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KOMMISSIONEN FOR DANMARKS FISKERI- OG HAVUNDERSØGELSER

SERIE: FISKERI · BIND IX

NR. 6. ANTON FR. BRUUN AND BØRGE HEIBERG: THE "RED DISEASE" OF THE EEL IN
DANISH WATERS.

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1. Introduction.

"Red Disease" in the Common Eel (*Anguilla vulgaris* TURTON) has been known for a long time. As early as the year 1718, according to HOFER, it was described by GIAN FRANCESCO BONAVERI from eels from the Comachio lagoons on the east coast of Italy, where also it was observed by SPALLANZANI in July 1790; on the latter occasion no less than 36,000 Kg. of eels were found dead in the course of 38 days. Further reports on the disease in Italy date from 1825, 1850, 1864, 1867, 1884, 1885, 1889, 1892, 1901 and 1903, as also from the Dalmatian coast and Herzegovina 1898 and 1899. Brief notices have also come from Danish waters about the end of last century and from Limhamn in Scania in 1906 and 1907; eels with Red Disease have also been described from Wismar 1898 and from Stralsund and Rügen in 1925 and 1926; these last reports will be referred to more closely below. Cases occurring in freshwater are not included here.

In 4 cases only (CANESTRINI 1892, INGHILLERI 1903, BERGMAN 1909, SCHÄPERCLAUS 1927) have the symptoms and cause of the disease been closely examined, especially bacteriologically; but these have shown already, that three specific diseases are probably in question, each due to its own microbe.

In the summer of 1931 numerous cases of Red Disease occurred in various parts of the Danish waters, e. g. at Copenhagen. This presented a favourable opportunity of obtaining copious material for the closer examination of the disease and its cause etc., as will be shown in the following pages.

The first-named author bears the main responsibility for the sections on the description of the disease, its distribution etc., whilst the second is responsible for the bacteriological investigation; but the report has resulted from close cooperation.

To our Chiefs, Professor JOHS. SCHMIDT, D. Ph., of the Carlsberg Laboratory and Director THORVALD MADSEN, M. D., of the State Serum Institute, we wish to express our sincere thanks for their interest in the investigation, for the conveniences placed at our disposal for the Laboratory work and for assistance during its progress. We would also thank the Fisheries Director, F. V. MORTENSEN, for the valuable information obtained through the Fisheries Bureau regarding the distribution and magnitude of the disease in the various parts of the country.

2. Description of the Disease.

The eel material examined came partly from the Sound at Copenhagen, kindly placed at our disposal by the Eel Export Co. "Østerbro" (managing director CHR. LAURSEN) and fish merchant EMILIUS CHRISTENSEN, partly from specimens sent us by fish exporter SVEND LARSEN of Kallehave. These specimens were caught at a number of different places in the waters south of Zealand, at Kallehave, in Guldborg Sound between Gedser and Nykøbing, Falster, at Banholm, Fæjølø, Fæmølø and Vejrlø. From Copenhagen we have seen about 200 specimens and from Kallehave, Guldborg Sound etc. 52 specimens.

The material contained both yellow eels from about 30 cm. in length, and silver eels.

Since the external examination of the material in fresh condition indicated and the later bacteriological

investigation confirmed, that only one type of disease occurred, we need only describe the general appearance of the disease and its epidemic nature in the eels from the localities mentioned. The conditions have not permitted us to carry out a minute anatomical or histological examination; yet the true nature of the disease could hardly have been recognized in such an investigation, the bacteriological study alone being effective, as will appear from a comparison later with the descriptions of the disease from elsewhere.

The first external symptoms are usually a red colouration at the end of the tail or on the body along the dorsal and anal fins; along with this the eel becomes less lively and swims round about, if stimulated, with stiff wriggling movements, not so powerful, however, that the fish cannot easily be taken in the hands again; left in peace it rests quietly on the bottom for the most part. The red colouration gradually increases along the fins until it appears as dark red streaks over longer or shorter distances, and a diffuse reddening spreads over most of the body; the lymph heart in the tail works more and more slowly until at last it quite ceases to beat.

The eel often dies before the red colouration is so well-marked, but in a number of cases it becomes even more distinct; for example, in most the anal opening is surrounded by a blood-red ring; the epidermis is loosened and the skin has a frayed appearance; if not earlier death now occurs and the fish rapidly takes on a grayish look, "sucking water" as the fishermen say, i. e. looks as if it had long been dead and drifting about in the water. The dead eels very quickly become rotten.

Macroscopic examination shows, that the blood-vessels of the intestine are very congested, the outer intestinal wall showing a diffuse and general reddening. Often the liver and sexual organs are obviously congested with blood, so much so that in parts of the liver one could speak of blood effusions into the tissues. Transverse sections in a number of cases show, that the muscle tissues within the anal and dorsal fins are strongly inspissated with blood. In the worst cases this infusion reaches almost to the vertebral column. Cysts or cyst-like formations have not been observed in our material, but they have been mentioned now and then in the reports from various parts of the country, to be discussed later.

As noted above, we have brief notices of earlier occurrences of the Red Disease epidemic among eels in Danish waters. FEDDERSEN in 1896 reported on a "remarkable eel"; it was a silver eel, in "quite good condition" when captured; it is stated further, that "over the whole body a carmine-red tint shines through the general eel-colour, and this is probably due to a basal colour below the epidermis, as the carmine-red is specially distinct at several places where the skin is torn. Owing to this basal colour the white belly and sides have the same sort of tint as one sees in fine, pale roses. Further the breast fins ("ears") are as red as cherries or blood, and the same colour is present on all the fins, dorsal, tail and anal. The colour is so strong on the dorsal, for example, that it looks like a streak of blood when the fish is lying in the water. The anus is also blood-red — —". The writer had never before seen or heard of such an eel, and compares it with earlier finds of quite white or yellow eels. In the following number of the same magazine, however, he reports briefly, that the eel died eight days after reception without any change in colour, but when the eel was skinned it was found that all the fin-rays in the dorsal and anal fins were blood-red. It seems very probable indeed that this eel was suffering from the above-mentioned disease; and this applies in still greater degree to the notices of LETH (1897) and FEDDERSEN (1897 a).

In addition to the red colouration of the skin and fins LETH mentions "that a no small part of the intestines also had an appearance of strong inflammation". FEDDERSEN's description of the symptoms and course of the disease is more detailed and agrees so exactly with our observations, that we have scarcely any doubt about the identity of the disease with the above-described; we are therefore using the name "Red Disease" introduced by FEDDERSEN. Apart from the "remarkable eel" mentioned, both LETH and FEDDERSEN seem to have known the disease only from the eel-boxes, where it can certainly appear only a few hours after the eels have been placed in them.

The general picture of the disease thus resembles to a considerable extent the accounts given of the

deadly epidemics among eels in Italy; but, as will be shown later, both the bacteria supposed to be the causes of the diseases there are quite different from that found by us, so far as one can judge. FEDDERSEN (1897 b) remarked that at least one of the Italian bacteria had "different habits" from the bacteria he supposed might be the cause of the Red Disease in Danish waters, and this supposition is confirmed by the present investigation.

BERGMAN'S (1909) description of an eel disease, on the other hand, is quite different from the Italian and the one described by us; the disease he has found in the eel he calls very significantly red cyst-disease, and bacteriological examination confirms the special nature of this disease. This has all the more interest in that the Red Disease examined by us had one of its most strongly infected areas just in the Sound near Copenhagen, thus not far from Limhamn. BERGMAN remarks, that the cyst disease can scarcely be of much importance for the fishery, whilst, as we shall show later, the epidemic Red Disease must be considered at certain places very harmful. Whilst the red cyst-disease may be regarded as a spring or early summer disease, the epidemic Red Disease appears in high and late summer.

SCHÄPERCLAUS (1927) has given a detailed account of a red disease occurring at Rügen and Stralsund from September to October 1925 and October 1926. Although the picture of the disease comes very closely to that found by us, SCHÄPERCLAUS comes to the conclusion after a careful bacteriological examination, that the disease was probably due to the microbe described by BERGMANN, *Vibrio anguillarum*, which thus apparently produces very diverse external symptoms as well as differences in the course of the disease, and may also occur at other seasons than the spring.

It may be noted further, that a high death-rate among eels has often been remarked in Danish waters during early spring, sometimes with a reddening of the skin; the occurrence and significance of this phenomenon has been closely described by A. C. JOHANSEN (1929) and has been ascribed by him, with good reason, to the direct influence of the cold on the eel during the hibernation; it is well-known that the eel is very sensitive towards cold.

WALTER (1910) has also referred to a "red disease" in the eel from quite a number of localities: Stettiner Haf, where at some places one-half to two-thirds of the catches were infected, Unterweser, Unterems and Kiel Canal, thus both in brackish and freshwater localities; here the disease or diseases are in all cases high summer phenomena, but their causes have not been investigated. This applies also to a notice by FEDDERSEN (1897 a) of the occurrence of a red disease in Holland, regarding which his informant, a Dutch skipper, stated "it is the heat that does it". SCHÄPERCLAUS (1927) likewise mentions a red disease from the Zuidersee and lastly, reference must be made to the fine work of SCHÄPERCLAUS (1930) on the red disease in eels from the freshwater (Havel district, in the tributaries of the Lower Elbe and in Pommern). In its symptoms the freshwater red disease has obvious resemblances to the forms of red disease described from the sea, but SCHÄPERCLAUS has proved, that it is due to the facultatively parasitic microbe *Pseudomonas punctata* f. *sacrowiensis*, SCHÄPERCLAUS.

According to all this the red disease of more or less deadliness is a very heterogenous collection, to be split up provisionally into 5 different diseases, the true nature of which must always be investigated bacteriologically. According to the available information any epidemic occurring in Danish waters may probably be due to one of 4 causes: 1. influence of the cold (great mortality, especially in shallow waters after hard winters; JOHANSEN 1929); 2. red cyst-disease (sporadic cases in the spring and early summer; BERGMAN 1909) or general symptoms of red disease (September-October, SCHÄPERCLAUS 1927) — in both cases caused by *Vibrio anguillarum*, BERGMAN); 3. Red Disease (mass mortality in high and late summer) caused by the microbe *Vibrio anguillcida* n. sp. to be described below; and lastly, 4. the freshwater red disease caused by *Pseudomonas punctata* f. *sacrowiensis*, SCHÄPERCLAUS.

A bacteriological examination of some cases of red disease found in freshwater in Denmark will be necessary, before we can determine if the epidemic saltwater form is able to penetrate into the freshwater courses and convey the disease from the sea.

3. Bacteriological Examination.

The bacteriological examination embraces 11 eels, all of which in more or less degree displayed the symptoms of the disease above-mentioned. Of these 5 came from places in the neighbourhood of Copenhagen, the other 6 from places in the south of Zealand (Kallehave etc.). All the eels were alive when they were received at the Laboratory.

Further, samples of the water from the fishing places at Copenhagen and Kallehave were examined.

Cultures from the dorsal fin, liver and blood of the heart were placed under aseptic precautions on gelatine plates and agar plates. The gelatine plates were kept at 22° C. for 24 hours, the agar plates at 37° for the same period.

From 4 of the eels examined no growth at all appeared on the plates, which remained sterile (observations over 4 days). On a gelatine plate from a 5th eel came a slight gelatine liquefaction at a single spot during the first day, but no bacteria could be isolated from this identical with that obtained from the other cases; possibly, the liquefaction was due to some foreign infection on the plate. From one eel a pure culture could not be isolated.

In the remaining 5 cases we got a pure culture of the bacteria to be described below; 3 of the eels yielding the pure culture came from the neighbourhood of Copenhagen, the other two from Kallehave.

Further, the success was attained of isolating from water samples from Kallehave a bacteria identical with that obtained from the eels.

In the case of the cultures from organs and blood, growths were obtained on the agar surface from liver and heart blood in all 5 cases, and in 4 cases also the liquefaction of the gelatine from the same places. The cultures from the dorsal fin yielded a growth on the agar surface in 2 cases and liquefaction of the gelatine in all 5 cases.

The isolated bacteria appeared under the microscope as a slightly curved rod, active in movements. It is a little smaller than the *Colibacillus*; it is gram-negative. Staining with dilute carbol-fuchsine often shows a stronger colouration at the ends of the bacteria than in the middle. Special staining shows up a single flagellum at the end.

The following investigation into the conditions connected with the bacteria was made on freshly isolated, or at most a few days old cultures.

On the different nutrient substrata the following conditions with regard to the growth were found.

Gelatine. Cultivation on plates of 10 % gelatine with NaCl content of $\frac{1}{2}$ % resulted in most cases in a strong, sharply marked liquefaction of the gelatine after standing 10—12 hours at 22° C. In a few cases the liquefaction took somewhat longer, which was also the case on using 15 % gelatine with $\frac{1}{2}$ % NaCl. Cultivation on 10 % gelatine free of NaCl caused the liquefaction to be weaker and somewhat more protracted.

Stab-culture in 10 % gelatine with $\frac{1}{2}$ % NaCl at 22° C. for 24 hours produced a funnel-shaped liquefaction of the gelatine extending to a depth of about 1 cm., but also a yellowish, flocculent growth along the whole of the deposit in the gelatine. After further standing 4—5 days at the room temperature the gelatine liquefied to a depth of 4—5 cm. with a bottom deposit of yellowish bodies about the size of millet-seed.

Agar. Cultivation on the agar surface yielded after 24 hours small, faintly yellowish, dense colonies, whether the cultivation took place at the room temperature, 22° or 37° C. The colonies were circular and regular.

On sloping plates of ascites-agar a rich growth of grayish yellow, dense colonies took place after standing 24 hours at 22° C.

Deposition in meat-extract agar for a couple of days at 22° C. gave a weak growth along the whole of the piece and growth on the surface of the substratum.

On blood-agar the bacteria grew copiously at 22° C. with grayish colonies.

Broth. In ordinary calf broth the bacteria yielded a foggy growth with a small, compact deposit at 22° or 37° C. in 24 hours.

In addition to the growths on the ordinary nutrient substrata investigation was also made of the conditions, with respect to acid-formation, resulting from the addition to broth of a number of different materials, partly sugar and partly alcohol. This resulted in a number of substances being found from which the bacteria were able to form acids, in contrast to another series of substances, which in spite of active growth of the bacteria were not split up by them within the period of observation. As substrata were used: aqueous solution of Liebig's meat-extract 1 ‰, Witte peptone 1 ‰ and NaCl 1/2 ‰; to these was added 1/2 ‰ of the substance to be operated upon. The cultivation proceeded at 22° C. Brom-thymol blue was used as indicator, and distinct yellow colouration of the substratum was required to make sure that acid formation had taken place. In none of the cases was there any emission of gas during the process. In the substrata where acid formation occurred this developed in the course of 24 hours, except in the case of a single sugar, maltose, where the reaction was only distinct after 48 hours. All the substrata, both those in which acid was formed and in the indifferent substrata, yielded a growth of the bacteria, showing itself as a foginess of the broth. This foginess developed generally in the course of 24 hours, but in the tartrate broth 72 hours passed before the substratum became clouded. The cultures were under observation for 4 days.

Acid formation took place in the above-mentioned substratum with the addition of the following substances: glucose, maltose, dextrine, glycogen, laevulose, starch, galactose.

Acid formation in the substratum did not take place on the addition of the following substances: mannite, lactose, saccharose, adonite, dulcite, sorbite, arabinose, xylose, rhamnose, inosite, salicin, erythrite, raffinose, melecitose, glycerine, inuline, amygdaline, tartrate.

Any indol reaction could not be detected. A 1 ‰ aqueous casein solution was used as substratum and as reagent a solution of the following composition: paradimethylamidobenzaldehyde 1 g., 96 ‰ alcohol 95 c. c., conc. HCl 20 c. c. The test was made after 1, 2 and 3 days at 22°.

With respect to the growths at different temperatures it appeared that the most active growth took place at temperatures between 15—22° C., on the gelatine most actively at 22°, in the case of agar without any noteworthy difference. At 4—5° C. there was a very slight liquefaction of the gelatine in the course of 10 days, but on transferring these cultures to 22° C., great liquefaction of the gelatine took place in 24 hours. After remaining at 4—5° C. for 5 weeks the bacteria were still living.

If the broth cultures were kept at 37° C. for a couple of days, they were found to be sterile. In a few cases this happened already after 24 hours. Hence the bacteria are very sensitive towards heat.

As one might expect in the case of a bacteria found in sea water, the NaCl concentration is of importance. To examine this more exactly, living bacteria from a gelatine culture were placed in 6 tubes with respectively distilled water, fresh water from the pipe and NaCl solutions of 0.9 ‰, 2 ‰, 5 ‰ and 10 ‰. On the addition of the bacteria the substrata became faintly cloudy. After 24 hours the cloudiness had either increased or diminished. After 6 days the tubes were apparently clear, but sewing on gelatine produced a liquefaction from the tubes containing water from the pipe, 0.9 ‰ and 2 ‰ NaCl. After 1 month the tube with 2 ‰ NaCl still contained gelatine-liquefying bacteria, whilst the other tubes were sterile. After 10 weeks the tube with 2 ‰ NaCl was again tested in respect to its power of liquefying gelatine, but this had come to an end. The cultures had stood the whole time at 15° C. It may be noted, that the water from the pipe was tested before the investigation with regard to its power of liquefying gelatine and found to have none. Further, as already mentioned, experiments were also made with the cultivation of bacteria on gelatine free of salt, which showed that the bacteria were able to liquefy the gelatine.

The bacteria live a long time in the different substrata; for example, they still lived after 14 weeks in the stab-cultures in gelatine and meat-extract agar and after 9 weeks in surface cultures on sloping plates of ascitesagar at room temperature.

To round off the bacteriological investigation some inoculation experiments were made on some eels partly living in fresh water partly in salt water, but as the research conditions were not uniform with regard to temperature etc., the investigation cannot be ascribed much importance, except that several of the fish became ill with undoubted clinical signs of Red Disease.

The results of earlier investigations of the disease in eels in salt water were published by CANESTRINI 1892, INGHILLERI 1903, BERGMAN 1907 and SCHÄPERCLAUS 1927.

CANESTRINI discovered, especially in the liver of diseased eels, a straight rod of $2\ \mu$ in length. It was gram-positive and grew aerobic. It liquefied gelatine, both saline and salt-free, the former, however, to a much greater extent than the latter. It was pathogenic towards eels after injection.

INGHILLERI found a gram-negative rod with rounded ends regularly in the blood, liver and serous fluids of the abdominal cavity. It was $2-3\ \mu$ long and provided with numerous flagella along the margin. It was active in movement and propagated in the ordinary nutrient substrata. The propagation occurred best at $18-20^{\circ}\text{C}$., but also took place at 35°C . It liquefied gelatine but had not the power to cause fermentation in carbonhydrates. It was pathogenic towards eels on injection.

The investigations of BERGMAN and SCHÄPERCLAUS have been of special interest to us because the waters, from which their eels were taken, are not far from our waters and the descriptions of the disease show many points of resemblance.

BERGMAN found a specific bacillus, which he calls *Vibrio anguillarum*, at the focus of infection, in the abdominal cavity, in the liver and sometimes the blood. It was a short, curved rod, active in movements with a flagellum at the end. The length was $1-3\ \mu$, breadth $\frac{1}{4}$ of the length. It was gram-negative. Stab-culture in gelatine for 24 hours at 20°C . gave a whitish thread along the deposit with a small depression at its upper end. In the following days the deposit became funnel-shaped with the expansion above; it was grayish in colour except at the base where there was a yellowish flocculence. In the course of a week all the gelatine was liquefied and on the bottom there was a layer of yellow floss, whilst the surface was covered by a thin grayish membrane.

Cultures placed in sterile freshwater became sterile after 3 days.

BERGMAN has investigated its fermenting power towards broth with dextrose, saccharose, lactose and maltose added. There was little or no growth in the substrata and no gas or acid was formed in any of the cases. In glucose agar the bacteria grew but slightly and without formation of gas or acid.

In salt-free gelatine the growth was slower than in saline.

The indol reaction was positive (substratum: weak alkaline 1% solution of Witte peptone with $\frac{1}{2}\%$ NaCl. A reddish brown colour appeared on addition of concentrated sulphuric acid or hydrochloric).

The bacteria grew at temperatures between 5° and 38°C . with an optimum apparently between 25° and 35° ; it remained living for a long time on agar and blood serum. With regard to oxygen requirements it appeared to be facultatively anaerobic.

SCHÄPERCLAUS in 1927 published a larger report on his investigations of diseased eels in the locality between Rügen and Stralsund. He succeeded in isolating a vibriion from the kidney or from the venous blood of the tail of 4 eels, and this vibriion SCHÄPERCLAUS considers identical with BERGMAN'S *Vibrio anguillarum*.

The bacillus in question is a gram-negative, active, comma-shaped rod, facultatively anaerobic. It has a single, terminal flagellum and is of the same size as BERGMAN'S. In gelatine cultures it grows like the cholera vibriion with a slightly darker, finely grained ring round the zone of liquefaction. Stab-cultures in gelatine melt the latter somewhat more slowly than seen by us, but no information is given regarding the concentration of the gelatine and temperature of cultivation, so that differences here cannot be ascribed much importance. Cultures in ordinary broth grow with a diffuse fogginess and with the for-

mation of a membrane on the surface. In peptone water with addition of 1 % glucose there was neither development of gas nor fermentation and SCHÄPERCLAUS writes: "zuckerspaltende Fermente können die Bakterien nicht bilden". On the other hand, the bacteria grew actively in the peptone solution with sugar.

Indol formation was soon apparent with Ehrlich's indol reaction.

Experiments on the vitality of the bacteria as regards NaCl concentration showed, that no living bacteria were found after 24 hours in peptone water free of NaCl or containing 8 % NaCl, and that the optimum NaCl concentration lay between 2 % and 2.5 %. It appeared also that the vibrios died very quickly in ordinary freshwater from the pipe, already inside 24 hours.

In concluding these references to earlier investigations mention should also be made of the work published by SCHÄPERCLAUS in 1930, regarding *Pseudomonas punctata* as the cause of disease in fish. This reports on some examinations of diseased eels from freshwater, with evidence that *Pseudomonas punctata* acts as a pathogenic bacteria. As in the earlier cited paper SCHÄPERCLAUS here brings together and compares the principal qualities of some pathogenic fish bacteria. Those who are specially interested may be referred to this paper, as we shall now restrict further comparison to the 4 bacteria mentioned above. These seem to be well-defined, according to SCHÄPERCLAUS 1930, from the other pathogenic fish bacteria.

Whilst BERGMAN maintained that the bacteria described by CANESTRINI, INGHILLERI and himself were different and stressed the disagreements found, SCHÄPERCLAUS maintains the view, that it is the same bacteria in all three cases, as well as in the case described by him. After referring to the different descriptions of the disease SCHÄPERCLAUS writes, with regard to the Rügen vibriion noted by him and the Limhamn vibriion noted by BERGMAN: "Bei einer sorgfältigen Differentialdiagnose ist die überraschende Tatsache festzustellen, daß der Erreger der roten Beulenkrankheit, die nach Bergmann sich wesentlich unterscheiden soll von der gewöhnlichen Rotseuche, genau denselben Erreger hat wie die bei Rügen und Stralsund beobachtete Erkrankung". Reference to the other two bacteria described earlier is made with greater reservation; SCHÄPERCLAUS writes: "... daß sehr viele Übereinstimmungen zwischen den vier untersuchten Fällen von Rotseuche bestehen . . . Ob die Identität wirklich besteht, das festzustellen muß weiteren Untersuchungen, besonders über die italienische Rotseuche des Aales, überlassen bleiben".

Comparing now the different characteristics of these 5 (4) pathogenic bacteria in the eel, we note the following:

1. CANESTRINI's bacteria was a straight, gram-positive, aerobic rod in contrast to ours, which is a slightly curved, gram-negative, facultative anaerobic rod.

2. INGHILLERI's bacteria was a straight rod with several flagella — number unknown; our bacteria is a vibriion with one flagellum.

3. In the case of the vibriion described by BERGMAN and later by SCHÄPERCLAUS a number of resemblances to the vibriion described by us are apparent. Both are curved, short rods, gram-negative and facultatively anaerobic with only a single, terminal flagellum. But with regard to the vitality in sterile water free of NaCl and acid-forming property, there are certain differences; similarly with regard to the indol formation, but as the reactions studied were not identical we cannot ascribe too much importance to this last point. Our bacteria kept alive in fresh water from the pipe for 6 days, whilst *Vibrio anguillarum* disappeared in 1—3 days after the sowing. Our bacteria produced always acid in the above-mentioned substratum to which was added glucose, maltose and dextrine, with which according to BERGMAN's express statement *Vibrio anguillarum* has no acid-forming power, and according to the above citation from SCHÄPERCLAUS this bacteria does not possess this power at all. Further, our bacteria was able to split up 4 others in addition to the 3 kinds of sugar mentioned. There seems also to have been a difference with regard to the rapidity of liquefaction of the gelatine, but no special weight can be attached to this owing to possible differences in the experimental conditions.

Summarizing the different characteristics of the bacteria schematically, we have the following¹:

	Bacillus anguillarum, Canestrini	Bacillus Inghillieri	Vibrio anguillarum Bergm. Schöp.	Vibron examined by us
Appearance.....	straight rod	straight rod	curved rod	curved rod
Activity.....	+	+	+	+
Flagella.....	+	+	+	+
Gram-staining.....	+	÷	÷	÷
O ₂ requirement.....	{ aerobic	facultatively anaerobic	facultatively anaerobic	facultatively anaerobic
Liquefaction of gelatine.....		+	+	+
Stab-culture in Gelatine.....	as cholera	as cholera	as cholera	as cholera
Acid-forming power towards } Glucose.....			÷	+
} other kinds of sugar.....			÷	+
Gas forming power with various kinds of sugar (Indol formed).....			÷	÷
Vitality in water free of salt.....			(+)	(÷)
Optimum temperature.....	16°	18—20°	(÷)	(+)
			25—35°	15—25° C.

We do not think that the relatively few experiments made with CANESTRINI'S and INGHILLERI'S bacteria justify a positive statement of difference between their bacteria and ours.

It is different with *Vibrio anguillarum*. Here we have numerous experiments dealing with the behaviour of the bacteria in definite media; and in several of these there is a distinct difference. For example, SCHÄPERCLAUS writes, that *Vibrio anguillarum* does not form an acid; that seems to be a clear difference. Certainly, in the reactions 1 % and 1/2 % of glucose were used, but the bacteria grew actively in both substrata (according to SCHÄPERCLAUS). *Vibrio anguillarum* seems much more sensitive than our bacteria towards the percentage of NaCl in water, a point that should not be underestimated where we are dealing with a bacteria living in sea water.

Whether one should ascribe greatest importance to the differences or to the common properties comparing one bacteria with another, is pretty much a matter of estimates. But as our bacteria showed very constant features just where the difference from *Vibrio anguillarum* appeared, we consider ourselves entitled to give it a special name *Vibrio anguillicida*.

The bacteria has been preserved in the State Serum Institute of Copenhagen, where an endeavour will be made to keep it alive by resowing several times yearly.

4. Distribution and features of the distribution in Danish waters.

Although we are quite aware, that great care has to be exercised in considering, whether reports about "Red Disease" arise from this or that cause, without bacteriological examination, we think we are justified in referring most of the cases of summer mortality to the epidemic Red Disease, so far as Danish waters are concerned. This applies naturally to a considerable stretch of water, the Sound and the waters south of Zealand, where the same bacteria has been discovered. We may add also that such an infectious disease will have very great possibilities of being carried far and wide in Danish waters owing to the transport of eels. The reports from round about the Danish waters are as a rule so much of the same kind, as regards time of year, localities, nature and infection of the disease, course of the disease and so on, that they afford a picture of the distribution of the disease which may be considered very near the truth; the spring mortality reported on in some cases may be referred to the influence of the cold.

¹ Scheme partly from BERGMAN and SCHÄPERCLAUS.

a. Earlier reports.

The first recorded case of Red Disease in Danish waters was that noted by FEDDERSEN (1896) as mentioned above; the eel in question was caught at "Sydspidsen af Amagerskoven" (Kongelunden) on the 8th of November, 1896.

LETH (1897) records the Red Disease from "some of our southern waters", where it is said to have caused both fishermen and fish exporters "considerable loss" in the summer of 1896; the disease was centred especially in Ulfsund and neighbouring waters as well as in a part of the SmaalandsHAVET, as at Bandholm. LETH mentions, that the same or very similar disease was said to have been prevalent some years earlier in the neighbourhood of Rødby, but was unable to obtain further information regarding it. In 1896 the disease also appeared in Horsens Fjord (FEDDERSEN, 1897 a).

In the following year 1897 the disease does not seem to have been specially worthy of note in these waters; on the other hand, it is now recorded from somewhat past the middle of July in the Limfjord (FEDDERSEN 1897 a), where it was rampant among the eels in the eel-boxes.

In the magazine of the Danish Fisheries Society for 1905 it is again reported, from the "Thisted Amtssavis", that a disease had broken out among the eels at some places in the Limfjord, especially about Fur and beyond Skive; it is said that the exporters were obliged to throw away up to two-thirds of the catch; whether this disease has been the Red Disease is perhaps a little doubtful, as it is stated, that the eels became "burnt-yellow under the belly and the eels so bitter that they were uneatable". According to experience later referred to, the eels attacked by Red Disease do not suffer in taste, if they are eaten in the fresh condition.

In 1906 again the same magazine contains a notice, cited from the paper "Nordjylland", regarding Red Disease in the Limfjord eels, where those from the eel grass were said to have been the most severely attacked "at the time when the seaweed was covered with a malodorous, grayish, sour fat or scum". At the same place a remarkable disease among the plaice with great mortality is mentioned; the plaice became spotted reddish brown on the belly; the connection of this disease with that of the eel is, however, not certain, though possible (cf. SCHÄPERCLAUS' investigation 1930 on *Pseudomonas punctata* as the cause of disease in different fishes).

To judge from these few reports, an infectious disease has been recognized among the eels for many years, in the more enclosed parts of the Danish waters, to some extent just from the locality where it was specially prevalent in 1931 and from which it has been more closely investigated. The probability is that it has been the same disease, as is indicated by the symptoms noted especially by LETH (1897) and FEDDERSEN (1897 a).

b. New information collected in 1931.

Through the Fisheries Bureau a scheme of questions was sent out in the autumn of 1931 to the local representatives of the Bureau round about the country; the answers gave quite a lot of interesting information, which may be summarized here.

The information is tabulated schematically in Table 1 and the following remarks from the answers may be added and commented on.

In the Ringkøbing-Fjord district the loss in 1930/31 is estimated to have amounted to about 750 kg., but in earlier years it is said to have been more than double this; a dry and warm summer seems to be specially favourable to the disease.

The Red Disease reported from Nissum Fjord is stated to be worst when the eels are stored in old, untarred boxes in the uppermost, fresh part of the fjord; only a close study of the conditions will be able to throw light on the exact cause of the disease.

Whilst the Red Disease in the Western Limfjord District has been of no importance in recent years, there were severe attacks in 1917 and 1918; a couple of men with leister or the bare hands could then take 15—20 Kg. in one day near the shore or in shallow water.

Table 1. Occurrence of the "Red Disease" in Danish waters

District	Informer	Occurrence	Disease observed in
Southern West Jutland.....	Fiskeriassistent J. M. Jensen, Højer-Sluse	scarcely remarked before 1931	silver eels
Esbjerg.....	Fiskeribetjent P. Tæbring, Esbjerg	each year; only known from shallow water	yellow eels, also some silver eels
Nymindegab and Holmsland.....	Fiskerifoged P. Jensen Pedersen, Nymindegab	everywhere, especially in deeper water but only occasionally	chiefly small yellow eels
Ringkøbing Fjord.....	Fiskeribetjent S. Jørg. Sørensen, Ringkøbing	frequent everywhere	both yellow and silver, perhaps more silver
Nissum Fjord.....	Fiskeribetjent H. Esager, Staby	known only in stored eels	chiefly silver
Western Limfjord.....	Fiskeribetjent P. Petersen Struer	slightly everywhere; chiefly in shallow water	yellow
Middlemost Limfjord.....	Fiskeriassistent J. Jensen, Nykøbing M.	not frequent, but seen at all eel-trap places	mostly large eels, both yellow and silver
Thisted Bredning.....	Fiskeribetjent Axel Jensen, Thisted	not frequent, but everywhere, especially in shallow water	mostly yellow, especially large and medium-large
Skagen—Sæby (Kattegat).....	Fiskeribetjent K. Nielsen, Frederikshavn	not known in district	
Eastern Limfjord.....	Fiskerikontrollør A. Jørgensen, Aalborg	a few everywhere especially on soft <i>Zostera</i> -ground	chiefly yellow
Hobro-Mariager Fjord.....	Fiskeribetjent A. S. Larsen, Mariager	only a few, especially in shallow water	yellow, seldom silver eels
Randers Fjord.....	Fiskeribetjent C. Skaaning, Randers	common everywhere, especially in shallow water	chiefly small and medium yellow
Grenaa and neighbourhood.....	Fiskeribetjent H. M. Jensen, Grenaa	not frequent, commonest in shallow water	only observed in silver eels
Aarhus Bugt.....	Fiskeribetjent O. Nørgaard, Aarhus	only now and then; mostly in shallow water	chiefly among small yellow eels
Northern Funen.....	Fiskeribetjent Chr. Larsen, Bogense	not frequent; mostly in shallow water	mostly small eels
Southern East Jutland.....	Fiskeriassistent J. M. Jensen, Aabenraa	fairly frequent everywhere, espec- ially in shallow water	mostly yellow, also silver eels from the beginning of the season
Southern Funen.....	Fiskeribetjent A. Gerhard, Svendborg	fairly frequent, especially in shallow water	mostly yellow, also silver eels in August
Southern Zealand.....	Fiskeribetjent Bagger Jensen, Næstved	observed at long intervals	mostly yellow but also silver
Nakskov Fjord.....	Fiskerifoged Julius Juul, Langø	variable, chiefly in shallow water	yellow, also silver eels
Smaalandsøhavet, Lolland-Falster.....	Fiskeribetjent Chr. Th. Larsen, Masnedesund	common everywhere, especially in shallow water	yellow eels
The Sound.....	Fiskeribetjent F. Jensen, København	every year, mostly in shallow water	yellow, and the first silver eels
Isefjord, Northern Zealand.....	Fiskeribetjent H. F. Frederiksen, Lynæs	not frequent; mostly in shallow water	yellow and the first silver eels
Great Belt.....	Fiskerikontrollør Trolle Thomsen, Nyborg	a few isolated cases; mostly in shallow water	especially yellow
Bornholm.....	Fiskeribetjent V. Kristiansen, Bornholm	very seldom	silver eels

according to information collected by the Fisheries Bureau.

Beginning	Season of disease		Remarks of reporter	Authors' remarks
	Height	End		
?	autumn	?	loss noted especially on storing in boxes after transport to Kolding	comes possibly from silver eels descending the Hvidaa; these eels have thus become diseased in freshwater
?	August and early Sept.	October	said to be specially common off gasworks; no fishing in deeper water; eel fishing unimportant	
in summer, especially August-September-October				
late May or June	July-August	November-December	the first caught silver or not quite silver eels chiefly affected	
?	August-September	?		
April	begin. of May	mid. May	occurs chiefly after long ice	certainly due to cold and not to epidemic Red Disease
March	varying, spring of autumn	mid. October	worst in silver eels of recent years	spring cases certainly due to cold; summer and autumn cases to Red Disease
spring	September	ca. 1st Nov.	loss especially among stored eels	
June	September	ca. 1st Nov.	greatest distribution after a warm summer	
March-April	March	mid. April	not regarded as infectious	due probably to cold, not to Red Disease
whole year round, but mainly in May, June and September			ca. 2 % mortality among silver eels in 1930	the first spring cases possibly due to cold
August-November, but worst in August			almost no fishery of yellow eels	some of cases reported from freshwater are perhaps not Red Disease
early April	April-May	?	the first silver eels especially infected	spring cases in part certainly due to cold
April	August	October		
mainly in spring and summer months			very frequent after cold winters	early spring cases certainly due to cold; summer cases to Red Disease
late July	August-September	October		
?	July	?	only known from a few fjords; also appears after long ice-winters	the cases after winter certainly due to cold
late April and May	June-July	September	specially frequent in warm summers, after a hard winter	
mid. July	?	late August	after long ice periods a few diseased eels also seen	these last certainly due to cold
?	July-August			
March	April	mid. May	most common in the southern parts of Roskilde and Holbæk Fjords	the diseased eels in spring certainly due to cold, but silver eel cases to Red Disease
June	July-August	September	only in more enclosed parts; seldom seen in the more open waters	
	July-August			probably an infectious disease, but cause unknown

In the Middlemost Limfjord District about 15 % of the silver eels were infected in September 1929; this was the worst year recorded for 20 years.

In the Thisted Bredning the Red Disease was specially noticeable in 1927, among both the yellow and silver eels; by spreading rapidly among the stored eels it caused great loss to the exporters.

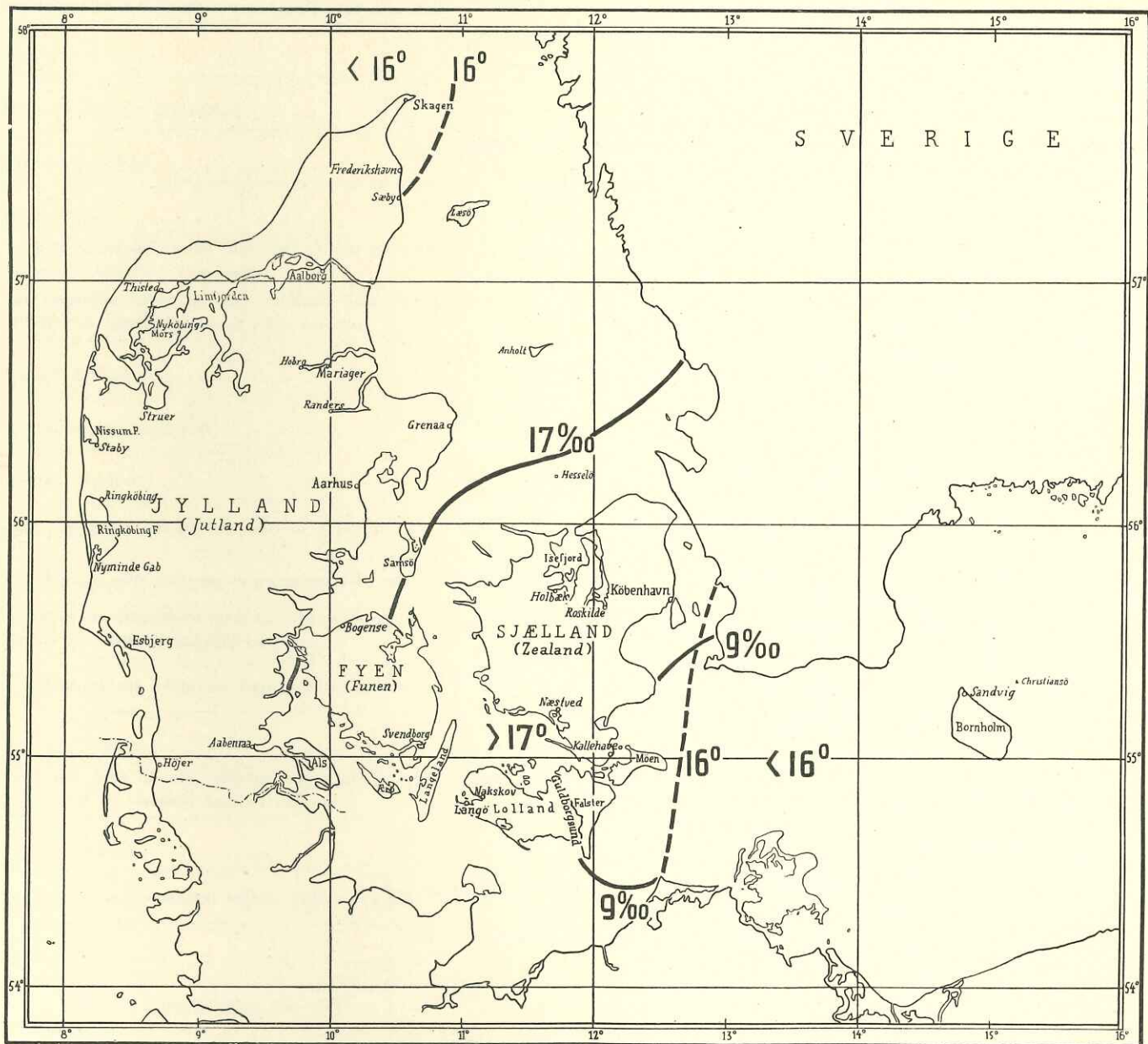


Fig. 1. Some surface isotherms and isohalines for August.

In the Eastern Limfjord District about 10 % of the silver eel catch in 1929 was estimated to have been lost through the disease.

From Randers Fjord District there are also reports of cases of disease from the part of the Gudena which lies below Tangesø, but none from the river above the lake; thus, eels in freshwater can be affected. Whether the disease has here extended up from the fjord and is due to the same bacteria, as the disease in salt water, has to be determined by further investigation. The same applies to the cases reported from the south

canal of Kolindsund in the Grenaa District; in August 1930 a loss occurred here of about 100 Kg. or 25 % of the total catch. In the same District the disease was common on the coast at Jernhatten in the years 1920—24; in 1924, the worst year, about 10 % of the catch or about 300 Kg. was lost.

In the Northern Funen District the disease was most marked in 1930, when the loss was estimated at 3—6 %, affecting mainly the exporters. It has been known here right back to about 1880 and is said to have been most widely distributed in the southern parts of the District.

In the Southern Jutland Fjord District the loss amounted to about 1 % in 1930—31.

In the Southern Funen District the Red Disease was common in 1930—31, especially in the waters between Rudkøbing—Vemmenæs, Marstal, Drejø and the other islands, whilst it only occurred sporadically in the Lunkebugt, Skaarupørbugt and on the north coast of Langeland; the disease does not seem to have been observed west of Faaborg or in Helnæsbugt. In 1930—31 the disease was not very deadly, yet one case is noted from Ærøskøbing where a sample of 30 Kg. was reduced to 8 Kg. in the course of a few days. In 1925 and 1928, however, there seems to have been a much greater loss, greatest in August and September. In the reports sent in a distinction is made between the diseased eels from early spring, most frequent after icy winters and thus probably due to the influence of the cold, and the eels attacked in the course of the summer. The first-named are so well-known by the fishing population that they are called in South Funen by a special name: Duninger.

In the Southern Zealand District the disease is considered not to have caused great loss; yet in July 1931 dead eels were found daily in the traps and nets in Næstved and Karrebæksminde Fjords.

In the Nakskov Fjord District the loss in June and July 1931 is said to have been about 4 % after a short time in store. The disease appears to have been known at least as far back as about 1882.

In the Smaalandshavet District the disease in 1931 was at its worst in the waters round about Banholm, Fæmø and Fæjø and in Guldborg Sund, especially in shallow water and off freshwater outlets. In the worst period it is impossible to keep the catch in the store-boxes, as the disease spreads rapidly with great mortality. In 1931 up to 30 % of the catch was dead or dying on being taken out. In 1890 a similar epidemic is said to have occurred, bringing the fishery to a standstill.

From the Sound the Red Disease is known chiefly from the waters about Copenhagen and in Præstø Fjord and now and then also from Faxe Bugt, whereas the northern part of the Sound seems to be free from it more or less.

In 1930—31 the economic loss seems in general to have been unimportant, as the infected eels were as a rule sold to the smoke-houses and obtained a fairly good price.

That in some cases, however, the disease has meant considerable loss and been very marked, is apparent from the following information received from the Manager of the Eel Export Co. "Østerbro", CHR. LAURSEN, who has kindly informed us, that the disease was first observed towards the beginning of August in the purchased catches; it became more and more general and culminated about 15. August, easing off gradually towards 25. August; it has to be noted, however, that the reception of affected eels was very soon restricted, so that the sudden ending in the store does not correspond with the conditions in nature, where even in September affected and dead eels were still to be found, e. g. in the traps on Bredegrunden; at the time when the Red Disease was raging in the basins no less than about 1000 Kg. died, and to this loss has to be added the indirect loss due to the restriction of the buying and the greatly increased work in sorting out the diseased eels. The present authors have several times had the opportunity of becoming personally acquainted with the conditions in the basins of the Eel Export Co. "Østerbro". The purchased eels are sorted out very quickly into the different qualities and placed in fine clean cement basins with a strong inflow of freshwater. All the eels showing signs of the disease were at once separated from the rest, yet the disease spread; for example, all the eels in one basin might seem to be fresh and sound in the evening, yet next morning display numerous dead and dying with marked signs of the disease; and if the eels continued to be kept, the disease continued to spread and cause loss.

At Bornholm the Red Disease is met with very rarely; in 1931 in spite of keen search only one case was detected; from earlier years only a few cases have been known from the shallow water of Sandvigbugten.

5. Concluding remarks.

From the above account of the distribution of the Red Disease in Danish waters it will be seen, that the disease has its main centre in the inner waters; it is less well-known from the open localities, but is most common in the shallow, enclosed bays, fjords etc. The Chart (Fig. 1) displays some of the surface isotherms and isohalines for August taken from I. P. JACOBSEN (1908). It will be seen that the curves for 9 and 17 ‰ embrace the most infected areas, which is in good agreement with the culture experiments with bacteria in different salt concentrations. Further, this area is the warmest in Danish waters, with temperatures of over 16° C.; in addition, we may note, that the SmaalandsHAVET which in 1931 at any rate were by far the most seriously affected, show a temperature maximum over 17° C., which sets in 3—4 days before the 1st of August according to JACOBSEN (l. c.); this agrees exactly both with the culture experiments and with the culmination period of the disease in nature.

In the second area where the disease at times has been common, Limfjord, the same outer conditions prevail in the main, shallow water, culmination with temperature and in salinities less than those of the open Kattegat or North Sea.

It follows from this, that in the storing of eels, where the disease is most prominent, one could probably lessen its further spread by not placing the storage boxes, which of course should be well-cleaned, in warm spots with a feeble stream of water. FEDDERSEN (1897 a) recommended the experiment of sinking the boxes in deeper water and this in all cases would prevent the further heating by the sun's rays. If it were possible to have cool, running freshwater, this would probably also be a preventive. As the disease is not produced by storing, but is introduced from infected eels of the catches, it would in all cases be advisable to separate the diseased and sound eels, and under no circumstances place the sound catches in the neighbourhood of the diseased. It must also be considered advisable to bring all dead and dying eels on land, and not throw them back again into the water. Similar regulations are also given by SCHÄPERCLAUS (1927); in the cases where *Vibrio anguillarum* BERGMAN is the cause of the red disease, freshwater will presumably be effective owing to the small resistance of this microbe towards freshwater.

The disease may be characterized as a running sepsis fatal as a rule; it is hardly caused by wound infection, its sometimes occurrence in masses speaks against this; the infection probably takes place through the gills or digestive canal from the bacteria living in the water.

Red Disease, caused by the microbe described by us, will undoubtedly be met with at all seasons of the year, but reaches a maximum in frequency in late summer, by which we do not mean that the eels in process of change from the yellow to silver condition are specially sensitive to the disease, but rather that the temperature conditions at this season are specially favourable for the cultivation of the bacteria, and it is a fact also that the yellow eels are attacked in very large numbers. It will be very difficult, however, to obtain a reliable, quantitative estimate of the influence of the disease among yellow and silver eels, owing to the different biology of these two forms. The extensive mortality among eels in certain places in the early spring seems therefore rightly ascribed by JOHANSEN (1929) to the direct influence of the cold, thus a "Pseudorotseuche" (SCHÄPERCLAUS 1929).

It is of considerable interest economically, that the disease is not dangerous to the health of human beings on eating the infected eels; we have tested this ourselves by eating fried eels with distinct signs of the disease; nor does the taste of the eel seem to suffer; nor is any case known where eating of such diseased eels has caused illness, assuming of course that the eels were in the fresh condition.

6. Danish summary.

„Rødsyge“ hos den almindelige Aal (*Anguilla vulgaris* TURTON) har været kendt gennem adskillige Aar, baade i Udlandet og i danske Farvande. Kun i 4 Tilfælde (CANESTRINI 1892, INGHILLERI 1903, BERGMAN 1909, SCHÄPERCLAUS 1927) er Sygdommen i Saltvand blevet nøjere undersøgt, især bakteriologisk, og det har vist sig, at der sandsynligvis er tre forskellige Sygdomme, som hver skyldes sin Mikrobe.

I Sommeren 1931 forekom talrige Tilfælde af Rødsyge i danske Farvande, bl. a. ved København og Syd for Sjælland, hvorved der frembød sig Lejlighed til den foreliggende Undersøgelse.

Sygdommen angriber saavel Gul- som Blankaal. De første synlige Tegn er oftest en Rødfarvning af Halespidsen eller Kroppen langs Ryg- eller Gatfinnen, samtidig med at Aalen bliver mat; Rødfarvningen tiltager, og efterhaanden breder sig over hele Kroppen en almindelig Rødmen; Lymfehjertet i Halen ophører efterhaanden at slaa. Ofte dør Dyret inden Sygdommen er saa udtalt, men i en Del Tilfælde bliver Rødfarvningen endnu kraftigere, bl. a. finder man hyppigt Gataabningen omgivet af en blodrød Ring; Overhuden løsner sig og Skindet faar derved et flosset Udseende. Forraadnelsen indtræder meget hurtigt hos de døde Aal.

Ved Sektion finder man altid, at Tarmens Blodkar er ret fyldt; ligeledes er Lever og Kønsorganer paa-faldende blodfyldt, og der forekommer ligefrem Blodudtrædninger i Leveren. Muskelvævet indenfor Gat- eller Rygfinne er ofte stærkt imbiberet med Blod; Bylder eller byldelignende Dannelser har vi ikke iagttaget, men de nævnes af og til i Indberetninger rundt fra Landet. Sygdommens Symptomer ligner saaledes i betydelig Grad de andetsteds fra beskrevne og i Virkeligheden kan kun en bakteriologisk Undersøgelse afgøre, hvilken Sygdom, det drejer sig om.

En „Rødsyge“ i danske Farvande vil efter det foreliggende kunne skyldes: 1) Kuldevirkninger (JOHANSEN 1929), 2) *Vibrio anguillarum* BERGMANN (BERGMAN 1909, SCHÄPERCLAUS 1927) og 3) *Vibrio anguillicida* n. sp., samt i Ferskvand *Pseudomonas punctata* f. *sacrowiensis* SCHÄPERCLAUS. Der er anlagt Bacteriekulturer fra Rygfinne, Lever eller Hjerteblood fra 11 Aal. Der er derved fra 5 Aal kommet Renkultur af en let krummet, livlig bevægelig Stav, som er undersøgt nærmere under forskellige Dyrkningsbetingelser. Den har vist sig at opføre sig forskelligt fra de hidtil undersøgte, hvorfor vi har givet den et særligt Navn, *Vibrio anguillicida*.

Gennem Fiskeridirektoratet indhentede Oplysninger viser, at „Rødsyge“ er kendt praktisk talt fra hele Landet. Indberetningerne har et meget ensartet Præg og synes at vise, at der i Almindelighed er Tale om to Tidspunkter for Massedødelighed, et om Foraaret især efter strenge Vintre, som uden Tvivl skyldes direkte Kuldevirkninger, dels et i Høj- og Eftersommeren, der skyldes Bakterieangreb. Sygdommen synes i 1931 afgjort at have haft Maximum i Smaalands havet, som er det varmeste Omraade i de indre danske Farvande med Saltholdighed mellem 9 ‰ og 16 ‰; dette er i god Overensstemmelse med Dyrkningsforsøgene med Bakterien.

Rødsygen spiller stedvis en meget stor Rolle for Aalefiskeriet i danske Farvande, dels ved at en betydelig Del af Aalene er død eller døende allerede ved Fangsten, dels ved at Sygdommen breder sig stærkt blandt lagrede Aal, ja i ondartede Tilfælde umuliggør Lagring i Hyttefade.

I det frie vil det naturligvis være umuligt at bekæmpe Sygdommen omend det maa formodes at være formaalstjenligt at ilandbringe alle døde og døende Aal fra Fangsterne og tilintetgøre dem.

Ved Lagring bør tilstræbes saa kraftigt strømmende og køligt Vand som muligt; efter Dyrkningsforsøgene at dømme vil fersk Vand have nogen hæmmende Virkning paa Bakterievæksten.

Rødsyge Aal er, naar de iøvrigt nydes i frisk Tilstand, ufarlige som Menneskeføde, og Kødet har ingen Afsmag.

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