Yolk-sac Dropsy in Newly Hatched Trout.

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This paper is a record of observations made in the hatcheries of the North Canterbury Acclimatisation Society at Christchurch, New Zealand, during the hatching seasons of 1928 and 1929. The Society kindly granted facilities for this study, and the author here wishes to record his thanks. Thanks are also due to the curator (Mr. D. Hope) for his most courteous assistance in many tedious observations. For the most part *S. fario* was used, and conclusions are meant to refer to *S. fario* in particular, although our experience in regard to this condition is that both *S. irideus* and *S. salar* differ in no fundamental respect.

Normal Development.

Newly-hatched trout absorb both yolk and the surrounding membranes or sac in about eight weeks from the time of fertilization, or 38 days from the time of eyeing, and 21 days from the time of leaving the egg. When the yolk is absorbed the fish rise to the surface and begin to feed. Until then they are entirely dependent on the food materials in the yolk together with water, salts and oxygen from the environment. The water in the hatching boxes in the Christchurch hatchery is aerated by falls between the upper and lower tiers of boxes and by an open race and fall from the artesian well-source to the upper tier. The temperature is maintained at a remarkably constant level, namely 54°F, during the whole period of hatching. In this paper, the term *alevin* means trout which have not yet absorbed the yolk, and the term *fry* will imply that the yolk has been completely absorbed.

Yolk-sac Dropsy.

Absorption of yolk and the surrounding membranes or sac, fails to occur in some of the fish, and this condition is variously known as *blue-swelling, blue-sac, hydrocele embryonale,* and *yolk-sac dropsy.* The latter term will be used in this paper. In this disease, the space between the inner and outer membranous coverings of the yolk becomes filled with opalescent fluid which gradually increases in amount until the bulk of the fluid-holding sac or *hydrocele* becomes as great as or even much greater than the yolk itself. Towards the fatal termination of the disease it is obvious that the yolk has not been absorbed as rapidly as in normal fish, but in the earlier stages this failure of absorption is not a prominent feature. Sooner or later, all the alevins which have any considerable degree of swelling die, but whether because of the mechanical disability of the dropsy, or for some reason of toxicity, is not known. The opinion of most hatchery curators in New Zealand is that all the fish which contract the disease die in the alevin stage, but our experience is that only a small proportion of affected fish develop the hugely swollen sac, and only these latter fail to reach the fry stage. The largest death-rate that author has seen is no more than one per cent. of the total hatching. Several curators, however, say that in some seasons as many as thirty or forty per cent. will die of the disease. Yolk-sac dropsy is apparently well-known in other parts of the world, but as to its percentage incidence elsewhere no information has been available to the author. The only printed account of the disease at hand is contained in the text-book "Handbuch der Fischkrankheiten," by Dr. Bruno Hofer. The substance of Hofer's account is as follows:—

> The disease appears sporadically; the first symptom being an enlarged sac, which, after a time, becomes so heavy that the fish are unable to rise to the surface. After a few days the sac usually bursts, resulting in the death of the fish in the first week after hatching. Many think that the disease is due to shock or pressure in the ova at stripping. The use of female fish that are too young is also held to be a cause, but without evidence. Von Betegh made a study of the disease and concluded that it is due to infection by a specific organism. He isolated a diplo-bacillus in pure culture in the contents of the sac and proposed to name it *diplo-bacillus liquefaciens piscium.*

In some respects Hofer's very brief account differs from our experience at Christchurch. We do not see marked cases in the first week. The second week is the time when the disease becomes noticeable, but the development of large sacs is only gradual. The author does not know whether the sacs are heavier than the rest of the fish or lighter, but any change in density would probably be in the latter direction, unless salts had been absorbed from the hatching water. It is not common to see diseased alevins burst. Although the author has seen thousands of cases he has not seen this. Again, our experience is that the fish appear to be surprisingly little distressed by the burden of their sacs. They move freely on the bottom with their normal companions, even in the more turbulent head waters of the hatching boxes, and this vitality is retained until the last few days. Comparative counts of the incidence of the disease in top-half and bottom-half of the race gave practically identical percentages. That they should be able to live for several weeks with this bulky impediment to movement suggests that the disease is not an intensely toxic process and that death is in the end due to a slowly progressing impairment of vital organs. When one pricks the sac with a needle the fluid escapes under pressure, but all the cases so treated by the author have died within one to three days.

Suggested Causes.

When little of fundamental importance is known about a disease the alleged causes and cures are correspondingly numerous. In New Zealand, overcrowding, deficiency of earth, lack of aeration, poor quality of parent fish, injury in stripping, irritative action of radium emanation, have all been regarded as causes by various observers.

That the disease is due to some abnormality in parental material or environment or both together, is obvious enough, but it is as yet impossible to say which will finally be blamed. Parental material will be cleared of suspicion only when all ova, from whatever source, can be induced to show the disease in response to known and deliberate alterations of the environment; and environmental causes will be discounted when all or some of the ova of certain fish show the disease no matter what variations of hatching water they are subjected to. But it may turn out that water itself unable to produce the disease in all ova is provocative only when presented with a certain kind of fertilised egg. A problem of this sort with so many possibilities which could only be eliminated, each in turn, by the most patient and laborious procedure would be most difficult of solution unless conspicuous features present attractive paths of exploration. It is the author's opinion that such clues are not likely to appear until statistical observation of the incidence of the disease is more thorough than hitherto.

Overcrowding.

This does not seem a likely cause in the Christchurch hatchery since, although derived from a poorly oxygenated artesian source, special means are taken to add air to the water. But overcrowding as a cause might mean something different from lack of oxygen. It might mean, for example, that waste products accumulate in the neighbourhood of the ova or fish, or that non-gaseous materials in the environment normally taken in by the fish are reduced by overcrowding to ineffective concentrations. When experimental overcrowding fails to produce a conspicuous crop of dropsies this line of investigation appears sterile. In the local hatchery one finds no evidence pointing to overcrowding as a cause, although a direct negative will not be possible until it is shown that no ingredient in the environment is tending to run short and no accumulation of waste products occurs. The water here is fairly well buffered, and by colorimetric methods the author could find no alteration in hydrogenion concentration when the entering flow and samples that had passed over 32,000 ova alevins were compared. This, of course, is due to the large volume of water flowing down the boxes, since the fish are actively respiring and adding carbon dioxide to the water, but in amounts too minute to estimate in these conditions. Alevins, however, are wont to collect together in large swarm masses, and it is conceivable that some of the innermost fish are subjected to unfavourable conditions sufficient to account for the cases of dropsy. When we placed, in the 1928 season, three-quarters of the usual number in one half-box and one-quarter in the other, the counts of marked cases of the disease turned out to be practically identical, namely, 1.3 per cent. for the former, and 1.4 per cent. for the latter. This, however, is not a clear proof, since the scattering of the fish was much the same in both boxes and the smaller number of fish appeared to make as dense a swarm mass (but less spread out) as the larger number. Probably a better way would be either to divide a box into many sub-divisions, or to try the effect of a number of pebbles as isolated swarm centres. The tentative conclusion of this observation, however, is that the ordinary percentage of yolk-sac dropsy is not attributable to overcrowding in the conventional sense. This leaves open the question whether an enormous overcrowding would produce exceptional percentages of the disease; but as will be seen later, the exceptional percentages (thirty or forty) actually found by some curators were not accompanied by exceptional overcrowding.

Lack of Aeration.

This aspect cannot be separated from the question of overcrowding, since it is part of the presumed inner meaning of the term. A number of New Zealand curators think that yolk-sac dropsy is due to deficient aeration, but no systematic estimations of the oxygen and carbon dioxide content of the local hatching water has been done except by Farr (1) and Farr and Florance (2) on the hatchery water in Christchurch about twenty years ago.* At that time the boxes were arranged and stocked differently from now, although the artesian source is the same in both cases. Quoting these figures a comparison of the top box with the bottom shows:—

TOP BOX. C.C. per Litre		BOTTOM BOX. C.C. per Litre.	DIFFERENCE.
Nitrogen, Argon } etc.	16.82	15.98	0.84
Oxygen	5.34	5.68	0.34
Carbon Dioxide	1.80	1.88	0.08
Temperature	12.7°C		

While it may be doubted whether the increase in carbon dioxide was detectable by the methods used, it is clear that rippling and falling from box to box removed an appreciable quantity of nitrogen and added oxygen at the same time.

The River Avon at Christchurch gave the following figures:—

Nitrogen, Argon } etc.	15.36 C.C. per Litre.
Oxygen	7.74 " " "
Carbon Dioxide	1.30 " " "
Temperature	11.4°C.

These figures are just about the ordinary saturation values at N.T.P.

*Since the above was written, the Department of Internal Affairs have supplied me with the following figures for the Tongariro Hatchery:—

p H value	6.8		
	Per Litre.		Parts per 100,000
Dissolved CO_2	4.48 cc.	Total Solids	12.9
Dissolved Oxygen	6.54 cc.	Volatile & Organic Matter	2.3
		Chlorine as Chlorides	0.82

In this hatchery, the death rate from dropsy is no more than 0.2%. Only Rainbow trout are hatched.

Taking the Avon figures as 100, the gas content of the two boxes is represented as follows:—

TOP BOX.		BOTTOM BOX.
Nitrogen } etc. Argon }	109.5	104.0
Oxygen	69.0	73.4
Carbon Dioxide	138.5	144.6

Note that carbon dioxide includes all the gas given off by boiling, i.e., both free and that combined with base are included.

The fact that special procedures are necessary to aerate artesian water to its full capacity suggests that deficiency of oxygen might cause yolk-sac dropsy. It should be noted, however, that the quantity of oxygen present is enormously more than could be used up by the developing eggs or alevins, and it will be seen that the water in the lowermost tier of five boxes increases its holding of the gas in spite of the fact that it flowed over many thousands of fish. To hypothecate an unfavourable effect one would have to assume that a solution pressure of oxygen equal to about 70 per cent. of the maximum provides too small a gradient for adequate interchange at the lung surface. There are various general grounds for rejecting this assumption, and in particular the fact that the majority of the fish do not contract the disease; and even if the assumption were true it would be true only for certain ova and alevins and not for others. In 1929 the author aerated a box of *S. fario* by special means but the results did not favour the idea that deficiency of oxygen was here a cause of the disease.

Counts of severe cases only:—

Specially aerated Box	1.2 per cent.
Non-aerated Box	1.0 per cent.

This experiment was done on pond fish, whose ova, according to some observers, are said to be particularly liable to give a high incidence of yolk-sac dropsy. As a final comment, it may be added that yolk-sac dropsy is not limited in New Zealand to poorly-aerated artesian water but occurs in all, or practically all, of our hatcheries, although most of them are supplied from small and presumably well aerated streams.

Radium.

It will be noticed in the gas analyses just given that Farr and Florance found that nitrogen and argon are present in the Christchurch artesian water in greater amount than in river water. With these inert gases is associated a considerable quantity of radium (1 and 2), and, like them, the emanation is decreased in amount by falls and ripples. In a series of five hatching boxes at the hatchery the quantity dropped from 126 arbitrary units at the top to 69 units at the bottom. It was also found by Farr and Florance that death in the ova stage and yolk-sac dropsy in the alevin stage were proportionately reduced as shown for the latter in this table:—

BOX NO.	PERCENTAGE OF FISH DEVELOPING BLUE-SWELLING.	RADIUM EMANATION.
1	19.5	126
2	13.0	111
3	15.4	95
4	13.0	83
5	13.0	69

For death of ova the decreases were much more nearly parallel. Farr and Florance, therefore, were of the opinion that radium emanation might be the destructive agent, but in 1909 they had no supply of radium which would permit them to add emanation to the hatching boxes to test out the theory. It will be noticed that their percentages of dropsy are much higher than the author has so far recorded. Presumably Farr and Florance counted all degrees of the condition. As is later shown in this paper the percentage incidence varies at different times, so that it was found desirable to compare only those which were so badly affected that they were almost certain to die of the disease. Since 7,500 ova went to the boxes used by Farr and Florance it is possible that the alevins were not all of the same age. The figure 13% is noticeable, as it occurs in the second box, although the quantity of emanation is relatively little reduced. In 1928 the author obtained a supply of emanation from the radon plant at Wellington Hospital for the purpose of testing out the theory of emanation as a cause of dropsy. The supply was obtained fresh each week and capillary tubes bearing the gas were broken each day deep in a twenty litre vessel of water which delivered by continuous drip into the head of a hatching box during the whole time of hatching and development right up to the fry stage. By a siphon attachment the emanation container was kept full of water so that no loss occurred except that due to decay of radio-activity. The emanation in a control trough was estimated by a gold leaf electroscope as $1.5 \times 10\text{–}12$ Curie; whereas in the trough into which emanation was delivered there was always from one hundred to one hundred and fifty times this amount. In spite of this great increase as compared with normal troughs, the death rate in the egg stage was unaltered and the percentage of alevins suffering from yolk-sac dropsy was practically identical, namely:—

(1) Ordinary hatching boxes .... 0.96 per cent.
(2) With emanation added .... 0.92 per cent.

It seems conclusive that emanation cannot be an ordinary cause of yolk-sac dropsy. This is the result one would expect when it is recalled that most of New Zealand hatcheries are supplied from small open streams where emanation could not be in such concentrations as occur in Christchurch but where dropsy is just as prevalent as with us.

Earth and Iodine.

For twelve or thirteen years the local curator (Mr. D. Hope) has added a slush of garden earth to the hatching boxes each morning, from the time the ova hatch right up to the rising of the fry. Before he commenced to do this great losses due to dropsy were occurring, amounting sometimes to thirty per cent. or more. While he was in charge of the Mataura hatchery, prior to coming to Christchurch, the boxes there were often silted over by a fresh in the river and sometimes the ova were quite buried by the silt. Thinking this might prevent dropsy he tried the effect of adding earth to the Christchurch water and was delighted to find that he was never again troubled by the disease to the former extent. The death rate since then has never been more than about one per cent. He found that rich garden loam was the most effective, while sandy soil was ineffective, and light soils gave an intermediate result. This was in the old hatchery where the boxes were smaller and the ova more crowded than now. In 1928 the author proposed to investigate this medicative practice but an expected supply of ova not coming to hand the experiment was not fully controlled. A weak solution of iodine was, however, added to one box during the whole hatching period and counts showed that the iodised water had practically the same number of diseased fish as a box to which earth had been added. This raised our hopes that a clue to the origin of the disease had been found. The reason for trying potassium iodide was that endemic goitre is very prevalent in Christchurch and with it is associated a relative deficiency of iodine in the soil and water of the district. In the Acclimatisation Society's ponds about one per cent. of the three-year-old trout contract goitre, and our idea was that yolk-sac dropsy might also be a deficiency disease due to lack of iodine. Analyses of soil for iodine content made by the author was found to have an average content of 0.022 mgm. of iodine per 100 gm., dry weight, while sandy soil as used had none or a faint trace only. In 1929 the effect of soil and iodine was again investigated, but this time with adequate controls. The iodine was added by continuous drip into the top of a race using the same arrangement as had been used for emanation. The solution was a 0.5 per cent. potassium iodide and the administration was maintained from the time of arrival of the ova until the fry rose to the surface—eight weeks in all. Estimation of iodine gave the following results:—

(1) Normal ova—6.0×10^{-7}gm. (dry).
(2) Normal alevins—2.5×10^{-7}gm. (dry).
(3) Normal trough water—2.3×10^{-9}gm.

It appears from this that the ova are surrounded by water containing only about one thousandth as much iodine and that during the process of hatching some iodine is lost, although the difference here recorded seems excessive and merits repetition. In iodised water the following results were obtained:—

(1) Iodised water—2.4×10^{-6}.
(2) Iodised ova—3.0×10^{-7} (dry).
(3) Iodised alevin—4.8×10^{-6} (dry).

So that in spite of the water containing about one thousand times as much iodine as uniodised water the ova did not gain iodine but apparently lost a little. The alevins, however, were able to take in and hold eight times as much iodine as uniodised fish, but this extra content made no difference to the incidence of yolk-sac dropsy, as the following counts show:—

(1)	Iodised box	1.9 per cent. (severe cases only)
(2)	With earth but no iodine	3.1 per cent. (severe cases only)
(3)	Neither earth nor iodine	1.3 per cent. (severe cases only)

It will be noticed that the counts vary unexpectedly, but this is probably due to the stage of the disease in the particular box. The ova are added to the boxes as they arrive from the stripping pen and each box holds alevins of different hatching age. That the counts vary with this age is shown by the figures for the same boxes four days later:—

(1)	Iodised box	0.9 per cent. (severe cases only)
(2)	With earth but no iodine	1.4 per cent. (severe cases only)
(3)	Neither earth nor iodine	0.7 per cent. (severe cases only)

Since few fish had died in these boxes it appeared that half of the alevins had recovered. This recovery was well illustrated in several boxes, of which the following is typical:—

(1) Second week after hatching—23 per cent. (mild cases)
(2) Fourth week after hatching—0.8 per cent. (severe cases)

While it is possible that a more meticulous examination would show even greater percentages, in general one found nearly thirty per cent. of mild cases in the early days, while at the end almost the only fish in which abnormality could be detected were the severe cases which would undoubtedly die of the disease. Counting mild cases is a most fatiguing task, and one has to look intently to determine which show or do not show excess of fluid in the yolk-sac, but the percentage in which abnormality is detectable steadily diminishes as the fish approach the fry stage.

To count the severe cases, weighing the fish in hauls was adopted, instead of directly counting the whole population. The conclusion is clear: that deficiency of iodine plays no part in the ordinary causation of the disease. As to the iodine-holding powers of ova and alevin, it is a point of interest to consider how the iodine might get into the body of the fish. At this stage they are still dependent on the yolk and have not yet taken food in by the gullet. It appears possible, however, that they might make swallowing movements from time to time and so ingest both water and salts. Although the author has watched for this he has not yet seen it occur. Towards the latter stages, when they are near to rising, it is probable that they try experimental swallowing movements before the reflex chain which is soon to be used in snapping at food has been perfectly established as a going concern. On the other hand, it would not seem impossible for the gill to absorb water and salts at the same time as dissolved gases. Sumner (3) describes experiments in which carp lost or gained weight according as the gill was supplied with salty or non-salty water. A further possible port of entry is the skin, which may be more pervious in these stages than later on. It should be noted that normal alevins hold about 500 times as much iodine as ordinary trough water, while iodised alevins have about ten times as much iodine as the iodised water of this experiment. This means that either the ingestive organ must specially select iodine and not water and dissolved salts as a whole, or else the excretory organs have to do work to maintain the iodine concentration of the internal environment at such a low level as compared with the hatching water. The ingestive organ, may, of course, be the thyroid gland itself, which in the trout is below the floor of the mouth and closely associated with the gill. It may be of interest to quote here my estimations of iodine in the goitres of *S. fario* pond fish as no record of this having been done is known to the author. The iodine of eight goitres averaged out as 2.8×10^{-5} (dry weight), which is approximately 160 times as much as was held by the whole body of normal alevins. These third-year trout weighed on the average three thousand times as much as each alevin. Even allowing that iodine may have been distributed over the whole body of the adult fish and not confined to the thyroid gland, it looks probable that iodine occurs in greater concentration in young trout tissue than in old. The goitres in these pond fish were 4.3 per cent. of the total fresh weight.

Infection.

In hatching boxes to which no earth is added, moulds grow on the bodies of dead alevins. A certain number of deformed fish occur in all hatchings and these soon die, together with those fish which have died of yolk-sac dropsy. The growth of moulds is reduced by removal of dead fish and by the practice of adding earth to the hatching boxes. Microscopic examination of dropsical fish shows no parasitic growth of moulds. Altogether, the author felt from the beginning that infection was unlikely to be the prime cause of the disease. The further one investigated the more it seemed that some degree of dropsy was so frequent as almost to give the appearance of normality. Certainly an infection which is chronically present in our hatcheries and which takes several weeks to affect the behaviour of highly sensitive young alevins must present peculiar aspects of morbidity. But in view of Von Betegh's claim to have isolated a specific bacillus it was deemed important to search for it in New Zealand, since, while its discovery would leave open the question whether the dropsy had preceded or followed microbic invasion, on the other hand, its absence would weaken the probability of its pathological specificity in Europe. Two dozen alevins, therefore, were punctured and the sac contents smeared on microscope slides and stained by Gram's method, but no single bacillus or mico-organism was detectable in the smears. A broth culture showed only the ordinary water organisms, apparently as contaminations.

Sac Contents.

Hofer gives no description or analyses of the dropsical fluid, and the author has made only a few elementary observations. As said before, the bluish appearance of the material is due to the colloids, mostly of protein nature. On allowing some of the fluid to evaporate in air clear gelatinous non-crystalline plates result which, when water or saline is added, becomes milky white and do not go readily into solution. Most of the protein is coagulable by heat and appears to be a mixture of serum albumen and serum globulin. Glucose and chlorides are also present, but quantitive comparisons with non-dropsical tissues have not been made. In the case of salts this, as well as the freezing point of the fluid, may be of interest, since osmotic effects seem to be involved.

Other Causes.

Curators in New Zealand are inclined to believe that the percentage of dropsy varies with different parent fish; and, as would be expected, attribute the abnormality to females rather than males. There is an idea abroad that fish in poor condition produce an excessive proportion of dropsies, but no evidence is known to the author. Injury in stripping and in transport has also been suggested, but again the evidence is in doubt. One would expect injury at stripping to produce death in the egg stage rather than dropsy six or seven weeks later; and it is to be remembered that the alevin is biologically remote from the ovum.

General Discussion.

Unless yolk-sac dropsy is studied from the point of view of the ionic arrangements which maintain and disturb the normal balance of osmotic forces in the tissues the clue to causation is likely to be stumbled on only by accident. In this case the membranes surrounding the yolk appear to have an increasing difficulty in maintaining osmotic equilibrium except in conditions which finally are incompatible with life processes in the cell. The dropsy could result from a mechanical blockage of the venous or lymph return, although examination with the microscope has failed to show any mechanical deformity. In human beings, excess water may be retained in the tissues in heart and kidney disease, but even here the water-logging cannot be simply a mechanical result like a break in a water-course, since that would not increase the total volume but merely shift the fluid from one part to another. Whatever the original departure from normal, the organised life-units, probably proteins, have acquired supernormal water-holding powers. Many workers agree that local alterations in the concentration of hydrogen-ions is an effective agent in making colloids more hydrophilic. Often, the local accumulation of hydrogen-ions is a result of deficient oxidation as when lactic acid accumulates in isolated muscle which is too poorly oxygenated. All vertebrate tissue fluids hold a rich assortment of substances which are available for automatic regulation of the internal medium, but the manner of this regulation is for the most part obscure and presents thorny problems for physical chemistry. It is not understood, for instance, why excess of sodium in tissues causes retention of water, while excess of potassium fails to do so. Again, young tissue is more water-holding than senescent tissue, yet young tissue has an intenser oxygen-use than the old. In the case of yolk-sac dropsy it is to be remembered that not only is water increasingly retained together with easily diffusible substances like chlorides and glucose, but colloids, like serum protein, are also held in the sac. There seems to be either an excessive permeability of capillary or lymphatic walls on the entrance side or a deficient permeability on the drainage side. But since the same membrane probably does both at the same time the separation of permeability from that of hydrophilic properties is artificial and misleading. Like the water-holding power of colloids, permeability of living membranes is generally regarded as varying with local oxidation and local concentration of hydrogen-ions, but again our knowledge of what actually occurs is inadequate. With oxidative processes one has to remember that concomitant reductive processes are inevitable and the one phrase must be held to include the other. Turning again to the oedema problem in these young trout, it is of interest to note that the condition is local, being prominent in the yolk-sac and not affecting the whole of the body. One imagines that unusual structural changes go on here since, unlike the rest of the fish, its tissue, though young, is shrinking in size. The area of the external sac-membrane is greater than that of the skin which will at first replace it. This regressive process is superficially like a local senescence in which we are accustomed to expect oxidative difficulties. Again, the yolk-sac is the most dependent part of the body, and is, therefore, a collection point for gravity drainage. Possibly, too, the digestive processes on the far side of the inner membrane, which must precede the assimilation of yolk, may have something to do with the limitation of dropsy to this area. The processes which give excessive changes in volume are generally irritative or injurious in nature, although these are no doubt quantitative excesses of what occurs in normal stimulation and response. Irritation of various sorts produces changes in the power to hold water, for example we have swelling in surface tissues as the result of sun-burn, or following a flea-bite, or pleuritic effusion following an infection by pus-forming bacteria. But however various the origins, in the end these new conditions must converge on and use the same mechanisms. It is conceivable that the digestive enzymes which are busy transforming the yolk act also as irritants to the membrane surrounding the yolk. This membrane would need to have a structure resembling the alimentary lumen to withstand intense digestive action. Whatever may be the local factors in this case one has to picture dropsy in general as a condition against which the normal organism is always with more or less difficulty on the defensive. That it occurs no more often is a tribute to the vigour of the adjustment. Most of the slightly affected dropsical fish recovered. Is this because the developing fish passes a crisis by evolving new structures which can transport or use the adjustive material already present in the ovum, or are certain necessary ingredients deficient in the egg now made available from the environment? These latter could only be diffusible salts or radiations such as light. Most tempting is the idea that the critical period for the new hatched trout is when the endocrime organs are not yet in a state of normal balance. Insulin and thyroxin are both known regulators of water balance in the mammalian organism, and no doubt they, with other autacoids, are potent in fish life also. Interesting observations probably lie along this line. At the same time, minute attention might be paid to differences in the ova themselves. Each ovum differs in its genetic constitution from all ova, but with the naked eye few differences are visible. They vary somewhat in bulk and colour and in thickness of the shell. According to the local curator thinner shelled ova burst before development has proceeded sufficiently far, and such alevins die. An attempt to separate ova into groups based on bulk or density might repay the trouble. The author, having no records on the changes in density during development of the egg, made some observations in 1929, but the results are as yet incomplete. As to the affected alevins, the author was unable to detect any constant stigma. Some were big, others small, some light yellowish green, others much darker, and all variations between seem to occur. If light had been an irritative cause of dropsy one would expect the colouration to present constant features. Among the lines of investigation one would like to look for the disease in other species and genera of fish. If it should turn out that the Salmonidae are singular among fishes in this respect one would be tempted to believe that a disproportion between the salt content of the upstream waters and the ova was playing a part, this again being a consequence of the evolutionary history of the species. In human embryos a condition of excessive ammotic fluid is well-known but the text-books give no information as to the cause. Altogether, one is inclined to believe that yolk-sac dropsy involves fundamental and not accidental processes and that inquiry must be deeply penetrative to give understanding. The problem of recovery from the disease is as interesting and important as the problem of causation.

Conditions at Christchurch Hatchery, 1929.

For the use of other investigators the fullest data available are here given, since it is possible that comparisons may automatically exclude false clues.

(1) Dimensions of boxes, 10 ft. $\times$ 10 in. The water is 7 in. deep.
(2) Rate of flow per box, 80 gallons per hour.
(3) Number of ova—8,000 per tray. Two trays per box.
(4) Temperature—12.7°.
(5) Gas content (assumed—see previously).

Nitrogen, Argon etc.	15.98 c.c. per litre.
Oxygen	5.68 c.c. per litre.
Carbon Dioxide	1.88 c.c. per litre.

(6) Radium emanation, 1.5 $\times$ 10^{-12} C (Milligan and Rogers 4).
(7) Reaction: pH equals 7.36.
(8) Iodine Content—2.3 $\times$ 10^{-9}.
(9) Water, Artesian bore 75 feet deep with content believed to be closely similar to the following figures which have been supplied by Mr. Geo. Gray, late lecturer in chemistry at Canterbury College. Mr. Gray's figures refer to a well deeper than the one used in the hatchery.

Total Solids 83	Volatile—18; Non-Volatile—65	Silicic Acid	20
Hardness 33.1		NH_3	Nil
Chloride 4.7		Albuminoid Nitrogen	0.01
P_2O_5 Nil		O_2 required to oxidise organic matter	0.01

All figures are in parts per million.

The chloride of our artesian supply is noted as being intermediate between that of the small Selwyn river (12.0) and the large mountain-derived Waimakariri (3.0), both streams being close to Christchurch. As regards hardness the Waimakariri is 26.0 at Courtneys and the Selwyn 31.2 at Coe's Ford. Trout are plentiful in both of these rivers but the reputation of the Selwyn as a trout stream is conspicuous all over New Zealand. It is from the Selwyn that the local Society gets its ova. The Selwyn discharges into the large brackish Lake Ellesmere, which abounds in natural trout food. If one suspected deficiency in salts in the environment to be a cause of dropsy one would not expect to find it in ova whose parents lived in the salt rich Selwyn. No figures of the proportions of the bases are available.

Questionnaire.

In October, 1929, the author circulated the following questionnaire to all the twenty-seven Acclimatisation Societies in New Zealand. Some of these did not hatch trout, but sixteen replies are available for summary:—

(1) Does yolk-sac dropsy occur in your district?
(2) (a) What percentage contract the disease?
(b) When does it first show and reach a maximum?
(c) What percentage die of it?
(3) Does it affect both brown and rainbow and salmon?
(4) What is the nature of the water supply in your hatchery? (Is it spring, artesian or river?)
(5) Have you any analyses of the gas and salt content of the water?
(6) What is the temperature of the water?
(7) What are the dimensions of the boxes?
(8) How many ova per box?
(9) What is the volume of inflow per hour?
(10) Where do you obtain your ova?
(11) Are ova from any particular source liable to contract yolk-sac dropsy?
(12) Do you know of any causes or conditions producing the disease?
(13) Do you know of any preventatives?
(14) Do you know of yolk-sac dropsy occurring in natural conditions, and if so, where?
(15) Do any other non-parasitic diseases of fish occur in your district (pop-eye, goitre, etc.?)

Replies Summarised.

(1) The disease was known in all the districts except one. The exception was Nelson, where, possibly, terminology was the difficulty.
(2) (a) 0.1 per cent. up to 4 per cent. Most about one.
(b) Shows on the average at about eleven days. Maximum is at four weeks.
(c) As in (a). Apparently they do not count minor grades.
(3) All three contract the disease. Three say that salmon is less susceptible.
(4) All kinds of water. Most are small streams.

(5) The Otago hatchery uses water of very low hardness. No other analyses are available.

(6) Most have variable temperature. The range is from 29°F to 58°F. The mean is 46°F.

(7) (8) (9) The number per cubic foot of water (not allowing for flow) varies between 3,000 and 8,300, but the two that are most crowded claim the lowest percentage of dropsy.

(10) (11) Ova from all localities have dropsy but the percentage varies from year to year. Three say that Southland ova (from Lake Te Anau) are the worst and they are inclined to believe that this is due to lack of attention in transport. The other thirteen replies do not blame locality.

(12) Causes are fairly freely suggested, sometimes confidently, such as:—
- (a) Locality (Southland up to 50 per cent. of deaths when transported)—three say this.
- (b) Pond fish—two say this.
- (c) Lack of aeration—two say this.
- (d) Injury in stripping—two say this.
- (e) Old fish—one says this.

(13) *Preventatives.*
- (a) Black earth—two say this. One says it increases the disease.
- (b) More aeration—two say this.
- (c) Salt solution—two say this.
- (d) Condy's Crystals—one says this.

(14) One says that he once saw in the Otapiri, Southland, two redds, side by side, in one of which were normal alevins and in the other several examples of yolk-sac dropsy. (The author unsuccessfully looked for specimens in the Selwyn district in 1929).

(15) Pop-eye in two districts, but no special incidence of dropsy.

Summary.

A number of alleged causes of yolk-sac dropsy in newly-hatched trout have been investigated, but without confirmation of any. The author inclines to the view that the disease is a quantitative accentuation of a process that occurs normally, and that excessive collection of fluid in the sac is the result of genetic differences in the ova.

REFERENCES.

(1) FARR, 1912. Effect of Gases on Trout, *Aust. Ass. Adv. Sci.*, vol. 13, p. 360.

(2) FARR and FLORANCE, 1909.
(a) Radioactivity of Artesian Water, *Trans. N.Z. Inst.*, vol. 42, p. 189.
(b) Influence of Ripples, *ibid*, p. 239.

(3) SUMNER, 1906. Effects upon Fishes of Changes in Salinity of Water, *Bulletin of Bureau of Fisheries, Washington.*

(4) MILLIGAN and ROGERS. 1928. Radium Emanation and Goitre, *Trans. N.Z. Inst.*, vol. 59, p. 389.