with crustacean feeds. Manu-

script 2 describes the effects of the previous diet treatment on the biochemical composition of sea urchin organs (test, lantern, gut, and gonad), the storage of macro-nutrients in these organs, and the histological evaluation of cellular (gametes and nutritive phagocytes) development of the gonad.

Manuscript 3 represents a progression to state-of-the-art recirculating system technologies, enhanced nutritional methodologies, and improved dry feed formulations. This study also examines protein: carbohydrate requirements for *L. variegatus* but utilizes an improved feed formulation and a more narrow range of protein levels. In this study the physical characteristics of the feed were changed to reflect a commercial-type pellet, a cold-extruded pellet of low moisture content. A new experimental design, allowing for the repeated measurements of individual sea urchins, was enlisted to increase our observations and statistical power. Sea urchins in each of the feed treatments were contained individually in newly-designed cages that optimize feeding and handling in a state-of-the-art semi-recirculating system with complete water temperature control, mechanical filtration, biological filtration, UV sterilization and protein skimming.

Manuscript 4 represents the first attempt to evaluate the effects of dietary lipid sources and levels on growth parameters in *L. variegatus*. The physical characteristics of the feeds, experimental design, and experimental systems were similar to those used for manuscript 3. The feeds used for this study were based on our best formulations from previous studies and supplemented with either marine-based menhaden oil (MF feeds) or non-marine soy oil (NMF feeds).

THE EFFECT OF DIETARY PROTEIN ON CONSUMPTION, SURVIVAL, GROWTH AND PRODUCTION OF THE SEA URCHIN *LYTECHINUS VARIEGATUS*

by,

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Aquaculture, in press

Format adapted for dissertation

Abstract

An increased understanding of dietary protein for growth and development of *Lytechinus variegatus* would increase our knowledge of the nutritional requirements of sea urchins and contribute to the development of formulated feeds for the aquaculture of commercially important sea urchin species. Previously starved urchins (ca. 36 mm diameter, $n = 12$) were held in replicated (3x) 80 l aquaria with artificial seawater at 22 $\pm$ 2 °C and 32 ‰ salinity. Urchins were fed one of three diets containing 9%, 20%, or 31% dry protein *ad libitum* for 65 days. Sea urchins fed diets containing 9% protein consumed more food than urchins fed 20% or 31% protein diets. Sea urchins fed the 9% protein diet had greatly decreased survival. Test diameters increased significantly from the initial sample only in those sea urchins fed the 20% protein diet. Sea urchins fed the 20% protein diet had larger test diameters than those fed the 9% but not the 31% protein diet at day 65. Urchins fed the 20% protein diet had significantly greater total wet and dry weights than urchins fed the 9% but not the 31% protein diet. At the conclusion of the study there were no significant differences in lantern, gut or gonad wet or dry weight for urchins fed the three diets (test dry weight was highest at 20% protein). Specific growth rate, estimated dry matter production and production efficiency in urchins fed the 20% protein diet were greater than in urchins fed the 9% but not the 31% protein diet. Urchins fed the 9% or 20% protein diet utilized dietary protein more efficiently than urchins fed the 31% protein diet. Gonad production was not different among diets but gonad production efficiency was significantly lower in urchins fed the 9% protein diet. Data suggest that adult *L. variegatus* utilize the 20% protein diet most efficiently and that this diet would be the most cost-effective of the diets tested.

1. Introduction

Sea urchin roe is a popular food in Japan, France, Chile and Barbados (Sloan, 1985; Hagen, 1996; Keesing and Hall, 1998; Lawrence, 2001). Annual world-wide fisheries of sea urchins was estimated to be over 120,000 metric tonnes (whole weight of sea urchin) in 1995 with the world's major producers being Chile, Japan and the United States (Andrews et al., 2002). Sea urchin roe production has decreased in recent years with the increased demand for roe and the over-fishing of sea urchin stocks world-wide (Andrews et al., 2002). Aquaculture of sea urchins is in its infancy but future demand, price incentives and breakthroughs in research may make closed-cycle aquaculture feasible (Hagen, 1996; Robinson, 2004).

Protein is a primary component of all multi-cellular animals. Lowe and Lawrence (1976) suggested that growth and reproduction rates of sea urchins may be more dependent on dietary protein than on dietary energy. Miller and Mann (1973) and Field (in Ebert 1975) have suggested that sea urchins in nature must ingest and process large quantities of protein-poor foods to meet nutritional requirements for protein. As protein is one of the most expensive components of aquaculture feeds, it is essential to determine the optimal levels of protein required to maximize growth and utilize protein most efficiently.

The sea urchin *L. variegatus* is a species frequently found in sea-grass ecosystems from North Carolina to Brazil (Moore et al., 1963; Beddingfield and McClintock, 1999; reviewed in Watts et al., 2001). *L. variegatus* is an omnivore that feeds on a wide variety of foods including but not limited to *Thalassia testudinum*, *Syringodium filiforme*, *Cymo-*

docea manatorum, *Haladule wrightii*, filamentous algae, sea grass epibionts, drift material, sand, shells, the mussel *Modiolus americanus*, and gastropods (Watts et al., 2001).

In the laboratory, high survival and weight gain of *L. variegatus* is supported by formulated feeds (Klinger, 1982; Klinger et al., 1988; Bishop and Watts, 1992; Beddingfield and McClintock, 1998; Hofer, 2002; Hammer et al., 2004). Formulated feed research has focused mainly on tissue growth, absorption efficiency and food preferences while few studies have reported the effect of specific dietary nutrients on total animal growth, tissue growth and tissue composition. The requirement for protein is not known for adult *L. variegatus*; however, this information is needed for future development of cost effective commercial formulated feeds for the aquaculture of sea urchins.

2. Materials and methods

2.1 *Collection and maintenance of sea urchins*

L. variegatus (35-45 mm diameter) were collected (n = 150) from Saint Joseph Bay, Florida and returned to the University of Alabama at Birmingham (UAB). Sea urchins were maintained on minimal prepared diets in 80-l aquaria with one feeding approximately every two weeks for a period of approximately 4 months after which the urchins were considered to be starved. The sea urchins (mean initial wet weight 22.8 ± 1.35) were then divided randomly into nine 80-l aquaria (n = 12 sea urchins per aquarium). Daily feeding was initiated and feeding parameters were recorded beginning five days after the first feeding. Any individuals that died during the first two weeks of the study were replaced with sea urchins from the same population. Sea urchins were maintained in 10-l aquaria supported within larger 80-l aquaria containing recirculated artificial seawater (30

‰ salinity and 21-23 °C), as described in Wallace (2001) (Fig. 1). This system allows easy collection of uneaten food and the removal of fecal material within a two-day period; a partial (less than 10%) water exchange was accomplished at each removal. The sea urchins were maintained on a 12h light: 12h dark photoperiod and water quality was maintained within optimal parameters.

Three semi-purified diets (Table 1) that vary in protein concentration were provided by Dr. Addison Lawrence (Texas A&M). These diets were constituted into a pellet by adding 10 g of dry formulated diet (14%, 32%, or 50% crude protein) to a solution of heated seawater (100 ml, 40 ‰, 60-70 °C) containing 2 g of agar binder. The wet slurry was allowed to solidify and was cut into small pellets of ca. 1 x 1 x 1 cm. The resulting percent protein on a dry weight basis of the diet following dehydration to constant dry weight (constituted diet) was calculated to be 9, 20, or 31% protein (Table 2). The resulting wet matter protein concentration to be fed to the urchins was calculated to be 1.3%, 2.9%, or 4.5 % protein. Sea urchins were fed *ad libitum* one of the three diets daily for 65 days (n = 12 individuals per aquarium, three aquaria per diet treatment). The wet weight of the food fed was weighed to the nearest milligram. Prior to the next feeding, uneaten food was removed (by siphon), placed on a paper towel to remove excess moisture, and weighed. Preliminary experiments have indicated that the feed does not change substantially in water content while in the aquarium (less than 2%, unpub. data).

2.2 *Consumption*

The total diet fed and the total uneaten diet removed was used to calculate the consumption rate. Mean wet feed consumption per individual per day was calculated as:

Table 1
Composition of the Texas A&M formulation used to produce diets varying in protein concentration

	% Protein		
	14	32	50
Component (% dry weight basis)			
Wheat starch	36.94	18.47	0.00
Algae kelp	30.00	28.50	26.99
Krill	15.00	15.00	15.00
Cellulose	4.00	4.00	4.00
Phospholipid	4.00	4.00	4.00
Fish oil (menhaden)	2.25	2.13	2.00
Vitamin premix[a]	1.50	1.50	1.50
Casein	1.10	9.50	17.90
PO_4	1.00	1.00	1.00
Soy	1.00	10.50	20.00
Cholesterol	1.00	1.00	1.00
Wheat gluten	1.00	3.20	5.40
Mineral premix[b]	1.00	1.00	1.00
Vitamin C	0.10	0.10	0.10
$ZnCO_3$	0.04	0.04	0.04
$CuCl*2H_2O$	0.03	0.03	0.03
$MnSO_4*H_2O$	0.03	0.03	0.03
Betacarotene	0.01	0.01	0.01

Final protein concentrations (after addition of agar binder) were calculated to be 9, 20 or 31% protein dry weight, respectively

[a] Vitamin premix contains on a per kg basis: vitamin A (retinol) 650,000 IU, vitamin D (cholecalciferol) 500,000 IU, vitamin E (tocopherol) 25,000 mg, vitamin K (menadione) 2,500 mg, vitamin B1 (thiamine) 5,000 mg, vitamin B2 (riboflavin) 10,000 mg, vitamin B6 (pyridoxine) 20,000 mg, niacin 12,500 mg, pantothenic acid 12,500 mg, biotin 187 mg, folic acid 5,000 mg, vitamin B12 (cyanocobalamine) 37.5 mg.

[b] Mineral premix contains: calcium 14.73%, phosphorous 11.39%, sodium 2.91%, potassium 10.76%, magnesium 11.45%, iron 994 ppm, zinc 876 ppm, 1,678 ppm, copper 667 ppm, selenium 4 ppm, chloride 101 ppm, iodine 8 ppm

Table 2
Calculated composition of the Texas A&M formulation used to produce diets varying in protein concentration

	Low protein (9%)		Medium protein (20%)		High protein (31%)	
	Calculated (%)[a]	Calculated final (%)[b]	Calculated (%)[a]	Calculated final (%)[b]	Calculated (%)[a]	Calculated final (%)[b]
Crude Protein	13.97	8.73	32.09	20.10	50.20	31.38
Moisture	4.85	-	5.29	-	5.73	-
Crude Fiber	6.72	4.20	6.66	4.16	6.59	4.12
Total Ash	8.35	30.22	8.66	30.41	8.96	30.60
Crude Fat	9.81	6.13	9.82	6.14	9.83	6.14
Carbohydrate	56.38	35.24	37.50	23.44	18.62	11.64
Energy[c]	3.95	2.9	4.42	3.01	4.49	3.44

Final protein concentrations (after addition of agar binder) were calculated to vary from 9% to 31% dry weight (as fed basis).

[a] Values provided by Texas A&M based on content of the formulated diet prior to incorporation into the food pellet.

[b] Constituted feed prepared from 10 g dry formulation, 2 g agar binder, and 100 ml salt water (40 ‰). Values represent calculated composition following dehydration to constant dry weight.

[c] Energy values in kcal/g calculated by bomb calorimetry.

$$\frac{\text{daily wet weight diet fed} - \text{daily wet weight diet removed}}{\text{number of sea urchins surviving daily}}$$

Mean dry protein consumption per individual per day (of the constituted diet) was calculated as:

$$\frac{\text{dry weight diet fed} - \text{dry weight diet removed}}{\text{number of sea urchins surviving daily}} \times \%\ \text{dry protein in constituted diet}$$

The two consumption parameters (mean wet consumption per individual per day and mean dry protein consumption per individual per day) were analyzed as the dependent variable in a repeated measures model with dietary protein level as the predictor. Although measurements on individual sea urchins were not available, the PROC MIXED procedure (SAS, Ver. 8.02) was used to analyze the relationship between each consumption parameter and time accounting for group differences. Group means in 7-day incre-

ments were compared using the Tukey's adjustment. A P value of < 0.05 was determined to be statistically significant.

2.3 *Survival*

Survival was calculated for the 65-day study using The Lifetest Procedure (SAS Ver. 8.02). Dead sea urchins were considered events and survival times were censored. Comparison of Kaplan-Meier survival curves were performed by log-rank test. A P value of < 0.05 was determined to be statistically significant.

2.4 *Dissection, growth and composition*

Immediately prior to the beginning of the study, a random sub-sample of sea urchins ($n = 15$) were removed from the initial population for dissection. Additional sub-samples of sea urchins ($n = 4$) were removed at random from each aquarium at day 32 and day 65 for dissection. Sea urchins were measured at two perpendicular points across the ambitus using calipers (test diameter), weighed to the nearest mg (wet weight), and dissected. Sea urchins were cut outside the peristomial membrane on the oral surface. During the dissection the test with spines, Aristotle's lantern, gut, and gonads were removed and separated. The gut (esophagus, stomach, and intestine) were rinsed in a finger bowl to remove excess food. Each of the components were blotted dry with a paper towel to remove excess water and weighed to the nearest mg (wet weight). Components were placed into a 60 °C oven, dried for several days to constant weight, and weighed again to the nearest mg (dry weight).

2.5 *Production*

The estimated specific growth rate (SGR; percent increase in body weight per day) was calculated as:

$$\frac{\text{individual ln final wet weight} - \text{ln mean initial wet weight}}{\text{time (days)}} \times 100$$

The estimated dry matter production (g) was calculated as:

individual final dry weight – mean initial dry weight

The production efficiency was calculated as:

$$\frac{\text{individual final dry weight} - \text{mean initial dry weight}}{\text{dry diet consumed}} \times 100$$

The estimated protein efficiency ratio (PERWET) was calculated as:

$$\frac{\text{individual final wet weight (no coelomic fluid)} - \text{mean initial wet weight (no coelomic fluid)}}{\text{dry weight protein consumed}}$$

The estimated protein efficiency ratio (PERDRY) was calculated as:

$$\frac{\text{individual final dry weight} - \text{mean initial dry weight}}{\text{dry weight protein consumed}}$$

The estimated gonad production (g) was calculated as:

individual final dry weight gonad – mean initial dry weight gonad

The estimated gonad production efficiency was calculated as:

$$\frac{\text{individual final dry weight gonad} - \text{mean initial dry weight gonad}}{\text{dry weight diet consumed}} \times 100$$

2.6 *Statistics summary*

All statistical comparisons, except consumption and survival, were completed using the SYSTAT 9 software package. All consumption parameters and survival analyses were performed using SAS (Ver. 8.02). If data were normal and had equal variance, pa-

rametric tests including ANOVA and ANCOVA were used. When significant differences were determined, a Tukey's test for pairwise group comparisons was completed. A P value of < 0.05 was determined statistically significant for all parametric tests. If data were non-normal, did not have equal variance, or ANCOVA analysis returned a significant relationship with covariates, non-parametric statistics (Kolmogorov-Smirnov two-sample, Kruskal Wallis, or Mann Whitney U tests) were completed. In order to maintain an overall acceptance criteria of $\alpha = 0.05$ during multiple comparisons, a Bonferroni's adjustment was adopted.

3. Results

3.1 *Consumption*

Consumption varied daily for all protein diets throughout the 65-day study period (Fig. 2A and B). For each consumption parameter (mean wet consumption per individual per day and mean dry protein consumption per individual per day), the predictive model obtained from The Mixed Procedure indicated a curvature in the data and the linear term was different for each group. There was a significant group effect, time effect, and group*time effect for each of the parameters in both consumption figures (Table 3).

The predicted model for mean wet consumption per individual per day (Fig. 2A and Table 4A) indicates that sea urchins fed the 9% protein diet consumed more wet food than sea urchins fed the 20% protein diet by day 14 ($P < 0.0001$) and the 31% protein diet by day 7 ($P < 0.0001$). Those sea urchins fed the 20% protein diet consumed more food initially than sea urchins fed the 31% protein diet but these differences disappeared by day 28. Those sea urchins that consumed the 9% protein diet consumed more food over

Table 3
Lytechinus variegatus: mixed model analysis of consumption data from repeated measures

The mixed procedure		
Type 3 tests of fixed effects		
Effect	*F* Value	*P*
Wet consumption per sea urchin per day		
Protein	21.67	<0.0001
Days	67.39	<0.0001
Days x protein	43.09	<0.0001
Days x days	56.16	<0.0001
Dry protein consumption per sea urchin per day		
Protein	104.10	<0.0001
Days	38.46	<0.0001
Days x protein	14.17	<0.0001
Days x days	36.64	<0.0001

all than sea urchins fed the 20% protein diet ($P < 0.001$) or the 31% protein diet ($P < 0.001$). There was no significant difference in overall consumption between those sea urchins fed the 20% protein diet or the 31% protein diet ($P = 0.2841$).

The predicted model for mean dry protein consumption per individual per day (Fig. 2B and Table 4B) indicated that sea urchins fed the 9% protein diet consumed less dry protein than sea urchins fed the 31% protein for the entire study ($P < 0.0001$) and less than the 20% protein diet until day 63 of the study ($P = 0.1457$). Those sea urchins fed the 20% protein diet consumed less dry protein than the 31% protein diet throughout the study ($P < 0.0001$). The overall dry protein consumed by the sea urchins fed each dietary protein level was significantly different from the other diets ($P < 0.0001$) and directly reflected the amount of dry protein present in the diets.

Table 4
Lytechinus variegatus: pair-wise comparison of (A) wet food consumed per individual per day and (B) dry protein consumed per individual per day

Days		9% vs. 20%	9% vs. 31%	20% vs. 31%
A				
	Overall	<0.0001	<0.0001	0.2841
	0	0.6162	0.0202	0.005
	7	0.1707	<0.0001	0.006
	14	<0.0001	<0.0001	0.0089
	21	<0.0001	<0.0001	0.0205
	28	<0.0001	<0.0001	0.0865
	35	<0.0001	<0.0001	0.4332
	42	<0.0001	<0.0001	0.8524
	49	<0.0001	<0.0001	0.3612
	56	<0.0001	<0.0001	0.1664
	63	<0.0001	<0.0001	0.0923
B				
	Overall	<0.0001	<0.0001	<0.0001
	0	<0.0001	<0.0001	<0.0001
	7	<0.0001	<0.0001	<0.0001
	14	<0.0001	<0.0001	<0.0001
	21	<0.0001	<0.0001	<0.0001
	28	<0.0001	<0.0001	<0.0001
	35	<0.0001	<0.0001	<0.0001
	42	<0.0001	<0.0001	<0.0001
	49	<0.0001	<0.0001	<0.0001
	56	0.0021	<0.0001	<0.0001
	63	0.1457	<0.0001	<0.0001

Consumption was analyzed as the dependent variables in a repeated measures model with dietary protein as the predictor. The PROC MIXED procedure was used to analyze the relationship between average consumption and time accounting for group differences. The group means at various points were compared using the Tukey's adjustment. The significance was determined for the entire 65-day study (overall) and for each 7-day period following the initiation of the study.

3.2 *Survival*

Survival was 100% for those sea urchins fed the 20% and 31% protein diets (Fig. 3). Survival decreased in sea urchins fed the 9% protein diet after day 35 and continued to decrease (to 57 %) for the duration of the study.

3.3 *Growth*

At day 65 of the study sea urchins fed the 20 % protein diet had test diameters that were larger than the sea urchins examined at day 0 ($P < 0.001$) (Table 5). At day 32 there were no significant differences in test diameter between sea urchins fed the three different diets. Sea urchins fed the 20% protein diet had a larger test diameter than those fed the 9% ($P = 0.007$) but not the 31% protein diet at day 65. Mean wet and dry weights increased in sea urchins fed the 9, 20, or 31% protein diets during the 65-day study (Table 4) ($P < 0.002$). At day 65 those sea urchins fed the 20% protein diet had a significantly greater total wet weight ($P = 0.010$) and total dry weight ($P = 0.032$) than sea urchins fed the 9% protein diet, but did not differ significantly from those fed the 31% protein diet.

3.4 *Component wet and dry weight*

The wet weight of the test in sea urchins fed the 20 and 31% protein diets increased by day 65 ($P < 0.001$) (Table 6); however, test wet weight was not influenced by diet. The dry weight of the test increased significantly for those sea urchins fed the 20% protein diet ($P < 0.001$). Sea urchins fed the 20% protein diet had a greater test dry weight than those fed the 9% protein diet ($P = 0.01$). The wet weight and dry weight of the Aristotle's lantern did not differ significantly with time or dietary protein level. Gut

Table 5
Lytechinus variegatus: growth parameters analyzed at day 0, 32, and 65 for sea urchins fed three different protein diets

Parameters	Day 0	Dietary pro-tein (%)	Day 32		Day 65	
Diameter (mm)	36.0 ± 0.80	9	38.6 ± 0.87	A	39.0 ± 0.84	B
		20	38.7 ± 0.62	A	42.2 ± 0.71	A*
		31	36.7 ± 0.49	A	40.6 ± 0.74	AB
Wet Weight (g)	22.8 ± 1.35	9	29.0 ± 2.00	A	31.6 ± 1.43	B*
		20	28.3 ± 1.44	A	38.4 ± 1.89	A*
		31	25.8 ± 0.88	A	33.9 ± 1.64	AB*
Dry Weight (g)	5.3 ± 0.33	9	7.0 ± 0.48	A	7.9 ± 0.46	B
		20	7.0 ± 0.39	A	9.6 ± 0.52	A*
		31	6.2 ± 0.31	A	8.2 ± 0.36	AB*

Asterisk indicates statistical significance from data collected at day 0.
Letters indicate statistical difference among diets.

wet weight and dry weight increased with time in animals fed all diets ($P < 0.001$). Gut wet weight or dry weight did not vary with dietary protein. Gonad wet weight and dry weight increased in all animals by day 32 ($P < 0.001$). At day 32 those sea urchins fed the 9% protein diet had lower gonad wet weight than those sea urchins fed the 20% ($P < 0.001$) or 31% ($P = 0.007$) protein diets. Sea urchins fed the 9% protein diet had lower gonad dry weight than sea urchins fed the 20% protein diet ($P = 0.001$). Gonad wet and dry weights did not differ with diet at day 65.

3.5 *Production*

The specific growth rate (SGR) was highest in animals fed the 20% protein diet and lowest in those fed the 9% protein diet at day 65 (Table 7). Estimated dry matter production (EDMP) was highest in sea urchins fed the 20% protein diet. The estimated pro-

Table 6
Lytechinus variegatus: the wet and dry weight (g) of the test, Aristotle's lantern, gut and gonad analyzed at day 0, 32, and 65 for sea urchins fed three different protein diets

Tissue	Parameter	Day 0	Dietary protein (%)	Day 32		Day 65	
Test	Wet Weight	8.5 ± 0.47	9	11.1 ± 0.74	A	11.5 ± 0.66	A
			20	10.6 ± 0.57	A	14.0 ± 0.69	A*
			31	9.8 ± 0.44	A	11.8 ± 0.56	A*
	Dry Weight	4.8 ± 0.29	9	6.0 ± 0.38	A	6.0 ± 0.37	B
			20	5.6 ± 0.31	A	7.6 ± 0.39	A*
			31	5.1 ± 0.26	A	6.3 ± 0.27	AB
Aristotle's lantern	Wet Weight	0.8 ± 0.06	9	1.1 ± 0.09	A	1.0 ± 0.06	A
			20	1.1 ± 0.07	A	1.1 ± 0.07	A
			31	1.0 ± 0.03	A	1.1 ± 0.06	A
	Dry Weight	0.5 ± 0.03	9	0.62 ± 0.06	A	0.59 ± 0.04	A
			20	0.61 ± 0.04	A	0.71 ± 0.04	A
			31	0.56 ± 0.03	A	0.66 ± 0.03	A
Gut	Wet Weight	0.12 ±.015	9	0.62 ± .07	A*	0.84 ± 0.07	A*
			20	0.69 ± .03	A*	0.83 ± 0.08	A*
			31	0.70 ± .03	A*	0.77 ± 0.04	A*
	Dry Weight	0.024 ± 0.003	9	0.15 ± 0.02	A*	0.17 ± 0.02	A*
			20	0.16 ± 0.01	A*	0.17 ± 0.02	A*
			31	0.15 ± 0.01	A*	0.17 ± 0.01	A*
Gonad	Wet Weight	0.07 ± 0.03	9	1.0 ± 0.19	B*	3.4 ± 0.39	A*
			20	2.4 ± 0.20	A*	4.4 ± 0.43	A*
			31	2.1 ± 0.28	A*	4.4 ± 0.46	A*
	Dry Weight	0.012 ± 0.001	9	0.25 ± .05	B*	1.1 ± 0.13	A*
			20	0.59 ± 0.06	A*	1.2 ± 0.12	A*
			31	0.41 ± 0.05	AB*	1.1 ± 0.09	A*

Asterisk indicates statistical significance from data collected at day 0.
Letters indicate statistical difference among diets.

Table 7
Lytechinus variegatus: production parameters analyzed at times day 0 to 32, day 32 to 65 and day 0 to 65 for sea urchins fed three different protein diets

Parameters	Dietary protein (%)	Day 0-32		Day 32-65		Day 0-65	
Estimated specific growth rate (SGR)	9	0.71 ± 0.20	A	0.52 ± 0.21	A	0.49 ± 0.07	B
	20	0.63 ± 0.15	A	1.0 ± 0.21	A	0.78 ± 0.08	A
	31	0.35 ± 0.10	A	0.82 ± 0.10	A	0.59 ± 0.07	AB
Estimated dry matter production (g)	9	1.7 ± 0.47	A	1.2 ± 0.30	A	2.6 ± 0.45	B
	20	1.7 ± 0.38	A	2.7 ± 0.51	A	4.3 ± 0.52	A
	31	0.9 ± 0.29	A	2.0 ± 0.36	A	2.8 ± 0.36	AB
Estimated production efficiency (%)	9	13.3 ± 3.69	A	6.8 ± 1.73	B	8.5 ± 1.51	B
	20	15.2 ± 3.52	A	28.5 ± 5.33	A	21.2 ± 2.55	A
	31	9.5 ± 2.98	A	20.0 ± 3.57	AB	14.4 ± 1.85	AB
Estimated protein efficiency ratio (PER) wet/dry	9	3.9 ± 0.92	A	2.1 ± 0.49	A	2.7 ± 0.37	A
	20	2.4 ± 0.36	A	3.0 ± 0.58	A	2.6 ± 0.27	A
	31	1.3 ± 0.21	A	1.4 ± 0.29	A	1.4 ± 0.15	B
Estimated protein efficiency ratio (PER) dry/dry	9	1.5 ± 0.42	A	0.75 ± 0.20	AB	0.97 ± 0.17	A
	20	0.76 ± 0.18	A	1.4 ± 0.27	A	1.1 ± 0.13	A
	31	0.30 ± 0.095	A	0.62 ± 0.11	B	0.46 ± 0.06	B

Letters indicate statistical difference among diets.

duction efficiency (EPEC) was highest in sea urchins fed 20% protein from day 32 to 65 ($P = 0.002$) and from day 0 to 65 ($P = 0.001$) compared to those fed the 9% protein diet.

Protein efficiency ratio (PER) for wet (PERWET) and dry (PERDRY) tissues were significantly lower in those sea urchins fed the 31% protein diet from day 0 to 65 (Table 7).

3.6 *Gonad Production*

Sea urchins fed the 9% protein diet had lower estimated gonad production (EGP) than sea urchins fed the 20% ($P < 0.001$) and 31% ($P < 0.048$) protein diets at day 32; the

differences in EGP disappeared by day 65 (Table 8). The estimated gonad production efficiency (EGPEC) was lower in sea urchins fed the 9% protein diet.

Table 8
Lytechinus variegatus: gonad production and gonad production efficiency analyzed at day 0, 32, and 65 for sea urchins fed three different protein diets

Parameter	Dietary protein (%)	Day 32		Day 65	
Estimated gonad production (g)	9	0.24 ± 0.05	B	1.1 ± 0.13	A
	20	0.58 ± 0.06	A	1.2 ± 0.12	A
	31	0.40 ± 0.05	A	1.1 ± 0.09	A
Estimated gonad production efficiency (%)	9	1.9 ± 0.41	B	3.6 ± 0.43	B
	20	5.3 ± 0.51	A	5.9 ± 0.60	A
	31	4.1 ± 0.55	A	5.5 ± 0.46	A

Letters indicate statistical difference among diets.

4. Discussion

Adult *L. variegatus* fed the 9% protein diet consumed more wet food overall than sea urchins fed the 20 and 31% protein diets in the present study. Hammer et al. (2004) demonstrated that small, rapidly growing *L. variegatus* fed a 9% protein diet consumed significantly more wet food per unit weight than urchins fed 14%, 20%, or 31% protein diets. Fernandez and Boudoresque (1998) found that consumption in adult *Paracentrotus lividus* was inversely correlated to the protein content of prepared feeds containing 13%, 29%, and 47% soluble protein. McBride et al. (1998) found that consumption decreased with increasing dietary protein in small *Strongylocentrotus franciscanus* fed prepared diets containing 30%, 40%, or 50% protein. *Eucidaris tribuloides* fed low nutrient density diets consumed more food than individuals fed a high quality diet (Brown and McClintock, 1990). In a communication with Ebert (1975), J. C. Field suggested that sea urchins consume and process large amounts of protein poor foods to

obtain sufficient dietary protein to meet nutritional needs. Additionally, Miller and Mann (1973) suggested that sea urchins may consume large amounts of carbohydrate rich (protein poor) material in order to process and obtain necessary dietary protein, only to later discharge carbohydrates as dissolved organic material (DOM). An interesting contrast is the study of Akiyama et al. (2001) in which small *Pseudocentrotus depressus* fed prepared diets of 10% to 51% protein had significantly greater daily consumption when fed the 10% or 51% protein diets compared to the 21% or 31% protein diets. These data suggest that adult sea urchins consume more food of low protein quality in an attempt to compensate for the lack of available protein in the diet. *L. variegatus* fed the 9% protein diet (*ad libitum*) consumed significantly less dry protein despite the increased consumption of wet food.

Data suggest that limited total protein and/or potentially the lack of essential amino acids, contributed to the decreased survival observed in sea urchins fed the 9% protein diet after week 5. Hammer et al. (2004) observed decreased survival in small *L. variegatus* fed 9% and 14% protein diets. De Jong-Westman et al. (1995) observed reduced survival in adult *Strongylocentrotus droebachiensis* fed a low protein diet without supplements (a 10% protein diet). Pearce et al. (2002) reported no significant difference in survival of adult *S. droebachiensis* fed diets with 19%, 24%, or 29% protein for 12 weeks. McBride et al. (1998) observed 100% survival in small *S. franciscanus* over a 10-month period fed diets of 30%, 40%, and 50% protein. Akiyama (2001) found 100% survival in small *P. depressus* held for 8 weeks on prepared diets of 10% to 51% protein.

Evaluation of growth in adult sea urchins is difficult to assess, particularly in studies of short duration. Weight gain and increases in test diameter are less obvious in adult

sea urchins and may vary among studies due to differences in the reproductive and nutritional condition of the experimental populations. Sea urchins in the current study were reduced to a minimal nutritional condition prior to the study, allowing an increased opportunity for evaluating protein utilization in growth.

The greatest increase in test diameter and highest weight gain was found in those sea urchins fed the 20% protein diet, suggesting that the 20% dietary protein content was more optimum for the omnivore *L. variegatus* than the 9% and 31% protein diets for the conditions of this study. Hofer (2002) observed a significant increase in weight gain and test diameter with adult *L. variegatus* fed a 20% protein diet, of similar composition to the present study, for a period of 8 weeks. De Jong-Westman et al. (1995) found no significant differences in test diameter of cultured adult *S. droebachiensis* fed diets of 10 and 20% protein for 9 months. Small *L. variegatus* fed 9%, 14%, 20%, and 31% protein diets, of similar composition to the present study, showed significantly reduced weight gain and test diameters if fed a 9% protein diet for 14 weeks (Hammer et al., 2004). Small *P. lividus* fed diets of 13% protein had significantly smaller test diameters than sea urchins fed 29% and 47% protein over 9 months (Fernandez and Boudouresque, 1998). Akiyama et al. (2001) reported that small *P. depressus* fed a 10% protein diet for 8 weeks had significantly smaller test diameters than sea urchins fed diets with 21% to 51% protein, but observed no significant differences in weight gain among animals fed the test diets. McBride et al. (1998) found no significant difference in total wet weight or test diameter among small *S. franciscanus* fed 30%, 40%, and 50% protein diets over 10 months (although all groups increased in wet weight). These data suggest that dietary

protein levels less than approximately 10% (dry weight) do generally not support maximal weight gain and increases in test diameter with small and adult sea urchins.

The dry weight of the test was significantly greater (covaried with total dry weight) in those sea urchins fed the 20% protein diet under the conditions of this study. Data suggest that the 9% protein diet provided insufficient protein for maximal test growth. Additionally, data suggest that high protein diets do not support maximal test growth, and that this is potentially related to the energetic cost of protein utilization. Similarly, Hofer (2002) observed a significantly greater test dry weight in adult *L. variegatus* fed a 20% protein diet for 8 weeks. Hammer et al. (2004) reported that test dry weight gain (covaried with test diameter) was significantly reduced in small *L. variegatus* fed a 9% protein diet relative to those fed diets of 15%, 21%, and 33% protein. Test index did not vary among diets containing 30%, 40%, or 50% protein fed for 10 months in small *S. franciscanus* (McBride et al., 1998). These data support the hypothesis that test growth is significantly affected by reduced dietary protein content. The Aristotle's lantern showed little change over the 65-day study period.

The gut wet and dry weight (covaried with total wet and dry weight, respectively) did not vary with dietary protein in adult *L. variegatus* under the conditions of this study. McBride et al. (1998) found no significant differences in wet or dry gut indices with small *S. franciscanus* fed diets containing 30%, 40%, or 50% protein diets after 10 months. Consequently, dietary protein did not affect the size of the gut, but it is not known whether the function of the gut was altered. In contrast, Hammer et al. (2004) observed that small *L. variegatus* fed a 9% protein diet had gut wet weights that were sig-

nificantly less than urchins fed 21% or 33% protein diets. These data suggest that dietary protein level may affect gut development during early growth of *L. variegatus*.

Several authors have reported excellent gonad production in sea urchins fed formulated feeds (de Jong-Westman et al., 1995; Fernandez et al., 1995; Lawrence et al., 1997; Klinger et al., 1997; Grosjean et al., 1998; Watts et al., 1998; McBride et al., 1999; Aikyama et al., 2001; Pearce et al., 2002). In *L. variegatus*, gonad weight gain was dependent on both dietary protein and the period of exposure to the diet. Similarly, Fernandez et al. (1995) reported that a mixed-base diet (29% protein) fed to adult *Paracentrotus lividus* for 1 month produced significantly greater gonad indices than a vegetable-base diet (13% protein) but not an animal-base diet (47% protein). No significant difference in gonad indices among animals fed the different diets as observed after 6 months. The authors remarked that, given enough time, all three diets appear to be adequate for gonad growth in *P. lividus*. Time-dependent changes in nutrient allocation to the gonad are most likely related to the attainment of gut competency. Bishop and Watts (1994) reported that, following starvation, the gut must reach a specific size (competency) before nutrients can be translocated to the gonad of *L. variegatus*.

Hofer (2002) demonstrated a significant increase in gonad wet and dry weight (covaried by total wet and dry weight, respectively) in adult *L. variegatus* fed the same 20% protein diet used in the current study for 8 weeks. Pearce et al. (2002) found no significant differences in gonad index with adult *S. droebachiensis* fed diets containing 19%, 24%, and 29% protein. Hammer et al. (2004) observed that small *L. variegatus* fed a 9% protein diet (of similar composition to the present study) had gonad wet and dry weights that were significantly smaller (covaried by test diameter) than urchins fed 15, 21 or 33%

protein diets. McBride et al. (1998) found no significant differences in gonad index with small *S. droebachiensis* fed diets containing 30%, 40%, or 50% protein diets for 10 months. Additionally, Akiyama et al. (2001) reported no significant differences in gonad index with small *P. depressus* fed diets containing 10%, 21%, 31%, 41%, or 51% protein for 8 weeks. Klinger et al. (1996) observed that formulated feeds fed to sea urchins for a period of 2 to 4 months were capable of producing maximal gonad production and that relative increases in gonad production probably reflect the sea urchin's nutritional and reproductive state at the onset of feeding.

Consumption of a moderate (20%) protein diet supports high specific growth rates, dry matter production, and production efficiency. Hofer (2002) reported a SGR of 0.61% body weight gain per day and a production efficiency of 23% for adult *L. variegatus* fed a 20% protein. Wallace (2001) reported SGRs for small, rapidly growing *L. variegatus* of a maximal 3-5% body weight per day that decreased over time as sea urchins approached adult size. Hammer et al. (2004) reported that small *L. variegatus* fed a 9% protein diet had significantly lower production efficiency than urchins fed 15%, 21%, or 33% protein diets. McBride et al. (1998) reported significantly lower production efficiency for small *S. franciscanus* fed a 30% protein diet compared to urchins fed 40 or 50% protein diets. Akiyama et al. (2001) found that feed efficiency (wet weight production) for small *P. depressus* as higher when fed > 21% protein but less than 51% protein. Fernandez and Boudouresque (1998) reported gross assimilation efficiencies were directly related to dietary protein content in small *P. lividus*.

Overall, sea urchins fed the 9% and 20% protein diets utilized dietary protein more efficiently than those fed the 31% protein diet under our experimental conditions. These

data suggest that excess protein is used as an alternative energy source. Akiyama et al. (1997) reported similar ranges of protein efficiency ratios in small *P. depressus* fed five different diets.

Each of the diets used in this study were adequate for gonad production if given enough time. Low protein intake may have limited the development of those metabolic processes associated with digestion, absorption and/or assimilation, resulting in limited gonad production in the first 32 days of the study. With time, sufficient development of those processes resulted in the compensatory allocation of nutrients to the gonads. Low protein diets can also limit the efficiency of gonad production. The higher gonad production efficiencies reported by Hofer (2002) for adult *L. variegatus* were most likely the result of differences in the nutrition condition of the sea urchins at the beginning of the respective studies. Hammer et al. (2004) reported that small *L. variegatus* fed a 9% protein diet (similar composition to the current study) had significantly lower gonad production efficiency compared to urchins fed 15%, 21%, or 33% protein diets. Those values for small *L. variegatus* were lower than values observed for large *L. variegatus* in the present study. Allocation of energy to gonad production in small sea urchins may be in conflict with allocation of energy to somatic non-gonadal growth (Lawrence, 2000).

This study has demonstrated that the 20% and 31% protein diets are superior to the 9% protein diet with respect to consumption, survival, specific growth rate, estimated dry matter production, production efficiency, and gonad production efficiency under our experimental conditions. The 9% and 20% protein diets outperformed the 31% protein diet with respect to protein efficiency. The present study also indicates that a moderate dietary protein level (20% to 40% dry weight, depending on the species) is utilized most ef-

ficiently by sea urchins. As protein is typically one of the most expensive components of formulated feeds, these data suggest that the 20% protein diet would seem to the most cost-effective and efficient diet for adult *L. variegatus* cultured under similar conditions.

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References

Akiyama, T., Unuma, T., Yamamoto, T., Furuita, H., Konishi, K., 1997. An evaluation of amino acid sources and binders in semipurified diet for red sea urchin *Pseudocentrotus depressus*. Fisheries Sci. 63, 881-886

Akiyama, T., Unuma, T., Yamamota, T., 2001. Optimum protein level in a purified diet for young red sea urchin *Pseudocentrotus depressus*. Fisheries Sci. 67, 361-363

Andrews, N.L., Agatsuma, Y., Ballesteros, E., Bazhin, A.G., Creaser, E.P., Barnes, D.K.A., Botsford, L.W., Bradbury, A., Campbell, A., Dixon, J.D., Einarsson, S., Gerring, P.K., Hebert, K., Hunter, M., Hur, S.B., Johnson, C.R., Juinio-Menez, M.A., Kalviss, P., Miller, R.J., Moreno, C.A., Palleiro, J.S., Rivas, D., Robinson, S.M.L., Schroeter, S.C., Steneck, R.S., Vadas, R.L., Woodby, D.A., and Xiaoqi, Z., 2002. Status and management of world sea urchin fisheries. Ocean. Mar. Bio. 40, 343-425

Beddingfield, S. D., McClintock, J. B., 1998. Differential survivorship, reproduction, growth and nutrient allocation in the regular echinoid *L. variegatus* (Lamarck) fed natural diets. J. Exp. Mar. Bio. Ecol. 226, 195-215

Beddingfield, S. D., McClintock, J. B., 1999. Food resource utilization in the sea urchin *Lytechinus variegatus* in contrasting shallow water microhabitats of Saint Joseph Bay, Florida. Gulf of Mexico Sci. 1, 27-34

Bishop, C. D., Watts, S. A., 1992. Biochemical and morphometric study of growth in the stomach and intestine of the echinoid *Lytechinus variegatus* (Echinodermata). Mar. Biol. 114, 459-467

Bishop, C. D. Watts, S. A., 1994. Two stage recovery of gametogenic activity following starvation in *Lytechinus variegatus* Lamarck (Echinodermata: Echinoidea). J. Exp. Mar. Biol. Ecol. 177, 27-36

Brown, B. A., McClintock, J. B., 1990. Effects of quality of diet on feeding rates, fecal production and gravimetric absorption efficiencies of the tropical sea urchin *Eucidaris tribuloides.* Northeast Gulf Sci. 11, 87

de Jong-Westman, M., March, B.E., Carefoot, T. H., 1995. The effect of different nutrient formulations in artificial diets on gonad growth in the sea urchin *Strongylocentrotus droebachiensis*. Can. J. of Zool. 73, 1495-1502

Ebert, T. A., 1975. Growth and mortality of post-larval echinoids. Am. Zool. 15, 755-775

Fernandez, C., Boudouresque, C., 1998. Evaluating artificial diets for small *Paracentrotus lividus* (Echinodermata: Echinoidea). In: Mooi, R., Telford, M. (Eds.) Echinoderms, San Francisco. Balkema, Rotterdam, pp. 651-656

Fernandez, C., Dombrowski, E., Caltagirone, A., 1995. Gonadic growth of adult sea urchins, *Paracentrotus lividus* (Echinodermata: Echinoidea) in rearing: The effect of different diet types. In: Emson, R., Smith, A., Campbell, A. (Eds.) Echinoderm Research. Balkema, Rotterdam, pp. 269-275

Grosjean, P., Spirlet, C., Gosselin, P., Vaitilingon, D., Jangoux, M., 1998. Land-based, closed-cycle echiniculture of *Paracentrotus lividus* (Lamarck) (Echinoidea: Echinodermata): A long-term experiment at a pilot scale. J. Shellfish Res. 17, 1523-1531

Hagen, N. T., 1996. Echinoculture: from fishery enhancement to closed cycle cultivation. World Aquacult. 27, 6-19

Hammer, B. W., Hammer, H. S., Watts, S. A., Desmond, R. A., Lawrence, J. M., Lawrence, A. L., 2004. The effects of dietary protein concentration on feeding and growth of small *Lytechinus variegatus* (Echinodermata: Echinoidea). J. Mar. Biol. 145, 1143-1157.

Hofer, S. C., 2002. The effect of temperature on feeding and growth characteristics of the sea urchin *Lytechinus variegatus* (Echinodermata: Echinoidea). M. S. Thesis, University of Alabama at Birmingham, Birmingham, AL.

Keesing, J. K., Hall, K. C., 1998. Review of harvests and status of word sea urchin fisheries points to opportunities for aquaculture. J. Shellfish Res. 17, 1597-1604

Klinger, T. S., 1982. Feeding rates of *Lytechinus variegatus* Lamarck (Echinodermata: Echinoidea) on differing physiognomies of an artificial food of uniform composition. In: Lawrence, J.M. (Ed.) Echinoderms: Proceedings of the International Conference, Tampa Bay. Balkema, Rotterdam, pp. 29-32

Klinger, T. S., Watts, S. A., Forcucci, D., 1988. Effect of short-term feeding and starvation on storage and synthetic capacities of gut tissues of *Lytechinus variegatus* (Lamarck) (Echinodermata: Echinoidea). J. Exp. Mar. Biol. Ecol. 117, 187-195

Klinger, T. S., Lawrence, J. M., Lawrence, A. L., 1996. Absorption and assimilation of prepared feeds by echinoids. In: Mooi, R. and Telford, M. (Eds.). Echinoderms: San Francisco. Balkema, Rotterdam. p. 713-721

Klinger, T. S., Lawrence, J. M., Lawrence, A. L., 1997. Gonad and somatic production of *Strongylocentrotus droebachiensis* fed manufactured feeds. Bull. Aquacult. Assoc. Can. 1, 35-37

Lawrence, J. M., 2001. Edible sea urchins. In: Lawrence, J. M. (Ed.) Edible Sea Urchins Biology and Ecology. Elsevier Science B. V., Amsterdam. pp 1-4

Lawrence, J.M., 2000. Conflict between somatic and gonadal growth in sea urchins: a review. The workshop on the coordination of green sea urchin research in Atlantic Canada, Moncton, New Brunswick. http://crdpm.umcs.ca/OURSIN.

Lawrence, J. M., Olave, S., Otaiza, R., Lawrence, A. L., Bustos, E., 1997. Enhancement of gonad production in the sea urchin *Loxechinus albus* in Chile fed extruded feeds. J. World Aquacult. Soci. 28, 91-96

Lowe, E. F., Lawrence, J. M., 1976. Absorption efficiencies of *Lytechinus variegatus* (Lamarck) (Echinodermata: Echinoidea) for selected marine plants. J. Exp. Mar. Biol. Ecol. 21, 223-234

McBride, S. C., Lawrence, J. M., Lawrence, A. L., Mulligan, T. J., 1998. The effect of protein concentration in prepared feeds on growth, feeding rate, total organic absorption, and gross assimilation efficiency of the sea urchin *Strongylocentrotus fransiscanus*. J. Shellfish Res. 17, 1563-1570

McBride, S. C., Lawrence, J. M., Lawrence, A. L., Mulligan, T. J., 1999. Ingestion, absorption, and gonad production of adult *Strongylocentrotus franciscanus* fed different rations of a prepared diet. J. World Aquacult. Soc. 30, 364-370

Miller, R. J., Mann, K. H., 1973. Ecological energetics of the seaweed zone in a marine bay on the Atlantic coast of Canada. III. Energy transformations by sea urchins. Mar. Biol. 18, 99-114

Moore, H. B., Jutare, T., Bauer, J. C., Jones, J. A., 1963. The biology of *Lytechinus variegatus*. Bull. Mar. Sci. Gulf and Caribbean 13, 23-53

Pearce, C. M., Daggett, T.L., Robinson, S.M.C., 2002. Effect of protein source ratio and protein concentration in prepared diets on gonad yield and quality of the green sea urchin, *Strongylocentrotus droebachiensis*. Aquaculture 214, 307-332.

Robinson, S.M., 2004. The evolving role of aquaculture in the global production of sea urchins. In: Lawrence, J, M., Guzmán, O. (Eds.). Sea Urchins: Fisheries and Ecology. DEStech Publications, Lancaster, Pennsylvania. pp. 343-357.

Sloan, N.A., 1985. Echinoderm fisheries of the world: A review. In: Keegan, B. F., O'Conner, B. D. S. (Eds.). Echinodermata, Proceedings of the Fifth International Echinoderm Conference, Galway. A.A. Balkema, Rotterdam. pp. 109-124

Wallace, B. D., 2001. The effects of dietary protein concentration on feeding and growth of small *Lytechinus variegatus* (Echinodermata: Echinoidea). M. S. Thesis, University of Alabama at Birmingham, Birmingham, AL.

Watts, S. A., Boettger, S.A., McClintock, J. B., Lawrence, J. M., 1998. Gonad production in the sea urchin *Lytechinus variegatus* (Lamarck) fed prepared diets. J. Shellfish Res. 17, 1591-1595

Watts, S. A., McClintock, J. B., Lawrence, J. B., 2001. The ecology of *Lytechinus variegatus*. In: Lawrence, J. M. (Ed.) Edible Sea Urchins Biology and Ecology. Elsevier Science B. V., Amsterdam. pp. 375-393.

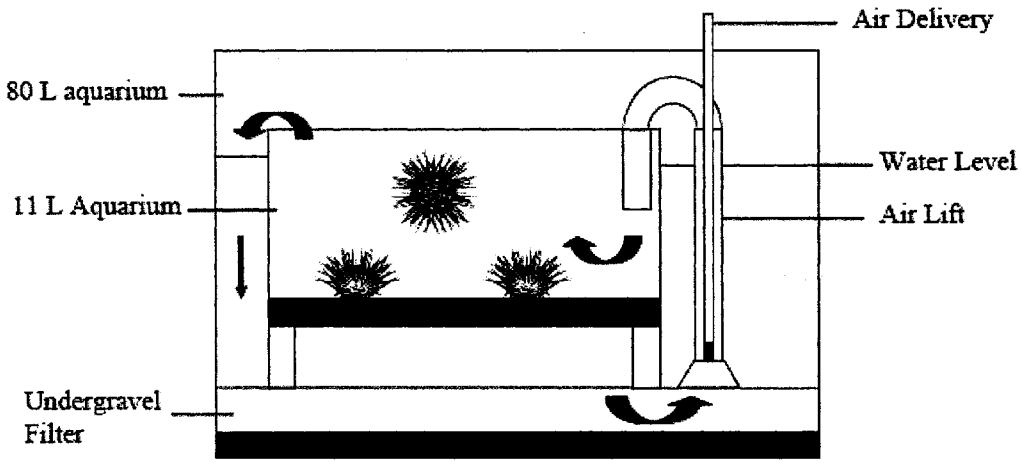

Fig.1. Schematic illustration of tank-within-a-tank system. An 11-l aquarium was elevated and placed inside a larger 80-l aquarium. Biofiltration occurred in the under-gravel dolomite filter. An air-lift circulated water through the under-gravel filter and delivered aerated seawater into the small aquarium. Overflow water returned to the larger aquarium and was then recirculated through the filter. Adapted from Wallace (2001).

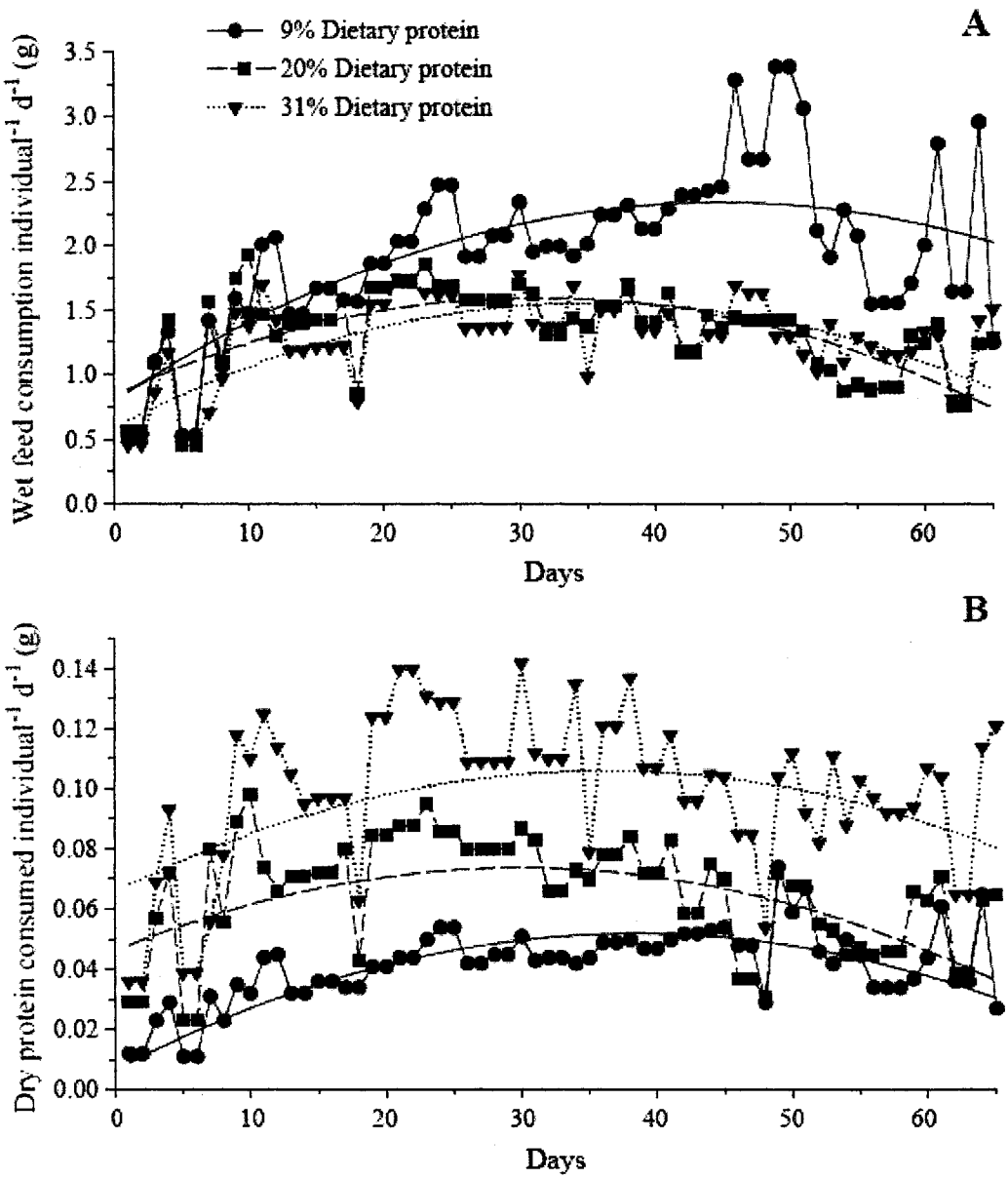

Fig. 2. *Lytechinus variegatus*. (A) Average consumption (g wet weight) per individual per day of sea urchins fed 9%, 21%, or 31% protein diets. Values were determined daily by dividing the total food consumed per tank by the number of surviving sea urchins (n = 36 sea urchins). Predicted values from mixed models are shown as arched lines. (B) Average protein consumption (g dry weight) per individual per day of sea urchins fed the 9%, 21%, or 31% protein diets. Values were determined daily by multiplying the average consumption (g dry weight) per individual per day by the percent dry matter protein in the food (n = 36 sea urchins). Predicted values from mixed models are shown as arched lines.

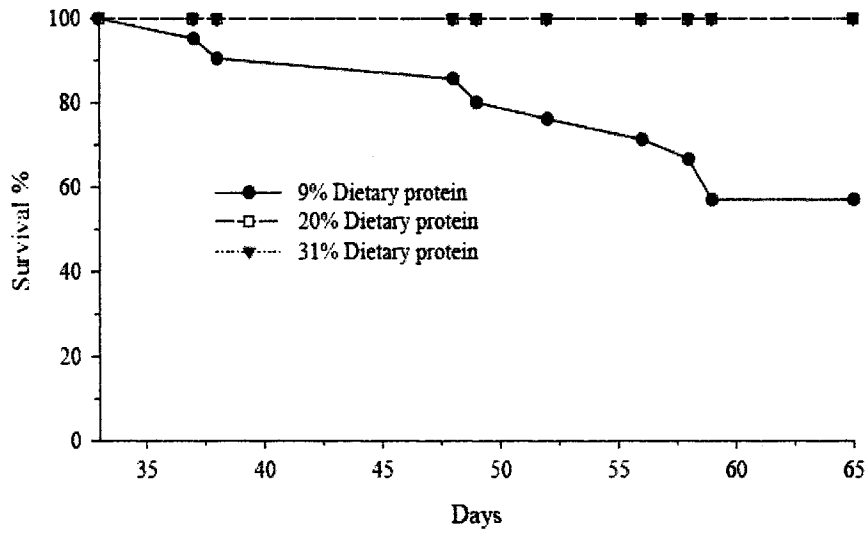

Fig. 3. *Lytechinus variegatus*. Survival of sea urchins fed one of three diets varying in protein concentration from 9% to 31%. Values represent the percentage of surviving individuals ($n = 36$ sea urchins). Survival was 100% in those fed 20% or 31% protein diets.

THE EFFECT OF DIETARY PROTEIN AND CARBOHYDRATE CONCENTRATION ON THE BIOCHEMICAL COMPOSITION AND GAMETOGENIC CONDITION OF THE SEA URCHIN *LYTECHINUS VARIEGATUS*

by,

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Abstract

Previously starved urchins, *Lytechinus variegatus*, (36.0 ± 0.8 (SE) mm test diameter) were held in replicated (3) 10-L aquaria with artificial seawater at 22 ± 2° C and 32 ‰ salinity and fed three diet treatments. Urchins were fed diets containing 9:35, 20:23 or 31:12 dry protein: % dry carbohydrate (P:C) *ad libitum* for a 65-day period. Gonads from urchins fed the 9:35 P:C diet had similar organic, lower ash, and lower water content than urchins fed the 31:12 P:C protein diet. Water content varied with both diet and nutritional history; consequently, water content may have limited value as a predictor of gonad nutritional status. Protein and carbohydrate concentrations in the gonad were directly related to the dietary composition of these nutrients; gonad lipids did not vary with diet. Excess carbohydrates are frequently stored as fats in fish and mammals but this does not appear to be the case in *L. variegatus*. Test carbohydrate storage and gut protein storage also reflected dietary composition. Image analysis of ovaries indicated decreased nutritive phagocyte volume, increased germinal epithelium volume and larger oocyte diameters in urchins fed high protein, low carbohydrate diets. Analysis of testes also indicated decreased nutritive phagocyte volume and increased gamete volume with urchins fed high protein, low carbohydrate diets, but differences among treatments were less obvious than in ovaries. This study suggests that high protein, low carbohydrate diets promote gamete growth and development. In addition, the biochemical and gametic composition of gonads can be altered by manipulating dietary composition. This could affect the quality and value of sea urchin roe for human consumption.

1. Introduction

Protein is an important dietary macro-nutrient that provides essential amino acids for maintenance, growth and reproduction in all animals (Morris, 1991). In addition, the oxidation of dietary carbohydrates is the primary source of energy for all herbivorous and many omnivorous animals and often comprises the bulk of the dry matter consumed by mature organisms (Morris, 1991). In sea urchins, both proteins and carbohydrates are major components of the gonads and as such provide important raw materials for reproduction (Lawrence et al., 2001; Marsh and Watts, 2001; Montero-Torreiro and Garcia-Martinez, 2003).

Previous studies have suggested that growth and development in sea urchins may be more dependent on the amount of protein in the diet than the amount of energy (Lowe and Lawrence, 1976). The effect of dietary protein on sea urchin gonad production has been reported in several studies (Fernandez et al., 1995; de Jong Westman et al., 1995; McBride et al., 1998; Akiyama et al., 2001; Pearce et al., 2002; Hammer et al., 2004). The effects of dietary protein and carbohydrate on biochemical composition and gametic condition of the gonad are less frequently studied but may directly influence the reproductive success of adults and their larvae in natural environments. These characteristics can also influence gonad color, taste, firmness and texture and, consequently, determine the quality and value of sea urchin roe in culture (Unuma, 2002; Pearce et al., 2002).

Lytechinus variegatus is an important species in many shallow seagrass beds from the coast of North Carolina to Brazil (reviewed in Watts et al., 2001). Overpopulation of *L. variegatus* can result in over-grazing and greatly reduced seagrass coverage (reviewed in Valentine and Heck, 1999). Though *L. variegatus* is an important grazer in seagrass

ecosystems, it feeds on a wide variety of foods including *Thalassia testudinum*, *Syringodium filiforme*, *Cymodocea manatorum*, *Haladule wrightii*, filamentous algae, sea grass epibionts, drift material, sand, shells, the mussel *Modiolus americanus*, and various gastropods (reviewed in Watts et al., 2001). *L. variegatus* spawns mainly in spring or summer when temperatures are rising toward annual highs (Beddingfield and McClintock, 2000).

Lytechinus variegatus can be easily and successfully reared in the laboratory and is frequently used as a model for nutritional research (Klinger, 1982; Klinger et al., 1988; Bishop and Watts, 1992; Watts et al., 1998; Beddingfield and McClintock, 1998; Hofer 2002; Hammer et al., 2004). This study examines effects of dietary protein and carbohydrate on the biochemical composition and gametic condition of the gonads and somatic tissues in adult *L. variegatus*.

2. Materials and Methods

2.1 *Collection and maintenance of sea urchins*

Lytechinus variegatus (35-45 mm test diameter, $n = 150$) were collected from Saint Joseph Bay, Florida (30.0°N, 85.5°W) in December and transported to the University of Alabama at Birmingham (UAB). Sea urchins were maintained on minimal levels of a formulated diet (Wenger Manufacturing, Inc., Sabetha, KS, USA) with one ration every two weeks for approximately 4 months to reduce stored nutrients. The sea urchins were divided randomly into nine 11-L aquaria ($n = 12$ sea urchins per aquarium). Daily feeding was initiated and feeding parameters were recorded beginning five days after the first feeding. Any individuals that died during the first 2 weeks of the study were replaced

with sea urchins from the same collection. Sea urchins were maintained in one 11-L aquaria submerged within one 80-L aquaria of recirculating artificial seawater (32‰ salinity and 21-23 °C, Instant Ocean Sea Salts, Aquatic Ecosystems, Inc., Apopka, FL), as described in Wallace (2001) (Fig. 1). Uneaten food and fecal material were removed every two days with a partial (less than 10%) water exchange. The sea urchins were maintained on a 12h light: 12h dark photo-period (over-head fluorescent lighting) and water quality was maintained within optimal parameters.

2.2 *Dietary treatments*

Three semi-purified diets (Table 1) that varied in protein: carbohydrate concentration were formulated. As the dietary protein level increased there was a concomitant decrease in dietary carbohydrate level. These diets were constituted into a pellet by adding 10 g of dry formulated diet 14%, 32%, or 50 % crude protein (56%, 38%, or 19% carbohydrate, respectively) to a solution of heated seawater (100 ml, 40‰, 60-70 °C) containing 2 g of agar binder. The wet slurry was allowed to solidify and was cut into small pellets of ca. 1 cm^3. The resulting dry matter protein: carbohydrate concentration of the diet composition following dehydration to constant dry weight (constituted diet) was calculated to be 9:35, 20:23, or 31:12 P:C (% protein: % carbohydrate) (Table 2). The resulting wet matter protein concentration fed the urchins was calculated to be 1.3%, 2.9%, or 4.5% (5.3%, 3.5%, or 1.7 % carbohydrate, respectively). Sea urchins were fed *ad libitum* one of the three diets daily for 65 days (n = 12 individuals per aquarium, three aquaria per diet treatment). The wet weight of the food fed was weighed to the nearest milligram. Prior to the next feeding, uneaten food was removed (by siphon), placed on a paper towel

Table 1
Composition of the Texas A&M formulation used to produce diets varying in protein concentration

	% Dry weight		
% Protein: Carbohydrate Component	14: 56	32: 38	50: 19
Wheat starch	36.94	18.47	0.00
Algae kelp	30.00	28.50	26.99
Antarctic Krill	15.00	15.00	15.00
Cellulose	4.00	4.00	4.00
Phospholipid	4.00	4.00	4.00
Fish oil (menhaden)	2.25	2.13	2.00
Vitamin premix[a]	1.50	1.50	1.50
Casein	1.10	9.50	17.90
$CaH(PO)_4$	1.00	1.00	1.00
Soy	1.00	10.50	20.00
Cholesterol	1.00	1.00	1.00
Wheat gluten	1.00	3.20	5.40
Mineral premix[b]	1.00	1.00	1.00
Vitamin C	0.10	0.10	0.10
$ZnCO_3$	0.04	0.04	0.04
$CuCl*2H_2O$	0.03	0.03	0.03
$MnSO_4*H_2O$	0.03	0.03	0.03
Betacarotene	0.01	0.01	0.01

Final protein concentrations (after addition of agar binder) were calculated to be 9%, 20%, or 31 % protein dry weight, respectively.

[a] Vitamin premix contains on a per kg basis: vitamin A (retinol) 650,000 IU, vitamin D (cholecalciferol) 500,000 IU, vitamin E (tocopherol) 25,000 mg, vitamin K (menadione) 2,500 mg, vitamin B1 (thiamine) 5,000 mg, vitamin B2 (riboflavin) 10,000 mg, vitamin B6 (pyridoxine) 20,000 mg, niacin 12,500 mg, pantothenic acid 12,500 mg, biotin 187 mg, folic acid 5,000 mg, vitamin B12 (cyanocobalamine) 37.5 mg.

[b] Mineral premix contains: calcium 14.73%, phosphorous 11.39%, sodium 2.91%, potassium 10.76%, magnesium 11.45%, iron 994 ppm, zinc 876 ppm, 1,678 ppm, copper 667 ppm, selenium 4 ppm, chloride 101 ppm, iodine 8 ppm.

Table 2
Proximate analysis and calculated mineral composition of the formulations used to produce diets varying in protein and carbohydrate concentration

Nutrient	Low Protein (9%) High Carbohydrate (35%)		Medium Protein (20%) Medium Carbohydrate (23%)		High Protein (31%) Low Carbohydrate (12%)	
	Calculated nutrient (%)[a]	Calculated constituted nutrient (%)[b]	Calculated nutrient (%)[a]	Calculated constituted nutrient (%)[b]	Calculated nutrient (%)[a]	Calculated constituted nutrient (%)[b]
Crude Protein	13.97	8.73	32.09	20.1	50.20	31.38
Moisture	4.85	-	5.29	-	5.73	-
Crude Fiber	6.72	4.20	6.66	4.16	6.59	4.12
Total Ash	8.35	30.22	8.66	30.41	8.96	30.60
Crude Fat	9.81	6.13	9.82	6.14	9.83	6.14
Carbohydrate	56.38	35.24	37.50	23.44	18.62	11.64
Energy[c]	3.95	2.9	4.42	3.01	4.49	3.44

Final protein and carbohydrate concentrations (after addition of agar binder) were calculated to vary from 9:35 to 31:12 % protein: % carbohydrate dry weight.

[a:] Values based on content of the formulated diet prior to incorporation into the food pellet.

[b:] Constituted feed prepared from 10 g dry formulation, 2 g agar binder, and 100 ml salt water (40‰). Values represent calculated composition following dehydration to constant dry weight.

[c:] Energy values in kilocalories per gram calculated by bomb calorimetry.

to remove excess moisture, and weighed. The feed changes less than 2% in water content while in the aquarium (unpub. data).

2.3 *Dissection and composition*

At the beginning of the study, a random sub-sample of sea urchins (n = 15) was removed from the initial population for dissection. Additionally, four sea urchins were removed at random from each replicate aquarium at days 32 and 65 for dissection (n = 12 per diet). Sea urchins from tanks within each treatment were combined (n = 12). Sea ur-

chins were measured at two perpendicular points across the ambitus (test diameter) using calipers, weighed to the nearest mg (wet weight), and dissected. Sea urchins were cut outside the peristomial membrane. The test with spines, Aristotle's lantern, gut, and gonads were separated. The gut (esophagus, stomach, and intestine) was rinsed in a finger bowl to remove food. Each of the components were blotted dry with a paper towel to remove excess water and weighed to the nearest mg (wet weight). The sex was determined by examining a gonad squash for the presence of spermatocytes or oocytes using a compound light microscope. A sub-sample of the gonad was placed in Bouin's fixative for histological analysis. Components were placed in a 60 °C oven, dried for several days to constant weight, and reweighed to the nearest mg (dry weight).

The moisture content (%) of each component (test, lantern, gut, gonad) was calculated as:

$$\frac{\text{component wet weight} - \text{component dry weight}}{\text{component wet weight}} \times 100$$

Dry body components were ground to a fine powder using a Wiley-Mill. Organic and ash content were measured by placing a known weight of powder (weighed to the nearest 0.1 mg) into a porcelain crucible and incinerating it in a muffle furnace at 500 °C for 4h. After incineration, the crucibles were cooled to room temperature in a desiccator and the ash was weighed to the nearest 0.1 mg.

The total organic material for each component was calculated as:

$$\frac{\text{sample dry weight} - \text{ash weight}}{\text{sample dry weight}} \times \text{total component dry weight}$$

The total ash for each component was calculated as:

$$\frac{\text{ash weight}}{\text{sample dry weight}} \times \text{total component dry weight}$$

Finely ground gonad, test, and gut were analyzed for protein (Lowry et al., 1951), carbohydrate (Dubois et al., 1956) and lipid (Freeman et al., 1957) when adequate material was available. Insoluble component was calculated by subtraction.

2.4 *Histology and image analysis*

Gonad samples were preserved in Bouin's fixative from each sea urchin dissected (when adequate material was available) and sectioned for histology, using hematoxylin and eosin as the primary stain. Slides were examined using a Leitz diaplan binocular compound microscope connected to a DAGE-MTI, digital signal processor (DPA-200) image analyzer (DAGE-MTI Inc., Michigan City, IN). An image analysis program, Optimas 6.2 (Media Cybernetics, Silver Springs, MD), was used to calculate the percent volume of germinal epithelium, nutritive phagocytes, and lumen with unattached oocytes in five acini of a single ovary in each female. In each section, the longest diameters of 50 oocytes with visible nuclei were measured to the nearest 0.1 μm. The percent volume germinal epithelium, percent volume nutritive phagocytes, and mean oocyte diameters were compared among diets. Another image analysis program, Image Tool 2.0 (Department of Dental Diagnostic Science, University of Texas Health Science Center, San Antonio, TX) was used to measure the percent volume of male gametes and nutritive phagocytes in five acini of a single testis in each male. The percent volume of nutritive phagocytes and percent volume of gametes were compared among diet treatments.

2.5 *Statistics*

All statistical comparisons were completed using the SYSTAT 9 software package (Systat Software Inc., Point Richmond, CA). If data were normal and were homoscedastic, parametric tests including ANOVA and ANCOVA were completed. When significant differences were determined, a Tukey's test for pairwise group comparisons was used. A P value of < 0.05 was determined statistically significant for all parametric tests. If data were non-normal or heteroscedastic, data transformations were attempted to make data normal and/or homoscedastic for ANOVA or ANCOVA analysis. When ANCOVA analysis was completed, duplicate tables indicate both actual means and least square means. If data transformations were not successful, non-parametric tests (Kruskal Wallis, Mann Whitney U, or Kolmogorov-Smirnov Two Way Tests) were employed. To maintain an overall acceptance criteria of $\alpha = 0.05$ during multiple comparisons, a Bonferroni's adjustment was adopted. Urchins from tanks within a dietary treatment were pooled. An ANOVA on total wet weight of urchins for separate tanks within dietary treatments was performed at day 65 to rule out the possibility of tank effects within dietary treatments.

3. Results

Observations from the initial dissection prior to the study, indicated urchins were nutritionally compromised with greatly reduced gut and gonad tissues. An ANOVA of total wet weight for urchins within treatments at day 65 indicated no differences among tanks within each dietary treatment (no tank effects). Initial dissection and growth parameters pertinent to this study, including total weight, test diameter, organ weights (Aris-

totle's lantern, test, gut and gonad) and production have been reported previously (Hammer et al., in press).

3.1 *Proximate composition of the gonad*

The organic content of the gonad was significantly greater at day 32 for sea urchins fed the 20:23 P:C diet ($F = 6.37$, $df = 2$, $P = 0.005$) than for sea urchins fed the other two diets (Tables 3A and 3B). No significant difference was apparent among the diets at day 65. Sea urchins fed the 9:35 P:C diet at day 65 had significantly lower gonad ash content than sea urchins fed the 31:12 P:C diet ($F = 3.77$, $df = 2$, $P = 0.035$)(Tables 3A and 3B). The total soluble protein of the gonad varied directly with dietary protein at day 65 ($F = 57.56$, $df = 2$, $P < 0.001$) (Tables 3A and 3B). The total carbohydrate in the gonad at day 65 varied directly with dietary carbohydrate (indirectly with dietary protein) ($F = 33.79$, $df = 2$, $P < 0.03$) (Tables 3A and 3B). The total lipid in the gonad varied with diet at day 32, with sea urchins fed the 9:35 and 31:12 P:C diets having significantly more total lipid than the 20:23 diet ($F = 6.92$, $df = 2$, $P < 0.017$) (Tables 3A and 3B). At day 65 the total lipid did not vary with diet (Tables 3A and 3B). The total insoluble component was significantly greater in sea urchins fed the 31:12 P:C diet than in the other diets at day 32 ($F = 19.51$, $df = 2$, $P < 0.001$) (Tables 3A and 3B). At day 65 the total insoluble component of the gonad did not vary significantly with diet.

3.2 *Proximate composition of somatic components*

The total organic content of the test was similar among the diets at day 65 (Tables 4A and 4B). The total ash content of the test was similar among the diets at day 32, but

Table 3
Lytechinus variegatus: (A) gonad composition actual means ± SE at day 0, 32 and 65 for sea urchins fed 9:35, 20:23 or 31:12 % protein: % carbohydrate diets; (B) gonad composition least square means ± SE at day 32 and 65 for sea urchins fed 9:35, 20:23 or 31:12 % protein: % carbohydrate diets.

Tissue	Parameter	Day 0	Dietary % Protein: % Carbohydrate	Day 32		Day 65	
A							
Gonad	Organic Material (mg)	-	9:35	303.0 ± 49.3		1030.8 ± 122.1	
			20:23	556.1 ± 52.2		1131.8 ± 114.1	
			31:12	381.6 ± 49.2		1022.6 ± 84.5	
	Ash (mg)	-	9:35	19.0 ± 2.8		53.7 ± 6.4	
			20:23	32.8 ± 3.1		70.9 ± 8.2	
			31:12	27.0 ± 4.3		67.5 ± 7.5	
	Protein (mg)	55.2 ± 0.7	9:35	62.9 ± 12.2		278.3 ± 34.1	
			20:23	169.0 ± 15.5		377.1 ± 41.3	
			31:12	152.0 ± 22.2		437.4 ± 26.4	
	Carbohydrate (mg)	23.4 ± 1.6	9:35	97.3 ± 21.8		522.9 ± 74.9	
			20:23	234.3 ± 23.0		482.5 ± 53.4	
			31:12	74.4 ± 9.0		267.1 ± 32.0	
	Lipid (mg)	-	9:35	73.6 ± 14.0		235.3 ± 25.1	
			20:23	116.4 ± 11.5		224.0 ± 21.9	
			31:12	91.5 ± 12.4		239.6 ± 19.7	
	Insoluble Component (mg)	-	9:35	38.0 ± 6.5		60.0 ± 17.5	
			20:23	71.2 ± 9.1		111.3 ± 21.8	
			31:12	77.3 ± 10.2		124.1 ± 18.8	
B	Organic Material (mg)		9:35	303.0 ± 56.2	B	1030.8 ± 117.6	A
			20:23	556.1 ± 48.7	A	1131.8 ± 101.9	A
			31:12	381.6 ± 48.7	B	1022.6 ± 101.9	A
	Ash (mg)		9:35	26.5 ± 2.1	A	55.8 ± 4.0	B
			20:23	24.5 ± 1.9	A	66.7 ± 3.5	AB
			31:12	29.6 ± 1.7	A	70.1 ± 3.5	A
	Protein (mg)[a]		9:35	-		258.0 ± 15.2	C
			20:23	-		348.4 ± 13.4	B
			31:12	-		490.0 ± 15.0	A
	Carbohydrate (mg)[a]		9:35	-		2.80 ± 0.04^{L}	A
			20:23	-		2.65 ± 0.03^{L}	B

Table 3 (Continued)

Tissue Parameter	Day 0	Dietary % Protein: % Carbohydrate	Day 32		Day 65	
		31:12	-		2.35 ± 0.04^{L}	C
Lipid (mg)		9:35	107.6 ± 5.0	A	237.5 ± 10.5	A
		20:23	80.2 ± 4.8	B	219.7 ± 9.9	A
		31:12	99.3 ± 4.2	A	241.5 ± 9.1	A
Insoluble Component (mg)		9:35	7.27 ± 0.41^{S}	B	60.4 ± 21.7	A
		20:23	6.58 ± 0.38^{S}	B	110.1 ± 18.9	A
		31:12	9.49 ± 0.33^{S}	A	125.1 ± 20.6	A

[a] The p value was less than 0.05 for the relationship between the parameter tested and the covariate, therefore ANCOVA was not performed at day 32.

[S] indicates that the data were square root transformed and [L] indicates that the data were natural log transformed.

Letters indicate significant ($P < 0.05$) differences among diets.

was significantly greater in sea urchins fed the 20:23 P:C diet ($F = 6.09$, $df = 2$, $P < 0.03$) than in those fed the other two diets at day 65 (Tables 4A and 4B). The total protein content of the test did not differ significantly among diets at day 65, but the total carbohydrate content of the test was significantly greater in sea urchins fed the 9:35 P:C diet than in those fed the other two diets ($F = 7.45$, $df = 2$, $P < 0.04$ Tables 4A and 4B). The organic and ash content of the Aristotle's lantern were not significantly different with respect to dietary protein concentrations (Tables 4A and 4B). The total protein content of the gut was significantly lower in those urchins fed the 9:35 P:C diet than in those fed 20:23 or 31:12 P:C diets ($F = 16.43$, $df = 2$, $P < 0.001$; Tables 4A and 4B). The total carbohydrate content of the gut did not differ significantly with dietary protein and carbohydrate concentrations (Tables 4A and 4B).

The water content of the gonad decreased significantly with time in all diet groups at day 32 and 65 (Kolmogorov-Smirnov $P < 0.014$; Table 5). At day 65 the gonad water

Table 4

Lytechinus variegatus. (A) actual means ± SE for the composition of somatic components analyzed at day 0, 32, and 65 for sea urchins fed 9:35, 20:23 or 31:12 % protein: % carbohydrate diets; (B) least square means ± SE for the composition of somatic components analyzed at day 32 and 65 for sea urchins fed 9:35, 20:23 or 31:12 % protein: % carbohydrate diets

Tissue	Parameter	Day 0	Dietary % Protein: % Carbohydrate	Day 32	Day 65
A					
Test	Total Organic Material (mg)	337.3 ± 29.4	9:35	686.8 ± 56.8	780.4 ± 41.0
			20:23	673.3 ± 38.7	921.8 ± 79.7
			31:12	679.9 ± 55.31	732.8 ± 50.1
	Total Ash Content (g)	4.4 ± 0.27	9:35	5.27 ± 0.33	5.24 ± 0.34
			20:23	4.92 ± 0.30	6.63 ± 0.33
			31:12	4.40 ± 0.22	5.52 ± 0.23
	Total Protein (mg)[a]	43.3 ± 8.4	9:35	-	222.9 ± 31.8
			20:23	-	275.6 ± 24.9
			31:12	-	210.6 ± 27.8
	Total Carbohydrate (mg)[a]	26.8 ± 4.7	9:35	-	145.4 ± 16.4
			20:23	-	127.4 ± 10.0
			31:12	-	119.4 ± 7.8
Aristotle's Lantern	Total Organic Material (mg)	58.2 ± 4.8	9:35	79.8 ± 7.4	71.4 ± 6.1
			20:23	74.1 ± 4.6	81.5 ± 4.9
			31:12	66.7 ± 4.1	75.3 ± 4.4
	Total Ash Content (mg)	456.0 ± 30.2	9:35	534.7 ± 49.8	516.3 ± 36.5
			20:23	537.6 ± 38.0	625.0 ± 36.8
			31:12	497.5 ± 26.9	587.2 ± 25.9
Gut	Total Protein (mg)[a]	7.7 ± 0.1	9:35	-	47.8 ± 6.7
			20:23	-	54.0 ± 4.5
			31:12	-	53.8 ± 2.5
	Total Carbohydrate (mg)[a]	0.81 ± 0.09	9:35	-	6.60 ± 0.94
			20:23	-	5.83 ± 0.51
			31:12	-	4.78 ± 0.26
B					
	Total Organic Material (mg)[b]		9:35	-	781.0 ± 44.5 A
			20:23	-	868.9 ± 53.1 A
			31:12	-	807.3 ± 48.7 A
	Total Ash Content (g)		9:35	5.3 ± 0.29 A	5.2 ± 0.33 B

Table 4 (Continued)

Tissue	Parameter	Day 0	Dietary % Protein: % Carbohydrate	Day 32		Day 65	
			20:23	4.9 ± 0.29	A	6.6 ± 0.29	A
			31:12	4.4 ± 0.29	A	5.5 ± 0.29	B
	Total Protein (mg)[a]		9:35	-		240.2 ± 30.1	A
			20:23	-		251.5 ± 28.6	A
			31:12	-		219.7 ± 25.4	A
	Total Carbohydrate (mg)[a]		9:35	-		160.1 ± 9.6	A
			20:23	-		107.4 ± 8.8	B
			31:12	-		128.4 ± 8.1	B
	Total Organic Material (mg)		9:35	4.29 ± 0.03[L]	A	80.1 ± 2.9	A
			20:23	4.38 ± 0.05[L]	A	75.7 ± 2.5	A
			31:12	4.31 ± 0.03[L]	A	74.6 ± 2.4	A
	Total Ash Content (mg)		9:35	6.24 ± 0.07[L]	A	516.3 ± 36.7	A
			20:23	6.26 ± 0.07[L]	A	625.0 ± 31.8	A
			31:12	6.19 ± 0.07[L]	A	587.2 ± 31.8	A
	Total Protein (mg)[a]		9:35	-		46.6 ± 1.2	B
			20:23	-		53.2 ± 1.0	A
			31:12	-		55.5 ± 1.1	A
	Total Carbohydrate (mg)[a]		9:35	-		2.46 ± 0.10[S]	A
			20:23	-		2.40 ± 0.08[S]	A
			31:12	-		2.21 ± 0.08[S]	A

[a] Parameter not tested at day 32.

[S] indicates that the data were square root transformed and [L] indicates that the data were log transformed.

Letters indicate significant ($P < 0.05$) differences among diets.

Table 5
Lytechinus variegatus: actual means ± SE for the water content of components analyzed at day 0, 32 and 65 for sea urchins fed 9:35, 20:23 or 31:12 % protein: % carbohydrate diets.

Tissue	Day 0	Dietary % Protein: % Carbohydrate	Day 32		Day 65	
Gonad (%)[a]	83.8 ± 1.99	9:35	75.4 ± 1.19	B	68.6 ± 0.52	B*
		20:23	75.5 ± 0.67	B	72.4 ± 0.92	A*
		31:12	80.1 ± 0.39	A	74.5 ± 1.04	A
Test (%)[a]	44.2 ± 0.61	9:35	46.3 ± 0.82	A	47.6 ± 0.70	A
		20:23	46.9 ± 0.97	A	46.2 ± 0.66	A
		31:12	48.7 ± 1.13	A	46.1 ± 2.87	A
Aristotle's lantern (%)[a]	38.6 ± 1.25	9:35	45.1 ± 1.37	A*	39.7 ± 1.00	A
		20:23	41.3 ± 1.5	A	36.8 ± 0.78	A
		31:12	41.4 ± 2.1	A	36.5 ± 3.73	A
Gut (%)	79.0 ± 1.04	9:35	75.3 ± 0.59	C	77.5 ± 0.54	B
		20:23	77.7 ± 0.22	B	79.4 ± 0.35	A
		31:12	79.2 ± 0.38	A	78.6 ± 0.32	AB

[a] indicates that data set was arcsine transformed.
Asterisk indicates significant difference from Day 0 and letters indicate significant differences among diets.

content appeared to be related directly to dietary protein and those sea urchins fed the 9:35 P:C diet had significantly less gonad water content than sea urchins fed the 20:23and 31:12 P:C diets ($F = 9.36$, $df = 2$, $P < 0.028$ Table 5). The water content of the test and Aristotle's lantern did not vary significantly among the diets (Table 5). The water content of the gut varied significantly with diet at day 32 (Kruskal Wallis = 22.72, $P < 0.002$, Table 5) and at day 65 urchins fed the 9:35 had less gut water content than urchins fed the 20:23 P:C diet ($F = 1.56$, $df = 2$, $P < 0.009$ Table 5).

3.3 *Gonad image analysis*

The volume of nutritive phagocytes in females at day 65 was inversely related to dietary protein level and directly related to dietary carbohydrate level ($F = 35.58$, $df = 2$, $P < 0.004$; Table 6). Volume of the germinal epithelium increased with increasing dietary protein and decreasing dietary carbohydrate ($F = 16.46$, $df = 2$, $P = 0.001$). Sea urchins

Table 6
Lytechinus variegatus: gonad development parameters analyzed from day 65 histological samples of gonad using image analysis software

Parameters	Dietary % Protein: % Carbohydrate	Day 65	
Female Nutritive Phagocytes (%)	9:35	88.5 ± 0.94	A
	20:23	82.9 ± 1.12	B
	31:12	68.9 ± 3.28	C
Female Germinal Epithelium (%)	9:35	8.91 ± 0.52	B
	20:23	12.9 ± 0.84	A
	31:12	15.06 ± 0.85	A
Egg Diameters	9:35	64.79 ± 1.67	B
	20:23	72.47 ± 1.39	A
	31:12	77.62 ± 1.32	A
Male Nutritive Phagocytes (%)	9:35	92.9 ± 0.50	A
	20:23	90.8 ± 0.70	B
	31:12	90.7 ± 0.49	B
Male Gametes (%)	9:35	7.1 ± 0.50	B
	20:23	9.2 ± 0.70	A
	31:12	9.2 ± 0.49	A

All data sets were arcsine transformed. Actual means ± SE, letters indicate significant differences among diets.

fed the 9:35 P:C diet had smaller oocyte diameters than sea urchins fed the 20:23 (Mann-Whitney U = 25,818, $df = 1$, $P = 0.001$) or 31:12 P:C diets (Mann-Whitney U = 17,634, $df = 1$, $P < 0.001$; Table 6). Oocyte diameters increased with increasing dietary protein

and decreasing dietary carbohydrate. A higher percentage of larger oocytes were observed with increasing dietary protein and decreasing carbohydrate (Fig. 2).

Male sea urchins fed the 9:35 P:C diet had a significantly greater volume of nutritive phagocytes than those fed the 20:23 and 31:12 P:C diets at day 65 ($F = 5.320$, $df = 2$, $P < 0.04$; Table 6). Those fed the 9:35 P:C diet also had a significantly smaller volume of gametes than those fed the 20:23 and 31:12 P:C diets ($F = 5.320$, $df = 2$, $P < 0.04$; Table 6).

4. Discussion

4.1 *Gonad composition*

The composition of the diet affects the proximate composition and somatic/gametic cell volume of the gonad in *L. variegatus*. Increasing dietary protein and decreasing dietary carbohydrate resulted in increased concentrations of stored protein in the gonad, and greatly decreased concentrations of stored carbohydrates. Additionally, females fed diets high in protein (low carbohydrate) had an increased volume of gametes and increased oocyte growth, resulting from an apparent increased allocation of nutrients to developing oocytes. Diets low in protein (high in carbohydrates) resulted in increased concentrations of stored carbohydrates and decreased volume of gametes. Lipid storage was not substantially affected by dietary protein.

A slight, but significant, increase in ash content with high dietary protein (low carbohydrate) may reflect the storage/utilization of essential minerals required for metabolism and/or growth. Fernandez (1997) did not find a significant difference in gonad ash content in adult *Paracentrotus lividus* fed diets containing 13%, 29%, or 47% protein

for 6 months. Gibbs (2005) reported 4.8% and 5.5% ash for ovaries and testes, respectively, for adult *L. variegatus* fed a 33% protein diet. Mineral requirements for sea urchin growth are essentially unknown, although recent studies have demonstrated a requirement for minerals including calcium, iron, and copper (Watts et al., unpub. data).

The total soluble protein stored in the gonads varied directly with dietary protein and indirectly with carbohydrate levels. These data indicate that protein is stored primarily in the gonad component and could contribute to the future reproductive success of the individual. Two proteins reported to occur in the ovaries and testes (major yolk protein, MYP, and YP30) comprise most of protein stored in these components (Unuma et al., 1998; Marsh and Watts, 2001; Unuma, 2002; Brooks and Wessel, 2003). MYP mobilized from nutritive phagocytes to oocytes reportedly results in increases in the size of vitellogenic oocytes (Brooks and Wessel, 2003), yet the role of MYP in male gametogenesis remains obscure (Unuma, 2002). Sea urchins fed the 31:12 P:C protein diet in the current study had smaller volumes of nutritive phagocytes, larger oocyte diameters, and more gametes (males and females) than the two lower protein diets suggesting increased nutrient mobilization to developing reproductive tissues when more dietary protein and less carbohydrate was available. In contrast to the current study, Fernandez (1997) reported that gonad protein did not vary with dietary protein in adult *Paracentrotus lividus* held in tanks for 6 months, although the source of the protein varied among the diets (vegetable versus animal origin). Similarly, McBride et al. (1998) indicated that gonad protein did not vary with dietary protein in small (34 mm) *Strongylocentrotus franciscanus* fed 30%, 40%, or 50% protein diets. Hill and Lawrence (1998) reported a decrease in gonad protein content for adult *L. variegatus* fed a formulated diet for 5 weeks

(composition of the formulated diet used for this study was not reported), further suggesting that diet affects gonad composition. In addition to dietary and seasonal effects, physical factors may influence protein accumulation in the gonad of *L. variegatus*. Protein content trended higher (but was not significantly different) in sea urchins cultured at median environmental temperatures for the Northern Gulf of Mexico compared to sea urchins cultured at temperatures above or below the median (Hill, 2000; Gibbs, 2005), and corresponded to increased gametic activity (Gibbs, 2005). These data suggest dietary protein and proximate environmental factors affect gonad composition and reproductive output.

At the conclusion of the study, the total gonad carbohydrate varied indirectly with dietary protein and directly with dietary carbohydrate. Fernandez (1997) reported that adult *Paracentrotus lividus* fed a vegetable-base diet (13% protein) had significantly higher carbohydrate concentrations in the gonad compared to the mixed-base (29% protein) and animal-base (47% protein) diets. These data suggest sea urchins can store nutrients relative to their availability in the diet. Carbohydrate is stored primarily in the gonads and to a lesser extent in the test, with minimal storage in the gut. Marsh and Watts (2001) concluded that glycogen was the major carbohydrate constituent of gonads accounting for 13-25% of the total dry weight and ca. 50%-75% of the total carbohydrate weight in the gonad (during early gonad growth phase). It has been suggested that glycogen is one of the primary energy sources utilized for gametogenesis in echinoderms (Zalutskaya et al., 1986; Marsh and Watts, 2001; Montero-Torreiro and Garcia-Martinez, 2003). In the current study, sea urchins fed the 9:35 P:C diets had greater amounts of carbohydrate stored in the gonads, larger volumes of nutritive phagocytes, smaller oocyte diameters and fewer

gametes (males and females) than urchins fed the other diets. These data suggest that, although energy reserves are adequate, gamete development is affected by limited protein availability.

Total lipid in the gonad did not vary with diet in the current study. Marsh et al. (1990) and Marsh and Watts (2001) reported that the ovaries and developing oocytes of many invertebrates exhibit lipid profiles that directly reflect dietary sources and that *de novo* synthesis of lipids is generally low. All of the diets in the current study contained nearly equivalent amounts of lipid and there was no significant differential lipid storage in the gonads. De Jong-Westman et al. (1995) did not find significant differences in gonad lipid concentrations among adult *Strongylocentrotus droebachiensis* fed diets containing 10% or 20% dietary protein. Similarly, McBride et al. (1998) indicated that gonad lipid concentrations did not vary among small *Strongylocentrotus franciscanus* fed formulated diets containing 30%, 40%, or 50% dietary protein. In contrast, adult *Paracentrotus lividus* had greater gonad lipid concentrations in sea urchins fed diets containing 47% (animal-base) and 29% (mixed-base) protein than urchins fed a 13% protein (vegetable-base) diet (Fernandez, 1997), apparently reflecting differences in the lipid content of the diets (15.5%, 12.8%, 10.7% lipid, respectively).

Lytechinus variegatus gonads had higher water content in individuals fed the high protein, low carbohydrate diet. The high water content of the gonad may be due to the differential hydration state of molecules stored in the gonad. Interestingly, we previously hypothesized the high water content of the initial population of starved individuals was a consequence of limited nutrient storage. Consequently, water content cannot be used as a predictor of gonad nutritional status. Pearce et al. (2002) and Hammer et al. (2004) indi-

cated that gonad water content varied significantly and directly with dietary protein in adult *Strongylocentrotus droebachiensis* and *Lytechinus variegatus*, respectively. In contrast, de Jong-Westman et al. (1995) reported no difference in water content with respect to diet in adult *Strongylocentrotus droebachiensis* fed formulated diets containing 10% or 20% protein for 9 months.

The preferential storage of gonad nutrients in direct relation to the composition of the diet suggests that sea urchins do not substantially alter the proximate composition of nutrients for storage. In the gonad, digested proteins may be assimilated into yolk proteins such as MYP or YP30 (Brooks and Wessel 2003) while carbohydrates may be stored as glycogen (Lawrence and Lane, 1982; Marsh and Watts, 2001; Montero-Torreiro and Garcia-Martinez, 2003) and lipids as triacylglycerides or sterols (Montero-Torreiro and Garcia-Martinez, 2003). In fish and mammals, excess carbohydrates are frequently modified and stored as fats; this does not appear to be the case with sea urchins.

4.2 *Somatic composition*

Sea urchins in the current study were held prior to experimentation for an extended time period on only a minimal maintenance diet. Energy reserves in the test of these individuals were severely depleted, but increased substantially when fed formulated diets. With feeding, the organic (including protein and carbohydrate) content of the test increased in all diets and reflects the storage of substantial nutrient reserves, second in total amount to the gonad. These nutrient reserves can be mobilized (through re-absorption of the test) during starvation (Lawrence and Lane, 1982). In some sampling periods, Fernandez (1997) reported greater soluble protein and carbohydrate concentrations in the test

of adult *Paracentrotus lividus* fed a 47% protein diet in open ocean enclosures as compared to those fed 13% protein or natural diets. Hill and Lawrence (1998) reported increased test carbohydrate content for adult *L. variegatus* fed a formulated diet for five weeks.

Changes in gut size of *Strongylocentrotus purpuratus* seasonally and with starvation suggest it is an important organ in the storage of nutrients (Lawrence et al., 1966). The gut is an important organ for short-term energy storage in *L. variegatus* (Klinger et al., 1988; Bishop and Watts, 1992; Klinger et al., 1996). The current study suggests that the protein content of the gut tissues can be directly influenced by diet. Carbohydrate stores were minimal and not affected by diet. The small size of the gut limits the amount of nutrients that can be stored and, as such, the gut contains only a small percentage of the stored protein and carbohydrate relative to the gonad and test. Fernandez (1997) reported no significant differences in the gut soluble protein with adult *Paracentrotus lividus* fed diets containing 13%, 29%, or 47% protein, but urchins fed the 47% protein diet had greater gut carbohydrate concentrations than urchins fed natural diets or those collected from the wild. We hypothesize that the size (storage capacity) of the gut is not influenced by the quality (proximate composition) as much as the quantity (food availability) of the diet.

4.3 *Gonad image analysis*

The reproductive status of the ovaries in adult *L. variegatus* is directly affected by the protein and carbohydrate content of the diet. Females fed the 9:35 P:C diet were in the mid-late stages of "pre-gametogenesis and nutritive phagocyte renewal" and very

early stages of "gametogenesis and nutritive phagocyte utilization" (Walker et al., 2001). These urchins had a larger volume of nutritive phagocytes, smaller, less active germinal epithelia, and smaller oocyte diameters consistent with previtellogenic oocytes. The nutritive phagocytes were large and filled with nutrients (probably glycogen and MYP) but translocation of nutrients to, and development of, the oocytes is apparently delayed due to the limited availability of dietary protein with relative higher availability of carbohydrate. In contrast, those sea urchins fed the 20:23 and 31:12 P:C diets indicated advanced characteristics of "gametogenesis and nutritive phagocyte utilization", having nutritive phagocytes of smaller volume, larger more active germinal epithelia, and larger early vitellogenic oocytes consistent with early MYP mobilization and storage (Walker et al., 2001; Brooks and Wessel, 2003). Based on these observations, reproductive success might be limited or delayed for urchins maintained on diets containing low levels of protein, although the absolute level of limitation was not determined. Similarly, Hammer et al. (2004) reported that oocyte diameters increased with increasing dietary protein and decreasing carbohydrate for small female *L. variegatus* fed 9%, 15%, and 21% protein diets, but no changes were observed in the percent volume of nutritive phagocytes or the germinal epithelium.

The effect of dietary protein and carbohydrate on the reproductive status of the testes is less obvious. Males fed the 9:35 P:C (high carbohydrate) diet were in the mid-late stages of "pre-gametogenesis and nutritive phagocyte renewal" and very early stages of "gametogenesis and nutritive phagocyte utilization" having slightly larger volumes of nutritive phagocytes and a smaller volume of gametes in the lumen (Walker et al., 2001) as compared to the 20:23 and 31:12 P:C diets. Although statistically significant, absolute

differences in cell populations among diets were small, minimizing the importance of dietary protein on male gamete development. Unuma (2002) and Brooks and Wessel (2003) have suggested that major yolk protein, the primary storage protein in the nutritive phagocytes, is translocated from nutritive phagocytes to oocytes, but not to spermatocytes (Unuma et al., 1998). Thus, the dependence of spermatogenesis on macronutrient composition of the diet may be limited. The role of protein and carbohydrate in spermatogenesis requires further study.

5. Conclusions

In field populations of sea urchins consuming natural diets, the biochemical composition of the gonad is largely dependent on the season and reproductive status of the sea urchin (Fernandez ,1997; Montero-Torreiro and Garcia-Martinez, 2003). However, Fernandez (1997) reported that the biochemical composition of sea urchin gonads cultured in the field or laboratory could be influenced by the composition of formulated diets. The current study supports this observation. *L. variegatus* preferentially stored nutrients (proteins and carbohydrates) in the gonads in relation to the composition of those nutrients in the diet. Additionally, gametic development was significantly advanced in individuals fed high protein, low carbohydrate diets. This study suggests that it might be possible to manipulate the quality and market value of sea urchin gonads through dietary manipulation, a concept with very useful implications for the culture of sea urchins (Fernandez, 1997: Pearce et al., 2002).

The determination of a dietary protein requirement in sea urchins is difficult. Protein sources used in feeds vary in the levels of specific indispensable amino acids and the

availability of nutrients. Additionally, the variation in dietary protein level using practical ingredients results in concomitant variations in other nutrients such as the levels of indispensable amino acids, carbohydrates or energy. All previous studies to date have used these approaches. Consequently, the ability to conclude that the observed effect is due to protein is limited. For this reason the conclusions made from the data in this paper are made with the realization that the effect may be the result of variations made to other nutrients when varying dietary protein.

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References

Akiyama, T., Unuma, T., Yamamota, T. (2001). Optimum protein level in a purified diet for young red sea urchin *Pseudocentrotus depressus*. Fisheries Science 67: 361-363

Beddingfield, S. D., McClintock, J. B. (1998). Differential survivorship, reproduction, growth and nutrient allocation in the regular echinoid *Lytechinus variegatus* (Lamarck) fed natural diets. Journal of Experimental Marine Biology and Ecology 226: 195-215

Beddingfield, S.D., McClintock, J.B. (2000). Demographic characteristics of *Lytechinus variegatus* Echinoidea: Echinodermata from three habitats in a North Florida Bay, Gulf of Mexico. Marine Ecology. 21: 17- 40

Bishop, C. D., Watts, S. A. (1992). Biochemical and morphometric study of growth in the stomach and intestine of the echinoid *Lytechinus variegatus* (Echinodermata). Marine Biology 114: 459-467

Brooks, J.M., Wessel, G.M. (2003). Selective transport and packaging of the major yolk protein in the sea urchin. Developmental Biology. 261: 353-370

de Jong-Westman, M., March, B.E., Carefoot, T. H. (1995). The effect of different nutrient formulations in artificial diets on gonad growth in the sea urchin *Strongylocentrotus droebachiensis.* Canadian Journal of Zoology 73: 1495-1502

Dubois, M., Gilles, K.A., Gilles, J.K., Hamilton, J.K., Rebers, P.A., and Smith, R., (1956). Colorimetric method for determination of sugars and related substances. Analytical Chemistry 28, 350-356

Fernandez, C., Dombrowski, E., Caltagirone, A. (1995). Gonadic growth of adult sea urchins, *Paracentrotus lividus* (Echinodermata: Echinoidea) in rearing: The effect of different diet types. In: Emson, R., Smith, A., Campbell, A. (eds.) Echinoderm Research. Balkema, Rotterdam, p. 269-275

Fernandez, C. (1997) Effect of diet on the biochemical composition of *Paracentrotus lividus* (Echinodermata: Echinoidea) under natural and rearing conditions (effect of diet on the biochemical composition of urchins). Comparative Biochemistry and Physiology. 118A: 1377 – 1384

Freeman, F., Lindgren, F.T., Ng, Y.S., and Nichole, A.V., (1957). Serum lipid analysis by chromatography and infrared spectrophotometry. Journal of Biological Chemistry 277, 449-464

Gibbs, V.K. (2005). The effect of temperature on the biochemical composition of the reproductive tissue from the sea urchin *Lytechinus variegatus*. M.S. Thesis University of Alabama at Birmingham. Birmingham, Alabama

Hammer, B. W., Hammer, H. S., Watts, S. A., Desmond, R. A., Lawrence, J. M., Lawrence, A. L. (2004). The effects of dietary protein concentration on feeding and growth of small *Lytechinus variegatus* (Echinodermata: Echinoidea). Journal of Marine Biology. 145: 1143-1157

Hammer, H., Watts, S., Lawrence, A., Lawrence, J., Desmond, R. (in press). The effect of dietary protein on consumption, survival, growth and production of the sea urchin *Lytechinus variegatus*. Aquaculture.

Hill, S.K., Lawrence, J.M. (1998). Effects of food and temperature on the energy budget of *Arbacia punctulata* and *Lytechinus variegatus*. In: Candia Carnevali, M.D., Bonasoro, F. (eds). Echinoderm research 1998. Proceedings of the fifth European conference on echinoderms. Milan, Italy. Balkema, Rotterdam. pp. 73-78

Hill, S.K. (2000). Effects of abiotic and biotic stress on the energy budgets of *Arbacia punctulata* and *Lytechinus variegatus* (Echinodermata: Echinoidea). PhD. Dissertation University of South Florida. Tampa, Florida.

Hofer, S. C. (2002). The effect of temperature on feeding and growth characteristics of the sea urchin *Lytechinus variegatus* (Echinodermata: Echinoidea). M. S. Thesis University of Alabama at Birmingham, Birmingham

Klinger, T. S. (1982). Feeding rates of *Lytechinus variegatus* Lamarck (Echinodermata: Echinoidea) on differing physiognomies of an artificial food of uniform composition. In: J. M. Lawrence (ed.) Echinoderms: proceedings of the international conference, Tampa Bay. Balkema, Rotterdam. pp. 29-32

Klinger, T. S., Watts, S. A., Forcucci, D. (1988). Effect of short-term feeding and starvation on storage and synthetic capacities of gut tissues of *Lytechinus variegatus* (Lamarck) (Echinodermata: Echinoidea). Journal of Experimental Marine Biology and Ecology 117:187-195

Klinger, T. S., Lawrence, J. M., Lawrence, A. L. (1996). Absorption and assimilation of prepared feeds by echinoids. Proceedings of the International Echinoderm Conference, San Francisco. A.A. Balkema, Rotterdam. pp. 713-721

Lawrence, J.M., Lawrence, A.L., Giese, A.C. (1966). Role of the gut as a nutrient storage organ in the purple sea urchin (*Strongylocentrotus purpuratus*). Physiological Zoology. 39: 281-290

Lawrence, J.M., Lane, J.M. (1982). The utilization of nutrients by post-metamorphic echinoderms. In: Jangoux, M., Lawrence, J. M. (eds.) Echinoderm nutrition. A. A. Balkema, Rotterdam, pp 331-371

Lawrence, J.M., Lawrence, A.L., McBride, S.C., George, S.B., Watts, S.A., Plank, L.R. (2001) Developments in the use of prepared feeds in sea-urchin aquaculture. World Aquaculture. September. 34-39

Lowe, E. F., Lawrence, J. M. (1976). Absorption efficiencies of *Lytechinus variegatus* (Lamarck) (Echinodermata: Echinoidea) for selected marine plants. Journal of Experimental Marine Biology and Ecology 21: 223-234

Lowry, O.H., Rosebrough, N.J., Farr, A.L., and Randall, R.J., (1951). Protein measurement with Folin reagent. Journal of Biological Chemistry 193, 265-275

Marsh, A.G., Harvey, H.R., Gremare, A., Tenore, K.R. (1990). Dietary effects on oocyte yolk-composition in *Capitella sp. I* (Annelida: Polycheata): Fatty acids and sterols. Marine Biology 106: 369-374

Marsh, A.G., Watts, S.A. (2001). Energy metabolism and gonad development. In: Lawrence, J. M. (ed.) Edible sea urchins: biology and ecology. Elsevier Science B. V. Amsterdam. pp. 27-42

McBride, S. C., Lawrence, J. M., Lawrence, A. L., Mulligan, T. J. (1998). The effect of protein concentration in prepared feeds on growth, feeding rate, total organic absorption, and gross assimilation efficiency of the sea urchin *Strongylocentrotus fransiscanus*. Journal of Shellfish Research 17: 1563-1570

Montero-Torreiro, M.F., Garcia-Martinez, P. (2003). Seasonal changes in the biochemical composition of body components of the sea urchin, *Paracentrotus lividus*, in Lorbe' (Galicia, north-western Spain). Journal of the Marine Biological Association of the United Kingdom. 83: 575-581

Morris, J. G. (1991). Nutrition. I: Prosser, C. L. (ed.) Environmental and Metabolic Animal Physiology. Wiley-Liss, New York, p. 231-276

Pearce, C. M., Daggett, T.L., Robinson, S.M.C., 2002. Effect of protein source ratio and protein concentration in prepared diets on gonad yield and quality of the green sea urchin, *Strongylocentrotus droebachiensis*. Aquaculture 214: 307-332

Unuma, T., Tohru, S., Tadahide, K., Yamamoto, T., Akiyama, T. (1998). A protein identical to the yolk protein is stored in the testis in male red sea urchin, *Pseudocentrotus depressus*. Biological Bulletin. 194: 92-97

Unuma, T. (2002). Gonadal growth and its relationship to aquaculture in sea urchins. In: Yokota, Matranga and Smolenicka (eds). The sea urchin: from basic biology to aquaculture. Swets & Zeitlinger, Lisse. pp. 115-127

Valentine, J.F. and Heck, K.L. (1999). Seagrass herbivory: evidence for the continued grazing of marine grasses. Marine Ecology Progress Series. 176: 291-302

Walker, C.W., Unuma, T., McGinn, N.A., Harrington, L.M., Lesser, M.P. (2001). Reproduction in sea urchins. In: Lawrence, J. M. (ed.) Edible sea urchins: biology and ecology. Elsevier Science B. V., Amsterdam. pp. 5-26

Wallace, B. D. (2001). The effects of dietary protein concentration on feeding and growth of small *Lytechinus variegatus* (Echinodermata: Echinoidea). M. S. Thesis. University of Alabama at Birmingham. Birmingham, Alabama.

Watts, S.A., Boettger, S.A, McClintock, J.B., Lawrence, J.M. (1998). Gonad production in the sea urchin *Lytechinus variegatus* (Lamarck) fed prepared diets. Journal of Shellfish Research. 17: 1591-1595

Watts, S.A., McClintock, J.B., Lawrence, J.M., 2001. The ecology of Lytechinus variegatus. In: Lawrence, J.M. (Ed.), Edible Sea Urchins Biology and Ecology. Elsevier Science B.V., Amersterdam, pp. 375-393.

Zalutskaya, E. A., Varaksina, G. S., and Khotimchenko, Y. S. (1986). Glycogen-content in the ovaries of the sea urchin *Strongylocentrotus intermedius*. Marine Biology (Vlad.): 38-44

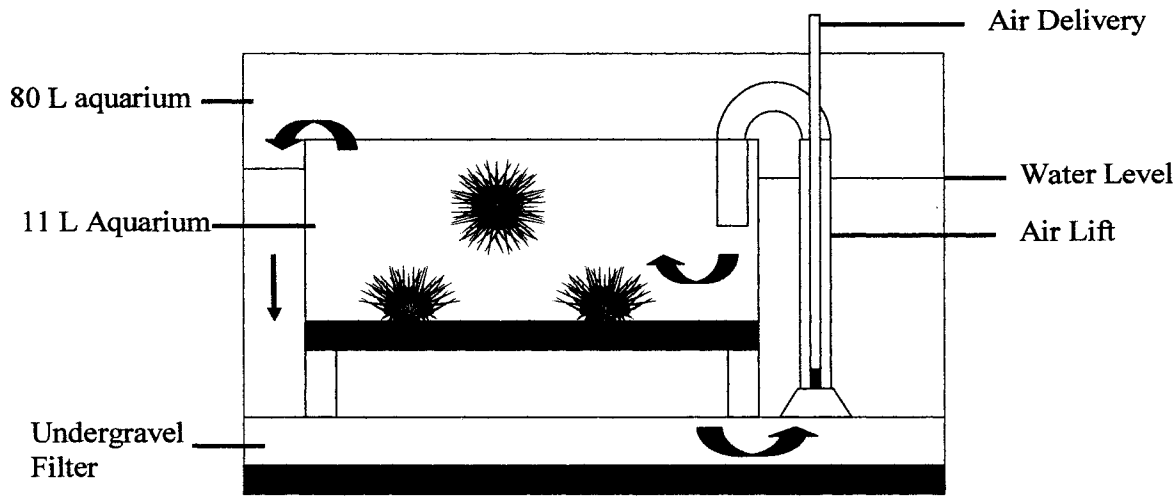

Fig. 1. Schematic illustration of tank-within-a-tank system. An 11-liter aquarium was elevated and placed inside a larger 80-liter aquarium. Biofiltration occurred in the under-gravel dolomite filter. An air-lift circulated water through the under-gravel filter and delivered aerated seawater into the small aquarium. Overflow water returned to the larger aquarium and was then recirculated through the filter. Adapted from Wallace (2001).

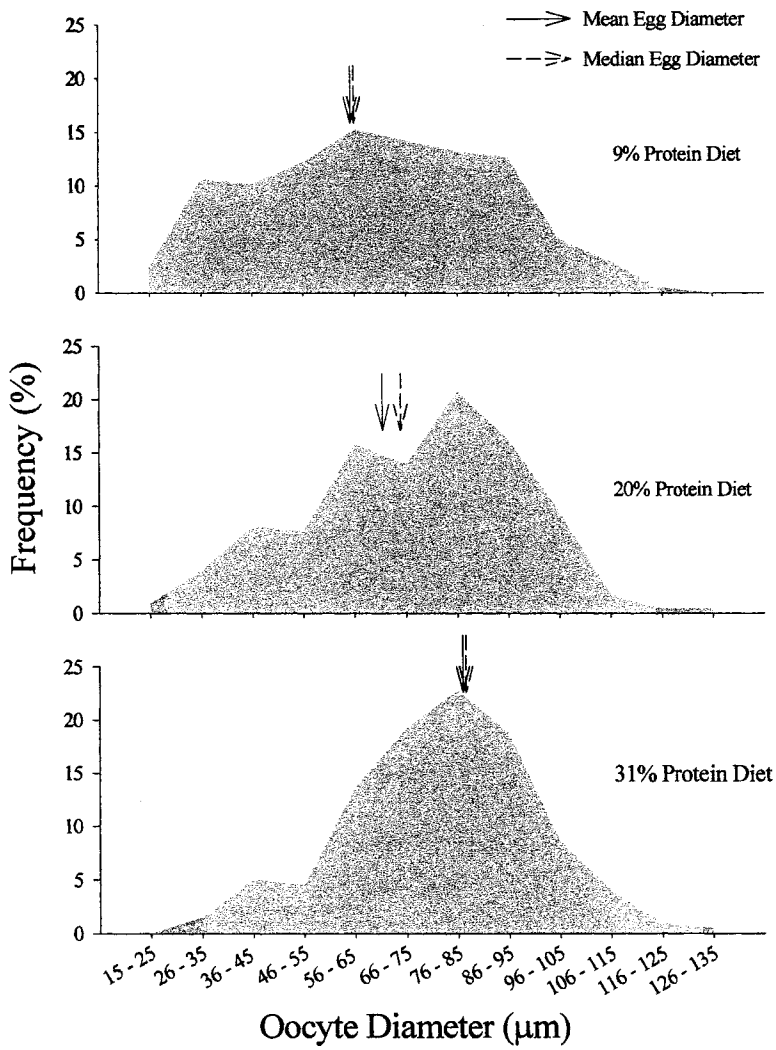

Fig. 2. *Lytechinus variegatus*. Oocyte diameter at day 65 for female sea urchins fed three different protein:carbohydrate diets. Lengths were measured by image analysis of histological sections. The mean and median sizes are indicated by solid and dashed arrows, respectively.

EFFECT OF FEED PROTEIN AND CARBOHYDRATE LEVEL ON CONSUMPTION, GROWTH AND EFFICIENCY OF THE SEA URCHIN *LYTECHINUS VARIEGATUS*

by,

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In preparation for *Aquaculture*

Format adapted for dissertation

Abstract

Adult sea urchins (12.6 ± 0.12 SE g wet weight, 29.5 ± 0.11 SE mm diameter) were collected from St. Joseph Bay, FL (30° N, 85.5°W) and transported to the Texas A&M Shrimp Mariculture Research Lab in Port Aransas, TX. The whole body weight and test diameter of a subpopulation were determined. These sea urchins were then dissected into component organs, weighed, dried, and weighed again. Sea urchins from the same population were held individually in replicated cylindrical enclosures contained within a recirculating natural seawater system (32 ± 2 ‰ and 22 ± 2 °C). Sea urchins (n = 16 urchins per feed treatment) were fed *ad libitum* one of four cold extruded feeds that differed relative to protein: carbohydrate levels (31:33%, 25:39%, 21:44%, and 17:47% dry weight) for 12-weeks. Consumption was measured daily and whole body weights and test diameters were taken at 0, 4, 8, and 12 weeks. At 12 weeks the study was terminated and sea urchins were dissected into component organs, dried, and reweighed. Survival was 100% in all feed treatments. Sea urchins fed the 31:33% protein: carbohydrate feed consumed less overall feed, more dry protein, less dry carbohydrate, less energy and had lower food conversion ratio (FCR) values than sea urchins in the other feed treatments. Sea urchins fed the 31:33% protein: carbohydrate feed also had higher specific growth rate (SGR) values for the first 8 weeks and overall higher test diameters, immersion weights, wet weights, production efficiencies, and gonad production efficiencies than urchins in the other feed treatments. There was no difference in gonad production among sea urchins fed the 31:33%, 25:39%, and 21:44% protein: carbohydrate feeds. Weight gain varied directly and significantly with protein consumption; however, no significant relationship was found between weight gain and energy consumption. Sufficient energy

was available for maximum weight gain as protein was apparently spared (protein efficiency ratios were similar among all feed treatments). Sea urchins in this study grew more efficiently and at higher rates than previous feeding studies with adult *L. variegatus*, suggesting that the feeds used in the current study are of higher quality. Determination of protein requirements in urchin culture will require an "optimization" of the levels of other nutrients in formulated feeds, balancing the needs for efficient growth with quality roe production. This area of nutrition research is essential to the development of cost-effective, commercially available feeds for future aquaculture of sea urchins.

1.0 Introduction

The over-fishing of natural sea urchin stocks and the associated ecological impacts, coupled with the increasing demand for the sea urchin gonad (uni, or roe), have stimulated research directed at the development of sea urchin aquaculture (Pearce et al., 2002; reviewed in Lawrence, 2001). Before sea urchin aquaculture can be feasible, cost-effective, high quality feeds must become commercially available to promote both rapid somatic growth and marketable roe production (Pearce et al., 2002). Quality nutrition, coupled with efficient feed management strategies, will promote rapid growth of the developing industry (Lawrence and Lawrence, 2003).

Protein is an important dietary macro-nutrient that provides essential amino acids and energy for maintenance, growth and reproduction in all animals (Morris, 1991). Since protein is one of the most expensive nutrients in aquaculture feeds, it is important to determine the nutritional requirement for optimum growth. In addition, the oxidation of dietary carbohydrates is the primary source of energy for all herbivorous and many

omnivorous animals (Morris, 1991). Knowledge of energy utilization is essential to the development of cost-effective feeds because energy must be supplied in sufficient amounts so that protein can be almost exclusively used for tissue synthesis (Cuzon and Guillaume, 1997).

Evaluation of nutrient performance is important for development of commercial aquaculture feeds. However, nutrient availability and response are maximized when a feed has optimal physical characteristics. The type of feed (extruded or pelleted), the ease of production, the costs of ingredients and manufacture, the ease of storage, and time to spoilage are all concerns that impact feed production and commercial culture.

Many previous nutritional studies with sea urchins have used natural feeds or natural feed components. These studies are important because they provide insight into dietary requirements of sea urchins. However, these feeds cannot be conveniently stored, may spoil rapidly, can be difficult to proffer, and inconsistent in quality. These diets would require significant costs and labor to collect and, consequently, would not be practical in commercial aquaculture. Moist semi-purified or purified feeds can be used to determine nutrient requirements, but are not feasible for commercial production because they are not cost effective. The current study used a cold-extruded feed pellet composed of purified and practical ingredients with low moisture content (7-9% moisture). The feeds are similar in physical characteristics and nutrient values to commercial aquaculture feeds. The purpose of this study is to evaluate the level of protein and carbohydrate in semi-purified feeds on growth and gonad production in *L. variegatus.*

2.0 Materials and Methods

2.1 Collection, culture and initial measurements

Lytechinus variegatus were collected in October 2004 from Port St. Joseph Peninsula State Park, FL (30° N, 85.5°W) and transported to the Texas A&M Shrimp Mariculture Facility in Port Aransas, TX. At the time of collection, gonads were minimal in size (gonads are not observed in field populations until individuals are 35-40 mm in diameter (Moore et al., 1963; Beddingfield et al., 2000). Sea urchins were held for 1 month in 750 L tanks at approximately 32 ± 2 ppt salinity and 22 ± 1 °C. Natural seawater was filtered using stratified sand filtration, a Diamond water filter (Diamond Water Conditioning, Horton, WI), and then piped to the systems under flow-through conditions (exchange rate approximately 150% daily). During this period sea urchins were fed a maintenance ration (approximately once every three days) consisting of a formulated feed containing 31% protein (Table 1).

To stock the experimental systems, sea urchins (12.6 ± 0.12 SE g wet weight, 29.5 ± 0.11 SE mm test diameter) were randomly selected (sexes were combined), weighed in saltwater (32 ppt salinity and 22 °C) by immersion according to Grosjean et al. (1999). The urchins were blotted dry with a paper towel to remove excess water, measured for test diameter at two perpendicular points across the ambitus using calipers, and weighed to the nearest mg with a Mettler balance (Mettler Toledo Scales Dublin, Ohio). A random sub-sample of sea urchins (n = 16) were removed from the initial population for dissection. These sea urchins were weighed as previously described and dissected. Sea urchins were cut outside the peristomial membrane on the oral surface. During the dissection the test with spines, Aristotle's lantern, gut, and gonads were removed

Table 1
Calculated nutrient composition of the Texas A&M formulation used to produce feeds varying in protein: carbohydrate level

	Values "As Fed"			
Ingredient	31:33 Feed	25:36 Feed	21:40 Feed	17:47 Feed
*Crude Protein	30.86%	24.80%	20.73%	17%
Carbohydrate	32.62%	35.50%	40.05%	47.43%
Crude Fiber	2.50%	2.50%	2.50%	2.47%
Crude Fat	7.52%	7.52%	7.52%	7.52%
Total Ash	23.52%	23.52%	23.52%	23.18%
*Moisture	9.35%	7.95%	7.03%	9.16%
Carotenoid	0.42%	0.42%	0.42%	0.42%
Lipid	6.88%	6.88%	6.88%	6.88%
Cholesterol	0.22%	0.22%	0.22%	0.22%
Calcium	2.35%	2.35%	2.35%	2.35%
Phospohorous	1.89%	1.89%	1.89%	1.89%
Sodium	1.29%	1.29%	1.29%	1.29%
Potassium	1.63%	1.63%	1.63%	1.63%
Magnesium	0.39%	0.39%	0.39%	0.39%
Iron	319 ppm	319 ppm	319 ppm	319 ppm
Zinc	91 ppm	91 ppm	91 ppm	91 ppm
Manganese	71 ppm	71 ppm	71 ppm	71 ppm
Copper	47 ppm	47 ppm	47 ppm	47 ppm
Selenium	0.228 ppm	0.228 ppm	0.228 ppm	0.228 ppm
Arginine	2.15%	2.15%	2.15%	2.15%
Histidine	0.62%	0.62%	0.62%	0.62%
Isoleucine	1.12%	1.12%	1.12%	1.12%
Leucine	2.00%	2.00%	2.00%	2.00%
Lysine	1.69%	1.69%	1.69%	1.69%
Methionine	0.49%	0.49%	0.49%	0.49%
Cystine	0.27%	0.27%	0.27%	0.27%
Phenylalanine	1.29%	1.29%	1.29%	1.29%
Tyrosine	0.96%	0.96%	0.96%	0.96%
Threonine	1.05%	1.05%	1.05%	1.05%
Tryptophan	0.26%	0.26%	0.26%	0.26%
Valine	1.19%	1.19%	1.19%	1.19%
Vitamin A	4800 IU	4800 IU	4800 IU	4800 IU
Vitamin D	3000 IU	3000 IU	3000 IU	3000 IU
Vitamin E	240 ppm	240 ppm	240 ppm	240 ppm
Vitamin C	349 ppm	349 ppm	349 ppm	349 ppm
Thiamine	36 ppm	36 ppm	36 ppm	36 ppm

Table 1. (Continued)

Ingredient	Values "As Fed" 31:33 Feed	25:36 Feed	21:40 Feed	17:47 Feed
Riboflavin	48 ppm	48 ppm	48 ppm	48 ppm
Pyridoxine	96 ppm	96 ppm	96 ppm	96 ppm
Niacine	96 ppm	96 ppm	96 ppm	96 ppm
Pantothenic Acid	36 ppm	36 ppm	36 ppm	36 ppm
Biotin	1 ppm	1 ppm	1 ppm	1 ppm
Inositol	0.10%	0.10%	0.10%	0.10%
Choline	0.10%	0.10%	0.10%	0.10%
Folic Acid	24 ppm	24 ppm	24 ppm	24 ppm
Vitamin B12	.2 ppm	.2 ppm	.2 ppm	.2 ppm
Protein: Energy Ratio (mg protein $Kcal^{-1}$)	80.79	65.96	56.49	47.09
*Energy (Kcal g^{-1})	3.82	3.76	3.67	3.61

Values on an "as fed" basis. Asterisk indicates that values were based on dry weight and derived empirically.

and separated. The gut (esophagus, stomach, and intestine) were rinsed in a finger bowl to remove excess food. Each of the organs were blotted dry with a paper towel to remove excess water and weighed to the nearest mg (wet weight). Organs were placed onto weighed aluminum pans and dried to constant weight at 60 °C. Dry weight was determined and moisture content calculated by subtraction. At the end of the study, sea urchins from each of the feed treatments were weighed and dissected as described earlier.

For the feed trials, sea urchins were placed individually into a cylindrical enclosure. The cylindrical enclosures were made from a plastic mesh (approximately 12 cm diameter, 30 cm height, and a 4 mm open mesh) secured by plastic cable ties. The mesh enclosures were fitted into 11.5 cm ID PVC couplings so that the floors of the cylindrical enclosures were approximately 5.5 cm above the bottom of the tank. Small plastic spacers (approximately 0.5 cm thick) were then placed under the bottom of each coupling to

allow water circulation underneath the enclosures. Four cylindrical enclosures were placed into a 0.07 m^2 bottom surface fiberglass tank with 20 L water volume. Water volume was held constant by a central standpipe (below the top of the enclosures to prevent escape) and seawater was supplied to each enclosure at a ca. rate of 25 L hr^{-1}. Each feed treatment incorporated four fiberglass tanks each containing four cylindrical enclosures (n = 16 individuals per feed treatment). The fiberglass tanks were connected within a temperature-controlled semi-recirculating aquaculture system with mechanical and biological filtration, foam fractionation and UV sterilization. Seawater was exchanged in the semi-recirculating systems at an approximate rate of 10% volume day^{-1}.

Salinity, temperature, and dissolved oxygen levels were maintained at 32 ± 2 ppt, 22 ± 2 °C, and 7 ± 2 ppm, respectively, during the experimental period. Photoperiod was maintained at 12 h light: 12 h dark. Ammonia, nitrite, nitrate and pH levels were checked weekly and were less than 0.1 ± 0.05 ppm, 0.1 ± 0.05 ppm, 5 ± 2 ppm, and 8 ± 0.3, respectively, during the 12 week trial period.

2.2 Feeds and feed preparation

Four semi-purified feeds (Table 1) that varied in levels of protein and carbohydrate were prepared from practical and purified ingredients, blended with a twin shell dry blender (Patterson-Kelley Co., East Stroudsburg, PA) for 10 minutes, and mixed in a Hobart mixer (Model A-200, Hobart Corporation, Troy, OH) for 40 minutes. Deionized water (500 ml kg^{-1}) was then added to the dry ingredients and mixed an additional 10 minutes to achieve a mash consistency appropriate for extrusion. Extrusion was accomplished using a meat chopper attachment (Model A-200, Hobart Corporation, Troy, OH)

fitted with a 4.8 mm die. Moist feed strands were dried on wire racks in a forced air oven at 35 °C to a moisture content of 7.0 to 9.4%, placed into zip-lock bags and stored in a refrigerator at 4 °C until used. Protein and carbohydrate levels were adjusted by adding different levels of soy protein isolate and pure starch. All other ingredient levels remained constant (Table 1).

Proximate analysis of feed protein content was 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. Energy content was determined by micro-bomb calorimetry (Parr Instrument Company, Moline, Illinois). All values presented are absolute dry values unless otherwise indicated.

2.3 Consumption

Approximately 50 g of feed (weighed to the nearest mg) was placed into labeled zip-lock bags assigned to each of the experimental urchins in each feed treatment ($n = 16$ bags per treatment). Sea urchins were fed one of the four feeds once daily at a rate that exceeded the estimated daily ration. Prior to the next feeding, uneaten food was removed by siphon. Daily consumption was estimated by visual inspection of the amount of feed remaining in each enclosure to nearest ¼ of the daily ration proffered. For each 4-week period, the amount of feed proffered and consumed was estimated for each individual.

Daily consumption (g food consumed individual^{-1} day^{-1}, as fed) was calculated as follows:

$$\frac{\text{Total feed proffered (g)} - \text{total estimated uneaten feed (g)}}{\text{Number of days}}$$

Total feed consumption (g individual^{-1}, as fed) over the 12-week study was calculated as:

Total feed proffered (g) – total estimated uneaten feed (g)

Protein consumption (g dry protein consumed individual^{-1}) over the 12-week period was calculated as:

[Total feed proffered (g dry feed) – total estimated uneaten feed (g dry feed)] x % dry protein

Carbohydrate consumption (g dry carbohydrate consumed individual^{-1}) over the 12-week period was calculated as:

[Total feed proffered (g dry feed)-total estimated uneaten feed (g dry feed)] x % dry carbohydrate

Energy consumption (kilocalories energy consumed individual^{-1}) over the 12-week period was calculated as:

[Total feed proffered (g dry feed) – total estimated uneaten feed (g dry feed)] x energy content of feed (Kcal g^{-1})

2.4 Growth

The test diameter, immersion weight and total wet weight were measured as previously described. Estimated specific growth rate (SGR; percent increase in body weight day^{-1}) was calculated as:

$$\frac{\text{Ln final wet weight (g)} - \text{ln initial wet weight (g)}}{\text{Time (days)}} \times 100$$

Feed conversion ratio was calculated as:

$$\frac{\text{Total feed consumed (g), as fed}}{\text{Wet weight final} - \text{wet weight initial}}$$

Wet weight gain over the 12-week period was calculated as:

Final wet weight – initial wet weigh

Protein: carbohydrate ratio was calculated at 12 weeks as:

$$\frac{\text{Dry protein consumption (g)}}{\text{Dry carbohydrate consumption (g)}}$$

Protein: energy ratio was calculated at 12 weeks as:

$$\frac{\text{Dry protein consumption (g)}}{\text{Energy content feed (Kcal)}}$$

2.5 Production

Estimated dry matter production was calculated as:

$$\text{Final dry weight (g)} - \text{initial dry weight (g)}$$

Production efficiency was calculated as:

$$\frac{\text{Final dry weight (g)} - \text{initial dry weight (g)}}{\text{Dry feed consumed (g)}} \times 100$$

Estimated gonad production was calculated as:

$$\text{Final dry weight gonad (g)} - \text{initial dry weight gonad (g)}$$

Estimated gonad production efficiency was calculated as:

$$\frac{\text{Final dry weight gonad (g)} - \text{initial dry weight gonad (g)}}{\text{Dry feed consumed (g)}} \times 100$$

Estimated protein efficiency ratio was calculated as:

$$\frac{\text{Final dry weight (g)} - \text{initial dry weight (g)}}{\text{Dry weight protein consumed (g)}}$$

2.6 Statistics

Statistical comparisons for feed consumption, protein consumption, test diameter, immersion weight and wet weight at 4, 8 and 12 weeks were completed using SAS (version 9.1). These parameters were analyzed as the dependent variable in a repeated measures model with feed protein level as the predictor. The PROC MIXED procedure was

used to analyze the relationship between each parameter and time accounting for group differences. Group means in 4-week increments were compared using the Tukey's adjustment. Statistical comparisons for feed consumption, protein consumption and specific growth rate at 4-week periods were performed on the Systat 11 software package (Systat Software Inc., Point Richmond, CA.). All other statistical comparisons were performed on the Systat 11 software package. If data were normally distributed and were homoscedastic, parametric tests including ANOVA and ANCOVA were completed. When significant differences were determined, a Tukey's test for pairwise group comparisons was used. A P value of < 0.05 was determined statistically significant for all parametric tests. If data were non-normally distributed or heteroscedastic, data transformations were attempted. When ANCOVA analysis was completed, tables of both actual means and least square means were presented. If data transformations were not successful, non-parametric tests (Kruskal Wallis, Mann Whitney U, or Kolmogorov-Smirnov two-way tests) were employed. To maintain an overall acceptance criteria of $\alpha = 0.05$ during multiple comparisons, a Bonferroni's adjustment was adopted.

3.0 Results

Water quality remained within stated levels during the 12-week study and was assumed not to be a factor affecting growth and efficiency parameters.

3.1 Survival

Survival was 100% in all feed treatments for the entire 12-week study.

3.2 Consumption

The predictive model from The Mixed Procedure indicated there was an overall significant time effect, group x time effect, but not a significant group effect for food consumed individual^{-1} day^{-1} and total consumption of food (Table 2A). Food consumed individual^{-1} day^{-1} and total food consumed was not different among the feed treatments at

Table 2A
Mixed model analysis of consumption data from repeated measures (mixed procedure type 3 tests of fixed effects)

The mixed procedure		
Type 3 tests of fixed effects		
Effect	*F* Value	P
Daily Consumption (g) Individual^{-1}Day^{-1}		
Protein	1.89	0.145
Weeks	12.68	<0.001
Weeks x protein	5.50	0.001
Total Feed Consumption (g)		
Protein	2.61	0.063
Weeks	18.11	<0.001
Weeks x protein	8.12	<0.001
Total Dry Protein Consumption (g)		
Protein	0.87	0.463
Weeks	18.08	<0.001
Weeks x protein	8.57	<0.001
Total Dry Carbohydrate Consumption (g)		
Protein	22.20	<0.001
Weeks	2058.24	<0.001
Weeks x protein	36.48	<0.001
Energy Consumption (Kcal)		
Protein	19.43	<0.001
Weeks	2066.04	<0.001
Weeks x protein	5.91	<0.001

Table 2B
Pairwise comparison of consumption parameters

Parameter	Week	Dietary Protein: Carbohydrate 17:47 vs. 21:44	17:47 vs. 25:39	17:47 vs. 31:33	21:44 vs. 25:39	21:44 vs. 31:33	25:39 vs. 31:33
Daily Feed Consumption Individual^{-1} Day^{-1} (g)	4	1.000	0.994	0.893	0.985	0.926	0.769
	8	1.000	0.262	0.015	0.290	0.018	0.576
	12	0.998	0.156	<0.001	0.216	<0.001	0.047
Total Dry Food Consumed (g)	4	1.000	1.000	0.876	0.999	0.893	0.870
	8	0.999	0.196	0.002	0.241	0.002	0.228
	12	0.997	0.057	<0.001	0.088	<0.001	0.009
Total Dry Protein Consumption (g)	4	0.675	0.106	<0.001	0.628	0.003	0.068
	8	<0.001	<0.001	<0.001	0.003	<0.001	<0.001
	12	<0.001	<0.001	<0.001	<0.001	<0.001	0.007
Total Dry Carbohydrate Consumption (g)	4	0.960	0.568	0.262	0.851	0.529	0.944
	8	0.060	<0.001	<0.001	<0.001	<0.001	<0.001
	12	0.032	<0.001	<0.001	<0.001	<0.001	<0.001
Energy Consumption (Kcal)	4	0.995	0.974	0.670	0.998	0.812	0.894
	8	0.838	1.000	0.124	0.826	0.018	0.131
	12	0.842	0.963	<0.001	0.563	<0.001	0.004

Consumption parameters were analyzed as the dependent variables in repeated measures models with feed protein as the predictor. The PROC MIXED procedure was used to analyze the relationship between average consumption and time accounting for group differences. The group means at various points were compared using the Tukey's adjustment. The significance was determined for the entire 12-week study (overall) and for each 4-week period following the start of the study.

week 4 (Table 2B and Table 3). At week 8, sea urchins fed the 31:33% protein: carbohydrate feed consumed significantly less food than those fed the 17:47% and 21:44% protein:carbohydrate feeds but not the 25:39% protein:carbohydrate feed. At week 12, sea urchins fed the 31:33% protein:carbohydrate feed consumed significantly less food than urchins fed the 17:47%, 21:44%, and 25:39% protein:carbohydrate feeds. Food

Table 3
Consumption parameters analyzed at 4, 8, and 12 weeks for sea urchins fed four different protein: carbohydrate feeds

Parameters	Dietary Protein: Carbohydrate (%)	Week 4		Week 8		Week 12	
Daily Feed Consumption (g) Individual^{-1} Day^{-1}	17:47	0.13 ± 0.01	A	0.18 ± 0.01	A	0.21 ± 0.01	A
	21:44	0.13 ± 0.01	A	0.18 ± 0.01	A	0.21 ± 0.01	A
	25:39	0.13 ± 0.01	A	0.17 ± 0.01	AB	0.19 ± 0.01	A
	31:33	0.14 ± 0.01	A	0.16 ± 0.01	B	0.17 ± 0.01	B
Total Feed Consumption (g)	17:47	3.85 ± 0.18	A	10.05 ± 0.41	A	18.27 ± 0.71	A
	21:44	3.88 ± 0.13	A	10.00 ± 0.45	A	18.17 ± 0.51	A
	25:39	3.82 ± 0.15	A	9.43 ± 0.31	AB	16.86 ± 0.53	A
	31:33	4.00 ± 0.21	A	9.21 ± 0.43	B	14.91 ± 0.67	B
Total Dry Protein Consumption (g)	17:47	0.60 ± 0.03	B	1.57 ± 0.06	D	2.86 ± 0.11	D
	21:44	0.74 ± 0.03	B	1.91 ± 0.09	C	3.47 ± 0.10	C
	25:39	0.88 ± 0.04	AB	2.18 ± 0.07	B	3.89 ± 0.12	B
	31:33	1.12 ± 0.06	A	2.58 ± 0.12	A	4.17 ± 0.19	A
Total Dry Carbohydrate Consumption (g)	17:47	1.66 ± 0.08	A	4.33 ± 0.18	A	7.87 ± 0.31	A
	21:44	1.56 ± 0.05	A	4.01 ± 0.18	A	7.29 ± 0.20	B
	25:39	1.37 ± 0.05	A	3.39 ± 0.11	B	6.06 ± 0.19	C
	31:33	1.18 ± 0.06	A	2.72 ± 0.13	C	4.41 ± 0.20	D
Energy Consumption (Kcal)	17:47	12.63 ± 0.59	A	32.94 ± 1.35	A	59.87 ± 2.32	A
	21:44	13.12 ± 0.45	A	33.79 ± 1.51	AB	61.42 ± 1.71	A
	25:39	13.35 ± 0.52	A	32.98 ± 1.07	AB	58.97 ± 1.85	A
	31:33	13.86 ± 0.73	A	31.90 ± 1.50	B	51.65 ± 2.31	B

Numbers represent means ± standard errors and letters indicate statistical differences among feeds.

consumed individual^{-1}day^{-1} and total food consumed did not differ significantly among the feed treatments until the last period of the study (weeks 8-12) at which time sea urchins fed the 31:33% protein:carbohydrate feed consumed significantly less food than sea urchins in the other feed treatments ($F = 10.604$, $df = 3$, $P \leq 0.007$ individual^{-1} day^{-1}; $F = 10.60$, $df = 3$, $P \leq 0.007$ total; Table 4).

Table 4
Consumption parameters analyzed at three different periods (0-4 weeks, 4-8 weeks, and 8-12 weeks) for sea urchins fed four different protein: carbohydrate feeds

Parameters	Dietary Protein: Carbohydrate (%)	Weeks 0-4		Weeks 4-8		Weeks 8-12	
Daily Feed Consumption (g) Individual^{-1} Day $^{-1}$	17:47	0.13 ± 0.01	A	0.22 ± 0.01	A	0.27 ± 0.01	A
	21:44	0.13 ± 0.01	A	0.22 ± 0.01	A	0.27 ± 0.01	A
	25:39	0.13 ± 0.01	A	0.20 ± 0.01	A	0.25 ± 0.01	A
	31:33	0.14 ± 0.01	A	0.19 ± 0.01	A	0.19 ± 0.01	B
Total Feed Consumption (g)	17:47	3.85 ± 0.18	A	6.20 ± 0.31	A	8.22 ± 0.42	A
	21:44	3.88 ± 0.13	A	6.11 ± 0.35	A	8.18 ± 0.40	A
	25:39	3.82 ± 0.15	A	5.61 ± 0.21	A	7.43 ± 0.30	A
	31:33	4.00 ± 0.21	A	5.21 ± 0.31	A	5.70 ± 0.32	B
Total Dry Protein Consumption (g)	17:47	0.60 ± 0.03	C	0.97 ± 0.05	C	1.29 ± 0.07	B
	21:44	0.74 ± 0.03	BC	1.17 ± 0.07	BC	1.56 ± 0.08	AB
	25:39	0.88 ± 0.04	B	1.29 ± 0.05	AB	1.71 ± 0.07	A
	31:33	1.12 ± 0.06	A	1.46 ± 0.09	A	1.60 ± 0.09	A
Specific Growth Rate (SGR)	17:47	1.08 ± 0.06	C	1.19 ± 0.06	C	0.57 ± 0.09	A
	21:44	1.38 ± 0.05	B	1.36 ± 0.04	BC	0.65 ± 0.06	A
	25:39	1.59 ± 0.05	AB	1.48 ± 0.05	AB	0.73 ± 0.11	A
	31:33	1.63 ± 0.07	A	1.61 ± 0.08	A	0.77 ± 0.04	A

Numbers represent means ± standard errors and letters indicate statistical differences among feeds.

The predictive model from The Mixed Procedure indicated an overall significant time effect, group x time effect but not a significant group effect for the total dry protein consumed (Table 2A). Sea urchins fed the 31:33% protein:carbohydrate feed consumed significantly more dry protein than the other treatments at week 4 (Tables 2B and 3). At week 8 the dry protein consumed was significantly different among all the treatments. Sea urchins fed the 31:33% protein: carbohydrate feed consumed significantly more dry protein than both the 17:47% and 21:44% protein:carbohydrate feeds ($F = 32.11$, $df = 3$, $P \leq 0.001$, first period; $F = 10.23$, $df = 3$, $P \leq 0.012$, second period; Table 4). In the final period of the study (weeks 8-12), sea urchins fed the 17:47% protein:carbohydrate feed

had consumed significantly less dry protein than the 25:39% and 31:33% protein: carbohydrate feeds but not the 21:44% protein:carbohydrate feed ($F = 5.83$, $df = 3$, $P \leq 0.025$). There were no differences in total dry protein consumed among sea urchins fed the 21: 44%, 25:39%, and 31:33% protein:carbohydrate feeds in the final period of the study.

The predictive model from The Mixed Procedure indicated an overall significant group effect, time effect and group x time effect for the total dry carbohydrate consumption (Table 2A). There was no significant difference in the total dry carbohydrate consumption among the feed treatments at week 4 (Table 3). Total dry carbohydrate consumed varied indirectly with feed protein content (directly with feed carbohydrate content) at weeks 8 and 12.

The predictive model from The Mixed Procedure indicated an overall significant group effect, time effect, and group x time effect for energy consumption (Table 2A). There was no significant difference in energy consumption among the feed treatments at weeks 4 or 8 (Table 2B and Table 3). At week 12, energy consumption was significantly less in urchins fed the 31:33% protein:carbohydrate feed but was not different among the other feed treatments.

3.3 Growth

The predictive model from The Mixed Procedure indicated an overall significant group effect, time effect but not a significant group x time effect for the specific growth rate (SGR) of sea urchins in the study (Table 5A). Sea urchins fed the 31: 33% protein: carbohydrate feed had a significantly higher SGR than sea urchins fed the 17: 47 or 21: 44% protein: carbohydrate feeds but not the 25: 39% protein: carbohydrate feed during

the first two periods (0-4 weeks and 4-8 weeks) of the study ($F = 18.071$, $df = 3$, $P \leq 0.019$ for weeks 0-4; $F = 9.077$, $df = 3$, $P \leq 0.02$ for weeks 4-8; Table 4). In the final period study (weeks 8-12), there were no significant differences in the specific growth rate among the feed treatments.

The predictive model from The Mixed Procedure indicated an overall significant group effect, time effect and group x time effect for test diameter (Table 5A). Sea urchins fed the 17:47% protein:carbohydrate feed had a significantly smaller test diameter than urchins fed the 31:33% or 25:39% protein: carbohydrate feeds but not the 21:44% protein: carbohydrate feed at week 4 (Tables 5B and 6). At both weeks 8 and 12, sea urchins fed the 31:33% protein: carbohydrate feed had a significantly larger test diameter than urchins fed the 17:47% or 21: 44% protein: carbohydrate feeds but not the 25: 39% protein: carbohydrate feed. The predictive model from The Mixed Procedure indicated an overall significant time effect, group x time effect, but not a group effect for both immersion weight and wet weight (Table 5A). At week 4, the immersion weight of sea urchins fed the 17:47% protein: carbohydrate feed was significantly less than sea urchins fed the 31:33% but not the 21:44% or 25:39% protein:carbohydrate feeds (Tables 5B and 6). At weeks 8 and 12, the immersion weight of sea urchins varied directly with protein level and sea urchins fed the 31:33% protein:carbohydrate feed had significantly higher immersion weights (except for the 25:39% protein:carbohydrate feed at week 12) than the other feed treatments (Tables 5B and 6). At week 4, the total wet weight of sea urchins fed the 31:33% protein:carbohydrate feed was significantly higher than sea urchins fed the 17:47% but not the 21:44% or 25:39% protein: carbohydrate feeds (Tables 5B and 6).

Table 5A
Mixed model analysis of growth data from repeated measures (mixed procedure type 3 tests of fixed effects)

The Mixed Procedure		
Type 3 tests of fixed effects		
Effect	F Value	*P*
Specific Growth Rate		
Protein	17.87	<0.001
Weeks	9.35	0.003
Weeks x protein	1.71	0.167
Test Diameter		
Protein	294.86	<0.001
Weeks	71.95	<0.001
Weeks x protein	6.71	<0.001
Immersion Weight		
Protein	0.29	0.833
Weeks	35.99	<0.001
Weeks x protein	9.06	<0.001
Wet Weight		
Protein	0.22	0.884
Weeks	52.47	<0.001
Weeks x protein	10.21	<0.001

At weeks 8 and 12 the total wet weight was different among all the feed treatments and varied directly with protein level.

There was no significant linear relationship ($R = 0.088$, $P = 0.488$) between the energy consumption and wet weight gain at week 12; however, there was a significant linear relationship ($R = 0.585$, $P < 0.001$) between protein consumption and wet weight gain (Fig. 1A and 1B). There were also significant curvilinear relationships between wet weight gain and both protein: carbohydrate ratio ($R = 0.692$, $P < 0.001$) and protein: energy ratio ($R = 0.694$, $P < 0.001$) at week 12 (Figures 2A and 2B).

Table 5B
Pairwise comparison of growth parameters

Parameter	Weeks	Dietary Protein: Carbohydrate 17:47 vs. 21:44	17:47 vs. 25:39	17:47 vs. 31:33	21:44 vs. 25:39	21:44 vs. 31:33	25:39 vs. 31:33
Test diameter (mm)	4	0.186	0.025	0.017	0.804	0.723	0.999
	8	<0.001	<0.001	<0.001	0.088	0.001	0.241
	12	<0.001	<0.001	<0.001	0.140	0.001	0.108
Immersion weight (g)	4	0.063	0.109	0.007	0.994	0.820	0.677
	8	<0.001	<0.001	<0.001	0.432	0.001	0.045
	12	<0.001	<0.001	<0.001	0.125	0.001	0.105
Wet weight (g)	4	0.282	0.104	0.018	0.952	0.582	0.877
	8	<0.001	<0.001	<0.001	0.045	<0.001	0.018
	12	<0.001	<0.001	<0.001	0.024	<0.001	0.013

Growth parameters were analyzed as the dependent variables in repeated measures models with feed protein as the predictor. The PROC MIXED procedure was used to analyze the relationship between average consumption and time accounting for group differences. The group means at various points were compared using the Tukey's adjustment. The significance was determined for the entire 12-week study (overall) and for each 4-week period following the start of the study.

Table 6
Growth parameters analyzed at 4, 8, and 12 weeks for sea urchins fed four different protein: carbohydrate feeds

Parameters	Initial		Dietary Protein: Carbohydrate (%)	4 Weeks		8 Weeks		12 Weeks	
Test Diameter	29.47 ± 0.20	A	17:47	31.89 ± 0.22	B	35.59 ± 0.24	C	38.07 ± 0.38	C
	29.67 ± 0.17	A	21:44	32.80 ± 0.23	AB	37.21 ± 0.27	B	40.43 ± 0.30	B
	29.27 ± 0.25	A	25:39	33.13 ± 0.26	A	38.03 ± 0.38	AB	41.36 ± 0.54	AB
	29.48 ± 0.25	A	31:33	33.16 ± 0.32	A	38.64 ± 0.45	A	42.37 ± 0.56	A
Immersion Weight (g)	1.70 ± 0.03	A	17:47	2.19 ± 0.04	B	3.00 ± 0.06	C	3.66 ± 0.08	C
	1.77 ± 0.04	A	21:44	2.47 ± 0.05	AB	3.54 ± 0.07	B	4.37 ± 0.09	B
	1.73 ± 0.04	A	25:39	2.49 ± 0.05	AB	3.55 ± 0.10	B	4.68 ± 0.16	AB
	1.76 ± 0.03	A	31:33	2.59 ± 0.05	A	3.81 ± 0.10	A	4.92 ± 0.19	A
Wet Weight (g)	12.61 ± 0.21	A	17:47	17.25 ± 0.25	B	24.09 ± 0.50	D	28.69 ± 0.88	D
	12.76 ± 0.25	A	21:44	19.03 ± 0.38	AB	27.81 ± 0.56	C	33.86 ± 0.76	C
	12.34 ± 0.27	A	25:39	19.56 ± 0.37	AB	29.64 ± 0.74	B	36.99 ± 1.25	B
	12.58 ± 0.26	A	31:33	20.21 ± 0.49	A	31.93 ± 1.25	A	40.26 ± 1.67	A

Numbers represent means ± standard errors and letters indicate statistical differences among feeds.

3.4 Organ wet and dry weight

Best wet weight did not vary among the feed treatments at the conclusion of the study (Tables 7A and 7B). Sea urchins fed the 31:33% protein:carbohydrate feed had a significantly higher test dry weight than urchins fed the 17:47% and 21:44% protein: carbohydrate feeds but not the 25:39% protein:carbohydrate feed at week 12 ($F = 5.293$, $df = 3$, $P \leq 0.038$). Sea urchins fed the 17:47% protein:carbohydrate feed had significantly lower test moisture content than the other feed treatments at week 12 ($F = 7.192$, $df = 3$, $P \leq 0.048$). Wet weight, dry weight or moisture content of the Aristotle's lantern did not vary significantly among the feed treatments. Gut wet and dry weight did not differ significantly among feed treatments (Tables 8A and 8B). Sea urchins fed the 31:33% protein: carbohydrate feed had significantly higher gut moisture content than the other feed treatments ($F = 8.697$, $df = 3$, $P \leq 0.008$). Gonad wet weight, dry weight and moisture content did not differ significantly among the feed treatments at week 12.

3.5 Production and Efficiency

The feed conversion ratio (FCR) of sea urchins varied indirectly with feed protein and directly with the carbohydrate level (Table 9). Sea urchins fed the 31:33% protein: carbohydrate feed had a significantly lower FCR than sea urchins fed the 17:47% or 21: 44% protein: carbohydrate feeds but not the 25:39% protein: carbohydrate feed at week 12 ($F = 39.054$, $df = 3$, $P < 0.001$). Sea urchins fed the 31:33% protein: carbohydrate feed had significantly higher production and production efficiency than sea urchins fed the 17: 47% or 21:44% protein: carbohydrate feeds ($F = 14.084$, $df = 3$, $P \leq 0.032$, production; $F = 19.309$, $df = 3$, $P \leq 0.003$, production efficiency; Table 9). Although production did not

Table 7A
Wet weight (g), dry weight (g) and moisture content (%) of the test and Aristotle's lantern at 12 weeks for sea urchins fed four different protein: carbohydrate feeds

Organ	Parameters	Initial	Dietary Protein: Carbohydrate (%)	12 Weeks
Test	Wet Weight (g)	5.74 ± 0.17	17:47	10.49 ± 0.22
			21:44	13.17 ± 0.28
			25:39	14.46 ± 0.52
			31:33	15.40 ± 0.73
	Dry Weight (g)	2.90 ± 0.08	17:47	5.47 ± 0.12
			21:44	6.58 ± 0.15
			25:39	7.05 ± 0.26
			31:33	7.46 ± 0.30
	Moisture (%)	49.34 ± 0.44	17:47	47.81 ± 0.60
			21:44	50.05 ± 0.49
			25:39	51.16 ± 0.60
			31:33	51.23 ± 0.68
Lantern	Wet Weight (g)	0.66 ± 0.02	17:47	1.03 ± 0.03
			21:44	1.11 ± 0.04
			25:39	1.08 ± 0.04
			31:33	1.05 ± 0.03
	Dry Weight (g)	0.38 ± 0.01	17:47	0.57 ± 0.02
			21:44	0.62 ± 0.02
			25:39	0.62 ± 0.02
			31:33	0.60 ± 0.02
	Moisture (%)	43.06 ± 1.00	17:47	44.66 ± 1.10
			21:44	44.11 ± 1.26
			25:39	42.20 ± 1.35
			31:33	42.97 ± 1.08
Gut	Wet Weight (g)	0.24 ± 0.01	17:47	0.83 ± 0.04
			21:44	0.85 ± 0.03
			25:39	0.90 ± 0.04
			31:33	1.06 ± 0.05

Table 7A (Continued)

Organ	Parameters	Day 0	Dietary Protein: Carbohydrate (%)	12 Weeks
	Dry Weight (g)	0.05 ± 0.01	17:47	0.20 ± 0.01
			21:44	0.21 ± 0.01
			25:39	0.22 ± 0.01
			31:33	0.25 ± 0.01
	Moisture (%)	79.23 ± 0.25	17:47	74.97 ± 0.48
			21:44	75.26 ± 0.47
			25:39	74.51 ± 0.33
			31:33	77.12 ± 0.21
Gonad	Wet Weight (g)	0.07 ± 0.02	17:47	3.75 ± 0.27
			21:44	4.98 ± 0.33
			25:39	5.45 ± 0.31
			31:33	6.20 ± 0.43
	Dry Weight (g)	0.02 ± 0.01	17:47	1.26 ± 0.08
			21:44	1.69 ± 0.12
			25:39	1.83 ± 0.10
			31:33	1.89 ± 0.10
	Moisture (%)	77.00 ± 0.90	17:47	66.15 ± 0.83
			21:44	65.98 ± 0.92
			25:39	66.03 ± 0.71
			31:33	68.93 ± 0.80

Numbers represent actual means ± standard errors.

Table 7B
Wet weight (g), dry weight (g) and moisture content (%) of the test and Aristotle's lantern at 12 weeks for sea urchins fed four different protein: carbohydrate feeds

Organ	Parameters	Initial	Dietary Protein: Carbohydrate (%)	12 Weeks	
Test	Wet Weight (g)	5.74 ± 0.17	17:47	13.18 ± 0.31	A
			21:44	13.38 ± 0.24	A
			25:39	13.57 ± 0.25	A
			31:33	13.38 ± 0.28	A
	Dry Weight (g)	2.90 ± 0.08	17:47	5.85 ± 0.29	B
			21:44	6.48 ± 0.25	B
			25:39	6.80 ± 0.25	AB
			31:33	7.48 ± 0.30	A
	Moisture (%)	49.34 ± 0.44	17:47	47.81 ± 0.60	B
			21:44	50.05 ± 0.60	A
			25:39	51.16 ± 0.60	A
			31:33	51.23 ± 0.60	A
Lantern	Wet Weight (g)	0.66 ± 0.02	17:47	1.10 ± 0.04	A
			21:44	1.12 ± 0.04	A
			25:39	1.06 ± 0.04	A
			31:33	1.00 ± 0.04	A
	Dry Weight (g)	0.38 ± 0.01	17:47	0.60 ± 0.03	A
			21:44	0.62 ± 0.02	A
			25:39	0.60 ± 0.02	A
			31:33	0.58 ± 0.03	A
	Moisture (%)	43.06 ± 1.00	17:47	44.66 ± 1.2	A
			21:44	44.11 ± 1.2	A
			25:39	42.20 ± 1.2	A
			31:33	42.97 ± 1.2	A
Gut	Wet Weight (g)	0.24 ± 0.01	17:47	0.97 ± 0.04	A
			21:44	0.86 ± 0.03	A
			25:39	0.86 ± 0.04	A
			31:33	0.94 ± 0..04	A
	Dry Weight (g)	0.05 ± 0.01	17:47	0.24 ± 0.01	A
			21:44	0.21 ± 0.01	A

Table 7B (Continued)

Organ	Parameters	Initial	Dietary Protein: Carbohydrate (%)	12 Weeks	
			25:39	0.22 ± 0.01	A
			31:33	0.21 ± 0.01	A
	Moisture (%)	79.23 ± 0.25	17:47	74.97 ± 0.39	B
			21:44	75.26 ± 0.39	B
			25:39	74.51 ± 0.38	B
			31:33	77.12 ± 0.39	A
Gonad	Wet Weight (g)	0.07 ± 0.02	17:47	5.20 ± 0.34	A
			21:44	5.10 ± 0.26	A
			25:39	4.97 ± 0.27	A
			31:33	5.12 ± 0.31	A
	Dry Weight (g)	0.02 ± 0.01	17:47	1.31 ± 0.14	A
			21:44	1.77 ± 0.11	A
			25:39	1.69 ± 0.12	A
			31:33	1.83 ± 0.13	A
	Moisture (%)	77.00 ± 0.90	17:47	66.15 ± 0.82	A
			21:44	65.98 ± 0.82	A
			25:39	66.03 ± 0.82	A
			31:33	68.93 ± 0.82	A

Numbers represent least squares means ± standard errors after ANCOVA analysis and letters indicate statistical differences among feeds.

vary between urchins fed the 31:33% and 25:39% protein:carbohydrate feeds, those fed the 31:33% protein: carbohydrate feed had significantly higher production efficiency (F = 19.309, df = 3, $P \leq 0.003$) than those fed the 25:39% protein: carbohydrate feed. Gonad production was significantly less in sea urchins fed the 17:47% protein: carbohydrate feed than in urchins fed the other three feeds (F = 8.112, df = 3, $P \leq 0.016$; Table 9).

Table 8A
Wet weight (g), dry weight (g) and moisture content (%) of the gut and gonad at 12 weeks for sea urchins fed four different protein: carbohydrate feeds

Organ	Parameters	Initial	Dietary Protein: Carbohydrate (%)	12 Weeks
Test	Wet Weight (g)	5.74 ± 0.17	17:47	10.49 ± 0.22
			21:44	13.17 ± 0.28
			25:39	14.46 ± 0.52
			31:33	15.40 ± 0.73
	Dry Weight (g)	2.90 ± 0.08	17:47	5.47 ± 0.12
			21:44	6.58 ± 0.15
			25:39	7.05 ± 0.26
			31:33	7.46 ± 0.30
	Moisture (%)	49.34 ± 0.44	17:47	47.81 ± 0.60
			21:44	50.05 ± 0.49
			25:39	51.16 ± 0.60
			31:33	51.23 ± 0.68
Lantern	Wet Weight (g)	0.66 ± 0.02	17:47	1.03 ± 0.03
			21:44	1.11 ± 0.04
			25:39	1.08 ± 0.04
			31:33	1.05 ± 0.03
	Dry Weight (g)	0.38 ± 0.01	17:47	0.57 ± 0.02
			21:44	0.62 ± 0.02
			25:39	0.62 ± 0.02
			31:33	0.60 ± 0.02
	Moisture (%)	43.06 ± 1.00	17:47	44.66 ± 1.10
			21:44	44.11 ± 1.26
			25:39	42.20 ± 1.35
			31:33	42.97 ± 1.08
Gut	Wet Weight (g)	0.24 ± 0.01	17:47	0.83 ± 0.04
			21:44	0.85 ± 0.03
			25:39	0.90 ± 0.04
			31:33	1.06 ± 0.05

Table 8A (Continued)

Organ	Parameters	Day 0	Dietary Protein: Carbohydrate (%)	12 Weeks
	Dry Weight (g)	0.05 ± 0.01	17:47	0.20 ± 0.01
			21:44	0.21 ± 0.01
			25:39	0.22 ± 0.01
			31:33	0.25 ± 0.01
	Moisture (%)	79.23 ± 0.25	17:47	74.97 ± 0.48
			21:44	75.26 ± 0.47
			25:39	74.51 ± 0.33
			31:33	77.12 ± 0.21
Gonad	Wet Weight (g)	0.07 ± 0.02	17:47	3.75 ± 0.27
			21:44	4.98 ± 0.33
			25:39	5.45 ± 0.31
			31:33	6.20 ± 0.43
	Dry Weight (g)	0.02 ± 0.01	17:47	1.26 ± 0.08
			21:44	1.69 ± 0.12
			25:39	1.83 ± 0.10
			31:33	1.89 ± 0.10
	Moisture (%)	77.00 ± 0.90	17:47	66.15 ± 0.83
			21:44	65.98 ± 0.92
			25:39	66.03 ± 0.71
			31:33	68.93 ± 0.80

Numbers represent actual means ± standard errors.

Table 8B
Wet weight (g), dry weight (g) and moisture content (%) of the gut and gonad at 12 weeks for sea urchins fed four different protein: carbohydrate feeds

Organ	Parameters	Initial	Dietary Protein: Carbohydrate (%)	12 Weeks	
Test	Wet Weight (g)	5.74 ± 0.17	17:47	13.18 ± 0.31	A
			21:44	13.38 ± 0.24	A
			25:39	13.57 ± 0.25	A
			31:33	13.38 ± 0.28	A
	Dry Weight (g)	2.90 ± 0.08	17:47	5.85 ± 0.29	B
			21:44	6.48 ± 0.25	B
			25:39	6.80 ± 0.25	AB
			31:33	7.48 ± 0.30	A
	Moisture (%)	49.34 ± 0.44	17:47	47.81 ± 0.60	B
			21:44	50.05 ± 0.60	A
			25:39	51.16 ± 0.60	A

Table 8B (Continued)

Organ	Parameters	Initial	Dietary Protein:Carb. (%)	12 Weeks	
			31:33	51.23 ± 0.60	A
Lantern	Wet Weight (g)	0.66 ± 0.02	17:47	1.10 ± 0.04	A
			21:44	1.12 ± 0.04	A
			25:39	1.06 ± 0.04	A
			31:33	1.00 ± 0.04	A
	Dry Weight (g)	0.38 ± 0.01	17:47	0.60 ± 0.03	A
			21:44	0.62 ± 0.02	A
			25:39	0.60 ± 0.02	A
			31:33	0.58 ± 0.03	A
	Moisture (%)	43.06 ± 1.00	17:47	44.66 ± 1.2	A
			21:44	44.11 ± 1.2	A
			25:39	42.20 ± 1.2	A
			31:33	42.97 ± 1.2	A
Gut	Wet Weight (g)	0.24 ± 0.01	17:47	0.97 ± 0.04	A
			21:44	0.86 ± 0.03	A
			25:39	0.86 ± 0.04	A
			31:33	0.94 ± 0..04	A
	Dry Weight (g)	0.05 ± 0.01	17:47	0.24 ± 0.01	A
			21:44	0.21 ± 0.01	A
			25:39	0.22 ± 0.01	A
			31:33	0.21 ± 0.01	A
	Moisture (%)	79.23 ± 0.25	17:47	74.97 ± 0.39	B
			21:44	75.26 ± 0.39	B
			25:39	74.51 ± 0.38	B
			31:33	77.12 ± 0.39	A
Gonad	Wet Weight (g)	0.07 ± 0.02	17:47	5.20 ± 0.34	A
			21:44	5.10 ± 0.26	A
			25:39	4.97 ± 0.27	A
			31:33	5.12 ± 0.31	A
	Dry Weight (g)	0.02 ± 0.01	17:47	1.31 ± 0.14	A
			21:44	1.77 ± 0.11	A
			25:39	1.69 ± 0.12	A
			31:33	1.83 ± 0.13	A
	Moisture (%)	77.00 ± 0.90	17:47	66.15 ± 0.82	A
			21:44	65.98 ± 0.82	A
			25:39	66.03 ± 0.82	A
			31:33	68.93 ± 0.82	A

Numbers represent least squares means ± standard errors after ANCOVA analysis and letters indicate statistical differences among feeds.

Gonad production efficiency was significantly higher in sea urchins fed the 31:33% protein:carbohydrate feed than in urchins fed the 17:47% and 21:44% protein: carbohydrate feeds but not the 25:39% protein:carbohydrate feed ($F = 17, 47.299$, $df = 3$, $P \leq 0.020$). There was no difference in the protein efficiency ratio among the feed treatments at week 12 (Table 9).

4.0 Discussion

The current study was conducted with feed pellets that were made by cold extrusion and dried to low moisture content. Protein and carbohydrate were varied using soy protein isolate and pure wheat starch, respectively, with all other ingredients held constant. All of the protein feeds tested in the current study supported 100% survival, weight gain, and gonad production for the duration of the experiment. Sea urchins fed the 31: 33% protein: carbohydrate feed had the lowest overall consumption and highest weight gain, production, production efficiency, and gonad production efficiency of the feeds examined. Gonad production was similar among urchins fed the 31:33%, 25:39%, and 21:44% protein: carbohydrate feeds.

4.1 Consumption

Sea urchins fed the highest protein feed consumed less feed than the other feed treatments. The high energy content of this feed suggests that the sea urchins consumed feed to satisfy an energy requirement. However, energy alone cannot easily explain all of the differences observed in feeding and production rates. Protein (or amino acid) levels, protein: energy ratios or, more specifically, protein: carbohydrate ratios may affect

Table 9
Production and efficiency analyses at 12 weeks for sea urchins fed four different protein: carbohydrate feeds

Parameters	Dietary Protein: Carbohydrate (%)	12 Weeks	
FCR	17:47	1.19 ± 0.07	A
	21:44	0.87 ± 0.04	B
	25:39	0.71 ± 0.04	BC
	31:33	0.56 ± 0.04	C
Estimated Total Production (g)	17:47	4.24 ± 0.21	C
	21:44	5.80 ± 0.28	B
	25:39	6.02 ± 0.31	AB
	31:33	7.06 ± 0.41	A
Estimated Total Production Efficiency (%)	17:47	27.31 ± 2.02	C
	21:44	35.32 ± 2.012	BC
	25:39	39.31 ± 2.45	B
	31:33	53.05 ± 3.04	A
Estimated Gonad Production (g)	17:47	1.24 ± 0.08	B
	21:44	1.68 ± 0.12	A
	25:39	1.81 ± 0.10	A
	31:33	1.87 ± 0.10	A
Estimated Gonad Production Efficiency (%)	17:47	6.90 ± 0.52	C
	21:44	9.36 ± 0.70	B
	25:39	10.86 ± 0.65	AB
	31:33	12.60 ± 0.42	A
Estimated Protein Efficiency Ratio	17:47	1.59 ± 0.12	A
	21:44	1.70 ± 0.10	A
	25:39	1.59 ± 0.10	A
	31:33	1.72 ± 0.10	A

Numbers represent means ± standard errors and letters indicated statistical differences among feeds.

consumption and, consequently, growth rates. Inherent differences in nutrient digestibilities may also affect production.Interestingly, the increased consumption by urchins fed the 17:47%, 21:44%, and 25:39% feeds did not become significant until the final period of the study (weeks 8 to 12) suggesting that dietary requirements were being satisfied early but were later insufficient to support maximal weight gain. Hammer et al. (2006) reported that adult *L. variegatus* (22.8 ± 1.35 g, 36.0 ± 0.80 mm) fed a 9% protein feed consumed less food than urchins fed 20% or 31% protein feeds. Similar results were observed with small *L. variegatus* (1.1 ± 0.35 g, 14.6 ± 0.15 mm, Hammer et al. 2004). Fernandez and Boudouresque (1998) and McBride et al. (1998) indicated an inverse relationship between consumption and protein content for small *Paracentrotus lividus* (5.8 ± 0.9 g, 23.2 ± 1.1 mm) and *Strongylocentrotus franciscanus* (19.9 ± 4.9 g, 34.8 ± 3.8 mm), respectively. Otero-Villanueva et al. (2004) indicated that ingestion rates were lowest for small *Psammechinus miliaris* (8-16 mm) fed a salmon feed (37% protein) than for urchins fed a mussel feed (31% protein), artificial (formulated) feed (27% protein) or algae feed (2% protein). Miller and Mann (1973) suggested that sea urchins in the field may consume large amounts of carbohydrate rich (protein poor) material to process and obtain necessary protein. Despite the increased consumption indicated in the current study, sea urchins fed the 17:47%, 21:44%, or 25:39% protein:carbohydrate feeds consumed less protein than urchins fed the 31:33% protein:carbohydrate feed.

4.2 Growth

Weight gain was directly proportional to the amount of protein consumed and differences were observed within the first 4 weeks of feeding. Weight gain was not directly proportional to the amount of energy consumed. Weight gain was higher than previously reported from laboratory and field observations for this species, with individuals increasing approximately 8.5 mm to 12.5 mm in test diameter and approximately 16 g to 28 g in wet weight in 12 weeks. We suggest the quality of these semi-purified feeds exceeds the quality of the feeds used in previous studies (Hammer et al. 2004; Hammer et al., 2006) with *L. variegatus*. Otero-Villanueva et al. (2004) reported that large *Psammechinus miliaris* (approximately 18 g wet weight and 33 mm test diameter) fed a salmon feed (28% protein) and a feed made from fresh mussel tissue (20% protein) had significantly higher total wet weights than urchins fed an algae feed (3% protein). Small *Paracentrotus lividus* fed 13% protein feeds for 9 months had smaller test diameters than urchins fed 29% or 47% protein feeds after 9 months (Fernandez and Boudouresque, 1998). Small *Pseudocentrotus depressus* (approximately 1.6 g wet weight and 15 mm test diameter) fed a 10% purified protein feed had significantly smaller test diameters than sea urchins fed 21% to 51% purified protein but no significant differences in wet weight were observed over the 8 week study (Akiyama et al., 2001). McBride et al. (1998) indicated no difference in test diameter or wet weight among *S. franciscanus* fed 30%, 40%, or 50% protein feeds for 10 months.

The rate of weight gain (specific growth rate [SGR]) varied with both protein content of the feed and with size of the individual. SGR was highest in those fed the high

protein feed, but decreased as individuals increased in size. Hammer et al. (2006) reported overall SGRs of 0.49%, 0.78%, and 0.59% body weight gain per day for adult *L. variegatus* fed 9%, 20%, and 31% protein diets, respectively. Hofer (2002) reported an SGR of 0.61% body weight gain per day for adult *L. variegatus* (35.8 ± 1.3 g, 42.1. ± 0.60 mm) fed a 20% protein feed at a similar temperature to the present study (22 °C). Wallace (2001) reported that small, rapidly growing *L. variegatus* (1.1 ± 0.35 g, 14.6 ± 0.15 mm) fed a 42% protein feed had higher SGRs (approximately 1.5%) than sea urchins fed 12, 19 or 27% protein feeds after 14 weeks. The high SGRs reported by Wallace (2001) for small sea urchins are indicative of small, rapidly growing sea urchins and are similar to those reported in the current study.

Trends observed for immersion weights were similar to those of wet weight. Grojean et al. (1998) suggested that immersion weights were more sensitive than total wet weight in determining differences in tissue production among sea urchins; however, immersion weights were not more sensitive in the current study. Immersion weights may be more valuable in determining differences in tissue production when gonads (or other organs) vary in size among individuals or experimental treatments. In the current study gonad production was similar in all sea urchins, regardless of protein in the feed.

4.3 Organ Wet Weight, Dry Weight and Moisture Content

Aristotle's lantern, gut and gonad weights did not vary with dietary protein content (when covaried for the weight of the individual), although total weight gain (and total organ production) was proportional to protein consumption. That is, organ allometry did not occur with protein consumption, but total organ biomass increased. These data

suggest that protein affects general metabolic processes related to organismal and organ growth. An exception was seen in the growth of the test, whereas the dry test of individuals fed the highest protein feed was relatively larger (ANCOVA) than the test of individuals fed lower protein feeds. We suggest that high protein levels enhance somatic components reflected in the skeletal formation of the test. Enhanced test growth would probably convey an advantage to individuals, by increasing their overall size, that potentially leads to an enhanced ability to compete for resources, protection from predation, and increased future gonad production (nutrient storage and gamete production). Hammer et al. (2006) reported that 31% and 20% protein feeds supported test growth while a 9% protein feed did not. In both studies, low protein did not support maximal test growth, but did support gonad production (albeit at a lower rate), suggesting that gonad growth is maintained even when feed protein levels are reduced. Hammer et al. (2004) reported that small *L. variegatus* fed a 9% protein feed had significantly lower test dry weight than sea urchins fed 15%, 21%, or 33% protein feeds. In contrast, McBride et al. (1998) reported no difference in the dry test index among *S. franciscanus* fed 30%, 40%, and 50% protein feeds over 10 months.

Lower moisture content of the test in sea urchins fed the 17:47% protein: carbohydrate feed and higher moisture content of the gut in urchins fed the 31:33% protein: carbohydrate feed may be due to the differential hydration state of stored molecules in these tissues (Hammer et al. in press; Hammer et al., 2004). Similarly, Hammer et al. (in press) reported gut moisture content was directly related to dietary protein. Hammer et al. (2004) reported that small *L. variegatus* fed a 9% protein feed had lower test and gut moisture content than urchins fed a 33% protein feed.

4.4 Production

The lower food conversion ratio, higher production, and higher production efficiency of urchins fed the 31:33% protein:carbohydrate feed suggests that this feed promotes efficient somatic growth. Total protein and/or indispensable amino acids may be limiting in feeds with lower protein levels. Energy content was apparently not limiting in any of the feeds. Production efficiencies in the current study were higher (25-48%) than those reported for large adult *L. variegatus* (9-26%) (Hofer, 2002, Hammer et al. 2006) and small *L. variegatus* (12-33%) (Hammer et al. 2004). The high production efficiencies in the current study resulted from a combination of lower consumption and higher production, strongly suggesting that the feeds were of higher nutritional quality. Additionally, the feeds used in the current study (extruded pellets) had much lower water content than those reported in previous studies (wet pellets) with *L. variegatus*. Fernandez and Boudouresque (1998) reported that production efficiency was directly related to protein content of the feed in small *P. lividus* cultured for 9 months. McBride et al. (1998) reported that reduced feed consumption in small *S. franciscanus* fed 40% and 50% protein feeds led to higher production efficiencies than for urchins fed a 30% protein feed. Otero-Villanueva (2004) reported highest production efficiencies in small and large *P. miliaris* fed high protein versus low protein feeds.

Higher dietary protein supports efficient gonad production. The gonad production efficiencies in the current study (6.9-12.6%) are higher than those reported for adult *L. variegatus* (1.9-9.3%) (Hofer, 2002; Hammer et al. 2006) and for small *L. variegatus*

(0.59-4.1%) (Hammer et al. 2004), further supporting the improved nutritional quality of the feeds.

Protein efficiency ratios were similar among the feeds examined, suggesting that adequate non-protein energy was not limiting for the growth rate obtained and that protein was probably not being utilized as a significant energy source. In contrast, Hammer et al. (2006) reported PER values that were significantly lower in a 31% protein feed (compared to 9% and 20% protein feeds) and suggested that the protein was used as an energy source, as carbohydrate was limited in the feed (12% vs. 33% in the current feed). The use of carbohydrate as the primary energy source in sea urchins is suggested by Marsh and Watts (in press). Akiyama et al. (1997) reported PER values ranging from 0.77 to 6.6 (based on wet weight gain) for small *Pseudocentrotus depressus* fed 4 diets of 26% to 30% crude protein. Schlosser et al. (2005) reported that protein efficiency (based on digestible protein) was much higher in adult *P. lividus* fed a prepared feed (23% protein) than that of urchins fed two different algae feeds (37% and 15% protein) because the prepared feed contained more digestible energy.

The protein: energy ratio of the 31:33% protein:carbohydrate feed was approximately 80.79 mg protein $Kcal^{-1}$ and is slightly lower than the ratio used in many commercial shrimp species (ranging from 90-160 mg protein $Kcal^{-1}$ reviewed in Cuzon and Guillaume, 1997). A better defined protein: energy ratio for this species might allow for a reduction in the amount of protein in the feed without compromising growth. Such a reduction in feed protein would reduce feed cost and pollution potential of the feed in aquaculture systems.

The determination of a dietary protein requirement in sea urchins is difficult without also looking at dietary changes in digestible energy (especially dietary protein and carbohydrates). In addition, protein sources used in feeds vary in the levels of indispensable amino acids and the availability of other nutrients if practical ingredients are being used. In this study all nutrients in the feeds were the same except indispensable amino acid and carbohydrate sources, which were derived from two purified ingredients. For this reason the conclusions of this paper are made with the realization that the effect may be the result of variations made to other nutrients when varying dietary protein.

In summary, sea urchins in this study grew at a rate higher than that reported for any other study using adult *L. variegatus*, suggesting that the feed formulations used were of high quality. These data indicate that we are approaching the upper requirement for feed protein levels, although it may be possible to improve upon the 31:33% protein: carbohydrate feed through evaluation of amino acid requirements and nutrient digestibilities. This study suggests that sea urchins are very efficient at converting protein to tissue (regardless of the protein level of the feed) as long as sufficient digestible energy is available (protein sparing). It has been suggested that sea urchins are unlikely to use fats as a direct energy source because of the high oxygen demand of lipid catabolism and their apparent limited ability to transport oxygen to internal organs (Marsh and Watts, in press). If this is the case, then the overwhelming majority of energy will be derived directly from carbohydrate sources in the feeds. We suggest that future feed research focus on protein: energy ratios, indispensable amino acid levels and requirements, and highly digestible protein and carbohydrate sources. These areas of research are essential to the develop-

ment of cost-effective, commercially available feeds for the future aquaculture of sea urchins.

From a commercial standpoint, determination of protein "requirements" in sea urchins is more complex than in finfish. The ultimate goal of commercial sea urchin culture is the production of consumer-preferred roe. Whereas certain protein levels may promote maximum weight gain and roe production, these same levels may negatively impact other important qualities (taste, texture, color) for the successful marketability of roe. Pearce et al. (2002) found that high levels of dietary protein increased the degree of bitterness in the roe of cultured gonads in *Strongylocentrotus droebachiensis*. Thus, determination of protein requirements in urchin culture will require an "optimization" of nutrient levels in formulated feeds, to balance the goal of efficient growth with the production of quality roe.

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References

Akiyama, T., Unuma, T., Yamamoto, T., Furuita, H., Konishi, K., 1997. An evaluation of amino acid sources and binders in semi-purified diet for red sea urchin *Pseudocentrotus depressus*. Fisheries Science 63, 881-886.

Akiyama, T., Unuma, T., Yamamota, T., 2001. Optimum protein level in a purified diet for young red sea urchin *Pseudocentrotus depressus*. Fisheries Science 67, 361-363.

Beddingfield, S.D., McClintock, J.B., 2000. Demographic characteristics of *Lytechinus variegatus* Echinoidea: Echinodermata from three habitats in a North Florida Bay, Gulf of Mexico. Marine Ecology 21, 17- 40.

Cuzon, G., Guillaume, J., 1997. Energy and Protein: Energy Ratio. In: D'Abramo, L., Conklin, D., Akiyama, D. (Eds.). Crustacean Nutrition. World Aquaculture Society, pp. 51-70.

de Jong-Westman, M., March, B.E., Carefoot, T.H., 1995. The effect of different nutrient formulations in artificial diets on gonad growth in the sea urchin *Strongylocentrotus droebachiensis*. Canadian Journal of Zoology 73, 1495-1502 .

Dubois, M., Gilles, K.A., Gilles, J.K., Hamilton, J.K., Rebers, P.A., and Smith, R., 1956. Colorimetric method for determination of sugars and related substances. Analytical Chemistry 28, 350-356.

Fernandez, C., Dombrowski, E., Caltagirone, A., 1995. Gonadic growth of adult sea urchins, *Paracentrotus lividus* (Echinodermata: Echinoidea) in rearing: The effect of different diet types. In: Emson, R., Smith, A., Campbell, A. (Eds.). Echinoderm Research. Balkema, Rotterdam, pp. 269-275.

Fernandez, C., Boudouresque, C., 1998. Evaluating artificial diets for small *Paracentrotus lividus* (Echinodermata: Echinoidea). In: Mooi, R., Telford, M. (Eds.). Echinoderms: San Fransico. Balkema, Rotterdam, pp. 651-656.

Grosjean, P., Spirlet, C., Jangoux, M., 1998. Comparison of three body-size measurements for echinoids. In: Candia, Carnevali, Bonasoro, (Eds.). Echinoderm Research. Balkema Rotterdam, pp. 31-35.

Hammer, B.W., Hammer, H.S., Watts, S.A., Desmond, R.A., Lawrence, J.M., Lawrence, A.L., 2004. The effects of dietary protein concentration on feeding and growth of small *Lytechinus variegatus* (Echinodermata: Echinoidea). Journal of Marine Biology 145, 1143-1157.

Hammer, H.S., Watts, S.A., Lawrence, A.L., Lawrence, J.M., Desmond, R.A., 2006. The effect of dietary protein on consumption, survival, growth and production of the sea urchin *Lytechinus variegatus*. Aquaculture 254, 483-495.

Hammer, H., Hammer, B., Watts, S., Lawrence, A., Lawrence, J., in press. The effect of dietary protein and carbohydrate concentration on the biochemical composition

and gametogenic condition of the sea urchin *Lytechinus variegatus*. Journal of Experimental Marine Biology and Ecology.

Hofer, S.C., 2002. The effect of temperature on feeding and growth characteristics of the sea urchin *Lytechinus variegatus* (Echinodermata: Echinoidea). M. S. Thesis University of Alabama at Birmingham, Birmingham, Alabama.

Lawrence, J.M., Lawrence, A.L., McBride, S.C., George, S.B., Watts, S.A., Plank, L.R., 2001. Developments in the use of prepared feeds in sea-urchin aquaculture. World Aquaculture September, 34-39.

Lawrence, A.L., Lawrence, J.M., 2003. Importance status and future research needs for formulated feeds for sea urchin aquaculture. In: Lawrence, J.M., Guzman, O. (Eds). Sea urchins: fisheries and ecology. DEStech Publications Inc. pp. 275-283.

Marsh, A.G., Watts, S.A., in press. Energy metabolism and gonad development. In: Lawrence, J.M. (Ed.). Edible sea urchins: biology and ecology. Elsevier Science B. V. Amsterdam.

McBride, S.C., Lawrence, J.M., Lawrence, A.L., Mulligan, T.J., 1998. The effect of protein concentration in prepared feeds on growth, feeding rate, total organic absorption, and gross assimilation efficiency of the sea urchin *Strongylocentrotus fransiscanus*. Journal of Shellfish Research 17, 1563-1570.

Miller, R., Mann, K., 1973. Ecological Energetics of the Seaweed Zone in a Marine Bay on the Atlantic Coast of Canada. III. Energy Transformations by Sea Urchins. Marine Biology 18, 99-114.

Moore, H., Jutary, T., Bauer, J., Jones, J., 1963. The biology of *Lytechinus variegatus*. Bulletin of Marine Science of the Gulf and Caribbean 13, 23-53.

Morris, J.G., 1991. Nutrition. In: Prosser, C.L. (Ed.) Environmental and metabolic animal physiology. Wiley-Liss, New York, pp. 231-276.

Otero-Villaneuva, M., Kelly, M., Burnell, G., 2004. How diet influences energy partitioning in the regular echinoid Psammechinus miliaris; constructing an energy budget. Journal of Experimental Marine Biology and Ecology 304, 159-181.

Pearce, C.M., Daggett, T.L., Robinson, S.M.C., 2002. Effect of protein source ratio and protein concentration in prepared diets on gonad yield and quality of the green sea urchin, *Strongylocentrotus droebachiensis*. Aquaculture 214, 307-332.

Schlosser, S., Lupatsch, I., Lawrence, J., Lawrence, A., Shpigel, M., 2005. Protein and energy and digestibility and gonad development of the European sea urchin *Paracentrotus lividus* (Lamarck) fed algal and prepared diets during spring and fall. Aquaculture Research 36, 972-982.

Wallace, B.D., 2001. The effects of dietary protein concentration on feeding and growth of small *Lytechinus variegatus* (Echinodermata: Echinoidea). M. S. Thesis. University of Alabama at Birmingham. Birmingham, Alabama.

Watts, S.A., McClintock, J.B., Lawrence, J.M., 2006. The ecology of *Lytechinus variegatus*. In: Lawrence, J.M. (Ed.) Edible Sea Urchins Biology and Ecology. Elsevier Science B. V., Amsterdam. pp. 375-393.

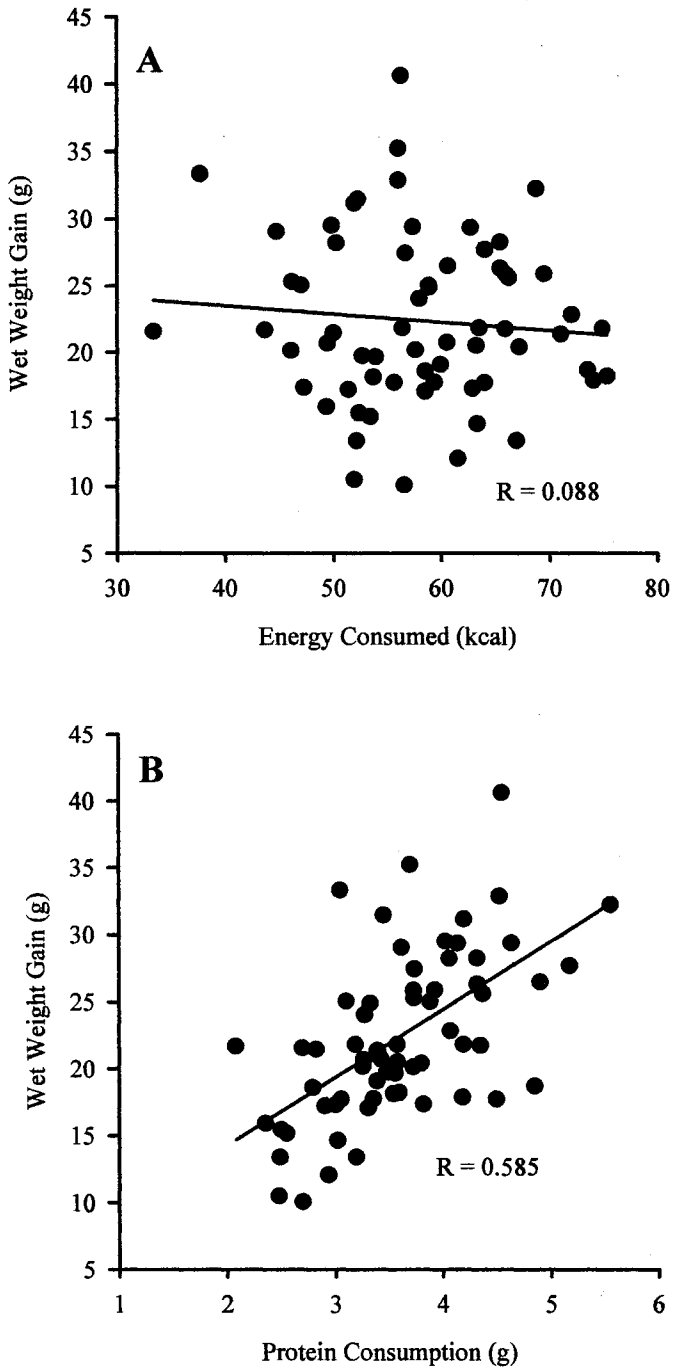

Fig. 1. Scatter plot of wet weight gain (A) versus energy consumption and (B) versus protein consumption at 12 weeks. The regression line of best fit indicates the relationship between the two parameters and the R value indicates the strength of the relationship.

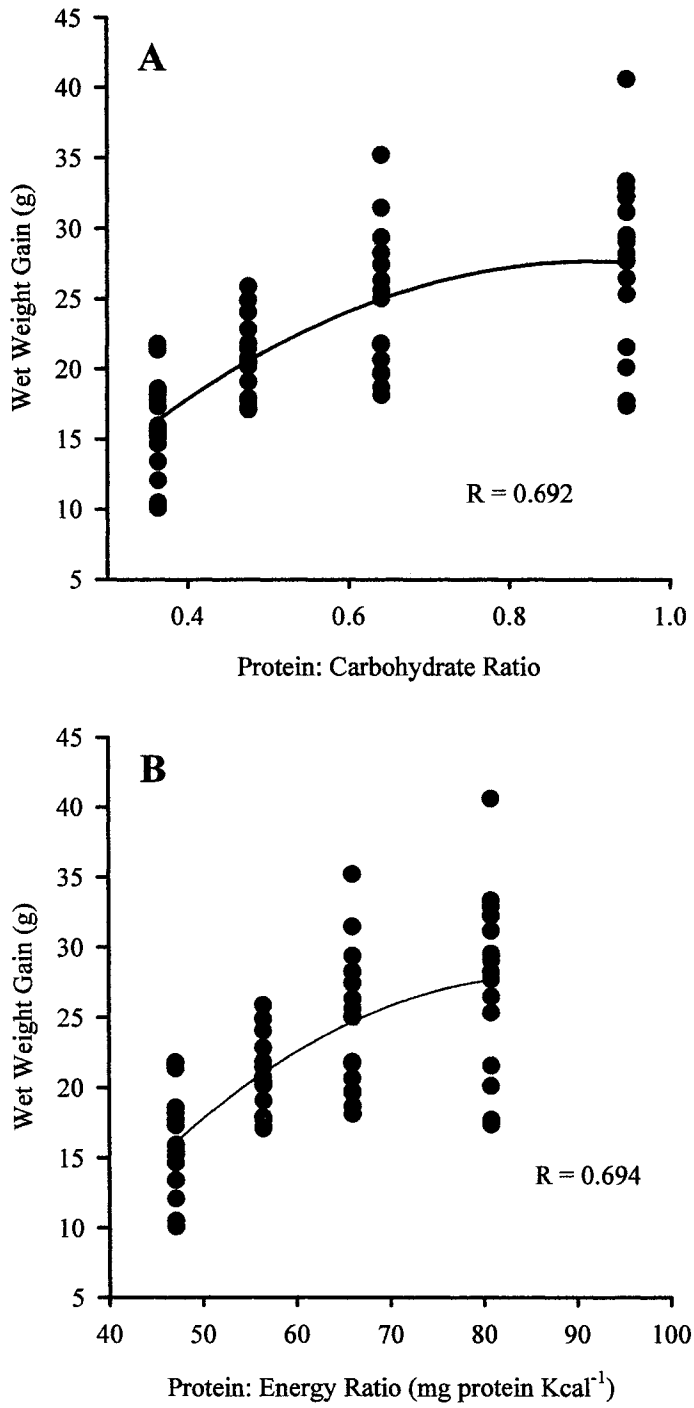

Fig. 2. Scatter plot of wet weight gain (A) versus protein: carbohydrate ratio and (B) versus protein: energy ratio at 12 weeks. The regression line of best fit indicates the relationship between the two parameters and the R value indicates the strength of the relationship.

EFFECT OF DIETARY MENHADEN OIL AND SOY OIL ON CONSUMPTION, SOMATIC GROWTH AND GONAD PRODUCTION OF THE SEA URCHIN *LYTECHINUS VARIEGATUS*

by,

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Format adapted for dissertation

Abstract

Adult sea urchins (12.6 ± 0.12 SE g wet weight, 29.5 ± 0.11 SE mm diameter) were collected from St. Joseph Bay, FL and transported to the Texas A&M Shrimp Mariculture Research Lab in Port Aransas, TX. An initial group of sea urchins (n = 16) were measured, weighed, dissected into component organs, and dried. Sea urchins from the same population were held individually in replicated cylindrical enclosures contained within a recirculating natural seawater system (32 ± 2 ‰ and 22 ± 2 °C). Sea urchins (n = 16 urchins per feed treatment) were fed *ad libitum* semi-purified, cold-extruded feeds that varied in neutral fat source (menhaden oil, MEN; or soy oil, SOY) and level (supplemented at 0, 1, or 4%, respectively) for 12 weeks. Consumption was recorded daily and sea urchins were weighed and diameters measured at 0, 4, 8, and 12 weeks. At 12 weeks sea urchins were dissected into component organs and dried. Digestibility was estimated for all the feeds using the gravimetric method over 7 to 13 continuous days. Survival was 100% in all feed treatments. Sea urchins fed the 1% MEN feed had the lowest consumption and FCR among the MEN feeds; however, there were no significant differences in SGR, immersion weight, wet weight, test diameter, total production, or gonad production among the MEN feed treatments. These data indicate that energy requirements were satisfied in all MEN feeds. Sea urchins fed 4% SOY feed had higher consumption, lower production efficiency and lower gonad production efficiency than the other SOY feed treatments. Sea urchins fed the 0% SOY feed had significantly higher SGR values, immersion weights, wet weights, and test diameters than the other SOY feed treatments. The apparent dry matter digestibility (ADMD), apparent crude protein digestibility (ACPD) and apparent carbohydrate digestibility (ACD) decreased significantly in sea ur-

chins fed both the 4% MEN feed and 4% SOY feed suggesting that digestibility is affected by lipid source and level. Data suggest that supplementation of SOY increased consumption and decreased SGR, weight gain, test diameter, and efficiency. However, production, growth efficiency, and digestibility parameters suggest that (1) a minimum level of marine source neutral fat is required for optimal growth, (2) high levels of soy-based and menhaden-based fatty acids negatively affect growth, (3) fatty acid profiles, not energy levels, affected growth, and (4) ADMD and macronutrient digestibilities are affected by lipid source and concentration. These studies are a necessary first-step to ultimately define essential fatty acid (EFA) requirements and neutral fat levels for sea urchin feeds.

1.0 Introduction

The recent over-fishing of sea urchin stocks world-wide, the ecological damage generated by over-fishing, and an increased demand for sea urchin roe or "uni" has led researchers to pursue aquaculture as an alternative supply for the uni market (reviewed in Lawrence, 2001). For aquaculture to become feasible, a commercially-available formulated feed must be developed (Lawrence and Lawrence, 2004). This feed must have a physical form that will facilitate storage, handling, and feed delivery. Many previous studies with sea urchins have utilized experimental or practical feed types that are not be feasible for use in production aquaculture (Lawrence, 1989; Fernandez et al., 1995; Hammer et al., 2004; Otero-Villanueva et al., 2004; Hammer et al., 2006).

Not only is the physical form of the feed important, but the formulated feed must also provide complete nutrition for rapid growth and quality gonad production (Pearce et

al., 2002). Much of the recent research on formulated feeds in sea urchins has focused on effects of dietary protein (McBride et al., 1998; Pearce et al., 2002; Hammer et al., 2004; Schlosser et al., 2005; Hammer et al., 2006). Few studies have addressed the effects of dietary fat sources or levels on somatic growth and gonad production in sea urchins (Pantazis et al., 2000; Castell et al., 2004). Marine animal and plant oils (e.g. menhaden and soy oil) can be an expensive component of formulated aquaculture feeds and have been shown previously to be required for optimal weight gain in aquacultured crustaceans and fish (reviewed in D'Abramo, 1997; reviewed in Sargent et al., 2002).

Formulated feeds can be well balanced with all of the dietary essential nutrients and still fail to produce favorable growth if the nutrients are not biologically available for use by the organism (Lee and Lawrence, 1997). The biological availability (digestibility) of a feed depends not only on the animal's digestive anatomy and physiology but also on the feed's physical and nutrient characteristics (Lee and Lawrence, 1997). The digestibilities (absorption efficiency) of natural and formulated feeds have been examined in sea urchins (reviewed in Lawrence et al., in press). With each new feed that is developed, detailed studies of feeding and growth parameters, including digestibility, are required to optimize feed formulations.

The sea urchin, *Lytechinus variegatus* has been an important model for the study of sea urchin nutrition due to rapid growth rates and the ease of culture in the laboratory (Lowe and Lawrence, 1976; Klinger et al., 1982; Klinger et al, 1986; Klinger et al., 1988; Klinger et al., 1994; Lares, 1999; Bishop and Watts, 1992; Bishop and Watts, 1994; Watts et al., 1998; Hammer et al., 2004; Hammer et al., 2006; Watts et al., in press). In a previous study, Hammer et al. (2006) described a semi-purified feed that resulted in high

survival, low consumption, rapid weight gain, and high production efficiency. This feed is a dried cold-extruded feed pellet of low moisture (8 to 10%) content that is similar in physical form to those used by the aquaculture industry (Pearce et al., 2002; Castell et al, 2004). Using this feed as a reference, we altered neutral fat sources and levels. Specifically, the concentration of marine source fat (menhaden oil) and non-marine source fat (soy oil) were varied to observe the effects on growth, survival, gonad production and digestibility in adult *Lytechinus variegatus.*

2.0 Materials and Methods

2.1 Collection, culture and initial measurements

Sea urchins, *Lytechinus variegatus*, were collected in October 2004 from Port St. Joseph Peninsula State Park, FL (30° N, 85.5°W) and transported to the Texas A&M Shrimp Mariculture Facility in Port Aransas, TX. At the time of collection, gonads were minimum size (gonads are not observed in field populations until individuals are 35-40 mm in diameter (Moore et al., 1963 Beddingfield and McClintock., 2000). The sea urchins were held for 1 month in 750 L tanks at approximately 32 ± 2 ppt salinity and 22 ± 1° C. Natural seawater was filtered using stratified sand filtration, a Diamond water filter (Diamond Water Conditioning, Horton, WI), and then piped to the systems under flow-through conditions (exchange rate approximately 150% daily). During this period sea urchins were fed a maintenance ration (approximately once every three days) of a formulated feed (31% crude protein, Tables 1 and 2).

To stock the experimental systems, sea urchins (12.6 ± 0.12 SE g wet weight, 29.5 ± 0.11 SE mm diameter) were randomly selected, weighed by immersion according

Table 1
Calculated nutrient values (protein level was determined empirically) on an "as fed" basis for the base feed

Nutrients	Feed Nutrients (% dry weight)
Crude Protein	30.86%
Carbohydrate	32.62%
Crude Fiber	2.50%
Crude Fat	6.52%
Total Ash	23.52%
Moisture	9.35%
Carotenoid	0.42%
Cholesterol	0.22%
Calcium	2.35%
Phospohorous	1.89%
Sodium	1.29%
Potassium	1.63%
Magnesium	0.39%
Iron	319 ppm
Zinc	91 ppm
Manganese	71 ppm
Copper	47 ppm
Selenium	0.228 ppm
Arginine	2.15%
Histidine	0.62%
Isoleucine	1.12%
Leucine	2.00%
Lysine	1.69%
Methionine	0.49%
Cystine	0.27%
Phenylalanine	1.29%
Tyrosine	0.96%
Threonine	1.05%
Tryptophan	0.26%
Valine	1.19%
Vitamin A	4800 IU
Vitamin D	3000 IU
Vitamin E	240 ppm
Vitamin C	349 ppm
Thiamine	36 ppm
Riboflavin	48 ppm
Pyridoxine	96 ppm
Niacine	96 ppm
Pantothenic Acid	36 ppm
Biotin	1 ppm
Inositol	0.10%

Table 1 (Continued)

Nutrients	Feed Nutrients (% dry weight)
Choline	0.10%
Folic Acid	24 ppm
Vitamin B12	.2 ppm

Table 2
Ingredient composition of experimental feeds on an "as fed" basis (MEN = menhaden oil; SOY = soy oil)

Experimental Feed	Menhaden Oil (%)	Soy Oil (%)	Total Neutral Fat (%)*	Purified Plant Starch (%)
0% MEN	0	1	2.3	19.2
1% MEN	1	1	3.3	18.2
4% MEN	4	1	6.3	15.2
0% SOY	1	0	2.3	19.2
1% SOY	1	1	3.3	18.2
4% SOY	1	4	6.3	15.2

All feeds contain approximately 28% marine ingredients, 34.6% plant ingredients, 5.5% crude fat, 1.1% carotenoids, 0.7% vitamin premix, 18.9% mineral premix, 10.2% binder-antifungal-antioxidant.

*Total neutral fat values represent the amount of menhaden oil, soy oil, and calculated marine fat contributed by the practical marine ingredients (1.3% for all feeds) added.

to Grosjean et al. (1999) in saltwater (32 ppt salinity and 22°C), blotted dry with a paper towel to remove excess water, measured for test diameter at two perpendicular points across the ambitus using calipers, and weighed to the nearest mg with a Mettler balance (Mettler Toledo Scales Dublin, Ohio). A random sub-sample of sea urchins (n = 16) were removed from the initial population for dissection. Sea urchins were weighed as described above and dissected. Sea urchins were cut outside the peristomial membrane on the oral surface. During the dissection the test with spines, Aristotle's lantern, gut, and gonads were removed and separated. The gut (esophagus, stomach, and intestine) were rinsed in a finger bowl to remove excess food. Each of the organs were blotted dry with

a paper towel to remove excess water and weighed to the nearest mg (wet weight). Organs were placed onto aluminum pans and dried to constant weight at 60°C. Dry weight was measured and moisture content determined by subtraction. At the end of the study, sea urchins from each of the feed treatments were weighed and dissected.

For the feed trials, sea urchins were placed individually into a cylindrical enclosure that was constructed from a plastic mesh (approximately 12 cm diameter, 30 cm height, and a 4 mm open mesh) secured by plastic cable ties. Mesh enclosures were fitted into 11.5 cm ID PVC couplings so that the floors of the cylindrical enclosures were approximately 5.5 cm above the bottom of the tank. Small plastic spacers (approximately 0.5 cm thick) were then placed under the bottom of each coupling to allow water circulation underneath the enclosures. Four cylindrical enclosures were placed into a 0.07 m^2 bottom surface fiberglass tank with 20 L water volume. Water volume was held constant by a central standpipe (below the top of the enclosures to prevent escape) and seawater was supplied to each enclosure at a ca. rate of 25 L hr^{-1}. Each feed treatment incorporated 4 fiberglass tanks (n = 16 individuals per feed treatment). Fiberglass tanks were connected within a temperature-controlled semi-recirculating aquaculture system with mechanical and biological filtration, foam fractionation and UV sterilization. Seawater was exchanged in the semi-recirculating systems at an approximate rate of 10% volume day^{-1}.

Culture conditions were maintained daily at 32 ± 2 ppt salinity, 22 ± 1 °C and dissolved oxygen 7 ± 2 ppm. Photoperiod was maintained at 12 h light: 12 h dark. Ammonia, nitrite, nitrate and pH levels were checked weekly and were maintained at or below the following levels: ammonia 0.1 ± 0.05 ppm, nitrite 0.1 ± 0.05 ppm, nitrate 5 ± 2 ppm, and pH 8 ± 0.3.

2.2 Feeds and feed preparation

Feeds that varied in fat source (menhaden oil, MEN; or soy oil, SOY) were prepared from a base feed (Table 1) containing semi-purified and purified ingredients (approximately 28% marine source ingredients, 34.6% plant source ingredients, 6.5% crude fat, 1.1% carotenoids, 0.7% vitamin premix, 18.9% mineral premix, 10.2% binder-antifungal-antioxidant). For the MEN feeds, the 6.5% crude fat level was supplemented with 0%, 1%, or 4% menhaden oil to provide 0% MEN, 1% MEN, and 4% MEN feeds, respectively (Table 2). For the SOY feeds, the 6.5% crude fat level was supplemented with 0%, 1%, or 4% soy oil to provide 0% SOY, 1% SOY, and 4% SOY feeds, respectively (total crude fat is 6.5%, 7.5%, and 10.5% for 0%, 1% and 4% MEN and SOY feeds, respectively). With the addition of 1% or 4% menhaden oil or soy oil an equivalent percentage of purified starch was removed. Dry ingredients were blended with a twin shell dry blender (Patterson-Kelley Co., East Stroudsburg, PA) for 10 minutes, and mixed in a Hobart mixer (Model A-200, Hobart Corporation, Troy, OH) for 40 minutes. Deionized water (500 ml kg^{-1}) was then added to the dry ingredients and mixed an additional 10 minutes to achieve a mash consistency appropriate for extrusion. Extrusion was accomplished using a meat chopper attachment (Model A-200, Hobart Corporation, Troy, OH) fitted with a 4.8 mm die. Moist feed strands were dried on wire racks in a forced air oven at 35 °C to a moisture content of 8-10%, placed into zip-lock bags and stored in a refrigerator at 4 °C until used.

2.3 Consumption

Approximately 50 g of feed (weighed to the nearest mg) was placed into labeled zip-lock bags assigned to individual urchins in each feed treatment (n = 16 bags per treatment). Sea urchins were fed one of the four feeds once daily at a rate higher than the estimated daily ration. Prior to the next feeding, uneaten food was removed by siphon. Daily consumption was estimated by visually inspecting the amount of feed remaining in each enclosure to nearest ¼ of the daily ration proffered. For each 4-week period, the amount of feed proffered was determined and the amount of feed consumed was estimated for each individual.

Daily consumption (g food consumed individual^{-1} day^{-1}, as fed) was calculated as follows:

$$\frac{\text{Total feed proffered (g)} - \text{total estimated uneaten feed (g)}}{\text{Number of days}}$$

Total feed consumption (g individual^{-1}, as fed) over the 12-week study was calculated as:

Total feed proffered (g) – total estimated uneaten feed (g)

Marine fat consumption (g dry marine fat consumed individual^{-1}) over the 12-week period was calculated as:

[Total feed proffered (g dry feed) – total estimated uneaten feed (g dry feed)] x % marine fat

Non-marine fat consumption (g dry non-marine fat consumed individual^{-1}) over the 12-week period was calculated as:

[Total feed proffered (g dry feed) – total estimated uneaten feed (g dry feed)] x % non-marine fat

2.4 Growth

The test diameter, immersion weight and total wet weight were measured as previously described. The estimated specific growth rate (SGR; percent increase in body weight per day) was calculated as:

$$\frac{\text{Ln final wet weight (g)} - \text{Ln initial wet weight (g)}}{\text{Time (days)}} \times 100$$

The feed conversion ratio was calculated as:

$$\frac{\text{Total feed consumed (g), as fed}}{\text{Wet weight final} - \text{wet weight initial}}$$

2.5 Production

The estimated dry matter production was calculated as:

$$\text{Final dry weight (g)} - \text{initial dry weight (g)}$$

The production efficiency was calculated as:

$$\frac{\text{Final dry weight (g)} - \text{initial dry weight (g)}}{\text{Feed consumed (g), as fed}} \times 100$$

The estimated gonad production was calculated as:

$$\text{Final dry weight gonad (g)} - \text{initial dry weight gonad (g)}$$

The estimated gonad production efficiency was calculated as:

$$\frac{\text{Final dry weight gonad (g)} - \text{initial dry weight gonad (g)}}{\text{Feed consumed (g), as fed}} \times 100$$

2.6 Digestibility Trials

Methods appear in Appendix A.

2.6 Statistics

Statistical comparisons for feed consumption, protein consumption, test diameter, immersion weight and wet weight at 4, 8 and 12 weeks were completed using SAS (version 9.1). These parameters were analyzed as the dependent variable in a repeated measures model with feed oil level as the predictor. The PROC MIXED procedure was used to analyze the relationship between each parameter and time accounting for group differences. Group means in 4-week increments were compared using the Tukey's adjustment. Statistical comparisons for feed consumption, oil consumption and specific growth rate at 4-week periods were performed on the Systat 11 software package (Systat Software Inc., Point Richmond, CA.). All other statistical comparisons were performed on the Systat 11 software package. Data were normal and homoscedastic, thus, parametric tests including ANOVA and ANCOVA were used. When significant differences were determined, a Tukey's test for pairwise group comparisons was used. A P value of < 0.05 was determined statistically significant for all parametric tests.

3.0 Results

3.1 Water Quality

The observed values for ammonia, nitrite, nitrate, temperature, salinity and pH remained within acceptable levels throughout the study and indicate that adequate water quality was maintained during the experiment.

3.2 Survival

Survival of sea urchins was 100% in all feed treatments.

3.3 Consumption, menhaden and soy oil feed treatments

There was no difference in total food consumption among the MEN feed treatments during the first period (0-4 weeks) (Table 3). In both the second and final periods (weeks 4-8 and 8-12) sea urchins fed the 1% MEN feed consumed significantly less total food ($F = 15.227$, $df = 2$, $P \leq 0.002$; $F = 24.310$, $df = 2$, $P < 0.001$, respectively) than sea urchins in other MEN feed treatments. There were no significant differences in total food consumed among the SOY feed treatments during the first period of the study (weeks 0-4; Table 3). In the final two periods (weeks 4-8 and 8-12), sea urchins fed the 4% SOY feed consumed significantly more total food ($F = 3.519$, $df = 2$, $P \leq 0.032$; $F = 10.397$, $df = 2$, $P \leq 0.029$, respectively) than sea urchins fed the 1% SOY feed (and the 0% SOY feed during the last period).

3.4 Growth, menhaden oil feed treatments

The predictive model from The Mixed Procedure indicated an overall significant group effect but no time effect or group x time effect for specific growth rates among the MEN feed treatments (Table 4A). Pairwise comparisons indicated no significant differences in the SGR among MEN feed treatments at 4, 8, or 12 weeks (Table 4B and Table 5).

The predictive model from The Mixed Procedure indicated an overall significant time effect but no group effect or group x time effect for immersion weight and wet weight among the MEN feed treatments (Table 4A). The predictive model from The Mixed Procedure indicated an overall significant group effect, time effect, but no group x time effect for test diameter among the MEN feed treatments. Pairwise comparisons indi-

cated that no significant differences in immersion weight, wet weight, or test diameter among the MEN feeds at 4, 8, or 12 weeks (Table 4B and Table 5).

Table 3
Consumption parameters analyzed at three different time periods (weeks 0-4, weeks 4-8, and weeks 8-12) for feeds that vary in menhaden oil (MEN) and soy oil (SOY)

Parameters	Feed	Week 0-4		Week 4-8		Week 8-12	
Total Food Consumed (g)	0% MEN	4.22 ± 0.15	A	6.63 ± 0.29	A	7.79 ± 0.33	A
	1% MEN	4.00 ± 0.21	A	5.21 ± 0.31	B	4.16 ± 0.26	B
	4% MEN	4.09 ± 0.10	A	7.36 ± 0.24	A	8.08 ± 0.33	A
Marine Fat Consumed (g)	0% MEN	0.05 ± 0.01	C	0.08 ± 0.01	C	0.09 ± 0.01	B
	1% MEN	0.08 ± 0.01	B	0.11 ± 0.01	B	0.09 ± 0.01	B
	4% MEN	0.20 ± 0.01	A	0.36 ± 0.01	A	0.39 ± 0.02	A
Nonmarine Fat Consumed (g)	0% MEN	0.20 ± 0.01	A	0.31 ± 0.02	A	0.37 ± 0.02	A
	1% MEN	0.19 ± 0.01	A	0.25 ± 0.02	B	0.20 ± 0.01	B
	4% MEN	0.20 ± 0.01	A	0.35 ± 0.01	A	0.39 ± 0.02	A
Total Food Consumed (g)	0% SOY	3.80 ± 0.12	A	5.55 ± 0.26	AB	5.93 ± 0.24	B
	1% SOY	4.00 ± 0.21	A	5.21 ± 0.31	B	4.16 ± 0.26	B
	4% SOY	4.03 ± 0.13	A	6.17 ± 0.20	A	6.92 ± 0.25	A
Marine Fat Consumed (g)	0% SOY	0.08 ± 0.01	A	0.12 ± 0.01	AB	0.12 ± 0.01	B
	1% SOY	0.08 ± 0.01	A	0.11 ± 0.01	B	0.09 ± 0.01	B
	4% SOY	0.09 ± 0.01	A	0.13 ± 0.01	A	0.15 ± 0.01	A
Nonmarine Fat Consumed (g)	0% SOY	0.15 ± 0.01	C	0.21 ± 0.01	B	0.23 ± 0.01	B
	1% SOY	0.19 ± 0.01	B	0.25 ± 0.02	B	0.20 ± 0.01	B
	4% SOY	0.30 ± 0.01	A	0.47 ± 0.02	A	0.52 ± 0.02	A

Numbers represent means ± standard errors and letters indicated significant differences among feed treatments.

Table 4A
Mixed model analysis of growth data from repeated measures (mixed procedure type 3 tests of fixed effects)

The Mixed Procedure		
Type 3 tests of fixed effects		
Effect	F Value	P
MEN Feeds SGR		
Diet	31.6	<0.001
Week	3.04	0.084
Week x diet	0.37	0.692
MEN Feeds Immersion Weight		
Diet	0.13	0.875
Week	28.56	<0.001
Week x diet	0.12	0.890
MEN Feeds Wet Weight		
Diet	1.23	0.316
Week	31.59	<0.001
Week x diet	0.10	0.905
MEN Feeds Diameter		
Diet	198.63	<0.001
Week	42.96	<0.001
Week x diet	0.34	0.712
SOY Feeds SGR		
Diet	63.26	<0.001
Week	3.43	0.066
Week x diet	3.01	0.053
SOY Feeds Immersion Weight		
Diet	0.57	0.570
Week	46.87	<0.001
Week x diet	2.74	0.069
SOY Feeds Wet Weight		
Diet	1.94	0.145
Week	55.26	<0.001
Week x diet	2.08	0.129
SOY Feeds Diameter		
Diet	257.39	<0.001
Week	58.54	<0.001
Week x diet	2.60	0.078

Table 4B
Pairwise comparisons of growth parameters

Parameter	Weeks	Feeds		
		1% MEN vs. 0% MEN	1% MEN vs. 4% MEN	0% MEN vs. 4% MEN
Specific Growth Rate (SGR)	4	0.744	0.871	0.970
	8	0.972	0.997	0.952
	12	0.901	0.829	0.988
Immersion Weight (g)	4	0.945	0.873	0.983
	8	1.000	0.958	0.958
	12	0.946	0.990	0.983
Wet Weight (g)	4	0.934	0.740	0.920
	8	0.992	0.780	0.844
	12	0.979	0.991	0.947
Test Diameter (mm)	4	0.553	0.251	0.834
	8	0.526	0.278	0.886
	12	0.931	0.921	1.000
		1% SOY vs. 0% SOY	1% SOY vs. 4% SOY	0% SOY vs. 4% SOY
Specific Growth Rate (SGR)	4	0.001	0.362	<0.001
	8	0.004	0.020	<0.001
	12	0.890	0.071	0.029
Immersion Weight (g)	4	0.068	0.627	0.361
	8	<0.001	0.980	<0.001
	12	<0.001	0.784	<0.001
Wet Weight (g)	4	0.075	0.667	0.352
	8	<0.001	1.000	<0.001
	12	0.002	0.656	<0.001
Test Diameter (mm)	4	0.002	0.113	0.209
	8	<0.001	0.625	<0.001
	12	<0.001	0.669	<0.001

Growth parameters were analyzed as the dependent variables in repeated measures models with feed as the predictor. The PROC MIXED procedure was used to analyze the relationship between average growth and time accounting for group differences. The group means at various points were compared using the Tukey's adjustment. The significance was determined for the entire 2-week study (overall) and for each 4-week period following the start of the study. (MEN = menhaden oil, SOY = soy oil).

Table 5
Growth parameters analyzed at 4, 8, and 12 weeks for sea urchins fed feeds that vary in menhaden oil (MEN) or soy oil (SOY)

Parameters	Day 0		Feed	Week 4		Week 8		Week 12	
Specific Growth Rate (SGR)	-		0% MEN	1.70 ± 0.11	A	1.60 ± 0.07	A	1.35 ± 0.04	A
	-		1% MEN	1.63 ± 0.07	A	1.62 ± 0.05	A	1.33 ± 0.04	A
			4% MEN	1.67 ± 0.06	A	1.61 ± 0.04	A	1.32 ± 0.03	A
Immersion Weight (g)	1.75 ± 0.03	A	0% MEN	2.61 ± 0.08	A	3.86 ± 0.14	A	4.76 ± 0.17	A
	1.76 ± 0.03	A	1% MEN	2.59 ± 0.05	A	3.81 ± 0.10	A	4.92 ± 0.19	A
	1.76 ± 0.04	A	4% MEN	2.62 ± 0.06	A	3.91 ± 0.10	A	4.77 ± 0.13	A
Wet Weight (g)	12.63 ± 0.20	A	0% MEN	20.80 ± 0.68	A	31.81 ± 1.24	A	39.47 ± 1.53	A
	12.58 ± 0.26	A	1% MEN	20.21 ± 0.49	A	31.93 ± 1.25	A	40.26 ± 1.67	A
	13.0 ± 0.27	A	4% MEN	21.15 ± 0.55	A	32.66 ± 0.93	A	39.71 ± 1.23	A
Test Diameter (mm)	29.13 ± 0.18	A	0% MEN	33.78 ± 0.33	A	38.87 ± 0.51	A	42.33 ± 0.59	A
	29.48 ± 0.25	A	1% MEN	33.16 ± 0.32	A	38.64 ± 0.45	A	42.37 ± 0.56	A
	29.28 ± 0.24	A	4% MEN	34.07 ± 0.35	A	39.07 ± 0.41	A	42.33 ± 0.49	A
Specific Growth Rate (SGR)	-		0% SOY	1.88 ± 0.08	A	1.71 ± 0.04	A	1.41 ± 0.03	A
	-		1% SOY	1.63 ± 0.07	B	1.62 ± 0.05	B	1.33 ± 0.04	AB
	-		4% SOY	1.56 ± 0.06	B	1.50 ± 0.04	C	1.24 ± 0.03	B
Immersion Weight (g)	1.79 ± 0.03	A	0% SOY	2.82 ± 0.05	A	4.22 ± 0.10	A	5.28 ± 0.12	A
	1.76 ± 0.03	A	1% SOY	2.59 ± 0.05	A	3.81 ± 0.10	B	4.92 ± 0.19	B
	1.83 ± 0.02	A	4% SOY	2.67 ± 0.05	A	3.87 ± 0.08	B	4.73 ± 0.10	B
Wet Weight (g)	13.3 ± 0.19	AB	0% SOY	22.55 ± 0.50	A	35.42 ± 1.00	A	43.54 ± 1.34	A
	12.58 ± 0.26	B	1% SOY	20.21 ± 0.49	A	31.93 ± 1.25	B	40.26 ± 1.67	B
	13.59 ± 0.27	A	4% SOY	21.1 ± 0.63	A	32.00 ± 0.95	B	38.68 ± 1.09	B
Test Diameter (mm)	29.64 ± 0.17	A	0% SOY	34.94 ± 0.31	A	40.45 ± 0.39	A	43.98 ± 0.50	A
	29.48 ± 0.25	A	1% SOY	33.16 ± 0.32	B	38.64 ± 0.45	B	42.37 ± 0.56	B
	29.79 ± 0.22	A	4% SOY	34.18 ± 0.37	AB	38.79 ± 0.48	B	41.76 ± 0.55	B

Numbers represent means ± standard errors and letters indicate statistical differences among feeds.

3.5 Growth, soy oil feed treatments

The predictive model from The Mixed Procedure indicated an overall significant group effect but no time effect or group x time effect for specific growth rates among the SOY feed treatments (Table 4A). The 0% SOY feed had a significantly greater SGR value than the other feed treatments at 4, 8, and 12 weeks (except the 1% SOY feed at 12 weeks, Table 4B and Table 5). The predictive model from The Mixed Procedure indicated an overall significant time effect but no group effect or group x time effect for both immersion weight and wet weight among the SOY feed treatments (Table 4A). There was no difference in immersion weight among the feed treatments at week 4 (Table 4B and Table 5). By week 4 there were no significant differences in wet weight among the feed treatments. Sea urchins fed the 0% SOY feed had significantly higher immersion weights and wet weights than the other feed treatments at 8 and 12 weeks (Table 4B and Table 5). The predictive model from The Mixed Procedure indicated an overall significant group effect and time effect but no group x time effect for test diameter among the SOY feed treatments (Table 4A). Sea urchins fed the 0% SOY feed had higher test diameters than sea urchins fed 1% SOY feed at 4, 8, and 12 weeks and higher test diameters than sea urchins fed the 4% SOY feed at 8 and 12 weeks (Table 4B and Table 5).

3.6 Organ growth, menhaden oil feed treatments

There were no significant differences in the wet or dry weight of the test among the MEN feed treatments at week 12 (Tables 6A and 6B). Sea urchins fed the 0% MEN feed had higher test moisture content than urchins fed the 1% MEN feed ($F = 4.049$, $df = 2$, $P \leq 0.025$) but not the 4% MEN feed. There were no significant differences in the wet

Table 6A
Wet weight (g), dry weight (g) and moisture content (%) of test, Aristotle's Lantern, gut and gonad at 12 weeks for sea urchins fed feeds that vary in menhaden oil (MEN)

Organ	Parameters	Initial	Feeds	Week 12
Test	Wet Weight (g)	5.74 ± 0.17	0% MEN	15.68 ± 0.56
			1% MEN	15.40 ± 0.73
			4% MEN	15.24 ± 0.53
	Dry Weight (g)	2.90 ± 0.08	0% MEN	7.31 ± 0.27
			1% MEN	7.46 ± 0.30
			4% MEN	7.33 ± 0.21
	Moisture (%)	49.34 ± 0.44	0% MEN	53.39 ± 0.40
			1% MEN	51.23 ± 0.68
			4% MEN	51.73 ± 0.57
Lantern	Wet Weight (g)	0.66 ± 0.02	0% MEN	1.06 ± 0.03
			1% MEN	1.05 ± 0.03
			4% MEN	1.07 ± 0.02
	Dry Weight (g)	0.38 ± 0.01	0% MEN	0.59 ± 0.02
			1% MEN	0.60 ± 0.02
			4% MEN	0.61 ± 0.01
	Moisture (%)	43.06 ± 1.00	0% MEN	44.46 ± 1.10
			1% MEN	42.97 ± 1.08
			4% MEN	42.99 ± 1.03
Gut	Wet Weight (g)	0.24 ± 0.01	0% MEN	1.03 ± 0.05
			1% MEN	1.06 ± 0.05
			4% MEN	1.06 ± 0.03
	Dry Weight (g)	0.05 ± 0.01	0% MEN	0.22 ± 0.01
			1% MEN	0.25 ± 0.01
			4% MEN	0.25 ± 0.01
	Moisture (%)	79.23 ± 0.25	0% MEN	78.41 ± 0.25
			1% MEN	77.12 ± 0.21
			4% MEN	76.20 ± 0.51
Gonad	Wet Weight (g)	0.07 ± 0.02	0% MEN	6.37 ± 0.34
			1% MEN	6.20 ± 0.43
			4% MEN	5.88 ± 0.32
	Dry Weight (g)	0.02 ± 0.01	0% MEN	1.86 ± 0.11
			1% MEN	1.89 ± 0.10
			4% MEN	1.76 ± 0.12
	Moisture (%)	77.00 ± 0.90	0% MEN	70.92 ± 0.63
			1% MEN	68.93 ± 0.80
			4% MEN	70.25 ± 0.82

Numbers represent actual means ± standard errors.

Table 6B
Wet weight (g), dry weight (g) and moisture content (%) of test, Aristotle's Lantern, gut, and gonad at 12 weeks for sea urchins fed feeds that vary in menhaden oil (MEN)

Organ	Parameters	Feeds	Week 12	
Test	Wet Weight (g)	0% MEN	15.38 ± 0.26	A
		1% MEN	15.40 ± 0.26	A
		4% MEN	15.54 ± 0.26	A
	Dry Weight (g)	0% MEN	7.35 ± 0.10	A
		1% MEN	7.36 ± 0.10	A
		4% MEN	7.36 ± 0.10	A
	Moisture (%)	0% MEN	53.39 ± 0.52	A
		1% MEN	51.23 ± 0.52	B
		4% MEN	51.73 ± 0.52	AB
Lantern	Wet Weight (g)	0% MEN	1.05 ± 0.03	A
		1% MEN	1.05 ± 0.03	A
		4% MEN	1.07 ± 0.03	A
	Dry Weight (g)	0% MEN	0.58 ± 0.02	A
		1% MEN	0.60 ± 0.02	A
		4% MEN	0.61 ± 0.02	A
	Moisture (%)	0% MEN	44.46 ± 0.98	A
		1% MEN	42.97 ± 0.98	A
		4% MEN	42.99 ± 0.98	A
Gut	Wet Weight (g)	0% MEN	1.02 ± 0.03	A
		1% MEN	1.05 ± 0.03	A
		4% MEN	1.08 ± 0.03	A
	Dry Weight (g)	0% MEN	0.23 ± 0.08	B
		1% MEN	0.24 ± 0.08	AB
		4% MEN	0.26 ± 0.08	A
	Moisture (%)	0% MEN	78.41 ± 0.35	A
		1% MEN	77.12 ± 0.35	B
		4% MEN	76.20 ± 0.35	B
Gonad	Wet Weight (g)	0% MEN	6.25 ± 0.29	A
		1% MEN	6.19 ± 0.29	A
		4% MEN	6.00 ± 0.29	A
	Dry Weight (g)	0% MEN	1.86 ± 0.10	A
		1% MEN	1.83 ± 0.10	A
		4% MEN	1.79 ± 0.10	A
	Moisture (%)	0% MEN	70.92 ± 0.79	A
		1% MEN	68.93 ± 0.79	A
		4% MEN	70.25 ± 0.79	A

Numbers represent least square means ± standard errors after ANCOVA analysis and letters indicate statistical differences among feeds

Table 7A
Wet weight (g), dry weight (g) and moisture content (%) of test, Aristotle's Lantern, gut and gonad at 12 weeks for sea urchins fed feeds that vary in soy oil (SOY)

Organ	Parameters	Initial	Feeds	Week 12
Test	Wet Weight (g)	5.74 ± 0.17	0% SOY	17.05 ± 0.53
			1% SOY	15.40 ± 0.73
			4% SOY	14.45 ± 0.40
	Dry Weight (g)	2.90 ± 0.08	0% SOY	8.07 ± 0.22
			1% SOY	7.46 ± 0.30
			4% SOY	7.25 ± 0.18
	Moisture (%)	49.34 ± 0.44	0% SOY	52.56 ± 0.37
			1% SOY	51.23 ± 0.68
			4% SOY	49.74 ± 0.51
Lantern	Wet Weight (g)	0.66 ± 0.02	0% SOY	1.11 ± 0.03
			1% SOY	1.05 ± 0.03
			4% SOY	1.03 ± 0.03
	Dry Weight (g)	0.38 ± 0.01	0% SOY	0.62 ± 0.01
			1% SOY	0.60 ± 0.02
			4% SOY	0.60 ± 0.01
	Moisture (%)	43.06 ± 1.00	0% SOY	44.41 ± 0.91
			1% SOY	42.97 ± 1.08
			4% SOY	41.60 ± 0.75
Gut	Wet Weight (g)	0.24 ± 0.01	0% SOY	1.01 ± 0.04
			1% SOY	1.06 ± 0.05
			4% SOY	0.88 ± 0.04
	Dry Weight (g)	0.05 ± 0.01	0% SOY	0.23 ± 0.01
			1% SOY	0.25 ± 0.01
			4% SOY	0.22 ± 0.01
	Moisture (%)	79.23 ± 0.25	0% SOY	77.80 ± 0.33
			1% SOY	77.12 ± 0.21
			4% SOY	75.06 ± 0.36
Gonad	Wet Weight (g)	0.07 ± 0.02	0% SOY	6.20 ± 0.31
			1% SOY	6.20 ± 0.43
			4% SOY	5.43 ± 0.32
	Dry Weight (g)	0.02 ± 0.01	0% SOY	2.02 ± 0.09
			1% SOY	1.89 ± 0.10
			4% SOY	1.82 ± 0.11
	Moisture (%)	77.00 ± 0.90	0% SOY	67.27 ± 0.60
			1% SOY	68.93 ± 0.80
			4% SOY	66.42 ± 1.02

Numbers represent actual means ± standard errors.

Table 7B
Wet weight (g), dry weight (g) and moisture content (%) of test, Aristotle's Lantern, gut and gonad at 12 weeks for sea urchins fed feeds that vary in soy oil (SOY)

Organ	Parameters	Feeds	Week 12	
Test	Wet Weight (g)	0% SOY	15.87 ± 0.31	A
		1% SOY	15.34 ± 0.29	A
		4% SOY	15.69 ± 0.31	A
	Dry Weight (g)	0% SOY	7.60 ± 0.11	A
		1% SOY	7.64 ± 0.10	A
		4% SOY	7.65 ± 0.11	A
	Moisture (%)	0% SOY	52.56 ± 0.52	A
		1% SOY	51.23 ± 0.52	AB
		4% SOY	49.74 ± 0.52	B
Lantern	Wet Weight (g)	0% SOY	1.08 ± 0.03	A
		1% SOY	1.05 ± 0.03	A
		4% SOY	1.06 ± 0.03	A
	Dry Weight (g)	0% SOY	0.61 ± 0.02	A
		1% SOY	0.61 ± 0.02	A
		4% SOY	0.59 ± 0.02	A
	Moisture (%)	0% SOY	44.41 ± 0.98	A
		1% SOY	42.97 ± 0.98	A
		4% SOY	41.60 ± 0.98	A
Gut	Wet Weight (g)	0% SOY	0.93 ± 0.03	B
		1% SOY	1.05 ± 0.03	A
		4% SOY	0.96 ± 0.03	AB
	Dry Weight (g)	0% SOY	0.21 ± 0.01	B
		1% SOY	0.25 ± 0.01	A
		4% SOY	0.23 ± 0.01	AB
	Moisture (%)	0% SOY	77.80 ± 0.35	A
		1% SOY	77.12 ± 0.35	A
		4% SOY	75.06 ± 0.35	B
Gonad	Wet Weight (g)	0% SOY	5.73 ± 0.32	A
		1% SOY	6.17 ± 0.30	A
		4% SOY	5.91 ± 0.33	A
	Dry Weight (g)	0% SOY	1.82 ± 0.11	A
		1% SOY	1.90 ± 0.10	A
		4% SOY	1.81 ± 0.11	A
	Moisture (%)	0% SOY	67.27 ± 0.79	A
		1% SOY	68.93 ± 0.79	A
		4% SOY	66.42 ± 0.79	A

Numbers represent least square means ± standard errors after ANCOVA analysis and letters indicate statistical differences among feeds

weight, dry weight, or moisture content of the Aristotle's lantern among the MEN feed treatments. There was no significant difference in the wet weight of the gut among the MEN feed treatments. Sea urchins fed the 4% MEN feed had a significantly higher gut dry weight ($F = 4.074$, $df = 2$, $P = 0.022$) than sea urchins fed the 0% MEN feed but not the 1% MEN feed. The moisture content of the gut was significantly higher in sea urchins fed the 0% MEN ($F = 10.094$, $df = 2$, $P \leq 0.035$) feed than the other feed treatments. There was no significant difference in the wet weight, dry weight or moisture content of the gonad among the MEN feed treatments.

3.7 Organ growth, soy oil feed treatments

There were no significant differences in the wet or dry weight of the test among the SOY feed treatments at week 12 (Tables 7A and 7B). Sea urchins fed the 0% SOY-feed had higher test moisture content than urchins fed the High SOY feed ($F = 7.051$, $df = 2$, $P = 0.001$) but not the 1% SOY feed. There were no significant differences in the wet weight, dry weight, or moisture content of the Aristotle's lantern among the SOY feed treatments. Sea urchins fed the 1% SOY feed had a significantly higher gut wet weight and dry weight ($F = 3.775$, $df = 2$, $P = 0.039$; $F = 4.961$, $df = 2$, $P = 0.01$, respectively) than sea urchins fed the 0% SOY feed but not the 4% SOY feed. Sea urchins fed the 4% SOY feed had a significantly lower gut moisture content ($F = 21.739$, $df = 2$, $P < 0.001$) than sea urchins fed the other SOY feeds. There was no significant difference in the wet weight, dry weight or moisture content of the gonad among the SOY feed treatments.

3.8 Efficiency, menhaden and soy oil feed treatments

The FCR was significantly lower for sea urchins fed the 1% MEN feed than for the other MEN feed treatments ($F = 8.305$, $df = 2$, $P \leq 0.004$; Table 8). There was no significant differences in total production or gonad production among the feed treatments; however, sea urchins fed the 1% MEN feed had significantly higher total production efficiency ($F = 10.996$, $df = 2$, $P \leq 0.004$) and gonad production efficiencies ($F = 15.717$, $df = 2$, $P \leq 0.001$) than the other MEN feed treatments.

The FCR for sea urchins fed the 0 and 1% SOY feed was significantly lower than the 4% SOY feed ($F = 11.433$, $df = 2$, $P \leq 0.002$; Table 8). Sea urchins fed the 0% SOY feed had higher total production than sea urchins fed the 4% SOY ($F = 3.848$, $df = 2$, $P = 0.024$) feed but not the 1% SOY feed. There was no significant differences in total gonad production among the SOY feed treatments. Sea urchins fed the 4% SOY feed had significantly lower total and gonad production efficiencies ($F = 12.163$, $df = 2$, $P \leq 0.001$; $F = 9.715$, $df = 2$, $P \leq 0.001$, respectively) than sea urchins in the other SOY feed treatments.

3.9 Digestibility of menhaden and soy oil feeds

Sea urchins that were dissected initially, to assess nutritional condition, had the following metrics: Wet weight (g) 40.53 ± 1.15, test diameter (mm) 45.18 ± 0.276, test dry weight (g) 8.14 ± 0.30, gut dry weight (g) 0.18 ± 0.01, Aristotle's lantern dry weight (g) 0.98 ± 0.05, gonad dry weight (g) 0.19 ± 0.05.

Table 8
Production and efficiency analyses at 12 weeks for sea urchins fed feeds that vary in menhaden oil (MEN) or soy oil (SOY)

Parameters	Feeds	Week 12	
FCR	0% MEN	0.73 ± 0.05	A
	1% MEN	0.54 ± 0.04	B
	4% MEN	0.75 ± 0.03	A
Estimated Total Production (g)	0% MEN	6.52 ± 0.44	A
	1% MEN	7.06 ± 0.41	A
	4% MEN	6.46 ± 0.34	A
Estimated Total Production Efficiency (%)	0% MEN	40.55 ± 3.49	B
	1% MEN	54.81 ± 3.14	A
	4% MEN	36.44 ± 1.81	B
Estimated Gonad Production (g)	0% MEN	1.84 ± 0.11	A
	1% MEN	1.87 ± 0.10	A
	4% MEN	1.74 ± 0.12	A
Estimated Gonad Production Efficiency (%)	0% MEN	11.08 ± 0.78	B
	1% MEN	14.35 ± 0.47	A
	4% MEN	9.65 ± 0.52	B
FCR	0% SOY	0.51 ± 0.02	B
	1% SOY	0.54 ± 0.04	B
	4% SOY	0.70 ± 0.03	A
Estimated Total Production (g)	0% SOY	7.69 ± 0.27	A
	1% SOY	7.06 ± 0.41	AB
	4% SOY	6.41 ± 0.29	B
Estimated Total Production Efficiency (%)	0% SOY	55.90 ± 1.97	A
	1% SOY	54.81 ± 3.14	A
	4% SOY	40.76 ± 1.97	B
Estimated Gonad Production (g)	0% SOY	2.00 ± 0.09	A
	1% SOY	1.87 ± 0.10	A
	4% SOY	1.80 ± 0.11	A
Estimated Gonad Production Efficiency (%)	0% SOY	14.41 ± 0.60	A
	1% SOY	14.35 ± 0.47	A
	4% SOY	11.36 ± 0.60	B

Numbers represent means ± standard errors and letters indicated statistical differences among feeds

The ADMD among the MEN feeds was ca. 77% overall and those sea urchins fed the 4% MEN feed had a significantly lower ADMD, ACPD, and ACD than those sea urchins fed the 0% MEN feed (ANOVA, $F = 13.152$, $df = 2$, $P \leq 0.004$; $F = 3.916$, $df = 2$, $P = 0.029$; Kruskal-Wallis = 28.181, $P < 0.001$, respectively, Table 9A). There were no significant differences in AOMD and AAD among the MEN feeds. The apparent lipid digestibility (ALD) was significantly higher for sea urchins fed the 4% MEN feed than for sea urchins fed the 0% MEN feed but not the 1% MEN feed ($F = 4.707$, $df = 2$, $P \leq 0.019$).

The ADMD among the SOY feeds was ca. 78% overall and those sea urchins fed the 4% SOY feed a significantly lower ADMD, AOMD, AAD, ACPD, and ACD than sea urchins fed the 0% SOY feed ($F = 11.218$, $df = 2$, $P \leq 0.009$; $F = 8.311$, $df = 2$, $P = 0.001$; $F = 6.431$, $df = 2$, $P = 0.00$; $F = 8.092$, $df = 2$, $P = 0.001$, $F = 36.038$, $df = 2$, $P < 0.001$, respectively, Table 9B). The apparent lipid digestibility (ALD) was not significantly different among sea urchins fed the SOY feeds.

4.0 Discussion

The growth rate of *L. variegatus* in the current study is greater than growth rates observed in the field (Moore et al., 1963; Beddingfield and McClintock, 2000; Watts et al., in press) and those previously reported in the laboratory (Hammer et al., 2004; Hammer et al., 2006). These results suggest that the culture conditions (water quality, experimental system and design) and experimental feeds were highly acceptable. In addition, *L. variegatus* held under optimal conditions require a greater daily nutrient requirement; that is, the higher the growth rate the higher the daily nutrient requirement.

Table 9
Digestibility parameters for sea urchins fed feeds that vary in menhaden oil

	Parameter	MEN Feeds	ADMD (%)	
A	ADMD (%)	0% MEN	78.77 ± 0.97	A
		1% MEN	78.56 ± 0.93	A
		4% MEN	72.74 ± 0.88	B
	AOMD (%)	0% MEN	83.5 ± 1.18	A
		1% MEN	83.2 ± 1.04	A
		4% MEN	79.9 ± 2.04	A
	AAD (%)	0% MEN	61.4 ± 3.97	A
		1% MEN	64.2 ± 2.12	A
		4% MEN	52.3 ± 4.78	A
	ACPD (%)	0% MEN	86.8 ± 1.08	A
		1% MEN	85.9 ± 0.70	AB
		4% MEN	83.2 ± 1.01	B
	ACD (%)	0% MEN	95.71 ± 0.28	B
		1% MEN	97.00 ± 0.20	A
		4% MEN	88.30 ± 1.66	C
	ALD (%)	0% MEN	78.11 ± 1.61	B
		1% MEN	83.58 ± 2.72	AB
		4% MEN	86.40 ± 1.16	A
	Parameter	SOY Feeds	ADMD (%)	
B	ADMD (%)	0% SOY	81.11 ± 1.48	A
		1% SOY	78.56 ± 0.93	A
		4% SOY	73.35 ± 1.06	B
	AOMD (%)	0% SOY	86.9 ± 1.14	A
		1% SOY	83.2 ± 1.04	AB
		4% SOY	80.8 ± 1.02	B
	AAD (%)	0% SOY	69.3 ± 3.70	A
		1% SOY	64.2 ± 2.12	AB
		4% SOY	54.5 ± 2.81	B
	ACPD (%)	0% SOY	87.8 ± 0.94	A
		1% SOY	85.9 ± 0.70	AB
		4% SOY	83.4 ± 0.70	B
	ACD (%)	0% SOY	96.75 ± 0.33	A
		1% SOY	97.00 ± 0.20	A
		4% SOY	93.54 ± 0.41	B
	ALD (%)	0% SOY	81.54 ± 3.14	A
		1% SOY	83.58 ± 2.72	A
		4% SOY	78.56 ± 1.42	A

Numbers represent means ± standard errors and letters represent statistical differences among feeds.

Dietary neutral fat provides energy and essential fatty acids for maintenance and growth of many animals. By increasing only the level of purified neutral fat with concomitant decreases in purified plant starch for either marine or non-marine sources, the contribution of neutral fat from either marine or non-marine sources can be evaluated. Furthermore, an evaluation of essential fatty acids can be accomplished by comparing the growth response of animals fed diets having equal levels of marine (menhaden oil) and non-marine (soybean oil) neutral fat because the fatty acid profiles of menhanden oil are significantly different from soybean oil.

Changing the concentration of menhaden oil had little effect on sea urchin weight gain and gonad production under the conditions of this study; however, the efficiency of production was highest in sea urchins fed 1% MEN feed (arising from differences in feed consumption). Those fed the 0% MEN feed were able to meet their fatty acid requirements by consuming more feed. Those fed the 4% MEN feed consumed much more marine neutral fat (and consequently energy) to obtain the same weight gain. These data suggest that energy requirements were being met in all MEN feeds, but that fatty acids levels or ratios (all MEN feeds contained soy oil and soy lecithin) affected the efficiency of weight gain. *Lytechinus variegatus* feed on many taxa of marine plants and invertebrates (reviewed by Watts et al., in press) and would be exposed to a wide variety of marine lipids at various concentrations.

Increasing the level of soy oil increased consumption, but decreased weight gain and production efficiencies. Soy oil did not affect organ weights (except gut dry weight) or gonad production. Energy levels did not appear to be limiting in these feeds. However, decreases in weight gain with increased soy oil suggest that excess linoleic and lino-

lenic fatty acids (or fatty acid ratios) negatively affect growth when levels are high (≥1% soy oil). Low levels of soy oil in feeds produced the highest weight gain and test diameter. Castell et al. (2004) suggested that *Strongylocentrotus droebachiensis* are able to elongate and desaturate 18:2 n-6 and 18:3 n-3 fatty acids and convert them into 20:4 n-6 (Arachidonic acid) and 20:5 n-3 (Eicosapentaenoic acid) fatty acids to meet EFA requirements; however, total crude lipid levels were less than those tested in the current study.

Although high MEN and SOY oil levels (total fat content 10.5% in each feed) had similar growth rates, individuals fed 0% soy oil (1% menhaden oil, total fat content 6.5%) had significantly higher weight gain that those fed 0% menhaden oil (1% soy oil, total fat content 6.5%; Proc Mixed $P = 0.009$). Elimination of supplemental soy oil from the feed resulted in an improved FCR (ANOVA, $F = 10.288$, $df = 4$, $P < 0.001$) and increased production efficiency (ANOVA, $F = 12.233$, $df = 4$, $P = 0.001$). These data suggest that high levels of neutral fat, regardless of the source, negatively affect growth. At low levels the quality (fatty acid composition or ratio) may affect growth, and marine source neutral fats are more desirable than non-marine source neutral fats. In contrast, Pantazis et al. (2000) suggested that feeds containing oleic and linoleic fatty acids (up to 9% crude fat) could support weight gain in *Psammechinus miliaris*. In addition, Castell et al. (2004) reported no significant differences in somatic growth (wet weight and test diameter) among juvenile *Strongylocentrotus droebachiensis* fed 6 different prepared feeds containing 5% lipid from the following sources: corn oil, linseed oil, menhaden oil, corn + linseed oil, corn + menhaden oil, and linseed + menhaden oil. Additionally, both studies suggested that EFA requirements could be supported by some combination of

18:2 n-6 and/or 18:3 n-3. This finding is unusual among marine crustaceans and finfish. Feeding trials with crustaceans consistently document that marine-derived lipids support superior growth compared to vegetable-derived lipids (reviewed in D'Abramo, 1997). In marine finfish, current estimates of the EFA requirements indicate that the n-3 EFA requirement can be met only by dietary sources of 20:5 n-3 and 22:6 n-3 (n-3 HUFA) (Sargent et al., 2002).

The decreased ADMD observed in the 4% MEN and 4% SOY feeds suggests that high levels of these lipid sources decreases digestibility of feed ingredients. This suggestion is supported by the lower digestibility of macronutrients (organic, ash, protein, carbohydrate) in the feeds with 4% lipid source supplementation. In the brown shrimp *Penaeus aztecus* the ADMD was affected by the source of lipid (lard vs menhaden oil, Borrer and Lawrence, 1989). The digestibility of total neutral lipids significantly decreased in the Tiger Prawn *Penaeus monondon* as the amount of dietary lipid increased over 105 mg g^{-1} or 10.5% (Glencross et al., 2002). This study also reported that higher levels of saturated fatty acids (SFA) in the feeds resulted in lower neutral lipid digestibilities. The digestibility of amino acids and total nitrogen was reduced in response to increased dietary lipid levels in the abalone *Haliotis laevigata* (van Barneveld et al., 1998).

The effect of supplemental neutral fat on consumption is complex. Feeds with high fat may not be as palatable or digestible, resulting in reduced production efficiencies. High levels of either supplemented marine source (menhaden oil) or non-marine source (soy oil) fat decreases the digestibility of other required nutrients. Further studies are necessary to determine the role of lipids in digestion, absorption, and assimilation.

The determination of essential fatty acid (EFA) requirements in sea urchins or other organisms is both difficult and expensive. Sargent et al. (2002) stated that the determination of EFA requirements requires consideration of both relative and absolute amounts of individual fatty acids, the animals' innate ability to metabolize fatty acids (whether anabolically or catabolically), and the animals' ability to biosynthesize fatty acids from shorter chain precursor fatty acids. Most commercial and experimental feeds contain practical or semi-purified fat sources, making precise and accurate quantification of specific fatty acids difficult. Furthermore, digestibility of fatty acids has not been investigated in any sea urchin species. Consequently, the ability to conclude that the observed effect is due solely to menhaden or soy oil is limited.

Ultimately, nutrient effects on gonad production and quality are of interest in commercial sea urchin culture. Under the conditions of this study, gonad wet weight, dry weight or production was not affected by the quantity or quality of neutral fat. However, the efficiency of gonad production was reduced at 4% supplemented levels of neutral fat, the apparent result of decreased assimilation of ingested nutrients. Hammer (2006, in press) reported that 4% levels of supplemented neutral fat reduced apparent dry matter digestibility and apparent carbohydrate digestibility in *L. variegatus*. The effects of neutral fat on gonad sensory (taste, color, texture) quality and, consequently, marketability has not been evaluated.

In summary, these data suggest that there is a minimal level of marine source neutral fat is required for optimal growth and development. In addition, high levels of soy oil in the feed decreased growth and growth efficiencies. We suggest that the fatty acid profile of the feed will affect growth of *L. variegatus*.

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References

Beddingfield, S.D., McClintock, J.B., 2000. Demographic characteristics of *Lytechinus variegatus* Echinoidea: Echinodermata from three habitats in a North Florida Bay, Gulf of Mexico. Marine Ecology 21, 17- 40.

Bishop, C.D., Watts, S.A., 1992. Biochemical and morphometric study of growth in the stomach and intestine of the echinoid *Lytechinus variegatus* (Echinodermata). Marine Biology 114, 459-467.

Bishop, C.D., Watts, S.A., 1994. Two stage recovery of gametogenic activity following starvation in *Lytechinus variegatus* Lamarck (Echinodermata: Echinoidea). Journal of Experimental Marine Biology and Ecology 177, 27-36.

Borrer, S., Lawrence, A.L. 1989. Effect of lipids and cellulose on the digestibility of penaeid shrimp diets. Journal of the World Aquaculture Society 20: 122-126. (Abstract)

Castell, J.D., Kennedy, E.J., Robinson, S.M., Parsons, G.J., Blair, T.J., Gonzalez-Duran, E., 2004. Effect of dietary lipids on fatty acid composition and metabolism in juvenile green sea urchins (*Strongylocentrotus droebachiensis*). Aquaculture 242 417-435.

D'Abramo L.R., 1997. Triacylglycerols and fatty acids. In D'Abramo, L.R., Conklin, D.E., Akiyama, D.M. (Eds). Crustacean Nutrition: Advances in World Aquaculture Volume 6. World Aquaculture Society, Baton Rouge, Louisiana pp. 71-84.

Fernandez, C., Dombrowski, E., Caltagirone, A., 1995. Gonadic growth of adult sea urchins, *Paracentrotus lividus* (Echinodermata: Echinoidea) in rearing: The effect of different diet types. In: Emson, R., Smith, A., Campbell, A. (Eds.) Echinoderm Research. Balkema, Rotterdam, pp. 269-275.

Gibbs, V.K., Watts, S.A., 2004. Exposure temperature affects nutrient absorption in *Lytechinus variegatus*. In: Echinoderms: Munchen, T. Heinzeller and J.H. Nebelsick (Eds.) A.A. Balkema Publishers, The Netherlands. pp. 187-192.

Glencross, B.D., Smith, D.M., Thomas, M.R., Williams, K.C., 2002. The effects of dietary lipid amount and fatty-acid composition on the digestibility of lipids by the prawn, *Penaeus monodon*. Aquaculture 205 157-169

Grosjean, P., Spirlet, C., Jangoux, M., 1999. Comparison of three body-size measurements for echinoids. In: Candia, Carnevali, Bonasoro, (Eds.) Echinoderm Research. Balkema Rotterdam, pp. 31-35.

Hammer, B.W., Hammer, H.S., Watts, S.A., Desmond, R.A., Lawrence, J.M., Lawrence, A.L., 2004. The effects of dietary protein concentration on feeding and growth of small *Lytechinus variegatus* (Echinodermata: Echinoidea). Journal of Marine Biology 145, 1143-1157.

Hammer, H.S., Watts, S.A., Lawrence, A.L., Lawrence, J.M., Desmond, R.A., 2006. The effect of dietary protein on consumption, survival, growth and production of the sea urchin *Lytechinus variegatus*. Aquaculture 254, 483-495.

Klinger, T.S., 1982. Feeding rates of *Lytechinus variegatus* Lamarck (Echinodermata: Echinoidea) on differing physiognomies of an artificial food of uniform composition. In: Lawrence, J.M. (Ed.) Echinoderms: Proceedings of the International Conference, Tampa Bay. Balkema, Rotterdam, pp. 29-32.

Klinger, T.S., Hsieh, H.L., Pangallo, R.A., Chen, C.P., Lawrence, J.M., 1986. The effect of temperature on feeding, digestion, and absorption of *Lytechinus variegatus* (Lamarck) (Echinodermata: Echinoidea). Physiological Zoology 59, 332-336.

Klinger, T.S., Watts, S.A., Forcucci, D., 1988. Effect of short-term feeding and starvation on storage and synthetic capacities of gut tissues of *Lytechinus variegatus* (Lamarck) (Echinodermata: Echinoidea). Journal of Experimental Marine Biology and Ecology 117, 187-195.

Klinger, T.S., Lawrence, J.M., Lawrence, A.L., 1994. Digestive characteristics of the sea urchin *Lytechinus variegatus* (Lamarck) (Echinodermata: Echinoidea) fed prepared feeds. Journal of the World Aquaculture Society 25, 489-496.

Lares, M.T., 1999. Evaluation of direct and indirect techniques for measuring absorption efficiencies of sea urchins (Echinodermata: Echinoidea) using prepared feeds. Journal of the World Aquaculture Society 30, 201-207.

Lawrence, J.M., 2001. Edible sea urchins. In: Lawrence, J.M. (Ed.) Edible Sea Urchins Biology and Ecology. Elsevier Science B. V., Amsterdam. pp. 1-4.

Lawrence, A.L., Lawrence, J.M., 2003. Importance status and future research needs for formulated feeds for sea urchin aquaculture. In: Lawrence, J.M., Guzman, O.

(Eds). Sea urchins: fisheries and ecology. DEStech Publications Inc. pp. 275-283.

Lawrence, J., Regis, M., Delmas, P., Gras, G., Klinger, T., 1989. The effect of quality of food on feeding and digestion in Paracentrotus lividus (Lamarck) (Echinodermata: Echinoidea). Marine Behavioral Physiology 15, 137-144.

Lee, P.G., Lawrence, A.L. 1997. Digestibility. In D'Abramo, L.R., Conklin, D.E., Akiyama, D.M. (Eds). Crustacean Nutrition: Advances in World Aquaculture Volume 6. World Aquaculture Society Baton Rouge pp 194-260.

Lowe, E.F., Lawrence, J.M., 1976. Absorption efficiencies of *Lytechinus variegatus* (Lamarck) (Echinodermata: Echinoidea) for selected marine plants. Journal of Experimental Marine Biology and Ecology 21, 223-234.

McBride, S.C., Lawrence, J.M., Lawrence, A.L., Mulligan, T. J., 1998. The effect of protein concentration in prepared feeds on growth, feeding rate, total organic absorption, and gross assimilation efficiency of the sea urchin *Strongylocentrotus fransiscanus*. Journal of Shellfish Research 17, 1563-1570.

Moore, H., Jutary, T., Bauer, J., Jones, J., 1963. The biology of *Lytechinus variegatus*. Bulletin of Marine Science of the Gulf and Caribbean 13, 23-53.

Otero-Villaneuva, M., Kelly, M., Burnell, G., 2004. How diet influences energy partitioning in the regular echinoid Psammechinus miliaris; constructing an energy budget. Journal of Experimental Marine Biology and Ecology 304, 159-181.

Pearce, C.M., Daggett, T.L., Robinson, S.M.C., 2002. Effect of protein source ratio and protein concentration in prepared diets on gonad yield and quality of the green sea urchin, *Strongylocentrotus droebachiensis*. Aquaculture 214, 307-332.

Sargent, J.R., Tocher, D.R., Bell, J.G., 2002. The lipids. In: Halver, J.E., Hardy, R.W. (Eds). Fish Nutrition Third Edition. Elsevier Science B. V., Amsterdam. pp. 181-257.

Schlosser, S., Lupatsch, I., Lawrence, J., Lawrence, A., Shpigel, M., 2005. Protein and energy and digestibility and gonad development of the European sea urchin *Paracentrotus lividus* (Lamarck) fed algal and prepared diets during spring and fall. Aquaculture Research 36, 972-982.

van Barneveld, R.J., Fleming, A.E., Vandepeer, M.E., Kruk, J.A., Hone, P.W., 1998. Influence of dietary oil type and oil inclusion level in manufactured feeds on the digestibility of nutrients by juvenile greenlip abalone (*Haliotis laevigata*). Journal of Shellfish Research 17 649-655.

Watts, S.A., Boettger, S.A., McClintock, J.B., Lawrence, J.M., 1998. Gonad production in the sea urchin *Lytechinus variegatus* (Lamarck) fed prepared diets. Journal of Shellfish Research 17, 1591-1595.

Watts, S.A., McClintock, J.B., Lawrence, J.M., in press. The ecology of *Lytechinus variegatus*. In: Lawrence, J.M. (Ed.) Edible Sea Urchins Biology and Ecology. Elsevier Science B. V., Amsterdam. pp. 375-393.

GENERAL DISCUSSION

Manuscript 1 was the first attempt to examine protein requirements in adult *Lytechinus variegatus*. The feeds used in this study were moist feeds that used agar as binder, had high moisture content, and low nutrient density. The dietary protein levels examined were 9%, 20%, and 31% protein. The 20% protein feed had good survival, moderate consumption, high weight gain, high production efficiency, and a high protein efficiency ratio. These data indicated that the 20% protein feed was used most efficiently by this species of sea urchin. The 31% protein feed had low consumption, high survival, good growth, and high production efficiency but had a lower protein efficiency ratio. It was suggested that the lower protein efficiency ratio was the result of protein being utilized for energy instead of growth. Marsh and Watts (in press) suggested that sea urchins do not use lipid, to any great extent, as an energy source due to the absence of an efficient circulatory system and the lack of oxygen available to internal organs (large amounts of oxygen are required to utilize lipid as an energy source). The 31% protein feed may be lacking in biologically available (digestible) non-protein energy sources (most likely carbohydrate), pressing the utilization of protein for energy production. Further research was needed to further evaluate protein requirements and to address protein requirements when energy was not limiting.

Manuscript 2 examined the effects of dietary protein on the biochemical composition and gametogenic condition of the gonad. The protein and carbohydrate composition of the gonad directly reflected the relative amounts of protein or carbohydrate consumed

in the feed (i.e. 31% protein feed resulted in more protein being stored in the gonad). Image analysis of histological sections from gonad tissue indicated that sea urchins fed the 31% protein feed had smaller volumes of nutritive phagocytes (nutrient storage cells) and larger volumes of germinal epithelium (gamete development cells) and gametes (oocytes or spermatogonia). Likewise, sea urchins fed the 9% protein feed had larger volumes of nutritive phagocytes and smaller volumes of germinal epithelium and gametes. Female sea urchins fed the 31% protein feed had a higher percentage of larger eggs present in histological sections. These findings have important implications for the aquaculture industry as they suggest that macronutrient storage and gametic condition can be manipulated by diet. In this case, the 31% protein feed provided undesirable characteristics for marketable uni because (1) the storage of protein in the gonads is linked to a bitter tasting uni, and (2) increased numbers of gametes in the gonad may result in an undesirable texture for uni.

The research presented in manuscripts 3 and 4 represent great improvements in feed formulation, the physical form of the feed, experimental systems, and experimental methods. Feed formulations were more complete and varied in only two purified ingredients that were changed concomitantly. The physical form of the feeds was also improved to resemble feed types used in commercial aquaculture settings. The feed pellets were made by cold extrusion and dried at low temperature resulting in a stable pellet with low moisture content and high nutrient density. The experimental systems used for these studies were technically advanced and featured automated temperature control, mechanical, biological, and chemical filtration with UV sterilization. The systems were designed for nutritional research with commercial marine shrimp, but were adapted to hold individual

sea urchins in plastic enclosures. The ability to follow individual sea urchins throughout growth trials greatly enhances experimental design, observation, and statistical analysis.

Manuscript 3 revisits protein requirements in adult *Lytechinus variegatus*. The protein: carbohydrate levels used in this study bracketed the "optimal" protein levels determined in manuscript 1. Protein and carbohydrate levels ranged from 17: 47% protein: carbohydrate to 31:33% protein: carbohydrate and varied only in the level of purified protein and purified starch. All of the feeds utilized had at least 30% carbohydrate energy and the total energy content of the feeds varied less than 6%.

The 31:33% protein: carbohydrate extruded feed promoted the best growth of the feeds examined and appeared to have sufficient energy to spare protein. Sea urchins in this study grew faster and more efficiently than any previous field or laboratory study with *Lytechinus variegatus*. Sea urchins fed the 31: 33% protein: carbohydrate feed had the lower total food consumption and lower energy consumption than the other feed treatments yet had the highest weight gain, production, production efficiency, gonad production, and gonad production efficiency. The production efficiency and gonad production efficiency was nearly 50% higher than that reported for the feeds examined in manuscript 1. There was no significant difference in protein efficiency ratio among the feed treatments, suggesting that dietary protein was used primarily for weight gain and not for energy production. The protein energy ratio for the 31: 33% protein: carbohydrate feed was ca. 81 mg protein $Kcal^{-1}$ and is slightly lower than those reported for many commercial shrimp species (ranging from 90 to 160 mg protein $Kcal^{-1}$ reviewed in Cuzon, 1997). The results of this study strongly suggest that further research in protein: energy ratios is needed to address questions regarding protein sparing and dietary energy utilization in

these organisms. Although these data provide an estimate of the protein requirement for this species, future evaluation of the protein: energy ratio may allow for a reduction in the amount of protein without compromising growth. This reduction in feed protein levels would reduce feed costs and potential pollution in an aquaculture setting.

Manuscript 4 was the first attempt to examine the effects of dietary lipid source and level on somatic growth, production, and digestibility in *Lytechinus variegatus*. Using the 31:33 protein: carbohydrate feed (a feed that provided high weight gain and production efficiency) from manuscript 3 as a reference, menhaden oil or soy oil were supplemented at levels of 0%, 1%, or 4%. Increasing levels of menhaden oil had no effect on growth but reduced efficiency above and below the 1% level, suggesting that some level of menhaden oil is necessary for optimal production efficiency. This is in contrast to research conducted with other sea urchin species that suggests there is no requirement for marine source fatty acids (Panazis et al., 2000; Castell et al., 2004). Increasing levels of soy oil had negative effects on growth, production and production efficiency. Under the conditions of this study, soy oil supplementation was not effective in sea urchin feeds. Digestibility of dry matter (ADMD), crude protein (ACPD), and carbohydrate (ACD) was reduced when either menhaden oil or soy oil was supplemented at the 4% level suggesting that high levels of lipid effect the digestion of dry matter and some macronutrients. Similar effects of lipid on digestibility have been suggested in marine shrimp and abalone species (Borrer and Lawrence, 1989; van Barneveld et al., 1998; Glencross et al., 2002). These data suggest that the observed effects of lipid source and concentration result from the fatty acids that comprise the supplemented oils. Soy oil contains high levels of polyunsaturated fatty acids (PUFA) such as linoleic and linolenic acid and menhaden

oil contains high levels of highly unsaturated fatty acids (HUFA) such as eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA) and arachidonic acid. Production, growth efficiency, and digestibility parameters of *L. variegatus* fed feeds supplemented with neutral fat suggest that (1) a minimum level of marine source neutral fat is required for optimal growth, (2) high levels of soy-based and menhaden-based fatty acids negatively affect growth and digestibility, and (3) fatty acid profiles, not energy levels, affected growth. These studies are a necessary first-step to ultimately define essential fatty acid (EFA) requirements and neutral fat levels for sea urchin feeds.

Clearly, manuscript 3 strongly suggests the need for additional research evaluating protein: energy ratios. The amount of protein required for feeds could also be potentially reduced (or the effects of the protein on growth enhanced) through research that supplements essential amino acids. Furthermore, digestibility studies are needed to identify practical protein and energy ingredients that could be utilized to produce least cost formulations for the first generation of commercial sea urchin feeds.

Identifying fatty acid requirements is complex due to an animal's ability to synthesize fatty acids de novo, alter dietary fatty acids, and the interactions that occur among fatty acids and other molecules (D'Abramo, 1997; Sargent et al., 2002). The fatty acid requirements for this sea urchin species (or any other species for that matter) are not known. Carefully designed studies are now needed to examine the effects of lipid sources and levels to better define optimal levels for growth. The interactions of phospholipids, carotenoids, and cholesterol and their affects on growth are not known and will have to be examined. Digestibility data presented in manuscript 4 has strong implications for the interactions of dietary lipids with other molecules and will require further research. Fatty

acid research with this and other sea urchin species will be an exciting area of future research.

This dissertation represents the first major work completed for nutritional requirements of adult *Lytechinus variegatus*. As is typical with many research efforts, this work presents more questions than answers and much work remains. Sea urchin aquaculture is in its infancy and the first successful closed-cycle commercial aquaculture operations have not yet arrived. High quality and cost-effective commercial aquaculture feeds will be necessary for the aquaculture of sea urchins to be feasible. This dissertation should help to refine experimental techniques, experimental methods, and experimental feeds for future research.

GENERAL LIST OF REFERENCES

Akiyama, T., Unuma, T., Yamamoto, T., Furuita, H., Konishi, K., 1997. An evaluation of amino acid sources and binders in semi-purified diet for red sea urchin *Pseudocentrotus depressus*. Fisheries Science 63, 881-886.

Akiyama, T., Unuma, T., Yamamota, T., 2001. Optimum protein level in a purified diet for young red sea urchin *Pseudocentrotus depressus.* Fisheries Science 67: 361-363.

Andrews, N.L., Y. Agatsuma, E. Ballesteros, A.G. Bazhin, E.P. Creaser, D.K.A. Barnes, L.W. Botsford, A. Bradbury, A. Campbell, J.D. Dixon, S. Einarsson, P.K. Gerring, K. Hebert, M. Hunter, S.B. Hur, C.R. Johnson, M.A. Juinio-Menez, P. Kalviss, R.J. Miller, C.A. Moreno, J.S. Palleiro, D. Rivas, S.M.L. Robinson, S.C. Schroeter, R.S. Steneck, R.L. Vadas, D.A. Woodby, and Z. Xiaoqi., 2002. Status and management of world sea urchin fisheries. Oceanography and Marine Biology: an Annual Review. 40: 343-425.

Beddingfield, S.D., McClintock, J.B., 1998. Differential survivorship, reproduction, growth and nutrient allocation in the regular echinoid *Lytechinus variegatus* (Lamarck) fed natural diets. Journal of Experimental Marine Biology and Ecology 226(2): 195-215.

Beddingfield, S.D., McClintock, J.B., 1999. Food resource utilization in the sea urchin Lytechinus variegatus in contrasting shallow water microhabitats of Saint Joseph Bay, Florida. Gulf of Mexico Science 1:27-34.

Beddingfield, S.D., McClintock, J.B., 2000. Demographic characteristics of *Lytechinus variegatus* (Echinoidea: Echinodermata) from three habitats in a north Florida Bay, Gulf of Mexico. Marine Ecology 21: 17-40.

Bishop, C.D., Watts, S.A., 1992. Biochemical and morphometric study of growth in the stomach and intestine of the echinoid *Lytechinus variegatus* (Echinodermata). Marine Biology 114: 459-467.

Bishop, C.D., Watts, S.A., 1994. Two stage recovery of gametogenic activity following starvation in *Lytechinus variegatus* Lamarck (Echinodermata: Echinoidea). Journal of Experimental Marine Biology and Ecology 177, 27-36.

Borrer, S., Lawrence, A.L., 1989. Effect of lipids and cellulose on the digestibility of penaeid shrimp diets. Journal of the World Aquaculture Society 20: 122-126. (Abstracct).

Briscoe, C.S., Sebens, K.P., 1988. Omnivory in *Strongylocentrotus droebachiensis* (Müller) (Echinodermata: Echinoidea): predation on subtidal mussels. Journal of Experimental Marine Biology and Ecology 115: 1-24.Bureau, D.P., Kaushik, S.J., Cho, C.Y., 2002. Bioenergetics. In: Halver, J.E., Hardy, R.W. (Eds.) Fish Nutrition. Academic Press, Boston, Massachusetts pp. 1-59.

Camp, D.K., Cobb, S.P., Van Breedveld, J.F., 1973. Overgrazing of seagrasses by a regular urchin, *Lytechinus variegatus*. Bioscience 23(2): 37-38.

Castell, J.D., Kennedy, E.J., Robinson, S.M., Parsons, G.J., Blair, T.J., Gonzalez-Duran, E., 2004. Effect of dietary lipids on fatty acid composition and metabolism in juvenile green sea urchins (*Strongylocentrotus droebachiensis*). Aquaculture 242 417-435.

Cuzon, G. Guillaume, J., 1997. Energy and Protein : Energy Ratio. In: D'Abramo, L.R., Conklin, D.E., Akiyama, D.M. (Eds.) Crustacean Nutrition. World Aquaculture Society, Baton Rouge, Louisiana, pp. 51-70.

D'Abramo L.R., 1997. Triacylglycerols and fatty acids. In D'Abramo, L.R., Conklin, D.E., Akiyama, D.M. (Eds). Crustacean Nutrition: Advances in World Aquaculture Volume 6. World Aquaculture Society Baton Rouge, Louisiana pp. 71-84.

de Jong-Westman, M., March, B.E., Carefoot, T.H., 1995. The effect of different nutrient formulations in artificial diets on gonad growth in the sea urchin *Strongylocentrotus droebachiensis*. Canadian Journal of Zoology 73: 1495-1502.

De Ridder, C., Lawrence, J.M., 1982. Food and feeding mechanisms: Echinoidea. In: Jangoux, M., Lawrence, J. M. (Eds.) Echinoderm nutrition. A. A. Balkema, Rotterdam, pp. 57-115.

Fernandez, C., Boudouresque, C., 1998. Evaluating artificial diets for small *Paracentrotus lividus* (Echinodermata: Echinoidea). In: Mooi, R., Telford, M. (Eds.) Echinoderms: San Francisco. Balkema, Rotterdam, pp. 651-656.

Fernandez, C., Dombrowski, E., Caltagirone, A., 1995. Gonadic growth of adult sea urchins, *Paracentrotus lividus* (Echinodermata: Echinoidea) in rearing: The effect of different diet types. In: Emson, R., Smith, A., Campbell, A. (Eds.) Echinoderm Research. Balkema, Rotterdam, pp. 269-275.

Field, J.C. (personal communication) in Ebert, T.A., 1975. Growth and mortality of post-larval echinoids. American Zoologist 15: 755-775.

Glencross, B.D., Smith, D.M., Thomas, M.R., Williams, K.C., 2002. The effects of dietary lipid amount and fatty-acid composition on the digestibility of lipids by the prawn, *Penaeus monodon*. Aquaculture 205 157-169

Hagen, N.T., 1996. Echinoculture: from fishery enhancement to closed cycle cultivation. World Aquaculture 27(4): 6-19.

Haley, M.P., Solandt, J.L. 2001. Population fluctuations of the sea urchin *Diadema antillarum* and *Tripneustes ventricosus* at Discovery Bay, Jamaica: a case of biological succession? Caribbean Journal of Science 37(3-4): 239-245.

Hammer, B.W., Hammer, H.S., Watts, S.A., Desmond, R.A., Lawrence, J.M., Lawrence, A.L., 2004. The effects of dietary protein concentration on feeding and growth of small *Lytechinus variegatus* (Echinodermata: Echinoidea). Journal of Marine Biology 145, 1143-1157.

Hofer, S.C., 2002. The effect of temperature on feeding and growth characteristics of the sea urchin *Lytechinus variegatus* (Echinodermata: Echinoidea). M. S. Thesis University of Alabama at Birmingham, Birmingham, Alabama.

Keesing, J.K., Hall, K.C., 1998. Review of harvests and status of word sea urchin fisheries points to opportunities for aquaculture. Journal of Shellfish Research 17(5): 1597-1604.

Klinger, T.S., 1982. Feeding rates of *Lytechinus variegatus* Lamarck (Echinodermata: Echinoidea) on differing physiognomies of an artificial food of uniform composition. In: J.M. Lawrence (Ed.) Echinoderms: Proceedings of the International Conference, Tampa Bay. Balkema, Rotterdam, p. 29-32.

Klinger, T.S., Hsieh, H.L., Pangallo, R.A., Chen, C.P., Lawrence, J.M., 1986. The effect of temperature on feeding, digestion, and absorption of *Lytechinus variegatus* (Lamarck) (Echinodermata: Echinoidea). Physiological Zoology 59, 332-336.

Klinger, T.S., Watts, S.A., Forcucci, D., 1988. Effect of short-term feeding and starvation on storage and synthetic capacities of gut tissues of *Lytechinus variegatus* (Lamarck) (Echinodermata: Echinoidea). Journal of Experimental Marine Biology and Ecology 117:187-195.

Klinger, T.S., Lawrence, J.M., Lawrence, A.L., 1994. Digestive characteristics of the sea-urchin *Lytechinus variegatus* (Lamarck) (Echinodermata: Echinoidea) fed prepared feeds. Journal of the World Aquaculture Society 25(4): 489-496.

Klinger, T. S., Lawrence, J. M., Lawrence, A. L., 1996. Absorption and assimilation of prepared feeds by echinoids. In: Mooi, R. and Telford, M. (Eds.). Echinoderms: San Francisco. Balkema, Rotterdam. pp. 713-721.

Klinger, T.S., Lawrence, J.M., Lawrence, A.L., 1997. Gonad and somatic production of *Strongylocentrotus droebachiensis* fed manufactured feeds. Bulletin of the Aquaculture Association of Canada 1: 35-37.

Lares, M.T., 1999. Evaluation of direct and indirect techniques for measuring absorption efficiencies of sea urchins (Echinodermata: Echinoidea) using prepared feeds. Journal of the World Aquaculture Society 30, 201-207.

Lawrence, J.M., 1975. On the relationship between marine plants and sea urchins. Oceanography Marine Biology Annual Review 13: 213-286.

Lawrence, J.M., Lane, J.M., 1982. The utilization of nutrients by post-metamorphic echinoderms. In: Jangoux, M., Lawrence, J.M. (Eds.) Echinoderm nutrition. A. A. Balkema, Rotterdam, pp. 331-371.

Lawrence, J.M., Sammarco, P.W., 1982. Effects of feeding on the environment: Echinoidea. In: Jangoux, M., Lawrence, J.M. (Eds.) Echinoderm nutrition. A. A. Balkema, Rotterdam, pp. 499-519.

Lawrence, J., Regis, M., Delmas, P., Gras, G., Klinger, T., 1989. The effect of quality of food on feeding and digestion in *Paracentrotus lividus* (Lamarck) (Echinodermata: Echinoidea). Marine Behavioral Physiology 15, 137-144.

Lawrence, J.M., Olave, S., Otaiza, R., Lawrence, A.L., Bustos, E., 1997. Enhancement of gonad production in the sea urchin *Loxechinus albus* in Chile fed extruded feeds. Journal of the World Aquaculture Society 28: 91-96.

Lawrence, J.M., Bazhin, A., 1998. Life-history strategies and the potential of sea urchins for aquaculture. Journal of Shellfish Research 17: 1515-1522.

Lawrence, J.M., 2001. Edible sea urchins. In: Lawrence, J.M. (Ed.) Edible sea urchins biology and ecology. Elsevier Science B. V. Amsterdam. pp. 1-4.

Lawrence, J.M. (Ed.), 2001. Edible sea urchins biology and ecology. Elsevier Science B. V. Amsterdam. pp. 1-416.

Lawrence, A.L., Lawrence, J.M., 2003. Importance status and future research needs for formulated feeds for sea urchin aquaculture. In: Lawrence, J.M., Guzman, O. (Eds). Sea urchins: fisheries and ecology. DEStech Publications Inc. pp. 275-283.

Lawrence, J.M., Lawrence, A.L., Watts, S.A., in press. Feeding, digestion and digestibility. In: Lawrence, J.M. (Ed.). Edible sea urchins: biology and ecology. Elsevier Science B. V. Amsterdam.

Lee, P.G., Lawrence, A.L., 1997. Digestibility. In: D'Abramo, L.R., Conklin, D.E., Akiyama, D.M. (Eds). Crustacean Nutrition: Advances in World Aquaculture Volume 6. World Aquaculture Society, Baton Rouge, Louisiana pp. 194-260.

Lessios, H.A., Robertson, D.R., Cubit, J.D., 1984. Spread of *Diadema* mass mortality through the Caribbean. Science 226:335-337.

Levin, V.S., Naidenko, V.P., 1987. Artificial diets for the laboratory-maintained sea urchin *Strongylocentrotus intermedius*. Marine Biology, Vladivostok 6: 50-56.

Lowe, E.F., Lawrence, J.M., 1976. Absorption efficiencies of *Lytechinus variegatus* (Lamarck) (Echinodermata: Echinoidea) for selected marine plants. Journal of Experimental Marine Biology and Ecology 21: 223-234.

Marsh, A.G., Watts, S.A., in press. Energy metabolism and gonad development. In: Lawrence, J.M. (Ed.). Edible sea urchins: biology and ecology. Elsevier Science B. V. Amsterdam.

McBride, S.C., Lawrence, J.M., Lawrence, A.L., Mulligan, T. J., 1998. The effect of protein concentration in prepared feeds on growth, feeding rate, total organic absorption, and gross assimilation efficiency of the sea urchin *Strongylocentrotus fransiscanus*. Journal of Shellfish Research 17(5): 1563-1570.

McEdward, L.R., Miner, B.G., 2001. Echinoid larval ecology. In: Lawrence, J.M. (Ed). Edible sea urchins biology and ecology. Elsevier Science B. V., Amsterdam. pp. 59-78.

Miller, R.J., Mann, K.H., 1973. Ecological energetics of the seaweed zone in a marine bay on the Atlantic coast of Canada. III. Energy transformations by sea urchins. Marine Biology 18: 99-114.

Moore, H.B., Jutare, T., Bauer, J.C., Jones, J.A., 1963. The biology of *Lytechinus variegatus*. Bulletin of Marine Science Gulf and Caribbean. 13(1): 23-53.

Morris, J.G., 1991. Nutrition. In: Prosser, C.L. (Ed.) Environmental and metabolic animal physiology. Wiley-Liss, New York, pp. 231-276.

Nestler, E.C., Harris, L.G., 1994. The importance of omnivory in *Strongylocentrotus droebachiensis* (Muller) in the Gulf of Maine. In: David, Guille, Feral, Roux (Eds.) Echinoderms Through Time. Balkema, Rotterdam, pp. 813-818.

Olave, S., Bustos, E., Lawrence, J.M., Carcamo, P., 2001. The effect of size and diet on gonad production by the Chilean sea urchin *Loxechinus albus*. Journal of the World Aquaculture Society. 32(2): 210-214.

Otero-Villaneuva, M., Kelly, M., Burnell, G., 2004. How diet influences energy partitioning in the regular echinoid Psammechinus miliaris; constructing an energy budget. Journal of Experimental Marine Biology and Ecology 304, 159-181.

Pantazis, P.A., Kelly, M. S., Conolly, J.G., Black, K.D., 2000. Effect of artificial diets on growth, lipid utilization, and gonad biochemistry in the adult sea urchin *Psammechinus miliaris*. Journal of Shellfish Research 19: 995-1001.

Pearce, C.M., Daggett, T.L., Robinson, S.M.C., 2002. Effect of protein source ratio and protein concentration in prepared diets on gonad yield and quality of the green sea urchin, *Strongylocentrotus droebachiensis*. Aquaculture 214: 307-332.

Sargent, J.R., Tocher, D.R., Bell, J.G., 2002. The lipids. In: Halver, J.E., Hardy, R.W. (Eds). Fish Nutrition Third Edition. Elsevier Science B. V., Amsterdam. pp. 181-257.

Scheibling, R., 1984. Echinoids, epizootics and ecological stability in the rocky subtidal off Nova Scotia, Canada. Helgolander Meeresunters. 37: 233-242.

Schlosser, S., Lupatsch, I., Lawrence, J., Lawrence, A., Shpigel, M., 2005. Protein and energy and digestibility and gonad development of the European sea urchin *Paracentrotus lividus* (Lamarck) fed algal and prepared diets during spring and fall. Aquaculture Research 36, 972-982.

Sloan, N.A., 1985. Echinoderm fisheries of the world: A review. Proceedings of the fifth International Echinoderm Conference, Galway. 109-124.

Tajima, K., Lawrence, J.M., 2001. Disease in edible sea urchins. In: Lawrence, J.M. (Ed). Edible sea urchins biology and ecology. Elsevier Science B. V., Amsterdam. pp. 139-160.

Tegner, M.J., Dayton, P.K., Edwards, P.B., Riser, K.L., 1995. Sea urchin cavitation of giant kelp (*Macrocystis pyrifera* C. Agardh) holdfasts and its effects on kelp mortality across a large California forest. Journal of Experimental Marine Biology and Ecology. 191: 83-99.

Valentine, J.F., Heck, K.L., 1999. Seagrass herbivory: evidence for the continued grazing of marine grasses. Marine Ecology Progress Series 176: 291-302.

Valentine, J.F., Heck, K L., Kirsch, K. D., Webb, D., 2000. Role of sea urchin *Lytechinus variegatus* grazing in regulating subtropical turtlegrass Thalassia testudinum meadows in the Florida Keys (USA). Marine Ecology Progress Series. 200: 213-228.

van Barneveld, R.J., Fleming, A.E., Vandepeer, M.E., Kruk, J.A., Hone, P.W., 1998. Influence of dietary oil type and oil inclusion level in manufactured feeds on the digestibility of nutrients by juvenile greenlip abalone (*Haliotis laevigata*). Journal of Shellfish Research 17 649-655.

Watts, S.A., Boettger, S.A., McClintock, J.B., Lawrence, J.M., 1998. Gonad production in the sea urchin *Lytechinus variegatus* (Lamarck) fed prepared diets. Journal of Shellfish Research 17, 1591-1595.

Watts, S.A., McClintock, J.B., Lawrence, J. M., 2001. The ecology of *Lytechinus variegatus*. In: Lawrence, J.M. (Ed.) Edible sea urchins biology and ecology. Elsevier Science B. V., Amsterdam. pp 375-393.

Watts, S.A., McClintock, J.B., Lawrence, J. M., in press. The ecology of *Lytechinus variegatus*. In: Lawrence, J.M. (Ed.) Edible sea urchins biology and ecology. Elsevier Science B. V., Amsterdam.

Wilson, R.P., 2002. Amino Acids and Proteins. In: Halver, J.E., Hardy, R.W. (Eds.) Fish Nutrition. Academic Press, Boston, Massachusetts pp. 143-179.

APPENDIX A

ASSESSMENT OF FEED DIGESTIBILITY

1.0 Materials and Methods

1.1 Collection, Culture, Initial Measurements

Adult *Lytechinus variegatus* were collected July 23, 2005, from Port St. Joseph Peninsula State Park, FL (30° N, 85.5°W) and transported to the Aquaculture Education and Development Center at Gadsden State Community College. Individual sea urchins were randomly assigned to a 3.8 L tank each with a single drain and incoming water line (n = 84 tanks). The incoming water line for each tank was fitted with an agricultural drip emitter to standardize water flow (15.2 Lh^{-1}). The recirculating system contained synthetic seawater (Instant Ocean, Mentor, OH) that circulated through mechanical and biological filters, a protein skimmer, and an activated carbon filter prior to being returned to each tank. The water salinity, temperature, and dissolved oxygen were maintained at 32 ± 2 ppt, 25 ± 2 °C and 7 ± 2 ppm, respectively. Ammonia, nitrite, nitrate, and pH levels were checked twice weekly and were maintained at 0.0 ± 0.05 ppm, 0.0 ± 0.05 ppm, 10 ± 5 ppm, and 8 ± 0.5, respectively. Photoperiod was maintained at 12 h light: 12 h dark.

Tanks were randomly divided into six feed treatments (n = 14 tanks per feed treatment, one urchin per tank). Each sea urchin was removed from the tank, blotted on a paper towel to remove excess water, weighed to the nearest mg with an Ohaus Explorer balance (Mettler Toledo Scales Dublin, Ohio), and measured for test diameter with calipers at two perpendicular points across the ambitus. An ANOVA was performed on the wet weight and test diameter to assure no differences among the feed treatments.

To assess the initial nutritional condition, a random sample of five sea urchins from the same population were weighed as described above and dissected. Sea urchins

were cut outside the peristomial membrane on the oral surface. During the dissection the test with spines, Aristotle's lantern, gut, and gonads were removed and separated. The gut (esophagus, stomach, and intestine) were rinsed in a finger bowl to remove excess food. Each of the organs were blotted dry with a paper towel to remove excess water and weighed to the nearest mg (wet weight). Organs were placed onto aluminum pans and dried to constant weight at 60 °C.

1.2 Feeds and Feed Preparation

Eight semi-purified feeds that varied in purified sources of either protein (soy protein isolate), and carbohydrate (purified starch) (Hammer, manuscript 3), or marine fat (menhaden oil) and non-marine fat (soy oil) (Hammer, manuscript 4) were prepared by blending practical and purified ingredients with a twin shell dry blender (Patterson-Kelley Co., East Stroudsburg, PA) for 10 minutes, and mixing in a Hobart mixer (Model A-200, Hobart Corporation, Troy, OH) for 40 minutes. Deionized water (500 ml kg^{-1}) was then added to the dry ingredients and mixed an additional 10 minutes to achieve a mash consistency appropriate for extrusion. Extrusion was accomplished using a meat chopper attachment (Model A-200, Hobart Corporation, Troy, OH) fitted with a 4.8 mm die. Moist feed strands were dried on wire racks in a forced air oven at 35 °C to a moisture content of 7% to 9.4%, placed into zip-lock bags and stored in a refrigerator at 4 °C until used.

1.3 Digestibility Trial

Sea urchins were held for 2 weeks prior to the initiation of the digestibility trial to standardize nutritional condition. During this time, all sea urchins were fed *ad libitum* a reference feed (31:33% protein: carbohydrate) that was shown previously to result in rapid weight gain and high production efficiency for this species (Hammer, manuscript 3). Uneaten feed and feces were removed daily by siphon. At 5-days prior to the beginning of the digestibility trial, sea urchins were fed daily one of eight different feeds *ad libitum* to allow complete egestion of the reference feed from the gut.

On the first day of the digestibility trial, the feed ration was weighed to the nearest mg (as fed) and recorded for each sea urchin; the ration was intentionally underestimated (ca. ½ the normal consumed ration) to reduce the amount of uneaten feed and facilitate clean fecal recovery. Feed was proffered to each sea urchin in the afternoon and fecal collection was initiated the following afternoon. Feces were removed completely from each tank by siphon and collected on a marked individual sieve (100 μm mesh). The feces in the sieve were rinsed quickly with distilled water to remove surface salt and moved to a pre-weighed aluminum pan for drying. Feces were dried at 70 °C overnight, cooled to room temperature, and weighed prior to the next day fecal collection. The same marked fecal sieve and aluminum pan were used for each individual sea urchin through the entire study. Sea urchins were fed and feces collected for each individual for 7 to 13 consecutive days.

At the conclusion of the digestibility trial, daily collections of fecal material were pooled for each individual, transferred into poly-ethylene vials, dried for 3 consecutive

days at 70 °C, allowed to cool to room temperature, and then capped for storage until analysis.

Feed and fecal samples were ground to a fine powder with a mortar and pestle. Approximately 100 mg of dry sample were ashed in a muffle furnace at 500 °C for 8 hours, cooled to room temperature in a desiccator, and weighed to determine ash content. Organic material was calculated by subtraction. The crude protein was determined by AOAC Method 990.3; FP-528 Nitrogen/Protein Determination; Leco Corporation, St. Joseph, MI. Carbohydrate was determined by the method of Dubois et al. (1956). Lipid was determined by the method of Freeman et al. (1957). Energy content was determined by micro-bomb calorimetry (Parr Instrument Company, Moline, IL.).

The apparent dry matter digestibility (ADMD) was calculated as:

$$\frac{\text{Dry food consumed (g) - Dry weight of feces (g)}}{\text{Dry food consumed (g)}} \times 100$$

The apparent digestibility of organic material (AOMD) was calculated as:

$$\frac{\text{Dry organic matter consumed (g)– Dry organic matter egested (g)}}{\text{Dry organic matter consumed (g)}} \times 100$$

The apparent digestibility of ash (AAD) was calculated as:

$$\frac{\text{Dry ash consumed (g)– Dry ash egested (g)}}{\text{Dry ash consumed (g)}} \times 100$$

The apparent digestibility of crude protein (ACPD) was calculated as:

$$\frac{\text{Dry protein consumed (g)– Dry protein egested (g)}}{\text{Dry protein consumed (g)}} \times 100$$

The apparent digestibility of carbohydrate (ACD) was calculated as:

$$\frac{\text{Dry carbohydrate consumed (g)} - \text{Dry carbohydrate egested (g)}}{\text{Dry carbohydrate consumed (g)}} \times 100$$

The apparent digestibility of lipid (ALD) was calculated as:

$$\frac{\text{Dry lipid consumed (g)} - \text{Dry lipid egested (g)}}{\text{Dry lipid consumed (g)}} \times 100$$

1.4 Statistical Analysis

Statistical comparisons were performed on the Systat 11 software package (Systat Software Inc., Point Richmond, CA.). If data were normal and homoscedastic an ANOVA parametric test was completed. When significant differences were determined, a Tukey's test for pairwise group comparisons was used. A P value of < 0.05 was determined statistically significant for all parametric tests. If data were non-normal or heteroscedastic, data transformations were attempted. When ANCOVA analysis was completed, tables of both actual means and least square means were presented. If data transformations were not successful, non-parametric tests (Kruskal Wallis or Mann Whitney U) were employed. To maintain an overall acceptance criteria of $\alpha = 0.05$ during multiple comparisons, a Bonferroni's adjustment was adopted.

Table A1
Ingestion and egestion rates of *Lytechinus variegatus* during digestibility trials.

Protein: Carbohydrate	Food g Day^{-1}		Feces g Day^{-1}	
17: 47	0.122 ± 0.001	B	0.028 ± 0.001	A
21: 44	0.123 ± 0.001	AB	0.027 ± 0.001	A
25: 39	0.128 ± 0.001	A	0.027 ± 0.95	A
31: 33	0.122 ± 0.002	B	0.026 ± 0.001	A
Feed	Food g Day^{-1}		Feces g Day^{-1}	
0% MEN	0.114 ± 0.003	AB	0.024 ± 0.001	B
1% MEN	0.122 ± 0.002	A	0.026 ± 0.001	AB
4% MEN	0.111 ± 0.001	B	0.031 ± 0.001	A
Feed	Food g Day^{-1}		Feces g Day^{-1}	
0% SOY	0.124 ± 0.006	A	0.023 ± 0.001	B
1% SOY	0.122 ± 0.002	A	0.026 ± 0.001	AB
4% SOY	0.114 ± 0.001	A	0.031 ± 0.001	A

Numbers represent mean ± standard errors and letters represent significant differences.
Note: sea urchins were purposely not fed at *ad libitum* levels during digestibility trials.

Table A2
Digestibility of protein: carbohydrate feeds fed to sea urchins, *Lytechinus variegatus*, during digestibility trials

Parameter	Protein: Carbohydrate	ADMD (%)	
ADMD (%)	17:47%	77.1 ± 1.00	A
	21:44%	78.1 ± 0.80	A
	25:39%	79.0 ± 0.95	A
	31:33%	78.6 ± 0.93	A
AOMD (%)	17:47%	81.5 ± 1.22	A
	21:44%	82.7 ± 0.42	A
	25:39%	84.3 ± 1.00	A
	31:33%	83.2 ± 1.04	A
AAD (%)	17:47%	61.1 ± 1.91	A
	21:44%	63.0 ± 1.26	A
	25:39%	63.23 ± 2.7	A
	31:33%	64.2 ± 2.12	A
ACPD (%)	17:47%	75.1 ± 1.36	C
	21:44%	80.3 ± 0.74	B
	25:39%	83.7 ± 0.89	AB
	31:33%	85.9 ± 0.70	A
ACD (%)	17:47%	97.36 ± 0.22	AB
	21:44%	97.76 ± 0.15	B
	25:39%	97.68 ± 0.15	B
	31:33%	97.00 ± 0.20	A

Numbers represent mean ± standard errors and letters represent significant differences.
Note: sea urchins were purposely not fed at *ad libitum* levels during digestibility trials.

APPENDIX B

INSTITUTIONAL ANIMAL CARE AND USAGE COMMITTEE FORM

THE UNIVERSITY OF ALABAMA AT BIRMINGHAM

THE UNIVERSITY OF ALABAMA AT BIRMINGHAM

Office of the Provost

NOTICE OF APPROVAL

DATE: April 4, 2003

TO: Stephen A. Watts, Ph.D.
CH-375 1170
FAX: 5-6097

FROM: Clinton J. Grubbs, Ph.D., Chairman
Institutional Animal Care and Use Committee

SUBJECT: Assessment of Sea Urchins as a Fishery and Aquaculture Candidate in the Gulf of Mexico (Internal) 030406738

On April 4, 2003, 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:

Species	Use Category	Number in Category
Invertebrates	A	300

Animal use is scheduled for review one year from April 4, 2003. 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) 030406738 when ordering animals or in any correspondence with the IACUC or Animal Resources Program (ARP) offices regarding this study. If you have concerns or questions regarding this notice, please call the IACUC office at 934-7692.

Institutional Animal Care and Use Committee
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DISSERTATION APPROVAL FORM
DOCTOR OF PHILOSOPHY

Name of Candidate Hugh Scott Hammer

Graduate Program Biology

Title of Dissertation Determination of Dietary Protein, Carbohydrate, and Lipid Requirements for the Sea Urchin *Lytechinus variegatus* Fed Semi-Purified Feeds

I certify that I have read this document and examined the student regarding its content. In my opinion, this dissertation conforms to acceptable standards of scholarly presentation and is adequate in scope and quality, and the attainments of this student are such that he may be recommended for the degree of Doctor of Philosophy.

Dissertation Committee:

Name		Signature
Stephen A. Watts	**, Chair**	
Louis D'Abramo		
Addison Lawrence		
James B. McClintock		
Timothy R. Nagy		

Director of Graduate Program

Dean, UAB Graduate School

Date