MANUSCRIPT REPORTS OF THE BIOLOGICAL STATIONS

No. 317

INVESTIGATIONS ON HALIBUT MEAL AS A HATCHERY FOOD

by

D.C.B. Duff

F I S H E R I E S   R E S E A R C H   B O A R D
O F   C A N A D A

MANUSCRIPT REPORTS OF THE BIOLOGICAL STATIONS

No. 317

Title

INVESTIGATIONS ON HALIBUT MEAL AS A HATCHERY FOOD

Author

D.C.B. Duff

INVESTIGATIONS ON HALIBUT MEAL AS A HATCHERY FOOD

D.C.B. Duff

Significance

In the late summer of 1930 a severe epizootic appeared among sockeye fry in the retaining ponds of the Smith Falls hatchery, Cultus Lake. The manner in which the daily deaths increased to a maximum and then fell off suggested that the disease might be of an infectious nature. Most of the affected fry exhibited a dull grayish film, appearing in patches on sides and back. Microscopic examination of scrapings from sides and gills of such fish showed the presence in nearly all cases of a small actively motile protozoan, corresponding closely to the description of Costia necatrix, a well-known parasite of trout and salmon. The organism was found on nearly all sick-appearing fish, frequently on newly dead, in a few cases on apparently healthy fry, and never on fry which had been dead more than a few hours. While conditions strongly suggested the association of the parasite with the disease, direct proof was lacking. It was possible that a the epizootic was due to some other and primary cause, and that the Costiasis was a secondary condition. Conversely Costiasis may have remained the primary cause, while other factors, such as physical conditions, improper food or a concurrent infection, may have aggravated the course of the disease.

It was suggested by Dr. R.E. Foerster that the halibut meal used as part of the feed in the ponds might have been a factor. Several possibilities are inherent in this hypothesis:

(i) The meal as fed may be directly harmful or poisonous, or may be lacking in essential nutritive qualities.

(ii) Decomposition of the meal in the ponds may give rise to poisonous diffusible substances.

(iii) One or more of the microorganisms present in the meal may be directly pathogenic to fry.

A consideration of item (i) is outside the scope of this investigation, and is being dealt with by Doctor Foerster in direct feeding experiments. The work conducted at Cultus lake from June to August 1931 is therefore limited to items (ii) and (iii).

Experimental Technique

In order to study the effect on sockeye fry of diffusible substances present in halibut meal and other foods, it was necessary that the fry be maintained in known volumes of water. Battery jars of 3 litres capacity, actually containing 2.5 litres during tests, were found satisfactory. These were maintained in the stream of a regulation hatchery trough. The contents were easier of access than in the case of ordinary fish culture jars, and the contained volume of water just came up to the water level of the hatchery trough in which they were held. The jar contents were thus constantly maintained at the temperature of the hatchery water supply, which kept fairly constantly within the limits of $11.5^{\circ} \pm 1^{\circ}C.$ throughout all experiments.

Each jar would maintain from five to ten sockeye fry in apparently normal condition for a period of weeks. The water in the jars was changed once daily, from one half to one hour after a single daily feeding of fresh liver. The required dose of test fluid was added immediately after changing the water. At the beginning of the series, samples of 10 fry were used. The symptoms and time of death due to poisoning from meal decomposition products proved so uniform, however, that the samples were later reduced to 6 fry each, without affecting the significance of results.

Test material: The term "filtrate" here designates the fluid from a single filtration, through a single thickness of Whatman No. 1 filter paper, of halibut meal or other food which had been mixed with water and allowed to stand

Sampling of test materials: In the case of halibut meal, samples were obtained from the centre of the barrel or sack at the hatchery. Canned salmon and liver were sampled from the ground mixtures as fed in the hatchery, freshly ground material was used as much as possible. In no case was material definitely "off" (by criteria of appearance and smell) utilized. Halibut meal, (hatchery sample), ether-extracted halibut meal, fresh ground beef liver, and canned salmon, both old pack and new pack, were allowed to decompose in water at room temperature for varying lengths of time, 20 gm. of material was mixed with 100 c.c. water in a 200 c.c. cylinder. It must be noted that while the halibut meal is fairly dry, ground liver and canned salmon contain large quantities of water. Thus any comparative results found with the different materials must be interpreted in terms of weight of food, as such, as fed in the hatchery, and not on a basis of the protein or oil content of each food. Graduated amounts of filtrate, 50 c.c., 20 c.c., 10 c.c., 5 c.c., and 1 c.c. were added to a series of experimental jars containing the fry.

Table I gives the results of one experiment only for filtrate from various decomposition periods for (Smith Falls) halibut meal. Other tests on that and on all other materials conducted in the same way. From Table I can be found the minimum lethal concentration for each period of decomposition, together with the time required for this dose to act.

The findings from all such experiments are tabulated in more concentrated form in Table II.

A point of interest is seen in comparing experiments 1, 2 and 3, performed in the latter part of June, with 7 and 8, carried out one month later. The M.L.D. for fry in 1, 2 and 3 is consistently.08 grm. per litre. In 7 and 8 growing sockeye fry become somewhat more resistant to the toxic principle.

TABLE I

MINIMUM LETHAL CONCENTRATION OF (SMITH FALLS) HALIBUT MEAL
decomposed for Different Periods - 10 fry per jar

Time of Death in days ± 6 hours

Time of Death in hours ± 1 1/2 hours

Concentration of Filtrate (in grams of meal per litre) / Dose of Filtrate	4 gm. 50 c.c.	1.6 gm. 20 c.c.	0.8 gm. 10 c.c.	0.4 gm. 5 c.c.	0.08 gm. 1 c.c.	0.016 gm. 0.2 c.c.
Time of Decomposition Hours (of meal)						
0	7 days	not in 10 dy.	not in 10 dy.	-	-	-
12	4 days	"	"	-	-	-
24	48 hr.	48 hr.	48 hr.	72 hr.	-	-
48		12 hr.	12 hr.	12 hr.	12 hr.	-
72				12 hr.	12 hr.	-
96				12 hr.	12 hr.	-
Control	-	-	-			

The values for concentration of filtrate given are expressed as the amount of filtrate per litre in the actual experimental jars, represented as grams weight of the material used to produce the filtrate.

TABLE II

RESULTS OF EXPERIMENTS WITH HALIBUT MEAL FILTRATES

Each division gives the Minimal Lethal Dose per litre of Filtrate together with the time required for the dose to act

Read Time of Death in days ± 6 hours

Read Time of Death in hours ± 1 1-2 hours

Time of Decomposition of Food	Fresh	12 hr.	24 hr.	48 hr.	72 hr.	96 hr.
Halibut Meal, Whole, in Tap Water. Commenced June 16	4.0 gm. 7 dy.	4.0 gm. 4 dy.	.40 gm. 48 hr.	.08 gm. 12 hr.	.08 gm. 10 hr.	.08 gm. 12 hr.
Same as 1. Commenced June 24	4.0 8 dy.	1.6 6 dy.	.40 45 hr.	.08 18 hr.	.08 18 hr.	- -
Same as 1, but used water from hatchery supply. Comm. June 29	4.0 7 dy.	4.0 6 dy.	.40 52 hr.	.08 12 hr.	.08 12 hr.	- -
Smith Falls Halibut Meal, ether-extracted (shaking). Comm. June 24			.40 19 hr.	.08 18 hr.	.08 15 hr.	.08 16 hr.
As 4, Meal extracted by Soxhlet. Commenced June 24			.40 45 hr.	.08 15 hr.	.08 15 hr.	.08 16 hr.
Same as 5. Commenced June 24			.40 52 hr.	.08 12 hr.	.08 12 hr.	.08 12 hr.
Smith Falls Halibut Meal, Whole, Tap Water, July 17			.80 24 hr.	.40 19 hr.	.40 2- hr.	.40 18 hr.
As 7			.80 30 hr.	.40 18 hr.	.40 18 hr.	.40 24 hr.
Taft Halibut Meal, Coarse Sample, Tap Water, July 22			.80 72 hr.	.80 23 hr.	.80 24 hr.	
Taft Halibut Meal, Fine Sample, Tap Water, July 22			.80 72 hr.	.80 26 hr.	.80 24 hr.	
Taft Halibut Meal, ether-extracted (shaking), Coarse Sample			.40 36 hr.	.40 30 hr.	.40 32 hr.	
Taft Halibut Meal, as 11, Fine Sample			.80 36 hr.	.80 24 hr.	.80 22 hr.	

Proof or disproof of this suggestion would require further experimentation.

In order to find whether the poison was formed by protein decomposition or by changes in the oil, samples of the Smith Falls halibut meal were extracted with ether, and the oil-free meal allowed to decompose under identical conditions with the whole meal. In Experiment 4 the meal was extracted by shaking in a drum, with three changes of ether. The meal used in Experiments 5 and 6 was Soxhlet extracted through the courtesy of Dr. A.S. Golding, of the Department of Dairying, University of British Columbia. In each of these experiments, the M.L.D. for the oil-free meal proved to be ± .08 grams per litre, or the same as for whole halibut meal.

Experiments 7 to 12 serve to compare the potency of the decomposition product in the Smith Falls halibut meal with that in two samples of halibut meal obtained from the plant at Taft, B.C. It is seen that there is little difference between these samples. If anything, the Smith Falls sample is more potent than the Taft samples. As with Smith Falls meal, the Taft meals did not lose their poison-producing power after ether extraction. This is interesting, since the coarse meal Taft sample was dark in colour and had a definitely rancid odour, indicating some change in the oil.

The lack of relation between the diffusible toxic product, obtained under laboratory conditions, to any situation occurring in retaining-pond practice, is evidenced as follows. The approximate capacity of a retaining pond is 18,000 gallons, and the approximate amount of halibut meal fed daily is 7 lbs. This is in the same ratio as 1 gram meal to 21.4 litres of water, or 0.05 grams meal per litre. Even this small concentration would only be possible providing the meal were all fed at once, instead of twice daily, and providing there was no change of water in the pond.

The lowest concentration of very highly (48 hr. at 20°C.) decomposed halibut meal, which would kill fry was 0.08 grams per litre, acting in non-flowing water in the experimental jars. In the case of filtrates made directly from fresh meal, it required a concentration equivalent to 4 grams meal per litre to produce death; and the fry did not die until after 7 days continuous exposure to such a dose. In the case of fresh meal, as fed in the ponds, we are thus contrasting the maximum possible pond concentration of diffusible substances, equivalent to 0.05 grm. per litre, with the 4.0 grm. per litre minimum of fresh filtrate required to kill in still water.

Cumulative Effect of Sub-Lethal Doses of Toxic Filtrate

The possibility of a cumulative injurious effect upon fry, from the continuous presence of small amounts of diffusible material from the meal, was next considered.

21-7-31 to 11-8-31: At the time of this experiment, the M.L.D. for the fry was somewhere between 0.4 grm. and 0.08 grm. of 48 hour filtrate. Exposure to 0.08 grm. per litre of filtrate was sufficient to cause slight symptoms, the fry remaining at the surface of the water and apparently having slight difficulty in respiration. Accordingly, 3 jars of 6 fry each were exposed continuously for three weeks to this dose. After the one daily feeding, the water in the jars was siphoned off, fresh water added, and a new dose of 48 hr. filtrate added. Next day, however, the fish, although still at the top of the water, appeared better. The following day they were quite well and occupying the bottom of the jars as usual. Two fry died on the 11th day; the remainder stayed in normal condition and fed well to the end (3 weeks) of the experiment.

It must again be noted that the conditions of the experiment were much more drastic than anything that might occur in hatchery practice. To begin with, the meal had decomposed under laboratory conditions at room temperature and had produced a definite poison. The fry were exposed to a concentration just short of that required to cause death, and no washing away of the substance by running water occurred. In spite of these conditions, the fry resisted the poison. Rather than a cumulative toxic effect being noted in time, instead the fry apparently became accustomed to a concentration of poisonous material which was at first injurious.

Further Experiments with Halibut Meal

26-6-31 to 11-7-31: Smith Falls halibut meal was rubbed over the surface of three experimental jars, and about 5 grm. of meal left in each jar. The jars were filled with water and kept overnight at hatchery temperature. Next day 5 fry were placed in each jar. In two days' time a light film, consisting of algal filaments, protozoa, and bacteria, together with entangled particles of meal, formed on the sides and bottom. The general nature of this film corresponded closely to the brownish film which covers the sides and bottom of hatchery troughs and retaining ponds. The fry were fed with liver and halibut meal once daily; the jars were siphoned off half an hour after feeding, and fresh water added, without disturbing the film. One fish died on the second day. None of the remaining fry showed any symptoms of distress, and were feeding well at the close of the experiment. There was here the condition of an initial film containing halibut meal, with the addition of fresh meal and liver daily, but with no attempt at removal of film. Since the water was changed only once daily, the chances of a concentration of poisonous diffusible substances from the material in the film, gathering in the water, were infinitely greater than would be the case in hatchery troughs or in retaining ponds, with running water. Fry kept in this environment for 15 days were apparently unharmed.

Test of Brown Scum from Troughs

1-7-31: Scrapings were obtained of the brown crust and scum which formed on the sides of a trough in which halibut meal was used and partially dried between filter papers. 20 grams was placed in each of three 100 c.c. quantities of water and left at room temperature. After 48 hours, (the time at which maximum toxicity of halibut-meal preparations is reached), 50 c.c. of the filtrate was placed in 3 experimental jars as usual. This amount was completely tolerated by the fry, which showed no symptoms. The fry were left in the jars, without food and without change of water, for four days, and were in good condition and ready for food at the end of this time.

Effect of Film on Trough upon Development of Fry

Using sections of hatchery trough, two small ponds were constructed, simulating in proportion the large outside retaining ponds. The rate of flow in the small ponds was cut down as far as possible.

Dimensions of actual retaining ponds	60' x 16' x 3'
Capacity	18,000 gallons
Approximate rate of flow	100 gal. per min.

The model ponds had a capacity of 30 gallons. It was found impracticable to use a rate of flow of less than 1 gallon per minute, or six times greater rate of change of water than would be strictly proportionate. While this condition is possibly a weak point in the experiment, it is considered that another condition acting in the opposite direction, would offset the advantage given to the fry to a great extent. This other condition was the comparatively crowded state of the experimental ponds - 500 fry in 30 gallons - which afforded much more intimate contact between the fry and the sides and bottom of the ponds than would ever occur in the large retaining ponds. Further, as will be noted below, the amount of film allowed to collect on the sides and bottom of Pond B was far in excess of anything that could collect in the large ponds in actual practice.

Five hundred fry were placed in each of the two experimental "ponds". Pond A was siphoned off, and was cleaned scrupulously twice daily with brush and cloth, so that no film was permitted to form. The imperceptible film which formed between cleanings was thus never more than 18 hours old. Pond B was cleaned only once in two days; the bulk of excreta and loose particles were siphoned off, but the film on the sides and bottom left intact. After five days this film became very thick and brown in colour, and portions of it kept breaking loose, and were siphoned off. During the entire 50 days of the experiment the film was left undisturbed. Both ponds were fed with equal portions of liver and halibut meal. Small portions of the film were examined weekly for the presence of any protozoa resembling Costia necatrix, with entirely negative results.

Detection of Effect of "Pond Film" on Development of Fry.

During the 50 days duration of the "pond" experiment, there was no mortality, except for the loss of four or five fish from each pond due to mechanical injury caused during cleaning operations. There was no morbidity; the reactions of the fry to feeding, frightening, etc., were about the same in each pond.

Sampling: A sample of 30 fry was taken from the supply trough at the beginning of the experiment. At the end of 50 days, samples of 30 fry were obtained from the supply trough and from the two experimental ponds. Samples were formalized, measured, and weighed.

	Supply trough July 1st	Supply trough Aug. 19th	Pond A Aug. 19th	Pond B Aug. 19th
Mean length	3.5 cm.	5.2 cm.	5.3 cm.	5.3 cm.
Mean weight	1.47 grm.	2.16 grm.	2.23 grm.	2.21 grm.

The mean length is the same for ponds A and B, the mean weights are significantly the same, and these values are slightly higher than the values for mean length and weight in the supply trough.

Standard Deviation

The standard deviation values for the three final samples was determined during the calculation of the coefficient of correlation (see below). These are:

Aug. 19th	Supply trough	Pond A	Pond B
length	± .376 cm.	± .352 cm.	± .362 cm.
weight	± .416 grm.	± 0.37 grm.	± 0.41 grm.

The values of are so close that it is unnecessary to calculate P.E. values for them. They indicate no significant differences between the mean values for length and for weight in the supply trough and in Ponds A and B.

Thus, considering the lengths and the weights separately, we have no evidence of a significant difference caused by different treatment in the ponds. It would, however, be advisable to investigate the relation existing between length and weight of the fry in the experimental ponds. Poorly nourished and ill-developed fry of the "pinhead" type have obviously a greater numerical value for the ratio length/weight than have normally developing fish. While no pinheads were present in the ponds, it is possible that smaller differences might be detected by considering this ratio. If, in a pond in which some inhibitive influence were active, all fish were equally affected, one would hypothecate a measurable difference in the length/weight ratio of the fry in this pond as compared with fry in another "normal" pond. Due to the complete agreement of the mean values from Ponds A and B, and of the approximate sameness of the standard deviation values for these figures, one would probably be justified in deciding at once that no change had been effected by the different treatments. The following calculations, i.e. of length/weight ratio comparison and coefficient of correlation comparison, may be regarded in this instance rather as an interesting exercise than as being of much value in determining differences. They are included because had there been sufficient difference in the mean values to justify further analysis, these methods would have been called into play, and might have given information as to the nature of the difference.

<u>Length/Weight Ratios</u>

	Supply trough	Pond A	Pond B
$\frac{\Sigma \cdot l/w}{N}$	2.48	2.44	2.42
σ	.291	.312	.295

<u>Coefficient of Correlation of Length and Weight</u>

While comparisons of length/weight ratios would indicate the type of difference where all fry in one pond were affected to the same extent by a harmful influence, while the fry in another pond remained normal, there is another conceivable type of difference which ought to be considered. Under ideal conditions all fry would be imagined to be developing uniformly as regards the relation of their length and weight. That is, there would be a very high correlation between length and weights. Under less favorable circumstances a certain number of fry, less suited in constitution to withstand harmful changes would be considerably stunted in growth, and their inhibition might manifest itself differently, as regards the length/weight ratio in individual fry. Further, a number of fry having thus been weakened by the supposed poisoning, the remaining fry, some of which would have a greater resistance to the harmful action, would be to a slight extent freed from competition against the remainder, and would flourish accordingly. Such a condition would result in a lower correlation between length and weight in the population, than would occur under ideal or "normal" conditions. Such a condition might be revealed by calculating the standard deviations of the ratio values for each pond. A more accurate measure would, however, be afforded by the calculation of the coefficient of correlation. This has been done by the use of Pearson's product-deviation method (Chaddock (1)), where

$$r = \frac{\Sigma xy}{N \sigma_x \sigma_y}$$

In this way we obtain

(Supply trough) r = .859 ± .032

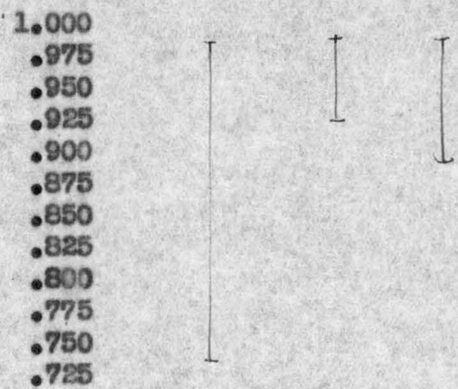
(Pond A) r = .973 ± .006

(Pond B) r = .952 ± .011

r = coefficient of correlation

The values given are ± 1 P.E. The following chart represents a comparison of the range of ± 4 P.E. for each of the ponds. It will be seen that the range of (Pond A) is contained within that of (Pond B), and that there is therefore no significant difference in the value of for the two ponds. It will be noted that the <u>range</u> of (A) is very slightly greater than that of (B), and that the range of (Supply) is very definitely greater. That is, there is practical uniformity of development in Ponds A and B, and a definitely poorer agreement between length and weight in the supply trough. Judging from this result -

Ranges of ± 4 P.E.



one would be tempted to say that the Pond B fish had undergone more uniform development than those in the supply trough, under standard hatchery conditions.

That halibut meal is not unique among hatchery foods in its capacity to produce, under laboratory conditions, substances poisonous to sockeye fry, is shown by the summary Table III.

TABLE III

Experiment No.	Time of Decomposition of Food	Fresh	12 hr.	24 hr.	48 hr.	72 hr.
1	Liver, July 23	-	4.0 24 hr.	.80 48 hr.	.80 30 hr.	.40 36 hr.
2	Canned Salmon "New Pack", July 26	-	-	.80 18 hr.	.40 18 hr.	.40 30 hr.
3	Canned Salmon "Old Pack", July 26	-	-	1.60 20 hr.	.80 18 hr.	.40 20 hr.
4	Liver, July 28	-	4.0 48 hr.	1.6 48 hr.	.40 36 hr.	.40 36 hr.

A comparison can be made of the strength of poison produced by these foods, with that produced by halibut meal in the experiments nearest the date of those given in Table III (Table II, 7 and 8). The decomposition product from canned salmon and from liver are only slightly less toxic than those from halibut meal.

No attempt has been made to determine the nature of the decomposition product in the filtrates responsible for the death of the fry.

Bacteriological Experiments

A bacteriological examination was made of the microflora present in properly sampled halibut meal. Isolations were effected by plating out suitable dilutions of the sample upon Difco nutrient agar plates, for aerobes, and by making shake tubes of dilutions in the search for anaerobes. Incubation was carried out at room temperature. By these methods, pure cultures of nine specimes of bacteria, one yeast, and two molds were obtained.

Microorganisms in Halibut Meal

Culture No.	Organism	Stain	Culture No.	Organism	Stain
1	Coccus	Gram +	7	Sarcina	Gram +
2	Coccus (fac. anaer.)	Gram +	8	Bacillus (fac. anaer.)	Gram +
3	Diplococcus	Gram ∓	9	Streptococcus	Gram +
4	Yeast	Gram +	10	Mold (1)	
5	Bacterium	Gram -	11	Mold (2)	
6	Sarcina	Gram +	12	Bacterium (fac. anaer.)	Gram -

Microorganisms Present in Hatchery Water Supply

W. 1. Bacterium, Gram +. W.2. Bacterium, Gram +. W. 3. Coccus, Gram +

No attempt was made to identify these cultures further. It will be shown below that no single organism can be directly blamed for the production of the toxic substance.

In an attempt to discover whether any single microorganism was responsible for the toxic product, all cultures were inoculated into beakers of sterilized halibut meal infusion. The same proportion of meal to water was maintained as in the fry experiments. It was found that the filtrate from sterilized halibut meal (15 lbs. for 20 minutes) was capable of poisoning fry to some extent. This poisoning effect was fairly strong; filtrate corresponding to 0.8 grams of meal per litre in the experimental jars produced death in from 24 to 31 hours. Actively growing pure cultures of the various bacteria were added to sterilized halibut meal, and filtrates were made after 48 hours.

Death of Fry Due to Pure Cultures of Bacteria Growing Aerobically in Halibut Meal Infusion. Dose - 0.8 grm. per litre

Sterilized Filtrate alone	Death in 30 hr.	Culture No. 5	Death in 24 hr.
" " "	" " 24 hr.	" " 6	" " 24 hr.
" " "	" " 31 hr.	"" " 7	" " 34 hr.
		" " 8	" " 34 hr.
Culture No. 1	Death in 31 hr.	" " 9	" " 24 hr.
" " 2	" " 31 hr.	"" " 10	" " 31 hr.
" " 3	" " 24 hr.	" " 11	
" " 4	" " 28 hr.	" " 12	" " 34 hr.

A comparison of the time of death in the (sterilized meal plus culture) experiments, with the time of death in the controls of sterilized meal filtrate alone, shows no appreciable difference. While this would indicate that none of these cultures are responsible, it might be argued that the meal had been so changed by sterilization that microorganisms were unable to effect further changes. To test this sterilized halibut meal was inoculated with a few drops of liquid from decomposing, unsterilized meal. After 48 hours the filtrate from this was compared with a 48 hour filtrate from unsterilized meal. In each case the lethal dose was 0.4 grm. per litre,- indicating that the mixed, natural flora were still capable of producing toxic products from meal even after its sterilization.

The three facultatively anaerobic cultures (2, 8, 12) were inoculated into halibut meal infusion under paraffin oil seal. The infusion was brought to boiling just before inoculation in order to drive off dissolved oxygen. Filtrates after 48 hours from these cultures killed fry in 24, 36 and 29 hours respectively, that is, in about the same time required by uninoculated sterile meal filtrate. Anaerobic growth of these three single species is apparently not responsible for formation of poisonous substances comparable to those produced by a mixed culture.

It is apparent that we have here a case of synergism, where the combined action of a number of microorganisms results in an end-product which cannot be formed by any of the species when acting alone. From the point of view of the retaining-pond problem, it is important to know whether the poisonous end product is the result of the action of aerobic bacteria alone, or the result of combined aerobic and anaerobic action. To investigate this, halibut meal mixed with the usual proportion of water was allowed to decompose in a deep vessel (Pyrex 200 c.c. cylinder, diameter 5 cm., height of fluid 10 cm.), and in a shallow layer, about 1 cm. in depth, in a battery jar. These were left at room temperature for 48 hours, and the filtrates tested against fry. The

Dose of filtrate per litre representing - grams meal		1.6 gm.	.80 gm.	.40 gm.	.08 gm.
Filtrate from deep cylinder	Time of death	22 hr. 3 dy.	24 hr. 4 dy.	48 hr. no deaths	48 hr. no deaths

fluid in the cylinder was heated to drive off dissolved oxygen, and was inoculated with 1 c.c. of fluid from previously decomposed fish meal, in case any of the organisms had been killed by heating. The shallow layer was of course not heated, but was inoculated, as a control measure, in a similar manner to the cylinder. This single experiment gives a definite suggestion that anaerobic decomposition ("putrefaction") may bear a part in the production of the toxic substance. This conclusion removes still further the possibility that these decomposition products are of practical significance in hatchery practice, since conditions in troughs and retaining ponds are quite definitely aerobic.

Pathogenicity of Bacterial Species

Fry in battery jars were exposed to a heavy dose (1 c.c. #4 McFarland suspension) of each of the bacterial species isolated. Two methods of dosage were used. The culture suspension was added directly to the water of the experimental jars; culture was also mixed directly with the liver fed to the fish, so that a proportion of the dose would be sure to reach the intestinal tract. Exposure to this dose was continued intermittently for two weeks. None of the species appeared to cause the least discomfort to the fry. Direct inoculation of fry with cultures was not attempted.

Discussion

While a substance, due to the decomposition of halibut meal under laboratory conditions, has been shown to be highly toxic for sockeye fry, a consideration of conditions as found in the Smith Falls retaining ponds shows that

(a) this toxic substance is not likely to be produced from halibut meal under hatchery conditions;

(b) even if it could be produced, it would never be present in sufficient concentration to have any effect, either immediate or cumulative, upon sockeye fry.

It would thus seem that the presence of diffusible substances from halibut meal, in the water of ponds or troughs, is not a likely factor in contributing to diseased conditions among the fry. Moreover, while the experiments on infection of fry by means of the different cultures isolated from meal are not quite complete, in that the fry were not held for a very long period after infection, these experiments at least point out that no very highly pathogenic bacterium is contained in the fish meal. The fact that the toxic substance produced in the laboratory is the result of the combined action of many microorganisms, rather than the work of one, and is formed equally well in whole meal and in ether-extracted meal, removes it from the class of bacterial toxins, and suggests that it is possibly some intermediate product of protein decomposition. It must be understood that the above experiments in no way rule out the possibility that halibut meal, when actually ingested by fry, exerts a harmful influence. Whether this influence is due to lack of vitamins or accessory food substances in the meal, or is due to some directly toxic principle, is matter for another investigation.

MEASUREMENTS OF SMITH FALLS SOCKEYE FRY SAMPLES

	Supply Trough Sampled July 1/31		Supply trough Sampled Aug. 19/31		Pond A Sampled Aug. 19/31		Pond B Sampled Aug. 19/31	
	Length	Weight	Length	Weight	Length	Weight	Length	Weight
	37 mm.	1.58 gm.	60 mm.	3.27 gm.	52 mm.	2.26 gm.	56 mm.	2.51 gm.
	35	1.47	52	2.31	53	2.25	57	2.72
	35	1.46	56	2.34	53	2.28	54	2.26
	34	1.42	51	1.80	45	1.43	56	2.30
	37	1.60	48	1.70	58	2.82	55	2.38
	36	1.50	57	2.73	52	2.22	52	1.94
	36	1.52	56	2.70	47	1.50	47	1.65
	35	1.48	53	2.27	50	1.81	57	2.75
	36	1.48	53	2.30	53	2.22	45	1.44
	36	1.53	48	1.89	53	2.17	50	1.90
	38	1.61	52	1.94	57	2.55	50	1.80
	34	1.44	48	1.68	53	2.20	56	2.68
	33	1.40	54	2.30	56	2.48	51	1.90
	36	1.48	48	1.70	52	2.05	50	1.78
	30	1.07	52	2.24	51	1.83	54	2.37
	32	1.39	54	2.24	57	2.58	53	2.27
	35	1.49	54	2.35	52	2.10	61	3.27
	35	1.44	47	1.73	58	2.78	54	2.43
	34	1.45	52	1.99	50	1.77	55	2.36
	36	1.55	51	1.88	55	2.32	59	2.98
	36	1.50	51	1.89	57	2.57	53	2.32
	33	1.44	53	2.40	55	2.35	53	2.27
	35	1.52	58	2.82	50	1.87	55	2.39
	34	1.46	48	1.67	58	2.75	54	2.42
	35	1.50	50	1.85	46	1.43	45	1.42
	37	1.56	52	1.96	56	2.48	51	1.98
	34	1.46	50	1.75	55	2.37	55	2.42
	35	1.48	51	2.00	52	2.16	51	1.91
	33	1.39	54	2.30	54	2.42	54	2.36
	35	1.53	59	2.86	58	2.90	53	2.30
Mean	3.5 cm.	1.47 gm.	5.2 cm.	2.16 gm.	5.3 cm.	2.23 gm.	5.3 cm.	2.21 gm.

APPENDIX TO REPORT ON HALIBUT MEAL INVESTIGATION

The experiments recorded above indicate definitely that diffusible poisonous substances from halibut meal and other hatchery foods are not responsible for diseased conditions in sockeye fry. This leaves two other possibilities open to investigation, having in mind the fact that heavy losses have actually been occasioned in retaining-ponds: (a) poisoning of the fry by direct action of ingested halibut meal, and (b) the causation of disease directly by parasites of pathogenic microorganisms.

As stated before, complete evidence was not obtained that the August, 1931, outbreak of disease in the Smith Falls ponds was due to Costiasis, although findings very strongly suggested this conclusion. During the summer of 1931, fry which had died from poisoning, as well as samples of normal fry from different ponds, were examined at least every two weeks for the presence of the <u>Costia</u>-like epithelial parasite found during the 1930 epizootic. Scrapings from gill surfaces and sides were used for this purpose. In no instance throughout the summer was any protozoon found resembling <u>C. necatrix</u>, even at the time of year when the 1931 outbreak had occurred. This still further indicates that the organism found during the epizootic must have been parasitic in nature, and not a saprophyte natural to the water.

During the summer of 1931, a number of yearling crosses, kept in a retaining-pond, became emaciated and inactive. The gills were generally covered with a growth of <u>Saprolegniaceae</u> and of bacteria. Numbers of the fish so affected died. Few or no deaths occurred among the healthy, clean-gilled fish in the same pond. A number of the emaciated fish were found to be without operculum on one or both sides, allowing a maximum condition for gill damage. But a number of fish also died in an emaciated, gill-molded condition, where the operculа were complete. Examination of all emaciated fish showed the one feature they had in common to be the presence, chiefly in the anterior intestine, and to a less extent in stomach and caeca, of an actively motile, pear-shaped protozoon. The organism was also found in one out of five apparently healthy fish taken from the same pond. Stained preparations showed that the parasites were of the genus Octomitus. Small forms of apparently the same protozoon were found in the gall bladder in several cases.

The presence of the organism, chiefly in the anterior intestine, and its definite place in the genus Octomitus, would at least suggest it to be Octomitus salmonis, the well-known parasite of trout. The fact that such species of protozoa are found in fish, under retaining-pond conditions, would suggest that a study be made of the normal protozoan fauna of the salmonids. In the case of future epizootics, one would then have a firmer basis for deciding upon the pathogenic or non-pathogenic characteristics of parasite found in the fish during the course of the disease.

REFERENCES

Chaddock	Principles & Methods of Statistics

Davis, H.S.: Bureau of Fisheries Doc. No. 988
"Octomitus salmonis, a Parasitic Flagellate of Trout".

Moore, Emmeline: Report of Bureau of Prevention of Stream Pollution, 1922.
"Diseases of Fish in State Hatcheries".

Wenyon, C.M.: Protozoology, Balliere, Tyndall & Cox, 1926.