Food Values of New Zealand Fish.

Part 10.—Seasonal Variations in Stewart Island Oysters.

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IN previous communications (1) it has been shown that the percentage of glycogen in these oysters falls at the time of spawning, and that the vitamin-A content is high but variable—thus the samples examined in June, 1926, were less rich in that respect than the October sample of the same year.

In the present research an attempt was made to follow the variations in chemical composition and in vitamin-A throughout the oyster season of 1927—March to October inclusive—and to find out whether there was any relationship between the data obtained such as has been found to exist between the fat percentage and the vitamin-A content of ordinary fishes. In contrast to the fishes, oysters and other molluscs store glycogen instead of fat, and what is usually termed a " fat " oyster is one rich in glycogen (Mitchell, (2)).

The project enlisted the interest and support of the Fisheries Department, and the writer has to thank Mr. A. E. Hefford, Chief Inspector of Fisheries, for facilitating the collection of samples, and for other help. The samples were taken monthly by Messrs. Dixon Bros. (Bluff) from a known area. A half-sack of the oysters was forwarded as expeditiously as possible to Dunedin, and was received usually within twenty-four hours of being dredged. For the first few months they were examined, sorted out, and measured by Mr. Maxwell Young, Marine Biologist to the Fisheries Department. Later samples were similarly treated by Mr. H. C. Manson, Chief Laboratory Assistant to the Physiology Department, acting under Mr. Maxwell Young's directions. The contents of the alimentary canal from June to October were examined by Dr. Harold J. Finlay; in practically all cases the canal was empty. Observations on seasonal variation in the composition of oysters in England and in America have been published in various reports and journals (3). No similar work on seasonal variation of the vitamins in oysters has come under my notice, but the subject is referred to in a comprehensive paper on the vitamins (A, B, D) in American oysters by Jones, Murphy, and Nelson (4) which appeared in February of this year.

CHEMICAL COMPOSITION.

Methods: From each monthly half-sackful, 250 oysters of approximately uniform size (2½ inches across), and average condition, were selected after being opened, were strained lightly in cheese cloth, weighed, minced, and the material so obtained was well mixed and samples were taken for water percentage and ash, glycogen, nitro-

gen, and fat. For the vitamin work six samples of 100 grm. each were put into cardboard containers and kept in cold storage till required.

The water percentage was obtained by drying in a hot air oven at about 100°C till approximately constant weight was attained. The same material was then incinerated to obtain the ash. At the end of the first stage of combustion a hot-water extract was made, filtered, evaporated, re-incinerated and weighed to give "soluble ash" separately from "total ash." Nitrogen was estimated by the Kjeldahl-Gunning method and the writer has to thank Mr. B. S. Irwin, B.Med. Sc., for carrying out these determinations. Glycogen was estimated in two samples of 25 grm. each by Pflueger's method, and fat by drying similar quantities, mixed with clean sand, grinding in a mortar and extracting with ether by the usual Soxhlet method—care being taken to use thoroughly dried ether to redissolve the extract before finally weighing.

The results of these estimations are given in Tables 1 and 2.

TABLE 1.—COMPOSITION OF OYSTERS.

	March	April	May	June	July	Aug.	Sept.	Oct.
Average weight	9.0g.	8.0g.	8.6g.	9.65g.	10.0g.	10.01g	12.1g.	11.45g
Water per cent....	74.00	74.40	75.73	77.08	76.50	76.13	76.04	76.11
Solids ,, ...	26.00	25.60	24.27	22.92	23.50	23.87	23.96	23.89
Nitrogen ,, ...	1.87	2.22	2.04	2.06	2.02	2.04	1.91	2.06
"Protein" % (N. × 6.25)...	11.68	13.87	12.78	12.91	12.62	12.75	11.93	12.87
Glycogen per cent.	6.71	6.39	5.07	4.69	4.92	4.69	5.22	4.36
"Fat" % (Ether Ext.) ...	2.66	2.40	2.15	2.21	2.28	2.10	2.16	1.91
Total Ash per cent.	1.48	(1.65)	1.94	2.03	2.50	2.03	2.03	2.03
Soluble Ash ,,	0.60	...	0.85	1.06	1.67	1.14	1.20	1.14
Insoluble Ash ,,	0.88	...	1.09	0.97	0.83	0.89	0.83	0.89
Unaccounted for ,,	3.47	(1.30)	2,33	1.08	1.18	2.30	2.62	2.72
Solids per oyster ,,	2.34g.	2.05g.	2.08g.	2.21g.	2.35g.	2.40g.	2.88g.	2.73g

TABLE 2.—PERCENTAGE DISTRIBUTION OF THE ORGANIC SOLIDS.

	March	April	May	June	July	Aug.	Sept.	Oct.
"Protein"	47.63	57.87	57.23	61.80	60.09	58.38	54.40	58.87
Glycogen	27.36	26.67	22.69	22.45	23.41	21.47	23.80	19.94
"Fat"	10.85	10.01	9.62	10.58	10.85	9.61	9.85	8.74
Residue	14.15	4.42	10.43	5.17	5.62	10.53	11.95	12.44

It will be noticed that there is at first a decrease in the average weight and in the solids per oyster. From May onwards these figures increase up to September. This may mean that these oysters continue to feed and grow during the winter months. On the other hand, the result may be due to the trawlers working along the oyster bed as will be mentioned later. That the September oysters were heavier than the October ones leads one to suspect that they were dredged from different ground.

The glycogen shows a more or less progressive fall but not to the same marked extent at the end of the season as was found in

previous work. Possibly the loss of spawn in the previous cases might account for the difference, for the 1927 oysters were late in spawning. The " fat " and " protein " (N x 6.25) show no significant variations—the fat in the September oysters (2.16% gross or 9.85% of the organic solids) is an average figure whereas the vitamin-A content of these oysters was higher than that of any of the others. From the point of view of chemical composition, the food-value of these oysters is much the same throughout the season, and the data supplied here do not indicate the advisability of any change in the limits of the season as at present defined.

Vitamin-A.

Methods: Litters of young rats reared in the laboratory were put on the basal diet when about three weeks old, and were weaned at the 28th day. They were then continued on the diet which contained purified casein, starch, " crisco," and salts as described in previous papers of this series. Vitamin-B was supplied in the form of " Marmite " and vitamin-D as oxidized cod-liver oil (2%). When the weights began to fall and eye symptoms began to appear, the oyster material was given. The form in which this was used requires special mention. When minced as already described the " mush " contained small lumps of adductor muscle, gills, etc., and it was obvious that some method of more uniform comminution was necessary. In the work done on oyster in 1926 (Paper 8) and on toheroa in 1927 (Paper 9 of this series (5)) the minced shellfish was incorporated with the constituents of the basal diet so as to form a stiff paste or leaven which, when partially dried, could be ground to a fine uniform meal. When so treated there was still clear evidence of the presence of vitamin-A, and in the earlier experiments reported here the same method was adopted. The oyster leaven was brought to such a degree of dryness that it corresponded weight for weight with the fresh oyster used, so that 1 grm. meal corresponded to 1 grm. fresh oyster. But while this method gave uniform sampling it allowed some destruction of vitamin to occur, probably to an uncertain and variable extent. The best results were obtained in the last series of experiments where the leaven prepared as described above was thoroughly pounded in a mortar, and weighed out in quantities sufficient for ten days. These rations were then packed closely in large clean test-tubes and sterilized in the boiling-water bath. Although opened daily to secure the daily feed, they kept clear of moulds for the whole ten-day period. In every case the oyster material was given for ten successive days, and the cage was not cleaned out for at least ten days thereafter, so that any vitamin-A in the faeces could be used over again by the rats. During this after-period they received the usual basal diet till death occurred.

In the earlier experiments as many as three or four rats were included in a group and fed together in the same cage. While this has the advantage of giving the average effect on several rats and of saving time and labour, there is always the possibility that the vitamin ration may not be equally divided. In the later experiments with the oyster " leaven " each rat was taken out of its cage and fed separately.

For ease of comparison the data are presented in tabular form rather than as charts. (Tables 3, 4, 5). In these tables some of the columns require a word of explanation. The designation of each rat is made according to the following method: The first letter indicates the year in which the litter was used, B = 1927 in this case; the second letter indicates a certain litter born and used that year; the Roman numerals I, II, etc. indicate the groups into which that litter was subdivided. When necessary the individual rat in a group received

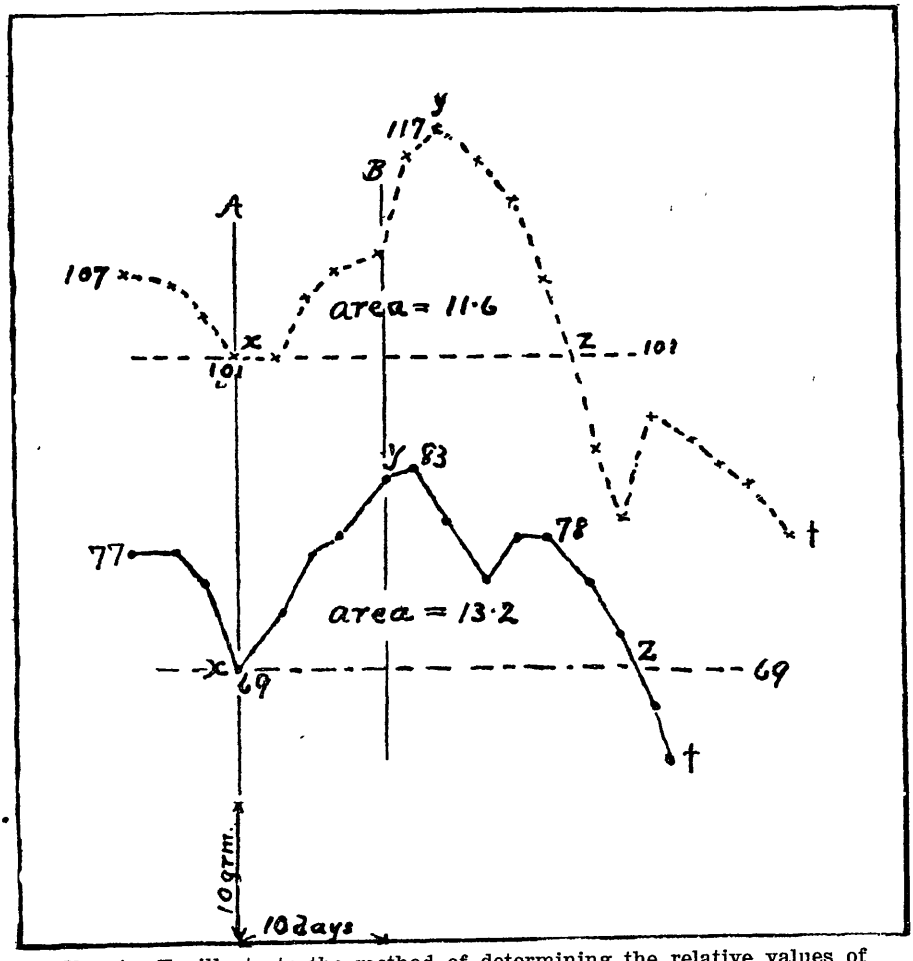

FIG. 1.—To illustrate the method of determining the relative values of effects on growth; XYZ = the "area of growth."

a number, 1, 2, etc. The second column gives the rise in weight, i.e. the difference between the weight when the special dieting began and the maximum attained during the feeding or soon after it ceased. For greater accuracy the actual weights are given in brackets. The "area of growth" in the third column was obtained by measurement of the growth-curves as follows: As stated already, after the ten

days of oyster feeding the rats were kept on basal diet till they died. Before death the weights fell usually to a lower point than that at which the feeding began. A horizontal line was drawn across the curve at this level and the included area was measured carefully in sq. cm. by a planimeter such as surveyors use for evaluation of areas on maps.* Figure 1 gives an example of this procedure. Rats Bu IV were given a meal containing oyster-spawn for ten days beginning at the point x; the maximum weight was at y, and at z the growth curve is back at the same level as x. The " area of growth " is the roughly triangular area xyz and in these cases measured 11.6 and 13.2 sq. cm. on the original chart. The advantage of this figure is that it indicates to some extent the amount of storage of vitamin-A that the rat was able to accomplish.

It is not claimed that this method can give a true mathematical measure of the amount of vitamin present in foodstuffs, but it gives a figure that can be used for comparative purposes.

The next column " Prolongation of life " gives in days the duration of life after the oyster feeding began. In the control groups (total = 30 rats) this figure averaged 20 days, and it will be noticed that the oyster feeding caused a distinct prolongation of life beyond this period whether growth had occurred or not. This figure may be regarded as an indication of a " maintenance " factor just as the " growth area" shows "growth + maintenance" factors.

The columns that refer to eye-symptoms need no further explanation, but note may be made of the fact that almost every case showed eye-trouble. That has been our experience in this laboratory during the past three years.

Comments on the Tables: Table 3 gives the results of *individual* feeding on *undried* oyster; this series was carried out late in the year, when all the samples had been received, and this made possible a certain amount of *overlapping,* i.e., the groups of one litter were given oyster material collected in different months. For these reasons the results are probably more reliable for comparative purposes than the others, and this method would have been adopted to a greater extent if the samples had all been available simultaneously, and if the best level of doseage had been known, for a great many experiments were done on the March, April, and May oysters at various levels ranging from 0.5 grm. to 3.0 grms. in order to determine the best level, viz., a dose that lay between the minimal and the just maximal. In the litter Bs three pairs (buck and doe) were fed March, May, and July oyster respectively. The area of growth and the prolongation of life indicate progressive falling off in vitamin-A content as the season advanced.

In litter Bt two pairs similarly received June and August oysters. and again there is evidence of less vitamin-A in August as compared to the earlier month, but it will be noticed Bt I showed a better result with June oyster than Bs III did on May oyster. This is probably due to a difference in the quality of the litter and not to a greater content of vitamin-A in the June as compared to the May oyster.

*I am indebted to my colleague Professor James Park, Director of the Otago School of Mines, for kindly giving me the loan of a planimeter, and for instruction in its use.

TABLE 3.

Rat.	Rise in weight.	"Area" of Growth.	Prolongation of life.	Initial State of Eyes.	Effect on Eyes.	REMARKS.
	grms.	*sq. cm.*	*days.*			
Bs I buck	33 (95-128)	69.6	56	slight	cured	*March* oyster as leaven.
Bs I doe	9 (90-99)	11.7	47	slight	cured	2 grms. each.
Bs III buck	23 (100-123)	34.2	50	marked	cured	*May* oyster as leaven.
Bs III doe	10 (85-95)	14.0	44	marked	almost cured	2 grms. each.
Bu II buck	20 (85-105)	29.2	38	marked	improved	
Bu II doe	16 (79-95)	26.5	57	marked	improved	
Bt I buck	28 (95-123)	66.7	57	marked	cured	*June* oyster as leaven.
Bt I doe	25 (72-97)	34.2	35	slight	cured	2 grms. each.
Bs II buck	no growth	—	33	marked	no cure	*July* oyster as leaven.
Bs II doe	no growth	—	26	slight	passed off	2 grms. each.
Bt II buck	26 (90-116)	33.5	38	marked	much improved	*August* oyster as leaven.
Bt II doe	12 (73-85)	10.9	43	slight	cured	2 grms. each.
Bv I buck	27 (92-119)	58.7	53	marked	cured	*September* oyster as leaven.
Bv I doe	19 (83-102)	37.0	56	slight	cured	1 grm. each.
Bw IV buck$_1$	31 (125-156)	51.6	47	marked	cured	*October* oyster as leaven.
Bw IV buck$_2$	24 (116-140)	36.0	37	marked	cured	1 grm. each.
Bu III buck	49 (77-121)	136.5	65	marked	cured	Tinned oyster as leaven.
Bu III doe	31 (74-105)	93.0	74	marked	cured	2 grms. each.

TABLE 4.

Rat.	Rise in weight.	"Area" of Growth.	Prolongation of life.	Initial State of Eyes	Effect on Eyes.	REMARKS.
	grms.	*sq. cm.*	*days.*			
Ba I buck$_1$	23 (87-110)	23.5	39	marked	almost cured	*March* oyster meal:
Ba I buck$_2$	22 (103-125)	33.8	50	slight	cured	4 grms. to two rats.
Bb IV doe$_1$	16 (76-92)	16.1	36	slight	cured	*April* oyster meal:
Bb IV doe$_2$	13 (81-94)	16.3	36	slight	cured	6 grms. to three rats.
Bb IV doe$_3$	15 (72-87)	16.0	40	slight	cured	
Be II buck$_1$	33 (80-113)	67.2	59	slight	cured	*May* oyster meal:
Be II doe$_2$	22 (71-93)	40.3	40	slight	cured	6 grms. to three rats.
Be II doe$_3$	18 (69-87)	23.1	33	slight	cured	
Bj I buck$_1$	11 (75-86)	9.8	?	moderate	improved	*June* oyster meal:
Bj I buck$_2$	14 (72-86)	17.4	?	moderate	improved	4 grms. to two rats.
Bu II buck$_1$	19 (85-104)	23.5	40	moderate	cured	*July* oyster meal:
Bu II buck$_2$	19 (75-94)	24.0	41	moderate	cured	4 grms. to two rats.
Bu I buck$_1$	5 (87-92)	—	26	marked	slightly improved	*August* oyster meal:
Bu I buck$_2$	4 (71-75)	—	26	marked	slightly improved	4 grms. to two rats.
Bq IV buck$_1$	55 (89-144)	134.8	65	moderate	nearly cured	*September* oyster meal:
Bq IV buck$_2$	48 (77-125)	131.4	66	moderate	cured	6 grms. to three rats.
Bq IV buck$_3$	41 (79-120)	104.2	58	slight	cured	
Bt III buck	19 (90-109)	28.9	42	moderate	nearly cured	*October* oyster meal:
Bt III doe	17 (81-98)	31.8	56	moderate	nearly cured	4 grms. to two rats.
Bt IV buck	13 (97-110)	19.5	44	moderate	cured	October oyster meal after spawn removed:
Bt IV doe	14 (67-81)	22.1	44	moderate	improved	4 grms. to two rats.

TABLE 5.

Rat.	Rise in weight.	"Area" of Growth.	Prolongation of life.	Initial State of Eyes	Effect on Eyes.	REMARKS.
	grms.	*sq. cm*	*days.*			
Ba I buck$_1$	24 (92-116)	23.6	?	moderate	almost cured	*March* oyster meal: 2 grms. to two rats, later increased to 4 grms. (See Table 4).
Ba I buck$_2$	13 (82-95)	8.0	?	moderate	cured	
Ba IV buck$_1$	15 (102-117)	16.4	46	slight	almost cured	*March* oyster meal: 2 grms. to two rats, reduced to 1 grm. after 4 days.
Ba IV buck$_2$	17 (93-110)	27.0	59	slight	almost cured	
Ba II buck$_3$	no growth	—	32	slight	improved	*April* oyster meal: 2 grms. to two rats, reduced to 1 grm. after 4 days.
Ba II buck$_4$	no growth	—	37	slight	improved	
Ba I buck$_1$	20 (81-101)	35.7	47	moderate	cured	*May* oyster meal: 3 grms. to three rats.
Be I buck$_2$	19 (80-99)	19.0	40	moderate	cured	
Be I buck$_3$	31 (82-113)	56.5	47	moderate	cured	
Bg II doe$_1$	10 (66-76)	11.4	34	slight	cured	*May* oyster meal: 4 grms. to four rats.
Bg II doe$_2$	11 (67-78)	21.1	54	slight	cured	
Bg II doe$_3$	11 (64-75)	3.2	51	slight	cured	
Bg II doe$_4$	9 (65-74)	11.5	54	slight	cured	
Bk I buck$_1$	no increase			slight	no cure	*June* oyster meal: 2 grms. to two rats.
Bk I buck$_2$	7 (81-88)	6.5	32	marked	no cure	
Bk II doe$_1$	14 (59-73)	10.2	?	slight	improved	*June* oyster meal: 3 grms. to three rats.
Bk II doe$_2$	12 (62-74)	15.1	?	moderate	improved	
Bk II doe$_3$	23 (66-89)	14.4	?	marked	nearly cured	
Bl I buck$_1$	18 (66-84)	20.8	48	moderate	cured	*July* oyster meal: 3 grms. to three rats.
Bl I buck$_2$	12 (61-73)	17.0	46	moderate	cured	
Bl I buck$_3$	16 (73-89)	22.2	56	moderate	cured	
Bm I buck$_2$	15 (58-73)	27.3	39	moderate	slight improvement	*August* oyster meal: 2 grms. to two rats.
Bm I buck$_4$	10 (70-89)	18.2	34	moderate	slight improvement	
Bq II buck	40 (82-122)	56.9	50	marked	nearly cured	*September* oyster meal: 2 grms. to two rats.
Bq II doe	10 (67-77)	11.3	50	moderate	nearly cured	
Bw IV buck$_3$	14 (132-146)	16.9	42	marked	cured	*October* oyster meal: 2 grms. to two rats.
Bw IV buck$_4$	22 (121-143)	28.7	53	marked	improved	

The only other striking feature is the marked improvement in the September and October oysters as compared to the July and August ones. At the 1 grm. level these are nearly as rich as any of the others at the 2 grm. level—this is shown by the "area of growth," by the prolongation of life, and the effect on the eye-symptoms, and similar results can be seen in Tables 4 and 5 when dried material was used.

In Tables 4 and 5 the only groups that "overlap" are Bu II on July oysters and Bu I on August oysters, where again the former proved the better sample, and Bt III on unspawned October oyster and Bt IV spawned oyster where weight for weight there was surprisingly little difference. There is clearly a tendency to low results in June, July, and August with a fairly high figure for March and May and a very high figure for September.

Tinned Oysters: A sample of tinned Stewart Island oysters was also examined. In making the leaven, care was taken to make the oyster solids correspond to the average of the fresh oyster. As in the case of the tinned toheroa the vitamin content was very high; thus the two rats Bu III (Table 3) fed on this material showed a much better result than their litter-mates, Bu II, fed on the same amount of May oysters. The canning of these oysters was said to have been carried out late in the season of 1926 but the exact date could not be ascertained.

Discussion: While these experiments show that when fed at the 2 grm. level these Stewart Island oysters nearly always show a high content of vitamin-A, the writer feels that the results of comparison of the oysters from month to month leaves much to be desired. If the comparison is again undertaken he would suggest that more of the "overlapping" method should be followed and that the method of comminution adopted by Jones, Murphy, and Nelson (4) should be followed—(grinding while frozen) as probably better than either the "leaven" or the "meal." As stated at the beginning of this paper it was hoped that some relationship between the gross chemical composition and the vitamin-A content might be found, but apparently such is not the case. The September oysters, though rich in vitamin, show no special peculiarity in composition, except for the fact that they were the heaviest of all. There are two likely sources of variation in the content of vitamin-A that occur to one—firstly, the oysters' food—secondly, the presence of the spawn: in regard to the first, September is the Spring in this, the Southern, Hemisphere, and with an increase in the intensity of sunlight there is likely to be a more or less rapid increase in the plankton on which the oysters feed, but in the same connection one has to remember that the successive samples could not be dredged always from exactly the same ground. The trawlers usually work the ground from east to west and there is said to be a considerable difference in the nature of the bottom and therefore probably of the food between the two extremes of the shoal.

Secondly, as to the growth of the spawn—this occurs steadily all through the winter up to the time of spawning (end of October). At this time there is a drop in the percentage of glycogen, but the vitamin content of oysters ripe for spawning and washed free of the

spawn is still a high figure (Table 4, Bt IV, last on table). The spawn also contains vitamin-A as shown by the following observation: the washings of the later October oysters was filtered and the spawn made into a meal and partially dried. Although the temperature during drying was kept low, the material browned considerably (presence of lecithin, iron salts, etc.). Two rats of litter Bu showed increase of weight—16 grm. (101-117) and 14 grm. (69-83), growth "areas" of 11.6 and 13.2 sq. cm. (Fig. 1) and prolongation of life = 37 and 30 days. Their eye-symptoms were far advanced in one case and moderate in the other when treatment began, but in the ten day period they improved greatly and if the feeding had been continued would probably have been cured. There is therefore clear evidence that both the spawn and parent oyster after losing the spawn contain considerable amounts of vitamin-A. This agrees with the results of the experiments on spawning and non-spawning oysters reported in part 8 of this series, where, weight for weight, the latter had more vitamin-A than the former.

With the growth of the spawn in the latter part of the season it is therefore natural to expect an increase in the vitamin-A content, but that the food of the oyster is in the end the more important factor is shown by the relative superiority of the March oysters, and the sudden rise in this value in the September oyster is more likely to be due to the food than to a sudden increase in the power of the spawn to store the vitamin.

SUMMARY.

Analyses of these oysters monthly from March to late in October show a high glycogen content early in the season and a more or less gradual fall up to October. Fat, protein, and ash showed comparatively little variation. The average weight of the oyster (soft parts) in oysters of approximately the same shell size increased during the season. The vitamin-A content was lower in the winter months of June, July and August than in March to May and showed a marked increase in September. No clear relationship was found between chemical composition and vitamin-A value. Both the spawn and the spawned oyster contain considerable amounts of vitamin-A as do also tinned oysters. A new method for expressing the value of the growth curves is described.

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