QUALITY CHARACTERISTICS OF GREEN SEA URCHIN (*Strongylocentrotus droebachiensis*) GONADS AS AFFECTED BY THE SEASON AND DIETARY FACTORS

CHANDRIKA M. LIYANAPATHIRANA

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BY

©CHANDRIKA M. LIYANAPATHIRANA

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Department of Biology

Memorial University of Newfoundland

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TO MY MOTHER

AND

TO THE MEMORY OF MY FATHER

ABSTRACT

The eating quality of sea urchin gonads from *Strongylocentrotus droebachiensis* harvested in the Newfoundland coasts as affected by the season and an artificial diet was assessed. Evaluations were performed on the basis of biochemical and fatty acid composition, total and free amino acid contents, and contents of nucleic acids and pigments, among others.

Noticeable changes occurred in the biochemical composition of wild and cultured sea urchin gonads. Moisture and lipid contents showed an inverse relationship in all samples examined. Sea urchin gonads had the highest amount of lipid and the lowest amount of moisture in the spring. Lipid content of cultured sea urchin gonads decreased with increased feeding time while the moisture content increased. Protein content, on the other hand, was highest in the fall in the wild sea urchins. The corresponding value for cultured sea urchins was observed 9 weeks after feeding on an artificial diet. Relative proportions of lipid and protein were quite low in the wild as well as cultured sea urchin viscera.

There were no qualitative differences in the lipid class composition between gonads and viscera of wild and cultured sea urchins. Major non-polar lipid classes were triacylglycerol (TAG), free fatty acids (FFA) and sterol (ST) while major polar lipid classes were phosphatidylcholine (PC) and phosphatidylethanolamine (PE). Triacylglycerol content was highest in the spring and fall in gonads and viscera, respectively. On the other hand, TAG content in cultured sea urchins was increased

with length of feeding period. Phosphatidylcholine was the dominant polar lipid class in wild sea urchin tissues. However, PE became dominant in cultured urchins on week 9 after feeding on an artificial diet.

The fatty acids 14:0 and 16:0 were the major saturated fatty acids (SFA) consistently present in total, polar and non-polar lipids of sea urchin gonads and viscera irrespective of their origin while 20:4n-6 and 20:5n-3 were the major polyunsaturated fatty acids (PUFA). However, in cultured sea urchins 18:2n-6 became dominant with increasing feeding period with a concurrent decrease in the content of 20:5n-3. The fatty acid 20:1n-15 was the dominant monounsaturated fatty acid (MUFA) in wild sea urchin tissues. The content of PUFA increased during cold climatic conditions with a simultaneous decrease in the content of SFA. These changes were reversed during warm seasons. In cultured sea urchins PUFA content increased with increasing feeding period with a concurrent decrease in SFA content.

Glycine was the dominant amino acid in the total amino acids (TAA) of wild sea urchin gonads and viscera. On the contrary, TAA of cultured sea urchin gonads was dominated by tyrosine on week 9 after feeding on the artificial diet. However, there were no qualitative differences in the TAA between wild sea urchins and their cultured counterparts. Glycine was also the dominant amino acid in the free amino acid (FAA) profiles of both wild and cultured sea urchins. The relative content of glycine was highest in the spring in both gonads and viscera of wild sea urchins. This was reduced considerably with increased feeding period in cultured sea urchins. There was a marked increase in the total FAA content of cultured sea urchin gonads and

viscera. Furthermore, the content of deoxyribonucleic acid (DNA) was higher than that of ribonucleic acid (RNA) in wild as well as cultured sea urchin tissues.

Total carotenoid content was highest in the spring in the gonads of wild sea urchins. However, this was reduced to a minimum in the viscera in the spring. Further, total carotenoid content was decreased in cultured sea urchin gonads and viscera with increased feeding period. Major carotenoids were echininone and fucoxanthin in gonads and viscera, respectively, irrespective of their origin. The relative proportion of echininone was highest in the spring and was lowest in the summer in the wild sea urchin gonads. In addition, β-carotene was present in all samples examined. In wild sea urchin gonads a maximum amount of β-carotene was detected in the summer. In cultured urchins the relative proportion of echininone increased with length of feeding period with a simultaneous reduction in β-carotene levels.

The study reported here thus demonstrates the importance of seasonal variations and feed supply on the quality characteristics of sea urchins. A number of biochemical indices will be examined to ascertain the eating quality of sea urchin gonads.

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LIST OF ABBREVIATIONS

AA	- Arachidonic acid
ACS	- American Chemical Society
AOAC	- American Oil Chemists' Society
CE	- Cholesterol ester
CL	- Cardiolipin
DAG	- Diacylglycerol
DNA	- Deoxyribonucleic acid
EPA	- Eicosapentaenoic acid
FAA	- Free amino acids
FAME	- Fatty acid methyl ester
FAO	- Food and Agricultural Organization
FFA	- Free fatty acids
Fig.	- Figure
GC	- Gas chromatography
HPLC	- High performance liquid chromatography
LA	- Linoleic acid
LPC	- Lysophosphatidylcholine
LPE	- Lysophosphatidylethanolamine
MAG	- Monoacylglycerol
MUFA	- Monounsaturated fatty acid

n-3	- Omega-3
n-6	- Omega-6
NMID	- Non-methylene interrupted diene
PC	- Phosphatidylcholine
PCA	- Perchloric acid
PE	- Phosphatidylethanolamine
PI	- Phosphatidylinositol
PS	- Phosphatidylserine
PUFA	- Polyunsaturated fatty acid
RNA	- Ribonucleic acid
SFA	- Saturated fatty acid
SM	- Sphingomyelin
ST	- Sterol
SURF	- Sea Urchin Research Facility
TAA	- Total amino acids
TAG	- Triacylglycerol
TBHQ	- t-Butylhydroquinone
TLC	- Thin layer chromatography
TLC-FID	- Thin layer chromatography-flame ionization detection
U/S	- Unsaturated/ Saturated fatty acid ratio
UV	- Ultraviolet
VLDL	- Very low density lipoprotein

CHAPTER 1

INTRODUCTION

1.1 Background

Sea urchin gonads, also known as roe or uni, are a highly valued food commodity in both the far east and Europe (Robbins and McKeever, 1990) where they are considered as delicacies (Anonymous 1989). Sea urchins are prized for their yellow gonad sacs which have a caviar-like appearance and a bitter-sweet flavour (Lignell, 1990). Sea urchin gonads are consumed raw in "sushi" or as "sashimi" and are one of the most popular and also most expensive items in sushi bars (Anonymous 1990a; Robbins, 1991).

Japanese and Koreans are the largest consumers of sea urchin gonads. In France sea urchins are also consumed as a delicacy to a considerable extent. Japan harvests and processes more gonads than any other country, but the demand is so high that it must also import to address its demands. Because of their commanding position in the marketplace, standards of quality and price are largely determined by the Japanese (Bruce, 1988; Anonymous 1995).

There are many grades of sea urchin gonads extracted from various species recognized in the Japanese market based on their size and quality (Anonymous 1990b, 1997). The quality of the gonads is basically assessed by evaluating colour, taste, texture and freshness (Anonymous 1990b). The success of a sea urchin industry is directly related to the quality of the gonads. Every level of the industry from

harvesting to the actual sale in Japan must reflect a sense of that quality (Anonymous 1991b).

Sea urchin gonads are harvested in many parts of the world. The Japanese market is the destination of most sea urchin products, both live and processed (Anonymous 1989, 1990a; Hooper and Cuthbert, 1994). Since there is an increasing demand for sea urchin gonads in Japan and elsewhere in the world, development of a sustainable sea urchin fishery in Newfoundland may be economically feasible (Anonymous 1997; Greenland, 1999). However, the harsh climatic conditions existing on the coastline of the province and the complexities of handling marketable sea urchin gonads have made this developing industry a challenging one (Anonymous 1997). On the other hand, due to the low yield and problems associated with the quality of wild populations an industry based on green sea urchin fishery has not yet been developed. Thus, research in the aquaculture field to develop methods to culture sea urchins in order to increase their gonad yield and quality is of paramount importance (Greenland, 1999).

The most common sea urchin, which is widely distributed in Newfoundland waters, is the green sea urchin *Strongylocentrotus droebachiensis*. Although they are abundantly distributed in the proximity of the coasts of Newfoundland, green sea urchins are underutilized (Anonymous 1990b, 1995). They occur from the intertidal zone through a depth of approximately 1 m to about 50 m (Anonymous 1995). In fact, green sea urchins are the dominant sublittoral herbivores along much of Newfoundland coasts (Keats *et al.*, 1983). Hence, there is a potential to develop a sea

urchin industry in Newfoundland in order to fulfil the continuing demand for sea urchin gonads (Dooley, 1994).

Generally, the culture of sea urchins depends on the availability of a proper commercially produced feed that is as successful as the sea urchin's preferred food. Hence, to develop an economically viable sea urchin industry it is important to evaluate the effect of feed on gonad indices and yield of sea urchin gonads (Butt, 1992). The naturally preferred diet of sea urchins is wild kelp so that an economical artificial feed that matches the nutrition of wild kelp will be required to fully expand the aquaculture of sea urchins (Lewis, 1999).

1.2 Objectives

The purpose of this study was to examine the eating quality of sea urchin gonads from *Strongylocentrotus droebachiensis* harvested in the Newfoundland coasts as affected by season and dietary factors. In order to achieve these objectives the study was focused on the following. Firstly, the seasonal effects on biochemical composition of sea urchin gonads from a wild stock were determined. In addition, seasonal changes on the fatty acid distribution, lipid classes, total and free amino acids, nucleic acids and pigments were investigated. Finally, the effect of a formulated diet based on soybean meal as a protein source over a period of 9 weeks on the same factors was evaluated.

CHAPTER 2

LITERATURE REVIEW

2.1 Biology of sea urchins

Sea urchins belong to the marine invertebrate phylum Echinodermata or spiny-skinned animals. These relatively small echinoderms have spherical bodies enclosed in a hard shell or "test" completely covered with numerous sharp spines (Anonymous 1991a). Sea urchins are omnivorous animals that live on the ocean floor feeding on small crustaceans and fish offal, but mainly seaweeds (Smith, 1980). Kelp is the main feed consumed by sea urchins so that their quality depends, to a certain extent, on the quality of the kelp in the harvesting areas (Anonymous 1990a). Sea urchins are normally found in aggregations and their combined feeding activities may remove all large plant materials from the rocks, resulting in barren patches along the coast (Wharton and Mann, 1981). It is known that the removal of urchins results in an increase in diversity and biomass of benthic algae and an increased abundance of some other herbivores (Keats *et al.*, 1983). It is also known that in areas where sea urchin densities are high macroalgal biomass is low or non-coralline algae are present (Himmelman, 1969). Sea urchins have only a few predators and are readily eaten by some sea stars and crabs. On the other hand, large adults appear to be less susceptible to predation by virtue of their size. However, even the largest sea urchins are eaten by marine mammals such as sea otters in the Pacific Ocean (Anonymous 1991a).

The green sea urchin *S. droebachinensis* occurs in cool, temperate waters in both the Pacific and Atlantic oceans. They occur intertidally and to depths of about 140 m, generally on rocky gravel or shell substrates (Miller and Mann, 1973). Due to decreasing amounts of sunlight in deeper areas of the ocean, less food is available for those urchins growing at the greater depths. Hence, there is a greater tendency to find quality sea urchins in shallow depths (Anonymous 1991b). Green sea urchin growth rates vary considerably depending on the availability of food (Foreman and Lindstrom, 1974). It takes about four years for a green sea urchin to reach a test diameter of 55 mm (Munk, 1992).

Food sources are extremely varied from one area to another and a high percentage yield of gonads is attributed to a good intake of food. An urchin feeding on good sources of food may bulk up fast while those with constant but small quantities of food may bulk up slowly. A good source of food may bulk up a sea urchin in three months or less while poor food sources might take the better part of the year (Anonymous 1991b). In addition to food quantity and quality, the growth of echinoids depends upon several abiotic factors such as temperature and photoperiod (Kenner, 1992; Guillou and Michel, 1994; Pearse *et al.*, 1995). In temperate waters growth is seasonal and is characterized by an increased growth rate during the spring and summer and a decreased growth in the fall and winter, thus reflecting the higher temperature during the spring and summer compared to that of fall and winter (Guillou and Michel, 1994).

S. droebachiensis has an annual reproductive cycle with major spawning period in late winter or early spring (Himmelman, 1978; Keats *et al.*, 1984; Munk, 1992). However, some spawning also has been observed in summer and fall off Newfoundland (Keats *et al.*, 1987). Numerous studies have shown that food quantity and quality strongly influence reproduction of *S. droebachiensis* and other sea urchins (Larson *et al.*, 1980). Phytoplankton blooms are increased in the spring and these induce the spawning in sea urchins (Starr *et al.*, 1993). Thus, growth and spawning in strongylocentroid sea urchins are directly related to the quantity and quality of available food to the larvae (Larson *et al.*, 1980; Thompson, 1982). Nevertheless, *S. droebachiensis* has an annual reproductive cycle that is synchronous across sites and habitats and between females and males (Meidel and Scheibling, 1998).

The product from sea urchins is their reproductive organs; ovaries or testes (Anonymous 1991). The gonad yield from sea urchins may vary with the time and the site of harvesting and generally ranges from 8 to 20% (Anonymous 1990a). Sea urchin gonads are considered mature and suitable for harvesting from mid November to March/ April when spawning occurs (Anonymous 1990b). In general, the gonad quality decreases after May as urchins begin to spawn (Anonymous 1991). The amount of gonad per urchin is generally greater in those with a larger body size. However, the apparent indicator of the best gonad yield is dictated by the month of harvest in the year (Smith, 1980).

Sea urchins may be harvested in a variety of ways such as by diving, dip nets, baited whelk pots, modified whelk pots or ring traps and by dragging. Among these

methods diving is most successful and is used worldwide. This method allows the selection of appropriate specimens (Anonymous 1990a, 1990b). The most common method of harvesting sea urchins in North America is diving. The sea urchin drag is one of the most effective means of harvest with some limitations; the drawback is mainly attributed to its non-selective nature. Also the drag cannot be operated in kelp beds as it tends to fill with kelp that reduces its fishing efficiency (Anonymous 1995). Thus, the least preferred method is dragging since this may cause damage not only the sea urchins but also the sea beds (Anonymous 1990b).

The green sea urchin *S. droebachiensis* is a small green, spiny echinoderm containing five gonad sacs. The gonad sacs are usually marketable when the yield is between 9 and 14%; the gonad colour varies from bright yellow to orange and the texture is firm. The quality of the gonad is determined by the feed on which urchins graze. *Laminaria* kelps are the preferred feed of sea urchins. It is known that spawning generally occurs in the early spring. In most areas the gonad is most marketable over the winter and in the early spring so that harvesting activities are usually concentrated over that time (Anonymous 1997).

2.2 Different varieties of sea urchins consumed

Keesing and Hall (1998) reported that there are at least 16 species of sea urchins harvested for food worldwide. However, the major species in Chile is *Loxechinus albus*, while *Strongylocentrotus intermedius* and *Strongylocentrotus nudus* are abundant in Japan. On the other hand *S. franciscanus* is abundant in the

Pacific while *S. droebachiensis* is common in the Atlantic region. Masson *et al.* (1988) reported that *L. albus* gonads are consumed in Chile as a customary food. The green sea urchin *S. droebachiensis* produces high quality gonads when the availability of fresh kelp is adequate (Kramer and Nordin, 1979). It is known that the edible green sea urchin *S. droebachiensis* is abundantly distributed in the North Atlantic, Arctic and North Pacific Oceans (Takagi *et al.*, 1980; Walker and Lesser, 1998). This species is currently exploited in the Northwest Atlantic and in the Northeast Pacific and the Northeast Atlantic to a lesser extent. Furthermore, *S. droebachiensis* is a target species for the development of commercial echiniculture (Hagen, 1996).

Two species of strongylocentroids are found in the near-shore coastal waters of California, the red sea urchin *S. franciscanus*, and purple sea urchin *S. purpuratus*. Both species are fished commercially for their high valued gonads although the proportion of purple urchin harvested is much less than that of the red sea urchin (Kalvass and Hendrix, 1997). Three species of edible sea urchins are known to occur in the coastal waters of the Shetland Island. The most common species is the common sea urchin *Echinus esculentus* which is abundant from the lower shore down to a depth of 50 m or more. In addition, there are the shore sea urchins *Psammechinus miliaris* and the northern sea urchin *Strongylocentrotus droebachiensis* that are much rarer than *E. esculentus* in Shetland waters (Penfold *et al.*, 1996). *Paracentrotus lividus* is another edible sea urchin species fished in the European coastal waters (Pearse and Cameron, 1991). Gonads of sea urchin species such as *Pseudocentrotus depressuss* and *Hemicentrotus pulcherrimus* are said to be of very good quality with

respect to their taste (James, 1990) and are known to be found commonly in Japan (Agatsuma, 1998). In addition, *Temnopleurus toreumaticus* (Yasuda and Fukamiya, 1977), *Mespilia globulus* and *Anthocidaris crassispina* (Kochi, 1976; Yasuda and Fukamiya, 1977) are also common in Japanese waters. There are some common edible strongylocentroids that are fished off in the waters of Japan. They include *S. pulcherrimus* (Kochi, 1968), *S. intermedius* (Chelomin and Svetashev, 1978; Kozhina *et al.*, 1978) and *S. nudus* (Kochi, 1968). *A. crassispina* and *P. depressus* are also known to be abundant in Korea (Kochi, 1976). The tropical edible white sea urchin *Tripneustes gratilla* is also one of the biggest and fastest growing sea urchins and is widely distributed in Japanese waters (Floreto *et al.*, 1996a; Agatsuma, 1998).

2.3 Sea urchin industry

Total annual world production of wholesale live weight sea urchin averages about 117,000 tons (Table 2.1) for 1995 (FAO, 1995). Hagen (1996) reported that prices for sea urchin gonads have reached up to 14,000 yen per kg. Thus, sea urchin gonad industry is a multimillion dollar a year industry for many countries around the world (Dooley, 1994). The major supply sources of sea urchin gonads are the United States of America, Chile, Korea and Canada, among other countries (Anonymous 1990a; Bruce, 1988). In both Canada and United States fresh sea urchin gonads are obtained primarily from the West coast (Anonymous 1990a). It has been reported that Chile, once the world's largest producer of sea urchins, is in a strong decline for its sea urchin production since the early 90s and this parallels production in Japan,

Table 2.1 World sea urchin landings in 1995.

Country	Weight (Tons)
Chile	54.609
USA	27.842
Japan	13.735
Canada	9178
Mexico	3000
South Korea	3707
Russia	2344
Iceland	923
New Zealand	804
Philippines	466
North Korea	150
China	150
Peru	131
Australia	93
France	79
Taiwan	63
Fiji	59
Martinique	15
Ireland	10
Total production	117.168

Adapted from FAO, 1995.

Northern California, Oregon and South Korea. On the other hand, Russia, China, North Korea and Norway are countries where they grow sea urchins. Furthermore, Southern California has a highly productive sea urchin resource based on strong recruitment and a good variety of sizes due to oceanographic conditions, currents and offshore transport, among other features (Anonymous 1993). In the last decade Canada has become a major supplier of sea urchin gonads to the Japanese market (Anonymous 1990a). At present the Atlantic sea urchin culture industry involves sea urchin gonad enhancement programmes where animals harvested from the wild are held in shallow raceways with adequate food supplies to enhance gonad quality and quantity (Hooper *et al.*, 1993; Cuthbert *et al.*, 1995).

It is proposed that proper sea urchin management strategies are important in developing ways and means of sustaining the industry. Thus, fishing practices to enhance the quality of the harvested product, methods by which production could reflect prices in the Japanese market in order to increase the value of the catch and methods to improve safety standards in the industry are considered essential (Anonymous 1993). On the other hand, practices such as closing the sea urchin fishery during summer prevents harvest of low quality post-spawned animals (Anonymous 1991).

Sea urchin fisheries are characterized by a lack of life history and biomass data which makes it difficult to develop a focused and sustainable fishery. However, emphasis has been placed on developing an industry which is defined by resource-

driven principles for managing the fishery rather than market-driven principles (Anonymous 1993).

A number of purely biological studies have been undertaken on sea urchin populations and these have limited applications to fisheries potential (Hooper and Cuthbert, 1994). In order to develop a sustainable sea urchin industry studies should be done to assess the percentage yield over a range of depths, time, temperature and food availability under the maximum yield and highest quality gonads, among others (Smith, 1980). Also more studies should be performed to evaluate the efficiency of certain feed formulations that produce good quality sea urchin gonads (Lewis, 1999).

With a decline in the availability of sea urchins in traditionally major producing countries, several countries with under-exploited sea urchin resources may be considered, but these are insignificant in terms of quantity to address world demand. The Russian Federation, Canada and North and South Korea are the only countries with significant potential for expansion of sea urchin fisheries (Keesing and Hall, 1998).

Despite a reduction in its resources, Chile remains the world's largest producer of sea urchins (*L. albus*) with landings of 54,609 tons in 1995 (FAO, 1995). The State of Maine on the eastern coast produces the highest harvest in the USA. Sea urchin (*S. droebachiensis*) harvests increased rapidly from 653 tons in 1987 to a peak of 19,115 tons in 1993 followed by a decline to less than 16,000 tons in 1997 (Keesing and Hall, 1998). In 1995 Canadian sea urchin catches in the Northwest

Atlantic were 2,850 tons (FAO, 1995) having increased from 109 tons in 1990 (Keesing and Hall, 1998).

It is known that the North American Pacific coast sea urchin fisheries are based principally on the red and green sea urchin *S. franciscanus* and *S. droebachiensis*, respectively. However, small amounts of purple sea urchin (*S. purpuratus*) are also caught. The combined catch from British Colombia in Canada and Alaska, Washington, Oregon and California in the US make up a very significant portion of the world sea urchin catch, producing over 18,000 tons in 1995. Maine fishery along with others mentioned above contribute over half of all sea urchin imports into Japan (Keesing and Hall, 1998). Kalvass and Hendrix (1997) reported that in California the sea urchin fishery is stable around 8,000 to 10,000 tons mark since 1985. FAO (1995) statistics also show modest harvests of sea urchins from Korea, China, Vietnam and Philippines, among other countries. Although the United States provides over 50% of the Japanese imports of sea urchins, declines in California and Maine catches are likely to have a significant impact on the world market for sea urchins (FAO, 1995).

Japan was the largest producer of urchins *S. intermedius* and *S. nudus* until 1984. These two species make up approximately 80% of the total Japanese sea urchin harvest while *S. pulcherrimus*, *A. crassispina*, *P. depressus* and *T. gratilla* are responsible for the rest (Keesing and Hall, 1998). Annual production of sea urchins in Japan in 1995 was 13,735 tons (FAO, 1995), indicating a decline from a peak production of 27,525 tons in 1969 (Hagen, 1996). The catches have fluctuated

between 27,000 and 23,000 tons up to 1987 and began to decline below 20,000 tons in 1990. However, the cause of decline in the Japanese catch is not well understood, but over-fishing, habitat degradation, disease or, most likely, a combination of these may be responsible for it (Hagen, 1996). France produced over 400 tons of *P. lividus* in 1992 (FAO, 1995). In general, the most important market, Japan, imports approximately 5,000 tons of sea urchin gonads per year, equivalent to 40,000 to 50,000 tons of live sea urchins (Hagen, 1996). Thus, Japan imported 6,200 tons worth US$ 243 million in 1996 (Keesing and Hall, 1998). Hagen (1996) further reported that the Japanese consumption of whole sea urchins was approximately 60,000 tons per year. It is known that Japanese prefer red sea urchin gonads harvested in the West. In fact, gonads from the US west coast are regarded to be of good quality (Anonymous 1990a). France is the second largest consumer of sea urchins after Japan with an annual consumption of approximately 1,000 tons (Le Gall, 1990; Hagen, 1996) as whole sea urchins (Le Gall, 1990).

Depending upon the respective gastronomic cultures sea urchin gonads may be considered as either a fine delicacy or absolutely inedible. However, their economic value is well established given the price consumers are willing to pay (Grosjean *et al.*, 1998). The wholesale price of live sea urchins in France ranged from 30 to 120 French Frank per kg and the price of fresh roe in Japan varied from 6000 to 14,000 yen per kg in 1990s (Hagen, 1996). Thus, sea urchin gonad is one of the most valuable seafoods in the world. In both markets, the lowest prices are those of imported sea urchins of proper quality (Grosjean *et al.*, 1998). In 1998 sea urchin

imports into Japan from Canada reached 457 tons at a value of Canadian $ 12.9 million (Anonymous 1990a). It has been reported that in 1994, Canada ranked fourth in the world's supply of sea urchin gonads to Japan at 535 tons (Anonymous 1997).

Increasing demand for gonads and steady rise in their prices have led to worldwide intensification of sea urchin fisheries (Conand and Sloan, 1989; La Gall, 1990). By 1998 the production of sea urchins reached a maximum, but this level of production could not be sustained because the declining productivity of the existing, but over-exploited stocks could no longer be compensated for by the harvest of new stocks (Grosjean *et al.*, 1998).

In general, wild sea urchin stocks constitute a limited resource and many are in a decline as a result of over-harvesting. However, there is a continued demand for sea urchin gonads and sea urchin aquaculture and gonad enhancement may create new commercial opportunities. However, sea urchin aquaculture is still in its infancy and considerable research is needed to overcome a range of obstacles to reach cost-effective commercial production of significant volumes (Keesing and Hall, 1998).

Aquaculture of echinoderms including sea urchins and sea cucumber is known as echiniculture (Le Gall, 1990, Hagen, 1996). The term echiniculture has been preferred for describing sea urchin aquaculture exclusively though this has not yet been fully developed (Grosjean *et al.*, 1998). Both the increasing demand for gonads and systematic over-exploitation of wild populations support the need for sea urchin cultivation (Grosjean *et al.*, 1998).

In temperate ecosystems several important biologically based problems affect the large scale natural harvest of sea urchins. As natural stocks dwindle and demand remains constant or increases harvesting of smaller, poorly fed natural populations may lead to production of low quality gonads. On the other hand, large populations of urchins exist in deeper waters (below 18 m) and harvesting them by scuba divers is potentially dangerous and impractical in mid winter when urchin gonads are supposed to be most valuable. However, during winter urchins may have poor quality gonads due to limited supply of algae which is their preferred natural diet (Keats *et al.*, 1984; Briscoe and Sebens, 1988; Larson *et al.*, 1980). Furthermore, smaller natural populations reduce the reproductively effective populations and this may subsequently affect fertilization success and recruitment of juveniles while the single annual gametogenic cycle limits the season when gonads are commercially valuable (Lesser and Walker, 1998). Therefore, sea urchin aquaculture may provide high quality and high dollar value products in the absence of large annual harvests of natural populations (Lesser and Walker, 1998).

2.4 Sea urchin industry in Newfoundland

Although sporadic attempts have been made to commercialize the resource since 1969 (Himmelman, 1969) sea urchin fishery is a new and developing industry in Newfoundland. Sea urchin locations, stock distribution and breeding seasons have not been well defined and this may hinder the development of a strong industry. Most of the earlier studies were mainly based on the data available on gonad yield and colour,

population density and feeding, among others. However, from experience it is known that most urchins have mature gonads and are therefore ready to be harvested in late fall through early spring. On the other hand, during this time of the year most areas of the province have adverse climatic conditions that may affect their harvest by scuba divers (Anonymous 1995).

The development of a green sea urchin industry in Newfoundland has recently gained much attention and continuing research has led to new developments in this field. A major problem associated with the development of such an industry refers to the gonads of the existing wild sea urchins. Thus, some wild sea urchins in Newfoundland waters have found to have non-commercial gonad yield. On the other hand, the existing commercial populations produce marketable gonads only during the fall and winter limiting the production only to a short period (Cuthbert *et al.*, 1995).

In 1995 to 1997 only three million pounds of sea urchins were harvested in Newfoundland (Hooper *et al.*, 1993; Cuthbert *et al.*, 1995). Presently, the demand for sea urchin gonads is much higher than what the industry can supply. Therefore, Newfoundland has the potential and capacity to develop a sea urchin culture industry with its existing resources (Caron, 1999). Furthermore, Newfoundland has the ability and technology to ranch sea urchins in the wild. However, there are a few factors that have continually held the industry back in this province (Dooley, 1994). Both the yield and quality of sea urchin gonads harvested in Newfoundland have been inconsistent in the past and inferior gonad quality has resulted in much lower prices in the Japanese market. Thus, in 1993 sea urchin gonads of lesser quality fetched a

wholesale price of only 6000 yen per kg (Hagen, 1996). However, Anonymous (1990b) reported that Newfoundland sea urchin gonads are well accepted in the Japanese market since the overall quality of the product is similar to the Japanese product in many ways. Thus, the taste, colour, colour uniformity and gonad segment size were within acceptable limits but with a soft texture which was not appropriate (Anonymous 1990b). On the other hand, harvesting of sea urchins is cumbersome in the late winter and is costly and time consuming. Consequently, the economic viability of this industry in Newfoundland has been questioned. Furthermore, any growth of coastal communities and their influence on the adjacent aquatic environment may also affect the recruitment of juvenile sea urchins in some of these areas (Thompson, 1999).

Despite all the aforementioned, there was a considerable growth in the sea urchin industry in Newfoundland from 1992 to 1996. The volumes of sea urchin purchased were 89, 283 and 680 tons in the years 1994, 1995 and 1996, respectively. The industry in Newfoundland is still developing and offers much potential since opportunity exists to respond to the increasing market demand in Japan (Anonymous 1997).

2.5 Mass production of sea urchins

There is much potential for the aquaculture of green sea urchins *S. droebachiensis*, as it represents a very profitable industry (Cuthbert *et al.*, 1995). For instance in the province of New Brunswick the wild sea urchin industry is worth

approximately Canadian $ 4 million per year (Morgan, 1998). Atlantic sea urchin culture industry is associated with gonad enhancement strategies using sea urchins harvested from the wild. In general, wild sea urchin stocks have shown great variability in gonad quality and yield. However, ranching was successful for enhancing gonad quality and yield (Hooper *et al.*, 1993; Cuthbert *et al.*, 1995). Many attempts have been made to develop an economically feasible method of producing top quality, high gonad content sea urchins. Thus, raceway partitioning has been used as a means of increasing gonad yield and quality in the green sea urchin. It has been reported that raceway culture could offer an effective means to improve gonadosomatic indices from wild stocks (Greenland, 1999). Furthermore, studies have been performed to evaluate the productivity of raceway held green sea urchin populations by feeding different artificial diet formulations. These formulations have increased the gonad yield considerably though they have not been efficient in enhancing their quality (Davidge, 1998). Moreover, the quality of sea urchin gonads held in cages has been compared with those taken from the wild. Thus, gonads obtained from urchins held in cages were firmer and deeper in their orange colour in addition to being easier to remove intact in comparison with wild counterparts (Smith, 1980). Food quality and quantity both affect sea urchin growth (Lawrence and Lane, 1982). Food limitation slows down growth rate and can lead to reabsorption of nutrients from the body wall leading to a reduction in test diameter (Levitan, 1989; 1991). Sometimes gametogenesis and maturation are also affected under poor nutritional conditions (Lawrence and Lane, 1982).

Commercial hatchery production of sea urchins is currently practiced in Japan and France. However, very little research has been carried out on the hatchery production of green sea urchins in Canada. Therefore, the culture practices of other commercially important invertebrates especially bivalves could be applied in the development of a sea urchin hatchery (Caron, 1999).

Since there is an existing lucrative market for sea urchin gonads, from an aquacultural perspective hatchery production of sea urchins may be beneficial (Hooper *et al.*, 1993; Cuthbert *et al.*, 1995). Although, there are advancements on the gonad enhancement and culture of sea urchins, very little information is available in the development of a suitable hatchery technique to ensure a consistent and large supply of hatchery-produced sea urchins (Thompson, 1999). Most of the information on hatchery production of sea urchins has focused on their nutritional requirements. Thus, studies have been done on sea urchin diets minimising their natural diets and/or using formulated diets (de Jong-Westman *et al.*, 1995a; Hagen, 1996; Blin, 1997). These diets seem either to be too simple or too expensive to be produced on a large scale. However, studies done with other commercially important organisms such as planktonic larvae (e.g. bivalves) have shown that mixed diets offer a more natural and a more complete diet with advantages for commercial scale production of marine animals in the hatchery (Epifanio, 1979). A study done with sea urchins revealed that mixed algal diets lead to better growth rates and survival; these included diatoms and flagellates as possible feeds (de Jong-Westman *et al.*, 1995a). Furthermore, the effect of ranching commercial quantities of sea urchins in tanks and feeding them seaweeds

to produce high quality gonads has been undertaken in order to verify the commercial viability of ranching sea urchins (Anonymous 1997). Investigations have also been carried out to culture sea urchins and enhance gonads using onshore methods which may enable exploitation of this natural resource. This has led to alleviating problems with late winter harvesting (Hatcher and Hatcher, 1997).

Walker and Lesser (1998) have performed research on promoting out-of-season gametogenesis in green sea urchin *S. droebachiensis* by manipulation of food and photoperiod that has significant implications for aquaculture. Hence, this has permitted production of sea urchins with significantly higher gonadal index ($18 \pm 6\%$) compared to wild sea urchins ($11 \pm 3\%$) during the months of March, April and May. Subsequently, experimental sea urchins had a mean gonadal index of 25 to 30% while the mean gonadal index for wild sea urchins was 11 to 13%.

In general, the knowledge of when gametogenesis initiates and ends will better define when urchin gonads are of high quality for harvest. The optimal firmness of urchin gonads for the market occurs prior to the ending of gametogenesis and cannot be predicted by the gonadal index alone (Walker and Lesser, 1998).

Russell (1998) demonstrated the ability of green sea urchin to rapidly build up gonadal tissue with practical applications for the sea urchin fishery. Lawrence and Lane (1982) showed that urchins are highly sensitive to food availability in their natural environments. Larson *et al.* (1980) and Minor and Scheibling (1997) found that reproductively mature urchins taken from the wild increased their test size and gonadal index more rapidly when they were fed algae in excess. Further, Walker and

Lesser (1998) observed that when adult urchins are fed a pelletized urchin diet rather than kelp they increased gonad size significantly without an apparent increase in their test size reflecting that most of the energy was allocated for gonadal growth. This is also advantageous in an aquacultural perspective since an increase in test size might require additional tank space in an aquaculture facility. A similar increase in test size has been observed in several studies with urchins fed natural algal diets (de Jong-Westman *et al.*, 1995a; Lawrence *et al.*, 1997; Russel, 1998). Beyer *et al.* (1998) reared juvenile red sea urchins under contrasting photoperiods feeding either kelp or an artificial palletized diet for one year. The animals fed kelp grew significantly larger in continuous dark than in continuous light, but their gonads were significantly smaller. However, in urchins that were fed on artificial pelletized feed body growth almost ceased while gonads were 2 to 3 times larger than those of kelp fed animals. Furthermore, unlike the gamete-filled gonads of kelp fed urchins the gonads of pellet fed animals were firm and full of nutrient cells, with an unusual pale tan colour. Hence, these findings are not only commercially promising, but also indicate that dietary content regulates both gametogenesis and nutrient allocation between body and gonad growth with major effects on reproductive efforts.

Walker and Lesser (1998) further suggested that land-based facilities could produce high quality urchin gonads at least twice a year. It has been further shown that use of abundant food and photoperiod manipulation offer the possibilities of shortening the time to reach the market condition and of coordinating the availability of a high quality product with market demand. Hence, such aquacultural techniques

would maximise the profit and reduce harvest pressure on natural populations. Furthermore, Walker and Lesser (1998) suggested that when urchins were fed prepared food and maintained on a summer photoperiod without changing to autumn photoperiod the gonads of green sea urchins may not initiate gametogenesis and may remain large and firm so that the product is highly marketable.

2.6 Experimental feeds for sea urchin culture

Sea urchins are widely distributed throughout the world's oceans and often play a major role in controlling macroalgal populations and organizing the structure of shallow subtidal communities (Harrold and Reed, 1985). The diets of these echinoderms consist largely of seaweeds (Lawrence, 1975). They also feed on microalgae, detritus, a variety of terrestrial and marine drift items, and small invertebrates (Himmelman and Steele, 1971; Harrold and Reed, 1985; Vadas *et al.*, 1986; Briscoe and Sebens, 1988). In many regions their intensive grazing and behavioural activities create barren grounds (Hagen, 1983; Andrew and Choat, 1985; Wharton and Mann, 1981). At high densities grazing becomes intense and food availability becomes limiting thereby reducing energy potential for growth and development (Keats *et al.*, 1984). On barren grounds sea urchins produce significantly less gonads than in areas dominated by macrophytes (Lang and Mann, 1976).

It has been shown that the main determinants of gonad yield are body size (Gonor, 1972), food availability (Hooper and Cuthbert, 1994) and seasonal variation in reproductive condition (Byrne, 1990). Body size determines the maximum possible

gonad size whereas food availability and reproductive conditions determine the actual proportion of gonad biomass (Hagen, 1998).

Kelp is a preferred natural food of exploited sea urchins (Larson *et al.*, 1980). Andrew (1986) stated that habitats with an abundant supply of kelp provide the best gonad yield; but their availability is limited (Mann, 1973). Feeding trials have been conducted on barren grounds, in bottom enclosures, in suspended cages, in laboratory tanks and in land-based rearing facilities. Feeding with either fresh macroalgae or formulated diets improved gonad yield (Keats *et al.*, 1983; de Jong-Westman *et al.*, 1995a). However, quality of gonads may be adversely affected by formulated diets deficient in carotenoids (Goebel and Barker, 1998). Too much fish meal or too little kelp also has an adverse effect on gonad quality (Hooper *et al.*, 1996; Klinger *et al.*, 1998).

The use of fresh algae is not always possible or profitable on a large scale. Hence, an artificial diet designed especially for sea urchins seems necessary for intensified echiniculture (de Jong-Westman *et al.*, 1995a & 1995b). Several studies have been performed to investigate the effects of formulated diets on gonadal growth (de Jong-Westman *et al.*, 1995a; Lawrence *et al.*, 1997; Goebel and Barker, 1998); in these urchins responded by an increase in growth compared with those on natural feeds (Lawrence *et al.*, 1992; Fernandez and Caltagirone, 1990; Fernandez and Pergent, 1998; Williams and Harris, 1998). Thus, encouraging results have been observed so far especially in terms of gonadosomatic index. However, results were unsatisfactory when considering the colour and palatability of the gonads (Grosjean *et*

al., 1998). Furthermore, trials with carotenoid-enriched artificial feed did not produce high quality gonads (Goebel and Barker, 1998). Thus, better formulation of the food is essential for achieving appropriate colour and taste for exploitation of the resource (Grosjean *et al.*, 1998).

The cultured kelp *Laminaria japonica* is a valuable diet for sea urchins (Sato and Notoya, 1988). Yields of kelp are high during spring and summer and gonadal indices of sea urchins fed this kelp in excess reached 18% within two months, the minimum level required for commercial harvesting (Agatsuma, 1997). On the other hand, the gonadal indices of sea urchins at traditional fishing grounds during winter and spring are only about half the maximum level in summer (Agatsuma *et al.*, 1988).

Very little work has been performed on the nutritional requirements of herbivorous marine animals. In general, the time and costs involved in the collection or culture of large amounts of microalgae are very high. Further, supply of macroalgae can be unreliable and the environmental consequences of removing them are also a concern. Thus, the focus of research has moved to the development of convenient and nutritious artificial diets that produce high growth rates (Fleming *et al.*, 1996) . Furthermore, due to the widening gap between supply and demand in the sea urchin market, interest has been growing in the development of nutritionally balanced and effective artificial feeds for sea urchin farming. Although wild kelps are the naturally preferred diet of sea urchins (Lewis, 1999), Hooper and Cuthbert (1994) on the other hand, stated that the best gonad quality was found in sea urchins fed on several different brown seaweeds. Studies have been carried out to determine the type of algal

species that can be used for enhancing gonad yield and quality in sea urchins. Thus, Keats *et al.* (1983) reported some six species of algae that performed well in producing good gonad yields from green sea urchins.

The advantages of using formulated feeds include elimination of problems linked to food availability and nutrient content observed for natural food sources (marine algae), limitation of environmental impacts associated with utilization of these natural food sources and facilitation of storage and transport of these feeds (Fernandez and Pergent, 1998).

The strategy for developing artificial feeds for urchins is to formulate diets that induce rapid growth of tests and gonads (Fernandez and Caltagirone, 1994; de Jong-Westman *et al.*, 1995a; Lawrence *et al.*, 1997). Most feeding trials have used formulation with protein levels between 15 and 20% which are considerably higher than that found in most seaweeds (2 to 10%). Urchins fed high protein diets consistently produced higher gonad yields than urchins fed low protein or algal diets (Lawrence *et al.*, 1992; de Jong-Westman, 1995b). Recent studies have suggested that utilizing artificial diets supports the hypothesis that protein levels in seaweeds control gonad yields in sea urchins (Vadas *et al.*, 2000). Even with abalone culture it has been shown that artificial feeds containing a high protein content were quite effective in the gonadal development compared to seaweed formulations (Lopez and Tyler, 2000). However, Klinger *et al.* (1997) have shown that the sea urchin *S. droebachiensis* produced better growth when fed on artificial diets containing kelp than those without it. Greater gonadal indices have been observed in *S. droebachiensis* in kelp beds than

on barren grounds (Lang and Mann, 1976; Keats *et al.*, 1984) which is generally attributed to differences in food availability in the two habitats (Lang and Mann, 1976). Several studies have shown that laminarian kelps are a preferred food for *S. droebachiensis* and support high rates of growth and reproduction (Keats *et al.*, 1984; Minor and Scheibling, 1997). Thus, Minor and Scheibling (1997) demonstrated that a diet of laminarian kelp may markedly increase gonad yield over 12 to 24 weeks. Nabata *et al.* (1999) showed that gonadal indices of *S. nudus* were highest when they were fed laminarian kelps, confirming their nutritional wholesomeness for growth of sea urchins. Furthermore, urchins need to be fed on a high ration in the latter part of their reproductive cycle in order to maximize gonad yield and improve the cost effectiveness of culturing *S. droebachiensis* and other commercially important sea urchins.

Uemura *et al.* (1986) aquacultured sea urchins, feeding them on fish meal and found that the gonads grew rapidly but their colour and taste became poor. Subsequently algal feed was used and this led to improved gonadal quality thereafter. Similarly, the sea urchin *S. nudus* was reared on a fish meal feed and produced large gonads with approximately 20% gonadal index (Hoshikawa *et al.*, 1998). Both the flavour and taste were adversely affected by fish meal. However, the adverse effects of fish meal on gonadal quality declined gradually when urchins were fed kelp prior to harvesting. On the contrary, Lawrence *et al.* (1997) showed that gonadal growth of sea urchins was much better when they were fed on an artificial diet containing menhaden meal than their natural food, laminarian kelps. Hence, gonadosomatic

indices were much larger when the urchins were fed on a protein rich meal than an algal meal.

Nestler and Harris (1994) suggested that non-algal foods can comprise a significant part of the diet in the aquaculture of echinoids. Lawrence *et al.* (1992) showed that sea urchins exhibit a higher somatic growth rate when fed on diets containing animal-derived proteins and lipids as compared to diets composed of either macroalgal or vegetable-derived materials. However, conclusive results have not been obtained. In case of gonadal growth animal-based artificial diets brought about a significant increase in the gonad size of *Paracentrotus lividus* (Lawrence *et al.*, 1992) and *S. intermedius* (Levin and Naidenko, 1987) when compared to a vegetable-based artificial diet. Fernandez *et al.* (1995) reported that regardless of the dietary source the majority of artificial diets led to a significant increase in gonadal growth when compared to a purely macroalgal diet, thus suggesting that the algal diet is lacking certain elements necessary for rapid gonadal growth. Cook *et al.* (1998) showed that both somatic and gonadal growth in *Psammechinus miliaris* are influenced by diets based on fish compared to macroalgal diets. Salmon aquaculture feeds contain a significantly higher proportion of protein as compared to *Laminaria saccharina* and *Ulva lactuca*. There is a large difference in the total protein content in the salmon feed and macroalgal diet. This may account for the significant difference in somatic growth rates observed in *P. miliaris*. Thus, the protein content of the diet has been identified as a major factor affecting production of echinoids (Frantiz and Gremare, 1992).

The exact protein requirements for somatic growth for any echinoid species remain to be elucidated. Cook *et al.* (1998) found that in sea urchins fed a macroalgal diet spawning lasted for eight weeks and this period was greater in sea urchins fed on a salmon feed. Further, the gonadal index declined considerably in the former urchins after spawning. Macroalgal diet contains considerably less lipid and protein than those found in the salmon feed although the carbohydrate content was higher in macroalgae as compared to salmon feed.

In general, protein is the most expensive nutrient to supply in an artificial diet and is essential for soft tissue growth. Hence, growth trials have to be performed to determine the best protein sources and levels of inclusion (ARC, 1981). Usually, protein requirements cannot be met by adding more and more quantities of an inappropriate protein to the diet. Thus, it is vital to ensure that the protein supplied is of high quality before its inclusion levels are determined (ARC, 1981). Hence, both quality and quantity of dietary protein strongly influence the growth rates of an animal. Increases of dietary lipid concentrations to between 12 and 24% on a dry weight basis have been shown to spare some dietary protein from energy utilization for use in growth for several fish species (Millikin, 1982). Amino acid composition is usually one of the main considerations in formulating test or commercial feeds. In fish it has been suggested that tissue amino acid patterns may be used as a starting point for preparing diets for fishes whose nutritional requirements are still unknown (Cowey and Luquet, 1983).

Several experimental techniques such as the essential amino acid index (EAAI), the protein efficiency ratio (PER) and net protein utilization (NPU) have been used to measure the nutritive value of proteins in fish (Wilson, 1989). These techniques require similar protein levels in the feeds from which the proteins are to be compared. This requirement is easily met in comparing formulated feeds but is not applicable in the evaluation of nutritional value of proteins in the natural feeds. For instance natural feeds such as algae are different in their protein content and digestibility. However, it is still possible to derive some useful information on protein and amino acid nutrition from growth trials and chemical analysis of natural feeds (Mai *et al.*, 1994). Therefore, further nutritional studies are required to determine the quantity of total protein or proportion of constituent amino acids that can enhance the somatic growth (Cook *et al.*, 1998).

Studies have been carried out to evaluate plant proteins as an alternative source to replace fish meal in the diet of a number of freshwater and marine fish species (Dabrowski *et al.*, 1989; Wee and Shu, 1989; Shiah *et al.*, 1990; Robinson and Li, 1994). Among plant proteins soybean meal is the most promising candidate for partial or total replacement of fish meal in fish feeds (Boonyaratpalin *et al.*, 1998). Hence, soybean meal has been extensively used in feeds for cultivation of fish on a commercial scale. The reasons for this are the high protein content available with a reasonably well balanced amino acid profile in soybean, the constant composition, the reasonable price and steady supply. However, soybean meal also contains approximately 30% indigestible carbohydrates in addition to several other compounds

that may disturb the digestive process (Storebakken *et al.*, 2000) which has limited its use in place of fish meal in culture of fish (Refstie *et al.*, 2000). However, echinoids in general are able to digest both soluble and structural carbohydrates efficiently (Frantzis and Gremare, 1992). On the other hand, it has been suggested that urchins fed on a macroalgal diet can produce gametes for a relatively short period of time so that a high level of carbohydrate in their diet is not as important as a high protein and high lipid content in influencing gonadal growth and gametogenesis (Cook *et al.*, 1998).

In general, adult diets potentially influence the egg quality. In fact, there is much evidence to show that differences in characteristics of echinoderm eggs are due to variations in food supply for adults (George *et al.*, 1990). Thus, interest on the development of nutritionally balanced and effective artificial feeds for sea urchin farming has been growing. This may be of great concern due to the existing gap between supply and demand in the sea urchin market in Japan.

Evaluation of egg quality in echinoderms could be done on the basis of biochemical composition and size. However, the relationship between these two parameters is unclear (McEdward and Chia, 1991). George *et al.* (1990) attributed variations in the size and protein and lipid contents of eggs of *Arabacia lixula* to differences in the diets of two adult sea urchin populations studied. Hence, they showed that differences in the adult diet of *A. lixula* produced significant differences in protein and lipid contents in eggs. When the urchins were given a natural seaweed diet it was rather difficult to observe a precise relationship between the nutritional

quality and biochemical composition of the eggs. On the other hand, nutritional uncertainties of natural seaweed diets can be avoided by using artificial diets (de Jong-Westman *et al.*, 1995a).

Emlet *et al.* (1987) have reported that protein and lipids are the most important energy reserves in eggs of echinoderms while Turner and Lawrence (1979) stated that in eggs of *S. droebachiensis* proteins and lipids represent 65 and 35% of the dry mass, respectively. Furthermore, Thompson (1982) noted that concentrations of these nutrient components in *S. droebachiensis* are influenced by the quality and quantity of the adults' diet. Hence, *A. lixula* having access to more abundant and diverse algal foods produced eggs with higher protein and lipid levels than did those having access to lesser algae (George *et al.*, 1990). Further, Thompson (1982) related differences in egg lipid levels in *S. droebachiensis* to various ration levels of kelp and mussel flesh fed to the adults. In contrast, de Jong-Westman *et al.* (1995b) attributed most of the differences to levels of proteins rather than lipids and found that lipid concentration in the gonads of *S. droebachiensis* varied by only 1.4% in a five month period from November to March. In support of this, George *et al.* (1990) also observed that while protein levels varied significantly in eggs of well-fed versus poorly-fed populations of *A. lixula* their lipid levels were relatively similar.

It is generally accepted that animals cannot produce omega-3 polyunsaturated fatty acids (PUFA) from saturated fatty acids or omega-9 fatty acids due to inability for methylene-interrupted desaturation on the terminal methyl side of a double bond. Further, many marine animals have only a limited capacity to chain elongate and

desaturate omega-3 C_{18} PUFA to the corresponding long chain C_{20} and C_{22} PUFA (Kanazawa *et al.*, 1979). Hence, these animals have a specific dietary requirement for eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3) that are normally the main PUFA in their tissues. The PUFA required for normal growth and reproduction must be obtained from the diet and are referred to as the essential fatty acids (Uki *et al.*, 1985). Thus, the supply of sufficient essential fatty acids is important in the formulation of a successful artificial diet (Dunstan *et al.*, 1996). The effects of an artificial diet rich in 18:2n-6 on the lipid composition of abalone against those fed a macroalgae diet rich in highly unsaturated omega-3 fatty acids have been evaluated (Dunstan *et al.*, 1996). The fatty acid composition of abalone fed artificial diet showed elevated levels of 18:2n-6 while the proportion of n-3 PUFA was reduced. Thus, lipid composition can be changed with diet (Uki *et al.*, 1985). On the other hand, lipids, especially long chain PUFA, are an important aspect of differentiating the flavour and odour of seafoods. It has been found that the use of artificial diets containing fish oils may give cultured abalone a much fishier flavour than those fed a diet containing vegetable oils. Further, the accumulation of lipid in abalone fed an artificial diet may lead to subtle changes in the gonad texture (Dunstan *et al.*, 1996). This also demonstrates the necessity of feeding abalone on microalgae for a period of time immediately prior to sale to ensure market acceptability and to maintain product quality, as the fishy flavour may disappear upon feeding the algal diet (Dunstan *et al.*, 1996).

2.7 Processing of sea urchin gonads

Sea urchins may be prepared for live shipment or the gonads are extracted and prepared for fresh shipment (Anonymous 1990b). When live sea urchins are being shipped they are placed in styrofoam boxes. Urchins are first pre-weighed into polyethylene bags and placed in styrofoam boxes. Previously frozen (-40°C) "gel-ice" freezer packs are kept together with the sea urchins. If the sea urchins are properly prepared for shipment they can live for up to four days (Anonymous 1990b). On the other hand, when sea urchin gonads are extracted and shipped fresh the processing is much more complicated (Anonymous 1990a, 1990b). Fresh sea urchin gonad is extracted from the shell in the exporting country, sorted on trays and shipped by air for sale in the Japanese wholesale markets (Anonymous 1990a).

The processing of sea urchin gonad is highly labour-intensive and some preliminary experiments done in Newfoundland revealed that the average production was 0.55 kg per hour per person while reports from Japan indicate the norm was 1.0 kg per hour per person (Anonymous 1990b). As the size of the gonad becomes smaller processing requires more highly skilled labour. For instance, the gonads of Atlantic green sea urchin are considerably smaller than that of the West coast species and extraction of gonads is much more laborious (Anonymous 1990a).

In general, the processing operation involves several steps from whole sea urchin to the final tray pack (Anonymous 1990a). Breaking the shell is the first step involved and this is done by inserting a shell cracking device into the centre of the top or bottom of the urchin. Then the handle of the urchin cracker is squeezed which

results in the opening of the shell so that the five gonad sacs are exposed (Anonymous 1997). The gonad sacs are located on the top side of the urchin just inside shell which must be carefully removed after cracking the live sea urchin. Then the cracked shells are allowed to drain for 10 to 15 minutes (Anonymous 1995). This is followed by careful removal of gonad sacs using a curved spoon-like device or a spatula that allows the gonads to be scooped out of the shell and placed in a saline solution (Anonymous 1990b, 1995, 1997). However, the gonad sacs have to be separated from the surrounding membranes using tweezers before they are rinsed in brine. Sea urchin gonads are generally rinsed with chilled sea water or chilled brine of similar strength (Anonymous 1990b). Hence, the gonad sacs are placed on screens and often rinsed in more than one of these solutions to facilitate cleaning. The cleaned gonadal sacs are then placed in an alum bath (aluminum potassium sulphate) for several minutes in order to increase the strength and firmness of the gonadal sac surface (Anonymous 1989, 1990b, 1995). Generally, the cleaned gonad sacs are soaked in alum for a period of 15 to 45 minutes. The alum strength and soaking duration are modified depending on the quality of the gonad and the desired end product (Anonymous 1997). Finally, cleaned and treated gonad sacs are arranged in small wooden trays and chilled at 2 to 4 ^{o}C prior to and during shipping (Anonymous 1990b). Alternatively, the gonads may be placed on styrofoam trays for shipment since shipping in wooden trays is unsatisfactory. In wooden trays the gonad sacs tend to shift with slightest movement. The gonad sacs, on the other hand, may also be prepared and shipped in jars containing chilled sterile sea water (Anonymous 1990b). Factors such as brine

strengths, alum bath times and final package type, among others, are critical in determining the processing procedures (Anonymous 1997). Drip drying of the gonad sacs and towel drying are also performed prior to packing. Both of these steps are aimed at obtaining the desired firm texture of the treated gonad sacs (Anonymous 1997). The actual arrangement of sea urchin gonads on the trays finally determines the acceptability of the gonads to the Japanese markets (Anonymous 1990b).

Sea urchin gonads are highly perishable. Even before bacterial degradation becomes apparent biochemical changes in the gonad apparently occur which detract from its appearance. The most common of these is termed "sweating" or "melting" which is undesirable at any stage. Thus, beads of fluid appear on the surface of the gonad sacs so that the entire surface appears somewhat fluid. It is reported that the susceptibility of gonad to sweating could be manipulated through proper strength and composition of solutions such as alum (Anonymous 1989).

2.8 Factors determining quality in sea urchin gonads

The sea urchin gonads are auctioned in Japan and the price is determined by the availability of the locally produced gonads, the amount available in the market on any given day and the gonad quality. The assessment of gonad quality is a subjective process and is rather difficult to quantify. However, the general components of quality judgment include consideration of gonad colour, size, taste, texture, uniformity of colour within the package and freshness (Anonymous 1989). Basically there are four major factors that determine the quality of sea urchin gonads (Anonymous 1990a,

1995). Thus, freshness, colour, gonadal yield and taste may be considered as major quality determining factors; freshness being the most critical among all factors (Anonymous 1990a). The freshness quality of a particular seafood refers to the degree of excellence referring to sensory variables such as appearance, texture, odour and flavour associated with it (Botta, 1994). And hence reflecting the high price paid for the prime freshness suitable of products for being eaten raw (Sikorski and Pan, 1994b). The second factor is the colour. There are three basic colours of sea urchin gonads harvested in Japan; yellow, red and pink. However, colour preference varies with the geographic location of the Japanese consumers. In general, it is the brightness of the colour that determines the price. Consequently, the darker the colour of the gonad, the poorer the quality (Anonymous 1990). The gonad colour in sea urchins ranges from dark brown through a series of dark to pale orange or yellow to white. The most desirable colour in Japan is bright orange to bright yellow. Darker and lighter shades of the same colours are generally less desirable while brown is least desirable. However, all of the less desirable gonads may be used in the "non-fresh" forms such as salted gonads or pastes that have much lower value than the fresh product (Anonymous 1989). Sea urchin gonads are presented and sold on delicate wooden or plastic trays. The individual gonad sacs are arranged in pleasing patterns and for greatest value all gonad sacs on each tray should be as uniform in colour as possible (Anonymous 1989). The third factor pertains to the yield rate while the fourth factor is the taste (Anonymous 1990). However, the gonad size does not seem

to be as important a marketing trait as other quality parameters, but clearly it has a marked influence on the processing costs (Anonymous 1989).

In general, sea urchin gonads produced from imported live sea urchins falls into four grades namely special high, high, middle and low. However, of the various grades of gonads produced from imported live sea urchins the special high grade accounts only for a mere 0.2% of production imports. The latter three grades vary and it could be 25, 50 and 25%, respectively, of the gonads produced (Anonymous 1990a).

Producing high quality sea urchin gonads begins with the divers. The ability to harvest sea urchins containing quality gonads requires harvesting expertise as well as a knowledge of sea urchin's environmental and feeding conditions (Anonymous 1997). On the other hand, evaluation of gonad quality in echinoderms could be often based on biochemical composition (McEdward and Chia, 1991).

Generally, the quality of the catch undergoes rapid changes especially with respect to aroma and flavour characteristics of fresh seafoods. It has been shown that in unfrozen seafood the quality deterioration proceeds mainly due to postmortem biochemical processes in proteins and non-protein nitrogenous compounds while sensory changes in frozen seafoods are primarily brought about by lipid oxidation (Sikorski and Pan, 1994a).

2.9 Pigmentation

Carotenoids are natural yellow and red pigments present in most living organisms (Goodwin, 1984; Matsuno and Hirao, 1989). A chromophore that consists of a chain of conjugated double bonds is responsible for the colour of carotenoids (Torrissen *et al.*, 1989). They occur in the free form as well as esters, glycosides, sulphates and as carotenoproteins (Matsuno and Hirao, 1989). In the case of animals these carotenoid pigments are often found within the reproductive organs (Matsuno and Hirao, 1989). Their distribution is widespread and is unlikely to be nonfunctional, but their function often remains unknown (Tsushima *et al*, 1997).

In the aquatic environment carotenoid pigments are synthesized by the phytoplanktons. The presence of characteristic animal carotenoids which utilize these compounds as major means of pigmentation should thus reflect the uptake and metabolism of pigments of dietary origin (Paanakker and Hallegraeff, 1978). Animals obtain their carotenoids either by assimilating some of the carotenoids in the food while rejecting others or by converting dietary carotenoids into related ones (Tsushima and Matsuno, 1990). In general, typical algal xanthophylls are almost completely absent in animals and most likely the majority of these compounds are either not absorbed or are rapidly oxidized into characteristic animal carotenoids or into colourless products (Goodwin, 1960). Katayama *et al.* (1973) reported that the algal pigment β-carotene is most consistently present in the animal tissues in small amounts while astaxanthin most commonly occurs as the principal carotenoid pigment.

Sea urchins usually feed on brown algae in temperate latitudes (Hagen, 1996). The characteristic principal carotenoid in brown algae is fucoxanthin accompanied by β-carotene and violaxanthin (Goodwin, 1971; Liaaen-Jensen, 1978; Goodwin, 1980; Matsuno and Hirao, 1989). In addition, diadinoxanthin, diatoxanthin, neoxanthin and fucoxanthinol are present in small amounts (Nitsche, 1974).

In sea urchins the gut wall is extremely rich in carotenoids. It contains 2 to 3 times as much carotenoid as the ovaries and this becomes 7 to 24 times on a dry weight basis. The gut wall appears to take up pigments of the food indiscriminately. However, most of the carotenoid is fucoxanthin, which cannot be utilized in the production of echininone. Consequently, only relatively small amounts of fucoxanthin find their way to the ovary and still smaller amounts into the eggs (Griffiths and Perrott, 1976). On the other hand, there is a considerable amount of β-carotene in the gut wall which is presumably the source of echininone (Griffiths and Perrott, 1976). Thus, it was thought that echininone in sea urchin eggs is derived by conversion of a dietary carotenoid; β-carotene being the best precursor present in food and in the gut wall (Griffiths and Perrott, 1976). It has been demonstrated that at the end of 3 months of fasting β-carotene content in the gut wall was much lower in the fasted animals than in a control set of urchins that were well fed, hence, it was expected that β-carotene may be converted to echininone (Griffiths and Perrott, 1976).

Carotenoids are important to flesh pigmentation and colour of both fish (Torrisen *et al.*, 1989) and echinoderms (Tsushima *et al.*, 1993a; 1993b). Gonad

colour is also important when it comes to sea urchin gonads as a gourmet food item (Kato and Schroeder, 1985). However, the natural variability in carotenoid content in the raw material makes fresh macroalgae or algae meal unsuitable in the role of carotenoid source in an aquaculture facility (Havardsson and Imsland, 1999). Astaxanthin is the most common carotenoid found in marine organisms and is found in crustaceans (Castillo *et al.*, 1982), fish (Torrisen, 1989) and in echinoderms (Tsushima *et al.*, 1993a). The carotenoids produced by certain microorganisms, fungi, algae and higher plants are passed into other organisms through the food chain (Havardsson and Imsland, 1999). These authors have further shown that when the feed contains excess pigment as a constituent there was accumulation of it in the gonads compared to feeds with low carotenoid content.

The presence of carotenoids in the eggs is a widespread phenomenon in the animal kingdom that it is generally assumed and these pigments play an important role in development of larvae. Thus, in certain instances animals transfer half or more of their total carotenoid reserves into their eggs (Herring, 1968). On the other hand, there are colourless animals who frequently have coloured gonads and eggs (Griffiths and Perrott, 1976). Generally, the specificity of the kinds of carotenoid present in an animal and particularly in its eggs is not well understood. Since animals cannot synthesize carotenoids *de novo* they are dependent upon dietary carotenoids for their carotenoid composition (Goodwin, 1984).

In sea urchin there is conversion of β-carotene only to the stage of echininone of which the significance is obscure (Griffiths and Perrot, 1976). However, Tsushima

et al. (1997) reported that β-carotene and echininone exert important influences on the early development of sea urchins. Further, Kawakami *et al.* (1998) have shown that fucoxanthin as well as β-carotene and echininone may play an important role in the biological defense and reproduction of sea urchins. Shina *et al.* (1978) showed that β-carotene is selectively ingested and metabolized to echininone: both being stored in the gonadal tissue while the minor pigments identified were ingested from the food of both plant and animal origin. Furthermore, these authors reported that during maturation there was increasing storage of echininone at the expense of β-carotene in the gonads, predominantly in the female.

Zagalaski *et al.* (1967) showed a pronounced accumulation of carotenoids in the reproductive organs of both sexes in many marine invertebrates; the specific major carotenoid being echininone and most often dominant in the mature female gonads. On the contrary, Hsu *et al.* (1970), Gilchrist and Lee (1972) and Katayama *et al.* (1973) reported that marine invertebrates typically have astaxanthin or canthaxanthin as the main carotenoid. Thus, Griffiths and Perrott (1976) stated that sea urchin is unusual in having echininone, a derivative of β-carotene, as the major carotenoid.

The high level of carotenoids in the gonads and their accumulation during maturation clearly suggests their potential function in reproduction (Shina *et al.*, 1978). Griffiths (1966) and Gilchrist and Lee (1972) suggested that one of the speculative roles of carotenoids was to act as heat and light shields. Griffiths (1966)

stated that gonadal pigments function to form structural components which stabilize the proteins in the developing animal. This was suggested owing to the different solubility of echininone that leads to different degrees of bonding between pigment and the tissue.

Many biological functions are associated with carotenoid pigments (Krinsky, 1994). However, only few reports are available about biological functions of carotenoids in marine animals. Mainly there is an increasing interest in the relationship between carotenoids and reproduction (Tsushima *et al.*, 1997). In Atlantic salmon diets supplemented with astaxanthin and canthaxanthin promoted growth rates during the early start-feeding period (Torrissen, 1984). In red sea bream swim-up egg rates and hatching larval fish rates were higher for fish fed an astaxanthin supplemented diet than for fish fed a carotenoid free diet (Miki *et al.*, 1992). Also there were abnormalities associated with eggs when the brood stock was fed a carotenoid free diet. In *Penaeus esculentus* (tiger prawn) it has been demonstrated that development and pigmentation of ovaries were very rapid suggesting a sudden increase in the intake of dietary carotenoids or having appreciable carotenoid reserves or both. Thus, carotenoids appear to have an important role in reproduction (Dall *et al.*, 1995). Chien and Jeng (1992) found that prawns showed higher survival rates when they were fed a diet supplemented with astaxanthin compared to prawns fed β-carotene or an algal meal. Hence, there is considerable body of evidence suggesting that carotenoids are closely connected with reproduction in marine animals. In sea urchins, the highest contents of carotenoid

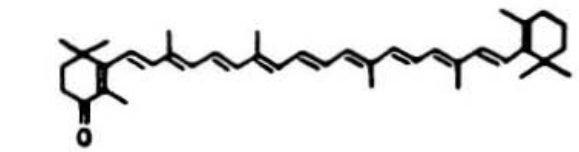
were found in the gonads, again suggesting their important role in reproduction (Tsushima *et al.*, 1997). Chew (1993) stated that carotenoids play a role in the biological defense function in mammals. In cattle β-carotene plays a specific role in relation to their fertility (Latthammer, 1979). Krinsky (1989) reported that carotenoid pigments play an important role in protecting cells and organisms against harmful effects of light, air and sensitizer pigments. The primary mechanism of action of this phenomenon is their ability to quench excited sensitizer molecules as well as quench singlet molecular oxygen. In addition, carotenoids may also serve as antioxidants under conditions other than photosensitization which is of even greater biological importance (Krinsky, 1989).

Pigmented diets have been used for feeding farmed salmon and the cost for supplementation of carotenoids is around 15% of the total feed cost (Hardy *et al.*, 1990). However, the cost may be reduced by way of better retention of dietary pigments and mainly depends on their digestibility, intestinal absorption, metabolism and excretion (Storebakken and No, 1992). Shahidi *et al.* (1993) reported that feeds supplemented with either astaxanthin or canthaxanthin brought about sufficient pigmentation that could be visualized in the fish Arctic char (*Salvelinus alpinus*). In sea urchins test, gut and gonad colour varied with the type of diet. When sea urchins were fed salmon the test and gut appeared reddish and this could be attributed to the incorporation of astaxanthin and canthaxanthin pigments present in the salmon feed. The gonads, on the other hand, were pale in colour and this suggests that the gonadal tissue is unable to absorb these dietary pigments (Cook *et al.*, 1998). The gonads of

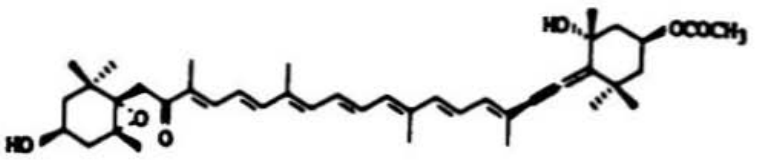
sea urchins fed on macroalgal diets were generally brighter in colouration before spawning than the gonads of sea urchins fed on a salmon feed. However, there was much greater variation in the colour when sea urchins were fed algae (Cook *et al.*, 1998). Barker *et al.* (1998) have shown that urchins fed prepared diets that did not contain kelp were consistently light cream to yellow in colour. However, commercial markets require yellow or orange roe.

2.10 Carotenoid pigments in sea urchins

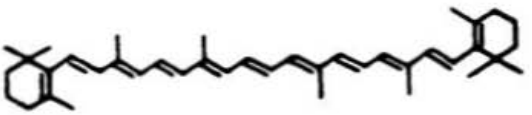
Many reports on carotenoids of Echinodermata and especially the carotenoids of sea urchins have been published (Hallenstvet *et al.*, 1978; Tsushima and Matsuno, 1990; Tsushima *et al.*, 1993a; 1993b; Tsushima *et al.*, 1995; Tsushima *et al.*, 1997). The class Echinoidea can be divided into two subclasses, Regularia (regular sea urchins) and Irregularia (irregular sea urchins). It has been reported that gonads of regular sea urchins have β,β-carotene and β-echininone as major carotenoids along with β,ε-carotene, β-isocryptoxanthin, isozeaxanthin, canthaxanthin, lutein, zeaxanthin, fucoxanthin, fucoxanthinol, among others (Griffiths and Perrott, 1976; Goodwin, 1984; Matsuno and Hirao, 1989). It was further reported that fucoxanthin and fucoxanthinol are the principal carotenoids in the viscera of regular sea urchins (Griffiths and Perrott, 1976; Matsuno and Hirao, 1989). In addition, the carotenoid paracentrone was discovered in the sea urchin *S. lividus* and was supposed to be formed via fucoxanthinol from dietary fucoxanthin abundant in the brown algae and diatom (Galasko *et al.*, 1969). Fig 2.1 represents some of the carotenoids commonly



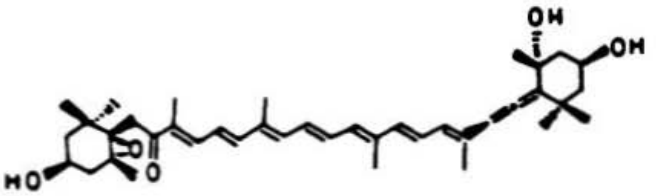
Echininone



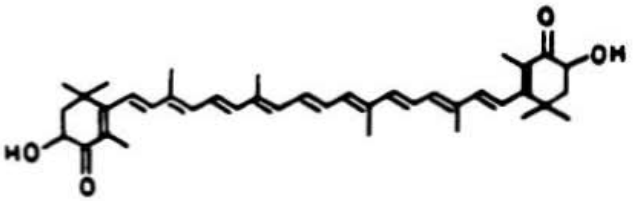
Fucoxanthin



β-Carotene

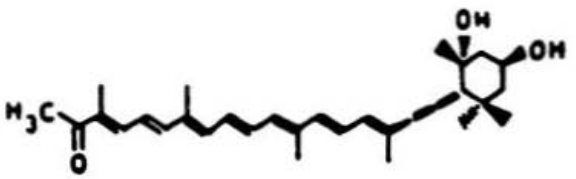


Fucoxanthinol



Astaxanthin

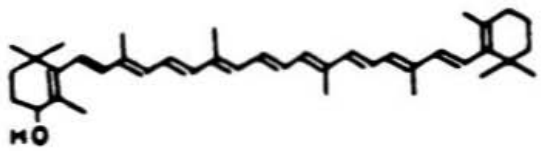
Zeaxanthin



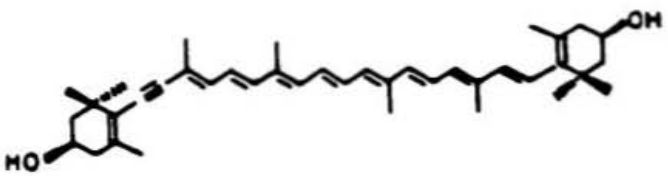
Paracentrone

Fig 2.1 Some carotenoids isolated from sea urchins.

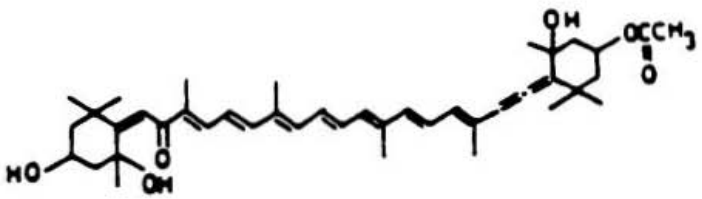
Fig 2.1 Continued.



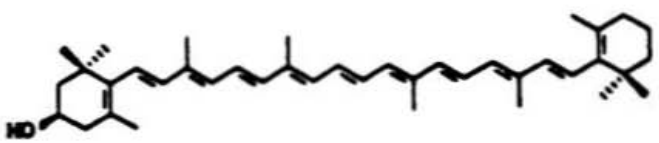
Isocryptoxanthin



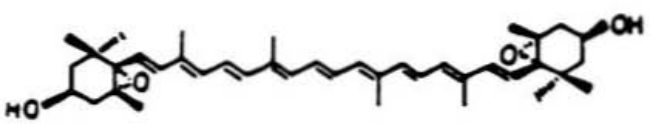
Diatoxanthin



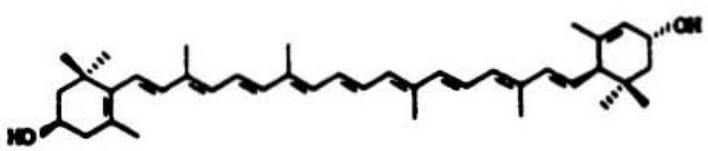
Isofucoxanthin



Crptoxanthin



Violaxanthin



Lutein

encountered in sea urchin tissues. Shina *et al.* (1978) found that the sea urchin *Tripneustes gratila* contained about 80% of its carotenoids as echininone while β-carotene constituted 8% of that in the gonads. Griffiths and Perrott (1976) reported that the gonads of *S. droebachiensis* contained echininone as the principal carotenoid with small amounts of β-carotene, isocryptoxanthin, zeaxanthin, fucoxanthin and isofucoxanthin (Table 2.2). Tsushima and Matsuno (1990) reported that in viscera of 11 species of sea urchins examined fucoxanthin, fucoxanthinol and fucoxanthinol ester were the common major carotenoids present and these contributed approximately 2 to 40, 2 to 32.3 and trace to 12.4% of the total carotenoids, respectively. The above carotenoids were found in both regular and irregular sea urchins. In addition to these, all irregular sea urchins examined contained β-echininone (14.1 to 51.4%) and α-echininone (3.0 to 17.2%) as their principal carotenoids. Furthermore, irregular sea urchins *Clypeaster japonicus* and *Peronella japonica* contained canthaxanthin (20.8%) and astaxanthin (39.0%), respectively as their major carotenoids. Furthermore, except for *P. japonica*, all other sea urchin species investigated had β,β-carotene (3.0 to 27.5%), β-echininone (31.4 to 71.0%) and α-echininone (3.0 to 17.0%) as their major gonadal carotenoids. *P. japonica* contained astaxanthin (60.5%) as the principal carotenoid along with small amounts of β,β-carotene, β-echininone, α-echininone, canthaxanthin, among a few others (Tsushima and Matsunao, 1990).

Table 2.2 Percentages of different carotenoids in different tissues of the sea urchin *Strongylocentrotus droebachiensis*.

Carotenoid	Ovary	Egg	Gut wall
β-Carotene	14.0	7.9	4.3
Echininone	77.0	87.1	1.9
Isocryptoxanthin	2.2	1.5	0
Zeaxanthin	3.5	1.5	8.5
Fucoxanthin and Isofucoxanthin	2.7	0.5	79.6

Adapted from Griffiths and Perrott, 1976.

Tsushima *et al.* (1995) carried out a comparative biochemical study in gonads, viscera and spines of 20 sea urchin species from seven orders and found that the levels of carotenoid in their gonads to be the highest among the organs investigated. Further, the total carotenoid level reached a maximum during the spawning season. Furthermore, Tsushima *et al.* (1995) reported that 17 species of urchins examined contained β,β-carotene and β-echininone as their main carotenoids. On the other hand, the carotenoids in sea urchin species *Asthenosoma ijimai* and *Araeosoma owstoni* were dominated by β,β-carotene, β-echininone, canthaxanthin and astaxanthin. Hallenstvet *et al.* (1978) showed that the whole animal *Psammechinus milliaris* contained β,β-carotene (10%), β,ε-carotene (2 to 5%), echininone (4 to 10%), lutein (6 to 8%) and fucoxanthinol (8 to 68%).

Tsushima *et al.* (1993a) carried out a comparative biochemical study of carotenoids from a few species of more primitive sea urchins and identified 30 known carotenoids in them. Among these more primitive sea urchins *Glyptocidaris crenularis* contained β-echininone (37.4 to 56.5%) as its dominant carotenoid followed by β,β-carotene, (21.4 to 35.6%) in the gonads. On the contrary, in gonads of *Prionocidaris baculosa*, *Phyllacanthus dubius* and *Eucidaris metularia* β,β-carotene (56.7 to 70.0%) was dominant followed by β-echininone (17.1 to 32.2%). In *As. ijimai* and *Ar. owstoni* the predominant carotenoids were β,β-carotene (11.7 to 16.1%), β-echininone (20.6 to 36.8%), canthaxanthin (5.8 to 10.1%) and astaxanthin (4.1 to 9.6%). Tsushima *et al.* (1993a) further reported that β,β-carotene (7.7 to

27.47), fucoxanthin (5.9 to 18.6%) and fucoxanthinol (8.8 to 45.0%) were the common major carotenoids in the viscera of *Pr. baculosa*, *Ph. dubius*, *E. metularia* and *Diadema savignyi*. In the case of *As. Ijimai* and *Ar. owstoni* β,β-carotene (7.3 to 13.6%), β-echininone (10.3 to 11.1%), canthaxanthin (4.1 to 6.3%) and astaxanthin (8.7 to 10.6%) were the main carotenoids present in the viscera. In addition, *Ar. owstoni* contained fucoxanthin (21.7%) and fucoxanthinol (5.2%). Hallenstvet *et al.* (1978) showed that the sea urchin *P. miliaris* has β,β-carotene (10%), β,ε-carotene (5%), echininone (10%), lutein (8%) and fucoxanthinol (68%) as the principal carotenoids in its adult stage while echininone (92%) was the major carotenoid accompanied by small amounts of β-carotene (8%) in the unfed larvae. Further Tsushima *et al.* (1993b) investigated the carotenoid composition of wild *Paracentrotus depressus* and found that most of the carotenoids present to be in common with those of other sea urchins (Table 2.3).

2.11 Biochemical composition

Sea urchins are often the dominant herbivores in many sublitoral communities and are frequently regarded as determinants of community structure in marine macrophyte habitats (Miller and Mann, 1973; Lawrence and Sammarco, 1982). They also play an essential role in the transfer of energy between benthic and pelagic environments (Fenaux *et al.*, 1977). The biochemical composition of Echinodermata has been studied in a number of marine species from tropical, temperate and polar

Table 2.3 Percentage composition of carotenoids in the viscera and gonad of wild *Paracentrotus depressus*.

Carotenoid	Viscera	Gonad
β,β-Carotene	9.2	3.8
β,ε-Carotene	3.1	1.3
β-Isocryptoxanthin	0.2	1.4
α-Isocryptoxanthin	0.1	0.7
β-Echinone	1.8	69.1
α-Echininone	0.9	10.5
Isozeaxanthin	Tr	0.3
Canthaxanthin	Tr	1.2
Astaxanthin	Tr	-
Lutein	0.7	0.3
Zeaxanthin	1.0	0.3
Fucoxanthin	40.0	Tr
Fucoxanthinol	32.3	Tr
Fucoxanthinol ester	1.4	Tr
Unidentified	9.3	11.1

Adapted from Tsushima *et al.*, 1993b.

environments (Giese 1966a; Sibuet and Lawrence, 1981; McClintock and Pearse, 1987; McClintock *et al.*, 1990a & b). Insight on biochemical composition is important at the tissue, organism and population levels and this may explain the nature and role of each tissue and the cells that compose it (Giese, 1966 a & b; Lawrence and Guille, 1982). Furthermore, biochemical composition of an organism may also be used to define nutritional requirements during the development of artificial diets (Floreto *et al.*, 1996b).

It has been found that in fish knowledge of the biochemical composition and factors affecting it allows assessment of fish health, determination of efficiency of transfer of nutrients from the feed to fish and makes it possible to predictably modify the carcass composition (Shearer, 1994). Cushing (1982) stated that seasonal growth and energy storage cycles are common among temperate fish and are related to environmental production cycles. Growth and energy storage occur in spring and summer when food is abundant while energy reserves are depleted in many temperate species for both metabolic needs and gonad development during cold seasons of low environmental productivity. A reduction in lipids and proteins has been observed in Arctic charr *Salvelinus alpinus* during winter (Boivin and Power, 1990). Thus, somatic growth is linked to higher environmental productivity while these temperate species spawn in late winter or spring. This seems to be disadvantageous since peak energy demands for gonad development come at a time when exogenous resources are at a minimum. However, this allows larvae and juveniles to use the seasonal maxima of food abundance during the critical period in their life history (Cushing, 1982).

Lipid content of sea urchin gonads has been found to be affected by temperature (Havardsson and Imsland, 1999). Thus, higher lipid contents have been observed with high temperatures. Further, temperature acts as a synchronising factor for gonad development (Himmelman, 1978; Starr *et al.*, 1993). Hence, animals may advance in their gonad development with a higher temperature. On the other hand, Elliot (1975) concluded that temperature had a significant effect on the body composition although the protein content was not altered. Brett *et al.* (1969) found that the maintenance energy requirement was highest at high temperature so that fish were unable to consume enough energy to accumulate body lipid. These authors further stated that lipid was stored at low temperatures.

Proteins are a very important component of fish as they affect nutritional as well as sensory properties of their products. Further, in the muscles and other organs of marine animals proteins may have associations or interactions with many other components such as water, mineral ions, lipids and carbohydrates (Sikorski, 1994). The crude protein content in different seafoods depends on many factors such as species and variety, the state of nutrition and the stage of reproductive cycle, among others (Sikorski, 1994). For instance, in cod a significant seasonal variation has been detected in the protein content thus decreasing it during spawning which may probably be due to the physiological processes that take place during reproductive activities (Dambergs, 1964).

Groves (1970) showed that in fish primary changes in the body composition occurred mainly in moisture and fat contents. This was attributed to additional energy

stored as fat by fish that simply replaced body water (Reinitz, 1983; Tidwell and Robinette, 1990). In fact, moisture and fat contents commonly exhibit an inverse relationship (Love 1970; Dygert, 1990). Church and Pond (1982) reported that most animals exhibit decreasing body protein levels with time and this was further supported by Tidwell and Robinette (1990). Reinitz (1983) showed that protein increased with size and age in salmonids and that changes in moisture and fat levels did not adversely affect the proportion of protein (Groves, 1970). Dygert (1990) revealed an increase in protein, lipid and ash levels in the gonads during sexual maturation of female English sole. Further, an increase in moisture content was apparent in sea urchin gonads during gametogenesis due to increased uptake of water (Miller and Mann, 1973; Munck, 1992).

McClintock and Pearse (1987) stated that there were no taxonomic differences in the composition of the gonads; high levels of protein and lipid were present in all sea urchin taxa. However, in many studies with different species it was found that the lipid content in the overies was higher than that in testes (Fenaux *et al.*, 1977; McClintock and Pearse, 1987). McClintock and Pearse (1987) further stated that lipid content in ovaries was higher than that in testes particularly in several Echinodermata species from the Atlantic region.

Since sea urchin is subject to intensive commercial fishing in several countries a considerable number of studies have been undertaken dealing with sea urchin nutrition and growth in order to improve its farming practices (Grosjean and Jangoux, 1994; Fernandez *et al.*, 1995). However, a relatively little research has been carried

out on sea urchin biochemical composition (Fernandez, 1997). Fernandez (1997) monitored the effect of diet on sea urchin biochemical composition in populations living in the field with or without access to an abundant food source and in rearing sites with individuals fed in excess with different artificial or natural feed. In fact, such information should allow for a better understanding of the nutrition of sea urchins, particularly on how feeds affect the biochemical composition of the body components and therefore how different nutrients are allocated to different tissues. Such results should enable one to define an artificial diet which is important for successful development of sea urchin farming.

Fernandez (1997) showed that in a natural population the biochemical composition of sea urchin organs did not depend on food availability and hence on the quantity of food consumed. However, a comparison between wild and reared sea urchin biochemistry revealed that organ biochemical composition was strongly influenced by the quality of the feed. Thus, it has been shown that use of artificial feed containing fish meal (rich in protein) favours a storage of reserves in gonad, gut and even test in the form of lipid and/ or carbohydrate. On the other hand, starvation is not an uncommon circumstance for marine invertebrates and affects metabolism and energy reserves in these animals (Gatty and Wilson, 1986). During food deprivation an organism's energy reserves are mobilized and its metabolism is altered. Thus, starvation has profound effects on an animal's physiological state, health and tissue quality and composition (Carefoot *et al.*, 1993).

2.12 Marine lipids, their fatty acid composition and significance

Sea urchins can endure rather long periods without apparent sources of nutrient; lipids serve as the primary storage materials for their energy during fasting. On the other hand, sea urchins can be an intermediate in the energy flow from seaweeds to the commercially important crustacea and other animals, and lipids are presumed to play an important role in this energy flow (Ferguson, 1969). Dietary lipids play an important role in the energy production process of animal tissues. In addition, they are significant as a source of essential fatty acids in animals. Recent studies in fish have demonstrated that essential fatty acid requirements differ considerably from species to species (Watanabe and Takeuchi, 1989).

Although the total amount of lipid deposited is important the fatty acid composition of lipids is critically important because it may influence flavour and storage characteristics of products (Gatlin and Stickney, 1982). In fish it has been shown that fatty acid composition reflects the fatty acid composition of dietary lipids (Yingst and Stichney, 1979). In general, the level of fat deposition in fish may influence both the nutritional and organoleptic properties of the product to the consumer (Ackman, 1989b). However, the sites of lipid storage are different from species to species (Ackman, 1980). When higher amounts of lipid in dietary formulations were used protein utilization was minimised as a source of energy, thus improving the growth performance. However, the use of high energy diets containing up to 33% dietary lipid has led to products having oily texture and strong flavour, in

addition to poor pigmentation. Furthermore, such a product was susceptible to rancidity (Bell *et al.*, 1998).

Kozhina *et al.* (1978) investigated the lipid composition of gametes of the sea urchin *S. intermedius* collected from the waters of Japan. Thus, it was reported that unfertilized eggs contained 53.7 and 9.4% of triacylglycerol (TAG) and cholesterol (CHOL), respectively, while corresponding values for spermatozoa were trace and 15.5%. The relative polar lipid contents of eggs and spermatozoa were 33.2 and 65.0%, respectively. Kozhina *et al.* (1978) further identified phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylserine (PS), phosphatidylinositol (PI), cardiolipin (CL) and lysophosphatidylcholine (LPC) as the polar lipid classes of both eggs and spermatozoa. Furthermore, lipid class composition did not show any qualitative changes during the development although PE, PS, LPC and CHOL contents increased while those of PC, PI and TAG decreased (Kozhina *et al.*, 1978). A marked increase in cholesterol and polar lipid concentrations during development and a related drop in TAG content may reflect more active catabolism of TAG (Kozhina *et al.*, 1978). The majority of marine invertebrates possess PC as their main polar lipid. Echinoderms are very rich in PC the proportion being over 60% and even more than 80% in some of these animals (Dembitsky, 1979). Phosphatidylethanolamine, the second essential polar lipid of marine organisms, usually amounts to 20-25% of the total polar lipids (Kostetsky and Shchipunov, 1983). de Koning (1966) found that PC exceeded PE in both mollusc and fish while Castledine and Buckley (1982) stated that the proportion of these two major lipid

classes is a characteristic of the polar lipids of marine organisms. Phosphatidylserine is a common polar lipid component in marine animals (Vaskovsky and Kostetsky, 1969). Komai *et al.* (1973) showed that both PS and PI are close in chromatographic behaviour, thus causing problems in separating them and data may be presented only as total amounts for both lipid classes. However, PI is less abundant than PS in marine animals (Kostetsky and Shchipunov, 1983). Furthermore, Kostetsky and Shchipunov (1983) reported that sphingomyelin (SM) is typical of animals at certain evolutionary stages. Kostetsky (1982) investigated the SM distribution in some marine invertebrates from 11 phyla and found that in the majority of Echinoidea SM is present but in low concentrations of 2% or less of the total polar lipids. During development the relative content of PC was decreased with a simultaneous increase in PE. Other polar lipid classes also underwent some alteration (Kozhina *et al.*, 1978). Since polar lipids localize mainly in membranes their class changes were associated with membrane changes, hence the polar lipid changes reflect the total results of biosynthesis and catabolism of lipids (Kozhina *et al.*, 1978). On the other hand, processing caused a significant change in lipids. Hence, Fujino *et al.* (1970) reported that salted sea urchin gonads contained a smaller amount of TAG, PC, and PE and a larger amount of free fatty acids (FFA) than the raw sea urchin gonads. However, Shimada and Ogura (1990) stated that TAG content remained constant while FFA content increased due to hydrolysis of polar lipids and glycolipids in salted sea urchin gonads.

Marine oils consist of saturated fatty acids (SFA), monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA). Polyunsaturated fatty acids belong to either omega-3 or omega-6 families. Although SFA and MUFA may be synthesised

by all animals including humans. PUFA cannot be synthesised by animals *de novo*. Therefore. PUFA must be supplied through the diet. The omega-3 and omega-6 PUFA may derive from linolenic and linoleic acid. respectively. Long-chain PUFA are also formed in unicellular phytoplankton and multicellular marine algae and subsequently pass into the food web to be incorporated into higher marine species (Yongmanichai and Ward. 1989). It has been shown that the primary purpose of omega-3 and omega-6 PUFA in the abalone tissues may be structural since they were purposely synthesised. Hence. Stewart *et al.* (1994) suggested that dietary fatty acids may have a limited function as an energy source in marine invertebrates as these organisms are incapable of efficiently oxidizing PUFA. The omega-3 PUFA are important components of cell membranes and are essential for normal human growth and development. Therefore. maintaining an optimum proportion of these fatty acids in the diet is of great importance. Indeed. man evolved on a diet with an approximate 1:1 ratio of omega-6 to omega-3 PUFA. However. this ratio has distorted to 10:1 if not 25:1 in today's society (Simopoulos. 1991). Hence. correction of the imbalance through dietary supplementation is likely to provide considerable clinical benefit (Ackman. 1986). Therapeutically omega-3 PUFA such as EPA and DHA have been characterized as hypolipidemic drugs. antiplatelet agents and cardioprotectants (Liebich *et al.*. 1991). Analysis of marine lipids such as fish oil and whale fat has shown that marine lipids contain high contents of EPA and DHA where one or the other fatty acid may predominate depending on the source (Ackman. 1986). On the other hand. seafoods. rich in omega-3 PUFA such as EPA and DHA. are very much susceptible to autoxidative degradation. Consequently. adequate antioxidant

protection must be provided to prevent postmortem rancidity (Hsieh and Kinsella, 1989). However, there is natural cellular antioxidant protection and α-tocopherol (vitamin E) is recognised as a major chain-breaking lipid-soluble antioxidant. It is commonly used as a feed additive (Buettner, 1993).

The principal interest of seafoods in human health is attributed to their high content of long-chain omega-3 PUFA that brings about protective effects in the treatment and prevention of a number of diseases (Diaz and Lezcano, 2000). In recent years fatty acids, especially PUFA, have attracted the attention of investigators (Isay and Busarova, 1984). These omega-3 fatty acids are known to be effective in the case of curing and treatment of certain cardiovascular diseases (CVD) by lowering serum TAG and CHOL and play an important role in the prevention and treatment of hypertension, arthritis and other inflammatory and autoimmune disorders (Simopoulos, 1991). Clinical studies have shown that fish oil derived omega-3 fatty acids can have a significant effect on the lipid profile with a consistent effect of dose dependent reduction in serum TAG both in normal and in subjects with hypertriglyceridemia (Flaten *et al.*, 1990; Nelson *et al.*, 1997; Green *et al.*, 1991). Furthermore, it is known that omega-3 fatty acids decrease the synthesis of very-low-density lipoproteins (VLDL) which accounts for their hypercholesterolemic effect (Connor, 1986; Nestel *et al.*, 1986). Numerous medical hypotheses connect many diseases with PUFA deficiency (Rudin, 1982). Thus, CVD are associated with a deficiency in PUFA (Mest *et al.*, 1977; Wennmalm, 1977; Constantinides and Kiser, 1981; Dyerberg *et al.*, 1982). Omega-3 PUFA are known to have significant

physiological effects in relation to heart and circulatory systems. Hence, prevention or treatment of atherosclerosis (Dyerberg, 1986), thrombosis (Kinsella, 1986), and high blood pressure (Dyerberg, 1986) are of much concern. There are numerous, but contradictory, data on the interconnection between unsaturated fatty acids and tumours. Thus, unsaturated fatty acids are known to accelerate tumour growth (Carrol, 1980; Hopkins *et al.*, 1981; McKenna and Radcliffe, 1981). However, McKenna and Radcliffe (1981) have reported on the role of unsaturated fatty acids as antitumoral agents. Further, there is evidence that unsaturated fatty acids participate in immune reactions of organisms (Rudin, 1982). Numerous physiological and pathophysiological reactions such as vascular resistance, thrombosis, wound healing, inflammation and allergy are modulated by oxygenated derivatives of arachidonic acid (AA, 20:4n-6) and EPA. These metabolites include prostaglandins, prostacyclins, thromboxanes, leukotrienes, lipoxins and hydroxy fatty acids and are collectively termed eicosanoids (Fisher, 1989). When orally taken these PUFA, such as EPA and DHA, are absorbed through lymphatic pathways, circulate largely in serum lipoproteins and distribute fairly widely in tissues. Further, they are incorporated into cellular membrane lipids and compete with AA in eicosanoid metabolism pathways (Hazra *et al.*, 1999). Eicosanoids produced from AA and EPA are different in structure and function (Fisher, 1989). Neuringer *et al.* (1988) reported that since eicosanoids are derived from PUFA in the diet both the qualitative and quantitative supply of PUFA by diets have a significant effect on the formation of eicosanoids. Thus, attention has grown on the study of the biochemistry of marine organisms as

they are rich in PUFA (Scheuer,. 1978-1981). In addition to being rich in PUFA marine organisms are also known to posess a great variety of fatty acids and serves as sources of many biologically active substances (Faulkner, 1977).

The presence of fatty acids with chain lengths of less than C_{20} is common in marine invertebrates. It includes fatty acids with odd numbers of carbon atoms in small quantities. On the other hand, the presence of palmitic acid (16:0) is a characteristic of almost all marine invertebrates (Isay and Busarova, 1984). Eicosapentaenoic acid is typical of marine invertebrates while the highest level of EPA content has been observed in echinoderms, among other invertebrates (Isay and Busarova, 1984). Arachidonic acid was present in smaller quantities in many marine invertebrates although in sea urchins and star fish this was more prominent. On the other hand, eicosatrienoic acid (20:3) was found in some molluscs and echinoderms and echinoderms were quantitatively richer than molluscs (Isay and Busarova, 1984). Furthermore, DHA is not characteristic of marine invertebrates (Isay and Busarova, 1984). Sea urchins of the genus *Strongylocentrotus* contained high amounts of 20:2 acids (Takagi *et al.*, 1980; Isay and Busarova, 1984) known as non-methylene interrupted dienes (NMID) and are commonly found in lipids of marine invertebrates (Ackman, 1989a). However, some of the sea urchin species obtained from Japanese seas either contained no such fatty acids at all or contained them only in small quantities (Isay and Busarova, 1984). Non-methylene interrupted dienes are still PUFAs but their chance of participation in biochemical processes, such as prostaglandin formation, seems to be remote (Ruggeri and Thoroughgood, 1985).

Initially, these NMIDs were found to be associated with seaweeds (Jamieson and Reid, 1968) and then presumed to pass into the lipids of marine invertebrates browsing on these seaweeds (Carballerira and Maldonada, 1986). Ackman and Hooper (1973) showed that the NMIDs 20:2 5,11, 20:2 5,13 and their chain extension products 22:2 7,13 and 22:2 7,15 were primarily associated with molluscs. However, Paradis and Ackman (1977) demonstrated that these are also associated with lipids of other marine invertebrates. Hence, biochemically interesting 20:2 and 22:2 NMIDs are formed in many marine invertebrates and the variety of fatty acids and derivatives in fats of invertebrate marine life is often surprising (Joseph, 1982).

Allen (1968) reported that EPA was the predominant fatty acid in *S. franciscanus* and *E. esculentus* tissues. Kaneniwa and Takagi (1986) found that sea urchin tissues analyzed (both raw and processed) contained 20:4n-6 and 20:5n-3 as their major PUFA. Among sea urchins, *S. intermedius* lipid was notable for its content of PUFA and contained considerable amounts of 18:3, 18:4, 20:3, 20:4 and 20:5 (Isay and Busarova, 1984). In fact, these authors showed that echinoderms are richest both in the variety and quantity of PUFA, among a large number of invertebrates analyzed from the Japan seas. Among PUFA, 20:5n-3 was the predominant fatty acid present. Wild sea urchins analyzed consumed mainly seaweeds, sea urchin's natural diet, especially *Laminaria* spp, that are known to be high in 18:3n-3, 18:4n-3, 20:4n-6 and 20:5n-3 (Pohl and Zurheide, 1979). Pohl and Zurheide (1979) further reported that green seaweeds are high in 16:4n-3, 18:3n-3 and 18:4n-3 while red seaweeds are high in 20:4n-6 and 20:5n-3. Takagi *et al.* (1980)

analyzed sea urchins collected from beds of *Laminaria digitata* and found that the the lipids of these sea urchins were rich in 20:4n-6. This seaweed is known to contain 20:4n-6 and 20:5n-3 in almost equal amounts (Ackman and McLachlan, 1977).

Kaneniwa and Takagi (1986) stated that sea urchin lipids contained approximately 10 - 17% of 5-olefinic acids. Takagi *et al.* (1986) reported 10 - 21% of 5-olefinic fatty acids in the total lipids of whole animals in 12 species of Echinoidea obtained in Japan. Hence, presence of these 5-olefinic fatty acids was thought to be a common and characteristic feature of Echinoidea lipids. The major 5-olefinic acids encountered were 20:1n-15, 20:2 5,11, 20:2 5,13 (Kaneniwa and Takagi, 1986), while 7-olefinic acids found in sea urchin lipids were 22:2 7,13 and 22:2 7,15 (Takagi *et al.*, 1980; Takagi *et al.*, 1982; Kaneniwa and Takagi, 1986). The major PUFA of sea urchin gonadal lipids were 18:4n-3, 20:4n-6 and 20:5n-3; contributing 6 to 30% to the total fatty acids. These fatty acids are known to serve as precursors of prostaglandins, the biological activity of which is well known in biochemistry and physiology (Kaneniwa and Takagi, 1986).

2.13 Taste-active components in sea urchins

Sea urchin has been favoured by consumers because of its distinctive aroma and good taste (Cruz-Garcia *et al.*, 2000). In general, the taste of fish is dependent on extractive components defined as water-soluble and low-molecular weight compounds. These extractive components may be either nitrogenous or non-nitrogenous in nature (Fuke and Konosu, 1991). Free amino acids (FAA) are known

to be one of the major nitrogenous extractive components (Haard *et al.*, 1994). The content of non-protein nitrogenous compounds (NPN) in marine animals depends on the species, the habitat, life cycle effects and freshness after catch while FAA may contribute a significant portion to the total NPN compounds (Sikorski, 1994).

Marine invertebrates are characterized by having a high intracellular concentration of FAA (Gilles, 1979). Yancey *et al.* (1982) reported that all phyla of marine invertebrates contain 200-500 mM of FAA in their tissue cells. The significance of having high FAA concentrations is that of their participation in osmoregulation (Fyhn, 1976; Pierce, 1981; Finne, 1992). This counteracts the dehydrating effect of the high ambient salinity of the sea water (Fyhn, 1976; Pierce, 1981). Fyhn (1989) also proposed that FAA in marine fish are important substrates for energy production during embryogenesis and early development.

Amino acids are precursors of many biological compounds, notably proteins, and may act as substrates for energy production. Deficiency or excess of one or more of the amino acids is known to limit protein synthesis, growth or both (Cowey, 1992; Murai, 1992). Therefore, amino acids should be present in proper balance in the body tissues in order to promote optimum growth and development. However, amino acids are not only the building blocks of proteins but also occur in the free form, thus playing a major role in the taste of numerous foods (Kato *et al.*, 1989). Each food has a characteristic taste which is determined by the balance of the primary and/ or secondary tastes and FAA and peptides are known to play an important role in the elicitation of each food taste (Kato *et al.*, 1989). Thus, FAA and peptides are very important as taste-active substances. Almost all FAA have some sweetness,

bitterness, sourness or umami taste (Solms, 1969). In fact, umami is a basic taste independent from the four basic tastes of sweetness, saltiness, sourness and bitterness (Komata, 1990). Komata (1990) has further reported that there is a unique synergistic effect between glutamate and 5'-nucleotides that may enhance the umami taste and the overall palatability of foods. In general, umami substances are widely distributed in seafoods such as sea urchin, abalone, crab, shrimp and lobster. Further, the umami substances contribute to the taste of seafood in association with other substances such as FAA, inorganic salts and organic acid (Komata, 1990). Thus, FAA are also responsible for the distinctive taste of many marine and other foods (Kato *et al.*, 1989).

Taste components of a number of seafood products have been examined by Kato *et al.* (1989) who found that each seafood product has its individual characteristic taste. Glycine, alanine and leucine were found to be the major amino acids in sea urchin (Komata *et al.*, 1962). Komata (1964) found that the characteristic components in sea urchin are glycine, alanine, valine, glutamine, methionine, inosine 5'-monophosphate (IMP) and guanosine 5'-monophosphate (GMP). Further, it was stated that glycine and alanine contributed to sweetness while valine was responsible for bitterness. Furthermore, glutamine, IMP and GMP contributed to the umami taste of sea urchin. Thus, FAA are one of the major extractive components that bring specific taste to seafoods (Fuke, 1994).

Glutamine and glycine are known to be taste-active in sea urchins, among other seafoods, irrespective of their amount. Sea urchin gonad seemed to be sweeter when it contained little or no glutamine. Alanine was found to occur in much higher

levels and function as a taste-active component in sea urchin. Furthermore, valine and methionine are taste-active only in sea urchin, while owing to its relatively high concentrations arginine is also taste-active in sea urchins (Fuke, 1994).

It has been found that different combinations of taste-active components as well as their relative amounts are of paramount importance in producing the characteristic flavour of each seafood. In addition pH of seafoods is a key factor since the taste of these components are pH dependent (Fuke, 1994). Shimada and Okajima (1989) reported that the taste of sea urchin gonads became more desirable when they were ripened by autolysis and these effects may be attributed to changes in FAA, among other substances, during processing. The contents of amino acids such as glycine, alanine, arginine and lysine are relatively high in sea urchin gonads. However, the content of alanine and lysine increased considerably with processing. In addition, there was an increase in other amino acid contents that were found originally in low quantities.

Sakaguchi and Murata (1989) have shown that in the body of oyster the FAA content underwent marked seasonal variations that could affect palatability. The FAA content was found to be higher in winter than in summer. On the other hand, Lee and Haard (1982) reported that optimum eating quality of sea urchin gonads was associated with low levels of total free amino acids. However, they stated that there was no direct relationship between sensory scores and total FAA content or glycine content. It is likely that the taste of sea urchin gonads is influenced in a complex way by the balance of FAA as well as other substances such as nucleotides (Hirano *et al.*, 1978).

The flavour of sea urchin gonads is affected by free glycine and alanine with a sweet taste that is abundant in the gonads of *S. nudus* that have grazed on kelp (Hirano *et al.*, 1978).When fish meal feeding period was prolonged the content of valine, with a bitter taste, increased with a simultaneous decrease in glycine and alanine contents. However, by shortening the fish meal feeding period and a subsequent change in the diet to kelp brought about an increase in glycine and alanine contents. Therefore, to improve the gonadal quality active grazing on algae of sea urchins should be maintained after fish meal feeding (Hoshikawa *et al.*, 1998).

2.14 Significance of RNA/DNA ratio

Traditional methods used for measuring growth rates in fish have yielded little insight into growth and environment relationships (Dutil *et al.*, 1998). In general, nutritional status of individuals assessed by proximate composition; as shown by Lambert and Dutil (1997), may be adequate (Holdway and Beamish, 1984). However, growth is a complex process influenced by many factors in the environment, some of them changing with days while others over longer periods and proximate composition is a static indicator of past growth performance (Dutil *et al.*, 1998). On the other hand this cannot be used to assess recent growth history unless measured periodically and on a suitable time scale (Dutil *et al.*, 1998). Nucleic acids reflect nutritional status and growth rate in several fish species (Bulow, 1970; Buckley, 1979; 1984; Mathers *et al.*, 1992; Foster *et al.*, 1993). In fish a positive correlation between growth rates and protein synthesis has been detected (Loughna and Goldspink, 1984). Thus, higher growth rates are expected to increase ribonucleic acid (RNA) concentrations and

thereby the ratio of RNA to deoxyribonucleic acid (DNA). The RNA content and RNA/DNA ratio have proven to be reliable indices of the nutritional condition of larval fish (Buckley, 1979, 1980, 1984; Wright and Martin, 1985; Buckley and Lough, 1987; Robinson and Ware, 1988; Canino *et al.*, 1991; Richard *et al.*, 1991; Clemmesen, 1993; Canino, 1994). Nucleic acid determination in marine habitats has attracted special attention because of the relationship between RNA/DNA ratios and the growth rates of a wide variety of marine organisms (Dortch *et al.*, 1983; Berdalet and Dortch, 1991; Berdalet and Estrada, 1993) such as bacteria (Mordy and Carlson, 1991; Jacobsen and Rasmussen, 1992), invertebrates (Sutcliffe, 1970) and fish (Buckley and Lough, 1987; Bulow, 1987).

Quantitative analysis of nucleic acids provides a relatively simple means of estimating recent growth rate of sea urchins. The processes of cellular growth and division require the synthesis of nucleic acids and proteins. The fact that RNA is an obligate precursor to protein synthesis has led to its use as an indicator of growth rate (Church and Robertson, 1966). The primary functions of RNA involve protein synthesis while DNA is the primary carrier of genetic information. As the bulk of the cellular DNA is chromosomal the quantity of DNA per cell is quasi-constant in somatic tissues and tissue DNA concentration therefore reflects cell numbers (Sulkin *et al.*, 1975; Bulow, 1987). Hence, DNA content has usually been used as an index of cell numbers or biomass (Regnault and Luquet, 1974). Thus, cellular RNA content is correlated with the rate of protein synthesis while DNA content may be used as an index of cell number since cellular DNA content remains constant in somatic tissues (Bulow, 1987). The RNA/DNA ratio has been employed as an estimate of growth for

a variety of invertebrates (Sulkin *et al.*, 1975). Thus, the RNA/DNA ratio is an index of the amount of protein synthetic activity per cell. Therefore, RNA/DNA ratio reflects the protein synthesising capacity and may be used for estimating recent *in situ* protein growth (Bulow, 1987; Robinson and Ware; 1988; Hovenkamp, 1990; Hovenkamp and Witte, 1991). Unfortunately, Mathers *et al.* (1994) showed that RNA/DNA ratios are not useful for estimating growth rates of wild fish larvae. In fact, correlation between RNA concentration or RNA/DNA ratio and growth rate has been observed for a wide variety of organisms (Bulow, 1970; Sutcliffe, 1970). It has been shown that RNA/DNA ratios generally reflect the growth conditions within the last few days prior to sampling thereby facilitating correlation between growth rate and environmental conditions measured at the time of sampling (Buckley, 1979; 1980; 1982; Buckley *et al.*, 1984). In general, the biosynthetic potential for growth of fish larvae can be determined from the quantity of RNA relative to DNA (Wright and Martin, 1985; Clemmesen, 1989; Richard *et al.*, 1991). Furthermore, RNA/DNA ratios allow comparison of nutritional status and recent growth of larvae thus a low RNA/DNA ratio indicating a lower growth potential (Robinson and Ware, 1988; Clemmeson, 1989). Hence, the RNA/DNA ratio has proven useful as an indicator of nutritional stress (Wright and Martin, 1985).

CHAPTER 03

MATERIALS AND METHODS

3.1 Materials

Wild sea urchins (*Strongylocentrotus droebachiensis*) were collected from the shore of Portugal Cove, Conception Bay in Newfoundland during the months of August and November (1999) as well as February and May (2000) to represent the four seasons summer, fall, winter and spring, respectively. These sea urchins were collected at a depth of 2 m by means of scuba divers. The cultured sea urchins were procured from Sea Urchin Research Facility (SURF) at Bonavista Bay, Newfoundland. Sea urchins captured from the wild were raised in raceways and fed on the grain based feed formulation shown in Table 3.1. Urchins were harvested for analysis at 0, 3, 6, and 9 weeks during the experimental feeding. In each case 125 sea urchins were procured. Sea urchins were transported in aquarium coolers and stored at 4 °C prior to the extraction of gonads and viscera. After extraction sea urchin gonads were homogenized for 2 min using a Waring Blender (Dynamics Corporation, New Hartford, CT). In this study sea urchin male and female gonads were pooled together for analysis. The tissues were frozen in liquid nitrogen and stored at -20 °C until used for further analysis. All chemicals used were obtained from either Fisher Scientific (Fair Lawn, NJ) or Sigma Chemical Co. (St Louis, MO). The solvents were of ACS grade, pesticide grade or HPLC grade.

Table 3.1. Composition of the grain based sea urchin feed formulation.

Constituent	Percentage (w/w)
Soy meal	19.625
Wheat middling	19.625
Barley	19.625
Corn meal	19.625
Laminaria longicruris	10.0
Gelatin	5.0
Lecithin	5.0
Sodium alginate	1.5

3.2 Proximate composition

3.2.1 Moisture content

Approximately 2 to 3 g of the homogenized samples were dried in a pre-weighed aluminum pan (Fisher Scientific, Unionville, ON) in a forced-air convection oven (Fisher Isotemp 300, Fair Lawn, NJ) at 105 °C overnight or until a constant weight was obtained. Moisture content was calculated as percentage weight loss of the sample during drying (AOAC 1990).

3.2.2 Ash content

Approximately 3 to 5 g of homogenized samples were placed in pre-weighed porcelain crucibles and charred using a Bunsen burner. The charred samples were heated in a muffle furnace (Thermolyne, F 62700, Dubuque, IA) at 550 °C overnight or until the entire sample turned grayish white. The weight of the residue was used to determine the ash content of each sample (AOAC 1990).

3.2.3 Crude protein content

Crude protein content of samples was determined by Kjeldhal method (AOAC 1990). Approximately 200 to 300 mg of homogenized samples were placed in pre-labeled digestion tubes with 20 mL of concentrated sulphuric acid (Fisher Scientific Co., Fair Lawn, NJ) and two catalyst tablets (Kjeltabs, Profamo Analytical Service Inc., Dorval, PQ). The samples were digested (Buchi 430 digester, Switzerland) for 50 to 60 min until the solution was clear or pale yellow. Fifty millilitres of distilled water and 150 mL of a

25% (w/v) solution of sodium hydroxide were added to the digested samples. The samples were then steam distilled (Buchi 321 distillation unit, Switzerland) and the distillate was collected into a 50 mL solution of 4% (w/v) boric acid containing 12 drops of methyl red/ methylene blue indicator (EM SCIENCE, Gibbstown, NJ). Distillation was continued until 150 mL of condensate was collected. The condensate was then titrated with a standardized 0.1 N sulphuric acid solution to a red end point. A blank determination was also carried out. The nitrogen content was calculated as a percentage using the equation given below and reported as crude protein content (N% x 6.25).

$$\text{Percentage N} = \frac{(V_{sample} - V_{blank}) \times N \times 14.0067 \times 100}{W}$$

where, V_{sample} = volume of titrant for sample (mL), V_{blank} = volume of titrant for blank, N = normality of H_2SO_4 solution used in the titration and W = weight of the sample (mg).

3.2.4 Total lipid content

Total lipids of samples were extracted and quantified by the Bligh and Dyer (1959) procedure. Approximately 25 g of each sample were homogenized with 25 mL of chloroform and 50 mL of methanol for 2 min using a Polytron PT 3000 (Brinkmann Instruments, Rexdale, ON) homogenizer. This was followed by the addition of another 25 mL of chloroform and blending for 30 s. To the mixture, 25 mL of water were added followed by blending for another 30 s. The homogenate was filtered through a Whatman No.1 filter paper using a Buchner funnel with slight suction. The residue and filter paper

were blended with an additional 35.5 mL of chloroform for 2 min and subsequently filtered as described above. The combined filtrates were transferred to a 250 mL separatory funnel and the two layers were allowed to separate. The chloroform layer containing total lipids was collected into a measuring cylinder and its volume recorded. Then a 10 mL of the chloroform layer containing the lipid were transferred to a pre-weighed 25 mL round bottom flask. The solvent was evaporated in a rotary evaporator (Buchi Rotavapor, Switzerland) at 40 °C (Buchi 461 water bath, Switzerland). The total lipid content of the sample was calculated gravimetrically.

3.2.5 Carbohydrate content

Carbohydrate content in each sample was determined by difference.

3.3 Separation of non-polar and polar lipid fractions by preparative thin layer chromatography (TLC)

Crude lipids extracted in section 3.2.4 were separated into non-polar and polar fractions by preparative-TLC using the solvent system hexane/ diethyl ether/ acetic acid (80:20:2,v/v/v) on silica gel G plates with UV indicator (20 x 20 cm, 250 µm, Aldrich Chemical Co. Inc., Milwaukee, WI) according to Christie (1982). A 0.1% (w/v) solution of 2,7'- dichlorofluorescein in 95% methanol was sprayed to render a better visibility to the simple and complex lipids. Then the silica gel was scraped off and the lipids eluted with an appropriate solvent. A 1 to 2% methanol in chloroform solution was used to elute

simple lipids while polar lipids were eluted with chloroform/ methanol/ water (5:5:1, v/v/v).

3.4 Analysis of fatty acid composition of lipids

Fatty acid composition of lipids was determined using gas chromatography (GC) according to Wanasundara and Shahidi (1997).

3.4.1 Preparation of fatty acid methyl esters

Fatty acid methyl esters (FAMEs) of total lipids (section 3.2.4) and polar and non polar lipids (section 3.3.5) were prepared by transmethylating approximately 10 to 20 mg of each lipid sample in 2 mL of freshly prepared transmethylating reagent {6% (v/v) sulphuric acid in 99.9 mole % HPLC-grade methanol containing 15 mg of t-butylhydroquinone (TBHQ)} at 65 °C for 15 h in a 6 mL Teflon-lined screw-capped conical vial. After incubation, the mixture was cooled and 1 mL of distilled water added to it. This was followed by extracting the FAMEs three times with 1.5 mL pesticide-grade hexane. A few crystals of TBHQ were added to each sample prior to extraction with hexane. The hexane layers were removed and combined in a clean test tube followed by washing twice with 1.5 mL of distilled water by vortexing. The aqueous layer was discarded at the first wash while the hexane layer was removed and placed in a GC vial after the second wash. Hexane was evaporated under a stream of nitrogen in a fume hood. The dried FAMEs were then dissolved in 1 mL of carbon disulphide and used for GC analysis.

3.4.2 Analysis of fatty acid methyl esters by gas chromatography

FAMEs were separated using a gas chromatograph (Hewlett-Packard 5890 Series II, Hewlett-Packard, Mississuaga, ON) equipped with a fused silica capillary column (SUPELCOWAX-10, 0.25 mm diameter, 30 m length, 0.25 μm film thickness; Supelco Canada Ltd., Oakville, ON). The sample was injected to the GC using a Hewlett-Packard 7673 autoinjector (Hewlett-Packard, Toronto, ON). The temperature of the oven was programmed at 220 °C for 10.25 min followed by ramping to 240 °C at 20 °C per min where it was held for 9 min. Helium at a flow rate of 2 mL per min was used as the carrier gas. The FAMEs were identified by comparing their retention times with those of authentic standard mixtures (GLC - 461, Nu-Check-Prep) or literature values (Takagi *et al.*, 1980; Takagi *et al.*, 1986). The relative content of fatty acids in the sample was determined using the peak areas of fatty acids.

3.5 Identification and quantification of major lipid classes by thin layer chromatography - flame ionization detection (TLC-FID) Iatroscan

3.5.1 Instrumentation

The crude lipids obtained from Bligh and Dyer (1959) extraction (section 3.2.4) were chromatographed on silica gel coated Chromarods - S III and then analyzed on an Iatroscan MK-5 (Iatroscan Laboratories Inc., Tokyo, Japan) analyzer equipped with a flame ionization detector (FID) connected to a computer loaded with TSCAN software (Scientific Products and Equipment, Concord, ON) for data handling. A hydrogen flow rate of 160 mL per min and an air flow rate of 2,000 mL per min were used in operating the FID. The scanning speed of rods was 30 s per rod.

3.5.2 Preparation of Chromarods

The Chromarods were soaked in concentrated nitric acid overnight followed by thorough washing with distilled water and acetone. The Chromarods were then impregnated with boric acid by dipping in a 3% (w/v) boric acid solution for 5 min in order to improve separation. Finally, the cleaned Chromarods were scanned twice to burn any remaining impurities.

3.5.3 Standards and calibration

A stock solution of each of the non-polar lipids: free fatty acid (FFA; oleic acid), cholesterol ester (CE), cholesterol (CHOL), monoacylglycerol (MAG; monoolein), diacylglycerol (DAG; diolein) and triacylglycerol (TAG; triolein) and the polar lipids: phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylinositol (PI), phosphatidylserine (PS), lysophosphatidylcholine (LPC), lysophosphatidylethanolamine (LPE) cardiolipin (CL) and sphingomyelin (SM) was prepared by dissolving them in chloroform/methanol (2:1, v/v) and stored at -20 °C. A range of dilutions of the stock solution from 0.1 to 10 µg per µL was prepared to be used as working standards. The Rf values were obtained for each standard (Sigma Chemical Co. St. Louis, MO) and using these Rf values and respective concentrations standard curves were obtained.

3.5.4 Iatroscan (TLC-FID) analysis of sea urchin lipids

The total lipids were extracted from samples as described in section 3.2.4. Lipids were dissolved in chloroform/methanol (2:1, v/v) in order to obtain a concentration of 1

µg lipid per mL. A 1 µL aliquot of sample was spotted on silica gel coated Chromarods - S III and conditioned in a humidity chamber containing saturated $CaCl_2$ for 20 min. The Chromarods were then developed in two solvent systems. According to the procedure described by Christie (1982) hexane/ diethyl ether/ acetic acid (80:20:2, v/v/v) were used as the solvent system for non-polar lipids (section 3.3.5). Following development the Chromarods were dried at 110 °C for 3 min and scanned completely to reveal non-polar lipids. For polar lipids (section 3.3.5) the Chromarods were first developed in the same solvent system used for non polar lipids and then dried at 110 °C for 3 min to remove solvents. This was scanned partially to a point just beyond the MAG peak to burn the non-polar lipids. These partially scanned Chromarods were developed in a second solvent system (Christie, 1982) of chloroform/ methanol/ water (80:35:2, v/v/v) for the separation of polar lipid classes. After development, the Chromarods were dried at 110 °C for 3 min and scanned completely to reveal polar lipids. The identity of each peak was determined by comparison with a chromatogram of standards acquired concurrently with the samples. The determination of weight percentages of individual lipid classes was achieved using the standard curves procured for each authentic standard.

3.6 Pigmentation

3.6.1 Extraction and determination of total and individual carotenoids

Carotenoids from each sample were extracted three times with a total of 50 mL acetone for 2 min. The homogenized samples were centrifuged (IEC Centra MP4 Centrifuge, International Equipment Co., Needham Heights, MA) for 5 min at 4000 x g.

Then the supernatant was filtered through a No 1 Whatman filter paper. Carotenoid pigments in acetone were then transferred to 40 mL of n-hexane in a 250 mL separatory funnel. One hundred millilitres of 0.5% sodium chloride solution were added to the mixture to maximise the transfer of carotenoids. The hexane layer was then transferred into a 50 mL volumetric flask and made up to volume. The absorption spectrum was recorded at 400 to 600 nm using a Spectronic spectrophotometer (Spectronic Genesis, Toronto, ON). The total and individual carotenoid contents were determined by the method of McBeth (1972). The total carotenoids present per 100 g of tissue were calculated using the following equation.

$$\text{mg Carotenoid per 100 g tissue} = \frac{A \times V \times 10^3}{E \times W}$$

where, A = absorbance at λ_{max}; V = total volume of the sample (mL); E = extinction coefficient and W = weight of the tissue (g). Since the crude extracts usually contained a variety of carotenoids an average coefficient of 2500 was used in the calculations.

The total pigment was separated into individual carotenoids by means of TLC. The crude carotenoids were separated by preparative TLC on silica gel G (20 x 20 cm, 250 µm, Aldrich Chemical Co. Inc., Milwaukee, WI) using acetone/ n-hexane (3:7, v/v) as the developing solvent. The various fractions obtained from TLC were scraped individually from the plate and eluted with 1-5% methanol in hexane. A visible absorption spectrum was determined for each fraction. The relative percentages of each fraction were calculated using the equation given below.

$$\text{Percentage caroteniod} = \frac{100 \times (V \times A)_{\text{for each fraction}}}{E \times (V \times A)_{\text{for all fractions}}}$$

3.6.2 Characterization of fractions

Co-chromatography on TLC provided the ultimate test for identification when authentic samples were available for comparison with unknown pigments. The unknown fraction and the authentic sample were spotted on either side in an equally proportionated mixture of the two pigments on silica gel G plates (20 x 20 cm. 250 µm. Aldrich Chemical Co. Inc., Milwaukee, WI) and unknown fractions were considered to be identical to the authentic sample if the two did not separate upon subsequent development of the plate. When authentic samples were not available the type of carotenoid in each fraction was tentatively identified according to its absorption maximum in n-hexane, ethanol and chloroform.

3.7 Determination of amino acid composition

3.7.1 Total amino acids

The amino acid composition of samples was determined according to the procedure described by Blackburn (1968). Samples were lyophilized and hydrolysed for 24 h at 110 °C with 6N HCl. Hydrochloric acid in the hydrolysate was removed under vacuum and the dried sample was reconstituted with a lithium citrate buffer (0.2 M, pH 2.2) for analysis. The amino acids in the hydrolysate were separated, identified and quantified using a Beckman 121 MB amino acid analyzer (Beckman Instruments Inc.,

Palo Alto, CA). Sulphur- containing amino acids were determined by oxidizing the samples with performic acid prior to their hydrolysis in a 6N HCl solution (Blackburn, 1978). Cysteine and methionine were measured as cysteic acid and methionine sulphone, respectively. To determine tryptophan samples were hydrolysed in 3M mercaptoethanesulphonic acid at 110 °C for 22 h under nitrogen and then neutralized with lithium hydroxide and adjusted to pH 2.2 (Penke *et al.*, 1974).

3.7.2 Free amino acids

Samples (10 g) were extracted with 20 mL of a 6% (v/v) perchloric acid (PCA) solution by homogenization using a Polytron homogenizer (Brinkmann Instruments, Rexdale, ON) at 10,000 rpm for 2 min in an ice bath. The homogenized samples were then incubated in an ice for 30 min. This was followed by centrifugation (IEC Centra MP4 Centrifuge, International Equipment Co., Needham Heights, MA) at 2000 x g for 15 min. The residue was re-extracted with another 20 mL of 6% PCA. The supernatants were combined and filtered through a Whatman No.4 filter paper. The pH of the filtrate was adjusted to 7.0 using a 33% KOH (w/v) solution. Potassium perchlorate precipitates were removed by centrifuging at 2000 x g for 10 min. The supernatant was then acidified to pH 2.2 using 10N HCl solution and the volume of the extract was brought to 50 mL with distilled water. Three millilitres of lithium citrate buffer (pH 2.2, 0.3M) were added to 1 mL of the extract and the resultant solution was analyzed on a Beckman 121 MB amino acid analyzer (Beckman Instruments Inc., Palo Alto, CA) for individual amino acids.

3.8 Determination of nucleic acid content

Deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) were extracted according to the method of Schmidt - Thannhauser (1945) as modified by Munro and Fleck (1969). Five grams of each of the sample were homogenized in 80 mL ice-cold deionized water using a Polytron homogenizer (Brinkman Instruments, Rexdale, ON) at 10.000 rpm. Five millilitres of the homogenate were allowed to stand for 10 min in ice. This was centrifuged (IEC Centra MP4 Centrifuge, International Equipment Co., Needham Heights, MA) at 2000 x g for 10 min. The residue was then washed with 2.5 mL of ice-cold 0.2N PCA and centrifuged at 2000 x g for 10 min. This was followed by digesting the residue in 4 mL of 0.3N KOH for 1 h at 37 °C in a water bath. The resultant solution was cooled in ice and mixed with 2.5 mL of 1.2N PCA and allowed to stand for 10 min which finally resulted in the coagulation of proteins. The mixture was centrifuged at 2000 x g for 10 min and the supernatant was recovered (Extract No.1). The precipitate was then washed twice with 2.5 mL of 0.2N PCA and centrifuged at 2000 x g for 5 min. The supernatant was combined with extract No. 1 and 10 ml of 0.6N PCA were added to the mixture. This was used for RNA determination after diluting it up to 100 mL level with distilled water. The residue was dissolved in 17 mL of 0.3N KOH at 37 °C and diluted to the 50 mL mark in an volumetric flask with distilled water. Deoxyribonucleic acid in the samples was estimated by determining the deoxyribose content in the extract using the indole procedure of Ceriotti (1952) while RNA was determined using ultraviolet spectrophotometric procedure by recording the absorbance values of nucleotide extracts at 260 nm using a Hewlett Packard diode array spectrophotometer

(Hewlett - Packard, Model 8452A, Hewlett-Packard (Canada) Ltd., Mississauga, ON). Protein interference at this wavelength was estimated by applying a correction factor of 0.001 absorbance unit per 1 µg per mL protein concentration in the extracts. The protein concentration of the extracts were measured using the Folin-phenol procedure of Lowry *et al.* (1951). Bovine serum albumin was used for standardization. Calf thymus DNA (containing 82% single stranded DNA) and calf liver RNA (96% purity) were used as the standards for DNA and RNA determinations, respectively.

3.9 Statistical analysis

All experiments except amino acid analysis were replicated 3 times and mean values ± standard deviations were reported for each sample. For statistical analysis mean values of experimental data were subjected to analysis of variance (One - way ANOVA) using GraphPAD Instat Version 1.0 (Motulsky, 1989). Significance was determined at 5% probability level.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Results

4.1.1 Proximate composition

4.1.1.1 Moisture content

Relative moisture contents of wild sea urchin gonads and viscera for the four seasons of spring, summer, fall, and winter are shown in Tables 4.1 and 4.2, respectively. The moisture content of both gonads and viscera varied significantly ($p<0.05$) with the season of catch. In gonads the amount of moisture varied from 72.5 $\pm$ 0.03 to 84.1 $\pm$ 0.1% while it ranged from 95.7 $\pm$ 0.1 to 96.6 $\pm$ 0.01% in the viscera. The moisture content was highest in the winter for gonads and was approximately 10, 11 and 14% more than that of the fall, summer and spring, respectively.

For sea urchins on artificial feed, the relative moisture content varied significantly ($p<0.05$) in the gonads with increased feeding time (Table 4.3). Moisture content initially increased by approximately 3% on week 3 followed by a subtle, but significant ($p<0.05$), increase on week 6. Thereafter, moisture level was significantly ($p<0.01$) decreased on week 9. The moisture content in the cultured sea urchin viscera was decreased significantly ($p<0.05$) from week 0 to week 9 (Table 4.4). Proximate composition of sea urchin's artificial feed is shown in Table 4.5.

Table 4.1 Proximate composition (weight %) of sea urchin gonads in spring, summer, fall and winter.

Constituent	Spring	Summer	Fall	Winter
Moisture	72.5 ± 0.03^{a}	75.0 ± 0.1^{b}	76.2 ± 0.1^{c}	84.1 ± 0.1^{d}
Ash	2.4 ± 0.1^{a}	1.8 ± 0.02^{b}	2.0 ± 0.1^{b}	2.9 ± 0.1^{c}
Protein	7.6 ± 0.2^{a}	11.1 ± 0.4^{b}	14.2 ± 0.3^{c}	9.5 ± 0.5^{d}
Lipid	6.1 ± 0.3^{a}	5.3 ± 0.1^{a}	2.8 ± 0.2^{b}	2.3 ± 0.2^{b}
Carbohydrate[\]	11.2 ± 0.2^{a}	5.5 ± 0.1^{b}	2.2 ± 0.1^{c}	0

Results are mean values of 3 replicates ± standard deviation. Values in each row with the same superscript are not different ($p>0.05$) from one another.

[\] Determined by difference.

Table 4.2 Proximate composition (weight %) of sea urchin viscera in spring, summer, fall and winter.

Constituent	Spring	Summer	Fall	Winter
Moisture	95.7 ± 0.1^{a}	96.6 ± 0.01^{b}	96.4 ± 0.02^{c}	96.0 ± 0.01^{d}
Ash	3.1 ± 0.06^{a}	2.4 ± 0.01^{b}	2.9 ± 0.01^{c}	3.2 ± 0.01^{a}
Protein	0.1 ± 0.1^{a}	0.2 ± 0.01^{b}	0.3 ± 0.01^{c}	0.2 ± 0.01^{b}
Lipid	0.1 ± 0.03^{a}	0.1 ± 0.02^{a}	0.1 ± 0.01^{a}	0.1 ± 0.01^{a}
Carbohydrate[x]	0.8 ± 0.1^{a}	0.6 ± 0.1^{a}	0.5 ± 0.03^{a}	0.2 ± 0.1^{b}

Results are mean values of 3 replicates ± standard deviation. Values in each row with the same superscript are not different ($p>0.05$) from one another.

[x] Determined by difference.

Table 4.3 Proximate composition (weight %) of sea urchin gonads at 0, 3, 6 and 9 weeks of feeding a grain based diet.

Constituent	Week 0	Week 3	Week 6	Week 9
Moisture	74.7 ± 0.04^{a}	77.4 ± 0.02^{b}	77.9 ± 0.04^{c}	75.5 ± 0.02^{d}
Ash	2.2 ± 0.2^{a}	1.2 ± 0.04^{bx}	1.0 ± 0.02^{bx}	1.4 ± 0.1^{c}
Protein	7.4 ± 0.2^{a}	6.0 ± 0.3^{b}	5.8 ± 0.1^{b}	7.9 ± 0.1^{a}
Lipid	4.7 ± 0.1^{a}	4.0 ± 0.2^{bx}	4.5 ± 0.1^{ab}	3.8 ± 0.2^{c}
Carbohydrate[x]	10.6 ± 0.2^{a}	11.6 ± 0.3^{a}	11.0 ± 0.2^{a}	11.2 ± 0.2^{a}

Results are mean values of 3 replicates ± standard deviation. Values in each row with the same superscript are not different ($p>0.05$) from one another.
[x] Determined by difference.

Table 4.4 Proximate composition (weight %) of sea urchin viscera at 0, 3, 6 and 9 weeks of feeding a grain based diet.

Component	Week 0	Week 3	Week 6	Week 9
Moisture	96.5 ± 0.03^{a}	96.2 ± 0.01^{b}	96.1 ± 0.0^{b}	95.2 ± 0.03^{c}
Ash	3.0 ± 0.02^{a}	2.8 ± 0.02^{b}	2.7 ± 0.1^{b}	2.6 ± 0.1^{b}
Protein	0.1 ± 0.02^{a}	0.1 ± 0.1^{a}	0.2 ± 0.1^{a}	0.5 ± 0.1^{b}
Lipid	0.1 ± 0.03^{a}	0.1 ± 0.02^{a}	0.1 ± 0.01^{a}	0.4 ± 0.02^{b}
Carbohydrate[x]	0.4 ± 0.1^{a}	0.9 ± 0.1^{b}	0.9 ± 0.1^{b}	1.3 ± 0.1^{c}

Results are mean values of 3 replicates ± standard deviation. Values in each row with the same superscript are not different ($p>0.05$) from one another.
[x] Determined by difference.

Table 4.5 Proximate composition (weight %) of artificial feed for sea urchins.

Component	Weight percentage (%)
Moisture	14.3 ± 0.03
Ash	3.3 ± 0.1
Total lipids	3.7 ± 0.3
Crude protein	13.6 ± 0.3
Carbohydrates[a]	66.7 ± 0.4

Results are mean ± standard deviation of three samples.
[a]Determined by difference.

4.1.1.2 Ash content

Ash content of wild sea urchin gonads is shown in Table 4.1. Results indicated an insignificant ($p>0.05$) change between summer and fall. Ash content was significantly ($p<0.05$) decreased from spring to fall followed by a significant ($p<0.05$) increase in the winter. The ash content of viscera from wild sea urchins also showed a somewhat similar pattern (Table 4.2).

In cultured sea urchin gonads ash level was significantly ($p<0.05$) decreased up to week 6 followed by an insignificant ($p<0.05$) increase on week 9 (Table 4.3). Cultured sea urchin viscera, on the other hand, showed a significant ($p<0.05$) decrease from week 0 to week 3. The ash content in the viscera further decreased with increased feeding period, but the decrease was not significant ($p>0.05$) among different harvests (Table 4.4).

4.1.1.3 Crude protein

The protein content of wild sea urchin gonads showed significant variations among different seasons (Table 4.1). The highest protein content ($14.2 \pm 0.3\%$) was attained in fall. The protein content increased from spring to fall significantly ($p<0.05$) followed by a significant ($p<0.05$) decrease in the winter. Protein content was lowest in the spring for gonads ($7.6 \pm 0.16\%$). The range of relative protein content in viscera was very narrow from 0.01 ± 0.02 to $0.3 \pm 0.01\%$ (Table 4.2).

However, a significant ($p<0.05$) increase was observed in the fall compared to other seasons.

Cultured sea urchin gonads showed a significant ($p<0.05$) decrease in the relative protein content from week 0 to week 6 followed by a significant ($p<0.05$) increase from week 6 to week 9. There were negligible differences in protein levels from week 0 to week 6 in cultured sea urchin viscera (Table 4.4). However, the protein content was significantly increased ($p<0.05$) from week 6 to week 9. As in wild sea urchin viscera, cultured sea urchin viscera also had very small amount of protein.

4.1.1.4 Total lipids

The total lipid content of wild sea urchin gonads in different seasons is shown in Table 4.1. There were no significant ($p>0.05$) differences in the total lipid content between spring and summer, although, gonads from animals harvested in the spring had the highest total lipid content ($6.1 \pm 0.3\%$). In the fall, total lipid content was significantly ($p<0.05$) decreased compared to that of the spring and the summer followed by an insignificant ($p>0.05$) decrease in winter. The viscera contained far less total lipids than gonads. Further, total lipids in sea urchin viscera did not show any significant ($p<0.05$) variation among seasons (Table 4.2).

The total lipid content of cultured sea urchin gonads was significantly ($p<0.05$) decreased by the end of week 9 (Table 4.3). Although, cultured sea urchin viscera, did not show any significant ($p>0.05$) variation from week 0 to 6 the total

lipid content was significantly ($p<0.05$) increased in week 9 (Table 4.4). Thus, total lipid content on week 9 was as much as 4 times that in week 0, 3 and 6. Both cultured and wild sea urchin viscera had very low levels of total lipid.

4.1.2 Identification and quantification of lipid classes of sea urchin lipids

4.1.2.1 Non-polar lipid classes

Major non-polar lipid classes present in wild sea urchin gonads (Table A.1) and viscera (Table A.2) were triacylglycerol (TAG), free fatty acids (FFA) and sterol (ST). Cultured sea urchin gonads (Table A.3) and viscera (Table A.4) also showed the same pattern of lipid class distribution. Triacylglycerols contributed the highest proportion to the total non-polar lipids of both wild and cultured sea urchin gonads and viscera. The content of TAG was highest in the spring for wild sea urchin gonads while viscera had its highest TAG levels in the fall (Fig 4.1). Thus, gonads and viscera showed contradictory patterns of variation of TAG among seasons. The content of TAG in gonads decreased significantly ($p<0.05$) from spring to winter although this decrease was not significant ($p>0.05$) among summer, fall and winter. In cultured sea urchin gonads, the content of TAG significantly ($p<0.05$) increased with increased feeding period (Fig 4.2). Triacylglycerol content increased from 66.7 ± 0.8 on week 0 to 77.4 ± 0.8% on week 9 in cultured sea urchin gonads. Cultured sea urchin viscera, on the other hand, showed a significant ($p<0.05$) increase in TAG from one harvest to another. The TAG content of cultured sea urchin viscera increased from 56.5 ± 0.7 on week 0 to 83.4 ± 1.2% on week 9.

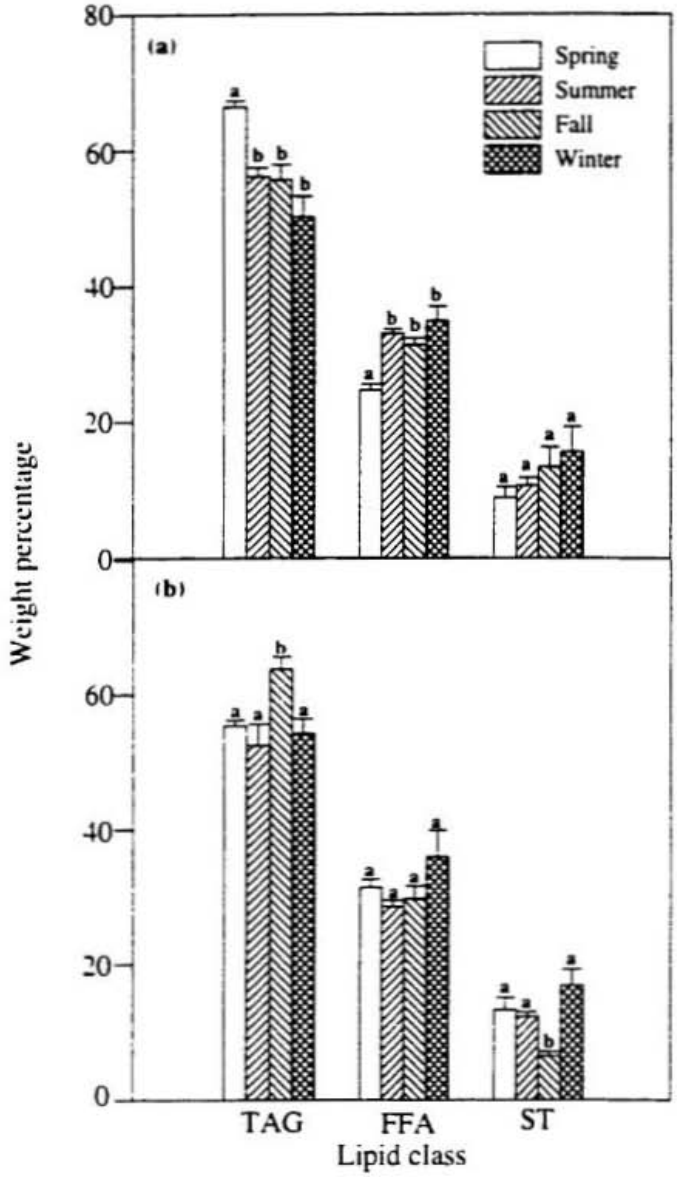


Fig 4.1 Contents of triacylglycerol (TAG), free fatty acids (FFA) and sterol (ST) in non-polar lipids of sea urchin (a) gonads and (b) viscera in spring, summer, fall and winter. Bars with the same letter within a group are not significantly ($p>0.05$) different from one another.

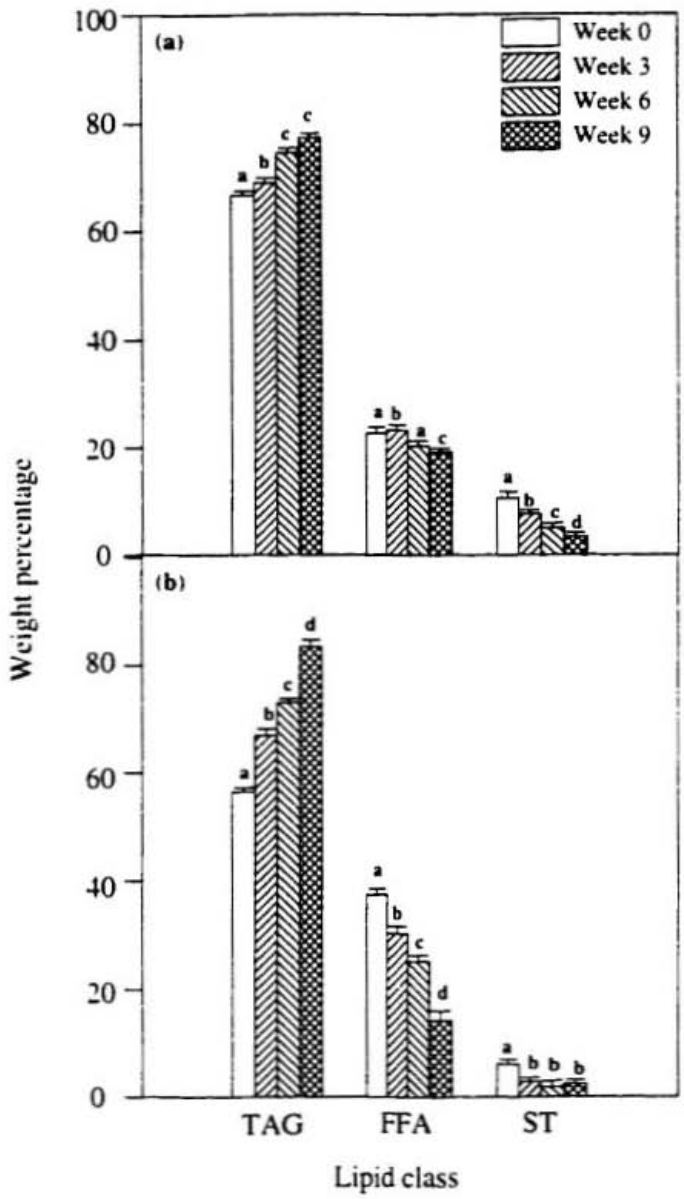


Fig 4.2 Contents of triacylglycerol (TAG), free fatty acids (FFA) and sterol (ST) in non-polar lipids of sea urchin (a) gonads and (b) viscera at 0, 3, 6 and 9 weeks of feeding on an artificial diet.
Bars with the same letter within a group are not significantly ($p>0.05$) different from one another.

Free fatty acids were the second predominating group that contributed to non-polar lipids in both wild and cultured sea urchin tissues. In wild sea urchin gonads FFA content was highest (34.9 ± 2.2%) in the winter while the lowest FFA content was observed for the spring harvest (24.7 ± 0.9%). Free fatty acid content did not show significant ($p>0.05$) differences among seasons in the wild sea urchin viscera. In cultured sea urchin gonads FFA content was significantly ($p<0.05$) increased from week 0 to week 3 (Fig 4.2). However, this was followed by a significant ($p<0.05$) decrease of FFA from week 3 to week 9. The FFA content of cultured sea urchin viscera, on the other hand, showed a significant ($p<0.05$) decrease from 37.4 ± 1.0 on week 0 to 14.2 ± 1.8% on week 9.

Sterol content was increased from spring to winter in the wild sea urchin gonads (Fig 4.1) although this increase was not significant ($p<0.05$) between consecutive seasons. Similar to gonads, sea urchin viscera had the highest ST content in the winter (Fig 4.1), but this was significantly ($p<0.05$) decreased from spring to fall.

The content of ST in cultured sea urchin gonads was highest on week 0 and this was significantly ($p<0.05$) decreased with increasing feeding period, reaching a minimum on week 9. Cultured sea urchin viscera also displayed a significant ($p<0.05$) decrease in its ST content from week 0 to week 9 although this was not significant ($p>0.05$) among consecutive harvests. The effect of artificial diet on major non-polar lipid components of sea urchin gonads and viscera is shown in Fig 4.3.

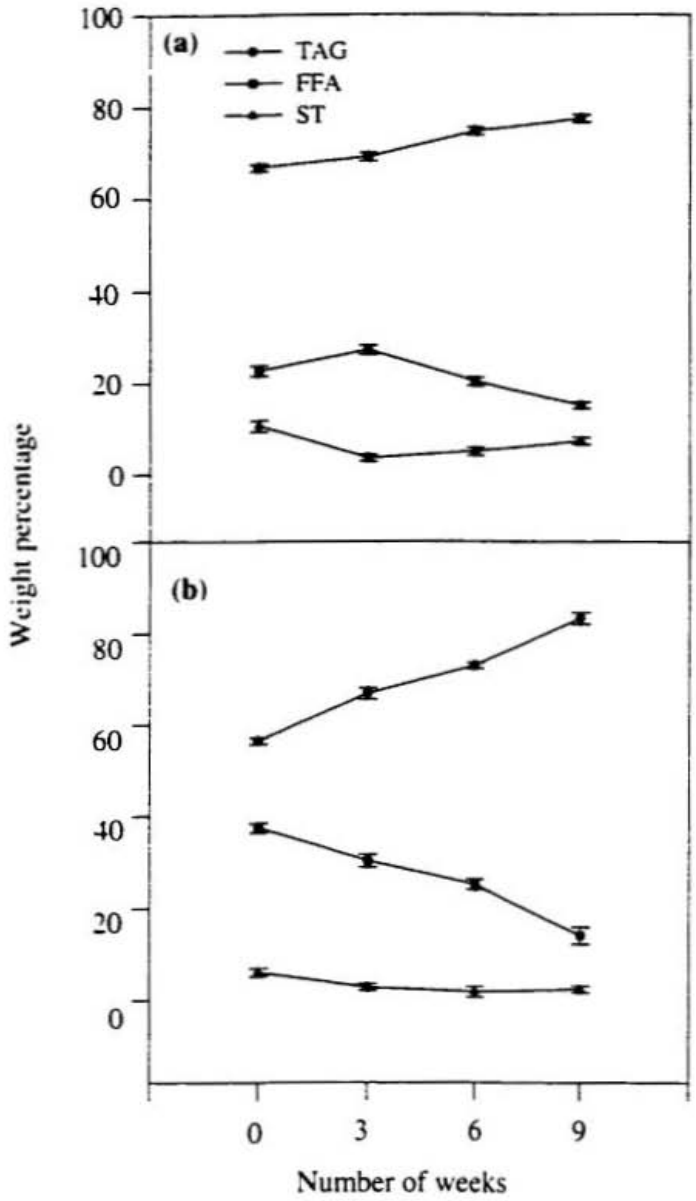


Fig 4.3. Effect of artificial diet on non-polar lipid constituents of sea urchin (a) gonads and (b) viscera.

4.1.2.2 Polar lipid classes

Major polar lipid classes of wild sea urchin gonads were phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylserine / phosphatidylinositol (PS/PI) and sphingomyelin/ lysophosphatidylcholine (SM/LPC) (Table A.5). Phosphatidylcholine was the dominant polar lipid class and contributed more than 50% to the total amount in each season (Fig 4.4). The content of PC decreased from spring to winter, but the change was not significant ($p>0.05$) between consecutive seasons. PC, PE and SM/LPC were the major polar lipid classes in the wild sea urchin viscera (Table A.6). The wild sea urchin viscera showed an insignificant ($p>0.05$) decrease in PC content from spring to summer followed by a significant ($p<0.05$) increase from summer to winter.

Similar to wild sea urchin tissues, PC and PE were the main polar lipid constituents in cultured sea urchin gonads (Table A.7) and viscera (Table A.8). In addition, cultured sea urchin gonads and viscera contained a considerable amount of SM/LPC. Cultured sea urchin gonads and viscera had very high levels of PC on week 0 (Fig 4.5). However, PC content decreased with increased feeding time compared to that on week 0. The PE content, on the other hand, increased from week 0 to week 9 in both gonads and viscera of cultured sea urchins. In gonads PE increased significantly ($p<0.05$) from one harvest period to another while in viscera the increase was not significant ($p>0.05$) between consecutive harvests. The influence of artificial feed on the major polar lipid constituents of sea urchin gonads and viscera is shown in Fig 4.6. Changes occurred in PC and PE content in the wild sea urchin gonads and

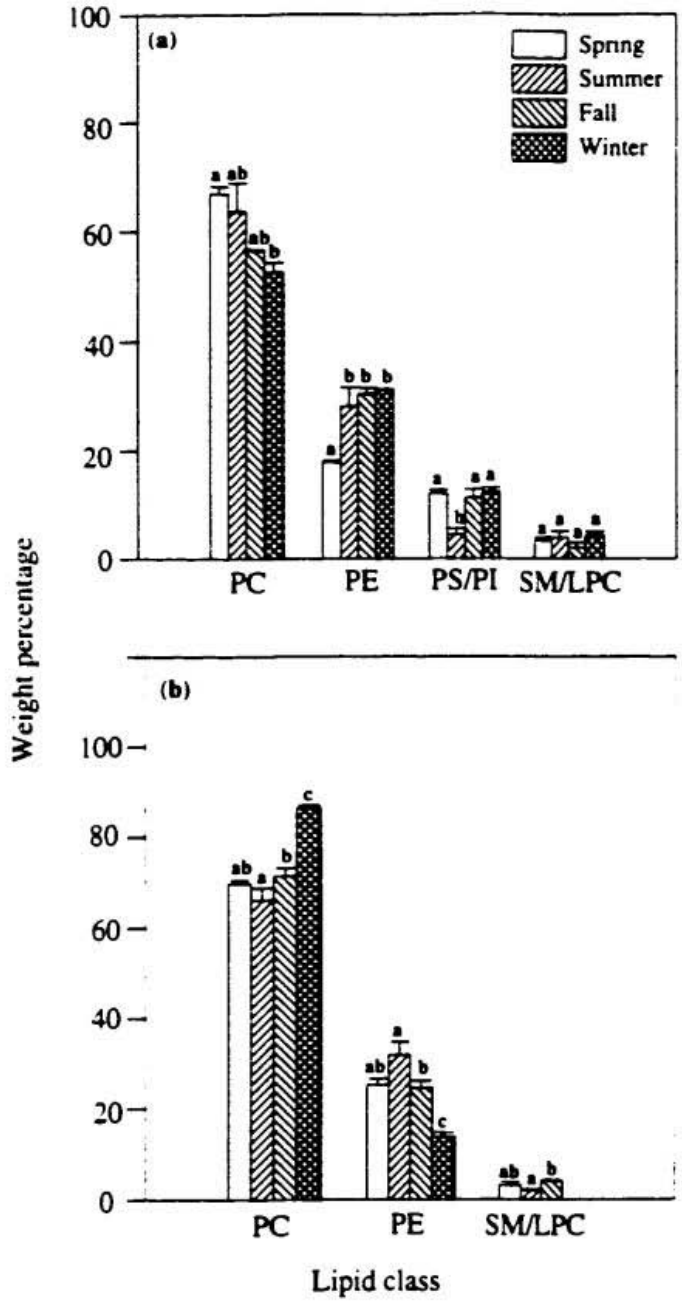


Fig 4.4. Contents of phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylserine/phosphatidylinositol (PS/PI) and sphingomyelin/lysophosphatidylcholine (SM/LPC) in the polar lipids of sea urchin (a) gonads and (b) viscera in spring, summer, fall and winter.
Bars with the same letter within a group are not significantly ($p>0.05$) different from one another.

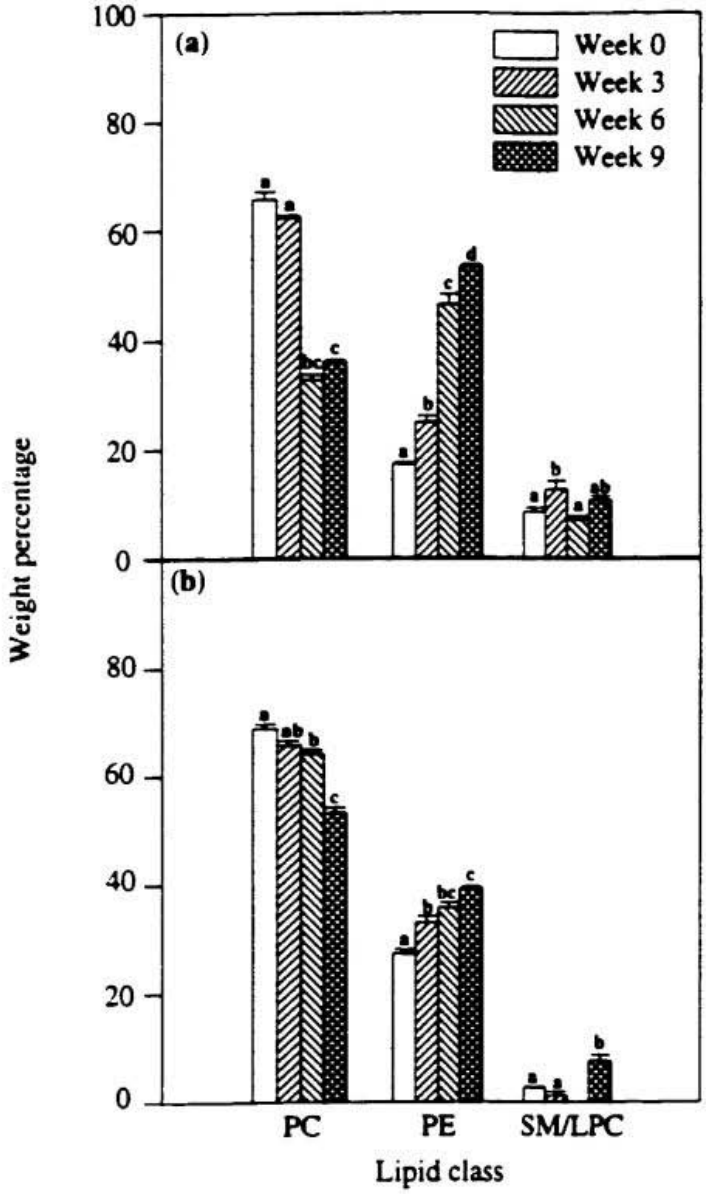


Fig 4.5. Contents of phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylserine/phosphatidylinositol (PS/PI) and sphingomyelin/lysophosphatidylcholine (SM/LPC) in the polar lipids of sea urchin (a) gonads and (b) viscera in spring, summer, fall and winter.
Bars with the same letter within a group are not significantly ($p>0.05$) different from one another.

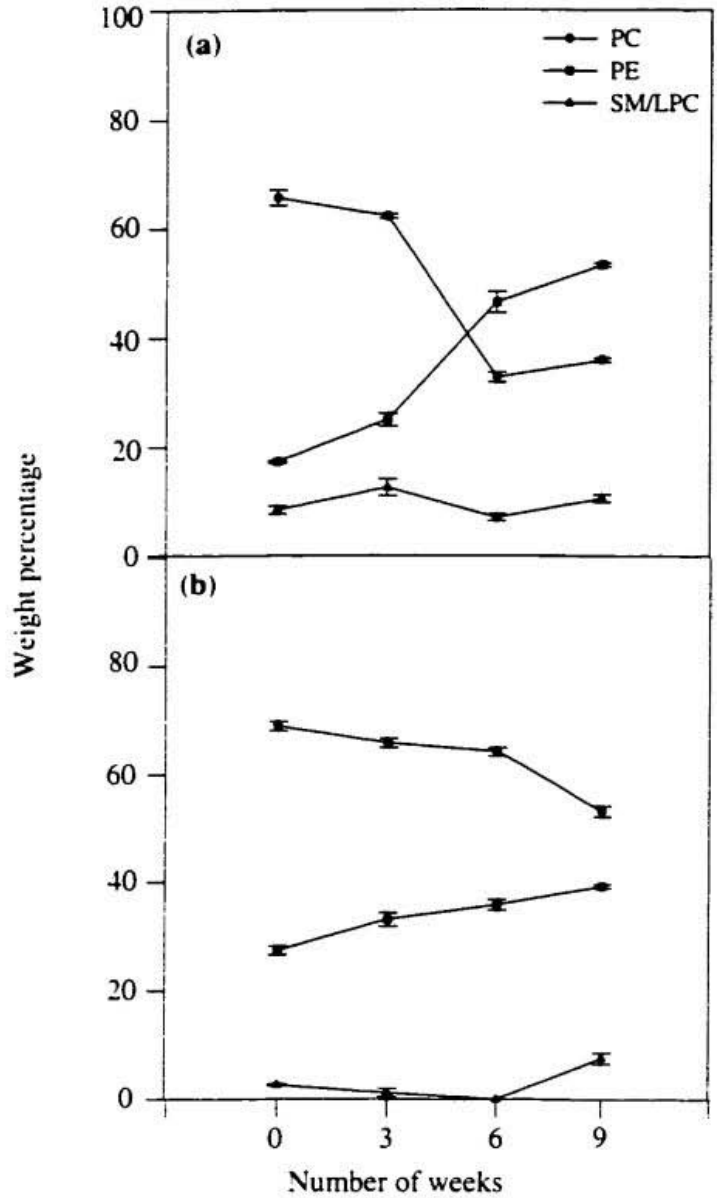


Fig 4.6. Effect of artificial diet on polar lipid constituents of sea urchin (a) gonads and (b) viscera over 9 weeks.

viscera, but trends were reversed in cultured sea urchin gonads and viscera. The SM/LPC content did not show a clear pattern of variation between consecutive harvests in gonads and viscera of cultured sea urchins.

4.1.3 Fatty acid composition of sea urchin lipids

4.1.3.1 Seasonal effects on fatty acid composition of total lipids

Fatty acid composition of total lipids of sea urchin gonads and viscera in the spring, summer, fall and winter is shown in Table A.9 and Table A.10, respectively. Fig 4.7 represents the major fatty acids obtained in the total lipids of sea urchin gonads (a) and viscera (b). All fatty acids listed in Fig 4.7 contributed more than 2% to the total fatty acids. The dominant saturated fatty acids (SFA) in total lipids of sea urchin gonads and viscera were 14:0 and 16:0. In addition, 18:0 occurred in considerable amounts in both tissues throughout the study period. The 5-monoenoic fatty acid 20:1 n-15 was the dominant MUFA in almost all the seasons.

The major polyunsaturated fatty acids (PUFA) were 18:4n-3, 20:2 5,11, 20:4n-6 and 20:5n-3 in both gonads and viscera of sea urchins with 20:5n-3 being the dominant fatty acid irrespective of the season. Further, sea urchin tissues had the non-methylene-interrupted eicosadienoic acid (NMID), 20:2 5,11 as a major fatty acid. Among other NMIDs 20:2 5,13 was also detected in both gonads and viscera throughout the study period. However, the proportion of 20:2 5,11 was always greater

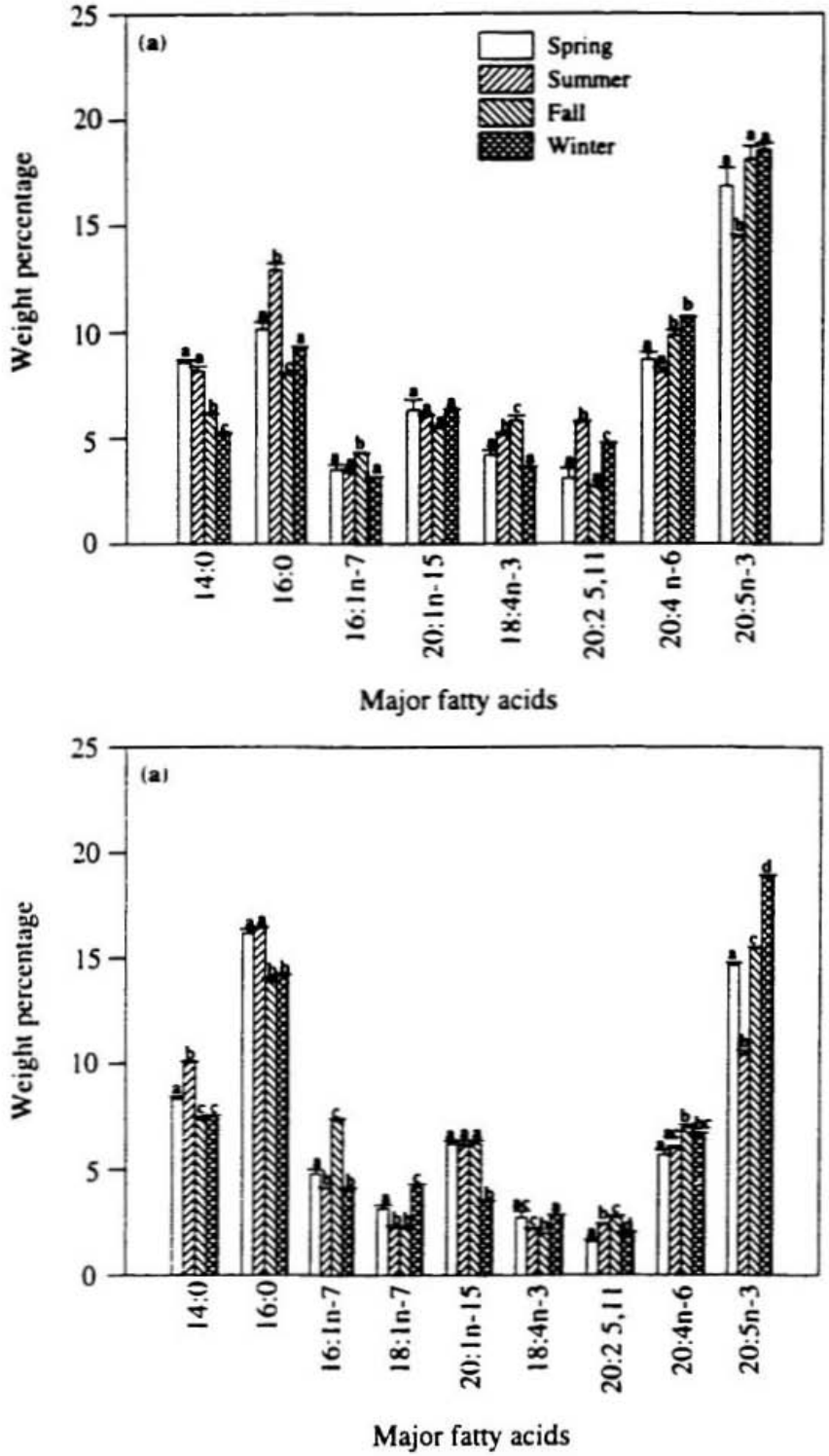


Fig 4.7. Major fatty acids of total lipids of sea urchin (a) gonads and (b) viscera in spring, summer, fall, and winter.
Bars with the same letter within a group are not significantly ($p>0.05$) different from one another.

than that of 20:2 5.13 in each instance. Other NMIDs detected as a minor component in gonads and viscera of sea urchins were 20:2 7.13 and 20:2 7.15. but their presence was not consistent.

Total lipids of both sea urchin gonads and viscera showed a predominance of total PUFA in each season (Fig 4.8). Total PUFA content was highest in the winter while the total SFA content was highest in the summer. Sea urchin viscera also showed the same pattern of variation in total PUFA and SFA content.

4.1.3.2 Seasonal effects on fatty acid composition of polar lipids

Fatty acid profiles obtained for polar lipids of sea urchin gonads and viscera are shown in Tables A.11 and A.12. respectively. The fatty acids that contributed more than 2% to the total fatty acids are shown as major fatty acids in Fig 4.9. Dominant SFA in both gonads and viscera were 14:0 and 16:0. Viscera, however, contained 18:0 in addition to the above fatty acids. Major MUFA in sea urchin gonads and viscera were 18:1n-7. 20:1n-15 and 20:1n-11 in each season. In polar lipids the 5-monoenoic acid. 20:1n-15. contributed the highest proportion to the total MUFA content. while being the dominant MUFA in each season. The dominant PUFA in gonads and viscera were 20:4n-6 and 20:5n-3 with gonads having 20:2 5.11 also in high amounts. In polar lipids 20:5n-3 was also dominant in each season. Fig 4.10 shows that polar lipids of sea urchin viscera and gonads were also dominated by PUFA in each season.

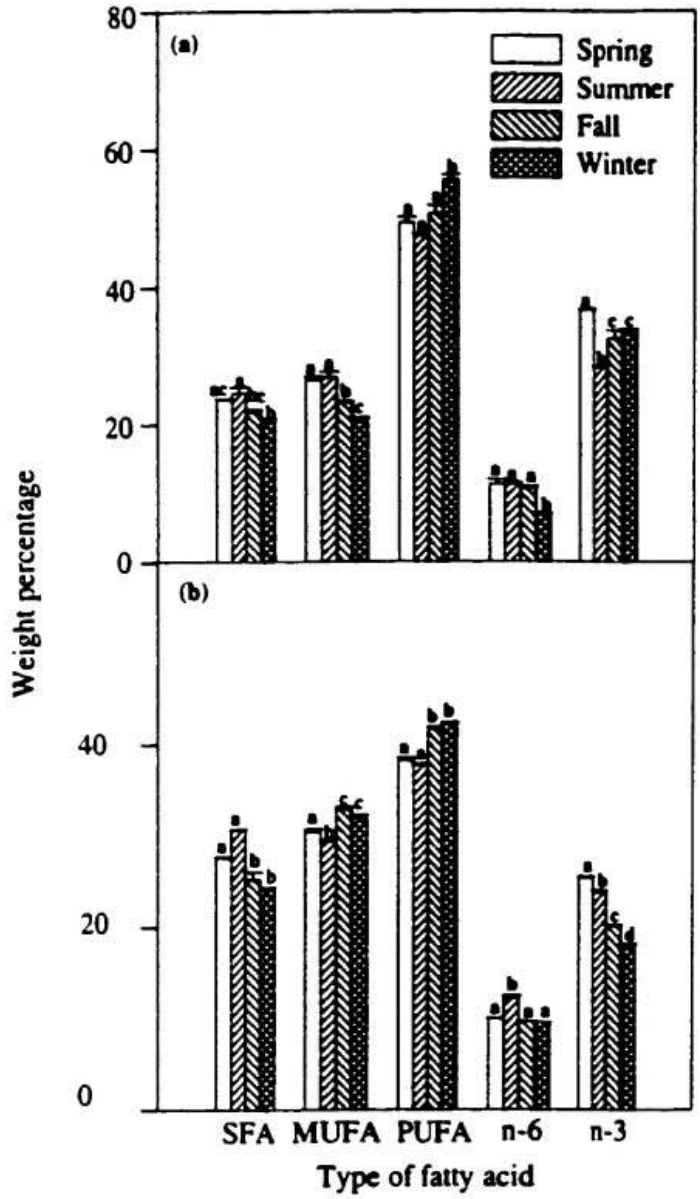


Fig 4.8. Contents of saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA), omega-6 (n-6) and omega-3 (n-3) fatty acids of total lipids of sea urchin (a) gonads and (b) viscera in spring, summer, fall and winter. Bars with the same letter within a group are not significantly ($p>0.05$) different from one another.

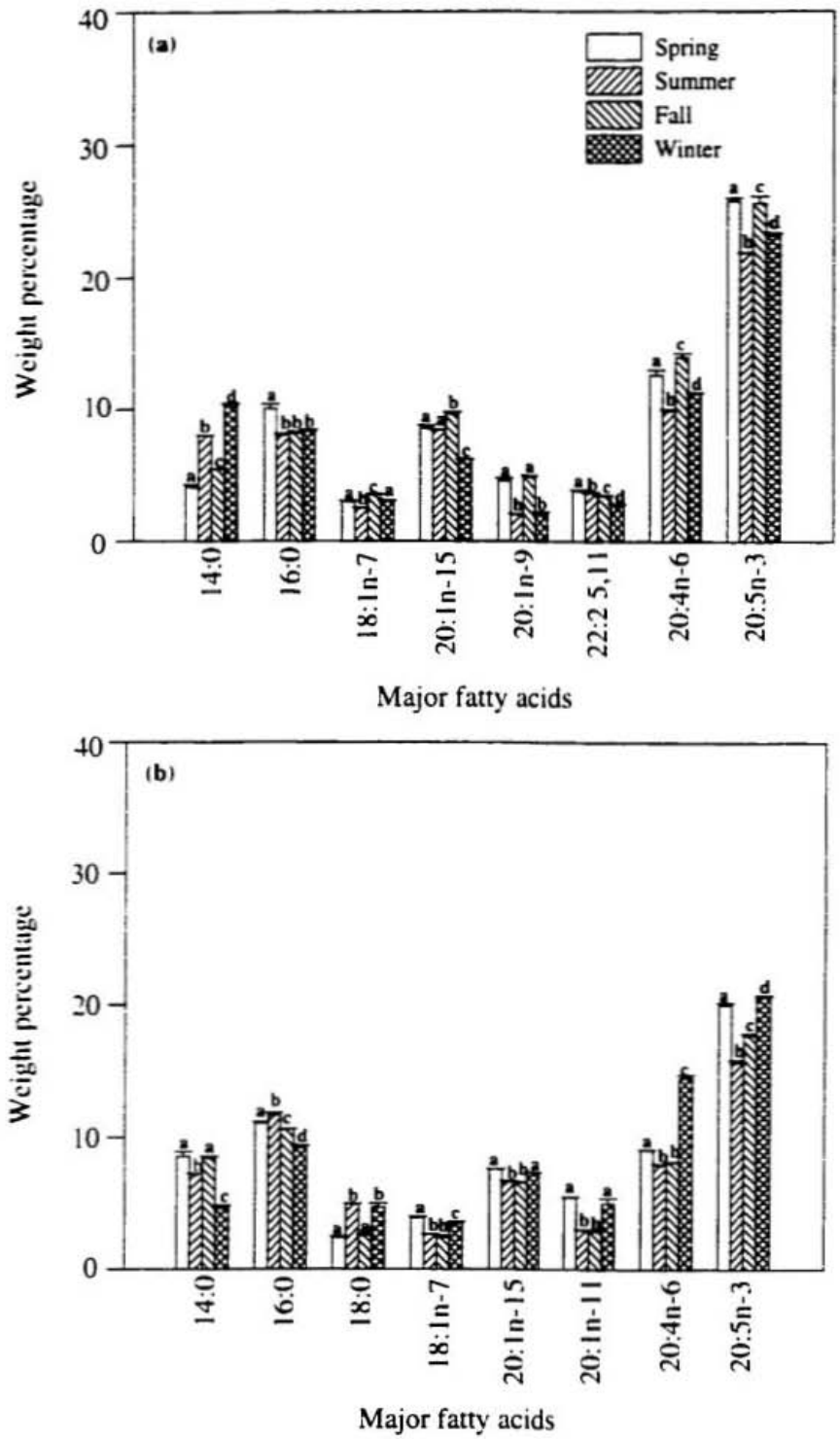


Fig 4.9 Major fatty acids of polar lipids of sea urchin (a) gonads and (b) viscera in spring, summer, fall and winter.
Bars with the same letter within a group are not significantly ($p>0.05$) different from one another.

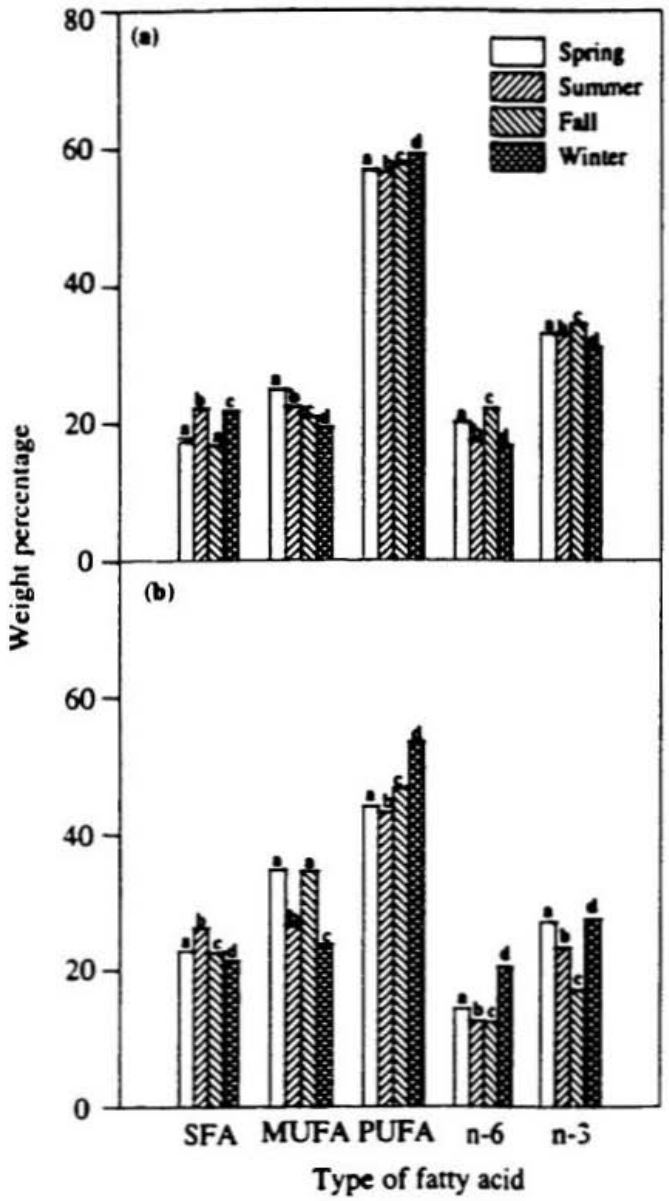


Fig 4.10. Contents of saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA), omega-6 (n-6) and omega-3 (n-3) fatty acids of polar lipids of sea urchin (a) gonads and (b) viscera at 0, 3, 6 and 9 weeks of feeding on an artificial diet.
Bars with the same letter within a group are not significantly ($p>0.05$) different from one another.

4.1.3.3 Seasonal effects on fatty acid composition of non-polar lipids

Tables A.13 and A.14 display the fatty acid compositions of non-polar lipids of sea urchin gonads and viscera, respectively. Further, Fig 4.11 is a representation of major fatty acids (those fatty acids that made a contribution above 2% to total fatty acids) of the non-polar lipids of sea urchin tissues analyzed. Non- polar lipids of sea urchin gonads and viscera contained 14:0 and 16:0 as the major SFA. Among the MUFA that were common to both gonads and viscera, 5-monoenoic acid 20:1n-15 contributed the highest proportion to the total MUFA content. In addition, gonads had 16:1n-7 while viscera had 18:1n-7 and 22:1n-9 as major MUFA. Major PUFA in sea urchin gonadal non-polar lipids were 18:4n-3, 20:2 5,11, 20:4n-6 and 20:5n-3 with the latter being the dominant PUFA in each season.

Major PUFA of sea urchin viscera were 20:2 5,11, 20:4n-6 as well as 20:5n-3 which was the dominant fatty acid irrespective of the season. Fig 4.12 depicts that even non-polar lipids of sea urchin gonads and viscera were characterized by having a predominance of total PUFA in their fatty acid compositions. Variations occurred in non-polar lipids were very much similar to those of total lipids of sea urchin tissues.

4.1.3.4 Dietary effects on fatty acid composition of total lipids

Fatty acid profiles of total lipids of cultured sea urchin gonads and viscera are summerised in Tables A.15 and A.16, respectively, while Fig 4.13 represents the major fatty acids (fatty acids that contribute more than 2% to total fatty acids except 18:2n-6 whose contribution was less than 2% initially, but became the dominant one

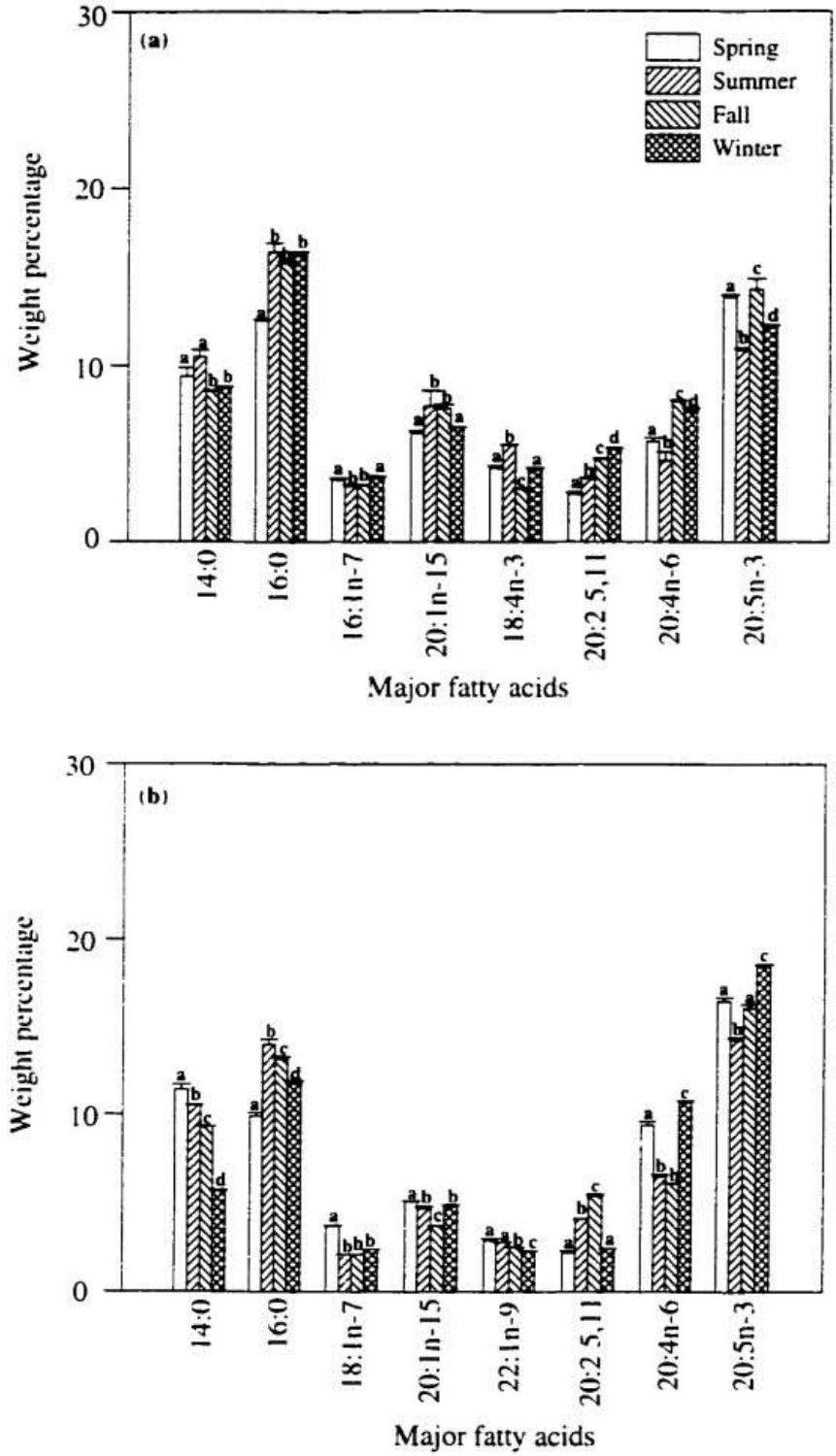


Fig 4.11 Major fatty acids of non-polar lipids of sea urchin (a) gonads and (b) viscera in spring, summer, fall and winter.

Bars with the same letter within a group are not significantly ($p>0.05$) different from one another.

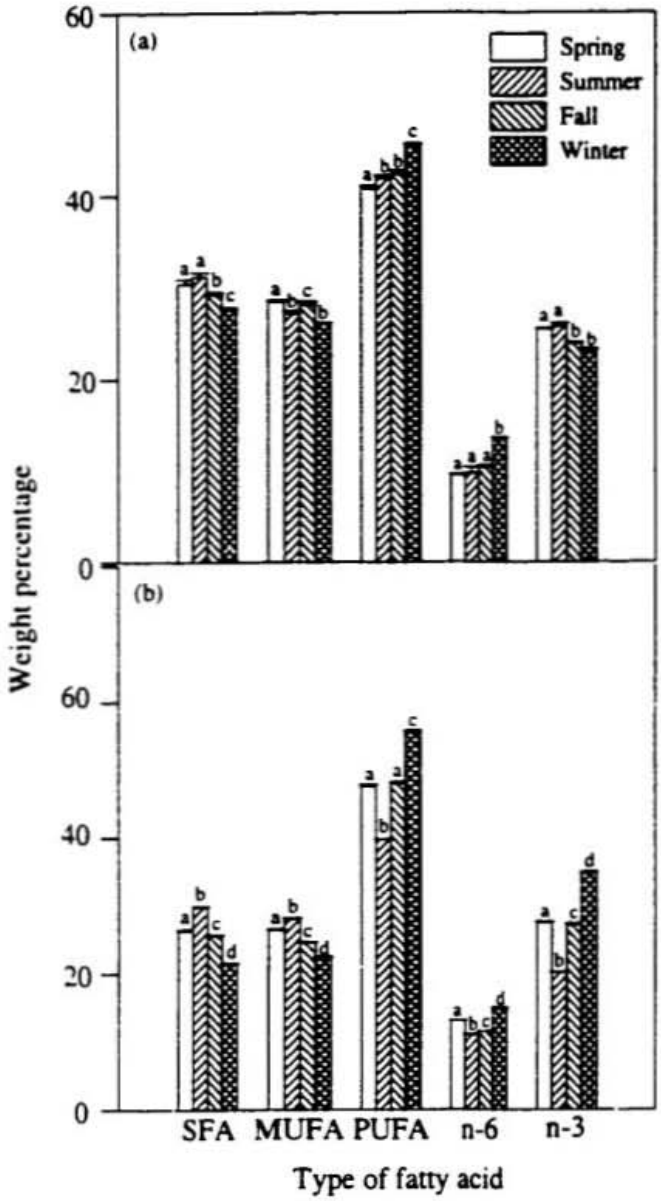


Fig 4.12. Contents of saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA), omega-6 (n-6) and omega-3 (n-3) fatty acids in non-polar lipids of sea urchin (a) gonads and (b) viscera in spring, summer, fall and winter. Bars with the same letter within a group are not significantly ($p>0.05$) different from one another.

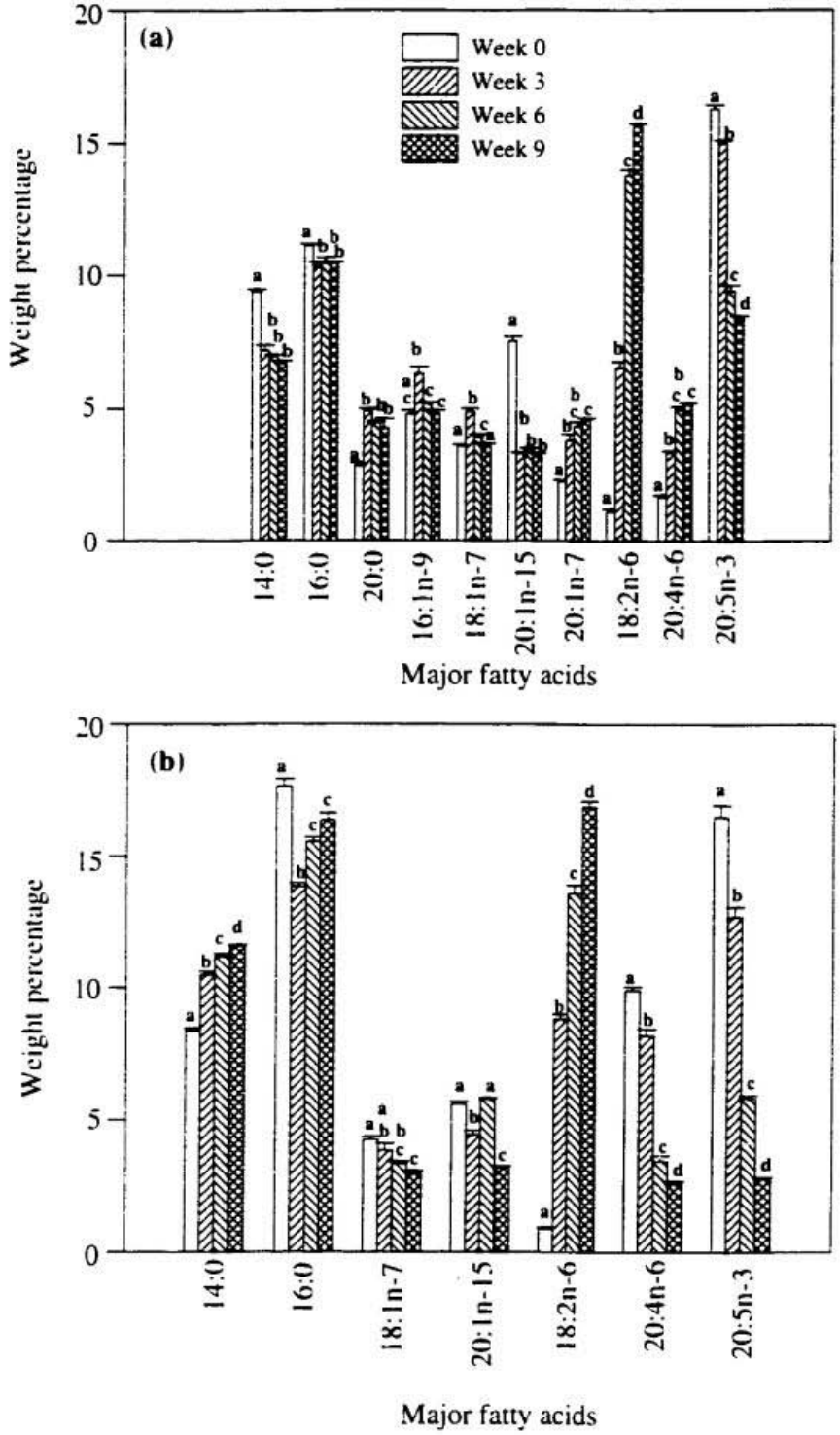


Fig 4.13. Major fatty acids of total lipids of sea urchin (a) gonads and (b) viscera at 0, 3, 6 and 9 weeks of feeding on an artificial diet.

Bars with the same letter within a group are not significantly ($p>0.05$) different from one another.

later on) to the total lipids. Total lipids of cultured sea urchin gonads had 14:0, 16:0 and 20:0 as their major SFA while viscera had 14:0 and 16:0. Major MUFA in cultured sea urchin gonads were 16:1n-9, 18:1n-7, 20:1n-15 and 20:1n-7 while 18:1n-7 and 20:1n-15 were the major MUFA in the viscera. In sea urchin gonads 20:1n-15 was dominant on week 0 while 16:1n-9 became dominant after feeding of sea urchins on the artificial feed. In cultured sea urchin viscera 20:1n-15 was the dominant MUFA before and after feeding the artificial diet. Major PUFA of cultured sea urchin gonads as well as viscera were 18:2n-6, 20:4n-6 and 20:5n-3. The dominant PUFA on week 0 was 20:5 n-3 while with increased feeding time 18:2n-6 became dominant. Thus, these two PUFA showed an opposite pattern of variation.

According to Fig 4.14 it is apparent that even in cultured sea urchins both gonads and viscera showed a predominance of total PUFA content. However, total PUFA content decreased significantly ($p<0.05$) by week 9 in sea urchin viscera. The content of total SFA in gonads decreased with increasing feeding period. In contrast, in viscera on week 9 total SFA content was significantly ($p<0.05$) increased. The effect of the artificial diet on the fatty acids 18:2n-6 and 20:5n-3 in the total lipids of gonads and viscera is shown in Fig 4.15.

4.1.3.5 Dietary effects on fatty acid composition of polar lipids

Fatty acid compositions of polar lipids of cultured sea urchin gonads and viscera are given in Tables A.17 and A.18, respectively. Further, major fatty acids of sea urchin tissues are shown in Fig 4.16. Major SFA were 14:0, 16:0 and 18:0 in both

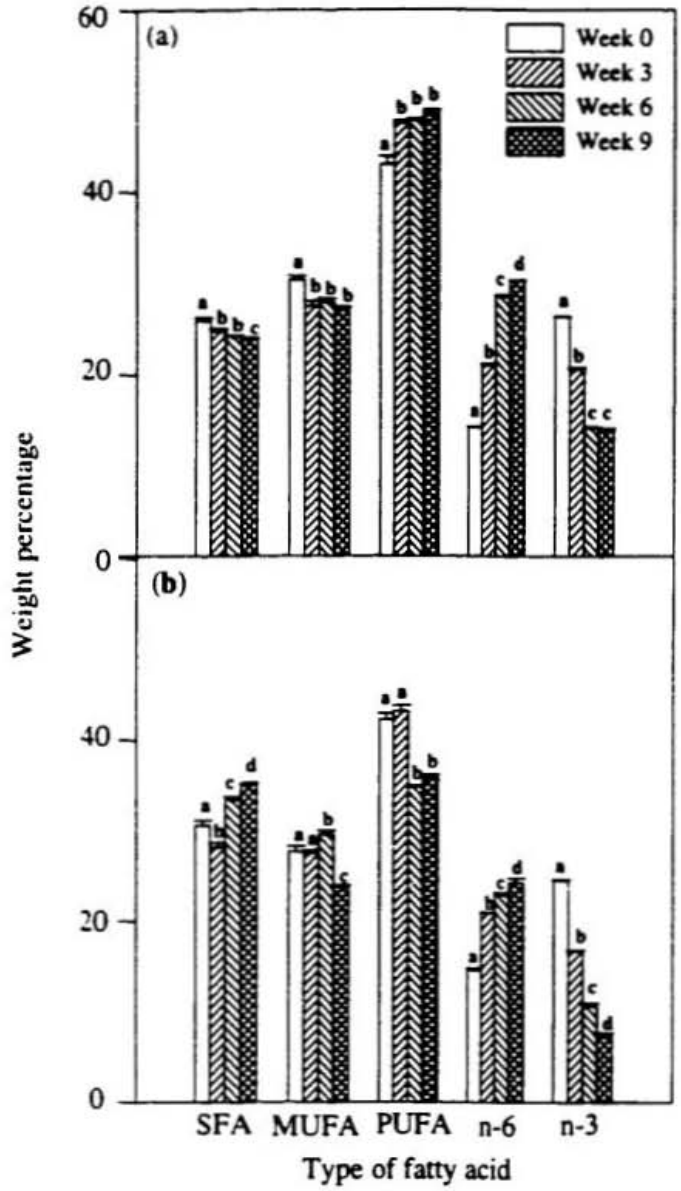


Fig 4.14. Contents of saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA), omega-6 (n-6) and omega-3 (n-3) fatty acids in total lipids of sea urchin (a) gonads and (b) viscera at 0, 3, 6 and 9 weeks of feeding on an artificial diet

Bars with the same letter within a group are not significantly ($p>0.05$) different from one another.

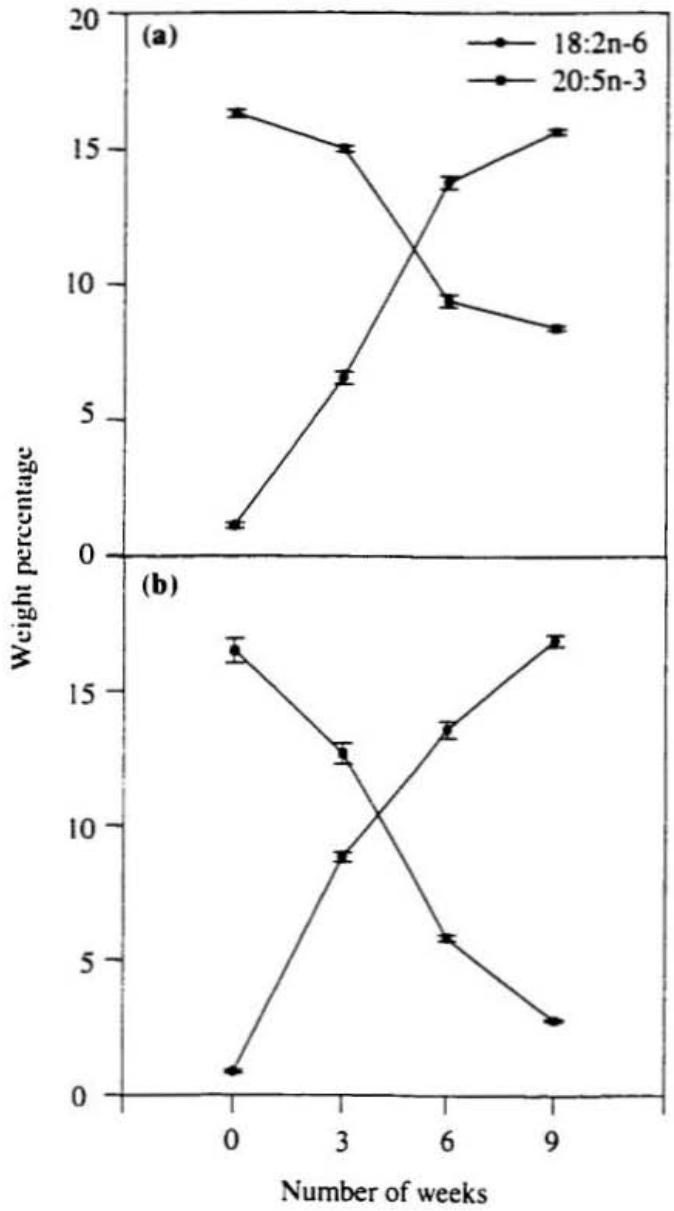


Fig 4.15. Effect of artificial diet on 18:2n-6 (LA) and 20:5n-3 (EPA) contents of sea urchin total lipids of (a) gonads and (b) viscera.

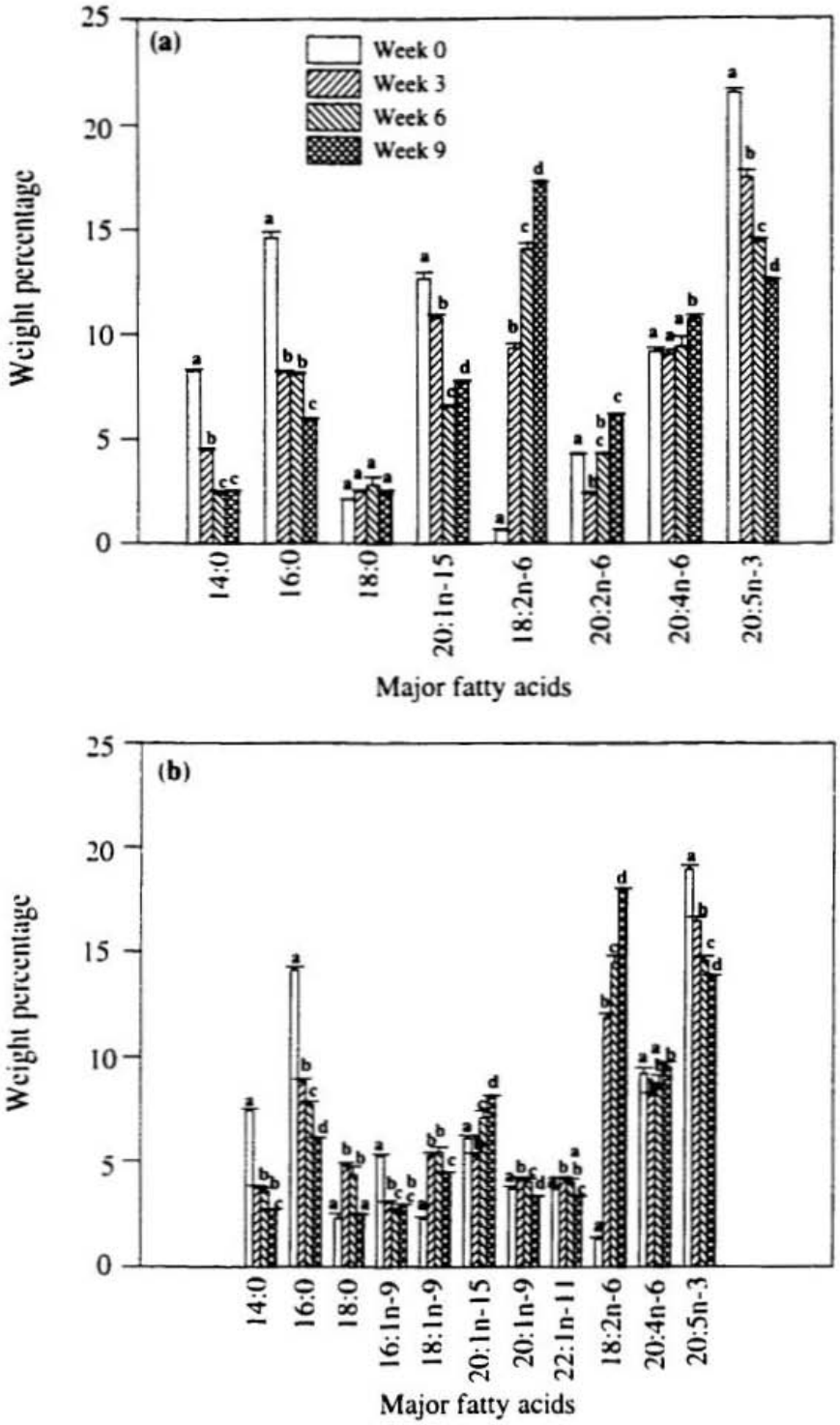


Fig 4.16. Major fatty acids of polar lipids of sea urchin (a) gonads and (b) viscera at 0, 3, 6 and 9 weeks of feeding on an artificial diet.
Bars with the same letter within a group are not significantly ($p>0.05$) different from one another.

gonads and viscera of cultured sea urchin polar lipids. Five-monoenoic acid 20:1n-15 was the major MUFA of polar lipids of cultured sea urchins. In addition, 16:1n-9, 18:1n-9, 20:1n-9 and 22:1n-11 contributed considerably to the polar lipids in cultured sea urchin viscera. The major PUFA in both gonads and viscera were 18:2n-6, 20:4n-6 and 20:5n-3. However, sea urchin gonads had a reasonably high level of 20:2n-6. The fatty acid 20:5n-3 was dominant in the PUFA in both gonads and viscera on week 0. With increased feeding period the content of 20:5n-3 decreased significantly ($p<0.05$) while that of 18:2n-6 increased significantly ($p<0.05$). On week 9, 18:2n-6 became the dominant PUFA in both gonads and viscera. Polar lipids of cultured sea urchin gonads and viscera also showed a predominance of total PUFA throughout the study period (Fig 4.17). The total SFA and total PUFA varied in a manner opposite to each other. The total SFA content decreased significantly ($p<0.05$) while that of PUFA content increased significantly ($p<0.05$) with increased feeding period. The effect of diet on major fatty acids, namely 18:2n-6 and 20:5n-3 in gonadal and visceral lipids is shown in Fig 4.18 a and b, respectively.

4.1.3.6 Dietary effects on fatty acid composition of non-polar lipids

Fatty acid profiles of non-polar lipids of sea urchin gonads and viscera are given in Tables A.19 and A.20, respectively. Further, those fatty acids that contributed 2% or more to the total fatty acids (except 18:2n-6 that was less than 2% on week 0 but became the dominant fatty acids later on) are shown in Fig 4.19. The major SFA acids were 14:0, 16:0 and 18:0 in both gonads and viscera of cultured sea

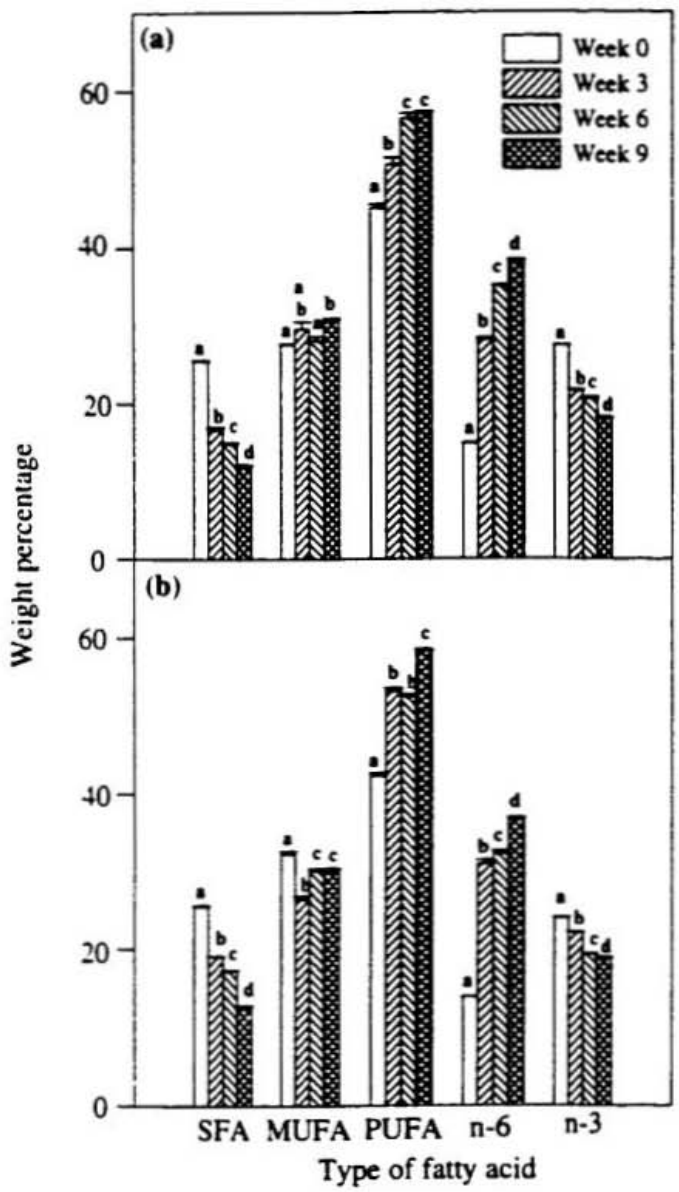


Fig 4.17. Contents of saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA), omega-6 (n-6) and omega-3 (n-3) fatty acids in polar lipids of sea urchin (a) gonads and (b) viscera at 0, 3, 6 and 9 weeks of feeding on an artificial diet

Bars with the same letter within a group are not significantly ($p>0.05$) different from one another.

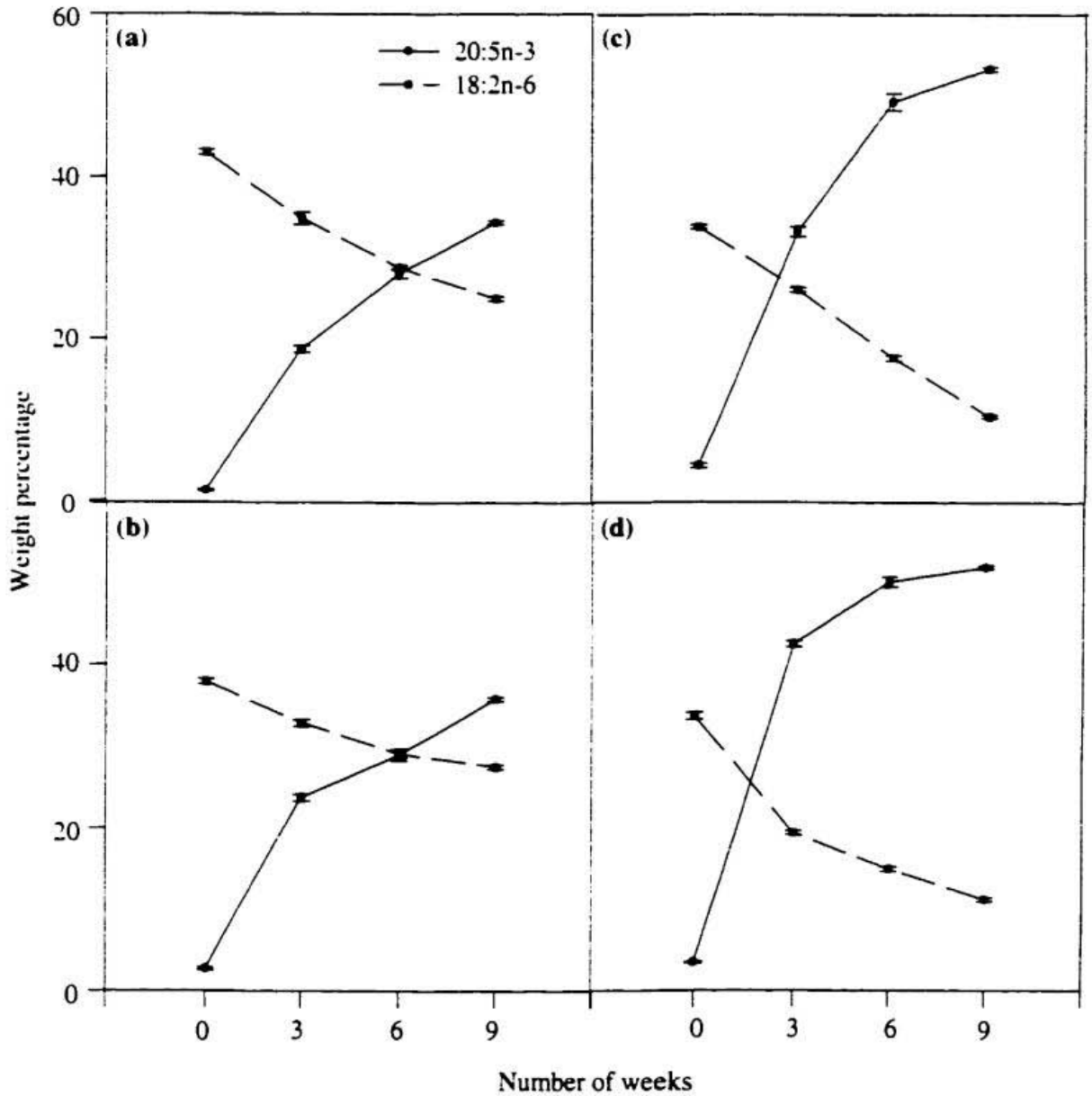


Fig 4.18. Effect of artificial diet on EPA and LA content of sea urchin lipids (a and b - polar lipids of sea urchin gonads, and c and d - non polar lipids of sea urchin gonads and viscera, respectively.)

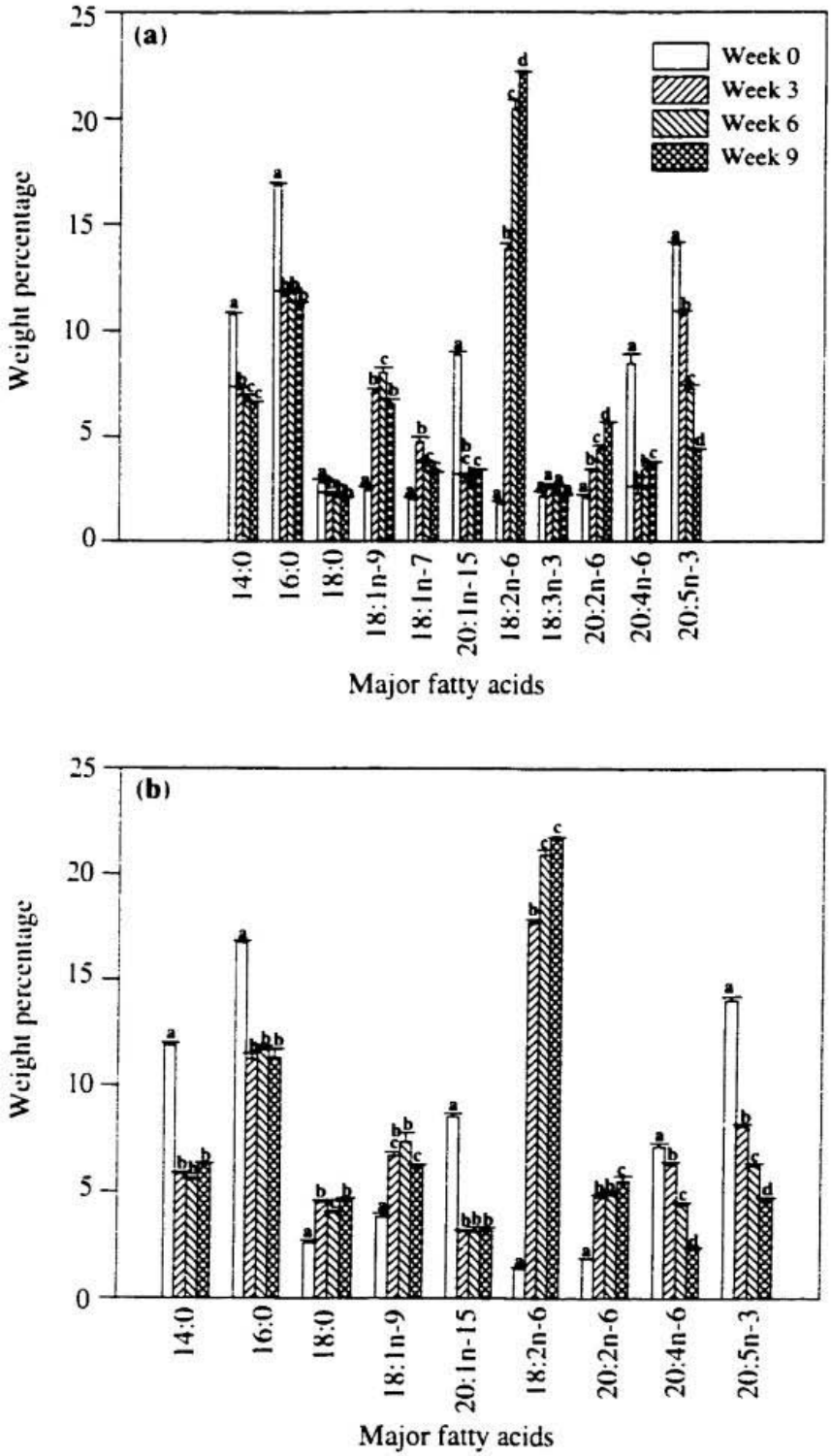


Fig 4.19. Major fatty acids of non-polar lipids of sea urchin (a) gonads and (b) viscera at 0, 3, 6 and 9 weeks of feeding on an artificial diet.
Bars with the same letter within a group are not significantly ($p>0.05$) different from one another.

urchins. In gonads, the major MUFA were 18:1n-9, 18:1n-7 and 20:1n-15, but only 18:1n-9 and 20:1n-15 were the major MUFA in the viscera and the latter was the dominant one on week 0 in viscera and the fatty acid 18:1n-9 became dominant in viscera with increased feeding period. The major PUFA of cultured sea urchin gonads included 18:2n-6, 18:3n-3, 20:2n-6, 20:4n-6 and 20:5n-3. In non-polar lipids of gonad the dominant PUFA was 20:5n-3 on week 0 and 18:2n-6 dominated the fatty acid profile with increased feeding time. The effect of the diet on these two major fatty acids of gonads and viscera is shown in Fig 4.18 c and d, respectively. Fig 4.20 depicts that non-polar lipids were also dominated by PUFA in their fatty acid profile. Both gonads and viscera of cultured sea urchins had the lowest amount of total PUFA on week 0. In gonads PUFA content increased significantly ($p<0.05$) from 40.5 ± 0.47 on week 0 to 50.4 ± 0.81% of total fatty acids on week 9. In viscera the total PUFA content increased significantly ($p<0.05$) from week 0 to week 3, but there were no significant changes thereafter. In contrast, total SFA of gonads and viscera decreased significantly ($p<0.05$) from week 0 to week 9.

4.1.3.7 Fatty acid composition of total, polar and non-polar lipids of artificial feed

Fatty acid composition of total, polar and non-polar lipids of sea urchin's artificial feed is shown in Table A.21 while Fig 4.21 presents the major fatty acids observed in these lipids. Major SFA of the feed were 16:0 and 18:0 in total, polar and non-polar lipids. The fatty acid 18:1n-9 was the only MUFA contributed above 2% to

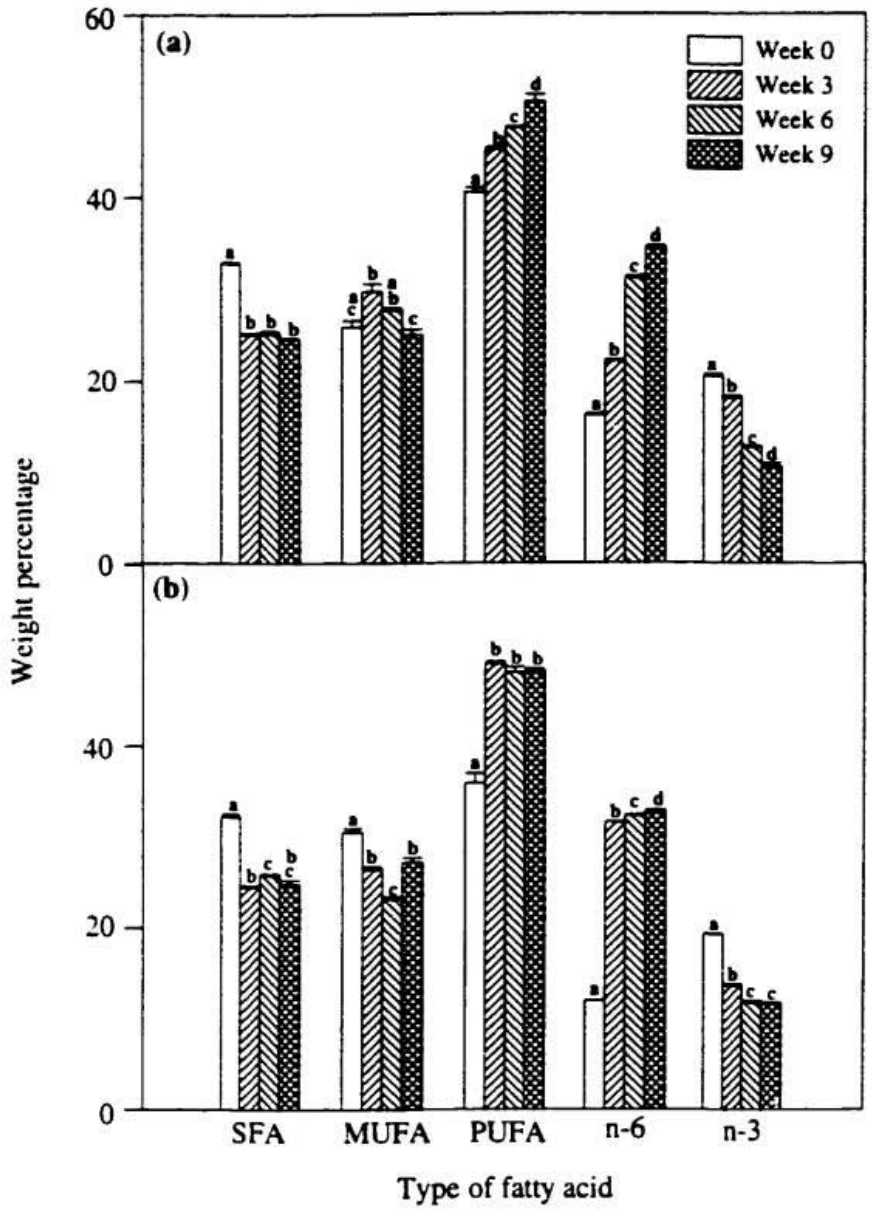


Fig 4.20. Contents of saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA), omega-6 (n-6) and omega-3 (n-3) fatty acids in non-polar lipids of sea urchin (a) gonads and (b) viscera at 0, 3, 6 and 9 weeks of feeding on an artificial diet

Bars with the same letter within a group are not significantly ($p>0.05$) different from one another.

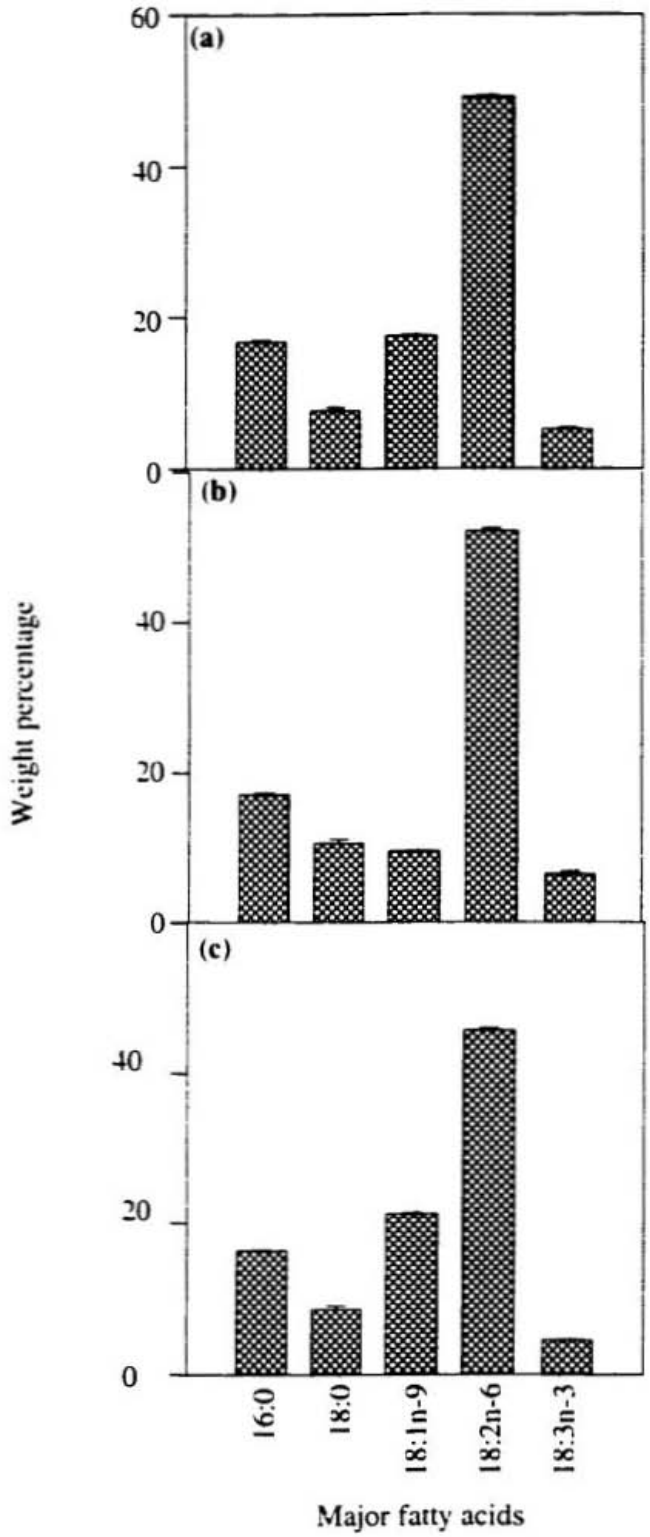


Fig 4.21. Major fatty acids of (a) total (b) polar and (c) non polar lipids of artificial feed.

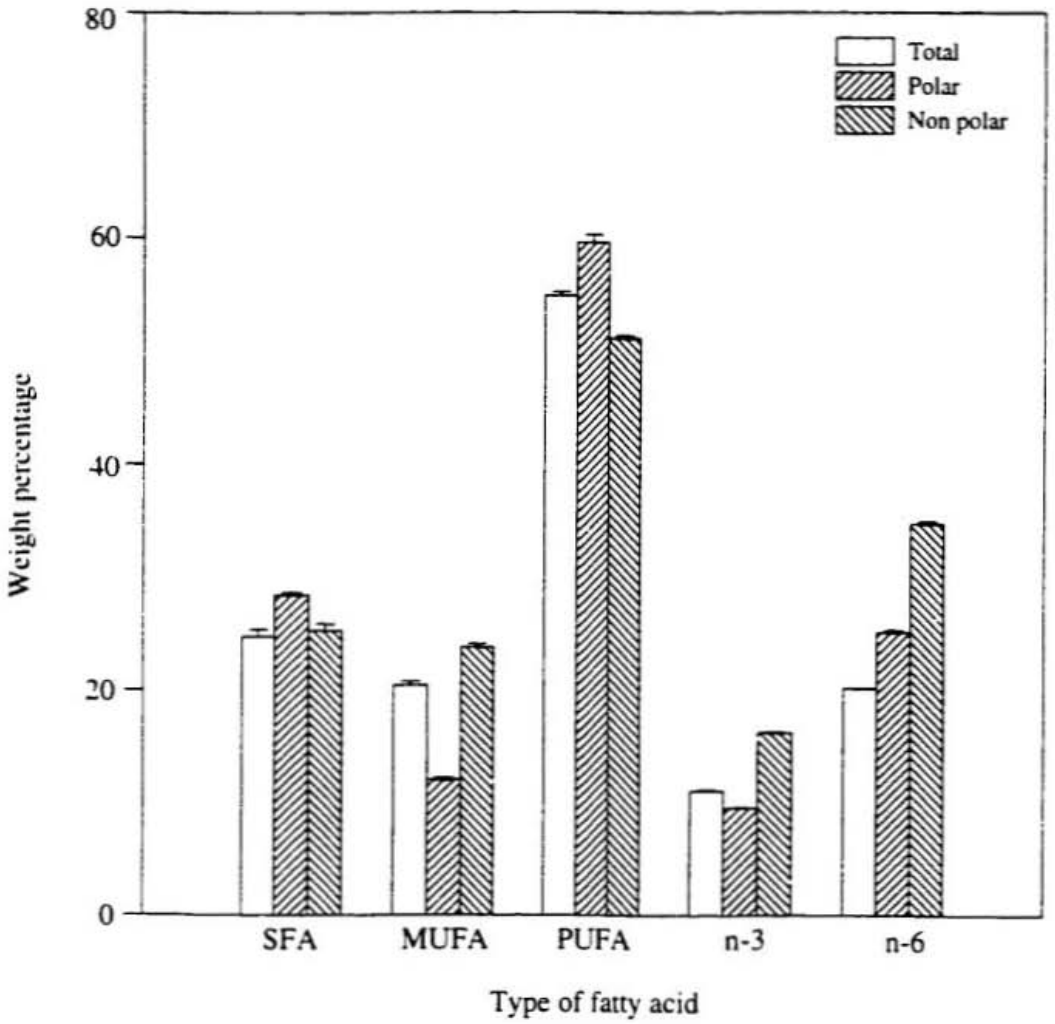


Fig 4.22. Contents of saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA), omega-6 (n-6) and omega-3 (n-3) fatty acids in artificial feed for sea urchins.

total fatty acids of the feed. On the other hand, the dominant PUFA was 18:2n-6 in total, polar and non-polar lipids. The fatty acid, 18:2n-6 contributed 49.1 ± 0.2, 52.1 ± 0.4 and 45. 7 ± 0.3% to total fatty acids in total, polar and non-polar lipids, respectively. Total PUFA content was more than 50% in each type of lipid and a major proportion was contributed by 18:2n-6 representing approximately 89% of polar lipids and 87% of each of the total and non-polar lipids. Polar lipids had the highest proportion of PUFA content followed by total and non-polar lipids. In addition to 18:2n-6, 18:3 n-3 contributed 5.2 ± 0.2, 6.5 ± 0.4 and 4.5 ± 0.1% of total fatty acids in total, polar and non-polar lipids, respectively. In the feed there was a predominance of total PUFA followed by SFA and MUFA, respectively (Fig 4.22).

4.1.4 Carotenoid pigmentation

4.1.4.1 Quantification

Total carotenoid content (dry weight basis) of wild and cultured sea urchins are shown in Table 4.6. In gonads the total carotenoid content was significantly increased ($p<0.05$) in the spring reaching 23.1 ± 0.3 mg per 100g. On the contrary, total carotenoid content in the viscera was significantly ($p<0.05$) decreased in the spring. In cultured sea urchins total carotenoid content was significantly ($p<0.05$) decreased with increasing feeding period. Hence, total carotenoid content of gonads was decreased from 23.2 ± 0.04 on week 0 to 9.2 ± 0.1 mg per 100g tissue on week 9. Similarly, the total carotenoid content in the viscera decreased significantly ($p<0.05$)

Table 4.6 Total carotenoid content (mg per100g) in sea urchin gonads and viscera for wild and cultured sea urchins.

Season/ Harvest time	Gonads	Viscera
WILD SEA URCHINS		
Spring	23.1 ± 0.3^{a}	1.2 ± 0.1^{a}
Summer	17.1 ± 0.2^{b}	3.7 ± 0.1^{b}
Fall	21.7 ± 0.1^{b}	2.2 ± 0.1^{c}
Winter	22.8 ± 0.1^{c}	0.9 ± 0.2^{a}
CULTURED SEA URCHINS		
Week 0	23.2 ± 0.04^{a}	3.7 ± 0.1^{a}
Week 3	20.1 ± 0.2^{b}	0.8 ± 0.1^{b}
Week 6	11.0 ± 0.2^{c}	1.2 ± 0.1^{c}
Week 9	9.2 ± 0.1^{d}	0.6 ± 0.1^{d}

Results are mean values of 3 replicates ± standard deviation. Values in each column with the same superscript are not different ($p>0.05$) from one another.

from 3.7 ± 0.1 mg to a minimum on week 9 (0.6 ± 0.1 mg per 100 g tissue). On the other hand, the total carotenoid content of the artificial feed was 38 mg per 100 g of the feed.

4.1.4.2 Fractionation of individual carotenoid pigments

Crude pigments from gonads of both wild and cultured sea urchins were separated by TLC into seven to eight individual fractions with two major bands; the corresponding members for the viscera were separated into six to seven fractions, again with two major bands (Fig 4.23 and Fig 4.24). In gonads fraction I (Rf = 0.96) and II (Rf = 0.88) ran close to the solvent front, but were adequately separated. For viscera fraction I (Rf = 0.94) ran almost close to the solvent front while fraction IV (Rf = 0.42) ran well behind.

4.1.4.3 Characterization of individual pigments

The carotenoid fraction I of both gonads and viscera of sea urchins was confirmed to be β-carotene using an authentic β-carotene sample and co-chromatography on silica gel TLC plates. Similarly, in gonads fractions III, IV, VI and VII were representative of astaxanthin ester (Rf = 0.57), zeaxathin (Rf = 0.51), canthaxanthin (Rf = 0.22) and free astaxanthin (Rf = 0.09), respectively. Furthermore, fraction II of each gonad sample corresponded to echininone by means of absorption maxima in hexane, chloroform and methanol. Thus, the observed λ_{max} were 484/460,

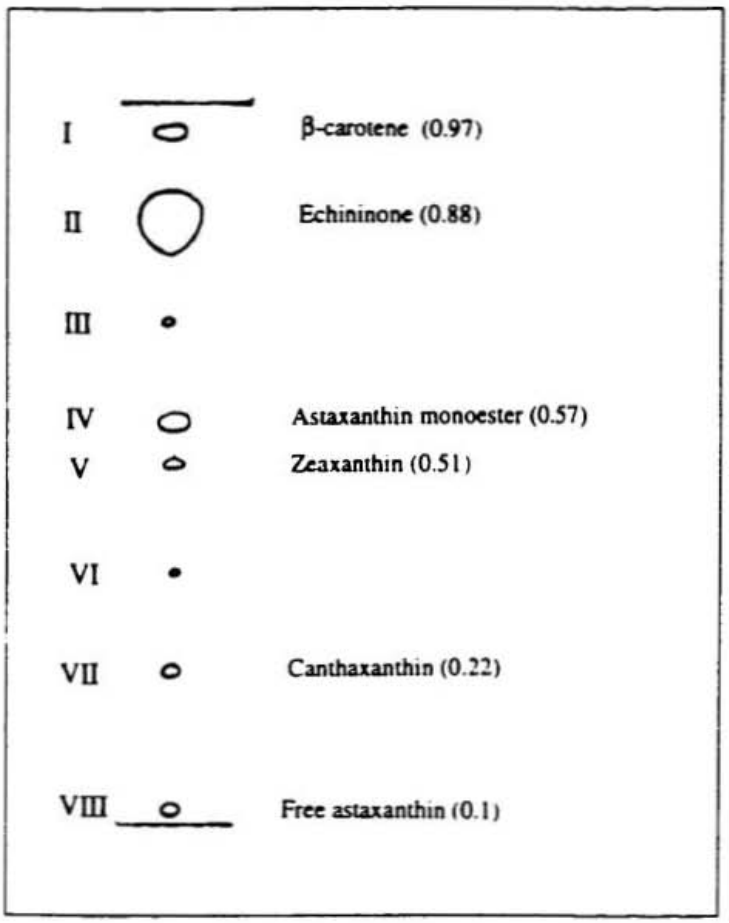


Fig 4.23 TLC chromatogram of total carotenoids of sea urchin gonads developed in acetone/n-hexane (3/7, v/v) on a silica gel plate.

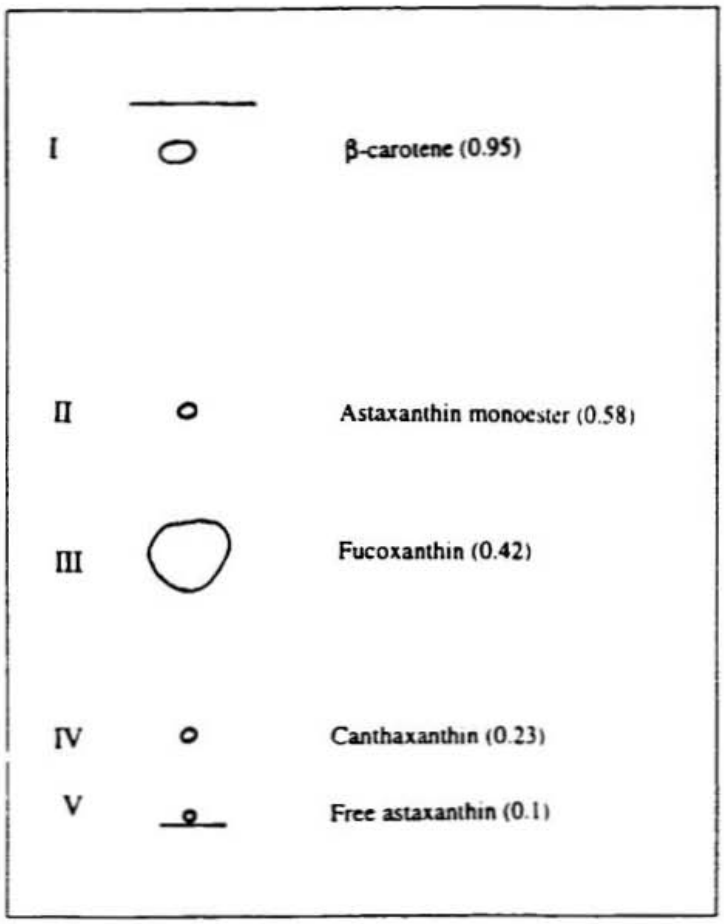


Fig 4.24 TLC chromatogram of total carotenoids of sea urchin viscera developed in acetone/n-hexane (3/7, v/v) on a silica gel plate.

466 and 475 nm in hexane, ethanol and chloroform, respectively. In viscera, fraction IV corresponded with fucoxanthin based on absorption maxima of 424/447/474, 423/446/472 and 454/488 nm in hexane, ethanol and chloroform, respectively. Other minor carotenoids in the viscera were astxanthin ester (Rf = 0.58), canthaxanthin (Rf = 0.23) and free astaxanthin (Rf = 0.07) that corresponded to fractions III, V and VI, respectively. The other minor carotenoids were not analysed because of their insufficient concentration to obtain absorption maxima and also lack of authentic samples.

4.1.4.4 Quantification of individual pigments

The relative contents of echininone and β-carotene in the gonads changed considerably with the season of catch. Echininone was the dominant pigment throughout the harvest period of one year. During spring the echininone content was approximately 76% of the total amount of carotenoids and this was the highest proportion detected. Corresponding values of echininone in winter, fall and spring were 71, 65 and 48%, respectively. On the contrary, β-carotene content was highest in summer with approximately 43% of the total amount of carotenoids followed by 23, 22 and 18% in fall, winter and spring, respectively. Thus, the content of the two pigments were inversely related in the spring in the gonads. The minor pigments in gonads were responsible for 6, 9, 11 and 7% of the total carotenoids in spring, summer, fall and winter, respectively.

In cultured sea urchins echininone remained the dominant carotenoid throughout the study period. On week 0 echininone content was approximately 64% while β-carotene content was approximately 24% of the total amount. On the other hand, when urchins were fed on artificial diet the echininone content was increased to approximately 79, 76 and 84% on weeks 3, 6 and 9, respectively. In cultured sea urchins the β-carotene content in the gonads was not quantified. In addition, fucoxanthin content in all sea urchin visceral samples was not quantified.

4.1.5 Amino acid composition

The total amino acid (TAA) composition of wild sea urchin gonads and viscera are listed in the Tables 4.7 and 4.8, respectively. Glycine was the dominant amino acid and contributed 12.9 to 16.6% and 14.6 to 20.3% to the TAA content in sea urchin gonads and viscera, respectively. The essential amino acids namely histidine, isoleucine, leucine, lysine, methionine, cysteine, phenylalanine, tyrosine, threonine, tryptophan and valine were present in both gonadal and visceral proteins. Other amino acids observed in considerable quantities included alanine, glutamic acid, arginine, serine, hydroxyproline, aspartic acid and proline in both tissues analysed. Taurine, cystathionine, α-aminoadipic acid and sarcosine were not present consistently. The diet had a noticeable effect on the TAA content of sea urchin gonads and viscera (Tables 4.9 and 4.10). In cultured sea urchin gonads glycine remained dominant until week 6 after introducing the artificial feed and its relative content was

Table 4.7 Total amino acid content (mg/g protein) of sea urchin gonads in spring, summer, fall and winter.

Amino acid	Spring	Summer	Fall	Winter
Glycine	139.6	129.0	146.2	164.3
Alanine	50.0	58.2	64.3	62.6
Glutamic acid	104.6	103.7	103.7	92.6
Arginine	84.4	86.0	99.0	61.0
Lysine	77.9	79.2	84.2	57.4
Cystathionine	2.9	3.2	5.1	-
Serine	40.5	39.9	42.1	62.7
Hydroxyproline	4.2	1.7	7.1	-
Threonine	51.8	51.3	47.6	66.1
Aspartic acid	77.9	90.5	87.1	123.5
Taurine	-	4.0	4.3	-
Leucine	73.1	68.6	68.3	59.0
Tyrosine	47.5	39.9	38.7	31.4
Proline	32.8	36.8	36.3	34.9
Valine	60.8	54.2	52.7	45.4
Isoleucine	46.2	44.5	43.3	37.3
Methionine	0.8	20.8	9.0	14.4
Phenylalanine	41.9	43.2	39.9	32.1
Histidine	19.4	20.8	21.2	17.2
Tryptophan	19.7	1.4	2.6	1.3
Cysteic acid	25.7	12.6	9.2	14.7
Sarcosine	-	-	1.4	10.1

Table 4.8 Total amino acid content (mg/g protein) of sea urchin viscera in spring, summer, fall and winter.

Amino acid	Spring	Summer	Fall	Winter
Glycine	144.5	201.1	155.4	185.8
Alanine	59.5	57.0	54.1	51.1
Glutamic acid	105.9	110.2	97.6	56.4
Arginine	75.9	60.1	58.4	61.3
Lysine	70.9	52.2	59.5	63.5
Cystathionine	2.6	2.6	2.6	-
Serine	42.1	35.7	41.3	52.9
Hydroxyproline	4.5	2.5	6.6	-
Threonine	55.4	43.8	49.8	59.6
Aspartic acid	86.1	87.1	98.7	21.3
Taurine	-	24.8	15.8	-
Leucine	70.2	54.7	64.1	79.1
Tyrosine	38.4	33.5	35.8	53.3
Proline	39.9	32.3	37.0	48.7
Valine	54.0	46.3	49.2	68.1
Isoleucine	42.7	37.1	39.9	51.7
Methionine	1.2	36.5	35.0	26.5
Phenylalanine	41.4	37.8	40.9	52.2
Histidine	59.9	19.9	22.9	26.1
Tryptophan	1.4	1.7	3.3	3.0
Cysteic acid	30.3	15.5	25.1	16.5
Sarcosine	-	0.5	3.2	18.8

Table 4.9 Total amino acid content (mg/g protein) of sea urchin gonads at 0, 3, 6 and 4 weeks of feeding on an artificial diet.

Amino acid	Week 0	Week 3	Week 6	Week 9
Glycine	118.0	110.8	115.1	94.1
Alanine	42.5	41.6	40.5	40.8
Glutamic acid	87.2	78.5	86.7	77.2
Arginine	80.7	69.7	84.5	70.1
Lysine	81.1	74.3	80.1	74.2
Cystathionine	3.0	2.1	2.0	2.2
Serine	47.1	43.0	39.5	40.2
Hydroxyproline	3.3	1.2	3.2	1.5
Threonine	47.8	53.4	54.7	52.9
Aspartic acid	85.4	60.3	66.9	60.2
Leucine	65.9	110.6	97.1	103.1
Tyrosine	36.3	66.2	73.1	105.6
Proline	40.2	36.9	28.3	32.5
Valine	78.3	73.3	69.9	72.5
Isoleucine	77.1	63.7	59.3	59.2
Methionine	1.2	0.6	0.1	0.7
Phenylalanine	53.2	61.2	50.7	65.1
Histidine	35.5	24.3	27.8	25.2
Tryptophan	1.4	2.3	2.5	2.1
Cysteic acid	7.2	21.4	21.7	18.5

Table 4.10 Total amino acid content (mg/g protein) of sea urchin viscera at 0, 3, 6 and 9 weeks of feeding on an artificial diet.

Amino acid	Spring	Summer	Fall	Winter
Glycine	143.6	173.3	181.0	139.3
Alanine	64.1	55.5	56.2	42.8
Glutamic acid	90.6	111.4	104.3	85.3
Arginine	74.2	79.0	58.8	70.3
Lysine	78.1	61.9	57.0	69.5
Cystathionine	2.4	1.2	1.7	1.6
Serine	46.5	40.6	31.1	35.9
Hydroxyproline	2.6	14.8	17.4	3.1
Threonine	47.2	46.6	51.1	56.1
Aspartic acid	78.1	76.4	67.0	71.5
Leucine	62.6	67.3	77.8	92.6
Tyrosine	38.6	31.6	37.7	64.4
Proline	48.4	58.1	50.8	30.8
Valine	76.1	50.3	57.1	68.0
Isoleucine	60.9	41.7	48.0	56.7
Methionine	0.9	0.1	0.3	0.8
Phenylalanine	42.1	39.2	45.7	51.8
Histidine	28.0	20.8	23.5	27.9
Tryptophan	1.1	1.8	2.1	2.4
Cysteic acid	5.7	25.1	24.4	25.0

reduced by week 9. Thus, by week 9 the amino acid tyrosine became dominant in the TAA of sea urchin gonads. Furthermore, the diet exerted an effect on increasing the relative content of lysine in sea urchin gonads. On the contrary, glycine remained to be the dominant amino acid throughout the study period in viscera although its relative content was reduced after introducing the artificial feed. In addition, relative proportion of tyrosine increased in viscera after the urchins were started feeding the artificial diet. It was apparent that viscera also showed the same pattern of variation in the total amino acid content. Qualitatively the amino acid profiles were the same in both wild stock and their cultured counterparts while there were marked differences in the quantity of individual amino acids.

The free amino acid (FAA) profiles of wild sea urchin gonads and viscera are shown in Tables 4.11 and 4.12, respectively. There were some similar aspects in FAA patterns in sea urchin tissues. The most abundant FAA was invariably glycine, which accounted for 30.3 - 61.4% and 39.1 - 58.5% of the total FAA in sea urchin gonads and viscera, respectively. There were marked seasonal differences in the total FAA content in sea urchin tissues. The total FAA contents for sea urchin tissues are shown in Table 4.13. The total FAA content was quite high in the spring especially in the gonads, and was 112.7 and 21.3 mg per g dry weight in gonads and viscera, respectively. Almost all the essential amino acids were detected in the wild sea urchin FAA pool of both tissues. In addition, a few amino acid derivatives such as cystathionine and taurine were detected consistently. However, the presence of other

Table 4.11 Free amino acid content (μg/g dry weight) of sea urchin gonads in spring, summer, fall and winter.

Amino acid	Spring		Summer		Fall		Winter	
	μg/g	%	μg/g	%	μg/g	%	μg/g	%
Glycine	34128	30.3	2484	34.1	10981	50.0	7827	61.4
Alanine	5804	5.2	637	8.6	2770	12.6	1293	10.1
Glutamic acid	3733	3.3	444	6.1	40	0.2	446	3.5
Glutamine	3035	2.7	330	4.5	512	2.3	113	0.9
Arginine	12809	11.4	319	4.4	82	0.4	280	2.2
Lysine	8519	7.6	317	4.4	321	1.5	252	1.9
Cystathionine	1202	1.1	58	0.8	430	1.9	77	0.6
Serine	2112	1.9	82	1.2	-	-	288	2.3
Hydroxyproline	1405	1.3	29	0.4	612	2.8	71	0.6
Threonine	3207	2.9	78	1.1	850	3.9	118	0.9
Aspartic acid	773	0.7	9	0.1	21	0.1	115	0.9
Taurine	1756	1.6	145	1.9	516	2.4	209	1.6
Leucine	6355	5.6	165	2.3	961	4.4	97	0.8
Tyrosine	7208	6.4	217	2.9	268	1.2	90	0.7
Proline	589	0.5	60	0.8	187	0.9	141	1.1
Valine	6927	6.2	161	2.2	939	4.3	82	0.6
Isoleucine	4302	3.8	108	1.5	652	2.9	86	0.7
Methionine	1895	1.7	48	0.7	372	1.7	69	0.5
Phenylalanine	2566	2.3	83	1.1	529	2.4	51	0.4
Asparagine	671	0.6	-	-	-	-	52	0.4
Histidine	1164	1.0	59	0.8	85	0.4	38	0.3
Cystine	606	0.5	31	0.4	165	0.8	210	1.6
Tryptophan	1632	1.5	54	0.7	205	0.9	-	-
α-aminoadipic acid	3103	0.3	8	0.1	108	0.5	-	-
Sarcosine	-		16	0.2	342	1.6	679	5.3

Table 4.12 Free amino acid content (µg/g dry weight) of sea urchin viscera in spring, summer, fall and winter.

Amino acid	Spring		Summer		Fall		Winter	
	µg/g	%	µg/g	%	µg/g	%	µg/g	%
Glycine	8347	39.1	3394	46.6	4117	48.9	5032	58.5
Alanine	1693	7.9	679	9.3	764	9.1	768	8.9
Glutamic acid	526	2.5	544	7.5	247	2.9	287	3.3
Glutamine	459	2.2	268	3.7	86	1.0	82	1.0
Arginine	2047	9.6	403	5.5	358	4.3	237	2.8
Lysine	1484	6.9	277	3.8	219	2.6	195	2.3
Cystathionine	191	0.9	44	0.6	69	0.8	Tr	-
Serine	82	0.4	47	0.7	-	-	145	1.7
Hydroxyproline	33	0.2	29	0.4	197	2.3	-	-
Threonine	192	0.9	94	1.3	178	2.1	150	1.7
Aspartic acid	-	-	88	1.2	-	-	71	0.8
Taurine	731	3.4	474	6.5	594	7.1	426	4.9
Leucine	971	4.6	156	2.1	247	2.9	171	1.9
Tyrosine	957	4.5	206	2.8	175	2.1	100	1.2
Proline	207	0.9	103	1.4	22	0.3	61	0.7
Valine	953	4.5	147	2.2	239	2.8	95	1.1
Isoleucine	658	3.1	88	1.2	167	1.9	111	1.3
Methionine	270	1.3	32	0.4	121	1.4	45	0.5
Phenylalanine	410	1.9	91	1.3	144	1.7	50	0.6
Asparagine	103	0.5	-	-	-	-	-	-
Histidine	235	1.1	71	0.9	94	1.1	55	0.6
Cystine	138	0.7	47	0.7	100	1.2	84	0.9
Tryptophan	150	0.7	-	-	147	1.8	-	-
α-aminoadipic acid	-	-	-	-	-	-	124	1.4
Sarcosine	496	2.3	-	-	136	1.6	321	3.7

Table 4.13 Content of total free amino acids (mg per g tissue dry weight) in wild and cultured sea urchin gonads and viscera.

Season/Harvest time	Gonads	Viscera
WILD SEA URCHINS		
Spring	112.7	21.3
Summer	5.9	7.3
Fall	13.9	8.4
Winter	12.8	8.6
CULTURED SEA URCHINS		
Week 0	20.6	12.0
Week 3	180.6	16.3
Week 6	153.8	21.2
Week 9	154.6	46.9

related types namely α-aminoadipic acid, glycerophosphoethanolamine and sarcosine was not consistent.

Free amino acid profiles of cultured sea urchin gonads and viscera are shown in Tables 4. 14 and 4.15, respectively. Glycine was the dominant amino acid in both sea urchin gonads and viscera. Glycine contributed 15.3 - 57.1% and 17.3 - 56.3% to the total FAA in sea urchin gonads and viscera, respectively. The relative proportion of glycine was more than 50% in both gonads and viscera on week 0 when the urchins were fed on a laminarian kelp diet during acclimation. The artificial diet brought about a noticeable effect on the total FAA content of sea urchin tissues. This effect was much prominent in the gonads, and the total FAA content in the gonads was increased from 20.6 mg per g dry weight on week 0 to 180.6 mg per g dry weight on week 3. However, the total FAA content in gonads was again reduced to153.8 and 154.6 mg per g dry weight on weeks 6 and 9, respectively. On the other hand, in viscera the total FAA content increased from 12.0 mg per g dry weight on week 0 to 46.9 mg per g dry weight on week 9. However, after introducing the artificial diet the relative proportion of glycine was noticeably reduced in both gonads and viscera. On the other hand, the amino acids arginine, lysine, leucine, tyrosine, valine, isoleucine and phenylalanine, among others, in the gonads increased their relative contents markedly as a consequence of feeding on the artificial diet. Furthermore, these effects were much similar in sea urchin viscera. With respect to FAA also there were no qualitative differences in the amino acid profiles between wild sea urchins and their

Table 4.14 Free amino acid content (mg/g dry weight) of sea urchin gonads at 0, 3, 6 and 9 weeks of feeding on an artificial diet.

Amino acid	Week 0		Week 3		Week 6		Week 9	
	µg/g	%	µg/g	%	µg/g	%	µg/g	%
Glycine	11751	57.1	27752	15.4	24204	15.7	28301	18.3
Alanine	2872	14	6097	3.4	5599	3.6	4239	2.7
Glutamic acid	874	4.2	3342	1.9	2799	1.8	2321	1.5
Glutamine	647	3.1	2899	1.6	2890	1.9	4389	2.8
Arginine	180	0.9	12128	6.7	10767	7.0	13701	8.9
Lysine	356	1.7	12196	6.8	11595	7.5	11681	7.6
Cystathionine	115	0.6	1056	0.6	1074	0.7	1128	0.7
Serine	316	1.5	6588	3.7	5321	3.5	4209	2.7
Hydroxyproline	124	0.6	459	0.3	596	0.4	1800	1.2
Threonine	521	2.5	7944	4.4	7712	5.0	6676	4.3
Aspartic acid	73	0.4	703	0.4	694	0.5	471	0.3
Taurine	214	1.0	1100	0.6	1011	0.7	792	0.5
Leucine	370	1.8	23755	13.2	10367	6.7	16854	10.9
Tyrosine	217	1.1	15331	8.5	16770	10.9	16493	10.7
Proline	140	0.7	4909	2.7	3706	2.4	617	0.4
Valine	273	1.3	15448	8.6	14229	9.3	12668	8.2
Isoleucine	313	1.5	12926	7.2	8788	5.7	9866	6.4
Methionine	68	0.3	3087	1.7	2438	1.6	1604	1.0
Phenylalanine	164	0.8	12046	6.7	13171	8.6	7114	4.6
Asparagine	13	0.1	1881	1.0	1543	1.0	1843	1.2
Histidine	100	0.5	5361	2.9	5187	3.4	5128	3.3
Cystine	214	1.0	478	0.3	247	0.2	303	2.2
Tryptophan	264	1.3	2742	1.5	2853	1.9	2044	1.3
α-aminoadipic acid	68	0.3	330	0.2	270	0.2	277	0.2
Sarcosine	332	1.6	-	-	-	-	71	0.1

Table 4.15 Free amino acid content (mg/g dry weight) of sea urchin viscera at 0, 3, 6 and 9 weeks of feeding on an artificial diet.

Amino acid	Week 0		Week 3		Week 6		Week 9	
	µg/g	%	µg/g	%	µg/g	%	µg/g	%
Glycine	6771	56.3	4372	26.9	5364	25.3	8141	17.3
Alanine	899	7.5	1078	6.6	1397	6.6	1852	3.9
Glutamic acid	478	4.0	866	5.3	1036	4.9	611	1.3
Glutamine	184	1.5	814	5.0	853	4.0	1222	2.6
Arginine	507	4.2	976	6.0	1153	5.4	3606	7.7
Lysine	312	2.6	822	5.1	1076	5.1	3200	6.8
Cystathionine	76	0.6	63	0.4	105	0.5	325	0.7
Serine	164	1.4	551	3.4	263	1.2	913	1.9
Hydroxyproline	88	0.7	156	0.9	320	1.5	812	1.7
Threonine	189	1.6	580	3.6	946	4.5	2255	4.8
Aspartic acid	80	0.7	534	3.3	285	1.3	16	0.03
Taurine	592	4.9	264	1.6	312	1.5	673	1.4
Leucine	293	2.4	1162	7.1	1937	9.1	5072	10.8
Tyrosine	212	1.8	522	3.2	998	4.7	4883	10.4
Proline	108	0.9	614	3.8	650	3.1	320	0.7
Valine	272	2.3	842	5.2	1356	6.4	4052	8.6
Isoleucine	175	1.5	688	4.2	1132	5.3	3130	6.7
Methionine	134	1.1	152	0.9	233	1.1	557	1.2
Phenylalanine	164	1.4	605	3.7	996	4.7	2471	5.3
Asparagine	-	-	135	0.8	-	-	523	1.1
Histidine	111	0.9	283	1.7	504	2.4	1462	3.1
Cystine	90	0.7	103	0.6	96	0.5	79	0.2
Tryptophan	140	1.2	82	0.5	188	0.9	653	1.4
α-aminoadipic acid	-	-	8	0.1	-	-	103	0.2

Table 4.16 Total and free amino acid content of artificial feed for sea urchins.

Amino acid	Total amino acid (mg/g protein)		Free amino acid (µg/g tissue dry wt)	
	mg/g	%	µg/g	%
Glycine	87.1	8.7	33	2.5
Alanine	61.4	6.1	100	7.6
Glutamic acid	151.9	15.2	79	6.0
Glutamine	-	-	32	2.4
Arginine	71.8	7.2	227	17.2
Lysine	52.0	5.2	47	3.6
Serine	45.9	4.6	30	2.3
Hydroxyproline	26.3	2.6	-	-
Threonine	38.1	3.8	29	2.2
Aspartic acid	85.6	8.6	33	2.5
Taurine	-	-	30	2.3
Leucine	73.3	7.3	39	3.0
Tyrosine	26.7	2.7	34	2.6
Proline	92.3	9.2	107	8.1
Valine	45.8	4.6	56	4.2
Isoleucine	36.2	3.6	19	1.5
Methionine	0.4	0.0	3	0.2
Phenylalanine	44.2	4.4	30	2.3
Asparagine	-	-	216	16.4
Histidine	-	-	18	1.4
Cystine	-	-	82	6.2
Tryptophan	22.3	2.2	58	4.4
α-aminoadipic acid	0.8	0.1	15	1.2
Cysteic acid	-	-	-	-
Sarcosine	22.7	2.3	-	-
			1217	

cultured counterparts, although the quantities varied considerably. The content of total and free amino acids in the artificial diet is shown in Table 4.16.

4.1.6 Content of nucleic acids

The content of nucleic acids (dry weight basis) in wild sea urchin gonads and viscera is presented in Table 4.17. The content of DNA in all samples was higher than that of RNA. Both RNA and DNA contents were considerably higher in the gonads than in the viscera. In gonads RNA/DNA ratio was significantly ($p<0.05$) increased in summer followed by fall, winter and spring. Wild sea urchin viscera also exhibited the same pattern of variation in RNA/DNA ratio.

The content of nucleic acids in cultured sea urchin gonads and viscera is shown in Table 4.18. The RNA/DNA ratio was significantly ($p<0.05$) increased on week 6 in both gonads and viscera with a subsequent decrease which was insignificant ($p>0.05$) on week 9.

Table 4.17 Content of deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) (µg per g tissue dry weight) in sea urchin gonads and viscera in spring, summer, fall and winter.

Season	RNA	DNA
GONADS		
Spring	2.1 ± 0.04^{a}	3.3 ± 0.06^{a}
Summer	2.8 ± 0.08^{b}	4.3 ± 0.08^{b}
Fall	3.1 ± 0.08^{c}	4.1 ± 0.11^{b}
Winter	3.3 ± 0.09^{c}	4.9 ± 0.1^{c}
VISCERA		
Spring	0.4 ± 0.02^{a}	1.0 ± 0.04^{a}
Summer	0.5 ± 0.04^{b}	0.7 ± 0.01^{b}
Fall	0.3 ± 0.04^{a}	1.6 ± 0.05^{c}
Winter	0.2 ± 0.03^{a}	2.9 ± 0.15^{d}

Results are mean values of 3 replicates ± standard deviation. Values in each column with the same superscript are not different ($p>0.05$) from one another.

Table 4.18 Content of deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) (μg per g tissue dry weight) in sea urchin gonads and viscera at 0, 3, 6 and 9 weeks of feeding artificial diet.

Harvest time	RNA	DNA
GONADS		
Week 0	2.6 ± 0.06^{a}	3.9 ± 0.08^{a}
Week 3	4.8 ± 0.05^{b}	5.2 ± 0.02^{b}
Week 6	6.6 ± 0.03^{c}	5.7 ± 0.16^{c}
Week 9	4.3 ± 0.08^{d}	6.3 ± 0.06^{c}
VISCERA		
Week 0	0.5 ± 0.03^{a}	1.0 ± 0.07^{a}
Week 3	1.0 ± 0.02^{b}	1.3 ± 0.03^{b}
Week 6	0.6 ± 0.02^{c}	1.0 ± 0.02^{a}
Week 9	0.5 ± 0.04^{d}	1.2 ± 0.03^{b}

Results are mean values of 3 replicates ± standard deviation. Values in each column with the same superscript are not different ($p>0.05$) from one another.

4.2 Discussion

In the present study the gonads were not separated into male and female sexes due to three different reasons. Firstly, echinoid gonads are a site of nutrient storage as well as gamete production (Fernandez *et al.*, 1995): the former being most important in these animals in a commercial perspective. Secondly, discrimination between male and female gonads during spring and summer is difficult since most of the tissue within gonadal sacs consists of nutritive phagocytes in both sexes and it would be difficult to distinguish these from one another prior to maturity. As the short day photoperiods trigger gametogenesis in these animals sexes become distinguishable at a later stage. However, the commercial industry does not distinguish between ovaries and testes in marketing them although the inadvertent separation based on colour and texture may take place. Hence, male and female gonads in this study were pooled together purposely as in the practice in the sea urchin processing industry (Hooper, 2001 personal communication).

4.2.1 Proximate composition

Major nutrient components of sea urchin gonads consist of polysaccharides, proteins and lipids (Fernandez *et al.*, 1995). *S. droebachiensis* in the present study also had the same nutrient components in considerably high levels. Shearer (1994) reported that both environment and diet affect the proximate composition of cultured fish. Similarly, it can be inferred for this study that sea urchin biochemical composition was affected by the season and diet. In

fact, the biochemical composition of the gonad of sea urchin *Paracentrotus lividus* exhibited considerable seasonal variations (Fernandez, 1998). Further, these seasonal variations were similar to those observed in various other temperate, tropical and polar Echinoidea species (Giese 1966a; 1966b; Lawrence and Guille, 1982; McClintock and Pearse, 1987). It is known that most similarities in the distribution of biochemical components in sea urchins are due to the organization of various organs which is similar in all Echinoidea regardless of species and geographical location (Lawrence and Guille, 1982; McClintock and Pearse, 1987).

During the present study there was an increase in the moisture level of sea urchin gonads with a simultaneous decrease in their lipid content. In fish an inverse relationship has been detected in the relative amount of lipid and moisture (Shearer, 1994). Hence, the same principle could be applied to sea urchin tissues. In general, gonadal growth occurs throughout fall and winter while maturing occurs in the spring in *S. droebachiensis* (Himmelman, 1978). It has been shown that initial gonadal enlargement in sea urchins results from an increase in the size and number of nutritive phagocytes involved in the storage of nutrients for gametogenesis. Further enlargement is due to an increase in the number and size of gametes with a proportionate decrease in the volume of the accessory cells and a large increase in moisture content (Gonor, 1973; Miller and Mann, 1973; Walker, 1982; Pearse *et al.*, 1986). de Jong-Westman *et al.* (1995b) reported the absence of a large change in lipid levels between November and March and indicated that the initial phase of nutrient build-up occurred before November

after which lipids were likely transferred to the developing gametes in *S. droebachiensis*. In this study also there were no significant changes between fall and winter in the lipid content which may correspond to the above period. De Jong-Westman *et al.* (1995b) further reported that the increase in gonad size after November was largely due to an increase in moisture content. Hence, water content increased from a mean of 70% in November to 82% in March. In the present study, the moisture level was significantly increased in the winter and was approximately 84% hence, leading further support to the findings of de Jong-Westman *et al.* (1995b).

In general, lipids serve as an energy store and correlate positively with food availability (Bailey and Robison, 1986). Both food quality and quantity affect sea urchin growth (Lawrence and Lane, 1982). During summer, plenty of food is available while winter is a period when food is sparse or not available at all. Hence, it is possible that these cold-water species may feed mainly in the summer. Klinger *et al.* (1997) have reported that sea urchin *S. droebachiensis* had reduced level of feeding over the cooler winter months which then increased during the spring and the early summer. Thus, in summer the excessive feeding may result in accumulation of nutrients as energy reserve to be used during the cooler temperatures when they are under nutritional stress. Some of the energy intake goes directly to the gonads while some passes to the pyloric caeca for storage (Pearse and Giese, 1966). This may be the reason for considerably higher lipid contents in the summer. It has been shown that in wild temperate fish growth and energy storage undergo seasonal changes (Jorgensen *et al.*, 1997) as energy

from diet and body reserves is partitioned between maintenance, somatic growth and reproduction (Smith and Paul, 1990). During somatic growth, lipid, protein and minerals are typically accumulated while protein and lipid are depleted during gonadal growth (Jorgensen *et al.*, 1997). In general, gonadal yield is strongly affected by the seasonal reproductive cycle of sea urchin. During spawning a high proportion of the gonad mass is released as gametes (Thompson, 1984). Once spawning occurred this may exert a significant effect on the biochemical composition of gonads. In this study the highest lipid composition was observed in the spring, hence sea urchin samples examined may have been caught prior to gamete release (pre-spawning stage). Larson *et al.* (1980) have reported that food quality and quantity strongly influence reproduction of *S. droebachiensis* and other sea urchins hence affecting their biochemical composition. In winter when food is sparse or nonexistent, reserves in the pyloric caeca may be used to fuel continuing gametogenesis (Clarke, 1988). Consequently, an increase in lipid content was observed in the gonads. This is further confirmed by the results obtained in this study. Hence, the highest lipid level is seen in the spring when urchins are at pre-spawning stage. The lipid levels in the gut are relatively high (in the range of 10.6 to 27.2%) thus revealing the importance of this for an organ for storing nutrients (McClintock and Pearse, 1987). It is also known that gut accumulates nutrient reserves only on a short term basis (Fenaux *et al.*, 1977; Klinger *et al.*, 1988). Hence, during winter when these animals are under nutritional stress accumulated lipids may be utilized for gametogenesis in addition to other needs.

Sea urchin gonads are known to contain high levels of soluble and insoluble protein. They also have considerably higher lipid levels while carbohydrate levels are low (Lawrence and Guille, 1982; McClintock and Pearse, 1987). However, echinoids are able to digest both soluble and structural carbohydrates efficiently (Frantzis and Gremare, 1992). *S. droebachiensis* in this study showed much variation in carbohydrate content. Hence, during winter there was no carbohydrate reserve while in the spring carbohydrate content was much higher than those of the other seasons. It has been revealed that gametogenesis is typically characterized by increased protein levels and glycogen storage in gonads (Moss and Lawrence, 1972; Fenaux *et al.*, 1977; Fernandez, 1998). However, lipid levels differ from species to species and may either increase with a subsequent depletion (Fenaux *et al.*, 1977) or may remain constant throughout the annual cycle (Moss and Lawrence, 1972). The increase in carbohydrate and lipid levels corresponds to an accumulation of nutrient reserves. However, these reserves are subsequently utilized during the process of gamete development (Fenaux *et al.*, 1977). Fenaux *et al.* (1977) further stated that there is a steady protein level while carbohydrate and lipid levels fluctuate. The decrease of carbohydrates and lipids may be due to ingestion of different food type or to the transformation of sugars and lipids to build up of energy reserves for gonad maturation. In fact, in this study relatively high levels of protein were observed in gonads in winter when gametogenesis was supposed to take place. On the other hand, in the spring carbohydrate level increased greatly, perhaps because gametes are rich in all these reserves which enables them to start another life cycle.

In general, growth and energy storage occur in spring and summer when food is abundant. In many species energy reserves are depleted for both metabolic needs and reproduction. It has been shown that in any analysis of seasonal fluctuations it is useful to consider the proximate components independently because lipids and proteins serve different functions. Hence, lipids primarily store energy whereas most stored proteins are used for gonad development (Love, 1970; Dawson and Grimm, 1980). In the sea urchin *S. droebachiensis* during winter the relative content of gonadal protein decreased and this corresponds to the time when gametogenesis may take place. Most of these changes were noticeable in the gonad, but not in the viscera. In fact, visceral content of protein and lipid was very much less compared to that of gonads.

In the present study a grain-based artificial feed was used for sea urchins which contained soybean as one of the main components. In general, soybean meal is extensively used in feeds for cultivation of fish on a commercial scale (Storebakken *et al.*, 2000). Cook *et al.* (1998) reported that the gonadal growth is very high when sea urchins are fed on a protein- and lipid-rich artificial feed compared to a macroalgal diet that resembles urchin's preferred natural diet. Another study on *P. lividus* suggested that diets rich in protein should induce high gonad production (Fernandez *et al.*, 1995). However, the precise nutritional requirements for gonadal growth in echinoids remain unknown.

Agatsuma (1998) showed that sea urchins fed on an artificial diet based on fish meal have increased moisture levels. On the other hand, Nishikiori (1989) observed that moisture content in the gonads of *S. nudus* fed *Laminaria japonica*

was below 70% when the urchins were fed in excess from fall to spring. In this experiment sea urchins were fed on *Laminaria* sp during acclimation and had the least amount of moisture on week 0. The biochemical composition of gonads of urchins fed on a protein- and algal-based diets were different. Thus, the moisture content increased significantly when urchins were given an artificial diet based on soybean meal as the major protein source. The protein content was significantly increased while lipids showed an inverse relationship with moisture. Furthermore, the carbohydrate level remained considerably high throughout the study. Similar to wild sea urchins, cultured urchin viscera did not show any marked changes in their biochemical composition.

Under rearing using the soybean-based feed noticeable effects were observed in the biochemical composition of sea urchins. For gonads, this effect was primarily observed for carbohydrate and/or lipid levels. Sea urchins fed artificial feed had low lipid levels while their carbohydrate level was more than 10% at each harvest time. In general, Echinoidea gonad is an important nutrient reserve tissue (Giese, 1966a; 1966b; Klinger *et al.*, 1988). This storage occurs in one of two ways: either through an increase in the gonadal size or through the storage of nutrients (lipid or carbohydrate) within specialized cells (Gonor, 1973). It has previously been shown that rearing using artificial feeds brings about an increase in the gonadal weight in *P. lividus* (Fernandez *et al.*, 1995), in part, due to accumulation of lipid and/or carbohydrate reserves in specialized gonadal cells.

When food resources are limiting, decreases in the proportions of lipids occur (Niimi, 1972) in order to meet the energetic demands of body metabolism

(Elliott, 1975). When depletion is severe protein reserves are mobilized (Wilkins, 1967) and changes in the proportion of minerals may also be observed (Love, 1970). In fish, it has been shown that during winter the content of muscle lipids decreases but protein and mineral levels are maintained, thus suggesting that they can withstand long periods of starvation (Wilkins, 1967; Love, 1970). Present study also demonstrated that there was a depletion in the lipid reserves during winter. However, the protein levels did not undergo drastic changes.

4.2.2 Lipid class composition

Biological studies of lipids as an energy source for metabolism in fish have revealed their importance during periods of stress (Jezierska *et al.*, 1982). In general, the growing season of the temperate zone organisms may be as short as four to five months and they consume only small amounts of food or none at all throughout the cold months of the year. During this overwintering period, they rely heavily on lipid reserves built up during the feeding season (El-Sayed *et al.*, 1984). The lipid composition of marine invertebrates is influenced by several factors including pattern of feeding, gametogenesis and probably also environmental conditions (Clarke, 1977; 1979; 1980; Hill-Manning and Blanquet, 1979; Jezierska *et al.*, 1982). Although numerous studies have demonstrated seasonal variations in the biochemical composition (Giese 1966a; 1966b; Shearer, 1994; Fernandez, 1998) almost none has documented the seasonal variation of both polar and non-polar lipid classes of sea urchins.

Wax esters have been reported to constitute energy reserves in various marine invertebrates (Lee *et al.*, 1971; Benson and Lee, 1972, Sargent, 1976). The sea urchin *S. droebachiensis* in this study did not possess energy reserves in the form of wax esters. However, TAG formed the main energy reserve in these animals and their gonads and viscera were composed of similar lipid class compositions qualitatively. The same non-polar lipid class compositions have been observed in *S. droebachiensis* shell contents (both viscera and gonads) collected from Nova Scotia (Takagi *et al.*, 1980).

The non-polar lipids of gonads and viscera of both wild and cultured sea urchins consisted mainly of TAG, FFA and ST. Triacylglycerols are usually considered to be a storage product rather than membrane lipids in eukaryotic cells (Sul *et al.*, 2000). Thus, sea urchin lipids contained much larger amounts of storage lipids principally triacylglycerols which constituted more than 50% of the total non-polar lipids of gonads and viscera of both wild and cultured sea urchins.

Swift *et al.* (1980) have shown that in the oyster *Crassostrea virginica* there is a possibility of TAG constituting an energy source in reproductive tissues. A study done with two species of clams (*Tapes decussatus* and *T. philippinarum*) demonstrated that there was a decrease in TAG content during the winter, exclusive of reproductive activity, which indicated that this lipid class may constitute an energy reserve at least during the winter period of nutritional deficiency (Beninger, 1984). Similarly, *S. droebachiensis* decreased its relative TAG content during winter and this lipid class may have contributed some of their energy needed for metabolism under nutritional stress. Beninger (1984) further

reported that in the two species of clam studied, seasonal variation of polar lipids followed that of TAG. Thus, both polar lipids and TAG showed maxima in spring and early summer corresponding to the period of active gametogenesis, while the decrease in summer coincided with the emission of gametes. The same principle may be applied to non-polar and polar lipids of sea urchins. Hence, PC, the major lipid class showed a maxima in the spring with a subsequent decrease in the summer. Relative content of TAG also was highest in the spring followed by a decrease in the summer. A net decrease in polar lipid content during winter indicates their catabolism and some of the polar lipids may be used for maintenance of energy beyond that supplied by TAG (Beninger, 1984). Thus, it was revealed that both TAG and polar lipid may contribute to the maintenance energy, but polar lipid may constitute a greater portion under severe stress conditions after the more labile reserves have been mobilized. In fact, Beninger and Lucas (1984) reported that decrease in protein content observed during nutritional deficiency indicates that it is likely that most of the polar lipids mobilized originated from membrane autolysis during winter.

The qualitative composition of non-polar lipids in gonads and viscera of wild sea urchins was shown not to change. However, relative content of individual classes suffers marked changes. Similarly, cultured sea urchins showed the same composition of non-polar lipid classes in gonads and viscera qualitatively. In cultured sea urchins relative content of TAG increased significantly at the end of the feeding period. On the other hand, ST content was decreased and reached a minimum after feeding for 9 weeks. However, FFA content remained constant

throughout this period. Thus, the artificial diet exerted a significant effect quantitatively on individual non-polar lipid classes, but did not have any significant qualitative effect.

Sea urchin lipids contain a considerable amount of FFA and some of these FFA may be artifacts arising from breakdown of sea urchin lipids by cell enzymes during the extraction process. The energy supplied to the animal by the breakdown of lipid reserves comes primarily from oxidation of fatty acids. It has been shown that the FFA content can be induced by stress (Frakas, 1979). Environmental temperature and diet can be specified as factors exerting a major impact on fatty acid metabolism and fatty acid composition of fish (Farkas *et al.*, 1978). A study on eels (*Anguilla anguilla*) has shown that starvation induced FFA formation probably as a result of an increased TAG hydrolysis (Larsson and Lewander, 1973). The sea urchin *S. droebachiensis* also had the highest amount of FFA in winter both in gonads and viscera.

Larsson and Fange (1969) investigated seasonal variations in plasma cholesterol levels in two species of fish (*Gadus virens* and *G. morrhua*). They found that cholesterol levels were associated with spawning and suggested that the high cholesterol level found at the end of the winter may reflect a redistribution of cholesterol from other tissues to the maturing gonads. Further, high levels of total cholesterol have also been observed in *Oncorhynchus* sp at the onset of spawning when the gonads were not fully developed (Idler and Tsuyuki, 1985). In *S. droebachiensis* the relative amount of sterol was greatly increased in winter and this may indicate the redistribution process from other tissues to the gonads while

viscera had a low proportion of sterol. During spawning the cholesterol levels are markedly reduced (McCartney, 1967). In fish it has been shown that cholesterol content depends on dietary level and stage of sexual development (Love, 1970). High cholesterol levels have been observed in gonads during spring when they are close to spawn (El-Sayed *et al.*, 1984). Diet and nutritional status (Larsson and Lewander, 1973; Dave *et al.*, 1975) and temperature (Umminger, 1969) are also known to influence cholesterol levels in fish.

Vaskovsky and Kostetsky (1969) have performed TLC on polar lipids of sea urchins *S. nudus* and *S. intermedius*. The polar lipid fraction was separated into five components of which PC, PE and SM constituted the major polar lipid classes present. It was further stated that lipid extracts of different organs of the same animal qualitatively have the same polar lipid composition (Vaskovsky and Kostetsky, 1969). In this study also both gonads and viscera showed qualitative similarities in the polar lipid fraction. Rainuzzo *et al.* (1992) found that most cold-water species of fish utilize polar lipids, mainly PC, as their primary energy source. This may also be a possible reason for the depletion of PC in sea urchins during the winter season.

Polar lipid composition obtained from cultured sea urchins showed a marked effect of the diet on its composition. The content of PC decreased with a concurrent increase in PE. Hence, PC was the dominant polar lipid before feeding on artificial diet while PE became dominant after 9 weeks of feeding. Cultured sea urchin viscera also showed a similar polar lipid composition. However, a decrease

in the content of PC and an increase in PE content were more pronounced in gonads than in the viscera.

Floreto *et al.* (1996a) demonstrated that sea urchin *Tripneustes gratilla* fed on a seaweed diet had PC and PE as the major polar lipid constituents. Furthermore, PC contributed a larger proportion than PE. When sea urchins are cultured on seaweed diets the lipid class composition may still have PC and PE as the major lipid classes.

4.2.3 Fatty acid composition of sea urchin lipids

The fatty acids of total, polar and non-polar lipids of sea urchins were typically similar to those of marine species with a dominance of 16:0 and 20:5n-3 (Gruger *et al.*, 1964). According to Gruger *et al.* (1964) 22:6n-3 is also a typical fatty acid in marine lipids although it contributed only 1 to 2.5% to the total fatty acids in the lipids of *S. droebachiensis*. In spite of the overall similarity in fatty acid constituents, several specific differences have been demonstrated among seasons for total, polar and non-polar lipids. Holland (1978) reported that the predominance of PUFA, 20:5n-3 and 22:6n-3, in typical marine fatty acids is a result of adaptation to the relatively low temperatures of the marine environment. This performs a significant role by contributing to the maintenance of cell membrane fluidity in those organisms living in the cold environment. Thus, accumulation of unsaturated fatty acids at low temperatures can be explained as an attempt to maintain proper physicochemical properties and membrane functions in the cold (McElhaney and Souza, 1976). The predominance of 20:5n-3

in the polar lipids of *S. droebachinensis* is in accord with this hypothesis. Lewis (1962) proposed several modes of adaptation to low temperatures involving fatty acids in a comparative study of numerous temperate and cold-water marine species. Among these were an increase in desaturation of fatty acids and a decrease in the level of SFA that can be applied to sea urchin lipids. Hence, in the winter the level of SFA was decreased to a minimum with an increase in total PUFA content in all sea urchin lipids except for the gonadal polar lipids that showed an increased SFA content in the winter. Further, Farkas and Herodek (1964) demonstrated that in temperate waters the above changes were seasonal and corresponding to the annual temperature cycle. Thus, the above pattern was reversed during warmer seasons (Farkas, 1979). Therefore, an increase of SFA with a simultaneous decrease of PUFA in summer is presumably an adaptation which prevents membrane hyperfluidity at warmer temperatures (Beninger and Stephan, 1985). However, these temperature-related variations have been observed mainly in the fatty acids of total lipids. Beninger and Stephan (1985) further demonstrated these seasonal variations in the total SFA and PUFA fractions and also found that in the case of PUFA these seasonal variations are mainly due to n-3 fatty acids. On the other hand, SFA and PUFA levels of polar lipids did not follow any clear seasonal variation. Hence, these authors concluded that seasonal variations are clearly reflected only in the TAG fraction. Thus, these variations are independent of the maintenance of membrane fluidity and are likely that their origin is dietary rather than endogenous. However, in this study polar lipids of sea urchin tissues behaved more or less in the same way as did total and

non-polar lipids, thus exhibiting decreased SFA levels during cold temperatures with a simultaneous increase in PUFA and vise versa. It is known that some fatty acids in the membranes are diet derived, while others are produced by the organisms themselves. For adaptation of membrane fatty acid composition to the temperature the fatty acids of biosynthetic origin are a more suitable means than those taken up from dietary sources. Fatty acids of dietary origin do not necessarily follow the changes of temperature (Farkas, 1979).

Considerable published data are available on the fatty acid composition of sea urchins (Tables 4.19 and 4.20). The fatty acid 16:0 was the major SFA in the sea urchin *S. droebachiensis* collected from Herring Cove, Nova Scotia (Takagi *et al.*, 1980). Fujino *et al.* (1971) analysed fatty acid composition of sea urchins *Anthocidaris crassispina*, *S. pulcherrimus*, *S. franciscanus*, *S. intermedius* and *Echinus esculentus*. In all these examples 16:0 was the prominent SFA followed by 14:0. The fatty acid 18:0 was also found in considerable amounts. Similarly, in this study the predominant fatty acids were 16:0 and 14:0 irrespective of their origin.

Among MUFA 20:1n-15 was present up to 11% in the total fatty acids of urchins (Takagi *et al.*, 1980). Ackman and Hooper (1973) reported that marine animals such as periwinkle (*Littorina littorea*), moon snail (*Lunata triseriata*) and sand shrimp (*Crangon septemspinosus*) contain 20:1n-15, but at much lower levels not exceeding 0.2% of the total fatty acids. However, this has not been commonly reported as being typical of marine lipids. In this study, 20:1n-15 was the main MUFA in wild as well as cultured sea urchin tissues in almost all

Table 4.19 Fatty acid composition of total, polar and non-polar lipids of the sea urchin *Strongylocentrotus nudus* harvested in Japan waters.

Fatty acid	Total lipids	Polar lipids	Non-polar lipids
14:0	16.5	11.2	18.8
15:0	1.6	1.6	0.4
16:0	0.4	0.3	19.4
18:0	17.6	14.6	1.33
20:0	0.2	-	0.5
16:1n-7	3.9	2.6	4.2
18:1n-13	0.4	0.3	0.5
18:1n-9	3.0	1.5	3.3
18:1n-7	3.9	1.9	3.9
18:1n-5	0.4	0.2	0.5
20:1n-15	3.1	5.6	2.4
20:1n-11	0.9	0.9	0.9
20:1n-9	4.7	3.9	4.9
20:1n-7	1.3	1.0	1.4
22:1n-9	3.1	5.3	2.5
18:2n-9	1.4	0.6	1.6
18:2n-6	1.2	0.9	1.3
18:3n-6	0.9	0.5	1.0
18:3n-3	1.2	0.7	1.3
18:4n-3	2.8	1.4	2.9
20:2 5,11	4.6	4.8	4.4
20:2 5,13	1.8	1.2	2.0
20:2n-6	2.0	2.3	1.6
20:3 5,11,14	0.1	0.2	0.1
20:3n-6	1.2	0.4	1.2
20:4n-6	5.9	15.4	3.6
20:3n-3	0.9	0.9	0.8
20:4 5,11,14,17	0.1	0.03	0.1
20:4n-3	1.1	0.5	1.3
20:5n-3	6.1	12.1	4.1
22:2 7,13	0.31	0.4	0.3
22:2 7,15	0.6	1.0	0.5
22:5n-3	0.2	0.1	0.1
22:6n-3	0.6	0.5	0.6

Adapted from Kaneniwa and Takagi, 1986.

Table 4.20 Composition (w/w%) of fatty acids recovered from lipids of the sea urchin *Strongylocentrotus droebachiensis* harvested in Nova Scotia.

Fatty acid	Total lipids	Non-polar lipids	Polar lipids
14:0	9.8	10.4	10.0
15:0	0.4	0.7	0.5
16:0	11.7	12.9	9.7
17:0	0.1	0.2	-
18:0	2.0	2.4	2.6
20:0	0.9	1.9	0.8
14:1n-5	1.0	3.2	1.1
16:1n-11	0.2	2.1	2.2
16:1n-9	0.1	0.9	0.1
16:1n-7	3.8	5.5	4.6
16:1n-5	2.3	1.3	2.6
18:1n-13	0.7	0.3	0.6
18:1n-9	2.7	1.1	1.9
18:1n-7	2.5	2.0	3.4
20:1n-15	6.5	4.2	5.7
20:1n-11	0.7	1.1	0.6
20:1n-9	3.6	3.6	4.2
20:1n-7	1.0	2.4	1.4
22:1n-11	1.8	2.9	2.4
18:2n-6	1.9	0.8	1.4
20:2 5.11	6.3	5.3	6.3
20:2 5.13	2.2	2.3	1.8
20:2n-6	2.0	1.5	1.9
22:2 7.13	0.3	0.7	0.1
22:2 7.15	1.0	1.2	0.7
18:3n-6	0.5	0.4	0.4
18:3n-3	2.2	1.3	1.9
20:3n-6	0.2	0.1	0.1
20:3n-3	2.0	1.1	2.0
18:4n-3	5.3	2.7	4.7
20:4n-6	8.1	7.2	6.5
20:4n-3	1.2	0.6	1.3
20:5n-3	15.0	14.7	17.7
22:5n-3	0.1	1.2	0.4
22:6n-3	0.4	0.8	0.9

Adapted from Takagi *et al.*, 1980.

instances. Hence, formation of 20:1n-15 in sea urchins may be biosynthetic in origin since it was not affected by the diet. This is further supported by the fact that 20:1 n-15 has not been reported in seaweeds, the natural diets of sea urchins (Ackman and McLachlan, 1977) and was also absent in the artificial diet given in this study.

Kochi (1976) found that in sea urchin lipids 16:0, 16:1n-7 and especially 18:1n-7 are synthesised *de novo* by the sea urchin and stored as energy sources. Since, 18:1n-7 occurred in proportions greater than that of 18:1n-9, this is considered to be an important indicator of the *de novo* synthesis of 18:1n-7 through the biosynthetic pathway 16:0 ----- > 16:1n-7 ----- > 18:1n-7. In the present investigation, similar results were observed as more 18:1n-7 was present in most instances in the lipids of wild sea urchins and results for cultured sea urchins also supported the above phenomenon.

The occurrence of unusual 5-olefinic fatty acids such as 18:1n-13, 20:1n-15, 20:2 5,11, 20:2 5,13, 20:3 5,11,14 and 20:4 5,11, 14, 17 has been noticeable in lipids of sea urchins, amounting to as much as 6 to 20% of total lipid fatty acids (Kaneniwa and Takagi, 1986). According to fatty acid details of sea urchin *S. droebachiensis* harvested in Nova Scotia the occurrence of 5-olefinic acids is noticeable amounting to as much as 10 to 21% of fatty acids of total lipids of whole animals. In a similar study, sea urchins from Japan have been reported to contain similar levels of 5-olefinic acid to those from the Atlantic region (Takagi *et al.*, 1982). In this study 5-olefinic acids were found in the lipids of all samples more or less in the same amounts. Takagi *et al.* (1986) investigated 5-olefinic fatty

acids in 12 species of Echinoidea collected in Japan and found them in all samples examined. The occurrence of 5-olefinic fatty acids has thus been established as a common and characteristic feature of sea urchin lipids. However, 20:3 5,11,14 and 20:4 5,11,14,17 have been reported to be minor components of total fatty acids. Takagi *et al.* (1986) further stated that 5-olefinic acids in polar lipids have an important role as constituents of lipids in membranes as well as serving as a supply source for physiologically active components and biological energy. Among these 5-olefinic fatty acids, the occurrence of NMID isomers 20:2 5,11 and 20:2 5,13 has been reported in several marine animals (Ackman and Hooper, 1973). Takagi *et al.* (1980) reported that *S. droebachiensis* collected from Nova Scotia had as much as 5 - 10% of 20:2 NMID in the total fatty acids. Allen (1968) reported that the sea urchin *E. esculentus* had 6.2 - 6.5% of NMID 20:2 8,11. However, this peak was very likely made up of several 20:2 NMID and not 20:2 8,11 alone (Takagi *et al.*, 1980). They, further reported that these NMID are characteristic of other marine species as well as the sea urchin genera. The lipids of *S. droebachiensis* in this study also showed comparative results with respect to NMIDs.

Among PUFA 20:5n-3 is notably high in sea urchin lipids and this is distinctly higher in polar lipids which are structural parts of membranes (Takagi *et al.*, 1980). Wild sea urchins analysed in this study consumed mainly seaweeds, sea urchin's natural diet, especially *Laminaria* sp. that are known to be high in 16:4n-3, 18:4n-3, 20:4n-6 and 20:5n-3 (Pohl and Zurheide, 1979). It has shown that in sea urchins fed on seaweeds, that were rich in linoleic family of fatty acids such as

18:2n-6 and 20:4n-6, these fatty acids may partly substitute the membranes for their linolenic-family fatty acid 20:5n-3. This may be supported by the results obtained for cultured sea urchin lipids. The artificial feed contained approximately 50% of the total fatty acids in the form of 18:2n-6, and very little of 20:5n-3. With time, the content of 18:2n-6 increased while that of 20:5n-3 decreased in sea urchin lipids. Hence, the diet of sea urchins may exert a major effect on their fatty acid composition. Thus, it may be assumed that 18:2n-6 is of dietary origin with a very low contribution in wild sea urchins. Further, cultured sea urchins initially had a high content of 20:4n-6 and 20:5n-3 in their total and non-polar lipids, possibly attributable to the diet that comprised *Laminaria* sp. which is high in 20:4n-6 and 20:5n-3. On the other hand, an increase in the content of 18:2n-6 in polar lipids with increased time of feeding may be due to the incorporation of 18:2n-6 into the membranes instead of 20:5n-3, hence reflecting an inverse relationship between the two families of fatty acids.

Fatty acid profiles of sea urchin tissues fed on a seaweed diet reflected that of their diets to a certain extent (Floreto *et al.*, 1996a). On the other hand, certain fatty acids such as 16:4n-3, 20:4n-6, 20:5n-3 and 20:1n-11 have been reported to be major fatty acids of sea urchin tissues even if these fatty acids are not detected or are present only in very small amounts in their diets. This suggests that sea urchins are capable of synthesising them from precursors, including lower fatty acids. Similarly in this study, 16:4n-3, 20:1n-9, 20:4n-6 and 20:5n-3, among others, may have been formed by elongation of precursors that include lower fatty acids. The major sea urchin fatty acids, namely 16:4n-3, 20:4n-6 and 20:5n-3, are

probably known to have structural functions and hence are purposely synthesised (Floreto *et al.*, 1996a). Furthermore, several studies have assessed the effects of algal diets on the growth of sea urchins, but there are very few that have investigated the effects on their biochemical composition. The present study is probably the first one assessing the effects of a grain-based diet on the lipid and fatty acid composition of sea urchins.

4.2.4 Pigmentation

In the sea urchin *S. droebachiensis* carotenoids were mainly concentrated in the gonadal tissues. The total carotenoid content varied noticeably with season. The total carotenoid content reached a maximum in the spring and this indicates that they were at the pre-spawning stage when caught. Griffith and Perrott (1976) observed that *S. droebachiensis* harvested near Friday Harbour, Washington, had reached maximal values of total carotenoids just prior to the spawning season. Thereafter, total carotenoid content fell to a minimum when most of the eggs had been shed (Fig 4.25). This may be supported by the total carotenoid values obtained for *S. droebachiensis* in the present study. Hence, sea urchins had the highest carotenoid levels in the spring and this may indicate pre-spawning while total carotenoid content was lowest in the summer after spawning. It has been shown that in sea urchins most carotenoids initially in the ovary were incorporated into the eggs (Griffith and Perrott, 1976). When the gametes were released, carotenoid content also diminished as a consequence leading to a low content of total carotenoids in the summer. Although total carotenoid content reached a

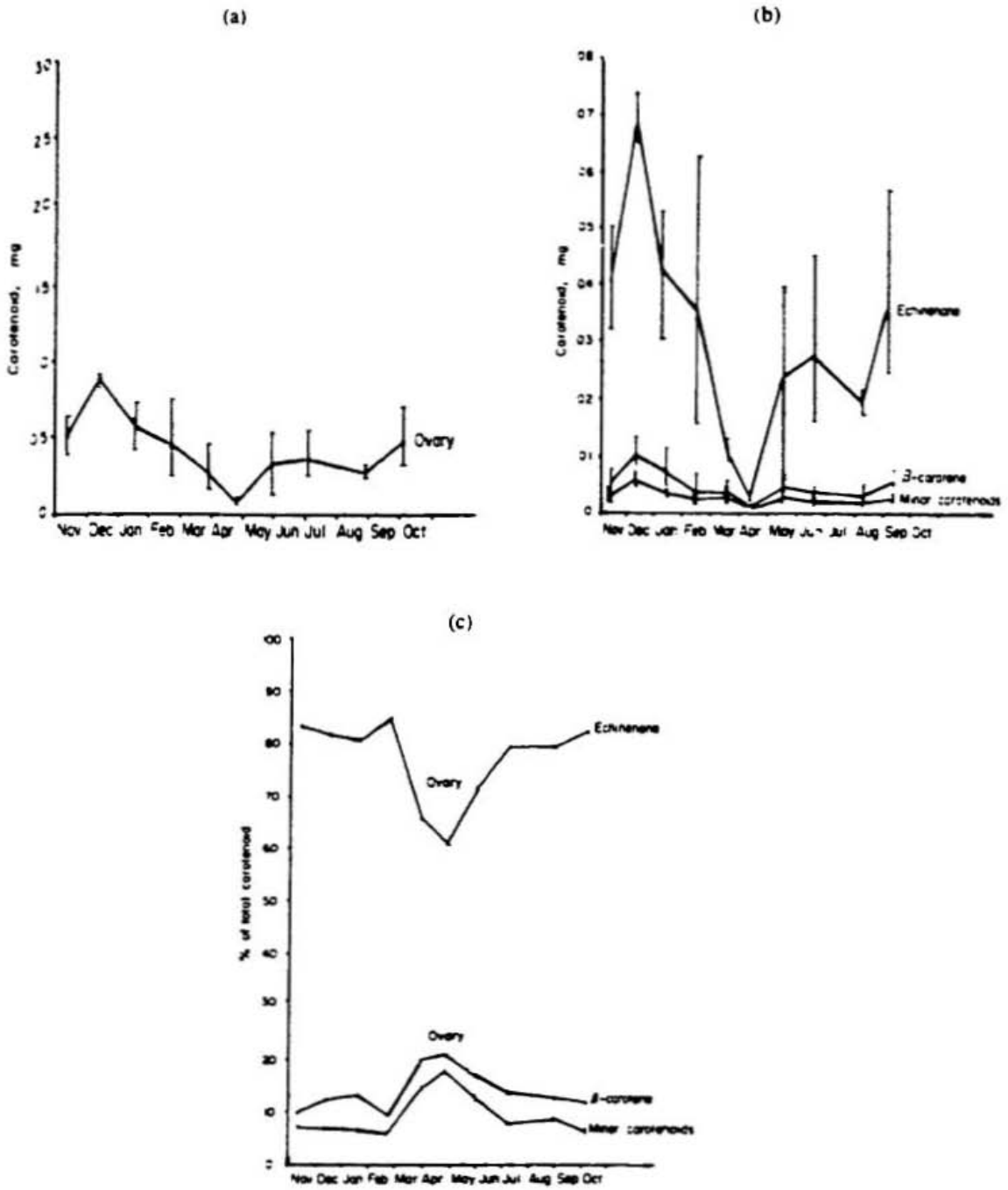


Fig 4.25 Seasonal changes in (a) total carotenoids (b) amount of echininone and (c) mean percentages of carotenoids, in the ovaries of the sea urchin, *Strongylocentrotus droebachiensis*. Adapted from Griffiths and Perrott, 1976.

maximum in gonads in the spring this was reduced to a minimum in the viscera. This supports the hypothesis that most of the carotenoids in the other tissues may also be transferred into the eggs. The corresponding results for cultured sea urchin gonads and viscera revealed that in *S. droebachiensis* the artificial diet had a significant effect on the total carotenoid content, reducing it with increasing feeding period. In fact, the total carotenoid level was almost halved in the gonads by the end of week 9 of feeding. This decrease in total content of carotenoids was apparent both in the gonads and viscera. The total carotenoid content of the feed was 0.38 mg per 100 g on a dry weight basis. In general, animals cannot synthesise carotenoids *de novo*, and are therefore dependent upon dietary carotenoids (Goodwin, 1984). Since the feed provides only a very low amount of carotenoids, this further brought about a significant reduction in the carotenoid content. Thus, supplementation of the artificial diet with carotenoids appears necessary.

Echininone and fucoxanthin were characterized as the major carotenoids present in the gonads and viscera, respectively, both in wild and cultured sea urchins. In addition, β-carotene was identified in both tissues. Echininone was found to be the main pigment with a lesser amount of β-carotene in the eggs of *S. purpuratus* (Griffith, 1966) and *S. droebachiensis* (Griffith and Perrott, 1976). Shina *et al.* (1978) also identified echininone as the major carotenoid both in the male and female gonads of tropical sea urchin *Tripneustes gratila*. Further, β-carotene was the second major carotenoid in sea urchin gonads examined. Tsushima *et al.* (1995) found that β-echininone and β-carotene were the major

carotenoids in the gonads of 19 out of the 20 sea urchin species examined. It has been reported that the major carotenoids of brown algae, the natural preferred diet of sea urchins, are β-carotene, violaxanthin and fucoxanthin (Jensen, 1966; Liaaen-Jensen, 1978; Goodwin, 1980; Matsuno and Hirao, 1989). Further, it has been reported that there is bioconversion of β-carotene to β-echininone via β-isocryptoxanthin in sea urchins; this taking place mainly in the gut wall and resulting in β-echininone to be incorporated into the gonads (Tsushima *et al.*, 1993). Kawakami *et al.* (1998) showed that fucoxanthin, the major carotenoid in brown algae, did not accumulate in the gonads. In fact, in the present study on *S. droebachiensis* fucoxanthin did not occur in the gonads. In contrast, viscera had fucoxanthin as its major carotenoid. Kawakami *et al.* (1998) further investigated the effect of these major pigments on the growth and reproduction and found that fucoxanthin, β-echininone and β-carotene may play an important role in the biological defense and reproduction of sea urchins.

Although the diet had a significant effect on the total content of carotenoids in sea urchin tissues, qualitatively there were no changes. Hence, cultured sea urchin gonads and viscera had the same pigment patterns seen in the wild sea urchins. The present study reveals that echininone and fucoxanthin are the dominant pigments in sea urchin gonads and viscera, respectively, in both wild and cultured animals.

Echininone content was highest in the spring and it can be inferred that the urchins were harvested prior to spawning. Griffith and Perrott (1976) have investigated seasonal variations of individual pigments of *S. droebachiensis* and

found that β-echininone was the predominant pigment in the gonad throughout the life cycle of animals. They also reported that sea urchins had the highest and lowest β-echininone content in the pre-spawning and post-spawning stage, respectively. Griffith and Perrot (1976) reported that β-echininone content varied between 79 and 85% most of the year except during the post-spawning stage when they constituted only 60% of their total carotenoids as β-echininone. On the other hand, β-carotene content ranged from approximately 10 to 20%. The β-echininone content obtained for *S. droebachiensis* in this study was somewhat less, but comparable to values obtained by Griffith and Perrott (1976).

Owing to the long conjugated double-bond system, the carotenoids show strong absorption bands in the UV-visible region. The position of the absorption maximum and the shape of the UV-visible spectrum are characteristic of each carotenoid. Hence, information on these data provide a means to identify different carotenoids (Vetter *et al.*, 1971). It has been shown that in light absorption properties a carotenoid is dependent on the organic solvent in which it is dissolved (Britton, 1995). In fact, UV-visible spectrum gives information about the "chromophore" that refers to the conjugated, unsaturated part of the molecule. In general, as the length of the chromophore increases the absorption occurs at a longer wavelength (Britton, 1995).

The absorption maxima values obtained for echininone and fucoxanthin in different organic solvents by various researchers are presented in Table 4.21. Further, the absorption maxima in hexane (section 4.1.4.3) obtained for fraction II and fraction IV from sea urchin gonads and viscera, respectively, are comparable

Table 4.21 UV/visible spectroscopic data for echininone and fucoxanthin in hexane, ethanol and choloroform.

Absorption maxima	Solvent	Reference
ECHININONE		
480/455	Hexane	Fox and Hopkins, 1966
461	Ethanol	Britton, 1995
470	Ethanol	Krinsky and Goldsmith, 1960
473	Chloroform	Goodwin, 1955
471	Chloroform	Britton, 1995
FUCOXANTHIN		
426/447/465	Hexane	Fox and Hopkins, 1966
426/449/465	Ethanol	Hager and Stransky, 1970
460/478	Chloroform	Bonnett et al., 1969
457/492	Chloroform	Goodwin, 1955
460/478	Chloroform	Britton, 1995

with those values. Hence, sea urchin gonads and viscera have echininone and fucoxanthin, respectively, as principal carotenoids. Furthermore, the UV spectra of echininone and fucoxanthin are shown in Fig 4.26 and Fig 4.27, respectively.

4.2.5 Amino acid composition

Although marine invertebrates characteristically contain a high intracellular concentration of free amino acids (FAA), the composition of the FAA pool may vary among species (Gilles, 1979). In the present study glycine was the dominant amino acid in both total amino acids (TAA) and free amino acids (FAA) in sea urchin gonads and viscera except that tyrosine was dominant in the gonads on week 9 after feeding on the artificial diet. Komata *et al.* (1962) reported that glycine was dominant in related green sea urchin *S. pulcherrimus* ranging from 35 - 41% of total FAA. Lee and Haard (1982) reported that glycine represented 18 - 60% of the FAA in sea urchin *S. droebachiensis* (Table 4.22). In this study sea urchin *S. droebachiensis* contained 12.9 - 16.6 and 14.6 - 20.3% glycine in the TAA profile throughout the year in gonads and viscera, respectively. On the other hand in the sea urchin *Paracentrotus lividus* glycine was not the dominant amino acid although it contributed a considerable amount to the TAA pool (Cruz-Garcia *et al.*, 2000). In the present study glycine was found to have the highest concentration in TAA and FAA of sea urchin gonad as well as viscera in the spring. According to Lee and Haard (1982) *S. droebachiensis* contained the highest FAA content when the gonads were well ripened and ready to release gametes. Srivastava and Brown (1991) reported the presence of a large

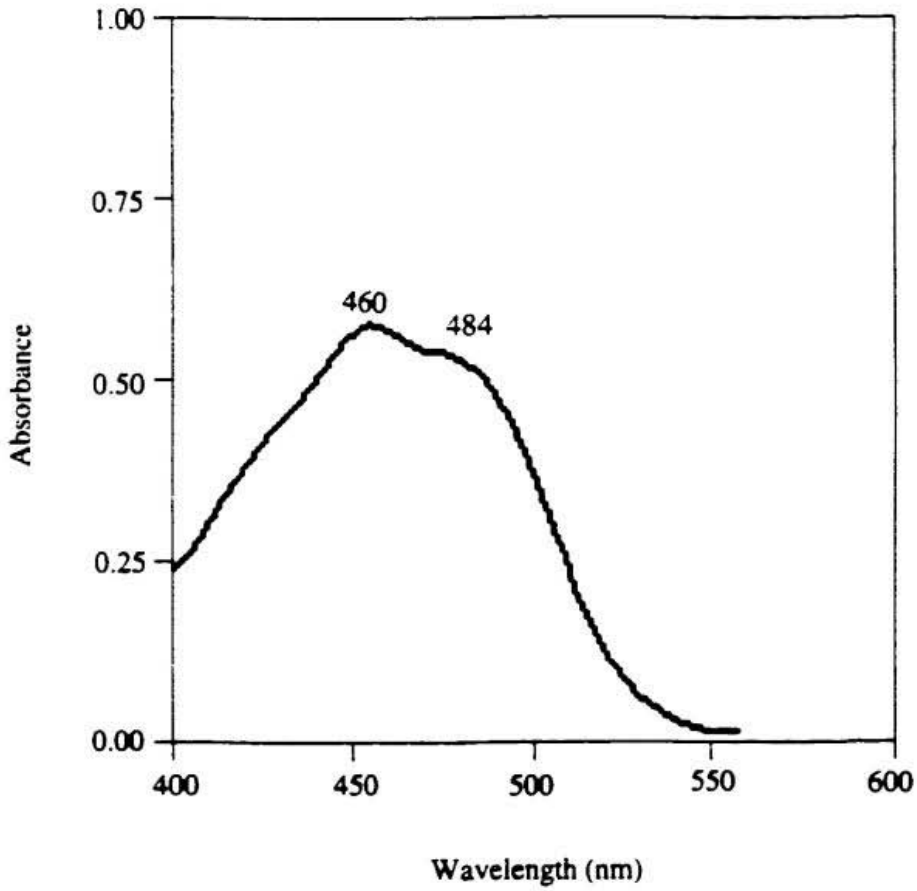


Fig 4.26 UV spectrum of echininone in hexane from the gonads of the sea urchin *Strongylocentrotus droebachiensis.*

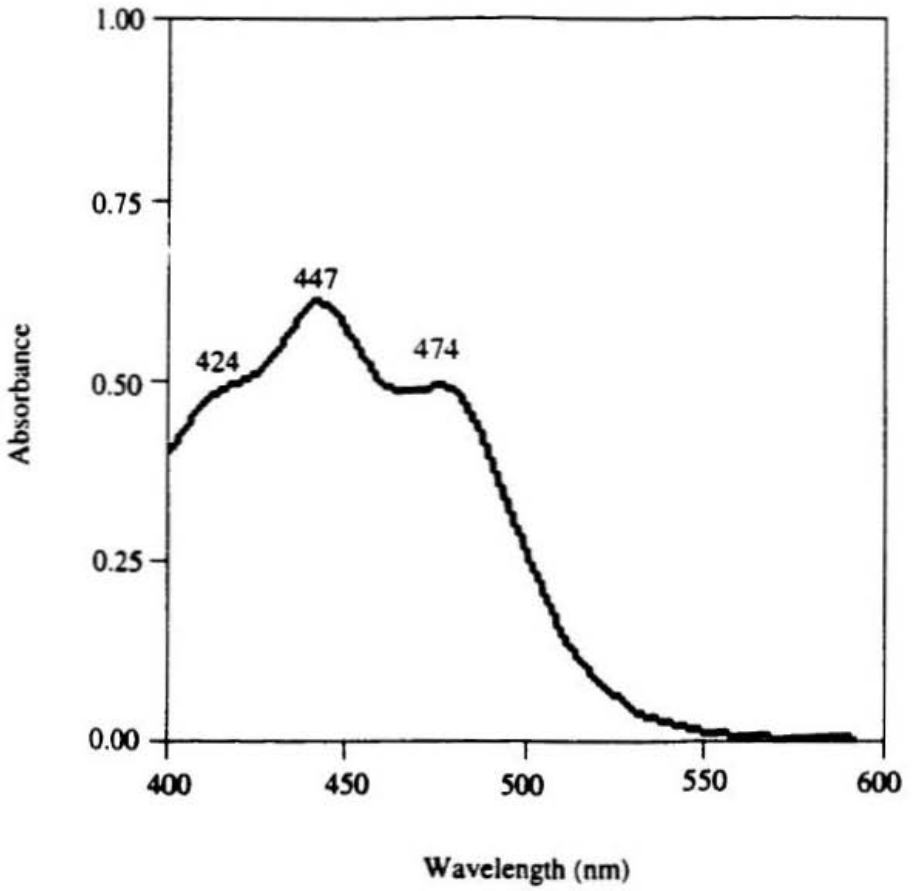


Fig 4.27 UV spectrum of fucoxanthin in hexane from the viscera of the sea urchin *Strongylocentrotus droebachiensis.*

Table 4.22 Content of free amino acids of eggs of the sea urchin *Strongylocentrotus droebachiensis*.

Amino acid	April		July		October	
	µg/g	%	µg/g	%	µg/g	%
Glycine	64.010	58.8	27.950	18.0	26.810	41.0
Alanine	9.450	8.7	5.520	3.6	3.620	5.5
Glutamic acid	1.740	1.6	2.370	2.9	2.960	4.5
Glutamine	560	0.5	1.900	1.2	-	-
Arginine	4.280	3.9	13.920	9.0	5.880	9.0
Lysine	3.320	3.0	14.240	9.2	4.540	6.9
Cystathionine	460	0.4	2.300	1.5	480	0.7
Serine	1.820	1.7	3.120	2.0	960	1.5
Hydroxyproline	880	0.8	410	0.3	340	0.5
Threonine	980	0.9	7.080	4.6	680	1.0
Aspartic acid	440	0.4	550	0.3	580	0.9
Taurine	1.280	1.2	500	0.3	780	1.2
Leucine	1.160	1.1	14.260	9.2	1.660	2.5
Tyrosine	960	0.9	12.880	8.3	2.060	3.2
Proline	Tr	Tr	950	0.6	260	0.4
Valine	1.120	1.0	11.430	7.3	1.500	2.3
Isoleucine	900	0.8	10.620	6.8	1.060	1.6
Methionine	480	0.4	6.540	5.2	540	0.8
Phenylalanine	660	0.6	7.960	5.1	850	1.3
Asparagine	360	0.3	980	0.6	330	0.5
Histidine	330	0.3	4.030	2.6	680	1.0
Phosphoethanolamine	320	0.3	Tr	Tr	Tr	Tr
Cystine	660	0.6	620	0.4	460	0.7
Trptophan	300	0.3	3.080	2.0	700	1.1
α-Aminoadipic acid	Tr	Tr	340	0.2	100	0.2
Glycerophosphoethanolamine	400	0.4	470	0.3	390	0.6
Cysteic caid	200	0.2	110	0.1	110	0.2
Sarcosine	10.440	9.6	140	0.1	990	1.5
Total Free Amino Acids (mg/g dry weight)	107.5		154.3		59.3	

Adapted from Lee and Haard, 1982.

amount of amino acids in Atlantic salmon, at spawning and this amino acid reserve depleted progressively when to resume feeding. Similarly, in the sea urchin *S. droebachiensis* amino acid reserves depleted after spawning as it started active feeding in summer. Other than glycine, the amino acids arginine, lysine, alanine, serine, glutamic acid and methionine are considered important for taste even though some of them are present in small quantities (Lee and Haard, 1982). *S. droebachiensis* in this study also contained considerable amounts of these amino acids, especially during the spring.

The total free amino acid pools of sea urchins were greatly affected by the soybean-based feed. The FAA content was obviously lower in week 0 when urchins were fed *Laminaria* sp compared to other harvests when they were fed on an artificial feed. Each amino acid showed a considerable increase following introduction of the artificial diet. Although glycine remained the dominant amino acid after feeding, its proportion was reduced since other amino acids increased their contribution to the total FAA pool. Srivastava *et al.* (1995) have shown that the amino acid content in fish is quantitatively higher in the eggs from the wild stock than the eggs from the cultured stock. However, qualitatively the amino acid composition of eggs from wild stock did not differ from that of the cultured stock. In this study *S. droebachiensis* did show qualitative similarities, but there were considerable quantitative differences in the amino acid profile; the amino acid content was greatly increased when urchins were fed on an artificial, soy-based diet.

4.2.6 Content of nucleic acids

Studies on fish have revealed that the biosynthetic potential for growth of fish larvae can be determined from the quantity of RNA relative to DNA (Wright and Martin, 1985; Clemmesen, 1989; Richard *et al.*, 1991). It has been shown that RNA/DNA ratio allows comparison of the nutritional status and recent growth of larvae; thus, a low RNA/DNA ratio indicates a lower growth potential (Robinson and Ware, 1988; Clemmesen, 1989). In the present study, *S. droebachiensis* showed significant seasonal variations in the RNA/DNA ratio, being highest in the summer both in gonads and viscera of the wild sea urchins. Fry (1971) reported that temperature and food availability are primary determinants of larval growth in the sea; temperature being the controlling factor that governs the chemical reactions. The RNA/DNA ratio appears to be a good indicator of the state of limiting factors, primarily food availability (Buckley, 1984). An increased ratio of RNA/DNA in sea urchin *S. droebachiensis* may be due to the availability of food with the onset of warmer environmental temperatures that prevail during summer. Relations between food availability and larval RNA/DNA ratio have been demonstrated (Buckley, 1979, 1980, 1982; Buckley *et al.*, 1984). However, less information is available on the effects of reproduction on RNA/DNA ratio. In general, gametogenesis involves intense synthetic activity and production of gametes (Robbins *et al.*, 1990). Hence, it may be assumed that in *S. droebachiensis* gametogenesis may also affect RNA/DNA ratios. In these urchins spawning occurs in late winter or early spring. Thus, comparatively high RNA/DNA ratio in the winter may reflect high synthetic activities associated with

gametogenesis. Skjoldal and Bamstedt (1976) found that in crustaceans there were prominent seasonal variations in the RNA/DNA ratio with respect to the whole animal. However, it was not determined if this variation was due to the effect of gametogenesis or not. On the other hand, Bulow *et al.* (1981) reported that RNA/DNA ratio in fish decreased during gonad maturation due to the reduced growth rate resulting from energetic demands of gonad maturation.

From the present study it is apparent that the RNA/DNA ratio was significantly affected by the artificial diet. The diet caused a significant increase in RNA/DNA ratio in the sea urchin tissues analysed. However, the increase was more prominent in gonads than that in viscera. Thus, it is obvious that the artificial diet had a positive effect on the growth of sea urchins. Increased RNA/DNA ratio which indicates increased growth rate may be due to the availability of food. Buckley *et al.* (1984) demonstrated that growth rate and RNA/DNA ratio in larval sand lance (*Ammodytes americanus*) increased with increasing feeding levels.

CHAPTER 5

SUMMARY AND CONCLUSIONS

The composition of sea urchin tissues were significantly affected by seasonal and dietary factors. The biochemical composition of gonads of urchins fed on the protein- and algal-based diets were different. The lipid content was highest in the spring while moisture level was lowest. In winter moisture level was increased with a decrease in the lipid content. Protein content was highest in the fall for sea urchin gonads. The dietary effects on the biochemical composition of sea urchin gonads were also prominent. Moisture and lipid contents displayed inverse relationships. Thus, lipid content decreased with increasing moisture levels at the end of a 9 week feeding period. The changes in the lipid and protein contents were less conspicuous in the visceral tissues owing to their very low contents.

Lipid class composition was not affected by the season or diet on a qualitative basis. However, there were noticeable quantitative differences. Triacylglycerol constituted the main energy reserve in these animals. Triacylglycerol, free fatty acids and sterol were the major non-polar lipid classes while phosphatidylcholine and phosphatidylethanolamine were the major polar lipid classes. The relative proportions of triacylglycerol, and sterol were increased in the spring. Phosphatidylcholine content was also highest in the spring. The free fatty acid content was highest in the winter when the animals were under nutritional stress. The diet brought about quantitative changes in the lipid classes. Triacylglycerol content was increased while the content

of phosphatidylcholine decreased at the end of the feeding period. Hence, PE was the dominant polar lipid class in cultured sea urchins at the end of 9 weeks of feeding.

Major saturated fatty acids were 14:0 and 16:0 in all the sea urchin samples analysed. The fatty acid 20:1n-15 remained the dominant monounsaturated fatty acid in the wild sea urchins. However, the dominant monounsaturated fatty acid of non-polar lipids of cultured sea urchin gonads and viscera was 18:1n-9 at the end of the feeding period. Among polyunsaturated fatty acids 20:5n-3 constituted a major proportion in wild sea urchins. On the contrary 18:2n-6 became dominant in the cultured sea urchins at the end of the feeding period. In addition all samples contained a relatively high amount of 20:4n-6.

Glycine was the dominant amino acid in both total and free amino acids in the wild and cultured sea urchin tissues, but tyrosine dominated the total amino acid profile in the gonads of cultured sea urchins at the end of a 9 week feeding period. In wild sea urchin gonads and viscera glycine levels were highest in the spring. Total and free amino acid pools in sea urchins were significantly affected by the artificial feed, especially the latter one. Free amino acid content was lowest on week 0 and increased manifolds after feeding on the artificial diet.

The RNA/DNA ratio was highest in the summer in the gonads and viscera of wild sea urchins. Furthermore, the RNA/DNA ratio was increased with increased feeding period. The increase was more prominent in the gonads than in the viscera.

Carotenoids were mainly concentrated in the gonadal tissue. The total carotenoid content varied significantly with the season. This was highest in the spring

while samples harvested in the summer had the lowest amounts. The diet also had a noticeable effect on the total carotenoid content. Thus, the total carotenoid content was approximately halved at the end of a 9 week feeding time. Echininone and fucoxanthin were the major carotenoid pigments in the gonads and viscera, respectively, in both wild and cultured sea urchins. In addition, β-carotene was present in all samples analysed. Seasonal and dietary factors did not bring about any qualitative changes in the carotenoid pigments.

The present study demonstrated that wild sea urchin tissues had much in common with those of their cultured counterparts. Further the study showed that the type and quality of feed greatly affects the nutrient composition of sea urchin tissues. In fact, for the gonad the accumulation of nutrient reserves would be beneficial as this contributes to the development of this organ, which is of economical interest to the sea urchin industry.

The gonads are of most acceptable eating quality during the early stages of ripening and least acceptable following early spent stages. On the other hand since natural populations are being depleted drastically around the world due to overfishing, development of an aquaculture industry for sea urchins is beneficial; this involves the formulation of feeds that could rapidly build up the gonadal tissue possessing desirable commercial characteristics.

The artificial feed used in this study was deficient in nutraceutically important long chain polyunsaturated fatty acids that are a major component in their natural diet. Thus, EPA content was gradually decreased by the end of 9 week feeding period.

However, almost all essential amino acids were present in the feed. Further, in the study reported here, it was apparent that feeding on an artificial diet brought about a significant reduction in the carotenoid content in cultured sea urchins after feeding for 9 weeks. In fact the artificial diet was also deficient in carotenoid pigments. Since carotenoids are important in bringing about protection against lipid oxidation and especially in acting as an effective antioxidant, inclusion of β-carotene, or relevant xanthophyll, in the feed of sea urchin is suggested. This may also help in preserving the polyunsaturated fatty acids which are abundant in sea urchin lipids in addition to imparting desirable colours. Thus, it is of prime importance to improve the feed composition of sea urchins in order to obtain faster growth in gonads with high quality. Indeed, additional studies are needed to confirm these observations, supported by sensory evaluation, and to evaluate the market potential of green sea urchins. However, precise nutritional requirements for gonadal growth in sea urchins remain unknown. Therefore, further research may also be directed in determination of the ways in which each nutrient affects the composition of sea urchin gonads. Furthermore, it would be beneficial if a similar analysis is done by separating the ovaries and testes, which enables to evaluate their effects on the biochemical composition.

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APPENDIX 1

TableA.1 Quantification of neutral lipids (weight %) of sea urchin gonads in spring, summer, fall and winter using Iatroscan (TLC - FID).

Lipid class	Spring	Summer	Fall	Winter
TAG	66.4 ± 0.9^{a}	56.2 ± 1.3^{b}	55.7 ± 2.2^{b}	50.2 ± 3.1^{b}
FFA	24.7 ± 0.9^{a}	33.1 ± 0.6^{b}	31.4 ± 1.0^{b}	34.9 ± 2.2^{b}
ST	8.9 ± 1.6^{a}	10.7 ± 1.1^{a}	13.4 ± 2.9^{a}	15.6 ± 3.8^{a}
MAG	ND	ND	ND	8.93 ± 0.95
DAG	Tr	Tr	Tr	ND

Results are mean values of 3 replicates ± standard deviation. Values in each row with the same superscript are not different ($p>0.05$) from one another.

Abbreviations are as follows: TAG, triacylglycerol; FFA, free fatty acids; ST, sterol; MAG, monoacylglycerol; DAG, diacylglycerol; ND, not detected; and Tr, trace.

Table A.2 Quantification of neutral lipids (weight %) of sea urchin viscera in spring, summer, fall and winter using Iatroscan (TLC - FID).

Lipid class	Spring	Summer	Fall	Winter
TAG	55.3 ± 0.9^{ab}	52.4 ± 3.2^{a}	63.8 ± 1.9^{b}	47.2 ± 2.2^{a}
FFA	31.4 ± 1.2^{a}	28.6 ± 0.9^{a}	29.7 ± 1.9^{a}	35.9 ± 4.0^{a}
ST	13.3 ± 1.8^{a}	12.3 ± 0.7^{a}	6.5 ± 0.6^{b}	16.9 ± 2.4^{a}
MAG	ND	6.8 ± 1.7	Tr	ND

Results are mean values of 3 replicates ± standard deviation. Values in each row with the same superscript are not different ($p>0.05$) from one another.

Abbreviations are as follows: TAG, triacylglycerol; FFA, free fatty acids; ST, sterol; MAG, monoacylglycerol; DAG, diacylglycerol; ND, not detected; and Tr, trace.

Table A.3 Quantification of non polar lipids (weight %) of sea urchin gonads at 0, 3, 6 and 9 weeks of feeding a grain based diet, using Iatroscan (TLC-FID).

Lipid class	Week 0	Week 3	Week 6	Week 9
TAG	66.7 ± 0.8^{a}	69.1 ± 0.9^{b}	74.6 ± 0.8^{c}	77.4 ± 0.8^{c}
FFA	22.7 ± 1.1^{a}	27.3 ± 1.0^{b}	20.3 ± 0.9^{a}	15.2 ± 0.7^{c}
ST	10.6 ± 1.2^{a}	3.6 ± 0.7^{b}	5.0 ± 0.8^{bc}	7.4 ± 0.8^{c}
MAG	Tr	Tr	Tr	Tr
DAG	Tr	ND	ND	ND

Results are mean values of 3 replicates ± standard deviation. Values in each row with the same superscript are not different ($p>0.05$) from one another.

Abbreviations are as follows: TAG, triacylglycerol; FFA, free fatty acids; ST, sterol; MAG, monoacylglycerol; DAG, diacylglycerol; ND, not detected; and Tr, trace.

Table A.4 Quantification of non polar lipids (weight %) of sea urchin viscera at 0, 3, 6 and 9 weeks after feeding a grain based diet, using Iatroscan (TLC-FID).

Lipid class	Week 0	Week 3	Week 6	Week 9
TAG	56.5 ± 0.7^{a}	66.9 ± 1.2^{b}	73.0 ± 0.6^{c}	83.4 ± 1.2^{d}
FFA	37.4 ± 1.0^{a}	30.3 ± 1.4^{b}	25.1 ± 1.1^{c}	14.2 ± 1.8^{d}
ST	6.1 ± 0.8^{a}	2.8 ± 0.7^{b}	1.8 ± 1.1^{b}	2.4 ± 0.7^{b}
MAG	Tr	ND	ND	Tr
DAG	Tr	Tr	Tr	ND

Results are mean values of 3 replicates ± standard deviation. Values in each row with the same superscript are not different ($p>0.05$) from one another.

Abbreviations are as follows: TAG, triacylglycerol; FFA, free fatty acids; ST, sterol; MAG, monoacylglycerol; DAG, diacylglycerol; ND, not detected; and Tr, trace.

Table A.5 Quantification of polar lipids (weight %) of sea urchin gonads in spring, summer, fall and winter using Iatroscan (TLC-FID).

Lipid class	Spring	Summer	Fall	Winter
PC	66.9 ± 1.3^{a}	63.7 ± 5.3^{ab}	56.5 ± 0.2^{ab}	52.6 ± 1.8^{b}
PE	17.9 ± 0.2^{a}	28.1 ± 3.5^{b}	30.5 ± 1.1^{b}	31.1 ± 0.1^{b}
PS / PI	12.0 ± 0.6^{a}	4.4 ± 1.1^{b}	11.2 ± 1.6^{a}	12.3 ± 0.7^{a} 7
SM/LPC	3.3 ± 0.5^{a}	3.8 ± 1.2^{a}	2.0 ± 1.0^{a}	4.2 ± 0.8^{a}
CL	ND	ND	ND	Tr

Results are mean values of 3 replicates ± standard deviation. Values in each row with the same super script are not different ($p>0.05$) from one another.

Abbreviations are as follows: PC, phosphatidylcholine; PE, phosphatidylethanolamine; PS, phosphatid ylserine; PI, phosphatidylinositol; SM, sphingomyelin; lysophosphatidylcholine, LPC; CL, cardiolipin; ND, not detected; and Tr, trace.

Table A.6 Quantification of polar lipids (weight %) of sea urchin viscera in spring, summer, fall and winter, using Iatroscan (TLC-FID).

Lipid class	Spring	Summer	Fall	Winter
PC	69.8 ± 0.7^{ab}	66.2 ± 2.6^{a}	71.4 ± 1.8^{b}	86.4 ± 0.6^{c}
PE	25.3 ± 1.3^{ab}	31.8 ± 2.9^{a}	24.5 ± 1.6^{b}	13.8 ± 0.9^{c}
SM/LPC	3.1 ± 0.7^{ab}	2.0 ± 0.3^{a}	4.1 ± 0.2^{b}	Tr
PS/PI	1.7 ± 0.6	Tr	Tr	Tr

Results are mean values of 3 replicates ± standard deviation. Values in each row with the same superscript are not different ($p>0.05$) from one another.

Abbreviations are as follows: PC, phosphatidylcholine; PE, phosphatidylethanolamine; PS, phosphatidylserine; PI, phosphatidylinositol; SM, sphingomyelin; LPC, lysophosphatidylcholine; and Tr- trace.

Table A.7 Quantification of polar lipids (weight %) of sea urchin gonads at 0, 3, 6 and 9 weeks of feeding a grain based diet, using Iatroscan (TLC-FID).

Lipid class	Week 0	Week 3	Week 6	Week 9
PC	65.7 ± 1.4^{a}	62.3 ± 0.4^{a}	32.8 ± 0.9^{bc}	36.0 ± 0.45^{c}
PE	17.3 ± 0.2^{a}	25.1 ± 1.2^{b}	46.5 ± 1.9^{c}	53.4 ± 0.3^{d}
SM/LPC	8.4 ± 0.7^{a}	12.6 ± 1.5^{b}	7.1 ± 0.6^{a}	10.6 ± 0.7^{ab}
PS / PI	8.5 ± 0.6	Tr	Tr	Tr

Results are mean values of 3 replicates ± standard deviation. Values in each row with the same superscript are not different ($p>0.05$) from one another.

Abbreviations are as follows: PC, phosphatidylcholine; PE, phosphatidylethanolamine; PS, phosphatidylserine; PI, phosphatidylinositol; SM, sphingomyelin; LPC, lysophosphatidycholine; and Tr, trace.

Table A.8 Quantification of polar lipids (weight %) of sea urchin viscera at 0, 3, 6 and 9 weeks of feeding a grain based diet, using Iatroscan (TLC-FID).

Lipid class	Week 0	Week 3	Week 6	Week 9
PC	68.9 ± 0.8^{a}	65.8 ± 0.8^{ab}	64.2 ± 0.7^{b}	53.2 ± 1.0^{c}
PE	27.3 ± 0.89^{a}	33.0 ± 1.2^{b}	35.8 ± 0.9^{bc}	39.2 ± 0.3^{c}
SM/LPC	2.6 ± 0.2^{a}	1.2 ± 0.7^{a}	Tr	7.6 ± 1.0^{b}
PS/PI	1.1 ± 0.5	Tr	Tr	ND

Results are mean values of 3 replicates ± standard deviation. Values in each row with the same superscript are not different ($p>0.05$) from one another.

Abbreviations are as follows: PC, phosphatidylcholine; PE, phosphatidylethanolamine; PS, phosphatidylserine; PI, phosphatidylinositol; SM, sphingomyelin; CL, cardiolipin; LPC, lysophosphatidycholine; and Tr, trace.

Table A.9 Fatty acid composition (weight %) of total lipids of sea urchin gonads in spring, summer, fall and winter.

Fatty acid	Spring	Summer	Fall	Winter
13 : 0	ND	ND	0.1 ± 0.01	ND
14 : 0	8.6 ± 0.1^{a}	8.2 ± 0.2^{a}	6.2 ± 0.02^{b}	5.2 ± 0.1^{c}
15 : 0	0.4 ± 0.2^{ab}	0.4 ± 0.02^{a}	0.8 ± 0.2^{b}	0.9 ± 0.1^{ab}
16 : 0	10.2 ± 0.3^{a}	12.9 ± 0.3^{b}	10.0 ± 0.1a^{b}	9.3 ± 0.1^{c}
17 : 0	0.2 ± 0.1^{a}	0.5 ± 0.1^{a}	0.6 ± 0.01^{a}	0.3 ± 0.2^{c}
18 : 0	2.0 ± 0.2^{a}	1.9 ± 0.04^{a}	1.6 ± 0.1^{a}	4.7 ± 0.1^{b}
19 : 0	0.4 ± 0.03^{a}	ND	0.3 ± 0.1^{a}	0.3 ± 0.04^{a}
20 : 0	2.0 ± 0.1^{a}	0.8 ± 0.1^{b}	2.3 ± 0.02^{a}	0.5 ± 0.02^{b}
Σ SFA	**23.7 ± 0.1^{a}**	**24.7 ± 0.9^{a}**	**21.9 ± 0.3^{b}**	**20.8 ± 0.2^{c}**
14 : 1 n-7	0.1 ± 0^{a}	ND	0.1 ± 0.02^{a}	0.1 ± 0.01^{a}
14 : 1 n-5	0.8 ± 0.0^{a}	0.6 ± 0.01^{a}	0.6 ± 0.20^{a}	0.6 ± 0.1^{a}
16 : 1 n-9	0.2 ± 0.03^{a}	ND	0.2 ± 0.01^{a}	0.1 ± 0.03^{a}
16 : 1 n-7	3.5 ± 0.2^{a}	3.4 ± 0.2^{a}	4.4 ± 0.01^{b}	3.1 ± 0.02^{a}
16 : 1 n-5	1.3 ± 0.1^{a}	1.6 ± 0.3^{a}	1.5 ± 0.1^{a}	1.2 ± 0.1^{a}
18 : 1 n-13	0.7 ± 0.1^{a}	0.5 ± 0.01^{a}	0.5 ± 0.1^{a}	0.2 ± 0.03^{b}
18 : 1 n-9	2.8 ± 0.03^{a}	1.9 ± 0.04^{b}	1.8 ± 0.1^{b}	0.3 ± 0.02^{b}
18 : 1 n-7	3.5 ± 0.1^{a}	3.0 ± 0.1^{b}	3.7 ± 0.02^{b}	1.2 ± 0.03^{d}
18 : 1 n-5	0.3 ± 0.1^{a}	ND	0.2 ± 0.01^{a}	0.2 ± 0.04^{a}
20 : 1 n-15	6.3 ± 0.5^{a}	6.1 ± 0.04^{a}	5.5 ± 0.03^{a}	6.3 ± 0.1^{a}
20 : 1 n-11	1.4 ± 0.3^{a}	ND	ND	4.5 ± 0.1^{b}
20 : 1 n-9	2. 3 ± 0.2^{a}	5.5 ± 0.1^{b}	1.5 ± 0.01^{c}	1.1 ± 0.03^{d}
20 : 1 n-7	0.3 ± 0.1^{a}	1.7 ± 0.03^{b}	0.9 ± 0.01^{c}	ND
22 : 1 n-11	1.7 ± 0.1^{a}	ND	1.9 ± 0.01^{b}	0.2 ± 0.04^{c}
22 : 1 n-9	1.5 ± 0.03^{a}	2.7 ± 0.05^{b}	1.2 ± 0.01^{c}	1.7 ± 0.02^{d}
22 : 1 n-7	0.1 ± 0.03^{a}	ND	0.2 ± 0.02^{a}	ND
Σ MUFA	**26.6 ± 0.4^{a}**	**26.9 ± 0.8^{a}**	**23.2 ± 0.2^{b}**	**20.7 ± 0.3^{c}**
16 : 2 n-6	0.4 ± 0.03^{a}	ND	0.2 ± 0.01^{b}	0.3 ± 0.01^{b}
16 : 4 n-3	1.9 ± 0.1^{a}	0.3 ± 0.03^{b}	1.1 ± 0.02^{c}	1.8 ± 0.02^{a}
18 : 2 n-9	0.3 ± 0.04^{a}	0.3 ± 0.01^{a}	0.3 ± 0.01^{a}	0.2 ± 0.1^{a}
18 : 2 n-6	1.0 ± 0.1^{a}	1.2 ± 0.03^{a}	1.1 ± 0.04^{a}	1.3 ± 0.1^{a}
18 : 3 n-6	0.2 ± 0.04^{a}	ND	0.3 ± 0.01^{a}	0.3 ± 0.1^{a}
18 : 3 n-3	1.8 ± 0.3^{ac}	1.7 ± 0.02^{a}	2.9 ± 0.1^{b}	2.5 ± 0.1^{c}
18 : 4 n-3	4.2 ± 0.2^{a}	5.1 ± 0.1^{b}	5.8 ± 0.2^{c}	3.6 ± 0.1^{d}
20 : 2 Δ5, 11	3.1 ± 0.5^{a}	5.8 ± 0.02^{b}	2.7 ± 0.03^{a}	4.7 ± 0.1^{c}
20 : 2 Δ5, 13	1.1 ± 0.1^{a}	1.8 ± 0.02^{b}	0.5 ± 0.1^{c}	1.6 ± 0.04^{b}
20 : 2 n - 6	1.7 ± 0.1^{a}	1.6 ± 0.04^{a}	1.5 ± 0.1^{a}	2.0 ± 0.04^{b}
20 : 3 n -9	0.4 ± 0.02^{a}	ND	0.6 ± 0.1^{a}	0.4 ± 0.01^{b}
20 : 3 n -6	0.5 ± 0.1^{a}	ND	0.59 ± 0.1^{a}	0.5 ± 0.04^{a}
20 : 4 n -6	8.7 ± 0.3^{a}	8.1 ± 0.2^{a}	9.8 ± 0.2^{b}	10.6 ± 0.1^{b}

Continued

20 : 3 n - 3	1.9 ± 0.1^{a}	2.2 ± 0.02^{a}	1.0 ± 0.04^{b}	2.1 ± 0.02^{a}
20 : 4 n - 3	1.9 ± 0.1^{a}	2.0 ± 0.01^{a}	1.5 ± 0.04^{b}	1.0 ± 0.1^{c}
20 : 5 n - 3	17.8 ± 0.9^{a}	14.5 ± 0.1^{a}	18.1 ± 0.6^{b}	18.6 ± 0.3^{b}
22 : 2 Δ7, 13	ND	ND	ND	0.4 ± 0.01
22 : 2 Δ7, 15	ND	ND	ND	1.3 ± 0.01
22 : 4 n - 6	0.3 ± 0.1^{a}	0.3 ± 0.04^{a}	0.2 ± 0.02^{ab}	0.1 ± 0.03^{b}
22 : 5 n - 6	0.3 ± 0.1^{a}	ND	0.3 ± 0.01^{ab}	0.2 ± 0.01^{a}
22 : 5 n - 3	0.6 ± 0.02^{a}	1.0 ± 0.01^{a}	0.6 ± 0.03^{b}	0.5 ± 0.01^{c}
22 : 6 n - 3	2.7 ± 0.1^{a}	1.5 ± 0.01^{a}	1.5 ± 0.1^{a}	1.3 ± 0.1^{a}
Σ PUFA	**49.3 ± 0.8^{a}**	**47.5 ± 0.3^{a}**	**50.6 ± 1.2^{a}**	**55.6 ± 0.7^{b}**
U / S	**3.2 ± 0.1^{a}**	**3.0 ± 0.1^{a}**	**2.7 ± 0.01^{b}**	**3.7 ± 0.02^{c}**
Σ n - 6	**11.2 ± 0.7^{a}**	**11.3 ± 0.2^{a}**	**10.7 ± 0.2^{a}**	**6.8 ± 0.2^{b}**
Σ n - 3	**36.8 ± 0.1^{a}**	**28.3 ± 0.1^{b}**	**32.6 ± 1.2^{c}**	**33.7 ± 0.3^{c}**
n - 6 / n - 3	**0.3 ± 0.1^{a}**	**0.4 ± 0.01^{a}**	**0.3 ± 0.01^{a}**	**0.2 ± 0.01^{a}**

Results are mean values of 3 replicates ± standard deviation. Values in each row with the same superscript are not significantly different ($p>0.05$) from one another.

Abbreviations are as follows: SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; U/S, ratio of unsaturated to saturated fatty acids; n-6, omega-6 fatty acids; n-3, omega-3 fatty acids.

Table A.10 Fatty acid composition (weight %) of total lipids of sea urchin viscera in spring, summer, fall and winter.

Fatty acid	Spring	Summer	Fall	Winter
13 : 0	ND	0.1 ± 0.01^{a}	ND	0.8 ± 0.04^{b}
14 : 0	8.3 ± 0.1^{a}	10.1 ± 0.03^{b}	7.4 ± 0.1^{c}	7.5 ± 0.1^{c}
15 : 0	0.7 ± 0.1^{a}	0.4 ± 0.04^{b}	1.6 ± 0.01^{c}	0.3 ± 0.1^{d}
16 : 0	16.2 ± 0.2^{a}	16.4 ± 0.03^{a}	13.2 ± 0.2^{b}	14.2 ± 0.1^{c}
17 : 0	0.6 ± 0.1^{a}	0.9 ± 0.01^{a}	1.0 ± 0.2^{b}	ND
18 : 0	1.2 ± 0.1^{a}	2.0 ± 0.02^{a}	1.5 ± 0.3^{ab}	1.0 ± 0.1^{b}
19 : 0	ND	ND	0.2 ± 0.02^{a}	0.3 ± 0.01^{b}
20 : 0	0.5 ± 0.1^{ab}	0.7 ± 0.02^{a}	0.4 ± 0.03^{b}	0.2 ± 0.01^{c}
Σ SFA	**27.6 ± 0.1^{c}**	**30.6 ± 0.1^{a}**	**25.2 ± 0.8^{b}**	**24.1 ± 0.2^{b}**
14 : 1 n-7	0.1 ± 0.1^{a}	0.2 ± 0.01^{a}	0.2 ± 0.03^{a}	0.1 ± 0.02^{b}
14 : 1 n-5	0.3 ± 0.1^{a}	0.6 ± 0.04^{b}	0.6 ± 0.02^{b}	0.5 ± 0.01^{b}
16 : 1 n-11	1.2 ± 0.2^{a}	0.8 ± 0.02^{b}	1.8 ± 0.02^{c}	2.6 ± 0.03^{d}
16 : 1 n-9	0.2 ± 0.1^{a}	1.6 ± 0.1^{b}	0.1 ± 0.02^{a}	0.1 ± 0.02^{a}
16 : 1 n-7	4.8 ± 0.2^{a}	4.1 ± 0.02^{b}	7.3 ± 0.1^{c}	4.1 ± 0.04^{b}
16 : 1 n-5	0.2 ± 0.1^{a}	0.5 ± 0.1^{b}	0.8 ± 0.1^{c}	0.8 ± 0^{c}
18 : 1 n-13	0.1 ± 0.1^{a}	0.3 ± 0.01^{a}	0.3 ± 0.03^{a}	0.5 ± 0.01^{b}
18 : 1 n-9	3.6 ± 0.1^{ab}	2.6 ± 0.01^{bc}	1.2 ± 0.04^{b}	2.6 ± 0.1^{c}
18 : 1 n-7	3.2 ± 0.2^{a}	2.3 ± 0.03^{b}	2.2 ± 0.02^{b}	4.2 ± 0.1^{c}
20 : 1 n-15	6.2 ± 0.1^{a}	6.1 ± 0.2^{a}	6.2 ± 0.1^{a}	3.5 ± 0.01^{b}
20 : 1 n-11	2.8 ± 0.1^{a}	0.6 ± 0.1^{b}	5.2 ± 0.1^{c}	2.2 ± 0.1^{d}
20 : 1 n-9	0.7 ± 0.1^{a}	1.6 ± 0.02^{b}	0.9 ± 0.01^{c}	0.8 ± 0^{c}
20 : 1 n-7	1.8 ± 0.1^{a}	1.5 ± 0.03^{b}	0.9 ± 0.1^{c}	1.6 ± 0.01^{ab}
22 : 1 n-11	1.4 ± 0.1^{a}	1.4 ± 0.01^{a}	1.6 ± 0.03^{b}	0.6 ± 0.1^{c}
22 : 1 n-9	0.5 ± 0.03^{a}	2.9 ± 0.04^{b}	0.5 ± 0.03^{a}	1.7 ± 0.04^{c}
22 : 1 n-7	0.5 ± 0.02^{a}	0.2 ± 0.04^{b}	ND	0.4 ± 0.01^{c}
Σ MUFA	**30.4 ± 0.2^{a}**	**29.4 ± 0.11^{b}**	**32.9 ± 0.2^{c}**	**32.0 ± 0.3^{c}**
16 : 2 n-6	0.2 ± 0.1^{a}	0.1 ± 0.03^{a}	0.2 ± 0.02^{a}	ND
16 : 4 n-3	0.8 ± 0.02^{a}	0.8 ± 0.04^{a}	2.0 ± 0.02^{b}	0.6 ± 0.01^{c}
18 : 2 n-9	1.6 ± 0.03^{a}	0.7 ± 0.01^{b}	0.5 ± 0.02^{c}	3.7 ± 0.04^{d}
18 : 2 n-6	0.4 ± 0.1^{a}	0.4 ± 0.1^{a}	1.1 ± 0.01^{b}	0.3 ± 0.03^{a}
18 : 3 n-6	1.6 ± 0.2^{a}	1.5 ± 0.01^{a}	ND	2.1 ± 0.1^{b}
18 : 3 n-3	1.4 ± 0.1^{a}	1.6 ± 0.01^{a}	0.9 ± 0.03^{b}	0.8 ± 0.1^{b}
18 : 4 n-3	2.7 ± 0.3^{ac}	2.2 ± 0.01^{ab}	2.0 ± 0.03^{b}	2.8 ± 0.03^{c}
20 : 2 Δ5, 11	1.6 ± 0.1^{a}	2.4 ± 0.04^{b}	2.8 ± 0.04^{c}	1.9 ± 0.1^{d}
20 : 2 Δ5, 13	0.7 ± 0.1^{a}	1.6 ± 0.1^{b}	1.0 ± 0.01^{c}	1.0 ± 0.1^{c}
20 : 2 n - 6	1.5 ± 0.2^{a}	1.3 ± 0.1^{ab}	1.1 ± 0.04^{b}	ND
20 : 4 n -6	5.7 ± 0.2^{a}	6.0 ± 0.1^{ac}	7.0 ± 0.03^{b}	6.6 ± 0.1^{bc}
20 : 3 n - 3	2.0 ± 0.04^{a}	1.6 ± 0.04^{b}	1.0 ± 0.1^{c}	1.0 ± 0.01^{c}
20 : 4 n - 3	1.0 ± 0.1^{a}	1.1 ± 0.01^{a}	0.4 ± 0.03^{b}	0.5 ± 0.01^{b}

Continued

20 : 5 n - 3	14.7 ± 0.1^{a}	10.4 ± 0.2^{b}	15.5 ± 0.04^{c}	18.9 ± 0.1^{d}
22 : 2 Δ7, 13	1.7 ± 0.1^{a}	1.8 ± 0.1^{a}	1.8 ± 0.04^{a}	ND
22 : 2 Δ7, 15	0.5 ± 0.1^{a}	1.1 ± 0.01^{b}	0.9 ± 0.1^{c}	ND
22 : 4 n - 6	0.2 ± 0.02^{a}	0.6 ± 0.01^{bc}	0.4 ± 0.02^{ab}	0.9 ± 0.1^{c}
22 : 5 n - 6	0.5 ± 0.02^{a}	0.4 ± 0.01^{b}	ND	0.8 ± 0.01^{c}
22 : 5 n - 3	1.0 ± 0.1^{a}	1.2 ± 0.01^{b}	1.1 ± 0.01^{ab}	1.2 ± 0.1^{b}
22 : 6 n - 3	2.0 ± 0.2^{a}	2.0 ± 0.03^{a}	2.6 ± 0.03^{b}	2.4 ± 0.1^{ab}
Σ PUFA	**38.3 ± 0.3^{a}**	**37.7 ± 0.2^{a}**	**41.7 ± 0.3^{b}**	**42.2 ± 0.2^{b}**
U / S	**2.5 ± 0.04^{a}**	**2.3 ± 0.01^{b}**	**1.7 ± 0.04^{c}**	**2.5 ± 0.01^{a}**
Σ n - 6	**10.1 ± 0.1^{a}**	**12.3 ± 0.2^{b}**	**9.7 ± 0.03^{a}**	**9.6 ± 0.01^{a}**
Σ n - 3	**25.4 ± 0.1^{a}**	**23.8 ± 0.2^{b}**	**20.2 ± 0.1^{c}**	**18.1 ± 0.2^{d}**
n - 6 / n - 3	**0.4 ± 0.04^{a}**	**0.5 ± 0.01^{b}**	**0.5 ± 0.01^{b}**	**0.5 ± 0.02^{b}**

Results are mean values of 3 replicates ± standard deviation. Values in each row with the same superscript are not significantly different ($p>0.05$) from one another.

Abbreviations are as follows: SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; U/S, ratio of unsaturated to saturated fatty acids; n-6, omega-6 fatty acids; and n-3, omega-3 fatty acids.

Table A.11 Fatty acid composition (weight %) of polar lipid fraction of sea urchin gonads in spring, summer, fall and winter.

Fatty acid	Spring	Summer	Fall	Winter
14 : 0	4.2 ± 0.2^{a}	8.1 ± 0.01^{b}	5.5 ± 0.01^{c}	10.4 ± 0.1^{d}
15 : 0	ND	0.2 ± 0.02^{a}	0.4 ± 0.01^{b}	0.2 ± 0.04^{a}
16 : 0	10.1 ± 0.3^{a}	8.1 ± 0.1^{b}	8.3 ± 0.1^{b}	8.4 ± 0.1^{b}
17 : 0	ND	0.3 ± 0.0^{a}	0.3 ± 0.01^{ac}	0.2 ± 0.03^{bc}
18 : 0	1.6 ± 0.2^{a}	1.4 ± 0.04^{a}	1.4 ± 0.01^{a}	1.8 ± 0.1^{a}
19 : 0	ND	0.3 ± 0.01^{a}	0.3 ± 0.02^{a}	ND
20 : 0	1.4 ± 0.1^{a}	0.8 ± 0.01^{b}	0.7 ± 0.00^{b}	0.7 ± 0.01^{b}
Σ SFA	**17.3 ± 0.6^{a}**	**22.0 ± 0.1^{b}**	**16.8 ± 0.1^{a}**	**21.7 ± 0.1^{c}**
16 : 1 n-9	1.7 ± 0.1^{a}	0.4 ± 0.01^{b}	ND	0.3 ± 0.01^{b}
16 : 1 n-7	1.0 ± 0.2^{a}	1.0 ± 0.04^{a}	0.8 ± 0.0^{a}	1.0 ± 0.01^{a}
16 : 1 n-5	ND	0.5 ± 0.0^{a}	0.4 ± 0.01^{b}	0.5 ± 0.04^{ab}
18 : 1 n-13	ND	0.9 ± 0.01^{a}	ND	0.3 ± 0.03^{b}
18 : 1 n-9	0.9 ± 0.03^{a}	1.3 ± 0.02^{b}	0.5 ± 0.01^{c}	0.4 ± 0.03^{c}
18 : 1 n-7	3.0 ± 0.1^{a}	2.6 ± 0.02^{b}	3.6 ± 0.01^{c}	3.1 ± 0.01^{a}
18 : 1 n-5	0.2 ± 0.02^{a}	0.4 ± 0.01^{b}	1.6 ± 0.00^{c}	0.7 ± 0.0^{d}
20 : 1 n-15	8.6 ± 0.2^{a}	8.5 ± 0.04^{b}	9.7 ± 0.10^{c}	6.2 ± 0.01^{a}
20 : 1 n-11	ND	1.6 ± 0.01^{a}	1.9 ± 0.01^{b}	ND
20 : 1 n-9	4.6 ± 0.2^{a}	2.1 ± 0.01^{b}	4.9 ± 0.10^{a}	2.1 ± 0.1^{b}
20 : 1 n-7	2.5 ± 0.1^{a}	2.3 ± 0.01^{ab}	ND	2.3 ± 0.04^{b}
22 : 1 n-9	1.7 ± 0.1^{a}	ND	1.5 ± 0.1^{a}	1.1 ± 0.1^{b}
Σ MUFA	**24.9 ± 0.01^{a}**	**21.6 ± 0.04^{b}**	**23.4 ± 0.1^{c}**	**19.4 ± 0.01^{d}**
16 : 2 n-6	1.5 ± 0.03	ND	ND	ND
16 : 4 n-3	1.2 ± 0.03^{a}	1.3 ± 0.01^{a}	1.3 ± 0.0^{a}	1.3 ± 0.1^{a}
18 : 2 n-9	ND	0.2 ± 0.01^{a}	ND	ND
18 : 2 n-6	1.4 ± 0.03^{a}	0.8 ± 0.01^{b}	1.3 ± 0.2^{c}	1.0 ± 0.01^{c}
18 : 3 n-6	0.8 ± 0.1^{a}	0.3 ± 0.00^{b}	0.5 ± 0.1^{c}	2.5 ± 0.03^{d}
18 : 3 n-3	0.3 ± 0.04^{a}	0.8 ± 0.01^{b}	0.5 ± 0.1^{a}	ND
18 : 4 n-3	0.7 ± 0.1^{a}	4.2 ± 0.1^{b}	0.5 ± 0.04^{c}	2.0 ± 0.04^{d}
20 : 2 Δ5, 11	3.9 ± 0.1^{a}	3.7 ± 0.03^{b}	3.5 ± 0.03^{c}	2.8 ± 0.1^{d}
20 : 2 Δ5, 13	1.4 ± 0.04^{a}	2.4 ± 0.04^{b}	0.9 ± 0.02^{c}	1.7 ± 0.04^{d}
20 : 2 n - 6	2.8 ± 0.1^{a}	2.7 ± 0.01^{a}	2.1 ± 0.01^{b}	1.3 ± 0.01^{c}
20 : 3 n -6	0.9 ± 0.04^{a}	0.7 ± 0.01^{b}	0.5 ± 0.03^{c}	0.8 ± 0.02^{b}
20 : 4 n - 6	12.6 ± 0.4^{a}	9.9 ± 0.1^{b}	14.0 ± 0.3^{c}	11.2 ± 0.1^{d}
20 : 3 n - 3	1.7 ± 0.1^{a}	1.0 ± 0.1^{b}	2.8 ± 0.03^{c}	2.4 ± 0.1^{d}
20 : 5 n - 3	25.8 ± 0.2^{a}	21.9 ± 0.04^{b}	25.6 ± 0.54^{a}	23.2 ± 0.2^{c}
22 : 2 Δ7, 13	ND	0.5 ± 0.04^{a}	0.5 ± 0.02^{b}	ND
22 : 2 Δ7, 15	ND	1.4 ± 0.1^{a}	1.1 ± 0.0^{b}	ND
22 : 4 n - 6	ND	0.5 ± 0.0^{a}	0.6 ± 0.1^{a}	0.9 ± 0.04^{b}
22 : 5 n - 6	ND	0.4 ± 0.02^{a}	0.4 ± 0.05^{a}	ND

Continued

22 : 5 n - 3	0.5 ± 0.1^{a}	0.56 ± 0.01^{a}	0.4 ± 0.04^{a}	0.9 ± 0.03^{b}
22 : 6 n - 3	2.8 ± 0.1^{a}	3.0 ± 0.1^{a}	0.8 ± 0.01^{b}	2.5 ± 0.02^{c}
Σ PUFA	**56.7 ± 0.2^{a}**	**56.5 ± 0.02^{b}**	**57.6 ± 0.3^{c}**	**59.0 ± 0.2^{d}**
U / S	**4.7 ± 0.1^{a}**	**4.2 ± 0.03^{b}**	**4.9 ± 0.1^{c}**	**3.6 ± 0.01^{d}**
Σ n - 6	**20.0 ± 0.1^{a}**	**17.3 ± 0.1^{b}**	**22.0 ± 0.1^{c}**	**16.7 ± 0.04^{d}**
Σ n - 3	**33.0 ± 0.1^{a}**	**32.8 ± 0.1^{b}**	**34.4 ± 0.1^{c}**	**30.8 ± 0.2^{d}**
n - 6 / n - 3	**0.6 ± 0.03^{a}**	**0.5 ± 0.01^{b}**	**0.6 ± 0.01^{c}**	**0.6 ± 0.01^{ab}**

Results are mean values of 3 replicates ± standard deviation. Values in each row with the same superscript are not significantly different ($p>0.05$) from one another.

Abbreviations are as follows: SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; U/S, ratio of unsaturated to saturated fatty acids; n-6, omega-6 fatty acids; and n-3, omega-3 fatty acids.

Table A.12 Fatty acid composition (weight %) of polar lipid fraction of sea urchin viscera in spring, summer, fall and winter.

Fatty acid	Spring	Summer	Fall	Winter
14 : 0	8.5 ± 0.4^{a}	7.2 ± 0.1^{b}	8.4 ± 0.1^{c}	4.6 ± 0.2^{e}
15 : 0	0.6 ± 0.03^{a}	0.6 ± 0.0^{a}	0.6 ± 0.02^{a}	1.7 ± 0.01^{b}
16 : 0	11.1 ± 0.1^{a}	11.7 ± 0.2^{b}	10.6 ± 0.04^{c}	9.3 ± 0.1^{d}
17 : 0	ND	0.8 ± 0.01^{a}	ND	0.3 ± 0.01^{b}
18 : 0	2.4 ± 0.1^{a}	4.9 ± 0.1^{b}	2.6 ± 0^{c}	4.7 ± 0.3^{d}
20 : 0	0.2 ± 0.04^{a}	1.1 ± 0.01^{b}	0.4 ± 0.02^{a}	0.7 ± 0.04^{d}
Σ SFA	**22.8 ± 0.04^{a}**	**26.2 ± 0.03^{b}**	**22.6 ± 0.03^{b}**	**21.3 ± 0.04^{c}**
14 : 1 n-7	0.8 ± 0.03	ND	ND	ND
14 : 1 n-5	0.2 ± 0.03	ND	ND	ND
16 : 1 n-11	ND	0.7 ± 0.01^{a}	0.6 ± 0.0^{b}	ND
16 : 1 n-9	2.3 ± 0.2^{a}	0.2 ± 0.01^{b}	0.6 ± 0.04^{b}	ND
16 : 1 n-7	5.7 ± 0.1^{a}	3.5 ± 0.1^{b}	9.6 ± 0.2^{c}	1.4 ± 0.01^{d}
16 : 1 n-5	0.3 ± 0.03^{a}	1.1 ± 0.03^{b}	1.2 ± 0.01^{c}	0.5 ± 0.03^{d}
18 : 1 n-13	ND	1.0 ± 0.01^{a}	0.7 ± 0.00^{b}	ND
18 : 1 n-9	2.5 ± 0.04^{a}	2.0 ± 0.03^{b}	2.5 ± 0.04^{a}	0.4 ± 0.01^{c}
18 : 1 n-7	3.9 ± 0.1^{a}	2.6 ± 0.01^{b}	2.4 ± 0.1^{b}	3.5 ± 0.1^{c}
18 : 1 n-5	0.4 ± 0.03^{a}	1.0 ± 0.01^{b}	ND	2.6 ± 0.03^{c}
20 : 1 n-15	7.6 ± 0.05^{a}	6.6 ± 0.1^{b}	6.6 ± 0.04^{c}	7.3 ± 0.01^{d}
20 : 1 n-11	ND	2.5 ± 0.02^{a}	2.4 ± 0.2^{a}	0.7 ± 0.1^{b}
20 : 1 n-9	5.5 ± 0.04^{a}	3.0 ± 0.04^{b}	2.9 ± 0.04^{b}	5.0 ± 0.4^{a}
20 : 1 n-7	2.4 ± 0.3^{a}	1.0 ± 0.01^{b}	2.0 ± 0.02^{a}	ND
22 : 1 n-11	ND	ND	0.4 ± 0.00^{b}	ND
22 : 1 n-9	2.8 ± 0.1^{a}	0.9 ± 0.1^{b}	2.1 ± 0.01^{c}	ND
22 : 1 n-7	0.5 ± 0.04^{a}	0.3 ± 0.01^{b}	0.6 ± 0.03^{a}	ND
Σ MUFA	**34.7 ± 0.1^{a}**	**26.4 ± 0.1^{b}**	**34.51 ± 0.30^{a}**	**23.45 ± 0.26^{c}**
16 : 2 n-6	0.7 ± 0.2^{a}	0.2 ± 0.01^{b}	ND	0.9 ± 0.01^{a}
16 : 4 n-3	1.3 ± 0.03^{a}	2.0 ± 0.01^{b}	2.1 ± 0.1^{b}	3.1 ± 0.03^{c}
18 : 2 n-9	ND	0.6 ± 0.02^{a}	0.8 ± 0.03^{b}	1.1 ± 0.1^{c}
18 : 2 n-6	1.5 ± 0.03^{a}	1.2 ± 0.02^{b}	0.9 ± 0.02^{c}	2.0 ± 0.01^{d}
18 : 3 n-6	0.4 ± 0.03^{a}	ND	1.0 ± 0.04^{b}	0.5 ± 0.03^{c}
18 : 3 n-3	1.5 ± 0.04^{a}	0.8 ± 0.01^{b}	1.9 ± 0.03^{c}	0.5 ± 0.0^{d}
18 : 4 n-3	0.7 ± 0.04^{a}	2.1 ± 0.03^{b}	1.5 ± 0.1^{c}	0.7 ± 0.01^{a}
20 : 2 Δ5, 11	1.5 ± 0.1^{a}	1.8 ± 0.01^{b}	4.6 ± 0.1^{c}	4.2 ± 0.04^{d}
20 : 2 Δ5, 13	0.4 ± 0.1^{a}	1.1 ± 0.0^{b}	1.0 ± 0.03^{b}	1.9 ± 0.02^{c}
20 : 2 n - 6	1.9 ± 0.1^{a}	1.4 ± 0.04^{b}	2.1 ± 0.1^{c}	2.5 ± 0.04^{d}
20 : 3 Δ5, 11, 14	ND	0.5 ± 0.1^{a}	ND	0.5 ± 0.02^{a}
20 : 3 n-9	0.6 ± 0.1	ND	ND	ND
20 : 3 n -6	0.5 ± 0.03^{a}	0.9 ± 0.01^{b}	ND	ND
20 : 4 n - 6	9.1 ± 0.04^{a}	7.9 ± 0.1^{b}	8.1 ± 0.1^{b}	14.6 ± 0.2^{c}

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20 : 3 n - 3	1.9 ± 0.03^{a}	1.2 ± 0.01^{b}	1.0 ± 0.01^{c}	1.8 ± 0.04^{a}
20 : 5 n - 3	18.1 ± 0.2^{a}	15.8 ± 0.1^{b}	17.8 ± 0.1^{c}	18.7 ± 0.03^{d}
22 : 2 Δ7, 13	ND	0.8 ± 0.01^{a}	0.3 ± 0.03^{b}	0.9 ± 0.02^{c}
22 : 2 Δ7, 15	ND	0.9 ± 0.01^{a}	1.6 ± 0.1^{b}	1.0 ± 0.01^{a}
22 : 4 n - 6	ND	0.7 ± 0.01	ND	ND
22 : 5 n - 6	0.2 ± 0.02^{a}	0.1 ± 0.00^{b}	ND	ND
22 : 5 n - 3	0.5 ± 0.02^{a}	0.3 ± 0.01^{b}	1.1 ± 0.0^{c}	ND
22 : 6 n - 3	1.0 ± 0.1^{a}	1.0 ± 0.01^{a}	0.6 ± 0.01^{b}	0.8 ± 0.01^{c}
Σ PUFA	**44.0 ± 0.1^{a}**	**43.2 ± 0.1^{b}**	**46.6 ± 0.2^{c}**	**51.3 ± 0.1^{d}**
U / S	**3.5 ± 0.03^{a}**	**2.7 ± 0.01^{a}**	**3.6 ± 0.02^{c}**	**3.6 ± 0.01^{c}**
Σ n - 6	**14.2 ± 0.1^{a}**	**12.4 ± 0.1^{a}**	**12.3 ± 0.1^{b}**	**20.4 ± 0.02^{c}**
Σ n - 3	**27.1 ± 0.17^{a}**	**23.2 ± 0.1^{a}**	**17.0 ± 0.1^{c}**	**27.5 ± 0.1^{a}**
n - 6 / n - 3	**0.5 ± 0.02^{a}**	**0.5 ± 0.01^{a}**	**0.7 ± 0.01^{b}**	**0.7 ± 0.01^{b}**

Results are mean values of 3 replicates ± standard deviation. Values in each row with the same superscript are not significantly different ($p>0.05$) from one another.

Abbreviations are as follows: SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; U/S, ratio of unsaturated to saturated fatty acids; n-6, omega-6 fatty acids; and n-3, omega-3 fatty acids.

Table A.13 Fatty acid composition (%) of non-polar lipid fraction of sea urchin gonads in spring, summer, fall and winter.

Fatty acid	Spring	Summer	Fall	Winter
14 : 0	9.4 ± 0.5^{ab}	10.5 ± 0.4^{a}	8.6 ± 0.04^{b}	8.8 ± 0.01^{b}
15 : 0	0.8 ± 0.03^{a}	0.5 ± 0.0^{b}	0.4 ± 0.02^{b}	0.5 ± 0.01^{b}
16 : 0	12.6 ± 0.6^{a}	16.4 ± 0.5^{b}	15.8 ± 0.1^{b}	16.3 ± 0.1^{b}
17 : 0	0.2 ± 0.01^{a}	0.2 ± 0.0^{a}	0.2 ± 0.1^{a}	0.2 ± 0.03^{a}
18 : 0	2.6 ± 0.2^{a}	2.8 ± 0.01^{a}	2.3 ± 0.03^{a}	0.4 ± 0.1^{b}
19 : 0	0.3 ± 0.02^{a}	0.3 ± 0.01^{a}	0.3 ± 0.01^{a}	0.2 ± 0.02^{b}
20 : 0	4.5 ± 0.3^{a}	0.7 ± 0.02^{b}	1.8 ± 0.1^{c}	1.3 ± 0.1^{d}
Σ SFA	$\mathbf{30.4 \pm 0.5^{ab}}$	$\mathbf{31.2 \pm 0.5^{a}}$	$\mathbf{29.3 \pm 0.2^{b}}$	$\mathbf{27.7 \pm 0.1^{c}}$
14 : 1 n-7	0.2 ± 0.02^{a}	0.1 ± 0.0^{b}	0.1 ± 0.01^{a}	0.1 ± 0.02^{ab}
14 : 1 n-5	0.2 ± 0.04^{a}	0.6 ± 0.0^{b}	0.7 ± 0.1^{b}	0.9 ± 0.02^{c}
16 : 1 n-11	ND	ND	ND	1.6 ± 0.04
16 : 1 n-9	1.1 ± 0.1^{a}	0.1 ± 0.02^{b}	0.1 ± 0.03^{b}	0.1 ± 0.0^{b}
16 : 1 n-7	3.5 ± 0.1^{a}	3.2 ± 0.03^{b}	3.2 ± 0.02^{b}	3.6 ± 0.1^{a}
16 : 1 n-5	0.1 ± 0.01^{a}	1.7 ± 0.1^{b}	1.5 ± 0.01^{c}	0.1 ± 0.01^{a}
18 : 1 n-13	0.5 ± 0.04^{a}	0.4 ± 0.1^{a}	0.5 ± 0.00^{a}	ND
18 : 1 n-9	3.4 ± 0.1^{a}	0.5 ± 0.01^{b}	1.8 ± 0.02^{c}	2.1 ± 0.02^{d}
18 : 1 n-7	3.8 ± 0.4^{a}	1.8 ± 0.01^{b}	2.6 ± 0.1^{c}	2.8 ± 0.01^{c}
18 : 1 n-5	0.3 ± 0.02^{a}	2.7 ± 0.01^{b}	0.2 ± 0.04^{c}	0.2 ± 0.01^{c}
20 : 1 n-15	6.2 ± 0.1^{a}	7.7 ± 0.9^{b}	7.6 ± 0.2^{b}	6.5 ± 0.01^{a}
20 : 1 n-11	1.1 ± 0.13^{a}	1.3 ± 0.03^{a}	1.9 ± 0.1^{b}	1.7 ± 0.04^{bc}
20 : 1 n-9	1.1 ± 0.1^{a}	3.4 ± 0.03^{b}	3.2 ± 0.04^{c}	1.9 ± 0.03^{d}
20 : 1 n-7	4.6 ± 0.2^{a}	0.5 ± 0.03^{b}	1.4 ± 0.01^{c}	0.6 ± 0.04^{b}
22 : 1 n-11	0.3 ± 0.03^{a}	0.3 ± 0.0^{a}	0.3 ± 0.01^{a}	2.1 ± 0.03^{b}
22 : 1 n-9	1.9 ± 0.1^{a}	2.44 ± 0.02^{b}	2.6 ± 0.04^{c}	0.5 ± 0.0^{d}
22 : 1 n-7	0.4 ± 0.04^{a}	0.49 ± 0.00^{a}	0.6 ± 0.04^{a}	1.5 ± 0.1^{b}
Σ MUFA	$\mathbf{28.5 \pm 0.1^{a}}$	$\mathbf{27.2 \pm 0.2^{b}}$	$\mathbf{28.2 \pm 0.3^{a}}$	$\mathbf{26.1 \pm 0.02^{c}}$
16 : 2 n-6	0.7 ± 0.02^{a}	ND	0.2 ± 0.04^{b}	0.5 ± 0.01^{a}
16 : 4 n-3	1.1 ± 0.1^{a}	2.5 ± 0.01^{b}	1.3 ± 0.04^{c}	2.3 ± 0.1^{d}
18 : 2 n-9	0.3 ± 0.04^{a}	0.2 ± 0.00^{a}	0.3 ± 0.1^{b}	0.3 ± 0.02^{a}
18 : 2 n-6	0.2 ± 0.02^{a}	1.2 ± 0.02^{b}	1.2 ± 0.01^{b}	1.4 ± 0.02^{c}
18 : 3 n-6	0.3 ± 0.02^{a}	0.3 ± 0.01^{b}	0.4 ± 0.02^{c}	0.4 ± 0.0^{c}
18 : 3 n-3	1.4 ± 0.1^{a}	1.8 ± 0.01^{a}	1.7 ± 0.04^{b}	1.6 ± 0.1^{b}
18 : 4 n-3	4.2 ± 0.1^{a}	5.5 ± 0.02^{b}	3.0 ± 0.02^{c}	4.1 ± 0.1^{a}
20 : 2 Δ5, 11	2.7 ± 0.1^{a}	3.6 ± 0.01^{b}	4.7 ± 0.04^{c}	5.3 ± 0.04^{d}
20 : 2 Δ5, 13	0.7 ± 0.03^{a}	1.7 ± 0.04^{b}	1.8 ± 0.02^{b}	1.8 ± 0.1^{b}
20 : 2 n-9	ND	ND	ND	0.2 ± 0.01
20 : 2 n-6	1.6 ± 0.04^{a}	1.7 ± 0.1^{a}	1.7 ± 0.1^{a}	2.3 ± 0.01^{b}

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20 : 3 n-9	0.3 ± 0.04^{a}	0.4 ± 0.0^{ab}	ND	0.5 ± 0.01^{b}
20 : 3 Δ5, 11, 14	0.4 ± 0.02^{a}	0.3 ± 0.0^{a}	0.4 ± 0.1^{a}	0.4 ± 0.02^{a}
20 : 3 n -6	0.5 ± 0.04^{a}	0.5 ± 0.01^{a}	0.5 ± 0.02^{a}	0.5 ± 0.03^{a}
20 : 4 n - 6	5.7 ± 0.2^{a}	4.6 ± 0.5^{b}	8.0 ± 0.05^{c}	7.5 ± 0.1^{c}
20 : 3 n-3	1.7 ± 0.1^{ab}	1.3 ± 0.2^{ac}	2.1 ± 0.02^{b}	1.1 ± 0.1^{c}
20 : 4 n-3	1.8 ± 0.1^{a}	1.9 ± 0.01^{a}	1.9 ± 0.1^{a}	ND
20 : 5 n-3	13.9 ± 0.1^{a}	10.9 ± 0.1^{b}	14.3 ± 0.6^{c}	12.2 ± 0.1^{c}
22 : 2 Δ7, 13	0.8 ± 0.04^{a}	0.5 ± 0.0^{b}	0.9 ± 0.01^{c}	0.2 ± 0.02^{d}
22 : 2 Δ7, 15	0.2 ± 0.03^{a}	0.2 ± 0.0^{a}	0.3 ± 0.04^{a}	0.3 ± 0.03^{a}
22 : 4 n - 6	0.1 ± 0.04^{a}	1.1 ± 0.03^{b}	ND	0.3 ± 0.02^{c}
22 : 5 n - 6	0.3 ± 0.02^{a}	0.3 ± 0.01^{a}	0.4 ± 0.0^{b}	0.3 ± 0.03^{a}
22 : 5 n - 3	0.3 ± 0.1^{ba}	0.5 ± 0.01^{bc}	0.4 ± 0.04^{ab}	0.6 ± 0.03^{c}
22 : 6 n - 3	1.0 ± 0.1^{a}	1.4 ± 0.03^{c}	0.8 ± 0.01^{a}	1.3 ± 0.04^{b}
Σ PUFA	**40.7 ± 0.3^{a}**	**41.9 ± 0.3^{b}**	**42.4 ± 0.4^{b}**	**45.5 ± 0.2^{a}**
U / S	**2.3 ± 0.04^{ab}**	**2.2 ± 0.04^{a}**	**2.4 ± 0.02^{b}**	**2.6 ± 0.01^{c}**
Σ n - 6	**9.4 ± 0.1^{a}**	**9.6 ± 0.7^{a}**	**10.3 ± 0.2^{a}**	**13.4 ± 0.2^{b}**
Σ n - 3	**25.6 ± 0.1^{a}**	**25.8 ± 0.4^{a}**	**23.9 ± 0.1^{b}**	**23.2 ± 0.1^{b}**
n - 6 / n - 3	**0.4 ± 0.03^{a}**	**0.4 ± 0.02^{a}**	**0.4 ± 0.01^{a}**	**0.6 ± 0.01^{b}**

Results are mean values of 3 replicates ± standard deviation. Values in each row with the same superscript are not significantly different ($p>0.05$) from one another.

Abbreviations are as follows: SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; U/S, ratio of unsaturated to saturated fatty acids; n-6, omega-6 fatty acids; and n-3, omega-3 fatty acids.

Table A.14 Fatty acid composition (weight %) of non polar lipid fraction of sea urchin viscera in spring, summer, fall and winter.

Fatty acid	Spring	Summer	Fall	Winter
14 : 0	11.4 ± 0.3^{a}	10.5 ± 0.04^{b}	9.3 ± 0.03^{c}	5.7 ± 0.04^{d}
15 : 0	0.9 ± 0.1^{a}	0.7 ± 0.02^{ac}	0.5 ± 0.0^{b}	0.6 ± 0.1^{cb}
16 : 0	9.9 ± 0.2^{a}	14.0 ± 0.3^{b}	13.2 ± 0.1^{c}	11.8 ± 0.1^{d}
17 : 0	0.1 ± 0.1^{a}	0.3 ± 0.01^{b}	0.2 ± 0.0^{c}	0.3 ± 0.03^{bc}
18 : 0	2.2 ± 0.1^{a}	2.5 ± 0.02^{b}	1.7 ± 0.01^{c}	2.0 ± 0.02^{d}
20 : 0	1.9 ± 0.1^{a}	1.8 ± 0.01^{a}	0.7 ± 0.02^{b}	1.1 ± 0.01^{c}
Σ SFA	**26.3 ± 0.1^{a}**	**29.8 ± 0.01^{b}**	**25.6 ± 0.1^{c}**	**21.5 ± 0.1^{d}**
14 : 1 n-7	1.1 ± 0.06^{a}	ND	ND	0.1 ± 0.0^{b}
14 : 1 n-5	0.3 ± 0.02^{a}	3.4 ± 0.1^{b}	1.0 ± 0.01^{c}	0.1 ± 0.0^{d}
16 : 1 n-11	ND	0.1 ± 0.0^{a}	0.1 ± 0.0^{b}	0.1 ± 0.01^{c}
16 : 1 n-9	1.8 ± 0.1^{a}	0.7 ± 0.0^{b}	0.7 ± 0.04^{b}	3.1 ± 0.02^{c}
16 : 1 n-7	2.1 ± 0.1^{a}	2.8 ± 0.01^{b}	3.9 ± 0.1^{c}	1.2 ± 0.01^{d}
16 : 1 n-5	0.3 ± 0.1^{a}	2.9 ± 0.03^{a}	2.3 ± 0.01^{c}	0.1 ± 0.03^{d}
18 : 1 n-13	0.6 ± 0.02^{a}	0.6 ± 0.0^{a}	0.4 ± 0.04^{b}	0.4 ± 0.01^{b}
18 : 1 n-9	2.6 ± 0.1^{a}	2.9 ± 0.01^{b}	2.5 ± 0.03^{a}	1.9 ± 0.1^{c}
18 : 1 n-7	3.7 ± 0.04^{a}	2.1 ± 0.01^{b}	2.1 ± 0.02^{b}	2.4 ± 0.01^{c}
18 : 1 n-5	0.3 ± 0.02^{a}	ND	ND	0.2 ± 0.0^{b}
20 : 1 n-15	5.1 ± 0.04^{a}	4.7 ± 0.1^{b}	3.7 ± 0.03^{c}	4.8 ± 0.1^{b}
20 : 1 n-11	0.7 ± 0.02^{a}	0.8 ± 0.04^{b}	0.7 ± 0.1^{ab}	1.6 ± 0.02^{c}
20 : 1 n-9	2.7 ± 0.3^{ac}	1.7 ± 0.1^{b}	3.1 ± 0.04^{cd}	2.0 ± 0.1^{bd}
20 : 1 n-7	0.6 ± 0.1^{a}	0.5 ± 0.01^{b}	0.8 ± 0.1^{c}	0.4 ± 0.02^{b}
22 : 1 n-11	1.4 ± 0.03^{a}	2.0 ± 0.02^{b}	0.6 ± 0.01^{c}	0.2 ± 0.01^{d}
22 : 1 n-9	2.9 ± 0.1^{a}	2.8 ± 0.01^{a}	2.5 ± 0.03^{b}	2.3 ± 0.01^{c}
22 : 1 n-7	0.1 ± 0.04^{a}	ND	0.2 ± 0.0^{a}	1.4 ± 0.02^{b}
Σ MUFA	**26.4 ± 0.1^{a}**	**28.1 ± 0.1^{b}**	**24.5 ± 0.1^{c}**	**22.3 ± 0.2^{d}**
16 : 2 n-6	0.9 ± 0.03^{a}	0.2 ± 0.0^{b}	0.4 ± 0.02^{c}	0.1 ± 0.01^{d}
16 : 4 n-3	1.4 ± 0.1^{a}	0.1 ± 0.01^{a}	0.5 ± 0.11^{b}	0.4 ± 0.1^{ab}
18 : 2 n-9	0.4 ± 0.02^{a}	ND	0.4 ± 0.02^{a}	1.5 ± 0.02^{b}
18 : 2 n-6	0.4 ± 0.1^{a}	0.8 ± 0.01^{b}	1.6 ± 0.02^{c}	1.5 ± 0.03^{c}
18 : 3 n-6	0.4 ± 0.1^{ab}	0.5 ± 0.04^{ac}	0.3 ± 0.0^{b}	0.5 ± 0.04^{c}
18 : 3 n-3	1.7 ± 0.1^{a}	1.0 ± 0.04^{a}	2.5 ± 0.21^{b}	6.3 ± 0.1^{c}
18 : 4 n-3	4.0 ± 0.04^{a}	0.5 ± 0.01^{b}	3.4 ± 0.02^{c}	4.5 ± 0.03^{d}
20 : 2 Δ5, 11	2.2 ± 0.1^{a}	4.1 ± 0.03^{b}	5.4 ± 0.1^{c}	2.4 ± 0.04^{a}
20 : 2 Δ5, 13	0.3 ± 0.02^{a}	1.6 ± 0.01^{b}	1.7 ± 0.04^{c}	1.4 ± 0.01^{d}
20 : 2 n - 6	1.7 ± 0.1^{a}	0.8 ± 0.02^{a}	1.9 ± 0.1^{c}	0.8 ± 0.04^{b}
20 : 3 Δ5, 11, 14	ND	ND	ND	0.2 ± 0.0
20 : 3 n-6	ND	ND	0.5 ± 0.03^{a}	0.3 ± 0.0^{b}
20 : 4 n-6	9.4 ± 0.2^{a}	6.5 ± 0.1^{b}	6.1 ± 0.02^{b}	10.7 ± 0.1^{c}
20 : 3 n-3	1.3 ± 0.1^{a}	0.1 ± 0.0^{b}	2.4 ± 0.01^{c}	2.3 ± 0.1^{c}

Continued

20 : 4 n-3	0.9 ± 0.01^{a}	1.5 ± 0.04^{b}	ND	1.0 ± 0.01^{c}
20 : 5 n-3	16.5 ± 0.2^{a}	14.3 ± 0.1^{b}	16.1 ± 0.2^{a}	18.5 ± 0.1^{c}
22 : 2 Δ7, 13	0.5 ± 0.04^{a}	0.9 ± 0.03^{b}	0.4 ± 0.01^{c}	0.2 ± 0.02^{d}
22 : 2 Δ7, 15	1.9 ± 0.1^{a}	1.9 ± 0.1^{a}	1.4 ± 0.04^{b}	0.8 ± 0.01^{c}
22 : 4 n-6	1.0 ± 0.1^{a}	0.8 ± 0.04^{b}	0.2 ± 0.0^{c}	0.3 ± 0.01^{c}
22 : 5 n-6	1.1 ± 0.1^{a}	1.4 ± 0.1^{b}	0.6 ± 0.1^{c}	0.1 ± 0.0^{d}
22 : 5 n-3	0.7 ± 0.1^{a}	0.31 ± 0.01^{b}	1.0 ± 0.03^{c}	0.5 ± 0.03^{d}
22 : 6 n-3	1.1 ± 0.1^{a}	2.4 ± 0.04^{b}	1.4 ± 0.03^{c}	1.2 ± 0.04^{a}
Σ PUFA	$\mathbf{47.6 \pm 0.2^{a}}$	$\mathbf{39.7 \pm 0.02^{b}}$	$\mathbf{48.0 \pm 0.2^{a}}$	$\mathbf{55.5 \pm 0.2^{c}}$
U / S	$\mathbf{2.8 \pm 0.04^{a}}$	$\mathbf{2.8 \pm 0.01^{b}}$	$\mathbf{2.7 \pm 0.02^{ab}}$	$\mathbf{3.6 \pm 0.03^{c}}$
Σ n - 6	$\mathbf{13.1 \pm 0.1^{a}}$	$\mathbf{11.0 \pm 0.1^{b}}$	$\mathbf{11.5 \pm 0.01^{c}}$	$\mathbf{14.9 \pm 0.1^{d}}$
Σ n - 3	$\mathbf{27.5 \pm 0.1^{a}}$	$\mathbf{20.2 \pm 0.01^{b}}$	$\mathbf{27.2 \pm 0.2^{c}}$	$\mathbf{34.8 \pm 0.2^{d}}$
n - 6 / n - 3	$\mathbf{0.5 \pm 0.02^{a}}$	$\mathbf{0.6 \pm 0.01^{b}}$	$\mathbf{0.4 \pm 0.01^{c}}$	$\mathbf{0.4 \pm 0.01^{ac}}$

Results are mean values of 3 replicates ± standard deviation. Values in each row with the same superscript are not significantly different ($p>0.05$) from one another.

Abbreviations are as follows: SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; U/S, ratio of unsaturated to saturated fatty acids; n-6, omega-6 fatty acids; and n-3, omega-3 fatty acids.

Table A.15 Fatty acid composition (weight %) of total lipids from sea urchin gonadal lipids at 0, 3.6 and 9 weeks of feeding the artificial diet.

Fatty acid	Week 0	Week 3	Week 6	Week 9
14 : 0	9.4 ± 0.1^{a}	7.2 ± 0.2^{b}	7.0 ± 0.1^{b}	6.7 ± 0.1^{b}
15 : 0	0.4 ± 0.02^{a}	0.4 ± 0.02^{a}	0.3 ± 0.1^{a}	0.4 ± 0.1^{a}
16 : 0	11.1 ± 0.1^{a}	10.3 ± 0.2^{b}	10.5 ± 0.2^{b}	10.5 ± 0.1^{b}
17 : 0	ND	0.2 ± 0.02^{a}	0.1 ± 0.03^{a}	0.1 ± 0.03^{a}
18 : 0	2.2 ± 0.04^{a}	1.8 ± 0.1^{a}	1.8 ± 0.04^{a}	1.2 ± 0.2^{b}
20 : 0	2.9 ± 0.1^{a}	4.9 ± 0.1^{b}	4.5 ± 0.03^{b}	4.3 ± 0.4^{b}
Σ SFA	**25.9 ± 0.3^{a}**	**24.8 ± 0.2^{b}**	**24.2 ± 0.1^{b}**	**23.9 ± 0.2^{c}**
14 : 1 n-7	0.8 ± 0.03^{a}	0.8 ± 0.03^{a}	0.7 ± 0.04^{a}	0.7 ± 0.03^{a}
14 : 1 n-5	0.2 ± 0.02^{a}	0.2 ± 0.03^{a}	0.2 ± 0.03^{a}	0.1 ± 0.02^{a}
16 : 1 n-9	4.8 ± 0.1^{ac}	6.3 ± 0.3^{b}	5.2 ± 0.1^{c}	4.8 ± 0.1^{c}
16 : 1 n-7	1.5 ± 0.1^{a}	1.9 ± 0.04^{b}	2.0 ± 0.1^{b}	1.8 ± 0.1^{b}
16 : 1 n-5	0.3 ± 0.03^{a}	0.3 ± 0.03^{a}	0.3 ± 0.02^{a}	0.3 ± 0.03^{a}
18 : 1 n-9	1.8 ± 0.1^{a}	2.3 ± 0.1^{b}	4.3 ± 0.1^{c}	4.9 ± 0.1^{d}
18 : 1 n-7	3.6 ± 0.1^{a}	4.9 ± 0.1^{b}	4.0 ± 0.1^{c}	3.6 ± 0.1^{a}
18 : 1 n-5	0.5 ± 0.02^{a}	0.2 ± 0.03^{b}	0.2 ± 0.03^{b}	ND
20 : 1 n-15	7.5 ± 0.2^{a}	3.3 ± 0.03^{b}	3.4 ± 0.1^{b}	3.2 ± 0.1^{b}
20 : 1 n-9	4.0 ± 0.03^{a}	1.8 ± 0.1^{b}	1.5 ± 0.04^{c}	1.4 ± 0.03^{c}
20 : 1 n-7	2.2 ± 0.1^{a}	3.8 ± 0.2^{b}	4.3 ± 0.2^{bc}	4.6 ± 0.05^{c}
22 : 1 n-11	2.9 ± 0.04^{a}	1.7 ± 0.1^{b}	0.2 ± 0.02^{c}	0.2 ± 0.03^{c}
22 : 1 n-9	0.4 ± 0.03^{a}	0.1 ± 0.03^{a}	1.7 ± 0.2^{b}	1.6 ± 0.1^{b}
Σ MUFA	**30.4 ± 0.4^{a}**	**27.5 ± 0.6^{b}**	**28.0 ± 0.4^{b}**	**27.2 ± 0.1^{b}**
16 : 2 n-6	0.5 ± 0.03^{a}	1.7 ± 0.03^{b}	1.3 ± 0.1^{c}	1.2 ± 0.04^{c}
16 : 4 n-6	1.9 ± 0.1^{a}	1.7 ± 0.1^{a}	1.2 ± 0.02^{bc}	1.1 ± 0.03^{c}
18 : 2 n-9	ND	0.1 ± 0.04^{a}	0.1 ± 0.02^{a}	0.1 ± 0.02^{a}
18 : 2 n-6	1.1 ± 0.1^{a}	6.5 ± 0.2^{b}	13.8 ± 0.2^{c}	15.7 ± 0.1^{d}
18 : 3 n-6	1.4 ± 0.03^{a}	0.6 ± 0.03^{b}	0.5 ± 0.1^{b}	0.4 ± 0.04^{b}
18 : 3 n-3	1.3 ± 0.04^{a}	1.2 ± 0.03^{a}	1.8 ± 0.1^{b}	1.8 ± 0.2^{b}
18 : 4 n-3	3.8 ± 0.03^{a}	2.4 ± 0.04^{b}	1.8 ± 0.1^{c}	1.7 ± 0.1^{c}
20 : 2 Δ5, 11	1.8 ± 0.1^{a}	2.8 ± 0.1^{b}	2.4 ± 0.2^{bc}	2.1 ± 0.04^{ac}
20 : 2 Δ5, 13	0.9 ± 0.1^{a}	0.4 ± 0.03^{b}	0.3 ± 0.03^{b}	0.4 ± 0.04^{b}
20 : 2 n - 6	1.7 ± 0.1^{a}	3.3 ± 0.1^{b}	5.0 ± 0.1^{c}	5.2 ± 0.1^{c}
20 : 3 n-9	ND	0.8 ± 0.03^{a}	0.9 ± 0.02^{b}	1.2 ± 0.04^{c}
20 : 3 Δ5, 11, 14	ND	0.2 ± 0.1^{a}	0.4 ± 0.1^{a}	0.5 ± 0.2^{a}
20 : 3 n-6	0.4 ± 0.1^{a}	0.75 ± 0.1^{b}	1.1 ± 0.1^{c}	1.1 ± 0.1^{c}
20 : 4 n-6	7.0 ± 0.1^{a}	5.8 ± 0.3^{b}	5.1 ± 0.2^{c}	5.0 ± 0.1^{c}
20 : 3 n-3	1.7 ± 0.2^{a}	0.8 ± 0.03^{b}	ND	ND
20 : 4 n-3	1.2 ± 0.02^{a}	0.6 ± 0.02^{b}	0.7 ± 0.1^{b}	1.8 ± 0.03^{c}
20 : 5 n-3	16.3 ± 0.1^{a}	15.0 ± 0.1^{b}	9.4 ± 0.2^{c}	8.4 ± 0.1^{d}
22 : 2 Δ7, 13	ND	0.3 ± 0.04^{a}	0.3 ± 0.03^{a}	0.3 ± 0.03^{a}

Continued

22 : 2 Δ7, 15	ND	1.3 ± 0.03^{a}	1.1 ± 0.03^{b}	1.1 ± 0.04^{b}
22 : 4 n-6	ND	0.5 ± 0.03^{a}	0.4 ± 0.03^{a}	0.4 ± 0.1^{a}
22 : 5 n-6	0.2 ± 0.1^{a}	0.3 ± 0.04^{a}	0.3 ± 0.03^{a}	0.3 ± 0.04^{a}
22 : 5 n-3	0.6 ± 0.1^{a}	0.4 ± 0.03^{ab}	0.3 ± 0.1^{b}	0.2 ± 0.03^{b}
22 : 6 n-3	1.4 ± 0.1^{a}	0.3 ± 0.03^{b}	0.2 ± 0.04^{b}	0.1 ± 0.1^{b}
Σ PUFA	**43.1 ± 0.8^{a}**	**47.6 ± 0.2^{b}**	**47.9 ± 0.1^{b}**	**48.7 ± 0.3^{b}**
U / S	**2.8 ± 0.02^{a}**	**3.0 ± 0.02^{b}**	**3.1 ± 0.1^{b}**	**3.2 ± 0.02^{b}**
Σ n - 6	**14.2 ± 0.1^{a}**	**21.1 ± 0.2^{b}**	**28.5 ± 0.1^{c}**	**30.3 ± 0.1^{d}**
Σ n - 3	**26.3 ± 0.04^{a}**	**20.6 ± 0.2^{b}**	**14.1 ± 0.1^{c}**	**14.0 ± 0.1^{d}**
n - 6 / n - 3	**0.5 ± 0.03^{a}**	**1.0 ± 0.02^{b}**	**2.0 ± 0.02^{c}**	**2.2 ± 0.02^{d}**

Results are mean values of 3 replicates ± standard deviation. Values in each row with the same superscript are not significantly different ($p>0.05$) from one another.

Abbreviations are as follows: SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; U/S, ratio of unsaturated to saturated fatty acids; n-6, omega-6 fatty acids; and n-3, omega-3 fatty acids.

Table A.16 Fatty acid composition (weight %) of total lipids from sea urchin visceral lipids at 0, 3, 6 and 9 weeks of feeding the artificial diet.

Fatty acid	Week 0	Week 3	Week 6	Week 9
14 : 0	8.4 ± 0.1^{a}	10.5 ± 0.1^{b}	11.2 ± 0.1^{c}	11.6 ± 0.04^{d}
15 : 0	0.7 ± 0.02^{a}	0.7 ± 0.1^{a}	0.5 ± 0.1^{b}	0.4 ± 0.04^{b}
16 : 0	17.6 ± 0.3^{a}	13.8 ± 0.1^{b}	15.6 ± 0.2^{c}	16.4 ± 0.3^{c}
17 : 0	0.2 ± 0.03^{a}	ND	ND	ND
18 : 0	2.3 ± 0.1^{a}	1.4 ± 0.1^{b}	3.8 ± 0.13	3.8 ± 0.02^{c}
19 : 0	0.2 ± 0.1^{a}	ND	0.2 ± 0.04^{a}	0.2 ± 0.1^{a}
20 : 0	1.2 ± 0.1^{a}	1.8 ± 0.1^{b}	2.1 ± 0.04^{c}	2.7 ± 0.04^{d}
Σ SFA	**30.5 ± 0.5^{a}**	**28.1 ± 0.5^{b}**	**33.4 ± 0.3^{c}**	**35.0 ± 0.2^{d}**
14 : 1 n-7	0.3 ± 0.03^{a}	0.2 ± 0.03^{a}	0.5 ± 0.04^{b}	$0.7 \pm 0.04c$
14 : 1 n-5	0.1 ± 0.02^{a}	0.2 ± 0.03^{ac}	0.3 ± 0.04^{bc}	0.2 ± 0.02^{ac}
16 : 1 n-9	1.6 ± 0.1^{a}	5.5 ± 0.2^{b}	3.4 ± 0.4^{c}	2.4 ± 0.12^{a}
16 : 1 n-7	3.4 ± 0.2^{a}	3.4 ± 0.04^{a}	2.2 ± 0.2^{b}	1.3 ± 0.04^{c}
16 : 1 n-5	0.6 ± 0.04^{a}	0.3 ± 0.03^{b}	0.9 ± 0.1^{c}	0.2 ± 0.03^{b}
18 : 1 n-9	2.2 ± 0.1^{a}	1.4 ± 0.1^{b}	3.2 ± 0.03^{c}	3.8 ± 0.04^{d}
18 : 1 n-7	4.2 ± 0.1^{a}	3.8 ± 0.3^{ab}	3.3 ± 0.1^{bc}	3.0 ± 0.1^{c}
18 : 1 n-5	3.4 ± 0.2^{a}	0.4 ± 0.2^{b}	1.3 ± 0.1^{c}	0.1 ± 0.0^{b}
20 : 1 n-15	5.6 ± 0.1^{a}	4.4 ± 0.2^{b}	5.8 ± 0.1^{a}	3.2 ± 0.1^{c}
20 : 1 n-9	1.2 ± 0.03^{a}	1.7 ± 0.1^{b}	1.7 ± 0.1^{b}	1.0 ± 0.03^{a}
20 : 1 n-7	1.9 ± 0.1^{a}	1.9 ± 0.1^{a}	2.5 ± 0.1^{b}	3.2 ± 0.1^{c}
22 : 1 n-11	1.2 ± 0.1^{a}	0.2 ± 0.02^{b}	1.6 ± 0.2^{c}	0.3 ± 0.1^{b}
22 : 1 n-9	1.1 ± 0.1^{a}	3.8 ± 0.1^{b}	2.9 ± 0.1^{c}	1.8 ± 0.1^{d}
22 : 1 n-7	0.3 ± 0.03^{a}	ND	ND	ND
Σ MUFA	**27.6 ± 0.5^{a}**	**27.4 ± 0.3^{a}**	**29.5 ± 0.4^{b}**	**23.7 ± 0.1^{c}**
16 : 2 n-6	0.2 ± 0.02^{a}	1.5 ± 0.04^{b}	2.2 ± 0.04^{c}	1.5 ± 0.1^{b}
16 : 4 n-6	ND	0.8 ± 0.02^{a}	0.3 ± 0.03^{b}	0.7 ± 0.02^{c}
16 : 4 n-3	2.8 ± 0.1^{a}	ND	ND	ND
18 : 2 n-9	0.5 ± 0.02^{a}	0.1 ± 0.04^{b}	0.4 ± 0.04^{a}	0.3 ± 0.02^{c}
18 : 2 n-6	0.9 ± 0.1^{a}	8.8 ± 0.1^{b}	13.6 ± 0.3^{c}	16.9 ± 0.2^{c}
18 : 3 n-6	1.3 ± 0.1^{a}	0.2 ± 0.02^{b}	$0.6 \pm 0.1c$	0.3 ± 0.03^{d}
18 : 3 n-3	0.5 ± 0.04^{a}	1.9 ± 0.03^{b}	1.7 ± 0.1^{bd}	1.5 ± 0.03^{d}
18 : 4 n-3	2.2 ± 0.03^{a}	1.5 ± 0.1^{b}	1.0 ± 0.04^{c}	0.8 ± 0.03^{b}
20 : 2 Δ5, 11	1.7 ± 0.1^{a}	2.7 ± 0.04^{b}	2.9 ± 0.1^{b}	2.9 ± 0.1^{b}
20 : 2 Δ5, 13	0.7 ± 0.03^{a}	0.8 ± 0.03^{b}	$0.3 \pm 0.03c$	0.5 ± 0.03^{d}
20 : 2 n - 6	1.7 ± 0.1^{a}	1.8 ± 0.1^{a}	2.8 ± 0.03^{b}	1.5 ± 0.1^{a}
20 : 3 n-9	ND	0.2 ± 0.03^{a}	1.1 ± 0.1^{b}	0.4 ± 0.04^{c}
20 : 3 Δ5, 11, 14	ND	0.1 ± 0.03	ND	ND
20 : 3 n-6	ND	0.3 ± 0.03^{a}	ND	0.8 ± 0.1^{b}
20 : 4 n-6	9.9 ± 0.1^{a}	8.2 ± 0.3^{b}	3.4 ± 0.2^{c}	2.6 ± 0.1^{d}
20 : 3 n-3	0.9 ± 0.04^{a}	0.2 ± 0.03^{b}	1.3 ± 0.03^{c}	1.4 ± 0.1^{b}

Continued

20 : 4 n-3	0.3 ± 0.02^{a}	ND	ND	ND
20 : 5 n-3	16.5 ± 0.5^{a}	12.7 ± 0.4^{b}	5.8 ± 0.1^{c}	2.8 ± 0.04^{d}
22 : 2 Δ7, 13	0.3 ± 0.03^{a}	0.2 ± 0.1^{b}	ND	ND
22 : 2 Δ7, 15	0.1 ± 0.02^{a}	0.6 ± 0.04^{b}	ND	ND
22 : 4 n-6	0.3 ± 0.02^{a}	ND	ND	ND
22 : 5 n-6	0.3 ± 0.03^{a}	ND	ND	ND
22 : 5 n-3	0.7 ± 0.04^{a}	0.3 ± 0.04^{b}	0.3 ± 0.1^{b}	0.4 ± 0.03^{b}
22 : 6 n-3	0.6 ± 0.1^{a}	0.3 ± 0.03^{a}	0.6 ± 0.2^{a}	0.7 ± 0.1^{a}
Σ PUFA	$\mathbf{42.3 \pm 0.7^{a}}$	$\mathbf{43.2 \pm 0.6^{a}}$	$\mathbf{34.8 \pm 0.1^{b}}$	$\mathbf{35.8 \pm 0.3^{b}}$
U / S	$\mathbf{2.3 \pm 0.04^{a}}$	$\mathbf{2.5 \pm 0.02^{b}}$	$\mathbf{1.9 \pm 0.1^{b}}$	$\mathbf{1.7 \pm 0.07^{c}}$
Σ n - 6	$\mathbf{14.6 \pm 0.2^{a}}$	$\mathbf{20.9 \pm 0.03^{b}}$	$\mathbf{22.9 \pm 0.2^{c}}$	$\mathbf{24.2 \pm 0.5^{d}}$
Σ n - 3	$\mathbf{24.5 \pm 0.1^{a}}$	$\mathbf{16.7 \pm 0.1^{b}}$	$\mathbf{10.7 \pm 0.3^{c}}$	$\mathbf{7.5 \pm 0.2^{d}}$
n - 6 / n - 3	$\mathbf{0.6 \pm 0.02^{a}}$	$\mathbf{1.3 \pm 0.02^{b}}$	$\mathbf{2.2 \pm 0.01^{c}}$	$\mathbf{3.2 \pm 0.03^{d}}$

Results are mean values of 3 replicates ± standard deviation. Values in each row with the same superscript are not significantly different ($p>0.05$) from one another.

Abbreviations are as follows: SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; U/S, ratio of unsaturated to saturated fatty acids; n-6, omega-6 fatty acids; and n-3, omega-3 fatty acids.

Table A.17 Fatty acid composition (weight %) of polar lipids from sea urchin gonadal lipids at 0, 3, 6 and 9 weeks of feeding the artificial diet.

Fatty acid	Week 0	Week 3	Week 6	Week 9
14 : 0	8.3 ± 0.1^{a}	4.5 ± 0.1^{b}	2.4 ± 0.1^{c}	2.5 ± 0.04^{c}
15 : 0	0.2 ± 0.1^{a}	0.5 ± 0.04^{ab}	0.5 ± 0.1^{b}	0.9 ± 0.04^{c}
16 : 0	14.6 ± 0.3^{a}	8.2 ± 0.04^{b}	8.1 ± 0.1^{b}	5.9 ± 0.1^{c}
18 : 0	2.1 ± 0.03^{a}	2.5 ± 0.03^{a}	2.8 ± 0.4^{a}	2.5 ± 0.1^{a}
20 : 0	0.3 ± 0.1^{a}	1.1 ± 0.2^{bc}	0.8 ± 0.04^{c}	0.3 ± 0.1^{a}
Σ SFA	**25.5 ± 0.1^{a}**	**16.8 ± 0.3^{b}**	**14.9 ± 0.2^{c}**	**12.1 ± 0.1^{d}**
14 : 1 n-7	0.2 ± 0.04^{a}	0.2 ± 0.03^{a}	0.3 ± 0.04^{ac}	0.4 ± 0.04^{c}
14 : 1 n-5	0.1 ± 0.02^{a}	0.1 ± 0.03^{a}	0.2 ± 0.04^{a}	0.1 ± 0.1^{a}
16 : 1 n-9	1.4 ± 0.1^{a}	2.0 ± 0.1^{b}	2.8 ± 0.1^{c}	3.2 ± 0.1^{d}
16 : 1 n-7	1.1 ± 0.1^{a}	0.7 ± 0.1^{b}	0.5 ± 0.1^{b}	0.2 ± 0.04^{c}
16 : 1 n-5	ND	ND	ND	0.2 ± 0.03
18 : 1 n-9	1.0 ± 0.3^{a}	4.5 ± 0.3^{b}	7.4 ± 0.3^{c}	5.0 ± 0.1^{b}
18 : 1 n-7	ND	1.5 ± 0.2^{a}	2.1 ± 0.1^{a}	2.0 ± 0.1^{a}
18 : 1 n-5	ND	0.2 ± 0.1^{a}	0.2 ± 0.1^{a}	0.4 ± 0.04^{a}
20 : 1 n-15	12.6 ± 0.3^{a}	10.8 ± 0.2^{b}	6.6 ± 0.1^{c}	7.8 ± 0.1^{d}
20 : 1 n-9	4.3 ± 0.0^{a}	2.4 ± 0.1^{b}	4.3 ± 0.04^{a}	6.2 ± 0.03^{c}
20 : 1 n-7	1.8 ± 0.1^{a}	4.1 ± 0.1^{b}	2.8 ± 0.1^{c}	1.8 ± 0.1^{a}
22 : 1 n-11	3.9 ± 0.1^{a}	1.2 ± 0.1^{b}	3.8 ± 0.2^{ac}	3.4 ± 0.1^{c}
22 : 1 n-9	1.1 ± 0.1^{a}	1.4 ± 0.1^{b}	1.1 ± 0.1^{a}	0.2 ± 0.1^{c}
22 : 1 n-7	0.3 ± 0.03^{a}	ND	ND	ND
Σ MUFA	**27.5 ± 0.1^{a}**	**29.5 ± 1.0^{ab}**	**28.0 ± 0.6^{a}**	**30.8 ± 0.1^{b}**
16 : 2 n-6	1.3 ± 0.03^{a}	3.6 ± 0.1^{b}	4.9 ± 0.1^{c}	2.5 ± 0.3^{d}
16 : 4 n-6	0.5 ± 0.02^{a}	0.5 ± 0.03^{a}	0.7 ± 0.2^{a}	0.4 ± 0.1^{a}
16 : 4 n-3	1.3 ± 0.1^{a}	ND	ND	ND
18 : 2 n-9	ND	ND	0.4 ± 0.04^{a}	0.4 ± 0.02^{a}
18 : 2 n-6	0.6 ± 0.04^{a}	9.3 ± 0.2^{b}	14.0 ± 0.3^{c}	17.2 ± 0.1^{d}
18 : 3 n-6	0.5 ± 0.1^{a}	0.5 ± 0.3^{a}	0.3 ± 0.1^{a}	0.2 ± 0.03^{a}
18 : 3 n-3	0.2 ± 0.04^{a}	0.5 ± 0.03^{b}	1.4 ± 0.1^{c}	1.7 ± 0.03^{d}
18 : 4 n-3	1.1 ± 0.1^{a}	0.6 ± 0.1^{b}	1.4 ± 0.04^{c}	0.7 ± 0.1^{b}
20 : 2 Δ5, 11	2.2 ± 0.1^{a}	0.8 ± 0.2^{b}	0.6 ± 0.1^{b}	ND
20 : 2 Δ5, 13	0.4 ± 0.1^{a}	0.1 ± 0.1^{b}	ND	ND
20 : 2 n-6	1.8 ± 0.1^{a}	4.7 ± 0.3^{b}	5.1 ± 0.3^{bc}	5.9 ± 0.1^{c}
20 : 3 n-9	1.3 ± 0.2^{a}	0.7 ± 0.2^{ab}	0.4 ± 0.1^{bc}	0.9 ± 0.04^{ac}
20 : 3 n-6	0.3 ± 0.03^{a}	0.5 ± 0.04^{b}	0.7 ± 0.1^{b}	1.3 ± 0.1^{c}
20 : 4 n-6	9.2 ± 0.2^{a}	9.0 ± 0.2^{a}	9.4 ± 0.5^{a}	10.7 ± 0.2^{b}
20 : 3 n-3	1.3 ± 0.1^{a}	0.9 ± 0.03^{b}	0.8 ± 0.1^{b}	0.9 ± 0.1^{b}
20 : 4 n-3	0.3 ± 0.02^{a}	0.7 ± 0.1^{b}	1.5 ± 0.1^{c}	0.8 ± 0.03^{b}
20 : 5 n-3	21.5 ± 0.2^{a}	17.5 ± 0.4^{b}	14.4 ± 0.1^{c}	12.5 ± 0.1^{d}
22 : 4 n-6	0.3 ± 0.02^{a}	ND	ND	ND

Continued

22 : 5 n-6	0.3 ± 0.03^{a}	ND	ND	ND
22 : 5 n-3	0.5 ± 0.04^{a}	0.4 ± 0.04^{b}	ND	0.3 ± 0.03^{c}
22 : 6 n-3	1.2 ± 0.1^{a}	1.2 ± 0.4^{a}	1.1 ± 0.4^{a}	1.2 ± 0.1^{a}
Σ PUFA	**45.1 ± 0.4^{a}**	**50.7 ± 0.9^{b}**	**56.5 ± 0.6^{c}**	**57.2 ± 0.2^{c}**
U / S	**2.9 ± 0.04^{a}**	**4.8 ± 0.1^{b}**	**5.7 ± 0.1^{c}**	**7.3 ± 0.2^{d}**
Σ n - 6	**14.9 ± 0.1^{a}**	**28.1 ± 0.2^{b}**	**35.0 ± 0.1^{c}**	**38.2 ± 0.2^{d}**
Σ n - 3	**27.4 ± 0.1^{a}**	**21.6 ± 0.1^{b}**	**20.6 ± 0.2^{c}**	**18.0 ± 0.2^{d}**
n - 6 / n - 3	**0.5 ± 0.02^{a}**	**1.3 ± 0.1^{b}**	**1.7 ± 0.04^{c}**	**2.1 ± 0.1^{d}**

Results are mean values of 3 replicates ± standard deviation. Values in each row with the same superscript are not significantly different ($p>0.05$) from one another.

Abbreviations are as follows: SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; U/S, ratio of unsaturated to saturated fatty acids; n-6, omega-6 fatty acids; and n-3, omega-3 fatty acids.

Table A.18 Fatty acid composition (weight %) of polar lipids from sea urchin visceral lipids at 0, 3, 6 and 9 weeks of feeding the artificial diet.

Fatty acid	Week 0	Week 3	Week 6	Week 9
14 : 0	7.4 ± 0.1^{a}	3.8 ± 0.1^{b}	3.6 ± 0.1^{b}	2.7 ± 0.04^{c}
15 : 0	0.6 ± 0.1^{a}	1.1 ± 0.04^{b}	1.2 ± 0.1^{b}	1.3 ± 0.04^{b}
16 : 0	14.1 ± 0.1^{a}	8.8 ± 0.1^{b}	7.7 ± 0.1^{c}	6.0 ± 0.1^{d}
18 : 0	2.3 ± 0.2^{a}	4.8 ± 0.1^{b}	4.3 ± 0.4^{b}	2.4 ± 0.1^{a}
20 : 0	0.8 ± 0.1^{a}	0.6 ± 0.1^{ab}	0.4 ± 0.04^{b}	0.3 ± 0.2^{cb}
Σ SFA	**25.3 ± 0.1^{a}**	**19.0 ± 0.1^{b}**	**17.2 ± 0.2^{c}**	**12.7 ± 0.1^{d}**
14 : 1 n-7	0.7 ± 0.04^{a}	0.4 ± 0.03^{b}	0.6 ± 0.04^{c}	0.3 ± 0.02^{b}
14 : 1 n-5	0.2 ± 0.03^{ab}	0.2 ± 0.03^{ab}	0.3 ± 0.04^{a}	0.1 ± 0.1^{b}
16 : 1 n-9	5.2 ± 0.1^{a}	3.0 ± 0.1^{b}	2.6 ± 0.1^{c}	2.8 ± 0.1^{bc}
16 : 1 n-7	3.1 ± 0.1^{a}	0.5 ± 0.1^{b}	0.7 ± 0.1^{b}	0.7 ± 0.04^{b}
16 : 1 n-5	0.2 ± 0.04^{a}	ND	ND	0.2 ± 0.03^{a}
18 : 1 n-9	2.3 ± 0.1^{a}	5.3 ± 0.1^{b}	5.5 ± 0.3^{b}	4.4 ± 0.1^{c}
18 : 1 n-7	3.8 ± 0.2^{a}	1.6 ± 0.2^{b}	2.3 ± 0.2^{b}	2.1 ± 0.1^{b}
18 : 1 n-5	0.3 ± 0.03^{a}	0.3 ± 0.1^{a}	0.2 ± 0.1^{a}	0.2 ± 0.04^{a}
20 : 1 n-15	6.1 ± 0.1^{a}	5.2 ± 0.2^{b}	7.1 ± 0.4^{c}	8.1 ± 0.1^{d}
20 : 1 n-9	4.4 ± 0.1^{a}	3.1 ± 0.1^{b}	3.9 ± 0.2^{c}	6.5 ± 0.2^{d}
20 : 1 n-7	2.2 ± 0.1^{a}	1.5 ± 0.1^{b}	1.3 ± 0.1^{b}	1.9 ± 0.1^{c}
22 : 1 n-11	3.7 ± 0.1^{ac}	4.2 ± 0.1^{b}	4.0 ± 0.2^{ab}	3.3 ± 0.1^{c}
22 : 1 n-9	0.3 ± 0.1^{a}	1.1 ± 0.1^{b}	1.8 ± 0.1^{c}	0.2 ± 0.2^{a}
Σ MUFA	**32.3 ± 0.4^{a}**	**26.4 ± 0.4^{b}**	**30.1 ± 0.2^{c}**	**30.1 ± 0.3^{c}**
16 : 2 n-6	0.8 ± 0.1^{a}	4.0 ± 0.1^{b}	3.2 ± 0.2^{c}	1.7 ± 0.1^{d}
16 : 4 n-6	0.9 ± 0.1^{a}	0.7 ± 0.1^{a}	0.7 ± 0.1^{a}	0.3 ± 0.03^{b}
18 : 2 n-6	1.3 ± 0.1^{a}	11.9 ± 0.2^{b}	14.5 ± 0.3^{c}	17.9 ± 0.1^{d}
18 : 3 n-6	0.3 ± 0.04^{a}	0.5 ± 0.03^{b}	0.4 ± 0.1^{a}	0.2 ± 0.03^{a}
18 : 3 n-3	1.7 ± 0.1^{a}	1.8 ± 0.03^{a}	1.6 ± 0.1^{a}	1.6 ± 0.1^{a}
18 : 4 n-3	0.9 ± 0.1^{a}	1.0 ± 0.04^{a}	0.9 ± 0.04^{a}	0.6 ± 0.1^{b}
20 : 2 Δ5, 11	2.9 ± 0.1^{a}	0.8 ± 0.2^{b}	0.3 ± 0.1^{c}	ND
20 : 2 Δ5, 13	1.0 ± 0.1^{a}	0.1 ± 0.1^{b}	ND	ND
20 : 2 n - 6	1.1 ± 0.1^{a}	4.7 ± 0.1^{b}	4.4 ± 0.3^{b}	5.7 ± 0.1^{c}
20 : 3 n-9	0.65 ± 0.1^{a}	0.5 ± 0.03^{a}	0.55 ± 0.05^{a}	1.0 ± 0.04^{b}
20 : 3 n-6	0.9 ± 0.1^{a}	0.5 ± 0.03^{b}	0.4 ± 0.1^{b}	1.3 ± 0.1^{c}
20 : 4 n-6	8.2 ± 0.3^{a}	8.2 ± 0.2^{b}	8.7 ± 0.5^{ac}	9.5 ± 0.3^{b}
20 : 3 n-3	ND	0.7 ± 0.03^{a}	0.7 ± 0.1^{a}	0.9 ± 0.1^{a}
20 : 4 n-3	ND	0.8 ± 0.1^{a}	0.8 ± 0.1^{a}	0.7 ± 0.03^{a}
20 : 5 n-3	22.9 ± 0.2^{a}	16.4 ± 0.2^{b}	14.6 ± 0.2^{c}	13.8 ± 0.1^{d}
22 : 5 n-6	0.2 ± 0.03^{a}	ND	ND	ND
22 : 5 n-3	0.6 ± 0.04^{a}	0.4 ± 0.1^{a}	ND	0.2 ± 0.03^{c}
22 : 6 n-3	0.9 ± 0.2^{a}	1.0 ± 0.1^{a}	0.8 ± 0.08^{a}	1.1 ± 0.1^{a}
Σ PUFA	**42.4 ± 0.3^{a}**	**53.2 ± 0.4^{b}**	**52.5 ± 0.4^{b}**	**58.4 ± 0.2^{c}**

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U / S	**3.0 ± 0.3[a]**	**4.2 ± 0.1[b]**	**4.8 ± 0.02[b]**	**6.9 ± 0.1c**
Σ n - 6	**13.9 ± 0.1[a]**	**31.0 ± 0.5[b]**	**32.3 ± 0.3[b]**	**36.7 ± 0.2[d]**
Σ n - 3	**24.0 ± 0.1[a]**	**22.1 ± 0.1[b]**	**19.3 ± 0.1[b]**	**18.8 ± 0.2[d]**
n - 6 / n - 3	**0.6 ± 0.01[a]**	**1.4 ± 0.04[b]**	**1.7 ± 0.03[b]**	**1.9 ± 0.1[d]**

Results are mean values of 3 replicates ± standard deviation. Values in each row with the same superscript are not significantly different ($p>0.05$) from one another.

Abbreviations are as follows: SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; U/S, ratio of unsaturated to saturated fatty acids; n-6, omega-6 fatty acids; and n-3, omega-3 fatty acids.

Table A.19 Fatty acid composition (weight %) of non polar lipids from cultured sea urchin gonadal lipids at 0, 3, 6 and 9 weeks of feeding the artificial feed.

Fatty acid	Week 0	Week 3	Week 6	Week 9
14 : 0	10.8 ± 0.1^{a}	7.3 ± 0.1^{b}	6.83 ± 0.16^{c}	6.62 ± 0.05^{c}
15 : 0	0.3 ± 0.1^{a}	0.7 ± 0.04^{b}	0.9 ± 0.1^{c}	0.7 ± 0.1^{b}
16 : 0	16.9 ± 0.1^{a}	11.7 ± 0.2^{b}	11.8 ± 0.1^{b}	11.3 ± 0.04^{b}
18 : 0	2.8 ± 0.2^{a}	2.2 ± 0.1^{b}	2.1 ± 0.1^{b}	2.1 ± 0.1^{b}
19 : 0	0.1 ± 0.1^{a}	0.1 ± 0.1^{b}	ND	0.1 ± 0.1^{a}
20 : 0	1.9 ± 0.1^{a}	3.3 ± 0.02^{b}	3.6 ± 0.4^{b}	3.9 ± 0.1^{b}
Σ SFA	**32.9 ± 0.1^{a}**	**25.1 ± 0.2^{b}**	**25.3 ± 0.3^{b}**	**24.6 ± 0.1^{b}**
14 : 1 n-7	0.5 ± 0.04^{a}	0.9 ± 0.03^{b}	0.6 ± 0.04^{ac}	0.7 ± 0.04^{c}
14 : 1 n-5	0.2 ± 0.03^{ac}	0.3 ± 0.03^{a}	0.2 ± 0.03^{ac}	0.1 ± 0.1^{c}
16 : 1 n-11	0.7 ± 0.04^{a}	ND	ND	ND
16 : 1 n-9	0.9 ± 0.1^{a}	4.8 ± 0.1^{bd}	4.1 ± 0.1^{c}	4.5 ± 0.1^{dc}
16 : 1 n-7	2.5 ± 0.1^{a}	2.1 ± 0.1^{b}	1.9 ± 0.1^{b}	1.6 ± 0.02^{c}
16 : 1 n-5	0.8 ± 0.1^{a}	0.4 ± 0.04^{b}	0.3 ± 0.03^{b}	0.3 ± 0.03^{b}
18 : 1 n-13	ND	0.5 ± 0.03^{a}	0.4 ± 0.03^{b}	ND
18 : 1 n-9	2.5 ± 0.2^{a}	7.1 ± 0.2^{b}	8.0 ± 0.3^{c}	6.6 ± 0.2^{b}
18 : 1 n-7	2.0 ± 0.1^{a}	4.7 ± 0.2^{b}	3.6 ± 0.2^{c}	3.2 ± 0.1^{c}
18 : 1 n-5	0.5 ± 0.03^{a}	0.2 ± 0.04^{b}	ND	0.2 ± 0.04^{b}
20 : 1 n-15	8.9 ± 0.1^{a}	3.1 ± 0.1^{bc}	2.7 ± 0.2^{b}	3.4 ± 0.1^{c}
20 : 1 n-9	3.3 ± 0.1^{a}	1.7 ± 0.1^{b}	0.8 ± 0.1^{c}	1.1 ± 0.03^{d}
20 : 1 n-7	0.7 ± 0.1^{a}	3.5 ± 0.1^{b}	2.9 ± 0.1^{c}	2.6 ± 0.1^{d}
22 : 1 n-11	1.6 ± 0.1^{a}	0.2 ± 0.1^{b}	0.2 ± 0.1^{b}	0.4 ± 0.1^{b}
22 : 1 n-9	0.3 ± 0.1^{a}	0.2 ± 0.1^{c}	1.3 ± 0.1^{bc}	1.1 ± 0.03^{c}
22 : 1 n-7	ND	0.1 ± 0.02	ND	ND
Σ MUFA	**25.9 ± 0.7^{ac}**	**29.8 ± 0.9^{b}**	**27.8 ± 0.2^{ab}**	**25.1 ± 0.6^{c}**
16 : 2 n-6	0.4 ± 0.1^{a}	0.6 ± 0.1^{ac}	1.1 ± 0.04^{b}	0.8 ± 0.1^{c}
16 : 4 n-6	1.4 ± 0.1^{a}	0.7 ± 0.04^{b}	1.8 ± 0.1^{a}	0.8 ± 0.03^{b}
18 : 2 n-9	0.4 ± 0.03^{a}	0.5 ± 0.04^{b}	0.2 ± 0.04^{c}	0.1 ± 0.02^{c}
18 : 2 n-6	1.8 ± 0.1^{a}	13.8 ± 0.3^{b}	20.4 ± 0.4^{c}	22.1 ± 0.1^{d}
18 : 3 n-6	0.7 ± 0.2^{a}	0.6 ± 0.1^{ab}	0.3 ± 0.04^{b}	0.3 ± 0.03^{b}
18 : 3 n-3	2.2 ± 0.2^{a}	2.6 ± 0.1^{a}	2.5 ± 0.2^{a}	2.2 ± 0.03^{a}
18 : 4 n-3	1.3 ± 0.1^{a}	1.3 ± 0.1^{a}	1.1 ± 0.04^{a}	1.2 ± 0.1^{a}
20 : 2 Δ5, 11	1.5 ± 0.3^{a}	1.2 ± 0.2^{a}	1.3 ± 0.1^{b}	1.9 ± 0.1^{a}
20 : 2 Δ5, 13	0.6 ± 0.04^{a}	0.3 ± 0.1^{b}	0.3 ± 0.1^{b}	0.3 ± 0.03^{b}
20 : 2 n - 6	2.1 ± 0.1^{a}	3.4 ± 0.1^{b}	4.4 ± 0.2^{c}	5.7 ± 0.1^{d}
20 : 3 n-9	0.2 ± 0.04^{a}	0.2 ± 0.04^{a}	0.3 ± 0.1^{ab}	0.4 ± 0.04^{b}
20 : 3 n-6	0.7 ± 0.1^{a}	0.7 ± 0.04^{a}	0.5 ± 0.04^{a}	1.1 ± 0.1^{b}
20 : 4 n-6	8.5 ± 0.5^{a}	2.3 ± 0.4^{b}	2.6 ± 0.1^{bc}	3.8 ± 0.1^{c}
20 : 3 n-3	1.2 ± 0.1^{a}	0.8 ± 0.1^{b}	0.6 ± 0.1^{b}	0.7 ± 0.1^{b}
20 : 4 n-3	0.8 ± 0.02^{a}	0.7 ± 0.1^{a}	0.3 ± 0.02^{b}	0.4 ± 0.04^{b}

Continued

20 : 5 n-3	14.1 ± 0.1^{a}	10.8 ± 0.1^{b}	7.3 ± 0.14^{c}	4.4 ± 0.1^{d}
20 : 2 Δ7,13	ND	0.2 ± 0.03^{a}	1.0 ± 0.06^{b}	0.3 ± 0.04^{a}
20 : 2 Δ7,15	ND	0.1 ± 0.03^{a}	0.2 ± 0.03^{a}	0.8 ± 0.04^{b}
22 : 5 n-6	0.6 ± 0.03^{a}	0.2 ± 0.03^{b}	0.2 ± 0.03^{b}	0.3 ± 0.04^{b}
22 : 5 n-3	0.3 ± 0.04^{a}	0.3 ± 0.04^{a}	0.3 ± 0.02^{a}	0.2 ± 0.04^{a}
22 : 6 n-3	0.7 ± 0.16^{a}	1.5 ± 0.1^{b}	0.5 ± 0.1^{a}	1.5 ± 0.3^{b}
Σ PUFA	**40.5 ± 0.5^{a}**	**45.3 ± 0.2^{b}**	**47.6 ± 0.1^{c}**	**50.4 ± 0.8^{d}**
U / S	**2.0 ± 0.1^{a}**	**3.0 ± 0.03^{b}**	**2.9 ± 0.1^{b}**	**3.1 ± 0.1^{b}**
Σ n - 6	**16.2 ± 0.2^{a}**	**22.2 ± 0.1^{b}**	**31.3 ± 0.2^{c}**	**34.5 ± 0.2^{d}**
Σ n - 3	**20.5 ± 0.3^{a}**	**18.1 ± 0.2^{b}**	**12.7 ± 0.1^{c}**	**10.5 ± 0.3^{d}**
n - 6 / n - 3	**0.8 ± 0.04^{a}**	**1.2 ± 0.1^{b}**	**2.5 ± 0.1^{c}**	**3.3 ± 0.1^{d}**

Results are mean values of 3 replicates ± standard deviation. Values in each row with the same superscript are not significantly different ($p>0.05$) from one another.

Abbreviations are as follows: SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; U/S, ratio of unsaturated to saturated fatty acids; n-6, omega-6 fatty acids; and n-3, omega-3 fatty acids.

Table A.20 Fatty acid composition (weight %) of non polar lipids from sea urchin visceral lipids at 0, 3, 6 and 9 weeks of feeding the artificial diet.

Fatty acid	Week 0	Week 3	Week 6	Week 9
14 : 0	11.9 ± 0.1^{a}	5.8 ± 0.1^{b}	5.6 ± 0.1^{b}	6.3 ± 0.1^{b}
15 : 0	0.6 ± 0.1^{a}	1.4 ± 0.04^{b}	0.9 ± 0.1^{c}	0.7 ± 0.1^{a}
16 : 0	16.8 ± 0.1^{a}	11.3 ± 0.3^{b}	11.7 ± 0.2^{b}	11.3 ± 0.4^{b}
18 : 0	2.2 ± 0.2^{a}	2.4 ± 0.1^{a}	3.4 ± 0.2^{b}	2.1 ± 0.1^{a}
19 : 0	ND	0.1 ± 0.1^{a}	ND	0.4 ± 0.2^{b}
20 : 0	0.7 ± 0.2^{a}	3.5 ± 0.1^{b}	4.2 ± 0.3^{bc}	4.3 ± 0.1^{c}
Σ SFA	**32.2 ± 0.2^{a}**	**24.4 ± 0.2^{b}**	**25.8 ± 0.1^{c}**	**24.8 ± 0.4^{bc}**
14 : 1 n-7	0.4 ± 0.03^{a}	0.7 ± 0.03^{b}	0.5 ± 0.03^{a}	0.7 ± 0.03^{b}
14 : 1 n-5	0.2 ± 0.04^{a}	0.2 ± 0.03^{a}	0.2 ± 0.03^{a}	0.2 ± 0.04^{a}
16 : 1 n-11	0.7 ± 0.1^{a}	ND	ND	ND
16 : 1 n-9	2.6 ± 0.1^{a}	4.5 ± 0.1^{b}	4.0 ± 0.04^{c}	4.6 ± 0.1^{b}
16 : 1 n-7	3.9 ± 0.1^{a}	1.3 ± 0.1^{bc}	1.2 ± 0.1^{b}	1.6 ± 0.04^{c}
16 : 1 n-5	0.3 ± 0.03^{ab}	0.3 ± 0.04^{ab}	0.2 ± 0.03^{a}	0.4 ± 0.04^{b}
18 : 1 n-13	ND	0.5 ± 0.03^{a}	0.5 ± 0.1^{a}	ND
18 : 1 n-9	3.8 ± 0.2^{a}	6.7 ± 0.2^{bc}	7.3 ± 0.4^{b}	6.2 ± 0.1^{c}
18 : 1 n-7	1.9 ± 0.1^{a}	2.9 ± 0.1^{b}	0.2 ± 0.2^{c}	3.3 ± 0.3^{d}
18 : 1 n-5	0.1 ± 0.04^{a}	0.1 ± 0.04^{a}	ND	0.2 ± 0.04^{a}
20 : 1 n-15	8.5 ± 0.2^{a}	3.1 ± 0.1^{b}	3.2 ± 0.2^{b}	3.2 ± 0.1^{b}
20 : 1 n-9	2.7 ± 0.1^{a}	0.9 ± 0.1^{bc}	0.8 ± 0.04^{b}	1.2 ± 0.1^{c}
20 : 1 n-7	0.7 ± 0.1^{a}	3.6 ± 0.1^{b}	3.6 ± 0.2^{b}	4.2 ± 0.1^{c}
22 : 1 n-11	3.2 ± 0.1^{a}	$0.1 \pm 0.1b$	ND	0.1 ± 0.1^{b}
22 : 1 n-9	0.6 ± 0.1^{a}	1.5 ± 0.1^{b}	1.6 ± 0.1^{b}	1.5 ± 0.1^{b}
22 : 1 n-7	ND	ND	ND	ND
Σ MUFA	**30.5 ± 0.4^{a}**	**26.4 ± 0.3^{b}**	**23.2 ± 0.2^{c}**	**27.17 ± 0.48^{b}**
16 : 2 n-6	0.5 ± 0.06^{a}	0.9 ± 0.4^{a}	0.5 ± 0.04^{a}	0.8 ± 0.04^{a}
16 : 4 n-6	0.3 ± 0.05^{a}	0.8 ± 0.04^{b}	0.6 ± 0.1^{b}	0.8 ± 0.1^{b}
18 : 2 n-9	ND	0.2 ± 0.04^{ab}	0.3 ± 0.1^{a}	0.1 ± 0.03^{b}
18 : 2 n-6	1.4 ± 0.04^{a}	17.7 ± 0.2^{b}	20.9 ± 0.3^{c}	21.7 ± 0.1^{c}
18 : 3 n-6	0.7 ± 0.04^{a}	0.3 ± 0.03^{b}	0.3 ± 0.04^{a}	0.4 ± 0.03^{b}
18 : 3 n-3	1.2 ± 0.1^{a}	2.6 ± 0.1^{b}	2.5 ± 0.2^{b}	2.5 ± 0.1^{b}
18 : 4 n-3	0.8 ± 0.1^{a}	1.3 ± 0.04^{b}	1.6 ± 0.1^{c}	1.3 ± 0.1^{bc}
20 : 2 Δ5, 11	2.2 ± 0.1^{a}	1.5 ± 0.1^{b}	1.5 ± 0.1^{b}	1.7 ± 0.1^{c}
20 : 2 Δ5, 13	0.9 ± 0.1^{a}	0.2 ± 0.04^{b}	ND	0.3 ± 0.03^{b}
20 : 2 n - 6	1.9 ± 0.04^{a}	4.8 ± 0.1^{b}	4.9 ± 0.1^{b}	5.5 ± 0.3^{c}
20 : 3 n-9	ND	0.3 ± 0.04^{a}	0.3 ± 0.02^{a}	0.4 ± 0.03^{b}
20 : 3 n-6	ND	0.7 ± 0.04^{a}	0.7 ± 0.04^{a}	1.1 ± 0.1^{b}
20 : 4 n-6	7.1 ± 0.2^{a}	6.3 ± 0.1^{b}	4.4 ± 0.11^{c}	2.3 ± 0.1^{d}
20 : 3 n-3	1.1 ± 0.1^{a}	0.5 ± 0.1^{b}	0.6 ± 0.1^{b}	0.6 ± 0.1^{b}
20 : 4 n-3	0.8 ± 0.04^{a}	0.3 ± 0.1^{b}	0.3 ± 0.02^{b}	0.4 ± 0.03^{b}

Continued

20 : 5 n-3	14.1 ± 0.2^{a}	5.1 ± 0.1^{b}	6.2 ± 0.1^{c}	4.6 ± 0.1^{d}
20 : 2 Δ7,13	0.7 ± 0.2^{a}	0.2 ± 0.04^{b}	ND	0.3 ± 0.04^{b}
20 : 2 Δ7,15	0.4 ± 0.04^{a}	0.7 ± 0.04^{b}	1.1 ± 0.1^{c}	0.7 ± 0.04^{b}
22 : 5 n-6	0.2 ± 0.1^{a}	0.2 ± 0.03^{a}	ND	0.2 ± 0.04^{a}
22 : 5 n-3	0.6 ± 0.04^{a}	0.1 ± 0.04^{b}	0.3 ± 0.02^{c}	0.2 ± 0.04^{bc}
22 : 6 n-3	0.8 ± 0.1^{a}	0.7 ± 0.1^{a}	0.4 ± 0.1^{b}	1.9 ± 0.3^{c}
Σ PUFA	$\mathbf{35.9 \pm 1.1^{a}}$	$\mathbf{49.1 \pm 0.2^{b}}$	$\mathbf{48.1 \pm 0.6^{b}}$	$\mathbf{48.3 \pm 0.3^{b}}$
U / S	$\mathbf{2.1 \pm 0.1^{a}}$	$\mathbf{3.1 \pm 0.1^{b}}$	$\mathbf{2.8 \pm 0.03^{c}}$	$\mathbf{3.1 \pm 0.1^{b}}$
Σ n - 6	$\mathbf{12.0 \pm 0.2^{a}}$	$\mathbf{31.6 \pm 0.3^{b}}$	$\mathbf{32.3 \pm 0.3^{c}}$	$\mathbf{32.8 \pm 0.1^{d}}$
Σ n - 3	$\mathbf{19.3 \pm 0.1^{a}}$	$\mathbf{13.7 \pm 0.2^{b}}$	$\mathbf{11.8 \pm 0.2^{c}}$	$\mathbf{11.7 \pm 0.1^{c}}$
n - 6 / n - 3	$\mathbf{0.6 \pm 0.04^{a}}$	$\mathbf{2.3 \pm 0.03^{b}}$	$\mathbf{2.7 \pm 0.04^{c}}$	$\mathbf{2.8 \pm 0.1^{c}}$

Results are mean values of 3 replicates ± standard deviation. Values in each row with the same superscript are not significantly different ($p>0.05$) from one another.

Abbreviations are as follows: SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; U/S, ratio of unsaturated to saturated fatty acids; n-6, omega-6 fatty acids; and n-3, omega-3 fatty acids.

Table A.21 Fatty acid composition (weight %) of grain based sea urchin feed.

Fatty acid	Total lipids	Polar lipids	Non polar lipids
14 : 0	0.3 ± 0.03	0.6 ± 0.1	0.3 ± 0.03
15 : 0	ND	1.2 ± 0.04	0.2 ± 0.1
16 : 0	16.7 ± 0.2	17.1 ± 0.2	16.3 ± 0.1
17 : 0	0.2 ± 0.03	ND	ND
18 : 0	7.6 ± 0.4	10.5 ± 0.6	8.5 ± 0.5
Σ SFA	**24.7 ± 0.6**	**28.4 ± 0.2**	**25.3 ± 0.6**
14 : 1 n-5	0.04 ± 0.04	0.04 ± 0.03	0.1 ± 0.1
16 : 1 n-9	0.3 ± 0.04	0.3 ± 0.1	0.2 ± 0.1
16 : 1 n-7	0.04 ± 0.03	0.1 ± 0.1	ND
18 : 1 n-9	18.8 ± 0.2	9.5 ± 0.1	21.2 ± 0.2
18 : 1 n-7	ND	1.1 ± 0.1	1.2 ± 0.02
18 : 1 n-5	0.04 ± 0.04	ND	0.04 ± 0.02
20 : 1 n-15	0.6 ± 0.03	0.4 ± 0.2	0.6 ± 0.04
20 : 1 n-9	0.1 ± 0.03	0.1 ± 0.02	0.04 ± 0.04
22 : 1 n-11	0.3 ± 0.04	0.2 ± 0.1	0.2 ± 0.1
22 : 1 n-9	0.3 ± 0.03	ND	0.3 ± 0.1
Σ MUFA	**20.4 ± 0.3**	**11.9 ± 0.2**	**23.8 ± 0.3**
16 : 2 n-6	0.1 ± 0.03	0.1 ± 0.1	0.1 ± 0.04
16 : 4 n-6	0.04 ± 0.04	0.03 ± 0.02	ND
18 : 2 n-6	49.1 ± 0.2	52.1 ± 0.4	45.7 ± 0.3
18 : 3 n-3	5.2 ± 0.2	6.5 ± 0.4	4.5 ± 0.1
18 : 4 n-3	0.1 ± 0.1	0.3 ± 0.1	0.1 ± 0.1
20 : 3 n-6	ND	0.1 ± 0.04	ND
20 : 4 n-6	ND	0.03 ± 0.02	ND
20 : 5 n-3	0.1 ± 0.1	0.03 ± 0.03	0.1 ± 0.1
22 : 5 n-3	0.1 ± 0.04	0.2 ± 0.1	0.1 ± 0.03
22 : 6 n-3	0.2 ± 0.1	0.2± 0.02	0.2 ± 0.1
Σ PUFA	**54.9 ± 0.3**	**59.61 ± 0.69**	**51.2 ± 0.2**
U / S	**2.8 ± 0.01**	**2.7 ± 0.02**	**4.1 ± 0.02**
Σ n - 6	**11.0 ± 0.1**	**9.5 ± 0.01**	**16.2 ± 0.1**
Σ n - 3	**20.2 ± 0.01**	**25.2 ± 0.2**	**34.8 ± 0.2**
n - 6 / n - 3	**0.6 ± 0.01**	**0.4 ± 0.01**	**0.5 ± 0.01**

Results are mean values of 3 replicates ± standard deviation.
Abbreviations are as follows: SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; U/S, ratio of unsaturated to saturated fatty acids; n-6, omega-6 fatty acids; and n-3, omega-3 fatty acids.

APPENDIX 2

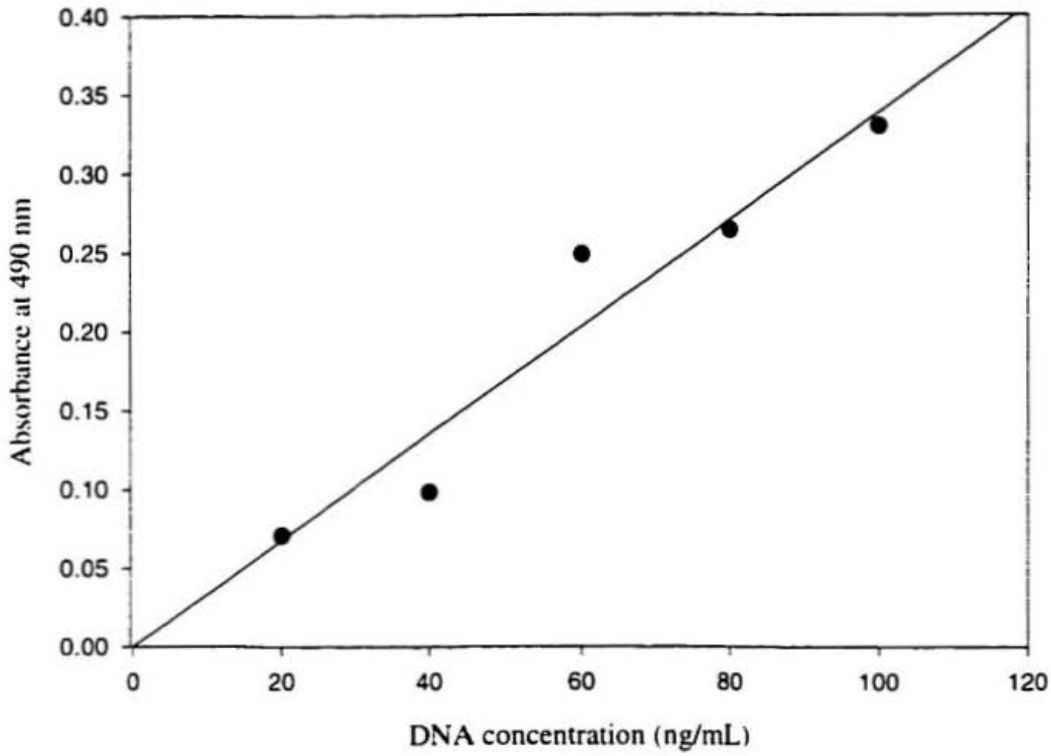

Fig A.1 Standard line of concentration dependance of deoxyribonucleic acid (DNA) at 490 nm

Regression coefficient (r) = 0.964
Equation of the line (Y = aX + b) where,
Y = absorbance at 490 nm (A_{490})
X = concentration of DNA (C)
a = 0.003
b = 0.0

$A_{490} = 0.003 * C$

Therefore, $C = A_{490}/0.003$

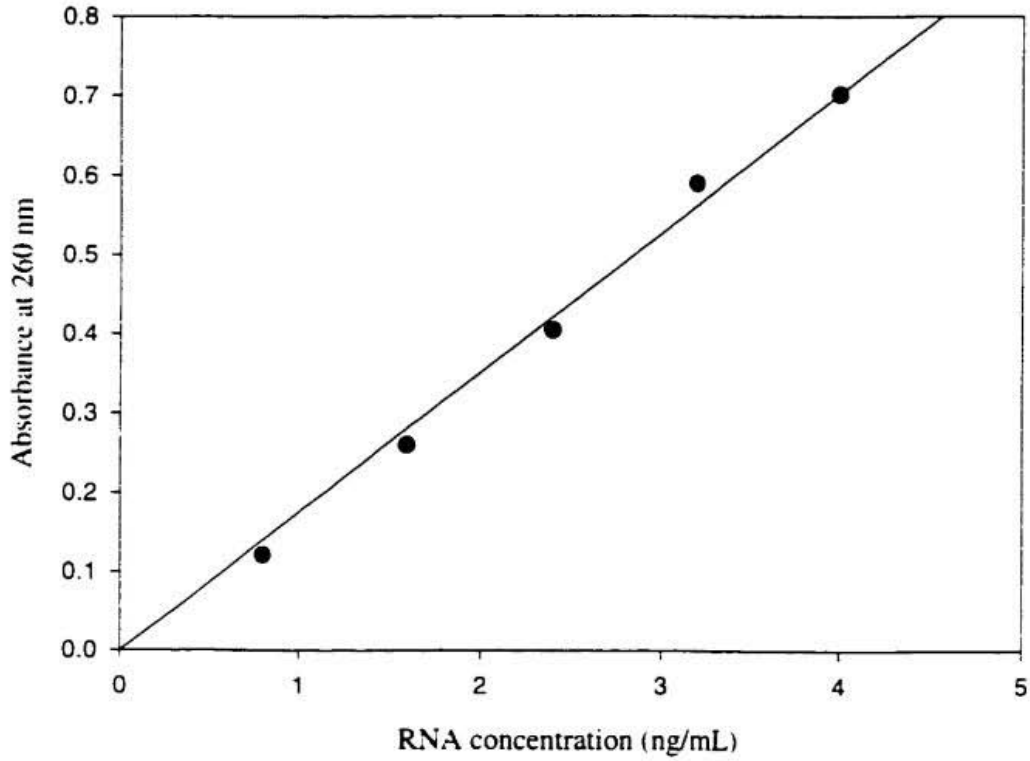

Fig A.2 Standard line of dependance of ribonucleic acid (RNA) at 260 nm

Regression coefficient (r) = 0.997
Equation of the line (Y = aX + b) where,
Y = absorbance at 260 nm (A_{260})
X = concentration of RNA (C)
a = 0.186
b = 0.0

$A_{260} = 0.186 * C$

Therefore, $C = A_{260}/0.186$

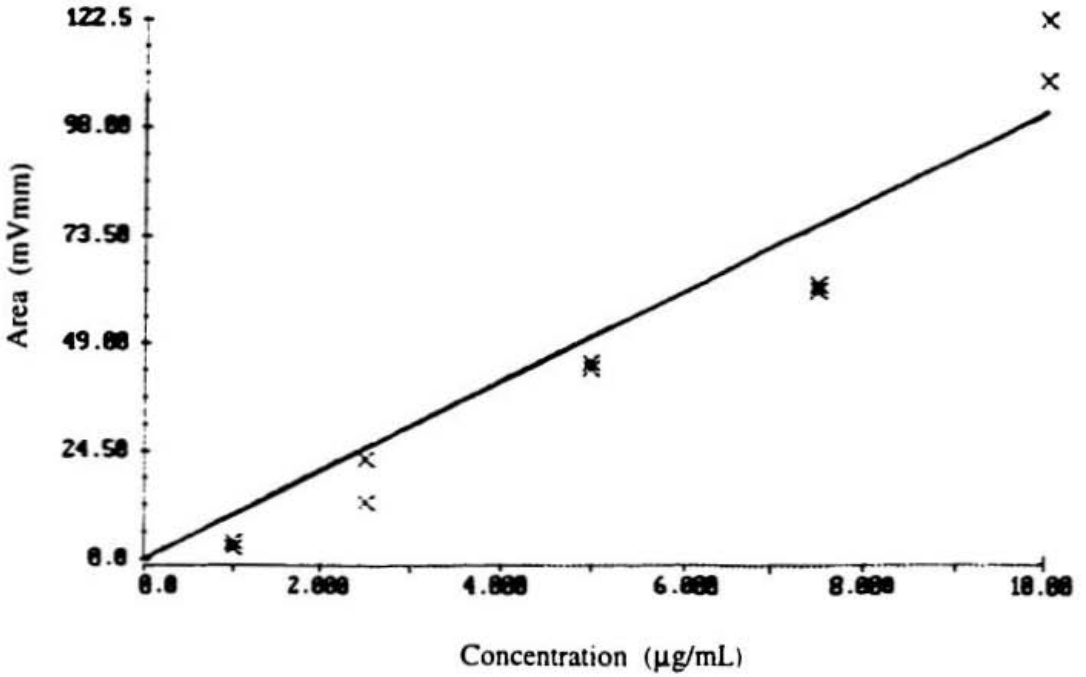

Fig A.3 Standard line for area under each peak at different concentrations of triacylglycerol.

Regression coefficient (r) = 0.97
Rf = 42.3

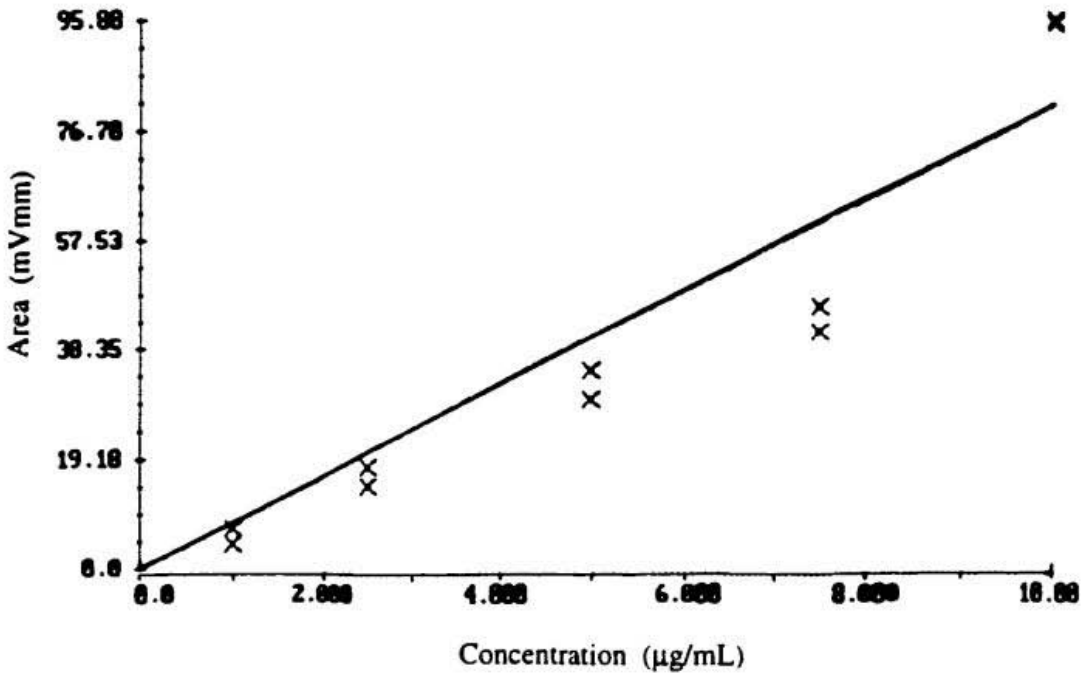

Fig A.4 Standard line for area under each peak at different concentrations of diacylglycerol.

Regression coefficient (r) = 0.95
Rf = 79.1

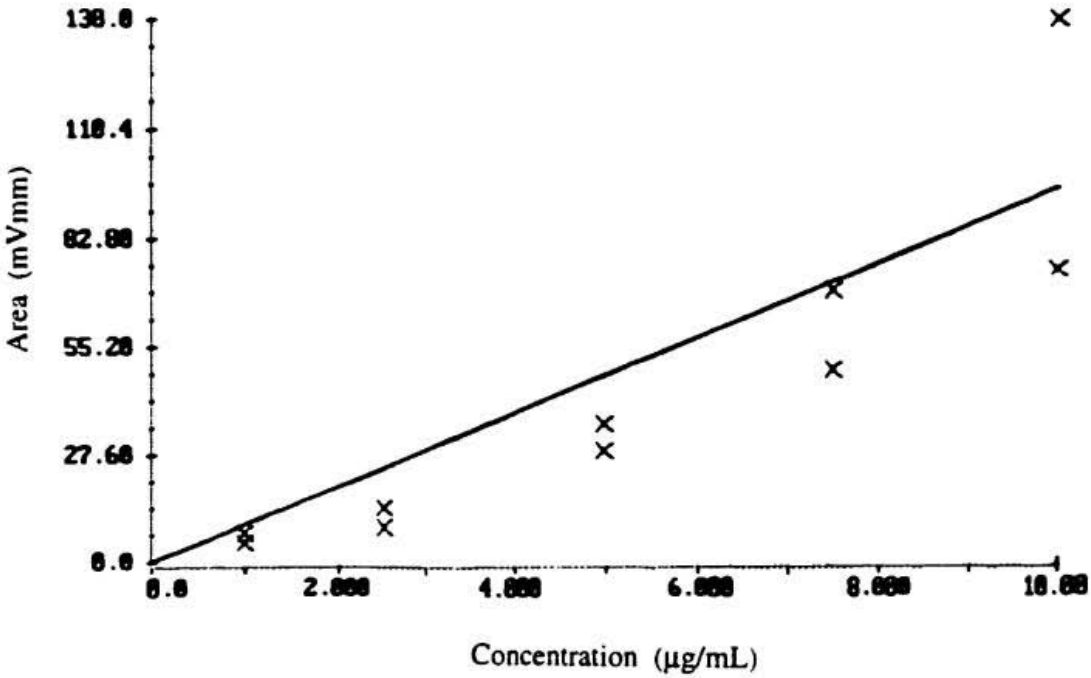

Fig A.5 Standard line for area under each peak at different concentrations of monoacylglycerol.

Regression coefficient (r) = 0.90
Rf = 106.0

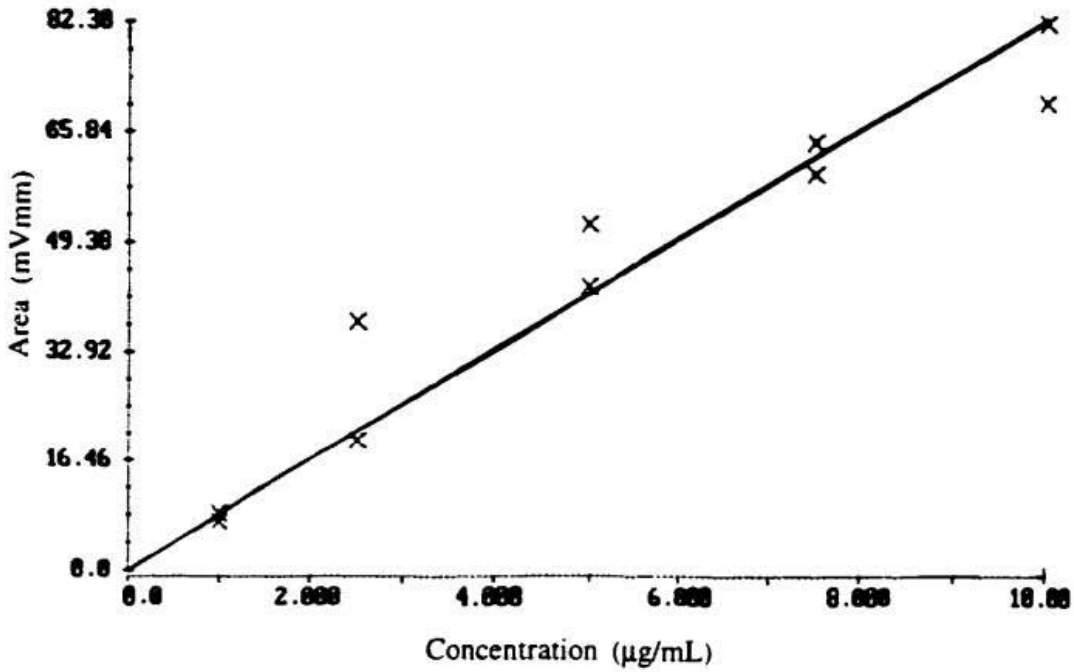

Fig A.6 Standard line for area under each peak at different concentrations of cholesterol.

Regression coefficient (r) = 0.96
Rf = 85.0

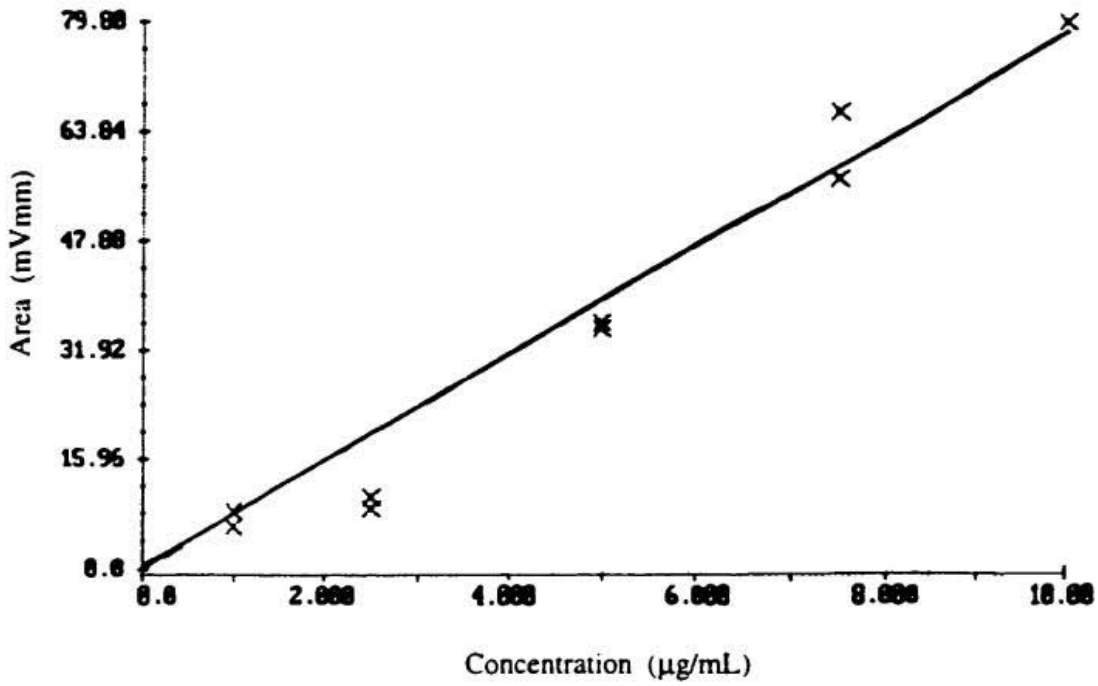

Fig A.7 Standard line for area under each peak at different concentrations of cholesterol ester.

Regression coefficient (r) = 0.99
Rf = 34.4

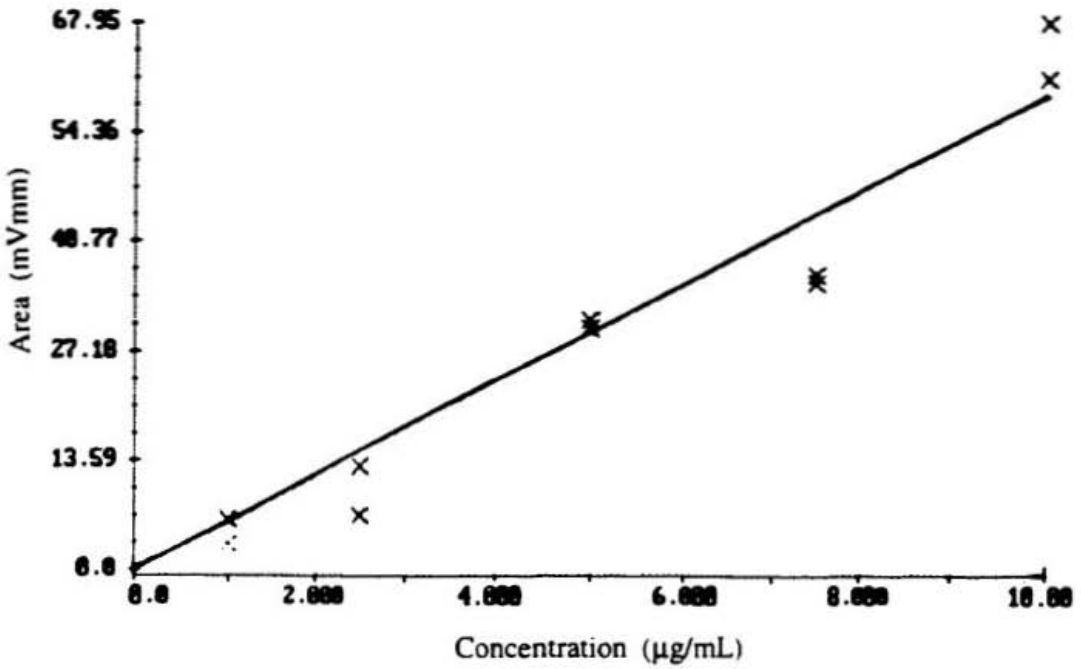

Fig A.8 Standard line for area under each peak at different concentrations of free fatty acids (oleic acid).

Regression coefficient (r) = 0.92
Rf = 45.5

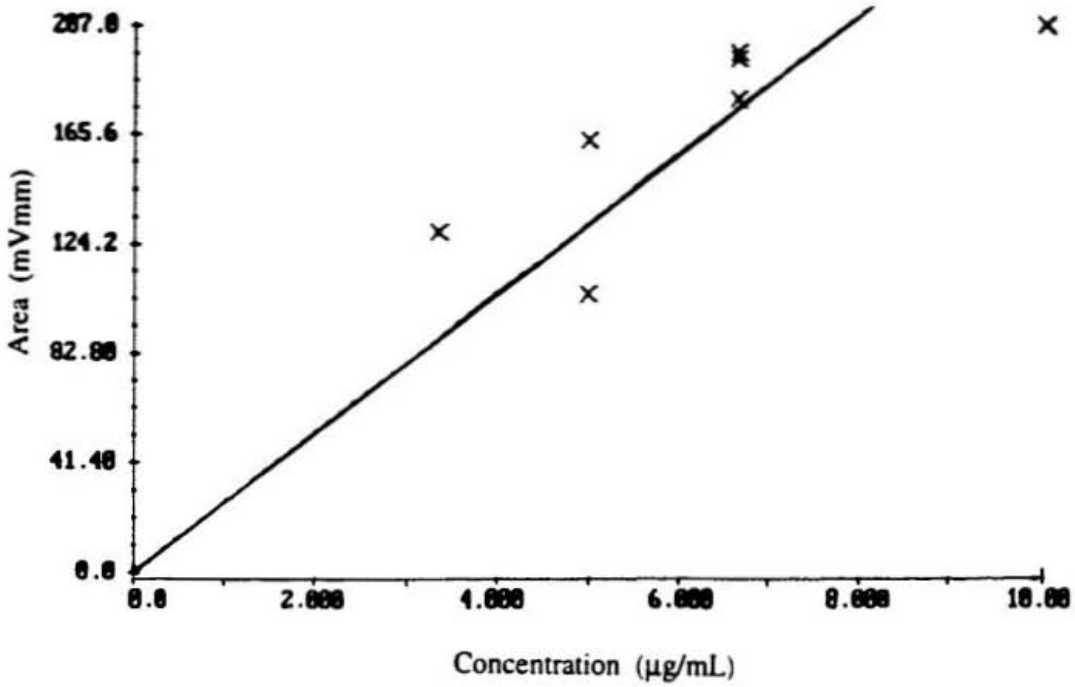

Fig A.9 Standard line for area under each peak at different concentrations of phosphatidylserine (PS).

Regression coefficient = 0.81
Rf = 36.2

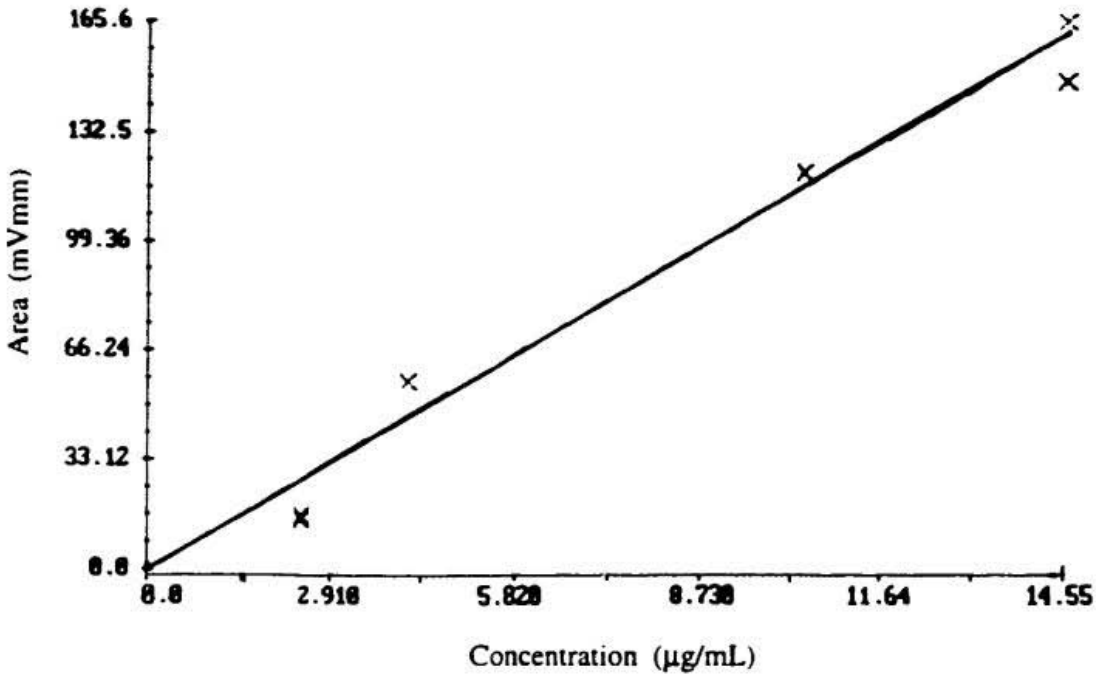

Fig A.10 Standard line for area under each peak at different concentrations of phosphatidylinositol (PI).

Regression coefficient = 0.98
Rf = 37.7

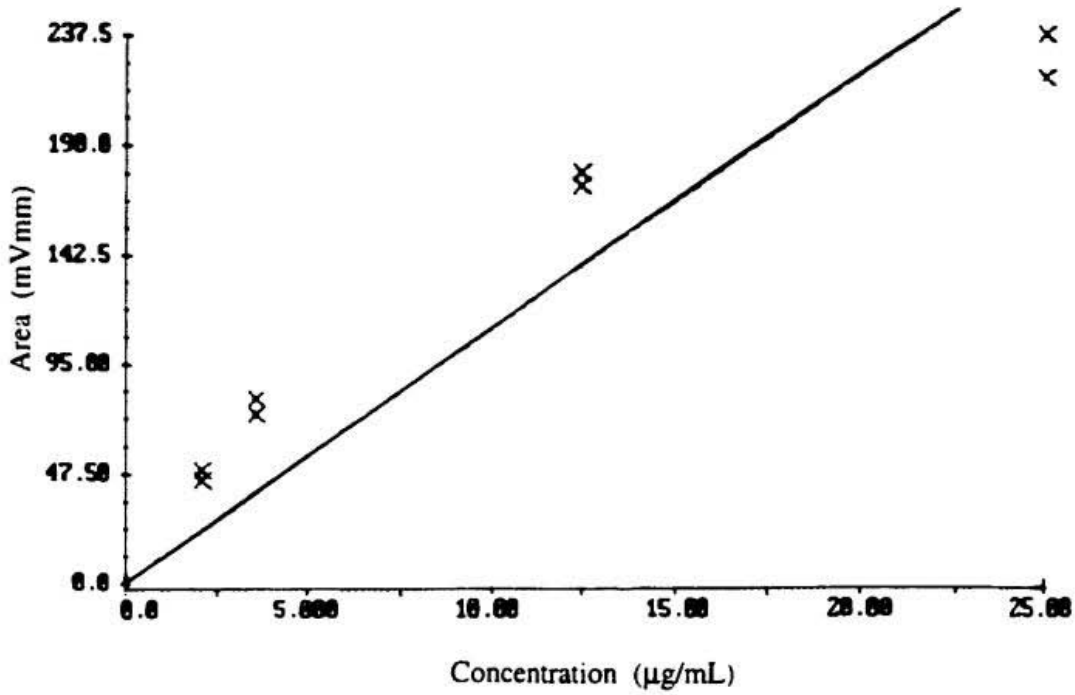

Fig A.11 Standard line for area under each peak at different concentrations of phosphatidylcholine (PC).

Regression coefficient = 0.95
Rf = 70.9

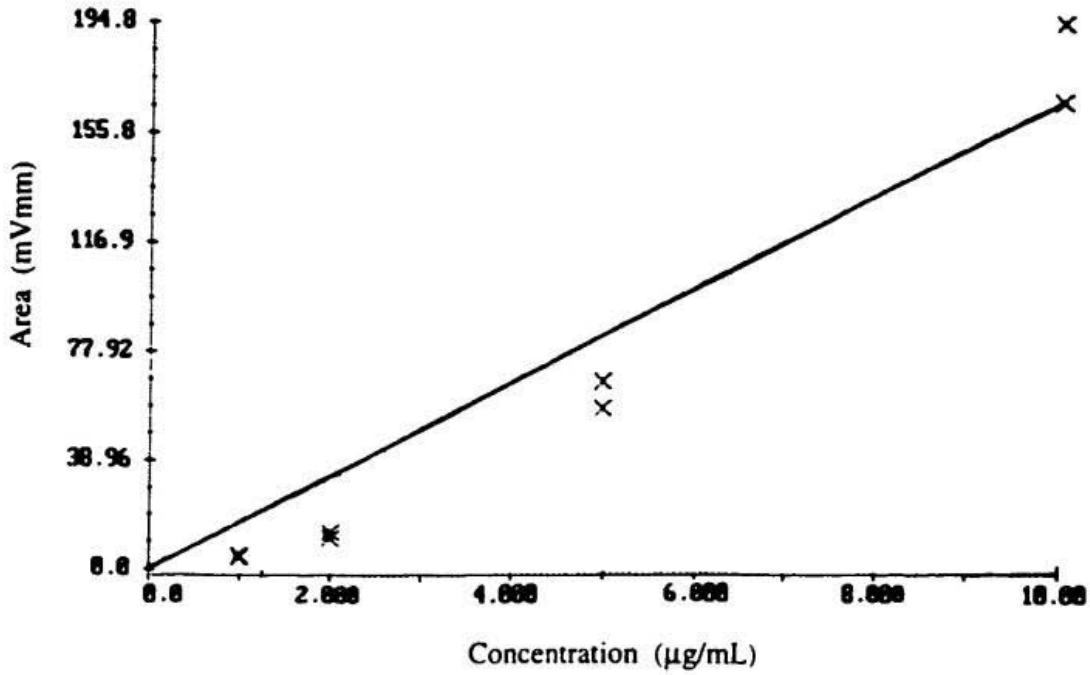

Fig A.12 Standard line for area under each peak at different concentrations of phosphatidylethanolamine (PE).

Regression coefficient = 0.99
Rf = 43.5

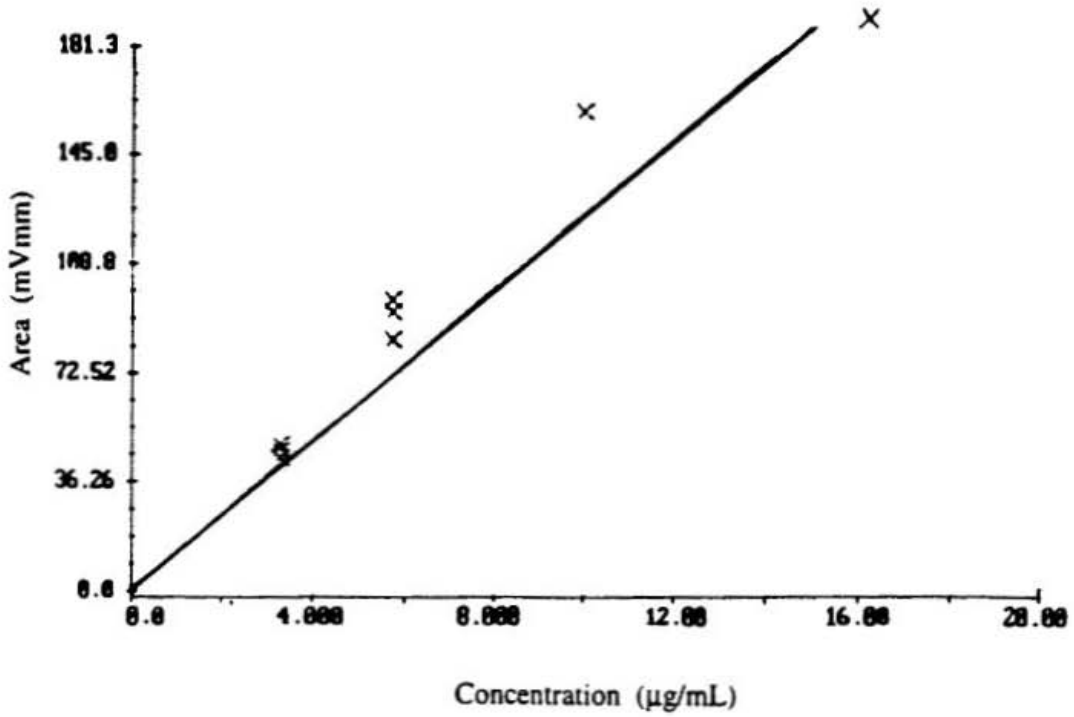

Fig A.13 Standard line for area under each peak at different concentrations of phosphatidic acid (PA).

Regression coefficient = 0.89
Rf = 21.4

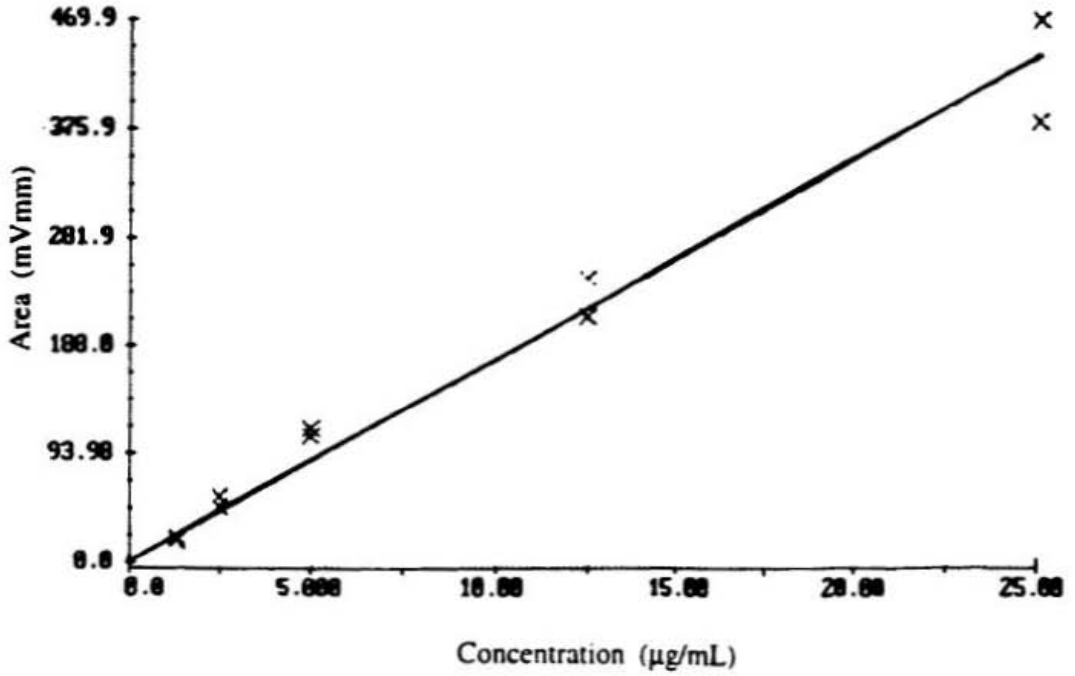

Fig A.14 Standard line for area under each peak at different concentrations of lysophatidylcholine (LPC).

Regression coefficient = 0.99
Rf = 94.8

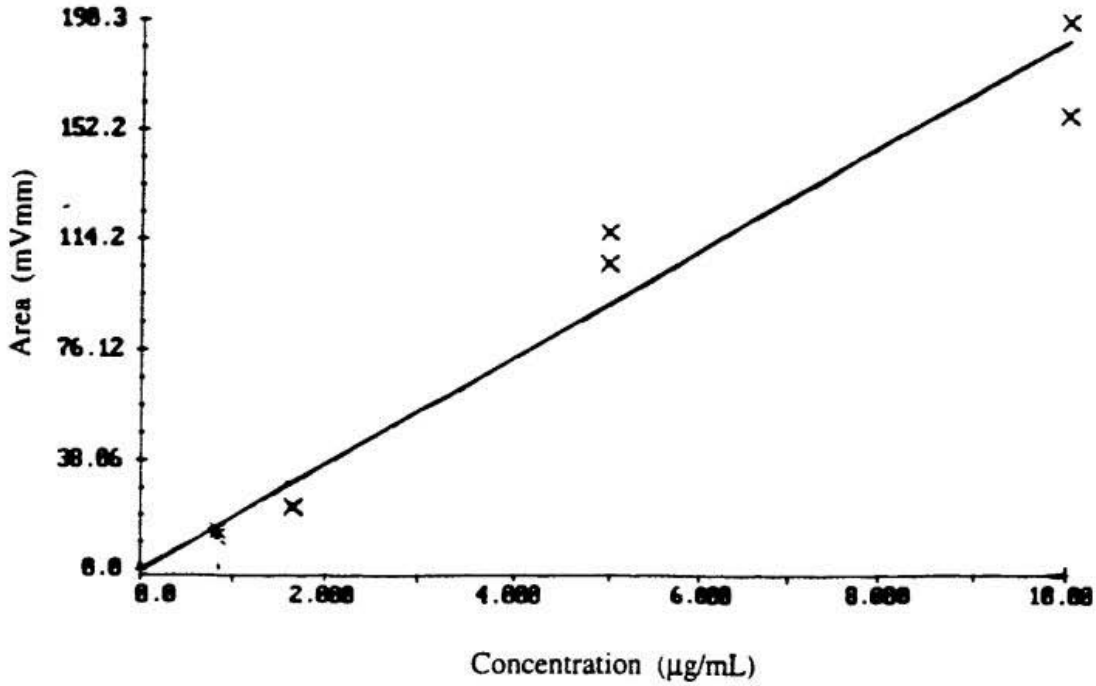

Fig A.15 Standard line for area under each peak at different concentrations of lysophatidylethanolamine (LPE).

Regression coefficient = 0.98
Rf = 51.4

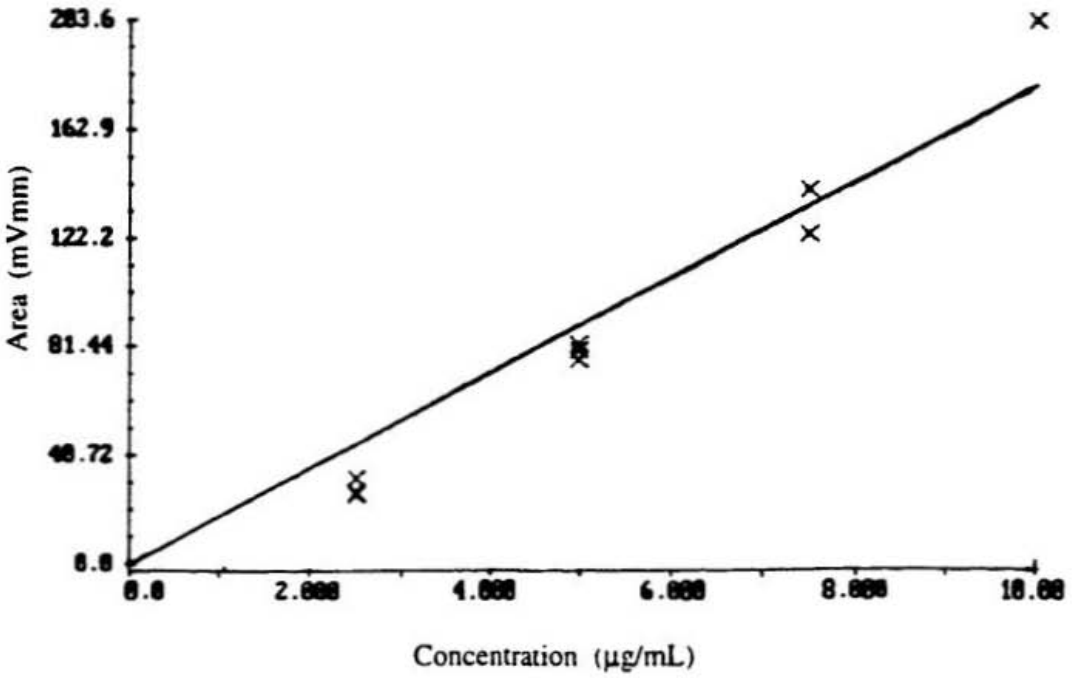

Fig A.16 Standard line for area under each peak at different concentrations of sphingomyelin (SM).

Regression coefficient = 0.99
Rf = 95.8

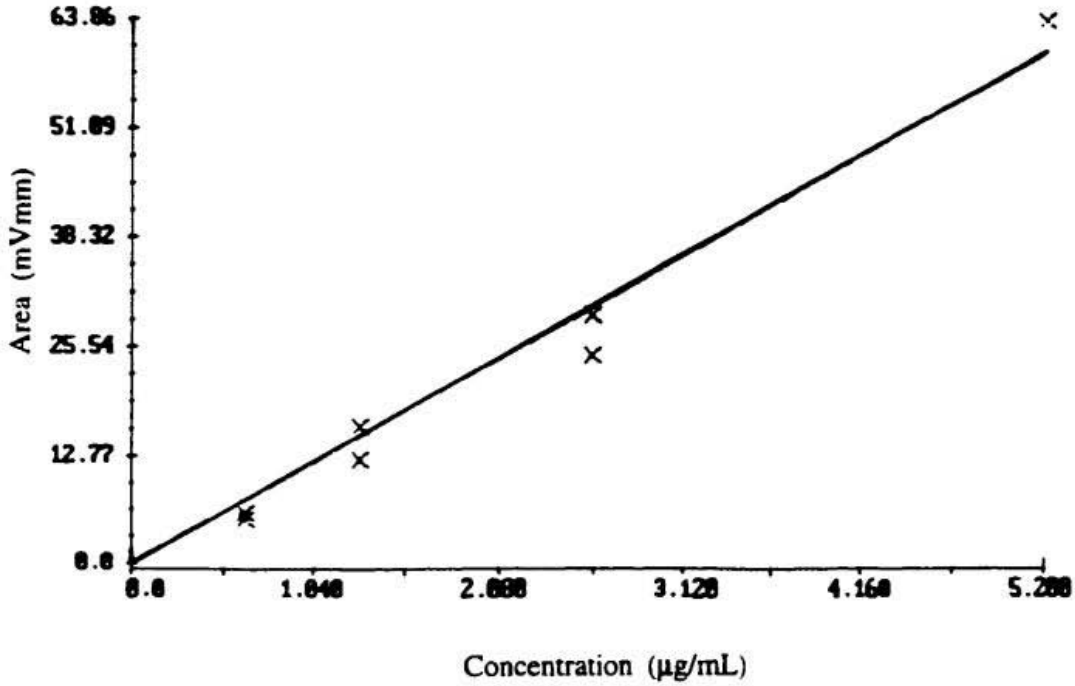

Fig A.17 Standard line for area under each peak at different concentrations of cardiolipin (CDL).

Regression coefficient = 0.99
Rf = 15.4