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AN EPIDEMIC DISEASE AFFECTING SALMON AND TROUT IN ENGLAND DURING THE SUMMER OF 1911[1].

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EPIZOOTICS of various Fish-diseases due to bacteria have been described by several observers, but most of these have affected coarse fish only, such as carp, tench and perch and the bacilli, which have been described as the causative organisms, have been quite different in their characters from those constantly found in the epizootic now under consideration.

Patterson (1898) described *Bacillus salmonis pestis* and showed that this bacillus was the essential microorganism present in "Salmon-disease," an infective disease occurring in the late Autumn and Winter months. Epidemics of the disease known as 'Furunculosis of the Salmonidae' have been observed in many parts of Europe, but the occurrence of this disease in the British Isles has not been hitherto recorded.

Previous observations by other writers.

Emmerich and Weibel (1894) published a detailed account of Furunculosis of the Salmonidae and described *Bacillus salmonicida* as the cause of the disease. They recorded an epidemic occurring in the Autumn (October) of 1888 in the tanks of a fish culture establishment. *Trutta fario* (trout) and *Salmo fontinalis* were attacked, but Rainbow-trout escaped. The disease was characterised by congestion of the

[1] This paper originally appeared as a Report (Arkwright, 1912) to the Board of Agriculture and Fisheries by whose permission it is now republished in a slightly altered form.

intestine, especially the mid and hind gut, and in many instances by haemorrhages in the muscles followed by patches of softening and ulceration with discharge of "pus" through an opening in the skin. Discharging sinuses often remained for some weeks. The affected fish usually died after one or two weeks, but occasionally recovered. *B. salmonicida* was isolated, often in pure culture, from the blood, the muscular haemorrhages and internal organs. This organism they described as a small non-motile Gram-negative bacillus which grew slowly on artificial media at the temperature of the laboratory. The optimum temperature for growth was between 10° C. and 15° C., but slight growth occurred almost down to freezing point. No growth occurred at 37° C. and the bacilli were rapidly killed at 60° C.

On gelatin the growth was slow and only very small colonies appeared during the first two or three days. Later, liquefaction of the gelatin with gas production occurred. They considered the appearance of stab gelatin cultures as characteristic. The growth on agar was whitish-grey at first and later became yellowish. After some weeks however the growth became brownish and the upper part of the agar in the culture tube also became brown. They claimed to have shown that the disease could be communicated to healthy fish by artificially contaminating the water of tanks with pure cultures. Marsh (1903) in the United States of America described an organism—*Bacterium truttae*—found by him in fish which had died of an epizootic disease occurring amongst trout in captivity. He does not mention the symptoms of the disease nor the pathological changes found. His description of *B. truttae* agrees very well with the characters of the bacillus isolated from fish which died during the English epidemic in 1911, and in particular, he mentioned the production of a diffusing brown pigment in the cultures.

Hofer (1904) in describing Furunculosis of the Salmonidae stated that he had repeatedly confirmed Emmerich and Weibel's observations on the disease and on *B. salmonicida*. He says that infected fish are not harmful when used as human food. Plehn (1909) in a short note stated that the *B. salmonicida* isolated from diseased fish examined during a recent epidemic corresponded to the original description of the bacillus, but also mentioned that the gelatin in cultures became slightly brown in ten days and "coffee-brown" in three weeks, also that broth cultures developed a brown colour though rather more slowly. On agar her strain made very scanty growth and showed involution forms; no mention is made of pigment formation. Plehn (1911) described

more fully the epizootic of 1909. Between 1894, the date of Emmerich and Weibel's paper, and 1909, fish from different parts of Germany which had died from Furunculosis were yearly examined at Munich and were found to be infected with *B. salmonicida.* These diseased fish were Trout and "Bach-saibling" but never Rainbow-trout. All the diseased fish were reported from fish-culture establishments and none from open water.

In 1909 and 1910 large numbers of fish died and many diseased fish were obtained from open streams and rivers in widely separated parts of Germany. Twenty-five rivers and streams were affected in Bavaria alone in 1909. Many grayling were attacked and a salmon from the Rhine was found to be suffering from this disease. Some tench in a tank were infected by diseased trout living in the same water, and a pike from an open stream was found to be infected. The haemorrhages and "abscesses" appeared to be less common than in former epidemics, and many of the fish had no lesions except congestion of the intestine. The tench had no macroscopic appearances of disease but the pike showed ulcers and abscesses. "Furunculosis" was also reported from Switzerland and France. The strains of *B. salmonicida* isolated by Plehn during these two years showed some characters on culture which differed from those of the bacillus isolated in former epidemics. Plehn calls this new strain *B. salmonicida-B.* The stab cultures in gelatin of this recent strain were less characteristic and the track of air bubbles along the line of liquefaction was less marked and less constant. A very characteristic brown colour appeared in the gelatin and broth cultures after the second week. This pigment was most marked near the surface of the culture. Pigment formation on agar was later and much less pronounced. A very slight acid reaction of the medium was found to inhibit growth markedly and an even smaller amount of acidity prevented the formation of pigment.

The bacteria were killed at 40° C. and growth was very much weakened at 30° C.

Trout were experimentally infected by feeding and by adding broth culture to the water, but all trout exposed to infection in this way did not succumb. Some yearling trout which had escaped infection by feeding were, six weeks later, put with healthy trout. Some of these latter became ill and died, and were found to be infected. The healthy trout which had introduced the infection were then killed and were also found to be infected. One trout which was exposed to infection by water containing culture, became ill and recovered. The water in

which it was kept was then raised in temperature from 8° C. to 15° C. with the result that this fish developed septicaemia with *B. salmonicida* and died. These latter experiments, if they were sufficiently controlled, show that apparently healthy trout can be carriers of *B. salmonicida* and can infect healthy fish.

The English Epidemic during 1911.

For the following Epidemiological facts I am indebted to Dr A. T. Masterman's report to the Board of Agriculture and Fisheries (1912). During the summer of 1911 there was a high mortality amongst fresh water fish in England. The Exe, the Dart, the Teign and the Wye were principally affected, and in these four rivers in the South-west, large numbers of Salmon and Trout died between the 12th of May and the end of July. Dead fish were not reported from the Exe after the 10th of July. The summer was unusually hot and dry and the general improvement at the end of July roughly coincided with some storms of rain and a temporary diminution of the drought, at any rate in some areas. During the epizootic large numbers of fish were found dead. In the Exe before the 16th of June 225 salmon and 718 trout were reported and during three days in June 521 dead trout were taken out of the river. Over 200 dead salmon were reported from the Wye between the 25th day of May and the 30th of July. The numbers must have been in reality larger as no doubt many dead fish were never reported. In the Wye besides salmon and trout, many grayling, eels, pike, chub and dace were found dead. For a more detailed account of the Epidemic the reader is referred to Dr Masterman's report.

Examination of dead fish.

The diseased fish, which were examined, were sent to the Lister Institute through the Board of Agriculture and Fisheries. They were received during the months of May to September, 1911, but the greater number came in during June. They were taken out of four rivers or their tributaries, the Exe, the Wye, the Teign and the Dart. The diseased fish were removed from the river either dead or in a dying condition, and were sent to London in a box or basket without ice, except in the case of six fish (Trout 2 to 7) which were packed in ice. The fish were generally received on the day after they were despatched, and usually, but not always, appeared to be fairly fresh

externally. The liver and spleen were, however, frequently diffluent in the case of diseased fish.

Besides the diseased fish which were sent because they were found dead or dying in the course of the epidemic, fish were also examined from other sources and served as controls.

In all, 50 fish were examined. Of these 14 were salmon[1], 34 were trout, one was a grayling, and one a bream. Thirteen of the salmon were sent because they were considered to be suffering from the epidemic disease, whilst the remaining salmon was caught alive in a net in the Wye and was not obviously diseased though it had lost some scales.

Of the 34 trout examined *post-mortem* 11 had been kept alive in the laboratory for varying lengths of time and attempts had been made to infect most of them. These are called "experimental trout" in the tables.

Of the remaining 23 trout, seven were taken dead or dying from the Exe and were supposed to be affected by the epidemic disease. Seven were sent from a breeding-station, having died in the midst of apparent health immediately after transference from one tank to another. Eight were healthy trout taken by rod in the North of Scotland, and one was a healthy trout taken by rod in the Dart in July during the epidemic.

Seven of the 11 experimental trout were sent from the same breeding-station as the seven trout mentioned above which died very rapidly without sufficient apparent reason.

For bacteriological reasons which are given below, this breeding-station cannot be considered above suspicion as regards freedom from the epidemic disease. The experiments by which it was attempted to infect trout with the disease in the laboratory were therefore less satisfactory than was desirable on account of the doubt as to how many of the fish used were infected before the experiment began.

The first four fish which were received (S. 1, S. 2, S. 3 and T. 1) were examined by Dr G. H. K. Macalister, who isolated from them the pigment-forming bacillus which will be described later.

[1] Dr Masterman informs me that some of these (*e.g.*, two from the River Teign on 12th June) were certainly sea-trout (or peal), though they were thought to be salmon when received in London.

Method of Examination.

The external naked-eye appearances of the surface of the body and of the internal organs were noted, and bacteriological cultures were made on ordinary neutral or slightly alkaline nutrient agar in Petri dishes. In some cases a special fish-broth agar was used, but it did not appear to have sufficient advantages to encourage its further use. Occasionally neutral-red-lactose-bile-salt agar was used, especially for plating the intestinal contents. The cultures were made from the heart-blood or vena cava-blood, from the liver, spleen, peritoneal fluid, intestinal contents, and mucus exuding from the vent. They were kept at the temperature of the laboratory, which varied from 10° to 22° C. The muscular tissue and any haemorrhages or softened patches discovered in the muscles were also used for inoculating Petri dishes. (In making cultures special care was taken by use of a searing iron to avoid contamination by the skin or other parts of the fish.)

Films for microscopical examination were made from the blood, liver, and spleen, and also from patches of muscular softening. The skin was stripped off, and the interior of the muscles was examined by means of numerous cross-sections. Portions of diseased organs and muscles were hardened and sections examined microscopically.

The examination of the subcutaneous tissues and muscles was not made so completely in the case of the fish examined at the beginning of the investigation as, at that time, its importance was not recognised.

Results of Examinations.

Naked-eye appearances.

Subcutaneous and muscular tissues.—Several of the diseased salmon presented abscess-like cavities under the skin, sometimes of large extent and full of opaque reddish liquid of the consistency of thin human pus. This discharge was often seen to be exuding from one or more small openings in the skin, or the cavity was closed. Some of these cavities extended deeply, laying bare the bones of the fins, and even the ribs, and in some cases the liquid matter had penetrated into the abdomen or thorax between the ribs, a secondary cavity being formed dorsal to the swim-bladder. In addition to these large cavities smaller dark foci were seen which were softened in the centre. These red spots in the muscles were of various sizes, some being as

small as a grain of wheat or a pin's head; when very small the spots presented no obvious softening. In no case were the spots and cavities very numerous, five or six being the largest number found in any one fish. The spots of softening and dark red areas closely resembled those described and figured as occurring in the disease known as Furunculosis of the Salmonidae (Emmerich and Weibel, 1894, and Hofer, 1904). The reddish-coloured foci and cavities were met with in any parts of the muscular or subcutaneous tissues, and sometimes they were observed on the head. They were, perhaps, most frequent in the neighbourhood of the lateral line, where the softening and pus-like liquid extended backwards and forwards in the subcutaneous and intermuscular tissues along the lateral line.

No cavities were found in the internal organs, but the liver was often unevenly patchy in colour and sometimes in part softened. The spleen was frequently diffluent and unrecognisable except as a dark red mass with no obvious attachments, looking like a mass of blood clot.

The intestines were frequently congested, but the congestion was not always most intense in the same region. The blood-vessels of the lower part of the gut appeared to be most frequently engorged. Occasionally the pylorus and pyloric caeca were deeply congested. The lower intestine of healthy trout was also sometimes the seat of congestion. As a rule, in the case of diseased fish, blood-stained mucus escaped from the vent when slight pressure was applied to the sides of the abdomen, but this was not quite constant, and occasionally occurred in healthy trout.

Intestinal parasites were not very numerous, but the species of worm which are commonly found in these fish occurred in small numbers. The interior of the swim-bladder areas usually appeared natural, but occasionally one or two nematodes were found.

The gills of the trout appeared healthy; those of the salmon were generally infected with Copepods (*Lernaepoda salmonis*).

Microscopic Pathology.

Films of the puriform matter from the "abscess-like" cavities showed very few cells or nuclei. Bacteria were very numerous and of various forms, chiefly very short oval or oblong Gram-negative bacilli, but longer bacilli were also seen. Films of the heart-blood and from the liver and spleen also showed numerous short bacilli of the same appearance and generally of very uniform size.

Sections of liver, kidney and diseased muscle all presented the same features, viz., patchy necrosis, and disintegration of the tissue cells, no increase in the leucocytes or connective tissue cells, whilst the blood vessels and capillaries were crowded with round or short oblong Gram-negative bacilli of uniform shape and about 1×1 or $1 \times 1{\cdot}5\,\mu$ in size. Sometimes the red corpuscles in the blood vessels in these diseased areas had almost all disappeared or were represented only by their nuclei.

Bacilli of the same type were also seen in great numbers in the connective tissue spaces in the neighbourhood of the diseased areas, but were absent elsewhere. In the liver similar oblong bacilli were seen in dense clumps of the size of two or three liver cells. Usually but not always, these clumps lay touching the wall of a blood vessel or capillary. In some places bacilli were also fairly numerous in and around the walls of small blood vessels.

Bacteriological Results.

From the diseased fish an easily recognised bacillus possessing well defined characters was obtained constantly and often in almost pure culture from the blood, liver, spleen and peritoneal fluid, and also from the deep red spots in the muscles. The most obvious characteristic of this bacillus was the production of a deep brown diffusible pigment on agar at the temperature of the laboratory. For the present, therefore, this bacillus will be referred to as the "pigment-producing bacillus."

As a rule, other bacteria were very few in cultures from the heart-blood, liver and peritoneal fluid. Those most frequently met with were cocci growing in white, yellow or red colonies. Sometimes also colonies of other bacilli, but in much smaller numbers than those of the pigment-forming bacillus.

On the other hand cultures from the pus of discharging abscesses, the mucus from the vent and the intestinal contents, contained a considerable variety of bacilli. A few colonies of these bacilli were isolated and they proved to be gelatin-liquefying organisms, some of which corresponded in their cultural characters to *B. fluorescens liquefaciens*, and others probably to the *Proteus* group. The latter, however, unlike *B. Proteus vulgaris* fermented mannite with production of gas.

Cultures from the blood and liver of healthy fish yielded as a rule only a few opaque colonies of cocci and moulds, or were sterile.

Two of the healthy control trout which were received dead from the North of Scotland were put in a bowl of tap water in the laboratory two days before they were examined, in order to compare the cultures which resulted with those obtained from other fish which did not arrive in a fresh condition. The organs yielded many more colonies than those of the trout from the same source which were examined at once. All the colonies were apparently of the *proteus* or *fluorescens* type of bacteria, and bacilli producing brown pigment were not found.

THE PIGMENT-FORMING BACILLUS.

Facts as to its occurrence.

This pigment-forming bacillus was found in all the diseased fish except the bream, *i.e.*, in 13 salmon, seven trout and one grayling. It was not found in any of the eight healthy trout from the north of Scotland, nor in the one healthy Dart trout, nor in six out of the seven dead control trout sent from the breeding-station. This bacillus was however found in one (Trout 9) of these seven trout from a fish-culture tank, but in this case only in very small numbers, three colonies appearing on the agar plate inoculated with heart-blood.

Of the 11 fish which were received alive at the laboratory, four which were bought in London gave negative results *post-mortem* as regards this bacillus. The remaining seven fish were none of them quite satisfactory for experimental purposes as some of them were probably infected before they were received. Attempts were made to infect four of them with material known to contain the bacillus. One of the seven fish died soon after arrival without having been infected in the laboratory, and yielded cultures of the bacillus. The remaining two also gave cultures of the bacillus after having been fed with part of a dead trout (Trout 17) from which, however, the bacillus was not isolated, although an attempt had been made to infect it with a pure culture.

However, of these seven fish which were received alive and which all yielded *post-mortem* cultures of the bacillus, only one (Trout 19) was certainly not infected in the laboratory. These seven fish came from the same breeding-station from which was sent Trout 9, which also was found to contain the bacillus, but in small numbers.

This pigment-forming bacillus occurred with such regularity, and, as a rule, in such preponderant numbers in the cultures from the

blood, &c., of the *diseased* fish that its characters will now be described at greater length.

It was no doubt identical with *B. salmonicida* Emmerich and Weibel and *B. truttae* Marsh, but at the time of the investigation the modified description of the former of these bacilli by Plehn (1911) had not been published and *B. truttae* Marsh was only reported from a culture tank in America and no description of the disease in which it occurred was available.

Characters of the Pigment-forming Bacillus.

The pigment-forming bacillus which was found with such regularity in the fish which died during the epidemic under consideration shewed the following characters.

Morphology.

In young cultures it was a small Gram-negative bacterium either round, oval or oblong, the long axis being equal to or at most twice as long as the short axis. In older cultures, however, and especially in broth cultures, a few long bacilli occurred which were four or five times as long as they were broad. The diameter of the coccal forms was about 1 μ or rather less.

No true motility was observed though the bacillus was examined at many different stages of growth.

When stained with Loeffler's blue or Carbol-thionin blue or with Leishman's stain, bacillary forms shewed a tendency to bipolar staining.

Cultural Characters.

Growth did not occur or was extremely scanty at 36° C. and agar cultures died at this temperature in two or three days. The optimum temperature for growth appeared to be about 18° to 20° C., but growth did not altogether cease at 5° C.

Ordinary nutrient agar which was slightly alkaline to litmus, gelatin, peptone beef-broth and peptone water all proved good culture media. An agar medium made with fish-broth was also used and gave a more vigorous growth than ordinary agar.

Fairly good growth took place on 0·5 % bile salt agar.

The first cultures direct from the fish were made on ordinary agar, and were grown at the temperature of the laboratory. In some cases the colonies were hardly visible in the first 48 hours. As a rule very small transparent colonies appeared after 24 hours, and in another day

or two they had become opaque and about 1 mm. in diameter. The surface of the colonies was moderately shiny and the shape slightly raised and flattened. After two to four days, if the culture was fairly pure and the colonies numerous, the characteristic pigment began to appear. It was of a deep brownish colour and diffused into the agar which remained transparent. In the course of the next week or two the colour deepened till the agar resembled strong black coffee in colour. The time of appearance of the pigment varied considerably and no doubt was very much dependent on the reaction of the medium and perhaps on other peculiarities in its composition. On one occasion when only three colonies of the bacillus were present with a few colonies of other bacteria, no pigment was seen for seven days. When colonies of other bacteria were numerous (*e.g.*, bacilli of the same group as *B. proteus vulgaris* or *B. fluorescens liquefaciens*) the brown pigment often did not appear at all though the pigment producing bacteria were present in considerable numbers. This occurred usually in the case of plates spread thickly with material from the discharging abscesses. The first plate spread would shew no brown pigment, but the second plate spread with the same rod and which contained fewer colonies shewed the brown pigment round some of the colonies.

On the other hand sometimes plates which were crowded with almost pure cultures of the pigment-forming bacillus remained without pigment for five days or more, and then the pigment appeared first round a colony of a coccus or a mould and gradually spread thence and coloured the whole plate. This appearance was probably due to the production of alkali by the contaminating colony.

Subcultures on agar generally shewed good confluent growth in 24 to 48 hours, and pigment in three to four days.

In seven to ten days the colonies themselves as a rule became a uniform pale brownish or buff colour, but occasionally they shewed a deeper brown area in the centre. The time of appearance of the diffusing pigment depended very much on the initial reaction of the agar. The pigment was produced most readily on agar with a reaction of -4 to $+8$ acid to phenolphthalein (Eyre's scale) and it did not appear when the reaction was -6 or $+16$ in one experiment made to test this point. Growth however still took place in broth of -6 and $+16$, but not of -8 nor at $+20$ acidity.

In peptone beef broth the pigment appeared very slowly, and the degree of acidity at which pigment was produced varied within narrower limits.

In peptone water (Witte 2 per cent.) the pigment was produced more readily than in broth, but it did not appear if the reaction was + 20, though pigment was noticed after a week when the initial reaction was + 16.

The pigment was soluble in water, but not in absolute alcohol, chloroform or ether. If absolute alcohol was added to an agar slope some of the pigment diffused out into the alcohol, but the pigment obtained dry from a watery solution was quite insoluble in absolute alcohol.

Indole was not produced in broth cultures.

On gelatin the bacillus grew fairly well, liquefaction usually commencing within the first 24 or 48 hours. Pigment was not usually formed in gelatin cultures, though with an initial acidity of + 15 (Eyre's scale) a slight brown colour was obtained in a week. Gelatin stab cultures shewed a funnel-shaped tract of liquefaction along a course of the needle, and some bubbles of gas were also formed in most cases. On potato the growth was very slight. Cultures made on solidified horse serum grew readily, while the medium became a deep brown colour and was slowly liquefied in 7 to 14 days.

Carbohydrate reactions.

All the strains of the pigment-forming bacillus which were isolated gave identical results when grown on certain carbohydrates in one per cent. peptone water coloured with litmus. The carbohydrates used were glucose, cane-sugar, lactose, mannite and dulcite, and they were added in a proportion of one per cent. to the peptone water. The cane-sugar, dulcite and lactose tubes became alkaline, but in about a week some pigment was produced in these tubes, especially in that containing dulcite, where the blue colour of the medium changed to a reddish tint, though the liquid was still distinctly alkaline to litmus paper. In the glucose and mannite tubes acid and gas were produced in 24 to 48 hours. The amount of gas produced in the case of the glucose was much less than that formed in the mannite tube, as judged by the height of the column of gas in the "Durham's tube." In some rare instances the amount of gas formed in the glucose tube was so slight as to be barely recognisable, none appearing in the small gas tube even after a week's growth. It often happened that no gas was visible in the glucose tube after 24 hours' growth though the acid production was quite distinct.

A variant of the bacillus was found in a colony picked off an agar

plate containing chloracetic acid 0·5 %. This variant produced acid without gas from glucose and mannite, in this respect somewhat resembling the variant forms of *B. coli* etc. produced on this medium by Penfold (1911). It is also of interest because it formed very little pigment, like *B. salmonicida* of Emmerich and Weibel and *B. salmonicida-A.* of Plehn. The growth of this variant strain was less vigorous than that of the parent strain.

Viability.

As has been stated already growth continues to go on slowly at 5° C. and sometimes for a short time to a very slight extent at 35° or 36° C. At 37° C. the culture soon dies. Cultures remain alive at 1° C. for at least four months.

From sterile tap water the bacilli could be recovered alive, in slightly diminished numbers, after five days. The experiment was made by emulsifying an agar culture in 100 c.c. of tap water and leaving the flask at room temperature. Two or three drops were removed and spread over the surface of a solid agar plate at varying intervals. An agar culture was also emulsified in the same way in 100 c.c. of sterilised *sea water*. In this case the bacilli rapidly died. After two hours the number of colonies which grew from a drop taken from the flask containing sea water was reduced from several hundred at the beginning of the experiment to 80, and after 19 hours no growth occurred. From a mixture of sea water and tap water in equal parts none of the bacilli could be recovered after 19 hours, but from a mixture containing sea water 25 per cent. and tap water 75 per cent. a fair growth was obtained after 45 hours, but not after 67 hours. Salt water (3 per cent. sodium chloride in distilled water) also had a destructive effect on the bacilli. The growth from one agar slope was emulsified in 500 c.c., of this 3 per cent. salt water. One drop taken after two days gave only one colony on an agar plate. On the other hand a flask containing an agar culture in 500 c.c. of tap water, when examined after two days, yielded unaccountable colonies from a drop plated in the same way.

Agglutination.

Emulsions of this bacillus made with normal salt solution (0·85 per cent.) very soon shewed complete clumping of the bacilli, and the same thing occurred if 0·2 per cent. salt solution was used. An emulsion in distilled water remained unclumped for 24 hours, and occasionally an

emulsion from a culture on agar of acidity + 20 (Eyre's scale) remained uniform in 0·85 per cent. salt solution.

Some agglutination experiments were made with emulsions in distilled water, but no distinct agglutination was obtained with the serum of diseased fish in higher dilution than 1 in 10.

Pathogenicity.

The experiments were not conclusive as the supply of healthy living trout or salmon available for experiment was not sufficient for the purpose.

The following experiments were, however, performed:—

Altogether 11 trout were kept alive in the laboratory and examined after death. Of these, four healthy trout were bought in London, and after they had lived three or four days in running water in the laboratory they were examined. These trout are numbered 15 to 18 inclusive.

T. 15.	Control ...	21st June. Killed.
	Post-mortem ...	Pigment-forming bacillus not found.
T. 16.	Control ...	21st June. Killed.
	Post-mortem ...	Pigment-forming bacillus not found.
T. 17.	19th June ...	Attempted infection by feeding and the addition of half an agar culture to the bowl.
	21st June ...	Died.
	Post-mortem ...	Pigment-forming bacillus not found.
T. 18.	19th June ...	Attempted infection by the same means.
	21st June ...	Died.
	Post-mortem ...	Pigment-forming bacillus not found.

Seven trout were received alive from a breeding station. These are numbered 19 to 23 inclusive, 32 and 33.

T. 19.	Control ...	No attempt at infection.
	23rd June ...	Died.
24th June.	*Post-mortem* ...	Almost pure culture of the pigment-forming bacillus obtained from the blood and liver.
T. 20.	21st June ...	Side was scratched and the scratch inoculated with a pure culture.
	23rd June ...	Died.
24th June.	*Post-mortem* ...	Much growth of the pigment-forming bacillus obtained from blood, liver and spleen.
T. 21.	21st June ...	Fed with liver of T. 17.
	24th June ...	Died.
24th June.	*Post-mortem* ...	Almost pure cultures of the pigment-forming bacillus.

T. 22.	21st June ...	Fed with liver of T. 17.
	26th June ...	Died.
26th June.	*Post-mortem* ...	Almost pure culture of the pigment-forming bacillus from blood.
T. 23.	21st June ...	Scratch on side and pure culture put in water.
	25th June ...	Died.
	Post-mortem ...	Pigment-forming bacillus cultivated from blood.
T. 32.	5th July ...	Attempted infection by feeding with pure culture.
	14th July ...	Attempted infection by adding pure culture to water.
	15th July ...	Attempted infection by adding pure culture to water. Fish remained well.
	27th Sept. ...	Scratch on side and pure culture rubbed in.
	30th Sept. ...	Died.
	Post-mortem ...	Local redness of skin and sloughy softening of tissues around wound. Many colonies of pigment-producing bacillus from heart-blood.
T. 33.	19th June ..	Attempted infection by adding pure culture to water.
	5th July ...	Attempted infection by adding pure culture to water.
	14th July ...	Attempted infection by adding pure culture to water.
	15th July ...	Fish died from an accident.
	Post-mortem ...	Pigment-forming bacillus found in blood, &c.

Five gold-fish were used experimentally. Two (Gf. 5 & 6) were inoculated with 0·25 c.c. of broth culture intraperitoneally. They both died in about 24 hours and the bacillus was recovered from the blood.

Gf. 1.	13th June ...	Received half an agar slope of a pure culture in water.
	5th July ...	Scratch on side and pure culture rubbed into wound.
	10th July ...	Injured region of skin has lost its scales and has a surrounding area of redness. Recovery.
	10th October...	0·2 c.c. of an emulsion of a pure culture from Trout 7 (one loop emulsified in 1 c.c. of water) injected into muscles near dorsum.
	12th October...	Swelling, desquamation and redness around.
	14th October...	Killed.
	Post-mortem ...	Pigment-forming bacillus found in spleen and muscles.
Gf. 2.	13th June ...	Attempt to infect by placing a piece of intestine of Salmon 4 into water.
	30th June ...	Attempt to infect by putting pure culture in water. Remained well.
	10th October...	Intramuscular injection of 0·1 c.c. of the same emulsion as that given to Gf. 1.
	12th October...	Swelling.
	14th October...	Swelling increasing, blood red ring on skin around swelling.
	18th October...	"Abscess" discharging. Died (8 days after injection).
	Post-mortem ...	Large cavity extending deeply amongst muscles. Pigment-forming bacillus found in "abscess" and spleen.
Gf. 3.	Attempt to infect by keeping in same bowl as Gf. 2 which had a discharging "abscess." Remained healthy for over three weeks.	
	10th November	Killed. Pigment-forming bacillus not found.

From these imperfect inoculation experiments it appears that it is possible to infect trout by scratching the side and rubbing in pure culture, and gold-fish by subcutaneous or intramuscular injection of small quantities of culture. In both cases cavities full of puriform matter are produced locally as in the natural disease.

Evidence of Pathogenicity.

In the absence of further attempts to produce the disease with pure cultures it is impossible to affirm, as far as this investigation is concerned, that the pigment-producing bacillus is the cause of the disease, but there is presumptive evidence that this is the case.

(1) The pigment-producing bacillus was present in 21 out of 22 diseased fish examined, *i.e.*, in 13 salmon, seven trout, and one grayling, but was not found in one bream sent up for examination.

(2) It was present in large numbers, and often almost in pure culture, in the blood from the heart or vena cava, and in the liver and spleen of these 21 diseased fish.

(3) It was absent from 13 healthy control trout and one control salmon. The salmon as well as one of the trout came from an infected river.

(4) From only one source, viz., a trout-breeding station, were fish which were sent as healthy controls found to be infected. Out of seven control fish which were sent dead from this source, only one yielded a very few colonies.

The seven control fish which were sent alive all eventually gave cultures of the pigment-forming bacillus, but only one of these certainly, and two others probably, had not been infected in the laboratory.

Therefore of ten fish from this doubtful source, which, however, were regarded as healthy controls, four yielded cultures of the bacillus.

(5) To sum up the results as regards healthy controls, out of 23 fish which were regarded as healthy controls, only four yielded cultures of the bacillus. These cultures all came from fish obtained from a single source, namely a fish-breeding station, and two of these fish were possibly infected in the laboratory.

(6) Laboratory experiments show that this pigment-forming bacillus is capable, when injected intramuscularly or intraperitoneally, of producing an "abscess" in gold-fish followed by septicaemia and death, and when inoculated into a trout through a scratch can produce deep ulceration, septicaemia, and death.

It remains to be shown whether this bacillus will infect trout or salmon with any degree of certainty when the bacillus is introduced into the water in which the fish are living, and whether a wound is necessary for the entrance of the bacillus. The experiments of Emmerich and Weibel, and of Plehn appear to show that the contamination of the water with a culture of the bacillus is sufficient to infect uninjured fish.

Remarks.

There seems to be no doubt that the epizootic in this country was due to the disease known on the continent as Furunculosis of the Salmonidae. This disease as has been pointed out by Emmerich and Weibel (1896), Hofer (1904), and Plehn (1911) is not always characterised by the muscular haemorrhages and foci of softening in the muscle to which its name is due. Apparently the fish frequently die at an early stage of the disease before localisation of the bacteria in the muscles has occurred and whilst no pathological change except septicaemia is discernible.

The strain of bacillus isolated from English fish almost exactly resembles that described by Marsh (1903) and called by him *B. truttae*. The only points of difference in his description are (1) his statement that acid is produced from glucose, but that this sugar is not fermented. This statement must be taken to mean that acid is produced but no gas. The English bacillus produced only a small amount of gas and sometimes this was so slight as to be unnoticed for two or three days. The variant mentioned above which occurred on a chloracetic acid agar plate and only produced a very small amount of pigment, formed no gas from either glucose or mannite. The production of neither acid nor gas from cane sugar is a character in which the English bacillus and Marsh's agree. (2) The brown pigment formed is said by Marsh to be soluble in alcohol. This was not found to be the case as regards the pigment formed by the English bacillus when the pigment was procured in a dry form. It was found however that if absolute alcohol was poured on to a moist pigmented agar culture, the watery solution diffused out into the alcohol and the pigment remained dissolved in the diluted alcohol.

Bacillus salmonicida of Emmerich and Weibel must be regarded as identical with *B. truttae* of Marsh and *B. salmonicida-B.* of Plehn and also with the English bacillus described in this paper. The German observers have not recorded the results of any carbohydrate tests and

the originally described character of liquefaction of gelatin with the formation of gas, which was considered as distinctive in the original description, is not sufficient to separate the bacillus from some of the Gram-negative non-motile bacilli which are found in the intestines of fishes. The very characteristic production of brown pigment is dependent on a correct reaction of the culture media, but was almost always obtained in my cultures with ordinary nutrient agar containing Witte's peptone and beef broth. Apparently, however, certain other less readily controlled conditions are necessary for a satisfactory and rapid pigment production. On one occasion ten or twelve of my stock cultures failed to form pigment on agar.

The distinctive characters of *B. salmonicida*, *B. truttae* and *B. salmonis pestis* are shown in Table IV. as well as the characters of some of the other bacilli found most frequently on the cultures made from the diseased fish.

Conclusions.

1. The epizootic disease which occurred amongst salmon and trout in the south-west of England during the summer of 1911 presented the same symptoms as the disease which occurs in Germany, especially Bavaria, and in Austria and France, and is called Furunculosis of the Salmonidae.

2. The most characteristic feature of the disease, *i.e.*, abscess-like cavities, softened haemorrhagic foci, and small haemorrhages in the muscles were of frequent occurrence, especially in the larger salmon, but also were frequently absent.

3. Congestion of the intestine, especially the lower intestine, and discharge of blood and mucus from the vent *post-mortem* were of constant occurrence.

4. No bacteriological evidence was obtained that fish other than members of the family of Salmonidae were attacked.

5. A pigment-forming bacillus occurred constantly in large numbers in the blood and internal organs and in the muscular haemorrhages of fish dying of the disease, but it was absent from the very large majority of healthy control fish.

6. This bacillus is probably identical with *B. salmonicida* described by Emmerich and Weibel, as the cause of Furunculosis of the Salmonidae, and with *B. truttae* described as causing an epidemic amongst trout in America.

7. It is almost certain that this bacillus is the cause of the epizootic disease.

8. This bacillus was present in some apparently healthy fish which had been bred in captivity. These infected fish may have been examined during the incubation stage of the disease, or before the symptoms became noticeable, or they may have been healthy carriers of the bacillus.

9. The bacillus associated with this epizootic was destroyed in a few hours by sea-water, and even by a mixture of equal parts of sea-water and tap-water, although it lived for at least five days in tap-water. It is therefore improbable that salmon usually become infected in the sea.

10. The infection probably spreads from fish to fish in fresh water. The spread is no doubt facilitated by conditions of low water.

11. Cultures of the bacillus soon died out at a temperature of 36° C. and therefore it is extremely improbable that this bacillus ever attacks warm-blooded animals.

12. The especially high mortality from the disease during hot weather and the experiments of Plehn suggest that a raised temperature of water favours the development of the disease, when *B. salmonicida* is already present.

The work on the epizootic disease of the Salmonidae (Furunculosis) which has already been accomplished leaves much still to be done before the natural history of this disease is understood sufficiently to control future outbreaks in rivers.

The following points especially require further investigation:—

1. Whether salmon coming up from the sea are infected before they enter the rivers.

2. Whether the spread of the disease from river to river is only due to the introduction of fish from other rivers and breeding stations.

3. Whether other fish besides the Salmonidae (salmon, salmon trout, trout, grayling, and rainbow trout) are naturally infected.

4. Whether salmon and trout frequently harbour the bacillus for long periods though apparently in good health, and whether any such infected fish survive the cold weather in the rivers, and so carry on the infection from year to year.

5. Whether infection is more easily obtained by feeding or by pollution of the water with the bacillus, and, if the latter, whether wounds are necessary as a predisposing cause for the infection.

6. Whether infected fish not suffering from discharging ulcers can readily communicate the disease to others.

7. The effect of the temperature of the water on the production and prevalence of the disease.

Though widespread, the distribution of this disease does not appear to be universal at present. Fresh rivers are probably infected by the introduction of fish from infected sources. By careful attention to this means by which the disease is spread, the infection of fresh waters might perhaps be avoided.

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TABLE I.

No.	Fish	Date	Source	Diseased or Control	Post-mortem	Organs from which pigment-forming bacillus cultivated	Pure culture
S. 1	Salmon ...	20. v. 11	Exe ...	Diseased	—	Blood ...	+
S. 2	,, ...	1. vi. 11	,, ...	,,	Haemorrhage in peritoneum	Blood, liver, muscle	+
S. 3	,, ...	3. vi. 11	Wye ...	,,	Haemorrhage in peritoneum	Peritoneal fluid	+
S. 4	,, ...	13. vi. 11	,, ...	,,	(Viscera removed) ...	Blood ...	+
S. 5	,, ...	13. vi. 11	Teign...	,,	Bloody fluid from vent	Blood, liver, gut, spleen	+
S. 6	,, ...	13. vi. 11	,, ...	,,	Bloody fluid from vent	Peritoneal fluid	+
S. 7	,, ...	13. vi. 11	,, ...	,,	Small ulcers of skin. Haemorrhage in peritoneum	Blood, peritoneal fluid	+
S. 8	,, ...	14. vi. 11	Exe ...	,,	(Dried up)	Blood ...	+
S. 9	,, ♂ 2 ft. 6 in.	28. vi. 11	Wye ...	Control	Bloody fluid from vent	—	–
S. 10	Salmon ♀ 3 ft. 3 in.	29. vi. 11	,, ...	Diseased	Large abscess ...	Blood abscess, peritoneal fluid	+
S. 11	Salmon ♂ 3 ft. 6 in.	7. vii. 11	Erwood	,,	Abscesses	Blood, muscle, peritoneal fluid	+
S. 12	Salmon ♂ 2 ft. 9 in.	18. vii. 11	Dart ...	,,	Abscesses. Haemorrhage in muscles	Blood, muscle	+
S. 13	Salmon...	1. viii. 11	? ...	,,	Abscesses	Blood ...	+
S. 14	,, 2 ft. 7 in.	3. viii. 11	Dart ...	,,	Abscesses	Liver	+
F. 1	Grayling ♀	18. vi. 11	Wye ...	,,	Blood from vent. Fluid in peritoneum	Peritoneal fluid	+
F. 2	Bream ...	18. vi. 11	,, ...	,,	—	—	–

TABLE II.

Ref. No.	Fish	Date	Source	Diseased or Control	Post-mortem	Organs from which pigment-forming bacillus cultivated	Pure culture
T. 1	Trout ...	1. vi. 11	? ...	Diseased	Macerated	Liver	+
T. 2	,, ...	10. vi. 11	Exe ...	,,	Fresh in ice ...	Blood ...	+
T. 3	,, ...	,,	,, ...	,,	Congestion round vent; in ice	Blood ...	+
T. 4	,, ...	,,	,, ...	,,	In ice	Blood, liver ...	+
T. 5	,, ...	,,	,, ...	,,	In ice	Blood ...	+
T. 6	,, ...	,,	,, ...	,,	In ice	Blood ...	+
T. 7	,, ...	,,	,, ...	,,	Bloody fluid from vent; in ice	Blood, liver, gut	+
T. 8	,, ...	13. vi. 11	Fish Culture Tanks	Control	Nil	—	–
T. 9	,, ...	,,	,,	,,	Bloody fluid from vent	—	–
T. 10	,, ...	,,	,,	,,	Nil	—	–
T. 11	,, ...	,,	,,	,,	Nil	—	–
T. 12	,, ...	,,	,,	,,	Bloody mucus from vent	Blood (three colonies)	+
T. 13	,, ...	,,	,,	,,	Bloody mucus from vent	—	–
T. 14	,, ...	,,	,,	,,	Nil	—	–
T. 24	,, ...	1. vii. 11	Scotland	,,	Bloody fluid from vent	—	–
T. 25	,, ...	,,	,,	,,	Nil	—	–
T. 26	,, ...	,,	,,	,,	Nil	—	–
T. 27	,, ...	,,	,,	,,	Nil	—	–
T. 28	,, ...	,,	,,	,,	Nil	—	–
T. 29	,, ...	,,	,,	,,	Nil	—	–
T. 30	,, ...	,,	,,	,,	Macerated; 2 days in water in laboratory	—	–
T. 31	,, ...	,,	,,	,,	—	—	–
T. 34	,, ...	26. vii. 11	Dart ...	,,	Fresh	—	–

TABLE III. (*Experimental Trout.*)

Ref. No.	Fish	Source	Experimental or Control	Method of infection	Date of experiment	Date of death	Post-mortem	Organs from which pigment-forming bacillus cultivated	Pure culture
T. 15	Trout	Shop	Control	—	—	21.vi.11	Nil	—	–
T. 16	,,	,,	,,	—	—	21.vi.11	Intestine congested	—	–
T. 17	,,	,,	Exp.	Culture in water	19.vi.11	21.vi.11	Intestine congested	—	–
T. 18	,,	,.	,,	Culture in water	19.vi.11	21.vi.11	Intestine congested	—	–
T. 19	,,	Fish culture tank	Control	—	—	24.vi.11	Petechiae on swim bladder	Blood, liver	+
T. 20	,,	,,	Exp.	Scratch on side inoculated pure culture	21.vi.11	24.vi.11	Intestine congested, large spleen	Blood, liver spleen	+
T. 21	,,	,,	,,	Fed with liver of T. 17	21.vi.11	24.vi.11	Inflamed spot on side	Liver, spleen blood, local lesion	+
T. 22	,,	,,	,,	Fed with liver of T. 17	21.vi.11	25.vi.11	Nil	Blood, liver, spleen	+
T. 23	,,	,,	,,	Scratch on side and culture in water	21.vi.11	25.vi.11	Inflamed wound, patches of congestion on liver	Blood, spleen, wound	+
T. 32	,,	,,	,,	Scratch and pure culture rubbed in	27.ix.11	30.ix.11	Large discharging wound	Blood, liver, wound	+
T. 33	,,	,,	,,	Water infected	19.vi.11	15.vii.11	Nil	Blood ...	+

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TABLE IV.

Bacillus	Morphology	Gram-staining	Motility	Agar	Potato	Serum	Glucose	Cane-sugar	Lactose	Mannite	Dulcite	Indole	Milk	Growth at 37° C.	Remains alive in sea water	Anaerobic (facultative)	Spores	Gelatine
B. salmonicida ... ... (England, 1911)	Short	–	–	Brown diffusing pigment	Very slight	Liquefaction deep brown	Acid and gas	–	–	Acid and gas	–	–	Acid digestion	Very slight	A few hours	+	–	+
B. truttae ... ... (Marsh)	Short	–	–	Brown diffusing pigment	Very slight	Liquefaction deep brown	Acid	–	–	...	...	–	Acid digestion	None	...	Slight	–	+
B. salmonicida ... ... (Emmerich and Weibel)	Short	–	–	Slightlate brown pigment	None	...	...	–	...	...	...	...	...	None	...	+	–	+
B. salmonis pestis ... (J. Hume Patterson)	Short	–	+	None	Profuse yellowish brown growth	No liquefaction	...	...	...	...	...	...	Acid digestion	Very slight	One month	–	–	+
Bacillus of Proteus group (Fish, 1911)	Irregular and long	–	+	None	Scanty	...	Acid and gas	Acid and gas	–	Acid and gas	–	+	Digested deodorised	+	...	+	–	+
B. fluorescens liquefaciens (Fish, 1911)	Long	–	+	Green diffusing pigment	Yellow	...	Acid	–	–	–	–	+ slight	Digested deodorised	+	...	–	–	+