

THE PRESENCE OF THE *B. COLI* AND *B. WELCHII*
GROUPS IN THE INTESTINAL TRACT OF FISH
(*STENOMUS CHRYSOPS*)

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The *B. coli* group, the *B. Welchii* group and other lactose-fermenting organisms have long been regarded as normal inhabitants of the intestinal tract of the homoiothermal or warm-blooded animals, and their presence outside of the body has been regarded as a very strong index of the presence of fecal pollution from those animals. The presence of these microorganisms in the intestinal tract of the poeciloothermal or cold-blooded animals, however, has been a subject for dispute. A review of the literature does not seem to help the situation in the least, for we find the authorities evenly divided on the subject. The tendency in England and America seems to be to regard the *B. coli* group as the normal inhabitants of the higher animals only, while on the continent of Europe, especially in Germany, the interpretation has not been so strict. No opinions concerning the status of the *B. Welchii* group could be found, and such would be difficult to form because of the spore-forming abilities and anaerobic conditions necessary for the growth of that group of organisms.

All workers seem to agree, however, that the *B. coli* group is much more constant in the intestinal tract of the higher animals than of the cold-blooded animals. Hoag (1899) was able to isolate members of the *B. coli* group from the fish which he examined. Amyot (1902) examined the intestinal contents of 23 fishes, representing 14 species taken from Lake Erie, and was unable to isolate any lactose fermenters of the colon type.

Houston (1903) found members of the colon group in 13 per cent of the fishes examined, but concludes that the *B. coli* group is "either absent or present in small numbers in the intestines of fishes dredged from the sea in localities remote from sewage pollution." Whipple (1904) was unable to find the colon group in the intestinal tract of the trout, perch, and sunfish, taken from unpolluted waters, while Johnson (1904) was able to isolate these organisms 47 times out of the 67 fishes examined from polluted waters. Eyre (1904) isolated the members of the *B. coli* group from the intestinal tract of the sprat, dab, smelt, mullet, sole, skate and dogfish taken from traps two miles from the shore in unpolluted waters. Van Mallannah in an unpublished work reports positive results in 37.5 per cent of 32 fish examined, while Fromme states that 41 per cent of fishes examined responded positively to the *B. coli* tests. He concludes, however, that the *B. coli* group is only rarely present in the intestinal tract of the cold-blooded animals, while they are much more constant in the higher vertebrates. Bettencourt and Borges (1908) isolated 29 cultures of colon-like bacilli from the intestinal tract of 17 fishes, reptiles and amphibia, of which only two proved to be members of the colon group, although in these tests no enrichment medium was employed.

The purpose of this paper is to present some results as to the presence of the *B. coli* group and the *B. Welchii* group in the intestinal contents of fishes which were obtained at the Woods Hole Laboratory of the Bureau of Fisheries during the summer of 1916, while the writer was making an intensive study of the Bacteriology of the Food Fishes, especially the scup (*Stenomus Chrysops*).

The scup belong to the family Sparidae and to that large group of fishes known as "bottom feeders" such as the porgies, sea robins, drum fishes, tautog and sea bass which obtain their food supply from the floor of the ocean; hence, the character of the bottom on which the fish feed will influence the bacterial flora of their intestinal tract. Peck (1895) found that the food of the scup was somewhat varied but generally characteristic of the bottom fauna and flora. He found "at times very many

sand dollars (*Echinarachnius parma*) ground up with the sand and deep black mud of the bottom from which they were feeding, just above which the amphipods are usually abundant." Young specimens have often been found near the shores of coves and harbors feeding on young fishes.

The scup used in the following experiments were taken from the waters in and around the Woods Hole Laboratory of the Bureau of Fisheries. In some cases the fishes examined came from the government traps situated in Buzzards Bay in waters which were always coli-negative, and in other cases they came from the traps of the Marine Biological Laboratory situated either in Buzzards Bay or Martha's Vineyard Sound, both of which were free from any bacteriological evidence of fecal pollution, while other fish were caught by hook and line in Laky's Bay, an arm of Martha's Vineyard Sound, south of the Marine Biological Laboratory traps. The depth of the water from which the fishes were taken varied from a few feet near the shore in case of the traps to over 40 feet in Laky's Bay. In general the bottom seemed to be rocky and the waters were always free from the presence of lactose fermenters of intestinal origin (as shown by formation of gas in lactose broth).

The fishes were brought to the laboratory in clean baskets just as soon as possible after they were caught, and the following examination was made. After the scales were removed, the skin was thoroughly washed with 95 per cent alcohol and ignited. An incision was then made through the skin into the peritoneal cavity and the intestinal tract carefully dissected out, using instruments which were sterilized by constantly dipping in 95 per cent alcohol and lighting in the flame. As soon as the intestinal tract was dissected out, its exterior was seared with a hot scalpel at a distance of about two inches from the anus and then an incision was made with sterile scissors and the intestinal content allowed to drop into tubes of lactose broth, which were incubated at 37°C. It was impossible to graduate exactly the amount of fecal material which dropped into the tubes, but generally 5 to 10 grams of the intestinal tract content was used. Tubes which showed a posi-

TABLE

	LOT NUMBER												TOTAL
	1	2	3	4	5	6	7	8	9	10	11	12	
Source.....	B. T.	A.	S. T.	S. T.	B. T.	B. T.	B. T.	B. T.	B. T.	V. S.	V. S.	V. S.	
Date.....	7/20	7/25	7/27	8/2	8/5	8/7	8/21	8/22	8/23	8/24	8/29	8/30	
Number of fish examined.....	10	7	2	8	8	6	5	6	5	8	21	7	93
Gas in lactose broth:													
Positive.....	4	6	2	6	7	4	4	4	4	7	19	6	73 (78.5%)
Negative.....	6	1	0	2	1	2	1	2	1	1	2	1	20 (21.5%)
<i>B. coli</i> group isolated.....	4	6	1	2	4	0	2	2	2	4	7	3	37 (39.8%)
Alone.....	4	5	1	2	4	0	2	2	2	1	1	3	27 (29.0%)
With <i>B. Welchii</i>	*	1	0	0	0	0	0	0	0	3	6	0	10 (10.8%)
<i>B. Welchii</i> group isolated.....	*	1	0	1	2	1	0	1	0	4	16	2	28 (30.1%)
Alone.....	*	0	0	1	2	1	0	1	0	1	10	2	18 (19.3%)
With <i>B. coli</i>	*	1	0	0	0	0	0	0	0	3	6	0	10 (10.8%)
Condition of water.....	0	+	0	0	0	0	0	0	0	0	0	0	
Total positive fish (<i>Welchii</i> or <i>Coli</i>).....	4	6	1	3	6	1	2	3	2	5	17	5	55 (59.2%)
Total negative fish.....	6	1	1	5	2	5	3	3	3	3	4	2	38 (40.8%)

* No examination.

S. T. = Sound trap; A. = Aquarium; B. T. = Bay trap; V. S. = Vineyard Sound; + = *B. coli* present.

tive gas production by the forty-eighth hour were plated out on litmus lactose agar, and transplants were identified as members of the *B. coli* group. A Gram-negative, rounded and aerobic bacillus, producing gas and acid in lactose broth, and no liquefaction of gelatine, was taken as the criterion of membership in the *B. coli* group. All tubes which showed gas were examined for the *B. Welchii* group by the following procedure. One cubic centimeter of the lactose broth tube showing gas production was inoculated into a tube of litmus milk which was then sealed with paraffin. These tubes were heated at a temperature of 80°C. for ten minutes after which they were incubated at 37°C. for forty-eight hours. Vigorous gas production, with the subsequent expulsion of the paraffin plug, acid reaction, "stormy fermentation" and odor of butyric acid were regarded as indicative of the presence of the *B. Welchii* group.

The results obtained by the above bacteriological examination of the intestinal content of the scup for the presence of the *B. coli* group and the *B. Welchii* group may be summarized in the accompanying table.

CONCLUSIONS

The intestinal content of 93 scup (*Stenopus chrysops*) taken from unpolluted waters in and about the Woods Hole Laboratory of the Bureau of Fisheries was examined bacteriologically for the presence of the *B. coli* and the *B. Welchii* group with the following results:

1. The intestinal content of 73 (78.5 per cent) scup examined contained lactose-fermenting organisms.
2. Members of the *B. coli* group were isolated from the intestinal content of 37 (39.8 per cent) of the scup examined, in 27 (29.0 per cent) cases alone and in 10 (10.8 per cent) cases in company with members of the *B. Welchii* group.
3. The members of the *B. Welchii* group were isolated from the intestinal content of 28 (30.1 per cent) of the scup examined, in 18 (19.3 per cent) cases alone and in 10 (10.8 per cent) cases in company with the members of the *B. coli* group.

4. The intestinal content of 55 (59.2 per cent) of the scup examined contained organisms of either the colon or the Welchii type.

5. The presence of bacteria in the intestinal tract of the scup seems to be correlated with the amount and type of food present.

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