

**FISHERIES RESEARCH BOARD
OF CANADA**

MANUSCRIPT REPORTS OF THE BIOLOGICAL STATIONS

No. 452

Title

The Chemistry of Fish Flesh
A Bibliography

Author

Lloyd H. Almy

1952

FISHERIES RESEARCH BOARD
OF CANADA

MANAGEMENT BOARD OF THE BUREAU OF FISHERIES



RESEARCH BOARD

OF CANADA

MANAGEMENT BOARD

The
CHEMISTRY
of
FISH FLESH

A BIBLIOGRAPHY

Submitted in Partial Fulfilment of
the Requirements for the Degree
of Master of Science at

The George Washington University

by

Lloyd H. Alay

Washington, D. C.

June, 1915.

CONTENTS

THE CHEMISTRY OF FISH	I.	Page 7
Introductory		
Chapter I		
Moisture, Fat, Protein and Ash Content of Fish Flesh		8
Food components of fresh fish		
Food components of cooked fish		
Loss of substances on cooking and drying fish		
The chemical composition of fish proteins		
Analytical constants of fish oils		
Ash constituents of fish flesh		
Chapter II		
Lesser important constituents of fish flesh		20
Purine bases		
Amino acids		
Creatin, Creatinin		
Isocreatinin		
Methyl, guanidin, Cholin, Neurin		
Muscarin, Trimethylamine oxide, Betaine, Carnin		
Carnosin, Lecithin		
Urea		
Inosinic acid, Taurin, Lactic acid, Glycogen		
Unknown compounds, Philothion		
Chapter III		
Some forms of preserved fish		26a
Frozen fish		
Marinated (herring), slated, pickled, broiled fish		
Canning of fish, fish sausage		
Fish meal and similar preparations		
Chapter IV		
The digestibility of fish flesh		32
Artificial digestion by means of pepsin and trypsin		
Metabolism experiments with fish		
Chapter V		
Putrefaction bases of fish flesh		34

Chapter VI

A consideration of fish waste	35
Fertilizer	
Feed for farm animals	
Fish glue and gelatine	
Fish oils	
Fish liver oils	
THE CHEMISTRY OF FISH II	
The chemical physiology of fish	42
The respiratory exchange of fish	42
Some conditions of water which affect fish	45
Changes in the density of water and their effects upon fish	47
Metabolism of fish	49
Differences in the body composition of fish	52
Parasites of fish	55
BIBLIOGRAPHY	56

THE CHEMISTRY OF FISH FLESH

Chapter I

-Introduction-

The value and importance of the fishing grounds of America in the vicinity of Newfoundland were recognized as early as 1504, when the fishermen of France began sending vessels to our shores and they still continue the practice after more than four centuries. According to McFarland (2), before the year 1522, houses had been erected for the accommodation of fishermen on Newfoundland. Local traditions have laid claim to the discovery of the banks of Newfoundland by the fishermen of Normandy and Brittany before 1492, but there is no positive evidence that these early voyages were really made.

The interest manifested in the fishing industry in the region of the Gulf of Maine increased steadily and resulted in the establishment of settlements along the New England coast, which were devoted almost entirely to this purpose. The town of Gloucester, Massachusetts, was founded in 1623 for the main purpose of conducting fishing operations. The state of Massachusetts has always been the largest single contributor of the fish products in the United States. The value of these in 1908 constituted 13% of the total, Alaska excepted.

A summary of statistics of the fisheries of the United States and Alaska for 1908 (6), and of Alaska for 1913 (7), follows.

Unfortunately, no complete statistics of the fisheries of the United States and Alaska have been compiled since 1908.

Table No. 1 SUMMARY OF STATISTICS

The General Statistics of Fisheries for the United States and for the Five Divisions

	Total	Atlantic coast division	Gulf of Mexico division	Pacific coast division	Humboldt River division	Great Lakes division
Number of persons employed	143,880	94,981	15,481	13,855	11,793	8,533
Capital	\$42,321,000	\$25,798,000	\$3,903,000	\$6,468,000	\$1,440,000	\$4,812,000
Vessels and boats including outfit	35,103,000	18,953,000	2,805,000	3,844,000	547,000	1,851,000
apparatus	6,999,000	3,822,000	574,000	2,549,000	514,000	1,831,000
shore and accessory property and cash	7,823,000	1,023,000	523,000	465,000	399,000	1,332,000
Value of products	54,030,000	35,476,000	4,825,000	6,879,000	3,125,000	3,767,000

Table No. 2 PRODUCTS OF ALASKA FISHERIES IN 1904

Southeast Alaska		Central Alaska		Western Alaska		Arctic Alaska		Total Value
Pounds	Value	Pounds	Value	Pounds	Value	Pounds	Value	
89,835,668	1,856,512	36,889,750	2,105,741	89,821,524	9,895,568	67,173	209,892	11,867,443

It is seen that the value of the total fish products of the United States, exclusive of Alaska, very nearly equals that of England, and far exceeds those of the other countries.

Canada supplied the greatest value of imports of fishery products into this country, contributing 37% of the total in 1908. Imports from Europe furnished 54% of the value of the total imports in 1908. Lack of import duty on fish now make these figures of little comparative value under present conditions. The European country which leads in the importation of these products, the United Kingdom, supplied imports valued at 17% of the total value of our imports of fishery products in 1908. The following table gives the statistics for 1913, (5):-

Table No. 5

FISH IMPORTS AND EXPORTS OF THE UNITED STATES IN 1913

Items	Imports		Exports	
	Quantity	Value \$	Quantity	Value \$
Fresh--Salmon and all others . . . pounds . .	52,968,301	2,331,822		
Cured or Preserved: Cod, Haddock, Hake and Pollock . . . pounds . .	29,761,996	1,646,073		
(dried, smoked, slated, or pickled)				
Anchovies and Sardines. (packed in oil or otherwise treated)		2,664,130		
Herring . . . pounds . . (pickled or salted)	76,838,454	3,165,748	14,539,140	208,880
Mackerel . . . pounds . . (pickled or salted)	26,204,735	1,319,363		
Mackerel and all others . . .pounds . . (pickled)			21,956	143,612
Other species		1,804,319		
Fresh--except Salmon-- pounds			5,517,248	291,332
Dried, smoked, or cured. Cod, Haddock, Hake, and Pollock . . .pounds . .			4,484,414	245,842
Dried, smoked, or cured. all others . . .pounds			6,681,334	272,649

Items	Imports		Exports	
	Quantity	Value \$	Quantity	Value \$
Salmon-canned-pounds. .			55,290,966	5,103,340
Salmon-others-fresh or cured. . .pounds. . .				2,122,566
Fish other than Salmon canned-pounds.				119,260
All other fish and fish products. . .pounds. . .				103,284
Total value		\$12,931,455		8,610,765

THE CHEMISTRY OF FISH I

Introductory

The value of fish as a food for the American people cannot be overestimated. The necessity for substituting fish for meat in order to solve the problem of meat shortage is discussed at length in a report by Pennington (2). The United States could profitably utilize more fish than it now consumes.

There are plenty of fish available to American fishermen, more than enough, at certain seasons, to supply the demand. Indeed, occasions often arise when it is necessary for the fish dealer to cart hundreds of pounds of fish to the dump, because of the lack of demand for this variety of food. This usually happens only to the small dealer when fish are too plentiful, for the larger firms can forestall any such great waste by placing the fish in cold storage and holding them until such time as fish are in greater demand. This freezing and storage of fish is now carried out on a larger scale by many firms and is a boon to the consumer, for it supplies him with a quantity and variety of fish which would otherwise be unavailable on account of the fish being out of season. The quality of a fish so stored apparently remains unimpaired, for the food value does not change, according to Smith, (3), and it is very difficult to detect the difference between the taste, in the cooked condition, of a fresh fish and one properly frozen and held in storage for several months. In addition to preserving fish in the iced and frozen condition there are, of course, the other methods, such as packing in oil, drying, smoking, salting, pickling, and canning, all of which latter methods somewhat modify the taste of the original fish. There is at present a somewhat unreasonable and widespread prejudice against the use of cold storage food products, but this attitude of the public will doubtless not prevail for any great length of time, because of the important role which the cold storage industry plays in maintaining the low price of certain perishable foodstuffs like fish, the supply of which is not under human control.

There are a great many varieties of both fresh and salt-water fish accessible to American fishermen. The common names given to certain species are quite varied and confusing. A complete list of the food fishes of the United States including their scientific and common names and their habitat, will be found in the Government publication, "Fisheries of the United States, 1908" (4).

A large amount of chemical research on fish has been done during several decades of the past century. The greater part of the work was carried out in Germany, a very small amount being done in this country. Considering the vast quantities of fish available to the American people, the food, for fertilizer, for oil, etc., the amount of chemical research on American fish is extremely limited. Atwater (1) who has done the greater part of the American work on this problem, stated in 1888 "that the work here reported can be regarded as only the beginning of a much-needed research." No systematic study of the chemistry of American food fish has been made since that reported by the above author. The extreme lack of reliable data on this subject makes its own plea for a more extended study of our food fishes.

Chapter I.

MOISTURE, FAT, PROTEIN AND ASH CONSTITUENTS OF FISH FLESH

Food Components of Fresh Fish

The value of a food depends upon its tissue-building and energy-producing powers. All foods contain more or less of the following nutrients,---protein, carbohydrate, lipins (fat and fat-like substances) mineral constituents and water. Fats and carbohydrates are the chief fuel ingredients, and the protein compounds, especially the albumin derivatives, are the principal tissue formers. In general, fish flesh is essentially a protein food, lower in fat and higher in water content than meat, and, like it, containing only a minute quantity of carbohydrate.

The earlier investigations on the chemistry of food fish are important only from a historical point of view. Among the first of these is the work of Morin, (27), in 1822, on the constituents of the smelt. Eighteen years later we have the report of Schlossberger, (39) on the quantitative separation of water soluble coagulable protein, alcohol soluble substances, fibrous matter with cell tissue, and salt, of the carp (Cyprinus nasus, Carassias barbus) and of the trout (Salmo fario L). Weidenbusch, (44) (1847) and Baumhauer, (8) (9) (1848), determined the elementary composition of the albumin separated from the flesh of the pike and of the tunny, flounder and haddock.

The first analyses of any importance of fish for water, protein, fat and ash are those of Payer (29) in 1854. These figures, together with analyses made by Koenig, (17), Buckland, (12) Alsen, (6) Kosty-tascheff (21), Popoff, (34) and Sempolowski, (41), are discussed in detail by W. C. Atwater, (1), in the report of the U. S. Commissioner of Fish and Fisheries for 1888. A complete report of these analyses in this place would be of slight value, but a few representative analyses of well known fish would perhaps not be out of place:-

Table No. 1

REPRESENTATIVE ANALYSES OF HERRING AND SALMON

No.	Name of Fishes	Author	Water	Water free substance	Albu- min- oids	Fats	Ash	Nit- rogen	Pro- tein N X 6.25	Water Protein - Fat - Ash
1.	Fresh Herring	Payen	70.00	30.00	17.80	10.30	1.90			
2.	Smoked Herring	Koenig	69.49	30.51	20.76	8.51	1.24	3.38	21.12	100.36
3.	Herring	Buckland	80.71	19.29	10.11	7.11	2.07			100.00
4.	Little Herring	Almen	73.25	26.75	19.23	5.87	1.65	3.01	18.83	99.60
5.	Fresh Herring	Kostyts- cheff	76.11	23.89	17.29	4.89	1.71			
6.	" "	Atwater	69.03	30.97	18.46	11.01	1.50	3.06	19.12	100.66
	Fresh Salmon	Payen	75.70	24.30	18.17	4.85	1.28			
	" "	Buckland	77.06	22.94	10.11	7.11	2.07			100.00
	" "	Almen	70.33	29.67	18.06	10.12	1.49	3.10	19.39	101.33
	" "									
	(average of 5 Specimens)	Atwater	63.61	36.39	21.60	13.38	1.41	3.59	22.39	100.79

The figures in the preceding table were obtained by Atwater after recalculating the original data into the present form, for the sake of comparison. On inspection of the analytical results above, it will be seen that, if the per cent fat is subtracted from the per cent water-free substance in the cases of Nos. 1, 5, and 6 for fresh herring, the per cent of fat-free solids is quite uniform, namely, 19.7%, 19.00%, and 19.96% respectively. This constancy in the amount of fat-free solids, holds true for the majority of fish and averages about 19.5%.

Insert I.

Miesher, (25) observed a decrease in the protein and fat content of the muscle of Rhine salmon at spawn time. According to Polimanti, (32) and (33) surface swimming fish have the highest fat content.

Bearing in mind the factors influencing the composition of fish flesh, one can readily see that an analysis of a fish of unknown history is liable to lead to an erroneous impression of its food value. As a case in point, note the analysis of bluefish in the following table (No. 8) of Atwater's. The fat content of this fish is given as 1.25%, with a moisture content of 78.46%. In a recent analysis (not published) by the author of a blue fish, the following figures for moisture and fat, respectively, were obtained, --- 63.65% and 16.13%. Table No. 2 is a copy of Atwater's (1), results, obtained on fresh food-fishes (averages only). It should be understood, however, that while the figures are correct in a general way for most of the fish, some of them particularly the surface swimming fish, are subject to wide variations in fat content. Until we know the extent of these changes, it is unwise to accept as final a single analysis of a fish, and judge therefrom as to its fuel value as a food.

Table No. 2

COMPOSITION OF FLESH OF AMERICAN FISHES

Kind of Fish	Number analyzed	Water	Water-free substance	Amino-acids, etc., by difference	Fats, Ether extract	Ash	Nitrogen	Protein N x 6.25	Water + protein + fats + ash
		%	%	%	%	%	%	%	%
Sturgeon (<i>Acipenser sturio</i>)		76.71	21.29	17.96	1.90	1.43	2.90	18.11	300.15
Small-mouthed red-bass (<i>Morone saxatilis</i>)		76.56	21.44	17.90	2.33	1.19	2.88	17.99	300.09
Herring (<i>Clupea harengus</i>)	1	69.03	30.97	18.44	11.01	1.90	3.04	19.12	300.64
Bluefish (<i>Clupea vermicilla</i>)	2	74.44	25.56	19.17	4.93	1.67	3.10	19.35	300.19
Shad (<i>Clupea savilliana</i>)	7	70.62	29.38	18.95	9.27	1.75	3.01	18.83	300.29
Smelt (<i>Osmerus mordax</i>)	2	79.16	20.84	17.37	1.79	1.66	2.83	17.99	300.23
Whitefish (<i>Coregonus clupeaformis</i>)	1	69.83	30.17	22.08	6.29	1.62	3.67	22.93	300.87
Salco (Carangid?) California Salmon (<i>Stenobrama chitonata</i>)	2	63.61	36.39	17.44	17.87	1.06	3.12
Salmon (Rainbow?)	9	63.61	36.39	21.60	13.38	1.41	3.59	22.79	300.79
Lake trout (<i>Salvelinus namaycush</i>)	2	69.14	30.86	18.33	11.38	1.35	3.97	18.53	300.30
Brook trout (<i>Salvelinus fontinalis</i>)	7	77.32	22.68	18.97	2.10	1.21	3.08	19.23	300.24
Pike (<i>Esox lucius</i>)	1	79.79	20.21	18.60	0.88	1.03	2.99	18.67	300.07
Flounder (<i>Paralichthys oblongus</i>)	2	79.68	20.32	18.64	0.90	1.18	3.00	18.73	300.09
Weakfish (<i>Cynoscion regalis</i>)	1	76.26	23.74	19.63	4.34	1.97	3.32	20.15	300.34
Hal, salt water (<i>Amblyraja rostrata</i>)	2	71.60	28.40	18.38	9.11	1.01	3.98	18.30	300.33
Willet (<i>Spizella albicollis</i>)	1	74.87	25.13	19.32	4.64	1.17	3.12	19.68	300.34
Maskarel (<i>Scophthalmus aquosus</i>)	6	73.77	26.23	18.28	7.09	1.38	2.99	18.64	300.38
Spanish Mackerel (<i>Scomber maculatus</i>)	1	68.10	31.90	20.97	9.43	1.30	3.43	21.45	300.48
Peopano (<i>Centropristis striata</i>)	2	72.78	27.22	18.61	7.97	1.00	3.02	18.83	300.38

Table No. 2 cont'd

	No. analyzed	Moisture %	Moisture Free substance %	Alberic acid, by difference %	Fats, ether extract %	ash	Starch %	Protein % 1, 5, 20 %	Moisture Free Protein + Fat + Ash
Bluefish (<i>Menhaden menhaden</i>)	1	78.26	21.24	19.02	1.22	1.22	1.21	79.41	100.39
Butterfish (<i>Centroscopus virgatus</i>)	1	75.03	24.98	17.81	12.03	1.24	2.88	17.93	100.18
Large-mouthed black bass (<i>Morone chrysops</i>)	1	78.64	21.36	19.24	2.10	1.29	1.21	79.44	100.20
Small-mouthed black bass (<i>Morone chrysops</i>)	1	78.82	21.18	19.30	1.84	1.24	1.27	81.75	100.25
Yellow perch (<i>Perca flavescens</i>)	2	79.23	20.77	18.88	1.89	1.24	1.00	80.74	100.06
Wall-eyed pike (<i>Esox lucius vitreum</i>)	1	79.71	20.29	18.42	1.29	1.27	2.97	80.58	100.11
Gray Pike (<i>Esox lucius canadensis</i>)	1	80.83	19.17	17.26	1.57	1.23	2.84	77.88	100.64
Striped bass (<i>Morone saxatilis</i>)	6	77.70	22.30	19.31	2.93	1.24	2.97	80.54	100.22
White perch (<i>Morone americana</i>)	2	75.71	24.29	19.03	4.07	1.29	1.88	79.24	100.22
Sea bass (<i>Centropristis striata</i>)	1	79.32	20.68	18.73	1.89	1.24	1.27	79.84	101.09
Croaker (<i>Micropogonias undulatus</i>)	2	79.43	20.57	18.80	1.66	1.15	1.84	79.25	100.40
Red snapper (<i>Lutjanus blackfordi</i>)	3	78.68	21.32	19.20	1.03	1.21	1.24	79.73	100.43
Forgy (<i>Halibut americanus</i>)	3	74.99	25.01	22.22	2.79	1.28	2.97	80.58	100.06
Sheepshead (<i>Morone chrysops</i>)	2	75.55	24.45	19.54	1.66	1.22	1.22	80.58	100.51
Red Bass (<i>Morone saxatilis</i>)	1	81.95	18.05	16.68	1.37	1.23	2.70	80.88	100.20
Kingfish (<i>Morone chrysops</i>)	1	79.21	20.79	18.64	2.15	1.18	1.03	80.86	100.28
Weakfish (<i>Cynoscion regalis</i>)	1	78.97	21.03	17.45	2.58	1.19	2.85	77.80	100.35
Blackfish (<i>Morone chrysops</i>)	4	79.10	20.90	18.47	1.43	1.08	2.99	80.71	100.22
Hake (<i>Merluccius</i>)	1	83.11	16.89	15.82	1.07	0.98	2.68	83.37	100.23
Cusk (<i>Merluccius</i>)	1	82.01	17.99	16.82	1.17	0.90	2.52	82.90	100.08
Sablefish (<i>Anchoa mitchilli</i>)	5	81.69	18.31	16.83	1.46	1.23	2.75	81.59	100.38
Cod (<i>Gadus morhua</i>)	1	82.64	17.36	15.77	1.58	1.23	2.54	80.31	100.74
Tuna (<i>Thunnus</i>)	1	81.33	18.67	17.08	1.58	0.99	2.70	81.24	100.24
Flounder (<i>Paralichthys</i>)	1	76.32	23.68	21.68	2.00	1.55	1.45	81.60	99.95
Halibut (<i>Paralichthys</i>)	3	75.43	24.57	22.22	2.35	1.06	2.97	80.38	100.23
Turbot (<i>Scophthalmus</i>)	1	71.79	28.21	24.92	3.29	1.28	2.36	81.75	101.83
Common flounder (<i>Paralichthys dentatus</i>)	2	81.21	18.79	17.82	0.97	1.20	2.26	81.07	100.25
Winter flounder (<i>Pleuronectes americanus</i>)	1	84.35	15.65	14.81	0.84	1.20	2.33	84.53	100.53
Langray cod (<i>Halargyreus</i>)	1	71.12	28.88	24.92	1.39	0.66	2.40	81.98	100.04
Skate (<i>Raja</i>)	1	82.15	17.85	15.34	1.51	1.14	2.91	80.27	100.89

The author follows the general custom of classifying the fish according to their proportions of fat into three classes: (1) containing over 5% of fat; (2) containing between 5% and 2% of fat; (3) containing less than 2% of fat. Practical use of such a classification is necessarily limited on account of the variations referred to above.

The following excerpts from Atwater's table are given in order to illustrate the principle that, as a rule, the amount of water in the flesh decreases as the fat increases and *vice versa*.

Table No. 3. CLASSIFICATION OF FISHES BY PROPORTIONS OF FAT
(percentages are on a wet basis)

Kinds of Fish	Water	Fats	Kinds of Fish	Water	Fats
Containing over 5% of fats.			Containing over 5% of fats.		
California Salmon	63.6	17.9	Sturgeon	78.7	1.9
Lake Trout	69.1	11.4	Blackfish	79.1	1.4
Shad	70.6	9.5	Kingfish	79.2	1.0
Porgy	75.0	5.1			
Containing between 5% and 2% of fats.			Common Flounders	84.2	0.7
Alewife	74.4	4.9	Sea Bass	79.3	0.5
Cisco	76.2	3.5	Tom Cod	81.6	0.4
Striped Bass	77.7	2.8			
Brook Trout	77.7	2.1			

Kianizyn, (16), determined the food constituents of the dried and salt cod; the fish so prepared contains 25.09% inedible portion.

In 1898 Balland, (7), reported analyses of 38 varieties of fish, crustaceans and molluscs for water, nitrogen constituents, fat, extractives and ash. He noted that fish containing a low per cent of fat are usually high in nitrogenous constituents.

Beythien, (11) determined the composition and food value of beef, pork and mutton, and compared them with those of salt herring and fresh cod. The relative food values of salt herring and fresh cod in Germany per mark are 3765.9 and 2000.0 respectively, while that of beef averages 1529 units.

That the nutritive value of a fish may be very low on account of its high proportion of water and refuse matter, as head, bones, etc., is shown by Milone (26), in his study of the food fishes of Italy, in which he calculated the price of different varieties of fish (whole) in lire and centimes per kilogram, and per 1,000 units of nutritive value. The nutritive value of fish per unit cost, as purchased in Italy is twice that of meat.

Holland, (15), found that the common fresh water fishes of France compare favorably in food value with those imported from foreign countries.

Vaillant, (43), found that eels vary in chemical composition, and hence in food value, at various stages of their growth. The eel fry, or "civelle", which are colorless, contain 19.35% fat. Then, when in the state of "montee", when the eel fry have turned black, the fat drops to 12.53% and in the half grown eel (6-10 inches in length) this constituent has increased to 17.13%. The explanation is that the eels are hatched with a rich amount of fat and also vitellin, which is exhausted when the eels arrive at the state of "montee". It then requires some time before this amount of fatty material can be replaced by means of food eaten.

Reuss, (37), analyzed carp, which had been living in hard water and soft water, and found slight variations, which could, however, be explained as being due to the greater amount of ash constituents in the fish living in hard water. When calculated to the ash-free basis these differences in composition between the two varieties of carp disappeared.

In a series of experiments on the oxygen requirement of fish, Futter, (36), estimates the food constituents, including carbohydrates, by fractional extraction of the flesh with hot water and ether.

The following table by Koenig and Splittgerber, (19), gives a means of comparing the food constituents of beef and veal (evidently lean meat) with those of fish-

Table No. 4 THE CHEMICAL COMPOSITION OF FISH, BEEF, AND VEAL
(Average values)

Kind of flesh	Water %	N X 6.25 %	Bas- ic Nit. %	Alb- umin %	Gel- atin %	Fat %	Ash %	Water sol.	Water sol. ash
Beef	74.45	22.20	0.44	1.81	0.97	3.12	1.32	4.32	1.14
Veal	78.16	20.23	0.31	0.78	0.70	1.07	1.07	2.91	0.96
Dried Cod	9.02	90.41	1.01	1.57	5.88	1.12	5.12	12.97	4.27
Haddock	76.18	23.94	0.38	0.84	0.94	0.27	0.84	4.43	0.71
Halibut	76.77	20.59	0.31	1.65	0.60	2.54	1.13	3.13	0.89
Herring	77.98	18.59	0.16	1.23	2.29	3.44	1.35	3.39	1.08
Pike	78.73	20.89	0.47	2.53	1.39	0.31	1.04	5.29	0.80
Carp	73.34	20.50	0.42	1.45	1.15	6.21	1.22	3.60	0.82
Elb Salmon	67.15	23.02	0.52	1.19	0.79	8.82	1.20	4.66	1.00

Food Components of Cooked Fish

Unique researches were carried on by Weigelt, (45), and by Schrupf, (40), in order to determine what proportion of the food constituents of the purchased fish is actually utilized by the consumer. For this purpose, the flesh of the fish after cooking was removed with a knife and fork, in order to be comparable with the usual deliberate eating of fish, and the flesh so obtained was analyzed for solids, protein, fat, etc. In the case of some small fish, the refuse matter exceeded the edible portion. In other cases, as with the cod, halibut, sea pike, and salmon, the inedible portion of the purchased fish varied from 11.4% in the sea pike to 18.8% in the cod. Certain fish are customarily sold gutted and with the head intact, some gutted and without the head, while still others are sold "in the round", that is, ungutted and with the head intact.

Ultimate analyses of the dried flesh of different cooked fish are reported by Williams, (47), with determinations of fat, protein, and reducing substances. The latter were obtained on the dried flesh after hydrolysis with hydrochloric acid, and varied from 2.17% in the smelt to 17.59% in salt herring.

Lehman, (22), recommends the use of fish along with added fat and carbohydrates as a ration for a soldier, basing his conclusion on analyses of several varieties of cooked fish. Canned fish has been made a part of the army ration in certain countries.

Ulrich, (42), records numerous analyses of fish under different conditions, such as cooked, smoked, salted, pickled, marinated, dried, etc. Fresh fish under different methods of preparation and cooking lose varying amounts of their chemical constituents.

The chemical composition of flesh of certain fish resembles that of beef and veal very closely, and in the case of such fat fish as the western salmon, and mackerel, the fuel value compares very favorably with that of the flesh of other animals. In general, however, fish contain more water and less fat than meat, and for this reason the fuel value is lower than meat. However, when fish are eaten with such supplemental foods as cereals and other vegetables, which supply carbohydrate and which are usually deficient in protein, a balanced diet is obtained which is equal in fuel value and muscle building power to a similar diet containing meat. An example of a whole nation living on such a diet is afforded by the Japanese with their nearly universal ration of fish and rice.

Loss of Substances on Cooking and Drying Fish

In an effort to determine the loss, if any, of substances in cooking fish, Peter, (31), cooked pieces of fish flesh in beakers placed on a steaming pot kept at 98.5°C. for one hour. The average loss in weight was 30.18%, being principally water, though some of the fish which were poor in fat lost considerable ether soluble material.

Ulrich's, (42), results on fresh and cooked fish seem to indicate a slight loss of food constituents of fish during cooking and after curing or preserving in pickle, oil, etc. Some of his figures for salmon and herring follow:

Table No. 5 ANALYSES OF FRESH AND COOKED FISH
(Per cent on dry bases)

	Fat %	Ash %	Nit. sub. %	Pure Ash %	N.free ext. matter %	P ₂ O ₅ %	NaCl %
Salmon - fresh	24.03	4.61	69.94	4.27	1.42	1.98	0.34
boiled	23.92	4.20	68.63	3.33	3.25	1.62	0.87
baked	20.59	8.34	69.38	4.62	1.69	1.75	3.72
smoked	24.32	20.77	51.06	3.50	3.87	1.62	17.27
Herring - fresh	29.14	3.88	63.94	3.65	3.04	1.54	0.23
smoked	29.79	12.84	56.50	6.64	1.47	1.12	5.60

The dogfish, (*Scyllium catulus*) whose flesh and internal organs are rich in urea, loses a substance of alkaline reaction on drying at 100°C. According to Buglia and Constantino, (13), this is due to ammonia formed from the decomposition of urea. If the heat is raised to 110-112°C, there is a loss of substances of acid reaction, presumably volatile fatty acids. The authors conclude that on drying such products the loss affects the nitrogen and moisture determination.

Schrumpf, (40), found that fish which were poor in fat seemed to lose nitrogenous substances when cooked. Likewise, the researches of Beythien, (10), on the water in which fish were cooked show that 8.8 to 11.3% of the total nitrogen is extracted by the water. This extract is not fit for food purposes on account of the odor.

It appears then, that losses occur when fish are cooked but those are so slight from a food standpoint as to be negligible, and that the losses occurring on drying might very easily vitiate the results in

The Chemical Composition of Fish Proteins

Weidenbusch, (44), in 1847, and Baumann, (89), in 1848, analyzed for its elementary constituents the albumin extracted from the flesh of the pike, tunny, flounder, and haddock, which is of historical interest only. Payen, (30), a few years later determined the composition of the fat-free flesh of the eel, mackerel, barbel and sole.

That the elementary composition of the fish flesh agrees very well with that of beef and veal is shown by the following figures of Koenig and Splittgerber, (19). Albumin separated from the fish by cold water extraction was found to have an average nitrogen content of 16%.

Table No. 6 ELEMENTARY COMPOSITION OF FISH FLESH

Flesh of	In the ash-free dry substance				
	Nitrogen	Carbon	Hydrogen	Sulphur	Oxygen
Beef	16.31	52.72	7.38	1.15	22.44
Veal	16.41	52.37	7.12	0.71	23.49
Dried Cod	16.57	53.15	7.46	1.09	21.37
Fresh Herring	16.50	51.50	7.24	0.86	22.90
Haddock	16.68	52.37	7.59	1.22	21.14
Halibut	16.57	53.08	7.57	0.94	21.84
Pike	16.48	52.16	7.47	1.92	22.97
Carp	16.48	53.18	7.49	1.34	21.51
Salmon	16.48	53.52	7.68	1.45	20.86

In an investigation of the proteins of the herring, dog salmon, pike, etc., Prutchinsky, (35), found that the phosphorus content of the myosins is higher than that of the myostromins, and in beef the reverse relation is true.

The ultimate analyses of cooked fish flesh recorded by Williams, (47), were performed on the dried crude protein before removal of the fat and so cannot be used for comparison with the results tabulated above. The only data we possess at present on the composition of the products of hydrolysis of fish muscle is that of Osborne and Heyl, (28), who compared the hydrolytic product of halibut muscle with those of chicken muscle. The mono-amino acids were determined by the Fischer esterification method and the hexone bases by the method of Kossel and Patten, (20), on the filtrate from the tyrosin determination:-

Table No. 7 PRODUCTS OF HYDROLYSIS OF HALIBUT AND CHICKEN MUSCLE

	Halibut	Chicken
Glycocoll	0000%	0.68%
Alanin	?	2.28
Valin	0.79	?
Leucin	10.33	11.19
Prolin	3.17	4.74

Continued-

	Halibut	Chicken
Phenylalanin	3.04	3.53
Aspartic acid	2.73	3.21
Glutamic acid	10.13	16.48
Serin	?	?
Tyrosin	2.39	2.16
Arginin	6.34	6.50
Histidin	2.55	2.47
Lysin	7.45	7.24
Ammonia	1.33	1.67
Tryptophane	Present	Present

Attention is called to the similarity in composition of the two kinds of muscle, although a slight difference may be noted in the proportions of alanin and glutamic acid. Note the high per cent of lysin.

Analytical Constants of Fish Oils

Fish oils are characterized by a high iodine number, usually over 100. Ulrich, (42), however, obtained values by the Hubl method which averaged considerably below 100. Those of Koenig and Splittgerber (Table No.) are also low. The low iodine values obtained by these observers might be due to any of three causes: (1) the oil has been heated excessively at one or another of the stages in its extraction; (2) the oil has been allowed to stand exposed to the air in thin layers and become oxidized; (3) too much oil has been used in the sample taken for the iodine number determination.

In considering the constants of a fish oil for comparison with other oils it is always necessary to know the history of the oil, i. e., how it was prepared, its age and under what conditions it was stored. For example, a commercial product such as Japanese sardine oil, usually prepared by allowing the oil to exude from a heap of decomposing fish, will have different constants from the oil separated from the sardine by extraction of the dried flesh with petroleum ether.

The following tables (Nos. 8 and 9) were compiled from tables in Lewkowitsch, Chemical Technology and Analysis of Oils, Fat, and Waxes, Vol. 1, (1913), pages 379-437, and vol. 11, (1914) pages 408-423, with additions from Koenig and Splittgerber, (19), Rosenfeld, (38), and White, (46).

A further consideration of fish oils will be made in the chapter of fish waste.

Ash Constituents of Fish Flesh

Levi (23) records the following analysis of fish bone ash:-

C_aO	52.53%	P_2O_5	34.09%	Na	2.54%
M_gO	1.19%	Cl	2.21%	K	1.90%
				CO_2	3.00%

Du Menil, (1836), (14), determined the mineral content of fish scales.

The following is a table compiled from analyses recorded by Albu and Neuberg, (5), and Koenig, (18).

Table No. 6

ANALYTICAL CONSTANTS AND VARIABLES OF FISH OILS

Kind of Oil	Specific Gravity	Index Number	Saponification No.	Acid Number	Reichert Value	Reiner No.	Viscosity at 50°C.	Unspecifiable matter %
Eschschol ¹	0.9250-0.9259	139 -273	190.6	"	1.5 ^b	"	"	"
Eschschol ²	0.9230	143.3	191.2	2.88	"	"	0.2707	"
Japanese Sardine ¹	0.924 -0.9247	129 -287	180 -196	"	"	94.5-95.3	"	0.32-0.35
Salmon ¹	0.9259	160.42	252.8	"	0.55 ^b	98.02	"	"
Salmon ²	"	97.87	232.2	"	4.3	"	"	"
Sardine ¹	0.9202-0.9203	153.5-167.0	172-184	"	"	98.54	"	"
Sturgeon ¹	"	125.3	186.3	"	"	"	"	1.78
Sardine ¹	"	160 -193	"	"	"	91.6-92.08	"	0.48-1.01
Salibet ³	"	83.06	155.85	"	3.4	"	"	"
Haddock ³	"	48.28	"	"	"	"	"	"
Pike ³	"	54.00	204.9	"	"	"	"	"
Carp ³	"	56.15	195.8	"	3.4	"	"	"
Seafiled ²	0.9181	130.9	195.2	0.74	"	"	0.2135	"
Squidbeard ²	0.9177	123.9	196.5	2.40	"	"	0.2402	"
Sea ²	0.9168	117.3	188.9	16.73	"	"	0.2143	"
Wallerfish ²	0.9090	91.1	190.4	42.87	"	"	0.2092	"
Sea ²	0.9135	117.4	190.4	1.82	"	"	0.2026	"
Sea-shepherd ²	0.9131	127.8	197.8	1.66	"	"	0.2452	"
Sea-shepherd ²	0.9153	121.6	197.5	0.80	"	"	0.1858	"
Wale ²	0.9152	116.6	192.8	6.70	"	"	0.1822	"
Blackfish head ²	0.9136	33.9	277.8	0.81	"	"	0.1132	"
Porpoise ²	0.9203	30.26	"	1.53	"	"	0.1206	"
Sole ⁴	"	156.49	200.4	"	"	"	"	"
Turbot ⁴	"	114.42	204.96	"	"	"	"	"

1. Leshowitzky; 2. White; 3. Eoenig and Spittsgerber; 4. Rosenfeld; a. Reichert value; b. Reichert - Beil value.

Table No. 9

ANALYTICAL CONSTANTS AND VARIABLES OF FISH LIVER OILS

Kind of Oil	Specific Gravity	Iodine Number	Saponification No.	Acid Number	Reichert Value	Schmer No.	Viscosity at 30°C.	Unsaponifiable matter %
Cod liver ¹	0.9331-0.9410	167 (mean)	171-189	"	0.40-0.75	"	"	0.8-5.1
Cod liver ²	0.9377	142.6	185.8	1.32	"	"	0.2001	"
Haddock liver ¹	0.939 -0.934	154.2	188.8	"	"	93.3	"	1.1
Shake liver ¹	0.9307	137.3	185.4	"	"	94.7	"	0.97
Bardooce Skate liver ²	0.9333	155.8	181.9	0.38	"	"	0.1970	"
Shark liver ¹	0.9263	114.6	141.0	"	"	86.9	"	10.2
Busy Shark liver	0.9337	118.2	185.2	0.81	"	"	0.2186	"
Sand Shark liver ²	0.9347	"	183.7	1.32	"	"	0.2843	"
Banner head shark liver ²	0.9343	135.5	174.1	3.32	"	"	0.2683	"
Coalfish liver ¹	0.9351	177 -163	177 -181	"	"	"	"	1.8
ling liver ¹	0.9340	139.6	184.1	"	"	"	"	1.0
Torpedo liver ¹	0.9344	131.0	164.0	0.92	"	"	0.2133	"
Dogfish liver ¹	"	"	"	"	"	"	"	"
(Squalus canis)	0.9364	124.8	189.9	1.80	"	"	0.1977	"
Dogfish liver ¹	"	"	"	"	"	"	"	"
(Squalus acanthias)	0.9387	142.7	171.4	1.84	"	"	0.2049	"

1: Lowiodolol

2: White

Table No. 9

ANALYTICAL COMPARISON AND VARIABLES OF FISH LIVER OILS

Kind of Oil	Specific Gravity	Iodine Number	Saponifi- cation No.	Acid Number	Reichert Value	Solner No.	Viscosity at 50°C.	Unsaponifiable Matter %
Cod liver (1)	0.9174-0.9410	147 (mean)	171-189		0.40-0.76			0.4-5.3
Cod liver (2)	0.9277	142.6	185.8	1.32			0.2001	
Maddock liver (1)	0.929-0.934	154.7	188.8			93.3		1.5
Shale liver (1)	0.9207	137.3	183.4			94.7		0.97
Barndoor Shale liver (2)	0.9253	151.8	184.9	0.38			0.1975	
Shark liver (1)	0.9163	124.6	181.0			86.9		10.2
Dusky shark liver	0.9127	128.2	183.2	0.81			0.2986	
Sand shark liver (2)	0.9127		183.7	1.52			0.1943	
Hammerhead shark liver (2)	0.9445	155.6	178.1	3.32			0.2582	
Coalfish liver (1)	0.9231	137-162	177-181					1.8
Ling liver (1)	0.9245	130.6	184.1					1.0
Torpedo liver (2)	0.9044	121.0	164.0	0.92			0.2133	
Dogfish liver (<i>Squalus mae</i>) (2)	0.9164	124.8	180.9	1.40			0.1977	
Dogfish liver (<i>Squalus acanthias</i>) (2)	0.9187	142.7	171.4	4.84			0.2049	

1: Laskowitch

2: White

Table No. 10

ASH CONSTITUENTS OF FISH FLESH

Water	Total ash	K ₂ O	Na ₂ O	CaO	MgO	Fe ₂ O ₃	P ₂ O ₅	SO ₃	Cl
Eel	1.74	0.18	9.48	45.83			43.18		0.17
Pike	6.13	23.92	20.45	7.38	3.81		38.16	2.50	4.74
(Fresh									
(Salmon	1.29	24.40	13.66	8.60	9.49		20.32		21.44
Haddock	11.26	13.84	36.51	3.39	1.90		13.70	0.31	38.11
(Anchovies									
(Salted	8.44	2.17	38.80	4.22	1.88	Trace	18.11	0.93	33.25

Albu and Neuberg

J. Koenig

Chapter II

Lesser Important Constituents of Fish Flesh

Extracts of fish flesh contain many compounds similar to those found in beef extracts. Those are present only in minute quantities, however, and large amounts of fish muscle must be extracted to obtain sufficient quantities of them for identification and quantitative estimation. Of these compounds, some are always present in the fresh fish flesh; others are the result either of bacterial or enzymic decomposition or, and this point cannot be over estimated, of a chemical change wrought by the action of the chemicals employed in isolating them. The following substances have presumably been isolated from fish, which are fit for food. The bases resulting from the decomposition of fish will be discussed in resulting from the Purine Bases.

Issac, (50), in 1904, examined herring brine that was one year old and reported xanthine present. Suzuki and Yoshimura, (71), likewise found this substance present in the hot water extract of dried bonito. Using the method adopted by Micko, (58), Koenig and Splittgerber, (19), found the following amounts of xanthine bases in the cold water extract of fish:-

Table No. 11

XANTHINE BASES IN FISH FLESH

	Water %	Pure Xanthine Bases (= N X 2.711)	
		Natural Substance	Dry Basis
		%	%
Dried cod	9.02	0.190	0.209
Haddock	76.18	0.081	0.340
Halibut	76.77	0.079	0.338
Pike	78.73	0.015	0.071
Carp	73.34	0.039	0.146
Salmon (Elb.)	67.15	0.045	0.137

Hypoxanthine is present in fish extract as shown by Suzuki and Yoshimura, (71), who found in dried bonito and fresh salmon 0.074% and 0.028%, respectively, calculated to the dry weight of the flesh. According to Krunkenberg, (51), this substance is also

present in the extract of carp and perch. Likewise, Isaac, (50), reports 5.0 gms. of hypoxanthine in 100 liters of one year old herring brine. He also separated 3.5 gms. of adenin as the picrate and 20 gms. of guanin as the hydrochloride from 100 liters of the same brine.

Amino Acids.

Koenig and Splittgerber, (19), determined the total mono-amino acids in the cold water extract of several fish. The amino acids were determined by the sodium nitrite method, using the apparatus designed by Böhmer. The mono-amino acids comprise only about 1% to 4% of the nitrogen in the filtrate from the phosphotungstic acid precipitate. The greater part of the nitrogen is in the form of other compounds, perhaps acid amides, with small amounts of creatin and creatinin:-

Table No. 12
MONO-AMINO ACIDS OF FISH FLESH
(per cent on wet basis)

	Water	Total Nitrogen in filtrate from Phosphotungstic acid precipitate.		Nitrogen as amino acids.		Remaining Nitrogen, in the form of other compounds.	
		%	%	%	%		
Dried cod	9.02	0.091	0.013	0.078			
Haddock	76.18	0.039	0.006	0.033			
Halibut	76.77	0.027	0.011	0.016			
Pike	78.73	0.009	0.003	0.006			
Salmon	67.15	0.034	0.007	0.027			

Wilson, (76), examined the water extract of a series of related vertebrate and invertebrate animals and found a definite relation between the amounts of mono- and di-amino acid nitrogen. The following table gives the per cent amino acid nitrogen on the dry basis.

Table No. 13
AMINO ACID NITROGEN OF
RELATED VERTEBRATE AND INVERTEBRATE MARINE ANIMALS
(Per cent on dry basis)

Kind of animal	Amino Acid Nitrogen		
	Total Amino Acid Nitrogen	Phosphotungstic acid filtrate, --- mono-amino acid Nitrogen fraction	Phosphotungstic acid precipitate, -di-amino acid Nitrogen fraction (by difference)
	%	%	%
Lamprey	1.31	0.212	1.10
Lisulus	2.98	0.514	2.46
Squid	1.78	0.799	0.99
Glan	2.14	1.18	0.97
Scallop	2.63	1.77	0.86
Periwinkle	1.94	0.598	1.34

X Böhmer, C. Koenig, J. Die Untersuchung lands. u. gewerbl. wichtiger Stoffe, Berlin, 1906, page 215.

The above results were obtained by the method of Van Slyke (74) while in the following table from Okuda (60) the amino acids were determined by the method of Sørensen (66). The feature of the latter's results is the relatively high per cent of mono-amino acid nitrogen in the molluscs and crustaceans, though the value of 0.485% for cuttlefish is lower than the figures obtained by Wilson above for squid.

Table No. 14 MONO-AMINO ACIDS IN SOME MARINE ANIMALS
(Per cent on dry basis)

Flesh of	Water	Mono-amino acid Nitrogen
	%	%
Carp	76.61	0.094
Tunny	73.52	0.072
Bonito	69.37	0.072
Porgy	76.79	0.069
Crussian carp	82.00	0.194
Spiny lobster	75.98	0.608
Cuttle fish	81.67	0.485

The following results on the torpedo and dog fish were obtained by Buglia and Costantino (13)

Table No. 15 AMINO ACID NITROGEN OF TWO SPECIES OF ELASMOBRANCHII

Flesh of	Amino Acid Nitrogen		
	Total amino acid nitrogen	Mono-amino acid nitrogen	Di-amino acid nitrogen (by difference)
	%	%	%
Torpedo (<i>Torpedo ocellata</i>)	0.82	0.35	0.47
Dogfish (<i>Scyllium catulus</i>)	0.19	0.11	0.08

Yoshimura and Kanai (77) found 0.05% anilin (air dry basis), and a trace of glutamic acid in the hot water extract of the cod. Suzuki and Yoshimura (71) found 0.01 gram alanine per 100 grams of dried muscle in the hot water extract of fresh salmon, and 0.13% leucine and 2.08% of α -amino valeric acid in the extract of dried cuttlefish. The authors also separated from the hot water extract of dried bonito, fresh bonito and tunney fish 1.50%, 0.17% and 0.47% respectively, (on dry basis) of histidine.

Creatin, $C_4H_9N_3O_2$, and

Creatinin, $C_4H_7N_3O$

Creatin is a normal constituent of all muscle, while creatinin is usually absent in the case of mammalian muscle, but occurs quite often in the flesh of certain fishes. The following table is a resume of the early work on the creatin and creatinin content of fish flesh and is valuable only from a historical point of view:-

Table No. 16

RESUME OF EARLY WORK ON THE CREATIN AND CREATININ CONTENT OF FISH FLESH
(Per cent on wet basis)

Investigator	Date	Fish	Creatin	Creatinin
Gregory (49)	1848	Cod	0.0935	Present
		Ray	0.0607	"
Valenciennes and Frey (73)	1855	Cuttle fish	Present	"
Stadler (67)	1857	Shark	"	"
Schulte (65)	1861	Electric ray	"	" ^x
Limpricht (56) (57)	1863	Roach	0.11	
	1865	Herring	Present	
Krunkenberg (51)	1880	Carp	"	None
		Perch	"	"
Krunkenberg (52)	1881	Sturgeon	"	"
		Ray	"	
		Shark		

x The electric organ of the ray.

x This was probably betaine.

The recent researches on creatin and creatinin in fish flesh, have been assembled in the table which follows. Attention is called to the wide variation in the results obtained on the same kind of fish. It is at once apparent that a great deal of work remains to be done on the determination of these substances in fish flesh.

Table No. 17

RECENT WORK ON THE CREATIN AND CREATININ CONTENT OF FISH FLESH.

Author	Fish	Grams per 100 grams of dried flesh	
		Creatin	Creatinin
Koenig and Splittgerber (19)	Dried cod	0.036	0.009
Yoshimura and Kenai (77)	Cod	None	0.140 x
Koenig and Splittgerber (19)	Haddock	0.013	0.273
			0.163
Suwa (69)	"	Present	Present
Koenig and Splittgerber (19)	Halibut		0.355
" " "	Pike	0.193	0.282
" " "	Carp	0.169	0.199
Okuda (60)	"	2.020	0.369
Koenig and Splittgerber (19)	Salmon	0.027	0.207
Suzuki and Yoshimura (71)	"	0.320	None

Continued on Page 24

Author	Fish	Grams per 100 grams of dried flesh	
		Creatin	Creatinin
Okuda (60)	Salmon	1.525	0.182
"	Bonito, fresh,	2.011	0.481
"	Steamed and dried	0.331	0.775
Suzuki and Yoshimura (71)	Bonito, fresh	0.10	None
"	Dried	Present	None
" " "	Tunny fish	0.30	None
Okuda (60)	" "	1.800	0.232
"	Snapper	3.327	0.308
Suzuki and Yoshimura (71)	Eel	0.065	None

0.140 grams in 100 grams air dry flesh.

Isocreatinin, $C_4H_7N_3O$.

In 1897, Thesen, (72), isolated from the water extract of the flesh of fresh haddock a substance which had an elementary composition similar to creatinin. The extract, freed from albumin was evaporated to a small volume, treated with an equal volume of alcohol, filtered and concentrated. On taking up the syrupy residue with alcohol and allowing it to cool, a compound crystallized out in fine yellow leaflets which he termed isocreatinin. One kilogram of fresh haddock flesh yielded one gram of substance. Properties of isocreatinin, $C_4H_7N_3O$: Crystallized from water diluted alcohol in yellow plates, from absolute alcohol in needles. At 12°C. one part is soluble in 4.2 parts of water. It forms salts with hydrochloric acid (needles), sulphuric acid (leaflets), oxalic acid (needles) and cadmium chloride (needles). Many doubt the existence of an isomer of creatinin and believe the supposed differences in the properties of the "isomer" to be due to insufficient purification.

Methyl guanidin, $C_2H_7N_3$.

Methyl guanidin is closely related to creatin, being obtained from it by boiling with mercuric oxide and dilute sulphuric acid. The base, in the organism, is probably derived from creatin. Suxa, (69), isolated methyl guanidin from the hot water extract of the haddock. The compound was identified by means of its gold salt. Yoshimura and Kanai, (77), obtained 0.07% (air dry basis) of this compound as the picrate in the extract from the cod.

Cholin, $C_5H_{15}NO_2$.

Boecklisch, (48), found cholin in herring brine, -- 10 grams of the hydrochloride per 30 liters of brine. Likewise, Mürner, (59), found this substance in the flesh of the "Gahrstromling", ("Surfick"), a food preparation eaten by the people of northern Sweden and prepared by allowing the fish, pickled with a small amount of salt, to ferment anaerobically. Yoshimura and Kanai, (77), examined the extract from the cod and failed to find cholin.

Neurin, $C_5H_{13}NO$.

Neurin, which may be prepared chemically from cholin by the removal of one molecule of water has been found in meat extracts, but has not been found in fish flesh and is not considered a normal constituent of muscle, but a product of putrefaction.

Muscarin, $C_5H_{15}NO_3$.

Muscarin is also related to cholin, in that a synthetic product, pseudo-muscarin,

may be prepared from it by oxidation, and this substance is chemically very similar to the natural muscarin prepared from *Amanita muscaria*, (the Fly Agaric), though, physiologically, it is much less toxic. Like neurin, this substance is a putrefactive product of muscle and has not been found in fresh fish.

Trimethylamine Oxide, C_3H_9NO

Suwa, (70), found 20 grams of the hydrochloric acid salt of trimethylamine oxide in the water extract from 18 Kg. of the flesh of the shark, (*Acanthias Vulgaris*)

Betaine, $C_5H_{11}NO_2 \cdot H_2O$

Betaine, or trimethylglycine, was found by Suzuki and Yoshimura, (71), in the cuttle-fish to the extent of 2.05% on the dry basis. According to Yoshimura and Kanai, (77), this substance is also present in the cod, 0.044 grams of betaine as the chloride being found in 100 grams of air dry flesh. Suwa, (70), isolated 15 grams betaine hydrochloride from 18 kilograms of the flesh of the shark (*Acanthias vulgaris*). Likewise, Kutscher, (54), reports finding 0.07% of betaine in the same kind of fish.

Carnin, $C_7H_8N_4O_3 \cdot H_2O$

Krunkenberg and Wagner (53), (1885), separated from the extracts of *Barbus fluviatilis*, *Abramis Brama*, and *Leuciscus dobula*, a substance resembling the hydrochloride of carnin. The basic lead acetate precipitate obtained from the extracts, when dissolved in hot water, filtered, and freed from lead by means of hydrogen sulfide, gave crystals which Barger, G. The similar Natural Bases. A Mon. on biochemistry, 1914 p. 65 decomposed at 230°C, and which appeared to be identical in form with those of the hydrochloric acid salt of the carnin-like substance prepared from the flesh of frogs and alligators. The double platinum chloride salt was prepared from it.

Carnosin, $C_9H_{14}O_3N_4$

Carnosin was determined by Suzuki and Yoshimura (71), in the extract from the flesh of a number of fish and the following results obtained:-

Table No. 18 CARNOSIN IN FISH

In hot water extract of the flesh of	Carnosin in per cent, of dried muscle.
Bonito, dried	0.36%
" fresh	0.00%
Tunny fish, fresh	0.20%
Salmon, fresh	0.055%
Hal fresh	0.067%

Treatment of the phosphotungstic acid precipitate of the protein free extract of the fish with silver nitrate and barium hydroxide solutions for the removal of Xanthin, hypoxanthin, and histidin, left a strongly alkaline solution which contained a free base, and which crystallized in colorless, prismatic crystals when the concentrated solution was treated with absolute alcohol. Heated in a capillary

tube, the crystals decomposed at 233-235°C. (uncor.). The platinum chloride salt was prepared. Analysis gave the formula $C_9H_{14}N_4O_3$ which corresponds to that of carnosin prepared from Liebig's extract of beef.

Lecithin.

In 1855 Valenciennes and Frey, (73), separated a phosphorus and nitrogen containing substance from the flesh of fish, which resembled the compound often found in the gray matter of the brain. It is possible that this substance was impure lecithin.

In an endeavor to ascertain the physiological action of hard and soft water on carp, Reuss, (37), determined the lecithin content of this fish under different conditions. 3.21% lecithin (dry fat-free basis) was found in a carp which had lived 2½ years in soft water, while 2.11% lecithin was found in a carp which had lived the same length of time in hard water. If the results are calculated to an ash-free basis, however, the difference in lecithin content is greatly reduced.

Urea was determined in the blood, muscle, and liver of a number of dogfish by Schroeder, (64). The constancy of the amount of urea in the blood leads to the assumption that the physiological processes in the body. When the blood content reaches a certain amount, the excess is excreted in the urine in such the same manner that sodium chloride is maintained at a constant proportion in the blood of warm-blooded animals by elimination of the excess through the kidneys.

Table No. 19 UREA IN THE DOGFISH (*Scyllium catulus*)
(Mean of seven analyses of different dogfish)

	Urea %	Maximum variations %
Blood	2.61	14.83
Muscle	1.95	18.68
Liver	1.36	87.12

The great amount of urea in the organs of selachians is due to its slow elimination. White and Crozier, (75), found 8.27% of the total nitrogen of dogfish flesh to be urea nitrogen. Rabuteau and Papillon, (62), found a urea-like substance in the peritoneum of the ray, which they conclude to be a mixture of urea and urea compound. The same phenomenon was noted by them in the case of the cramp fish and dogfish.

Inosinic Acid, $C_{10}H_{13}N_4PO_8$.

Gregory, (49), in his work on the cod and ray, and Limpricht, (57), in the examination of the herring, failed to find inosinic acid. We have no record that his compound has ever been separated from the flesh of fish.

Taurin, $C_2H_7NSO_3$.

Valenciennes and Frey, (73), in 1855 isolated a substance from cuttle-fish, which according to its chemical constitution, was probably taurin. Three years later Stödler and Frerichs, (68), found it in the flesh of the ray and shark. Limpricht, (56), (1863), found 0.106% (fresh basis) of taurin in the flesh of the roach. Suzuki and Yoshimura, (71), separated 1.3% (air dry basis) of taurin from the cod, while Yoshimura and Kanai, (77), found 1.74% (dry basis) in the flesh of dried cuttlefish.

Lactic Acid $C_3H_6O_3$

Liebig (55) (1847) separated lactic acid from the pike and prepared the calcium salt therefrom. In 1863 Limpricht (56) found 0.064% lactic acid in the water extract from fresh roach.

Glycogen $C_6H_{10}O_5$

The glycogen content of the muscle of fish varies from 0 to 0.59%, according to Schöndorff and Wachholder (63). Fresh water fish contain more glycogen than do salt water fish. The content in this substance changes rapidly after death of the fish. The fresh carp contains 0.527%, after one hour 0.359%, after one day 0.145%, and on third and fourth days the glycogen has disappeared. The muscle of fresh salmon and cod contain no glycogen. In the fish livers the glycogen varies from 2.5 to 12.94%.

Unknown compounds.

Occasionally compounds are separated from the extracts of fish which cannot be identified with any previously known substance. Suwa, (69), isolated a substance from the hot water extract of haddock, from which he prepared, first, the mercury salt, then the gold chloride salt. The latter salt resembled neurin gold chloride in composition, except that the carbon content was somewhat low. Buglia and Costantino, (13), obtained a crystalline deposit on the inside surface of a flask, in which the muscle of the dogfish had been heated at 100-20°C. for three or four days. The platinum chloride compound could not be prepared in sufficient amount for identification of the substance. Limpricht, (56), obtained a substance from the roach on addition of sulfuric acid to the filtrate from his creatin determination, which he called "protosure." When in solution in ammonia, most metallic salts precipitated it. It also formed a barium salt.

Philothion (Hydrogenase)

According to Ray-Pailhade (61), fish flesh preserved in oil or salt contains a substance which has the property or power of reduction. It is sometimes called a hydrogenase, mainly because of its ability to form hydrogen sulfide from sulfur. Whether the substance is actually an enzyme or whether the reducing properties observed are due to unsaturated compounds is not known.

Chapter III

Some Forms of Preserved Fish

Fish are prepared for market in many different ways. In some cases the purpose is merely one of preservation. When long voyages to the fishing grounds are necessary the catch must be preserved by salting or pickling, or when fish are caught in large numbers during a very short natural season they are canned as in the case of salmon or they are frozen and held in cold storage, as in the case of a large number of the fish caught off both coasts of the United Coast. In other cases, however, the mode of preparation is governed by the popular demand.

In the present chapter will be mentioned a few of the commoner varieties of preserved fish (except canned) on which we have chemical data.

Frozen Fish.

Preservation of fish by freezing is perhaps the best method in many respects. It comes the nearest to preserving the natural appearance and taste of the fish. As soon as the fish are brought to shore they are gutted (if large,) washed, arranged in rows, heads and tails alternating, in shallow iron pans, and these pans placed for 24 to 48 hours on the cold brine pipes in a freezing room. This freezer is maintained at from -2° to 4°F . When the fish have been frozen hard, they are removed from the pan in a solid cake and glazed with ice by being dipped two or three times at intervals into a trough of water in a room at about 10° - 15°F . The cakes are then boxed and placed in permanent cold storage rooms maintained at a low temperature not exceeding 15°F .

According to Smith, (3), and also Perlzweig and Gies, (84), flounders did not undergo any chemical change of importance from the standpoint of nutritive value, at any time during a period of two years storage, as shown by the following table:-

The methods of preserving fish by "artificial cold", as used by the Dutch Commission, (80), in an investigation of this subject, differed from those used in this country in that no attempt was made to prevent the fish from drying out. As a consequence, the taste, odor, and appearance of the fish was unfavorably influenced by this one factor, 12% to 24% loss in weight having occurred in the case of soles kept hard frozen for 4 - 5 weeks. An interesting discussion of the physical and histological changes occurring in the tissues is given in the report of the above commission. It appears that in freezing fish, the water is withdrawn from the cells and then crystallized outside of them in the intervening spaces, and is not frozen in its original position within the cells. In thawing, it is supposed that if the fish is thawed slowly the water from the melting ice will be reabsorbed by the cells and the flesh return to its natural state.

According to Wall, (90), frozen fish keep as well, or even better in the round than when gutted. These fish, however, kept their original qualities for only one month and at the end of three months mold growths were observed. The putrefaction was observed to begin in the skin and spread internally. Under proper conditions of storage, however, this decomposition would not have occurred.

A new method has recently been introduced in Halifax, N. S., which deserves special notice. The principle of the method consists in placing the fish for 2 hours in a brown sugar and salt (17.8%) solution lowered to -10°C . The hard frozen fish are then removed and placed in the regular shipping package to await transportation to market. It is claimed that fish so prepared can be brought to the market in exactly the same condition as when they were drawn from their native water, (83).

Table No. 20

GENERAL AVERAGE DATA PERTAINING TO THE COMPOSITION OF FLOUNDER IN COLD STORAGE

Months in cold storage	Summer flounder (flake.)				Winter flounder.				Lipins (ether extract.)		Reaction of aqueous extract. (2)
	Water	Nitrogen		Ash	Water	Nitrogen		Non- coagulable	%	Acidity (1)	
		Total	Ammonium			Total	Soluble				
	%	%	%	%	%	%	%	%			
0	78.56	3.44	0.0195	1.28	81.73	2.89	0.964	0.383	0.379	136	1.57
6	78.69	3.39	0.0142	1.29	81.58	2.79	0.887	0.351	0.366	136	1.17
9	78.45	3.60	0.0213	1.26	82.04	2.83	0.937	0.353	0.443	127	1.23
17	78.91	3.46	0.0177	1.23	-----	-----	-----	-----	-----	-----	-----
20	-----	-----	-----	-----	79.14	-----	-----	-----	0.431	134	1.28
21	79.24	3.51	0.0205	1.26	-----	-----	-----	-----	-----	-----	-----
22	-----	-----	-----	-----	76.93	3.39	0.876	0.433	-----	-----	1.20
23	79.19	3.26	0.0231	1.25	81.23	-----	-----	-----	0.301	147	-----
24	75.74	3.28	0.0217	-----	76.32	3.55	1.008	0.379	-----	---	1.32

(1) Milligrams KOH required to neutralize 1 gram of the total lipin mixture.

(2) Cubic centimeters N/5 NaOH solution required to neutralize 100 c.c. of extract.

Marinated (Herring), salted,
pickled, broiled fish.

Koenig and Splittgerber, (19), found that in three common methods of preserving herring, broiled, marinated and pickled, a perceptible amount of food constituents and stimulants are lost during the course of their preparation. The following table will give an idea of the extent and character of this loss. The basic nitrogen of the broiled and the marinated herring consisted mainly of purine bases, creatin, creatinin, diamino acids and mono-amino acids.

Table No. 21

LOSS OF FOOD CONSTITUENTS IN THE PREPARATION OF COMMERCIAL BROILED, MARINATED AND
PICKLED HERRING.

(In per cent of the fresh weight of the fish)

Kind of Preparation	Albumin	Basic Nitrogen	Ammonia Nitrogen	Residue on evaporation	Ash	Organic matter
	%	%	%	%	%	%
Broiled Herring	0.01	0.47	-----	6.60	2.53	4.04
Marinated "	0.03	0.26	0.005	3.47	1.48	1.99
Pickled "						
(grams in 1000 c.c. of brine.)	5.9	5.27		322.82	289.19	33.63

In northern Sweden is found a variety of pickled fish called "Gårströmling". The peculiar flavor brought about by the bacteria which thrive in dilute salt solution, is considered a delicacy by the inhabitants but is despised by people in other lands. In the water extract of this preparation Mürner (59), found the following (a) acids and bases:-

- | | | | |
|----|-------------------|----|----------------|
| a. | succinic acid | b. | Ammonia |
| | butyric " | | Methylamine |
| | acetic " | | Dimethylamine |
| | valerianic " | | Trimethylamine |
| | formic " | | Cholin |
| | solid fatty acids | | |
| | leucine | | |

Indol, skatol, phenol, putrescin and cadaverin were absent.

Schmidt-Nielsen, (86), (87), (88), concluded from a number of experiments that the changes produced in the fat and flesh of herring in pickle for long periods of time, were due to enzyme action. As the following tables show, there are many constituents present in herring brine which are not present in fish meat, due to the fermentation processes which are carried on in saturated sodium chloride solution. Note the high proportion of nitrogen as amides and amino-acids.

Table No. 22-A

ANALYSES OF HERRING-BRINE OF DIFFERENT AGES

Age of Brine	Total nitrogen	Nitrogen		Xanthin	(NH ₃)-N
		Split off by HNO ₂	NaBrO		
	%	%	%	%	%
1 month	3.7	0.7	0.7	0.6	0.1
1 year	5.3	1.2			
2½ years	8.8	3.7			
5 years	12.0	5.7	1.6	1.3	

Table No. 22-B

ANALYSES OF FAT OF PICKLED HERRING OF DIFFERENT AGES

Kind of Herring	Time after pickling	Characteristics of fat (obtained by leaching out with boiling water.)		
		Acid No.	Acetyl. No.	Iodine No.
Norwegian	0	0.6	8.88	131.2
"	0	0.8		
Holland	14-30 days	10.6		
	Age unknown	30.2	25.5	134.
				122.
Norwegian	9 months	37.0	21.0	127.9

The increase in the acetyl number, as shown in Table No. 22-B indicates an increase in the proportion of oxy fatty acids, or an apparent increase in -OH groups.

The extent to which varying amounts of salt will preserve meat and fish is clearly shown in the following table by Peterson, (85). Fish decomposes much quicker than meat. At 15% concentration of the salt, a fungus growth was observed in both kinds of meat. In all concentrations at 15% or below, ammonia, peptones and butyric acid was proven to be present.

Table No. 23

COMPARATIVE RATE OF DECOMPOSITION OF FISH AND BEEF PRESERVED IN SALT SOLUTION

% NaCl	F I S H			M E A T		
	H ₂ S	Indole	Phenol	H ₂ S	Indole	Phenole
5	6	16	55	6	26	---
8	14	29	75	12	75	---
10	28	68	--	40	75	---
12	58	75	--	--	--	---

The same author, (85), in a bacteriological study of the question of meat preservation by means of salt, found that 5% solutions of salt stopped the growth of obligatory anaerobes, while the higher per cent solutions permit only the presence of facultative anaerobes and aerobes. By using the smallest amount of salt possible to prevent putrefaction, certain organisms continue their action and change the appearances, odor and taste in the desired direction (for a certain kind of fish preparation).

The number of days necessary to produce a confirmative test.

Isaac, (50), found 20 grams guanin as the hydrochloride, 3.5 grams adenine as the picrate, 5.0 grams of hypoxanthin, and a trace of xanthin in 100 liters of year-old herring brine.

The technical processes employed in curing, pickling, "water-horsing", drying, etc., of salt cod is well described in an article by Bitting (79). The author suggests a number of sanitary methods to prevent the spoilage of the fish by "reddening", a condition brought about by infection of the fish with a chromogenic organism, said to be found in the bogs, marshes and lowlands in the vicinity of Gloucester, and also in the salt employed in preserving fish.

Canning of Fish.

The canning of fish is a very large industry in this country. The estimated peck of canned salmon in the United States and Alaska during 1914 exceeded eight million cases. In 1908, a little over five million dollars worth of sardines were canned. No discussion of the chemistry of this kind of preserved fish will be made in this paper.

Fish sausage.

Bitter (78), prepared a number of fish sausages from the flesh of the cod and sea salmon. The finely chopped fish flesh and some kind of fatty material (originally hog lard was used but later chopped bacon was adopted) were filled into bladders in the usual manner. Analysis of this sausage gave the following values:-

Table No. 24
ANALYSIS OF FISH SAUSAGE

	Water-free basis	Original basis
Water		66.64%
Protein	65.70%	21.90%
Pat	28.00%	9.33%
Ash	6.03%	2.01%

Comparing the food value of the above sausage with those of other forms of food, the author found fish sausage to be an economically cheap food. He recommends that the sausage be cooked at 80°C. for at least thirty minutes to kill existing bacteria and to destroy the toxins formed by them.

Fish meal and similar preparations.

A number of processes for the preparation of fish meals, fish extracts, and so forth, have been recorded in the literature in recent years. Among these is a process for making a food from pulped flesh of fish and starch powder, patented by Davidson, (82). Schwickerath, (89), makes what he calls a proteose preparation by subjecting the fish flesh to the action of superheated steam at 120-30°C. for two hours, the expressed solution therefrom being treated with milk of lime, heated until a flocculent precipitate separates, filtered, and the filtrate evaporated to dryness. By digesting the albuminous matter extracted from fish with alkali at 90-100°C, neutralizing, filtering, then flavoring and concentrating in vacuo, Danilevsky, (81), obtains a tasty fish broth.

Chapter IV

The Digestibility of Fish Flesh
Artificial digestion by means of pepsin and trypsin

Among the earliest experiments on the relative digestibility of fish are those of Honigsberg, (94), in 1882, who found that cooked white fish was digested less rapidly by pepsin than raw beef and more rapidly than cooked beef.

The work of Chittenden and Cummins, (92), on a number of different kinds of meats and fish flesh showed, however, that cooked beef was more readily digested than cooked fish. A few of their results are given below:-

Table No. 25

RELATIVE DIGESTIBILITY OF FISH FLESH IN GASTRIC JUICE.

Kind of Meat	Per cent of solid matter.	Solid matter in 20 grams	Av. amt. digested from 20 grams	Relation of amounts digested to amounts digested from 20 grams of cooked beef. (Average 4.461)
Beef	26.03	5.206	4.1167	100.00
Lake whitefish	25.56	5.112	3.8350	94.78
Salmon	31.06	6.212	3.7345	92.29
Bluefish	19.84	3.968	3.5885	88.69
Flounder	23.04	4.608	3.4525	85.32
Striped bass	20.73	4.164	3.2770	80.99
Weak fish	19.78	3.956	2.9180	72.11
Eel	21.78	4.356	2.9062	71.82

Herter and Popoff, (93), on finding that cooked fish was less digestible than raw, explained this phenomenon as being due to two factors; firstly, the heat coagulates the protein and thus renders it more resistant to the action of the gastric juice; secondly, the steaming tends to tear apart the fibres, thus favoring the entrance and action of the digestive fluids. In meat, the protein is coagulated into impenetrable and tenacious lumps, while in fish where the connective tissue readily changes to gelatin, the flesh is quickly disintegrated. The relative digestibility of raw fish flesh and raw beef is as 70:100. As, however, the boiling water affects the digestion of meat more than of fish the ratio for cooked

flesh is not as great, --86:100. In some later experiments, (95), Popoff found that smoked eel and plaice were more digestible than the raw, but that smoked beef was less digestible than raw.

Working on the fat-free dry flesh of fish, Koenig and Splittgerber (19), found that there is no marked difference between the digestibility of beef and of fish flesh with pepsin.

A method for comparing the digestibility of various protein substances has been devised by White and co-workers, (75), (102), who followed the rate of hydrolysis by means of amino acid determinations, using the methods of Van Slyke, (74), and Scranson, (66). They found that trypsin digested boiled codfish and dogfish much more readily than it did boiled beef. Figures for weakfish are also given. White suggests that rapid hydrolysis of some proteins is not beneficial to the organism, as the organism is not capable of building up into more complex bodies, the product of rapid and complete hydrolysis, absorbed by it. Otherwise stated, the slowly digested foods are more thoroughly catabolized because the organism can more readily transform into higher complexes the intermediary products of hydrolysis, such as polypeptides, than it can the final products--amino acids.

Metabolism Experiments with Fish.

Kianikyn, (16), (1887), found that when salt cod was fed to human subjects, alone, 90.02% of the nitrogenous substances was absorbed, but when fed with a mixed diet, 94.44% was absorbed. A corresponding increase was noted in the degree of assimilation of dried cod alone with a mixed diet.

According to Rossoff, (96), the fat of smoked smelt is more completely absorbed than that of the fresh fish. But since the amount absorbed was approximately 98% in the former and 97% in the latter, we do not think the author was justified in drawing this conclusion.

Atwater, (91), fed meat and fish in periods of three days each to a man and a dog and concluded therefrom that beef and fish are equally well absorbed as regards protein, fat and ash. Likewise, Rosenfeld, (97), experimenting with the sea pike and sea salmon, found that fish flesh was equal to beef in maintaining nitrogen equilibrium. It gives the same satisfaction of appetite for an equal length of time, produces the same or a little less uric acid, and has the same strength-giving power as the best beef. Furthermore, according to Slowoff, (98, 99) and also Slowoff and Kraitschenko (100) the organism assimilates the fresh fish flesh nitrogen better than the nitrogen of beef, while the nitrogen of dried or salt fish is as easily, or a little less easily, assimilated than that of beef. On substituting fish for beef in a human diet, the urine showed a decrease in urea, uric acid, creatinin, ammonia, phosphoric acid and sodium chloride, and an increase in residual nitrogen. The absorption of mineral salts in the intestine was better on a diet of fresh and salt fish and poorer on dried fish than on beef. In order to confirm some results, (75), on the artificial digestion of fish flesh, Van Slyke and White, (101) performed metabolism experiments with boiled cod, fried cod, boiled beef, boiled weakfish, boiled mussel, etc., and found as in the previous case that boiled cod was digested the most rapidly. The rate of excretion in the urine is taken as an index of the readiness with which proteins are digested, absorbed from the alimentary canal, and metabolized in the body. The nitrogen of the feces is taken as an index of the relative completeness with which the proteins of the different foods are absorbed. The results show that the proteins are more capable of

amintaining nitrogen equilibrium when they are slowly digested than when the digestion is rapid. Addition of starch to the diet caused a retardation of the absorption of the protein, from which it is apparent that the amount of other foods in the diet affects the rate of absorption. When arranged according to the rates at which nitrogen was digested and absorbed, the flesh proteins (boiled) appear in the following order: fresh cod, beef, tautog, eel, weakfish, mussel, salt cod, periwinkle. But when ranked according to the amounts of nitrogen retained from each, the order is practically reversed.

Taking the foregoing facts into consideration, there is not reason to believe that fish are not fully as digestible, wholesome and palatable as other forms of flesh foods. Their ability to supply the protein needs of the body is unquestionable.

Chapter V.

Putrefaction Bases of Fish Flesh.

Some fish are always poisonous; others are poisonous only during spawning time; still others are subject to epidemic bacterial diseases, and those affected with certain of these diseases furnish flesh that is toxic to man. Lastly, fish, like other kinds of meat, may become infected with saprophytic germs that may be harmful to man.

In Russia and Japan, certain species of fish, particularly the Tetradon and Orthogoricus, have been known to cause sickness and death. The toxin is apparently natural to the fish and though it is distributed throughout the body, the amount in the flesh is small compared to that which is concentrated in the ovaries. The maximum of toxicity coincides with the maximum of ovarian activity. The toxin is destroyed only after prolonged heating (boiling for at least three hours). There are no such naturally poisonous fish, however, in the waters of the United States.

A great amount of illness has been caused by poisoning from eating fish, either poisonous species or edible fish partially decomposed. The poisoning effects are ascribed to the toxic action of ptomaines. Very little is known at present concerning the exact nature of these toxic substances. Quite a few of the cases reported as ptomaine poisoning are undoubtedly due to acute indigestion and bacterial infection. According to a classification of fish poisons by Blanchard, the poison due to putrefactive changes is called botulism. Barger (104) states that the term ptomaine has fallen into disfavor and recommends in its stead a more comprehensive term, putrefaction base.

A partial decomposition takes place in herring in pickle. According to Schmidt-Nielsen (86), (88), these changes are the result, for the most part, of autolytic processes which complete themselves in saturated salt solution. It is not entirely impossible that some of the toxic substances produced in fish flesh are the result of autolysis as well as of bacterial action.

Another example of an edible variety of fish in partial decomposition is the Gehrströmling, a peculiar Swedish fish preparation made by allowing the fish to ferment under anaerobic conditions in a dilute salt solution. Mörner, (59), detected amines, choline, volatile fatty acids and leucine, but failed to find indol, scatol, phenol, putrescin and cadaverin in the flesh of fish treated in this way.

Brieger, (106), isolated from spoiled cod the following bases: neuridin, ethylene diamine, muscarin, gadamin and triethylamine. Wacklisch, (48) (105), obtained from spoiled perch and herring, cadaverin, putrescin, neuridin, gadamin, methylamine, dimethylamine and trimethylamine. Both Brieger and Wacklisch believe that the most poisonous substances are produced in the earlier stages of decomposition, and that oxidation in the later stages reduces the toxicity. "In some cases people like spoiled fish and eat it with no apparent sign of trouble. In fact, certain dwellers on the sea coast in Asia bury the fresh fish and allow them to remain until they develop a bad odor before eating. However, we must not conclude from this that spoiled fish are harmless. The question must be more thoroughly investigated" from the chemical and pharmacological standpoint.

Anrep, (103), separated two bases from the salted sturgeon which had caused death in Charkow in 1885. One of the bases resembled the ptomatropin of Kobert and was toxic, while the other base was non-toxic. Quoted from Smolenski (112). Furthermore, Lieventhal, (110), obtained from salt sturgeon by the Stas-Brieger method, two bases, one solid and the other oily. The former when tested on dogs proved to be non-toxic. Wassiljew and Wotschaloff, (114) obtained poisonous bases from ordinary frozen and salted sturgeon and salmon in St. Petersburg, and also from the same fish which had caused sickness in Charkow. On feeding the substances to frogs and dogs, the fact was established that the poisoning effect was due to the combined action of the whole group of bases, rather than to any single base. In an investigation of the toxic substance of the fish of Russia, Konstanov and Manov (109) found that this substance did not lose its toxicity for mice when digested with erepsin, but lost it when digested with pepsin and with trypsin. It was, therefore, a protein and not a proteose or peptone.

According to Vaughan and Novy, (113) the former found in canned salmon, which had caused sickness, a micrococcus, which, when grown for twenty days in a sterilized egg under anaerobic conditions produced a potent poison. Ten drops of the white of the egg sufficed to kill white rats.

Gautier and Etard, (108), obtained a base called hydrocollidine, and a base, isomeric with parvolin, $C_9H_{13}N$, from decomposed mackerel.

Desgres and Cains, (107), isolated by the Stas-Otto method from canned fish certain bases, more or less oily liquids, which were only slightly toxic and which acted favorably in stimulating the appetite.

Sieber-Schomow, (114), separated from dead and infected fish, and from the water in an aquarium where several fish had died in the course of two days, a highly toxic germ, which was given the name of B. piscicidus agilis. From cultures of this bacillus, cadaverin and other known putrefactive bases were obtained.

Chapter VI

A Consideration of Fish Waste. Fertilizer.

The first known use of fish as a fertilizer occurred before the advent of early settlers in this country. For fertilizing purposes the Indian placed one or two fish in each hill of corn. Carried to extremes, the practice had a deleterious effect upon the soil, due to the accumulation in it of undecomposed oil. Later, the oil was removed from the fish by allowing them to decompose while suspended in water, whereby the oil rose to the surface of the water and was skimmed off. The residue

was spread upon the land. At the present time, however, steam presses are used in all new factories for the separation of the oil from the scrap. The latter is dried in artificial hot air driers for shipping.

The recovery of fertilizer and oil from cannery waste is a comparatively new industry in this country. The production of fertilizer and oil from this source deserves more attention than it has been given in the past. The beef packer relies to a considerable extent upon the manufacture of marketable products from the inedible portion of the steer for the success of his business. The majority of the salmon packers, however, throw away 30 per cent of their raw material, the value of which, though it does not equal in importance the by-products of the meat packing business, is of sufficient value to pay for working it up into fertilizer and oil. A few of the progressive canneries of the Pacific coast are awake to the value of these products and have installed machinery for their recovery.

The following table gives the output of crude and dried fertilizer by the states of the Atlantic coast during the year 1912, from a report by Turrentine, (135).

Table No. 26

OUTPUT OF FISH SCRAP BY STATES OF THE ATLANTIC COAST, 1912.

State	Acid tons	Dry tons	State	Acid tons	Dry tons
Maine	100	256	Delaware	6,312	500
Connecticut	1,500	6,500	Virginia	-----	34,000
New York	19,800	-----	South Carolina	-----	7,250
New Jersey	530	1,500	Florida	-----	160

Also during 1913, a total of 1,630 tons, (136), of dried fish scrap were manufactured from the waste from salmon canneries on the Pacific coast of the United States.

The principal source of fish scrap fertilizer is the menhaden, other sources being insignificant in comparison to it. The United States Government has been investigating the problem of the feasibility of using the dogfish as a food. The fish is not popular as a food and it will probably in the future be used extensively as a fertilizer, as it yields a scrap high in nitrogen, (129). Fertilizer is also made from herring, salmon, and sardine cannery waste. Scrap could also be prepared from the refuse from the dressing of cod and other fish, but the individual plants are scattered over a long stretch of shore, and at present no attempt is made to save it, for now it is thrown away as fast as produced.

The following analyses made under the direction of J. W. Turrentine, (135), serve to show the value of fish scrap as fertilizer,

Table No. 27
ANALYSIS OF FISH SCRAP.

No.	Location	Description	Nitro- gen.	P ₂ O ₅ %	Moist- ture. %	Oils %
1	Kilmarnock, Va.	Dry scrap from 6 sacks.	8.93	6.17	6.48	5.91
2	Taft, Va.	Dry scrap. Sample of 525 tons.	8.96	7.75	6.18	6.61
3	Irvington, Va.	Dry scrap, dried in hot air and steam driers (From one sack.) Fall product.	7.7	5.22	11.68	6.62
4	Cape Charles, Va.	Scrap, ground from 3 sacks.	9.29	6.12	7.86	5.38
5	" " "	Dust from grinders.	8.80	5.21	7.17	7.55
6	Beaufort, N. C.	Dry scrap, hydraulic pres- ses, sample from heap.	8.82	5.95	6.13	8.57
7	Morehead City, N. C.	Dry scrap from open heap.	8.49	5.95	9.12	8.23
8	" "	Dry scrap from hydraulic presses.	7.76	9.65	8.15	7.56
9	Lenoxville, N. C.	Ground scrap, sun dried, hydraulic presses. Sample from heap.	7.81	5.85	7.46	7.89
10	" "	Dry scrap found, hydraulic presses. Sample from heap.	8.29	9.00	7.00	5.40

According to Turrentine, (196), on the Pacific coast, the salmon canneries of the United States, in 1913, produced a total of approximately 4,000,000 cases of sockeye salmon, and 2,700,000 cases of other kinds of salmon, from which it is calculated there resulted 119,900 tons of waste, valued at \$1,798,650.00.

In this same year only 1,630 tons of dry scrap, and 280,000 gallons of oil, were manufactured from the waste from salmon canneries on the Pacific coast of the United States, this amount being the output of five plants. From the above figures, it is plain that the greater amount of the cannery waste is thrown away. The fertilizer industry could easily make room for several more rendering plants to take care of the waste that is not being thrown away. The difficulty attending the rendering of fish scrap is in procuring the raw material, which often must be collected from several canneries scattered over a great length of shore. In addition to this

difficulty, the refiner is never sure of a steady supply of cannery waste, the amount being great at times and negligible at others. It has been suggested that the fish-scrap fertilizer plants be used in dull seasons for the preparation of fertilizer from the giant kelps found all along the Pacific coast. A centrally located rendering plant is not as desirable as the small by-products plant operated as an integral part of the cannery, as by the latter method all of the waste of the cannery may be utilized whereas with the former much of the waste of the canneries upon which it depends for its supply is thrown away because the barge or vehicle for conveying it to the plant is not available at the proper time. The following tables (136) will show the composition of raw and dried scrap obtained from this source:-

Table No. 28.

ANALYSES OF SAMPLES OF RAW MATERIAL PRODUCED AS WASTE IN THE MECHANICAL DRESSING OF "HUMPBACK SALMON."

Character of sample	Moisture.	Nitrogen.	P ₂ O ₅	Ca ₃ (PO ₄) ₂	Oil	Oil per ton
Roe and Milt. (50% of each.)	68.7	3.68	1.08	2.35	3.18	8.24
Heads.	63.2	2.65	1.54	3.36	13.70	35.51
Fins and tails.	63.26	3.11	2.20	4.80	11.16	28.94
Average-	64.6	3.02	1.59	3.46	10.43	27.05

Table No. 29.

ANALYSES OF DRIED FISH SCRAP PREPARED FROM SALMON CUTTINGS.

No.	Description	Nitrogen	P ₂ O ₅	Moisture	Oil ¹
1.	Steam Drier	9.39	5.32	5.36	12.96
2.	" "	8.26	7.91	5.21	17.36
3.	Hot air drier	9.49	9.26	5.26	8.32
4.	Steam drier	8.76	7.00	3.91	20.02
5.	Hot air drier	7.63	12.08	5.11	10.96

(1) More accurately, ether extract. This consists principally of oils.

Many analyses of fish guano or fertilizer appear in the literature from time to time. Interesting information may be obtained in regard to the fish fertilizer industry of Norway, Japan, Russia, Germany, and England by consultation of the works of Cligny (117), Audebert (116), Kossovich and Kolotov (127), Weibull (137), and Douglas (118). Additional analyses of fish scrap in this country are reported by Lindesuth and Parker (128), Atwater (115), and Johnson (125).

Feed for Farm Animals.

That fish scrap may also be used successfully as a food for farm animals was shown by Schenke, (113), Winberg, (139) and Kleeman, (126). According to Henry (123) two kinds of feeds are on the market; (1) fish scrap, which is the dried and ground refuse in the manufacture of dried codfish, and (2) fish cake, which is the whole fish dried and ground. Both feeds are used as cattle feeds in the coastal regions of Europe. Fish scrap has been used in this country as early as 1864 as a food for sheep, swine and poultry.

On feeding fish scrap to cattle, milk and butter normal quality are produced on a daily allowance of 2.3 pounds of fat-free scrap supplied with a variety of other feed without deleterious effect. Kellner calculates that the maximum daily allowance of fish scrap for sheep, in the beginning, should be from $\frac{1}{2}$ to $\frac{2}{3}$ of a pound. Chickens fare well on a diet of fish scrap and seem to prefer it to corn, but as yet, very little of this kind of chicken feed is being used. The universally satisfactory results of all the recorded experiments with fish scrap as a food for farm animals leaves little room for doubt as to its efficiency.

Fish Glue, Gelatin, and Isinglass.

A product known as fish glue is prepared from the scales and skins of large fish like the carp by acting on them with hydrochloric acid and the extracting with water.

In making isinglass, the swimming bladders of sounds of fish are taken and thoroughly washed in water to remove all fatty and bloody particles. They are then removed and cut longitudinally in sheets and allowed to dry, with the outer face turned down, upon boards of linden or bass-wood. The inner face of the bladders is pure isinglass, which can be removed from the muscular coat when the bladders are partially dry. The isinglass layer is bleached with sulphurous acid and then thoroughly dried.

In 1908, \$150,000 worth of isinglass and \$632,000, worth of glue were produced in the United States, most of which was manufactured in Massachusetts.

Kellner, Landw. Versuchs. 1877, p. 430, quoted by Henry, (135).

Fish Oils.

The best known varieties of fish oils of commercial importance are the menhaden, Japanese sardine, salmon, herring and a European sardine. The oils are obtained from the entire fish by boiling with water and removing the oil which rises to the surface.

The odour, colour and taste of fish oils are very much affected by contact with the decomposing matter of the fish. This difficulty is overcome to a great extent by removing the oil from the fish before the latter have had a chance to putrefy. An improvement in the quality of fish oils prepared in the last decade has been due largely to the substitution of steam trawlers and motor boat for the slow-going sailing vessels, for with the former type of fishing-boat there results less decomposition of fish and a consequent better quality of oil. The oils prepared under the best condition are paler in colour and have a much less disagreeable smell.

Menhaden oil is an American fish oil of great importance. In 1912 the United States produced from this source 6,651,203 gallons of oil valued at \$1,551,990. This oil is prepared by boiling the fish with live steam and skimming the oil from the surface of the water. The flesh is then pressed by hydraulic force, a little more

oil recovered, and the flesh dried and sold as fertilizer. Menhaden oil is used in the leather industry for currying, as a lubricant in soap making, in the paint and varnish industry as a substitute for linseed oil, for tempering steel, for the manufacture of linoleum, rubber substitutes and water-proofing solutions, and for adulterating cod liver oil. Japanese sardine oil is usually prepared by allowing the fish to decompose in heaps, whereby the oil flows out, the remainder of the oil being expressed from the residue by force. Oil so prepared does not lend itself easily to refining methods. This oil is used for tanning as a linseed oil substitute, in making soft soap, etc.

Salmon oil is rated as a high-grade fish oil, but very little of this oil is prepared in the United States, 286,000 gallons being the output in 1913. This oil is light and sweet, and in this respect resembles the edible oils. It may be used for the identical purposes for which menhaden oil is now used.

Attempts are being made in Europe to use sardine oil, obtained from the refuse of the sardine canneries by pressure, for the manufacture of varnishes. Herring oil is used somewhat in the leather industry. It is not as good a drying oil as menhaden oil.

For most uses of fish oils it is particularly desirable that the objectional odor be removed by some means or other. The odor and consistency of fish oils may be favorably altered by a process of hydrogenation. According to Ellis (119), by hydrogenation, oils which formerly made only soft soaps, now yield hard soaps, and in the case of fish oils the characteristic disagreeable odor is also removed. Whale oil treated in this manner yields a satisfactory substance for lard. Other processes and various foreign patents are recorded which deal with the problem of deodorizing fish oil, among them being those of Heyden, (124), Sandverg, (132), and de Hemptinne, (122). Okada, (130) found on dividing fish oil into solid and liquid that the odor persisted in the portion of the oil which remains liquid at -10°C .

According to Tsuyimato, the odor of fish oil is due almost entirely to a fatty constituent and not to so-called impurities. This fatty constituent is clupanodonic acid, having the formula $\text{C}_{27}\text{H}_{52}\text{O}_2$, which therefore by the addition of 8 H atoms becomes stearic acid.

Valenciennes and Frey, (73), isolated what they called salmonic acid from the fat of salmon. It has all the properties of a fatty acid and contained most of the colouring matter of the fish.

Payen (30), noted that the fluidity of the fat varied with different fish, being greatest with fat-rich fish and least with fish-poor in fat, as the cod, whiting and sole. In this connection, White, (102) found that the fluidities of mixtures of fish oils is additive; that is from the fluidity the percentage composition of the mixture can be calculated.

That there is no direct relation between the body fat of a fish and the amount of fat consumed by it in its food is shown by Rosenfeld, (38), who found that fat-rich fish will thrive on a diet poor in fat, and *vice versa*.

According to Weill, (138), the fatty material of the body of fish is distributed among the various tissues in proportions varying with the species and with the season.

In the carp and trout the accumulation of fat is in the subcutaneous tissue and muscles; in the whiting and sole the reserve of fat is in the liver, and in the mackerel the supply is concentrated in both muscles and liver. The following tables will show the variation in the fatty acid content of the organs and muscles of the carp at different times (specified times are not given.)

Table No. 30.

FAT DISTRIBUTION IN THE CARP AT DIFFERENT TIMES.

Fatty acids in:		
Kidney	Liver	Muscles
11.30%	7.59%	-----
9.43%	8.92%	30.91%
12.38%	16.84%	13.77%
-----	5.29%	7.23%

From the above table it appears that there is an interchange of fat from the muscles to the liver, and vice versa, and but little change in the fat content of the kidneys of the carp at different times.

Fish Liver Oils.

Cod liver oil possesses a medicinal value from the fact that it is easily hydrolysed or digested, this property being caused by the peculiar constitution of its unsaturated fatty acids. The therapeutic properties of this oil have been ascribed to a number of causes of doubtful worth. By some its action is attributed to the presence of a small amount of iodine, by others to the presence of such bases as butyl amine, isoamyl amine, hexylamine, dihydrolutidine, etc., separated from the oil by Gautier and Mourgues, (121).

Certain diets consisting of isolated proteins, starch "protein-free milk" and commercial lard were found by Osborne and Mendel (131) to be lacking in some substance which was necessary to maintain growth in white rats. The addition of cod liver oil to these diets, however, caused the animals to resume their normal rate of growth. It is therefore quite possible that the real value of cod liver oil as a medicine is due to the fact that it contains one or more of the necessary accessory food substances. The latter substances may be considered as carrying radical which the animal body is unable to synthesize of itself, but which it must receive preformed.

These oils are obtained from the fish livers in a manner very similar to the method used for the extraction of fish oil.

For the analytical constants of fish oils and fish liver oils see Chapter II, Tables Nos. 14 and 15, facing page 18.

It is hoped that the foregoing resumé will be of help to those engaged in investigation of fish problems. A further contribution of a similar nature but dealing primarily with the chemical pathology and physiology of fish, will appear in a future number of the Biochemical Bulletin.

THE CHEMISTRY OF FISH II.

The Chemical Physiology of Fish.

An intelligent campaign for the purpose of protecting fish from conditions causing their decrease necessitates primarily a thorough study of their habits and requirements. While considerable work has been done (in this country, principally by the U. S. Bureau of Fisheries) a great deal remains to be accomplished. Research of this nature supplies information which may be used not only as a contribution to pure science, but also to increase our food supply and to propagate new species of food fish. The fishing industries recognize the economic value of these investigations.

The Respiratory Exchange of Fish.

The respiration of fish is fundamentally the same as that of warm-blooded animals. The gases of the water come into contact with the vascular filaments of the gills where they are combined with the gases of the blood. The breathing rhythm of fish is affected more by a decrease in the oxygen content of the water (with consequent alterations in the blood) than by an accumulation of carbon dioxide in the blood. A thorough discussion of this question may be found in a review by Babak (140) and in an article by Petrik (201).

Fish suffocate when placed in boiled water because such water is practically free from dissolved oxygen. Kupsis (180) found that signs of asphyxiation appeared in fish when placed in water containing 0.91 c.c. oxygen per liter, and that death occurred when the oxygen content became as low as 0.66 c.c. per liter.

Clark and Adams (153) estimated that a one-pound fish will use 10 p. P.m. of oxygen per gallon of water per hour.

The determination of the oxygen consumption of aquatic animals is fraught with many difficulties. Many methods have been devised for accomplishing this purpose, among the earliest being those of Humboldt¹ and Provencal and of Bauert (144) (1855).

1/ Humboldt G. and Provencal - Mem. de la Soc. de phys. et chem. d'arcueil, Paris. 2: 359 (1807).

In the method employed by the former, and by Vernon (22), the animal is confined for a definite time in a closed receptacle containing water. The water is subsequently drawn off in order to determine the gases. This procedure is open to criticism because the absence of aeration causes the metabolic and respiratory processes of the animal to greatly alter the composition of the water by diminishing the available oxygen. The method of Jolyet and Regnard (176), based upon that of Regnault and Reiset, for land animals, consists in pumping air through the water in a hermetically sealed jar. The amount of oxygen used and the amount of carbon dioxide produced by the animal in a given time can be calculated from the composition of the entering and issuing air and water. Table No. 31 presents the figures in connection with the respiratory exchange of a number of fish. It will be noted that

Table No. 31
THE RESPIRATORY EXCHANGE OF FISH UNDER VARIOUS CONDITIONS

Kind of Fish	Total Weight of Fish	Oxygen Consumption per Ellipten and Hour in cc.	Respiratory Quotient	Temperature	Remarks	Observer
Tench (<i>cyprinus labeo</i>)		75.0				Quinquest (208)
"		11.53	0.70	5°		Baumert (144)
Carp (<i>cyprinus auratus</i>)		36.9	0.79	5°		"
"		41.3	0.76	11-12°		"
Brown trout, (<i>salmo fario</i>)	276.0	89.04	0.75	7.5-7.8°	12 fish-4" long	Gardner & Leithen
"	264.0	117.45	1.09	15.8-16°	11 "	(144)
"	300	213.80	0.84	21.3-22.4°	13 "	"
"	220	298.87	0.81	25-25.5°	11 "	"
"	423	144.76	0.33	4.1-4.5°	4 "	"
"	453	78.20	0.98	6.3-7.4°	5 "	"
"	285	230.	0.75	10°	3 "	"
"	361	186.44	0.63	16.1-16.6°	4 "	"
"	367	253.1	0.91	21.6-22.7°	4 "	"
Carp (<i>cyprinus auratus</i>)	300	50.4	0.43	12.5°	9 fish	Jolyet and Bagnard
"	398	41.0	0.80	11.0°	10 "	(177)
"	130	79.2	0.68	12°	1 "	"
"	145	37.0	0.83	12°	4 "	Fresh Water
"	130	36.0	0.87		4 "	"
Tench (<i>cyprinus labeo</i>)	447	55.7	0.64	14°	2 "	"
Sal (<i>salmo gairdneri</i>)	125	40.5	0.79	14°	8 "	"
"	150	48.0	0.60	17.5°	4 "	"
Carp (<i>cyprinus auratus</i>)	235	143.0	0.64	18.0°	3 fish	"
Gardner (<i>trichia bryonia</i>)	320	54.5	0.71	14°	1 "	"
Sal (<i>salmo gairdneri</i>)	515	50.8	0.72	17°	1 "	"
"	425	75.5	0.67	18°	3 "	Salt Water
Torpedo (<i>salmo torpedo</i>)	365	45.3	0.61	15°	1 "	"
"	450	48.8	0.56	14°	1 "	"
Sal (<i>salmo gairdneri</i>)	370	73.5	0.81	14°	2 "	"
Turbot (<i>sluwa marma</i>)	300	80.0	0.60	15°	1 "	"
Flounder	5.9	192.0	----	20.5°	normal fish	Bergelin (194)
Flounder	3.27	92.	----	17.5°	same fish as above after fasting 600 hours.	"

the temperature of the water has a very marked effect upon the oxygen consumption.

According to Jolyet and Regnard (177), the oxygen consumed by the carp, (in cubic centimeters per kilogram, per hour,) varies considerably in the same animal, according to changes in the temperature of the surrounding water. At 2°C, this amounted to 14.8 c.c.; at 10°C, 37.8 c.c.; at 30°C, 147.8 c.c. The respiratory quotients for the period stated above, were 0.89, 0.96 and 0.75 respectively, which does not indicate a definite relation between the CO_2/O_2 factor and the temperature. Gardner and Laethan (164), however, found a lower respiratory quotient for fish kept in cold water, than for those kept at higher temperatures, and concluded that in cold water the fish in a state of hibernation or starvation, may live on their fat, partially converting it into glycogen and sugar, and thus lowering the CO_2/O_2 ratio.

Zuns (228) also experimenting with the carp, observed that partaking of food raises the gaseous exchange of fish during the digestive process, the greatest rise being caused by protein-rich food, especially the natural diet of the fish.

In comparing the respiratory activity of the lower and higher marine animals (teleosts), Vernon (222) found a considerable difference between them, the latter having a higher respiratory quotient than the former.

Variations in the relation between the oxygen consumption and the body surface of fish, were noted by Putter (204), whose figures are present in Table No. 32. The figures in column (II) more nearly approximate a constant value than those in column (I), from which he concluded that the oxygen consumption of fish, per unit of body surface is very nearly constant.

The oxygen consumption of flounders was found by Morgulis (195) to have no direct bearing upon the weight or surface of the fish, the consumption per gram-hour diminishing, and per square centimeter-hour increasing, as the flounders became larger. The surface of normal flounders can be calculated from the usual formula $S = K \sqrt{W}$ where $K = 13.44$. The value K , however, is varied by changes in the nutritive state of the organism, and in the case of fasting animals, is considerably higher than normal.

Table No. 32.

Relation of the Oxygen Consumption of Fish to Mass and Body Surface.

Fish	Temp. C.	Weight of animals in grams.	Oxygen Consumption per	
			Kg. dried or- ganic matter per hour	Sq. meter of body surface per hour.
Salt Water				
Hippocampus	23	0.348	1050	335
Balistes	23	178.0	463	672
Scorpaena	23	16.3	535	408
Gobius	23	10.5	840	374
Hbliastes	23	13.2	980	625
Fresh Water				
Goldfish	23	4.2	562 at 16°C.	360
Stickleback	23	0.716	2750 at 16°C.	375

Some Conditions of Water Which Affect Fish.

.. In the same way that human beings are affected by abnormal condition of the atmosphere, such as a deficiency in oxygen, the presence of dust and of carbon monoxide and ethylene from illumination gas, etc., fish are also affected by alteration in the gas content, mineral composition, etc., of the water in which they live.

Marsh (189) found that aerated distilled water was fatal to fish. The cause is probably the inability of fish to maintain the proper concentration of the body fluids, owing to the great difference in the osmotic pressure between the external and internal fluids. And in the distillation of the water, poisonous substances may be generated which prove injurious to the fish.

Occasionally waters are found which have an abnormal gas content. Two waters of this nature were described by Marsh, one of which (189) contained at 15°C., 52.8 c.c. CO₂, 55.2 c.c. N₂, and 0.71 c.c. O per liter and the other (190) contained at 10°C, 18.79 c.c. N₂ and 8.54 c.c. O per liter. The former was obtained from a driven well 83 feet deep, intended to supply the water for the trout aquaria. It was soon discovered that trout, when placed in this water, at once exhibited great distress and within a few minutes turned over, apparently suffocated. By pouring the water through several perforated pans in succession, thus producing through aeration, it was possible to lower the carbon dioxide and nitrogen content of the water, and raise the oxygen content, to concentrations which would support the life of the trout.

In the second water, mentioned above, the abnormal condition was caused by a faulty pump, air having entered through holes in the pipe on the suction sides of the pump. An excess of 6.31 c.c. of nitrogen and of 2.09 c.c. of oxygen, in addition to the amount which would normally saturate the water at the same temperature and pressure, proved fatal to fish which died rapidly when placed in such water. An autopsy revealed that the heart was distended with gas, the larger blood vessels particularly the main vessel of the gill filament, was filled with gas, and the walls of the auricle and ventricle were possibly emphysematous. A fatal embolism resulted, death being due to stasis.

This disease of fish is termed by Marsh "the gas disease," the cause being explained as follows: The gills have a temperature almost the same as that of the water but lower than that of the venous blood, which derives its heat from oxidations incident to metabolic processes. Under normal conditions, the blood does not become completely saturated at the gills, as otherwise the gas would be constantly thrown out, owing to the higher temperature of the systemic circulation. It is probable, that under conditions of supersaturation, the blood at the gills collects more gas than it can hold at the higher temperature encountered in the blood which circulates through the body, and thus gas emboli are formed. Under conditions of mild supersaturation of gases in the water, there results, in certain fish, an accumulation of gas behind the eye, causing it to protrude. This condition, called "pop-eye", is not always due to excess of dissolved gases, however, as it may be caused by a blow on the head and by other conditions not definitely known.

It will be seen by comparing the oxygen content of ordinary air-saturated water, (507 c.c. oxygen per liter,) with the oxygen consumption of fish, that unless air is continually supplied to water in which they live, either by natural or artificial aeration, the fish will suffocate. Decomposing organic matter, and certain chemicals and bacteria will also lower the oxygen content of the water. The waste of the sulphite-cellulose factories (160) consists partly of inorganic matter, such as sodium sulphite and, at times, calcium carbonate, and the remainder, consists of wood particles and cellulose fibres. The oxygen content of water into which the waste pours is very low. The fine wood particles cover the bottom of the water courses, making them unsuitable as breeding places for fish, which avoid such waters, as the wooden particles also tend to clog up their gills.

According to Butterfield (152), tarred roads are a menace to fish life because surface waters carry impurities from them into the streams. Seydel (217) determined the effect produced upon fish, by various common fertilizers. He expressed the opinion no injury will result, if normal dressings of calcium nitrate, calcium superphosphate, basic slag, potassium chloride, potassium sulphate, kainite, sodium nitrate, or guano are applied to land in the vicinity of streams, since gudgeon were unaffected by 18 hours exposure to these solutions. Lime (except in small quantities) and cyanamide kill the gudgeon rapidly, and ammonium sulphate and stable manure also cause the death of fish. For this reason it is inadvisable to dress the borders of streams with these fertilizers.

Clark and Adams (153) found that pure aerated sewage is fatal to fish, a dilution of the sewage to more than 90% being required to maintain them alive. This shows the effect of concentration of the toxic principle upon the life of the organism. In investigating problems of this nature it is important to determine the proportion of pollution in the water. When factory waste reaches the streams it is enormously diluted, and, in the majority of cases, this dilution is sufficient to render the dissolved impurities innocuous. Marsh (189) states that the waste from tannery and dye works and from the sulphite-paper and the soda-paper pulp processes, in dilutions of 1-200, failed to kill fish. Illuminating gas is the most toxic factory waste, as dilutions of one part to several hundred thousand parts of water prove fatal to fish. Acidity of the water caused by sulphuric acid in the waste from coal mines is also fatal. Wells (224) found that fresh water fish prefer slight acidity to neutrality or alkalinity. This finding is rather unusual as Oswald (199), Garrey (166), and others have stated that a moderate alkalinity is preferable. According to Loeb and Wasteneys (186), the toxicity of acids, both mineral and organic, can be partially neutralized by sodium and calcium chlorides.

Salmon ascend rivers a considerable distance for the purpose of spawning. Roule (214) observed that salmon prefer water having an oxygen content equal to or slightly above the saturation point. Any condition such as contamination with sewage, etc., which would lower the oxygen content of the water, might result in driving the salmon from the stream.

Although these wastes, when highly diluted, are not immediately fatal, it is not known what effect a prolonged sojourn in such water might have upon the life cycle of fish. Might not a time eventually come when such conditions, (if maintained) would result in the extinction of the less hardy species? There is good reason to believe, that the almost complete disappearance of shad and salmon from certain rivers in New England, where they were formerly abundant is largely owing to the pollution of the water by harmful chemicals and dyestuffs from numerous factories, as well as to the presence of dams and barriers in the streams.

Copper is normally present in the systems of some marine animals. Dubois (159) gives the following figures, showing the copper content in the blood of fish:

Table No. 33.

Copper in the Blood of Fish
(weight of copper per 100 grams)

Fish	Fresh Basis	Dry Basis
Herring	Trace	Trace
Sardine	.00182 grams	.00535 grams
Tench	Trace	Trace
Carp	Trace	Trace

The copper content in the blood and muscle of molluscs and crustaceans is much higher than in the blood of that fish referred to above. In the blood of the crab, the ingredient which corresponds to the hemoglobin in mammals, is called hemocyanin and contains copper instead of iron. However, although copper is occasionally present in the blood of fish, it is known that copper salts in the smelter waste may cause their death.

The work of Thiergardt (221) shows that fish are unusually resistant to the action of arsenic and lead, in the proportion of one part of metal to 20,000 parts of water. Goldfish absorbed as much as .0099 grams of the former and .0557 grams of the latter, per 100 grams of fish, before succumbing.

Changes in the Density of Water and their Effects upon Fish.

A condition having a very great effect upon fish is the relative concentration of the mineral constituents of the water. If the gills are excellent organs for the diffusion of gases, may they not also be instrumental in the diffusion of salts? A number of investigations have been made to determine the cause of distress and death occurring in fish which have been transferred from a medium of low density to one of high density, and vice versa.

Rodier (213), observed that the high osmotic pressure of the body fluids in elasmobranch fish, which is very nearly equal to that of sea water, is more largely owing to the organic constituents, than to the presence of mineral salts. The limiting membranes of the teleosts constitute an effective barrier against osmotic changes, and it was found by Quinston (210, 211), Garrey (165) and Dakin (156) that the body fluids of marine invertebrates are isotonic with the surrounding water. Garrey (167), noted that in fresh-water teleosts and ganoids the freezing point of the blood is practically the same.

According to Frederica (163), the three kinds of aquatic animals may be classified as follows: (1) molecular concentration and salt content, the same in the body fluids of the animal as in the surrounding water (marine invertebrates); (2) molecular concentration the same in both body fluids and water, but the proportion of salts less in the body fluids than in the water, the deficiency being compensated for by organic matter in solution (elasmobranch); (3) molecular concentration and salt content of the body fluids varying greatly from those of the external water (all teleosts, and fresh-water invertebrates).

The following figures were obtained by Botazzi (149):

Elasobranch	2.26°C.
Teleost	1.04 - 0.76°C.
Reptile, Turtle	0.61°C.
Mammal, Whale	0.65 - 0.70°C.

We find that as animals ascend in the scale of evolution, the blood is maintained at a nearly constant osmotic pressure by regulative mechanisms. The finer adjustment of the osmotic pressure of the body fluids, in the higher animals, may be explained as an effort of the body to maintain a constant condition most favorable for the delicate processes carried on in it.

Sumner (22) found that certain fish die when transferred abruptly to sea water, and certain species of brackish or salt-water fish cannot survive even a gradual transfer to pure fresh water. In confirmation of similar results obtained by Giard (168) it was observed that some salt-water fish can survive daily alterations in the salinity of the aquarium water, from fresh to salt, for fairly long periods of time (two weeks). The osmotic changes in the bony fish take place by means of the thin membranes covering the gill filaments, and not through the skin. This was proven in the case of fresh-water teleosts when a loss of weight was produced by passing salt water through the gills and fresh water over the body. Salt-water animals gained in weight when transferred for a short time to fresh water. Although osmotic phenomena are evident in most of the cases observed, other considerations of possibly a physiological or chemical nature may modify the effects produced.

Scott (216) investigated the effect upon the blood of the killifish (Fundulus heteroclitus), caused by changes in the density of water. The change in the number of corpuscles per cubic centimeter, the percentage of hemoglobin, the specific gravity, and the depression of the freezing point, corresponded exactly with what might have been expected from the dilution or concentration of the blood, following transfer of the fish to water of higher or lower salinity than that to which the fish had been accustomed. The results would indicate that there is neither destruction nor increased formation of red corpuscles under these conditions.

On the other hand, according to Mosso (196) and Bert (147), fish will die when

placed in fresh water, death being due to asphyxiation as a result of the gill capillaries being blocked by disintegrated blood cells. There appears to be a relation between the resistance of blood of fish to the hemolysing action of dilute salt solution, and the ability of the fish to withstand changes in the salinity of the medium. The results obtained by Mosso in this connection agree in principle with those obtained by Hamburger (172) and Botazzi and Ducceschi (150).

The death of fish which have been placed in water deficient in salts is believed by Ringer (212) to be caused either by the withdrawal of mineral constituents from the body, (mainly through the gills,) or by the fact that the water, acting osmotically on the tissues of the gills destroys by interfering with the respiration.

Siedlecki (219) observed that the stickleback is, ordinarily, able to protect itself against the action of salts in the external medium. But when the body resistance of the animal is lowered, the limiting membranes no longer prevent osmotic change, and the animal very easily succumbs to the action of salts. The changes in the blood of salmon at spawning time may be due to the same cause. The average depression of the freezing point of the blood of salmon is, (according to Greene (169)) in sea salmon, 0.762°C .; in brackish-water salmon, 0.737°C .; and in spawning-ground salmon, 0.628°C . Greene expressed the opinion, however, that the lowering of the concentration of the blood salts, at spawning time, is owing to absence of food and to metabolic processes which occur during the several weeks sojourn in fresh water, rather than to osmotic changes.

Siedlecki (218) noted the important fact that the toxic action of potassium chloride can be neutralized with calcium chloride, in certain concentrations. Solutions which were inactive when employed alone became toxic when mixed with others. The importance of Magnesium salts in reducing the toxicity of other salt mixtures to be used for the fresh-water fish, *Notropis blennioides*, was observed by Garrey (166), who found that these fish could not live long in Ringer's solution alone, but could live indefinitely in Ringer's solution to which small amounts of magnesium chloride had been added. Loeb (185) found that by slowly raising the concentration of $\text{NaCl} + \text{KCl} + \text{CaCl}_2$, the resistance of fish to a pure NaCl solution could be increased. It may be that the adaption of the animal involves a "tanning" of its surface by the higher concentrations of the salts in sea-water, especially the CaCl_2 . According to Loeb and Westeneys (188), non-balanced solutions, which, in this instance, are those solutions with mineral constituents which are unsatisfactory in kind or amount for supporting fish life, will increase the permeability of the cells. In the case of a very toxic salt, the fish dies before a marked change in the osmotic pressure of the body fluid has taken place; while in the case of the less toxic NaCl great osmotic changes may take place in the fish before the salt kills it. It is possible therefore, that certain proportions of the salts in sea-water may change the cell membranes, either to the advantage or detriment of the fish.

Metabolism of Fish.

In the table below, as given by Lipschütz (183), the variation in composition of the flesh of the carp is explained on the basis of the fish being in various stages of hunger. The points to be noted are the decrease in solids, proteins, extractive nitrogen, and fat and the increase in water and ash. He found a somewhat similar relation to be true in the case of young eels, kept in conduit water, without food, for more than six weeks. The results obtained by Morgulis (194) in experiments on fasting flounders, show the same general trend, i.e. a decrease in

protein and fat and an increase in water as the fasting proceeds.

Table No. 34.

"Influence of the State of Nutrition on the Chemical Composition of the Carp".

Class	Length	Dry Weight	100 parts live weight contain		100 parts of solids contain					
			Water	Solids	Ash	Nitrogen containing extractive matter.	Protein	Fat	Soluble Carbohydrates.	Insoluble free substances.
A	54.5	0.980	76.1	23.9	24.2	9.7	46.2	2.9	1.0	16.0
B	51.7	0.712	77.4	22.6	29.8	9.3	42.3	0.7	1.0	16.9
C	65.0	0.666	79.3	20.7	41.2	8.9	34.3	0.9	0.9	13.8

^oAverage of several fish

^{oo}A-normal. B and C - progressive stages of starvation.

Putter (207) carried out an elaborate series of experiments on aquatic animals, to ascertain the nature of the organic food material utilized by them, and concluded that the animals procure their food from two sources, namely, from the soluble organic matter of the sea-water (thru the gills) and from particulate organic matter. Putter's views are not shared by other investigators in this field. Hanzel (174) was unable to detect organic matter in sea-water above 3 or 4 milligrams per liter, it can be stated that sea-water contains a practically negligible quantity of dissolved organic matter. Moore *et al.* (192, 193) were also able to prove that fish supplied with sea water during fasting do not abstract any organic matter from it. Lipschuts (182) found that certain compounds, like asparagin, glucosamine, tyrosine and glucose, placed in aquarium water, are not observed and retained, as such, by the organisms of the carp and eel. Furthermore, according to Kerb (188) grape sugar dissolved in water is not absorbed by aquatic animals. The high values obtained by Putter for the oxygen consumption of fish are explained by Morgulis (194) as being due to a restless condition, incident to their being placed in the apparatus for measurement of the respiratory activity. In this excited state their respiratory exchange might be increased 50 per cent. By measuring the oxygen consumption of flounders during a fasting period of 671 hours, it was found that the difference between the amount of oxygen consumed and the oxygen in the wasted body material was small enough to be within the limits of experimental error and shows, in agreement with other opponents of Putter, that aquatic animals subsist only on particulate food.

Knuthe (170), experimenting with fasting carp, noted an increased nitrogen elimination on raising the temperature of the water. As in the case of warm blooded animals, a carbohydrate diet when fed to fish reduced the nitrogen elimination below the starvation value. It was also found that mineral salts were necessary in the food, their absence causing disturbance in the digestion and an increased nitrogen excretion. Cooked starch is not readily utilized by young carp unless protein is fed with it; the digestion gradually becomes less complete until the entire amount of carbohydrate which has been given is eliminated in the feces.

In regard to the enzymes of fish, the evidence indicates that at low temperatures their action is much greater than those of warm-blooded animals. Basing their con-

clusion on the greater activity of the proteolytic enzymes of the pike and trout than those of pigs and dogs at 0°C., Murisier and Fick (198) state that the pepsin of cold-blooded animals is an enzyme distinct from that of warm-blooded animals. Experimenting with the pike and carp, Müller (197) found that the enzyme of these fish (diastase from the liver, pepsin, rennin, and trypsin) are slowly active at 0°C. Hoppe-Seyler (175) noted that the optimum temperature for the action of pepsin from the stomach of the pike was approximately 20°C.

According to Gohnheim (154,155) the surviving intestines of fish (Grenilabrus pavo and Labrus festinus) have a deaminizing effect on the end products of protein digestion. The intestinal mucosa of cats and dogs did not react in this manner. This phenomenon may account for the high ammonia figures obtained by Denis (see below) from the blood of fish.

The metabolism of the elasmobranch fish is different from that of the teleosts, as may be seen by comparing the composition of the blood of these two classes of fish. In the table below, (158), the percentage of non-protein nitrogen accounted for by the urea fraction, in the case of teleosts, is much smaller than in the blood of man, or of any mammal so far examined.

Table No. 35.

Non-Protein Nitrogen Fractions of the Blood of Fish.
(The figures represent milligrams per 100 grams of blood)

	Non-Protein N	Urea N	Ammonia N	Uric Acid N	Creatine & Creatinine
Elasmobranch					
Dogfish (<u>Mustelis canis</u>)	1000	800	1.4	0	4.0
Sand shark (<u>Carcharias littoralis</u>)	1160	1000	2.5	0	4.0
Skate (<u>Raja erinacea</u>)	1100	868	1.6	0	3.1
Teleosts					
Butterfish (<u>Poronotus tracanthus</u>)	50	9	5.1	1.4	16.0
Mackerel (<u>Scomber scombrus</u>)	86	10	3.8	1.1	7.5
Squeeteague (<u>Scymnoscion regalis</u>)	66	20	1.0	0.7	6.0

The composition of the urine of the goosefish, a teleostean, is shown by the same author, in the table below. In the urine of a bony fish the creatinine is almost entirely replaced by creatine.

Table No. 36.

Analysis of a Composite Sample of Urine from Six Goosefish.

	Total Urea		Ammonia	Uric Acid	Great- inine	Creatine	Phos- phates P ₂ O ₅	Chlorides	Total sul- phur	Inor- ganic SO ₃
	N	N	N	N						
Milligrams per liter,	830	120	12	1	7	140	440	10600	108	92
Per cent of total N.	---	14	2.7	0.1	0.8	16.6				

Sp. Gr. - 1.616 Albumen and reducing sugar absent.

On examining the urine of the dogfish (*Mustelis canis*), Denis (157) found that the urea-N amounts to 80-89% of the total nitrogen of the urine. The amount of ammonia nitrogen varied from 1.9 to 6.1% of the total nitrogen of the urine, except in one case, where it was 10.6%. Very little creatinine and no creatine, sugar, nor albumen were found.

According to Baglioni (143), the physiological retention of urea in the blood of the dogfish, is analogous to the retention of sodium chloride and other blood salts in a definite percentage concentration in the blood of other vertebrate animals. This agrees with the findings of Schroeder (215) who noted a constancy in the urea concentration of the blood of the dogfish, together with a somewhat more varied concentration of urea in the muscle and a decidedly variable urea content in the liver. Schroeder believes that this condition is due to slow elimination of urea by the organism. Urea raises the tonus of heart muscle, and in elasmobranch fish its action is contrary to that of NaCl, which reduces the tonus. Urea and NaCl both of which are present in the blood of elasmobranch fish to the extent of 2 grams per 100 c.c. are, therefore, compensating factors in the regulations of the heart. Baglioni (141, 142) believes that this specific action of urea is not dependent upon the fact that it is a non-electrolyte, as sugar does not replace it, but that its action depends entirely on its chemical composition.

Differences in the Body Composition of Fish.

The major portion of the amino acids in the water extract of marine vertebrates is in the form of diamino acids, while in molluscs the monamino acids are in excess. This is shown in the table below by Wilson (227). Another noteworthy feature is the small amount of amino acid nitrogen in per cent of total extractive nitrogen in the higher marine forms, the lamprey and limulus as compared to that in the lower forms, the clam and scallop. The lamprey has a very low organization of skeleton, which is cartilaginous without vertebral segmentation; the limulus, squid clam, scallop and periwinkle are true invertebrates.

Table No. 37

Nitrogen Distribution in the Protein-free Water Extract of Muscle in a Series of Related Marine Animals.

Kind of Marine Animals.	In % of Total N Water Extract	Amino-acid nitrogen					
		Grams of nitrogen per 100 gms. of fresh muscle			Grams of nitrogen per 100 gms. of dry muscle		
		Total Extract	PTA ^o filtrate, mono-amino acid fraction	PTA precipitate di-amino acid fraction ^{oo}	Total Extract	Mono-amino acid fraction	Di-amino acid fraction
Lamprey	4.03	0.010	0.004	0.006	0.053	0.021	0.032
Limulus	13.8	0.061	0.031	0.030	0.412	0.209	0.203
Squid	35.6	0.151	0.127	0.024	0.635	0.534	0.101
Clam	51.1	0.285	0.266	0.019	1.100	1.020	0.073
Scallop	56.2	0.283	0.268	0.015	1.470	0.400	0.078
Periwinkle	27.0	0.146	0.118	0.028	0.524	0.423	0.100

^o Phosphotungstic Acid

^{oo} By Difference

Wely (225) found that the electric organ of the torpedo has a peculiar proportion of mineral salts, the ratio of KCl to NaCl in two cases being 1.35; 100 and 0.69; 100. The potassium, in very small amount, is practically insignificant as compared to the sodium, whereas in ordinary muscle the potassium is the largest mineral constituent.

Polimanti (202) noticed a marked difference between the appearance of the liver in a pregnant female elasmobranch fish and that of a male; the former, though the same weight, was more voluminous and yellower in colour.

The bile of the shark (*Scymnus borealis*) is free from cholesterol and contains, instead of the usual taurocholic acid, two ethereal sulphates which Hammarsten (173) calls a- scymnosulphuric acid and b- scymnosulphuric acid. The latter have a lower sulphur content than taurocholic acid and a practically negligible nitrogen content. Similar acids were isolated from the bile of the roach. No ethereal sulphates could be found in the bile of the mackerel. Hammarsten observes that the presence of sulphuric acid in other bile acids than taurocholic acid renders necessary a revision of views in regard to the content in taurocholic acid of the bile of other animals.

Polimanti (203) believes that there is a definite relation between the depth in which a fish habitually swims and its specific gravity, and that the specific gravity is influenced largely by the amount of fat. The following classification is given by him:

Table No. 38.

Classification of Fish according to their Fat Content and Habitat.

Class	Water %	Fat %
A- Wandering sea fish	71.82	4.07
B. Living "inshore" between cliffs at various depths from 30m. to 1000m.	70.77	2.67
C- Living in the sand of the shore at a maximum depth of 100m.	70.33	1.44
D- Living among the seaweeds of the shore at a depth of not more than 50m.	70.15	1.03
E- Living in mud at a depth of 100m. to 800m.	75.03	0.57
F- Living in the deep sea at a depth of 1000 to 2000m.	86.14	0.035

As an example of Class A the mackerel may be cited, though the fat content of this fish averages much higher than 4%. The flounder, a typical bottom fish, has an average fat content of about 0.40%.

The alterations in the fat content of the same species during a season is closely connected with the physiological changes attendant upon spawning. Miescher (191) found that there is a great decrease in the quantity of fat as the spawning season approaches when the salmon ascend the rivers. His results were confirmed by Noel Paton (200) and Greene (170). Miescher's interpretation of his findings was incorrect in one particular. He believed that the considerable quantity of fat which he observed in the muscle fibres was a stage of fatty degeneration, preliminary to the use of the fat for building up the immense amount of food materials present in the salmon ova at spawning time. Both Paton and Greene contend that the fat stored in the body during the period of feeding and growth is the immediate source of energy expended by the salmon during the spawning migration and is not, in any sense a fatty degeneration.

The following table illustrates the changes in the composition of the flesh of shad at the period of spawning:

(Author's unpublished data)^o

Table No. 39.

Variation of the Fat Content of the Flesh of the Shad at the Spawning Period.

Description	Locality	Date	% Fat (wet basis)
Roe shad (not very ripe)	North Carolina Coast	4-5-15	14.43
Roe shad (ripe)	" " "	4-15-15	13.93
Roe shad (ready to spawn)	Delaware River	4-24-15	5.87
Roe shad (after spawning)	New Jersey Coast	6-19-15	2.95

^oE. D. Clark and L. H. AlayJ. Biol. Chem. Vol. 33; 483-498
(1918)

Parasites of Fish.

It is not the purpose of this paper to go into the question of the occurrence and effects of the various parasites found on fish. In this country much work on this subject has been done by Linton (181) whose papers contain numerous references to the literature. Other contributions have been made by Wilson (226), Ward (223), Hahn (171), and others.

B I B L I O G R A P H Y

Introductory

1. Atwater, W. C. The Chemical Composition and Nutritive Values of Food-Fishes and Aquatic Invertebrates. Report of the Commissioner of Fish and Fisheries. 1888, pp. 679-868.
- McFarland, R. A History of the New England Fisheries. Published by University of Pennsylvania, 1911.
2. Pennington, M. E. Supplementing our Meat Supply with Fish. Year book of United States Department of Agriculture for 1918. Yearbook separate No. 623.
3. Smith D. S. A Study of the Influence of Cold Storage Temperatures upon Chemical Composition and Nutritive Value of Fish. Biochem. Bull. 3; 54-68 (1913). Special Report of the Commissioner of Fisheries (1914).
4. Bureau of the Census. United States Department of Commerce and Labour Fisheries of the United States. 1908. United States Department of Commerce, Bureau of Fisheries. Alaska Fisheries and Fur Industries in 1913.
- Webb. New Dictionary of Statistics. Complement to "Mulhall". 682 pages. London, 1911.

Chapter I

5. Albu, A. and Nauberg, C. Physiologie Und Pathologie des Mineralstoffwechsels, pp. 240-5, Berlin, 1906.
6. Almen A. Analyse des Fleisches einiger Fische. Mitgeteilt der Kgl. Ges. der Wissenschaft. su Upsala, April 7, 1887. Published by E. Bertling.
7. Balland Sur la Composition des Poissons, des crustaces et des mollusques. Compt. rend. de l'acad. des Sciences 126; 1728-1731 (1898).
8. von Baumhauer, Ueber die Zusammensetzung des Muskelgewebes der Fische. J. prakt. Chem. 44; 506-511 (1848).
9. von Baumhauer. Ueber des losliche Eiweiss der Fische. J. f. prakt. Chem. 45; 120-122 (1848).
10. Beythien, A. Kochwasser von Seefischen. Pharm. Centr. 47; 140 (1906).
11. Beythien A. Ueber die chemische Zusammensetzung und den Nahtwert verschiedener Fleischsorten. Z. Nahr. Genussm. 4; 1-9 (1901)

12. Buckland, F. Bestandtheile des Fleisches von Lachsen und Heringen im Vergleich zu dem von Rindfleisch. Arch. Pharm. Ser. 3, 53; p. 178 (1874). Also Centr. Agrickulturchemie. 2, 75, (1874).
13. Biglia, G. and Costantino, A. Beitrage zur Nuckolchemie IV. Der extractive Stickstoff und der freie durch Formol titrierbare Aminostickstoff in der Muskulatur verschiedener Tierarten. A. physiol. Chem. 86; 137-140 (1913).
14. Du Menil. Ueber den Metallgehalt der Fischechuppen. Arch. pharm. Ser. 2, 6; 155-157 (1836).
15. Hollande, A. Valeur nutritive de la chair de quelques poissons exotiques importes en Francee durant ces dernieres annees. Bull. sci. Pharmacol. 20; 405-406 (1913).
16. Kianizyn, N. J. Food value of the Cod. Dissertation (Russian). St. Petersburg, 1887.
17. Koenig, J. Der Gehalt der menschlichen Nahrungsmittel an Naturungstoffen im Vergleich zu ihren Preisen, A. Biol. 12; 497-512 (507) (1876).
18. Koenig, J. Chemie der menschlichen Nahrungs und Genussm. v. II, 125, (1893).
19. Koenig, J. and Splittgerber, A. Die Bedeutung der Fischerei fur die Fleischversorgung im Deutschen Reich. Landw. Jahrb. 38, Ergansungsb. IV, 1-169, (1909).
20. Kossel and Patten. A. S. Zur Analyse der Hexonbasen, Z, physiol. Chem. 38; 39-45 (1903).
21. Kostytschoff, P. The Chemical Composition of Fish Products, with some remarks on their nutritive Value. (Russian) 144; part II, (1883).
22. Lehman, F. Die Bedeutung der Seefische fur Militar- und Volksernahrung. Mitt. Deut. Seefischereivereins. (Berlin) 12; 301-312 (1896).
23. Levi, E. Boden- und Aschenanalysen. Analyse der Fisch-Graten. Ann. Chem. 50; 426 (1844).
24. Lichtenfeld, H. Chemische Zusammensetzung einiger Fisch Arten, warum und wie sie periodic wechselt. Arch. ges. Physiol. 103; 353-402 (1904).
25. Miesher. Ueber das Leben des Rheinlachs im Susswasser. Arch. fur Anat. und Physiol. (Anat.) 193-218 (1881).

26. Milone, Ugo. Composizione, valore nutritive ed assimilabilita della carne muscolare dei pesci. Bolletino della Societa di Naturalisti di Napoli. Ser. I, 10: 311 (1896).
27. Morin. Examen chimique de l'Eperlan, Salmo eperlanus. Jour. Pharm. 8: 61 (1822).
28. Osborne, T. B. and Heyl, F. W. Hydrolysis of Fish muscle. Am. J. Physiol. 23: 81-89 (1908).
29. Payen A. Precis des Substances Alimentaires, 4th. Ed. pages 45 and 488 (1865).
30. Une Communication a l'Academie des Sciences. Comp. rend. 39: 318-321 (1854).
31. Pters, F. Ueber den Gewichtsverlust des Fisch-fleisches beim Dunsten. Arch. Hyg. 54: 101-106 (1905).
32. Polimanti, Osw. Ueber den Fettgehalt und die biologische Bedeutung desselben fur die Fische und ihren Aufenthaltsort. Biochem. Z. 56: 439-445 (1913).
33. Polimanti, Osw. Ueber den Fettgehalt u die biologische Bedeutung desselben fur die Fische u ihren Aufenthaltsort sowie uber den Fettgehalt je nach dem Alter die Fische. Biochem. Z. 69: 145-54 (1915).
34. Popoff. Determination of the Food Value of the Most common Kinds of Fish. (Russian) Dissertation. St. Petersburg. (1882).
35. Pruchnitsky, L. On the Characteristics of Fish Proteins. (Russian). Dissertation. Mil. Med. Acad., St. Petersburg, (1912).
36. Putter, A. Die Ernahrung der Fische. Z. allgen, Physiol. 9:173 (1909)
37. Reuss, E. Untersuchungen uber die chemische Zusammensetzung des Fischkorpers unter dem Einfluss seines Wachstums und des Wassers. Ber. K. Bayer. Biol. versuchs. unchen, 1: 185-220 (1908).
38. Rosenfeld, G. Studien uber das Fett der Merresorganismen. Wissenschaft liche Meeresuntersuchungen, new series, part V, No. 2. Kiel und Leipsig, (1904).
39. Schlossberger. Vergleichende Chemische Untersuchungen uber das Fleisch verschiedener Thiere. Dissertation. Stuttgart, (1840).
40. Schrupf, P. Das Fischfleisch als Nahrungsmittel. Z. phusik. diat. Ther. 15: 392-401 and 470-480 (1911).
41. Sempolowski L. Untersuchungen von Seetieren auf ihren Gehalt an Agricult-urchemisch wichtigen Stoffen. Lnadw. Vers. Sta. 36: 61-64. (1889).

42. Ulrich, C. Fischfleisch. Arch. Pharm. 249: 68-92 (1911).
43. Vaillant, L. Remarques sur la Composition chimique de l'anguille, a differentes etats de son developpement. Compt. rend. doc. biol. 55: 749-750 (1903).
44. Weidenbusch, H. Ueber die Analyse des Fisch- und Huhnerrfleisch albumins und eine neue Bestimmungsmethods des Schwefels. Ann. Chem. 61: 370-376 (1847).
45. Weigalt, C. Wie viel Fischfleisch essen wir von einem Kilogramm unserer Wichtigsten Marktfische Allgem. Fischerei Zt. 21. 135-138. (1896) also Die abfalle der Seefischerei Berlin 1891.
46. White, G. K. The properties and Utilization of some Marine Animal Oils. J. Ind. Eng. Chem. 4: 106-267 (1912). Also Trans. Am. Fish. Soc., December 1914.
47. Williams, K. I. The Composition of Cooked Fish. J. Chem. Soc. London, 71 649-653 (1897).

Chapter II.

48. Bocklisch, O. Ueber Paulnisbasen (Ptonaine) aus Fischen II. Ber. 18: 1922-1927 (1885).
49. Gregory, W. Ueber den Gehalt einiger Fleischarten an Kreatin. Ann. 64: 100-108 (1846).
50. Issac, S. Die Purinbasen der Heringslake. Beitr. Chem. Physiol. Path. 5: 500 (1904).
51. Krunkenberg. Untersuchungen aus dem physiologischen Institute der Univ. Heidelberg. 3: 197 (1880).
52. Krunkenberg. Untersuchungen aus dem physiologischen Institute der Univ. Heidelberg. 4: 47, (1881).
53. Krunkenberg, C.F.W. and Wagner, H. Ueber Besonderheiten des chemischen Baues contractiler Gewebe. Z. Biol. 21 (New Series, 3): 31 (1885).
54. Kutscher. Ueber sinige Extraktivstoffe, Sitz. Ges. Naturwiss. Marburg, 91-94 (1910).
55. Liebig, J. Ueber die Bestandtheile des Flussigkeiten des Fleisches. Ann. 62: 257-369 (1847).
56. Limpricht, H. Vorlaufige Notiz uber einige Bestandtheile der Fleisch flussigkeit von Fischen, Ann. 127: 185-189 (1863).
57. Limpricht, H. Ueber einige Bestandtheile der Fleischflussigkeit. Ann. 133: 293-305 (1865).

58. Micko, K. (a) Vereichende Untersuchung von Fleischextrakten und deren Ersatzmitteln. A. Mahr. Genussm. 5: 193-210 (1902).
(b) Untersuchung von Fleisch-, Hefen-, und anderen Extrakten auf Xanthinkörper. I, Die Xanthinkörper des Fleischextraktes. A. Mahr. Genussm. 6: 781-791 (1903).
59. Morner, C.T. Ueber ein eigentümliches Nahrungsmittel, nebst einiger Beobachtungen über darin angetroffene Faulnisbasen. Z. physiol. Chem. 22: 514 (1897).
60. Okuda, Y. Quantitative Determination of Creatin, Creatinin, and Mono-amino acids in some Fishes, Mollusca, and Crustacea. Orig. Com. 8th. Intern. Congr. Appl. Chem. 18: 275-281 (1912).
61. Failhade, de Rey. Le philothion dans l'alimentation par les poissons. Bull. gen. Therap. 164: 699-701, 1912.
62. Rebuteau and Papillon. Observations sur quelques liquides de l'organisme des Poissons des Crustacés, et des Cephalopodes. Compt. rend. 77: 135-138 (1873).
63. Schonodorff B. and Washholder, K. Ueber den Glykogenstoffwechsel der Fische I. der Glykogen gehalt von Susswasser fischen. Arch. ges. Physiol. 157: 147-164 (1914).
64. Schroeder, W. Ueber die Linnrstoffbildung der Haifische. A. physiol. Chem. 14: 576-598 (1890).
65. Schulte, M. Ergebnisse Einiger die elektrischen Organe von Torpedo und das Schwanzorgan von Raja betreffender chemischer Untersuchungen. J. prakt. Chem. 82: 1-12 (1861).
66. Sorensen, S. P. L. Enzymstudien, I Ueber die quantitative Messung proteolytischer Spaltungen. "Die Formoltitrimung".
67. Stadeler, G. Ueber das Vorkommen und seine einfache Darstellungsweise des Kreatins. J. prakt. Chem. 72: 256 (1857).
68. Stadeler, G. and Frerichs, F. T. Ueber das Vorkommen von Harnstoff, Taurin, und Scyllit in den Organen der Plagiostomen. J. prakt. Chem. 73: 48 (1858).
69. Suwa, A. Untersuchungen über die Extraktstoffe des Fischfleisches Zentr. Physiol. 22: 307-310 (1908).
70. Suwa, A. Untersuchungen über die Organextrakte der Selachier. I, Die Muskelextraktstoffe des Dornhai (Acanthias Vulgaris). Arch. ges. Physiol. 128: 421-426 (1909).

71. Suzuki U. and Yoshimura, K. Ueber die Extraktivstoffe des Fischfleisches. Z. physiol. Chem. 62; 1-35 (1909). Also J. Coll. Agri., Imp. Univ. Tokyo, 1; 21-58 (1909).
72. Thesen, J. E. Ueber Isckreatinin, eine neue stickstoffhaltige Verbindung in Fischfleisch. A. physiol. Chem. 24; 1-17 (1897).
73. Valenciennes and Frewy. Recherches sur la composition des muscles dans la serie des amineux. J. Pharm. Chem. 3rd. Ser. 28; 401-406 (1855).
74. Van Slyke, D. D. A Method for the Quantitative Determination of Alifatic Amino Groups. I, J, Biol. Chem. 9; 185 (1911).
75. White, G. F. and Crozier, W. Comparative Proctolysis Experiments with Trypsin. J. Am. Chem. Soc. 33; 2042-2048 (1911).
76. Wilson, D. W. Wilson, D. W. The Comparative Chemistry of Muscle; The Partition of non-protein, water-soluble nitrogen. J. Biol. Chem. 17; 385-400 (1914).
77. Yoshimura K. and Kanai, M. Beitrage sur Kenntnis der stickstoffhaltigen Bestandteile des getrockneten Kabeljau. (Gadus Brandtil) Z. physiol. Chem. 88; 346-351 (1913).
- Chapter III.
78. Bitter, L. Untersuchungen uber Fischwurst, Hyg. Rundschau 21; 181-169 (1911).
79. Bitting, A. W. Preparation of the Cod and other salt fish for the Market. Including a Bacteriological Study of the Causes of Reddening. U. S. Dept. Agric., Bu. of Chem. Bull 133. (1911).
80. Bottemanne, J. M. Notes on the Investigation of Preserving Fish by Artificial Cold. Third Int. Congr. Refrig., Washington-Chicago, 1913. Third Section; Application of Refrigeration to Foods.
81. Danilebsky, A. Preparation from Albuminous Extract from Fish. British Patent 18, 365. Aug. 14, 1911.
82. Davidson, J. Making a Food from the Pulped Flesh of Fish and Starch Powder. U. S. Patent 1,047,760. Dec. 17, 1912. British Patent 23,604.
83. New Method for Curing Fish. Fishing Gazette, 31; 1264 (1914).
84. Perlzweig and Gies. A Further Study of the Chemical Composition and Nutritive value of fish subjected to Prolonged Periods of Cold Storage. Biochem. Bull. 3; 69-71 (1913).

85. Fetterson, A. Experimentelle Untersuchungen über das Conserviren von Fleisch und Fisch mit Salzen. (a) Berl. klin. Wochschr. 36; 915-916 (1899). (b) Arch. hydf. 37; 171-238 (1900).
86. Schmidt-Nielsen, S. On the Ripening Process in the Pickling of Herring. Det. kgl. norske videnskagers selskabs skrifter, (1910) No. 5, Thondkjes, 1902.
87. Schmidt-Nielsen S. Report on Norwegian Fishery and Marine Investigations. Vol. I, No. 8, pp. 99 (1900).
88. Schmidt-Nielsen, S. Zur Kenntnes der Autolyse des Fischfleisches. Beitr. chem. Physiol. Pathol. 3; 266-276 (1902).
89. Schwickerath, K. Proteose Preparation from Fish. U. S. Patent 954,050.
90. Wall, S. The Preservation of Fresh Fish Kept on Ice or After Freezing. Handlingar till Landbruksveekan ar. p. 264 (1912).

Chapter IV.

91. Atwater, W. C. Jahresberichte der Thier Chemie, Maly. 17; 418 (1887). Also Z. Biol. 24; 16 (1888).
92. Chittenden, R. H. and Cummins, G. W. The Relative Digestibility of Fish Flesh in Gastric Juice. Am. Chem. J. 6; 318-332 (1884).
93. Hertter and Popoff, M. Ueber den Einfluss der Zubereitung auf die Verdaulichkeit von Rind und Fischfleisch. Arch. Physiol. p. 561-563. (1889).
94. Honigsberg, P. Untersuchungen über die Verdaulichkeit des Fleisches. Wiener med. Blätter. 5; 582-585 and 614-616, (1882).
95. Popoff, M. Ueber Verdauung von Rind und Fischfleisch bei verschiedener art der Zubereitung. A. physiol. Chem. 14; 524-532 (1890).
96. Rosanoff. Comparative Utilization of the Fat of Fresh and Smoked Fish. (Russian) Dissertation. St. Petersburg, (1891). For Abstract see Smolenski P. O. Hyg. Rundschau 7,11,7.
97. Rosenfeld, G. Nahrungswert des Fischfleisches. Zentr. inn. Med. 27; 169-176 (1906).
98. Slowoff, B. Die Ausnutzung des Fischfleisches im Vergleich mit der des Rindfleisches auf die Zusammensetzung des Harnes A. physik. diat. Ther. 14; 22-34 (1910).
99. Slowoff, B. J. Fish as Food and Its Effect on the Metabolism of P, Ca, and Vg. Verh. Ges. Russ. Ärzte Wdv., - Dec. (1908-9) Abstract in Zentr. Ges. Physiol. 4; 669 (1909).

100. Slowsoff, B. J. and Krawtschenko, A. S. On the Action of Fish Food upon Metabolism. *Verh. Ges. Russ. Arzte.* (1907-8) Abstract in *Zentr. Ges. Physiol. Pathol.* 4:49 (1909).
101. Van Slyke, D. D. and White, G. F. The Relation between the digestibility and the Retention of Ingested Proteins. *J. Biol. Chem.* 9; 219-229 (1911).
102. White, G. F. and Thomas, A. A New Method for the Determination of the Food Value of Proteins, with Application to Cynoscion Regalis. *Bull. Bu. Fisheries Doc.* 784 Vol. 32, 1912. Issued Feb. 18/14.

Chapter V.

103. von Anrep, E. Fish Poisoning and Fish Poison. (Russian). *Wratsch*, pp. 213-216 (1885).
104. Barger, G. The simple Natural Bases. A Monograph on Bio-chemistry. Longmans, Green & Co., London, 1914.
105. Bocklisch, O. Ueber Fraulnisebasen (Ptomaine) and Fischen. *Ber.* 12; 26-89 (1885).
106. Brieger, L. Ueber Ptomaine, I. August Hirschwald, Berlin, (1885).
107. Desgrez, S. and Caius, P. Sur le ptomaines des conserves de poissons et de crustaces. *Compt rend.* 152; 893-895 (1911).
108. Gautier and Etard Sur le mecanisme de la fermentation putride et sur les alcooldes qui en resultent. *Compt. rend.* 94; 1601 (1885).
109. Konstansoff, S. W. and Manoiloff, E. O. Ueber die Einwirkung der verdauungsfermente auf das sogenannte Fischgift. *Wien Klin. wachschr.* 27; 883-6 (1914).
110. Lieventhal, Alkaloid Substances Occurring in Salt Sturgeon. (Russian). *Pharm. J. (of Russia)*. pp. 542-546 (1886).
111. Sieber-choumow, N. C. Contribution a l'etude des Poissons venimeux. Sur le bacillus poscidus agilis, microbe pathogene pour les poissons. *Arch. Sci. biol.* 3; 226-256 (1894).
112. Smokenski, P. C. Das Fischfleisch in Hygienischer Beziehung, *Hyg. Rundschau.* 7; 1105, 1166, 1226 (1897).
113. Vaughan and Novy. Ptomines, Leucomaines, Toxins, and Antitoxins. Lea Bros. Co., Philadelphia and New York, (1896). Also Cellular Toxins, Lea Bros & Co., Philadelphia and New York, (1902).

114. Wassilijew and
Wotschaloff. An Investigation of Fish Poison. (Russian) *Newasti
Weterinarnoi Literatury*. II, No. 3, (1885).
Chapter VI
115. Atwater, W. C. *Conn. Agr. Sta. Report*, p. 63, (1876).
116. Audebert, P. *Fish Fertilizer in Japan*. *L'Engrais*. 24; 1141 (1909)
117. Cligny A. *Les Engrais de Poisson Ann. Sci. agron.* 1: 166-187, (1906).
118. Douglas, L. W. *Fish Guano*. *Irish Framing World*, 22; 176 (1909).
119. Ellis, C. *The Hydrogenation of Oils*. Van Nostrand & Co., New York,
(1914).
120. Gautier and Mourgues. *Alcaloides volatils de L'huile de foie de morue;*
butylamine, anylamine, hexylamine, dihydrolutidine. *Compt.*
rend. 107; 254-257 (1888).
121. Gautier and Mourgues. *Sur les alcaloides de l'huile de foie de morue. (suite)*.
Compt. rend. 107; 626-629 (1888).
122. de Hemptinne, A. *Deodorizing Fish Oil*. *British Patent* 12, 525 (1905).
German Patent 169,410.
123. Henry, W. A. *Feeds and Feeding*. *Madison Wis.* (1906).
124. Heyden, H. *Deodorizing Fish Oils*. *French Patent* 392, 530, Nov. 29, 1907
125. Johnson, *Conn. Agr. Esp. Sta. Report*, p. 41, (1877).
126. Kleeman, A. *Vergleichende Fütterungsversuch mit Schweinen über die
wirkung von Fleischmehl und Fischmehl*. *Landw. Vers
Sta.* 73; 186-219 (1919).
127. Kossovich, P. S.
and Kolotov, G. J. *The Composition of Russian and Foreign Fish Fertilizer
and other marine manures. (Russian.) Bull. Agr. Intell-*
igence, 4: 377-378, (1913).
128. Lindemuth, J. R.
and Parker, F. C. *Some Analyses of Fish Scrap*. *J. Ind. Eng. Chem.*,
5: 368-369, (1913).
129. Martell, L. H. *Dogfish and how it is made into fertilizer*. *American
Fertilizer* 42: No. 8, 54-8 (1915).
130. Okada, H. *Ueber flüssige und feste Bestandteile des Japantrans*.
Chem. Zt. 31: 1135 (1907).
131. Osborne, T. H. and
Mendel, L. B. *The Influence of Cod and Liver oil and some other Fats
on Growth*. *J. Biol. Chem.* 17: 401 (1914).

132. Sandberg, G. Methods for the Preparation of an Odorless Fatty Acid Mixture from Blubber and Fish Fats. German Patent 162,638. May 30, 1903.
133. Schenke, V. Fleischfutttermehl, Kadavermehl, Fischfutttermehl, Landw. Ver. Sta. 58: 9-35, 36-54 and 55-64. (1903).
134. Tsujimoto, Jour. Coll. of England, Tokoyo Imperial Univ. Vol. IV. No. 1, (1908) No. 5
135. Turrentine, J. W. The Fish-Scrap Fertilizer Industry of the Atlantic Coast. U. S. Dept. Agric. Bull. 2, Dec. 27, 1913.
136. Turrentine, J. W. Utilization of Fish Waste of the Pacific Coast for the manufacture of Fertilizer. U. S. Dept. Agri. Bull. 150, (1915).
137. (a) Weibull, M. Zur Analyse von Fischguano, Poudrette, Knochenmehl und dergl. Substanzen. Chem. Zt. 16: 1689-1690 (1892).
- (b) Weibull, M. Weitere Beitrage Zur Analyse von Fischguano, Poudrette und dergl. Substanzen. Chem. Zt. 18; 31-32 (1894).
138. Weill, J. Sur le teneur an acides gras et en cholesterine des tissus d' animaux a sang froid. Compt. rend. 138; 642-645, (1914).
139. Winberg, H. Feeding experiments with Fish Press Cake from Alnarpzin, Sweden. (Sedish). Tidskrift for landman 12: 522-526 (1891) Biederman's Contr. Agric. Chem. 20; 736-740, (1891).
140. Babak, Ed. Ueber die Tatigkeit der Atenzentren bei den Fischen. (A Review) Rev. ges. Hydrobiol. 6: 69 (1913-14).
141. Baglicioni, S. Beitrage sur allgemeinen Physiologie des Hersen. Der Einfluss der chemischen Lebensbedingungen auf die Tatigkeit des Salachierhersens. A. allg. Physiol. 6: 72-98 (1906-7).
142. Baglicioni, S. Die Bedeutung des Harnstoffes als chemische Lebensbedingung fur das Salachierherz. Z. saag. Physiol. 6: 213-216 (1906-7).
143. Baglicioni, S. Sur Kenntnis des N-Stoffwechsels des Fische. (Die Bedeutung des Harnstoffes bei Selachiern. Zentr. Physiol. 20: 105-108 (1906).
144. Baumert. Chem. Untersuch., u. d. Respir. d. Schlammpeitzgers. Breslau, Page 24, 1855.
145. Bert, P. Sur les phenomenes et les causes de la mort des animaux d'eau douce que l'on plonge dans l'eau de mer. Compt. Rend. 73: 362-385, 464-467 (1871).

146. Bert, P. La mort des animaux d'eau douce que l'on immerge dans l'eau de mer. de mer. Comp. rend. soc. biol. Series 5. Vol. 3; 59-61 (1873).
147. Bert, P. Sur la cause de la mort des animaux d'eau douce qu'on plonge dans l'eau de mer et reciproquement. Compt. rend. 97; 133-136 (1883).
148. Bottazzi, F. La pression osmotique du sang des animaux marins. Arch. italiennes Biol. 28; 61-72 (1897).
149. Bottazzi, F. Osmotische Druck und elektrische Leitfähigkeit der Flüssigkeiten der einzelligen, pflanzliche und tierische Organismen. Ergebnisse Physiol. 7; 161-402 (1908).
150. Bottazzi, F. and Ducceschi. Resistance des erythrocytes, alsalinite du plasma et pression osmotique du sang dans les differentes classes der vertebrees. Arch. italiennes Biol. 26; 161-172 (1896).
151. Bottazzi and Enriques, P. Über die Bedingungen des osmotischen Gleichgewichts und des Gleichgewichtsmangels zwischen den organischen Flüssigkeiten und dem ausseren Medium bei den Wasserthieren. Arch. Anat. Physiol. Suppl. 109-170 (1901).
152. Butterfield, W. J. A. The Effects of Ears and other road-dust Abating Substances on Fish Life. Eng. News 67; 689-690 (1912).
153. Clark, H. W. and Adams, S. C. Studies of Fish Life and Water Pollution. Eng. News 69; 300-302 (1913).
154. Cohnheim, O. Versuche über Eiweiss resorption, Z. physiol. Chem. 59; 239-246 (1909).
155. Cohnheim, O. and Makita, F. Zur Frage der Eiweiss resorption. Z. physiol. Chem. 61; 189-193 (1909).
156. Dakin, W. J. The Osmotic Concentration of the Blood of Fishes taken from sea water of naturally varying concentration biochem. J. 3; 258-278 (1908).
157. Denis, W. Metabolism Studies on Cold-Blooded Animals. I. The Urine of the fish. J. Biochem. 13, 225-532 (1912).
158. Denis, W. Metabolism Studies on Cold-Blooded Animals. II. The Blood and Urine of Fish. J. Biol. Chem. 16; 389-393 (1913-14).
159. Dubois, R. Sur le Cuivre normal dans la serie animale. Compt. rend. Soc. biol. 52; 392-394 (1900).

160. Finnish Sulphate Cellulose Committee.
Der Einfluss der Abwasser der Sulphat-cellulose-fabriken auf die Fischerei. Papier-fabrikant. 8; 61 (1912).
161. Fredericq, L.
Influence du milieu ambiant sur la composition du sang des animaux aquatiques, Arch. Zool. Exper. 2nd. Series Vol. 3 Notes and Review. pp XXIV-XXXVIII (1885).
162. Fredericq, L.
Sur la physiologie de la branchie. Arch. Zool. Exper. 2nd. series, Vol. 9; 117-123 (1891).
163. Fredericq, L.
Sur la concentration moleculaire du sang et des t'issues chez les animaux aquatiques. Arch. Biol. 20; 709-730 (1904).
164. Gardner, J. A. and Leethen. C.
On the Respiratory Exchange in Fresh Water Fish. Part I; On Brown Trout. Biochem. J. 8; 374-390 (1914).
165. Garrey, W. E.
The Osmotic Pressure of Sea Water and of the Blood of Marine Animals. Biol. Bull. 8; No. 4, (1905).
166. Garrey, W. E.
The Resistance of Fresh Water Fish to Changes of Osmotic and Chemical Conditions. Am. J. Physiol. 39; 313-329 (1916).
167. Garrey, W. E.
Some Cryoscopic and Osmotic Data. Biol. Bull. 28; 77-86 (1915).
168. Giard, A.
Sur l'adaptation brusque de l'epinoche (Gasterosteus trachurus Cuv. et Val.) aux eaux alternativement douces et marines. Compt. rend. soc. biol. 52; 46-48 (1900).
169. Greene, C. W.
Physiological Studies of the Chinook salmon; I. Relation of the Blood Pressure to the Functional Activity. II. A Study of the Blood and Serous Liquids by the Freezing Point Method. U. S. Bur. Fisheries. Doc. 584 Issued 1905.
170. Greene, C. W.
The Storage of Fat in the Muscular Tissue of the King Salmon and its Resorption During the fast of the Spawning Migration. Bull. U. S. Bur. Fish. 33; 69-138 (1913).
171. Hahn, G. W.
Sporozoon Parasites of Certain Fishes in the Vicinity of Woods Hole, Mass. U. S. Bur. Fish. Doc. No. 810, Issued 1915.
172. Hamburger, H. J.
Über die Sals- und Rohr-zucker Lösungen bewirkten Veränderungen der Blutorperchen Arch. Anat. Phys. 31-50 (1887).
173. Hammarsten, O.
Über eine neue Gruppe gepaarter Gallensäuren. Z. physiol. Chem. 24; 322-350 (1896).
174. Henze, M.
Bemerkungen zu den Anschauungen Putter's über den Gehalt des Meeres an gelösten organischen Kohlenstoffverbindungen und deren Bedeutung für den Stoffhaushalt des Meeres. Arch. ges. Physiol. 123; 487-490 (1908).
175. Hoppe-Seyler, F.
Über Unterschiede im Chemischen Bau und der Verdauung höherer und niedriger Thiere. Arch. ges. Physiol. 14; 395-400 (1877).

176. Jolyet, F. and Regnard, P. Sur une nouvelle methode pour l'etude de la respiration des animaux aquatiques. *Compt. rend.* 82; 1060-1062 (1876).
177. Jolyet, F. and Regnard, P. Recherches Physiologiques sur in Respiration des Animaux Aquatiques. *Arch. Physiol. Ser. 2. Vol. 4.* 44-62, 504-633 (1877).
178. Kerb. W. Uber den Nahrwert der in Wasser gelosten Stoffe. *Int. Rev. ges. hydrobiol. Hydrogr.* 3; 495-505. (1910).
179. Knuthe, K. Zur Kenntinis des Stoffwechsels der Fische. *Arch. ges. Physiol.* 73; 490-500 (1898-99).
180. Kupsis, J. Uber den Niefriigsten fur das Leven der Fische notwendigen Sauerstoffgehalt des Wassers, und uber die fur dieselben giftigen mengen in Wasser geloster Kohlensaure. *Z. Nahr. Genussm.* 4; 631-638 (1901).
181. Linton, E. Parasites of Fishes of Beaufort, North Carolina. U. S. Bur. Fish. *Doc. No. 583*, Issued 1905.
182. Lipschutz, A. Zur Frage uber die Ernahrung der Fische. *Z. allgem. Physiol.* 12; 99-117 (1911).
183. Lipschutz, A. Uber den Hungerstoffwechsel der Fische, *Z. allgem. Physiol.* 12; 118-124. (1911).
184. Loeb, J. The Toxicity of Sugar Solution upon Fundulus and the Apparent Antagonism between Salt and Sugar. *J. Biol. Chem.* 11; 415 (1912).
185. Loeb, J. Uber die Anpassung von Fundulus an höhere Konzentrationen Biochem. *Z.* 53; 391-405. (1913).
186. Loeb, J. and Wasteneys, H. Die Entgiftung non Sauren durch Salze. *Biochem. Z.* 33; 489-502 (1911).
187. Loeb, J. and Wasteneys, H. Die Entgiftung von Natriumchlorid durch Kaliumchlorid. *Biochem. Z.* 33; 503 (1911).
188. Loeb, J. and Wasteneys, H. On the influence of balanced and Non-balanced Salt Solutions upon the osmotic pressure of the body liquids of Fundulus. *J. Biol. Chem.* 21; 223-238, (1915).
189. Marsh, M. C. Notes on the Dissolved Content of Water in its Effect on Fishes. U. S. Bur. Fisheries *Coc. No. 688*. Issued April 1910.
190. Marsh, M. C. and Gorham, F. P. The Gas Disease in Fishes, U. S. Bur. Fish. *Doc. No. 578*, Issued 1905.

191. Miescher, F. Statistische und biologische Beiträge zur Kenntnis vom Leben des Heilbaches in Süßwasser. Schweizer cher Fischerei-Ausstellung in Berlin, p. 154, 1880. Also Die histochemischen und physiologischen Arbeiten von Frederick Miescher, p. 116, Leipzig, 1897.
192. Moore, B., Edie, E. S. and Whitley, E. The Nutrition and Metabolism of Marine Animals; The Rate of Oxidation and output of Carbon dioxide in Marine Animals in Relation to the Available Supply of Food in Sea Water. Report for 1913 on the Lancashire Sea-Fisheries. Liverpool. 22; 297-320 (1914).
193. Moore E., Edie E. S., Whitley E., and Dakin, W. J. The Nutrition and Metabolism of Marine Animals in Relationship to (a) Dissolved organic matter and (b) Particulate Organic Matter of Sea Water. Biochem. J. 6; 255-296 (1912).
194. Morgulis, S. Studies on Fasting Flounders. J. Chem. Biol. 20; 37-46 (1915).
195. Morgulis, S. The Body Surface of Flounders and its relation to the Gaseous Metabolism. Am. J. Physiol. 36; 207-216 (1914-15).
196. Mosso, A. Über verschiedene Resistenz der Blutkörperchen bei verschiedenen Fischarten. Biol. Centralb. 10; 570 (1890).
197. Müller, M. Über das Wachstum und die Lebenstätigkeit von Bacterien, sowie den ablauf fermentativer Prozesse niedriger Temperatur unter spezieller Berücksichtigung des Fleisches als Nahrungsmittel. Arch. Hyg. 47; 127-193 (1903).
198. Murisier and Fick, A. Über das Magenferment kaltblütiger Thiere. Verhand physikal-med. Gessel. Warzburg. New Series 4; 120-121 (1873).
199. Oswald, Wolfgang. Versuche über die Giftigkeit des Seewassers für Süßwassertiere (Gammarus pulex De Geer). Arch. ges. Physiol. 106; 568-598 (1905).
200. Paton, D. Noel. Report of the Fishery Board for Scotland, 1898, p. 143.
201. Petrik, J. Über die reflektorische Einwirkung des Sauerstoffgehaltes im Wasser (insbesondere) auf die Atembewegungen der Fische. Arch. ges. Physiol. 161; 555-576 (1915).
202. Polimanti, Osw. Über den Fettgehalt der Leber einiger Selachier während der Zeit der Schwangerschaft. Biochem. Z. 38; 497-500 (1912).
203. Polimanti, Osw. Über den Fettgehalt und die biologische Bedeutung desselben für die Fische und ihren Aufenthaltsort, sowie über den Fettgehalt je nach dem Alter der Fische. Biochem. Z. 69; 145-154 (1915).

204. Putter, A. Aktive overflache und organfunktion. Z. allgem. Physiol. 12; 125-214 (1911).
205. Putter, A. Die Ernährung der Wassertiere. Z. allgem. Physiol. 7; 283-320 (1907-8).
206. Putter, A. Der Stoffhaushalt der Meeres. Z. allgem. Physiol. 7; 321-368 (1907-8).
207. Putter, A. Die Ernährung der Fische. Z. allgem. Physiol. 9; 147-242 (1908-9).
208. Quinquand. Experiences relatives a la respiration des poissons Compt. rend. 76; 1141 (1873).
209. Quinton, R. Communication osmotique chez L'Invertebre marin non seulement a l'eau mais encore aux sels. Comp. Rend 131; 905-908 (1900).
210. Quinton, R. L'eau de mer milieu organique. Constance du milieu marin original comme milieu vital des cellules a travers la serie animale. Paris 1904. 503.
211. Quinton, R. Permeabilite de la paroi exterieure de l'Invertebre marin, non seulement a l'eau mais encore aux sels. Compt. rend. 131; 952-955 (1900).
212. Ringer, S. Concerning the influence of Saline Media on Fish. etc. J. Physiol. 5; 98-115 (1884).
213. Rodier, E. Sur la pression osmotique du sang et des liquides internes chez les poissons Selaciens. Compt. rend. 131; 1908-1010 (1900).
214. Roule, L. Sur l'influence exercee sur la migration de montee du saumon (*salmo salar* L.) par la proportion d'oxygene dissous dans l'eau des fleuves. Compt. rend. 158; 1364-1366. (1914).
215. Schroeder, W. Uber die Harnstoffbildung der Halfische. Z. Physiol. Chem. 14; 576-598 (1890).
216. Scott, G. G. Effect of changes in the density of water upon the blood of Fishes. U. S. Bur. Fish. Doc. 712. Issued 1910.
217. Seydel, E. Uber die Wirkung von Mineralionen auf Fischwasser. Mitteilung des Fischereivereins d. Provinz Brandenburg 5; 26 (1914). also Wasser und Abwasser 7; 479 (1914).
218. Siedlecki, M. L'action des solutions des sels alcalins et alcalino-terreux sur les epinoches. Compt. rend. 137; 525-527 (1903).
219. Siedlecki, M. Sur le resistance des epinoches aux changements de la pression osmotique du milieu ambiant. Compt. rend. 137; 469-471 (1903).

220. Sumner, F. B. The Physiological effects upon fishes of changes in the density and salinity of water. U. S. Bur. Fish. Doc. No. 596, Issued 1906.
221. Thiergardt, A. Uber Aufnahme und Ansammlung von Arsen und Blei im Korper der Fische. Dissertation. Wurzburg (1897).
222. Vernon, H. M. The Respiratory Exchange of the Lower Marine Invertebrates. J. Physiol. 19; 18 (1895-6).
223. Ward, R. B. Internal Parasites of the Sebago Salmon. U. S. Bur. Fisheries Doc. No. 713, Issued 1910.
224. Wells, M. W. Reactions and resistance of Fishes in their natural Environment to Acidity, Alkalinity, and Neutrality. Biol. Bull. 29; 221-257 (1915).
225. Weyl, Th. Physiologische und chemische Studien an Torpedo. Z. physiol. chem. 7; 541-552 (1882-3).
226. Wilson, C. B. The Fish Parasites of the Genus Argulus Found in the Woods Hole Region. U. S. Bur. Fish. Doc. 561, Issued 1904.
227. Wilson, D. W. The comparative Chemistry of Muscle; The partition of Non-protein Water Soluble Nitrogen. J. Biol. Chem. 17; 385-400 (1914).
228. Zuns, N. Ein Respirations apparet fur Wasserthiere. Arch. Anat. Physiol. 543-551. (1901).

1000

1000

1000