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Dove Marine Laboratory, Cullercoats

The Effect of Gas Content of Water on Larval and Young Fish

By

H. M. Bishai[1]

Mit 1 Abbildung

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1. Review of literature

The importance of the gas content of water and its effect on the survival and distribution of fish has been extensively studied especially in relation to fresh water fish. Whilst marine fish (except inshore and littoral) are subjected to comparatively small changes in gas content of the water in which they live, inland surface waters differ considerably in reaction in different localities and wide seasonal fluctuations occur in many rivers and bodies of fresh water.

Water in which fish live is either saturated, supersaturated or of a low oxygen content. In the first case it is favourable to fish life; in the last two cases it may not be favourable for maintaining fish populations.

Supersaturation (mainly oxygen and nitrogen) occurs in still water in localities where active photosynthesis takes place or when the temperature of the saturated water rises

Death of fish caused by supersaturation of water with gases has been observed by many authors (MARSH 1904, OSGOOD 1904, MARSH and GORHAM 1905; SHELFORD and ALLEE 1913; SEMPER (cited from HENLY 1952); ROTH 1922; PLEHN 1922; MRSIC 1933; WOODBURY 1941; ALF DANNEVIG and G. DANNEVIG 1950; ALF DANNEVIG and HANSEN 1952; HENLY 1952 etc.).

MARSH 1904 noticed that all adult marine fish died when the water was supersaturated with gases (nitrogen and possibly oxygen).

MARSH and GORHAM 1905 in their study of the gas disease attributed the death of fish with such disease to supersaturation, although gas disease may also arise as a result of decompression.

[1] Present address: Dr. H. M. Bishai, Khartoum, Sudan. University of Khartoum, Dept. of Zoology.

SEMPER (cited from HENLY 1952) noticed the presence of gas in the veins and muscles of fish kept in water of rich gas content. PLEHN 1922 found that trout died in ponds exhibiting high photosynthesis. He showed that the "gas embolism" occurring can be avoided if these fish were placed in normal water. WOODBURY 1941 recorded high mortality among several species of fish which was accompanied by extremely high oxygen content caused by phytoplankton (oxygen content 30—32 ppm.). He noticed the presence of "gas emboli" in the gill capillaries and gas bubbles in the subcutaneous tissue, and attributed death to the blocking of the circulation through the gills by gas bubbles with consequent respiratory failure. WOODBURY 1941 suggested that oxygen was the gas forming the bubbles. HAEMPEL 1928, 1937 assumed an increase in the oxygen content of water to be responsible for the death of young salmon.

MRSIC 1933 attributed the high mortality of trout larvae in his investigation to supersaturation with carbon dioxide.

Mortality of sea-fish larvae due to supersaturation of water with gas has been correlated with the presence of gas bubbles in the water. ALF DANNEVIG 1948 observed high mortality among herring larvae which he attributed to their having swallowed gas bubbles. Similar observations were made by KOTTHAUS 1939; SCHACH 1939; SOLEIM 1940; ALF DANNEVIG and G. DANNEVIG 1950; ALF DANNEVIG and HANSEN 1952 and HENLY 1952. These authors noticed the presence of gas bubbles in the intestine of herring and plaice larvae which they reared. Cod, however have a swollen swimbladder. Also gas bubbles attached themselves to the small fry and caused their death.

SCHACH 1939 succeeded in rearing herring larvae by eliminating gas bubbles from the water. This was achieved by an indirect water circulation.

ALF DANNEVIG and G. DANNEVIG 1950 attributed mortality of their reared fish larvae to supersaturation, and by using sand filters succeeded in rearing these larvae as this decreased the gas content of the water. The rearing of cod was not however successful and was only possible in deep aquaria.

ALF DANNEVIG and HANSEN 1952 pointed out that the "gas pressure" and not the "gas content" is the chief factor. HENLY 1952 emphasised the importance of the water pressure and oxygen concentration. The former authors concluded that larvae living in deeper water (10 metres) probably tolerate a gas content in the sea-water which would be detrimental near the surface.

The symptoms due to supersaturation which fish develop have been termed "gas disease" (MARSH and GORHAM 1905; HENLY 1952) or "gas embolism". The symptoms of this disease are the appearance of gas bubbles inside the blood vessels, tissues, fins, behind the eyes and even covering the body of the fish. Gas disease is not only caused by supersaturation of water with gas but is also caused by decompression (this is dealt with in a separate paper).

Many theories were put forward to explain the cause of this "gas disease" which can be briefly stated as follows:

1. Nitrogen excess (MARSH 1904; MARSH and GORHAM 1905).
2. Oxygen excess (PLEHN 1922, 1924, HAEMPEL 1928, 1937; WOODBURY 1941; HENLY 1952).
3. Carbon dioxide (MRSIC 1933).
4. pH variations.
5. Temperature variations.
6. Irritation caused by associated plants or compounds liberated from plants.

(The last three are cited from HENLY 1952 — no reference).

MARSH and GORHAM 1905 showed that although excess of air causes the gas disease, nitrogen chiefly if not solely plays the most important part in the disease, HARRIS *et al* 1945 concluded that carbon dioxide is the important factor in the initiation of the formation of bubbles in the early stages but which are then maintained by nitrogen.

PLEHN 1922, 1924; HAEMPEL 1928, 1937 and HENLY 1952 attributed gas disease to increased content of oxygen.

That fish can live under high oxygen concentration either at atmospheric or higher pressures (about two atmospheres) was shown by WIEBE 1931; 1933; and WIEBE and MCGAVOCK 1932. These authors concluded that no harm is likely to result from any ordinary excess of oxygen (up to 250 per cent air saturation) as long as this was not associated with a total pressure of dissolved gases exceeding the hydrostatic pressure. WIEBE 1931, 1933 has shown that increases of oxygen dissolved in water was accompanied by slowing down of the respiratory movements and observed no instance of exophthalmus, opaqueness of the lens or gas bubbles. WIEBE and MCGAVOCK 1932 concluded that certain sizes of several species of fish can survive indefinitely at high oxygen tension (250 per cent).

PLEHN 1924 attributes the occurrence of exophthalmus to supersaturation of blood with oxygen.

HAEMPEL 1928 however maintained that excess of oxygen was fatal to fish. He noticed one case of opaque lenses, but no sign of exophthalmus or gas bubbles. Using Salmonidae he noticed that they end by a marked increase of respiration (dyspnoea). This author showed that young salmonids soon turn on their backs and pass into a condition of paralysis "oxygen Narkose". He concluded that young salmonids were more sensitive to excess oxygen than adults.

MRSIC 1933 attributed the gas disease appearing in trout to excess of carbon dioxide.

SHELFORD and ALLEE 1913 attributed the occurence of gas bubbles to excess of gas dissolved in the water (1 to 2cc/litre of both oxygen and nitrogen). Curing the diseased fish was done either by bubbling oxygen or nitrogen in the water in which the fish lived. They stated that there is "no suggestion that the fish develop the disease as a result of a simple increase of gas when one gas is displaced by another, under one atmosphere of pressure, but rather the disease appears only when the gases are so much in excess that bubbles collect on any rough or warm object in the water".

HENLY 1952 maintained that the gas disease appearing among sea fish larvae (cod, plaice, herring etc.) originates from the oxygen concentration and water pressure obtained under experimental conditions.

The effect of low oxygen content on the survival and distribution of fish was extensively studied both under natural and laboratory conditions (KUPZIS 1901; PATON 1902, 1904; PACKARD 1907; WINTERSTEIN 1908; GARDNER and LEETHAM 1914, 1914a; POWERS 1921, 1922a; GARDNER and KING 1922; PLEHN 1924; THOMPSON 1925; GARDNER 1926; JOHANSEN 1927; GUTSELL 1929; HALL 1930; ROOT 1931; SOUTHGATE 1933; WIEBE *et al* 1934; TOMLINSON 1925; SEARS 1936; HUTCHINSON 1936; ELLIS 1937; WILDING 1939; SCHACH 1939; MOORE 1942; PRIVOLNEV 1947; GRAHAM 1949; MOOKERJEE and BHATTACHARYA 1949; GANAPATI *et al* 1950; FRY 1951 and many others).

Low oxygen content occurs in nature from many causes e. g. pollution; organic decay; ice covering; high consumption of oxygen by aquatic animals in a large population etc. In many cases the polluting substance is not in itself poisonous to fish but

during its decomposition a great reduction of dissolved oxygen in the water takes place, thus causing high mortality of fish (SOUTHGATE 1933).

JUDAY and WAGNER 1909 attributed the absence of oxygen from the lower layers of some lakes to decay of organic matter (which partly depends on the amount of plankton).

The depletion of oxygen resulting from the formation of ice on the surface and its effect on fish distribution was observed by THOMPSON 1925 and JOHANSEN 1927.

From the extensive studies on the effect of dissolved gases on fishes and their need for oxygen which have been made it is difficult to arrive at the amount of the minimum concentration of dissolved oxygen which is necessary to support fish life. This amount depends on many factors i. e. Species (SHELFORD and ALLEE 1913; GARDNER *et al* 1922; KROGH and LEITCH 1919; HALL 1930 etc.); alkaline reserve of the blood (PACKARD 1907; BIRGE and JUDAY 1911; WELLS 1913; MCCLENDON 1917; KROGH and LEITCH 1919; HAGGARD and HENDERSON 1919; POWERS 1922a; POWERS and LOGAN 1925; ROOT 1931; FRY and BLACK 1938; HALL and MCCUCHEON 1938; BLACK 1940); Age and Size (LOEB 1894; PACKARD 1907; PATON 1902, 1904; WELLS 1913; SHELFORD 1918; WIEBE *et al* 1934; SUMNER and DOUDOROFF 1938; MOORE 1942; PRIVOLNEV 1947; SOUTHGATE 1948); temperature (GARDNER and KING 1922; GARDNER *et al* 1922; PLEHN 1924; THOMPSON 1925; GARDNER 1925; MOORE 1942; FRY *et al* 1947; GRAHAM 1949; SOUTHGATE 1948 etc.); acclimatisation to low oxygen tensions (GUTSELL 1929; SOUTHGATE 1948; GRAHAM 1949; SHEPARD 1951 and FRY 1951); p_H (WELLS 1913; POWERS 1922, 1923; PEREIRA 1924; ROOT 1930; WIEBE *et al* 1934; FRY *et al* 1937 etc.); Presence or absence of certain substances in water (WELLS 1913; SOUTHGATE 1933 etc.).

The alkaline reserve of the blood of fish was shown by various authors to affect their ability to stand low oxygen tensions. Thus sluggish fish have blood of higher oxygen capacity than active ones.

That large individuals will survive for longer periods in water of low oxygen content than smaller ones of the same species was observed by many investigators. PATON 1902 working with young salmonids, concluded that larger individuals can live longer in water of low oxygen content than smaller ones. WELLS 1913; and SHELFORD 1918 found that on the whole larger fish survived longer than smaller ones in the majority of solutions of different compounds they used, although much variation did occur and some of the largest fish died first.

WIEBE *et al* 1934 reported that larger fish can reduce oxygen tension of water in a sealed vessel to lower levels than smaller fish.

MOORE 1942 in his field experiments found a distinct tendency towards longer survival of larger individuals under low oxygen tensions. He showed that smaller fish were less tolerant to low oxygen tensions than were larger fish of the same species at both summer and winter temperatures. This was attributed by him to the lower metabolic rate of large fish.

SUMNER and DOUDOROFF 1938 found that small individuals have higher respiratory rhythm and died more rapidly in boiled water than larger ones.

PRIVOLNEV 1947 found that whilst the critical oxygen tension of 36 days-old *Salmo salar* was 30—35% saturation it was 11—13% at an age of 107 days-old at 15° C.

PACKARD 1907 confirming LOEB's 1894 findings found that a decrease in the resistance of *Fundulus heteroclitis* embryos to lack of oxygen took place as the embryo

grew. He attributed this to the using up of material stored in the egg (probably carbohydrates).

SHELFORD 1917 pointed out the importance of the study of the effect of different factors on the more sensitive (i.e., young) stages. He considered this stage as the weakest link in the life-history chain and represents its strength. He states that "it is the stage on which minimum fatal concentration must be worked out". SOUTHGATE 1948 emphasises the importance of the study of the resistance of different stages of fish to low oxygen, as little is known of this subject. He pointed out that the effect of lack of oxygen on fish of different sizes — under natural conditions — is complicated by the fact that the extent to which fish of the same species swim away from de-oxygenated water is known to differ with age of fish.

In their studies on the effect of temperature, various authors concluded that the minimum oxygen tension necessary for fish life increases with increase of temperature. FRY 1951a not agreeing with BREDER 1927 showed that if the water is completely air saturated, the oxygen content is adequate at any temperature up to the "ultimate lethal level".

The effect of pH will be dealt with in another paper. It was shown by many authors that high carbon dioxide content increases the lethal effect of low oxygen tensions.

Acclimatization to low oxygen tensions affects the lethal oxygen level. GUTSELL 1929 showed that trout gradually accustomed to reduced oxygen survived oxygen contents considerably below those which were fatal to trout from well oxygenated water. SHEPARD 1951 reached the same conclusion and gave lower figures for the lethal oxygen levels for acclimatized fish than those given by GRAHAM 1949 who used fish living in water nearly saturated with air. Acclimatization to low oxygen exerts a marked effect on the tolerance of low oxygen content (FRY 1951a). FRY pointed out that at low oxygen tensions the fish were not very active and that active fishes could not compete with those fish which were adapted by thier heredity to conditions of moderately low oxygen.

The oxygen relationship of fishes has been given many terms: critical oxygen tension; lethal level; asphyxial oxygen tension etc. GRAHAM 1949 distinguishes between two levels of significance to the well being of the organism i. e. the "incipient limiting level" which is the level at which the activity of the organism begins to be restricted by decreasing oxygen; and the "lethal level".

The effect of low oxygen content on the distribution of fish was mentioned by many investigators (JUDAY and WAGNER 1909; BIRGE and JUDAY 1911; THOMPSON 1925; JOHANSEN 1927; ELLIS 1937; HILE and JUDAY 1941 etc.).

JUDAY and WAGNER 1909 concluded that dissolved oxygen was an important factor in determining the distribution of fish in some Wisconsin lakes. They found that oxygen distribution is very important for the success or failure of introduced trout in lakes, as during summer the fish is confined to cooler water. If this water is deprived of oxygen, or the amount of oxygen is not sufficient to supply the requirement of the fish, the fish cannot occupy the region in question.

JOHANSEN 1927 attributed the fluctuations in the quantity of young fry of plaice and certain other fish to depletion of oxygen in water which has been covered with ice for long periods. Low oxygen content of such water results in high mortality among eggs and larval stages.

ELLIS 1937 in his study of American streams pointed out that the oxygen tension may be a factor in fish distribution. However he showed experimentally that dissolved oxygen is definitely one of the determining factors of fish distribution.

HILE and JUDAY 1941 concluded that deficiency of oxygen or high concentration of free carbon dioxide or a combination of both might limit the depth to which fish penetrate in the lake investigated during mid summer. This distribution barrier may operate directly by "eliciting" an avoidance reaction on the part of the fish or indirectly by affecting the distribution of food organisms. The effectiveness of this barrier may possibly have been increased by the sharp gradient of temperature.

2. Methods of studying the direct effect of gas content on fish larvae

A. Previous methods

Most investigators studying the effect of oxygen tensions on fishes were largely concerned with those tensions which are very rapidly lethal to fishes. Experiments dealing with this problem have been of two kinds. In the first, the fish were placed in a closed container of water and allowed to remain undisturbed until asphyxiation took place, the oxygen content of the water in the vessel being then taken as the lethal figure for such fish. In the second method partially de-oxygenated water was passed over the experimental animals for varying periods of time and the oxygen tension at which the fish showed symptoms of asphyxia was taken to be b e l o w the lethal tension, whilst the tension giving no ill effects on the fish for the same period of time was taken to be a b o v e the critical level.

In the first method, which was used by many authors (PATON 1902; SOUTHGATE 1933; WIEBE *et al* 1934; WILDING 1939; and recently by PRIVOLNEV 1947), accumulation of carbon dioxide and waste products took place in the water. Further MOORE 1942 pointed out that under the conditions of this method the fish continues to respire and extract further oxygen from the water already having a lethal oxygen tension. Thus the oxygen tension determined after the death of the fish will be lower than that which will cause death when the fish is exposed for longer periods under more normal conditions.

MOORE 1942 working on the same fishes used by WIEBE *et al* 1934 and WILDING 1939 but using method 2, gave higher figures for the lethal oxygen tension than those given by the latter author. MOORE 1942 in order to study the effect of low oxygen tensions on different species of fish, adopted the method used by SMITH 1924, in which fishes could be subjected to various oxygen tensions for long periods of time under more or less natural conditions. This was done by placing the fish in live-boxes suspended from floats at various levels in the lakes.

In his experiments to determine the oxygen threshold for various species, MOORE 1942 took the survival of experimental fish for twenty four hours to indicate the presence of adequate oxygen for metabolic require-

ments. This length of time was chosen as being the minimum for which the oxygen content of different water strata would be suspected to remain roughly constant.

In the second method — which was a continuous water flow of known oxygen tension — the difficulty arises of maintaining a constant low oxygen tension for long periods. A fish can live for a short time under a certain low oxygen tension, but may die if it is exposed to the same oxygen tension for a longer period.

WILDING 1939 in some of his experiments, used a continuous flow of water having a low oxygen tension, but found it difficult to keep it constant for more than four hours, resulting in lower values for the lethal oxygen tension for *Perca flavescens* than those given by MOORE 1942.

To overcome the above mentioned difficulties an apparatus is necessary which can provide a continuous flow of water of constant low oxygen tension.

Water of low oxygen concentration can be prepared by boiling normal water, by passing through normal water a stream of nitrogen or hydrogen, or by subjecting normal water to low pressure. SHELFORD 1918a, designed an elaborate apparatus which could deliver a maximum of 4 to 5 litres per minute in a dissolved gas-free condition but of pH 9,0. In either of the two cases boiling the water was the principle of preparing the deaerated water. BASU 1949 and later 1952 used to boil the water to obtain water of low oxygen content. By his apparatus water of 0.05 ppm. of oxygen was obtained. His experiment lasted for 24 hours utmost. PRIVOLNEV 1949 in his study on the relation of the age and critical oxygen pressure for *Salmo salar* used to mix boiled water with river water.

PACKARD 1907 used hydrogen which had been passed over sodium hydroxide, potassium permanganate and then distilled water, to remove oxygen. Recently FRY 1951 devised a fractionating column which supplies about one litre per minute of water of various dissolved oxygen tensions, nitrogen being used to deaerate the water. Graham 1949 used this column in his experiments with the speckled trout *Salvelinus fontinalis*. The minimum period of his experiments was 24 hours. JOHANSEN and KROGH 1914 used a filter pump to provide water of low oxygen tensions. While both boiling the water and using a filter pump was later used by ERICHSEN JONES 1952.

B. Methods Used in the Present Investigation

In experiments on the effect of oxygen tension on the survival of fish, many other factors should be taken into consideration, i. e. tempera-

ture, hydrogen ion concentration, dissolved carbon dioxide, metabolic materials, and the size of fish. Starvation or heavy feeding may also be factors.

In the present study an attempt was made to control as many factors as possible in order that results shown vary only with the amount of dissolved oxygen. Thus a more or less constant temperature was maintained which fluctuated if at all, not more than 1 to 2° C. The fluctuation of temperature of 1 to 2° C. will not affect the incipient limiting level of oxygen very much.

To keep the hydrogen ion concentration more or less constant the use of nitrogen to deoxygenate the water was adopted. With this method variation in the hydrogen ion concentration was not exceeding ±0,05. Furthermore it has been shown by SHELFORD and ALLEE 1913 that excess of nitrogen in water has no ill effects on fishes. Boiled water was only used in very few experiments when gas-free water was required. It has been shown by many authors (SHELFORD and ALLEE 1913, WILDING 1939) that boiling the water causes a change in hydrogen ion concentration of the water, as well as in the carbonate — bicarbonate content. SHELFORD and ALLEE 1913 found that fishes react differently in boiled and in tap water. WILDING 1939 found that the hydrogen ion concentration of water changed from 7,0 (of tap water) to 8,0 and 8,8 when boiled and he used sulphuric acid to neutralize it. To avoid accumulation of carbon dioxide and other waste products a continuous water flow was maintained. Larval fish used in these experiments were of known age and size. When in feeding stage, they were fed once a day during the period of the experiment. FRY's fractionating column (1951) was used as the source of water of known oxygen tension.

Description of Apparatus

The apparatus consists essentially of an arrangement for providing a downward flow of water through a series of flasks meeting a slow upward flow of nitrogen. Water of different low oxygen tensions could be drawn off at different levels. GRAHAM 1949 used a fractionating column of large test tubes on this principle, but in the present investigation a series of Erlenmeyer flasks was used. GRAHAM used his culumn to study only the effect of low oxygen tensions on the metabolism and activity of speckled trout, whilst I used the column to investigate the effect of high and low oxygen tensions as well as different hydrogen ion concentrations on both marine and fresh water fish in early larval and post-larval stages.

The column consists of a 4-foot length of 2-inch Pyrex tubing on top of a series of ten Erlenmeyer flasks from which water of different oxygen tensions can be drawn off.

The two inch tube has three openings along its side through which alternate water inlets are inserted. The top is fitted with a two-hole stopper carrying the upper water inlet and a tube for the escape of gas. The bottom end of the tube narrows to a one-inch neck with a B 29 fitting which fits tightly into the top flasks of the series forming the rest of the column (in the original apparatus this connection was rubber tubing). Whilst glass marbles were recommended to fill the 2-inch tube, it was found more efficient and practicable to fill it with glass bubbles which were prepared in the laboratory by sealing off the ends of short pieces of one cm. diameter glass tubing. To prevent these glass bubbles from falling into the flasks there is a two inch perforated porcelain disc at the bottom of the tube.

The column flasks were of 350 ml. capacity (originally 250 ml. flasks were used), and were connected together through sealing the neck of one into the bottom of the one above it as shown in fig. 1. Water is withdrawn from each flask through a side outlet connected to the experimental flasks by a rubber tubing provided with a screw clamp fixed at the places of greatest hydrostatic pressure to regulate the rate of flow. Although the original author (FRY 1951) recommends the use of a glass tee for each outlet to prevent air locks, such tubes were not used in the beginning of the present investigation. The formation of air pockets was not frequent, and their presence did not interfere either with the water flow or with the gas content of the water. Although T-glass pieces were used later in the present investigation, air-locks were still formed. The lowest flask was provided with a glass tee short enough to act as an overflow as well as an air trap. It was found necessary to allow some water to go to waste through this overflow to ensure sealing in the apparatus.

The experimental flasks were 350 ml. Erlenmeyer flasks. To ensure thorough circulation and renewal of the water in them, the water was allowed to flow in near the bottom, and out through the top. In experiments on larval fish, the overflow-tubes were fitted with nylon netting to prevent the escape of larvae.

In order to obtain a constant gradient of the gas used, a continuous and constant water inflow as well as a continuous and constant gas inflow must be maintained in the column for the whole period of the experiment which lasted sometimes up to five days.

Commercial gas cylinders (either oxygen or nitrogen) were used, and by the aid of a regulating valve and pressure gauge the pressure was main-

tained constant for long periods, by opening the cylinder widely and regulating the gas flow with the valve. In the present investigation a pressure of 1,5 to 2,5 pounds per square inch was used but occasionally 5 pounds per square inch was used as well. In the event of the fall of pressure during the night, it could again be regulated by opening the valve. This however rarely happened and only when the cylinder was nearing exhaustion. The gas cylinder was connected to the bottom of the column by rubber tubing. It was sometimes observed that water filled this tube and hindered the gas flow. To avoid this, the rubber tube was interrupted by a Y-shaped tube connected to a water trap as shown (Fig. 1), so

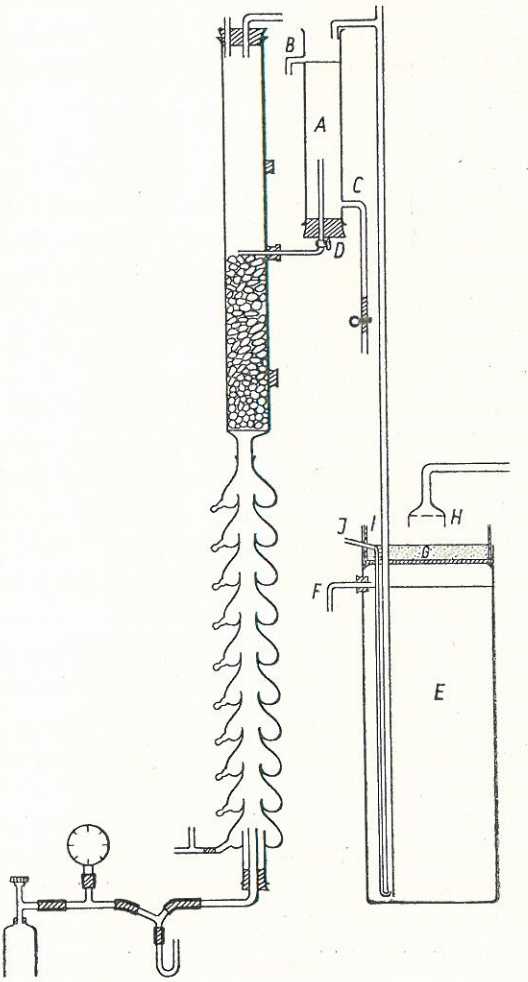

Fig. 1. Fractionating column with a constant water-level supply

that any water flowing into the gas tube was caught in the water trap, and never interfered with the gas flow. This water trap consisted of a U-shaped tube with its free end short enought to allow the water to overflow when full. The trap contained some water before the experiment started to prevent the escape of gas used.

Due to daily changes in pressure of the supply of fresh (tap) and sea-water (aquarium water) a direct supply was undesirable. A constant water supply device was therefore set up using the constant level tube A. The tube was two inches in diameter and 18 inches in length with an inflow C and overflow B. The bottom of the tube was fitted with a two inch stopper through which a glass tube D passed to feed the fractionating column. This glass tube D was provided with a stop-cock to regulate the water inflow to the desired rate. In the present investigation the use of the middle inlet in the fractionating column was found satisfactory. The inflow C was only used for tap water; with sea water another device was necessary to maintain a constant water level in A as the sea-water supply pressure fell very considerably during the night. For this purpose a large tank E (13 inches wide, 19 inches long, 38 inches high) was used. This tank provided with an overflow F and a water inflow consisting of a filter funnel H placed over a sand filter G with a glass cloth bottom. From the tank E water was lifted into the constant level tube A through the tube I by means of compressed air led to the base of I by the tube J from the laboratory compressed-air system. The latter remained constant throught the day and night and thus ensured a constant sea-water supply to A. When using the apparatus for long periods it was necessary to protect it from light to prevent algal growth on the walls of the column.

To test the efficiency of the column, daily oxygen determinations were done for long periods, as well as measurements of pH. These showed that the variation of the oxygen saturation was 1 to 2% for a period of 5 days.

The determination of the oxygen content of water was done by Winkler's method as given by SUTTON 1924. Bottles of 140 ml. capacity were used for sampling. For low oxygen content water was allowed to flow into the sampling bottle for about 20 minutes, after which analysis was done quickly. Using a large volume of the sample decreases the error of diffusion of gas from the atmosphere. In addition the narrow neck of the sampling bottle decreases gas diffusion. In all cases the percentage saturation of oxygen was adopted.

Species of fish used for these experiments were the following: herring (*Clupea harengus* L.); lump-sucker (*Cyclopterus lumpus* L.); salmon (*Salmo salar* L.); sea trout (*Salmo trutta* L.) and brown trout (*Salmo trutta*

f. fario L.), all larval and postlarval stages (except herring only larval stages were used). Young plaice of 1,5 to 2,0 inches long were also used. Methods of rearing these fish are given in a separate paper (BISHAI in print).

C. Experimental Results

(i) Herring Larvae

1. Low oxygen content

Experiments were carried out with the fractionating column using ten larvae in each experiment. In some a continuous water flow was used, in others the flow was stopped after 6 hours. This was to prevent the injury of the larvae by crushing against the overflow. The oxygen content of the well stoppered flasks in the latter case was found to remain more or less constant. Results of experiments show that the incipient limiting level of oxygen of newly hatched larvae in 34‰ salinity is 26,9 to 32,2% oxygen saturation at 14° C. This level is that at which the activity of the organism begins to be restricted by decreasing oxygen. As the larvae get older the incipient limiting level increases; and is 55 to 64% for 3 and 4 days old larvae respectively. In all cases experiments were carried out for 24 hours at 14 to 14,6° C. at 34‰ salinity.

All the larvae used in these experiments had been previously living in the rearing tanks at 83% oxygen saturation. Death of the larvae under low oxygen tensions takes place after several hours (6 hours at 11,6% saturation) and although the heart continues to beat, but the larvae remain on the bottom of the flask and will not recover again if transferred to aerated water.

2. High oxygen content

Two experiments were carried out in which the larvae were subjected to a sudden increase in oxygen. Although the oxygen saturation reached 411,4% the water was not supersaturated in respect to the total gas content. All the larvae either newly hatched or 4 days old lived under the high oxygen tensions for at least 24 hours without any apparent ill effects. On circulating normal aerated water the larvae continued to live healthily as controls without any mortality.

3. Effect of bubbling air in the rearing waters

In order to study the effect of aeration on the early stages of herring larvae experiments were conducted in tanks 8,5 inches long, 7 inches wide

and 15,5 inches high. In each case two experiments were carried out, one with aerated water the other non-aerated as control.

In the former, aeration was achieved by the use of diffusers which cause the air to be emitted in the form of fine bubbles. The aim of these experiments was to investigate the effect of the water saturated with gas and the presence of tiny air bubbles on herring larvae i.e. whether they cause any gas disease or not and whether the saturated water is thus unsuitable for rearing the larvae or not.

The two tanks (aerated and non-aerated) were left in the aquarium room at room temperature and under the same light conditions. Food was supplied in both tanks in the form of nauplii of *Artemia salina,* and *Tigriopus fulvus* and phytoplankton. There was no water renewal and the experiment was carried out in the same water. Two experiments were carried out and showed that herring larvae lived better and for a longer period in non aerated still water. High mortality in the aerated water may be due to mechanical injury caused by bubbling. In no case the presence of air-bubles inside the intestine of the larvae was observed.

(ii) Lump-sucker larvae

Low oxygen content

In the beginning of this investigation experiments were carried out for a few hours (1 to 6 hours). Results show that larvae lived for at least four hours at 27% oxygen saturation (18-days old larvae) at 12,7° C. and 34‰ salinity. Older larvae (20-days old) however lived at 30,6% for at least 6 hours. Larvae of 21-days die within one hour if subjected to 19,1% oxygen saturation. Most of the larvae after manifesting distress by gulping, gasping, coughing and quick respiratory movements turned on their backs. The time taken for overturning to occur was taken as the maximum time for which these larvae can live at that oxygen saturation. Most of the larvae recovered when transferred to aerated water. Recovery, however was impossible if the larvae were left for appreciable periods after turning on their backs.

More experiments were carried out for 24 hours with 22 and 24-days old larvae. Experiments showed that the incipient limiting level of oxygen is about 42,6% oxygen saturation where 80 per cent of 22 days old larvae lived for 24 hours without any apparent ill effect. At 42,6 per cent oxygen saturation 50 per cent of the 24 days old larvae lived for 24 hours. Thus it seems that the incipient limiting level of oxygen increases with increase of age.

The short duration experiments are not reliable for the determination of the incipient limiting level. Thus while larvae lived for 6 hours at 30,6% oxygen at 13° C., they died if the exposure time was longer.

No experiments were carried out with high oxygen content.

(iii) Young plaice

1. Low oxygen content

Two young plaice (1,5 to 2 inches long) were used for each oxygen content experiment.

Results show that young plaice turned on their backs within 6 hours at an oxygen saturation of 14,6% after which they could recover. However individuals can live at 14% saturation for three days, showing the occurrence of individual differences. The incipient limiting level of oxygen is 14% oxygen saturation at 15,2° C. When the water flow stopped overnigth all fish living at 14—21,6% saturation died. This shows that if the oxygen content dropped below 14% saturation it will be lethal to young plaice.

2. High oxygen content

Young plaice lived at high oxygen contents of 333% saturation and 17° C. at least 5 days without any apparent ill effects. When transferred to ordinary aerated water at the end of the experiment the fish behaved normally and no mortality took place. During all the experiments with young plaice the fish were fed once a day.

(iv) Salmon, sea trout and brown trout

1. Low oxygen content

Experiments which lasted for 5 days were carried out with the fractionating column using 15 larvae (5 of each species), at different ages. Results show that the larvae in the yolk-sac stage are resistant to low oxygen tensions, but this resistance decreases as the yolk is consumed. Thus while the incipient limiting level of oxygen for salmon was 2,8% oxygen saturation (5 days experiment) when newly hatched; only 20% of the larvae lived for 48 hours in water of the same oxygen content (2,8%) when 40 days old. The incipient limiting level at this age is 5,8 per cent. When 54-days old the incipient limiting level is 15,6%; at 73 days old it is 23,8% and at 80 days old 24,4% oxygen saturation.

For sea trout and brown trout the incipient limiting level also increases with age. The following table shows the incipient limiting level of oxygen saturation at different ages. (% saturation) for salmon; sea trout and brown trout.

(Feeding stage for sea trout and brown trout 50-days old and for salmon 55 days old.)

Figures representing oxygen saturation under which 100% of the fish lived for the duration of the experiment.

Age Days	Temp. °C.	Duration of Exp. Hours.	Salmon	Sea trout	Brown trout
Newly Hatched	6.6	120	2.8	5.0	2.8
10	5.1	72	5.4	8.4	5.4
18	5.0	96	4.9	6.7	4.9
40	5.0	48	5.8	9.4	9.4
54	6.1	72	15.6	15.6	15.6
73	8.0	48	23.8	23.8	
80	9.0	72	24.4	22.4	
82	12.4	24	19		
117	12.4		27.3		
127	15.9	72			19
132	15.8	48	26.5		
			18.1		
135	15.9	48	22.3		
163	15.9	48			22.3
177	15.9	48			27.3
180	15.9	48			18.1

From this table specific differences can be seen. Thus while the incipient limiting level of newly hatched salmon and brown trout is 2,8 per cent oxygen, it is 5,0 per cent for sea trout; when 18-days old it is 4,9 per cent for salmon and brown trout and 6,7 per cent for sea trout. Thus salmon and brown trout alevins are more resistant than sea trout alevins.

An experiment was also carried out to investigate the effect of total oxygen lack on the newly hatched alevins of the three species. Tap water which was boiled for an hour, then cooled and nitrogen bubbled for 15 minutes, was used. The experiment lasted 20 hours at 5° C. All salmon and 80 percent of brown trout alevins were alive, while all sea trout alevins were dead.

The increase of the incipient limiting level takes place almost at the same time in the three species (at 54-days old i. e. the pre-feeding stage for salmon and at the feeding stage for sea trout and brown trout).

In 1954 fish of all three species were used up to 80 days old only; the experiments on older fish were performed on the fish reared in 1953. The effect of temperature on the incipient limiting level of oxygen was not studied due to technical difficulties. However it can be seen from the table

that the incipient limiting level of oxygen increases from 2,8% oxygen (newly hatched) to 15,6% (54 days old) for salmon and brown trout at about 6° C. All experiments were made at room temperature using fish reared in tap water of 90 per cent oxygen saturation.

When the fish were 117 days old and onwards, two fish of each kind were used in each experiment instead of the 5 used in younger stages.

2. High oxygen content

The effect of this was studied on larvae in the yolk-sac stage (3 days to 24 days old) and in the feeding stage (102 days old salmon and 147 days old brown trout).

Results show that salmon, brown trout and sea-trout larvae in the yolk-sac stage lived at oxygen saturations as high as 353 per cent for at least three days at 6° C. When returned to ordinary aerated water at the end of the experiment they all lived normally and no apparent ill-effects or gas diseases was observed.

Experiments with young fish (salmon and trout) show that these fish lived for at least 5 days at an oxygen saturation of 388 percent without any apparent ill effects at 15° C.; when transferred to aerated water they lived normally. All fish were fed once daily.

Discussion and Conclusions

Many authors have shown that water supersaturated with gas is unfavourable to both larval and adult fish. In Flødevigen fish hatchery where sea fish were reared it was difficult at the beginning to rear fish in water which was supersaturated with gases (nitrogen and oxygen). This was injurious to the tiny larvae which mostly develop a gas disease under these conditions. Successful rearing took place only when lowering the gas content of the water by passing it through sand filters. Under such conditions however rearing of cod larvae beyond a month old was impossible unless a deep rearing tank was used. A. DANNEVIG 1948; A. DANNEVIG and G. DANNEVIG 1950; A. DANNEVIG and HANSEN 1952 and HENLY 1952 all working at Flødevigen attributed the failure to rear sea-fish to the high gas content of the water. HENLY 1952 pointed out that the oxygen concentration is an important factor in the survival of sea fish and concluded that it may be also important in nature. She stated that "if newly hatched larvae are brought into contact with sea water containing high amount of oxygen they will be seriously affected". MARSH and GORHAM 1905 who attributed the death of fish to supersaturation noticed that cod fry could live in water which was fatal to adults.

Supersaturated water which is fatal to larval fish may prove to be fatal to adults as shown by SEMPER (*Pace* HENLY 1952); OSGOOD 1904; MARSH & GORHAM 1905; SHELFORD and ALLEE 1913; ROTH 1922; PLEHN 1924; HAEMPEL, 1928, 1937 and HENLY 1952.

Saturated water is not unfavourable for either fish larvae or adult and it is only when it becomes supersaturated that it may become so. Thus fish larvae living in nature at any depth if they come to the surface which has a higher oxygen content than the water in which they are living, are not affected by the high oxygen content. Oxygen concentrations (as high as 411% and 333% or high nitrogen content) are not injurious to fish larvae within 24 hours at least.

It is concluded that fish larvae and young fish can live in water of a high oxygen or nitrogen content. When one gas is increased by displacing another under N. T. P. larval fish do not develop gas disease or other ill symptoms. A similar conclusion was reached by WIEBE and MCGAVOCK 1932. My results however do not agree with HAEMPEL's findings 1928, who found that excess of oxygen was fatal to young salmonids. Gas disease may appear only when the water is supersaturated with gas in such a way that gas bubbles collect on any rough object in the water. SHELFORD and ALLEE 1913 reached the same conclusion with regard to adult fish they experimented with.

My experiments show that gas disease develops in salmonid fry if the water is made supersaturated with gas by compression followed by decompression, but young plaice are not affected. It seems that the presence or absence of a swimbladder is important in this connection and fish larvae having a closed swimbladder (e. g. cod) react in a different way to those with an open one (e. g. herring).

The time of closure of the swimbladder in cod larvae (when they are about 3 to 4 weeks old DE JOUNG 1936) is the most important factor in determining the depth at which they live and is irrespective of the gas content of the water. Water pressure may therefore be the limiting factor of their survival and distribution. Thus while they live at the surface during the early stages they are found in deeper water later on (WIBORG 1948; HENLY 1952).

The importance of the study of the development and the first filling of the swimbladder has been pointed out by many investigators (A. DANNEVIG and G. DANNEVIG 1950, HARDEN JONES and MARSHALL 1953 etc.). In the present study due to the difficulty of obtaining cod larvae such investigation was not carried out although an apparatus for the collection and analysis of very small samples of gas (1 $mm.^3$ or less) was designed for this purpose.

The mechanism of gas secretion and resorption in the swimbladder is under nervous control (HARDEN JONES and MARSHALL 1953 and cit.). Thus while the vagus nerve leads to gas secretion, the sympathetic inhibitis it. It is probable that when the swimbladder of the cod is developing, the branch of the vagus nerve to it is well developed while the sympathetic is not yet effective. Thus when cod larvae reach a certain stage (3 weeks or more) gas secretion starts and if the larvae are not under pressure to equilibrate the secreted gas, a continuous gas secretion takes place causing inflation of the swimbladder, floating then death (symptoms which are termed gas disease, HENLY 1952). When the sympathetic nerve develops later on, the continuous gas secretion is stopped and regulation of its mechanism takes place. MOREAU 1865; 1876 and FRANZ 1937 showed that the gas secretion occurs only when the inhibiting influence of the sympathetic nerve is overcome (this was shown experimentally by section of the sympathetic nerve).

Supersaturated water is unfavourable for early stages of cod when reared under normal atmospheric pressure, but they can live under pressure in this same water when it will not be supersaturated. At the stage when the swimbladder begins to function, cod larvae need deep water if they are to survive.

The effect of gas content of water has been well studied in herring larvae (which are physostomous). The presence of gas bubbles in the intestine of the larvae has been observed by many authors (ERDMANN 1934; KOTTHAUS 1939; SCHACH 1939; SOLEIM 1940; A. DANNEVIG and G. DANNEVIG 1950; A. DANNEVIG and HANSEN 1952 and HENLY 1952).

I have shown that herring larvae do not develop disease or illness nor they develop air bubbles in the intestine so long as the water is not supersaturated with gas. They can live in water of 411% oxygen saturation provided that the total gas pressure in the water equals the atmospheric pressure. Thus a high oxygen (even a high nitrogen) content is not injurious to these larvae.

The formation of air bubbles inside the intestine of herring larvae has been given many explanations.

1. The assimilating activities of monads in the intestine (ERDMANN 1934). This view was rejected by KOTTHAUS 1939 and SCHACH 1939 because the intestine of the diseased larvae was always empty. I have confirmed these findings and also noticed that even if the intestine contained phytoplankton gas bubbles were not observed inside the intestine.

2. Active swallowing of air bubbles present in the water (ERDMANN 1934 and KOTTHAUS 1939). I however confirmed SCHACH's finding 1939 that

the larvae do not swallow gas bubbles, although they are present in the water (due to direct aeration). In addition I noticed that the mouth of these larvae remains widely open up to at least 10 mm. stage, and is not capable of actively siezing air bubbles in the water. KUPFFER 1878 and SCHACH 1939 also observed that herring larvae are unable to close their mouths before the 10 mm. stage.

3. Breaking down of the yolk material. This was given by KOTTHAUS 1939 who noticed that no further bubbles appeared after the absorption of the yolk-sac.

4. Lack of dissolved oxygen. This causing the larvae to swallow air-bubbles even at low oxygen (40% saturation). While KUPFFER 1878 said that the interchange of gases takes place through the intestinal epithelium of herring larvae, SCHACH 1939 assumed that respiration takes place over the whole surface of the body.

The lack of oxygen may not be the cause of these bubbles is shown in the present investigation where herring larvae lived for at least 24 hours at 30—50% oxygen saturation at 14° C. without any apparent ill effects. Other investigators have also showed that gas bubbles appear in water nearly saturated with gas (KOTTHAUS 1939 and SCHACH 1939 etc.).

5. Supersaturation of water with gas. KOTTHAUS attributed the presence of gas bubbles inside the intestine of herring larvae to supersaturation with oxygen. In the present experiments herring larvae survived at 411% oxygen saturation for at least 24 hours at 14,7° C. Thus high oxygen concentrations are not injurious to herring larvae unless the water is supersaturated.

When water is supersaturated with gas, gas bubbles are liable to separate and attach themselves to any object in the water. Larvae present in such water will be enclosed by these air bubbles which eventually will cause death. Gas bubbles may also enter the mouth of these larvae either when it is widely opened or actively moving. In the first case (e. g. in the early stages of herring larvae) the gas bubbles get into the intestine of the larvae through the gaping mouth as an i n v o l u n t a r y consequence of the swallowing movements of the oesophagus. By the movement of the cilia of the intestinal epithelium the air bubbles are moved towards the anus where they keep joining up and they settle at the posterior end of the intestine.

In the second case the gas bubbles find their way either by entering the mouth while it is moving (ie. being swallowed) or they are formed while the water is passing through the mouth. This was observed in all

experiments with salmonoid fry, in which the water was supersaturated as a result of decompression. The presence of these bubbles inside the mouth of fish hinders their respiratory movements and eventually the fry die. In addition gas bubbles may be formed inside the tissues under these conditions. This will be discussed later when dealing with the effects of decompression.

6. Swallowing air bubbles by herring larvae may be related to the first filling of the air bladder (WUNDER 1935; LEDEBUR 1938 and JACOBS 1938, 1938 a). SCHACH 1939 was uncertain whether the swallowed gas filled the air bladder or stimulated gas secretion.

This swallowed air cannot however be related to the first filling of the swimbladder of herring larvae since this organ does not develop until a later stage. My observations show that even till a 10 mm. stage the swimbladder could not be seen. MAIR and SCHEURING 1923 show that the swimbladder bud appears in the winter herring when the larvae reach a length of 9—10 mm. It starts growing when the winter larvae are about 10 mm. and the spring larvae 15 mm. long. These authors pointed out that even at this stage there is a large individual variation either in the length of larvae or the young bud. Thus sometimes larvae of 10—15 mm. have the swimbladder developed not further than those of 10 mm. The presence of gas bubbles in the intestine of herring larvae has been observed in larvae less than 10 mm. length (KOTTHAUS 1939 and SCHACH 1939).

Thus the 'gas disease' observed in herring larvae during their rearing may be attributed to supersaturation of water with gas. This is supported by earlier observations (A. DANNEVIG and G. DANNEVIG 1950; A. DANNEVIG and HANSEN 1952 and HENLY 1952). KOTTHAUS 1939 observed the formation of gas bubbles in the intestine in the afternoon (17—19 hours). This may be due to the saturated water in which the larvae were reared becoming supersaturated when it has been warmed during the day, with the subsequent formation of tiny gas bubbles, which are then swallowed involuntarily by the larvae.

Furthermore the successful rearing of herring larvae was achieved in all cases in which elimination of the causes of formation of bubbles in the water was carried out (SCHACH 1939; A. DANNEVIG and G. DANNEVIG 1950; A. DANNEVIG and HANSEN 1952 and HENLY 1952).

Salmonid larvae and fry on the other hand live under high concentrations of oxygen ($< 300\%$ saturation). While alevins are not affected by supersaturated water (resulting from decompression) young fry develop gas bubbles inside the viscera, the mouth and in some cases inside the

fin rays. Thus supersaturated water which proves to be fatal to the fry is not so to the alevins. This will be discussed in a separate paper.

Sea fish larvae without a swimbladder — as those of most flat fishes — are also visibly affected by supersaturated water. Although they swallow gas bubbles formed in such water, unlike the herring they can get rid of them and therefore suffer no harmfull ill-effects.

The resistance of fish larvae to low oxygen content decreases as they age. While the lower incipient oxygen limiting level of newly hatched herring is 30% saturation, it is 60% when 4 days old (at 14° C.; for salmon, sea trout and brown trout it is 6% till 35 days old; 9% when 40 days old and 15,6% during the pre feeding stage at 6° C. (54 days old). For salmon and brown trout the lower incipient limiting oxygen level probably remains near this level at least until the fry is 6 months old. Specific differences between salmonid species were observed, salmon and brown trout being about equally resistant to oxygen lack whilst the sea trout is less so.

The decrease in the resistance of fish embryos and larvae as they grow older was observed by LOEB 1894; PACKARD 1907; TRIFANOV 1937; PRIVOLNIEV 1938 and OLIFAN 1940; (Pace PRIVOLNEV 1947). LOEB 1894 attributed the resistance of young embryos to oxygen lack to the difference in the chemical constitution of the early formed cells as compared to those formed later on. PACKARD 1907 attributed it to the presence of carbohydrates which act as a depolarizer in the process of protoplasmic respiration, and this enables respiration to be carried on to a certain extent in the absence of oxygen. The decrease in the resistance to oxygen lack as the embryo ages is due to the using-up of the carbohydrate material stored in the egg. PRIVOLNEV 1947 regards this as resulting from the increased oxygen demand of older individuals.

The presence of carbohydrates may be important in the resistance of fish larvae to oxygen lack. Experiments show that salmon and sea trout alevins (till 15 days old at 6° C.) live at least 20 hours in deoxygenated water. This resistance to oxygen content can be explained as due to the mode of respiration, the activity of such larvae, and the presence of carbohydrates. Early larval stages of most fishes respire through thin body wall or by the aid of the blood vessels round the yolk sac (or by both) in the absence of functioning gills (KRYZANOWSKY 1934, KROGH 1941 and GRODZINSKI 1948). Thus fish larvae (in the yolk-sac stage) can live at comparatively low oxygen tensions as they can obtain the oxygen they need through a wider surface by diffusion through the thin body wall. In addition the early larval stages — especially in salmonoids are inactive and mostly lie at the bottom showing little activity. Thus the

amount of oxygen needed is low as compared to that of older stages. As the larvae become older the yolk is absorbed thus decreasing the carbohydrate available; the skin covering the body becomes thicker thus restricting the cutaneous respiration and becomes confined to the gills, furthermore the fish become more active and need more oxygen for their metabolism. This will lead to a higher demand of oxygen resulting in a higher figure for the lower incipient level of oxygen. Once the respiration by the gills is established the lower incipient oxygen level remains more or less the same.

In comparing my results on *Salmo salar* with those of PRIVOLNEV 1947 who studied the relation between the critical oxygen tension and age of the same fish at 15° C. it will be seen that while PRIVOLNEV 1947 mentions that the critical oxygen tension is 30—35% when 36 days old and 11—13% when 107 days old, at 15° C. my results show that from the feeding stages the lower incipient limiting level of oxygen at 12,4° C. is 19% and from 117 days old onwards it is about 19—27% at 15,9° C. The difference between the present results and PRIVOLNEV's is probably owing to the method used by the latter author, who used closed containers in which fish were left, till they die. A discussion of this method is given on page 42 which shows that under such conditions the values obtained are lower than those actually lethel for the fish. Thus at 107 days old PRIVOLNEV 1947 found that the critical oxygen tension was 10%, but my results showed that it was 19%. In the present experiments a continuous flow of water of low gas content was used and experiments lasted for 3 to 5 days. The high figure which PRIVOLNEV 1947 obtained for young fish (36 days old at 15° C.) can be explained by the inability of young stages to extract oxygen at low tensions (especially when present in a group). In addition the presence of 5 fish in 250 ml. of water may cause a change in the carbon dioxide content and a decrease in the oxygen consumption. An experiment was carried out using a combination of high carbon dioxide and low oxygen content. In this experiment it was found that the lower incipient limiting oxygen level increases with decrease in pH caused by carbon dioxide (from 6% at pH 7,2 to 12,5% at pH 6,20). Several authors (WELLS 1913; POWERS 1922; SHELFORD 1923, 1929; O. HALL 1925; F. HALL 1931 etc.) have pointed out that the ability of fish to absorb oxygen at low tensions is more or less dependent on the hydrogen ion concentration. PATON 1902, 1904 concluded that large salmonoids can live longer in water of low oxygen content than smaller ones. The same conclusion but with other fish was reached by many others (WELLS 1913; SHELFORD 1918 a; WIEBE *et al* 1934; SUMNER and DOUDOROFF 1938; MOORE 1942) who found that large fish can reduce the oxygen tension

of water in a sealed vessel to lower levels than smaller ones. This may explain the lower value of critical oxygen tension which PRIVOLNEV 1947 obtained when using old salmonids (107 days critical tension was 10%).

The previous history of the fish (i. e. the oxygen concentration under which it was living, temperature, pH, nature of the water) is very important in determining the lower oxygen levels under which fish live. GUTSELL 1929 showed that trout gradually accustomed to low oxygen survived in water of oxygen content considerably lower than that which was fatal to trout from well oxygenated water. Fry 1951 mentions the effect of acclimatisation to low oxygen tensions as in important factor.

Summary

1. A review of the effect of gas content of water (oxygen and nitrogen) on fish is given.
2. An apparatus is used which gives a continuous flow of water of a known gas content. A comparison between the method used in the present investigation and those previously known is made.
3. Herring, lump-sucker, plaice, and salmonoid larval and young stages are used.
4. The resistance to water of low oxygen content decreases with increase of age. This is explained as due to: mode of respiration in which exchange of gases takes place through the body wall and the blood vessels surrounding the yolk sac; carbohydrate content — which acts as a depolarizer — which decreases as the fish ages; and to the increased activity of the growing larvae.
5. All fish used at all stages live at high oxygen concentrations ($< 300\%$ saturation) without any apparent ill effects provided that the total gas pressure does not exceed the hydrostatic pressure.
6. The presence of air bubbles either attached to or inside the intestine of herring larvae was not observed. In all cases the water was either saturated or nearly so but never supersaturated. Although herring larvae were kept in aerated tanks swallowing air bubbles was not observed. A brief discussion of the 'gas disease' in herring larvae is given.

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