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The effect of an artificial diet on the biochemical composition of the gonads of the sea urchin (*Strongylocentrotus droebachiensis*)

Chandrika Liyana-Pathirana[a], Fereidoon Shahidi[a,b,*], Alan Whittick[a]

[a]*Department of Biology, Memorial University of Newfoundland, St. John's, NF, Canada A1B 3X9*
[b]*Department of Biochemistry, Memorial University of Newfoundland, St. John's, NF Canada A1B 3X9*

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Abstract

Green sea urchins (*Strongylocentrotus droebachiensis*), taken from the wild were fed on a grain-based artificial diet over a 9-week period. At intervals, the animals were harvested and the gonads analysed for proximate composition, lipid class distribution, fatty acid and carotenoid pigment compositions. During the feeding period the lipid content decreased and the moisture content increased. Meanwhile, the protein content was highest after 9 weeks of feeding on the artificial diet. Major non-polar lipid classes were triacylglycerol (TAG), free fatty acids (FFA) and sterols (ST) while dominant polar lipid classes were phosphatidylcholine (PC) and phosphatidylethanolamine (PE). The content of PC was much higher than that of PE on week 0. However, PE became dominant on week 9 after feeding on the artificial diet. The fatty acids 14:0 and 16:0 were the major saturated fatty acids (SFA), consistently present in the total, non-polar and polar lipids of sea urchin gonads. Polyunsaturated fatty acid (PUFA) 20:5n-3 (eicosapentaenoic acid, EPA) was the highest on week 0 while 18:2 n-6 became dominant with increased feeding period with a concurrent decrease in the content of 20:5n-3. Further, 20:1n-15 was the major monounsaturated fatty acid (MUFA) at week 0, but its content decreased significantly ($P<0.05$) after introducing the artificial diet. The total carotenoid content decreased by 50% at the end of the feeding period. Major carotenoids present in sea urchin gonads were echininone and β-carotene. The relative content of echininone was lowest at week 0, but increased when the urchins were fed on the artificial diet with a simultaneous reduction in β-carotene levels. The study demonstrates the importance of feed supply in lipid composition of sea urchin gonads.

Keywords: Artificial diet; Carotenoids; Fatty acids; Green sea urchins; Proximate composition

1. Introduction

Sea urchin gonads, also known as roe or uni, are a highly valued seafood commodity and are considered as delicacies in many parts of the world. Sea urchins are prized for their yellow gonad sacs which have a caviar-like appearance and a bitter-sweet flavour (Robbins & McKeever, 1990).

The most common sea urchin, which is widely distributed in Newfoundland waters, is the green sea urchin *Strongylocentrotus droebachiensis*. However, despite their abundance, green sea urchins remain underutilized. Sea urchin gonads are harvested in many parts of the world, but used primarily in Japan and marketed as both live and processed (Hooper & Cuthbert, 1994). Since there is an increasing demand for sea urchin gonads in Japan and elsewhere in the world, the development of a sustainable sea urchin fishery in Newfoundland may be economically feasible (Greenland, 1999). However, because of harsh climatic conditions and challenges arising from the low yield and quality of wild populations, an industry based on green sea urchin fishery has not yet been fully developed (Greenland, 1999). Thus, research on developing methods to culture sea urchins in order to increase their gonadal yield and quality could offer opportunities to Newfoundland coastal communities.

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

* Corresponding author. Tel.: +1-709-737-8552; fax: +1-709-737-4000.
E-mail address: fshahidi@mun.ca (F. Shahidi).

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gonadosomatic index, yield and quality of sea urchin gonads (Butt, 1992). The preferred diet of sea urchins is the naturally occurring algal species, mainly *Laminaria* kelp (Lewis, 1999). Hence, an economical artificial feed that matches the nutrition of wild kelp is required to fully develop the sea urchin aquaculture (Lewis, 1999). Since, green sea urchins are the dominant sublittoral herbivores along much of the Newfoundland coasts (Keats, Steel, & South, 1983), there is a great potential to develop a suitable industry to address the continuing market demands (Dooley, 1994).

The use of fresh algae is not always possible or profitable on a large scale. Several studies have been performed to investigate the effects of formulated diets on gonadal growth (de Jong-Westman, March, & Carefoot, 1995; de Jong-Westman, Qian, March, & Carefoot, 1995; Goebel & Barker, 1998; Lawrence, Olave, Otaiza, Lawrence, & Bustos, 1997). Sea urchins exhibited an increase in growth compared with those on natural feeds (Lawrence et al., 1992; Fernandez & Caltagirone, 1990; Fernandez & Pergent), 1998. Thus, encouraging results have emerged so far, especially in terms of gonadosomatic index. However, results have been unsatisfactory with respect to the colour and palatability of the gonads (Grosjean, Spirilet, Gosselin, Vaitilingon, & Jangoux, 1998).

The purpose of this study was to examine the effects of an artificial diet on the proximate composition, lipid class distribution, fatty acid composition and carotenoid pigments of the gonads of the Newfoundland green sea urchin *Strongylocentrotus droebachiensis*. Thus, the effects of a grain-based diet over a 9-week period were evaluated.

2. Materials and methods

2.1. Materials

Sea urchins were collected at a depth of 2 m at Bonavista Bay, Newfoundland and were cultured in the Sea Urchin Research Facility (SURF) at the same area. Urchins were cultured in raceways using sea water to maintain salinity level. The temperature in the facility was adjusted to simulate temperature effects in nature. Initially, urchins were fed a *Laminaria* kelp diet for three weeks. After three weeks of algal feeding, urchins were harvested for analysis and this was considered as the control (week 0) harvest. Thereafter, a grain-based feed formulation (Table 1) was introduced and feeding was continued for 9 weeks. Urchins were harvested for analysis after 3, 6, and 9 weeks during the experimental feeding on the artificial diet. Fifty sea urchins were procured from each of the three batches of sea urchins. Sea urchins were transported in aquarium coolers and stored at 4 °C prior to the removal of gonads. The wet mass and test diameter across the widest portion were recorded for 25 animals of similar size from each batch before extraction of gonads. (Three different sub samples were drawn from each batch of urchins for analysis.) Sea urchins were cracked into two halves using a specially devised sea urchin cracking tool. The shells were left for a few minutes to drain the coelomic fluid and gonads were scooped out and blotted to dryness on an adsorbent towelling for a few minutes before recording their masses. The gonadosomatic index of sea urchins was calculated as the ratio of gonad mass to the wet mass of the whole body and recorded as a percentage (Table 2). Sea urchin gonads were homogenized for 2 min using a cooled Waring blender (Dynamics Corporation, New Hartford, CT). In commercial perspectives sea urchin gonad is considered as a nutrient storage tissue rather than a reproductive tissue. Since male and female gonads are not differentiated during marketing, male and female gonads were pooled together for analysis in our study. The tissues were flushed with liquid nitrogen and stored at −20 °C until used for further analyses. All chemicals used were obtained from either Fisher Scientific (Fair Lawn, NJ) or Sigma Chemical Co. (St Louis, MO). The solvents used were of ACS-, pesticide- or HPLC-grade.

Table 1
Composition of the grain-based sea urchin feed formulation

Constituent	Content (%)
Soy meal	19.6
Wheat middling	19.6
Barley	19.6
Corn meal	19.6
Laminaria longicruris	10.0
Gelatin	5.0
Lecithin	5.0
Sodium alginate	1.5

2.2. Determination of proximate composition

The moisture and ash contents of sea urchin gonads were determined according to the standard procedures of AOAC (1990). Crude protein content was evaluated

Table 2
Gonadosomatic indices of cultured sea urchins at different harvest levels

Harvest level	Average diameter (cm)	Average weight (g)	Gonadosomatic index (%)
Week 0	3.3±0.3a	43.7±3.9a	9.1±2.1a
Week 3	3.4±0.1a	45.0±2.7a	11.7±2.7a
Week 6	3.3±0.2a	44.3±2.1a	15.7±1.9ab
Week 9	3.2±0.1a	43.3±2.0a	21.3±1.8b

Results are mean values of 25 animals±standard deviation. Values in a column with the same letter are not different ($P<0.05$) from one another.

by Kjeldhal method (AOAC, 1990) while total lipids were extracted and quantified by the method of Bligh and Dyer (1959). Carbohydrate content of each sample was determined by difference.

2.3. Separation of non-polar and polar lipid classes by preparative thin layer chromatography (TLC)

Crude lipids, extracted according to the Bligh and Dyer (1959) procedure, 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 (20×20 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 both the simple and complex lipids. The silica gel was subsequently scraped off and the lipids were dissolved in a 1–2% methanol in chloroform solution for non-polar lipids while polar lipids were dissolved in chloroform/methanol/water (5:5:1, v/v/v).

2.4. Analysis of lipid classes by Iatroscan

2.4.1. Instrumentation

The crude lipids obtained from the Bligh and Dyer (1959) extraction were chromatographed on silica gel-coated Chromarods-S III and then analysed on an Iatroscan MK-5 (Iatroscan Laboratories Inc., Tokyo, Japan) analyser 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 2000 ml per min were used in operating the FID. The scanning speed of rods was 30 s per rod.

2.4.2. 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 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.

2.4.3. Standards and calibration

A stock solution of each of the non-polar lipids, namely 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, namely phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylinositol (PI), phosphatidylserine (PS), lysophosphatidylcholine (LPC), lysophosphatidylethanolamine (LPE) cardiolipin (CL) and sphingomyelin (SM), was prepared by dissolving them in a chloroform/methanol (2:1, v/v) solution and stored at −20 °C. A range of dilutions of the stock solution, from 0.1 to 10 μg per μl, was prepared for use as working standards. Each compound was developed individually and run on the Iatroscan-FID to determine its purity and R_f values. For each compound, peak area was plotted against a series of known concentrations to obtain the calibration curve.

2.4.4. Iatroscan (TLC–FID) analysis of sea urchin lipids

The total lipids extracted 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. The solvent system hexane/diethyl ether/acetic acid (80:20:2, v/v/v) was used for separation of non-polar lipids (Christie, 1982). Following their development, Chromarods were dried at 110 °C for 3 min and scanned completely to reveal non-polar lipids. For polar lipids the Chromarods were first developed in the same solvent system as 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 of chloroform/methanol/water (80:35:2, v/v/v) for the separation of polar lipid classes (Christie, 1982). 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 obtained for each authentic standard.

2.5. Analysis of fatty acid composition of lipids

The fatty acid composition of lipids was determined using gas chromatography (GC), as described by Wanasundara and Shahidi (1997). The fatty acid methyl esters (FAMEs) of total lipids and polar and non-polar lipids 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 mol% HPLC-grade methanol containing 15 mg of t-butylhydroquinone (TBHQ)] at 65 °C for 15 h in 6 ml Teflon-lined screw-capped conical vials. 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. 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 into 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, Elysian, MN) and literature values (Takagi, Eaton, & Ackman, 1980; Takagi, Kaneniwa, & Itabashi, 1982). The relative content of fatty acids in the sample was determined using the peak areas of fatty acids.

2.6. Carotenoid pigmentation

2.6.1. Extraction and determination of total and individual carotenoids

Carotenoids from each gonad 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 × *g*. The supernatant was subsequently filtered through a Whatman No. 1 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 a 0.5% sodium chloride solution were added to the mixture to maximize 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 400–600 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.

$$\text{mg Carotenoid per 100 g tissue} = \left(A \times V \times 10^3\right)/(\varepsilon \times W)$$

where, A = absorbance at λ_{max}; V = total volume of the sample (ml); ε = molar 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 extracted was separated into individual carotenoids by means of TLC. The crude carotenoids were separated by preparative TLC on silica gel G (20×20 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 off individually from the plate and dissolved in 1–5% methanol in hexane. The visible absorption spectrum for each fraction was obtained and relative percentages of each fraction calculated from the following equation.

$$\text{Percentage of carotenoids} = \left\{100 \times (V \times A)_{\text{for each fraction}}\right\}/\left\{\varepsilon \times (V \times A)_{\text{ for all fractions}}\right\}$$

2.7. 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 (20×20 cm, 250 μm, Aldrich Chemical Co., 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 (Britton, 1995; Fox & Hopkins, 1966; Goodwin, 1955; Krinsky & Goldsmith, 1960).

2.8. Statistical analysis

The significance of differences among mean values was determined at 5% probability level using analysis of variance (ANOVA), followed by Tukey's multiple range test.

3. Results

3.1. Proximate composition

The artificial diet had a significant effect on the proximate composition of sea urchin gonads (Table 3). The relative moisture content was increased significantly ($P < 0.05$) while this paralleled a significant ($P < 0.05$)

decrease in the total lipids by the end of the 9-week feeding period. Ash and protein levels were also affected slightly after feeding on the artificial diet. However, the effect on carbohydrate content was insignificant ($P > 0.05$). It appears that the composition of the artificial feed (Table 4) had a direct influence on the compositional characteristics of the gonads.

3.2. Identification and quantification of lipid classes

Major non-polar lipid classes were TAG, FFA and sterols ST; (Table 5) while major polar lipid classes included PC and PE (Table 6). In addition, gonadal lipids contained a considerable amount of SM/LPC. Triacylglycerols contributed the highest proportion of the non-polar lipids in sea urchin gonads. Free fatty acids were the second predominating group that contributed to non-polar lipids.

A high level of PC was present in sea urchin gonads prior to feeding on the artificial diet. However, PC content decreased with progression of feeding on the grain-based diet. The PE content, on the other hand, increased significantly ($P < 0.05$) from week 0 to week 9. Thus, the trends for changes in PC and PE contents in sea urchin gonads changed in opposite directions.

Table 3
Proximate composition (wt.%) 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)bc	1.0 (0.02)bc	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)bc	4.5 (0.1)ab	3.8 (0.2)c
Carbohydrate[a]	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 three replicates ± standard deviation.
Values in each row with the same letter are not different ($P > 0.05$) from one another.
[a] Determined by difference.

Table 4
Proximate composition (wt.%) 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.

3.3. Dietary effects on the fatty acid composition

Fatty acid profiles of sea urchin total, non-polar and polar lipids are shown in Tables 7, 8 and 9, respectively. The major saturated fatty acids (SFA) of sea urchin total lipids were 14:0, 16:0 and 20:0. On the other hand, 14:0, 16:0 and 18:0 were the major SFA of both polar and non-polar lipids (the fatty acids that contributed more than 2% to the total fatty acid profile were considered as the major ones). The fatty acid 20:1 n-15 was the dominant monosaturated fatty acid (MUFA) on week 0 while 16:1n-9 and 18:1n-9 became dominant in total and non-polar lipids, respectively, after feeding of sea urchins on the artificial feed. However, 20:1n-15 remained dominant in polar lipids, even after introducing the artificial feed, but its content was significantly ($P < 0.05$) decreased.

The polyunsaturated fatty acids (PUFA) that contributed more than 2% to the total lipids during the entire feeding period were 20:4 n-6 (arachidonic acid) and 20:5 n-3 (eicosapentaenoic acid, EPA). However, the fatty acid 18:2 n-6 (linoleic acid, LA) became the dominant fatty acid at the end of the experimental feeding although its relative content was less than 2% on week 0. Thus, LA and EPA showed opposite

Table 5
Quantification of non polar lipids (wt.%) 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	76.7 (0.8)a	79.1 (0.9)b	84.6 (0.8)c	87.4 (0.8)c
FFA	12.7 (1.1)a	17.3 (1.0)b	10.3 (0.9)a	5.2 (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 three replicates (standard deviation).
Values in each row with the same letter are not different ($P > 0.05$) from one another. Abbreviations are: TAG, triacylglycerol; FFA, free fatty acids; ST, sterol; MAG, monoacylglycerol; DAG, diacylglycerol; ND, not detected; and Tr, trace.

Table 6
Quantification of polar lipids (wt.%) 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 three replicates ± standard deviation.
Values in each row with the same letter are not different ($P > 0.05$) from one another. Abbreviations are: PC, phosphatidylcholine; PE, phosphatidylethanolamine; PS, phosphatidylserine; PI, phosphatidylinositol; SM, sphingomyelin; LPC, lysophosphatidycholine; and Tr, trace.

patterns of variation with progression of feeding period (Fig. 1). With increased length of feeding the content of EPA decreased significantly ($P<0.05$) in both polar and non-polar lipids, while the content of LA increased significantly ($P<0.05$). In non-polar lipids 18:2 n-6 was the dominant fatty acid since the first harvesting level after introducing the diet. Moreover, there was a predominance of total content of PUFA in all total, polar and non-polar lipids of sea urchin gonads (Fig. 2).

3.4. Carotenoid pigments

3.4.1. Contents

The total carotenoid contents (on a dry weight basis) of green sea urchin gonads are shown in Table 10. Total carotenoid content was significantly ($P<0.05$) decreased as feeding on the artificial diet progressed. Hence, the total carotenoid content decreased from 23.2 ± 0.04 on week 0 to 9.2 ± 0.10 mg per 100 g tissue on week 9. The total content of carotenoids in the feed was 0.38 mg per 100 g.

Table 7
Fatty acid composition (wt.%) 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
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
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
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

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

Table 8
Fatty acid composition (wt.%) 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
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
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
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

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

3.4.2. Fractionation of individual pigments

Crude pigments were separated by TLC into seven or eight individual fractions with two major bands. In gonads, fractions I ($R_f = 0.96$) and II ($R_f = 0.88$) ran close to the solvent front, but were adequately separated from one another.

3.4.3. Characterization of individual pigments

The carotenoid present in fraction I of sea urchin gonads was β-carotene, using an authentic β-carotene sample as established by co-chromatography on silica gel TLC plates. Similarly, fractions III, IV, VI, and VII were representative of astaxanthin monoester ($R_f = 0.57$), zeaxanthin ($R_f = 0.51$), canthaxanthin ($R_f = 0.22$) and free astaxanthin (0.1), respectively. Furthermore, fraction II of sea urchin gonads corresponded to echininone, as determined by means of absorption maxima in hexane, chloroform and ethanol (Britton, 1995; Fox & Hopkins, 1966; Goodwin, 1955; Krinsky & Goldsmith, 1960). Thus, the observed λ_{max} were 484/460, 466 and 475 nm in hexane, ethanol and chloroform, respectively.

3.4.4. Quantification of individual pigments

The relative content of echininone and β-carotene in sea urchin gonads varied significantly ($P < 0.05$)

Table 9
Fatty acid composition (wt.%) 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
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 (0.1)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
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
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

Results are mean values of three replicates ± standard deviation.

Values in each row with the same letter are not significantly different ($P > 0.05$) from one another.

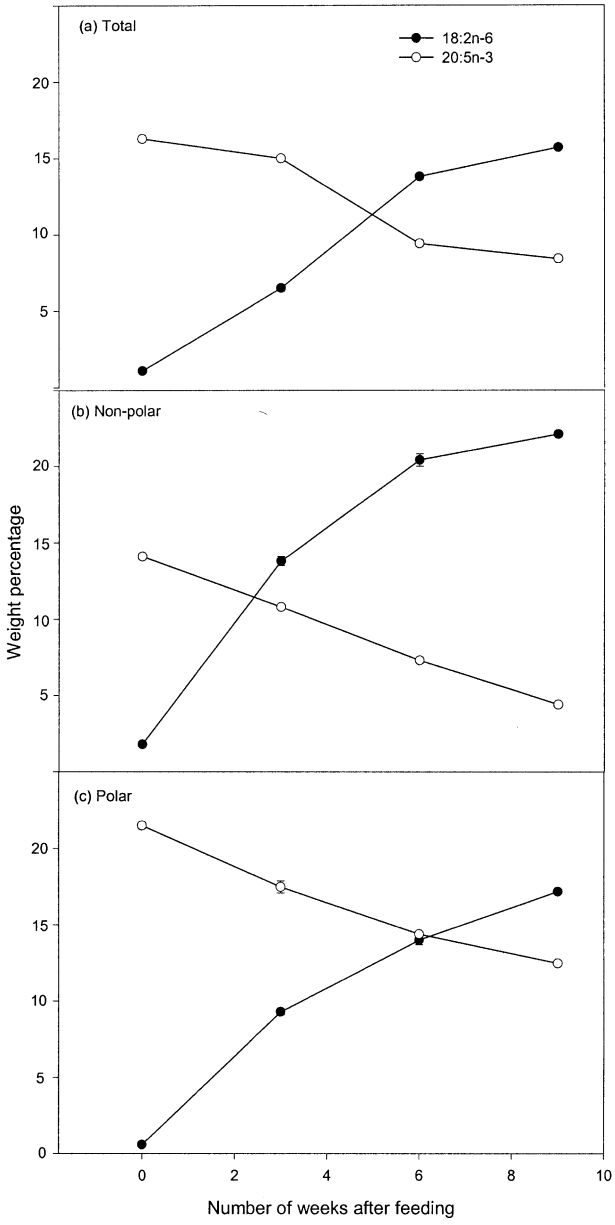

Fig 1. Effect of grain-based artificial diet on contents of 18:2n-6 and 20:5n-3 of (a) total (b) non-polar and (c) polar lipids of sea urchin gonads.

after introducing the artificial feed. The carotenoid pigment echininone remained dominant throughout the study period. On week 0 echininone content was approximately 64±5% while β-carotene content was approximately 24±4%. On the other hand when urchins were fed on the artificial diet the echininone content was increased significantly ($P<0.05$) and constituted approximately 76±6, 79±7 and 84±5% of the total on weeks 3, 6 and 9, respectively.

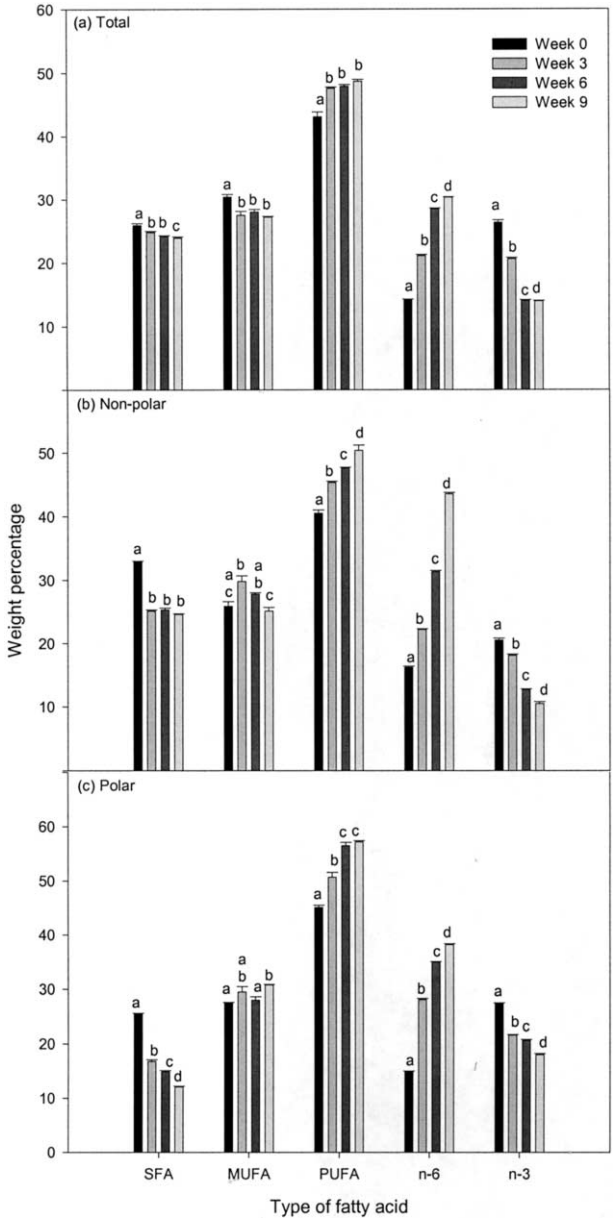

Fig 2. Content of saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA), n-3 (omega-3) and n-6 (omaega-6) fatty acids of (a) total (b) non-polar and (c) polar lipids of sea urchin gonads.

4. Discussion

4.1. Proximate composition

There are relatively few research reports available on the effects of artificial diet on the biochemical composition of sea urchins. Shearer (1994) reported that diet affects the proximate composition of cultured fish. Similarly, the present study revealed that biochemical composition of sea urchins was significantly affected by the diet. An increase in moisture level with a simultaneous decrease in lipid content, was observed in cultured sea urchin gonads. In fish, an inverse relationship existed in the relative amounts of lipid and moisture (Shearer, 1994). The grain-based feed used in this study contained soybean as one of its main components. In general, soybean meal is used extensively in feeds for cultivation of fish on a commercial scale (Storebakken, Refstie, & Ruyter, 2000). Cook, Kelly, and McKenzie (1998) reported that the gonadal growth is very high when urchins are fed on protein- and lipid-rich artificial feeds compared to a macroalgal diet resembling urchin's preferred natural diet. Another study, on the sea urchin *Paracentrotus lividus*, suggested that diets rich in proteins induce high gonad production (Fernandez, Dombrowski, & Caltagirone, 1995).

Agatsuma (1998) showed that sea urchins fed on an artificial diet containing fish meal had increased moisture levels in gonads. On the other hand, Nishikiori (1989) observed that the moisture content in the gonads of *Strongylocentrotus nudus* fed *Laminaria japonica* was below 70% when urchins were fed to satiation. In this experiment, sea urchins were fed on *Laminaria* sp. for a 3-week period and had the least amount of moisture at the end, which corresponds to the value at week 0. The biochemical composition of gonads of urchins fed on protein-and algal-based diets were different. Thus, in this study the moisture content in sea urchin gonads increased significantly upon feeding on a grain-based diet.

Table 10
Total carotenoid content (mg per 100 g) in sea urchin gonads and viscera for wild and cultured sea urchins

Harvest time	Gonads
Week 0	23.2 (0.04)a
Week 3	20.1 (0.2)b
Week 6	11.0 (0.2)c
Week 9	9.2 (0.1)d

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

4.2. Lipid class composition

Wax esters have been reported to constitute energy reserves in various marine invertebrates (Benson & Lee, 1972; Lee, Nevenzel, & Paffenhoffer, 1971; Sargent, 1976). In sea urchin *S. droebachiensis,* in our study, the TAG served as the main energy reserves and no wax esters were present. This was the same when both viscera and gonads of *S. droebachiensis* collected from Nova Scotia were examined (Takagi et al., 1980). The non-polar lipids of sea urchins fed on the artificial feed consisted mainly of TAG, FFA and ST. Triacylglycerols are usually considered to be storage products rather than membrane lipids in eukaryotic cells (Sul, Kaneshiro, Jayasimhulu, & Erwin, 2000). Thus, sea urchin lipids contained much larger amounts of storage lipids, principally TAG, which constituted more than 75% of the total non-polar lipids of gonads of cultured sea urchins. In cultured sea urchins the relative content of TAG increased significantly at the end of the feeding period. On the other hand, the relative content of FFA decreased and reached a minimum after 9 weeks of feeding. The artificial diet brought about a significant effect, quantitatively, on individual non-polar lipid classes, but with insignificant qualitative effects.

Sea urchin lipids contained a considerable amount of FFA at certain harvest levels 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 (Farkas, 1979). Also, diet can be a specified factor, having a major impact on fatty acid composition land metabolism (Farkas, Csengeri, Majoros, & Olah, 1978). Moreover, sterol level may depend on dietary evel and stage of sexual development (Love, 1970). Therefore, diet and nutritional status are known to influence sterol levels in animals (Dave, Johansson-Sjobeck, Larsson, Lewander, & Lidman, 1975; Larsson & Lewander, 1973).

The polar lipid composition was affected considerably by the consumption of the artificial feed. 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. Floreto, Teshima, and Ishikawa (1996) demonstrated that sea urchin, *Tripneustes gratilla*, fed on a seaweed diet, had PC and PE as the major polar lipid constituents and PC was present in a larger proportion than PE. In cultured sea urchins, fed on seaweed diets, the lipid class composition may still be dominated by PC and PE, and similar to that in the present study where PC and PE were dominant in the polar lipids and PC was more prevalent than PE on week 0 when urchins were fed the *Laminaria* that constituted their natural diet.

4.3. Fatty acid composition of sea urchin lipids

The fatty acids of total, non-polar and polar lipids of sea urchin gonads in week 0 were typically similar to those of marine species with a dominance of 16:0 and 20:5n-3 (Wanasundara, 1996). Although, 22:6n-3 is also a typical fatty acid in marine lipids, it contributed only 1–2.5% to the total fatty acid content in *S. droebachiensis*. On the other hand, after introducing the artificial diet, the 18:2n-6 content was significantly increased and finally became dominant in the fatty acid profile of total, non-polar and polar lipids.

The major SFA in sea urchin gonadal lipids were 16:0 and 14:0. This is in accord with the available published data on the fatty acid composition of wild sea urchins (Kaneniwa & Takagi, 1986; Takagi et al., 1980). Thus, diet did not have a significant effect on the two major SFA. On the other hand, 20:1n-15 was the dominant MUFA in sea urchin gonadal lipids on week 0. Takagi et al. (1980) stated that sea urchins may contain 20:1n-15 up to 11% of their fatty acid content. However, Ackman and Hooper (1973) reported that marine animals, such as periwinkle (*Littorina littorea*), moon snail (*Lunata triseriata*) and sand shrimp (*Crangon septemspinosus*), contained 20:1n-15, but at much lower levels, not exceeding 0.2% of the total fatty acid content. Thus, the presence of 20:1n-15 has not been commonly reported as being typical of marine lipids. Hence, the formation of 20:1n-15 in sea urchin gonadal lipids may be biosynthetic in origin since it was not affected by the diet. This is further supported by the fact that 20:1n-15 has not been reported in seaweeds, the natural diet of sea urchins (Ackman & McLachlan, 1977). This fatty acid was also absent in the artificial diet employed in this study.

Floreto et al. (1996) demonstrated that fatty acid profiles of sea urchin tissues reflected that of their diets to some extent. 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 the major contributors in sea urchin tissues, even if these fatty acids were not detected in the diet. Thus, it appears that sea urchins are capable of synthesising these fatty acids from lower fatty acid precursors. 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. The major fatty acids of sea urchin gonadal lipids, namely 16:4n-3, 20:4n-6 and 20:5n-3, are probably known to have structural functions and hence are purposely synthesised by urchins (Floreto et al., 1996).

On week 0, the 20:5n-3 content was quite high in sea urchin gonadal lipids and was present in distinctly higher amounts in the polar lipids which serve as structural parts of membranes (Takagi et al., 1980). These sea urchins consumed the seaweed *Laminaria*, which is known to be high in 16:4n-3, 18:4n-3, 20:4n-6 and

20:5n-3 (Pohl & Zurheide, 1979). It has been shown that, in sea urchins fed on seaweeds 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, mainly 20:5n-3. This may be supported by the results obtained for cultured sea urchin gonadal lipids in the present study. The artificial feed contained approximately 50% of its total fatty acids in the form of 18:2n-6 and very little of 20:5n-3 (Table 11). Over time the content of 18:2n-6 increased while that of 20:5n-3 decreased in the sea urchin gonadal 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 dietary in its origin and was present at less than 2% in week 0. Further, the urchins initially had a high content of 20:4n-6 and 20:5n-3 in their total and non-polar lipids, possibly attributable to their diet, that is comprised of *Laminaria* sp. which is high in 20:4n-6 and 20:5n-3. On the other hand, the 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 membranes instead of 20:5n-3, hence reflecting an inverse relationship between the two types of fatty acids.

Several studies have assessed the effects of algal diets on the growth of sea urchins and some have investigated the effects on biochemical composition of gonads. The present study is probably the first one to assess the effects of a grain-based diet on the lipid and fatty acid composition of sea urchin gonadal lipids.

4.4. Carotenoid pigments

In the sea urchin, *S. droebachiensis,* carotenoids were mainly concentrated in the gonadal tissues. The total carotenoid content varied noticeably after introducing the artificial diet and its total content was reduced significantly with increasing feeding period. In fact, the total carotenoid level was almost halved in the gonads by the end of the 9 weeks of feeding. The total carotenoid content of the artificial feed used in our study was 0.38 mg per 100 g on a dry weight basis. In general, animals cannot synthesize carotenoids de novo, and are therefore dependent upon dietary carotenoids (Goodwin, 1984). Since the feed provided 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 was characterized as the major carotenoid present in the gonads of cultured sea urchins. In addition, β-carotene was identified in small amounts. Numerous studies have been carried out on carotenoid pigments in wild sea urchins (Griffith, 1966; Griffith & Perrott, 1976; Shina, Gross, & Lifshitz, 1978; Tsushima, Byrne, Amemiya, & Matsuno, 1995), but none has been reported on the effect of artificial diets on carotenoid spectrum in the gonads. Although, the diet had a significant effect on the total content of carotenoids in sea urchin gonads, carotenoids exhibited the same qualitative profile before and after introducing the artificial diet. In our study, the relative content of echininone increased significantly with a simultaneous decrease in β-carotene. Thus, at week 0 there was the lowest amount of echininone and the highest amount of β-carotene, when urchins were fed *Laminaria* sp. which is a good source of carotenoids. However, the artificial diet was deficient in carotenoids and a gradual reduction in carotenoids occurred in the gonads. Tsushima et al. (1995) showed 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 allowing β-echininone to be incorporated into the gonads. Hence, at the end of the 9-week feeding period the content of echininone in sea urchin gonads was significantly increased.

5. Conclusion

The diet used in this study brought about significant changes in all the compositional parameters tested. Thus, the type and quality of the feed greatly affected

Table 11
Fatty acid composition (wt.%) of grain based sea urchin feed

Fatty acid	Total lipids	Polar lipids	Non polar lipids
4:0	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)
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)
20:1 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)
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)

Results are mean values of three replicates ± standard deviation.

the nutrient composition of sea urchin gonads. In fact, gonads serve as a reservoir for the accumulation of nutrients and this is of economical interest to the sea urchin industry. Further, the diet used in this study was deficient in nutritionally important long chain PUFA that are a main component of the urchin's natural diet. Thus, the EPA content in the urchin gonads was gradually decreased by the end of the 9-weeks of feeding. Furthermore, a significant reduction in the carotenoid content in cultured sea urchins occurred after feeding on an artificial diet for 9 weeks. Since the diet used was deficient in carotenoid pigments, it is necessary to supplement the diet with carotenoids. These carotenoids, not only provide a desirable colour to the product, but also protect their lipid constituents against oxidation. Therefore, inclusion of β-carotene or relevant xanthophylls, in the feed of sea urchins is suggested. It is also of prime importance to improve the feed composition of sea urchins in order to obtain faster growth in gonads and without a loss in quality.

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