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Seasonal Variations in Sterol Content of the Oyster, *Crassostrea virginica* (Gmelin), from Natural Reefs in the Mississippi Sound

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Sterol composition of *C. virginica* was assayed by gas-liquid chromatography. Total sterol levels were determined by analyzing colorimetrically the sterol digitonides prepared from single whole oysters. The predominant sterols (%) were cholesterol (33), campesterol (20), brassicasterol (18) and 22 dehydrocholesterol (12); also found were β-sitosterol (6) and a C_{26} sterol (9). Percentage composition of sterols in oysters was not significantly different in relation to season or sex. Total sterol level varied with season and sex. Lowest levels were seen during winter (February) at which time the sexes could not be distinguished. Male oysters had higher sterol levels than females in the spring (April) and fall (October) while females had higher levels during summer when the spawning season was ending. Total sterol content decreased with an increase in size of the oysters during all seasons; the relationship followed a polynomial function. Comparison with values reported indicated that southern oysters have higher fat but lower cholesterol than their northern counterparts.

Relationship between the total sterol content and the meat weight of oyster, *Crassostrea virginica*, was nonlinear and followed a 5th degree polynomial function with the sterol level decreasing with growth[1]. This observation was made on so called lean oysters collected from natural reefs during October. It was also observed that the polynomial parameters were sex-dependent, with males showing a higher sterol content than females. It was not known whether this relationship persisted in the oysters during each season. With this in view the sterol composition and seasonal variations in sterol levels of *C. virginica* from a reef in the Mississippi Sound were studied.

Materials and Methods

Oysters above 25 mm shell length were collected from the bay of St. Louis, Mississippi during October 1977 and February, April and July 1978. The mean salinity and temperature conditions at the reef during the time of each collection were: October 1977, 11.6‰, 18°C; February 1978, 19‰, 13.5°C; April 1978, 14‰, 21°C; and July 1978, 17‰, 31°C. The live oysters were brought to the laboratory, shucked, and separated by sex and size. Sexes were distinguished by examining a gonadal smear under the microscope. The oyster meat was removed from the shell, blotted dry, and weighed prior to sterol analysis.

For isolation and estimation of sterol levels each oyster meat was minced, mixed with acetone-alcohol (1:1) by volume at the rate of 20 ml/5 g meat and homogenized in a tissue grinder to extract the 3 β-OH sterols. The solution was separated by centrifugation at 600 × g. Supernatant (2 ml) was saponified with 1 ml of 6 *M* methanolic KOH at 45° for 4 hr and neutralized with 10% acetic acid followed by a drop of 1% phenolphthalein as an indicator. The 3 β-OH sterols present in the crude saponified extract were precipitated after the addition of 3 ml of 0.4% Digitonin (Sigma Co.) at room temperature. The precipitant was allowed to settle over night. The digitonide was separated by centrifugation at 1500 × g, purified by repeated washings of acetone-alcohol and centrifuged again. A final washing was given with ether. The separated digitonide was subjected to the Libermann-Burchard colour reaction[2] using a Spectronic 20 (Bausch and Lomb) colorimeter. Cholesterol (Sigma Co.) was used as the standard.

To make sure of the recovery of sterols by this treatment, a test was run on a sample containing a known cholesterol standard. The routine colorimetric assay of sterol digitonide was found to be sensitive and was estimated to recover 98.59% of sterols from oysters.

The purified digitonide was dissolved in 3 ml of pyridine according to the method of Bergmann[3] and the freed sterols separated by column chromatography on deactivated Florisil (12 g, 60-100 mesh, Sigma Co.). The eluent used was 85:15 hexane-ether. The solvent in the fraction was then evaporated under nitrogen; the pure sterol residue was weighed, dissolved in methylene chloride, and stored on dry ice.

The analysis of sterols was done by analytical gas-liquid chromatography (GLC) according to the

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AOAC[4] method on 3% SP-2250 on SLCOPORT mesh 100/120 using a flame ionization detector (Model 3920, Perkin-Elmer Corp., Norwalk, Connecticut). Equilibrated columns of 2 mm ID and 2.44 m (8 ft) length were used. The temperatures used were: column 270°C, detector cell 300°C, and flash heater 300°C. Nitrogen was the carrier gas at a flow rate of 38 ml/min.

Total fat content in dried oysters was assessed gravimetrically after extracting the liquid with 3:1 chloroform-methanol for 6 hr in a Soxhlet apparatus.

Methods of Steel and Torrie[5] were used for statistical analyses. Computations were carried out with an IBM 1130 subroutine package.

Results

Seven sterol components (Fig. 1) were detected in the extracts of oysters of different size, of both sexes, and during different seasons. Four sterol components were predominant and were identified to be cholesterol, brassicasterol, campesterol, and 22 dehydrocholesterol. Cholesterol was the major component constituting 32.9% of total sterols. The relative percentage composition of sterols appeared to be independent of sex, size and season and so the curve showing sterols from female oysters during the spring (Fig. 1) is considered representative.

Table 1 compares the relative retention time of the sterols obtained in the present study and the percentage composition of sterols reported by earlier workers. The number of sterols reported varied but all the reports agreed with the predominance of cholesterol: the percentage of cholesterol obtained in this study was the lowest of all cholesterol values reported by earlier workers (Table 1). The quantity of campesterol recorded was high in the present study.

Variations in the total sterol levels of the oysters collected during different seasons are given in Figs 2A-D. The relationship between the total sterol content and meat weight was non-linear and decreased with size of the oyster in both sexes but varied with season. The general trend was best explained by a polynomial regression analysis whose parameters differed with season and sexes (Figs 2A-D).

The sex influences were recognizable only in certain seasons. Oysters collected during fall (October) showed significant differences in sterol content with relation to sex for sizes above 5 g meat weight; males showed higher sterol levels (Figs 2A and 3) than the females. Males (above 15 g meat weight) were not abundant on the reef during fall; the larger individuals were

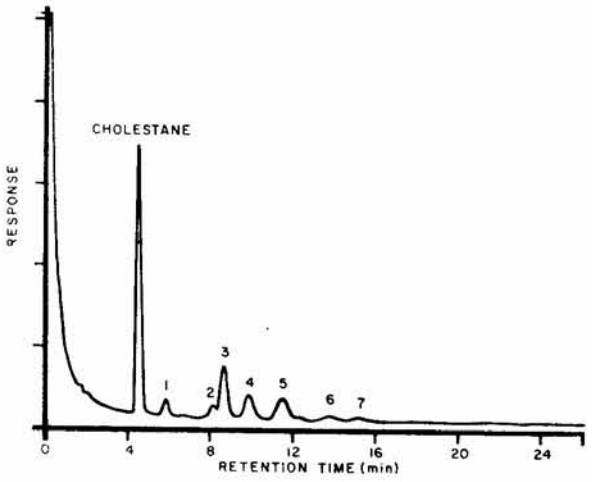

Fig. 1—Representative GLC on 3% SP-2250 on SLCOPORT mesh 100/120 of sterols isolated from spring oyster (females) [Numerals indicate sterol components (see Table 1). Cholestane is the reference internal standard]

Table 1—Percentage of Sterols in Spring Oysters (April) as Determined by GLC

Sterol	Systematic nomenclature[a]	Relative retention time		Composition[b] %			
		In ref. cholesterol	In ref. cholestane	Present study	Idler & Wiseman[6]	Kritchevsky *et al.*[23]	Tamura *et al.*[13]
C_{26} Sterol	24-*nor* Cholesta-5,22(E)-dien-3β-ol	0.66	1.32	9.4	4.6	5.6(?)	4
22 Dehydrocholesterol	Cholesta-5, 22(E)-dien-3β-ol	0.94	1.86	11.8	7.5	2.9	8
Cholesterol	Cholest-5-en, 3β-ol	1	1.96	32.9	34.1	41.4	40
Brassicasterol	24-Methyl cholesta-5, 22-dien-3β-ol	1.13	2.23	17.8	28.1	16	27
Campesterol	24-Methyl cholest-5 en-3β-ol	1.34	2.62	20	1.7	—	—
β-Sitosterol	24-Ethyl cholesta-5, 22-dien-3β-ol	1.59	3.13	5.8	3.7	—	—
Unknown	Unknown	1.78	3.45	2.3	20.3[c]	34.1[c]	21[c]

[a]Nomenclature is in accordance with the IUPAC/IUB[22]
[b]Based on measurement of areas under GLC peaks
[c]Constitutes other sterols both identified and unidentified

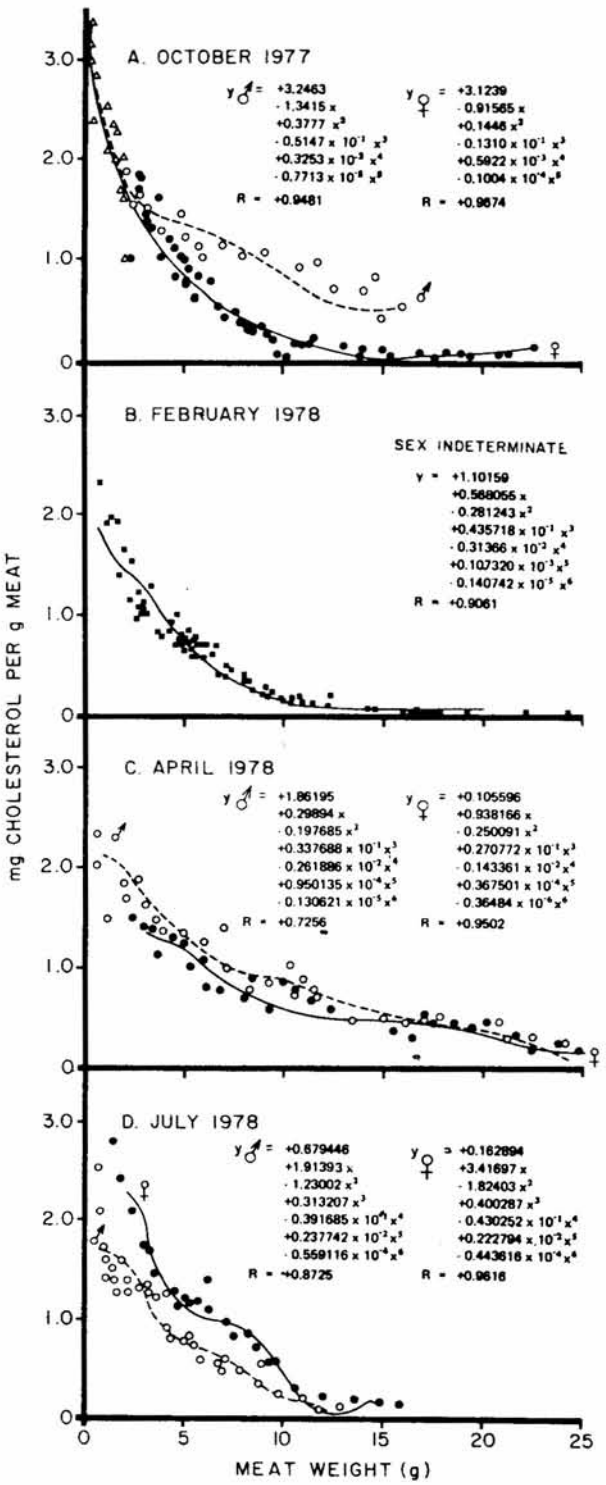

Fig. 2—Variations in sterol levels in relation to size, sex and season in *C. virginica* from a natural reef in Mississippi Sound

invariably females. For oysters of 10-15 g meat weight, the sterol level was 4-5 times greater in males than females (Figs 2A and 3). During winter season a greater number of large oysters were collected but the sexes could not be determined because the gonadal tissue was not differentiated. The sterol content of these oysters decreased with an increase in size (Figs 2B and 3). When compared to fall oysters, the winter animals showed considerably decreased cholesterol content (Fig. 3).

In spring, the sexes became distinguishable. The sterol levels decreased with increasing size but there were no significant differences in the levels between sexes (Figs 2C and 3, Table 2). Statistical comparison (Fig. 3) of the data from the spring group with that from the winter group revealed an increased cholesterol content in the spring oysters.

As in the other seasonal groups, the summer oysters showed decreased sterol levels with increased size (Fig. 2D). Sex differences in the sterol levels were obvious; however, as contrasted with spring and fall oysters, the summer female showed a greater sterol content than the males. The sterol content in the group decreased significantly compared to levels in the spring (Fig. 3). Fig. 3 draws a clear comparison of the variations in meat weight and cholesterol content in the reef oyster population with reference to season and sex. Greater meat yields and lower cholesterol content were observed in female oysters of April. Lower meat yields and higher cholesterol were obtained in male oysters of October.

Cholesterol content of oysters as reported (Table 2) was inconsistent and values were higher compared to the present study. However, many authors did not mention the species they used nor the season when the cholesterol content was assayed.

Total fat content varied with season (Table 3) only in fall, significant differences between male and female were noted, when females showed a higher fat content ($P<0.001$). During spring the fat content was the highest and varied between 4.5 and 5.25% meat. The content decreased to a minimum in fall (Table 3).

Discussion

Idler and Wisemann[6,7] reported the percentage composition of 12 sterol fractions of *C. virginica* from Prince Edward Island (Canada), and cholesterol constituted 34% of total sterol. The present study on the same species from Mississippi coast demonstrated the occurrence of 7 sterol fractions, in which the cholesterol constituted nearly 33%. The variation in number of sterol fractions may be due to the geographic distribution of the species and also partly due to the methods of isolation and analyses of sterols[7]. The present GLC analysis was done on the isolated digitonides which represent only the 3 β-OH sterols. However, variations in total fat, unsaponifiable matter, and sterols are not surprising as they have been obtained for various species of molluscs[8-10]. It is interesting to note that *C. virginica* from southern reefs

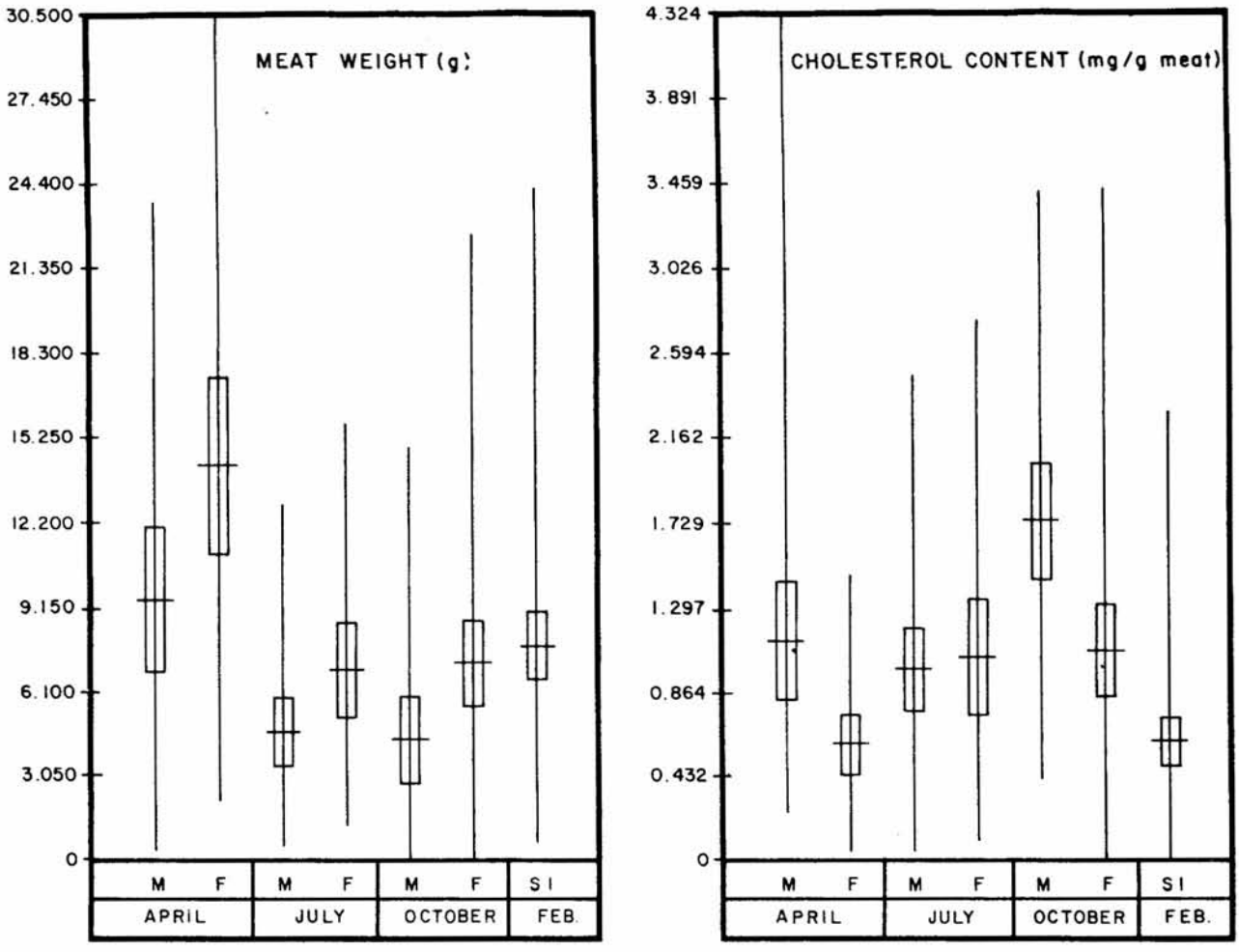

Fig. 3—Hubbs-Hubbs type diagram indicating seasonal variations in the meat yields and cholesterol content in the reef populations of *C. virginica* at Bay of St Louis, Mississippi [For each month the range of variation is shown by a vertical line; the mean by the horizontal line, while the hollow bar indicates the upper and lower limits of 95% confidence interval. M = male; F = female; SI = sex indeterminates]

Table 2—Cholesterol Content of Oysters

Reference	Cholesterol mg/100 g meat
Archard *et al.*[24]	215
Okey[25]	230-470
Kritchevsky and Tepper[26]	112
Thompson[12]	37-58
Kritchevsky *et al.*[23]	150
Present study (market size)	20-50[a]

[a]Varies with seasons and sexes—range between 8 and 15 g meat weight

Table 3—Seasonal Variations in the Total Fat Content of Marketable Oysters (80-90 mm Shell Length) of Mississippi Sound

Date of collection	No. of observations	Fat content g fat/100 g meat[a] Male	Female
October 1977	7	1.15 ± 0.04	1.4 ± 0.12
February 1978	12	4.03 ± 0.91[b]	
April 1978	11	5.25 ± 1.02	4.53 ± 0.92
July 1978	14	3.51 ± 0.68	3.59 ± 0.98

[a]Values are mean ± SD
[b]Sexes undetermined

has a complex mixture of β-OH sterols with cholesterol being the principal one.

C. virginica from the Mississippi Sound differs somewhat from its northern and eastern counterparts both in reproductive behaviour and biochemical composition[11]. Thompson[12] showed that *C. virginica* from eastern waters contained a sterol level of 0.06% meat, although its fat content was reported to be much lower than that of its Mississippi counterpart. Tamura *et al.*[13] reported a sterol level equivalent to 0.14% of the meat weight in *C. virginica* from Prince Edward Island (Canada) and a very low fat content (2%) compared to that of eastern species. Comparison of the present figures with these studies, cannot be made because the

sterol level is size and sex dependent and the earlier authors did not mention the size of the oysters nor the sex. Considering the minimum market size for oysters (8-10 g meat weight, winter oysters) a sterol level of about 0.02% meat would be present in the oysters of the Mississippi Sound, a figure which is fairly lower than that of eastern oyster is reported by Thompson[12].

The variations in the fat (lipid) content of these oysters also follow a seasonal cycle with the lowest level recorded in fall and the highest in the spring, when it constitutes 4-5% of the meat weight (Table 3). The fat content of the northern oysters is 2% meat[13] and the eastern species contain 2.4% (ref. 12). These reports did not mention the size and season of harvest. However, these values are lower than the values obtained for winter and spring oysters in the Mississippi Sound (Table 3). During these seasons they are largely harvested. The sterol level, however, was in reduced levels in the winter and increased substantially in spring in the present study. It could also be reasonable to generalize from these observations that *C. virginica* shows a high fat and low sterol content as it is distributed southwardly.

The sex-related differences observed in the present study may be due to differences in the metabolism of sterols in spawning activity since the importance of cholesterol for gonadal activities has been greatly emphasized by Kritchevsky[14]. Unlike some marine anthropods, oysters are capable of synthesizing cholesterol from simple precursors[13]. Through filter feeding activity the oyster absorbs dissolved free fatty acids from the sea water[15] and by the same mechanism it may absorb the dispersed free sterols which occur in fairly large quantities in sea water[16]. Whether the endogenous metabolism or exogenous absorption contribute towards seasonal changes in the levels of sterols is still not known.

Seasonal changes in sterol content in oysters and other bivalves, do not appear to be extreme, but such studies have been limited. So far, seasonal changes in the biochemical composition of oysters, such as glycogen, fat, protein, etc. have been demonstrated[9,11,17,18]. Idler *et al.*[9] demonstrated that the cholesterol content per unit weight of flesh in the scallop *Placopecten megellanicus* increased in March. They correlated the low sterol levels to the pre-spawning period (August) when active transfer of cholesterol to gonadas occurs. Ashikaga[8] studied seasonal variations of the sterol content in the meat of the pearl oyster *Pinctada martensi* and reported that the sterol level remained steady throughout the year (0.6-0.7%) except in July when it dropped to 0.45%. According to the present study, sterol levels became low in winter and increased in spring. Oysters in the Mississippi Sound start spawning in late spring and continue to early fall[11,19-21]. Such activity probably is the cause for the observed variations in sterol levels. The reduction in the sterol level with growth may be due to changes in the biosynthetic or metabolic pathways of sterols with age.

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