EFFECT OF PROTEINS, LIPIDS, MINERALS, AND PIGMENT IN PREPARED DIETS ON THE SOMATIC GROWTH OF JUVENILE GREEN SEA URCHINS, STRONGYLOCENTROTUS DROEBACHIENSIS

EDWARD J. KENNEDY

Your file Votre référence
ISBN: 0-612-84017-4
Our file Notre référence
ISBN: 0-612-84017-4

EFFECT OF PROTEINS, LIPIDS, MINERALS, AND PIGMENT IN PREPARED DIETS ON THE SOMATIC GROWTH OF JUVENILE GREEN SEA URCHINS, *STRONGYLOCENTROTUS DROEBACHIENSIS*

by

© Edward J. Kennedy

A thesis submitted to the

School of Graduate Studies

in partial fulfilment of the

requirements for the degree of

Master of Science (Aquaculture)

School of Fisheries, Marine Institute

Memorial University of Newfoundland

October 2002

St. John's Newfoundland

Abstract

This study investigated the effects of proteins (fishmeal and soybean), lipids (corn, linseed, and menhaden oils), minerals (modified Bernhart-Tomerelli and Shur-Gain salt mixes), and pigment (beta-carotene) over a range of concentrations in moist-extruded prepared diets on the somatic growth performance of juvenile green sea urchins, *Strongylocentrotus droebachiensis*, in five feeding experiments (ranging in length from 159 to 300 days). The growth of juveniles (ranging in size from 1 mm to 20 mm initial test diameter (TD)) fed prepared diets was compared to the growth of similar sized juveniles fed kelp, *Laminaria longicruris*. Juveniles fed the diets with the different sources and concentrations of proteins and lipids had smaller, poorly pigmented tests with short, stubby spines compared to the juveniles fed kelp after each experiment. Those fed kelp allocated more energy towards test production, whereas those fed the prepared diets allocated more energy to gonad production. The dietary protein treatments used in this study had no effect on growth and survival of the juvenile sea urchins. The lipid source treatments, which differed in the major essential fatty acids (i.e., n-3 and/or n-6), also had no effect on juvenile growth and survival in this study, but juveniles fed diets with lower lipid concentrations (i.e., 1% and 3%) had larger test sizes, but similar survival, than those fed diets with a high lipid concentration (i.e., 10%). The poor growth and physical appearance of the juveniles fed the protein and lipid diets were attributed to nutrient deficiencies in the prepared diets and the associated stress in the juveniles. Juveniles fed pigmented diets grew to a larger size than those fed non-pigmented diets. Similarly, dietary mineral concentration had a positive effect on juvenile test growth.

Juveniles (1-2 mm initial TD) fed a pigmented diet with high mineral concentration (15%) grew to a larger size than kelp-fed juveniles. The data indicate there were no differences in the nutritional needs of the various sizes of juvenile green sea urchins used in this study. Hence, nutritionally balanced prepared diets can be used for a wide size range of green sea urchins to increase juvenile test growth while maintaining health and survival.

Acknowledgements

Drs. Shawn Robinson, Jay Parsons, and John Castell have spent many unselfish and dedicated hours advising, supporting, and assisting in all aspects of my studies. This thesis would not have been possible without their never-ending guidance and dedication. Dr. Bob Hooper also has provided his time and guidance ensuring the content of this thesis was appropriate and kept it at a manageable level. I would like to thank the Canadian Centre for Fisheries Innovation (CCFI) and Memorial University of Newfoundland for the financial support they provided during my studies. I also thank Shur-Gain/Maple Leaf Foods Inc. and the Department of Fisheries and Oceans Canada, for their financial assistance towards costs to attend aquaculture conferences. I also would like to thank Shur-Gain/Maple Leaf Foods Inc. (Dr. Adel El Mowaffi, Alan Donkin, Dr. Dan McPhee, and Tom Taylor) for donating the feed ingredients used in most of the prepared diets as well as expert advice in diet formulation and preparation. In addition, some feed ingredients and the chemicals required for diet and tissue analyses were generously donated by Chris Pearce (Ross Island Salmon, Grand Manan, New Brunswick), Jay Parsons (Marine Institute of the Memorial University of Newfoundland), John Castell and Shawn Robinson (Fisheries and Oceans Biological Station, St. Andrew's, New Brunswick). I also would like to thank the past and present staff of the St. Andrews Biological Station, especially the Shellfish Aquaculture section (Jim Martin, Robbie Longmire, Lisa Peters, Tammy Blair, Steve Pomerleau, Captain Wayne Minor and crew of **Pandalus III**, Rabindra Singh, and Joy Wade), for their assistance and support during lab preparation, diet preparation, feeding trials, sampling periods, and

final analyses. Finally, I would like to thank my friends who willing helped in feeding and measuring sea urchins especially Bethan Lawson who also spent countless hours encouraging me to write.

Table of Contents

List of Tables

List of Figures

List of Appendices

Chapter 1

Introduction

1.1 Biology and ecology

The green sea urchin, *Strongylocentrotus droebachiensis,* belongs to a group of organisms called the echinoderms, Latin for "spiny-skin", and classified into the Phylum Echinodermata, Class Echinoidea, Order Echinoida and Family Strongylocentrotidae. The outer surface of the animal is composed of a test ("shell"), which is covered by an epidermal layer. Numerous spines radiate from the test and are used for motility, protection against predators, food gathering and manipulation (Mottet, 1976). Extending from pores in the test are tube feet, which compose part of the water vascular system, one of the characteristic features of the phylum. These tube feet allow attachment to the substrate and aid in food detection and motility (Mottet, 1976). The body of a sea urchin has a "flattened apple" shape with the oral end facing the substrate and the aboral end facing the water column. The feeding apparatus, located in the oral region, is called the Aristotle's Lantern and is composed of 5 teeth-like structures that manipulate food particles. Regular sea urchins share a pentaradial symmetry depicted by the 5 'teeth', the 5 sets of plates that form the test and the 5 gonad sacs. The diet mainly consists of seaweeds, especially kelp when available, but also diatom and bacterial films (important for young juveniles), detritus, dead fish, mussels, and other sea urchins (Dawson, 1868; Bedard, 1973; Mottet, 1976). Green sea urchins have a circumpolar distribution and are usually found on rocky/gravel, sub-tidal substrates from the low tide mark to 15 m, but can extend down to 90 m or beyond (Mottet, 1976). Sea urchins are cannibalistic and

other predators include sea stars, Atlantic wolfish, lobsters, and humans (Bedard, 1973; Mottet, 1976). In the past decade, humans have caused increased pressure on sea urchin populations.

1.2 Economic importance

Sea urchin roe, or "uni", has become a very valuable commodity within the past decade, especially in the Japanese seafood market. There is also a market for sea urchin roe in France and a growing interest in North America. Of the 500 species worldwide, only 18 species are commercially important based on animal size, gonad size, and the quality of the roe (Mottet, 1976; Anonymous, 1989). The single most important species in Japan is *Strongylocentrotus intermedius* (Hagen, 1996), but since wild fishery production has peaked, market supply now relies heavily on imported sea urchins. Another important uni marketed in Japan comes from the largest commercial sea urchin, the red sea urchin, *S. franciscanus,* found from Baja, California northward to the Aleutians and across to Hokkaido, Japan's northern most island (Mottet, 1976; Anonymous, 1989). The most harvested sea urchin is *Loxechinus albus,* the Chilean sea urchin, while the most widely distributed variety worldwide is the green sea urchin, *S. droebachiensis* (Anonymous, 1989; Hagen, 1996). Other important sea urchin species include *Paracentrotus lividus* (the European sea urchin) and the purple sea urchin, *S. purpuratus* (Mottet, 1976; Anonymous, 1989). These species have large, high quality gonads, which are desirable in the market-place.

The gonad of the sea urchin serves two functions, nutrient storage and gamete production. Nutrients are stored throughout the year in specialised cells called nutritive phagocytes and during gametogenesis the stored nutrients are utilised in the production of gametes (Mottet, 1976). High quality roe depends upon the concentration of the nutritive phagocytes in the gonad with quality directly related to the numbers of nutritive phagocytes (Hagen, 1996). Gonad quality depends on the colour (yellow-orange being superior) (Havardsson et al., 1996), sweet taste and firm texture. Sea urchins are one of the most valuable seafoods in the world and a higher quality product attains higher market prices. Fresh Japanese roe can fetch up to $180 per kilogram, while fresh imported roe can attain a price of $70 - $80 per kilogram (Hagen, 1996). The market demand for high quality sea urchin roe in major markets and the associated high stable market prices has created a global fishery for sea urchins.

1.3 Sea urchin fisheries

The Japanese catch of wild sea urchins had peaked at 27,500 tonnes per year in the late 1960's, and by 1991, the catch dropped to 14,000 tonnes (Hagen, 1996). Similarly, in Maine, USA, wild fishery landings of *S. droebachiensis* peaked in 1993 at 18,600 tonnes (US$26 million), but have declined by 40% between 1996 and 1999 (Lesser and Walker, 1998; Vadas et al., 2000). In addition, the California fishery (primarily *S. franciscanus*) peaked at over 20,870 tonnes (US$20 million), which was followed by an 80% decline from 1988 to 1991 (Morgan et al., 2000).

The demand for sea urchins in Japan greatly exceeds the supply offered by the local wild fishery; thus markets depend heavily on imports from other regions including Chile, France, and both coasts of North America. Problems with the wild harvest include overfishing of the marketable sea urchins in many countries, which has caused reductions in their natural populations. In Japan, for example, six species of sea urchins have become over-exploited due to overfishing (*Pseudocentrotus depressus, Heterocentrotus pulcherrimus, Strongylocentrotus droebachiensis, S. nudas, Anthocidaris crassispina,* and *Tripneustes gratilla*) (Hagen, 1996). The green sea urchin fishery in southwest New Brunswick, Canada and in Maine, USA has become more dependent on yearly recruitment to the wild populations rather than on the surplus of individuals from past generations (Pers. Comm., Dr. Shawn Robinson, Biological Station, St. Andrews, NB). These trends have resulted in a total production capacity of 60,000 tonnes per year, while the demand still increases (Hagen, 1996). Also associated with the wild fishery is variable roe quality and roe yield resulting in lower percentages of the harvested biomass actually becoming processed (Keats et al., 1984; Hooper et al., 1994; Cuthbert et al., 1995; Walker and Lesser, 1997). The removal of mature sea urchins from a population ultimately limits the potential spawning stock of that population, thus reducing the potential recruitment in future generations. In addition, some sea urchin species require at least four years to reach reproductive maturity (Fugi, 1963; Mottet, 1976; Abe and Tada, 1994), thus once an area has been fished, long-term growth is necessary for the adult population to rebuild (Hooper et al., 1996). In addition, the wild fishery for green sea urchins in the northwest Atlantic is limited to a few months during the winter season

when quality and size of the gonads are optimal (Walker and Lesser, 1997). It is during this time when weather conditions are most unfavourable and the dangers associated with harvesting are amplified, especially for scuba divers. For these reasons, alternatives to the wild fishery must be developed to ensure sustainable supplies of quality sea urchins remain available.

1.4 Aquaculture as a solution

The growth of sea urchins has been studied since the 1920s (Swan, 1961). However, a concentrated effort on sea urchin culture has occurred only in the past few years coincident with the rapid decline in wild populations, the lack of suitable natural habitat and food supply, combined with an increase in market demand (Hagen, 1996; Lesser and Walker, 1998). Sea urchin culture also avoids the problems of the wild fishery, such as season length, off-season harvests, roe quantity and quality, and long-term sustainability (Vadas et al., 2000).

One approach to sea urchin culture is to simply collect adult sea urchins from the wild and increase gonad yield by providing the animals with a constant supply of natural feed, such as kelp (Cuthbert et al., 1995; Hooper et al., 1996). This method, however, still relies on adults from wild populations and thus will be regulated by natural reproductive rates. Another approach is to collect juveniles from the wild by using suitable collectors that offer a settling substrate for the excess supply of larvae produced by broadcast spawning. This method again relies on the adult populations to supply the settling larvae,

as well as hydrodynamics of the local water body, which transports potential settling larvae to the collectors. A third approach would be to use a closed life-cycle system, raising the sea urchins from fertilised eggs to mature adults, which greatly reduces dependence on wild populations. A closed system also would allow selection of superior strains, as well as offer the potential to inhibit sexual maturation through manipulation of photoperiod and temperature (Hagen, 1996). Therefore, a closed system could extend the marketable season and improve gonad quality and yield.

Control of somatic growth is essential in culture operations since the size of the test ultimately limits the size (amount) of the gonad that can be produced. Studies have shown that very little energy is allocated to test growth in adult populations (de Jong-Westman et al., 1995; Fernandez et al., 1995; Lawrence et al., 1997; Klinger et al., 1998). For adult sea urchins (*S. droebachiensis*), at a minimal size of approximately 20 mm test diameter (Raymond and Scheibling, 1987), most of the food energy is channelled into gonad production by incorporating excess nutrients into the nutritive phagocytes (Lozano et al., 1995). This represents the source of energy for gamete production during gametogensis. Juvenile nutrition is important for maximising growth since development and growth are dependant on the quality of the diet consumed and not on previous parental nutrition (Lawrence and Lane, 1982; Klinger et al., 1983; Minor and Scheibling, 1997; Fernandez and Boudouresque, 1998; Meidel and Scheibling, 1998b; Meidel et al., 1999; Lamare and Mladenov, 2000). Studies by Lawrence et al. (1997) with *Loxechinus albus* and by Fernandez and Boudouresque (1998) with *Paracentrotus lividus* have

shown that approximately 46 to 50% of the available assimilated energy in juvenile sea urchins is used for test production and somatic growth. Therefore, to maximise the output of roe from individual adult sea urchins in culture operations, concentration must be focused upon maximising somatic growth during the juvenile stages. Without evidence that juvenile somatic growth can be increased to allow for sufficient roe production, a closed life-cycle approach to sea urchin aquaculture may not be economically feasible and research into hatchery development would become unwarranted. Understanding the nutritional requirements of the juveniles is essential in the success of the sea urchin aquaculture industry.

1.5 Manufactured diets

Over the past 20 years, much effort has been expended on achieving a comprehensive understanding of the relationships between sea urchins and their food (Emson and Moore, 1998). Most of the nutritional research to date on sea urchins has been focused on gonad production in adult sea urchins. However, some studies have considered the nutritional requirements for somatic growth of juvenile sea urchins (Gonzalez et al., 1993; Cook et al., 1998; Fernandez and Pergent, 1998; McBride et al., 1998; Kennedy et al., 1999; Akiyama et al., 2001; Wallace et al., 2001). Such studies have focused on feed type (i.e., wild and prepared diets) and feed ingredients of prepared diets. It is important to consider the prepared diets utilised for juvenile somatic growth separately from those utilised for adult gonad growth because dietary requirements for each process may be quite different (Kelly et al., 1998).

The preferred wild diets of green sea urchins (*S. droebachiensis*) are the laminarian kelps (Vadas, 1977; Hooper et al., 1997), such as *L. longicruris* and *L. digitata* (Larson et al., 1980; Keats et al., 1984; Scheibling et al., 1999; Scheibling and Hatcher, 2001), which are sufficient for supporting sea urchin somatic growth (Himmelman and Steele, 1971; Vadas, 1977; Himmelman, 1978; Larson et al., 1980; Thompson, 1982; Munk, 1992). The use of these wild diets (i.e., seaweeds) in sea urchin aquaculture has potential problems. The dependency on the natural environment to supply the seaweeds for harvesting and the time required for the rejuvenation of seaweeds once an area has been harvested are major concerns. Other problems include variable seasonal nutrient composition (Lobban and Harrison, 1994), wild supplies are inconsistent (George et al., 2000), harvesting seaweeds conflicts with other industries (such as the lobster industry) (Robinson and Colborne, 1997), and they are expensive to collect and store fresh (Lawrence et al., 1997; George et al., 2000). Furthermore, some juvenile sea urchins may not have the digestive capabilities to digest some wild seaweeds (Vadas et al., 2000). Morris and Campbell (1996) found that juvenile red sea urchins did not grow when fed eel grass because they did not produce pectinase, required to breakdown the pectin within the eel grass. In addition, protein supplements to a kelp diet have been found to enhance sea urchin growth and improve overall food conversion (Hagen, 1996). Although kelp has been found to provide good flavour and colouring of gonads it may not be the best choice for sea urchin feeds in culture operations.

An alternate source of nutrients for sea urchin growth is in manufactured feeds, which eliminate the dependency of wild food availability, variability of nutrient content, and possible impacts of harvesting natural foods. Prepared feeds are readily digested, absorbed, and assimilated by sea urchins (Klinger et al., 1998), are relatively inexpensive compared to the costs associated with wild feeds, and they are easier to store (Hagen, 1996). As well, manufactured diets can be manipulated to supply sea urchins with the optimal concentrations of nutrients required for growth and survival (Lawrence et al., 1997). Some studies in the literature (Lasker and Giese, 1954; Klinger et al., 1994; Morris and Campbell, 1996) have examined the digestive characteristics of sea urchins. To ensure the utilisation of diet components, the digestive characteristics of sea urchins must be considered during diet formulations. Manufactured feeds for sea urchins generally consist of a carbohydrate source (corn, wheat, or potato starch), protein source (soybean or fish), lipid source (fish oil, corn oil, cholesterol), and vitamin and mineral mixes (Klinger et al., 1986; Klinger et al., 1988; Lawrence et al., 1989; John et al., 1990; Lawrence et al., 1991; Klinger et al., 1994; de Jong-Westman et al., 1995; Klinger et al., 1995; Lares and Lawrence, 1995; Lawrence et al., 1995; Pearse et al., 1995; Lawrence et al., 1997).

The various nutritional compositions available in manufactured diets should support higher growth rates than those attained by natural foods since the optimal nutritional requirements of juvenile sea urchins can be provided in the diet. At present, most juvenile sea urchin growth rates using prepared diets are not greater than those from

seaweeds. A natural growth rate of 0.02 mm test diameter (TD) per day was observed for juvenile *Hemicentrotus pulcherrimus* (Fugi, 1963), and *S. intermedius* (3 mm to 12 mm initial TD) showed growth rates of 0.04 mm to 0.07 mm TD per day held in aquaria with *Laminaria japonica* (Fugi, 1967). Ebert (1968) also observed similar growth rates (0.04 mm/day) for juvenile *S. purpuratus* (5 mm to 20 mm initial TD) held at 11°C and fed brown algae. As well, juvenile *S. droebachiensis* (15 mm to 25 mm initial TD) showed growth rates from 0.04 mm to 0.05 mm TD per day with *L. longicruris* and *Chrondrus crispus* as the diet (Larson et al., 1980) and 0.05 mm TD per day when fed *L. digitata* (Swan, 1961). Growth rates of juvenile sea urchins using manufactured feeds have ranged from 0.018 mm to 0.026 mm TD per day for *Paracentrotus lividus* (20 mm to 25 mm initial TD) (Fernandez and Boudouresque, 1998) and from 0.05 mm to 0.13 mm TD per day for *S. droebachiensis* (1 mm to 10 mm initial TD) (Williams and Harris, 1998). Williams and Harris (1998) also noted that a wild diet achieved similar growth rates as the manufactured diets. Studies to examine the impacts of specific diet components on juvenile growth are required to develop diets that maximise sea urchin growth production.

Some literature suggests that varying the source and concentration of protein in the diet can affect the growth production within different species of sea urchins. Lawrence et al. (1991) found that diets consisting of fish and soybean protein gave greater test growth in small *Loxechinus albus* than diets containing only soybean protein. For adult *S. droebachiensis*, de Jong-Westman et al. (1995) found no increase in test growth among

sea urchins fed diets differing in protein concentration, but did find greater gonad production using diets containing higher fish protein. Fugi (1967) found that the ability of *S. droebachiensis* to use protein nitrogen for growth declined in a curvilinear fashion with increasing test diameter. These studies suggest that test production primarily occurs in small sea urchins and may be influenced by protein source and concentration. Lowe and Lawrence (1976) support this and suggest that growth and reproduction are more dependent on protein content than diet energy.

1.6 Important nutrients for growth

In determining the optimal diet for juvenile sea urchin growth, it is important to study the effects of components that have the greatest impact on juvenile test production. Proteins, which function as the basic building blocks for cellular growth, and lipids, which function in cell membrane development, are two major dietary components that potentially impact juvenile growth. In addition, minerals, which are important for test production and osmoregulation (Marsh and Watts, 2001; Wasson and Watts, 2001), and pigments, which can act as antioxidants and precursors to certain vitamins (Matsuno and Tsushima, 2001), can affect the production of somatic growth for juvenile sea urchins.

There are two possible protein sources for manufactured sea urchin diets, either plant or animal proteins. The optimal concentration of proteins for sea urchin diets is unknown. For abalone, Uki et al. (1986) tested a range of protein levels from 0% to 55% and found the optimal protein level for growth to be 38% of the diet dry mass. Sea urchin diets in

the literature consist of 10 to 50 % protein from a variety of sources (Klinger et al., 1998; de Jong-Westman et al., 1995), but there is no evidence of the optimal protein source and concentration for maximal juvenile sea urchin somatic growth. In a study by Wallace et al. (2001), 32% was suggested as the optimal concentration for juvenile *Lytechinus variegatus*, but only one protein source was tested and the duration of the experiment was short (i.e., 14 weeks). Furthermore, a study by Akiyama et al. (2001), suggested that there were no growth differences for young red sea urchins (*Pseudocentrotus depressus*) fed prepared protein diets with 20-50% protein, but sea urchins fed 10% protein had lower growth. Again, only one protein source was tested for a short duration of 8 weeks. More work is required to determine the optimal protein source and concentration in a prepared diet for maximal sea urchin somatic growth throughout the juvenile period of culture.

The dietary lipid sources and concentrations for sea urchins are not as well reviewed in the literature as dietary protein. The ability of juvenile green sea urchins to produce omega-3 and/or omega-6 fatty acids (essential fatty acids in higher order animals) has not been thoroughly examined. It is plausible that because sea urchins are lower in the food chain, they may have the ability to produce these fatty acids from precursors. Bell et al. (2001), showed *Psammechinus miliaris* converted linolenic acid (18:3n-3) to eicosapentaenoic acid (20:5n-3), but how these fatty acids affect the somatic growth of juvenile sea urchins is unknown. In the literature, the amount of lipid ingredients added to manufactured diets was usually low, ranging from 2% (de Jong-Westman et al., 1995)

to 6.5 % (Nagai and Kaneko, 1975). Kochi (1969) studied the fatty acid composition of sea urchin gonads, but, like most other studies on sea urchin lipids, did not examine the impacts on somatic growth.

Minerals and pigments are often considered minor elements in manufactured diets because they only represent a small percentage of the diet and they may not be a necessity for survival. In salmon culture, for example, pigments can be added towards the end of the production cycle only for the purpose of flesh coloration to meet market expectations (Torrissen et al., 1989). In the sea urchin industry, gonad quality also hinges on colour (i.e., pigment) (Tsushima et al., 1993; Matsuno and Tsushima, 2001), but the function of pigments for the growth of sea urchin juveniles has not been investigated. The importance of minerals in manufactured diets for juvenile somatic growth has also been neglected in the literature. There have been studies that suggest calcium and magnesium are the major minerals used in test structure (Fernandez, 1998; Chen et al., 2000), but the effect of mineral concentration in the diet on test growth is unknown. According to Klinger et al. (1998), little information exists on the manufactured foods suitable for sea urchin mariculture.

1.7 Research objectives

The research objective of this study will be to examine various manufactured diets designed to maximise juvenile sea urchin somatic growth. The primary emphasis will be the growth rate of juvenile sea urchins and how different nutrient sources and

concentrations affect it. By supplying the nutritional requirements of the sea urchins in the prepared diet, it is possible to optimise juvenile growth rate (Gonzalez et al., 1993). Results will be collected for different sizes of juvenile sea urchins so that the effects of juvenile size on the performance of the different diets can be determined.

The study will consist of four major sets of experiments (encompassed in four chapters) that will isolate the effects of proteins (fishmeal and soybean), lipids (corn, linseed, and menhaden oils), minerals (modified Bernhart-Tomerelli and Shur-Gain salt mixes), and pigment (beta-carotene) over a range of concentrations on juvenile green sea urchin growth. Chapter 2 will focus on dietary protein source (either plant or plant and animal), protein concentration in the diets, and the effect of both source and concentration on juvenile somatic growth compared to juvenile growth when fed a preferred wild kelp diet. Chapter 3 will examine sea urchin lipid requirements for somatic growth by comparing the growth of juveniles fed prepared diets with different lipid sources over a range of concentrations. Growth of juveniles fed prepared diets will also be compared to growth of kelp-fed juveniles. Chapter 4 will determine the effect of minerals and pigment on juvenile somatic growth and suggest an optimal mineral concentration to produce maximum juvenile test growth. Growth of juvenile sea urchins fed the best diet from the study will be compared to growth of kelp-fed juveniles to determine if prepared diet can be superior to wild kelp diet for test production. Hence, the objective of this study is to develop a manufactured (prepared) diet that yields the maximum survival and body growth for juvenile green sea urchins.

Chapter 2

The effect of protein source and concentration on the somatic growth of juvenile green sea urchins (*Strongylocentrotus droebachiensis*)

2.1 Introduction

Success in rearing juvenile sea urchins to market-size animals using a prepared diet requires extensive knowledge of sea urchins' nutritional and energy requirements throughout the stages of juvenile growth (Watts et al., 1998). Juvenile growth is dependent on food quality and quantity, but food sources in natural populations differ as the juveniles increase in size (Fugi, 1967; Williams and Harris, 1998). Therefore, the nutritional and energy requirements of juvenile sea urchins change as body size increases. Juvenile growth rates are a critical factor in the success of a full-cycle operation and knowledge of these growth rates, using appropriate prepared diets at various stages of development, will provide valuable information regarding the feasibility of sea urchin culture and the sustainability of the industry (Williams and Harris, 1998).

An important step in formulating a diet specifically designed for optimal somatic (i.e., test) growth is to determine the nutritional components of the diet responsible for major changes in somatic growth. Proteins are one of the important nutrients for sea urchins (Lilly, 1975; Lowe and Lawrence, 1976; de Jong-Westman et al., 1995). They are major constituents of the body wall (Lawrence and Guille, 1982; Lawrence and Byrne, 1994; Shimizu et al., 1994; Fernandez, 1997) and they are important in many physiological

functions. Dietary protein has been identified as a major factor affecting growth production in echinoids (Lawrence et al., 1991; Frantzis and Gremare, 1992).

Although green sea urchins are omnivores, kelp is the preferred wild diet (Larson et al., 1980). Since kelps are generally lower in protein than animal sources (per unit volume), sea urchin juveniles may grow well on prepared feeds with low concentrations of plant protein. Previous studies have shown juvenile sea urchins grow well when fed algal diets (Morris and Campbell, 1996; Chang et al., 1999; Agatsuma, 2000), when fed prepared diets with plant protein (Cuthbert et al., 2000), and when fed prepared diets with both plant and animal protein (Lawrence et al., 1991; Fernandez and Pergent, 1998). A study by Wallace et al. (2001) also suggested that formulated diets for *Lytechinus variegatus* should contain at least 32% protein to maximise both growth and survival of juveniles. In addition, it is important to consider the combined effect of protein source (either plant or animal) and protein concentration in prepared diets on juvenile growth.

Protein is available for use in prepared feeds from both animal and plant sources. Most protein used in aquaculture feeds, especially aquaculture salmon feeds, is animal protein contained in fishmeal. However, it is more feasible for commercial feed producers to use plant protein in feed production since plant protein is less expensive and more available than animal protein (Tidwell and Allan, 2001). Hence, the culture of marine species, which can survive and grow on plant material (such as sea urchins), will benefit the aquaculture industry by reducing production costs, particularly feed costs.

Different sources of protein at various concentrations were incorporated into a grain-based diet and fed to juvenile green sea urchins, *S. droebachiensis*, to determine whether dietary protein can significantly affect their somatic (i.e., test) growth compared to juveniles fed a kelp, *L. longicruris*. Prepared diets consisted of two protein sources (i.e., plant protein and animal protein) at three different proportions. Commercially available diets containing a low concentration of animal protein would be desirable for the sea urchin culture industry to minimise feed costs (McBride et al., 1998), as well as lower water pollution due to digestion products (i.e., ammonia) in the rearing environment. The exact protein requirements for somatic growth in any species of echinoid remains unknown (Cook et al., 1998), but since protein is such an important nutrient for growth processes, higher concentrations of dietary protein are expected to elicit an increased growth response in juvenile sea urchins.

This study investigated the effect of three factors (protein source, protein concentration, and juvenile size) on the somatic growth rates of juvenile green sea urchins. The main objective was to determine the optimal protein source and concentration in prepared diets that maximised the somatic growth of juvenile green sea urchins (*S. droebachiensis*). In addition, the somatic growth of the juvenile sea urchins fed the different prepared diets was compared to the somatic growth of those fed kelp (*L. longicruris*).

2.2 Materials and methods

2.2.1 Laboratory set-up

The experiment used twenty-six out of thirty tanks (49 x 53 x 33 cm), erected in columns standing three tanks high (Figure 2.1a). Each tank contained four black, plastic, hydroponic baskets (22 x 22 x 22 cm) with a mesh size of 2 mm (Figure 2.1b). Thus, 104 treatment baskets, within a randomised block design, were used in the following growth experiment. Each tank had aeration as well as a flow-through seawater system with a separate inflow and outflow that ensured no mixing of water between tanks. Flow rates for each tank averaged 3 - 4 L/min. All tanks were supplied with the same seawater source at ambient temperatures, filtered to 37-$_{\mu}$m using a rotating drum filter. Water temperatures were recorded routinely using either a thermograph or a glass thermometer.

2.2.2 Sea urchin source

Juvenile green sea urchins (*S. droebachiensis*), between 4 mm and 20 mm test diameter (TD), were collected off Tongue Shoal in Passamaquoddy Bay, New Brunswick (45° 03.747' N, 067° 00.600' W), on November 20, 1998, by SCUBA then transported to the laboratory at the Department of Fisheries and Oceans Biological Station in St. Andrews, New Brunswick, Canada. The sea urchins were graded into two sizes: cohort 1 = 4 mm – 8 mm TD; and cohort 2 = 12 mm – 20 mm TD. Thirty sea urchins were randomly selected from each cohort and placed into each rearing basket. Each tank contained two baskets of cohort 1 juveniles and two baskets of cohort 2 juveniles. The sea urchins were

starved for two weeks prior to the growth experiment to standardise their nutritional condition.

2.2.3 Diet preparation

Diets contained one of two protein sources (i.e., plant (soybean protein concentrate) and animal (commercial grade fishmeal)) at one of three different proportions (i.e., 100% soybean protein (SBP), 95% SBP:5% fishmeal protein (FMP), and 50% SBP:50% FMP). These three protein source combinations were added to the diet formulation at 20%, 30%, 40%, and 50% dry mass. All ingredients were supplied by Shur-Gain/Maple Leaf Foods Inc. Table 2.1 summarises the composition of the different prepared diets used in the experiment.

Diet pellet preparation involved mixing the ingredients (Table 2.1), excluding agar, using a Hobart mixer for approximately one hour. As protein concentration increased in the diets, it was mirrored by a decrease in starch concentration in order to keep the percentage of the other ingredients equal among diets. After mixing the ingredients, the binder (i.e., agar) was dissolved in boiling water at a mass ratio of 50:50 (water : total mass of ingredients). The dissolved agar was then added and the mixture was further mixed for an additional 10 minutes until a doughy paste was formed. The doughy feed was removed from the mixer and extruded through a 2 mm-extruding die using a Hobart moist extruder to form 2 mm diameter cords approximately 300 mm in length. The cords were laid on a foil-covered mesh tray, then frozen in a –20°C freezer. The cords were

broken into pellets of approximately 5 mm in length, then bagged, and stored in a –20°C freezer until fed to sea urchins. The feed preparation process was repeated every 3 months to ensure diet freshness. The kelp reference diet (*L. longicruris*) was periodically harvested from wharves and local fishing structures (e.g., herring weir poles) in the area and stored in a tank with running seawater to keep it fresh. Prior to feeding the sea urchins, the stipes were removed and the blades were torn into squares measuring approximately 50 mm^2.

The digestible energy values for each of the diets were calculated by multiplying the level of each organic constituent by its energy equivalent (Brody, 1945; Beukema and DeBruin, 1979). The calculations used to determine the energy budgets of the sea urchins were based on the assumptions that protein, lipid, and carbohydrate digestibilities were 80%, 45%, and 62%, respectively (Lowe and Lawrence, 1976; Klinger et al., 1994; Klinger, 2000).

2.2.4 Diet analyses

Each diet was analysed for water, ash, lipid, and protein. It was assumed that the weight of ash, lipid, protein, and carbohydrate for a particular diet sample equalled 100% of the dry weight of the sample. Based on this assumption, carbohydrate content of each diet was estimated by subtraction. The diet samples used to determine the percentage of ash lipid, and protein were frozen then dried in a freeze-dryer for 3 days before analysis to eliminate the effect of water on the analysis.

2.2.4.1 Water

Three replicate samples (approximately 3 g/sample), for each diet, were dried in a drying oven at 85°C for 24 hours then reweighed. The initial and final sample weights were used to calculate the percent water in the sample using the equation:

$$\% \text{ water} = [(\text{initial weight (g)} - \text{final weight (g)})/\text{initial weight (g)}] \times 100$$

For the kelp samples, the surface water was initially dried off and the residual salt was removed with a damp towel to eliminate inconsistencies generated by the residual sea salt after the drying process. The percent water in each of the diet samples were averaged together to give the average percent water of the diet.

2.2.4.2 Ash

Three replicate samples of pre-dried (i.e., freeze-dried) diet, each weighing approximately 1 g, were combusted in a muffle furnace for 24 hours at 550°C, cooled in a dessicator, then reweighed. The initial and final sample weights were used to calculate the percent ash in the sample using the equation:

$$\% \text{ ash} = [(\text{initial weight (g)} - \text{final weight (g)})/\text{initial weight (g)}] \times 100$$

The percentages of ash of the diet samples were averaged together to give the average percent ash of the diet.

2.2.4.3 Lipid

Lipid was extracted from the freeze-dried replicate diet samples using the Folch extraction method (Folch et al., 1957) (Appendix 1). Two replicate samples, each weighing approximately 1 g, were homogenised in 15 ml of chloroform: methanol (2:1) lipid solvent. The homogenate was filtered to remove the solids. The liquid (which contained the lipid) was mixed with 3.75 ml of 0.88% potassium chloride (KCl) solution to separate the aqueous and lipid layers which facilitated water extraction. The top lipid layer was pipetted off into a clean test-tube then evaporated under nitrogen gas for approximately 30 minutes to retrieve the dietary lipid. The initial and final sample weights were used to calculate the percent lipid in the sample using the equation:

$$\% \text{ lipid} = [(\text{initial weight (g)} - \text{final weight (g)})/\text{initial weight (g)}] \times 100$$

The percentages of lipid of the diet samples were averaged together to give the average percent lipid of the diet.

2.2.4.4 Protein

The nitrogen content of the different prepared diets, determined by the Duman method (Ebling, 1968) using a FP-228 Nitrogen Determinator (Leco®Corp., St. Joseph, Michigan, USA), was multiplied by the factor 6.25 to give the estimate of percent crude protein (Jones, 1941; Schakel et al., 1997). Kelp protein values used in this experiment were referenced from the literature (Chapman and Craigie, 1977; Chapman, 1986) and not determined analytically.

2.2.4.5 Carbohydrate

The estimated carbohydrate content for each of the diets was determined using the equation:

$$\% \text{ carbohydrate} = 100 - (\% \text{ lipid} + \% \text{ ash} + \% \text{ protein})$$

2.2.5 Growth trial

Each dietary treatment consisted of four replicate baskets (30 sea urchins per replicate for a total of 120 sea urchins per treatment) up to the sixth sampling period. At this time, two replicates for all treatments were terminated to allow room for a second growth experiment. Individual tanks were allocated a specific dietary treatment based on a randomised block design and all the baskets in a tank received the same diet. The sea urchins were fed to excess daily from December 4, 1998 to September 10, 1999. Each week the juvenile sea urchins were removed from the baskets and both the rearing baskets and tanks were sprayed clean with hot fresh water and rinsed with cold sea water to remove faeces, uneaten food, and accumulating diatom films.

The test diameters (TD) of all sea urchins were measured monthly. All sea urchins in each treatment basket were transferred to a gridded petri dish and videotaped individually using silhouette imagery by placing the petri dish over a light source. This gave a clear outline of the sea urchins' test between the radiating spines and tube feet. Using Optimas™ image analysis software (from Media Cybernetics, Inc., Maryland, USA), three replicate measurements of the test (from one ambulacral plate to the opposite inter-

ambulacral plate) were recorded for each animal, from which an average TD was calculated. This reduced the variability of the measurements. To eliminate initial size differences, the smallest treatment TD for each cohort was used to standardise the average TD for all sea urchins in the other treatments, using the equation:

$$z = 1-([x-y]/x),$$

where x = average initial TD for treatment A,

y = average initial TD for the smallest treatment, and

z = standardising coefficient.

The individual juveniles for each cohort in treatment A were multiplied by the standardising coefficient for all sample periods. This was repeated for all diet treatments to standardise the initial test measurements for all treatments. In addition, average growth rates were calculated for the juveniles in each treatment basket using the equation:

$$GR = (TD_f - TD_i)/ t$$

where GR = growth rate (mm TD/ day)

TD_f = final test diameter (mm)

TD_i = initial test diameter (mm)

t = time (days)

For each treatment, the growth rates for the juveniles in each of the treatment baskets were averaged together to give the average growth rate of the juveniles in that treatment.

2.2.6 Sea urchin analyses

2.2.6.1 External observations

Following the growth trial, a sample of 30 sea urchins from each treatment, as well as a wild sample of 10 sea urchins, were compared based on physical characteristics of the individuals (i.e., relative spine lengths, test colour, test formation, and other abnormalities). The wild sea urchins were collected by SCUBA on September 20, 1999 from the same location where the experimental juvenile sea urchins were collected (i.e., Tongue Shoal) and ranged in size from 20 mm to 24 mm TD.

2.2.6.2 Internal observations and analyses

Ten sea urchins from cohort 2 were sacrificed and the gonads were removed and measured. Cohort 2 juveniles were used in the internal analyses because they were larger in size with larger gonads, which facilitated gonad removal and decreased measurement errors. Average gonad yield was calculated for the juveniles from each diet treatment as well as from the wild sample using the equation:

Gonad yield (%) = (Gonad weight (g) / Total sea urchin weight (g)) x 100

The gonad yields of all the juveniles in a treatment were averaged together to give the average gonad yield for that treatment.

2.2.7 Statistical analyses

Data were tested for homogeneity of variances using the Levene statistic ($\alpha = 0.05$). When variances were homogeneous, Analysis of Variance (ANOVA) using Tukey's

multiple comparisons were used to analyse for differences among treatments. However, when variances of the data were not homogeneous, the non-parametric Kruskal-Wallis statistic using the Tukey-type Nemenyi test for multiple comparisons ($\alpha = 0.05$) was used (Sokal and Rohlf, 1995; Zar, 1999). Arcsine transformations were calculated for ratios to normalise the data prior to statistical analysis. All statistical analyses were performed using the SPSS statistical software package.

2.3 Results

2.3.1 Diet analyses

2.3.1.1 Protein

The soybean and fishmeal protein sources from Shur-Gain, were not pure protein sources. The fishmeal contained 76% protein, while the soybean contained 66% protein (according to the manufacturer specifications). Thus, the protein concentration for each diet had to be quantified to ensure it was the actual protein concentration desired. From the analyses, all protein concentrations within the diets matched the desired protein concentrations for all treatments (i.e., the 100% SBP at 20% diet contained 20% protein and the 50% SBP:50% FMP at 50% diet contained 50% protein).

2.3.1.2 Lipid

For the diets containing 100% SBP and 95% SBP:5% FMP, the average lipid concentration ranged from 5.9% to 6.4% of the diet dry mass. For the diets containing 50% SBP:50% FMP, the average lipid concentration was significantly higher ($P<0.001$)

than the diets containing the other protein sources, and ranged from 10.5% to 11.6% of the diet dry mass (Figure 2.2). The increase in dietary lipid in the prepared diets correlated with an increase in fishmeal, indicating it had a higher lipid content than the soybean meal per unit volume. The average lipid concentration of the kelp was 2.4% dry mass, which was significantly less than that in the prepared diets ($P<0.001$).

2.3.1.3 Water

The average water content of the kelp used in this experiment was 87% and was significantly higher than that of all the prepared diets, which ranged from 46% to 50% water ($P<0.001$).

2.3.1.4 Ash

The average percent ash for the kelp (38% dry mass) was significantly higher than that of all the prepared diets, which ranged between 4% and 8% of the diet dry mass ($P<0.001$) (Figure 2.3). The average percent ash of the prepared diets increased with an increase in protein concentration for all protein sources. Diets containing 50% protein concentration were significantly higher in ash than the diets containing 20% and 30% protein concentration ($P<0.001$). Likewise, the diets containing 40% protein concentration were significantly higher in ash than the diets containing 20% protein concentration for all protein sources ($P<0.001$). There were no significant differences in average ash content among the different protein sources ($P=0.551$).

2.3.1.5 Carbohydrate

The percentage of carbohydrate in each diet was estimated by difference. Those diets that were low in protein were high in carbohydrate because carbohydrate offset the changes in protein concentration for the diets. The estimated carbohydrate concentrations ranged from 70% dry mass in diet 1 to 31% dry mass in diet 12. The carbohydrate level in kelp was estimated to be 50% of the dry mass.

2.3.2 Energy budgets

The total energy per 1 g of prepared diet ranged from 11.67 kJ for diets 1 and 5 to 13.52 kJ for diet 12 (Table 2.2). The energy content of the kelp reference diet (7.47 kJ/g) was lower than the energy contents of the prepared diets due to lower protein and lipid concentrations and a higher concentration of crude, indigestible fibres in the kelp compared to the prepared diets.

2.3.3 Growth trial

All replicate baskets were pooled together since the growth performances of the juveniles in the basket replicates for each treatment were not significantly different ($P>0.080$). The juvenile sea urchins in cohort 1 that were fed the kelp diet had a significantly larger average TD than those juveniles fed the prepared protein diets ($P<0.05$ for all diets) after the 280 day growth experiment (Figure 2.4; Table 2.3). The initial average size of all the juveniles in cohort 1 was 6.3 mm TD. At the end of the growth experiment the juveniles fed kelp had an average TD of 20.7 mm, whereas the average TD for those fed the

prepared diets ranged from 13.2 mm to 16.2 mm TD (Figure 2.4; Table 2.3). The range in the final average test diameters between the juveniles fed the prepared diets was only 3.0 mm. However, this range did not correlate with the differences in dietary protein (Table 2.3). The only strong observable pattern in the experiment was the significant increase in TD for the kelp-fed juveniles especially noticeable when water temperature increased in early spring (Figure 2.5). After 35 days of the growth experiment (January 8, 1999), the kelp-fed juveniles had a larger average TD than diet 1 and diet 7 ($P=0.012$), and after 150 days (May 3, 1999) and continuing throughout the study, the kelp-fed juveniles had significantly larger tests than all the juveniles fed the prepared diets ($P<0.05$) (Figure 2.4).

The growth trends of the juvenile sea urchins in cohort 2, seen in Figure 2.6, resembled those of cohort 1. All treatments of cohort 2 juveniles had an average initial size of 13.8 mm TD. After the 280 day growth experiment the average test diameter of the kelp-fed juveniles (i.e., 24.5 mm) was significantly larger than all the average TDs for the juveniles fed the different prepared diets, which ranged from 20.4 mm to 22.9 mm ($P<0.001$) (Table 2.4). As for cohort 1, differences in TD of the juveniles fed the prepared protein diets were not correlated to dietary protein (Table 2.4).

For both cohorts, the juveniles fed the prepared diets had significantly smaller average growth rates than those fed kelp for both intervals observed (i.e., from December 4, 1998 to March 30, 1999; and from March 30, 1999 to September 10, 1999) (Table 2.5)

($P<0.001$ for all tests). The average growth rate in both cohorts of juveniles fed the prepared diets up to March 30, 1999 (i.e., 0.022 (+/- 0.001 SE) mm TD/d) was significantly smaller than the average growth rate for the kelp-fed juveniles in both cohorts over the same time period (i.e., 0.030 (+/- 0.002 SE) mm TD/d) ($P<0.001$). From March 30, 1999 to September 10, 1999, which corresponded to an increase in water temperature, the average growth rate of the cohort 1 juveniles fed the prepared diets increased to 0.039 (+/- 0.002 SE) mm TD/d, but was significantly smaller than the average growth rate for cohort 1 juveniles fed kelp over the same time period (i.e., 0.069 (+/- 0.005 SE) mm TD/ day) ($P<0.001$). Similarly, the average growth rate for the cohort 2 juveniles fed the prepared diets increased to 0.035 (+/- 0.001 SE) mm TD/d, but was significantly smaller than the average growth rate for cohort 2 juveniles fed kelp (i.e., 0.052 (+/- 0.003 SE) mm TD/d) ($P<0.001$).

In some treatments of cohort 2 juveniles, test diameter deceased between sample periods (Figure 2.6). The decrease was only observed in the juveniles fed the prepared diets, but it was not correlated to the dietary protein source or concentration. The juveniles responded from a decrease in TD with an increase during the next sample period. Test shrinkage has also been observed in other species when food supply is in short supply (Ebert, 1967; Levitan, 1988; Levitan, 1991; Constable, 1993), or perhaps when essential ingredients for test production are lacking in the diet.

2.3.4 Sea urchin analyses

2.3.4.1 External observations

At the end of the growth trial, the juvenile sea urchins fed kelp were larger than the juveniles fed the prepared diets. As well, all the kelp-fed juveniles had a dark green test with long green spines (Figure 2.7a). These juveniles resembled the juveniles removed from the wild. In contrast, many of the juveniles fed the prepared diets had a pale coloured test, short stubby spines, and raised areas of the test around the aboral region (Figure 2.7b). Also, there were juveniles that suffered from test necrosis where spines would fall off leaving blackened areas of the test (Figure 2.7c). However, there were no significant differences in survivorship (ca. 95%) among any of the treatments resulting from diet influence ($P>0.05$).

2.3.4.2 Internal observations and analyses

The gonads of the kelp-fed juveniles were small (see gonad yield below) and yellow/orange in colour (Figure 2.7d). The gonads of the juveniles fed the prepared diets were larger (see gonad yield below), but pale white in colour (Figure 2.7e). The gonads of the kelp-fed juveniles closely resembled the gonads of the wild juveniles.

The gonad yields of cohort 2 juveniles fed the prepared diets were all significantly greater ($P<0.001$) than the gonad yields of juveniles fed kelp as well as those of juveniles collected from the wild (Figure 2.8). The average gonad yield for the juveniles fed kelp was 4.2%, which was similar to the average gonad yield of 4.0% from the wild sea

urchins (P=0.900). The average gonad yield of the juveniles fed the prepared diets ranged from 13.2% to 21.8% (Figure 2.8). In Figure 2.8, a trend was observed in the 100% SBP and 95% SBP:5% FMP treatments that showed the juveniles fed the diets with 30% protein had higher gonad yields (i.e., 20.9% and 21.6%, respectively) than those fed diets with either 40% or 50% protein concentration (i.e., gonad yield ranged from 13.2% to 17.4%). The juveniles fed the diets with a high fishmeal source (i.e., 50% SBP:50% FMP) did not show any significant differences in average gonad yield with differences in dietary protein concentration (P=0.957).

2.4 Discussion

Sea urchin somatic growth (i.e., test growth) results from complex interactions among several factors including size, feeding behaviour, physical environment, food availability, and food quality (Hatcher and Hatcher, 1997). By varying food quality, while keeping the other factors constant, an optimal diet for juvenile growth may be developed. The first steps in the development of the optimal juvenile diet for sea urchins should first involve maximising the nutrients required for sea urchin somatic growth in a stable (i.e., retaining form and consistency when exposed to sea water) prepared pellet, which is readily ingested, digested, absorbed, and assimilated by juveniles (Klinger et al., 1998). In this experiment, the prepared diets retained their shape and consistency in water, thus diet stability did not appear to have an effect on feeding or growth. Most studies dealing with formulated feeds have examined the effect on either gonad quantity or quality, but studies concerning the effect on somatic growth are less numerous (Fernandez and

Pergent, 1998). Also, there are few studies conducted on the nutritional requirements of sea urchins as they grow from post-metamorphosis to market size (Cook et al., 1998; McBride et al., 1998). Understanding the response of sea urchins to individual nutrients in prepared diets is one way to assess the importance of feed components for growth potential (McBride et al., 1998) since differences in somatic growth have been attributed to differences in food quality (Lawrence and Lane, 1982; Klinger et al., 1983; Andrew and Choat, 1985; Raymond and Scheibling, 1987; Levitan, 1988; Rowley, 1990; Lamare and Mladenov, 2000). The determination of the optimum protein source and concentration in a sea urchin diet is an important step for sea urchin culture because of the importance of protein to sea urchins (Lilly, 1975; Lowe and Lawrence, 1976; de Jong-Westman et al., 1995).

Experiments to determine the optimal diet for juvenile somatic growth occurred because available food supply is a major factor affecting both body growth rates and upper size limits reached by sea urchins (Ebert, 1968). The development of a growth enhancing diet for juvenile green sea urchins is also necessary for the success of the industry due to the relatively slow growth of the species living in the wild (Ebert, 1975; Robinson and MacIntyre, 1997). In the growth trials, the brown kelp (*Laminaria longicruris*) was the superior diet for increasing somatic growth in the juvenile sea urchins, while the juveniles fed the prepared diets that contained the different protein sources and concentrations did not grow as large as those fed kelp over the course of the experiment. As a result, protein source and concentration were not the only major factors affecting juvenile growth.

Similar growth trends for all the juveniles, regardless of diet and size, suggested that additional protein or different sources of protein did not affect growth as long as there was a minimum concentration of dietary protein available to meet the basic requirements for growth.

The juvenile sea urchins in cohort 1 fed diet 9 (50% SBP:50% FMP at 20%) had the largest average TD after 10 months. However, the average TD for the juveniles fed diet 4 (100% SBP at 50%) was smaller by only 1 mm. Growth production of the juveniles fed these two diets was similar even though the diets had different protein sources and concentrations. In addition, juveniles in cohort 2 fed diets 12, 9, and 1 (50% SBP:50% FMP at 50%; 50% SBP:50% FMP at 20%; and 100% SBP at 20%, respectively) produced similar test diameters of 23 mm, but once again these diets had different protein sources and concentrations. The growth performances of all these sea urchins, however, were dwarfed by the performance of the juveniles fed kelp, which consisted of plant protein ranging from 6.25% to 22.5% dry mass (depending on the section of the kelp blade, the time of year, and water depth) with an average of 13.1% protein (Chapman and Craigie, 1977; Chapman, 1986). In general, the somatic growth of juvenile sea urchins fed prepared diets was slow in contrast to those fed kelp. Similar results were found for *Evechinus chloroticus* and *Strongylocentrotus franciscanus* (Barker et al., 1998; McBride et al., 1998). In addition, the growth rates of small sea urchins in other studies were similar when fed prepared diets consisting of different protein sources (Klinger, 2000). As well, small *S. franciscanus* (Fernandez and Pergent, 1998), *Lytechinus variegatus*

(McBride et al., 1998), and *P. lividus* (Wallace et al., 2001), fed prepared diets with various protein concentrations had similar growth rates. Young red sea urchins (*Paracentrotus depressus*) also showed no difference in test diameter growth when fed prepared diets consisting of 20% - 50% protein, but those fed 10% protein had significantly lower growth (Akiyma et al., 2001). Therefore, growth differences do not seem related to protein concentration as long as there is a minimum concentration of protein available to meet basic growth requirements. Differences in growth production for the juveniles in this experiment seemed to be linked to other factor(s) besides dietary protein.

It was unlikely that individual differences (e.g., genetic differences) resulted in growth differences since all the juveniles were collected from the same source population at the same time of the year, all juveniles were randomly distributed among treatments at the same density, and all juveniles were treated equally throughout the experiment. It also was unlikely that differences in juvenile somatic growth were due to physical differences among treatments (e.g., light intensity) since the treatments were randomly assigned to the tanks, thus theoretically eliminating tank effects between treatments.

The results suggest the prepared diets were lacking some essential ingredient(s) for somatic growth (i.e., test growth) that was present in the kelp. An interesting pattern in somatic growth, especially seen for the juveniles in cohort 1, was the decrease in growth rate for those fed the prepared diets after 116 days of the growth (March 30, 1999) and

continuing for the duration of the experiment. This decrease in growth was not observed for the juveniles fed kelp. A similar pattern was identified for small *S. franciscanus* (McBride et al., 1998) and for juvenile *S. droebachiensis* (Williams and Harris, 1998) fed wild and prepared diets. In addition, the kelp-fed juveniles had an increase in somatic growth that coincided with an increase in ambient sea water temperatures (Tajima and Fukuchi, 1991; Hooper et al., 1997; Fernandez and Pergent, 1998). However, the juveniles fed the prepared diets did not mirror this increase in somatic growth. One explanation for this lack of somatic growth for the juveniles fed the prepared diets was that these diets were missing or had insufficient amounts of nutrients necessary for assimilation into, and growth of, the test (Klinger et al., 1998; Klinger, 2000) that were being supplied by kelp. These elements may have been lost to the seawater from the prepared diet by leaching or they may not have been incorporated in the diet formulation. For example, in studies comparing amino acids in prepared diets and macro-algae, arginine was found to be the limiting amino acid for *Pseudocentrotus depressus* (Akiyama et al., 1997). Thus, some macro-nutrients and/or micro-nutrients necessary for growth may have been lacking from the prepared diets. However, individual components (i.e., amino acids, fatty acids, minerals and pigments) of the diet were not quantified in this experiment, thus specific factors required for somatic growth could not be identified.

Other components in kelp, besides protein, may be responsible for its success in growth production. For example, according to de Jong-Westman et al. (1995), high gonad growth rates recorded for kelp were not due to protein, but probably to other nutrient

components like algin, a key carbohydrate store that makes up more than 20% of the kelp's dry mass and is readily absorbed by sea urchins (Boolootian and Lasker, 1964). This is not surprising since *S. droebachiensis* have evolved to grow and survive on sea plants, of which *L. longicruris* is preferred (Larson et al., 1980). In addition, the balance of dietary nutrients, for example dietary amino acid balance, is an important factor for improving somatic growth of sea urchins (Akiyma et al., 2001). The kelp diet supplied the juveniles with balanced levels of the essential ingredients required for somatic growth throughout the growth trial compared to the ingredients supplied by the prepared protein diets.

Having evolved to survive and grow on sea plants suggests that the digestive system of sea urchins is able to effectively utilise crude indigestible plant material like cellulose. Some studies have identified the presence of N_2-fixing bacteria in the intestinal region of sea urchins (Lasker and Giese, 1954; Fong and Mann, 1980; Guerinot and Patriquin, 1981). These bacteria are an important transformer serving to create a more stable nutrient source for sea urchins (Lasker and Giese, 1954; Burkholder et al., 1971). However, if the bacterial colony were reduced in the sea urchins fed the prepared diets, due to unfavourable conditions caused by the diets, the nutrients normally supplied by the bacteria would be unavailable to these sea urchins. On the other hand, kelp may have been a source of the natural beneficial bacteria, and eliminating kelp from the diet would, therefore, reduce the internal bacterial colonies. Another possibility is that the juveniles fed kelp may have been less stressed throughout the experiment probably due to the

presence of a wild diet and/or a cleaner environment. The increased waste and lower water quality generated from using prepared diets, caused by the disintegration and biodegradation of the food along with sea urchin metabolic wastes, represented conditions that could have lead to high stress and inhibition of growth (Fernandez and Pergent, 1998; McBride et al., 1999). Kelp may also have provided refuge for the juveniles who are naturally cryptic and inhabit the crevices and undersides of rocks (Keats et al., 1984; Raymond and Scheibling, 1987), and, therefore, simulated the wild environment more closely than the prepared diets.

The decrease in test diameter near the latter stages of the growth trial for the larger juveniles in cohort 2 may also have been an indicator of food stress (i.e., depletion of nutrient reserves). Studies have shown that food-stressed sea urchins or those fed a low quality diet (i.e., high concentrations of unusable material) may exhibit shrinkage in test diameter (Ebert, 1968; Lawrence, 1975; Ebert, 1980; Black et al., 1984; Russel, 1987; Lewis et al., 1990; Edwards and Ebert, 1991; Morris and Campbell, 1996) caused by a possible decrease in suture width (Constable, 1993). The prepared protein diets, which lacked ingredients for optimal test growth, may have stressed the larger juveniles thereby facilitating test shrinkage.

Since food quality can alter the basic feeding patterns and growth rates of sea urchins (Vadas, 1977; Fernandez and Boudouresque, 1998), diet analyses were completed on all diets to gain an understanding of the growth promoting qualities of kelp compared to the

prepared diets used in this experiment. The biochemical composition of kelp varied with season and harvest location since it is a product of its environment (Chapman and Craigie, 1977). Kelp was harvested monthly throughout the experiment to maximise freshness and quality, but this also may have resulted in nutrient compositional differences among the monthly samples. Even with compositional variation, average protein and lipid concentrations in kelp were lower than those found in the prepared diets. The nutritional components with a higher concentration in kelp than in the prepared diets that could be identified in this experiment were ash (up to 40% dry mass of kelp), pigment, and water.

Dietary ash (a measure of the inorganics in the diet) may be an important factor in juvenile sea urchin growth since the test is comprised mainly of inorganic compounds. One essential mineral in physiological processes is calcium, which is indispensable in spine and tooth regeneration as well as in the overall growth process (Ebert, 2001). Obtaining such minerals from the seawater environment may not be adequate to promote rapid growth (Grosjean et al., 1998), suggesting required minerals supplied to the sea urchin through the diet may enhance test production. The ash content in the prepared diets showed a trend of increasing value as the protein concentration increased, especially with an increase in fishmeal concentrations. This trend reflected the minerals in the protein sources especially the fishmeal. However, the types of minerals and the lower concentration in the prepared diets (i.e., 3% to 6.5% dry mass) may have hindered optimal sea urchin somatic growth. On the other hand, the type and concentration of

minerals available in kelp seemed to satisfy the requirements of increased juvenile test growth.

The function of pigments in the growth processes of juvenile sea urchins has not been widely studied. Juveniles obviously absorb pigment from the diet because the gonad of the sea urchins fed the non-pigmented prepared diets were pale white (i.e., they had no pigment), while the gonads from the sea urchin fed the kelp (with naturally occurring pigments) were yellowish orange (Figures 2.7d and 2.7e). *Evechinus chloroticus* juveniles fed non-pigmented prepared diets also had white gonads, while those fed algal food had coloration resembling wild sea urchins (Barker et al., 1998). Thus, sea urchin gonad colour can be attributable to different diet ingredients (Watts et al., 1998). Pigments also function as antioxidants and, therefore, may increase the ability of sea urchins to utilise lipids in test production (Matsuno and Tsushima, 2001). Pigments were incorporated by the juvenile sea urchins fed kelp in this experiment and therefore may have been beneficial for test production.

The excess energy available to the sea urchins fed the prepared diets was apparent from their large gonad yields compared to the gonad yields of the kelp-fed juveniles. The gonad is both a sex organ responsible for gametogensis and the major energy storage organ (Gonor, 1973; Walker, 1982; Tajima et al., 1986), thus it can be an indicator of the reproductive stage as well as the energy available to the individual (Keats et al., 1984). Since the juveniles fed the high-energy prepared diets did not show rapid test growth,

compared to the kelp treatment, the excess metabolised energy was rapidly converted to reserves (i.e., glycogen) and stored in the gonad (Klinger et al., 1998; Russel, 1998). Assuming the juveniles were reproductively mature, it was unlikely that the large gonad yields resulted from increased reproductive capacity since the sampling time (i.e., mid-September) was outside the adult reproductive season of winter and early Spring (Walker, 1982; Pearse and Cameron, 1991; Fernandez and Pergent, 1998; Klinger, 2000). In addition, no spawning activity (release of eggs or sperm) was observed upon dissection of these juveniles. Therefore, it was assumed that the large gonads were indicative of high nutrient storage as opposed to gamete production.

Comparing the gonad yields of the juvenile sea urchins fed the prepared diets consisting of either 100% SBP or 95% SBP:5% FMP showed that the juveniles fed the diets with 30% protein had the largest gonad yields, while those fed diets with higher or lower protein concentrations had lower gonad yields. In addition, juveniles fed diets with 95% SBP:5% FMP at 40% and 50% dry mass had larger average gonad yields than juveniles fed diets with 100% SBP at the same concentration (i.e., 40% and 50%). Thus, a decrease in gonad yield seemed to be correlated with an increase in plant protein above 30%. On the other hand, juveniles fed diets with 50% SBP: 50% FMP had high gonad yields for all the protein concentrations. The protein content in these diets, however, did not exceed 30% plant protein, even in the 50% protein diet (i.e., the diet consisted of 25% soybean protein and 25% fishmeal protein). Thus, in this experiment, 30% dry mass of plant protein in the diet appeared to be optimal for the digestion, assimilation and storage

of excess energy by sea urchin juveniles. A possible reason for these trends in gonad yield may be an inability of sea urchin juveniles to process a large percentage of terrestrial plant protein (i.e., soybean protein) in a prepared diet. This may result from the impact of soy toxins, such as protease inhibitors and phytates in soybean protein (Anonymous, 2002) that were ingested with the diets by the juveniles. Protease inhibitors prevent protein metabolism, while phytates act as chelating agents that bind to metal ions (e.g., calcium) making them less available for biological functions. It is possible that the levels of soy toxins in the diets containing 40% and 50% protein for both the 100% SBP and the 95% SBP:5% FMP protein sources inhibited protein digestion in the juvenile sea urchins and, therefore, showed reduced gonad yield. However, there was no effect on test production, which suggests protein concentration (under the conditions of the experiment) did not affect test growth. Other dietary factors, therefore, must be required to maximise juvenile somatic growth.

The final aspect of the study was the physical appearances of the juveniles immediately following the growth trial. The juveniles fed kelp had healthy looking tests with dark green coloration and long, green spines. The juveniles fed the prepared diets, however, had unhealthy looking tests that varied in coloration from pale green to purple/red and had short, pale spines. Other studies with *S. droebachiensis* (Williams and Harris, 1998) and *Psammechinus miliaris* (Cook et al., 1998) had similar findings with those fed prepared diets having shorter spines and paler test coloration compared to sea urchins fed macroalgae. Since diet was the only variable between the kelp and prepared diet

treatments in this experiment, the abnormalities in physical appearance were assumed to result from poor nutrition. The incorporation of nutrients, like minerals and pigments, may have been limiting in the juveniles fed the prepared diets compared to the kelp-fed juveniles resulting in poor health, reduced growth, and inconsistent test pigmentation (Williams and Harris, 1998). Improper test formation due to limiting ingredients would ultimately increase stress upon the sea urchins. Thus, the abnormalities in physical appearance may be an indicator of such stress. The kelp diet provided the juvenile sea urchins with the required nutrients for growth thereby minimising stress caused by nutrient deficiencies.

The juvenile green sea urchins (*S. droebachiensis*) fed the prepared protein diets had smaller test diameters than those fed kelp after the 280 day growth experiment. The energy gained from the prepared diets was directed to gonad production for storage. Minimal gonad production occurred in the kelp-fed juveniles suggesting that most of the energy gained from the kelp diet was directed to test production. Neither protein source nor protein concentration in the prepared diets affected juvenile test production in this experiment. Other dietary ingredients that were deficient in the prepared diets, but present in the kelp, are important for optimal test production in juveniles green sea urchins.

Chapter 3

The effect of lipid source and concentration on the somatic growth of juvenile green sea urchins (*Strongylocentrotus droebachiensis*)

3.1 Introduction

Understanding the effect of prepared diets on juvenile sea urchin growth at various stages of development will provide valuable information regarding the feasibility of sea urchin aquaculture and the sustainability of the industry. The major nutrient components of the prepared diets must reflect the major nutrient requirements of sea urchins, which are polysaccharides, proteins, and lipids (Cook et al., 1998). It was suggested in Chapter 2 that the protein sources and concentrations tested did not affect test production (providing there was sufficient protein available to meet the basic metabolic requirements of the sea urchins). This experiment will determine the effect of lipid source and concentration on the somatic growth production of juvenile sea urchins.

Lipids are important for sea urchin growth because they are a concentrated source of energy (i.e., they can store more energy per unit volume than either proteins or carbohydrates) (Montero-Torreiro et al., 1998). Lipids are also important structural components for cellular membrane production (e.g., phospholipids and cholesterol), which is an essential process for somatic growth (Takagi et al., 1980; Voogt, 1982; Ebert, 2001; Marsh and Watts, 2001). As well, certain lipids can serve as vitamins, hormones, and pigments (e.g., carotenoids) as well as precursors to essential substances such as

eicosanoids, which function in internal processes such as osmoregulation and immune responses (Hwang, 1992). However, no studies have identified optimal dietary lipid concentration for sea urchin somatic growth.

It is important to determine the dietary lipid requirements for the growth of juvenile sea urchins because, depending on the animal, certain lipids are essential (i.e., have to be supplied by the diet), while others can be synthesised within the body. For example, most animals have an essential requirement for the n-3/n-6 fatty acid groups for growth and survival (Chapkin, 1992). The fatty acids found in adult *S. droebachiensis* were described by Takagi et al. (1980), but these may differ from the fatty acids that support optimal juvenile somatic growth due to differences in adult and juvenile diets (Kelly et al., 1998). Takagi et al. (1980) also found both n-3 and n-6 fatty acids in sea urchin tissues that were not present in the diet and suggested that the sea urchins had the ability to synthesise required fatty acids from dietary precursors (Bell et al., 2001). Although fatty acids in sea urchin tissues have been identified, lipid requirements for juvenile growth are still unknown. For example, in *Psammechinus miliaris*, Cook et al. (2000) suggested that DHA (a long-chain n-3 fatty acid) is important for growth since it was present in the diet that gave superior growth among the diets tested. However, Pantzis et al. (2000) suggested that vegetable diets, which are high in short-chain n-6 fatty acids, provide the required lipids to increase growth in *P. miliaris*. Understanding the responses of juvenile sea urchins to dietary lipids is essential to formulating diets that maximise somatic growth.

The concentration of dietary lipids also is important because it affects diet energy (Montero-Torreiro et al., 1998), efficiency of lipid metabolism, the concentration of other diet ingredients, and diet cost. However, in some species of echinoids the concentration of dietary lipids is not reflected in the tissue lipid concentration (de Jong-Westman, 1995; Watts et al., 1998). Therefore, the combination and concentration of dietary lipids are important factors that must be considered when optimising sea urchin somatic growth.

This study tests the effects of three factors (lipid source, lipid concentration, and sea urchin size) on the somatic growth production of juvenile green sea urchins. In addition, the somatic growth of the juveniles fed the prepared diets was compared to the somatic growth of the those fed a naturally preferred kelp diet, *Laminaria longicruris*. It's expected that juveniles fed prepared diets that are high in both n-3 and n-6 fatty acid concentration will have the best growth production due to the importance of lipids for overall health, growth, and energy, and because neither essential fatty acid group would be limiting.

3.2 Materials and methods

The study was conducted at the St. Andrews Biological Station (SABS) in St. Andrews, NB, Canada from May 8, 1999 to June 5, 2000. The lipid source experiment was conducted from May 8, 1999 to March 3, 2000 and the lipid concentration experiment was conducted from November 25, 1999 to June 5, 2000.

3.2.1 Laboratory set-up

The laboratory set-up was identical to the one used in Chapter 2 (Figures 2.1a & 2.1b). During the feeding trials, sea urchins were housed in plastic mesh baskets (22 x 22 x 22 cm) with a mesh size of 1-2 mm. Four of these baskets were suspended in each experimental tank (49 x 53 x 33 cm). Each tank had aeration as well as a flow-through water system with a separate inflow and outflow to eliminate cross-contamination between tanks. All tanks were supplied with ambient temperature, filtered sea water (filtered through a 32-$_{\mu}$m rotating drum filter) at flow rates that averaged between 3 – 4 L/min.

Each prepared diet treatment (6 treatment diets for the lipid source experiment and 8 treatment diets for the lipid concentration experiment) consisted of four replicate baskets for cohort 1 and four replicate baskets for cohort 2 juveniles. The kelp reference treatment consisted of two replicate baskets for cohort 1 and two replicate baskets for cohort 2 juveniles. Each replicate basket contained 30 randomly selected juvenile sea urchins, thus each prepared diet treatment had a total of 120 juveniles from each cohort, while the kelp reference treatment had a total of 60 juveniles from each cohort. Each experimental tank housed two baskets of cohort 1 juveniles and two baskets of cohort 2 juveniles, with all juveniles in the tank fed the same diet. Individual tanks were allocated a specific diet based on a randomised block design.

3.2.2 Sea urchin source

All the animals for this study were collected by SCUBA from Tongue Shoal, Passamoquoddy Bay, New Brunswick (45° 03.747'N, 067° 00.600'W), 2 weeks prior to each experiment. The population occupied a gravel/sandy area with the smaller sea urchins hidden cryptically under small rocks or shell debris. Divers used dip nets (1mm mesh) to scoop the juveniles from the bottom and place them in collection bags. The juvenile sea urchins were then transferred to water-filled containers at the surface and transported to the laboratory at the Department of Fisheries and Oceans Biological Station in St. Andrews, New Brunswick. The juvenile sea urchins were graded by size into two cohorts; cohort 1 juveniles were between 6 mm and 9 mm test diameter (TD), and cohort 2 juveniles were between 13 mm and 16 mm TD. The juveniles were acclimated to laboratory conditions for two weeks and were starved during this time to ensure a similar nutritional condition among all juveniles.

3.2.3 Diet preparation

Shur-Gain/Maple Leaf Foods Inc supplied all diet ingredients.

3.2.3.1 Lipid source experiment

Cylindrical pellets (2 mm x 5 mm) were prepared by moist extrusion. For each prepared diet, the dry ingredients (Table 3.1), with the exception of gelatin, were weighed and mixed with a Hobart mixer for 10 minutes. The required lipid source (the lipid sources used in the experiment are described in Table 3.2) was then added and mixed for another

10 minutes. When the ingredients were thoroughly mixed, dissolved gelatin (at a mass ratio of 45:55, water : dry ingredients) was added and the ingredients were further mixed for approximately 5 minutes until a doughy paste formed. The moist feed was removed from the mixer and transferred to an extruder where it was pressed to form cylindrical rods. These rods were placed onto foil-covered mesh trays, frozen in a –20°C freezer for 4 hours, then cut into 5 mm pieces and stored in 8 L plastic bags in the –20°C freezer. After the materials used in the diet preparation process were thoroughly cleaned, the process was repeated until each diet was made. Weekly rations of each prepared diet were kept in plastic containers in the –20°C freezer for daily feeding of the animals. This minimised the thawing of the diet.

3.2.3.2 Lipid concentration experiment

Diet preparation was the same as the lipid source experiment. The lipid sources used in this experiment (menhaden oil and corn + linseed oils) were those that were being fed to the fastest growing juveniles in the lipid source experiment. Table 3.3 shows the concentration of ingredients used in diet preparation. As the concentration of dietary lipid increased, the concentration of starch decreased in order to keep the percentage of the other ingredients equal among diets.

3.2.3.3 Reference diet

Laminaria longicruris (i.e., brown kelp) was used as the reference diet in this study. The kelp was collected periodically from several areas within Passamoquoddy Bay and stored

at the St. Andrews Biological Station in tanks with running water and aeration. This kept the kelp fresh throughout the feeding experiments. The juveniles were fed only the blade portion of the kelp frond torn into squares approximately 50mm^2; the stipe portion was discarded.

3.2.4 Diet analyses

Diet analyses were only performed for the diets prepared for the lipid source experiment. One week after diet preparation 50 g samples from each diet (including kelp) were freeze-dried for 4 days to remove water content. Prior to freeze-drying the kelp sample, the surface was cleaned with a moist cloth then blotted to remove the remaining droplets of seawater. The freeze-dried samples were then used to determine the percentage of lipid and ash in the various diets.

3.2.4.1 Lipid

Two replicate samples from each diet, weighing approximately 1 g, were analysed for lipid using the Folch extraction method (Folch et al., 1957). Each sample was homogenised in 15 ml of chloroform: methanol (2:1) lipid solvent, filtered, then mixed with 3.75 ml of 0.88% potassium chloride (KCl) solution to facilitate water extraction. The top lipid layer was pipetted off into a clean test-tube then evaporated under nitrogen gas for approximately 30 minutes to retrieve the dietary lipid. Refer to Appendix 1 for the step-by-step procedure of the lipid extraction method. The initial and final sample weights were used to calculate the percent lipid in the diet sample using the equation:

% lipid = [(initial weight (g) – final weight (g))/initial weight (g)] x 100

The percent lipid values for the diet samples were averaged to give the average percent lipid of the diet.

3.2.4.2 Ash

Two replicate samples from each diet, weighing approximately 1 g, were combusted in a muffle furnace for 24 hours at 550°C, cooled in a dessicator, and reweighed. The initial and final sample weights were used to calculate the percent ash in the diet samples using the equation:

% ash = [(initial weight (g) – final weight (g))/initial weight (g)] x 100

The percent ash in each of the diet samples were averaged together to give the average percent ash of the diet.

3.2.5 Growth trial

All sea urchins were fed to excess for the duration of each experiment. The tanks were cleaned weekly by removing the sea urchins from the baskets and spraying the empty baskets and tanks with hot, fresh water to remove uneaten food and accumulated diatom films. The tanks were refilled with seawater, baskets were returned to their original location, and the sea urchins were returned to the appropriate baskets.

The juvenile sea urchins were measured monthly for changes in somatic growth. The indicator of somatic growth in this study was test diameter (a measure from the center of

one ambulacral plate to the center of the opposite interambulacral plate). Test diameter was measured using video imaging. The sea urchins from each basket were transferred to a gridded petri-dish containing seawater, which was placed over a light source to create a silhouette image of the individual sea urchins. This image allowed a clear outline of the test between the radiating spines and tube feet. The images of the juveniles were videotaped with a Hi-8 video camera mounted above the light source. The grids on the petri-dish allowed calibration of the video image. Using Optimas™ image analysis software (from Media Cybernetics, Inc., Maryland, USA), three linear measures of the test diameter were recorded and averaged for each sea urchin, to improve estimating accuracy of the test diameter. This procedure was repeated for all the sea urchins at each sample period.

To quantify the growth pattern of the sea urchins in the different treatments, the specific growth rate (SGR) of the juveniles in each replicate basket for the different treatments was calculated using the equation described by Busacker et al. (1990):

$$SGR = 100 * [Ln(TD_2) - Ln(TD_1)] / (t_2 - t_1),$$

where SGR = specific growth rate of sea urchins in basket replicate X

Ln = natural logarithm

TD_1 = average test diameter of sea urchins in basket replicate X at time 1

TD_2 = average test diameter of sea urchins in basket replicate X at time 2

t_1 = time 1 in days

t_2 = time 2 in days

The SGRs for the replicate baskets in each treatment were averaged to give the SGR for that treatment. In the lipid source experiment, SGRs were determined for each treatment from May 8, 1999 to June 10, from June 10 to August 12, and from August 12 to March 3, 2000 (i.e., day 0 to 33, day 33 to 96, and day 96 to 300, respectively). In the lipid concentration experiment, SGRs were determined for each treatment from November 25, 1999 to March 6, 2000, from March 6 to April 5, and from April 5 to June 5, 2000 (i.e., day 0 to 102, day 102 to 132, and day 132 to 193, respectively).

3.2.6 Sea urchin analyses

3.2.6.1 External and internal observations

At the end of each experiment the external appearances of the sea urchins (i.e., test coloration, spine length, spine colour, and any physical abnormalities) fed the different diets were observed. In addition, a representative sample of juveniles fed each treatment diet were sacrificed to observe the appearance of the gonad (i.e., size and colour).

3.2.6.2 Test, gonad, and gut yields

Following the lipid source experiment, a sample of twenty cohort 2 juveniles from each of the prepared diet treatments and a sample of ten cohort 2 juveniles from the kelp diet treatment were weighed and sacrificed. Tissue yields were not conducted on juveniles

from the lipid concentration experiment. Only cohort 2 juveniles were used because they were larger in size, which facilitated gonad removal and decreased measurement errors. The test, gonads, and gut (i.e., remaining internal tissues) were separated from each animal. The average test, gonad, and gut yields were determined for each animal using the equation:

Tissue Yield = (wet tissue weight (g)) / total sea urchin weight (g)) x 100

The test, gonad, and gut yields of all the juveniles in a treatment sample were averaged to give the average test, gonad, and gut yield, respectively, for that treatment.

3.2.6.3 Tissue lipid

A random sample of four sea urchins from each of the diet treatments were analysed for the lipid content in the internal soft tissue (i.e., gonad and gut combined) using the Folch extraction procedure (Folch et al., 1957) described above in section 2.4.1 Dietary lipid analysis. In addition, a random sample of three sea urchins from the corn + menhaden oil, the linseed + menhaden oil, and the kelp treatments were analysed to determine the percent lipid in each of the test, gonad, and gut. However, the percent lipid in the gonad and gut of the kelp-fed juveniles was lost and not recorded.

3.6.6.4 Tissue ash

A random sample of three sea urchins from the corn + menhaden oil, linseed + menhaden oil, and kelp treatments were each analysed for ash content in the test, gonad, and gut using the procedure described above in section 2.4.2 Dietary ash analysis.

3.2.7 Statistical analyses

The data analyses for this study were performed using the "SPSS 8.0 for Windows" statistical software package. The Levene statistic (α=0.05) was used to test for homogeneity of variances for all data. Data with homogeneous variances were statistically analysed using Analysis of Variance (ANOVA) with Tukey's multiple comparisons to determine differences among independent factors (Sokal and Rohlf, 1995; Zar, 1999). Data with heterogeneous variances were analysed using the non-parametric Kruskal-Wallis statistic (α = 0.05) with the Tukey-type Nemenyi test for multiple comparisons (Mosteller and Rourke, 1973; Sokal and Rohlf, 1995; Zar, 1999). Arcsine transformations were calculated for ratios to normalise the data prior to statistical analyses.

3.3 Results

3.3.1 Diet analyses

3.3.1.1 Lipid

There were no significant differences in the lipid concentration among any of the diets used in the lipid source experiment (P=0.051), which ranged from 5.1% lipid for kelp to 9.9% lipid for the corn oil + menhaden oil diet (Figure 3.1).

3.3.1.2. Ash

There were no significant differences in ash content among the prepared diets (P=0.110), which ranged from 13.0% dry mass for the corn + linseed oil diet to 18.3% dry mass for

the linseed oil diet (Figure 3.2). However, the average percent ash of the kelp diet (i.e., 54.1% dry mass) was significantly larger than the average percent ash values of all the prepared diets ($P<0.001$).

3.3.2 Water temperature

The temperature of the incoming water, shown in Figure 3.3, was recorded throughout the duration of this experiment. The water temperature increased from 7.5°C on May 20, 1999 to a maximum of 14°C on September 13, 1999. The temperature them decreased to a low of 1.7°C on February 23, 2000 and then increased to 8.4°C on June 14, 2000.

3.3.3 Growth trial

There were no significant differences in juvenile test diameter among replicate baskets in each treatment over the duration of the lipid source experiment ($P=0.145$) and the lipid concentration experiment ($P=0.066$), thus replicate baskets in each treatment for both experiments were pooled. In addition, the juvenile sea urchins in both experiments had high survival rates that ranged from 95% to 100% for all treatment groups.

3.3.3.1 Lipid source experiment

3.3.3.1.1 Cohort 1

The growth patterns of the juvenile sea urchins in cohort 1 fed the different diets are shown in Figure 3.4. At the start of the experiment, the average test diameter (TD) of the kelp-fed juveniles and those fed the linseed oil diet were significantly larger than the

average TDs of the juveniles fed the other diets ($P<0.001$). However, the average juvenile TDs for all treatment groups only ranged from 6.3 mm to 7.7 mm.

The juveniles fed the prepared diets had similar growth rates up to August 12, 1999 (day 96) (all tests; $P>0.05$) (Figure 3.4). From May 8 to June 10 (day 0 to 33), the average specific growth rate (SGR) of juveniles fed kelp (0.30) was significantly smaller than the average SGRs of the juveniles fed corn oil (0.50), corn + linseed oil (0.57), and linseed + menhaden oil (0.61) diets ($P=0.021$, $P=0.007$, and $P<0.001$, respectively) (Figure 3.5a). From June 10 to August 12 (day 33 to 96), the average SGR of kelp-fed juveniles (0.53) was also significantly smaller than the average SGRs of those fed the menhaden oil (0.63), corn + menhaden oil (0.61), and linseed + menhaden oil (0.63) diets ($P=0.013$, $P=0.006$, $P=0.002$, respectively) (Figure 3.5a). From August 12 to March 3, 2000 (day 96 to 300), the average SGRs of the juveniles fed the prepared diets, which ranged from 0.11 for those fed the linseed oil diet to 0.13 for those fed the linseed + menhaden oil diet, were significantly lower than the average SGR of the kelp-fed juveniles (0.28) ($P<0.001$) (Figure 3.5a). As a result, the average TD for the kelp-fed juveniles after the 300 day feeding experiment (i.e., 21.0 mm) was significantly larger than the average TDs of the juveniles fed the prepared diets ($P<0.001$), which ranged from 15.0 mm for those fed the corn + linseed oil diet to 16.2 mm for those fed the linseed oil diet. There were no significant differences in the SGRs among the juveniles fed the prepared diets throughout the experiment ($P=0.096$), thus differences in TD among these juveniles at the end of the experiment were due to the initial TD differences among the treatment groups.

3.3.3.1.2 Cohort 2

The growth patterns of the sea urchins in cohort 2 fed the different diets are shown in Figure 3.6. The initial average test diameter of the juveniles fed kelp (i.e., 13.7 mm) was significantly smaller than the average TDs of juveniles fed the various prepared diets ($P<0.001$), which ranged from 15.1 mm for those fed the menhaden oil diet to 15.8 for those fed the corn + menhaden oil diet. There were no significant differences among the initial average test diameters for the juveniles fed the prepared diets ($P=0.234$).

From May 8, 1999 to June 10 (day 0 to 33), the kelp-fed juveniles had a significantly lower average SGR (0.10) than the juveniles fed the prepared diets ($P=0.023$), which ranged from 0.28 for those fed the linseed oil diet to 0.35 for those fed the linseed + menhaden oil diet (Figure 3.5b). There were no significant differences in the average SGRs among the juveniles fed the prepared diets during this period ($P=0.786$).

From June 10 to August 12 (day 33 to 96) the average SGR of the kelp-fed juveniles increased to 0.40, and was similar to the average SGRs of the juveniles fed the prepared diets ($P=0.292$), which ranged from 0.31 for those fed the linseed + menhaden oil diet to 0.37 for those fed the menhaden oil diet.

From August 12 to March 3, 2000 (day 96 to 300) the average SGRs of the juveniles fed the prepared diets, which ranged from 0.04 for the juveniles fed the corn + menhaden oil

diet to 0.05 for those fed the menhaden oil diet, were similar (P=0.869), but significantly lower than the average SGR of 0.17 of the kelp-fed juveniles (P<0.001) (Figure 3.5b). During this period, and similarly throughout the experiment, there were no significant differences in SGRs among the juveniles fed the prepared diets (P=0.989).

At the end of the experiment the kelp-fed juveniles, with an average TD of 25.7 mm, was significantly larger than the average TDs of the juveniles fed the various prepared diets (P<0.001), which ranged from 23.1 mm for those fed the menhaden oil diet to 23.6 mm for those fed the linseed + menhaden oil diet (Figure 3.6). Throughout the feeding experiment there were no significant differences among the average test diameters of the juveniles fed the prepared diets (P=0.234).

3.3.3.2 Lipid concentration experiment

3.3.3.2.1 Cohort 1

The growth patterns of the sea urchins in cohort 1 fed the different diets are shown in Figure 3.7. The initial average TD for the juveniles were statistically similar among treatment groups (P=0.200) and ranged from 8.0 mm for those fed the 10% menhaden oil diet to 8.5 mm for those fed the 7% linseed + corn oil diet. From November 25, 1999 to March 6, 2000 (day 0 to 102), the kelp-fed sea urchins had a lower average SGR of 0.18, but not significantly different than the average SGRs of the juveniles fed the prepared diets (P=0.083), which ranged from 0.21 for those fed the 7% corn + linseed oil diet to 0.27 for those fed the 1% corn + linseed oil diet (Figure 3.8a). Hence, after 102 days, the

average TD of the kelp-fed juveniles (i.e., 9.7 mm) was significantly smaller than the average TDs of the juveniles fed the different prepared diets (P=0.002), which ranged from 10.3 mm for those fed the 10% menhaden oil diet to 10.8 mm for those fed the 3% menhaden oil diet. There were no significant differences in the average TDs of the juveniles fed the prepared diets (P=0.557).

From March 6 to April 5 (day 102 to 132) there were no significant differences among the average SGRs of the juveniles fed the various diets (P=0.053). The average SGRs of the juveniles fed the prepared diets decreased during this period compared to those from day 0 to 102, whereas the kelp-fed juveniles showed an increase in average SGR (Figure 3.8a). As a result, on April 5 the average TD of the kelp-fed juveniles (i.e., 10.5 mm) was similar to the average TDs of the juveniles fed the different prepared diets (P=0.080), except for the significantly larger juveniles fed the 3% Menhaden oil diet (i.e., 11.6 mm TD) (P<0.001).

From April 5 to June 5 (day 132 to 193) the average SGR of the kelp-fed juveniles (i.e., 0.65) was significantly higher than the average SGRs of the juveniles fed the various prepared diets (P<0.001), which ranged from 0.26 for those fed the 7% corn + linseed oil to 0.39 for those fed the 1% menhaden oil diet (Figure 3.8a). There were no significant differences among the average SGRs of the juveniles fed the prepared diets (P=0.076), which had increased compared to the average SGRs from day 102 to 132. Hence, the average test diameter of the kelp-fed sea urchins (i.e., 15.4 mm TD) was significantly

larger than the average TD of the juveniles fed the prepared diets (P<0.001), which ranged from 12.5 mm for those fed the 10% menhaden oil diet to 13.8 for those fed the 3% menhaden oil diet (Figure 3.7).

There were also some trends in the SGR data that were not statistically identified due to the low statistical power of the SGR data (i.e., n = 4). For example, from March 6 to June 5 (day 102 to 193) the average SGRs of the juvenile sea urchins fed the 1% and 3% lipid diets were larger than the average SGRs of those fed the 7% and 10% lipid diets (Figure 3.8a). As a result, the average TDs of the juveniles fed either the 1% or 3% lipid diets were significantly larger than the average TDs of the juveniles fed the 10% lipid diets (P<0.001).

3.3.3.2.2 Cohort 2

The average initial TDs of the juveniles ranged from 14.7 mm for the 10% menhaden oil treatment to 15.6 mm for the 1% menhaden oil treatment (Figure 3.9). The average TDs of the juveniles fed these two diets were significantly different from each other (P=0.022), but both were similar to the other treatments (P=0.160). From November 25, 1999 to March 6, 2000 (day 0 to 102), the sea urchins in the kelp treatment had a significantly lower average SGR (0.08) than the average SGRs of juveniles fed the prepared diets (P=0.001), which ranged from 0.14 for those fed the 10% corn + linseed oil diet to 0.18 for those fed the 10% menhaden oil diet (Figure 3.8b). There were no

significant differences in average SGRs among the prepared diet treatment groups (P=0.233).

From March 6 to April 5 (day 102 to 132), there were no significant differences in average SGRs among the diet treatments (P=0.344). However, the average SGR of the juveniles fed the prepared diets decreased, while the average SGR of the kelp-fed juveniles increased compared to day 0 to 102 (Figure 3.8b). As a result, after 132 days, there were no significant differences among the average TD of the kelp-fed juveniles (i.e., 17.4 mm) and the average TDs of juveniles fed the prepared diets (P=0.071), which ranged from 17.8 mm for those fed the 10% menhaden oil diet to 18.5 mm for those fed the 1% menhaden oil diet (Figure 3.9).

From April 5 to June 5 (day 132 to 193) the average SGRs for all treatment groups increased, but the average SGR of the kelp-fed juveniles (0.32) was significantly higher than the average SGRs of the juveniles fed the prepared diets (P<0.001), except for those fed the 1% menhaden oil diet (P=0.148) (Figure 3.8b). There were no significant differences among the average SGRs of the juveniles fed the prepared diets (P=0.080), which ranged from 0.16 for those fed the 7% menhaden oil diet to 0.24 for those fed the 1% menhaden oil diet.

There were some trends in the SGR data, similar to those in the cohort 1 juveniles, that were not statistically identified due to the low statistical power of the data (i.e., n = 4).

For example, from April 5 to June 5, the juveniles fed the 1% and 3% lipid diets had larger average SGRs than the juveniles fed the 7% and 10% lipid diets. As a result, the average TDs of juveniles fed the 1% and 3% menhaden oil diets or the 1% corn + linseed oil diet were significantly larger than the average TDs of the juveniles fed the 10% lipid diets ($P<0.001$). The kelp-fed juveniles had a larger average TD than the juveniles fed the 7% and 10% lipid diets ($P=0.021$), but it was similar to the average TDs of the juveniles fed the 1% and 3% lipid diets ($P=0.123$) (Figure 3.9).

3.3.4 Sea urchin analyses

3.3.4.1 External and internal observations

At the start of both experiments the juvenile sea urchins had green tests, relatively long green spines, and active transparent tube feet. At the end of both experiments, the sea urchins fed the kelp diet had a similar healthy appearance to the initial sea urchins (except for a larger average test diameter) (Figure 3.10a). On the other hand, the majority of the juveniles fed the prepared diets had different appearances than the kelp-fed juveniles. For example, the sea urchins fed the prepared diets had various test colours ranging from pale green to dark green to pale red, and their spines and tube feet were shorter and less active than those on the kelp-fed juveniles. As well, fluid-filled sacs surrounded the anus on the aboral surface of the sea urchins fed the prepared diets, which caused an irregular appearance of the test (Figure 3.10b). Internally, the juveniles fed the prepared diets had large white gonads (see gonad yield below) (Figure 3.10c), while the

kelp-fed juveniles had smaller (see gonad yield below, orange/yellow gonads (Figure 3.10d)

3.3.4.2 Test, gonad, and gut yields

The average test, gonad, and gut yields of juvenile sea urchins fed the various diets were used as additional indicators of somatic growth. There were no significant differences in the average test yields among juveniles fed the different prepared diets, which ranged from 74.0% for the juveniles fed the linseed oil diet to 77.4% for those fed the corn + linseed oil diet (P=0.730) (Figure 3.11a). The average test yield for the kelp-fed sea urchins was 91.9% and was significantly larger than the average test yields of the juveniles fed the prepared diets (P<0.001).

The average gonad yields of the juvenile sea urchins fed the prepared diets, which ranged from 16.3% for those fed the corn + linseed oil diet to 19.4% for those fed the corn + menhaden oil diet (Figure 3.11b), did not differ significantly from one another (P=0.647), but were significantly larger than the average gonad yield of the kelp-fed juveniles (i.e., 4.1%) (P=0.001).

Similarly, there were no significant differences in the average gut yields among the juveniles fed the prepared diets (P=0.431), which ranged from 5.7% for those fed the corn + menhaden oil diet to 6.7% for those fed the linseed oil diet (Figure 3.11c). The

sea urchins fed the kelp diet had an average gut yield of 3.9%, which was significantly smaller than the average gut yields of the juveniles fed the prepared diets ($P<0.001$).

3.3.4.3 Tissue lipid

The lipid content of the internal tissue (i.e., gut and gonad combined) ranged from 17.6 % lipid in the juvenile sea urchins fed the linseed + menhaden oil diet to 23.4 % lipid in the kelp-fed juveniles (Figure 3.12). There were no significant differences in the internal tissue lipid content among the juveniles fed the various diets ($P=0.140$).

The average test lipid content of juvenile sea urchins fed the corn + menhaden oil diet, the linseed + menhaden oil diet, and the kelp diet (i.e., 1.5%, 1.5%, and 1.2%, respectively) were not significantly different ($P=0.051$) (Figure 3.12a). Similarly, the average gonad lipid content of the juveniles fed the corn + menhaden oil diet and the linseed + menhaden oil diet (i.e., 14.3% and 20.7%, respectively) were not significantly different ($P=0.050$) (Figure 3.13b). As well, the average percent gut lipid content of the juveniles fed the corn + menhaden oil diet and the linseed + menhaden oil diet (i.e., 24.0% and 30.7%, respectively) were not significantly different ($P=0.065$) (Figure 3.13c). However, the gut lipid content was significantly larger than the gonad lipid content of juveniles fed the corn + menhaden oil diet ($P<0.001$) and the linseed + menhaden oil diet ($P=0.043$). [Note: Data for the gonad and gut lipid content of the kelp-fed juveniles were lost]

3.3.4.4 Tissue ash

The average test ash content of the juveniles fed kelp, linseed + menhaden oil diet, and corn + menhaden oil diet (i.e., 74.3%, 71.5%, and 69.7% dry mass, respectively) (Figure 3.14a) were not significantly different from one another (P=0.481). However, a trend showing higher percent ash in the tissues of kelp-fed juveniles was observed.

The kelp-fed juveniles had a significantly higher gonad ash content of 9.7% dry mass compared to the gonad ash content of the juveniles fed the corn + menhaden oil diet and the linseed + menhaden oil diet (i.e., 4.2% and 5.7%, respectively) (P<0.001) (Figure 3.14b). There was no difference in the average gonad ash content among the juveniles fed these two prepared diets (P=0.061).

Similarly, the gut tissue in the kelp-fed juvenile sea urchins had an average of 8.8 % ash (Figure 3.14c). This was significantly higher than the gut ash content of the juveniles fed the corn + menhaden oil diet and the linseed + menhaden oil diet (i.e., 4.5% and 4.7%, respectively) (P<0.001). Like the other tissues, there was no significant difference in the average gut ash content among the juveniles fed the two prepared diets (P=0.895).

3.4 Discussion

3.4.1 Lipid source experiment

From the start of the experiment to August 12, 1999 (day 96), the average specific growth rates (SGRs) of the sea urchins fed the prepared diets were either greater than or similar

to the average SGRs of the juveniles fed the kelp diet, for both cohorts. Hence, during this time the prepared diets were as sufficient as the preferred wild kelp diet (i.e., *Laminaria longicruris*) in satisfying the growth requirements of the juvenile sea urchins. Most studies on small sea urchins also have shown prepared diets satisfy the requirements for test growth (Klinger et al., 1998), but these studies occurred over intervals of less than five months. After the fifth month of this feeding trial, the average SGRs of the juveniles fed the prepared diets decreased, similar to the findings of McBride et al. (1998) and Lamare and Mladenov (2000). However, the growth rate of the kelp-fed juveniles remained high after 5 months similar to other studies (Barker et al., 1998; Klinger et al., 1998; McBride et al., 1998; Williams and Harris, 1998). Although differences in somatic growth have been attributed to differences in food quality and quantity (Lawrence and Lane, 1982; Klinger et al., 1983; Andrew and Choat, 1985; Raymond and Scheibling, 1987; Rowley, 1990), the exact differences in food quality have been, up to now, unknown.

From the literature, *Laminaria* species of kelp are high in arachidonic acid (20:4n-6) and EPA (20:5n-3) (Paradis and Ackman, 1977), which are different than the major fatty acids in the various prepared diets used in this study (Table 3.2). However, the juveniles fed the different diets (including kelp) had similar growth rates for the first 96 days of the experiment. In addition, the prepared diets containing the corn oil + menhaden oil and the corn oil + linseed oil provided the juveniles with the precursors required to synthesise the major fatty acids present in kelp. It has been suggested that some species of sea

urchins can synthesise required fatty acids (Takagi et al., 1980; Bell et al., 2001) since the sea urchin lipids can differ from the fatty acid composition of their diets (Kochi, 1969). Therefore, the reduction in growth rate common to all the juveniles fed the prepared diets was unlikely the result of essential fatty acid deficiencies. The juveniles fed the prepared diets with different lipid sources (i.e., different major fatty acid groups) had similar growth rates throughout the feeding trial, which suggests that the sea urchins could utilise all lipid sources equally for somatic growth. This implies that some other essential component(s) necessary for growth, that was present in the kelp diet, was deficient in all the prepared diets (Klinger et al., 1998; Williams and Harris, 1998; Klinger, 2000).

The common pattern of growth shown by the juveniles fed the prepared diets (i.e., initial rapid growth followed by a sudden decline after 96 days that continued for the duration of the feeding trial) was unlikely the result of water temperature fluctuations. Although water temperature affects the growth rate of sea urchins (Fernandez and Pergent, 1998), the kelp-fed juveniles that also were subjected to the same water temperature did not show the characteristic growth trend of those fed the prepared diets. The water temperature was similar among all treatments, but the results show that the average SGRs of the juveniles fed the different diets were not always similar. Thus, water temperature is unlikely to be the major cause of growth differences between the juveniles fed the prepared diets and those fed kelp.

This common pattern of growth was similar for both cohorts suggesting that juvenile size was not a major factor in the growth patterns observed. The nutritional deficiencies of the prepared diets had a similar impact on the small and large juveniles suggesting that differences in developmental processes or food manipulation between the cohorts (if any existed) did not impede growth performance of the sea urchins. In addition, the larger SGRs of the smaller juveniles in cohort 1, which also has been observed in other sea urchins studies (Raymond and Scheibling, 1987; Meidel and Scheibling, 1998b; Chapter 2 of Thesis), showed that the smaller juveniles could manipulate, digest, and assimilate the prepared diets and kelp as efficiently as the larger juveniles.

The rapid growth rate over the initial 96 days of the juveniles fed the prepared diets compared to the juveniles fed kelp (for both cohorts) suggests that there were no nutritional deficiencies for somatic growth during this period (McBride et al., 1998). This balanced supply of nutrients, combined with the high-energy prepared diet (Chapter 2 of Thesis), provided the factors necessary for rapid test growth. The juveniles fed the kelp, although they also had a balanced supply of required nutrients, did not have a high-energy food source (Vadas et al., 2000). The balanced supply of nutrients that was initially utilised by the juvenile sea urchins likely originated prior to the experiment while the sea urchins were living in the wild. Nutrients assimilated by the sea urchins from diets in the wild that were not allocated to growth and maintenance would be stored in the gonad (Gonor, 1972; Fernandez and Boudouresque, 1998; Russel, 1998) making them available for future use. Therefore, during the initial period of rapid test growth, the

energy from the prepared diets was complemented by the stored nutrients in the juveniles previously acquired from the natural environment. Since the prepared diets were high in energy compared to the kelp, the juveniles fed the prepared diets showed higher initial growth rates.

The decrease in growth rate common among the juveniles fed the prepared diets likely resulted from a depletion of the stored nutrients in the gonad and an inability of the juveniles to replenish these reserves. As discussed above, the prepared diets were likely deficient in some nutrients required for somatic growth, thus could not supply the juveniles with these nutrients. The kelp, on the other hand, had a balanced supply of required nutrients for sea urchin somatic growth, hence no dramatic decrease in somatic growth was observed in the juveniles fed kelp. Maintaining a well-balanced diet that adequately supplies the juvenile sea urchins with the required nutrients for growth appears to be more effective in achieving larger sea urchins over a longer time interval than just supplying the sea urchin with an unbalanced, high-energy diet. The ideal prepared diet would provide both well-balanced nutrients and high-energy to maximise juvenile somatic growth throughout the juvenile stage of the life-cycle.

The effect of the high-energy prepared diets on the juvenile sea urchins compared to the kelp diet was observed in the tissue yields. Sea urchin gonads are the primary storage tissues where most energy not utilised for growth and maintenance is stored (Holland and Giese, 1965; Gonor, 1972; Gonor, 1973; Walker, 1982; Tajima et al., 1986; Pearse and

Cameron, 1991). However, the gut yield is also a sensitive indicator of the nutritional condition of the sea urchin (Keats et al., 1984; Bishop et al., 1994) since gut tissue is the immediate storage site for assimilated nutrients (Klinger, 2000). Although the allocation of resources to different tissues (i.e., test, gonad, and gut) may vary with the level of food quality and quantity (Minor and Scheibling, 1997), the lack of test growth in the juveniles fed the prepared diets after day 96 implied that most of the assimilated nutrients from the prepared diets were being allocated to the gonad and gut (Klinger et al., 1998). This was verified by the tissue yields of the sea urchins fed the prepared diets as well as those fed kelp. The kelp-fed sea urchins directed more energy towards test growth than to either the gonad or gut tissues, whereas those fed the prepared diets directed more energy towards gonad and gut tissues than to the test. The juveniles fed the prepared diets did not produce rapid somatic growth thus the excess energy was being allocated to the storage cells in the gonad and gut tissues.

One of the major nutrients that may affect the somatic growth performance of the juveniles is minerals. Diets analyses has shown that the kelp diet had significantly higher ash (a measure of mineral content) than the prepared diets, and this difference may have played a role in juvenile growth performance. Minerals are required nutrients for sea urchins because they are important components for test construction (Okazaki, 1956; Wilbur, 1976; Grosjean et al., 1998) as well as other physiological functions (Bishop et al., 1994). The kelp-fed juveniles were supplied with high mineral concentration and did not appear deficient in essential minerals for test growth. Minerals are also abundant in

the seawater, and sea urchins may derive some of the essential minerals from the seawater environment (Grosjean et al., 1998). However, it was evident that the juveniles fed the kelp diet, high in mineral concentration, grew to large sizes, thus additional essential minerals attained from the diet seem to complement the growth performance of the juveniles. The higher ash content of the kelp diet also was reflected in the internal storage tissues (i.e., gonad and gut) of the juveniles fed kelp, suggesting that the sea urchins uptake and store the dietary minerals that may be later required for somatic growth. Hence, the nutritional deficiency of the prepared diets may be linked to the low mineral concentration of the prepared diets.

In addition, the absence of specific minerals in the prepared diets may have lead to further nutritional deficiencies. For example, magnesium, an important mineral for test construction (Grosjean et al., 1998), was absent from the mineral source used in formulating the prepared diets (Pers. Comm., Adel El Mowaffi, Shur-Gain, Missassauga, Ontario). Magnesium, however, was present in the kelp diet (Cho et al., 1995). The absence of magnesium from the prepared diets combined with the gradual utilisation of stored reserves may have lead to the sudden decrease in SGR for those juveniles fed the prepared diets.

Another major difference between the kelp diet and the prepared diets was presence of pigment in the kelp (Haugan and Liaaen-Jensen, 1994; Matsuno and Tsushima, 2001) and a total absence of pigment in the prepared diets. Pigments are important in many sea

urchin physiological functions (Lukyanova and Khotimchenko, 1995; Kawakami et al., 1998; Matsuno and Tsushima, 2001) and may be related to the potential nutritional deficiency of the prepared diets. Similar to minerals, pigments would not be limiting in juveniles in the wild since pigments would be obtained from the wild diet. However, after 96 days being fed the non-pigmented prepared diets, the natural pigments in the juveniles accumulated prior to the study likely became depleted and the prepared diets could not replenish the pigment stores. The kelp diet, however, continuously supplied the juveniles with pigments, thus the kelp-fed juveniles did not become pigment deficient. This pigment deficiency was easily observed in the gonads of the juveniles. The gonads of the sea urchins fed the prepared diets, similar to those observed by Barker et al. (1998), were white in colour (i.e. no pigments), whereas the gonads of the kelp-fed juveniles had an orange/red coloration accumulated from the kelp pigments (Tsushima and Matsuno, 1990; Matsuno and Tsushima, 2001). The absence of dietary pigments would affect physiological processes in the sea urchins, which may have directly affected somatic growth or indirectly affected somatic growth by increasing stress within the animals.

In addition to somatic growth, physical appearance was also an indicator of the nutritional deficiencies in the prepared diet (Cook et al., 1998). Similar to the observations in Chapter 2 and by Williams and Harris (1998), the short spines of the juveniles fed the prepared diets, compared to the spines of the kelp-fed juveniles, were indicative of poor nutrition and stress. In addition to poor nutrition, pollution can be a

causative agent of stress in sea urchins (Fernandez and Pergent, 1998). Leaching of the water soluble nutrients from the prepared diets would have resulted in a reduction of the nutrients ingested by the sea urchin as well as an increase in the organic loading of the surrounding water. Kelp is not prone to leaching (since it is evolved to live in seawater) thus nutrients are not lost to the water column and pollution is minimal. Therefore, the juveniles fed the prepared diets were subjected to poor nutrition and increased pollution, which are capable of inhibiting growth (Bottger et al., 2001) and reducing the animals' health by suppressing feeding and digestive functions (Lares and McClintock, 1991). The prepared diets used in this study seemed to induce stress in the juveniles that resulted in dwarfed spine growth, poor test appearance, and reduced growth.

3.4.2 Lipid concentration experiment

In the lipid concentration feeding experiment, as in the previous experiment, both cohorts of juveniles had similar growth trends (i.e., high initial growth for the first 102 days followed by minimal growth for 30 days followed by high growth for the duration of the feeding trial). This suggested that the nutritional needs of the two size groups tested were similar. As well, all the prepared diets over the range of lipid concentrations tested (i.e., 1% to 10%) provided sufficient lipids to increase somatic growth, though some differences in the effectiveness of the prepared diets to increase somatic growth became evident.

The differences in the initial growth rates between experiments (i.e., the juveniles in the lipid source experiment had higher initial SGRs than the juveniles in the lipid concentration experiment) was likely caused by differences in ambient seawater temperatures. This experiment was started in late autumn while the lipid source experiment was started in late spring; thus the ambient seawater temperatures were lower at the start of this experiment. Since sea urchin growth is temperature dependent (over the natural range of the sea urchin) (Fernandez and Pergent, 1998), the average SGRs of the juveniles in this experiment were lower than those of the juveniles in the lipid source experiment.

Similar to the lipid source experiment, after 102 days into this experiment there was a general decrease in test growth for those juveniles fed the prepared diets, but a general increase for those fed kelp. These trends for the juveniles fed the prepared diets were likely due to the factors described above, such as nutrient(s) depletion, increased stress, and increased organic loading.

The increased growth rates exhibited by all the juveniles fed the various diets from day 132 to the end of the experiment, which was not observed in the juveniles in the lipid source experiment, most likely resulted from a change in the surrounding environment and independent of treatment effects because the increased SGRs were common for all juveniles. An increase in diatom films on the basket walls may have provided an additional source of essential nutrients for the growing juveniles (Ebert, 1968; Raymond

and Scheibling, 1987; Tajima and Fukuchi, 1991), but the effect would be minimal since the baskets were cleaned weekly. Therefore, it is probable that the increase in temperature, and to a lesser extent phytoplankton density, promoted juvenile somatic growth during the final months of the experiment. However, the increase in average SGRs was still higher for the kelp-fed juveniles compared to those fed the prepared diets (except for the juveniles fed the 1% menhaden oil diet). In addition, the physical appearances of the juveniles fed the prepared diets and kelp were similar to those in the lipid source experiment (i.e., the kelp-fed juveniles resembled wild sea urchins whereas those fed the prepared diets had pale coloured tests with short, stubby spines). This further suggests that the prepared diets were deficient in the specific nutrients required for maximum test growth that were present in kelp.

Similar to the lipid source experiment and to the observations by Klinger et al. (1998), there were no differences in growth among the juveniles fed the different lipid sources in this experiment (i.e., menhaden oil and corn oil + linseed oil). However, there were differences in the final test diameters of the juveniles fed the prepared diets and these differences were related to lipid concentrations in the prepared diets. The juvenile sea urchins fed the lower lipid diets (i.e., 1% and 3% lipid) grew to a larger average size than the juveniles fed the high lipid diets (i.e., 10% lipid). For comparison, the lipid levels in fresh *Laminaria saccharina* range from 0.10% to 0.39% from June to August (Vadas et al., 2000), whereas the average lipid content of local *L. longicruris* ranged from 2.4%

(Chapter 2 of the Thesis) to 5.1% (Figure 3.1). This suggests that juvenile sea urchins do not require high lipid concentration in their diet for optimal somatic growth.

This negative effect of high dietary lipid concentration may be related to the excess energy storage function of the gonad. Lipids, which have more energy per unit volume than either proteins or carbohydrates (Chow, 1992; Montero-Torreiro et al., 1998), have high absorption efficiency in sea urchins (42% to 72%) (Klinger et al., 1998). Therefore, increasing the dietary lipid would increase the energy available to the sea urchin. Since excess energy is stored in the gonad, gonads in the juveniles fed the high lipid diets would grow faster than the gonads in the juveniles fed the low lipid diets. According to Marsh and Watts (2001), most of the energy stored in the gonads may not be available to the sea urchins due to the limited availability of oxygen necessary for aerobic metabolism. Hence, large gonads in growing juveniles would required increased energy requirements for tissue maintenance and likely would increase stress in the juveniles. In addition, animals that have a small requirement for lipids, such as sea urchins, the high-lipid diets may depress the digestibility of other nutrients (e.g., amino acids) by restricting the action of digestive enzymes (van Barneveld et al., 1998). Thus, high-energy, high-lipid diets may decrease the availability of other nutrients required for growth as well as increase juveniles stress.

In addition, an increase in lipid concentration increases the potential for lipid peroxidation within the diets as well as within the sea urchins, and peroxides have a

negative effect on growth, health and survival of sea urchins (Lukyanova and Khotimchenko, 1995). Lipid peroxidation is minimised by antioxidants (such as ethoxyquin or pigments) (Lukyanova and Khotimchenko, 1995), but the prepared diets used in this study did not contain antioxidants. As the stores of pigment in the juvenile tissues decreased over time (Havardsson and Imsland, 1999), the high lipid diets may have increased the stress in the juveniles (through increased peroxide concentration), which eventually would lead to reduced feeding and growth (Lares and McClintock, 1991).

Dietary lipids are an important nutritional component for juvenile sea urchins in promoting somatic growth because of their roles in membrane structure, cellular energy, and other physiological processes. It is still questionable whether green sea urchins have the ability to elongate and/or desaturate to synthesise required fatty acids or whether the required fatty acids have to be obtained from the diet. However, the lipid types tested in this study, which differed in n-3 and n-6 fatty acids, did not result in significant differences in the somatic growth patterns of the juvenile sea urchins. Also, juvenile size did not affect the growth promoting qualities of the prepared diets since both cohorts had similar growth patterns when fed the same diets. Providing there are lipids available to satisfy the metabolic and maintenance requirements of the sea urchins, the rate of juvenile somatic growth was not dependent on lipid source under the conditions of the study. However, the concentration of lipids in the diet did affect the somatic growth of the juvenile sea urchins. The juveniles fed the prepared diets with low lipid

concentrations (i.e., 1% and 3% lipid) had larger test diameters at the end of the experiment than those fed diets with the high lipid concentration (i.e., 10% lipid). Other factors that may have affected the somatic growth of the juveniles were nutritional deficiency in the prepared diets, juvenile stress, and seawater temperature. The juveniles fed the low-energy, low-lipid kelp diet outperformed the juveniles fed the prepared diets in test growth production over the duration of the feeding experiments. Kelp provided the sea urchins with a continuous supply of nutrients required for somatic growth in a well-balanced diet.

Chapter 4

The effect of minerals and pigment on the somatic growth of juvenile green sea urchins (*Strongylocentrotus droebachiensis*)

4.1 Introduction

Recent studies have established that juvenile prepared diets require at least 20% plant and/or animal protein (McBride et al., 1998; Akiyama et al., 2001; Wallace et al., 2001; Chapter 2 of Thesis), a low concentration of lipids with n-3 and/or n-6 essential fatty acids (Chapter 3 of Thesis), a carbohydrate energy source, as well as minerals and vitamins (Nagai and Kaneko, 1975; Lawrence et al., 1991; Fernandez and Pergent, 1998; Klinger et al., 1998; Williams and Harris, 1998) to provide the required nutrients for growth. However, in some studies kelp-fed juveniles had superior growth compared to the growth of juveniles fed prepared diets (McBride et al., 1998; Williams and Harris, 1998; Chapters 2 and 3 of Thesis). Some of the major differences between *L. longicruris* and the prepared diets were low mineral and low pigment concentrations in the prepared diets compared to the kelp (William and Harris 1998, Chapters 2 and 3). Therefore, minerals and pigments may be essential nutrients for continued juvenile sea urchin somatic growth.

The effect of minerals and pigments in prepared diets on juvenile sea urchin somatic growth has not been thoroughly investigated. Minerals, especially calcium and magnesium, are important in test construction (Okazaki, 1956; Wilbur, 1976; Grosjean et

al., 1998), and other physiological processes (Bishop et al., 1994). The function of pigments in sea urchins, on the other hand, is not well documented. In general, animal pigments function in light absorption, camouflage, deactivating reactive species, immune functions, and reproduction (Hallenstvet et al., 1978; Lukyanova and Khotimchenko, 1995; Kawakami et al., 1998; Matsuno and Tsushima, 2001), but how they are involved in sea urchin somatic growth has not yet been investigated. In addition, the effect of minerals and pigments on somatic growth may differ depending on the size of the sea urchin since smaller juveniles may have different somatic requirements than larger juveniles (Hooper et al., 1997; Kelly et al., 1998; Meidel and Scheibling, 1998a). Thus, various prepared diets may be necessary to meet the growth requirements of different sizes of juvenile sea urchins.

This study was designed to show the effect of minerals and pigments in moist-extruded diets on somatic growth of juvenile green sea urchins (*S. droebachiensis*). The study will attempt to (1) show the effect of mineral source on juvenile somatic growth, (2) determine the optimal mineral concentration for maximum juvenile somatic growth, (3) examine the effect of dietary pigment on juvenile somatic growth, (4) investigate the effect of juvenile size on diet performance, and (5) compare the growth performances of the juveniles fed the prepared diets with those fed kelp. In addition, the effect of dietary minerals and pigment on the internal tissues will be investigated. It was anticipated that minerals and pigment would have a positive effect on juvenile somatic growth because of their importance in test structure and internal physiological functions.

4.2 Materials and methods

4.2.1 Experiment 1

4.2.1.1 Juvenile collection

Juvenile sea urchins were collected by SCUBA on February 11, 2000 in Brandy Cove adjacent to the government wharf at the federal department of Fisheries and Oceans Biological Station in St. Andrews, New Brunswick, Canada (45° 04.935'N, 067° 05.102'W). Using commercially available aquarium dip nets, juveniles were scraped off the ocean bottom and transferred to collection bags. At the surface the sea urchins were further transferred to buckets of sea water, taken into the lab, and placed in a holding tank with running sea water at ambient temperature and aeration. The sea urchins were measured and only those with test diameters (the linear distance from one ambulacral plate to the opposite interambulacral plate of the sea urchin) between 13 mm and 15 mm test diameter (TD) were used in the experiment.

4.2.1.2 Laboratory set-up

The laboratory set-up consisted of a header tank with temperature controlled sea water, 18 experimental tanks (40 cm x 55 cm x 34 cm), 2 floating baskets (22 cm x 22 cm x 22 cm with 2 mm mesh size on all sides and bottom) per tank, and a water filtration system (Figure 4.1). Incoming sea water at ambient temperature was filtered through a 32-μm sand filter and two cartridge filters (30-μm and 5-μm), then mixed with heated filtered sea water (30-μm and 5-μm cartridge filters) in a header tank (92 cm x 92 cm x 64 cm).

The water was mixed in the header tank using aeration, and water temperature was maintained at 10°C using the computer software "ADI Process Monitor 5" (Analog Devices Inc., MA, USA). Water from the header tank supplied each of the experimental tanks at 2 L/min. All tanks were flow-through with aeration. The tanks were connected in groups of three by a collecting pipe for water outflow. Photoperiod in the lab was set at a constant mid-August 14:10 (L:D) for the duration of the experiment.

Thirty juvenile sea urchins (13 mm – 15 mm TD) were randomly assigned to each of the thirty-six baskets. The sea urchins were starved for 10 days to standardise the nutritional condition of the animals. Water temperature in the tanks was increased from the initial ambient temperature of 0°C to 10°C over these 10 days at 1°C every 24 h.

4.2.1.3 Treatments

In this experiment a modified Bernhart-Tomerelli salt mix at 0%, 1.5%, 3%, 6%, and 10% dry mass, and a Shur-Gain/Maple Leaf Foods mineral mix at 3% and 6% dry mass were incorporated into pigmented diets containing 1.25% Algro™ (a sprayed-dried nutritional ingredient derived from the alga, *Dunaliella salina* that is high in natural beta-carotene) (Robinson et al., 2002). The concentration of beta-carotene in the diets was 250 mg/kg. In addition, 3% mineral from each mineral source was added to non-pigmented diets. These nine treatment diets (Table 4.1) were used to test the effect of dietary minerals and pigments on juvenile sea urchin somatic growth. The pigmented diet with 0% mineral was used to test for the effect of mineral concentration as the

control treatment diet. The effect of mineral source was determined by comparing the growth performance of the two mineral sources at a constant 3% mineral concentration. The effect of pigment was determined by comparing the growth performance of the pigmented and non-pigmented diets with a constant 3% mineral concentration. Each of the 9 treatment diets was fed to two tanks (i.e., 4 baskets) of juveniles based on a randomised block design.

4.2.1.4 Diet preparation

Diets were prepared one week prior to the start of the experiment following the procedure outlined in Chapters 2 and 3. The diet ingredients (Table 4.1) were mixed using a Hobart mixer for 10 minutes. Dissolved gelatin was then added to form a doughy paste that was extruded into 2 mm diameter strands of approximately 30 mm in length. There was a 40:60 ratio of boiling water to dry ingredients. The strands were frozen at –20°C, cut into 5 mm length pieces, then stored in bags at –20°C.

4.2.1.5 General procedures

The feeding experiment started in February 2000 and ended in July 2000 (i.e., 154 days). The juvenile sea urchins were fed to excess daily (i.e., feed was always available for consumption). Approximately one food pellet (2 mm x 5 mm) was made available for each sea urchin daily. Water temperature was monitored daily, as was the physical appearance and health of the sea urchins. Dead sea urchins were recorded and removed from the baskets immediately to avoid contamination. The tanks were cleaned weekly;

this involved removing the sea urchins from the baskets, emptying the tanks and spraying the tanks and baskets clean with hot, fresh water. The tanks were then refilled with seawater from the header tank and the sea urchins were replaced in the appropriate baskets. As well, the sand filter was back-washed and the cartridge filters were either sprayed clean or replaced with new filters depending on sediment load.

4.2.1.6 Somatic growth

The growth performance of the treatment diets was determined by monitoring the increase in test diameter of the juveniles fed the different diet treatments. Test diameter was measured for all sea urchins on days 0, 51, 88, 112, and 154 of the feeding experiment. Test diameter (TD) was measured following the procedure outlined in Chapters 2 and 3. Silhouette images of the individual sea urchins were video-taped then measured with an image analysis system using Optimas™ computer software. The average juvenile TDs for each basket were compared within each treatment and among treatments.

To quantify the growth patterns indicated by the test diameter data, specific growth rates (SGRs) were calculated for each treatment basket from day 0 to 51 (February 14 to April 5, 2000), from day 51 to 112 (April 5 to June 5, 2000), and from day 112 to 154 (June 5 to July 17, 2000) using the equation described by Busacker et al. (1990):

$$SGR = 100 * (Ln(TD_2) - Ln(TD_1)) / (t_2 - t_1), \text{ where}$$

SGR = specific growth rate

Ln = natural logarithm

TD_1 = average test diameter of sea urchins in basket replicate X at time 1

TD_2 = average test diameter of sea urchins in basket replicate X at time 2

t_1 = time 1 in days

t_2 = time 2 in days

The SGRs for the sea urchins in replicate baskets in each treatment were averaged to give the average SGR for all the juveniles in that treatment.

4.2.1.7 Sea urchin analyses

4.2.1.7.1 External and internal observations

Qualitative measures based on observations were made for the juveniles fed the different diets at the end of the experiment. The appearance of the test (i.e., colour, necrosis, and abnormal features), spines (i.e., colour, relative length, and erectness), and gonad (i.e. colour) for the juveniles fed the different diets were noted.

4.2.1.7.2 Test, gonad, and gut yields

Following the feeding experiment, twenty sea urchins, randomly selected from each treatment, were sacrificed to determine the effect of diet on tissue growth. The test, gonad, and gut (i.e., remaining viscera) were separated, dried at 80°C for 48 h, and weighed for each sea urchin. The test, gonad, and gut were each expressed as a percentage of the total dry weight of the animal using the equation:

Tissue yield (%) = (weight of dry tissue (g) / total dry weight of the sea urchin (g))*100

The test, gonad, and gut yields for the twenty sea urchins were averaged to give the average test, gonad, and gut yields for the juveniles fed that diet treatment.

4.2.1.7.3 Tissue ash

At the end of the feeding experiment, a random sample of four sea urchins were removed from the treatments fed the pigmented diets containing 0% mineral, 3%, and 15% modified Bernhart-Tomerelli salt mix and the non-pigmented diet with 3% modified Bernhart-Tomerelli salt mix, to determine tissue ash content. These sea urchins were sacrificed and the test, gonad, and gut (i.e., remaining viscera) were separated and weighed. The samples were transferred to preweighed clay crucibles and burned in a muffle furnace at 550°C for 24 h, following the procedure used in Chapters 2 and 3. The remaining ash for each tissue sample was weighed and the replicates were averaged to obtain a measure of the inorganic material within the test, gonad, and gut for each treatment.

4.2.2 Experiment 2

4.2.2.1 Sea urchin source

The juveniles used in this experiment were produced in the hatchery at the St. Andrews Biological Station. Adults were spawned in March-April 2000 and samples of the settled juveniles were collected in late October 2000. The juveniles were separated into two

cohorts by size; cohort 1 had an average TD of 1.3 mm and cohort 2 had an average TD of 2.3 mm.

4.2.2.2 Laboratory set-up

The laboratory set-up consisted of six tanks with each tank holding two floating baskets, as outlined above. The mesh size of the baskets was too large (i.e., 2 mm) to hold the juvenile sea urchins, thus each basket was lined with 250-$_{\mu}$m mesh. The other aspects were the same as the laboratory set-up for Experiment 1.

4.2.2.3 Treatments

The treatment diets in this experiment were the best diet (in terms of somatic growth production) from Experiment 1 (i.e., the pigmented diet with 15% Bernhart – Tomerelli salt mix) and kelp, *Laminaria longicruris*. Each treatment diet was randomly assigned to three of the six tanks. The two baskets in each tank were randomly assigned to either cohort 1 or cohort 2. Each basket held 40 juvenile sea urchins, thus each treatment contained a total of 120 sea urchins.

4.2.2.4 Diet preparation

The prepared diet was prepared prior to the start of the experiment, using the same procedure as described above in Experiment 1. The pellet size was reduced to 2 mm x 1 mm to facilitate feeding by the smaller juveniles. Kelp that was less than 300 mm in blade length was collected weekly from wharves in the local area and stored at the St.

Andrews Biological Station in a holding tank with flow-through seawater and aeration. Only the blades were fed to the sea urchins; the stipes were removed and discarded.

4.2.2.5 General procedures

The feeding experiment lasted from November 2, 2000 to April 10, 2001 (i.e., 159 days). Laboratory procedures were similar to those outlined in Experiment 1.

4.2.2.6 Somatic growth

The performance of the diet treatments was measured by increases in juvenile sea urchin test diameter. The test diameter for all sea urchins was measured at day 0, day 40, day 119, and day 159. As in Experiment 1, the silhouette image of each sea urchin was video-taped and the diameter was measured with an image analysis system using Optimas™ computer software.

As in Experiment 1, the growth patterns that were identified by the test diameter data were quantified by calculating SGRs for the juveniles in each treatment basket, using the equation above in Experiment 1. SGRs were calculated from November 2 to December 12, 2000 (day 0 to 40), from December 12 to March 1, 2001 (day 40 to 119), and from March 1 to April 10, 2001 (day 119 to 159).

4.2.2.7 External observations

The juveniles fed the different diet treatments were observed at the end of the experiment for differences in test colour, test abnormalities, spine colour, and relative spine length.

4.2.3 Statistical analyses

The data for both experiments were analysed using the SPSS version 8.0 for Windows statistical software package. The default Levene statistic ($\alpha = 0.05$) was used to test for homogeneity of variances. Data sets with homogeneous variances were further analysed using Analysis of Variance (ANOVA) (α=0.05) with Tukey's multiple comparisons to determine differences among independent factors (Sokal and Rohlf, 1995; Zar, 1999). Data sets with heterogeneous variances were analysed using the non-parametric Kruskal-Wallis statistic (α=0.05) with Tukey-type Nemenyi test for multiple comparisons (Mosteller and Rorke, 1973; Sokal and Rohlf, 1995; Zar, 1999). Arcsine transformations were used to normalise ratio data sets prior to analyses.

4.3 Results

4.3.1 Experiment 1

4.3.1.1 Somatic growth

There were no significant differences in the average juvenile sea urchin test diameter between the replicate baskets in each treatment for all sample periods (P=0.477). Hence, the juveniles in the replicate baskets for each treatment were pooled.

4.3.1.1.1 Effect of mineral concentration

The juveniles fed the pigmented diets that differed in the concentration of the modified Bernhart-Tomerelli salt mix (i.e., 0% to 15%) showed differences in average test diameter over the 154 day experiment (Figure 4.2a). There were no significant differences in test diameter ($P=0.091$) observed among treatments up to May 12 (day 88) and no significant differences in SGR ($P=0.079$) up to June 5 (day 112) (Figure 4.3). However, there was a general trend that showed the juveniles fed the higher mineral diets (i.e., 6% and 15%) had higher SGRs than those fed the lower mineral diets for the duration of the experiment. Hence, on June 5 (day 112) and July 17 (day 154) the juveniles fed the 15% mineral diet were significantly larger than the juveniles fed the 0% and the 1.5% mineral diets ($P<0.001$). In addition, the juveniles fed the 6% and 3% mineral diets were significantly larger than those fed the 0% mineral diet ($P<0.001$) at the end of the experiment. The juveniles fed the 15%, 6%, and 3% mineral diets were similar in size ($P=0.282$), and those fed the 0% and 1.5% mineral diets were similar in size ($P=0.659$) after the 154 day experiment.

4.3.1.1.2 Effect of mineral source and pigment

The juveniles fed the pigmented and non-pigmented diets with 3% mineral (the modified Bernhart – Tomerelli salt mix and the Shur-Gain mineral mix) showed differences in growth over the 154 day experiment (Figure 4.2b). Again, there were no significant differences in juvenile size among treatments up to May 12 (day 88) ($P=0.160$) and no significant differences in SGR among treatments up to June 5 (day 112) ($P=0.079$)

(Figure 4.3). After 112 days there were no differences in size among the juveniles fed the pigmented diets (P=0.080) or among those fed the non-pigmented diets (P=0.110). However, on day 112, the juveniles fed the non-pigmented diets were significantly smaller than the juveniles fed the pigmented diets (P<0.001).

From June 5 to July 17 (day 112 to 154) the SGRs of the juveniles fed the non-pigmented diets were significantly smaller than the SGRs of those fed the pigmented diets (P<0.001) (Figure 4.3). Hence, after the 154 day experiment, the juveniles fed the non-pigmented diets were similar in size (P=0.960), but significantly smaller than the juveniles fed the pigmented diets (P<0.001) (Figure 4.2b). In addition, the juveniles fed the pigmented diet with the modified Bernhart-Tomerelli salt mix was significantly larger than the juveniles fed the pigmented diet with the Shur-Gain mineral mix (P<0.001).

4.3.1.1.3 Effect of mineral concentration and pigment

From February 14 to June 5, 2000 (day 0 to 112), there were no significant differences in size among juveniles fed the diets containing the Shur-Gain mineral mix (i.e., 3% non-pigmented diet and the 3% and 6% pigmented diets) (P=0.643 on day 0, P=0.183 on day 51, P=0.278 on day 112) (Figure 4.2c). However, on day 154, the juveniles fed the non-pigmented diet were significantly smaller than those fed the pigmented diets (P<0.001). There were no significant differences in size among the juveniles fed the pigmented diets with different mineral concentrations (P=0.853).

4.3.1.2 Sea urchin analyses

4.3.1.2.1 External and internal observations

Besides differences in test growth, there were other differences in physical appearance among the juveniles fed the different diets. The most prominent were differences in test colour and spine length between some of the juveniles fed the pigmented and non-pigmented diets. Those fed the non-pigmented diets had pale coloured tests and short stubby spines compared to the sea urchins fed the pigmented diets (Figure 4.4a and b). There were also differences internally. The juveniles fed the non-pigmented diets had white coloured gonads compared to the golden orange gonad colour of juveniles fed the pigmented diets (Figure 4.4c and d). There were no physical differences among those juveniles fed the pigmented diets with different mineral sources and concentrations.

There were no significant differences in survival among any of the treatments at the end of the experiment (P=0.113), which ranged from 94.2% for the juveniles fed the pigmented diet with 1.5% modified Bernhart-Tomerelli salt mix to 100% for those fed the pigmented diets with 0% mineral, 6% modified Bernhart-Tomerelli salt mix, and 3% Shur-Gain mineral mix.

4.3.1.2.2 Test, gonad, and gut yields

There were no significant differences in the average test yields of juveniles fed the pigmented diets, which ranged from 84.5% for those fed the 6% modified Bernhart-Tomerelli salt mix diet to 87.6% for the juveniles fed the 3% Shur-Gain mineral mix diet

(P=0.118) (Figure 4.5a). The average test yields of juveniles fed the non-pigmented diets were statistically similar to those of the juveniles fed the 6% and 15% modified Bernhart-Tomerelli salt mix diets (P=0.079), but were significantly lower than the test yields of those fed the other pigmented diets (P<0.001).

The average gonad yield of juveniles fed the 3% Shur-Gain mineral mix non-pigmented diet (13.9%) was significantly larger than the average gonad yields for the juveniles fed the pigmented diets that ranged from 7.8% for those fed the 3% Shur-Gain mineral mix diet to 10.7% for those fed the 6% modified Bernhart-Tomerelli salt mix diet (P<0.001) (Figure 4.5b). There were no significant differences in gonad yields among the juveniles fed the non-pigmented diets (P=0.063) or among those fed the pigmented diets (P=0.081).

The average gut yield of juveniles fed the non-pigmented 3% modified Bernhart-Tomerelli salt mix diet (5.8%) was significantly larger (P=0.024) than the average gut yields of the juveniles fed the pigmented diets with 1.5% and 3% modified Bernhart-Tomerelli salt mix and 6% Shur-Gain mineral mix (4.2%, 4.2%, and 4.2%, respectively) (Figure 4.5c). All juveniles, except those fed the non-pigmented 3% Bernhart-Tomerelli salt mix diet, had similar gut yields (P=0.747).

4.3.1.2.3 Tissue ash

The juveniles fed the non-pigmented diet had significantly lower test ash content (79.1%) than those fed the pigmented diets (P=0.003), which ranged from 83.3% for those fed the 15% modified Bernhart-Tomerelli salt mix to 82.2% for those fed the 0% mineral diet (Figure 4.6a). There were no significant differences in the average test ash values of juveniles fed the different pigmented diets (P=0.555).

The average gonad ash values of juveniles fed the non-pigment diet (4.1%) were significantly smaller than those of the juveniles fed the pigmented diets with 0% mineral (6.6%) and 15% modified Bernhart-Tomerelli salt mix (6.8%) (P=0.001) (Figure 4.6b). There were no significant differences in the gonad ash values of the juveniles fed the pigmented diets (P=0.325).

There were no significant differences in the gut ash values of juveniles fed the different diets (P=0.309), which ranged from 9.6% for those fed the non-pigmented diet to 13.3% for those fed the pigmented 0% mineral diet (Figure 4.6c).

4.3.2 Experiment 2

The best diet from Experiment 1 for increasing juvenile sea urchin somatic growth was the pigmented diet with 15% modified Bernhart-Tomerelli salt mix. Hence, this experiment compared the somatic growth production of smaller juveniles fed this prepared diet to the growth production of those fed kelp, *Laminaria longicruris*·

4.3.2.1 Somatic growth

For both cohorts there were no significant differences in the average juvenile sea urchin test diameter between the replicate baskets in each treatment for all sample periods (P=0.980). Hence, for each cohort the juveniles in the replicate baskets for each treatment were pooled.

4.3.2.1.1 Cohort 1

There were no initial significant differences in the average test diameters (TD) of the juveniles fed the prepared diet (1.3 mm TD) and those fed kelp (1.4 mm TD) (P=0.070) (Figure 4.7a). From November 2 to December 12, 2000 (day 0 to 40) the specific growth rate (SGR) of the juveniles fed the prepared diet (0.63) was statistically similar to the SGR of those fed kelp (0.66) (P=0.690) (Figure 4.8a). Hence, on day 40, the average TD of the juveniles fed the prepared diet (1.71 mm TD) was statistically similar to the average TD of those fed kelp (1.84 mm TD) (P=0.190). From December 12 to March 1, 2001 (day 40 to 119), the juveniles fed the prepared diet had a significantly larger average SGR (1.09) than those fed kelp (0.560) (P=0.008) (Figure 4.8a), thus the average TD of the juveniles fed the prepared diet (4.0 mm TD) was significantly larger on day 119 than the average TD of the kelp-fed juveniles (2.9 mm TD) (P<0.001) (Figure 4.7a). From March 1 to April 10, 2001 (day 119 to 159), the average SGR for the juveniles fed the prepared diet (0.92) was again significantly larger than the average SGR for those fed kelp (0.31) (P=0.001). Thus, the average TD of the juveniles fed the prepared diet (7.4

mm TD) was significantly larger at the end of the experiment than the average TD of the kelp-fed juveniles (4.4 mm TD) ($P<0.001$).

4.3.2.1.2 Cohort 2

There were no significant differences in TD between the juveniles fed the prepared diet and those fed kelp on day 0 (2.2 mm and 2.3 mm TD, respectively) ($P=0.124$) or on day 40 ($P=0.139$) (2.9 mm and 3.1 mm TD, respectively) (Figure 4.7b). From December 12 to March 1, 2001 (day 40 to 119), the average SGR for the juveniles fed the prepared diet (0.82) was significantly larger than the average SGR for the kelp-fed juveniles (0.33) ($P=0.006$) (Figure 4.8b), which lead to a significant difference in average TD for juveniles fed the prepared diet (5.5 mm TD) and the kelp-fed juveniles (4.0 mm TD) on day 119 of the experiment ($P<0.001$). Similarly, from March 1 to April 10, 2001 (day 119 to 159) the average SGR for the juveniles fed the prepared diet (0.71) was significantly larger than the average SGR for those fed kelp (0.023) ($P<0.001$). Hence, the juveniles fed the prepared diet (7.4 mm TD) were significantly larger than the kelp-fed juveniles (4.4 mm TD) ($P<0.001$).

4.3.2.2 External observations

There were no major differences in the physical appearance (except for test diameter) between the juveniles fed the different diets. All juveniles had dark green tests with long, radiating spines characteristic of those found in the wild.

There were no significant differences in survival between the cohort 1 juveniles fed the prepared diet (72%) and those fed kelp (82.5%) (P=0.187). The cohort 2 juveniles fed either the prepared diet or kelp also had similar survival rates (72% and 77.5%, respectively) throughout the experiment (P=0.404).

4.4 Discussion

The quality of the diets used in these experiments was assessed based on the growth of the juveniles. Sea urchin growth rates are also affected by water temperature, thus to minimise the effect of water temperature and to maximise the growth rates of juveniles independent of diet, the water temperature for both experiments was maintained at 10°C (Tajima and Fukuchi, 1991).

4.4.1 Experiment 1

The results of this experiment showed that the somatic growth of juvenile green sea urchins was dependent on the minor ingredients (i.e., those in addition to proteins, lipids, and carbohydrates) of prepared diets. The addition of essential minerals and pigments to the diets led to significant increases in juvenile test diameter. In past studies juvenile sea urchins fed prepared diets had slower growth rates over time than those fed wild diets (Nagai and Kaneko, 1975; Williams and Harris, 1998; Chapters 2 and 3). The prepared diets used in these past studies, however, had low concentration of required minerals (less than 5% dry mass) and were deficient in required pigments, such as beta-carotene. Hence, minerals and pigment seem to be important factors for juvenile somatic growth.

The growth of juveniles fed the different diets in this experiment showed increased juvenile growth with an increase in the dietary modified Bernhart-Tomerelli salt mix. Thus, test production appeared to be related to the mineral concentration in the diet. However, an increase in the dietary Shur-Gain mineral concentration did not coincide with an increase in juvenile test diameter. In addition, the juveniles fed the pigmented Shur-Gain mineral diet had less test growth that those fed a pigmented diet with similar concentration of the modified Bernhart-Tomerelli salt mix. The Shur-Gain diets, therefore, appeared to lack minerals that affected test production. This suggests the importance of individual minerals within the diet as well as the overall dietary mineral concentration for sea urchin test production. Two of the more important minerals used in test production by sea urchins are calcium and magnesium (Okazaki, 1956; Pearse and Pearse, 1975; Shimizu et al., 1994; Grosjean et al., 1998; Chen et al., 2000; Ebert, 2001). The modified Bernhart-Tomerelli salt mix contained these major minerals, but the Shur-Gain mineral mix lacked magnesium (Pers. Comm., Adel El Mowaffi, Shur-Gain, Mississauga, Ontario). Hence, the larger test size attained by the juveniles fed the pigmented diet with the modified Bernhart-Tomerelli salt mix compared to those fed the pigmented Shur-Gain mineral mix diet may have been due to the magnesium present in the modified Bernhart-Tomerelli salt mix diet.

The juveniles in this study derived minerals from other sources in addition to the modified Bernhart-Tomerelli salt mix or the Shur-Gain mineral mix for their growth and

maintenance. For example, juveniles fed the pigmented diet with 0% mineral grew to a similar size as the juveniles fed the pigmented diets with the Shur-Gain minerals. As well, the juveniles fed the pigmented diet with 0% mineral source had better test growth than those fed the non-pigmented diets, which consisted of 3% mineral. Hence, juveniles were deriving some of their minerals from other sources, such as the surrounding seawater (Grosjean et al., 1998), accumulating diatom films (Tajima and Fukuchi, 1991), and/or other diet ingredients (such as the Algro™ pigment source). Mineral contribution from ingested diatom films would be expected to be small because the water was filtered down to 5-μm, thus preventing the passage of most diatoms, which range from 3 μm to over 1 mm in size (Lebour, 1930), through the filters. The mineral contribution from seawater also may have been small due to the importance of magnesium and calcium carbonates as natural pH buffers in seawater. According to Grosjean et al. (1998), the actual fraction of carbonates the sea urchins can use from seawater is probably under 10% of the total carbonate alkalinity. Thus, other dietary ingredients, such as the Algro™ pigment source, appeared to be an important mineral source for the juvenile sea urchins.

According to Cognis Australia Pty Ltd., the company that manufactures Algro™ (a sprayed-dried nutritional ingredient derived from the alga, *Dunaliella salina*), the ash content of Algro™ is 61.9% with 3% of the mineral component being magnesium. Therefore, in this experiment, Algro™ contributed 0.8 percent mineral to the pigmented diets, and this additional mineral source appeared to have a positive influence on sea

urchin test production. However, the juveniles fed the non-pigmented diets deficient in specific minerals (i.e., the Shur-Gain mineral mix), must have acquired these minerals for test production through sources such as the surrounding seawater. The incorporation of these minerals into test growth was at a slower and less efficient rate than the juveniles fed the pigmented diets, shown by the different growth rates. Hence, juvenile test production was enhanced by the addition of required minerals at increased concentrations suggesting that mineral uptake by sea urchins for test production is partly dependent on the concentration of required minerals in the diet.

A second important dietary nutrient for juvenile somatic growth found in this study was pigment. Although the Algro™ appeared to be an additional source of dietary minerals for juvenile growth, it is also an important source of the pigment beta-carotene (Robinson et al., 2002). The major pigments in the gonad, test, and spines are beta-echinenone and beta-carotene, while beta-carotene, fucoxanthin, and fucoxanthinol are the major carotenoids in the viscera (Tsushima et al., 1993; Matsuno and Tsushima, 2001). Beta-carotene is an important pigment for sea urchins because it acts as the precursor for beta-echinenone within sea urchins (Griffiths and Perrott, 1976; Tsushima et al., 1993; Matsuno and Tsushima, 2001). Pigments are accumulated in sea urchins by direct accumulation and/or by modifications of precursors in metabolic processes.

Beta-carotene had a positive effect on the somatic growth production of juvenile sea urchins shown by the differences in growth between the juveniles fed the pigmented and

non-pigmented diets. It is unlikely the minerals supplied by the Algro^{TM} were solely responsible for the differences in growth since the non-pigmented diet with the modified Bernhart-Tomerelli salt mix contained required minerals (such as Ca and Mg) for test production. As well, the juveniles fed this non-pigmented diet did not perform as well as those fed the pigmented diets, including the pigmented diet with 0% mineral added. Pigment effect on juvenile sea urchin growth was also seen in the relative tissue production (i.e., gonad, gut, and tissue yields) of the juveniles fed the different diets. The juveniles fed the non-pigmented diets had large gonad yields that represented the excess energy stores, from the high-energy prepared diets, not used in test growth. Somatic growth is a major energy expenditure for sea urchins, thus the juveniles with low growth rates store the excess energy in their gonads (Marsh and Watts, 2001). In contrast, the juveniles fed the pigmented diets utilised more energy for somatic growth and, therefore, produced smaller gonads. Pigments seemed to enhance some physiological function within sea urchins that enhanced test production.

The functions of pigments in sea urchin test production, however, are not well understood. Pigments have been shown to be important in sea urchin development (Tsushima et al., 1995) as antioxidants (Lukyanova and Shmidt, 1994), as precursors to vitamins (de Jong-Westman, 1995), and in biological defence systems (Kawakami et al., 1998). The increase in test diameter by juvenile sea urchins fed pigmented diets may not have resulted from a direct role of pigments in test production, but rather indirectly by

increasing the health and minimising the stress of the animals. Healthy animals can allocate more energy to new growth as opposed to maintenance of existing body tissues.

Pigments may also play a role in the incorporation of minerals into tissues. The gonad and test mineral concentrations (which are directly related to the ash contents) of the sea urchins fed the pigmented diets did not change as the dietary mineral concentration increased. However, the juveniles fed the non-pigmented diet with 3% modified Bernhart-Tomerelli salt mix had a lower mineral content in the gonad and test than those fed the pigmented diet with the same mineral source and concentration. Hence, the increases in gonad and test mineral concentrations coincided with the addition of pigment and not the addition of minerals to the diets. On the other hand, the gut mineral content of juveniles fed the different diets were not significantly different from one another. However, the major pigments of the gonad and test (i.e., beta-echinenone and beta-carotene) are different than the major pigments in the gut or viscera (i.e., beta-carotene, fucoxanthin, and fucoxanthinol). Hence, specific pigments may play an important role in mineral uptake by juvenile sea urchins that would lead to increased mineral availability for test production.

The test growth of the juveniles fed the different diets did not differ significantly until June 5 (day 112), which indicated pigment and minerals did not have an initial effect on growth. The slow growth at the start of the experiment for all juveniles may have been due to a period of acclimation to a change in diet (from a wild algal diet to a prepared

diet) and water temperature (from 0°C to 10°C) (McClintock et al., 1982; Tajima and Fukuchi, 1991). The similar initial growth rates for all juveniles were probably due to the use of stored nutrients for test growth, suggested in Chapters 2 and 3. Sea urchins have the ability to store excess nutrients in their tissues, especially the gonad (Holland and Giese, 1965; Gonor, 1972), thus it is likely the juveniles used in this experiment had nutrients stored in their tissues that they had accumulated from diets in the wild prior to collection. These stored nutrients would have included minerals and pigments since algae, the preferred wild diet of sea urchins, contain these nutrients (Goodwin, 1980; de Jong-Westman et al., 1995; Munilla et al., 1995; Kawakami et al., 1998). However, once these stores were depleted, test growth was reduced in the juveniles fed the nutritionally deficient diets. The juveniles fed the pigmented diets with high mineral concentration did not have any reduction in test growth because the diets were able to replenish the reserves used in test production. Sea urchins have the ability to respond physiologically and morphologically to fluctuating resources (Ebert, 1980; Black et al., 1984; Russel, 1987; Levitan 1988, Edwards and Ebert 1991; McBride et al., 1999), therefore, as the juvenile nutrient stores change in composition over time, those that retain the essential nutrients for somatic growth continue to have rapid growth as opposed to the slow growing juveniles that have depleted nutrient reserves.

The juveniles fed the non-pigmented diet with 3% Shur-Gain mineral mix had similar growth patterns and physical appearances as the juvenile sea urchins fed the pigment and mineral deficient prepared diets described in Chapters 2 and 3. The S-shaped growth

pattern, consistent between studies, was neither characteristic of the kelp-fed juveniles in the previous studies nor the juveniles in this experiment fed the pigmented diets. The average specific growth rate of the kelp-fed juveniles from Chapters 2 and 3 (with an average TD of 14.5 mm and 10°C water temperature) was 0.41. In comparison, the average SGR for the juveniles in this experiment from April 5 to July 17 (day 51 to 154) fed the pigmented diets was 0.48. The average SGR for those fed the pigmented diets with 3% and 6% Shur-Gain mineral mix was 0.42, and the average SGR for those fed the pigmented diets with 6% and 15% modified Bernhart-Tomerelli salt mix was 0.54. In addition, the physical appearance of the juveniles fed the pigmented diets were more characteristic of kelp-fed juveniles observed in Chapters 2 and 3 with well-pigmented green tests and long, radiating spines. Those juveniles fed the non-pigmented diets looked unhealthy with pale tests and short spines (Williams and Harris, 1998; Chapters 2 and 3). The addition of required pigment and minerals to the diet increased the growth rate and maintained the healthy physical appearances of juveniles comparable to those attained by the kelp-fed juveniles.

4.4.2 Experiment 2

This experiment showed that the prepared pigmented diets with a high concentration of required minerals supplied juvenile sea urchins with the nutrients for growth more efficiently than the wild kelp diet, *Laminaria longicruris*. There were no differences in test production between the juveniles in the different cohorts suggesting there are no differences in the nutritional requirements between the two cohorts of small juvenile

green sea urchins. The cultured juveniles used in this experiment efficiently consumed and assimilated the prepared diet, shown by their significantly greater growth over the kelp-fed juveniles, thus juveniles that are 1 mm in size and larger can manipulate, ingest, and assimilate the prepared diet for somatic growth production.

The differences in test growth of the juveniles fed the different diets may have resulted from the possible inability of small juveniles to effectively feed upon kelp since in the wild, sea urchins below 3 mm TD usually feed on diatom films and fleshy algae (Lamare and Mladenov, 2000). However, as the kelp-fed juveniles grew and became more effective as kelp grazers, test growth rates remained significantly smaller than the juveniles fed the prepared diet. In addition, survival was similar among the juveniles fed the different diets, which indicated the juveniles obtained sufficient nourishment for survival from both diets. A more likely explanation for the differences in growth between the juveniles fed the different diets was the energy differences between the prepared diet and kelp. Kelp is a low energy, low nutritional food source (Lasker and Giese, 1954; Vadas et al., 2000), whereas the prepared diet, modified from the diets used Chapters 2 and 3, is a high energy, high digestible diet (Klinger et al., 1998; Chapter 2 of Thesis). Hence, juveniles fed the prepared diet were supplied with the required nutrients and abundant energy for maximal somatic growth.

In conclusion, minerals and pigments have important functions in the somatic growth processes of juvenile green sea urchins (*S. droebachiensis*). Pigmented diets, regardless

of mineral source or concentration, increased juvenile test growth. Growth performance was further enhanced with the addition of minerals at high concentrations to the pigmented diets. There also seems to be an interaction between minerals and pigments in the tissues of sea urchins since the test and gonad have higher mineral concentration in juveniles fed pigmented diets. The best diet from Experiment 1, the pigmented diet with 15% modified Bernhart-Tomerelli salt mix, was better than kelp for increasing somatic growth of small (1-2 mm initial TD) juvenile sea urchins. Hence, feeding juveniles this prepared diet throughout the juvenile period of sea urchin culture can optimise juvenile growth. Further studies on juvenile diets should focus on determining optimal pigment concentration, alternate pigment sources, as well as the importance of individual minerals for maximising juvenile somatic growth.

Chapter 5

General discussions and conclusions

Sea urchin somatic growth results from complex interactions among several factors including size, feeding behaviour, physical environment, food availability, and food quality (Hatcher and Hatcher, 1997). By varying food quality, while keeping the other factors constant, a diet specifically formulated to maximise juvenile sea urchin somatic growth can be developed. There have been few studies on the nutritional requirements of sea urchins as they grow from post-metamorphosis to market size (Cook et al., 1998; McBride et al., 1998). This study investigated the effects of different dietary nutrients (i.e., proteins, lipids, minerals, and pigment) in prepared diets on the somatic growth of juvenile green sea urchins (*S. droebachiensis*). The somatic growth of juveniles fed the prepared diets was also compared to the somatic growth of juveniles fed kelp, *Laminaria longicruris*.

The juvenile sea urchins fed the various prepared protein diets (Chapter 2) and the various prepared lipid diets (Chapter 3) had similar growth patterns, unlike the juvenile sea urchins fed kelp. The general growth pattern of the juveniles fed the prepared diets was characterised by an initial period of rapid test growth (greater than the test growth of those fed kelp) followed by a decrease in test growth approximately 4 to 5 months into the feeding trials. The juvenile sea urchins fed kelp did not experience this decrease in

test growth, which was likely due to nutritional deficiencies in the diets compounded by a depletion of stored reserves within the gonad that initially benefited test production.

The juveniles in the protein and lipid experiments were also consistent in the relative sizes of the gonads between those fed the prepared diets and those fed kelp (i.e., those fed the prepared diets had larger gonads than those fed kelp). This suggested that the sea urchins fed the high-energy, highly digestible prepared diets were directing most of the dietary energy to storage in the gonads and not to test production. The juveniles fed kelp, on the other hand, allocated most of the dietary energy to test production and little was stored in the gonads. The protein and lipid prepared diets were high-energy diets (Chapter 2), but nutritionally deficient for juvenile sea urchins. The kelp diet was a low-energy diet (Vadas et al., 2000), but nutritionally balanced to provide the required materials for juvenile test production.

Somatic growth of the juvenile sea urchins was not effected by differences in dietary protein source or concentration or by differences in lipid source (over the ranges tested in the experiments). This suggests that the juveniles could utilise plant sources of proteins and lipids and that they possibly have the ability to synthesise required proteins and fatty acids for growth and maintenance from precursors in the dietary plant sources (Klinger et al., 1994; de Jong-Westman, 1995; Pantzis et al., 2000; Akiyma et al., 2001; Bell et al., 2001). In addition, test size was similar for juveniles fed prepared diets with 20% dietary protein and 50% dietary protein, which indicates that juvenile sea urchins do not have

high dietary protein requirements for test production (Akiyma et al., 2001; Wallace et al., 2001). Lipid concentration, on the other hand, did have an effect on juvenile growth. Test diameter of the juveniles fed the prepared diets increased as the dietary lipid concentration decreased from 10% to 1% dry mass. High lipid concentration had a negative effect on test growth probably due to higher dietary energy and the increased stress upon the juveniles. The kelp, with lipid concentrations ranging from 0.1% - 0.4% (Vadas et al., 2000) to 2.4% - 5.1% (Chapters 2 and 3), provided the juveniles with a well-balanced supply of nutrients including lipids required for somatic growth. Stress can cause reduced feeding as well as reduced growth, health, and survival (Bottger et al., 2001), thus factors that increase stress in juveniles will likely lead to small, unhealthy sea urchins.

Two of the major differences between the prepared diets (protein and lipid diets) and kelp consistent between experiments were mineral concentration and pigments. The prepared diets were non-pigmented and had significantly lower mineral concentrations compared to kelp. In Chapter 4 it was shown that pigments and minerals in prepared diets had a significant effect on juvenile sea urchin somatic growth. In addition, juveniles fed a similar diet to those in Chapter 3 (i.e., non-pigmented using the Shur-Gain mineral mix) had similar growth trends and physical appearances to the juveniles in Chapter 3 fed the prepared diets. Juveniles fed the pigmented diets with high concentrations of modified Bernhart-Tomerelli salt mix, however, had growth trends and physical appearances similar to the juveniles in Chapters 2 and 3 fed kelp. Hence, the addition of pigment and

high mineral concentration to the prepared diets increased the growth performance and health of the juvenile sea urchins fed the prepared diets.

The use of the modified Bernhart-Tomerelli salt mix also enhanced juvenile growth compared to the Shur-Gain mineral mix. This may be related to the presence or absence of specific minerals in each mineral mix and the importance of these minerals for juvenile growth. For example, magnesium, which has been found to be an important mineral for test construction (Okazaki, 1956; Pearse and Pearse, 1975; Shimizu et al., 1994; Grosjean et al., 1998; Chen et al., 2000; Ebert, 2001), was absent from the Shur-Gain mineral mix (Pers. Comm., Adel El Mowaffi, Shur-Gain, Missassauga, Ontario), but it was present in the modified Bernhart-Tomerelli salt mix. Therefore, magnesium, and/or some other mineral(s), may have been limiting in the juveniles with minimal test growth that were fed the prepared diets containing the Shur-Gain mineral mix. The specific minerals and pigments required for maximum test production, as well as their functions in somatic processes, must be investigated in order to gain a better understanding of the growth requirements of juvenile sea urchins.

In this study, somatic growth and physical appearances were maximised in juvenile sea urchins that were fed the pigmented diet with 15% mineral concentration (modified Bernhart-Tomerelli salt mix) described in Chapter 4. The juveniles fed this diet did not appear to have nutrition deficiencies apparent in juveniles from previous experiments. The juveniles fed the high mineral, pigmented prepared diet had similar growth rates and

similar physical appearances as the kelp-fed juveniles in the protein and lipid experiments. Thus, the nutritional deficiencies of the prepared diets that were suggested as the major reasons for reduced juvenile growth were likely due to pigment and mineral deficiencies.

The success of the pigmented, high-mineral prepared diet in increasing test growth in small juveniles compared to kelp suggests prepared diets could be utilised by sea urchins of at least 1 mm in test diameter to promote growth and survival. The small juveniles fed the kelp remained physically healthy (i.e., no degradation in test colour, relative spine length, and/or tube-feet activity), but they had less test growth than the small juveniles fed the prepared diet. Although kelp was an efficient diet for test production in larger juveniles, it was not as efficient as the prepared diet for increasing test production in smaller juveniles (1-2 mm test diameter). Prepared diets can be utilised by a larger range of juveniles, compared to *Laminaria longicruris*, to provide the required nutrients for body maintenance and somatic growth production. In addition, the nutritional requirements for small and large juveniles appeared to be similar since the same diet formulation, tested on both sizes, resulted in high growth rates and healthy physical appearances.

This study demonstrated that juvenile green sea urchins have definite nutritional requirements for growth and survival beyond the major nutrient groups of proteins, lipids, and carbohydrates. Minerals and pigments play an important role not only in somatic

growth processes, but also in other physiological processes important in the test and spine maintenance, and tube-feet functioning. Minerals and pigments appeared to reduce the stress experienced by the juvenile sea urchins fed various prepared diets enabling increased allocation of energy and nutrients to somatic growth. More research is required in fine-tuning the specific nutrient requirements of juvenile green sea urchins before a prepared diet can be formulated to maximise juvenile health, survival, and somatic growth. The complete life-cycle production of green sea urchins is a biological feasible option for sea urchin aquaculture, with the potential of being economically feasible, due to the success of using prepared diets to promote juvenile somatic growth.

References

Abe, E., M. Tada. 1994. The ecology of the sea urchin, *Strongylocentrotus intermedius* (A. Agassiz). *Science Report, Hokkaido Fisheries Experimental Station* **45**: 45-56.

Agatsuma, Y. 2000. Food consumption and growth of the juvenile sea urchin *Strongylocentrotus intermedius*· *Fisheries Science* **66**: 467-472.

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

Akiyama, T., T. Unuma, T. Yamamoto, H. Furuita, K. Konishi. 1997. An evaluation of amino acid sources and binders in semipurified diet for red sea urchin *Pseudocentrotus depressus*· *Fisheries Science* **63**: 881-887.

Andrew, N.L., J.H. Choat. 1985. Habitat related differences in the survivorship and growth of juvenile sea urchins. *Marine Ecology Progress Series* **27**: 155-161.

Anonymous. 1989. Sea Urchin Echinoidae, Strongylocentrotidae. *Seafood Leader* (March-April): 206-217.

Anonymous. 2002. Soy Toxins. www.soyonlineservice.co.nz/soytox.htm. (visited February 7, 2002).

Barker, M.F., J.A. Keogh, J.M. Lawrence, A.L. Lawrence. 1998. Feeding rate, absorption efficiencies, growth, and enhancement of gonad production in the New Zealand sea urchin *Evechinus chloroticus* Valenciennes (Echinoidea: Echinometridae) fed prepared and natural diets. *Journal of Shellfish Research* **17**(5): 1583-1590.

Bedard, R.W. 1973. Sea Urchin. Prepared for the Canadian Sea Urchin Fisheries Industry, Fisheries and Fish Production Division; Agriculture, Fisheries and Food Products Branch; Dept. of Industry, Trade and Commerce, Ottawa, Canada.

Bell, M.V., J.R. Dick, M.S. Kelly. 2001. Biosynthesis of eicosapentaenoic acid in the sea urchin *Psammechinus miliaris*· *Lipids* **36**(1): 79-82.

Beukema, J.J., W. DeBruin. 1979. Calorific values of the soft parts of the tellinid bivalve *Macoma baltica* (L.) as determined by two methods. *Journal of Experimental Marine Biology and Ecology* **79**: 19-37.

Bishop, C.D., K.J. Lee, S.A. Watts. 1994. A comparison of osmolality and specific ion concentrations in the fluid compartments of the regular sea urchin *Lytechinus*

variegatus Lamarck (Echinodermata: Echinoidea) in varying salinities. *Comparative Biochemistry and Physiology* **108A**(4): 497-502.

Black, R., C. Codd, D. Hebbert, S. Vink, J. Burt. 1984. The functional significance of the relative size of Aristotle's lantern in the sea urchin *Echinometra mathaei* (de Blainville). *Journal of Experimental Marine Biology and Ecology* **77**: 81-97.

Black, W.A.P. 1950. The seasonal variation in weight and chemical composition of the common British *Laminariaceae*· *J. Mar. Biol. Ass. UK.* **29**: 45-72.

Boolootian, R.A., R. Lasker. 1964. Digestion of brown algae and the distribution of nutrients in the purple sea urchin *Strongylocentrotus purpuratus*· *Comparative Biochemistry and Physiology* **11**: 273-289.

Bottger, S.A., J.B. McClintock, T.S. Klinger. 2001. Effects of inorganic and organic phosphates on feeding, feeding absorption, nutrient allocation, growth and righting responses of the sea urchin *Lytechinus variegatus*· *Marine Biology* **138**: 741-751.

Brody, S. 1945. Bioenergetics and growth. Hafner Publ. Co. Inc., New York. 1023pp.

Burkholder, P.R., L.M. Burkholder, L.R. Almordoran. 1971. Nutritive constituents of some Caribbean marine algae. *Botanica Marina* **14**: 132-135.

Busacker, G.P., I.A. Adelman, E.M. Goolish. 1990. Growth. Pages 363-364. In: Schreck, C.B., P.B. Moyle (eds.). *Methods for Fish Biology*· American Fisheries Society: Maryland.

Chang, Y., Z. Wang, G. Wang. 1999. Effect of temperature and algae on feeding and growth in sea urchin *Strongylocentrotus intermedius*· *Journal of Fisheries of China* **23**(1): 69-76.

Chapkin, R.S. 1992. Reappraisal of the essential fatty acids. Pages 429-436. In: Chow, C.K. (ed.). *Fatty acids in foods and their health implications*· Marcel Dekker, Inc.: New York.

Chapman, A.R.O. 1986. The wild harvest and culture of *Laminaria longicruris* in eastern Canada. Pages 193-237. In: Doty, M.S., J.F. Caddy, B. Santelices (eds.). *Case studies of seven commercial seaweed resources*· No. 281 FAO fisheries technical paper: Rome.

Chapman, A.R.O., J.S. Craigie. 1977. Seasonal growth in *Laminaria longicruris*: relations with dissolved inorganic nutrients and internal reserves of nitrogen. *Marine Biology* **40**: 197-205.

Chen, W., Y. Zhao, J. Wang, S. Tong. 2000. Mineral element and protein levels of the sea urchin *Strongylocentrotus intermedius* and its diets. *Journal of Dalian Fisheries College / Dalian Shuichan Xueyuan Xuebao* **15**(1): 41-44.

Cho, D.M., D.S. Kim, D.S. Lee, H.R. Kim, J.H. Pyeun. 1995. Trace components and functional saccharides in seaweed. 1. Changes in proximate composition and trace elements according to the harvest season and places. *Journal of the Korean Fisheries Society* **28**(1): 49-59.

Chow, C.K. 1992. Biological effects of oxidised fatty acids. Pages 689-706. In: C.K. Chow (ed.). *Fatty Acids in Foods and their Health Implications*. Marcel Dekker, Inc.: New York, 868pp.

Constable, A.J. 1993. The role of sutures in shrinking of the test in *Heliocidaris erythrogramma* (Echinoidea: Echinometridae). *Marine Biology* **117**: 423-430.

Cook, E.J., M.V. Bell, K.D. Black, M.S. Kelly. 2000. Fatty acid compositions of gonad material and diets of the sea urchin, *Psammechinus miliaris*: Trophic and nutritional implications. *Journal of Experimental Marine Biology and Ecology* **255**(2): 261-274.

Cook, E.J., M.S. Kelly, J.D. McKenzie. 1998. Somatic and gonadal growth of the sea urchin *Psammechinus miliaris* (Gmelin) fed artificial salmon feed compared with a macroalgal diet. *Journal of Shellfish Research* **17**(5): 1549-1555.

Cuthbert, C., G. Burnell, J. Connolly. 2000. Preliminary trials with "green" artificial diets for juvenile abalone and sea urchins. *Journal of Shellfish Research* **19**(1): 505-506.

Cuthbert, F.M., R.G. Hooper, T. McKeever. 1995. Sea urchin feeding and ranching experiments. Report for Canadian Centre for Fisheries Innovation. St. John's, NF. 61pp.

Dawson, L.W. 1868. The food of the common sea urchin. *The American Naturalist* **1**: 124-125.

de Jong-Westman, M., B.E. March, T.H. Carefoot. 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.

Ebert, T.A. 1967. Negative growth and longevity in the purple sea urchin *Strongylocentrotus purpuratus* (Stimpson). *Science* (N.Y.) **157**: 557-558.

Ebert, T.A. 1968. Growth rates of the sea urchin *Strongylocentrotus purpuratus* related to food availability and pine abrasion. *Ecology* **49**(6): 1075-1091.

Ebert, T.A. 1975. Growth and mortality of post-larval echinoids. *American Zoologist* **15**: 755-775.

Ebert, T.A. 1980. Relative growth of sea urchin jaws: an example of plastic resource allocation. *Bulletin of Marine Science* **30**: 467-474.

Ebert, T.A. 2001. Growth and survival of post-settlement sea urchins. Pages 79-102. In: J.M. Lawrence (ed.) *Edible Sea Urchins: Biology and Ecology*. Elsevier: Amsterdam.

Ebling, M.E. 1968. The Duman method for nitrogen in feeds. *J. Assoc. Off. Anal. Chem.* **51**: 766-770.

Edwards, P.B., T.A. Ebert. 1991. Plastic responses to limited food availability and spine damage in the sea urchin *Strongylocentrotus purpuratus*. *Journal of Experimental Marine Biology and Ecology* **45**: 205-220.

Emson, R.H., P.G. Moore. 1998. Diet and gonad size in three populations of *Echinus esculentus*. Pages 641-644. In: Mooi R. and M. Telford (eds.). *Echinoderms : San Francisco. Proc. 9th International Echinoderm Conference*. A.A. Balkema: Rotterdam.

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

Fernandez, C. 1998. Seasonal changes in the biochemical composition of the edible sea urchin *Paracentrotus lividus* (Echinodermata: Echinoidea) in a lagoonal environment. *Marine Ecology* **19**(1): 1-11.

Fernandez, C., C.-F. Boudouresque. 1998. Evaluating artificial diets for small *Paracentrotus lividus* (Echinodermata: Echinoidea). Pages 651-656 In: Mooi R. and M. Telford (eds.). *Echinoderms : San Francisco. Proc. 9th International Echinoderm Conference.* A.A. Balkema: Rotterdam.

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

Fernandez, C., G. Pergent. 1998. Effect of different formulated diets and rearing conditions on growth parameters in the sea urchin *Paracentrotus lividus*· *Journal of Shellfish Research* **17**(5): 1571-1581.

Folch, J., M. Lees, G.H. Sloane-Stanley. 1957. A simple method for the isolation and purification of total lipids from animal tissues. *Journal of Biological Chemistry* **226**: 497-509.

Fong, W., K.H. Mann. 1980. Role of gut flora in the transfer of amino acids through a marine food chain. *Canadian Journal of Fisheries and Aquatic Science* **37**: 88-96.

Frantzis, A., A. Gremare. 1992. Ingestion, absorption and growth rates of *Paracentrotus lividus* fed different macrophytes. *Marine Ecology Progress Series* **95**: 169-183.

Fugi, A.R. 1963. On the growth of the sea urchin, *Hemicentrotus pulcherrimus*· *Bulletin of the Japanese Society of Scientific Research* **29**(2): 118-126.

Fugi, A. 1967. Ecological studies on the growth and food consumption of Japanese common littoral sea urchin, *Strongylocentrotus intermedius*. *Memoirs of the Faculty of Fisheries Hokkaido University* **15**: 83-160.

George, S.B., J.M. Lawrence, A.L Lawrence, J. Ford. 2000. Fertilisation and development of eggs of the sea urchin *Lytechinus variegatus* maintained on an extruded feed. *Journal of the World Aquaculture Society* **31**(2): 232-238.

Gonor, J.J. 1972. Gonad growth in the sea urchin, *Strongylocentrotus purpuratus* (Stimpson) (Echinodermata: Echinoidea) and the assumptions of gonad index methods. *Journal of Experimental Marine Biology and Ecology* **10**: 89-103.

Gonor, J.J. 1973. Reproductive cycles in Oregon populations of the echinoid, *Strongylocentrotus purpuratus* (Stimpson). I. Annual gonad growth and ovarian gametogenic cycles. *Journal of Experimental Marine Biology and Ecology* **12**: 45-64.

Gonzalez, M.L., M.C. Perez, D.A. Lopez, C.A. Pino. 1993. Effects of algal diet on the energy available for growth of juvenile sea urchins *Loxechinus albus* (Molina, 1782). *Aquaculture* **115**: 87-95.

Goodwin, T.W. 1980. The Biochemistry of the Carotenoids, Vol. I, Plants. Chapman and Hall: London.

Griffiths, M., P. Perrott. 1976. Seasonal changes in the carotenoids of sea urchin *Strongylocentrotus droebachiensis*· *Comparative Biochemistry and Physiology* **55B**: 438-441.

Grosjean, P., C. Spirlet, P. Gosselin, D. Vaitilingon, M. Jangoux. 1998. Land-based, closed-cycle echinoculture of *Paracentrotus lividus* (Lamarck) (Echinoidea: Echinodermata): A long-term experiment at a pilot scale. *Journal of Shellfish Research* **17**(5): 1523-1531.

Guerinot, M.L., D.G. Patriquin. 1981. The association of N_2-fixing bacteria with sea urchins. *Marine Biology* **62**: 197-207.

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

Hagen, N.T. 1998. Effect of food availability and body size on out-of-season gonad yield in the green sea urchin, *Strongylocentrotus droebachiensis*· *Journal of Shellfish Research* **17**(5): 1533-1539.

Hallenstvet, M., E. Ryberg, S. Liaaen-Jensen. 1978. Animal carotenoids – XIV Carotenoids of *Psammechinus miliaris* (Sea-urchin). *Comparative Biochemistry and Physiology* **60B**: 173:175.

Hatcher, B.G., A.I. Hatcher. 1997. Research directions and management options for sea urchin culture in Nova Scotia. *Bulletin of the Aquaculture Association of Canada* **97**(1): 62-65.

Haugan, J.A., S. Liaaen-Jensen. 1994. Algal carotenoids 54. Carotenoids of brown algae (Phaeophyceae). *Biochem. Sys. Ecol.* **22**: 31-41.

Havardsson, B., A.B. Imsland, R. Christiansen, N.T. Hagen, S. Stefansson. 1996. The effect of carotenoids in feed and environment temperature on the gonad development and gonad colour of the green sea urchin (*Strongylocentrotus droebachiensis* Muller 1776). ICES 1996, Mariculture committee. ICES C.M. 1996/F:15.

Havardsson, B., A.K. Imsland. 1999. The effect of astaxanthin in feed and environmental temperature on carotenoid concentration in the gonads of the green sea urchin *Strongylocentrotus droebachiensis*· *Journal of the World Aquaculture Society* **30**(2): 208-218.

Himmelman, J.H. 1978. Reproductive cycle of the green sea urchin, *Strongylocentrotus droebachiensis*· *Canadian Journal of Zoology* **56**: 1828-1836.

Himmelman, J.H., D.H. Steele. 1971. Foods and predators of the green sea urchin, *Strongylocentrotus droebachiensis* in Newfoundland waters. *Marine Biology* **9**: 315-322.

Holland, N.D., A.C. Giese. 1965. An autoradiographic investigation of the gonads of the purple sea urchin *Strongylocentrotus purpuratus*· *Biological Bulletin* **128**: 241-258.

Hooper, R., F. Cuthbert, T. McKeever. 1996. Ration size, seasonal growth rates, mixed kelp diets, fish diet, old sea urchin growth and baiting confinement experiments. Report for Canadian Centre for Fisheries Innovation, St. John's, NF. 37pp.

Hooper, R., F. Cuthbert, T. McKeever. 1997. Feasibility trials for sea urchin aquaculture using natural feeds. *Bulletin of the Aquaculture Association of Canada* **97**(1): 5-7.

Hooper, R., F. Cuthbert, D. Patey. 1994. Studies on roe quality, yield and maturation in relation to specific habitats around the coasts of Newfoundland. Report for Government of Newfoundland and Labrador, Department of Fisheries, March 9, 52pp.

Hwang, D. 1992. Dietary fatty acids and eicosanoids. Pages 545-558. In: Chow, C.K. (ed.). *Fatty acids in foods and their health implications*· Marcel Dekker, Inc.: New York.

John, D.A., T.J. Menken, T.S. Klinger. 1990. Feeding and digestion of prepared protein and carbohydrate rich diets by *Strongylocentrotus droebachiensis* (O.F. Muller) (Echinodermata: Echinoidea). *Northeast Gulf Science* **11**: 89.

Jones, D.B. 1941. Factors for converting percentages of nitrogen in foods and feeds into percentages of protein. USDA Circular 183. Washington, DC: US Department of Agriculture.

Kawakami, T., M. Tsushima, Y. Katabami, M. Mine, A. Ishida, T. Matsuno. 1998. Effect of β,β- carotene, β-echinenone, astaxanthin, fucoxanthin, vitamin A and vitamin E on the biological defence of the sea urchin *Pseudocentrotus depressus*· *Journal of Experimental Marine Biology and Ecology* **226**(2): 165-174.

Keats, D.W., D.H. Steele, G.R. South. 1984. Depth-dependant reproductive output of the green sea urchin, *Strongylocentrotus droebachiensis* (O.F. Muller), in relation to the nature and availability of food. *Journal of Experimental Marine Biology and Ecology* **80**: 77-91.

Kelly, M.S., C.C. Brodie, J.D. Mckenzie. 1998. Somatic and gonadal growth of the sea urchin *Psammechinus miliaris* (Gmelin) maintained in polyculture with the Atlantic salmon. *Journal of Shellfish Research* **17**(5): 1557-1562.

Kennedy, E.J., S.M.C. Robinson, G.J. Parsons, J. Castell. 1999. Somatic growth trials for juvenile green sea urchins fed prepared and natural diets. *Bulletin of the Aquaculture Association of Canada* **99**(4): 52-54.

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

Klinger, T.S. 2000. Absorption and assimilation of prepared feeds by echinoids. www.calacademy.org/research/izg/echinoderm/conference/abst118.htm. (last visited on January 7, 2002).

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

Klinger, T.S., J.M. Lawrence, A.L. Lawrence. 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., J.M. Lawrence, A.L. Lawrence. 1998. Digestion, absorption, and assimilation of prepared feeds by echinoids. Pages 713-721 In: Mooi R. And M. Telford (eds.). *Echinoderms : San Francisco. Proc. 9th International Echinoderm Conference*. A.A. Balkema: Rotterdam.

Klinger, T.S., J.M. Lawrence, A.L. Lawrence, K.L. Price, R. Boudreau, M. Koder. 1995. Somatic and gonadal growth of *Strongylocentrotus droebachiensis* (Echinodermata: Echinoidea) fed manufactured feeds. *American Zoologist* **35**: 109A.

Klinger, T.S., B.M. McCarthy, J.M. Lawrence. 1983. The effects of food quantity and quality upon somatic and gonadal growth of *Lytechinus variegatus* Lamarck (Echinodermata: Echinoidea). *American Zoologist*. **23**(4): 964.

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

Kochi, M. 1969. Studies on "Uni-Shiokara" - VI. Fatty acid composition of lipid from sea-urchin gonad. *Fisheries Research Board of Canada Translation Series* No. 1262, 15p.

Lamare, M.D., P.V. Mladenov. 2000. Modelling somatic growth in the sea urchin *Evechinus chloroticus* (Echinoidea: Echinometridae). *Journal of Experimental Marine Biology and Ecology* **243**: 17-43.

Lares, M.T., J.M. Lawrence. 1995. The effects of feeding frequency on feeding and digestion of sea urchins (Echinodermata). *American Zoologist* **35**: 109A.

Lares, M.T., J.B. McClintock. 1991. The effects of temperature on the survival, organismal activity, nutrition, growth, and reproduction of the carnivorous, tropical sea urchin *Eucidaris tribuloides*. *Marine Behavioural Physiology* **19**: 75-96.

Larson, B.R., R.L. Vadas, M. Keser. 1980. Feeding and nutritional ecology of the sea urchin *Strongylocentrotus droebachiensis* in Maine, USA. *Marine Biology* **59**: 49-62.

Lasker, R., A.C. Giese. 1954. Nutrition of the sea urchin, *Strongylocentrotus purpuratus*. *Biological Bulletin* (Woods Hole, Mass.) **106**: 328-340.

Lawrence, J.M. 1975. On the relationship between marine plants and sea urchins. *Oceanog. Mar. Biol. Ann. Rev.* **13**: 213-286.

Lawrence, J.M., M. Byrne. 1994. Allocation of resources to body components in *Heliocidaris erythrogramma* and *Heliocidaris tuberculata* (Echinodermata : Echinoidea). *Zoological Science* **11**: 133-137.

Lawrence, J.M., L. Fenaux, A. Lawrence, M. C. Corre. 1991. The effect of quantity and quality of prepared diets on production in *Paracentrotus lividus* (Echinodermata: Echinoidea). Pages 107-110 In: L. Scalera-Liaci and L. Canicatti (eds.). *Echinoderm Research*. A.A. Balkema: Rotterdam.

Lawrence, J.M., A. Guille. 1982. Organic composition of tropical, polar, and temperate water echinoderms. *Comparative Biochemistry and Physiology* **72B**: 283-287.

Lawrence , J.M., J.M. Lane. 1982. The utilisation of nutrients by post-metamorphic echinoderms. Pages 331-371 In: Janoux, M., Lawrence, J.M. (eds.). *Echinoderm Nutrition*. A.A. Balkema: Rotterdam.

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

Lawrence, J.M., S. Olave, R. Otaiza, A.L. Lawrence, E. Bustos. 1995. A comparison of gonad production in *Loxechinus albus* in Chile fed algae and prepared feeds. *American Zoologist* **35**: 109A.

Lawrence, J.M., S. Olave, R. Otaiza, A.L. Lawrence, E. Bustos. 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.

Lebour, M.V. 1930. The planktonic diatoms of northern seas. Dulau & Co., Ltd., London, 244pp.

Lesser, M.P., C.W. Walker. 1998. Introduction to the special section on sea urchin aquaculture. *Journal of Shellfish Research*· **17**(5): 1505-1506.

Levitan, D.R. 1988. Density-dependent size regulation and negative growth in the sea urchin *Diadema antillarum* Philippi. *Oceologia* **76**: 627-629.

Levitan, D.R. 1991. Skeletal changes in the test and jaws of the sea urchin *Diadema antillarum* in response to food limitation. *Marine Biology* **111**: 431-435.

Lewis, C.A., T.A. Ebert, M.E. Boren. 1990. Allocation of 45Calcium to body components of starved and fed purple sea urchins (*Strongylocentrotus purpuratus*)· *Marine Biology* **105**: 213-222.

Lilly, G.R. 1975. The influence of diet on the growth and bioenergetics of the tropical sea urchin, *Tripneustes ventricosus*· Ph.D. thesis, University of British Columbia, Vancouver, Canada.

Lobban, C.S., P.J. Harrison. 1994. Seaweed ecology and physiology. Cambridge University Press, Cambridge, England, 366pp.

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

Lozano, J., J. Galera, S. Lopez, X. Turon, C. Palacin, G. Morera. 1995. Biological cycles and recruitment of *Paracentrotus lividus* (Echinodermata: Echinoidea) in two contrasting habitats. *Marine Ecology Progress Series* **122**: 179-191.

Lukyanova, O.N., Y.S. Khotimchenko. 1995. Lipid peroxidation in organs of the scallop *Mizuhopecten yessoensis* and sea urchin *Strongylocentrotus intermedius* during the reproductive cycle. *Comparative Biochemistry and Physiology* **110B**: 371-377.

Lukyanova, O.N., T.Y. Shmidt. 1994. Concentration of carotenoids in marine invertebrates under conditions of contamination. *Russian Journal of Marine Biology* **19**(2): 92-101.

Marsh, A.G., S.A. Watts. 2001. Energy metabolism and gonad development. Pages 27-42. In: J.M. Lawrence (ed.) *Edible Sea Urchins: Biology and Ecology*. Elsevier: Amsterdam.

Matsuno, T., M. Tsushima. 2001. Carotenoids in sea urchins. Pages 115-138. In: J.M. Lawrence (ed.) *Edible Sea Urchins: Biology and Ecology*. Elsevier: Amsterdam.

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

McBride, S.C., J.M. Lawrence, A.L. Lawrence, T.J. Mulligan. 1999. Ingestion, absorption, and gonad production of adult *Strongylocentrotus franciscanus* fed different rations of a prepared diet. *Journal of the World Aquaculture Society* **30**(3): 364-370.

McClintock, J.B., T.S. Klinger, J.M. Lawrence. 1982. Feeding preferences of echinoids for plant and animal food models. *Bulletin of Marine Science* **32**(1): 365-369.

Meidel, S.K., R.E. Scheibling. 1998a. Resource allocation in juvenile and adult sea urchins (*Strongylocentrotus droebachiensis*) in kelp beds and barren grounds off Nova Scotia. Page 735. In: Mooi R. And M. Telford (eds.). *Echinoderms : San Francisco. Proc. 9th International Echinoderm Conference.* A.A. Balkema: Rotterdam.

Meidel, S.K., R.E. Scheibling. 1998b. Size and age structure of the sea urchin *Strongylocentrotus droebachiensis* in different habitats. Pages 737-742. In: Mooi R. And M. Telford (eds.). *Echinoderms : San Francisco. Proc. 9th International Echinoderm Conference.* A.A. Balkema: Rotterdam.

Meidel, S.K., R.E. Scheibling, A. Metaxas. 1999. Relative importance of parental and larval nutrition on larval development and metamorphosis of the sea urchin *Strongylocentrotus droebachiensis*. *Journal of Experimental Marine Biology and Ecology* **240**(2): 161-178.

Minor, M.A., R.E. Scheibling. 1997. Effects of food ration and feeding regime on growth and reproduction of the sea urchin *Strongylocentrotus droebachiensis*. *Marine Biology* **129**: 159-167.

Montero-Torreiro, M.F., P. Garcia-Martinez, J.L. Catoira, G. Mosquera. 1998. Seasonal variation in biochemical composition in gonads of the sea urchin, *Paracentrotus lividus* Lmk. Pages 753-758 In: Mooi R. And M. Telford (eds.). *Echinoderms : San Francisco. Proc. 9th International Echinoderm Conference.* A.A. Balkema: Rotterdam.

Morgan, L.E., L.W. Botsford, S.R. Wing, B.D. Smith. 2000. Spatial variability in growth and mortality of the red sea urchin, *Strongylocentrotus franciscanus*, in northern California. *Canadian Journal of Fisheries and Aquatic Sciences* **57**: 980-992.

Morris, T.J., A. Campbell. 1996. Growth of juvenile red sea urchins (*Strongylocentrotus franciscanus*) fed *Zostera marina* or *Nereocystis luetkeana*· *Journal of Shellfish Research* **15**(3): 777-780.

Mosteller, F., R.E.K. Rourke. 1973. Sturdy Statistics. Nonparametrics and order statistics. Addison-Wesley Publishing Company, Reading Massachusetts, 395pp.

Mottet, M.G. 1976. The Fishery Biology of Sea Urchins in the Family Strongylocentrotidae. State of Washington Dept. of Fisheries, Shellfish Division. Technical Report No. 20.

Munilla, M.A., I. Gomez-Pinilla, S. Rodenas, M.T. Larrea. 1995. Determination of metals in seaweeds used as food by inductively coupled plasma atomic-emission spectrometry. *Analusis* (Paris) **23**(9): 463-466.

Munk, J.E. 1992. Reproduction and growth of the green sea urchin *Strongylocentrotus droebachiensis* (Muller) near Kodiak, Alaska. *Journal of Shellfish Research* **11**(2): 245-254.

Nagai, Y., K. Kaneko. 1975. Culture experiments on the sea urchin *Strongylocentrotus pulcherrimus* fed an artificial diet. *Marine Biology* **29**: 105-108.

Okazaki, K. 1956. Skeleton formation of sea urchin larvae. I. Effect of Ca concentration of the medium. *Biological Bulletin* **110**: 320-333.

Pantzis, P.A., M.S. Kelly, J.G. Connolly, K.D. Black. 2000. Effect of artificial diets on growth, lipid utilisation, and gonad biochemistry in the adult sea urchin *Psammechinus miliaris*· *Journal of Shellfish Research* **19**(2): 995-1001.

Paradis, M., R.G. Ackman. 1977. Potential for employing the distribution of anomalous non-methylene-interrupted dienoic fatty acids in several marine invertebrates as part of food web studies. *Lipids* **12**: 170-176.

Pearse, J.S., D. Beyer, M.E. Steele. 1995. Both photoperiod and diet influence resource partitioning between somatic and gonad growth in sea urchins (*Strongylocentrotus franciscanus* and *S. purpuratus*) *American Zoologist* **35**: 109A.

Pearse, J.S., R.A. Cameron. 1991. Echinodermata Echinoidea. Pages 513-662. In: A.C. Giese, J.S. Pearse, and V.B. Pearse (eds.). Reproduction of Marine Invertebrates, vol. VI. Echinoderms and Lophophorates. The Boxwood Press: Pacific Grove, CA.

Pearse, J.S., V.B. Pearse. 1975. Growth zones in the echinoid skeleton. *American Zoologist* **15**: 731-753.

Raymond B.G., Scheibling R.E. 1987. Recruitment and growth of the sea urchin *Strongylocentrotus droebachiensis* (Muller) following mass mortalities off Nova Scotia, Canada. *Journal of Experimental Marine Biology and Ecology* **108**: 31-54.

Robinson, S.M.C., J.D. Castell, E.J. Kennedy. 2002 Developing suitable colour in the gonads of cultured green sea urchins (*Strongylocentrotus droebachiensis*). *Aquaculture* **206**(3-4): 289-303.

Robinson, S.M.C., L. Colborne. 1997. Enhancing roe of the green sea urchin using an artificial food source. *Bulletin of the Aquaculture Association of Canada* **97**(1): 14-20.

Robinson, S.M.C., A.D. MacIntyre. 1997. Ageing and growth of the green sea urchin. *Bulletin of the Aquaculture Association of Canada* **97**(1): 56-60.

Rowley, R.J. 1990. Newly settled sea urchins in a kelp bed and urchin barren ground: a comparison of growth and mortality. *Marine Ecology Progress Series* **62**: 229-240.

Russel, M.P. 1987. Life history traits and resource allocation in the purple sea urchin, *Strongylocentrotus purpuratus* (Stimpson). *Journal of Experimental Marine Biology and Ecology* **108**: 199-216.

Russel, M.P. 1998. Resource allocation plasticity in sea urchins: rapid, diet induced, phenotypic changes in the green sea urchin, *Strongylocentrotus droebachiensis* (Muller). *Journal of Experimental Marine Biology and Ecology* **220**: 1-14.

Schakel, S.F., I.M. Buzzard, S.E. Gebhardt. 1997. Procedures for estimating nutrient values for food composition databases. *Journal of Food Composition and Analysis* **10**: 102-114.

Scheibling, R.E., B.G. Hatcher. 2001. The ecology of *Strongylocentrotus droebachiensis*. Pages 271-305. In: J.M. Lawrence (ed.) *Edible Sea Urchins: Biology and Ecology*. Elsevier: Amsterdam.

Scheibling, R.E., A.W. Hennigar, T. Balch. 1999. Destructive grazing, epiphytism, and disease: the dynamics of sea urchin – kelp interactions in Nova Scotia. *Canadian Journal of Fisheries and Aquatic Sciences* **56**: 2300-2314.

Shimizu, M., I. Naoshi, Y. Watanabe. 1994. Protein, calcium levels and acid phosphatase activity in regenerative test tissues of the sea urchin, *Strongylocentrotus intermedius*. *Comparative Biochemistry and Physiology* **109A**(3): 721-726.

Sokal, R.R., F.J. Rohlf. 1995. Biometry. The principles and practice of statistics in biological research. W.H. Freeman and Company, New York, USA, 887 pp.

Swan, E.F. 1961. Some observations on the growth rate of sea urchins in the genus *Strongylocentrotus*. *Biological Bulletin* **120**: 420-427.

Tajima, K., M. Fukuchi. 1991. Studies on the artificial seed production of the sea urchin, *Strongylocentrotus intermedius* I. The growth of early juveniles in winter. *Canadian Translation of Fisheries and Aquatic Sciences* No. 5528. 17pp.

Tajima, K., T. Sugimoto, N. Nishikawa, S. Torii, T. Ushiro, M. Yuki, A. Ebisu, I. Isozaki. 1986. Comparison of the gonadal growth of six groups of sea urchins, *Strongylocentrotus intermedius*, reared by different quantities of *Laminaria* in polyethylene net cages on shallow reef of Rumoi, Hokkaido. *Canadian Translation of Fisheries and Aquatic Sciences* No. 5244. 14pp.

Takagi, T., C.A. Eaton, R.G. Ackman. 1980. Distribution of fatty acids in lipids of the common Atlantic sea urchin *Strongylocentrotus droebachiensis*. *Canadian Journal of Fisheries and Aquatic Science* **37**: 195-202.

Thompson, R.J. 1982. The relationship between food ration and reproductive effort in green sea urchin, *Strongylocentrotus droebachiensis*. *Oecologia* **56**: 50-57.

Tidwell, J.H., G.L. Allan. 2001. Maintaining and managing the wealth from the seas – Ecological and economic impacts and contributions of fish farming and capture fisheries. *European Molecular Biology Organisation reports* **2**(1): 958-963.

Torrissen, O.J., R.W. Hardy, K.D. Shearer. 1989. Pigmentation of salmonids – carotenoid deposition and metabolism. *CRC Critical Reviews in Aquatic Science* **1**: 209-225.

Tsushima, M., M. Byrne, S. Amemiya, T. Matsuno. 1995. Comparative biochemical studies of carotenoids in sea urchins. 3. Relationship between developmental

mode and carotenoids in the Australian echinoids *Heliocidaris erythrogramma* and *H. tuberculata* and a comparison with Japanese species. *Comparative Biochemistry and Physiology* **110B**(4): 719-723.

Tsushima, M., T. Kawakami, T. Matsuno. 1993. Metabolism of carotenoids in sea urchin *Pseudocentrotus depressus*· *Comparative Biochemistry and Physiology* **106B**(3): 737-741.

Tsushima, M., T. Matsuno. 1990. Comparative studies of carotenoids in sea urchins. 1. *Comparative Biochemistry and Physiology* **96B**(4): 810-810.

Uki, N., A. Kemuyama, T. Watanabe. 1986. Optimum protein level in diets for abalone. *Bull. Jpn. Soc. Fish.* **52**: 1005-1012.

Vadas, R.L. 1977. Preferential feeding: An optimisation strategy in sea urchins. *Ecological Monographs* **47**: 337-371.

Vadas (Sr.), R.L., B. Beal, T. Dowling, J.C. Fegley. 2000. Experimental field tests of natural algal diets on gonad index and quality in the green sea urchin, *Strongylocentrotus droebachiensis*: a case for rapid summer production in post-spawned animals. *Aquaculture* **182**: 115-135.

van Barneveld, R.J., A.E. Fleming, M.E. Vandepeer, J.A. Kruk, P.W. Horne. 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**(3): 649-656.

Voogt, P.A. 1982. Steroid metabolism. Pages 417-436. In: Jangoux M. and J.M. Lawrence (eds.) *Echinoderm Nutrition.* A.A. Balkema: Rotterdam.

Walker, C.W. 1982. Nutrition of gametes. Pages 449-468 In: Jangoux M. and J.M. Lawrence (eds.) *Echinoderm Nutrition.* A.A. Balkema: Rotterdam.

Walker, C.W., M.P. Lesser. 1997. Prepared food coupled with manipulation of photoperiod yield an out-of-season crop for the green sea urchin. *Bulletin of the Aquaculture Association of Canada* **97-1**: 31-34.

Wallace, B.D., H.S. Hammer, S.A. Watts, J.M. Lawrence, A.L. Lawrence. 2001. The effects of dietary protein on growth of juvenile sea urchins. Page 674. In: *Aquaculture 2001: Book of Abstracts*· World Aquaculture Society: Louisiana State University, Baton Rouge, LA.

Wasson, K.M., S.A. Watts. 2001. Reproductive endocrinology of sea urchins. Pages 43-58. In: J.M. Lawrence (ed.) *Edible Sea Urchins: Biology and Ecology*. Elsevier: Amsterdam.

Watts, S.A., S.A. Boettger, J.B. McClintock, J.M. Lawrence. 1998. Gonad production in the sea urchin *Lytechinus variegatus* (Lamarck) fed prepared diets. *Journal of Shellfish Research* **17**(5): 1591-1595.

Wilbur, K.M. 1976. Recent studies of invertebrate mineralization. Pages 79-108. In: Watabe, N., K.M. Wilbur (eds.). *The Mechanisms of Mineralization in the Invertebrates and Plants*. University of South Carolina Press: Columbia, South Carolina.

Williams, C.T., L.G. Harris. 1998. Growth of juvenile green sea urchins on natural and artificial diets. Pages 887-892 In: Mooi R. And M. Telford (eds.). *Echinoderms : San Francisco. Proc. 9th International Echinoderm Conference.* A.A. Balkema: Rotterdam.

Zar, J.H. 1999. Biostatistical analysis (4th edition). Prentice-Hall, Inc., New Jersey, USA, 929pp.

Tables

Table 2.1. Composition (percent dry weight) of prepared protein diets fed to juvenile green sea urchins (*Strongylocentrotus droebachiensis*) to test the effect of dietary protein on somatic growth.

Prepared Diets	Ingredients (% dry mass)				Protein Concentration
	Protein Sources[a]				
	Fishmeal	Soybean	Starch	Other[b]	
1	0	30	54	16	20
2	0	45	39	16	30
3	0	60	24	16	40
4	0	75	9	16	50
5	1.5	29	53.5	16	20
6	2	43	39	16	30
7	3	57	24	16	40
8	4	71	9	16	50
9	13	15	57	15[c]	20
10	20	23	42	15[c]	30
11	27	30	28	15[c]	40
12	33	38	14	15[c]	50

[a] fishmeal and soybean contain 75% and 66% protein, respectively
[b] includes lecithin (2%), soybean oil (2%), fish oil (1%), Shur-Gain vitamin mix (2%) and mineral mix (3%), agar (4%), sodium alginate (2%)
[c] no fish oil was included in these diets

Table 2.2. Approximate energy analysis of prepared protein diets and kelp (*Laminaria longicruris*) based on the assumptions that protein, lipid, and carbohydrate have digestibilities of 80%, 45%, and 62%, respectively, and have digestible energies of 20.9 KJ/g, 37.7 KJ/g, and 16.7 KJ/g, respectively.

	Diet Composition[a] (% dry mass)				Energy (kJ) / 1 g diet (dry mass)			Total E (KJ)	P/E [c]
Diet	Protein	Lipid	Ash	CHO	Protein	Lipid	CHO	per 1 g diet	(mg/KJ)
1	20	6.2	3.5	70.3	3.3	1.1	7.3	11.67	17.1
2	30	6.1	4.5	59.4	5.0	1.0	6.2	12.20	24.6
3	40	6.4	5.2	48.3	6.7	1.1	5.0	12.78	31.3
4	50	6.4	6.1	37.5	8.4	1.1	3.9	13.33	37.5
5	20	6.3	3.6	70.1	3.3	1.1	7.3	11.67	17.1
6	30	5.9	4.5	59.6	5.0	1.0	6.2	12.19	24.6
7	40	6.0	5.5	48.5	6.7	1.0	5.0	12.73	31.4
8	50	6.1	6.3	37.6	8.4	1.0	3.9	13.28	37.6
9	20	10.7	4.1	65.2	3.3	1.8	6.8	11.91	16.8
10	30	10.5	5.3	54.2	5.0	1.8	5.6	12.41	24.2
11	40	11.0	6.5	42.5	6.7	1.9	4.4	12.95	30.9
12	50	11.6	7.6	30.8	8.4	2.0	3.2	13.52	37.0
kelp	13.1[b]	2.7	37.6	46.6	2.2	0.5	4.8	7.47	17.5

[a] diet composition was determined from proximate analysis

[b] protein composition of kelp (13.1%) from Chapman and Craigie (1977)

[c] P/E = the amount of protein energy (KJ) in 1 mg of diet.

Table 2.3. Summary statistics from Tukey's post-hoc test highlighting subsets of similar average test diameters (mm) (identified by the P-values) of cohort 1 juvenile green sea urchins (*Strongylocentrotus droebachiensis*) fed different prepared protein diets over 280 days (from December 4, 1998 to September 10, 1999). The average test diameter (mm) for the juvenile sea urchins fed kelp (*Laminaria longicruris*) was included for comparison.

Diet [a]	Protein Source [b]	Protein Concentration	Subsets of test diameters (mm) with a P-value > 0.05					
			1	2	3	4	5	6
12	C	50%	13.22					
2	A	30%	13.45	13.45				
6	B	30%	13.61	13.61	13.61			
8	B	50%	13.78	13.78	13.78			
7	B	40%	13.80	13.80	13.80			
10	C	30%	14.14	14.14	14.14	14.14		
5	B	20%	14.22	14.22	14.22	14.22		
1	A	20%		14.53	14.53	14.53		
11	C	40%			14.78	14.78		
3	A	40%				15.05	15.05	
4	A	50%				15.11	15.11	
9	C	20%					16.17	
kelp	kelp	13.1% [c]						20.74
P-value			0.17	0.09	0.16	0.21	0.06	1.00

Diets are arranged by the corresponding ascending test diameter (mm) of the juvenile sea urchins to which they were fed.

[a] refer to Table 2.1 for the composition of the diets

[b] A = 100% soybean protein (SBP); B = 95% SBP:5% fishmeal protein (FMP); C = 50% SBP:50% FMP

[c] from Chapman and Craigie (1977)

Table 2.4. Summary statistics from Tukey's post-hoc test highlighting subsets of similar average test diameters (mm) (identified by the P-values) of cohort 2 juvenile green sea urchins (*Strongylocentrotus droebachiensis*) fed different prepared protein diets over 280 days (from December 4, 1998 to September 10, 1999). The average test diameter (mm) for the juvenile sea urchins fed kelp (*Laminaria longicruris*) was included for comparison.

Diet [a]	Protein Source [b]	Protein Concentration	Subsets of test diameters (mm) with a P-value > 0.05			
			1	2	3	4
6	B	30%	20.41			
4	A	50%	20.62	20.62		
5	B	20%	20.64	20.64		
7	B	40%	21.60	21.60	21.60	
3	A	40%		21.71	21.71	
10	C	30%			22.13	
11	C	40%			22.35	
8	B	50%			22.40	
2	A	30%			22.51	
9	C	20%			22.61	
1	A	20%			22.66	
12	C	50%			22.86	
kelp	kelp	13.1% [c]				24.51
P-value			0.06	0.14	0.15	1.00

Diets are arranged by the corresponding ascending test diameter (mm) of the juvenile sea urchins to which they were fed.

[a] refer to Table 2.1 for the composition of the diets

[b] A = 100% soybean protein (SBP); B = 95% SBP:5% fishmeal protein (FMP); C = 50% SBP:50% FMP

[c] from Chapman and Craigie (1977)

Table 2.5. Average daily growth rates for juvenile green sea urchins (*Strongylocentrotus droebachiensis*) in both cohorts fed either protein prepared diets or kelp (*Laminaria longicruris*) during Interval 1 (from December 4, 1998 to March 30, 1999) and Interval 2 (from March 30, 1999 to September 10, 1999).

Cohort	Interval	Treatment	Growth Rate [a] (mm TD/day)
1	1	Pellet	0.022 (0.001)
1	1	Kelp	0.030 (0.002)
1	2	Pellet	0.039 (0.002)
1	2	Kelp	0.069 (0.005)
2	1	Pellet	0.022 (0.001)
2	1	Kelp	0.030 (0.002)
2	2	Pellet	0.035 (0.001)
2	2	Kelp	0.052 (0.003)

Cohort 1 = 4-8 mm TD initial size; Cohort 2 = 12-20 mm TD initial size.
[a] values in "( )" represent +/- 1 standard error

Table 3.1. Composition of prepared diets used to test the effect of lipids on the somatic growth of juvenile green sea urchins (*Strongylocentrotus droebachiensis*). The concentrations of the diet ingredients represent the basic diet formulation in the lipid source experiment.

Ingredients [a]	**Percent dry mass**
Soybean protein concentrate	44
Potato starch	39
Shur-Gain vitamin mix [b]	2
Shur-Gain mineral mix [b]	3
Gelatin	5
Sodium alginate	2
Lipid source	5
Water	50

[a] all dry ingredients were supplied by Shur-Gain
[b] composition unknown because it is company information

Table 3.2. The major fatty acids of the lipid sources used in the prepared diet treatments formulated to test the effect of lipid source on the somatic growth of juvenile green sea urchins (*Strongylocentrotus droebachiensis*).

Lipid Source	Major Fatty Acids
Corn Oil	18:2n-6 (Linoleic acid)
Linseed Oil	18:3n-3 (Linolenic acid)
Menhaden Oil	22:6n-3 (Docosahexaenoic acid)
Corn Oil + Linseed Oil	18:2n-6 + 18:3n-3
Corn Oil + Menhaden Oil	18:2n-6 + 22:6n-3
Linseed Oil + Menhaden Oil	18:3n-3 + 22:6n-3
Kelp (*Laminaria longicruris*)	22:4n-6 + 20:5n-3 (Arachidonic acid + Eicosapentaenoic acid)

Table 3.3. The concentration (percent dry mass) of ingredients used to formulate prepared diets used to test the effect of lipid concentration on the somatic growth of juvenile green sea urchins (*Strongylocentrotus droebachiensis*).

Lipid Source			
Menhaden Oil	Corn Oil + Linseed Oil	Starch	Other [a]
1	-	43	56
3	-	41	56
6	-	37	56
10	-	34	56
-	1	43	56
-	3	41	56
-	6	37	56
-	10	34	56

[a] includes soybean protein (44%), Shur-Gain vitamin mix (2%), Shur-Gain mineral mix (3%), gelatin (5%), sodium alginate (2%)

Table 4.1. Composition of prepared diet treatments used to test the effect of dietary minerals and pigment on the somatic growth of juvenile green sea urchins (*Strongylocentrotus droebachiensis*).

Treatment Name [a]	Concentration of Ingredients (% dry mass)				
	Starch	Shur-Gain Mineral Mix [b]	Modified Bernhart-Tomerelli Salt Mix [c]	Algro™	Other [d]
0% Min + Pig	39.55	0	0	1.25	59.2
1.5% BT + Pig	38.05	0	1.5	1.25	59.2
3% BT + Pig	36.55	0	3	1.25	59.2
6% BT + Pig	33.55	0	6	1.25	59.2
15% BT + Pig	24.55	0	15	1.25	59.2
3% BT	38.05	0	3	0	58.95
3% SG	38.05	3	0	0	58.95
3% SG + Pig	36.55	3	0	1.25	59.2
6% SG + Pig	33.55	6	0	1.25	59.2

[a] Min = minerals; Pig = pigment (250 mg beta-carotene/kg dry diet); BT = modified Bernhart-Tomerelli salt mix; SG = Shur-Gain mineral mix

[b] composition unknown

[c] contains $CaCO_3$ (2.1%), $Ca(PO)_4$ (73.5%), citric acid (0.205%), cupric acid (0.046%), ferric citrate (0.558%), MgO (2.5%), $Mn_3(C_6H_5O_7)_2$ (0.835%), KI (0.001%), K_2HPO_4 (8.1%) NaCl (6.8%), Na_2HPO_4-$2H_2O$ (2.14%), $Zn_3(C_6H_5O_7)_2$-H_2O (0.133%), NaF (0.002%), $CoCl_2$ (0.02%)

[d] consists of soybean protein concentrate (45%), sodium alginate (2%), Shur-Gain vitamin mix (2%), corn oil + linseed oil (4%), ethoxyquin (0.2%), gelatin (5%)

Figures

(A) (B)

Figure 2.1. Laboratory set-up used to test the effect of protein in prepared diets on the somatic growth of juvenile green sea urchins (*Strongylocentrotus droebachiensis*). Figure A shows 12 of 30 experimental tanks three tiers high. Figure B shows the four baskets in a tank. Each tank has its own flow-through system and aeration. Juvenile sea urchins are feeding on the pale coloured prepared diet (arrows).

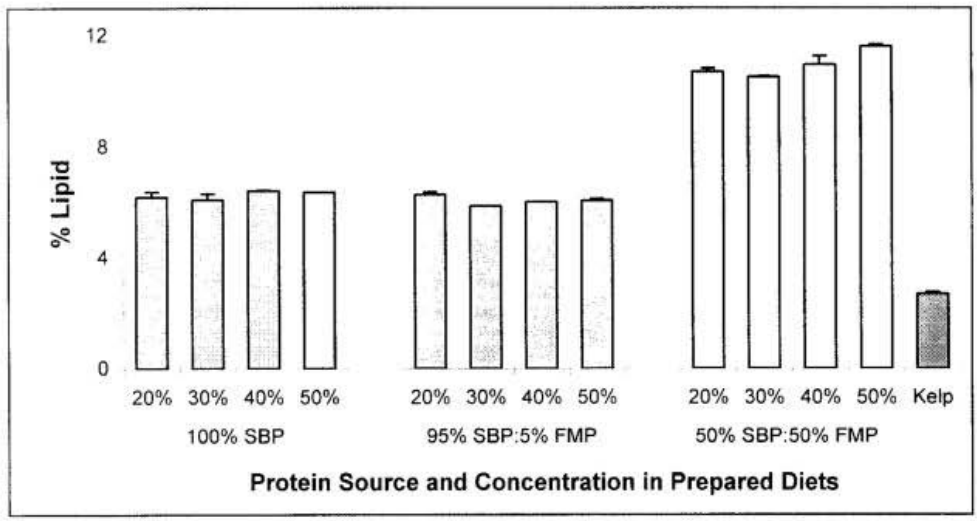

Figure 2.2. Average percent lipid in the prepared protein diets and kelp (*Laminaria longicruris*) (n=2). Protein sources consisted of soybean protein (SBP) and fishmeal protein (FMP). Bars represent one standard error.

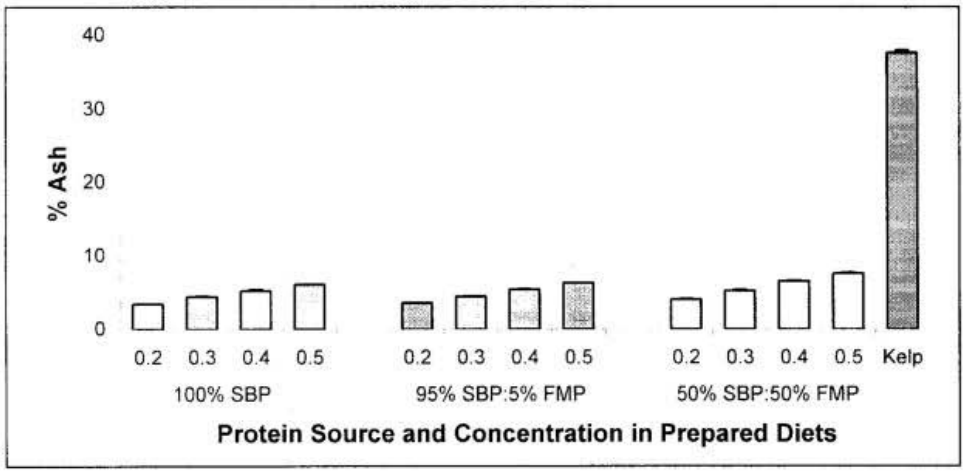

Figure 2.3. Average percent ash in the prepared protein diets and kelp (*Laminaria longicruris*) (n=2). Protein sources consisted of soybean protein (SBP) and fishmeal protein (FMP). Bars represent one standard error.

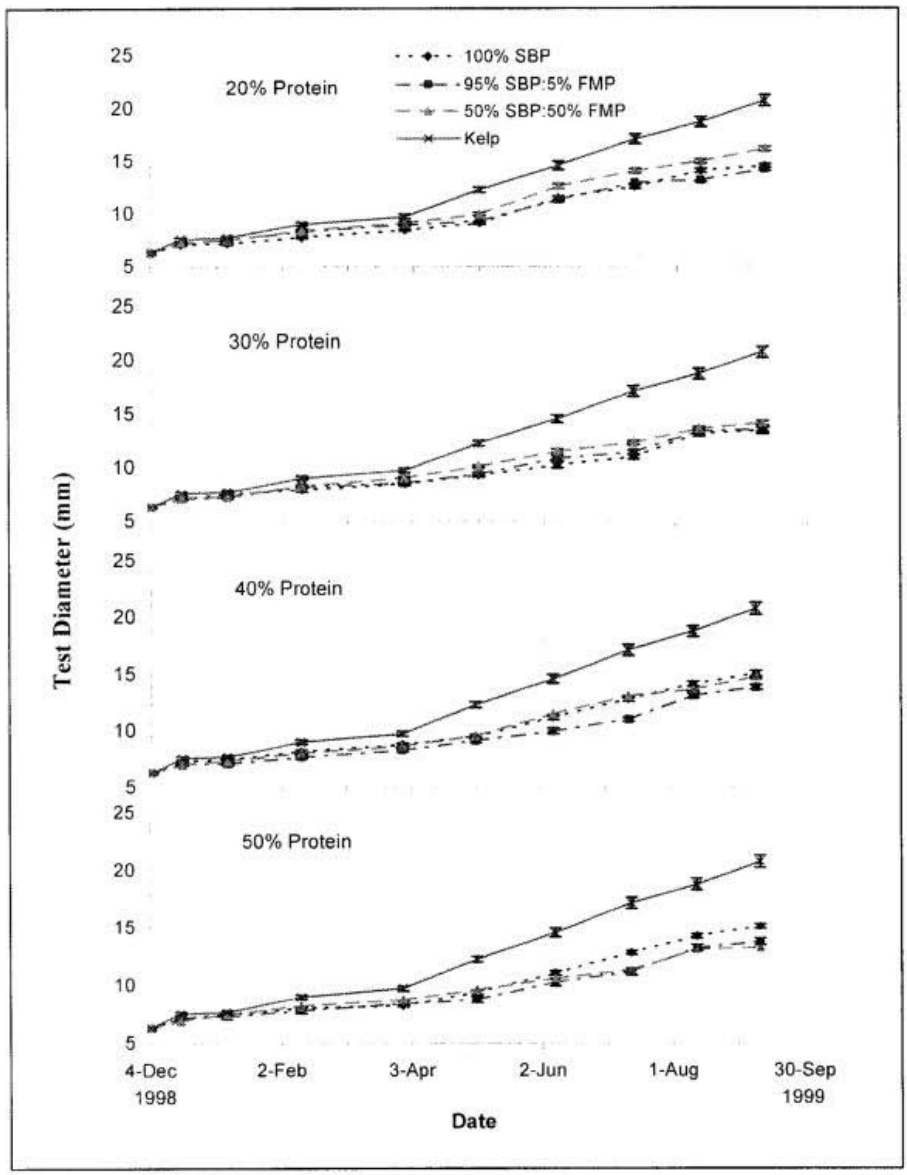

Figure 2.4. Somatic growth of cohort 1 juvenile green sea urchins (*Strongylocentrotus droebachiensis*) fed either prepared diets or kelp (*Laminaria longicruris*) from December 4, 1998 to September 10, 1999 (n=120). Protein sources consisted of soybean protein (SBP) and fishmeal protein (FMP). Protein concentrations consisted of 20%, 30%, 40%, and 50% protein. Kelp data have been replicated for each protein concentration. Bars represent +/- 1 standard error.

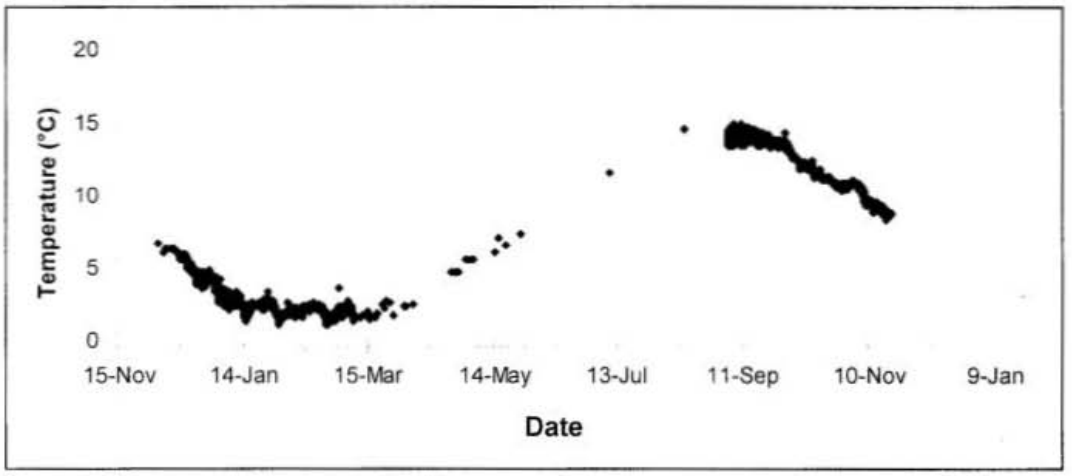

Figure 2.5. Ambient seawater temperatures (°C) in the experimental tanks at the Department of Fisheries and Oceans Biological Station in St. Andrews, New Brunswick from December 1998 to December 1999.

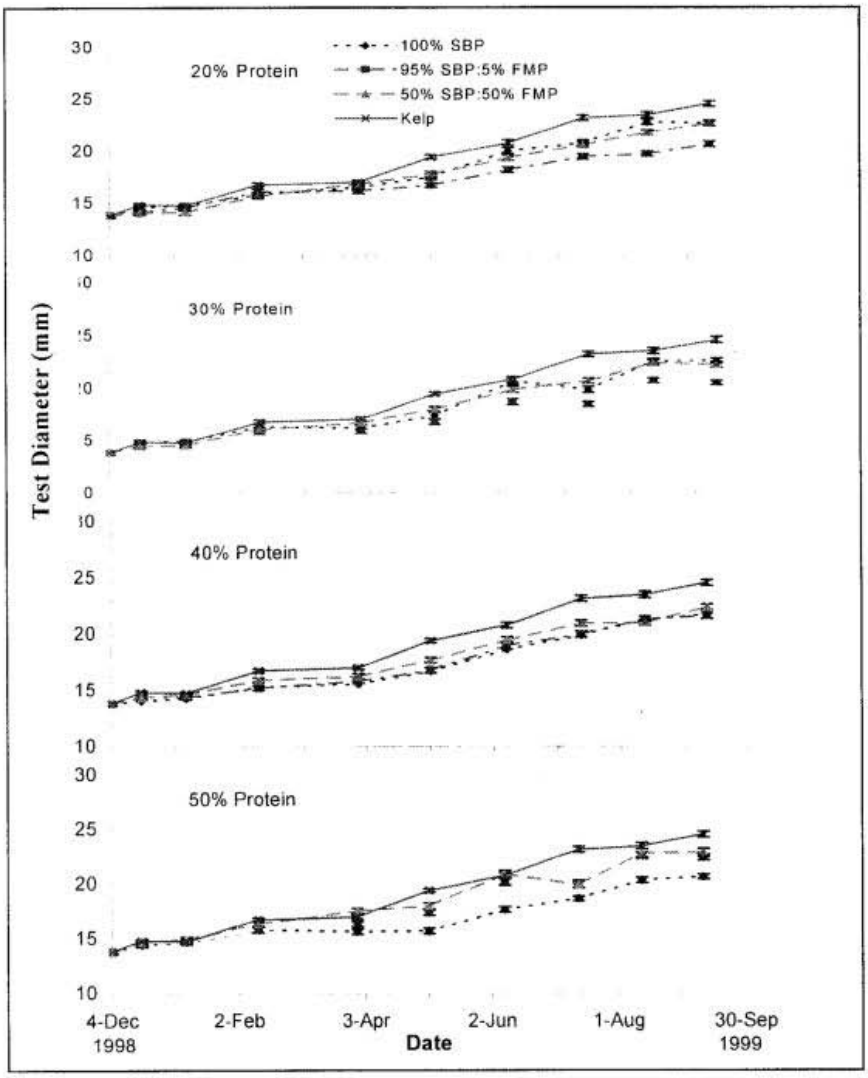

Figure 2.6. Somatic growth of cohort 2 juvenile green sea urchins (*Strongylocentrotus droebachiensis*) fed either prepared diets or kelp (*Laminaria longicruris*) from December 4, 1998 to September 10, 1999 (n=120). Protein sources consisted of soybean protein (SBP) and fishmeal protein (FMP). Protein concentrations consisted of 20%, 30%, 40%, and 50% protein. Kelp data have been replicated for each protein concentration. Bars represent +/- 1 standard error.

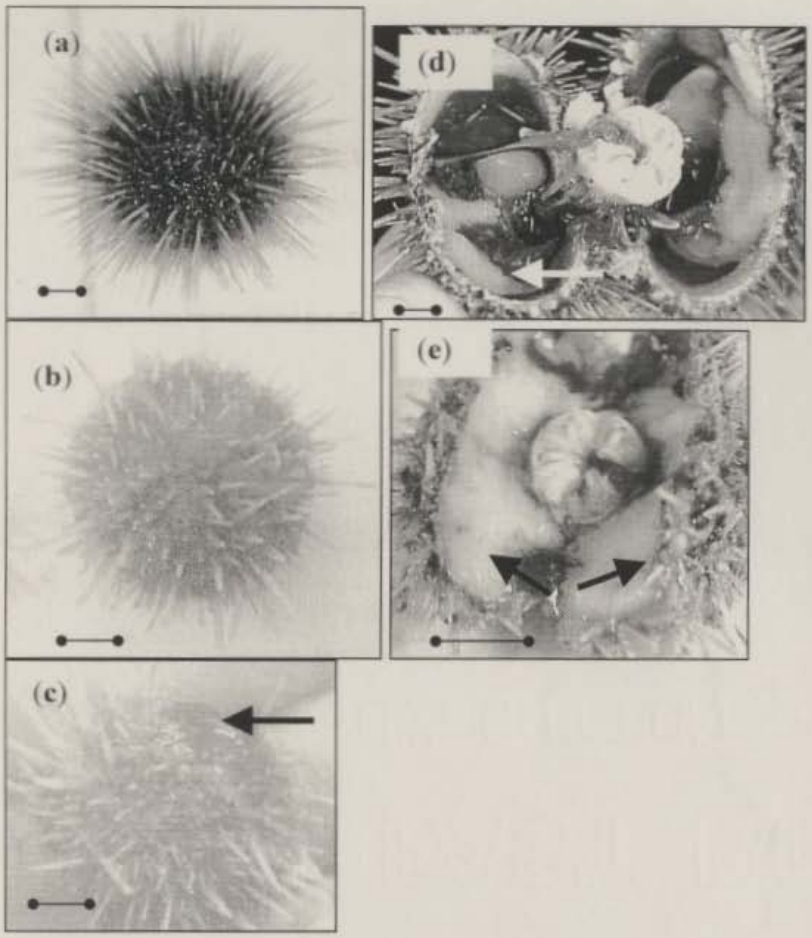

Figure 2.7. The external and internal physical appearances of juvenile green sea urchins (*Strongylocentrotus droebachiensis*) fed either prepared protein diets or kelp (*Laminaria longicruris*) over a 280 day feeding trial that tested the effect of protein on somatic growth (a. external appearance of a typical juvenile sea urchin fed *L. longicruris*; b. external appearance of a typical juvenile fed a prepared diet; c. test necrosis (arrow) on a juvenile sea urchin fed a prepared diet; d. internal tissue from a typical juvenile sea urchin fed *L. longicruris* (arrow showing small mass of orange/yellow coloured gonad); e. internal tissue from a typical juvenile sea urchin fed a prepared diet (arrow showing large mass of white coloured gonad)). Scale line represents 5 mm.

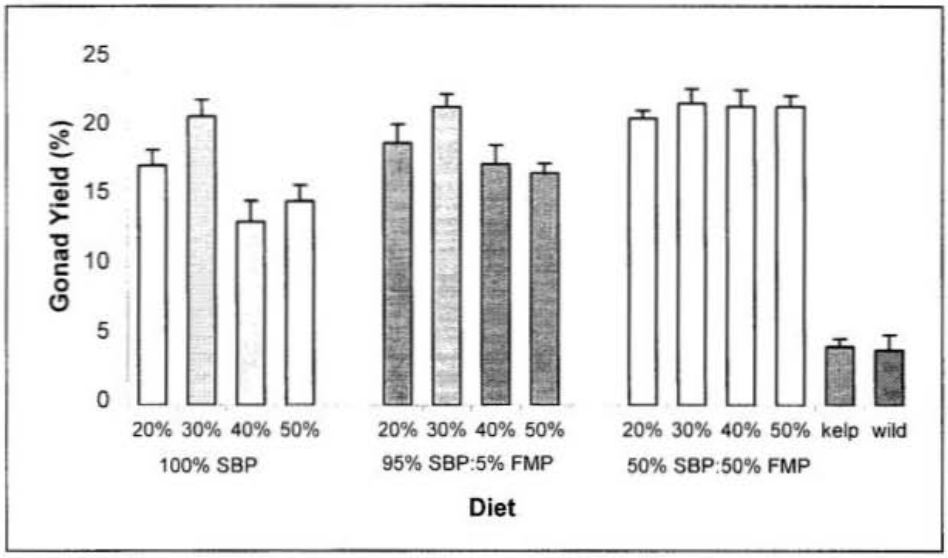

Figure 2.8. Average gonad yields of cohort 2 juvenile green sea urchins (*Strongylocentrotus droebachiensis*) fed either prepared protein diets or kelp (*Laminaria longicruris*) compared to the average gonad yield of similar size wild juvenile green sea urchins (n=10). Protein sources consisted of soybean protein (SBP) and fishmeal protein (FMP). Bars represent one standard error.

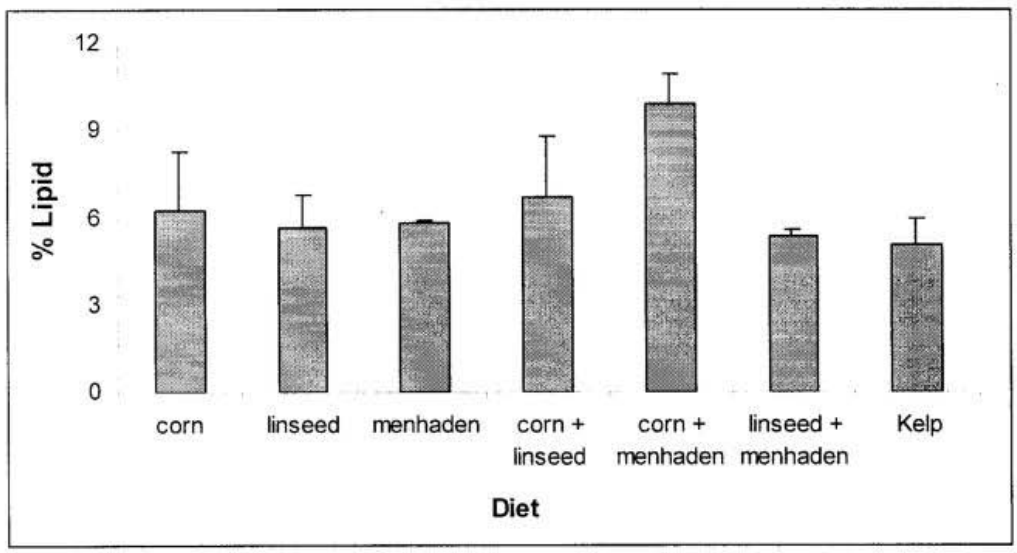

Figure 3.1. Percent lipid (n=2) in the diets fed to the juvenile green sea urchins (*Strongylocentrotus droebachiensis*) in the lipid source feeding experiment. The prepared diets (excluding kelp, *Laminaria longicruris*) are identified by the lipid source. Bars represent one standard error.

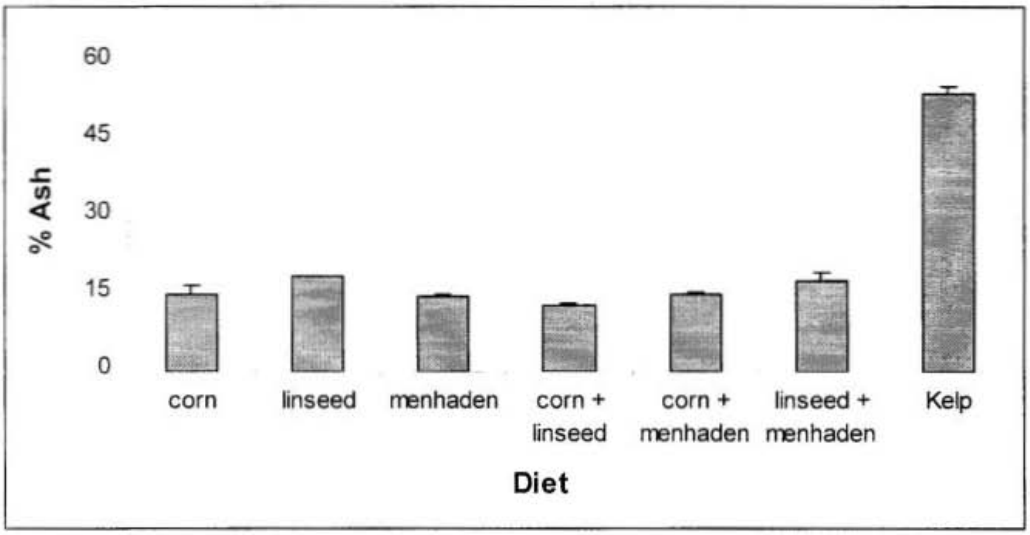

Figure 3.2. Percent ash (n=2) in the diets fed to the juvenile green sea urchins (*Strongylocentrotus droebachiensis*) in the lipid source feeding experiment. The prepared diets (excluding kelp, *Laminaria longicruris*) are identified by the lipid source. Bars represent one standard error.

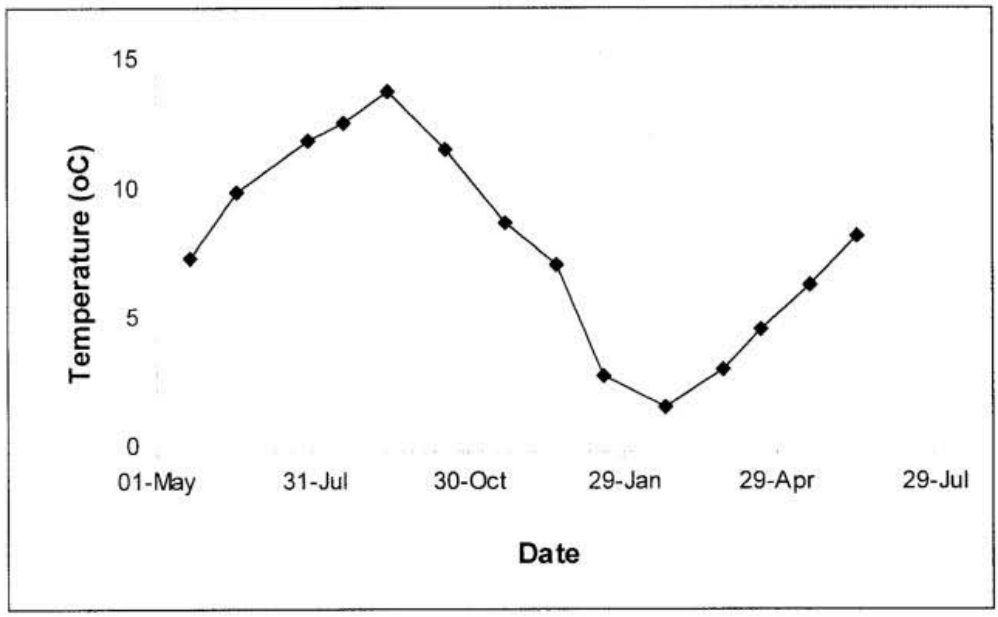

Figure 3.3. Ambient seawater temperature (°C) at the Department of Fisheries and Oceans Biological Station in St. Andrews, New Brunswick from May 20, 1999 to June 14, 2000. Water temperature was recorded at the Station's intake pipes.

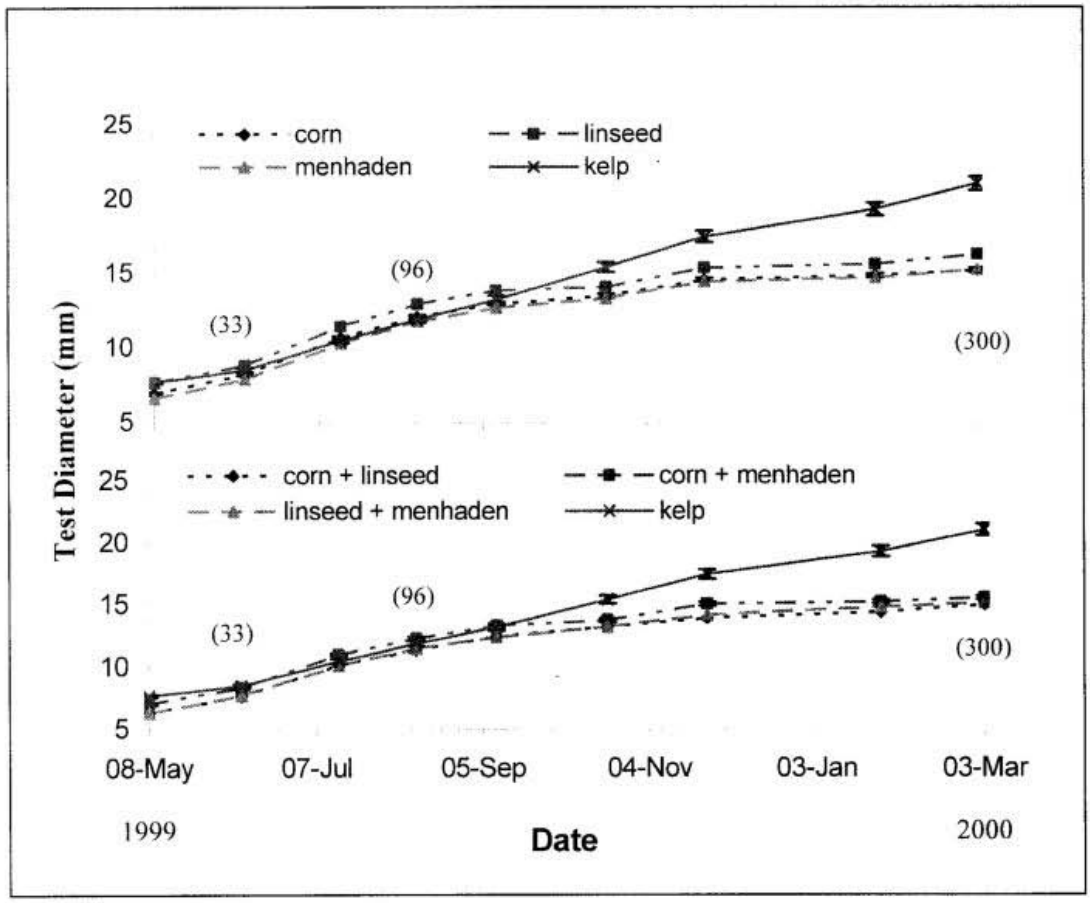

Figure 3.4. Somatic growth of cohort 1 juvenile green sea urchins (*Strongylocentrotus droebachiensis*) fed either prepared diets (with different lipid sources) (n=120) or kelp (*Laminaria longicruris*) (n=60) from May 8, 1999 to March 3, 2000. Kelp data are replicated for each graph. Numbers in brackets represent consecutive days into the experiment. Vertical bars represent +/- one standard error.

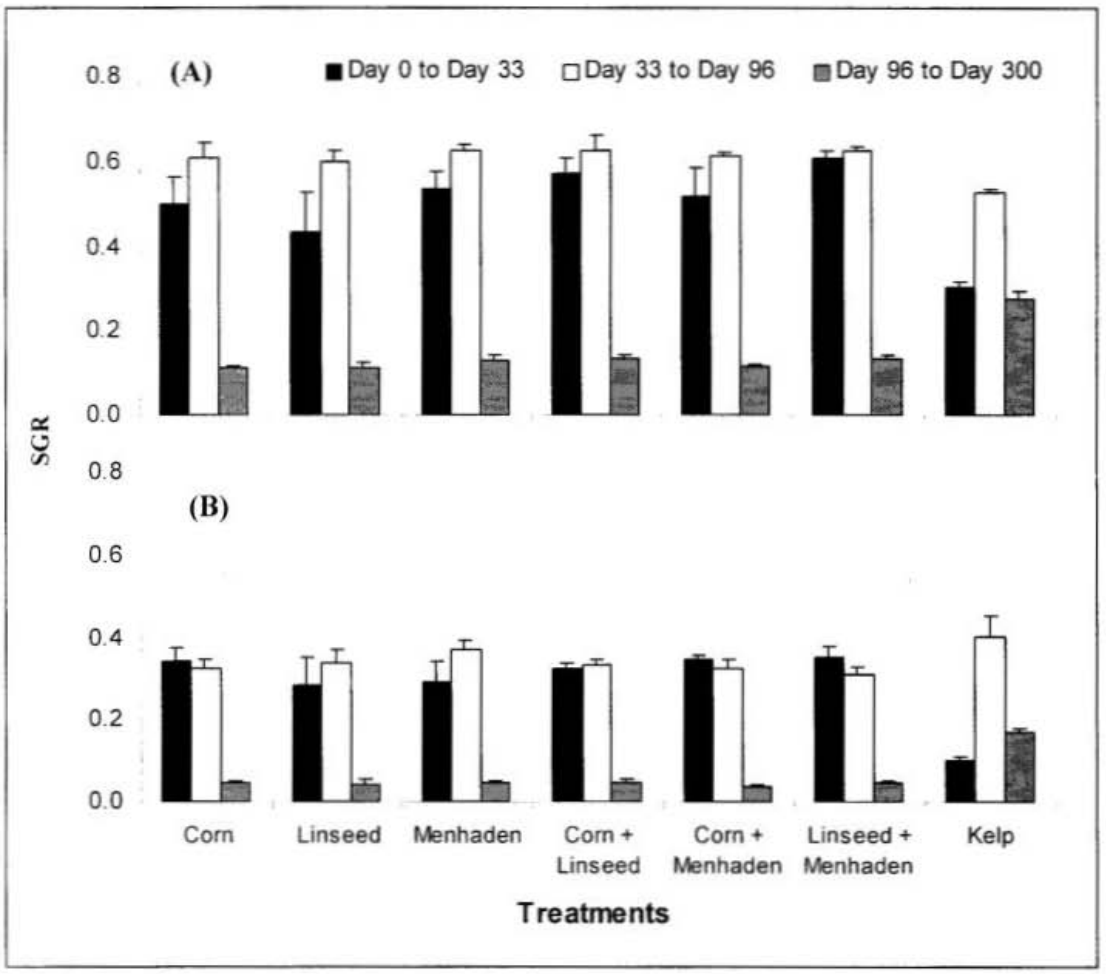

Figure 3.5. Specific growth rates (SGR) of cohort 1 (A) and cohort 2 (B) juvenile green sea urchins (*Strongylocentrotus droebachiensis*) fed either prepared diets (with different lipid sources) (n=4) or kelp (*Laminaria longicruris*) (n=2) from day 0 to 33, day 33 to 96, and day 96 to 300 of the lipid source feeding experiment. Bars represent one standard error.

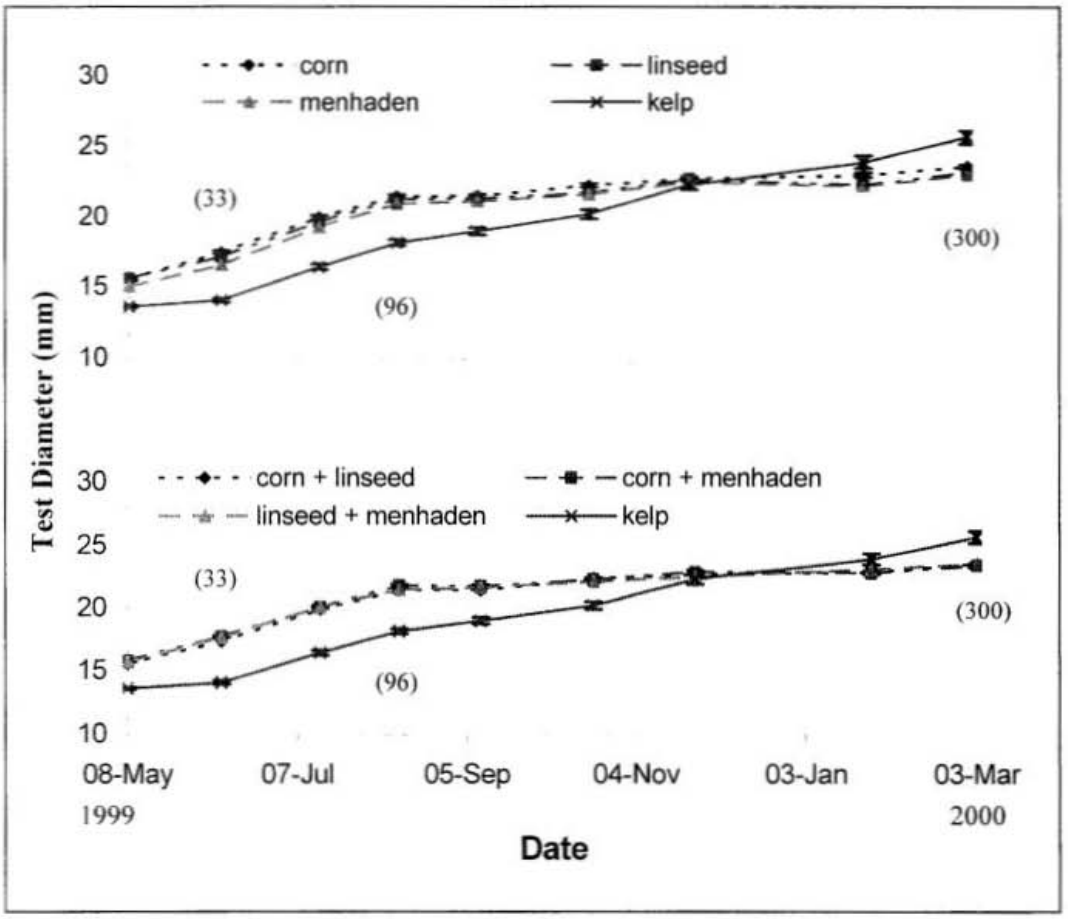

Figure 3.6. Somatic growth of cohort 2 juvenile green sea urchins (*Strongylocentrotus droebachiensis*) fed either prepared diets (with different lipid sources) (n=120) or kelp (*Laminaria longicruris*) (n=60) from May 8, 1999 to March 3, 2000. Kelp data are replicated for each graph. Numbers in brackets represent consecutive days into the experiment. Vertical bars represent +/- one standard error.

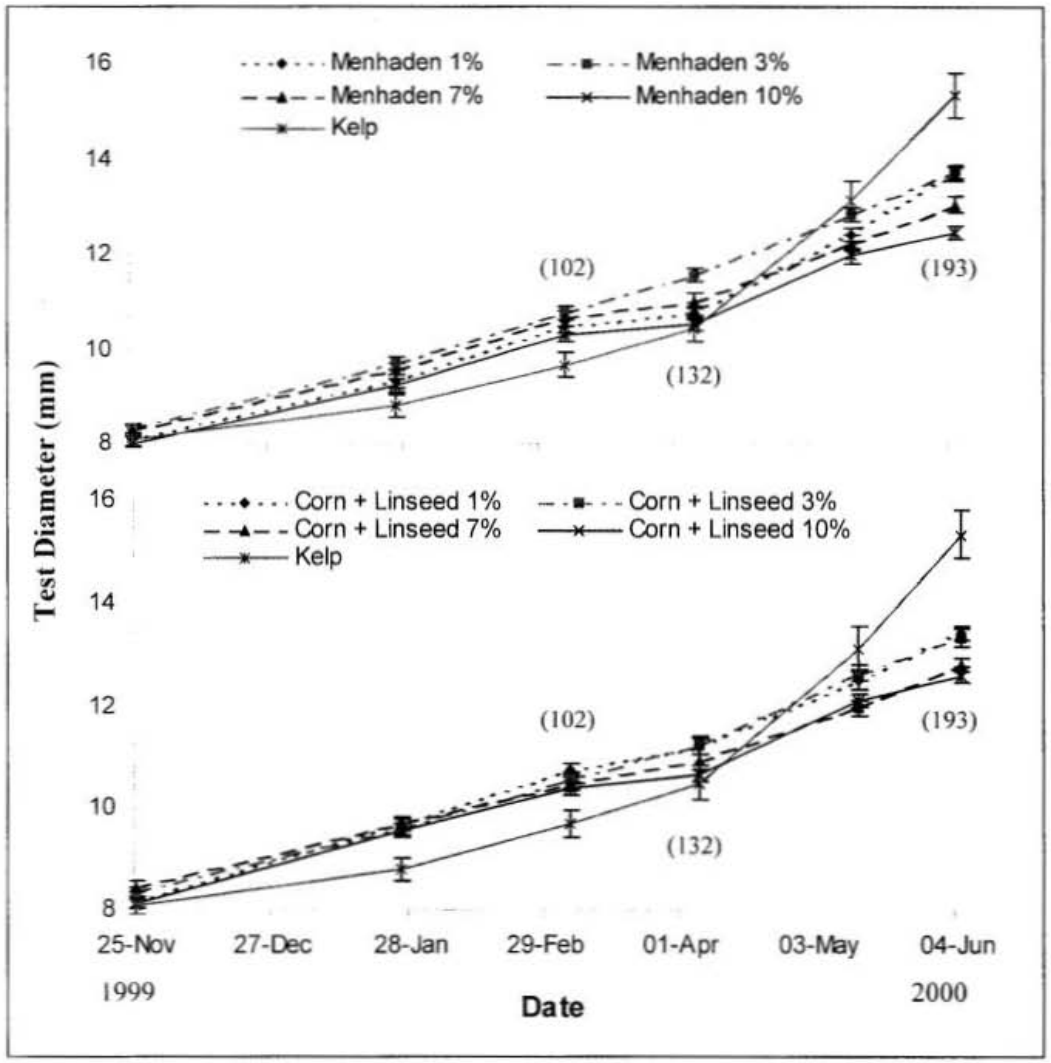

Figure 3.7. Somatic growth of cohort 1 juvenile green sea urchins (*Strongylocentrotus droebachiensis*) fed either prepared diets (with different lipid concentrations) (n=120) or kelp (*Laminaria longicruris*) (n=60) from November 25, 1999 to June 5, 2000. Kelp data are replicated for each graph. Numbers in brackets represent consecutive days into the experiment. Vertical bars represent +/- one standard error.

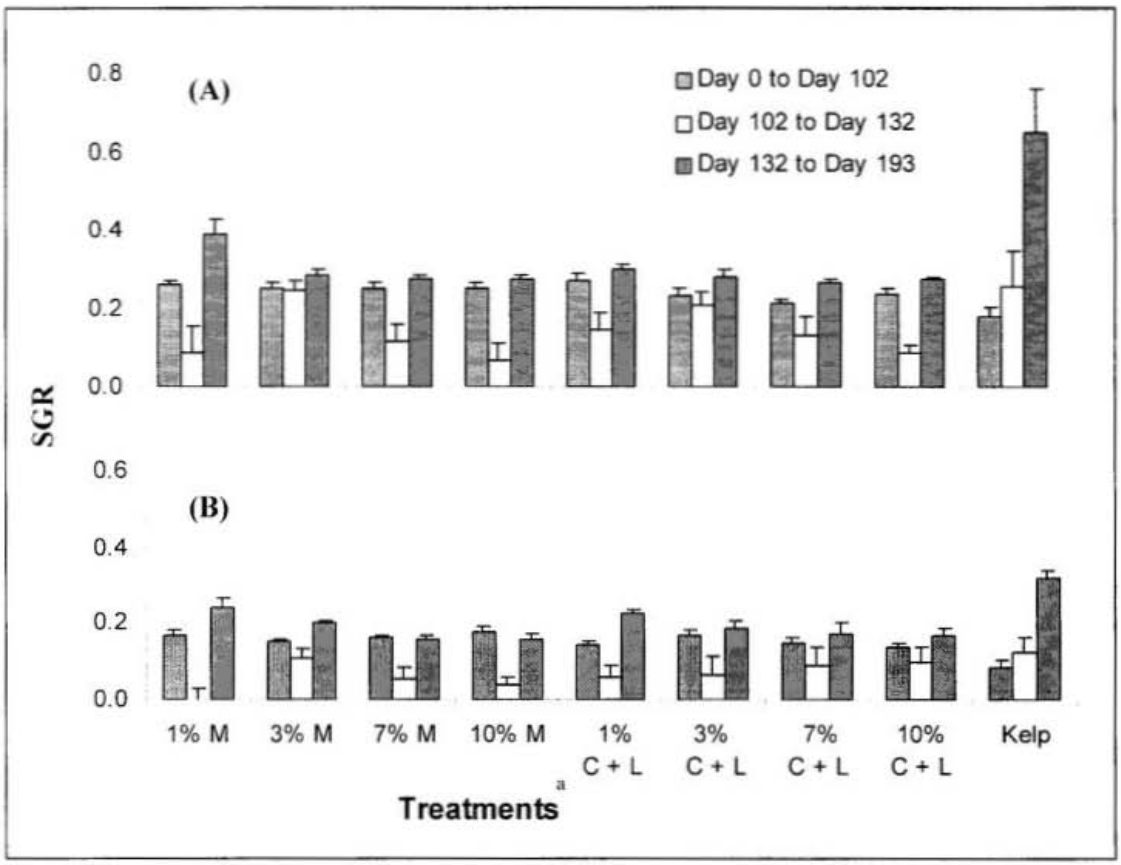

Figure 3.8. Specific growth rates (SGR) of cohort 1 (A) and cohort 2 (B) juvenile green sea urchins (*Strongylocentrotus droebachiensis*) fed either prepared diets (with differed lipid concentrations) (n=4) or kelp (*Laminaria longicruris*) (n=2) from day 0 to 102, day 102 to 132, and day 132 to 196 of the lipid concentration experiment. Bars represent one standard error. ([a] "M" represents menhaden oil treatments; "C + L" represents corn oil + linseed oil treatments)

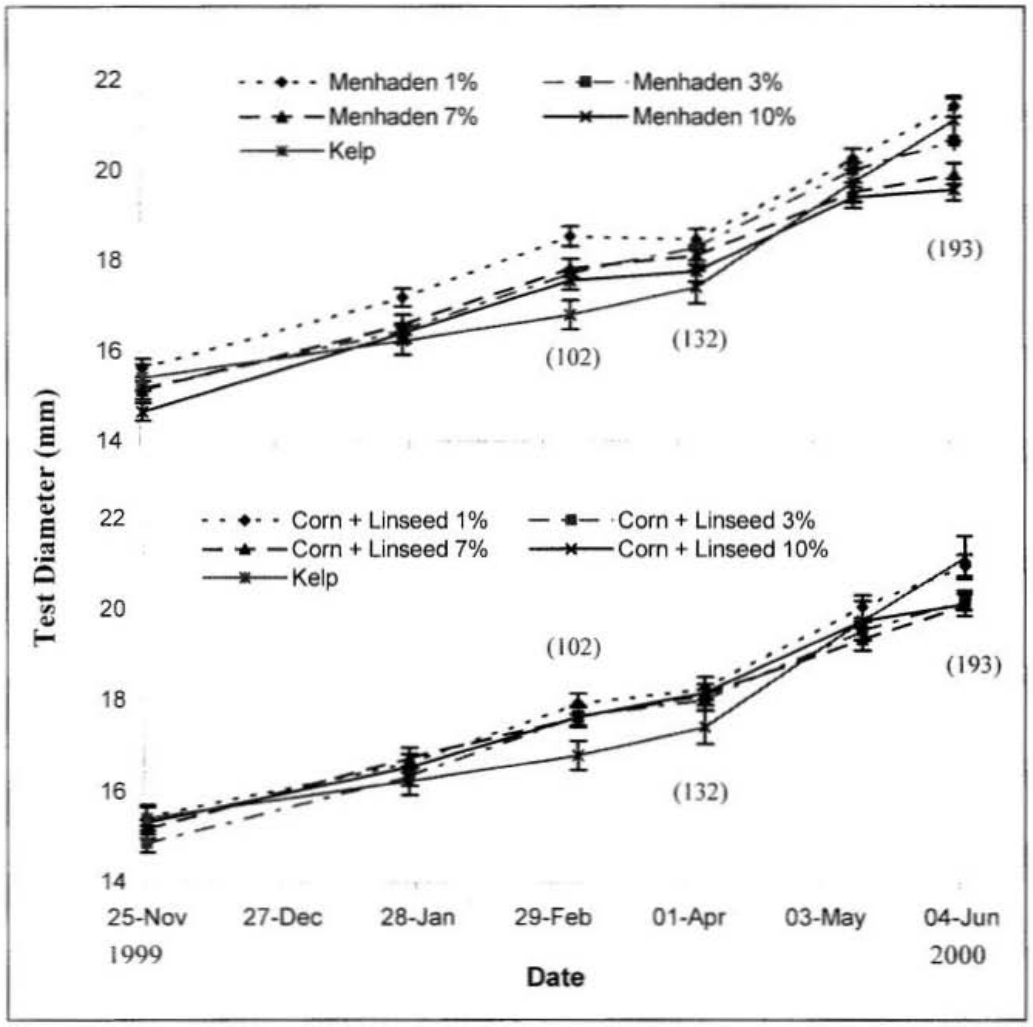

Figure 3.9. Somatic growth of cohort 2 juveniles green sea urchins (*Strongylocentrotus droebachiensis*) fed either prepared diets (with different lipid concentrations) (n=120) or kelp (*Laminaria longicruris*) (n=60) from November 25, 1999 to June 5, 2000. Kelp data are replicated for each graph. Numbers in brackets represent consecutive days into the experiment. Vertical bars represent +/- one standard error.

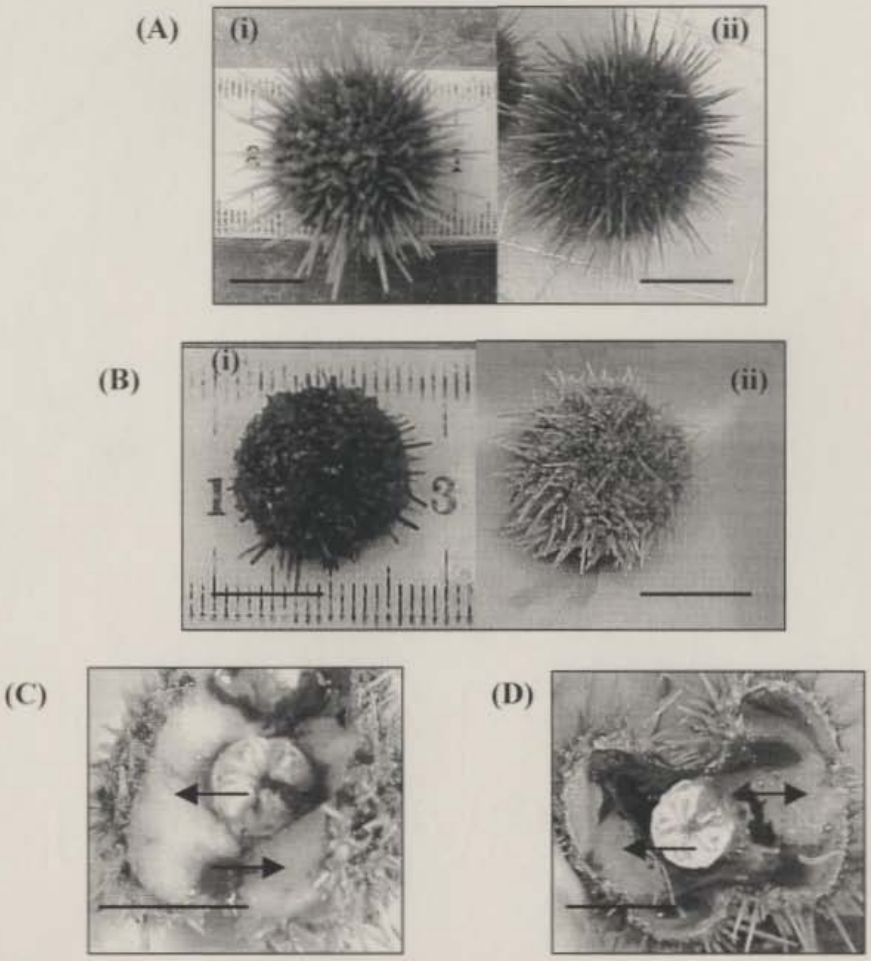

Figure 3.10. The external and internal physical appearances of the juvenile green sea urchins (*Strongylocentrotus droebachiensis*) fed either prepared diets or kelp (*Laminaria longicruris*) in the lipid source (i) and lipid concentration (ii) experiments. The kelp-fed juveniles (A) have larger, greener tests with longer, radiating spines than those fed the prepared diets (B). The juveniles fed the prepared diets had large, white gonads (C) compared to smaller, orange gonads of those fed kelp (D). Gonads are indicated by arrows. (Scale bar represents 10 mm)

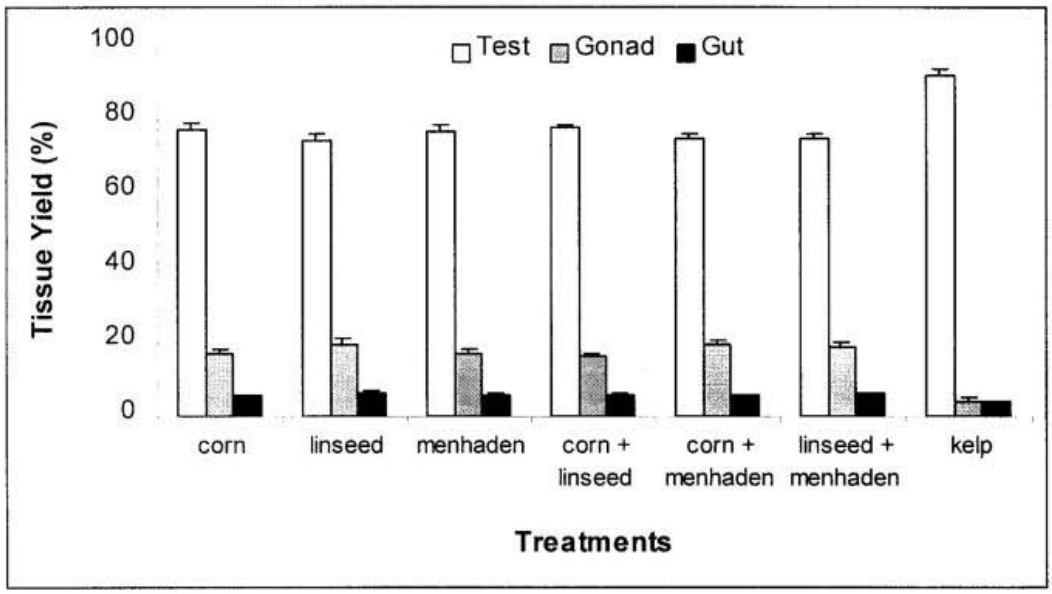

Figure 3.11. Average test, gonad, and gut yields of cohort 2 juvenile green sea urchins (*Strongylocentrotus droebachiensis*) fed either prepared diets (with different lipid sources) (n=20) or kelp (*Laminaria longicruris*) (n=10). Bars represent one standard error.

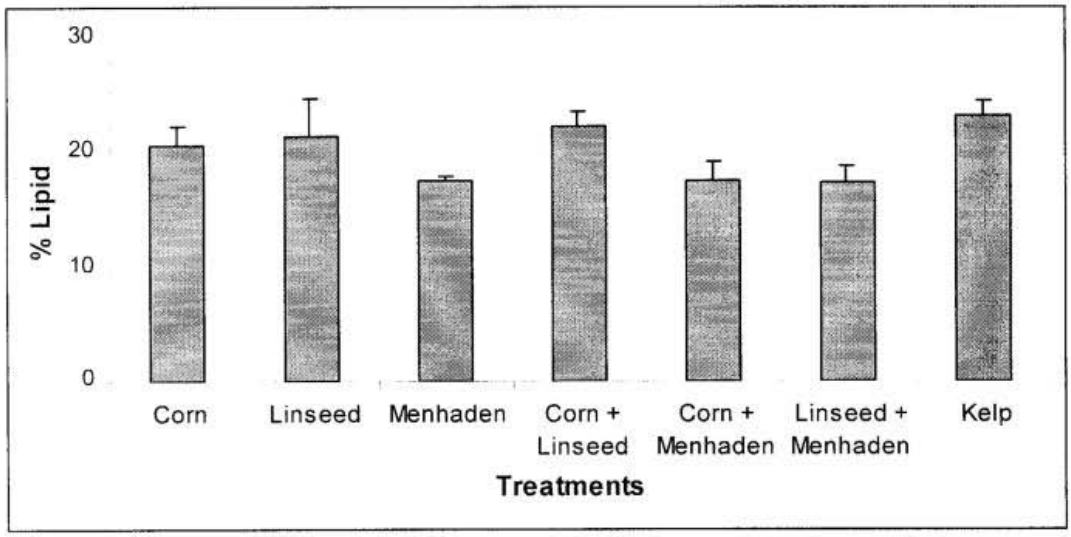

Figure 3.12. Average percent lipid (n=4) in the internal tissues (i.e., combined gonad and gut) of cohort 2 juvenile green sea urchins (*Strongylocentrotus droebachiensis*) fed either prepared diets (with different lipid sources) or kelp (*Laminaria longicruris*). Bars represent one standard error.

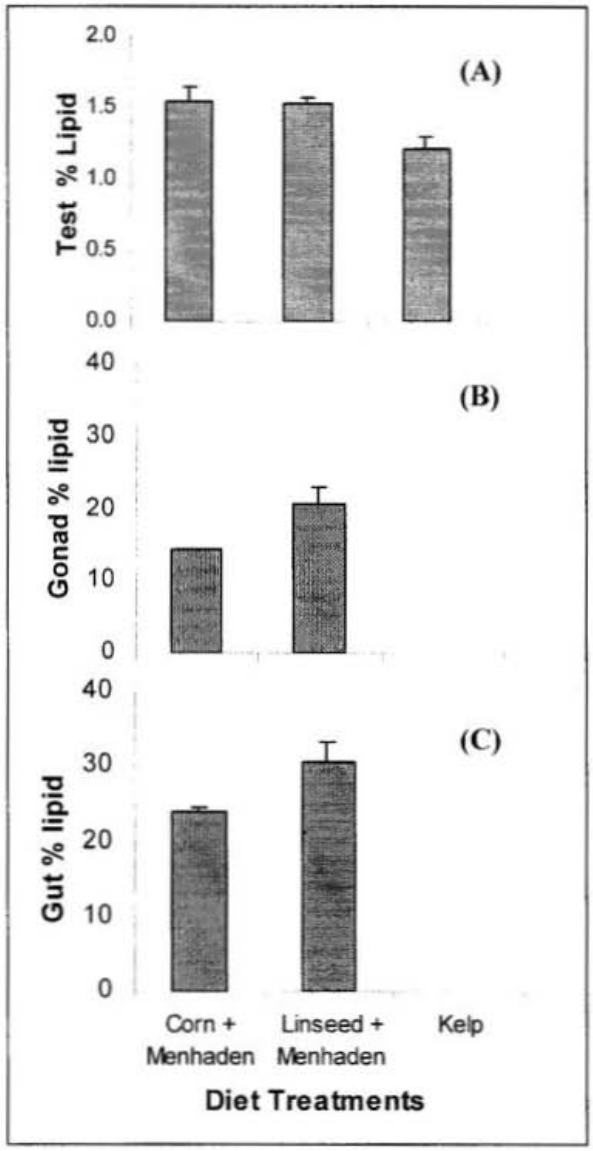

Figure 3.13. Average percent lipid in the test (A), gonad (B), and gut (C) of cohort 2 juvenile green sea urchins (*Strongylocentrotus droebachiensis*) (n=3) fed either prepared diets (with corn oil + menhaden oil or linseed oil + menhaden oil) or kelp (*Laminaria longicruris*). Bars represent one standard error. (Note: Gonad and gut lipid data for the kelp fed juveniles were unavailable)

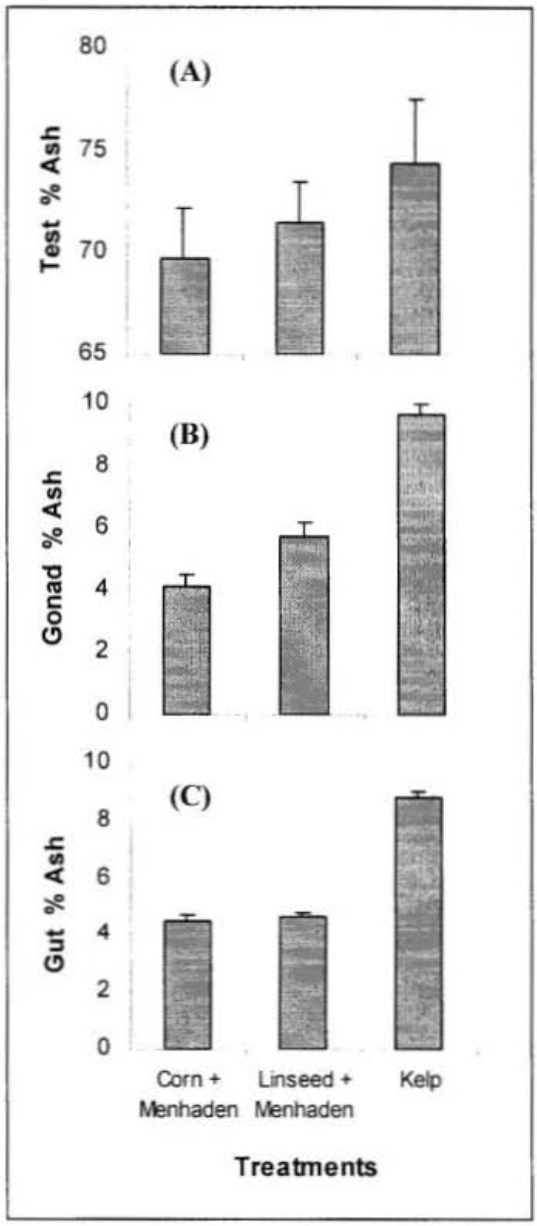

Figure 3.14. Average percent ash in the test (A), gonad (B), and gut (C) of cohort 2 juvenile green sea urchins (*Strongylocentrotus droebachiensis*) (n=3) fed either prepared diets (with corn oil + menhaden oil or linseed oil + menhaden oil) or kelp (*Laminaria longicruris*) reference diet. Bars represent one standard error.

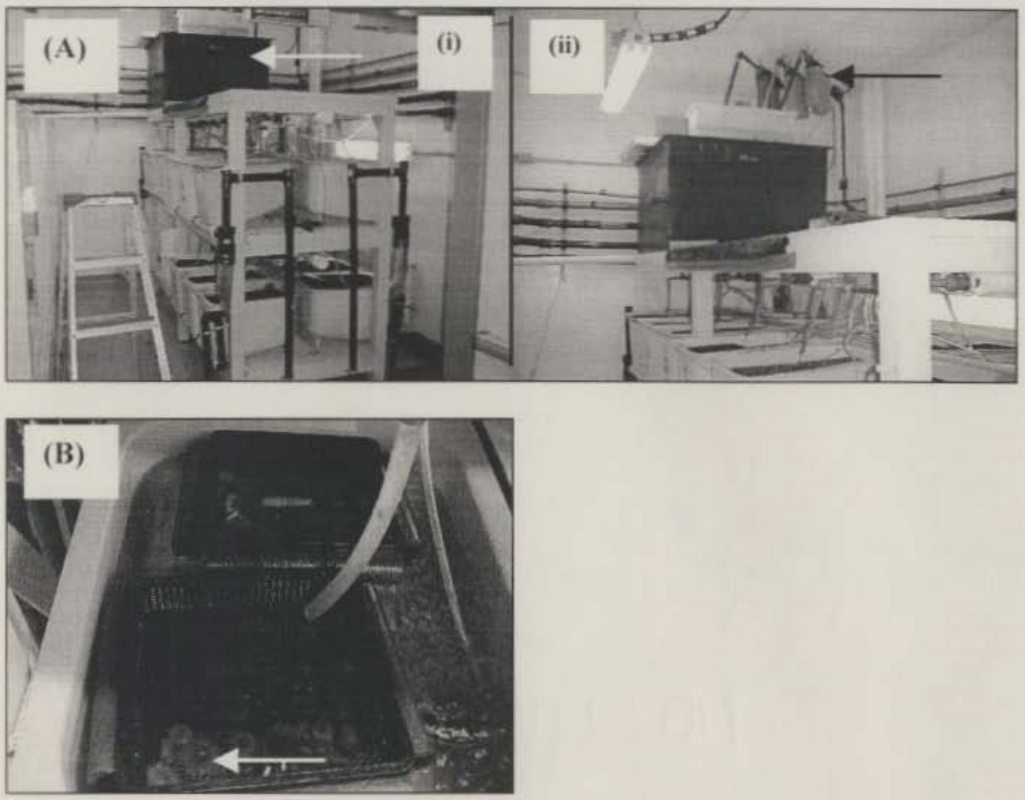

Figure 4.1. The laboratory set-up used to test the effect of minerals and pigments in prepared diets on the somatic growth of juvenile green sea urchins (*Strongylocentrotus droebachiensis*). Figure A(i) shows the experimental tanks with the header tank (arrow) at the top, while figure A(ii) shows the in-line cartridge filters (arrow) that filter the water before it enters the header tank. Figure B shows an experimental tank with two baskets, separate water inflow, and aeration. Sea urchins can be seen in the baskets (arrow).

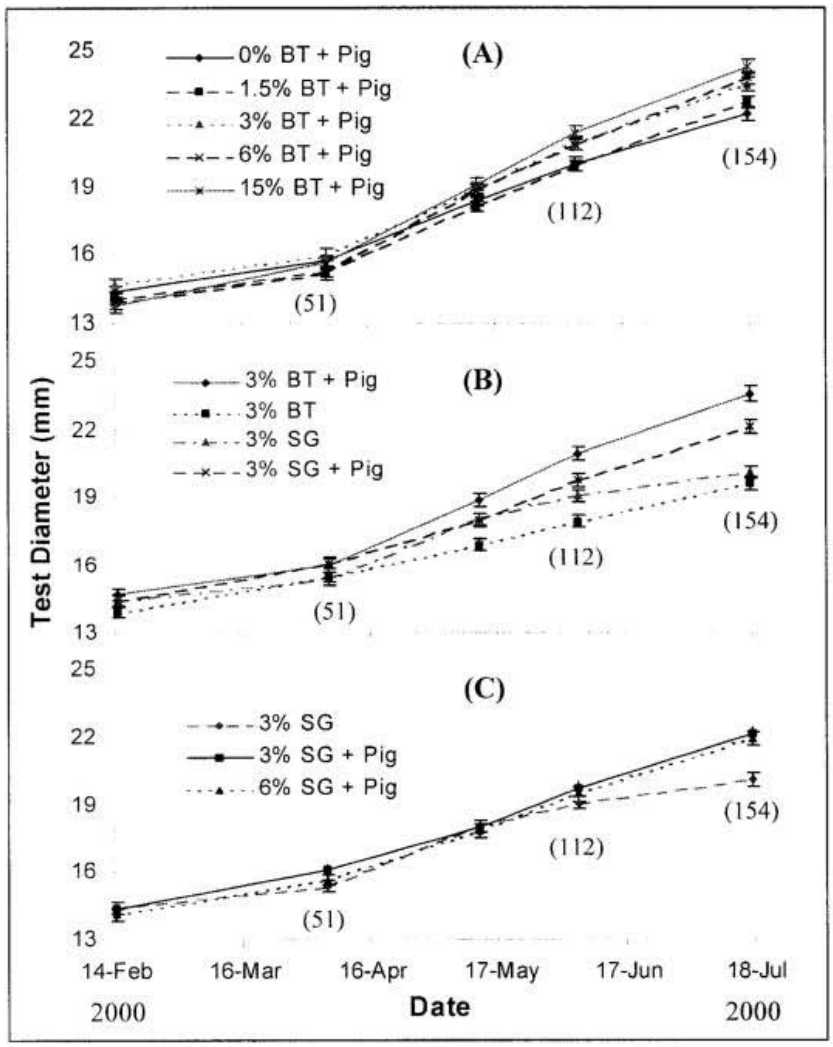

Figure 4.2. Somatic growth of juvenile sea urchins (*Strongylocentrotus droebachiensis*) (n=120) fed prepared diets from February 14 to July 17, 2000. In Figure A, pigmented diets [Pig] differed in modified Bernhart-Tomerelli salt mix [BT] concentration. In Figure B, diets differed in mineral source (BT or Shur-Gain mineral mix [SG]) and Pig. In Figure C, diets differed in SG concentration and Pig. Numbers in brackets represent consecutive days into the experiment. Vertical bars represent +/- one standard error.

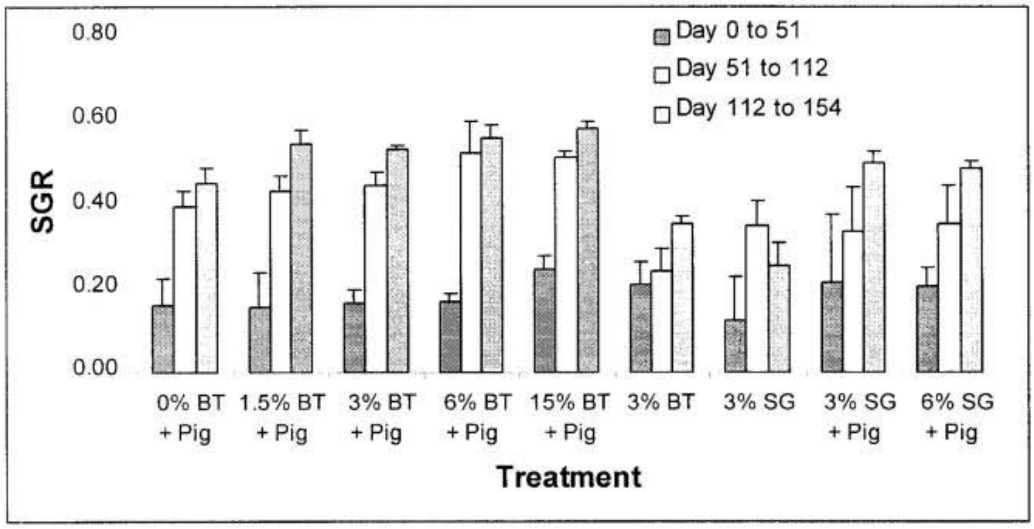

Figure 4.3. Specific growth rates (SGR) of juvenile green sea urchins (*Strongylocentrotus droebachiensis*) fed prepared diets that differed in mineral source (i.e., either modified Bernhart-Tomerelli salt mix [BT] or Shur-Gain mineral mix [SG]), mineral concentration (0% to 15%), and pigment (250 mg β-carotene/kg dry diet [Pig]) (n=4) from day 0 to 51, day 51 to 112, and day 112 to 154 of the feeding trial. Bars represent one standard error.

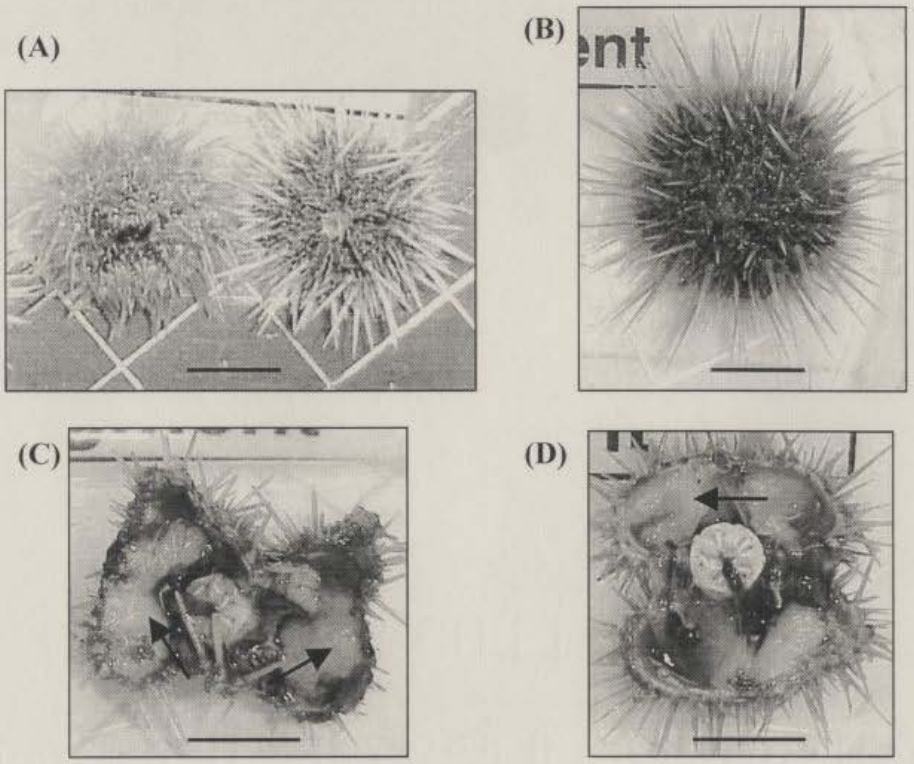

Figure 4.4. External (A & B) and internal (C & D) appearances of juvenile green sea urchins fed either non-pigmented (A & C) or pigmented (B & D) prepared diets that contained 3% modified Bernhart-Tomerelli salt mix. The juveniles fed the non-pigmented diet have pale tests, short spines, and white gonads (arrow). The juveniles fed the pigmented diet have dark green tests, long spines, and orange/yellow gonads (arrow). Scale bar represents 10 mm.

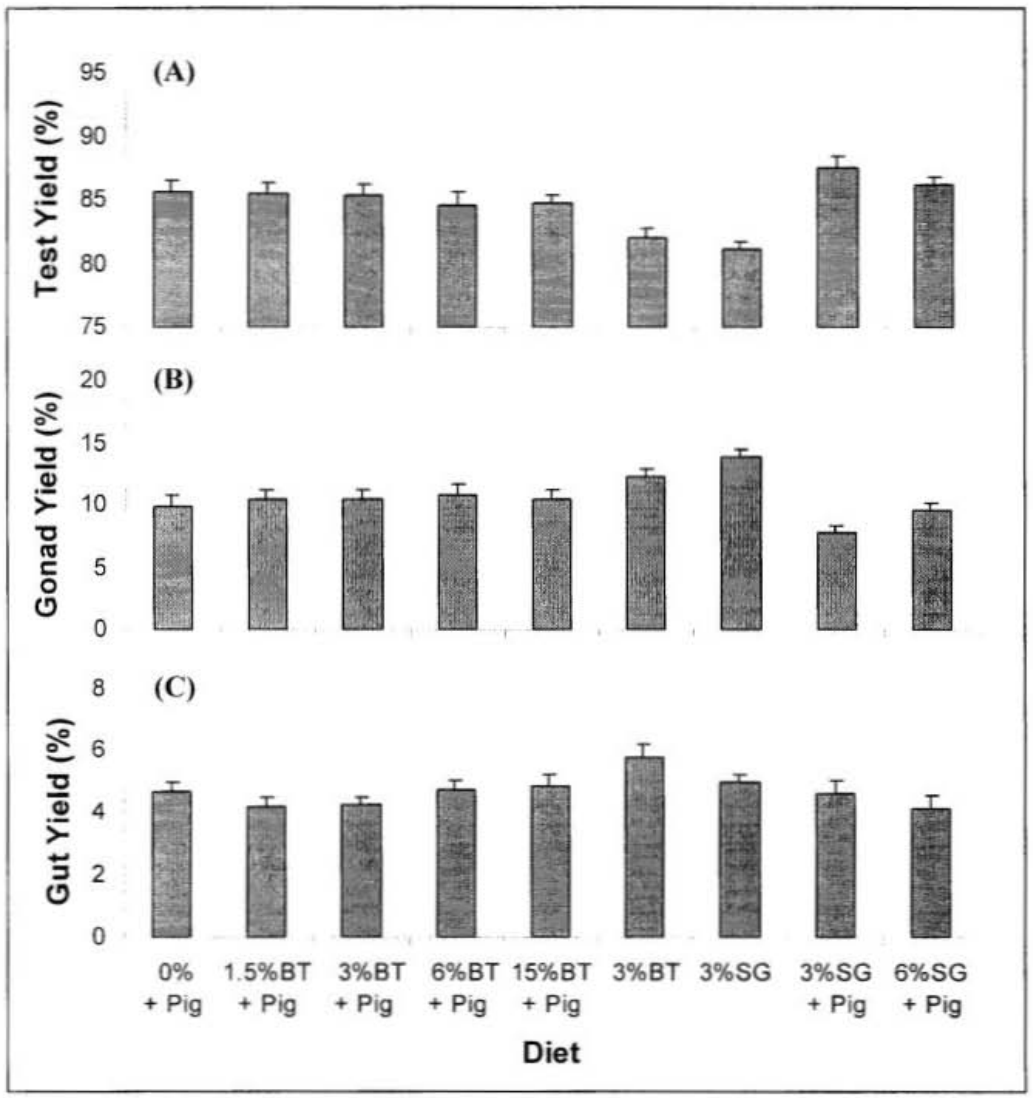

Figure 4.5. Test (A), gonad (B), and gut (C) yields from dried tissues of juvenile green sea urchins (*Strongylocentrotus droebachiensis*) (n=20) fed prepared diets that differed in mineral source (i.e., either modified Bernhart-Tomerelli salt mix [BT] or Shur-Gain mineral mix [SG]), mineral concentration (0% to 15%), and pigment (250 mg β-carotene/kg dry diet [Pig]) over 154 days. Bars represent one standard error.

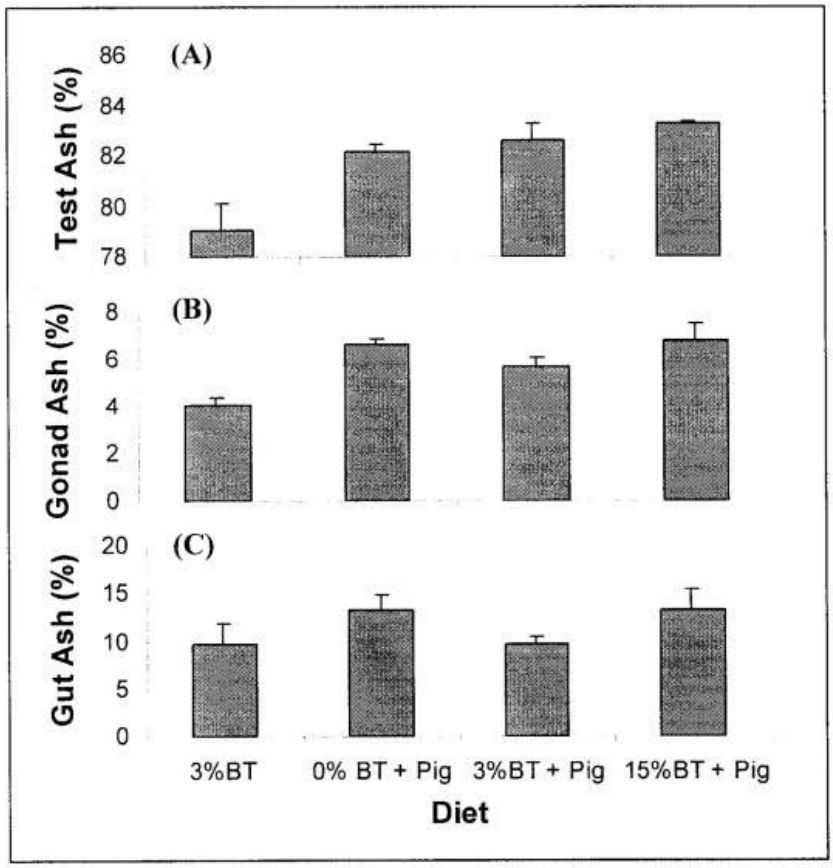

Figure 4.6. Average percent ash in the test (A), gonad (B), and gut (C) tissues of juvenile green sea urchins (*Strongylocentrotus droebachiensis*) (n=4) fed either pigmented (250 mg β-carotene/kg dry diet [Pig]) prepared diets (that contained modified Bernhart-Tomerelli salt mix [BT] at 0%, 3%, and 15% concentration) or a non-pigmented prepared diet (that contained BT at 3%) over 154 days. Bars represent one standard error.

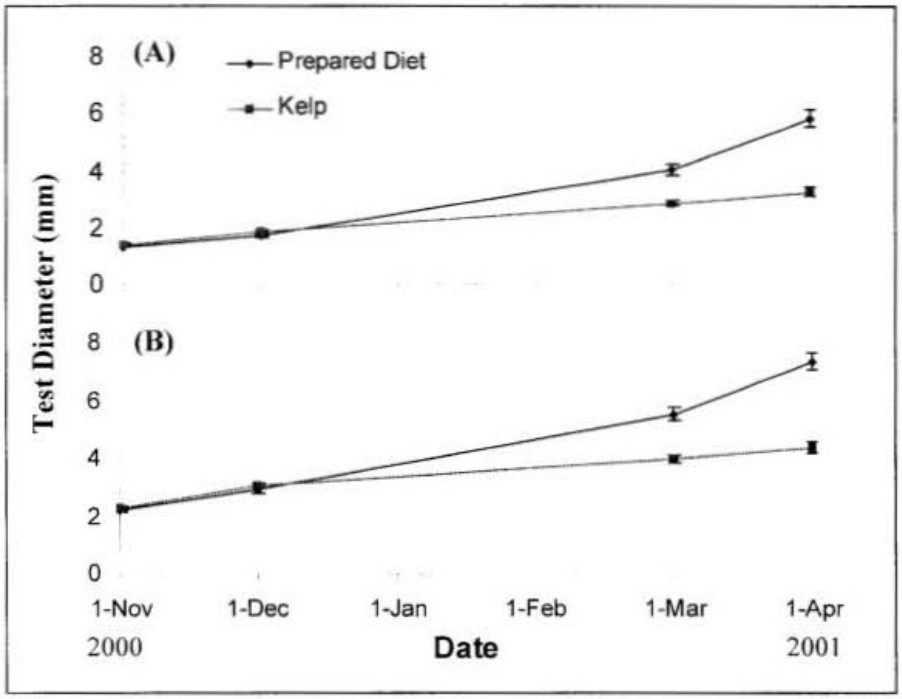

Figure 4.7. Somatic growth of hatchery-reared juvenile green sea urchins (*Strongylocentrotus droebachiensis*) (n=120) from cohort 1 (A) or cohort 2 (B) fed either the prepared diet (pigmented with 15% Bernhart-Tomerelli salt mix) or kelp (*Laminaria longicruris*) over 159 days. Numbers in brackets represent consecutive days into the experiment. Vertical bars represent +/- one standard error.

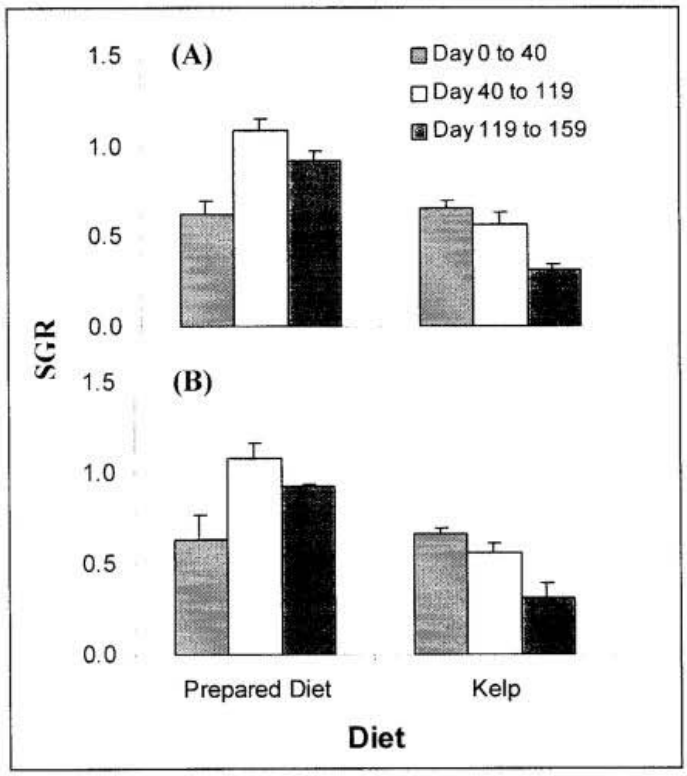

Figure 4.8. Specific growth rates (SGR) of hatchery-reared juvenile green sea urchins (*Strongylocentrotus droebachiensis*) from cohort 1 (A) and cohort 2 (B) fed either the prepared diet (pigmented with 15% Bernhart-Tomerelli salt mix) or kelp (*Laminaria longicruris*) from day 0 to 40, day 40 to 119, and day 119 to 159 of the feeding trial. Bars represent one standard error.

Appendices

Appendix 1. The step-by-step lipid extraction method used to determine the lipid content in diet and tissue samples.

1. Weigh dry sample and transfer to 20 ml culture tube
2. Add 5 ml chloroform:methanol (2:1) and homogenise using a homogenizer
3. Transfer homogenate to 15 ml screw cap test tube
4. Rinse culture tube with 5 ml chloroform:methanol (2:1) and add to 15 ml screw cap test tube
5. Vacuum filter through Buchner funnel into 50 ml screw cap test tube
6. Rinse filter paper with 5 ml chloroform:methanol (2:1)
7. Add 3.75 ml 0.88% (w/w) potassium chloride solution to 50 ml culture tube and shake
8. Allow separation of layers (lipid and aqueous layers)
9. Pipette off the top aqueous layer (H_2O and methanol) and discard
10. Add ~ ½ inch of sodium sulphate (anhydrous) to bottom layer (chloroform + lipid) and shake
11. Filter lipid layer through pipette packed with glass wool and sodium sulfate into a 15 ml screw cap test tube
12. Rinse sodium sulfate (2x) with ~5 ml chloroform
13. Evaporate under a stream of nitrogen gas until a couple of ml of lipid remain
14. Transfer by pipette to a pre-weighed 4 ml glass vial
15. Follow with 3 rinses of chloroform
16. Evaporate under a stream of nitrogen to dryness
17. Transfer tubes to vacuum dessicator for 10 minutes
18. Weigh lipid in vial using analytical balance
19. Add 4 ml chloroform:methanol (2:1) flush with nitrogen and seal with a Teflon lined cap for storage.