SEASONAL CHANGES IN THE NORMAL BACTERIAL FLORA OF FRESH-WATER FISH

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(With 1 Figure in the Text)

INTRODUCTION

The problem of bacterial infection in cold-blooded animals is a complex one. Evidence has been presented that infections of aquatic animals are frequently caused by saprophytic water bacteria (Williamson, 1929; Bisset, 1946), although specific pathogens may also be responsible, as in the case of 'furunculosis' of the Salmonidae (Mackie, Arkwright, Pryce-Tannatt, Mottran, Johnston, Menzies & Martin, 1930). External conditions, especially temperature, appear to influence very greatly the nature of the infection (Bisset, 1947*b*). It has been shown (Bisset, 1946, 1947*a*), that there exists, at low temperatures, a balance between the body defences of cold-blooded vertebrates and the pathogenic powers of their bacterial parasites. At higher temperatures the mutual tolerance is upset by increases in both these factors and disease and death of some of the animals may result, but the survivors usually clear themselves completely of infection. The balance is also affected by the fact that whereas acquired immunity in these animals is very much greater at higher temperatures, natural immunity to new infection is somewhat greater at lower temperatures (Bisset, 1947*c*). Antibody production is especially sensitive to changes in temperature and is inhibited below about 10° C. (Widal & Sicard, 1897; Pliszka, 1939; Bisset, 1947*a*).

The object of the present study was to determine the nature and extent of bacterial infection in fresh-water fish under natural conditions, and the effect upon it of seasonal changes in temperature.

TECHNIQUE

The fish studied were Powan (*Coregonus clupeoides*) from Loch Lomond and Perch (*Perca fluviatilis*) from Lake Windermere, and from Whitacre Reservoir, Warwickshire. The fish were examined bacteriologically immediately after being caught, and in most cases had not been dead for more than a few minutes. It is therefore assumed that the bacteria isolated from them represented the flora of the fish during life. The perch were trapped in wire traps, on the lobster-pot principle; the powan were netted. The fish were not in either case subjected to gross damage or mishandling.

Cultures were made upon Lemco agar and were incubated at 20° C. aerobically. As sterilization of instruments was impracticable, where large numbers of fish were examined under field conditions, the following technique was adopted for taking inocula from the peritoneal cavity. A transverse incision was made in the abdominal wall near the posterior end of the cavity. The opening was held apart with forceps, and the inoculating wire was passed forward into the cavity, so as to avoid touching the sides of the incision, or the part of the cavity which might have been liable to contamination through the opening. The wire was, of course, sterilized before and after each culture was made.

POWAN (LOCH LOMOND)

The Powan, which is an aberrant member of the Salmonidae, is fished commercially in Loch Lomond during the summer months. Samples of about 100 fish were taken on three occasions in 1947, in the third week in July, the second week in August, and the second week in September. Cultures were made from the peritoneal cavity and the opercular cavity. The bacteria isolated from the latter being assumed to represent the external flora of the fish, uncontaminated by handling.

After incubation for 48 hr. at 20° C. the cultures were classified as profuse, if consisting of numerous colonies or confluent growth; slight, where the number of colonies was small; and nil, if there was no growth. Cultures from the opercular cavity were invariably profuse. The results of cultures from the peritoneal cavity were as shown in Table 1.

Table 1

Date	Water temp. (° C.)	No. of fish	Cultures from peritoneal cavity		
			Profuse	Slight	Nil
July	14·0	118	91	23	4
August	16·5	99	50	36	13
September	18·0	83	41	28	14

Thus the degree of infection showed a steady decrease throughout the warm season.

In any batch of fish the bacteria isolated from the peritoneal cavity were morphologically and culturally identical with those from the opercular cavity. A batch consisted of the fish taken in a particular cast of the net, of which five or six were represented in each monthly sample. There was some variation between the batches. In the July cultures an easily recognized Gram-negative cocco-bacillus, producing a canary yellow colony, may be taken as an example. This organism occurred in all the cultures from both gill and peritoneal cavity in two batches, in three-quarters of the cultures from both sites in one other batch, and was almost completely absent from the remaining two batches. Each batch, or cast of the net, may reasonably be taken to represent a portion of a single shoal, feeding in one place.

The species of bacteria in each monthly sample were very much the same, differing only in the proportions in which they occurred. They resembled common species of water bacteria; chromogenic organisms were common, and both Gram-positive and Gram-negative forms were represented.

PERCH (LAKE WINDERMERE)*

The technique of examination of these fish differed from that described above, because a large proportion of them were alive when examined. A drop of peritoneal fluid was aspirated from the cavity with a sterile syringe, and cultivated upon agar. Cultures from the gill were made in the usual manner. Samples, each of about 120 fish, were taken at the beginning and end of May 1947. The average temperature of the lake for the week preceding the first sample was 5·6° C. Preceding the second sample it rose from 14 to 17° C. during the week. Cultures were made from the opercular cavity and peritoneal cavity, and from the gut contents in twenty-five cases. All cultures from the opercular cavity and gut were profuse, in both samples. The results of cultures from the peritoneal cavity are as shown in Table 2.

Table 2

Date	Water temp. (° C.)	No. of fish	Cultures from peritoneal cavity: Profuse	Slight	Nil
28 April	5·8	118	46	44	28
28 May	17·0	120	19	56	45

The considerable increase in water temperature over this short period was thus paralleled by a marked decrease in the degree of bacterial infection of the fish. The bacteria were of about a dozen different species, and were the same whether derived from the peritoneal cavity, gill or gut content.

* A preliminary note upon some of these observations has been included in another paper: Bisset, 1948.

Dr C. B. Taylor, who kindly examined a representative selection of these organisms, expressed the opinion that they were water bacteria of types commonly occurring in Lake Windermere. All were Gram-negative bacilli and pigmented and fluorescent species were common.

It was noticeable that in the second sample several bacterial species were absent which had been conspicuously present in the first sample. This was very obvious in the case of an organism producing a dark indigo pigment, which was to be found in almost every culture from the first sample, and in none from the second. The majority, however, of bacterial types were present in both samples.

PERCH (WHITACRE RESERVOIR)

Weekly samples, varying in number from 20 to 60 fish, were trapped in this water from the second half of July to the end of October 1947. The water was filtered and was consequently of a much lower bacterial content than the natural waters. This was reflected in the degree of infection in both the peritoneal and opercular cavities of these fish, which was much less than in the foregoing examples. Cultures from the gut contents were invariably profuse. The species of bacteria cultured from the various sites were the same. Only three or four species were present, all of which were Gram-negative bacilli; the usual chromogenic and fluorescent bacteria were absent from the fish and also from the water.

Water temperatures were high throughout the summer months, ranging from 18 to 20° C. By the end of September the temperature had fallen to 15° C. and by the end of October to 11° C. The percentage of fish in each sample showing profuse, slight, and nil cultures from both opercular and peritoneal cavities, is shown in Table 3. The proportion of negative cultures is shown in Fig. 1.

It can be seen that the degree of infection fell rapidly from July to early September, and thereafter rose again as the temperature fell. The opercular cavity was always more heavily infected than the peritoneal cavity, but unlike the preceding examples, it gave more than 10 % of sterile cultures throughout August and September. Cultures were taken from the muscles of occasional, small numbers of fish. These showed a slight degree of infection with the same bacteria in about 20 % of the fish examined.

DISCUSSION

These observations indicate that considerable seasonal changes occur in the bacterial flora of fresh-water fish. High temperatures encourage the development of immunity in these animals, and enable them to free themselves progressively of

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Table 3

Date	Water temperature (° C.)	No. of fish	Cultures from peritoneal cavity: Profuse	Slight	Nil	Cultures from opercular cavity: Profuse	Slight	Nil
22. vii. 47	19·0	34	68	26	6	88	12	0
29. vii. 47	21·0	67	48	25	27	42	52	6
6. viii. 47	18·5	47	21	36	43	36	51	13
19. viii. 47	20·0	46	13	41	46	39	39	22
26. viii. 47	20·0	43	14	11	75	47	40	14
2. ix. 47	19·5	21	5	0	95	9	58	33
9. ix. 47	18·5	56	22	19	59	14	39	47
16. ix. 47	18·0	37	24	30	46	27	43	30
23. ix. 47	15·5	47	15	15	70	45	38	17
7. x. 47	12·0	34	30	47	23	76	18	6
14. x. 47	12·0	34	18	53	29	70	24	6
22. x. 47	11·0	24	13	50	37	29	67	4

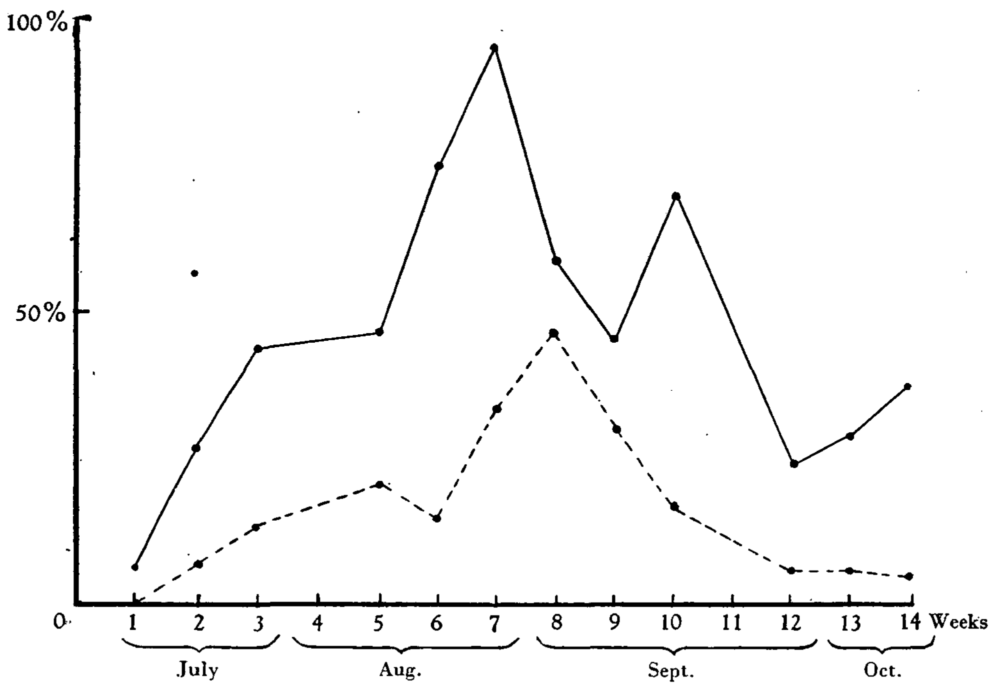

Fig. 1. Perch from Whitacre Reservoir. The full line shows the percentage giving sterile cultures from the peritoneal cavity, and the broken line the percentage of sterile cultures from the opercular cavity.

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infection, exactly as has been shown to occur under laboratory conditions (Bisset, 1946, 1947*a*). When the rise in temperature is large and rapid the reduction in infection may be correspondingly rapid; but even when the increase is small, continued warm conditions induce a gradual increase in immunity and a reduction in infection. When the temperature falls infection at once increases, indicating that the recently acquired immunity is lost or greatly reduced. This has been shown to occur, under experimental conditions, with amphibia (Bisset, 1948).

The similarity of the bacterial species isolated from various sites, inside and outside the body of the fish, and the apparent identity of these, where they can be identified, with bacteria from the same water, confirms previous observations that water bacteria which are usually saprophytic may parasitize aquatic animals. It is especially interesting to observe the small number of bacterial species isolated, and the low degree of infection of the opercular cavity of Perch living in filtered water; whereas the same species of fish, from the unpurified water of Lake Windermere, gave uniformly profuse cultures from this site, even when the proportion of sterile cultures from the peritoneal cavity had risen to almost half of the total number of fish in the sample.

SUMMARY

1. Bacteriological examination of large samples of Perch and Powan from natural and artificial waters has shown that a considerable decline in the normal bacterial flora of the fish occurs during the summer months. Reinfection occurs when the temperature falls.

2. These variations are largely attributable to the effect of temperature upon the immunological responses of the fish.

3. The bacteria concerned appear to be water bacteria, which invade the muscles and body cavity of the fish, as well as the gill and gut-content.

4. The degree of infection is also influenced by the bacterial content of the water.

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