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COMPARISON OF NUTRIENT COMPOSITION OF GONADS AND COELOMIC FLUID OF GREEN SEA URCHIN *STRONGYLOCENTROTUS DROEBACHIENSIS*

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ABSTRACT The compositional characteristics of sea urchin gonads and coelomic fluid from *Strongylocentrotus droebachiensis* harvested in the coasts of Newfoundland and thereafter reared in an aquaculture facility and fed on a *Laminaria* diet for a 3-week period, were assessed. Evaluations were performed on the basis of proximate composition, lipid class distribution, fatty acid composition, total and free amino acid composition, and contents of nucleic acids and carotenoids. Noticeable changes existed between proximate composition of sea urchin gonads and coelomic fluid. Moisture content was 74.7 ± 0.04 and 96.5 ± 0.03% in gonads and coelomic fluid, respectively. Gonads contained very high levels of lipids, proteins, and carbohydrates; whereas, these were present at very low levels in the coelomic fluid. Major nonpolar lipid classes were triacylglycerols (TAG), free fatty acids (FFA), and sterols (ST) while dominant polar lipid classes were phosphatidylcholine (PC), phosphatidylethanolamine (PE), sphingomyelin/ lysophosphatidylcholine (SM/LPC), and phosphatidylserine / phosphatidylinositol (PS/PI) in both the gonads and the coelomic fluid. Major saturated fatty acids (SFA) were 14:0 and 16:0; whereas, 20:1n-15 was the main monounsaturated fatty acid (MUFA) present. Furthermore, 20:5n-3 (eicosapentaenoic acid, EPA) was the dominant polyunsaturated fatty acid (PUFA) in the gonads and the coelomic fluid. The total amino acid (TAA) and free amino acid (FAA) profiles were dominated by glycine. The total FAA content was much higher in the gonads than in the coelomic fluid. In addition, the total carotenoid content of sea urchin gonads was approximately 6.4 times greater than that of coelomic fluid. Hence, most of the carotenoids were concentrated in the gonadal tissue. Echininone and fucoxanthin were the dominant carotenoids in the gonads and the coelomic fluid, respectively. The content of deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) was much higher in the gonad than in the coelomic fluid, thus indicating greater biomass and protein synthetic activity in the former tissue. The present study demonstrates that sea urchin gonads have much in common with sea urchin coelomic fluid on a qualitative basis. However, there were marked quantitative differences between the two tissues.

KEY WORDS: amino acid composition, carotenoids, fatty acid composition, lipid class distribution, nucleic acids, *Strongylocentrotus droebachiensis*

INTRODUCTION

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. Sea urchins are omnivorous animals that live on the ocean floor, feeding on small crustaceans, fish offal, but mainly seaweed (Smith 1980). Thus, the eating quality of sea urchin gonads is dictated, to a certain degree, by the quality of kelp consumed. *Laminaria* kelps are the preferred source of feed for sea urchins. Kramer and Nordin (1979) reported that the green sea urchin *Strongylocentrotus droebachiensis* produces high-quality gonads when the availability of fresh kelp is adequate. The edible green sea urchin *S. droebachiensis* is abundantly distributed in the North Atlantic, Arctic, and North Pacific Oceans, but this species is currently exploited to a much lesser extent in the Northwest Atlantic and in the Northeast Pacific, and Northeast Atlantic (Walker & Lesser 1998). Furthermore, *S. droebachiensis* is a target species for the development of commercial echiniculture (Hagen 1996).

The edible portions of the sea urchin body are its reproductive organs; ovaries, and testes. Gonad yield from sea urchin may vary with the time and the site of harvest and generally ranges from 8–20% of the total body mass. When sea urchins are processed for gonads, the initial step is to break the shell and open it so that the five gonad sacs are exposed. The cracked shells are then allowed to drain for several minutes to dispose of coelomic fluid. Thus, during extraction of sea urchin gonads, large amounts of coelomic fluid are obtained. So far, there are no effective means of using sea urchin coelomic fluid in a useful manner. Furthermore, no information is available on the nutrient composition of sea urchin coelomic fluid. In fact, knowledge of nutrient composition may be useful to determine whether sea urchin coelomic fluid could serve as a potential source of a flavoring in fabricated seafood.

The objective of this study was to assess the nutrient composition of sea urchin coelomic fluid as compared with that of the gonads. Thus, proximate composition, lipid class distribution, fatty acid composition, amino acid composition, and contents of carotenoids and nucleic acids of gonads and coelomic fluid were determined. This may lead to potential commercial utilization of the processing byproducts from sea urchins, which would otherwise be discarded.

MATERIALS AND METHODS

Materials

One hundred twenty-five sea urchins were procured from the Sea Urchin Research Facility (SURF) at Bonavista Bay, Newfoundland and subsequently transported in aquarium coolers to our laboratory at Memorial University of Newfoundland. Urchins were captured from the wild (June 2000) and raised in raceways feeding on a *Laminaria* diet. Urchins were harvested for analysis after three weeks of feeding on a purely algal diet. Live urchins were stored at 4°C before the extraction of tissues. The gonads and coelomic fluid of sea urchins were separated after breaking the shell, using a specially devised sea urchin cracking tool. After extraction, sea urchin gonads were homogenized for 2 min using a cooled Waring blender (Dynamics Corporation, New Hartford, CT), and coelomic fluid was used for analysis as it is. In this study,

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sea urchin male and female gonads were pooled together for analysis. The tissues (both gonads and coelomic fluid) 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 were of ACS-, pesticide-, or HPLC-grade.

Determination of Proximate Composition

Moisture and ash contents of sea urchin tissues were determined according to the standard AOAC (1990) procedures. Crude protein content was obtained by Kjeldhal method (AOAC 1990), and total lipids were extracted and quantified by the Bligh and Dyer (1959) procedure. Carbohydrate content of each sample was determined by difference.

Analysis of Lipid Classes by Iatroscan

Instrumentation

The crude lipids obtained from Bligh and Dyer (1959) extraction were chromatographed on silica gel-coated Chromarods - S III and then analyzed using an Iatroscan MK-5 (Iatroscan Laboratories Inc., Tokyo) 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 airflow rate of 2,000 mL per min were used in operating the FID. The scanning speed of rods was 30 sec per rod.

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 five minutes to improve separation. Finally, the cleaned Chromarods were scanned twice to burn any remaining impurities.

Standards and Calibration

A stock solution of each of the nonpolar 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 each 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 Rf value. For each compound peak, area was plotted against a series of known concentrations to obtain the calibration curve.

Iatroscan (TLC-FID) Analysis of Sea Urchin Lipids

The total lipids extracted were dissolved in chloroform/methanol (2:1, v/v) 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 nonpolar lipids (Christie 1982). Following their development, Chromarods were dried at 110°C for three minutes and scanned completely to reveal nonpolar lipids. For polar lipids, following the same procedure and drying, the Chromarods were scanned partially to a point just beyond the MAG peak to burn the nonpolar 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) followed by drying at 110°C for three minutes. Finally, the Chromarods were 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.

Analysis of Fatty Acid Composition of Lipids

Fatty acid composition of lipids was determined using gas chromatography (GC) as described by Wanasundara and Shahidi (1997). Fatty acid methyl esters (FAMEs) of total lipids of sea urchin gonads and coelomic fluid were prepared by transmethylating approximately 10 to 20 mg of each lipid sample in 2 mL of freshly prepared transmethylating reagent [6% (v/v) sulfuric acid in 99.9 mol% 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 vials. After incubation, the mixture was cooled, and 1 mL of distilled water was 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 before 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 after the first wash, while the hexane layer was removed and placed in a GC vial following 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 disulfide 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 analyzer 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 nine minutes. 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) and 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.

Carotenoid Pigments

Extraction and Determination of Total and Individual Carotenoids

Carotenoids from each tissue were extracted three times with a total of 50 mL of acetone for two minutes. The homogenized samples were centrifuged (IEC Centra MP4 Centrifuge, International Equipment Co., Needham Heights, MA) at 4000 × g for five minutes. 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 milliliters 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 then recorded (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 content of carotenoids present per 100 g of tissue was calculated using the following equation.

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

where, A = absorbance at λ_{max}; V = total volume of the sample (mL); ε = molar extinction coefficient, and W = weight of the tissue (g). Because the crude extracts usually contained a variety of carotenoids an average coefficient of 2,500 was used in the calculations.

The total pigment extracted was separated into individual carotenoids by means of thin-layer chromatography (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.

Characterization of Fractions

Cochromatography 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., Inc., Milwaukee, WI); 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 unavailable, the type of carotenoid in each fraction was tentatively identified according to its absorption maximum in n-hexane, ethanol, and chloroform (Goodwin 1955, Krinsky & Goldsmith 1960, Fox & Hopkins 1966, Britton 1995).

Determination of Total Amino Acids

The amino acid composition of sea urchin gonads and coelomic fluid was determined according to the procedure described by Blackburn (1968). Samples were lyophilized and then hydrolyzed for 24 h at 110°C with 6M HCl. Hydrochloric acid in the hydrolyzate 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 hydrolyzate were separated, identified and quantified using a Beckman 121 MB amino acid analyzer (Beckman Instruments Inc., Palo Alto, CA). Sulfur-containing amino acids were determined by oxidizing the samples with performic acid before their hydrolysis in a 6M HCl solution (Blackburn 1968). Cysteine and methionine were measured as cysteic acid and methionine sulphone, respectively. To determine tryptophan, samples were hydrolyzed in 3M mercaptoethanesulfonic 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).

Determination of 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 two minutes in an ice bath. The homogenized samples were then incubated in an ice bath for 30 min. This was followed by centrifugation (IEC Centra MP4 Centrifuge, International Equipment Co., Needham Heights, MA) at 2,000 × 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. Precipitates of potassium perchlorate were removed by centrifugation at 2000 × g for 10 min. The supernatant was then acidified to pH 2.2 using a 10 M HCl solution, and the volume of the extract was brought to 50 mL with distilled water. Three milliliters of lithium citrate buffer (pH 2.2, 0.3M) were added to 1 mL of the extract, and the resultant solution was analyzed using a Beckman 121 MB amino acid analyzer (Beckman Instruments, Inc., Palo Alto, CA) for individual amino acids.

Determination of Nucleic Acids

The DNA and RNA contstituents of gonads and coelomic fluid of sea urchins were extracted according to the method of Schmidt and Thannhauser (1945) as modified by Munro and Fleck (1969). Five grams of each sample were homogenized in 80 mL ice-cold deionized water using a Polytron homogenizer (Brinkman Instruments, Rexdale, ON) at 10,000 rpm. Five milliliters of the homogenate were allowed to stand for ten minutes in ice and then centrifuged (IEC Centra MP4 Centrifuge, International Equipment Co., Needham Heights, MA) at 2000 × g for ten minutes. The residue was subsequently washed with 2.5 mL of ice-cold 0.2 M PCA and centrifuged at 2,000 × g for ten minutes followed by digestion of the residue in 4 mL of a 0.3 M KOH for one hour at 37°C in a water bath. The resultant solution was cooled in ice and mixed with 2.5 mL of 1.2 M PCA and allowed to stand for ten minutes, which finally resulted in the coagulation of proteins. The mixture was centrifuged at 2,000 × g for ten minutes, and the supernatant was recovered (Extract No.1). The precipitate was then washed twice with 2.5 mL of a 0.2 M PCA solution and centrifuged at 2,000 × g for five minutes. The supernatant was combined with extract No. 1 and 10 mL of a 0.6 M PCA were added to the mixture. This was used for RNA determination after diluting it up to 100 mL with distilled water. The residue was dissolved in 17 mL of a 0.3 M KOH solution at 37°C and diluted to the 50 mL mark in a volumetric flask with distilled water. The content of DNA in the samples was estimated by determining the deoxyribose content in the extract using the indole procedure of Ceriotti (1952), while RNA was determined by recording the absorbance of the 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 eliminated by employing a correction factor of 0.001 absorbance unit for each 1 μg per mL protein concentration in the extracts. The protein concentration of the extracts was measured using the Folin–phenol procedure of Lowry et al. (1951). Bovine serum albumin (BSA) was used as a standard. 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.

Statistical Analysis

Each experiment was replicated three times and mean values ± standard deviations reported for each sample. For statistical analy-

ses, mean values of the experimental data were subjected to one way analysis of variance (ANOVA) using GraphPAD Instat Version 1.0. Significance was determined at 5% probability level.

RESULTS

Proximate Composition

Proximate composition of sea urchin gonads and coelomic fluid is shown in Table 1. The moisture and ash contents of sea urchin coelomic fluid were much higher than those of the gonads. On the other hand, the levels of protein, lipid, and carbohydrate in the coelomic fluid were much lower than those in the gonads on a fresh weight basis.

Lipid Class Distribution

The nonpolar and polar lipid classes of gonads and coelomic fluid of sea urchin *S. droebachiensis* are shown in Table 2. Major nonpolar lipid classes were TAG, FFA, and ST; whereas, main polar lipids classes were PC, PE, SM/LPC, and PS/PI in both gonads and coelomic fluid. Triacylglycerols constituted the main energy reserve in both tissues, contributing more than 65% to the total nonpolar lipids. On the other hand, PC was the dominant polar lipid, accounting for more than 60% in both gonads and coelomic fluid. The polar lipid classes SM and LPC as well as PS and PI did not show a clear chromatographic separation from each other during Iatroscan analysis.

Fatty Acid Composition

Fatty acid composition of sea urchin gonads and coelomic fluid is presented in Table 3. Qualitatively, the fatty acid compositions were the same in both tissues, while there were significant ($P < 0.05$) quantitative variations. In both tissues, 14:0 and 16:0 were the main saturated fatty acids. In addition, 18:0 and 20:0 were present in considerably high levels. The fatty acid 20:1n-15 was the dominant MUFA in both gonadal and coelomic fluid lipids. Furthermore, 16:1n-7, 16:1n-9, 18:1n-7, 20:1n-7, 20:1n-9, and 22:1n-11 were detected in noticeable amounts. Among PUFA, 20:5n-3 contributed the highest proportion to the total fatty acid content in both gonadal and coelomic fluid lipids.

Carotenoid Pigments

The total carotenoid content, on a dry weight basis, of sea urchin gonads and coelomic fluid was 23.2 ± 0.04 and 3.7 ± 0.1 mg per g tissue, respectively. Crude pigments from gonads and coelomic fluid were separated by TLC into eight and seven fractions, respectively. Crude pigments of both tissues, upon TLC separation, exhibited two major bands. In gonads, fractions I (Rf = 0.96) and II (Rf = 0.88) ran close to the solvent front, but they were adequately separated. For coelomic fluid, fraction I (Rf = 0.94) ran almost close to the solvent front; whereas, fraction IV (Rf = 0.42) ran well behind. The carotenoid fraction I of both gonads and coelomic fluid of sea urchin *S. droebachiensis* was confirmed to be β-carotene using an authentic β-carotene sample as established by cochromatography on silica gel TLC plates. Similarly, gonadal fractions III, IV, VI, and VII contained astaxanthin ester (Rf = 0.57), zeaxanthin (Rf = 0.51), canthaxanthin (Rf = 0.22), and free astaxanthin (Rf = 0.1), respectively. Furthermore, fraction II of gonadal crude pigments corresponded to echininone by means of absorption maxima in hexane, chloroform, and ethanol (Goodwin 1955, Krinsky & Goldsmith 1960, Fox & Hopkins 1966, Britton 1995). Thus, the observed λ_{max} values of echininone were 484/460, 466, and 475 nm in hexane, ethanol, and chloroform, respectively. In coelomic fluid, the pigment in 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 coelomic fluid were astaxanthin etser (Rf = 0.58), canthaxanthin (Rf = 0.23), and free astaxanthin (Rf = 0.1), which corresponded to fractions III, V, and VI, respectively. The other minor carotenoids were not analyzed because of their insufficient concentration to obtain absorption maxima and also lack of authentic samples.

Amino Acid Composition

The total and free amino acid compositions of sea urchin gonads and coelomic fluid are shown in Tables 4 and 5, respectively. Results so obtained did not show any clear variation on a qualitative basis for tissues examined. Thus, the spectrum of amino acids, both total and free, present was nearly the same for both gonads and coelomic fluid. Total amino acid profile indicated the

TABLE 1.

Proximate composition of sea urchin gonads and coelomic fluid after feeding urchins on a *Laminaria* diet for three weeks.

Constituent	Gonads	Coelomic Fluid
Moisture	74.7 (0.04)	96.5 (0.03)
Ash	2.2 (0.2)	3.0 (0.02)
Protein	7.4 (0.2)	0.1 (0.02)
Lipid	4.7 (0.1)	0.1 (0.03)
Carbohydrate[a]	10.6 (0.2)	0.4 (0.1)

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

[a] Determined by difference.

TABLE 2.

Quantification of non-polar and polar lipids (weight %) of sea urchin gonads and coelomic fluid after feeding the urchins on *Laminoria* diet for three weeks.

Lipid Classes	Gonad	Coelomic Fluid
Non polar lipids		
TAG	66.7 (0.8)	56.5 (0.7)
FFA	22.7 (1.1)	37.4 (1.0)
ST	10.6 (1.2)	6.1 (0.8)
MAG	tr	tr
DAG	tr	tr
Polar lipids		
PC	65.7 (1.4)	68.9 (0.8)
PE	17.3 (0.2)	27.3 (0.9)
SM/LPC	8.4 (0.7)	2.6 (0.2)
PS/PI	8.5 (0.6)	1.1 (0.5)

Results are mean values of three replicates (standard deviation). Values in each row with the same superscript are not different ($P > 0.05$) from one another. Abbreviations: TAG, triacylglycerol; FFA, free fatty acid; ST, sterol; MAG, monoacylglycerol; DAG, diacylglycerol; PC, phosphatidylcholine; PE, phosphatidylethanolamine; SM, sphingomyelin; LPC, lysiphosphatidylcholine; PS, phosphatidylserine and PI, phosphatidylinositol; and tr, trace.

TABLE 3.

Fatty acid composition (weight %) of total lipids sea urchin gonads and coelomic fluid after feeding the urchins with *Laminaria* diet for three weeks.

Fatty Acid	Gonads	Coelomic Fluid
14:0	9.4 (0.1)[a]	8.4 (0.1)[a]
15:0	0.4 (0.02)[a]	0.7 (0.02)[a]
16:0	11.1 (0.1)[a]	17.6 (0.3)[a]
18:0	2.2 (0.04)[a]	2.3 (0.1)[a]
20:0	2.9 (0.1)[a]	1.2 (0.1)[a]
14:1n-7	0.8 (0.03)[a]	0.3 (0.03)[a]
16:1n-9	4.8 (0.1)[ac]	1.6 (0.1)[a]
16:1n-7	1.5 (0.1)[a]	3.4 (0.2)[a]
16:1n-5	0.3 (0.03)[a]	0.6 (0.04)[a]
18:1n-9	1.8 (0.1)[a]	2.2 (0.1)[a]
18:1n-7	3.6 (0.1)[a]	4.2 (0.1)[a]
18:1n-5	0.5 (0.02)[a]	3.4 (0.2)[a]
20:1n-15	7.5 (0.2)[a]	5.6 (0.1)[a]
20:1n-9	4.0 (0.03)[a]	1.2 (0.03)[a]
20:1n-7	2.2 (0.1)[a]	1.9 (0.1)[a]
22:1n-11	2.9 (0.04)[a]	1.2 (0.1)[a]
22:1n-9	0.4 (0.03)[a]	1.1 (0.1)[a]
16:2n-6	0.5 (0.03)[a]	0.2 (0.02)[a]
16:4n-6	1.9 (0.1)[a]	ND
16:4n-3	ND	2.8 (0.1)[a]
18:2n-9	ND	0.5 (0.02)[a]
18:2n-6	1.1 (0.1)[a]	0.9 (0.1)[a]
18:3n-6	1.4 (0.03)[a]	1.3 (0.1)[a]
18:3n-3	1.3 (0.04)[a]	0.5 (0.04)[a]
18:4n-3	3.8 (0.03)[a]	2.2 (0.03)[a]
20:2Δ5, 11	1.8 (0.1)[a]	1.7 (0.1)[a]
20:2Δ5, 13	0.9 (0.1)[a]	0.7 (0.03)[a]
20:2n-6	1.7 (0.1)[a]	1.7 (0.1)[a]
20:4n-6	7.0 (0.1)[a]	9.9 (0.1)[a]
20:3n-3	1.7 (0.2)[a]	0.9 (0.04)[b]
20:4n-3	1.2 (0.2)[a]	0.3 (0.02)[a]
20:5n-3	16.3 (0.1)[a]	16.5 (0.5)[a]
22:5n-6	0.2 (0.1)[a]	0.3 (0.03)[a]
22:5n-3	0.6 (0.1)[a]	0.7 (0.04)[a]
22:6n-3	1.4 (0.1)[a]	0.6 (0.1)[a]

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

TABLE 4.

Total amino acid content (mg/g protein) of sea urchin gonads and coelomic fluid after feeding urchins on a *Laminaria* diet for three weeks.

Amino Acid	Gonads	Coelomic Fluid
Alanine	42.5 (0.4)[a]	63.1 (0.5)[b]
Arginine	80.7 (0.5)[a]	74.2 (0.6)[b]
Cysteine	7.2 (0.1)[a]	5.7 (0.2)[b]
Glutamic acid	87.2 (0.2)[a]	90.6 (0.6)[a]
Glycine	118.0 (0.7)[a]	143.6 (0.7)[b]
Histidine	35.5 (0.3)[a]	27.0 (0.8)[b]
Hydroxyproline	3.3 (0.2)[a]	2.6 (0.2)[a]
Isoleucine	77.1 (0.1)[a]	60.9 (0.9)[b]
Leucine	65.9 (1.0)[a]	62.6 (0.3)[b]
Lysine	81.1 (0.9)[a]	77.1 (0.9)[a]
Methionine	1.2 (0.3)[a]	0.9 (0.1)[b]
Phenylalanine	53.2 (0.9)[a]	42.1 (0.6)[b]
Proline	40.2 (0.5)[a]	48.4 (0.1)[b]
Serine	47.1 (0.1)[a]	46.5 (0.7)[b]
Threonine	47.8 (0.4)[a]	47.2 (0.5)[b]
Tryptophan	1.4 (0.1)[a]	1.1 (0.2)[b]
Tyrosine	36.3 (0.8)[a]	38.6 (0.4)[b]
Valine	78.3 (0.4)[a]	75.1 (1.0)[b]
Total (mg/g protein)	989.4 (2.2)	985.4 (1.3)

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

dominance of glycine in both tissues analyzed. However, on a dry weight basis, the content of glycine in sea urchin coelomic fluid was significantly ($P < 0.05$) higher than that of gonads. Almost all the essential amino acids were present in both gonads and coelomic fluid of *S. droebachiensis*. With respect to FAA content of sea urchin tissues, the total FAA content was much higher in sea urchin gonads than that in the coelomic fluid. However, in both tissues glycine was the dominant FAA contributing 57.1 and 56.3% to the total amount in the gonads and coelomic fluid, respectively.

Content of Nucleic Acids

The content of nucleic acids in sea urchin gonads and coelomic fluid, on a dry weight basis, was different. The content of DNA of sea urchin gonads and coelomic fluid was 3.93 ± 0.1 and 1.02 ± 0.07 μg per g tissue, respectively; whereas, corresponding values for the content of RNA were 2.63 ± 0.06 and 0.49 ± 0.03 μg per g tissue, respectively. Thus, the content of DNA was higher than that of RNA in both tissues analyzed. Furthermore, the ratio of RNA/DNA was 0.7 ± 0.1 and 0.5 ± 0.03 for sea urchin gonads and coelomic fluid, respectively.

DISCUSSION

Reproductive State of Sea Urchins

For feeding experiments, sea urchins were obtained from the wild in the month of June, representing the spring season. In general, gonad development in sea urchins may include five different stages of resting, growing, premature, mature, and spawning (de Jong-Westman et al. 1996). In resting, gonad size is at a minimum, which usually occurs after spawning. *S. droebachiensis* has an annual reproductive cycle with major spawning period in the late winter or early spring (Keats et al. 1984). Therefore, at this stage, urchins were presumably in the state of resting; hence, they have undergone a large drop in gonad size following spawning.

In general, gonadal yield is strongly affected by the seasonal reproductive cycle of sea urchins. 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. Because urchins were fed on a *Laminaria* diet after harvesting and consequently subject to intense feeding, this may have a significant effect on the nutrient composition of sea urchin tissues. Generally, both food quality and quantity affect sea urchin growth (Lawrence & Lane 1982), thus excessive feeding resulting in the accumulation of nutrients in the tissues.

Proximate Composition

The major nutrients of sea urchin *S. droebachiensis* gonads were polysaccharides, proteins, and lipids, similar to that reported

TABLE 5.

Free amino acid content (μg/g dry weight) of sea urchin gonads and coelomic fluid after feeding urchins on *Laminaria* diet for three weeks.

	Gonads		Coelomic Fluid	
Amino Acid	μg/g	%	μg/g	%
Alanine	2,872 (77)[a]	14	899 (17)[b]	7.5
α-aminoadipic acid	68 (5)[a]	0.3	——	
Arginine	180 (3)[a]	0.9	507 (19)[b]	4.2
Asparagine	13 (1)[a]	0.1	——	——
Aspartic acid	73 (8)[a]	0.4	80 (3)[b]	0.7
Cystathionine	115 (2)[a]	0.6	76 (39)[b]	0.6
Cysteine	214 (12)[a]	1.0	90 (7)[b]	0.7
Glutamic acid	874 (12)[a]	4.2	478 (14)[b]	4.0
Glutamine	647 (34)[a]	3.1	184 (8)[b]	1.5
Glycine	11,751 (223)[a]	57.1	6,771 (430)[b]	56.3
Histidine	100 (6)[a]	0.5	111 (10)[b]	0.9
Hydroxyproline	124 (11)[a]	0.6	88 (6)[b]	0.7
Isoleucine	313 (11)[a]	1.5	175 (6)[b]	1.5
Leucine	370 (23)[a]	1.8	293 (7)[b]	2.4
Lysine	356 (7)[a]	1.7	312 (11)[b]	2.6
Methionine	68 (4)[a]	0.3	134 (8)[b]	1.1
Phenylalanine	164 (2)[a]	0.8	164 (12)[b]	1.4
Proline	140 (10)[a]	0.7	4,909 (147)[b]	2.7
Sarcosine	332 (14)	1.6	——	——
Serine	316 (5)[a]	1.5	163 (10)[b]	1.4
Threonine	521 (27)[a]	2.5	189 (12)[b]	1.6
Tryptophan	264 (11)[a]	1.3	140 (3)[b]	1.2
Tyrosine	217 (16)[a]	1.1	212 (5)[b]	1.8
Valine	273 (16)[a]	1.3	272 (9)[b]	2.3
Total (mg/g)	21 (1.0)[a]		12.0 (0.8)	

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

by Fernandez et al. (1995). However, coelomic fluid contained 96.5 ± 0.03% moisture; thus, its contents of lipid, protein, and polysaccharide were extremely low. On the other hand, the ash content of sea urchin coelomic fluid was much higher than that of the gonads. In general, sea urchin gonads are known to contain high levels of protein. They also have considerably high lipid levels; whereas, carbohydrate levels are low (McClintock & Pearse 1987). However, in our study, the carbohydrate content was high and accounted for approximately 10% of the total amount, on a fresh weight basis. Furthermore, in coelomic fluid, although the relative proportion of carbohydrate was only 0.4 ± 0.1%, this was four times more than that of its protein and lipid contents. From this study, it is apparent that content of lipid, protein and carbohydrate in the coelomic fluid was much less than that of the gonads. The urchins in this work were given a diet purely comprised of *Laminaria* kelp to resemble the urchin's preferred natural diet. In general, the diet plays a very important role in the compositional characteristics of these animals (Nishikiori 1989, Fernandez et al. 1995, Agatsuma 1998) and almost all studies have only determined compositional characteristics of the gonads. Thus, Agatsuma (1998) showed that a diet of fishmeal increased moisture levels, and Nishikiori (1989) observed that moisture content in the gonads of *S. nudus* was below 70% when the urchins were fed *Laminaria japonica* to satiation. None of these studies reported compositional characteristics of sea urchins' coelomic fluid. In our study, when urchins were fed on a *Laminaria* diet, gonads contained 74.7 ± 0.04% moisture, which was significantly ($P < 0.05$) lower than that of the coelomic fluid.

Lipid Class Composition

The lipid composition of marine invertebrates is influenced by several factors, including pattern of feeding, gametogenesis, and possibly environmental conditions (Jezierska et al. 1982). Wax esters have been reported to constitute energy reserves in various marine invertebrates (Sargent 1976), but this was not the case for sea urchin *S. droebachiensis*. TAG formed the main energy reserve in these animals, and their gonads and coelomic fluid were qualitatively composed of similar lipid classes. The same nonpolar lipid class distribution has been observed in *S. droebachiensis* gonads and coelomic fluid collected form Nova Scotia (Takagi et al. 1980). The nonpolar lipids of gonads and coelomic fluid consisted mainly of TAG, FFA, and ST. Triacylglycerols are usually considered to serve as storage lipids in eukaryotic cells (Sul et al. 2000). Thus, sea urchin lipids contained much larger amounts of storage lipids, principally TAG, which constituted more than 60% of the total nonpolar lipids of gonads and coelomic fluid.

Although, qualitative composition of nonpolar lipids of gonads and coelomic fluid of *S. droebachiensis* was similar, relative content of individual classes differed. Hence, both sea urchin gonads and coelomic fluid were composed of the same major lipid classes, both nonpolar and polar, but their relative contents were markedly different. Thus, relative content of TAG in sea urchin gonads was much higher than that of coelomic fluid; whereas, that of FFA in gonads was much less than that of coelomic fluid. It was apparent in the preliminary experiments that both gonads and coelomic fluid contained high levels of FFA. Therefore, it was thought that partial hydrolysis of TAG may lead to an underestimation of TAG content. However, the impact of this on a nutritional value of the fatty acids involved is inconsequential. To verify the above fact, fatty acid content was determined in a set of freshly harvested sea urchins. The gonads were extracted as quickly as possible at 0°C immediately after homogenization. It was assumed that hydrolysis of lipids because of the activity of endogenous enzymes is minimized under these conditions. The FFA content was 15.5 ± 1.7% of the total nonpolar lipids upon Iatroscan analysis. Hence, the high levels of FFA observed for stored sea urchin tissues following homogenization could be attributed to the hydrolysis of TAG during storage of samples at −20°C.

In general, the energy supplied to the animal by the breakdown of lipid reserves comes primarily from oxidation of fatty acids. Farkas (1979) has shown that the production of FFA can be induced by stress. Thus, the environmental temperature and diet can be specified as factors exerting a major impact on the content and metabolism of fatty acids in animals (Farkas et al. 1978).

There were noticeable differences in the relative content of sterols in sea urchin tissues. In general, cholesterol level may depend on dietary level and stage of sexual development (Love 1970). In fact, diet and nutritional status are known to be the main factors that influence cholesterol levels (Dave et al. 1975). On the other hand, during gametogenesis, a redistribution of cholesterol takes place that may lead to high levels of cholesterol in the gonads (Idler & Tsuyuki 1985). Therefore, all relevant factors must be considered to explain the content of cholesterol in different tissues.

In the present study, the relative content of ST in the gonads was significantly ($P < 0.05$) higher than that in the coelomic fluid.

Vaskovsky and Kostetsky (1969) have performed TLC on polar lipids of sea urchin *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. Furthermore, lipid extracts of different organs of the same animal had a similar qualitative polar lipid composition (Vaskovsky & Kostetsky 1969). In this study, both gonads and coelomic fluid showed qualitative similarities in their polar lipid fractions. Thus, PC, PE, SM/LPC, and PS/PI constituted the polar lipids of *S. droebachiensis* gonads and coelomic fluid, and PC was dominant in both tissues, with a contribution of more than 65% to the total content of polar lipids. Similarly, Floreto et al. (1996) demonstrated that sea urchin *Tripneustes gratila* fed on a seaweed diet had PC and PE as the major lipid constituents, and PC contributed a greater proportion than PE.

Fatty Acid Composition

The fatty acids of total lipids of sea urchin gonads and coelomic fluid were typically similar to those of other marine species with a dominance of 16:0 and 20:5n-3 (Wanasundara 1996). Although, 22:6n-3 is a typical fatty acid in marine lipids, it contributed only 1.4 ± 0.1 and 0.6 ± 0.1% to the total fatty acids in the lipids of sea urchin gonads and coelomic fluid, respectively. Holland (1978) reported that the predominance of 20:5n-3 and 22:6n-3 in typical marine fatty acids is a result of low- temperature adaptation. This helps in the maintenance of cell membrane fluidity in organisms living in the cold environments.

Considerable data are available on the fatty acid composition of sea urchins (Takagi et al. 1980, Kaneniwa and Takagi 1986). The fatty acid 16:0 was the major SFA in the sea urchin *S. droebachiensis* harvested from Herring Cove, Nova Scotia (Takagi et al. 1980). Fujino et al. (1970) analyzed fatty acid composition of sea urchins *Anthocidaris crassispina*, *S. pulcherrimus*, *S. franciscanus*, *S. intermedius*, and *Echinus esculentus*. In all these samples, 16:0 was the prominent SFA followed by 14:0. The fatty acid 18:0 was found to occur in considerable amounts. Similarly, in the present study, the predominant SFA were 16:0 and 14:0 in the lipids of both gonads and coelomic fluid of *S. droebachiensis*.

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 such marine animals as periwinkle (*Littorine littorea*), moon snail (*Lunata triseriata*), and sand shrimp (*Crangon septemspinosus*) contain 20:1n-15, but at much lower levels of up to 0.2% of the total fatty acids. However, this has not been commonly reported as being typical of marine lipids. In our study, 20:1n-15 was also the major MUFA in both tissues analyzed. On the other hand, seaweeds, the natural diet of sea urchins, have not been reported to contain 20:1n-15 (Ackman & McLachlan 1977); hence, the formation of 20:1n-15 in sea urchin tissues may be biosynthetic in origin, because this was not dependent on the diet.

The occurrence of such unusual 5-olefinic fatty acids as 18:1n-13, 20:1n-15, 20:2Δ5,11, 20:2Δ5,13, 20:3Δ5,11,14, and 20:3Δ5,11,14,17 has been noticeable in lipids of sea urchins accounting for as much as 6–21% of the fatty acids of total lipids (Takagi et al. 1980, Kaneniwa & Takagi 1986). In this study, the amount of 5-olefinic acids found in the lipids of both gonads and coelomic fluid was in the range of 7–10%. The presence of 5-olefinic fatty acids has been reported in 12 species of Echinoidea collected in Japan (Takagi et al.1986); thus, they serve a common and characteristic feature of sea urchin lipids.

The amount of eicosapentaenoic acid (20:5n-3) was quite high in sea urchin lipids (Takagi et al. 1980). Pohl and Zurheide (1979) reported that urchins that consumed *Laminaria* had a high content of 16:4n-3, 18:4n-3, 20:4n-6, and 20:5n-3. Similarly, sea urchin *S. droebachiensis* in our study consumed *Laminaria* for only a three week period, and their gonadal and coelomic fluid lipids contained quite high levels of these fatty acids. Thus, the fatty acid profiles of sea urchin tissues somewhat reflect that of their diets as was also observed by Floreto et al. (1996). However, certain fatty acids, such as 16:4n-3, 20:4n-6, 20:5n-3, and 20:1n-11, which constitute the major fatty acids of sea urchin tissues, were not detected in their diets; therefore, suggesting that sea urchins are capable of synthesizing them from lower fatty acid precursors. Similarly, in the present study 16:4n-3, 20:1n-11, 20:4n-6, and 20:5n-3, among others, may have been formed by chain elongation of precursors. In general, the sea urchin fatty acids; namely, 16:4n-3, 20:4n-6, and 20:5n-3, may possibly confer some structural function and, hence, are purposely synthesized by the animal (Floreto et al. 1996).

Carotenoid Pigments

In the sea urchin *S. droebachiensis*, carotenoids were mainly concentrated in the gonadal tissue. Hence, the total content of carotenoids in the gonads was about 6.3 times more than that of the coelomic fluid. However, the content of carotenoids in different tissues may vary with the reproductive stage of urchins. Hence, during gametogenesis most of the carotenoids in other tissues may be transferred into gonads, consequently increasing their carotenoid content (Griffiths & Perrott 1976).

Echininone and fucoxanthin were characterized as the major carotenoids present in the gonads and the coelomic fluid, respectively. 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 gonads of *S. purpuratus* (Griffiths 1966), *S. droebachiensis* (Griffiths & Perrott 1976) and *Tripneustes gratila* (Shina et al. 1978). Tsushima et al. (1995) found that β-echininone and β-carotene were the major carotenoids in the gonads of 19 out of 20 sea urchin species examined. Meanwhile, the major carotenoids of brown algae, the natural preferred diet of sea urchins, consist of β-carotene, violaxanthin, and fucoxanthin (Matsuno & Hirao 1989). Furthermore, there is bioconversion of β-carotene to β-echininone via β-isocryptoxanthin in sea urchins; which takes place mainly in the gut wall, and the resultant β-echininone is 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. On the contrary, coelomic fluid had fucoxanthin as its major carotenoid.

Amino Acid Composition

Although marine invertebrates characteristically contain a high intracellular concentration of 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 TAA and FAA

profiles in both sea urchin gonads and coelomic fluid. Komata et al. (1962) reported that glycine was dominant in the gonads of sea urchin *S. pulcherrimus*, and its content ranged from 35–41% of total FAA. Lee and Haard (1982) reported that glycine constituted 18–60% of the FAA in the gonads of sea urchin *S. droebachiensis*. The gonads and coelomic fluid of sea urchin *S. droebachiensis* in this study contained 11.9–14.6% glycine in the TAA profile, respectively. However, glycine was not the dominant amino acid in the gonads of the sea urchin *Paracentrotus lividus*, although it contributed a considerable amount to the TAA pool (Cruz-Garcia et al. 2000). Other than glycine, alanine, arginine, glutamic acid, lysine, and methionine are considered important for taste, even though some of them were present in small quantities (Lee & Haard 1982). These amino acids were present in considerable amounts in both gonads and coelomic fluid of sea urchins in this study.

It has been found that different combinations of taste-active components (substances that influence the taste of any food) as well as their relative amounts are of paramount importance in producing the characteristic flavor of each seafood (Fuke 1994). In general, glutamine and glycine, which were present in higher amounts in the gonads than coelomic fluid, are known to be taste-active in sea urchins and other seafoods, regardless of their quantity. Sea urchin gonads seemed to be sweeter when little or no glutamine was present, and alanine was found in considerably high levels. Alanine is a taste-active component in sea urchin tissues, contributing noticeably to both TAA and FAA contents. Furthermore, valine and methionine are known to be taste-active only in sea urchins; whereas, arginine was also taste-active in sea urchins because of its high content (Fuke 1994). Both methionine and arginine were present at a higher proportion in the coelomic fluid than in the gonads. Similarly, the contents of aspartic acid, histidine, and especially proline were much higher in the coelomic fluid than those in the gonads. Thus, amino acids play a major role in the taste of sea urchin gonads. In our study, various amino acids contributed differently to both the TAA and FAA of sea urchin gonads and coelomic fluid.

Contents of Nucleic Acids

In general, quantitative analysis of nucleic acid 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 a precursor to protein synthesis led to its use as an indicator of growth rate (Church & Robertson 1966). The primary function of RNA involves protein synthesis; whereas, DNA is the primary carrier of genetic information. Because the majority of cellular DNA is chromosomal, the quantity of DNA per cell is quasiconstant in somatic tissues; the tissue DNA concentration reflects cell numbers (Sulkin et al. 1975; Bulow 1987). Therefore, DNA content has usually been used as an index of cell numbers or biomass (Regnault & Luquet 1974). In this study, the DNA content in the gonads was approximately four times higher than that in the coelomic fluid. Although the gonad is a tissue with a higher biomass as compared with coelomic fluid, the latter contains mostly coelomic fluid with a lower biomass. On the other hand, the RNA/DNA ratio has been used as an estimate of growth for a variety of invertebrates (Sulkin et al. 1975). Thus, the RNA/DNA ratio is an index of protein synthetic activity per cell and reflects the protein synthesizing capacity for estimating recent *in situ* protein increase (Bulow 1987, Hovenkamp & Witte 1991). In fact, correlation between RNA concentration or RNA/DNA ratio and growth rate has been observed for a wide variety of organisms (Sutcliffe 1970). Furthermore, the gonadal RNA content was about 5.4 times higher than that in the coelomic fluid, thus demonstrating higher protein synthetic activity in the gonads. In general, gonad is the site of gametogenesis, which involves much protein synthesis. Furthermore, the RNA/DNA ratio was much lower in coelomic fluid than that in gonads, indicating greater protein synthetic activity per cell in the gonads. This is an indication that gonad is a tissue with greater *in situ* protein growth as compared with coelomic fluid.

CONCLUSIONS

The present study demonstrated that sea urchin gonadal and coelomic fluid tissues had many common compositional characteristics. Most of the parameters analyzed did not show qualitative differences; whereas, there were quantitative differences. In fact, gonads of sea urchins are a site of nutrient storage in addition to being the reproductive organs. The accumulation of nutrient reserves contributes to the growth and development of the commercially important sea urchin gonads. Although sea urchin coelomic fluid has not yet been exploited commercially, evaluation of its composition may lead to its potential use as a flavoring source.

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