Effect of Dietary Lipids on Growth, Tissue Composition and Metabolism of the Oyster (*Crassostrea virginica*)[1]

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ABSTRACT Hatchery reared oysters (*Crassostrea virginica*) were fed six different diets for 30 weeks. The diets contained cod liver oil (CLO), corn oil (CO), corn oil and cod liver oil (1:2) (CO + CLO), hyrogenated coconut oil (HCO), ethyl esters of cod liver oil fatty acids (SF) or cod liver oil ethyl esters supplemented with cholesterol (CH). Data on growth and tissue composition imply that oysters have an essential fatty acid (EFA) requirement for both linolenic or ω3 and linoleic or ω6 series fatty acids, with the former playing a more significant role. The feeding study supported by a ^{14}C-acetate metabolism experiment suggests that these oysters were unable to synthesize sterols and excessively high dietary sterol (1% of the dry weight of the diet) inhibited growth. J. Nutr. *110:* 1303–1309, 1980.

INDEXING KEY WORDS oysters · fatty acids · sterols

Attempts to find a food for oysters based on common foodstuffs have met with varying degrees of success. The best non-algal feeds to date have been carbohydrates such as corn starch, corn meal, dextrose and wheat flour as found by Haven and Turgeon [2] (1, 2). This is not surprising since many glycolytic enzymes are present in the oyster's digestive system (3). Growth of oysters fed feeds composed of common foodstuffs (or mixed algal diets) in the laboratory has not approached that of oysters in the wild (4).

Castell and Trider (4) fed oysters artificial diets of known composition made from purified ingredients. Their experimental oysters showed a growth response not only to dietary carbohydrate but also to lipid type (cod liver oil versus corn oil) and level. Other researchers had earlier demonstrated the importance of lipid in early larval development of oysters (5, 6).

Sterol composition and sterol synthesizing ability of marine mollusks have been investigated by Idler and Wiseman (7) and Teshima and Kanazawa (8). They found that cholesterol was a major sterol in oysters and that it could be synthesized from squalene. Reports on oysters have shown that their sterols were at least in part of dietary origin (9, 10). However, it is difficult to draw any conclusions about molluscan sterol metabolism at present since reported results are often conflicting (11–13).

The limited data on bivalve sterol synthesis and lipid requirements, despite the latter's importance in embryonic development, pointed to a need for more information on oyster lipid requirements. This study was initiated to determine these requirements by feeding oysters, *Crassostrea*

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[2] Haven, D. S. & Turgeon, K. W. (1967) Influence of small quantities of corn starch and dextrose in glycogen levels of *Crassostrea virginica*. Proc. Nat. Shellfish Assoc. *58*, 4 (abs.).

TABLE 1

Artificial diets (percent composition by weight)[1,2]

Ingredient	CO	CLO	CO and CLO	HCO	SF	CH
Cornstarch	45	45	45	45	45	45
Casein (vitamin free)	37.8	37.8	37.8	37.8	37.8	37.8
Vitamin mix[3]	2	2	2	2	2	2
Choline chloride (70%)	1 ml	1 ml	1 ml	1 ml	1 ml	1 ml
DL-α-Tocopherol acetate	0.2	0.2	0.2	0.2	0.2	0.2
Corn oil	15	—	5	—	—	—
Cod liver oil	—	15	10	—	—	—
Hydrogenated coconut oil	—	—	—	15	—	—
Cod liver oil ethyl esters[4]	—	—	—	—	15	15
Cholesterol	—	—	—	—	—	1
ω 6 PUFA (%)	61.7	2.3	22.1	0	2.3	2.3
ω 3 PUFA (%)	0.4	22.2	14.9	0	22.2	22.2

[1] Dietary ingredients from Nutritional Biochemical Co., Cleveland, OH, unless stated otherwise. [2] A mineral mix was not included because of results obtained feeding a mineral-free diet in a previous study (Castell and Trider 1972, unpublished data). Oysters fed such a diet grew as well as those receiving a diet containing minerals. This suggests that requirements are satisfied by absorption of dissolved minerals from the surrounding seawater. [3] Thiamin, 0.32%; riboflavin, 0.72%; niacinamide, 2.56%; D-biotin, 0.008%; Na-pantothenate, 1.458%; pyridoxine (HCL), 0.24%; folic acid, 0.967%; menadione, 0.08%; cobalamine, 0.2665%, myo-inositol, 12.5%; ascorbic acid, 6.0%; para-amino benzoic acid, 2.0%; ergocalciferol, 0.0235%; BHA, 0.075%; BHT, 0.075%; retinyl acetate, 0.5%; celite, 73.095%. [4] Purchased from Nuchek Prep., Elysian, MN.

virginica, diets containing either ω6 series polyunsaturated fatty acids (as corn oil), ω3 series polyunsaturated fatty acids (as cod liver oil) or saturated fatty acids (as hydrogenated coconut oil) to indicate possible essential fatty acid requirements. Sterol-supplemented diets and sterol-deficient diets were also fed to determine sterol requirements.

MATERIALS AND METHODS

Oysters, *Crassostrea virginica*, were hatched in May 1973 and raised in 400-liter tanks at the Biological Station in Ellerslie, Prince Edward Island. Spat were collected on concrete-coated wood veneer rings and collectors were suspended from floats so the oysters grew under natural conditions for 1½ years. At this time, 840 of the larger animals were selected, cleaned of fouling organisms, air dried and labeled with a non-water soluble marker. Each animal was weighed and held in unfiltered ambient temperature (5°) for 2 months. The oysters were distributed equally among 14 rearing trays, as previously described (4), each of which was supplied with heated seawater (32°/00, 20°) filtered to 10 μ, at a rate of 0.75 liters/minute. Before entering the trays, water was "degassed" in reservoir tanks to avoid incidence of gas bubble disease (14). Water heated in a closed heat exchanger tends to become supersaturated with air. Excess dissolved gases were removed by agitating the water in the reservoir tanks using aquarium type air stones.

Each of six diets of differing lipid type—corn oil (CO), cod liver oil (CLO), corn oil + cod liver oil, 1:2 (CO + CLO), hydrogenated coconut oil (HCO), sterol free (SF) and sterol supplemented with free cholesterol (CH), (table 1)—were fed to two trays of oysters and two duplicate trays served as unfed controls. The diets were fed as a suspension, 3 g of dry diet in 1 liter of filtered seawater, through ⅛-inch i.d. surgical tubing by means of a peristaltic pump (4). The diet suspensions were dripped into each tray at the water inlet and in the middle, over a 6–7 hour period every day. Diet suspensions were maintained in 1 liter erlenmeyer flasks by magnetic stirrers and no separation of aqueous and lipid phases was noted. The oysters were fed for 30 weeks in a darkened room. Fresh diets were prepared every 8–10 weeks and stored in a freezer

at −20° until fed. Trays and animals were cleaned every 10–14 days and oysters were randomly replaced with their openings facing into the current. Six oysters were randomly sampled from each tray initially and after 4, 10 and 20 weeks of feeding. All surviving animals were killed at 30 weeks. Animals were not fed the day before sampling and shells of all oysters were carefully cleaned prior to analyses.

Growth was measured by comparison of average final whole animal weight (shell plus meat) and meat weight with average initial whole animal weight and meat weight. Tissue analyses for moisture, ash, lipid sterol and glycogen were performed on individual animals, expressed as a percentage of the tissue dry weight and the results averaged. For moisture, tissues were oven dried at 100–110° for 4 hours. Lipid was extracted using the Bligh and Dyer method (15). Sterol was measured in initial and final samples by the method of Momose et al. (16). Glycogen was extracted as described by Good et al. (17) and its concentration measured using the procedure of Montgomery et al. (18).

Data were analyzed by nested analysis of variance techniques and mean values were compared using the Student-Neuman-Keuls multiple range test (19).

^{14}C acetate metabolism. Mantle and gill tissue were removed from two oysters selected from each of four treatments (CLO, SF, CH, unfed) upon termination. These tissues were incubated in separate flasks (Respirometer, Gilson Medical Electronics, Madison, WI) in a 0.1% solution of sodium acetate in autoclaved seawater (20). ^{14}C-acetate (5 mCi) was added directly to the incubating medium and filter paper saturated with 0.1 ml of 10% potassium hydroxide was placed in the flask centerwell to trap respired CO_2. Flasks were incubated at 20–22° and O_2 uptake readings were taken at 30-minute intervals for 4 hours. Tissue metabolism was halted by addition of 1.0 ml of 100% (w/v) trichloroacetic acid from the flask side arm (21). Lipid was extracted from the tissue and separated on an alumina packed column (13, 22). Column fractions and all tissues and solutions were analyzed for radioactivity. Samples were put directly into scintillation vials with 10 ml of scintillation fluid (Aquasol, New England Nuclear, Boston, MA) and placed in a scintillation counter (Tri-Carb, Model 3003, Packard Instruments, Downers Grove, IL).

RESULTS

The importance of unsaturated fatty acids in the diet was clearly shown by the total weight gain (table 2). The unfed (UN) and hydrogenated coconut oil (HCO) fed oysters showed little weight gain after 30 weeks. The diets with high levels of ω3 type fatty acids (CLO) produced significantly greater weights than the one with mainly ω6 type fatty acids (CO). A mixture of ω3 and fatty acids (CO + CLO) was not significantly different from CLO alone. The diets containing cod liver oil ethyl esters, either sterol free (SF) or supplemented with 1% cholesterol (CH), produced significantly poorer growth than natural CLO (fig. 1).

Adaption from 5 to 20° induced shell formation in oysters on all treatments during the first 4 weeks as evidenced by new deposits at shell margins (fig. 2). The UN and HCO-fed oysters both had meat weights significantly lower than those oysters fed a diet containing cod liver oil after 30 weeks. The superiority of combined ω3 and ω6 lipids (CO + CLO) was seen in the greater percent dry weight of oysters fed these lipids. Oysters receiving SF or CH diets had meat weights that were not significantly different from oysters fed a diet containing cod liver oil. The average meat weight of the initial sample was greater because these oysters were larger, 2–4 g, than those used in the study.

The total mortality for all treatments over the experimental period was less than 1% with no mortality-diet correlation.

Biochemical composition. The percent composition of the dry oyster meats in the initiation and termination of the feeding study are given in table 2. The initial dry weight was 12.3±1.0% which indicated that the oysters were in poor (starved) condition when the study commenced. The percent dry weight of the UN group was just as low after 30 weeks. Oysters fed

TABLE 2

Whole animal weight, meat weight and percent composition (dry weight basis) of meats after 30 weeks[1]

Treatment	Total live weight (Shell and meat)		Meat weight	% Dry weight	% Ash	% Glycogen	% Lipid
	Initial	Final					
	g	*g*	*g*				
Initial	—	—	2.5±1.2 (36)	12.3±1.0 (10)	17.6±1.7 (10)	10.6±6.6 (13)	5.6±1.3 (13)
CO	9.8±3.4 (120)[a]	11.6±3.9 (83)[b]	1.3±0.5 (83)[a,b]	18.6±2.3 (24)[b]	10.5±3.4 (24)[a]	41.2±13.9 (24)[a]	6.3±2.1 (6)[a]
CLO	9.9±3.3 (120)[a]	12.9±4.2 (84)[a]	1.6±0.6 (84)[a]	19.7±2.3 (23)[a]	10.1±2.3 (23)[a]	40.1±11.8 (24)[a]	8.3±1.4 (6)[a]
CO + CLO	10.2±3.4 (120)[a]	13.0±4.5 (84)[a]	1.6±0.7 (84)[a]	20.7±5.4 (23)[a]	10.3±2.3 (23)[a]	44.0±13.4 (24)[a]	8.0±1.3 (6)[a]
HCO	9.9±3.3 (102)[a]	10.5±3.6 (82)[c]	1.1±0.5 (82)[b,c]	14.4±1.6 (22)[d]	13.4±4.4 (22)[b]	24.9±15.4 (34)[b]	6.4±2.2 (6)[a]
SF	9.5±3.1 (102)[a]	11.8±3.9 (83)[b]	1.4±0.5 (83)[a,b]	17.0±2.5 (22)[c]	13.3±2.9 (22)[b]	29.5±10.5 (24)[b]	7.2±1.1 (6)[a]
CH	9.8±3.1 (120)[a]	12.0±4.0 (83)[b]	1.4±0.5 (83)[a,b]	17.6±2.3 (23)[a]	10.5±2.1 (24)[a]	27.6±13.4 (24)[b]	8.1±0.9 (6)[a]
UN	9.7±3.8 (120)[a]	9.9±3.9 (83)[c]	0.9±0.4 (83)[c]	12.5±2.2 (24)[e]	18.4±3.6 (24)[c]	9.7±5.9 (24)[c]	5.9±2.9 (6)[a]

[1] Data expressed as Mean±SD; numbers in parenthesis indicate number of animals tested. Means with same superscript were not significantly different ($P \leq 0.05$).

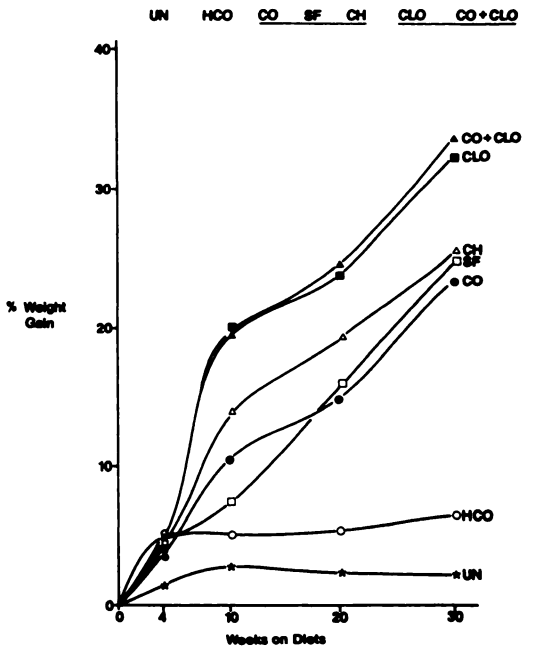

Fig. 1 Average percent weight gain of oysters fed different dietary lipids. Any diets in the inset not joined by a straight line were significantly different at the 95% confidence level. This refers only to analyses at 30 weeks. The average initial weight was 10.7±4.0 g for 36 animals. (UN unfed, HCO hydrogenated coconut oil, CO corn oil, SF sterol free, CH cholesterol supplemented, CLO cod liver oil, CO + CLO corn oil plus cod liver oil, 1:2).

diets containing cod liver oil had percent dry weights significantly higher than the other groups. The percent dry weights of the CH and SF-fed groups, while not significantly different from each other, were lower than the CO-fed group but higher than the UN and HCO-fed animals.

Ash levels were significantly higher in UN group. The ash of animals fed HCO and SF were not significantly different from each other but were significantly higher than other in dietary treatments.

Glycogen content increased in all fed oysters over the experimental period. At 30 weeks oysters receiving diets with corn oil or cod liver oil contained significantly higher glycogen levels (table 2). Both the sterol-deficient and sterol-supplemented diets with ethyl esters of CLO fatty acids produced significantly lower glycogen levels than either CO, CLO or a mixture of the two but not significantly lower than

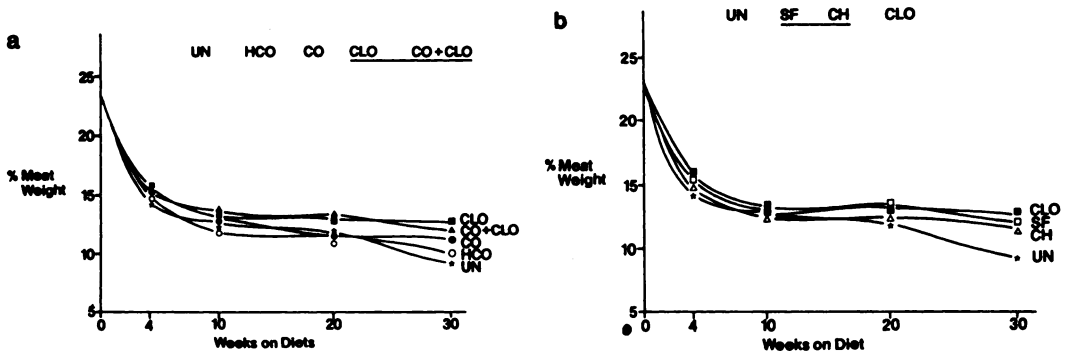

Fig. 2a Comparison of average percent meat weights of oysters fed diets of different fatty acid composition. Any dietary treatments not joined by a straight line were significantly different from each other at the 95% confidence level. This applies only to analyses at 30 weeks. The initial average percent meat weight was 23.2±2.7 for 36 animals. 2b Comparison of average percent meat weights of oysters fed diets of different sterol content. (UN unfed, SF sterol free, CH cholesterol supplemented, CLO cod liver oil).

the HCO-fed animals. The UN group had a glycogen content lower than the initial sample after 30 weeks.

Cod liver oil-fed oysters had the highest lipid levels after 30 weeks but these were not significantly higher than levels in any of the other dietary treatments (table 2). After 30 weeks there were no significant differences among lipid levels of oysters on any of the dietary treatments.

Significant differences among dietary treatments did not appear until after 20–30 weeks of feeding, particularly in meat composition.

The sterol content was not significantly different among any treatments after 30 weeks. The CO, HCO, SF and UN groups had levels of 1.4–1.7 mg sterol per gram of wet meat which was similar to that obtained in the initial sample. Animals fed the CLO, CO + CLO and CH diets had a sterol content of 2.1–2.3 mg sterol per gram of wet meat. No radioactivity was found in the sterol portion of any lipid samples even though 30–40% of the ^{14}C-acetate was metabolized as indicated by the labeled CO_2 recovered. No differences in acetate metabolism were observed among the oysters from the different dietary treatments.

DISCUSSION

It appears that polyunsaturated fatty acids (PUFA) are essential to oysters for growth and maintenance, ω3 more so than ω6 PUFA. HCO produces a limited growth response that may be due either to a lack of essential fatty acids or to poor digestibility since this oil is solid at room temperature. Oysters not receiving ω3 PUFA had higher moisture contents (lower dry weights and lower glycogen weights). High tissue moisture content is associated with EFA deficiency in mammals and trout (23, 24) and lobsters.[3] Engle (25) has stated that glycogen level in oysters is the best index of condition. Since all diets contained the same amount of starch, it seems that PUFA are needed for optimum glycogen production and storage. The presence of both CLO and CO in the diet produced slightly faster growth rates, higher tissue dry weight and glycogen content than CLO alone. Both ω3 and ω6 PUFA are required by some animals like carp (26). Oysters held in storage for 6 months retained 20:4ω6 along with 20:5ω3 at the expense of other fatty acids (27). The retention of both ω6 and ω3 fatty acids as well as growth and composition data suggests that the oyster may also have a dual EFA requirement. The exact level and ratio of ω6 and ω3 fatty acids required by oysters will be the subject of future research.

Oysters fed cod liver oil ethyl esters, CH and SF diets grew better than those fed HCO but not as well as oysters receiving CLO. This suggests that the oysters were

[3] Castell, John D., unpublished results.

able to utilize the PUFA of the ethyl esters but that some nutrient was lacking which was present in whole CLO. Since oysters were not able to synthesize sterol de novo under the present experimental conditions, perhaps the proper amount of sterol was missing from the SF and CH diets. Comparison of the CLO, SF and CH diets indicates that some sterol is required but not as 1% of the diet. It appears that the HCO and UN animals may be selectively retaining sterol when the initial and termination values are compared. Perhaps the 0.1–0.2% sterol naturally present in the CLO diet (28) is nearer the optimum level. Castell et al. (29) have shown that another invertebrate, the lobster (*Homarus americanus*) has an optimum level for dietary sterol and that an excess suppresses growth. Excess dietary cholesterol has also been reported to decrease tissue EFA of other animals (30, 31) which may explain the decrease in lipid of the CH-fed oysters. Although the authors believe oysters have a dietary requirement for sterol at some level below 1% of the dry weight, as implied by the present data, another explanation for the difference between the CLO and SF or CH diets may be that the fatty acids of the ethyl esters may not have satisfied the lipid requirements of oysters as well as natural cod liver oil because they may not be as easily absorbed, or if peroxides were formed in the esters they could have been toxic. Perhaps mono-, di- or triglycerides or other factors (i.e., α-tocopherol) destroyed during esterification are needed.

The initial oyster growth observed in the feeding trial reported above was primarily shell formation as indicated by decreases in meat weight, visible shell growth and increases in total weight. This may be in response to the increase in water temperature, 5–20° (32), or to the annual growth cycle proposed by Walne (33) where new shell forms first, followed by spawning and then tissue synthesis. There was no indication of spawning observed in any of the experimental oysters. Shell growth may also have been stimulated by the prolonged periods of darkness (34).

This study has shown that oysters require PUFA in their diet. Oysters appear to have a major requirement for ω3 and a possible minor requirement for ω6 PUFA. Under the present experimental conditions, oysters were not able to synthesize sterols. This work has opened several areas for further research such as the level of lipid required by oysters, the optimum ratio of ω3/ω6 PUFA, level of sterol required, ethyl esters versus mono-, di-, triglycerides, etc. Microencapsulation techniques, such as those developed by Jones et al. (35), may prove beneficial in future work to overcome some of the problems encountered feeding artificial diets to oysters.

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LITERATURE CITED

1. Dunathan, J. P., Ingle, R. M. & Havens, W. K. Jr. (1969) Effects of artificial foods upon oyster fattening with potential commercial applications. Fla. Dept. Nat. Res. Mar. Res. Lab. Ser. *58*, 1–39.
2. Willis, S. A., Havens, W. K. & Ingle, R. M. (1976) Quality improvement of oysters, *Crassostrea virginica* (Gmelin), using artificial foods. Fla. Marine Res. Publications No. *20*, 16p.
3. Galtsoff, P. J. (1964) The American oyster, *Crassostrea virginica* Gmelin. Fish. Bull. U.S. Fish Wildlife Ser. *64*, 340p.
4. Castell, J. D. & Trider, D. J. (1974) Preliminary feeding trials using artificial diets to study the nutritional requirements of oysters (*Crassostrea virginica*). J. Fish. Res. Board Can. *31*(1), 95–55.
5. Helm, M. M., Holland, D. L. & Stephenson, R. R. (1973) The effect of supplementary algal feeding of a hatchery breeding stock of *Ostrea edulis* L. on larval vigour. J. Mar. Biol. Ass. U.K. *53*, 673–684.
6. Holland, D. L. & Spencer, B. E. (1973) Biochemical changes in fed and starved oysters, *Ostrea edulis* during larval development, metamorphosis and early spat growth. J. Mar. Biol. Ass. U.K. *53*, 287–298.
7. Idler, D. R. & Wiseman, P. (1972) Molluscan sterols: a review. J. Fish. Res. Board Can. *29*, 385–398.
8. Teshima, S. & Kanawawa, A. (1972) Comparative study on the sterol composition of marine mollusks. Bull. Jap. Soc. Sci. Fish *38*(11), 1299–1304.
9. Tamura, R., Truscott, B. & Idler, D. R. (1964) Sterol metabolism in the oyster. J. Fish. Res. Board Can. *21*(6), 1519–1522.

10. Salaque, A., Barbier, M. & Lederer, E. (1966) Sur la biosynthèse des stérols de l'hûitre (*Ostrea gryphea*) et de l'oursin (*Paracentrotus lividus*). Comp. Biochem. Physiol. *19*, 45–51.
11. Teshima, S. & Kanazawa, A. (1973) Biosynthesis of sterols from demosterol in a mussel, *Mytilus edulis*. Bull. Jap. Soc. Sci. Fish. *39*(12), 1309–1314.
12. Teshima, S. & Kanazawa, A. (1974) Biosynthesis of sterols in abalone, *Haliotis gurneri*, and mussel, *Mytilus edulis*. Comp. Biochem. Physiol. *47B*, 555–561.
13. Walton, M. J. & Pennock, J. F. (1972) Some studies on the biosynthesis of ubiquinone, isoprenoid alcohols, squalene and sterols by marine invertebrates. Biochem. J. *127*, 471–479.
14. Malouf, R., Keck, R., Maruer, D. & Epifanio, C. (1972) Occurrence of gas-bubble disease in three species of bivalve molluscs. J. Fish. Res. Board Can. *29*, 588–589.
15. Bligh, E. G. & Dyer, W. J. (1959) A rapid method of total lipid extraction and purification. Can. J. Biochem. Physiol. *37*, 911–917.
16. Momose, R., Ueda, Y., Yamamoto, K., Masumura, T. & Ohta, K. (1963) Determination of total cholesterol in blood serum with perchloric acid-phosphoric acid-ferric chloride reagent. Anal. Chem. *35*(11), 1751–1753.
17. Good, C. A., Kramer, H., & Somogyi, M. (1933) The determination of glycogen. J. Biol. Chem. *100*, 485–491.
18. Montgomery, R. (1957) Determination of glycogen. Arch. Biochem. Biophys. *67*, 378–386.
19. Sokal, R. R. & Rolf, F. J. (1969) Biometry, W. H. Freeman & Co., San Francisco & London.
20. Simpson, J. W. & Awapara, J. (1966) The pathway of glucose degradation in some invertebrates. Comp. Biochem. Physiol. *18*, 57–548.
21. Hammen, C. S. & Wilbur, K. M. (1959) Carbon dioxide fixation in marine invertebrates. I. The main pathway in the oyster. J. Biol. Chem. *234*, 1268–1271.
22. Teshima, S. & Kanazawa, A. (1971a) Sterol compositions of marine crustaceans. Bull. Jap. Soc. Sci. Fish. *37*(1), 63–67.
23. Holman, R. F. (1968) Essential fatty acid deficiency. Progress Chem. Fats and Other Lipids *9*(2), 275–348.
24. Castell, J. D., Sinnhuber, R. O., Lee, D. J. & Wales, J. H. (1972) Essential fatty acids in the diet of rainbow trout (*Salmo gairdneri*): physiological symptoms of EFA deficiency. J. Nutr. *102*, 87–92.
25. Engle, J. B. (1950) The condition of oysters as measured by the carbohydrate cycle, condition factor and percentage dry weight. Natl. Shellfisheries Assoc. Conv. Add. 20–25.
26. Watanabe, T., Takeuchi, T. & Ogino, C. (1975) Effect of dietary methyl linoleate and linolenate on growth of carp-II. Bull. Jap. Soc. Sci. Fish. *41*, 263–269.
27. Watanabe, T. & Ackman, R. G. (1977) Effects of storage on lipids and fatty acids of oysters. Can. Inst. Food Sci. Technol. J. *10*, (1), 40–42.
28. Bailey, B. E., ed. (1952) Marine oils with particular reference to those of Canada. Fish. Res. Bd. Canada Bull. *89*, 335p.
29. Castell, J. D., Mason, E. G. & Covey, J. F. (1975) Cholesterol requirements of juvenile American lobsters (*Homarus americanus*). J. Fish. Res. Bd. Canada *32*(8), 1431–1435.
30. Morin, R. J., Bernick, S., Mead, J. F. & Alfin-Slater, R. G. (1962) The influence of exogenous cholesterol on hepatic liver composition of the rat. J. Lipid Research *3*, 432–438.
31. Alfin-Slater, R. B. & Aftergood, L. (1968) Essential fatty acids reinvestigated. Physiol. Rev. *48*(4), 758–784.
32. Yonge, C. M. (1960) Oysters, William Brothers and Haram Ltd. Birkenhead for Collins Clear-Type Press, London and Glasgow.
33. Walne, P. R. (1970b) The seasonal variation of meat and glycogen content of seven populations of oysters *Ostrea edulis* L. and a review of the literature. Fishery Investigations Ser. III *26*(3), 1–35.
34. Medcof, J. C. & Kerswill, C. J. (1965) Effects of light on growth of oysters, mussels and quahaugs. J. Fish. Res. Board Can. *22*(2), 281–288.
35. Jones, D. A., Munford, J. G. & Gabbot, P. A. (1974) Microcapsules as artificial food particles for aquatic filter feeders. Nature *247*, 233–234.