[CONTRIBUTION FROM THE ANIMAL PHYSIOLOGICAL CHEMICAL LABORATORY, BUREAU OF CHEMISTRY, U. S. DEPARTMENT OF AGRICULTURE.]

THE FOOD OF THE SMALL SEA HERRING AND AMMONIA AND AMINES AS END PRODUCTS OF ITS DECOMPOSITION.

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On the Atlantic Coast of the United States, canned sardines are prepared entirely from the sea herring (*Clupea harengus*). With the exception of a few canneries in Massachusetts, the industry is located in the State of Maine, the principal center of the industry being the towns of Eastport and Lubec, which lie at the entrance to Passamaquoddy Bay.

In the investigation of the sardine industry of Maine, conducted by the Animal Physiological Chemical Laboratory during the seasons of 1913 to 1916, decomposing food in the alimentary tract of these small fish was found to be one of the most important factors in rendering them unsuitable for packing. The term "feedy" is applied to those fish which have undigested and partly digested food remaining in the alimentary tract when caught. "Feedy" fish is one of the most troublesome factors in the sardine industry. Fish more or less gorged with food when taken from the water deteriorate very rapidly, whereas those having their digestive tracts free from food when taken from the water remain in good condition for a comparatively long time. The rate of deterioration depends upon the quantity and stage of digestion of the food material contained in the digestive tract and the bacteria accompanying it. Food recently eaten and present in the stomach of the fish appears to cause a greater degree of deterioration than that which has been partially digested. In confirmation of this, bacteriological studies[1] showed that the stomach portions of the digestive tract were sterile when free from feed, though digestion was incomplete in the intestines. By the time "feedy" fish which have been 3 to 6 hours out of water reach the cannery, they have deteriorated to such an extent and are so badly broken that a large percentage is entirely unfit for packing.

The physical evidence of this deterioration is plainly seen by the ruptures of the stomach walls in earlier stages and later in the rupture and sloughing away of the belly portion of the fish. Fish brought to the canneries in such condition are termed "belly broken" or "belly-blown."

Food of the Sea Herring.

The young herrings from which sardines are made, constantly rove the seas in search of food. A plentiful supply of these fish, therefore, can

[1] The bacteriological work was done by M. M. Obst, Bacteriological Chemist, Microbiological Laboratory, Bureau of Chemistry, Washington, D. C. A discussion of this work may be found in the *J. Infect. Dis.*, [2] **24**, 158 (1919).

always be found in regions where food abounds, as the Passamaquoddy Bay region, where the largest number of sardine canneries have been built.

A number of observers, Moore,[1] Scott,[2] Hanson,[3] Wheeler,[4] MacDonald,[5] Wright,[6] and Bigelow,[7] have explored the Atlantic Coast waters and have shown that the herring live chiefly upon minute crustaceans. Of these the chief forms are copepods, amphipods, schizopods, also the embryos of gasteropods and lamellibranchs.

The schizopods collected and used in this investigation were *Meganyctiphanes norwegica* (M. Sars). The copepods were identified by the U. S. National museum as mostly *Calanus finmarchicus* with smaller numbers of *Pseudocalanus elongatus* and *Temora longicornis*. These undoubtedly are the chief food of the herring of the Passamaquoddy Bay region. Fish which have been feeding extensively on either of the 2 kinds of food known to the fisherman as "red feed" and "shrimp" may be designated as "feedy" fish, which are most unsuitable for packing.

Examination of the Feed.

In order to determine the cause and the products formed in the decomposition of "feedy" fish, both the fish and the feed were subjected to chemical and bacteriological examination. Through the courtesy of the Bureau of Fisheries the authors were able to collect a sample of plankton at the Biological Station at Woods Hole, Massachusetts. The determinations made on this sample are recorded in the results of the chemical analyses. All other samples were obtained from the waters of Passamaquoddy Bay, off Eastport, Maine.

Collection of Samples.

Plankton.—The sample of plankton, taken at Woods Hole, Massachusetts, was obtained by tying a net made of bolting cloth just off the wharf at a point where a tidal current would run through it. About 4 hours later, and after the tide had begun to slacken, the net was brought in. The contents of the net were washed into a bolting cloth sieve with sea water, cleansed from seaweed and other foreign material, then shaken

[1] H. F. Moore, "Observations upon the Herring and Herring Fisheries of the Northeast Coast, with special reference to the vicinity of Passamaquoddy Bay." *U. S. Commissioner of Fish and Fisheries Report*, 1896.

[2] Scott, "Observations on the Food of Fishes." *20th Ann. Rept. Fishery Board of Scotland*, 1901, Pt. 3, 486–541.

[3] H. H. Hanson, *Proc. 8th Intern. Cong. Appl. Chem.*, 18, 131 (1912).

[4] W. M. Wheeler, "The Free Swimming Copepods of the Woods Hole Region," *U. S. Commission of Fish and Fisheries Bulletin*, 1899.

[5] D. L. MacDonald, *Contributions to Canadian Biology*, 1906–10, 83.

[6] "The Plankton of Eastern Nova Scotia Waters." *Contributions to Canadian Biology. 39th Ann. Rep. Dept. Marine and Fisheries, Fisheries Branch*, Ottawa, Canada, 1907.

[7] H. B. Bigelow, *Bull. Museum Comp. Zoöl., Harvard University*, 59, No. 4.

until practically all the water was removed. This sample was transferred to a glass bottle, and kept at the temperature of the laboratory until after the second analysis was made. The samples of plankton taken at Eastport, Maine, were obtained by the method employed for copepods.

Copepods (Feed or "Red Feed").—A net about 3 meters long, 1.23 meters in diameter at one end, and tapering to about 15 cm. in diameter at the other end, was used. The first 1.2 meters of the net consisted of coarse scrim; the remaining portion was of bolting cloth. A sterile Mason jar was fastened to the smaller end by means of a draw-string, and the net attached to the stern of the launch by a strong line. About 60 meters of rope was let out, and the net was dragged through the water at a depth ranging from 3 to 9 meters, just fast enough to keep the rope taut. Occasionally the net was brought in, the contents of the jar were transferred to another sterile jar, and the water was drained off. At the laboratory the sample was dried by shaking in a sieve of sterile bolting cloth, transferred to another sterile jar, and mixed for use.

Schizopods (Shrimp).—The schizopods were caught just as the tide began to go out, from a tender, among the rocks, behind breakwaters and other places more or less protected from the waves, by means of a small scrim scoop net, fitted with a long handle, and having an opening at the small end to which was fastened, by a draw-string, a sterile Mason jar. They were transferred to another sterile jar, and brought in sea water to the laboratory, where the water was drained off as completely as possible, the sample ground in an Enterprise meat grinder, and transferred to a sterile jar for use.

Feed from "Belly-Blown" Fish.—The stomach of each fish was removed with sterile instruments, slit with a scalpel, and the feed transferred to a sterile jar by means of a pair of tweezers previously sterilized in the flame.

Preparation of Samples.

With the exception of the plankton taken at Woods Hole, all the samples, during the intervals between analyses, were kept in an incubator at 37.5° at periods of time noted. Immediately after the preparation of the sample, samples for the determination of total volatile nitrogen and amines were removed by means of a sterile spatula, care being taken that the sample was not contaminated by material from the outside. At the end of each interval of time noted the material was removed from the incubator just long enough to weigh out the samples, and returned at once. The samples were placed in sterile jars and handled under aseptic conditions in order that they could also be used for bacteriological work.

Methods of Analysis.

Only the total volatile alkaline material, ammonia and total amines was determined in this investigation. At the time these studies were made

the method for the separation of the amine fraction of the total volatile material had not been perfected. These methods have been reported in detail[1] in connection with a study of the formation of ammonia and amines in canned sardines.

As a result of the bacteriological work it was found that 2 organisms were associated with the feed (copepods and shrimp) of the small herring. One of these so closely resembles *Bacillus Walfischrauschbrand* that it has been so designated, and the other has been tentatively designated as *Bacillus B*. *Bacillus Walfischrauschbrand* is the name applied by Nielsen[2] to the organism found in diseased whales. *Bacillus Walfischrauschbrand* was also found to be responsible for the "swelling" of canned sardines. In fact, these organisms were first isolated from "swells," and later trace to the fish, where they were found upon the gills and in the intestinal tract with the feed.

The bacteriological examination of the feed showed that the bacterium (*Bacillus B.*) commonly associated with copepods grows faster and produces a slightly greater amount of gas than the organism *Bacillus Walfischrauschbrand* found chiefly on the shrimp.

In the French sardine industry[3] bait is employed in the catching of the fish. So particular are the fisherman and the manufacturers to avoid the decomposition caused when fish are "feedy" that the strictest attention is given to the quality of the bait. In early times in this industry, owing to decomposition caused in the fish, the use of a prepared bait containing especially powdered prawn and shrimp was forbidden by royal decree. In this connection it is interesting to note that *Bacillus Walfischrauschbrand* found most commonly in shrimp is capable of decomposing fish tissue. This organism forms spores which are resistant to drying, but which are capable of growth and reproduction when conditions again become favorable. The bait prepared from dried shrimp doubtless contained the spores of this organism (*Bacillus Walfischrauschbrand*) which were responsible for the decomposition of the fish when this bait was employed.

In addition to the analyses made to determine the volatile alkaline constituents formed when the feed (copepods and schizopods—shrimp) was allowed to decompose, the same determinations were made upon culture media of the *Bacillus Walfischrauschbrand* and *Bacillus B*. When these bacteria were grown in pure cultures.

[1] F. C. Weber and J. B. Wilson, "The Formation of Ammonia and Amines in Canned Sardines During Storage." *J. Ind. Eng. Chem.*, **11**, 121 (1919).

[2] Ivar Nielsen, "Ein Stück Moderner Bakteriologie aus dem 12. Jahrhundert," *Centr. Bakt. Parasitenk.*, **7**, 267 (1890).

[3] Hugh M. Smith, "French Sardine Industry," *U. S. Fish Commission Bull.*, **1901**, 9–10.

Composition of Copepods.

In an investigation of the chemical composition of plankton, Brandt[1] reports the analysis of material which consisted almost entirely of copepods as follows:

TABLE I.

Chemical Composition of Copepods (dry basis).

	%
Protein	58.80
Fat	7.40
Carbohydrates (by difference)	22.88
Ash	10.92
Composition of ash:	
SiO_2	2.31
NaCl	1.49
Other salts	7.12

The composition of the dry substance of copepods, secured in the investigation made by Brandt, and which included varieties taken from fresh water, averaged as follows:

TABLE II.

Average composition of Copepods (dry basis)

	%
Protein	59.0
Chitin	4.7
Fat	7.0
Carbohydrates	20.0
Ash	9.3

Table III shows the results of the determination of ammonia and amines, as products of the decomposition of the feed of the herring, when the feed, copepods and schizopods (shrimp), were allowed to decompose under the most favorable conditions. The determination of the total volatile alkaline material in a catch of plankton, which consisted almost entirely of diatoms, taken at Woods Hole, Massachusetts, is also given. In all cases as many determinations were made on each sample as was possible with the amount of material obtained, which was not often large, as it was difficult to obtain any kind of feed except when the water was very quiet. The amount of total volatile nitrogen found before incubation was so small that it was not considered necessary to determine amine nitrogen.

Both ammonia and amines in very large quantities were found in the decomposing feed (schizopods and copepods). When allowed to spoil under these conditions and to the extent that took place during a period of 48 to 72 hours, ammonia was found in much larger amounts than amine.

[1] K. Brandt, Beiträge zur Kenntnis der Chemischen Zusammensetzung des Planktons. Wissenschaftliche Meeres untersuchungen herausgegeben von der Kommission zur wissenschaftlichen Untersuchung der deutschen Meere in Kiel. *Abt. Kiel Bd.*, 3, 43–90 (1898).

TABLE III.—AMMONIA AND AMINES IN SAMPLES OF FEED KEPT AT INCUBATOR TEMPERATURE (37.5°).[1]

Sample No.	Description.	Volatile nitrogen per 100 g. When taken. Total. Mg.	When taken. Ammonia. Mg.	When taken. Amines. Mg.	24 hrs. in incubator. Total. Mg.	24 hrs. in incubator. Ammonia. Mg.	24 hrs. in incubator. Amines. Mg.	48 hrs. in incubator. Total. Mg.	48 hrs. in incubator. Ammonia. Mg.	48 hrs. in incubator. Amines. Mg.	72 hrs. in incubator. Total. Mg.	72 hrs. in incubator. Ammonia. Mg.	72 hrs. in incubator. Amines. Mg.
1	Plankton from Woods Hole, Mass., kept at 30°. Sample mostly composed of diatoms	2.82	..	..	...	...	...	...	...	...	4.14	...	...
3	Plankton from St. Croix River, off Campobello Island. Mostly composed of copepods	6.04	..	..	...	...	...	...	...	...	402.2	...	...
7	Copepods from east and south of Campobello Island	3.94	..	..	272.4	206.2	66.2	468.2	375.1	93.1	...	...	...
13	Copepods from shallow water east of Grand Manan Island	...	..	..	216.6	153.8	62.8	...	...	...	...	...	...
18	Copepods from north end Grand Manan Island in very deep water	9.50	..	..	191.7	143.4	48.3	296.8	260.9	35.9	...	...	...
5	Schizopods from Wilson's Beach, off Campobello Island	1.98	..	..	...	...	...	...	...	...	822.9	602.3	220.6
14	Schizopods from Wilson's Beach, off Campobello Island	3.95	..	..	220.4	88.2	132.2	787.6	573.7	213.9	1009.2	832.6	176.6
21	Feed taken from "belly-blown" fish at wharf, Eastport, Me.	37.7	17.1	20.6	...	...	...	...	...	...	...	...	...

[1] Through the courtesy of the United States National Museum the copepods composing the different samples examined were identified for us as follows:

Nos. 3 and 13 were identified as *Calanus finmarchicus* (Gunner).

No. 7 contained *Calanus finmarchicus* (Gunner) and *Pseudocalanus elongatus* (Boeck) in about equal numbers.

No. 18, *Pseudocalanus elongatus* (Boeck) with a very few *Calanus finmarchicus* (Gunner).

Nos. 5 and 14 were identified as *Meganyctiphanes norwegica* (M. Sars).

From the number of determinations it was possible to make, it was apparent that little, if any, difference exists in the rate of decomposition or in materials formed between copepods and schizopods. Considering the similarity of composition of the two forms, it is quite probable that no difference in degree or kind of decomposition would be found. The examination of the feed taken from the viscera of "belly-blown" fish gave results confirming those found under artificial conditions. Both ammonia and amines were present in appreciable amounts, the quantity of amine in this instance being in excess of the ammonia.

Laboratory Experiments with Bacillus Walfischrauschbrand and Bacillus B.—To establish firmly the conclusion that the decomposition of the feed and of the fish, which results in the condition termed "belly-blown," is due to the presence of *Bacillus Walfischrauschbrand* and *Bacillus B.*, which were found in great numbers in the samples of feed and swelled cans examined, and to show that the presence of these bacteria is indicated by finding volatile nitrogen bases in "belly-blown" fish and swelled cans, these bacteria were grown in pure culture upon a medium containing fish protein, and the volatile alkaline products resulting from their growth determined. The fish used for preparing Cultures 24, 25, 31 and 32 (Table IV) were fresh Boston mackerel. Freshly caught Potomac bass were used for Cultures 27, 28, 29 and 30. After removing the skin, solid masses of meat were passed through a meat grinder and mixed with a solution of dextrose of such strength that the final mixture, of a uniform paste consistency, contained 0.2% of dextrose. The whole was then sterilized under 15 pounds pressure. Portions of this paste were inoculated with 24-hour dextrose agar anaerobic cultures of bacteria, and covered with an inch layer of sterilized fish broth made firm with 1.5% agar and no nutriment, and then incubated at 37.5° until removed for analysis. Whenever a sample was removed for analysis, the presence of the bacterium with which it has been inoculated was determined positively. The determinations are reported in Table IV.

The results show that both ammonia and amines are formed when *Bacillus Walfischrauschbrand* and *Bacillus B.* are grown in pure cultures on fish media. Under the conditions which obtained when these experiments were made, amines are formed in smaller amounts than when the different lots of feed are decomposed at incubator temperature. It would appear that a larger proportion of amines are formed during the decomposition of the feed and the fish under natural conditions. In the cultures of *Bacillus Walfischrauschbrand* and *Bacillus B.* used for the determination of ammonia and amines, positive tests for both indole and skatole were obtained.

These results confirm those shown in Table III that ammonia and amines are produced by these organisms during the decomposition of the feed.

TABLE IV.—DETERMINATION OF AMMONIA AND AMINES IN PURE CULTURES OF BACILLUS WALFISCHRAUSCHBRAND AND BACILLUS B. GROWN IN THE LABORATORY.

Culture. No.	Bacteria.	Time of incubation. Days.	Volatile nitrogen per 100 g.			Percentage of total volatile nitrogen.		Nitrogen determined by the amino-acid method per 1.0 g.[a]	Alkalinity (0.05 *N* acid per 1.0 g.)
			Total. Mg.	Ammonia. Mg.	Amines. Mg.	Ammonia. %.	Amines. %.	Mg.	Cc.
30	Sterile	7	18.5	...	..	..	..	0.55	0.0
28	*B. Walfischrauschbrand*	2	259.5	215.2	44.3	82.9	17.1	5.34	...
28		4	...	...	..	..	..	5.65	...
31		7	510.1	447.1	63.0	87.7	12.3	9.95	2.1
32		7	334.6	286.2	48.4	85.6	14.4	5.16	1.5
24	*B. B.*	2	146.8	...	..	..	..	..	1.2
24		4	208.4	160.3	48.1	76.9	23.1	..	1.9
25		3	159.1	127.7	31.4	80.3	19.7	..	...
29		2	145.8	132.6	13.2	91.0	9.0	2.52	...
27[b]		2	225.2	198.8	26.4	88.2	11.8	5.07	...
27		3	...	...	..	..	..	5.59	...

[a] These determinations made by the Van Slyke method and apparatus (D. D. Van Slyke, *J. Biol. Chem.*, 16, 121 (1913).

[b] A few micrococci in this culture.

Conclusions.

The copepods and schizopods (shrimp) constitute the chief forms of feed of the small sea herring of the Passamaquoddy Bay region. Of the copepods *Calanus finmarchicus*, *Pseudocalanus elongatus*, and *Temora longicornis* appear to be the chief species found in this region, and may be classed as the "red feed," as distinguished from shrimp.

The results of the examinations made show that ammonia and amines are found in very appreciable quantities when the different forms of food decompose. These results also show with what rapidity and to what extent the food decomposed. When the 2 kinds of bacteria, so commonly found associated with the feed, are grown in pure culture it is shown that ammonia and amines are formed in the media.

Skatole and indole were also detected in the culture media on which these bacteria were grown. Ammonia and amines were also determined in the contents of the digestive tract of "belly-blown," feedy fish. The results show that the formation of ammonia and amines in the decomposing food is due to the action of the 2 bacteria always found asssociated with the 2 forms of food (copepods and schizopods), whether taken directly from the water or from the digestive tracts of the fish. This decomposition, also accompanied by the evolution of gas when *Bacillus B* is present, is responsible for the bursting of the bellies ("belly-blown") of "feedy" fish.

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SOME DERIVATIVES OF 2,4-DINITRO-BENZALDEHYDE.[1]

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The work upon the derivatives of 2,4-dinitro-benzaldehyde was under taken for the following reasons: (1) Upon examination, the literature revealed that relatively few derivatives of this compound have been prepared.[2] (2) Research upon trinitro-benzaldehyde was already under way in these laboratories and the results of this work, to be published in the near future, showed the desirability of a parallel investigation upon 2,4-dinitro-benzaldehyde. (3) Since benzaldehyde has long been an important intermediate in the dye industry it seemed probable, reasoning by analogy, that 2,4-dinitro-benzaldehyde might also find considerable use in the manufacture of dyestuffs. (4) But perhaps the most im-

[1] This report represents a part of a thesis presented by Blaine B. Wescott in partial fulfillment of the requirements for the degree of Master of Science, January, 1920.

[2] *Ber.*, **35**, 1224 (1902); *ibid.*, **35**, 2704 (1902); *Monatsh.*, **23**, 1003 (1902); *Ber.*, **37**, 1861 (1904); *ibid.*, **39**, 2754 (1906); *ibid.*, **40**, 3230 (1907); *Monatsh.*, **23**, 554 (1902); *Ber.*, **41**, 109 (1908); *ibid.*, **42**, 601 (1909); *J. prakt. Chem.*, **2**, 89.