# A consideration of the problems involved in rearing rainbow trout in recirculating water.

Philip John Parkinson B.A.Hons.

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# DECLARATION

I hereby declare that this thesis has been composed entirely by myself and that the contents are my own work, except where it has been clearly stated otherwise.

P.J.PARKINSON

# SUMMARY

This thesis examines the problems involved in rearing rainbow trout in recirculated water, with special reference to the particular problems encountered with the equipment at Edinburgh University.

There is a thorough review and discussion of the relevant literature. The primary problem encountered when fish are reared in recirculated water is that of the toxic effect of high free-ammonia levels on the fish and compounded with this is the toxic effect of a low oxygen concentration. The literature review looks closely at these problems and those of reconditioning the recirculating water, with the main emphasis being placed on a reconditioning of the water by biological filtration.

Following these topics, is a review of the recirculation based, fish rearing equipment available at Edinburgh University and there are details of the Modifications which were made to this equipment and those which still should be made, so that valuable research work could be facilitated.

Experimental results are included where appropriate and the thesis is concluded by suggestions for further experimental work.

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# INTRODUCTION

If the water in a fish rearing establishment is recirculated, instead of being discarded after it has passed through the rearing ponds, then a tremendous water economy is achieved.

However, before the water is recirculated and thus reused, it must be reconditioned. This reconditioning process basically involves passing, say, 95% of the recirculating water through a biological filter, in order to remove the highly toxic ammonia which the fish excrete. Also the water must also be aerated in order to raise it's dissolved oxygen level. The 5% of recirculating water which is not in this case reconditioned is discarded, and this volume is made up with fresh water in order to avoid the long term build up of nitrates and dissolved organic compounds.

So, a fish farm which is at present supporting the maximum number of fish for a single-pass system (i.e. no water reuse), would be able to increase it's production by twenty times, if it installed the necessary equipment for a recirculation system. Water reuse also means that fish can be reared at sites which previously have been discounted, due to the shortage of an adequate natural water supply.

Another advantage of water reuse is that it offers the possibility of a greater degree of control over the fishes' environment. Two of the most important environmental factors which are more easily controlled in a water reuse system are:

water temperature and water hygiene. In the case of water temperature, if only 5% of the water in the system is being changed every cycle, then it may well become an economic proposition to heat the water in winter and if necessary, cool it in summer. The ability to manipulate the temperature, means that the fish may be reared at the optimum temperature for food conversion or for growth, the former perhaps becoming increasingly important due to the high costs of foodstuffs. However, if the fish are reared at the optimum temperature for growth, then a greater annual throughput of fish through the farm is possible, and so the capital invested in the farm construction would be made to work harder. Burrows (1971) gives details of how salmon smolts are produced in one year instead of the usual two years, if the fish are reared in heated, recirculated water.

In a water reuse system, as opposed to a single-pass system, it would be a better economic proposition to provide the fish with a disease-free environment in which to live, because only the make-up water would need to be sterilised by an ultra-violet filter (this make-up water being perhaps only 5% of the amount that would need to be sterilised in a single-pass system). Ultra-violet filters are relatively expensive to buy and run, for example a filter system which would sterilise 1000 gallons per minute, cost 30,000 dollars in 1972 (Flatow R.E. and Mooney D.Y. 1972); and so clearly, the less water there is to sterilise, thecheaper the costs would be.
It is pertinent to point out that contrary to what might be expected, results from adult chinook salmon reared in a disease-free recycle system, indicate that these fish are not more susceptible

to diseases when released, than are fish reared in a non-sterilised single-pass system (Burrows 1971).

So, the reasons why the field of rearing fish in recirculated water is important and therefore worthy of having a research project directed towards it, have now been briefly mentioned. From October 1973 until January 1975, an investigation was carried out, which examined the problems involved in rearing rainbow trout in recirculating water. This work was facilitated by the use of equipment which had been assembled at the Department of Forestry and Natural Resources, Edinburgh University, two years previously. It was intended to test the system which had been installed, prove that it worked satisfactorily, and then carry out experiments on the themes suggested towards the end of this thesis. However, as is revealed in the following pages, the problems involved in this work were much more extensive than had been expected, and after twelve months it was decided to terminate any further development of the project, because of the high costs which would be involved in correcting the shortcomings of the equipment.

# CHAPTER I

## The sources of ammonia, and the ways in which environmental factors affect it's toxicity to the fish.

The principal problem presented by a water re-use system is that unless the recirculating water is reconditioned, the level of metabolic products in the water would increase and the oxygen level would decrease, to such levels that they would produce a damaging effect on the fish. Different fish species vary in their susceptibility to various metabolic products, and different metabolic products vary in their toxicity to any particular fish species. The metabolic product which possess the most serious threat to the Salmonid farmer is ammonia, (these fish are sensitive to very low levels of ammonia)and both this and the factors which influence it~~'~~s toxicity are discussed below.

### (i) The sources of ammonia

The metabolic products referred to, are waste products produced by the chemical processes occuring within the fish's body; these products being excreted primarily via the gills and secondarily through the kidneys. The main function of the latter organ is osmoregulatory, removing excess water from the body, which floods in primarily through the gills. (Warren 1971). The waste products excreted via the gills include ammonia, urea, amine, amine-oxide derivatives and carbon dioxide; waste products excreted via the kidneys include creatine, creatinine, uric acid (Brockway D.R. 1950 and Warren 1971). As well as these the fish excretes faeces which are expelled from the alimentary canal through the anus, and unless these faeces and any waste food are removed from a recirculation system, their breakdown leads to a source of ammonia.

## (ii) The chemistry of ammonia when dissolved in water; and the particular chemical state of ammonia which is toxic to fish.

The ammonium radical $NH_4^+$ is present as ammonium hydroxide as a product of excretion, and in water it dissociates reversibily into water and ammonia (Burrows 1964). That the toxic effect of an ammonia solution is only caused by the free or unionised ammonia ($NH_3$) was shown first by Whurmann and Woker 1948; and Downing and Merkens in 1955. So, any parameter present in the aquatic environment which influences the equilibrium between the concentrations of ammonium hydroxide and ammonia and water, will affect the toxicity of the solution and so warrants careful study. It is important to know how varying each of the parameters individually and in combination will change the equilibrium, so that if a measurement of the total ammonium nitrogen concentration ($NH_4$-N) in the environment is made, together with a measurement of the environmental parameters which will affect the $NH_4$ - $NH_3$ equilibrium, then the level of free ammonia present can be calculated. (Unfortunately there is no method available for directly measuring the quantity of free ammonia in a solution, but the total $NH_4$-N can be measured. )

It has been shown that the important relevant environmental parameters are:-

pH;
temperature;
dissolved oxygen concentration;
free carbon dioxide content of the water;
bicarbonate alkalinity;
salinity.

In every rearing situation all these parameters will have a value and so they will have a contributory effect towards the level of free ammonia present in the environment. Naturally they all differ in the magnitude of their effect; and by their very nature their effect may be weak, or their effect may be weak in a particular circumstance because of the value that another parameter holds.

### (iii) The effect of the pH of the water on ammonia's toxicity.

pH is the major factor determining the toxicity of an ammonium solution (Lloyd 1961) and a change in pH of 0.3 units, that is 7.0 to 7.3 results in a doubling of the amount of free ammonia present (EIFAC 1970and Trussel 1972). It has been suggested that it is the pH at the gill surfaces as opposed to the pH level of the whole environment that is really important in determining the toxicity of an ammonia solution. Lloyd and Herbert (1960) say that if the carbon dioxide level in the aqueous environment is low, then the amount of respiratory carbon dioxide excreted by the fish will considerably reduce the pH at the gill surface thereby reducing the toxicity of the ammonia solution. They contrast two examples:

(a) The pH value of the the general environment is high and the carbon dioxide level is very low.

(b) The pH level is lower and the carbon dioxide level is very high.

They suggest that the effect of carbon dioxide excretion at the gill surfaces is such that the level of free ammonia which would have a toxic effect on the fish, would be five times less in the case of the former compared to that of the latter, which is explained by the effect of the respiratory carbon dioxide being much greater in the former case than in the latter. So, an assessment of the level of free ammonia in an ammonia solution when the pH is known but not the level of carbon dioxide, could give a misleading indication as to the toxicity of that solution to the fish.

(iv) The effect of the temperature of the water on ammonia's toxicity.

A rise in temperature of 10°C doubles the amount of free ammonia present in a solution of an ammonium salt. (EIFAC 1970) However, the relationship between ammonia toxicity and temperature is more complicated than this might suggest, because it would seem that temperature actually influences the speed with which rainbow trout show an ill effect, when in a potentially damaging concentration of free ammonia. Herbert (1962) found in experiments conducted above 10°C that survival times of rainbow trout at a constant level of un-ionized ammonia, decreased with a rise in temperature, but the threshold concentration remained the same. Below 10°C, the relationship appears to be different, and there is evidence to suggest that as the temperature falls, the level of free ammonia required to cause a toxic effect decreases. Brown (1968) suggests that at 3°C, the threshold value for rainbow trout is one half it's value at 10°C; (it is worth noting that this approximately cancels out the effect of rising temperature on the $NH_4$ - $NH_3$ equilibrium). Burrows (1964) found that free ammonia became increasingly toxic to chinook salmon, Oncorhynchus Tshawytscha, fingerlings, as the temperature fell. This apparent anomaly between the results from above 10°C and below 10°C can be explained as follows: Burrows (1964) tells of the major excretory waste being urea at 8°C, ammonia excretion being more important at higher temperatures. Lloyd and Orr (1969) suggest that a reduced ability to excrete ammonia at lower temperatures may well mean that the fish are more susceptible to ammonia at lower temperatures. This would seem to explain the findings of Brown (1968) and Burrows (1964) and Herbert (1962); water temperature could be having an effect on a possible ammonia detoxification system. The increasing temperatures used by Herbert (1962) may have resulted in matching increasing rates of ammonia detoxification and so the threshold concentration remained at the same value. It is relevant to add that Burrow's work goes on to show that by increasing the fish loading of ponds, one finds that ammonia, not urea is the principal excretory waste, so both stress and temperature influence which waste nitrogen product is formed.

The pH of a solution can in it's own right cause a toxic effect on fish, and the EIFAC 1968 report advises that a pH of below 5 and

and above 9 should be avoided, Lloyd and Jordan (1964) found that if the pH of the fishes' environment was 4.5, and the free carbon dioxide concentration of the water was 50ppm, then the pH value of the fish's blood dropped by 0.55 units, and the fish died. In considering the significance of the carbon dioxide concentration it would seem likely that apart from the carbon dioxide concentration influencing the pH of the water, it may also aggravate the effect of a high hydrogen ion concentration on the fish as follows: a high hydrogen ion concentration in the fish's environment will, by diffusion, mean an increased hydrogen ion concentration in the fish's blood and so a lower pH; now if the carbon dioxide concentration in the environment is also relatively high, this will lower the pH of the fish's blood even further by reducing the fish's ability to excrete carbon dioxide by diffusion outwards across the gill membranes.

Unfortunately, there has been very little work done in this field and so the effect of pH per se, and in conjunction with various free carbon dioxide levels can not really be predicted, especially at levels not causing death within a short period of time.

### (v) The effect of the dissolved oxygen concentration of the water on ammonia's toxicity.

Lower dissolved oxygen levels in the fishes' environment, means an increased toxicity of free ammonia (Wuhrmann 1952, Downing and Merkens 1955, 1957). N.B. unlike the two environmental parameters mentioned so far changing levels of dissolved oxygen do not alter the $NH_4 - NH_3$ equilibrium.

Downing and Merkens (1955) showed that when the oxygen content of the water was half the value of when water was saturated with air, then if several fish species were put in an environment containing a threshold level of free ammonia, their survival time was cut by two thirds. Brockway (1950) stated that as the ammonia concentration in the environment rises, then the fish progressively lose the ability to use the oxygen available in the water. He says that when the ammonia concentration in the water increases to 1 ppm (it should be noted that he gives no mention of pH etc.) , the oxygen content of the blood decreases to one-seventh of it's normal value; but he does not suggest how the ammonia causes this effect. However, if this is true, the effect of lowering the dissolved oxygen concentration of the water can readily be understood, if the fish are already having trouble in utilising the available oxygen.

It would seem that there are two points to be considered when looking at the oxygen concentration in a fish tank:

(i) An oxygen concentration of x ppm will in the presence of 0 ppm free ammonia, limit the fish's activity.

(ii) As soon as free ammonia is present in the environment, then the minimum oxygen concentration for unaffected growth rises to x + y ppm.

Where the value of x will depend on water temperature and the associated food consumption rate; the former having a direct effect on activity both physical and chemical and hence on respiration, Warren (1971) states that when food is freely available to the young coho salmon, then the food consumption of the fish may be limited even if the oxygen concentration in the water was to fall a little below the air saturation value. As food consumption of the fish increases, then the growth rate also increases, but not as greatly because respiration increases too. In his discussion, Warren (1971) explains that 4 ppm is the usual minimum acceptable oxygen level and this is due to the prevailing conditions and limited food availability. A value of 4 - 5 ppm is also suggested by Willoughby (1968), Burrows and Coombes (1968), Larmoyeux and Piper (1973).

So at each different free ammonia concentration there will be a certain minimum dissolved oxygen concentration which must be maintained if the fish is not to suffer any effects due to a low oxygen level in the blood. However, even though this minimum oxygen concentration may be maintained, the particular level of free ammonia present may be sufficiently high to cause damage to the fish in it's own right. (A much fuller discussion of this free ammonia - oxygen interaction is discussed in the following sections.)

### (vi) The effect of the free carbon dioxide concentration of the water on ammonia's toxicity.

The EIFAC 1968 report suggests that as long as the carbon dioxide concentration is below 20 ppm at pH 5.0 - 6.0, and below 100 ppm at pH 6.0 - 6.5 there should be no harmful effects. Carbon dioxide's involvement with the effect of other parameters is discussed in sections (iii) and (iv).

### (vii) The effect of the alkalinity of the water on ammonia's toxicity.

The effect of alkalinity is bound up with that of carbon dioxide in influencing the pH of the solution.

### (viii) The effect of the salinity of the water on ammonia's toxicity.

Lloyd and Orr (1969) give results from experiments involving rainbow trout 17 - 23 centimetres long, which indicates that the rate of urine excretion increases with a rise in the concentration of free ammonia in the environment. They suggest that this diuresis is caused by an increase in the permeability of the fish to water. They add that concentrations of free ammonia below 12% of the lethal threshold value may not produce this effect, which calculated from their results means 0.047 ppm. Rainbow trout culturists consider that such a level of free ammonia is too high for rearing fish, and so it can be deduced that the range of free ammonia concentrations found in fish rearing establishments would not be expected to increase the permeability of the fish to water.

Herbert and Shurben (1965) state that a rise in salinity up to 30% sea water (isotonic with the blood), causes a corresponding decrease in ammonia toxicity. This would seem logical for free ammonia concentrations above 0.047 ppm because if one takes a free ammonia concentration which causes an increase in the fish's permeability to water, then if the salinity of the water is increased, this will mean that the difference in osmotic pressure between the fish's body fluids and it's environment will be reduced, and so the net diffusion of water into the fish will also be reduced.

However if the free ammonia concentration is below 0.047 ppm, then as the ammonia concentration is not affecting the fish's permeability to water, then one would expect salinity to have no effect on ammonia toxicity.

# CHAPTER 2

## The effect of ammonia on the fish.

Now; having discussed how certain chemical and physical parameters of the fishes' environment affect the $NH_4^+ - NH_3$ equilibrium and also to some extent how these physical parameters themselves affect the fish, it would be valuable to look at how free ammonia elicits it's effect on the fish.

## The effect of fluctuations in the ammonia concentration on it's toxicity.

A suggestion of some importance, relevant to this point was put forward by Lloyd and Orr (1969). They ran two experiments:

(i) Fish in an environment with a free ammonia level fluctuating (on a two hourly cycle) from 1½ to ½ times the two day threshold concentration, were found to exhibit a greater mortality than would have been expected if they had been kept in the non-fluctuating two day threshold concentration.

(ii) Fish in a similar environment but with the same fluctuation based on a one hourly cycle, produced the mortality expected. That is, they were reacting to the average concentration.

This evidence led them to conclude that whatever the effect of the ammonia is, it takes somewhere between one hour and two hours to have a physiological effect on the fish. However, I would add that this particular time relationship may be relevant only to the free ammonia concentration tested, and that at another free ammonia concentration the same principle may apply, but the times may be different. However, this experiment only tested the effect of high free ammonia concentrations, especially high when compared to those found in fish rearing establishments, and it could possibly be misleading if these results were applied directly to investigations made into the mode of action of ammonia levels which are found in fish farming situations. Lloyd and Orr (1969) were looking for death as the effect of the treatment, whereas trout farmers are looking for much more subtle changes in the fish. High levels of free ammonia could well upset different physiological systems in the fish than lower levels of free ammonia, so one must be careful in extrapolating conclusions from studies on the effects of higher concentrations of free ammonia, to studies on the effect of much lower concentrations of free ammonia.

The level of ammonia in a rainbow trout raceway has been shown to fluctuate considerably during a twenty four hour cycle by Burrows (1964) and it would be of value to know whether there was a peak value of free ammonia of a certain length of time, that was potentially damaging to the fish; because as long as the average free ammonia concentration measured over a time interval which included the time plus a certain time interval either side, was below the free ammonia value which would cause the particular damage concerned, then there would be no need to worry about any ill effect. Whether or not this can be deduced, depends entirely on whether the effects produced by long term low ammonia exposure add up to produce a similar effect to that of short term high ammonia exposure.

TABLE 1 The experimental results of Larmoyeux and Piper (1973).

| Reuse | Increase in size during experiment. | Exposure time | Free $NH_3$* | ppm. $NH_4$-N | pH | Conversion ratio | % Mort. | ppm. $O_2$ | ppm. $CO_2$ | Comments |
|---|---|---|---|---|---|---|---|---|---|---|
| 0 | 1.2" to 6.1" | 230 days | 0.0009 | 0.1 | 7.7 | 1.4 | 11.1 | 7.7 | 4.4 | See page 17 |
| 1st. | 1.3" to 6.4" | | 0.0028 | 0.3 | 7.7 | 1.4 | 7.9 | 6.5 | | " |
| 2nd. | 1.2" to 6.4" | | 0.0037 | 0.4 | 7.7 | 1.4 | 10.7 | 5.6 | | " |
| 3rd. | 1.2" to 6.1" | | 0.0046 | 0.5 | 7.7 | 1.4 | 15.4 | 4.9 | | " |
| 4th. | 1.3" to 5.8" | | 0.0055 | 0.6 | 7.7 | 1.5 | 15.5 | 4.2 | | See page 20 |
| 5th. | 1.2" to 5.5" | | 0.0064 | 0.7 | 7.7 | 1.7 | 12.7 | 3.8 | | See page 21 |
| 6th. | 1.2" to 5.1" | | 0.0074 | 0.8 | 7.7 | 2.0 | 15.7 | 3.3 | 9.4 | " |

* Figures corrected using Trussel's (1973) table, assuming a temperature of 10°C

Burrows (1964) asserts that his experiments show that the period of exposure to an ammonia concentration is more important than the peak exposure. He found that salmon fingerlings could tolerate free ammonia levels of 0.0126 ppm for 1 hour per day with apparent impunity, but when the exposure exceeded 12 hours per day at 0.0018 ppm the growth rate was reduced, and continual exposure led to reduced stamina and disease resistance.

In any case, further experimentation is needed on the lines of Lloyd and Orr (1969) but using different free ammonia concentrations.

## The effect of long term exposure to unfavourable ammonia and oxygen levels.

The most valuable paper on the effects on long term low ammonia concentration exposure would seem to be that of Larmoyeux and Piper (1973) based on the original work of Piper (1970). They record changes in:- growth rate; red blood cell number; gill condition; disease resistance; liver, kidney and spleen histology; appearance; behaviour; stamina and food conversion. The detailed data of the experiment can be seen in table I . The basic equipment was a series of seven troughs all containing fish; fresh water entered the top trough and was serially reused as it flowed from trough to trough, until it was discarded after the seventh trough.

This equipment design meant that the fish in each of the troughs were living in different environments as regards the concentration of $NH_4$-N, oxygen and carbon dioxide. However, it is unfortunate that no mention is made of the temperature, which means that one cannot calculate the free ammonia concentration in each trough with accuracy, but the statement that the maximum level of free ammonia in trough seven was 0.0144 ppm, means that using the tables of Trussel (1972), the temperature can be back-calculated to be about 10°C. The values of free ammonia based on this temperature are thus entered in table I . It should be noted that Larmoyeux and Piper had calculated the amount of free ammonia from their recorded levels of $NH_4$-N, by using the tables provided by Burrows (1964). However, Trussel (1972) has shown that these tables produced by Burrows are not at all accurate and so this means that the free ammonia level in reuse trough six was in fact 0.0074 ppm and not 0.0144 ppm. It can be seen from these two tables that in no case does anyone give details of all the parameters which should be considered when calculating the free ammonia concentration at the gill surface. (See page 3 )

Returning to the results of Larmoyeux and Piper (1973), they found that levels of free ammonia up to 0.0046 ppm accompanied by oxygen levels down to 4.9 ppm led to significant differences in the fish after eight months (Larmoyeux and Piper's data only permits one to say that the oxygen concentration was 4.9 ppm + (0 to 0.7) and that the free ammonia concentration was 0.0046 ppm - (0 to 0.0009) ). Larmoyeux and Piper's results show evidence of the types of changes caused by free ammonia levels from 0.0046 ppm up to 0.0074 ppm with accompanying oxygen levels from 4.9 ppm down to 3.3 ppm, and these could valuably be discussed further :-

### (i) Growth rate

The growth rate was lower where the oxygen concentration was below 4.9 ppm and the free ammonia concentration was above 0.0046 ppm. This was accompanied by a progressive decrease in the efficiency of food conversion as the oxygen concentration fell below 4.2 ppm and the $NH_4$-N concentration rose above 0.6 ppm (0.0055 ppm free ammonia).

### (ii) Histology

The red blood cell number increased by some 20% where the oxygen concentration was less than 4.9 ppm and the $NH_4$-N concentration was greater than 0.5 ppm (0.0046 ppm free ammonia). After four months, gill hyperplasia was observed in the sixth reuse trough where the oxygen concentration was 3.3 ppm and the $NH_4$-N concentration was 0.8 ppm (0.0074 ppm free ammonia). These fish also showed a reduction in lymphoid tissue in the spleen; and in the kidneys there was a reduction in haematopoietic tissue and glomeruli were sometimes congested with red blood cells. Liver cells were less vacuolated than similar cells from healthy trout, which indicated that less glycogen was present. In the low oxygen environments, thyroid tissue was less active.

### (iii) Appearance and behaviour

Where the oxygen concentration was less than 3.8 ppm and the $NH_4$-N concentration was greater than 0.7 ppm (0.0064 ppm free ammonia) the fish were lethargic, grouping near the tail of the troughs.

### (iv) Stamina

If fish from the environments where the oxygen concentration was less than 3.8 ppm and the $NH_4$-N concentration was greater than 0.7 ppm (0.0064 ppm free ammonia), were placed in a clean water environment for forty eight hours prior to being tested in a stamina tunnel, then their performance was superior to that of fish from the better environments. Larmoyeux and Piper (1973) suggest that these fish may have adapted a more efficient cardiorespiratory system so that they could survive in a low oxygen environment. This suggestion is reinforced by the observation that when at the end of the experiment, fish from the first reuse trough were introduced into the sixth reuse trough, they showed signs of stress within thirty minutes. The fish from the low oxygen environments have 20% more blood cells, but this alone would not seem to be an explanation, because fish from the third and fourth reuse troughs have the same 20% increase in red cell numbers and yet they perform no better than "control"fish.

### (v) Disease resistance

Resistance to bacterial gill infections was lowered when the oxygen concentration was less than or equal to 4.2 ppm and the $NH_4$-N concentration was greater than or equal to 0.6 ppm (0.0055 ppm free ammonia); this being demonstrated by the fourth, fifth and sixth reuse fish populations experiencing an outbreak of bacterial gill disease durihg the experiment, whereas the other fish populations did not.

## Discussion of the effects noted in (i) to (v) above.

In analysing these findings, and attributing the effects observed to their cause, the problem is that as the free ammonia level changes, then so does the oxygen concentration. It would be of great value to have results to examine, which were drawn from experiments which first of all tested the effect of different levels of free ammonia at the same fixed oxygen concentration, so that the effect of the free ammonia concentration per se, under the experimental regime could be examined. Then the experiment should carry on to test the effect of varying the oxygen concentration at fixed ammonia concentrations. This series of experiments should lead to a true understanding of the separate and combined effects of various oxygen and free ammonia levels. In this experiment the effect of the two are intertwined, but with the help of experimental findings from other workers, the separate effects of ammonia and oxygen can at least be partially separated. The following data is taken from Larmoyeux and Piper (1973)

| Reuse Number | Oxygen Conc. | Free ammonia Conc. | Symptoms |
|---|---|---|---|
| Control | 7.7 ppm | 0.0009 ppm | No effect on the fish after eight months. |
| 1st | 6.5 ppm | 0.0028 ppm | |
| 2nd | 5.6 ppm | 0.0037 ppm | |
| 3rd | 4.9 ppm | 0.0046 ppm | (i) Lower growth rate.<br>(ii) Increased red blood cell number. |

Reuse Number:- The trough number indicating the number of times the water has been reused.

(i) Warren (1971) states that if oxygen is limiting, the fish may be able to reduce their food intake and hence their growth rate, so that they would use less oxygen. So this would suggest that the lower growth rate exhibited by fish in the third reuse tank, is due primarily to a low oxygen level per se and that the free ammonia level of 0.0046 ppm is not having any real adverse effect. In fact Smith's (1972) results would suggest that if oxygen levels had been kept above 5 ppm in all the reuse troughs by aeration, then the free ammonia levels found should have caused no problems.

(ii) Red blood cells have haemoglobin associated with them, which binds with oxygen in the gill capillaries, and then transports it to a site of oxygen demand in the body; so an increase in the red blood cell number could be seen as an attempt to secure the same amount of oxygen from a lower oxygen environment. This may be affected by a greater density of red blood cells in the gills meaning that any oxygen present there, would be bound and transported away faster, and so the rate of oxygen diffusion into the gills would be increased.

The results of the stamina trials which show that fish from reuse environments three and four perform no better than "control" fish, despite the former having 20% more red blood cells, may not necessarily destroy the mentioned theory but may simply suggest that when oxygen is freely available the normal complement of red blood cells is perfectly adequate to supply all the oxygen for the fishes' needs. The superior performance of the fish from the fifth and sixth reuse environments being due not to their increased red blood cell number but due to the more efficient cardiorespiratory system which they have developed due to the prevailing oxygen conditions in their environment, being worse than in environments three and four.

It would seem that an increased red blood cell number would also confer some advantage, although probably marginal, on fish in an environment with a higher carbon dioxide level (as is the case as the reuse level increases). This would be because as the carbon dioxide concentration of the environment rose the proportion of the fishes' haemoglobin that was present in the form of carboxyhaemoglobin would increase, due to a reduced ability of the fish to excrete carbon dioxide outwards across the gill membranes by diffusion. The extra 20% of red blood cells would act as a "reserve" to compensate for this trend.

It would seem likely that both effects (i) and (ii) are due to a low oxygen level, and this may be due to the oxygen level per se, or it may be due to a free ammonia level of 0.0046 ppm reducing the blood's ability to utilize the oxygen available. Brockway (1950) states that free ammonia reduces the blood's ability to utilize the oxygen available. It would be interesting to know the oxygen level in the blood of the "control", first and second reuse tanks, in order to see whether the free ammonia concentrations up to 0.0037 ppm were in fact having this effect, but under the prevailing oxygen levels the effect was harmless.

Smith (1972) says that if the oxygen concentration is greater than 5 ppm, growth is not significantly reduced until the average $NH_4$-N concentration reaches 1.6 ppm (0.0186 ppm free ammonia) and then only after six months. (However, from the accuracy of his results it is clear that this limiting $NH_4$-N value should in fact be stated as being somewhere between the free ammonia values of 0.0139 ppm and 0.0186 ppm.)

| Reuse Number | Oxygen Conc. | Free ammonia Conc. | Symptoms |
|---|---|---|---|
| 4th | 4.2 ppm | 0.0055 ppm | (i) Lower growth rate.<br>(ii) Increased red blood cell number.<br>(iii) Reduced resistance to bacteria gill infections. |

(i) and (ii) have been explained previously. (See page 18)

(iii) Larmoyeux and Piper (1973) suggest that ammonia may be a predisposing factor in bacterial gill disease, but oxygen stress could also be a contributory factor. Burrows (1964) states that when fish are continuously exposed to measurable amounts of ammonia, bacterial gill disease epidemics are prevalent and he suggests that this is due to ammonia irritation of the gills, gill hyperplasia being an extreme example of this. However, he makes no mention of the oxygen level in the data from which he draws this conclusion, and so one cannot judge whether oxygen stress might have been a contributory factor. There appears to be no real understanding of how the free ammonia physiologically irritates the gills.

| Reuse Number | Oxygen Conc. | Free ammonia Conc. |
|---|---|---|
| 5th | 3.8 ppm | 0.0064 ppm |
| 6th | 3.3 ppm | 0.0074 ppm |

Symptoms

(i) Lower growth rate.

(ii) Increased red blood cell number.

(iii) Reduced resistance to bacterial gill infections.

(iv) Dark coloured, poorly marked, lethargic after eight months.

(v) Reduced scope for activity, but greater stamina than "control" fish if first acclimatized for forty eight hours in clean water.

(vi) Gill hyperplasia, congestion of kidney glomeruli, reduction in lymphoid tissue of spleen, reduction in haematopoietic tissue of liver, and reduction in vacuolation of liver cells.

(vii) Increase in conversion.

(i), (ii) and (iii) have been explained previously. (see pp 18 - 20)

(iv) It is suggested that dark colouring and poor marking of the fish is simply a sign of stress. The reduction in thyroid activity, the lethargy of the fish, the reduced scope for activity, and the increased conversion efficiency of the food consumed, could all be seen as a response to a low oxygen environment. The reduced thyroid activity would produce a lower metabolic rate and so the reduced scope for activity and lethargy would follow. This would lead to a great oxygen saving on the fish's part, thereby adapting it to manage on the low oxygen level available. The decreased conversion efficiency of the food taken in would follow as a consequence of the metabolic processes of the fish involved in digestion being less efficient at lower oxygen levels. The lower glycogen levels of the liver may well just be a reflection of a lower daily food intake. This would not be surprising because it seems sensible if the fish is trying to economise on oxygen, that it should only eat sufficient food for it's daily maintenance, and as has been mentioned earlier, the latter has been reduced.

(vi) There is no clear indication of how the high ammonia or low oxygen levels cause the observed physical changes in liver, spleen and kidney tissue. As previously mentioned, Lloyd and Orr (1969) state that the rate of urine excretion in rainbow trout increases with a rise in the ambient level of un-ionised ammonia, and they suggest that this diuresis is caused by an increase in the permeability of the fish to water. They conclude by saying that free ammonia levels below 12% of the threshold value (0.0047 ppm) are without any effect on permeability. So, it would seem that Larmoyeux and Piper (1973) are not correct in suggesting that a congestion of the kidney glomeruli could be due to the kidney straining to cope with an increasing influx of water into the fish's body, caused by the prevailing level of free ammonia. However, it should be borne in mind that Lloyd and Orr (1969) were looking for an effect to show itself in a short period of time, and it seems feasible that free ammonia concentrations below the level that they

| cies | Size | Exposure Time | Free $NH_3$ (ppm) | $NH_4$-N (ppm) | | pH | | Temp. (°C | |
|---|---|---|---|---|---|---|---|---|---|
| | | | $T_{end}$ | $T_o$ | $T_{end}$ | $T_o$ | $T_{end}$ | $T_o$ | $T_{end}$ |
| ok<br>out | 3" - 4" | 50 mins. | | 0 | 3.00 | | | 8 | 10 |
| ok<br>out | 3" - 4" | 1 hr. 45 mins. | 0.008* | 0 | 3.25 | 7.9 | 7.0 | 8 | 13 |
| ok<br>out | 3" - 4" | 2 hr. 30 mins. | 0.0149* | 0 | 5.50 | 7.8 | 7.0 | 8 | 15 |
| ok<br>out | 3" - 4" | 4 hr. 50 mins. | 0.0131* | 0 | 0.50 | 7.9 | 7.8 | 8 | 20 |
| nbow<br>out | - | 6 weeks | - | 0.8 - 1.0 | | - | | - | |
| nbow<br>out | 23g. initially<br>Control 64.9g. )at end<br>Test 1 59.4g. )of<br>Test 2 50.8g. )expt. | 4 months | Control 0.0058<br>Test 1 0.0139<br>Test 2 0.0186 | Control 0.5<br>Test 1 1.2<br>Test 2 1.6 | | 7.75 | | 10 | |
| nbow<br>out | 23g initially<br>Control 64.9g. )at end<br>Test 1 59.4g. )of<br>Test 2 50.8g. )expt. | 6 months | As above | As above | | 7.75 | | 10 | |
| nbow<br>out | 23 g. initially<br>Control 147.4g. )at end<br>Test 1 134.3g. )of<br>Test 2 90.7g. )expt | 12 months | As above | As above | | 7.75 | | 10 | |
| inook<br>almon | Fingerlings | 1 hr. per day | 0.0126** | 0.7 | | 7.8 | | 16 | |
| inook<br>almon | Fingerlings | 12 hrs. per day | 0.0018** | 0.1 | | 7.8 | | 16 | |
| inook<br>almon | Fingerlings | 24 hrs. per day | 0.0018** | 0.1 | | 7.8 | | 16 | |
| inook<br>almon | Fingerlings | 6 weeks | 0.0026**<br>0.0043**<br>0.0060** | 0.3<br>0.5<br>0.7 | | 7.8 | | 6 | |
| inook<br>almon | Fingerlings | 6 weeks | 0.0047**<br>0.0079**<br>0.0101** | 0.3<br>0.5<br>0.7 | | 7.8 | | 14 | |

The Cortland Hatchery free $NH_3$ peak levels must have been far in excess of the free ammonia values found at the end of the experiment, because these peak values will be determined by the relative rates of fall of pH, rise of temperature and rise of the $NH_4$-N level.

Burrows (1964) figures for the free $NH_3$ concentrations are inaccurate (see Trussel 1972), and so they have been corrected.

TABLE 2 The results of various workers.

| $O_2$ (ppm) | | Reference | Comments |
|---|---|---|---|
| $T_o$ | $T_{end}$ | | |
| 10.3 | 7.4 | | |
| 10.1 | 7.0 | Cortland Hatchery 1950 | See page 23 |
| 8.8 | 5.6 | | |
| 8.6 | 5.0 | | |
| 7 - 8 | | Speece (1973) quoting 1970 Bozeman hatchery data. | Growth unaffected. |
| - | | | |
| - | | Smith, C.E. 1972 | See page 23 |
| - | | | |
| - | | | No ill effect. |
| - | | | Reduced growth rate. |
| - | | | Reduced growth rate, stamina and disease reisitance. |
| - | | Burrows 1964 | All developed gill hyperplaia which showed a corresponding increase in severity as the free $NH_3$ level increased. |

suggest, may have an effect in the longer term. It is interesting that Lloyd and Swift (1974) suggest that at low dissolved oxygen levels (3.5 ppm) there is an increase in the permeability of rainbow trout to water. So the kidney could be strained for this reason.

## Discussion of further experimental data concerning the effect of unfavourable ammonia and oxygen levels.

Table 2 shows the experimental results from other workers. The results from the Cortland Hatchery (1950) are very difficult to interpret because only the change in pH, temperature, oxygen concentration, and the $NH_4$-N concentration over the experimental period are given. I have calculated the free ammonia concentration present at the end of the experiments, but from the data given it is certain that during the experiments various combinations of pH, temperature, $NH_4$-N concentration, would have yielded much higher free ammonia concentrations than were recorded at the end. So, one has no idea of the free ammonia concentrations to which the fish have been subjected, and of course this makes a meaningful interpretation of the results impossible.

The results of Smith's (1972) six week experiment on rainbow trout are again limited in their value, because from the incomplete details given the free ammonia concentration can not be calculated. one could guess from other experiments of his that the temperature might be **10°C** and the pH might be 7.7, thus giving a free ammonia concentration range of 0.0074 ppm - 0.0092 ppm; but this could be very misleading and it really is not very satisfactory.

The results from his twelve month experiment are somewhat more informative. He says that he maintained the oxygen concentration at or above 5 ppm and the experimental free ammonia concentrations he tested were 0.0014 ppm and 0.0186 ppm . (The free ammonia concentrations that Smith calculated have been corrected according to Trussel (1972)). The "control" he used was based on a free ammonia concentration of 0.0058 ppm. But if one looks at Larmoyeux and Piper's (1973) results on table 1 it is clear that a free ammonia concentration of 0.0037 ppm coupled with an oxygen concentration of 4.9 ppm, caused the fish in that tank to show both a reduced growth rate and more red blood cells per unit volume of blood, after eight months. The consequence of this is that Smith (1972) has used a "control" environment which in fact is more than likely producing a detrimental effect on the fish living in it. This means that it is quite likely that a lower free ammonia concentration than is suggested by Smith (1972) might cause an ill effect on the fish after six months, but it is impossible to say how much lower the damaging free ammonia concentration might be, because it is not known to what degree the "control" environment was damaging the fish. So, it would have been much better if he had based the "control" on a safer free ammonia concentration of perhaps 0.0025 ppm.

His results indicate that after six months, fish kept in a free ammonia concentration of 0.0186 ppm when compared with his "control" fish showed a reduction in growth rate and showed signs of lethargy;

their gills showed evidence of hyperplasia and their livers showed reduced glycogen storage. After twelve months, there was an outbreak of bacterial gill disease in fish maintained in this free ammonia concentration, and fish maintained in a free ammonia concentration of 0.0139 ppm showed a reduced growth rate as compared to his "control" fish. Despite the shortcomings, Smith's observations are a valuable addition to those of Larmoyeux and Piper.

## Conclusions

Smith's (1972) results show that if the oxygen concentration is maintained at 5 ppm or above, then when the free ammonia concentration is at a level of 0.0186 ppm for six months, fish are noticed to be lethargic, have gill hyperplasia, increased incidence of bacterial gill disease, reduced glycogen storage in the liver, reduced growth rate, affected stamina. Larmoyeux and Piper's (1973) results show that if the oxygen concentration is maintained at this sort of level, (their free ammonia concentration was very low, that is up to 0.0046 ppm) then there is no detrimental effect on the fish. So, Smith has shown that a high ammonia level can cause the symptoms mentioned above. Larmoyeux and Piper observed extremely similar symptoms in their fish in a similar period of time to Smith if the oxygen level was lowered to 3.3 ppm (free ammonia concentration was 0.0074 ppm). Now, Larmoyeux and Piper's free ammonia level was less than half that which Smith had found necessary to cause the symptoms at 5 ppm oxygen concentration; and so it looks as though broadly speaking, moderate free ammonia levels at low oxygen levels will cause the same symptoms as high free ammonia levels at moderate oxygen levels.

From this information it would seem that the effects of free ammonia and low oxygen are inseparable, as suggested on page 7 Brockway (1950) suggests that a high free ammonia concentration reduces the blood's ability to use the available oxygen, and the symptoms observed (except for gill damage) would seem most likely to be caused by a low oxygen effect.

Free ammonia causes gill hyperplasia (Burrows 1964) with the consequence that the surface area of the gills is reduced. This in turn means that the gills have a reduced ability for gaseous exchange and so the rate of inward diffusion of oxygen is reduced, thereby causing or adding to an oxygen stress in the fish.

A point of value which this study has revealed is that as the oxygen level falls, then the gills become more susceptible to ammonia damage, or conversely, as the oxygen level rises, the gills become more resistant to gill damage. (Evidence:- gill hyperplasia recorded after four to six months under the following conditions:

(i) 0.0186 ppm free ammonia, oxygen of 5.0 ppm or above.
(ii) 0.0074 ppm free ammonia, oxygen of 3.3 ppm.)

This phenomenon may be partially or wholly due to the fact that at low oxygen levels, water is pumped over the gills more rapidly in order to help the fish gain the necessary amount of oxygen from the water, and so the amount of free ammonia which is brought in contact with the gill surfaces is increased. This idea is forwarded for a similar relationship with other poisons in the EIFAC technical report number 19.

In rounding off the discussion on ammonia toxicity, it seems relevant to mention that the gill damage caused by high free ammonia levels may not be permanent. Burrows (1964) gives results on the recovery of fish which have been exposed to damaging levels of free ammonia. His work shows that young chinook salmon, reared at 6°C in an environment which, due to it's free ammonia levels caused the gills to show hyperplasia, showed no recovery after they had been kept in clean water at the same temperature for three weeks. However, similar fish with similar symptoms, reared at 14°C showed a marked recovery from the hyperplasia after three weeks. These observations show the temperature dependance of tissue repair.

# CHAPTER 3

## The fundamentally important points that must be considered when assessing the quantity of fish which can be safely raised in a particular rearing system.

### The necessary physical details to ensure efficient water use in a fish rearing system.

In examining any rearing system, there are two main points to consider, one being the volume of water in the tank and the other being the rate of inflow of fresh water into the tank. If both these are known, then the rate of replacement or turnover of water in the tank can be assessed, and this leads to an understanding of the fish carrying potential of the tank. The rate of inflow of fresh water determines the rate of dilution of metabolic wastes produced by the fish and the rate of addition of dissolved oxygen to the tank. Clearly for the inflowing fresh water to have it's optimum effect, it must thoroughly mix with the tank water. If the tank water is relatively deep, there may be a tendency for the fresh water to stratify and lie on top of the water profile. So, in a system where the tank drain takes water off the top of the water profile, the beneficial effect of the fresh incoming water may be seriously reduced. In addition to this, excretory products ranging from faeces to ammonia are heavier than water and so tend to sink to the bottom of the tank, making conditions there less hospitable for the fish unless there is thorough mixing of the water.

## The real limitation to the fish carrying capacity of an efficient water reuse system.

In a single-pass system, the carrying capacity (i.e. the weight of fish of a particular size which can safely be held in a particular rearing system) is normally limited by the oxygen available rather than the accumulation of metabolic products (Kramer Chin and Mayo 1972), but if the water is being serially reused through a number of tanks or if the water is being recycled using an inefficient filter, then the level of metabolic products, especially ammonia may become limiting.

It is clear then, that in a well managed rearing system, it is the fishes' progressive lowering of the tank water's oxygen level as their loading increases that limits the carrying capacity.

## Consideration of the effect of fish density on the optimum carrying capacity of a fish rearing system.

Piper (1972) states that his experience has shown that water flow is of greater importance than water volume (space factor). He quotes an example which shows that although the density of rainbow trout in a rearing tank increased from 1 lb/cu ft to 5.6 lb/cu ft over a ten month period, there was no effect on the rate of length increase or food conversion. However, both he and Burrows and Coombes (1968) agree that the density cannot be raised indefinitely without there being a harmful effect on the fish, such as reduced growth rate due to stress and poor food utilization. Piper (1972) suggests a rule of thumb for not exceeding the maximum advisable density, that states that rainbow trout should not be kept above half their length in inches, in pounds per cubic foot of tank water (although he now feels this may be safely exceeded but he is awaiting the results of further experiments). Burrows and Coombes (1968) give advisable maximum densities which work out to be about one half of Piper's values, but they were working on salmon, and as salmon usually lie only at the bottom of the water profile whereas rainbow trout lie throughout the water profile, the difference in values is understandable.

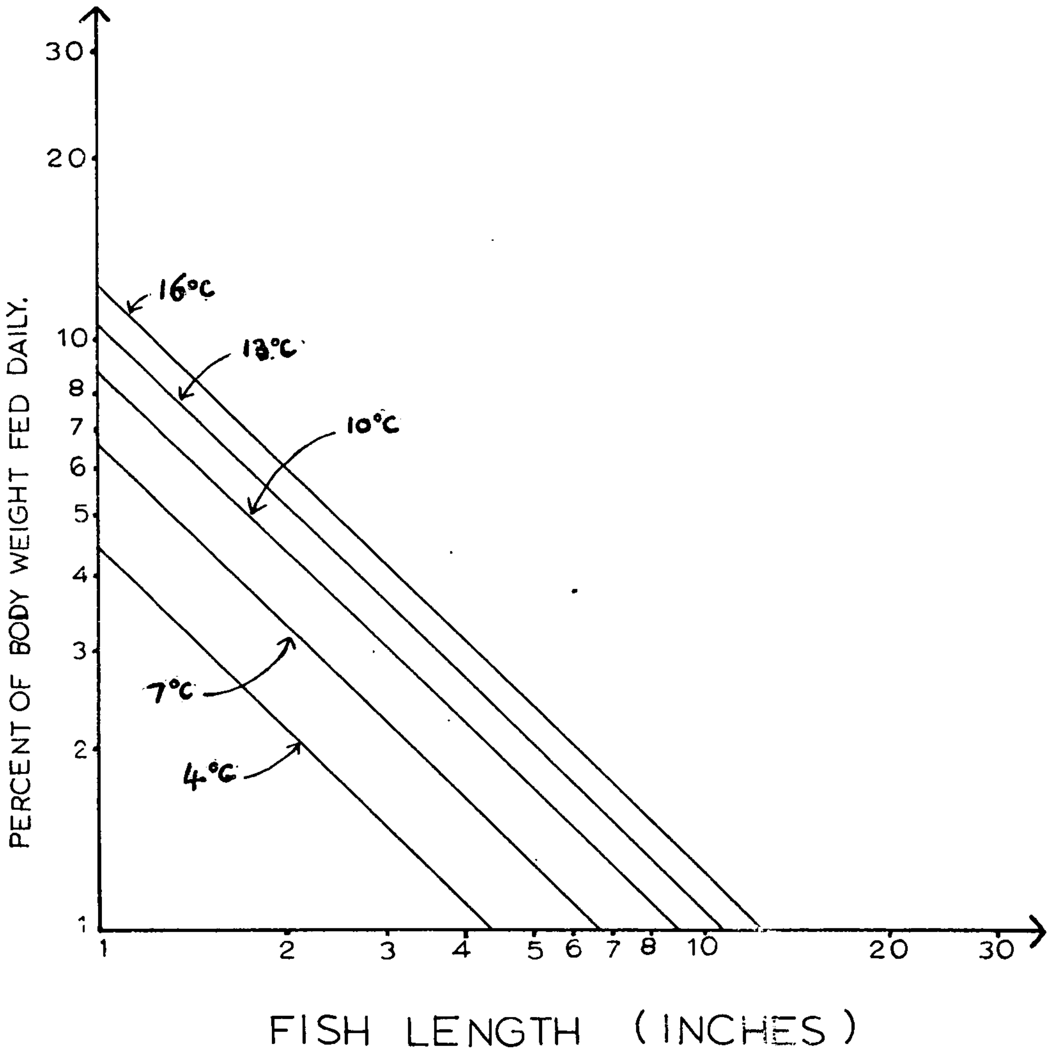
The oxygen uptake of fish is mainly dependent upon fish species, feeding rate, fish size, water temperature and activity; with sex, season and the presence of catabolic products having a lesser effect. (Liao 1971)

### (i) The effect of fish species on oxygen uptake.

Different fish species have innate differences in their diet, quantity of food eaten in relation to fish size and so metabolism. Thus as the oxygen requirements of a fish depend on the metabolic activity of the fish, then the oxygen requirements of different species must differ.

### (ii) The effect of the feeding rate, fish size and water temperature on oxygen uptake.

These three factors have to be considered together, as will be seen shortly. Basically, different sizes of fish are given different feeding rates (expressed as weight of food per unit weight of fish



Feeding rate as a function of length and temperature. (From: Speece 1973)

FIGURE 1.

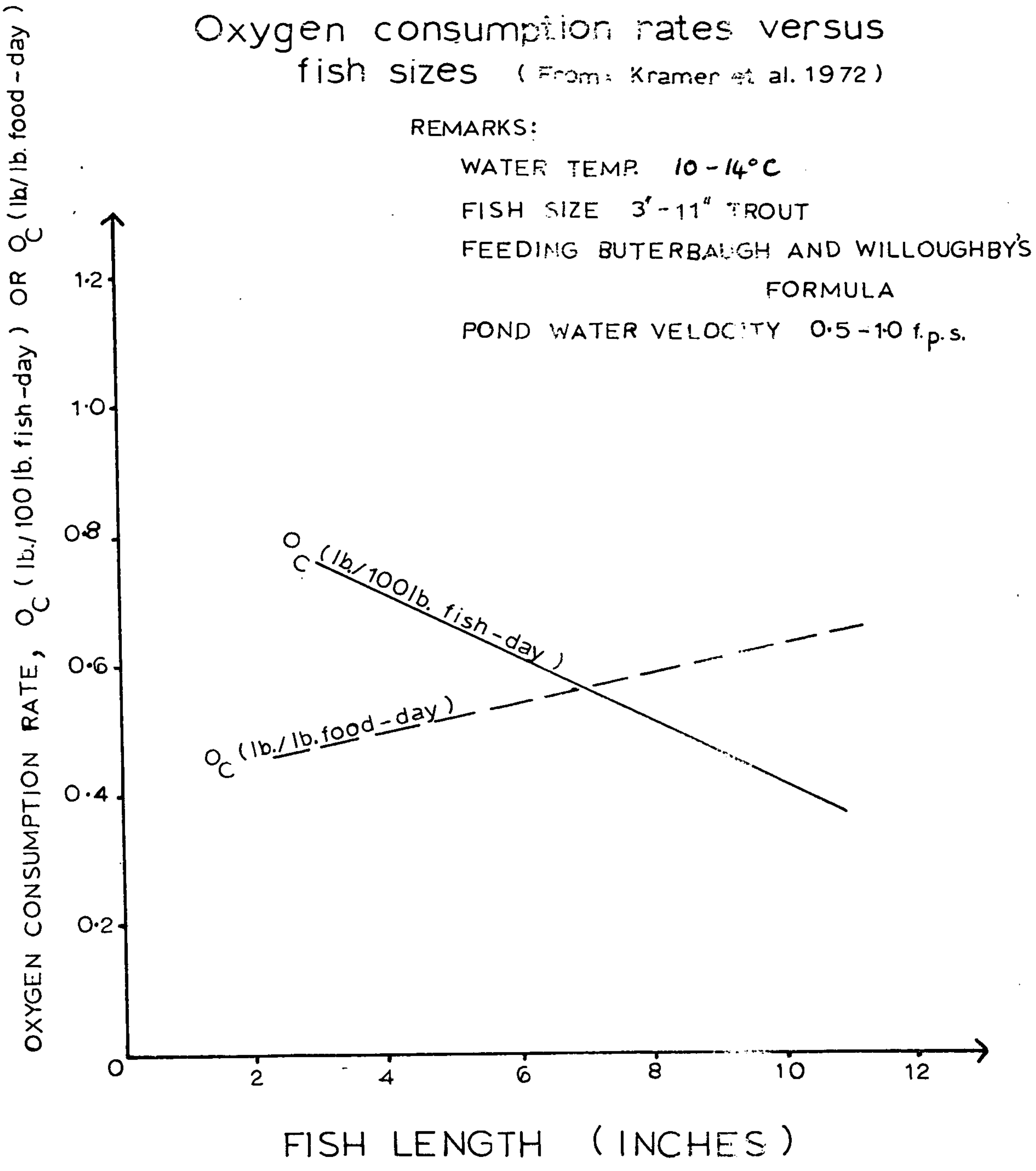

Oxygen consumption rates versus fish sizes (From: Kramer et al. 1972)
REMARKS:
WATER TEMP. 10 - 14°C
FISH SIZE 3"-11" TROUT
FEEDING BUTERBAUGH AND WILLOUGHBY'S FORMULA
POND WATER VELOCITY 0·5 - 1·0 f.p.s.
OXYGEN CONSUMPTION RATE, O_C (lb./100 lb. fish-day) OR O_C (lb/lb. food-day)
1·2
1·0
0·8
0·6
0·4
0·2
0
2
4
6
8
10
12
O_C (lb./100 lb. fish-day)
O_C (lb./lb. food-day)
FISH LENGTH (INCHES)

FIGURE 2.

per day or percentage body weight fed per day), and the feeding rate for a given fish size is further influenced by temperature. Feeding rates with respect to fish size and temperature can be found in tabular form in the following papers: Freeman, Haskell, Longacre, Stiles (1967) and Buterbaugh and Willoughby (1967).

These feeding rates have been worked out from the results of years of experimentation and experience, and they are designed to encourage the maximum growth rate in the fish and yet also give a favourable food conversion efficiency (that is, food intake in grams divided by growth in grams (EIFAC 1971)). The feeding rates are generally calculated so that the fish are fed to satiation, as this gives the maximum growth rate (EIFAC 1967); but Freeman, Haskell, Longacre, and Stiles (1967) express a view that perhaps trout growth is very nearly independant of feeding levels above a certain minimum level, and that feeding more than this simply increases cost and reduces the conversion efficiency. So, clearly more work is needed in this field.

Speece (1973), used Buterbaugh and Willoughby's (1967) feeding rates and produced a graph (see figure 1 ) to show how the feeding rates vary at different fish sizes and temperatures. The feeding rate declines as:
(a) the fish size increases; and (b) the temperature falls.

(a) Figure 2 (Kramer Chin and Mayo 1972) shows that as the feeding rate declines as the fish size increases, the oxygen consumption per

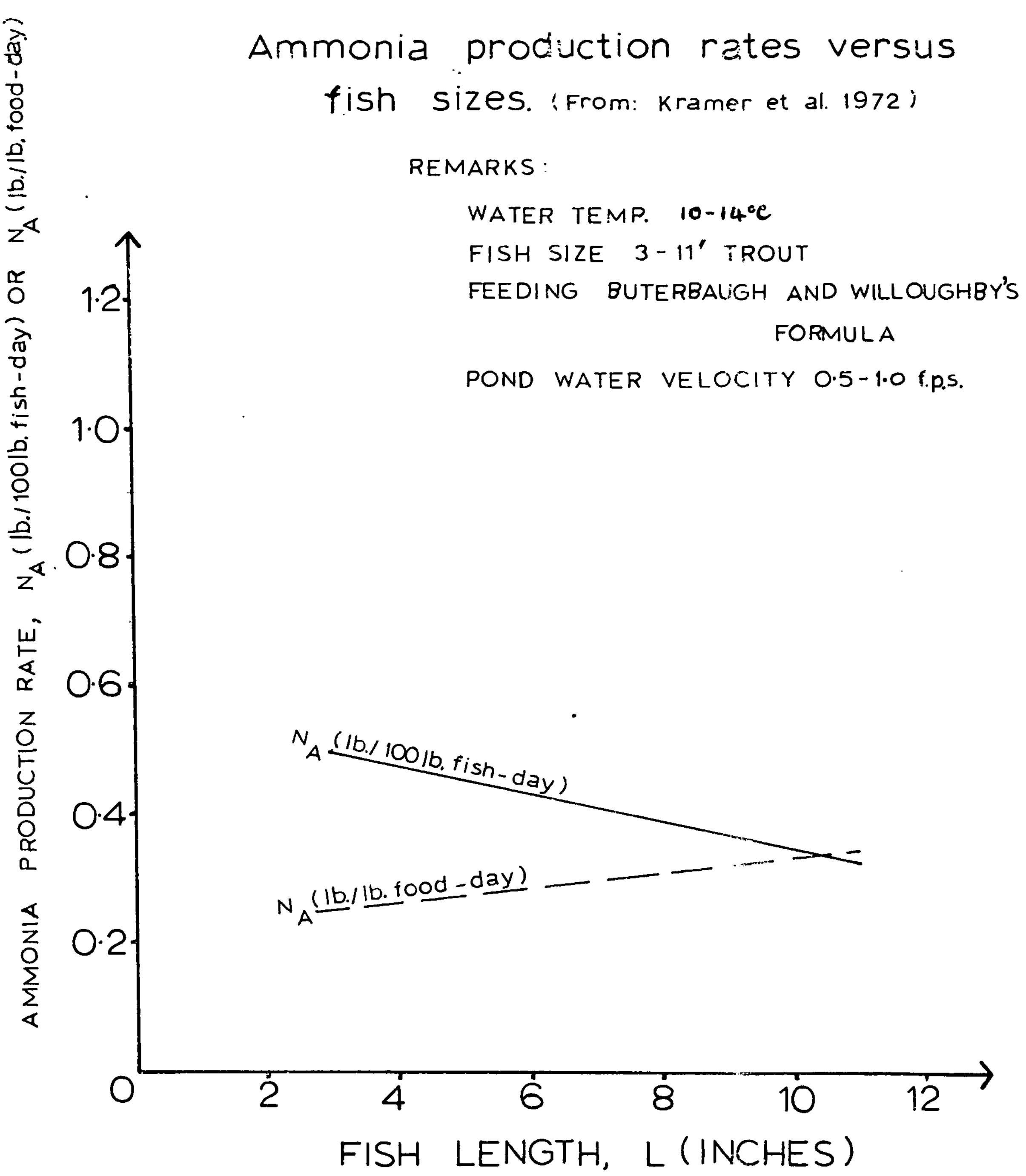

Ammonia production rates versus fish sizes. (From: Kramer et al. 1972)
REMARKS:
WATER TEMP. 10-14°C
FISH SIZE 3-11' TROUT
FEEDING BUTERBAUGH AND WILLOUGHBY'S FORMULA
POND WATER VELOCITY 0·5-1·0 f.p.s.
AMMONIA PRODUCTION RATE, $N_A$ (lb./100lb. fish-day) OR $N_A$ (lb./lb. food-day)
1·2
1·0
0·8
0·6
0·4
0·2
0
$N_A$ (lb./100lb. fish-day)
$N_A$ (lb./lb. food-day)
2
4
6
8
10
12
FISH LENGTH, L (INCHES)

FIGURE 3.

Oxygen consumption and metabolite production rates versus feeding rates. ( From: Kramer et al. 1972 )

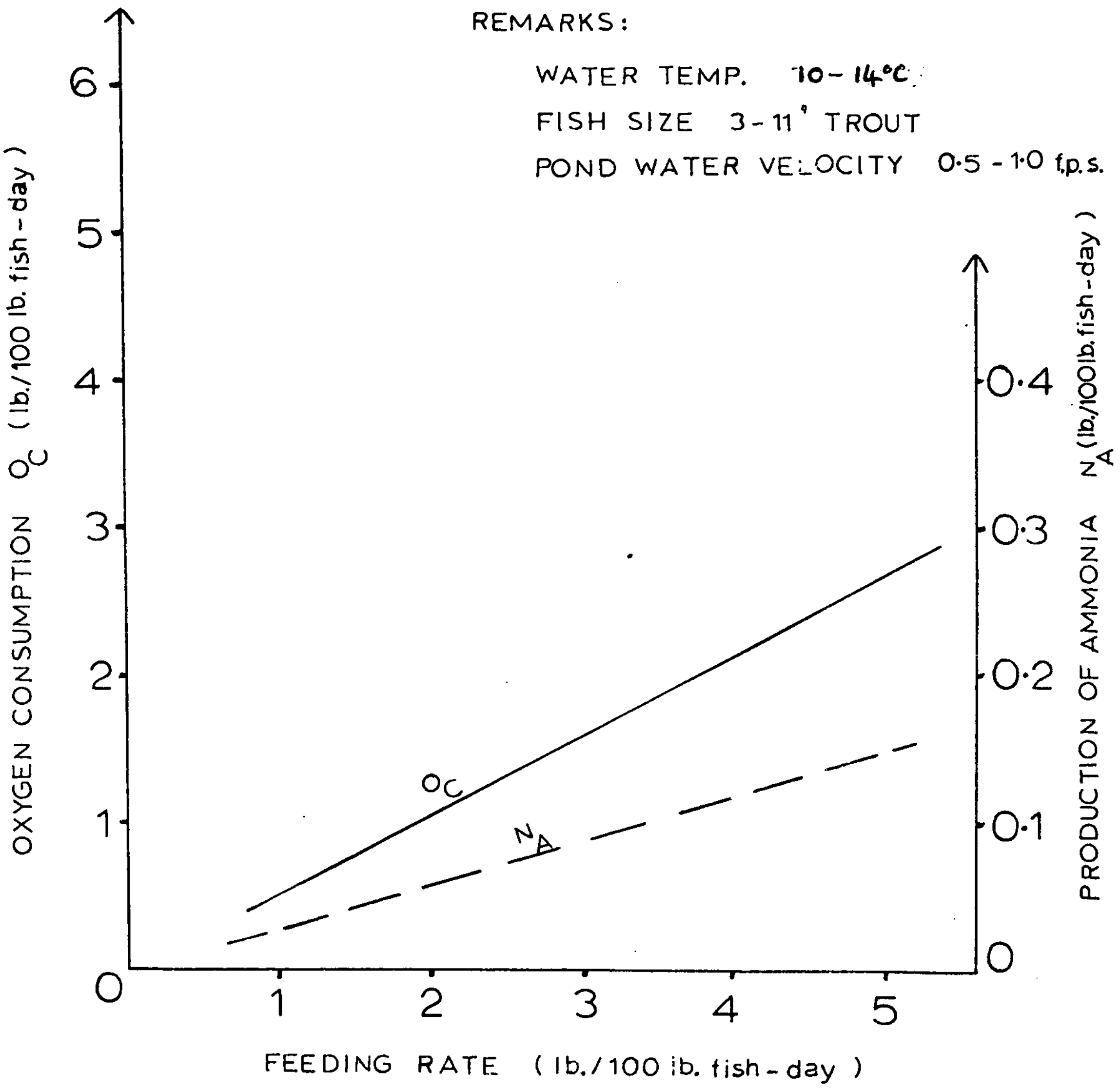


FIGURE 4.

unit weight of fish falls, despite the oxygen consumption per unit weight of food fed to larger fish, being higher, due to larger fish having a more efficient food metabolism. This more efficient metabolism of larger fish is shown again by figure 3 of (Kramer Chin and Mayo 1972), which shows that although the weight of $NH_4$-N produced per unit weight of food fed, was greater in larger fish than smaller ones, the decline in the feeding rate of larger fish is attributed partially to their greater efficiency of food metabolism, but primarily to the fish being less metabolically active per unit weight of fish than smaller fish.

This difference between larger and smaller fish in oxygen consumption per unit weight of fish clearly means that a fish tank with a given change over-rate of water can support a greater weight of larger fish than smaller fish.

(b) Elliott(1974) noted in his experiments with brown trout that the appetite increased with rising temperature, until the appetite reached it's maximum in the range 13.3°C - 18.4°C. This increased appetite with it's associated increase in oxygen consumption and ammonia production (see figure 4 from Kramer Chin and Mayo 1972), is attributed to the metabolic rate of the fish increasing as the temperature rises. So, clearly as the temperature rises, the weight of fish of a given size, that can be supported in a fish tank with a given change over rate of water, decreases. (An increase in temperature will also mean that the oxygen content of the inflowing freshwater will be reduced, assuming that this water will be saturated with air as it enters the tank. This is a well known physical phenomenon.) To summarize, Wester (1970) indicates that there is a 25% decrease in carrying capacity for a rise in temperature.

### Calculating the carrying capacity of a rearing system, from a knowledge of the quantity of oxygen available in the water.

The discussion so far has paved the way for an understanding of the ways in which the carrying capacity of a tank can be calculated. Haskell (1955) stated that the amount of oxygen consumed by fish was proportional to the amount of food consumed (see figure 4 from Kramer Chin and Mayo (1972)), and Willoughby (1968) bearing this in mind, tackled the problem of calculating carrying capacity by first of all looking at the amount of oxygen available for use. This he could do because he could measure the oxygen level of the inflowing water, subtract 5 ppm from this to give him the available oxygen, and then relate this quantity to the inflow rate of fresh water. (Workers in this field are agreed that the oxygen level of the tank water must not drop below 5 ppm, and their reason for stipulating this minimum value is that they have found through experience that an oxygen level below 5 ppm causes a reduced growth rate and other problems.) (Willoughby (1968), Larmoyeux and Piper (1973) and Burrows and Coombes (1968))

Having calculated the amount of available oxygen, Willoughby could use Haskell's relationship to calculate the feeding level, which if applied, would reduce the oxygen level of the water to 5 ppm.

The following equation was developed by Willoughby (1968).

$$O^a - O^b \times \frac{5.45}{100} \times \text{gpm} = \text{lb. food per day}$$

where:-

$O^a$ is the oxygen concentration in the inflowing water.

$O^b$ is the 5 ppm oxygen in the outflowing water.

5.45 is the metric tons of water in 1 gpm flowing for 24 hours.

100 is the grams of oxygen needed to metabolise 1 lb. of food (1200 cals.)

gpm is the rate of inflow of fresh water, in gallons per minute.

Willoughby then argued that the pond could safely carry the number of fish of any particular size which would correspond to the calculated feeding rate.

N.B. He does not make any mention of how changes in water temperature would affect the weight of fish of a given size that would be appropriate to a given feeding level.

Piper (1970) took up Willoughby's results and said that because there is a straight line relationship between the length of the fish in inches and the feeding rate, when plotted on logarithm paper (see figure 1 from Speece 1973); the fish size in inches can be used instead of the weight of food fed daily, to calculate the safe carrying capacity of a tank. He created the term "loading factor" for calculating the carrying capacity, which is defined as follows:-

## TABLE 5

## Loading factors as related to water temperature and altitude for trout and salmon. (Piper 1970)

| | Water Temp. °F | 0 | 1000 | 2000 | 3000 | 4000 | 5000 | 6000 | 7000 | 8000 | 9000 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 40 | 2.70 | 2.61 | 2.52 | 2.43 | 2.34 | 2.25 | 2.16 | 2.09 | 2.01 | 1.96 |
| 5°C | 41 | 2.61 | 2.52 | 2.44 | 2.35 | 2.24 | 2.18 | 2.09 | 2.02 | 1.92 | 1.87 |
| | 42 | 2.52 | 2.44 | 2.35 | 2.27 | 2.18 | 2.10 | 2.02 | 1.95 | 1.88 | 1.81 |
| | 43 | 2.43 | 2.35 | 2.27 | 2.19 | 2.11 | 2.03 | 1.94 | 1.88 | 1.81 | 1.74 |
| | 44 | 2.34 | 2.26 | 2.18 | 2.11 | 2.03 | 1.95 | 1.87 | 1.81 | 1.74 | 1.68 |
| | 45 | 2.25 | 2.18 | 2.10 | 2.03 | 1.95 | 1.88 | 1.80 | 1.74 | 1.68 | 1.61 |
| | 46 | 2.16 | 2.09 | 2.02 | 1.94 | 1.87 | 1.80 | 1.73 | 1.67 | 1.61 | 1.55 |
| | 47 | 2.07 | 2.00 | 1.93 | 1.86 | 1.79 | 1.73 | 1.66 | 1.60 | 1.54 | 1.48 |
| | 48 | 1.98 | 1.91 | 1.85 | 1.78 | 1.72 | 1.65 | 1.58 | 1.53 | 1.47 | 1.42 |
| | 49 | 1.89 | 1.83 | 1.76 | 1.70 | 1.64 | 1.58 | 1.51 | 1.46 | 1.41 | 1.36 |
| 10°C | 50 | 1.80 | 1.74 | 1.69 | 1.62 | 1.56 | 1.50 | 1.44 | 1.39 | 1.34 | 1.29 |
| | 51 | 1.73 | 1.67 | 1.62 | 1.56 | 1.50 | 1.44 | 1.38 | 1.34 | 1.29 | 1.24 |
| | 52 | 1.67 | 1.61 | 1.56 | 1.50 | 1.44 | 1.39 | 1.33 | 1.29 | 1.24 | 1.19 |
| | 53 | 1.61 | 1.55 | 1.50 | 1.45 | 1.39 | 1.34 | 1.29 | 1.24 | 1.20 | 1.15 |
| | 54 | 1.55 | 1.50 | 1.45 | 1.40 | 1.34 | 1.29 | 1.24 | 1.20 | 1.16 | 1.11 |
| | 55 | 1.50 | 1.45 | 1.40 | 1.35 | 1.30 | 1.25 | 1.20 | 1.16 | 1.12 | 1.07 |
| | 56 | 1.45 | 1.40 | 1.35 | 1.31 | 1.26 | 1.21 | 1.16 | 1.12 | 1.08 | 1.04 |
| | 57 | 1.41 | 1.36 | 1.31 | 1.27 | 1.22 | 1.17 | 1.13 | 1.09 | 1.05 | 1.01 |
| | 58 | 1.36 | 1.32 | 1.27 | 1.23 | 1.18 | 1.14 | 1.09 | 1.05 | 1.07 | 0.99 |
| 15°C | 59 | 1.32 | 1.28 | 1.24 | 1.19 | 1.15 | 1.10 | 1.06 | 1.02 | 0.99 | 0.95 |
| | 60 | 1.29 | 1.24 | 1.20 | 1.16 | 1.11 | 1.07 | 1.03 | 0.99 | 0.96 | 0.92 |
| | 61 | 1.25 | 1.21 | 1.15 | 1.13 | 1.08 | 1.04 | 1.00 | 0.97 | 0.93 | 0.90 |
| | 62 | 1.22 | 1.18 | 1.14 | 1.09 | 1.05 | 1.01 | 0.97 | 0.94 | 0.91 | 0.87 |
| | 63 | 1.18 | 1.14 | 1.11 | 1.07 | 1.03 | 0.99 | 0.95 | 0.92 | 0.88 | 0.85 |
| | 64 | 1.15 | 1.12 | 1.08 | 1.04 | 1.00 | 0.96 | 0.92 | 0.89 | 0.86 | 0.83 |

$$\text{Loading Factor} = \frac{\text{Weight of fish}}{\text{Fish length in inches x gpm inflow of fresh water}}$$

N.B. He states that the optimum loading factor for a given inflow of water in gpm, is affected by the temperature and altitude, which both affect the solubility of oxygen in water (see table 3 from Piper 1970 ). As an example, he states that at 10°C and 5000 feet altitude, the optimum loading factor at which rainbow trout growth was not impaired was 1.5. (His maximum advisable density in pounds per cubic feet of trough space as mentioned on page 29, must be borne in mind.)

## The correct way to express the fish density in a particular rearing system.

Many workers report the densities of fish that they can hold in their tanks as x lb/cu ft of tank water, or y lb/gpm inflow. Findings expressed in one of these forms, without reference to the other, and preferably water changeover, can be very misleading. The potential maximum value of x in a tank is limited by Piper's (1972) maximum advisable density for each size, but below this value, it is always proportional to the rate of inflow of freshwater, and so attaching any meaning to it's value israther pointless if the value of x only is given. If the value of only y is given this is much more meaningful, but if the tank is small in relation to a high rate of fresh water inflow, then the value of y may be limited again by the advisable optimum density of fish in pounds per cubic feet of tank water given by Piper (1972). Hence the importance of giving the values of both x and y can be seen. The information provided by x and y can be combined to give the expression:

Pounds of fish per cubic foot of tank water per single change over of water per hour.

Apart from an increase in the inflow rate enabling an increased weight of fish to be supported in the tank until the weight is limited by Piper's maximum advisable density ruling, there will be an increased rate of water velocity in the tank and so increased activity and oxygen consumption by the fish.

# CHAPTER 4

## The reconditioning of recirculating water.

As has been mentioned in the introduction, when water is recirculated in a fish rearing system, for use over again, it must be purified, or reconditioned to stop the dissolved oxygen level falling too low and the level of metabolic products rising too high. The dissolved oxygen level in the water can be raised simply by vigorous aeration, the degree of the oxygen level increase depending on the amount of aeration and the temperature. This aeration should also lower the level of carbon dioxide. But removing the metabolic products, chiefly ammonia and dissolved organic matter, is a much more complicated business. A thorough survey of the possible water treatment processes is given by Kramer Chin and Mayo (1972), and the possible processes which they suggest might valuably be briefly discussed.

### Possible reconditioning methods.

#### (i) Air Stripping

This method can be very efficient in ammonia removal but the dis-advantages involved, can clearly be seen. It depends on the addition of lime to the water to be treated to raise the pH to 10 so that virtually all the ammonia in the water will be present in the unionised form. This water is then passed down a tower filled with plastic media, and air rushes in a counter flow upwards direction, carrying away the ammonia gas. If the water flow to air flow ratio is correct and the pH is greater than ten and the temperature is 16°C - 21°C then 98% ammonia removal is possible. Clearly the water then needs to be treated again to lower the pH to the desired level for fish culture.

### (ii) Ion Exchange

This method involves passing the water to be treated, through a bed of cation exchange resin. As most of the ammonia in the water is present as $NH_4^+$, it can be removed quite efficiently. The size of bed needed is proportional to the quantity of $NH_4^+$ per gpm to be removed, and in these calculations it must be borne in mind that the ammonium ions will compete for adsorption with all the other cations in the water.

The disadvantages of this method are that it is both relatively expensive and the bed may become "exhausted" when not expected. (Changing fish loads in the equipment make the "exhaustion" time difficult to predict.) Of course the exchange resin can be regenerated.

### (iii) Chemical coagulation

Chemical coagulants are used to coagulate waste materials in suspension or in colloidal form, by neutralising the charges on the particles. Some of the ammonia is associated with solids and physical removal of the fine solids can be very difficult. The problems with this method are that the treated water needs to be retained for approximately three hours for coagulation, sedimentation and filtration which means large water holding facilities; and also errors in adding the chemicals could be serious.

### (iv) Activated sludge process

This method relies on a biological oxidation of ammonia by the same ways as are described in method (v) (Kramer, Chin and Mayo 1972); (Bruce and Merkens 1970). The only difference is that the biological oxidation takes place in an aerated fluidised suspension as compared with a filter bed. The suspended solids concentration and the degree of aeration necessary is calculated, and the total retention time is five to ten hours. This process is not as efficient as biological filtration. (Kramer, Chin and Mayo 1972)

### (v) Biological filter system

This method involves removing solids by mechanical screening or settling, and then passing the water to be treated through a filter bed. The filter media may be one or more of the following:
sands, gravels, rocks, plastic media, polystyrene beads, anthracite, oyster shells etc., and the purpose of the bed is :

(a) to provide a medium on which micro-organisms will grow;these micro-organisms removing ammonia and dissolved organic matter from the water.

(b) to remove any remaining solids from the water.

The filter can be operated in two main ways. One way is to have the water flowing upwards through the filter, and this submerged filter has the advantage (as will be explained later) that the retention time within the filter of the water to be treated, can be controlled simply be altering the hydraulic loading. However, this method has the disadvantage that the water needs to be thoroughly aerated before entering the filter, in order to satisfy the oxygen demand of the bacteria. The other way of operating the filter is to disperse the water to be treated, over the surface of the filter and allow it to trickle or percolate through, continually re-aerating itself as it does this, by means of the air currents which flow through the filter voids.

Experience has shown that if well managed, this latter method is the most efficient, simple and easy to install (Kramer Chin and Mayo 1972), and has a low operating cost (Bruce 1969). For these reasons, biological percolating filters are by far the most popular method of water treatment in fish propogation systems and also in fact in sewage treatment systems, where this method has been used for some sixty years (Bruce 1969).

As this method is the most widely used, a detailes explanation of the filter's specification and how it works will be of value.

## The essential criteria for an efficient biological filter.

As has already been mentioned, the prime purpose of the biological filter is to remove ammonia from the water and so the structure of the filter bed is designed to achieve this in the most efficient manner. The somewhat conflicting criteria that one has to consider when designing a filter are:

(i) The filter should be efficient at removing ammonia.

(ii) The filter should not clog and so severly restrict the water flow.

(iii) The filter should for the sake of economy be as compact in size as possible.

The solution must be a compromise and the reason for this will clearly be seen when the criteria for each of these requirements are examined in more detail.

(i) The filter should be efficient at removing ammonia.

Many research workers discuss the micro-organisns which remove ammonia from the water in a biological filter, but the most thorough coverage seems to be given by Painter (1970) in a literature review on inorganic nitrogen metabolism in micro-organisms. He explains how ammonia as $NH_4^+$ is oxidised in two steps, firstly to nitrite $NO_2^-$ and secondly to nitrate $NO_3^-$, this oxidation process being termed nitrification. Both steps can be carried out by a limited number of autotrophic bacteria, the main genera carrying out the first step being Nitrosomonas and Nitrosococcus; and the main genera carrying out the second step being Nitrobacter and Nitrocystis. (Nitrosomonas and Nitrobacter are the chief nitrifying bacteria found in biological filters.) A large number of heterotrophic fungi and bacteria can also form $NO_2^-$ or $NO_3^-$.

Buswell et al. (1954) give the optimum temperature for Nitrosomonas growth as 30°C - 36°C with little growth below 5°C. Deppe and Engel (1960) report the optimum temperature for Nitrobacter as being 34°C - 35°C with no growth below 4°C, whereas Laudelont and van Tichelen (1960) report the optimum as being 42°C. There is similar vagueness with regard to pH optima, with Winogradski and Winogradski (1933) reporting six strains of Nitrosomonas with pH optima ranging from six to nine, and seven strains of Nictrobacter with pH optima varying from 6.3 - 9.4. From this information, it is clear that the optimum environmental conditions for the filter will vary, depending on the particular strains of nitrifying micro-organisms that colonise it. However, it can be said that the pH optimum for nitrification is usually on the alkaline side of 7.0 (Lees (1952) reported that the nitrification rate fell dramatically when the pH was less than 7.0), and the temperature optimum tends to be above 30°C and below 40°C.

Both Nitrosomonas and Nitrobacter have minimum requirements for : carbon dioxide (carbon source), $NH_4^+$ or $NO_2^-$, and oxygen concentration (4 mg/litre, Eikum 1967), and trace elements eg: magnesium, iron, calcium, copper and molybdenum. The supply of trace elements is often ensured by having broken oyster shells covering the top of the filter; these shells are rich in trace elements and also release calcium carbonate which stabalises the pH. Burrows and Coombes (1968) found that if they had no oyster shells on top of their filter, in three weeks the pH dropped to 5.7 and the salmon fingerlings died. This drop in pH was due to a build up of nitrate from the nitrification process, causing the formation of nitric acid; and they found that the presence of shell releasing calcium carbonate bound the nitrate as calcium nitrate, thereby stabalising the pH at 7.8 - 8.0 which is about the optimum for salmonid production. A relevant footnote here, is that although a pH of 7.8 - 8.0 is the optimum for salmonid production, it also means that at 50°F, 1.2% - 1.8% of the ammonium nitrogen present in the water will be present as free ammonia Trussel (1972), and this is in fact a relatively high percentage. So, having the pH so high means that in order to keep the free ammonia concentration at a safe level, the water may have to be filtered more often or the fish loading may have to be lower, or the amount of water lost from the system daily and made up with freshwater may have to be higher than would be the case if the pH was lower. Whether the increased level of free ammonia constitutes a threat to the fishes' health depends to a great extent on the oxygen level of the water (see pages 7 and 8).

The nitrate which has been produced by the micro-organisms is then potentially available for assimilation into cellular organic nitrogen, or dissimilation. This latter process involves the oxidation of carbon compounds, using $NO_3^-$ as an alternative hydrogen acceptor to oxygen. Denitrification is a special case of this process, where $NO_3^-$ is reduced to yield nitrogen gas or $N_2O$. However, dissimilation does not occur to a significant extent in a biological filter because conditions are aerobic (they have to be for the nitrification process) and the aerobic decomposition of carbon compounds is energetically more efficient. The presence of oxygen prevents the formation of nitrate reducing enzymes Painter (1970), and so dissimilation can only occur under anaerobic conditions.

Denitrification could be carried out as a separate process, by means of an anaerobic flooded denitrification filter. McCarthy, Beck and Amant (1969) describe how methanol can be mixed with the water, thereby both making it anaerobic and providing a carbon source. They provide an equation for calculating the quantity of methanol needed:

$$C_m = 2.47N_o + 1.53N_i + 0.87D_o$$

where: $C_m$ = the quantity of methanol in mg/litre necessary for denitrification.

$N_o$ = the mg/litre of $NO_3$-N present.

$N_i$ = the mg/litre of $NO_2$-N present.

$D_o$ = the mg/litre of initial dissolved oxygen concentration present.

This method has been reported to remove in excess of 90% of the nitrate present in the water. In practice, fish propagation establishments find this a somewhat expensive process, but as high nitrate levels could be toxic, a long term nitrate build up is avoided by discarding a quantity of the water every time it is recycled and making up this loss with fresh water. The quantity of water discarded every cycle is recommended to be in the order of 5% of the total water volume (Burrows and Coombes 1968). They suggest that 2% would be adequate, but 5% would allow a margin of safety.

In the early stages of a filter's life, there is a risk of a nitrite build up, and this is very toxic to fish. Smith and Williams (1971) found that when trout were exposed to a nitrite concentration as low as 0.15 mg/litre for forty eight hours, about 72% of the haemoglobin in the blood was converted to methaemoglobin, compared to only 2% methaemoglobin in the blood of fish living in a nil nitrite concentration. So this work shows the mode of nitrite's action.

However, there seems to be no information on the effect of long term low nitrite exposure and so one cannot really advise safe nitrite levels.

Kramer Chin and Mayo (1972) state that the start up time for a new filter is usually one to two months and during this time the way to avoid high nitrite levels is to increase the fish loading on the filter gradually; once the reconditioning system has been seasoned and is in a steady state condition, then there should be no danger from excessive nitrite.

From what has been said so far, it might seem as though nitrifying bacteria are the only micro-organisms present in the filter, but this is certainly not true. Degradable organic matter (normally expressed in terms of BOD) is also present in the water as it enters the filter, and this is removed by heterotrophic bacteria and fungi. These carbonaceous compound degrading micro-organisms are more successful than the nitrifying bacteria at competing for living space within the filter, and so although zones of carbonaceous

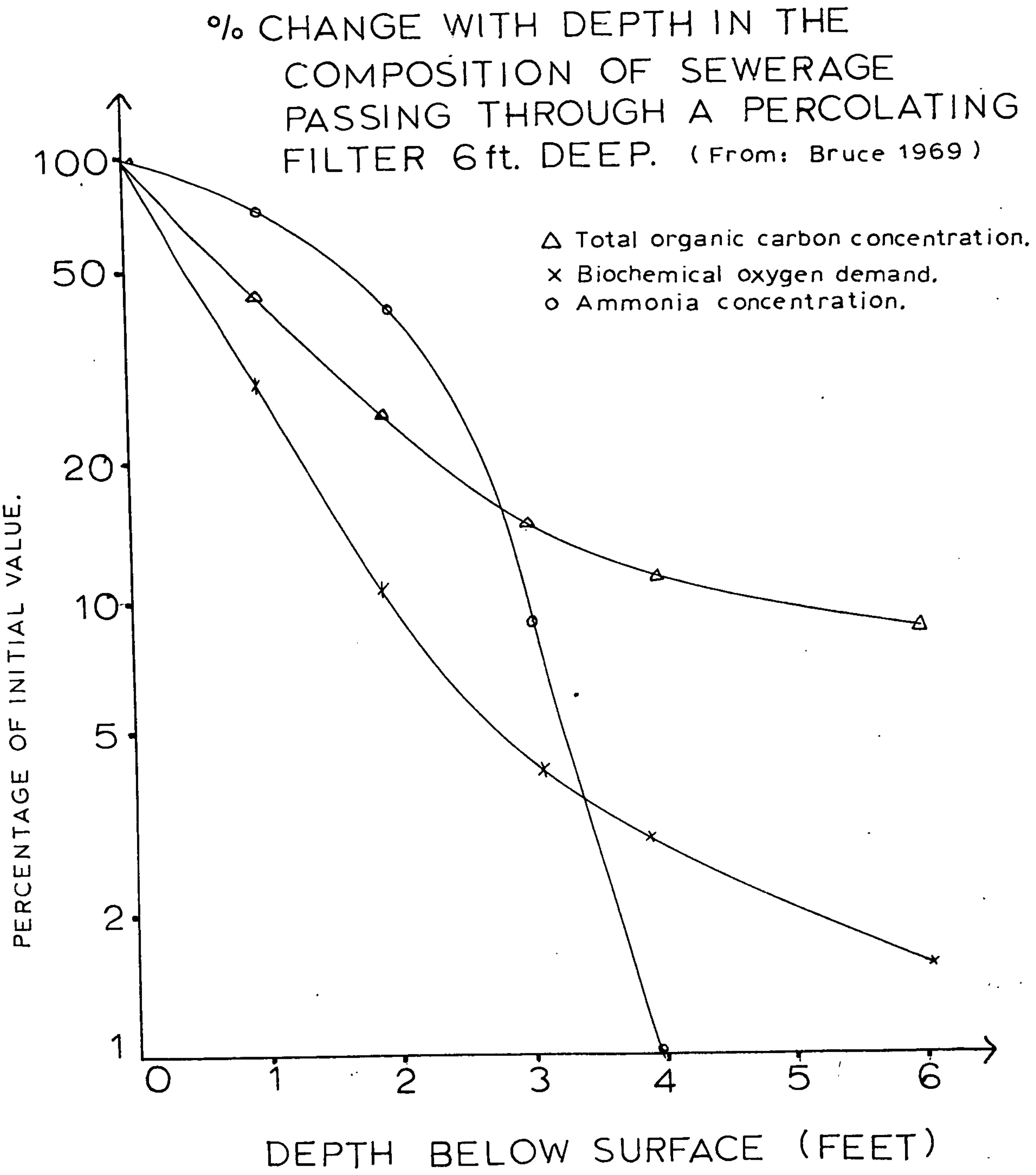

% CHANGE WITH DEPTH IN THE COMPOSITION OF SEWERAGE PASSING THROUGH A PERCOLATING FILTER 6ft. DEEP. (From: Bruce 1969)
△ Total organic carbon concentration.
× Biochemical oxygen demand.
○ Ammonia concentration.
PERCENTAGE OF INITIAL VALUE.
100
50
20
10
5
2
1
0
1
2
3
4
5
6
DEPTH BELOW SURFACE (FEET)

FIGURE 5.

oxidation and nitrification are not sharply defined, the former process tends to take place towards the top of the filter profile and the latter process tends to occur lower down. The extent of the carbonacious compound oxidation zone will depend on the success of the removal of these compounds before they reach the filter.

Burrows and Coombes (1968) stated that they had found by experimentation that no matter what the surface area of the filter was, a minimum depth of five feet was an essential requirement for a biological filter if it was to be efficient at removing ammonia; and Bruce (1969) gives a similar depth for filters involved in sewage effluent purification, which is analagous. (see figure 5 from Bruce 1969) One must be careful here, in that although the technology of sewage effluent purification is very relevant to hatchery water treatment, the levels of undesirable compounds are different in the former effluent compared to the latter (Kramer, Chin and Mayo 1972).

The implications of this minimum filter depth are not very clear, in that one would have thought that if a particular hydraulic water load of a given ammonia concentration was dispersed, in one case over a filter of surface area 100 square feet and depth five feet, and in another case over a filter surface area of 200 square feet and depth 2.5 feet, then the resulting filtrate should be chemically the same; the ammonia load per unit volume of filter being the same in both cases. However this does not appear to be the case.

To suggest that the minimum depth for a filter is related to the size of media used would seem to be sensible. After all, the larger the media, the greater the percentage of the filter volume that is void; and the greater the percentage voidage, the deeper one would expect the filter to have to be in order to ensure that all the water entering the filter, comes in contact with a bacterial film on the media, and that there is no hydraulic short circuiting. This is purely a physical phenomenon. So, this minimum filter depth of five feet, noted by Burrows and Coombes (1968), may be peculiar to the size of media used. This point of view is supported by evidence from Parisot (1967); who built a filter out of smaller media than Burrows and Coombes, and found that a depth of two feet was adequate for efficient performance. Unfortunately, neither Burrows and Coombes nor Parisot quantify what they mean by "efficient performance" and so one does not know what percentage of the total $NH_4$-N entering the filter is removed in a single pass. (This point is discussed in more detail on pages 52 - 55.)

(ii) The filter should not clog and so severly restrict the water flow.

and

(iii) The filter should for the sake of economy be as compact as possibl

These two criteria are bound up together as will be seen. The first step to take, in order to help ensure that the filter does not clog, is to remove as much of the solid matter as possible, from the water to be treated. (Solids should also be removed because they become secondary sources of ammonia production if allowed to decompose in the system - Kramer Chin and Mayo 1972).

This can best be done by having a settling tank or a series of settling troughs (as at Unilever's Laboratory at Findon) where, because of their high viscosity, many of the solid particles settle out (Kramer Chin amd Mayo 1972 recommend a basin with a retention time of 15 - 30 minutes and a depth greater than 3 feet). There will nevertheless still be some colloidal particles in suspension which will be carried into the filter where they will probably be adsorbed onto bacterial films or fungal mycelium and so add to the organic bulk in the filter.

An alternative to the settling tank would be a rapid sand filter in which the water is drawn through a bed of fine sand in order to remove particulate matter. This filter has the advantage of being compact, but being composed of quickly clogged fine sand, it has the disadvantage of needing frequent back flushing for cleaning purposes.

The removal of suspended solids from the water may well be important not only in removing sources of ammonia production, but also in reducing the turbidity of the water. Olson Chase and Hanson (1973) found in their experiments that fish kept in water of a high turbidity due to a silt suspension showed physical irritation of the gill lamellae, resulting in gill hyperplasia, reduced growth rate, and inactivity.

As the filter bacteria grow, their films on the surfaces within the filter increase in thickness (Bruce 1969) and this leads to a

SPECIFIC SURFACE AREA PER UNIT VOLUME VERSUS SIZE OF FILTER ROCK ( From: Speece 1973 )

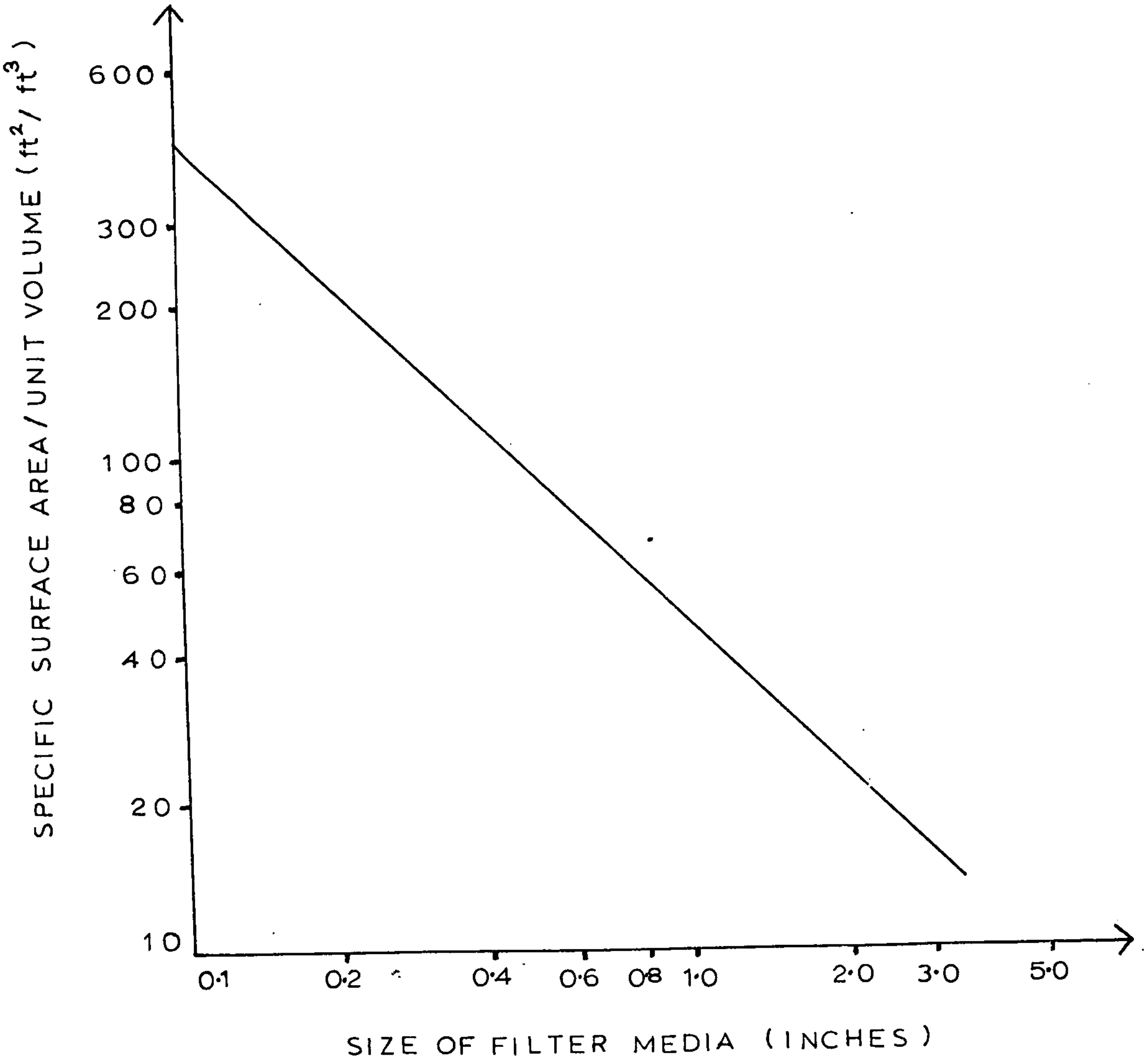


FIGURE 6.

risk of clogging. When calculating the volume of filter needed to cope with a particular load of water and nutrients (BOD and ammonia), it is the total surface area of all the rock surface within the filter that is important*, because this figure describes the space available for bacterial colonisation (Bruce 1969, 1970, Bruce and Merkens 1973, Speece 1973). Speece (1973) produced a graph (see figure 6 ) which shows that as the rock size used decreases, the surface area per unit volume of filter increases. Thus, to make the filter as compact as possible, one would use fine sand as the filter media, but this would clog in a very short time. (Dewitt and Salo 1960, McCrimmond and Berst 1966, describe sand filters which had to be cleaned often.) So, a compromise has to be made that embraces the following consideration: the larger the media size, the larger the filter will have to be, but the longer it will take to clog.

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*There are two publications which are set out in an authorative manner, advising on various matters, including the size of the filter needed to cope with a particular daily load of ammonia. These publications are a paper by Speece (1973) and a lengthy report by Kramer Chin and Mayo (1972); this report being the more important document. As will become more apparent in the following pages, Speece looks upon a biological filter as being a body containing a certain specific surface area of filter media suitable for bacterial colonisation,whereas Kramer Chin and Mayo look upon a biological filter as being a body which represents a certain retention time i.e. the water passing into it is held within the filter for a specific length of time before being released.

Both these attitudes are different ways of describing the same filter and it is unfortunate that nowhere in the literature does there seem to be any work which tells one that a filter containing a specific surface area of x square feet, provides a retention time of y minutes. So, cross references between Speece's advise and that of Kramer Chin and Mayo is not really possible.

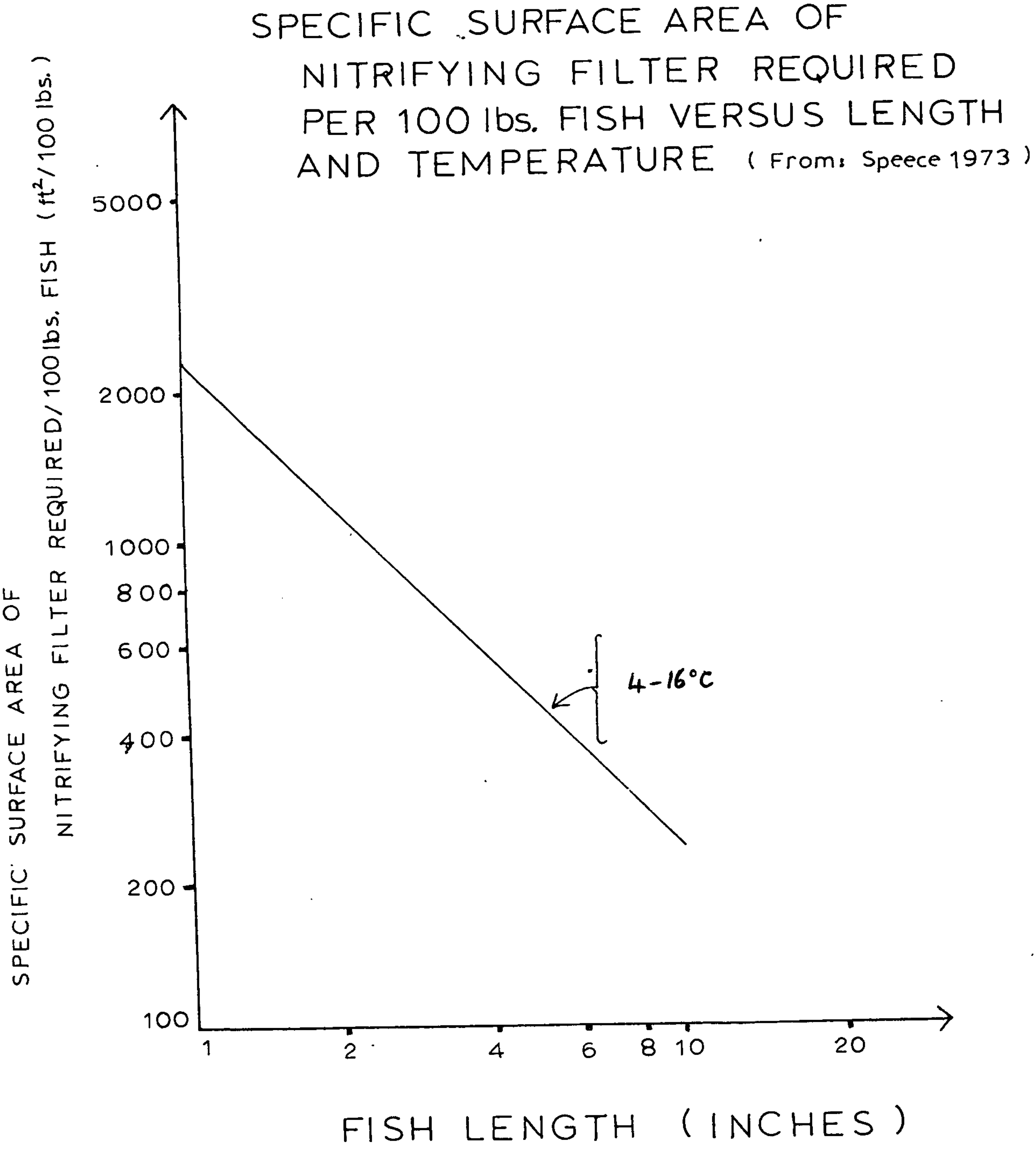
Using the approach of Speece, one has the problems of calculating the specific surface area per unit volume of filter media, because it is unlikely that the media will be conveniently round so that his figures may be used; whereas using the approach of Kramer Chin and Mayo, one has the problem of calculating the retention time per unit volume of filter media.

It is important that the filter, if possible, should be designed so that it does not need cleaning often, because the cleaning process which usually involves reversing the flow of water through the filter (termed backwashing), apart from being an inconvenient operation, dislodges a proportion of the bacterial film from the media surfaces and so can impair the filter's efficiency at ammonia removal. Kramer Chin and Mayo (1972) advise that the filter should be designed so that it will run for an indefinite period without needing cleaning, thereby providing a steady performance.

Another important point to consider here, is that the larger and more angular the media size, then the larger will be the size of the channels and voids within the filter (Bruce 1968). These voids are important because they allow free circulation of air throughout the bed (this is important because nitrification is an aerobic process, see page43) and they facilitate the widespread distribution of liquid flow (Bruce 1968). As mentioned earlier, it would seem reasonable if these void sizes determine the minimum depth of the filter. Burrows and Coombes (1968) whose paper is authoritative on filter design, recommend that the compromise relating to rock size should be resolved by using graded crushed rock from a quarter of an inch to three inches in diameter. (Bruce 1969 recommends two inches diameter.)

Returning to filter clogging, Bruce (1969) states that the critical condition which means that the filter has to be cleaned, seems to be reached when 50% of the void space is filled with bacterial film. However, he does describe how biological filters at sewage works can be run indefinitely without the necessity of physical cleaning. These filters have populations of macro-invertebrates such as midge larvae, mite larvae, collembola, nematode worms, and oligochaete worms which eat the biological films, thereby keeping them in check. A balanced system seems to result, with maybe a succession of dominant species as the season changes and as the filter bed matures. In the winter there may be a problem in that due to low water temperatures the growth of the macro-invertebrates is reduced to a greater extent than that of the film and so clogging occurs. However, if the water used in the fish tanks was maintained at a steady temperature then this problem should not occur. This controlling of the bacterial film by "grazing" does not seem to have been referred to by fish culturists, but it would seem to be possibly useful if the voids are large enough to permit the macro-invertebrates to thrive in the filter.

The actual method of distribution of water over the surface of the filter can reduce or increase the filter's tendency to clog. The "Notes on Water Pollution" (1959) states that water distribution as a fine spray commonly causes heavy growths in the surface layers of the bed. Hawkes (1963) found that if the travel of the water distributor arm is slowed as it moves over the filter bed so that each section of the bed receives a higher instantaneous dose, then the performance of the filter is improved. This he attributes to two factors:



SPECIFIC SURFACE AREA OF NITRIFYING FILTER REQUIRED PER 100 lbs. FISH VERSUS LENGTH AND TEMPERATURE (From: Speece 1973)

FIGURE 7.

(i) A higher instantaneous dose means the nutrients surge further down into the bed and so a more even film distribution is achieved.

(ii) The time interval between dosings is increased to 5 - 12 minutes, thus the micro-organisms are then starved of nutrients and so enter a stage of endogenous respiration and declining bulk, thereby slowing down the growth of the surface film.

## An explanation of the physical and chemical details which characterize a biological filter's performance.

If one is to learn from research papers, the size to build a biological filter, one would need to know in the research worker's case and one's own case:

(a) The free ammonia concentration.

(b) The BOD.

(c) The temperature of the water, especially if it is above 16°C or below 4°C as this will, apart from it's effect on the fishes' ammonia production rate, have an effect on the nitrification rate within the filter (see figure 7 , Speece 1973). The most sensible move is to design the filter size to be adequate for the lowest temperature likely to be experienced.

(d) The pH of the water, as this will also have an effect on the nitrification rate within the filter.

(e) The "retention time", the "hydraulic loading" and the "ammonia loading" of the filter.

AMMONIA REMOVAL VERSUS RETENTION TIME AT VARIOUS AMMONIA LOADING RATES ( From: Kramer et al. 1972 )

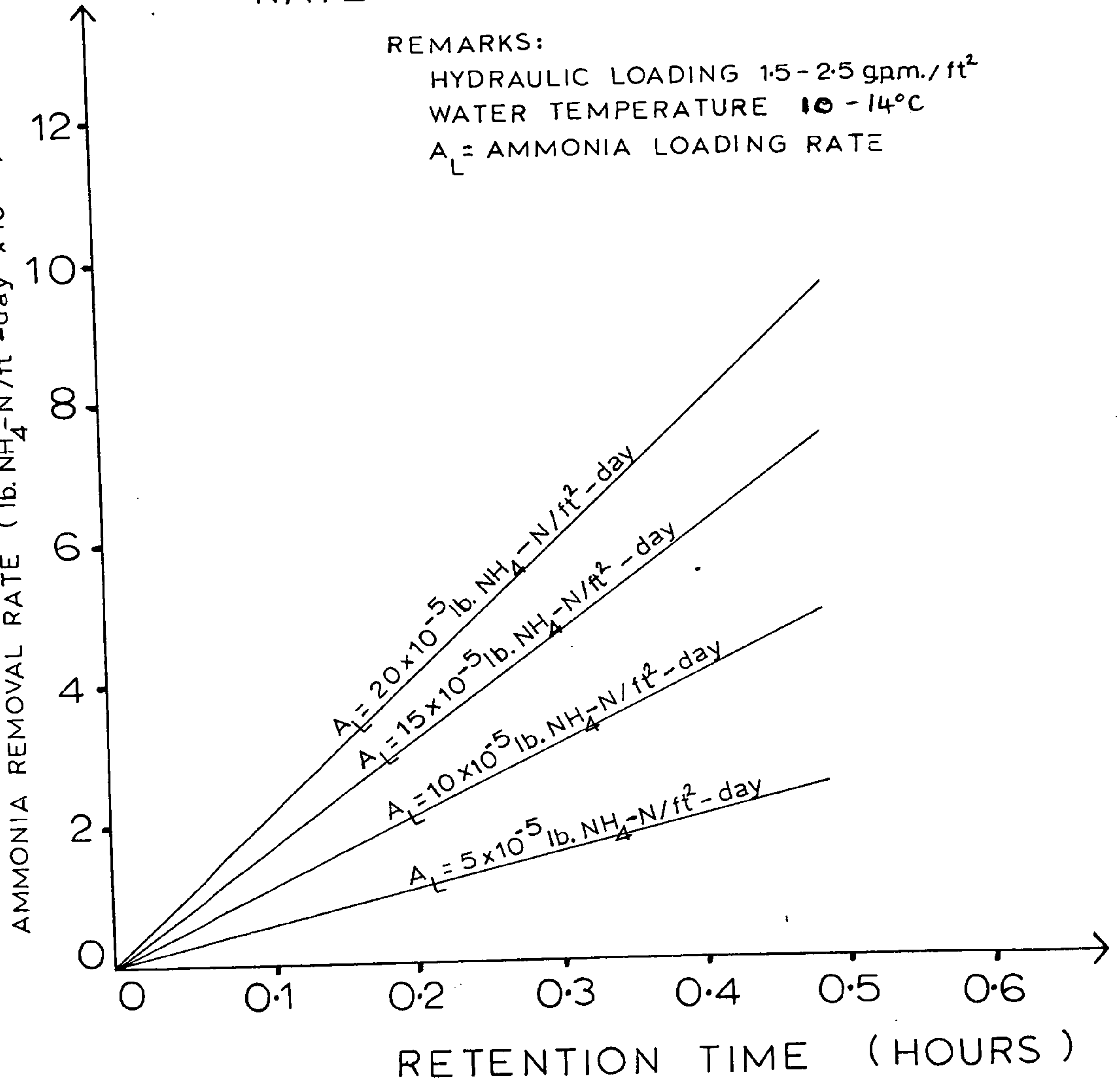


FIGURE 8.

AMMONIA REMOVAL VERSUS FILTER HYDRAULIC LOADING (From: Kramer et al. 1972

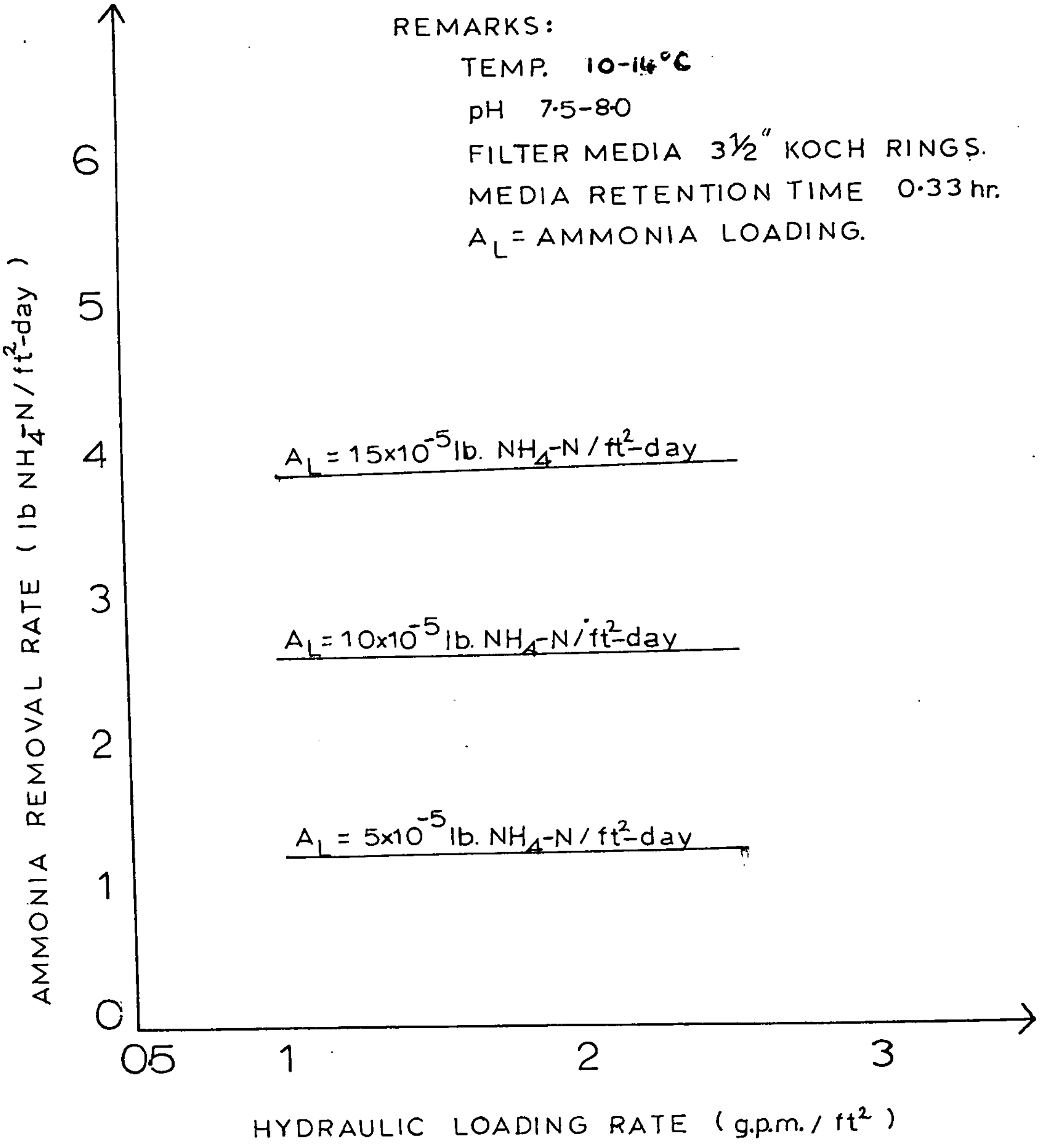


FIGURE 9.

### (i) Retention time

Figure 8 taken from the study of Kramer Chin and Mayo (1972), shows clearly that at a given ammonia loading, increasing the length of time that the water (to be treated) is held within the filter, increases the filter's efficiency of removing ammonia from that water. This is to be expected, because the water is held in contact with the bacterial films for a longer period of time, and so the chances of each ammonia molecule being removed are increased. Increasing the retention time could be looked upon as being synonomous with increasing the size of the filter. (See page 49)

### (ii) Ammonia Loading

Both figures 8 and 9 taken from Kramer Chin and Mayo (1972) show that at a given retention time, increasing the ammonia loading in lb.$NH_4$-N x $10^{-5}/ft^2$/day, leads to an increase in the amount of ammonia being removed per square foot per day. This would seem to be straight forward, because by increasing the ammonia loading, one is increasing the density of ammonia molecules in the water to be treated, and so this increases the number of ammonia molecules in contact with the nitrifying bacteria, and/unless the bacteria are already working at full capacity at nitrifying ammonia, they will be able to capitalise on this increased ammonia availability. The nitrifying bacteria may well also respond to this increased ammonia availability by multiplying in number.

### (iii) Hydraulic loading

Figure 9 shows clearly that at a fixed retention time, a particular ammonia loading rate (lb.$NH_4$-N x $10^{-5}$/ft$^2$/day) can be applied at various hydraulic loadings (1.0 - 2.5 gpm/ft$^2$) and the filter's efficiency at removing ammonia, expressed as lb.$NH_4$-N x $10^{-5}$/ft$^2$/day, remains the same. It took me quite a while to fully understand this, but when grasped, it made sound sense, and it is probably best explained as follows.

At all the hydraulic loadings, the amount of ammonia passed into the filter per square foot per day is the same; so an increase in hydraulic loading means the same daily quantity of ammonia is being applied to the filter in a more dilute ammonia solution. The following picture can be imagined:

(a) Lower hydraulic loading, therefore $NH_4$-N concentration higher.
(b) Higher hydraulic loading, therefore $NH_4$-N concentration lower.

The retention time is the same, therefore a gallon of (a) must, by definition, spend the same time in the filter as a gallon of (b).

Now, if the retention time is the same for various hydraulic loadings, this must mean that as the hydraulic loading of the filter increases, then the amount of water actually present inside the filter at any given time equally increases. (N.B. There must be a certain hydraulic loading for each filter (depending on the media size used), which if exceeded would result in the filter surface flooding due to the filter having physical limits as to the flow rate it can pass.)

Hence from this picture one can see that in the case of (a) and (b) a given weight of ammonia spends the same time in the filter, and so the same proportion of the daily $NH_4$-N load is removed in both cases.

So, the effect of changing retention time, and/or ammonia loading and/or hydraulic loading can be examined by considering the time a given weight of ammonia spends in the filter.

## Sources of variability in the performance of similar biological filters.

It must be stressed that even when consideration has been given to all the factors discussed in this thesis, the performance of a biological filter can not be predicted with absolute certainty. An obvious reason for this statement at this point in time, is that the literature is scanty in this field; but always there will be random differences in the grading and packing of conventional media, and the biological material which is the main feature of the filter is by it's very nature variable.

## Possible ways of improving an inadequate biological filter's performance.

Due to the uncertain efficiency of the filter, any research worker or fish farmer operating a closed system fish culture unit, may find that although the filter seems to be working properly, it is not removing sufficient ammonia from the water. Listed below are the four main possibilities for solving this problem:

(i) The filter should be built larger.

(ii) The retention time of the water within the filter should be increased.

(iii) The quantity of water removed daily from the system and exchanged for freshwater should be increased.

(iv) The fish loading of the tank(s) should be reduced.

Possibility (i) is self-explanatory, but (ii) needs further discussion:

If one is interested in how the performance of a filter with respect to a particular daily $NH_4$-N load is related to the retention time of the filter; then it is not simply the retention time of the filter that one wishes to know, but it is the numerical product of the filter's retention time and the number of times that the total body of water in the rearing system passes through the filter each day. This figure gives the effective length of time that a unit volume of water spends in the filter, during a day. So, the possibility numbered (ii), of increasing the retention time, could be accomplished by speeding up the recirculation rate of the water.

However, although this might provide a relief of the problem, it would be expensive in the long term in pumping costs and so building the filter bigger as suggested by (i) would be better as it is a once and for all capital cost.

Possibility (iii) defeats the object of building a biological filter and a recirculation system (i.e. water economy), and so should only be used as an emergency procedure.

Possibility (iv) would provide an answer to the problem, but to limit the stocking density of fish in a tank so as to suit the inadequate nitrifying capacity of the filter, is clearly not a satisfactory solution. The filter should be designed to cope with the expected maximum fish loading.

# CHAPTER 5

## Conclusions

The discussion so far has been principally directed to show how varying environmental parameters such as temperature, pH and dissolved oxygen affect not only the fishes' growth but also the performance of the biological filter. The problem is that the optimum level for the fish, of a particular parameter, may not be the optimum level for the filter. For example, the temperature optimum for the filter's performance would appear to be between 30°C and 40°C (Deppe and Engel 1960; Laudelont and van Tichelin 1960) whereas the temperature optimum for salmonid growth is 10°C to 15°C (Burrows 1968). So, clearly compromises have to be made in determining the levels of environmental parameters which will be employed in the fish rearing system.

### (i) Temperature

The actual optimum temperature for the filter's performance will depend on the particular strains of bacteria that colonise the filter, but it is clear from the evidence stated above that the fishes' optimum temperature will always be below that of the filter. A sound viewpoint would be that the fish are the most important, and so if rearing the fish at their optimum temperature means that the filter is less efficient; then a larger filter should be built. To build a larger filter is a once and for all capital expenditure, whereas to limit the suitability of the fishes' environment so that the filter will perform more efficiently, will merely result in permanent frustration for the fish culturist.

The advisability of employing a water temperature which is at the optimum for the fishes' growth is in fact open to question for the following reasons:

(a) Does the increased growth rate more than compensate for the cost involved in maintaining the water at the optimum temperature? An advantage of a water reuse system, would be that the cost of heating the water would clearly not be as high as in a single-pass system.

(b) The higher the temperature, the more $NH_4$-N will be present as free ammonia. However, this effect is not very important because it takes a 10°C rise in temperature to double the proportion of $NH_4$-N present as free ammonia. However, an increase in temperature does mean an increase in the feeding rate in pounds of food per hundred pounds of fish per day, and this means an increase in the $NH_4$-N production per hundred pound of fish per day. This will give the filter more $NH_4$-N to cope with but of course as the temperature rises, then so does the filter's efficiency at removing $NH_4$-N from the water passing through it. The extent to which all these effects cancel each other out is uncertain, Speece (1973) suggests that as the temperature increases the filter's increased efficiency at removing $NH_4$-N matches the increased $NH_4$-N production by the fish, (<u>N.B.</u> Speece's results may be innacurate in this matter, because for his tests, he used a synthetic effluent which contained only $NH_4$-N, no organic load. In reality, an increase in the feeding rate would also lead to an increase in the filter's BOD load and so the relationship may not be as simple as Speece suggests, but his results do give us an approximate indication. (See figure 7 ) However, any result of these effects can be coped with by building the filter big enough in the first place.

(c) It must also be borne in mind that the optimum temperature for fish growth rate is above the optimum temperature for food conversion, (Elliott,personal communication), the latter being quite low. This may be important during a time of high food costs. A higher growth rate would therefore (if the temperature was above the optimum for food conversion) result in an increased food cost per pound of fish sold, but if the fish grew faster then they would be ready for sale after a shorter time in the expensive rearing equipment and so although the profit per fish would be reduced, the farm's annual turnover of fish would be increased.

It is clear that the decision on the best temperature to use, can be made only after a careful consideration of the effects listed, and a study of the economics involved. However, it can be said that the temperature will not be above 15°C.

(ii) pH

As in the case of temperature, the optimum pH for a biological filter's performance will depend on the strains of bacteria that colonise the filter, but it can be said that it will be on the alkaline side of 7.0 probably nearer 7.5 - 8.0 (Lees 1952). The EIFAC (1968) report states that pH values of between 9.0 and 5.0 will not in themselves be harmful to salmonids. (N.B. Extreme pH values, may have a harmful effect in conjunction with dissolved oxygen by influencing the pH of the fish's blood, See page 6 .) On page 6 it was mentioned that there seems to be no information on whether there is any pH value within the 5 - 9 range that would be, per se, an optimum value; but one would perhaps expect the middle of the range, that is pH of 7, to be most suitable for growth, if the effect of pH, per se, is being considered.

An important effect of a change in pH is it's influence on the equilibrium between $NH_4$-N and free ammonia; a change of 0·3 pH units results in a two fold increase in the proportion of $NH_4$-N which is present as free ammonia. So, as the pH increases, then so does the percentage of $NH_4$-N which is in the form of free ammonia. However, similarly increasing is the filter's efficiency at removing $NH_4$-N from the water passing through it. The extent to which these conflicting influences cancel out each other is not certain, but Burrows and Coombes (1968) recommend a pH of 7.8 - 8.0 as being a suitable compromise. Of course, as in the case of temperature, any adverse consequence of these conflicting influences can be allowed for by building the filter big enough in the first place.

It would seem that the advisable limits for the pH of a recirculating rearing system are 7.0 - 8.0, but the actual optimum pH for a particular system is best determined by experimentation on that system.

### (iii) Dissolved oxygen

Willoughby (1968), Burrows and Coombes (1968), Larmoyeux and Piper (1973) as well as many others, state that if the dissolved oxygen content of the water falls below 5 ppm then this will, per se, lead to a lower growth rate in the fish, as well as other more involved changes. It has also been shown by Larmoyeux and Piper (1973) that as the oxygen concentration rises, then so does the level of free ammonia which the fish can safely tolerate. However, in a well managed system, the oxygen concentration is limiting before free ammonia becomes limiting.

The minimum oxygen concentration needed in the filter for nitrification is 4 ppm (Eikum 1967), and there should be no problem in maintaining this as long as there is free access for air to enter the bottom of the filter and so create reoxygenating air currents within the filter.

Thus there is no conflict in interest between the filter and the fish with regard to the dissolved oxygen level of the water.

### (iv) Free carbon dioxide

The effects of the free carbon dioxide concentration of the water have been discussed fully on pages 3 to 6, especially it's effect associated with pH. The EIFAC (1968) report advises that at a pH as low as 6.5, a carbon dioxide concentration as high as 100 ppm should not be harmful, therefore in the pH range of 7 - 8 which would be used in a fish rearing system, the carbon dioxide concentration should present no problems, especially as the process of aerating the water also drives free carbon dioxide out of solution.

It seems relevant to add that in a recirculation system, carbon dioxide does have a useful function in that it is an essential carbon source for the nitrifying bacteria in the filter.

It is difficult to recommend an advisable carbon dioxide level which should not be exceeded, due to the lack of information in this field, but in the pH range being used, there should be no need to worry.

### (v) Salinity

Altering the salinity of the water in which the fish are reared could well have beneficial effects. Rainbow trout when reared in fresh water, are living in a hypertonic environment, that is an environment of a lower osmotic pressure than their body fluids. This means that water is continually flooding into the fish through it's semi-permeable membranes, primarily the gills. In order to maintain the osmotic pressure of their fluids above that of their aqueous environment, the fish's kidney is continually filtering the blood to produce a dilute urine which is then excreted. This work requires the expenditure of energy and this could be saved if the salinity of the environment was increased so that it's osmotic pressure became equal to that of the fish's body fluids, termed iso-osmotic. Infact any increase in the salinity of the environment up to that of the fish's body fluids, would produce a saving in energy expenditure which may then be directed towards growth.

There would seem to be other beneficial effects of increasing the salinity of the environment. Lloyd and Swift (EIFAC 1973) suggest that low oxygen concentrations (3.3 ppm) increase the permeability of rainbow trout to water. Now, low oxygen concentrations are quite possibly encountered in intensive fish culture, and so if the osmotic concentration of the fish's environment was the same as that of it's body fluids, this would mean that if a low oxygen concentration was encountered, this would not mean an increased energy demand from increased kidney activity. Lloyd and Orr (1969) state that a free ammonia concentration above 0.047 ppm leads to an increase in the permeability of rainbow trout to water, but such levels of free ammonia should not be present even for short times in a well managed recirculation system.

# CHAPTER 6

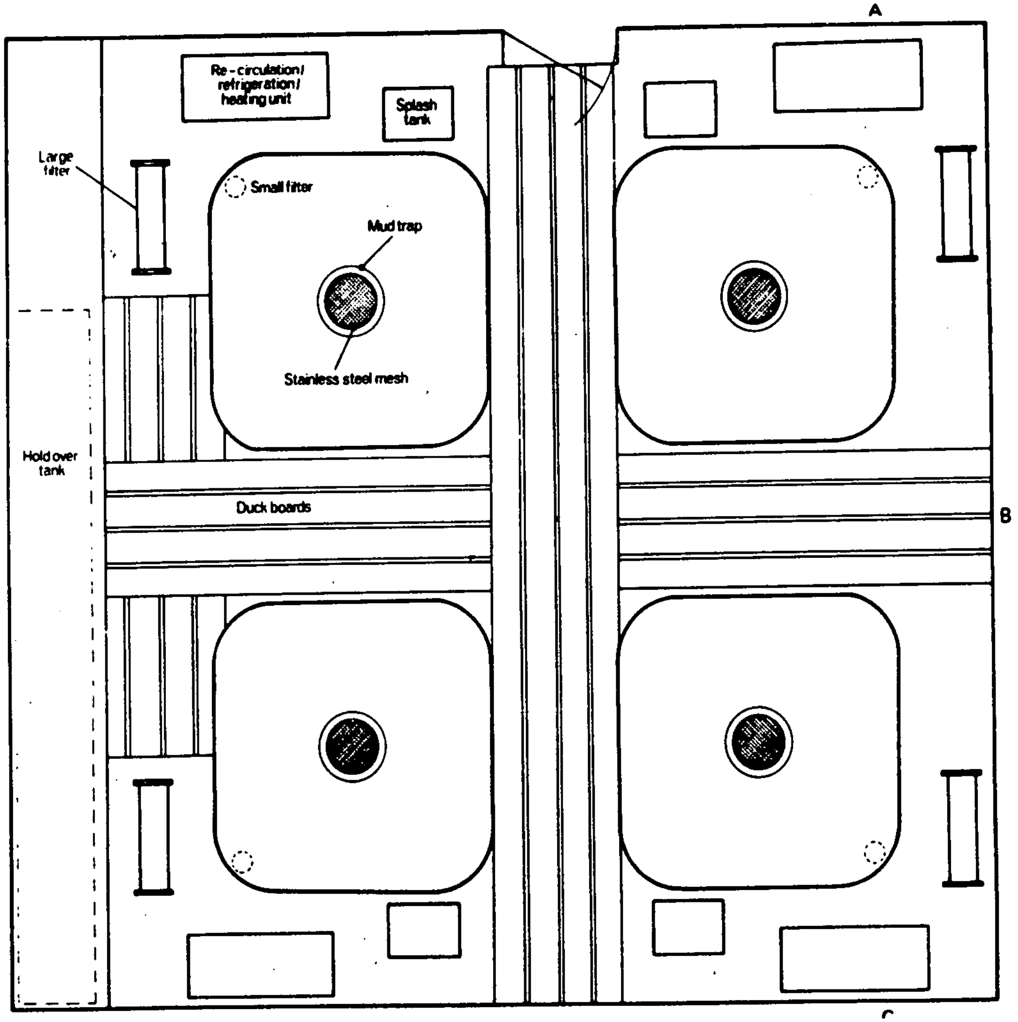
## Details of the fish rearing equipment provided for the research work, the reasons why modifications were necessary, and the ways in which these modifications were carried out.

The fish rearing equipment is contained in a building which is 24 feet wide, 22 feet long and 9 feet high. The roof is constructed of corrugated asbestos sheeting with several translucent skylights; the fish tanks thereby protected from rain and direct sunlight. Three of the four walls are outside walls, and are constructed of open slatted boarding so that, (i), the fish tank area can be locked up, in order to deny entry to intruders; and (ii) so that security can be achieved whilst still allowing a free circulation of air through the building, thereby ensuring that the room does not heat up in summer. However, it has been found that on hot still summer days the gaps between the boarding are not wide enough to ensure that the latter requirement is met.

Inside the building are four fish tanks, which are designed to be identical, each being constructed of moulded asbestos, 6 feet in diameter and 1½ feet deep. The tank floor is virtually flat and in the centre is a 14 inch diameter drain which leads into a mud trap and then out to the recirculation system, splash tank or the drain. (see figure 11 ). As can be seen from the drawing, the fish tank outlet to the splash tank is in the form of an adjustable arm which can be raised or lowered, in order to decrease or increase the rate

## PLAN OF FISH REARING UNIT

DEPARTMENT OF FORESTRY AND NATURAL RESOURCES

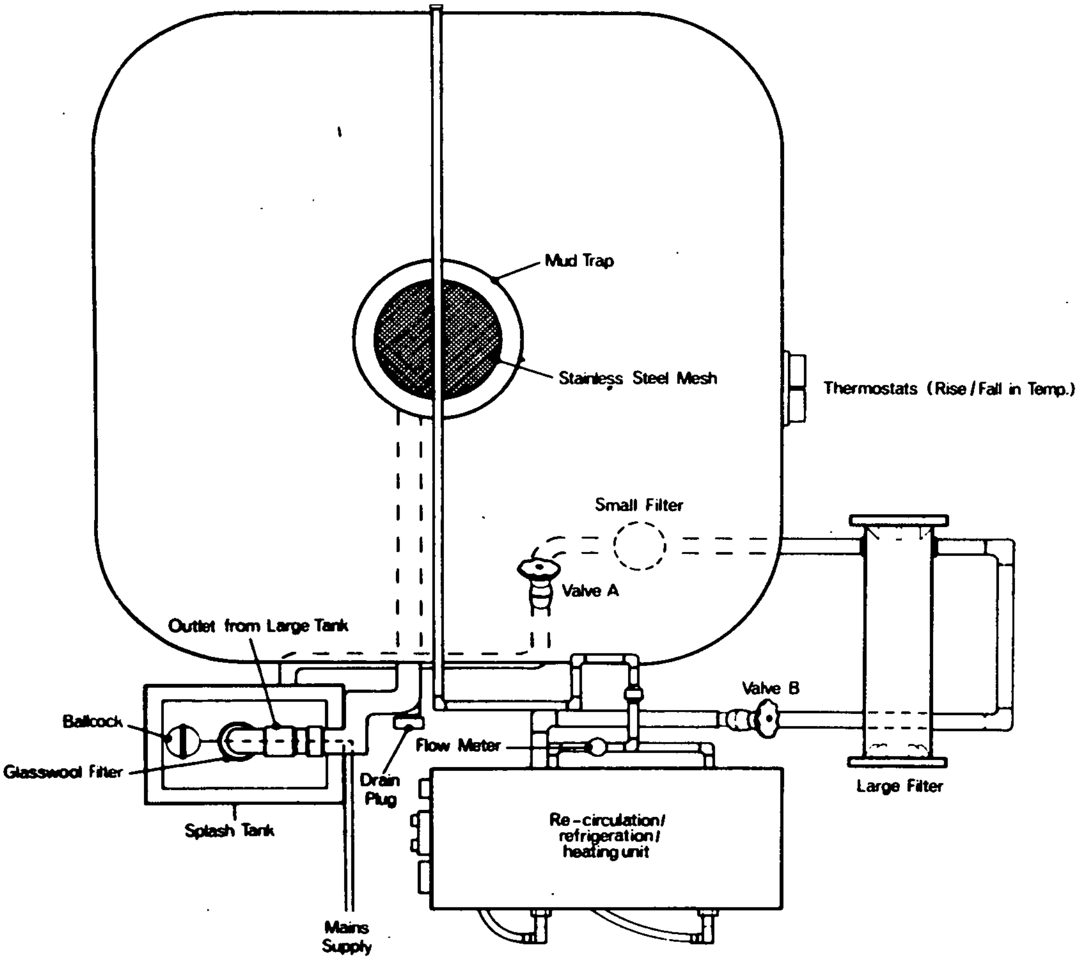


A,B&C slatted walls to allow air movement through the building

FIGURE 10

FIGURE 11

# DETAILED PLAN OF ONE TROUT REARING UNIT



of flow of water out of the fish tank, and into the splash tank. A pump then draws the water out of the splash tank through a polypropylene sleeve filter to remove coarse particulate matter and then through a large filter to remove finer particulate matter and the ammonia excreted by the fish. The same pump then drives the water up through the temperature control unit and back into the fish tank, in a manner which aerates the water, and rotates the body of water in the tank in a clockwise or anti-clockwise direction.

From figure 11 , it would appear that the system is designed to operate as an entirely closed system, without replacing a certain percentage of the water every cycle. The only fresh water entering the system would be in the splash tank, via the mains supply, if the rate at which the water entering the splash tank was lower than the rate at which it was being drawn out by the pump. In such a case, the difference between supply and demand is compensated for by the ballcock mechanism on the main water pipe. If the water level in the splash tank falls, then the ballcock falls, so permitting the addition of mains water.

Set out below is a detailed discussion of each of the features of this system, and also discussed is the way in which they were or should be altered before full scale experiments on "closed systems" could begin.

### (i) The fish tanks

Having only four tanks severly limits the number of different "treatments" and replicates possible in any experiment. It would really mean that the whole experiment would have to be repeated again to achieve proper replication if there were to be more than two different "treatments". Ways of subdividing each tank with screens were examined, so that effectively one could have, for example, four tanks within one tank; but in no way could this be seen to be satisfactory. It would be difficult to feed the fish in each section exactly the same amount of food if the fish were at the same density, or different amounts of food if the fish were at different densities. The screens would have to be fine to stop the food drifting from one section to the next, and such fine screens would restrict the circular flow of the water in the tank. If this happened, then the phenomenon of fish in subsequent sections being subjected to lower oxygen levels and higher metabolic product levels than fish in preceeding sections, would be exaggerated. The screens would also undoubtedly be detrimental to the self-cleaning action of the tank, by slowing down the current and becoming covered in biological growth themselves.

Another problem with the tanks is their size, because with units which are so large, with so much pipework, and all the recirculation system, it is most unlikely that any two rearing units will provide exactly the same environment for the fish. Apart from the pipework lay-out differing in the four units, the inside surfaces of the pipes were observed to become coated with a considerable film of micro-organisms, and these films may well differ in thickness and micro-organism composition in different units,

# A CONSIDERATION OF VARIOUS FACTORS INFLUENCING: FISH LOADING, $NH_4$-N PROD$^{\underline{N}}$, BIOLOGICAL FILTER SIZE etc.

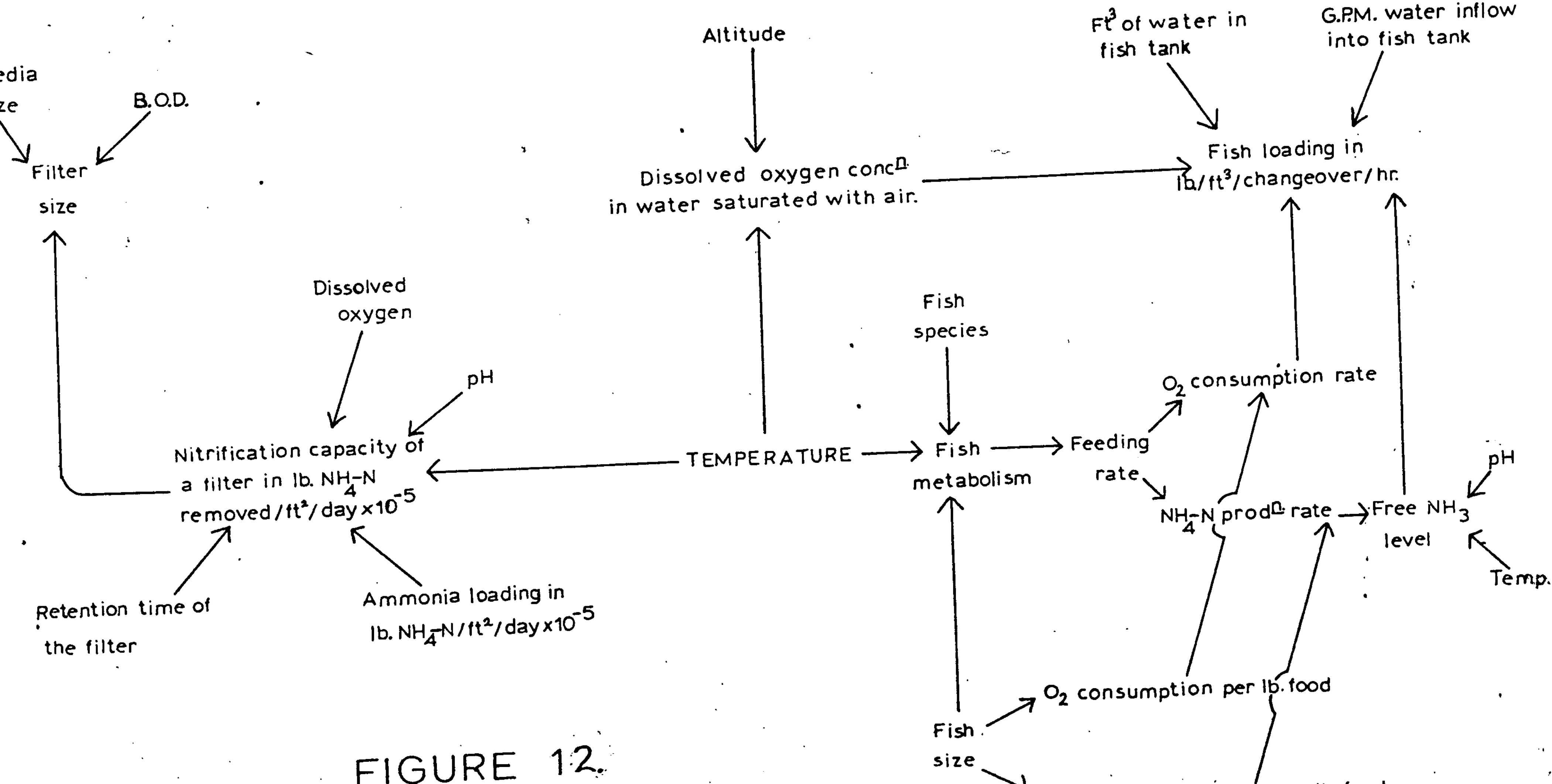


FIGURE 12.

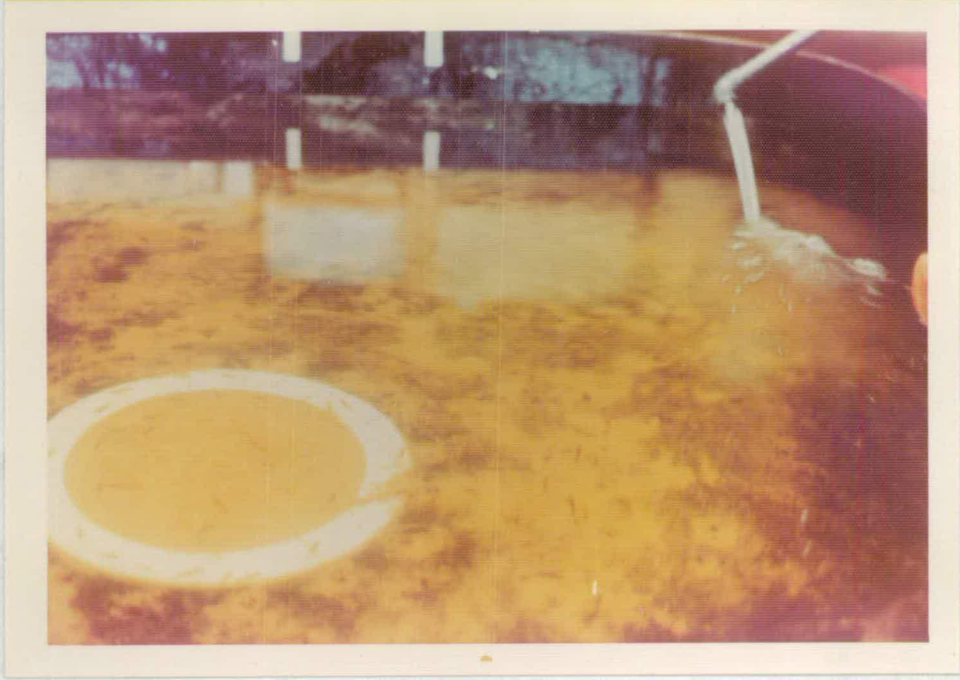


Figure 13

Surplus food was thrown into this fish tank, and then photographed one hour later. This clearly shows that the tank is not self-cleaning.

and so this may result in the dissolved oxygen concentration being different in different tanks. Each of the units will have different amounts of decaying matter trapped in various parts of the system, and this will influence the dissolved oxygen concentration, and the $NH_4$-N concentration. There are many other possible sources of variation between the units, and the larger the tanks and their supports systems are, then the greater the variations are likely to be. This variation could make the interpretation of any results open to question, and so the first experiment to be performed on the equipmentshould be to rear identical populations of fish in all four tanks for a period of time (perhaps two months), in order to see whether in fact they do grow equally well in all the tanks. This should answer the question of how significant the difference in the environments provided by the different units is.

Another advantage of having smaller tanks in the building would be that there would be room for more than four of them, and so there could be more extensive replication of different experimental treatments of fish populations.

The floor of the fish tank was virtually flat, and this meant that any uneaten food and fish faeces tended to lie on the bottom of the tank and not move towards the drain in the middle of the tank.(fig 13) If the flow of reconditioned water into the tank had been higher, then the circular current may well have been fast enough to sweep waste particles into the centre of the tank, despite the floor being flat,

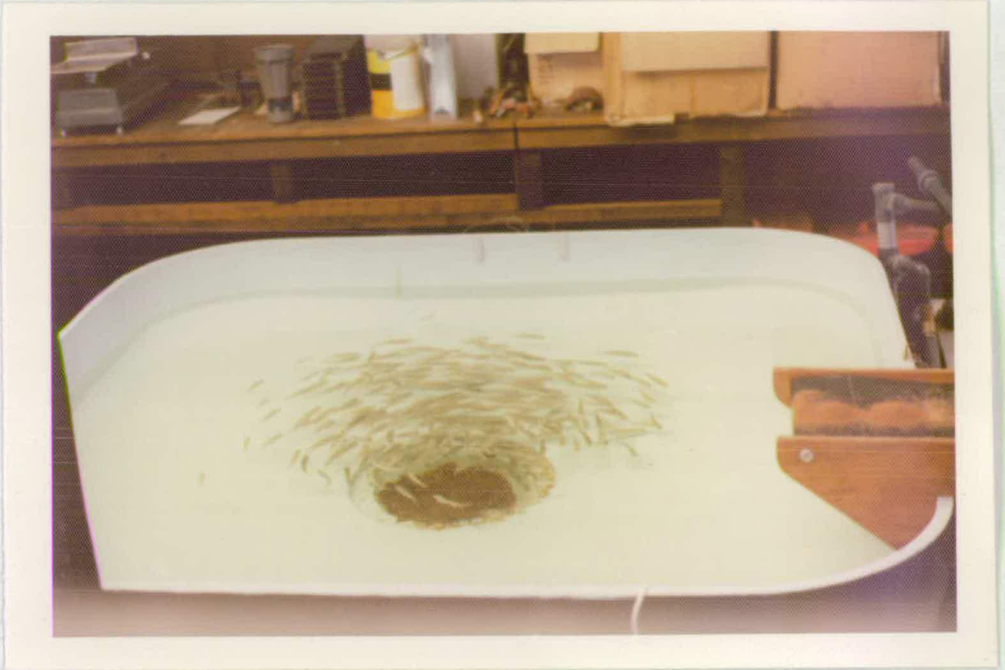


Figure 14

The sloping concrete floor in a fish tank, coated with an impermeable plastic membrane.

but the prevailing recirculation rate was inadequate to ensure this. As has been mentioned previously, uneaten food and faeces decompose and are a source of ammonia production and oxygen consumption, and so they should be removed from the system as soon as possible. So, a sealed concrete floor was constructed in each tank, giving a 17 degree slope out to the tank sides from the drain in the middle. (fig 14

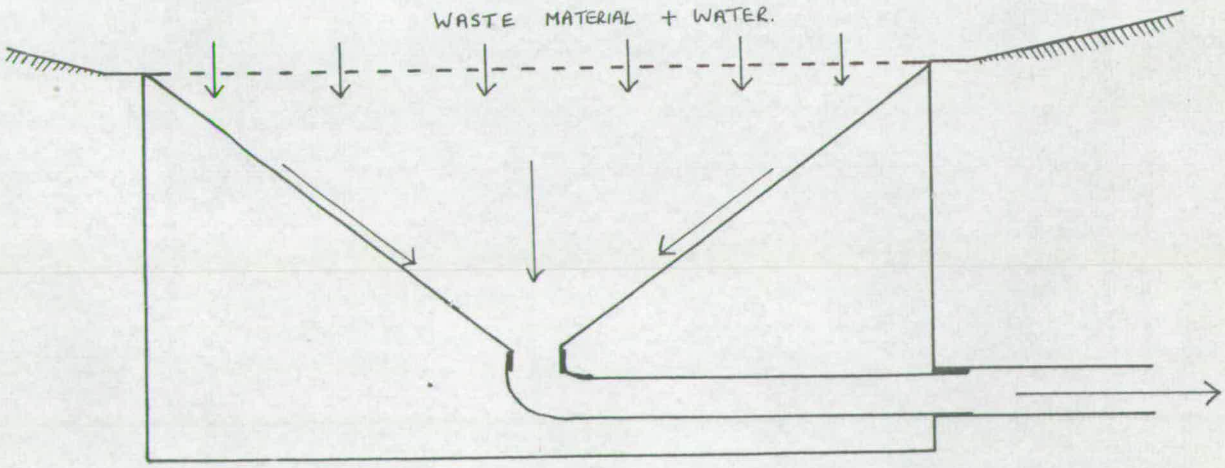
A moulded fibreglass floor had been considered as a possibility, but if the floor was not solid, water would inevitably seep underneath it and so an unhealthy pocket of water containing decomposing organic matter would be trapped there. A solid floor seemed to provide the best solution and so as the tank could withstand the weight, concrete mixed with a lightweight breeze block material was used.

The new sloping floor, coupled with the circular current in the tank proved enough to ensure that all waste particles rolled down into the middle of the tank. However, the stainless steel mesh grid in the middle of the tank was so fine that most of the waste material built up on the grid and did not pass through it. After a few days, the accumulated material restricted the flow of water out of the fish tank into the splash tank, and so mains water was automatically added to compensate for the falling level of water in the splash tank. This caused the level of the water in the fish tank to rise until it overflowed. Larger holes were drilled through a spare stainless steel grid, but this left rough metal edges which would damage the fish if the tank was to be drained and the fish netted off the grid surface. So, a new grid was made by drilling

$\frac{1}{4}$" diameter holes in a $\frac{1}{4}$" thick, disc of perspex which was the same overall size as the stainless steel grid. The smooth perspex surface ensured that no damage could be inflicted on the fish, and also any waste materials slid over the grid surface until they were sucked down through a hole.

Once through the grid, much of the waste then settled in the flat bottomed mud trap and did not leave the tank via the drain pipe. The tank was of an old design for a single-pass system and would have been much better if it had not had the mud trap. However, this was an integral part of the tank and so it had to be modified in order to make it self-cleaning. Unfortunately, the mud trap was not very deep (7$\frac{1}{2}$") in relation to it's diameter (14") and so this limited the degree of slope which could be constructed, in order to direct the waste to the drain pipe. Two possible solutions came to mind:

(1) to fit a funnel made of stainless steel into the mud trap, with a length of flexible hosing connecting the bottom of the funnel to the drain pipe. (See diagram below.)



(2) To build a sealed, smooth sloping concrete floor within the

Figure 15

The mud trap in a fish tank.

Figure 16

The mud trap in a fish tank, fitted with a sloping concrete floor for self-cleaning purposes.

mud trap (see figure 16). This method, apart from being by far the cheaper alternative, also seemed to be the most promising, because as can be seen from the picture, a considerable part of the slope down to the drain pipe is vertical, whereas the lowest degree of slope found is no lower than that found on all parts of the funnel.

However, when tested, the sloping floor was found to be rather ineffective, despite the lowest degree of slope being 40 degrees. The waste particles lay deep all over the slope as can be seen by the dark brown area in figure 16. The reasons for the failure seem most likely to be as follows:

(a) The perspex grid was very effective in stopping any currents from the main tank being transmitted down to the mud trap region. This was unfortunate, because any such currents would have been helpful in moving waste particles down the slope to the drain pipe.

(b) The volume of water being drawn down the drain pipe by the pump is very small in relation to the body of water around the opening of pipe, and so there was very little tendency to draw an appreciable current of water down the slope and hence draw waste particles down the slope with it.

(c) Although the slope was smooth, it was not as smooth as the surface in a stainless steel funnel would have been; the lower friction in the case of the latter may enable waste particles to move down the slope.

The only possible solution would be to try the funnel.

### (ii) The splash tank

When the fish tank is not being drained, the water from the tank flows through the adjustable tank outlet into the splash tank. As it flows into the splash tank, the water is poured through a simple glasswool filter which is there to remove a proportion of the waste matter that is washed out of the fish tank. The smaller waste particles were found to wash through the glass wool and infact some of the larger particles must have been broken down and washed through, by the action of the water pouring incessantly onto the wool. The wool was rinsed every day but the amount of waste material which passedthrough this filter was still considerable, especially over a period of a week or more.

The splash tank acted as an inefficient settling tank for some of these waste particles, and this resulted in a deposit of decomposing waste matter on the bottom of the splash tank. There seemed no solution to this problem, if a splash tank was used.

An advantage of the splash tank was that it automatically made up for water loss due to evaporation, by adding mains water via the ballcock mechanism.

But it also presented disadvantages:

(a) A sediment of decomposing waste matter collected on the bottom of the splash tank.

(b) If the grid over the mud trap restricted the flow of water to the splash tank, then mains water was continually added to the system and the fish tank overflowed.

(c) If the rate at which the pump drew water out of the splash tank was reduced, due to the resistance across the small filter and/or the large filter increasing as they became dirty, then the splash tank would overflow until the quantity of water lost from the system meant that the flow rate of water out of the adjustable outlet arm had dropped to the new rate at which the pump was drawing water.

So, the splash tank can be looked upon as a clever mechanism for making up the water lost from the system by evaporation but the problems associated with it, especially the last one which was itemised, limit it's real value.

(iii) The small physical filter:

This unit (price £18 in 1970) is constructed of moulded asbestos and inside, it contains a polypropylene tubular open mesh element which has fitted over it a replaceable polypropylene sleeve. The design of the filter unit is such that the water is forced to flow across this sleeve, and particles exceeding a certain size are prevented from passing through it. The filter can be made more efficient or less efficient by changing the polypropylene sleeve for one made of a different mesh size.

The purpose of this filter is, as suggested, to remove particulate matter exceeding a specific size; this particulate matter being drawn with the water out of the splash tank. However, judging by how dirty the filter elements in the large

Figure 17

A photograph of the carbon filled cartridges, used in the large activated charcoal filter. On the right is the type of cartridge originally supplied, and on the left is the improved cartridge which contains more activated charcoal. Note the build up of biological "jelly-like" growth on the left hand cartridge, which has been in use for one week.

filter became, there would seem to be evidence to suggest that particles too large to pass through the sleeve could be physically broken down on the sleeve surface and pulled through it, by the sucking action of the pump.

It was found that it was usually advisable to clean the small filter every day, (this would vary with the fish loading in the tank), in order to remove the accumulated waste material. This cleaning was achieved by turning off the pump, closing valve 'A', lifting up the adjustable tank outlet arm in order to stop water flowing from the fish tank, and unscrewing the filter unit so that the sleeve could be taken out and scrubbed. Removing this waste material daily reduced:

(a) Any build up in the resistance across the sleeve.

(b) The amount of $NH_3$ production from the decomposing waste.

(c) The quantity of waste material that was physically broken down and sucked through the sleeve.

### (iv) The large activated charcoal filter.

This unit is constructed of P.V.C., and it is fitted with three banks of two 10" long carbon filled cartridges (see **figure 17**), across which the water is made to flow. The engineers who advised this unit, stated that these carbon filled cartridges would both clarify the water and remove metabolic products (especially ammonia). The clarification of the water would be carried out by the sheath of fine synthetic wool which enshrouded the layer of activated charcoal;

this layer of activated charcoal being present to remove any metabolic products. It was suggested that a set of six of these carbon filled elements (price £25 in 1970) would last for two months, and then they should be discarded and replaced.

During this period of study, it has become clear that the engineers had little insight into the problems that they were trying to solve, the evidence for this statement being as follows:

(1) They made no allowance for the effect of different fish loadings on the life of the carbon filled filter elements. Now, the higher the fish loading, the more ammonia the activated charcoal will have to remove, and the more particulate matter the synthetic wool will have to remove. So, the higher the fish loading, the shorter must be the life of the carbon filled elements.

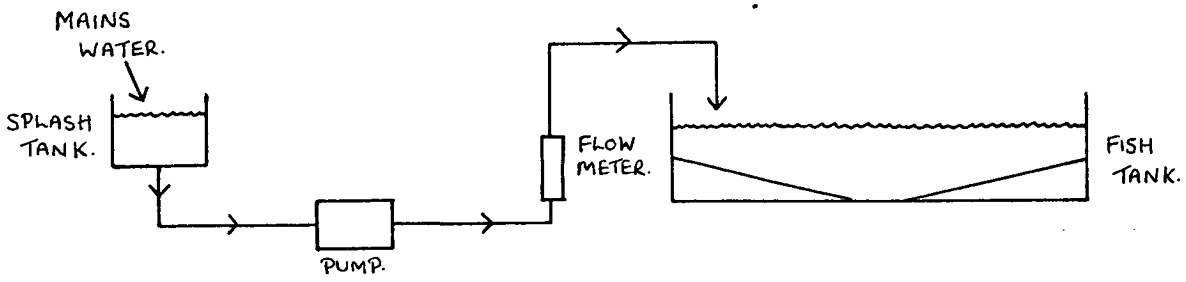
When the recirculation system was running it was found that it was advisable to scrub the carbon filled elements approximately once a week in order to remove both the waste material which had accumulated on the elements' surface, and the prolific biological growth which developed there. This biological growth which resembles a brown jelly in appearance (see figure 17) must be similar to the biological films present in a biological filter. The growth was also found to be present on all the internal surfaces of the recirculation equipment, deriving it's food from the enriched recirculating water. However, it's presence on the outer surface on the the carbon filled elements, together with accumulated particulate matter, increased the resistance across the elements and so the rate at which the pump could recirculate water dropped. Scrubbing the elements helped to reduce this resistance, but some particulate matter and biological growth built up, and/or was sucked into the element and so this could not be removed.

(2) The main fault with this filter was that from the very time new cartridges were put in the unit, there was no removal of metabolic products. The amount of activated charcoal in each of the cartridges was very small indeed and in any case activated charcoal is very poor at removing ammonia molecules, (Macdowell, Norit-Clydesdale personal comment). Macdowell explained that due to the charged nature and small size of the ammonia molecule, it is poorly adsorbed by activated charcoal; and this problem is further aggravated in the case of the particular filter being used, because due to the very small quantity of activated charcoal present, the contact time between the water to be treated and the activated charcoal, is very small. He sent a sample of small granular activated charcoal, which is the best type of carbon for adsorbing ammonia; and the engineers produced a cartridge which could contain extra charcoal, but even so, the whole filter unit contained only 1500 cc. of activated charcoal, and the performance was not measurably better than before. This inadequacy of the activated charcoal filter was proved by conducting the following experiment.

## An experiment designed to test the need for some treatment of the recirculating water, and also to test the performance of the activated charcoal filter.

### Methods

The most reliable of the four recirculation units was chosen for this experiment. The experiment involved several trials, which were performed in succession in the same tank, so that any effect of different systems on the final result, could be ruled out.



The tank chosen was one of the two still possessing a splash tank, and this splash tank proved to be most useful in enabling one to put a known quantity of water into the fish tank (see diagram above). Mains water was fed into the splash tank so that it was kept full, the pump was turned on, and the reading on the flow meter was recorded (300 gallons per hour). During the next three quarters of an hour, the data for the following table was collected:

| Depth of water, as measured above the edge of the concrete floor. | Time taken to reach given depth from time 0. | Gallons of water in the tank. | Cubic feet of water in the tank. |
|---|---|---|---|
| 0 inches | 21 minutes | 105 | 16.85 |
| ½ inch | 23 " | 115 | 18.46 |
| 1 " | 25 " | 125 | 20.06 |
| 4 inches | 38 " | 190 | 30.50 |
| 5 " | 42 " | 210 | 33.71 |

In this experiment, the tank was filled to a depth of 3½", above the concrete floor at the edge of the tank, which provided 28.09 cubic feet of water or 70 gallons of water in the tank.

The total number of fish used was 230, and weighing samples amounting to one third of the population, their average weight was found to be 26.1 grams. This meant that the total weight of the population was approximately 6003 grams or 13.23 lb. Using Cooper Nutrition's feeding chart as a guide, it was decided to feed the fish 100 grams of pellets daily, as the water temperature was 10°C.

Water samples were taken from the fish tank at the times shown in the table of results and the method used for the determination of the level of $NH_4$-N present is described later (see page 121), and this was found to be quite simple and very reliable.

Table 4 Ammonia levels found in trials X, Y and Z.

| Time in hours | Trial | Temp °C | Recircn. rate in g./hr. | pH | Spectroph -atometer reading | $NH_4$-N mg/l | % free $NH_3$ | Free $NH_3$ mg./l |
|---|---|---|---|---|---|---|---|---|
| 0 | X | 9 | 270 | 7.3 | 0.014 | 0.014 | 0.34 | 0.0000 |
| | Y | 9 | 240 | 7.1 | 0.072 | 0.078 | 0.22 | 0.0002 |
| | Z | 10 | 240 | 7.3 | 0.069 | 0.073 | 0.37 | 0.0003 |
| 3 | X | 9 | 270 | 7.6 | 0.021 | 0.021 | 0.68 | 0.0001 |
| | Y | 10 | 230 | 7.2 | 0.237 | 0.256 | 0.29 | 0.0007 |
| | Z | 10 | 210 | 7.4 | 0.198 | 0.208 | 0.47 | 0.0010 |
| 21 | X | 8 | 270 | 7.7 | 0.024 | 0.024 | 1.00 | 0.0002 |
| | Y | 10 | 220 | 7.2 | 1.020 | 1.150 | 0.29 | 0.0034 |
| | Z | 9 | 200 | 7.3 | 0.875 | 0.926 | 0.34 | 0.0032 |
| 24 | X | 9 | 270 | 7.7 | 0.033 | 0.033 | 0.86 | 0.0003 |
| | Y | 10 | 220 | 7.2 | 1.170 | 1.320 | 0.29 | 0.0038 |
| | Z | 9 | 200 | 7.3 | 1.300 | 1.300 | 0.34 | 0.0044 |
| 27 | X | 10 | 270 | 7.7 | 0.030 | 0.032 | 0.92 | 0.0003 |
| | Y | 10 | 230 | 7.2 | 1.220 | 1.370 | 0.29 | 0.0040 |
| | Z | 10 | 190 | 7.3 | 1.700 | 1.700 | 0.37 | 0.0063 |
| 45 | X | 8 | 270 | 7.9 | 0.024 | 0.024 | 1.25 | 0.0003 |
| | Y | 10 | 240 | 7.3 | 0.775* | 2.200 | 0.37 | 0.0081 |
| | Z | 9 | 180 | 7.3 | 0.675* | 2.150 | 0.34 | 0.0073 |
| 48 | X | 8 | 270 | 7.9 | 0.022 | 0.022 | 1.25 | 0.0003 |
| | Y | 10 | 240 | 7.3 | 0.660* | 2.565 | 0.37 | 0.0095 |
| | Z | 9 | 180 | 7.3 | 0.750* | 2.400 | 0.34 | 0.0087 |

* Sample diluted to one third strength.

## Results

The results obtained from the trials are shown in table 4 and figure 18., and the coding of the trials is explained as follows:

Trial X represented the control, being simply a tank of recirculating water.

Trial Y represented the fish population present, but no activated charcoal filter.

Trial Z represented the fish population present and also the activated charcoal filter.

## Discussion

The results show quite clearly that the activated charcoal filter does not remove any appreciable quantities of ammonia. Therefore, the activated charcoal filters can not be used in association with the recirculation system, and an alternative must be found. The obvious choice is a biological filter, and the reasons for this suggestion have been explained on pages 29 - 30.

The need for water treatment is clearly shown by the data. After 48 hours, even at the very low fish loading used in these trials (4" to 5" fish at 0.47 lb. per cubic foot, which is a quarter to a fifth of that advised by Piper 1972), the free ammonia concentration is above the safe level recommended by Kramer, Chin and Mayo (1972). If this recommendation is to be adhered to, then this trial has also shown that at Piper's (1972) recommended fish loading , rainbow trout being fed normally can be safely kept in non-filtered recirculating water for approximately 12 hours; provided the recirculating water is adequately re-aerated. This information is valuable to know, in case a filtration system has to be closed down for a while, or more fundamentally, if the water supply to a single pass hatchery fails.

## Conclusions

So, the system provided by the engineers is entirely unsuitable for a recirculating trout rearing unit. The only viable solution is to build a biological filter.

## (v) The temperature control unit.

As can be seen in figure 11, the temperature control unit consists essentially of two separate units, lying side by side. The heating unit is composed of an insulated box of 3.5cubic feet capacity, containing a kettle-type heating element which has been nickel plated so that the copper does not come in contact with the water. The cooling unit is again an insulated box of the same dimensions, and contains a cooling unit constructed on the same principal as that found in a fridge. The water temperature in the fish tank is continually being measured, and if the temperature falls below or rises above the level at which the thermostat has been set, then solenoid controlled valves are opened or closed so that the recirculating water passes either through the heating or the cooling chamber, thereby correcting the water temperature in the fish tank. This temperature control unit means that the temperature of the fish tank water can be controlled to within several degrees. The ability to exercise control over the temperature of the fish's environment is obviously of great value in fisheries research work.

However, this type of temperature control unit was found to present problems, in that the heating chamber especially, served as a culture chamber for prolific growths of micro-organisms, and it was noticed that when a unit had been turned off for several days and was then restarted, the water which was pumped out of the chamber smelt foul. This situation could easily arise when the unit is in operation, for example, during a warm spell the refrigeration unit would be in use, and then if there was a cold day, the heating unit which may have been standing idle for a considerable time, full of water and sewage fungus, would be called upon. The first 3.5 cubic feet of water discharged, would be deoxygenated and absoloutely foul, quite unsuitable for addition to a fish tank.

A much better way of controlling the water temperature, is to either contain the equipment in a closed building and control the air temperature, or to pass the recirculating water through a heat exchanger. This involves passing the recirculating water through a complex of pipework which is in intimate contact with other pipes containing heated.water. The pipework can be imagined to be arranged in the same manner as when the fingers of the right hand are held inbetween the fingers of the left hand. This pipework is

THE RESULTS FROM THE TRIALS WHICH TESTED THE PERFORMANCE OF AN ACTIVATED CHARCOAL FILTER.

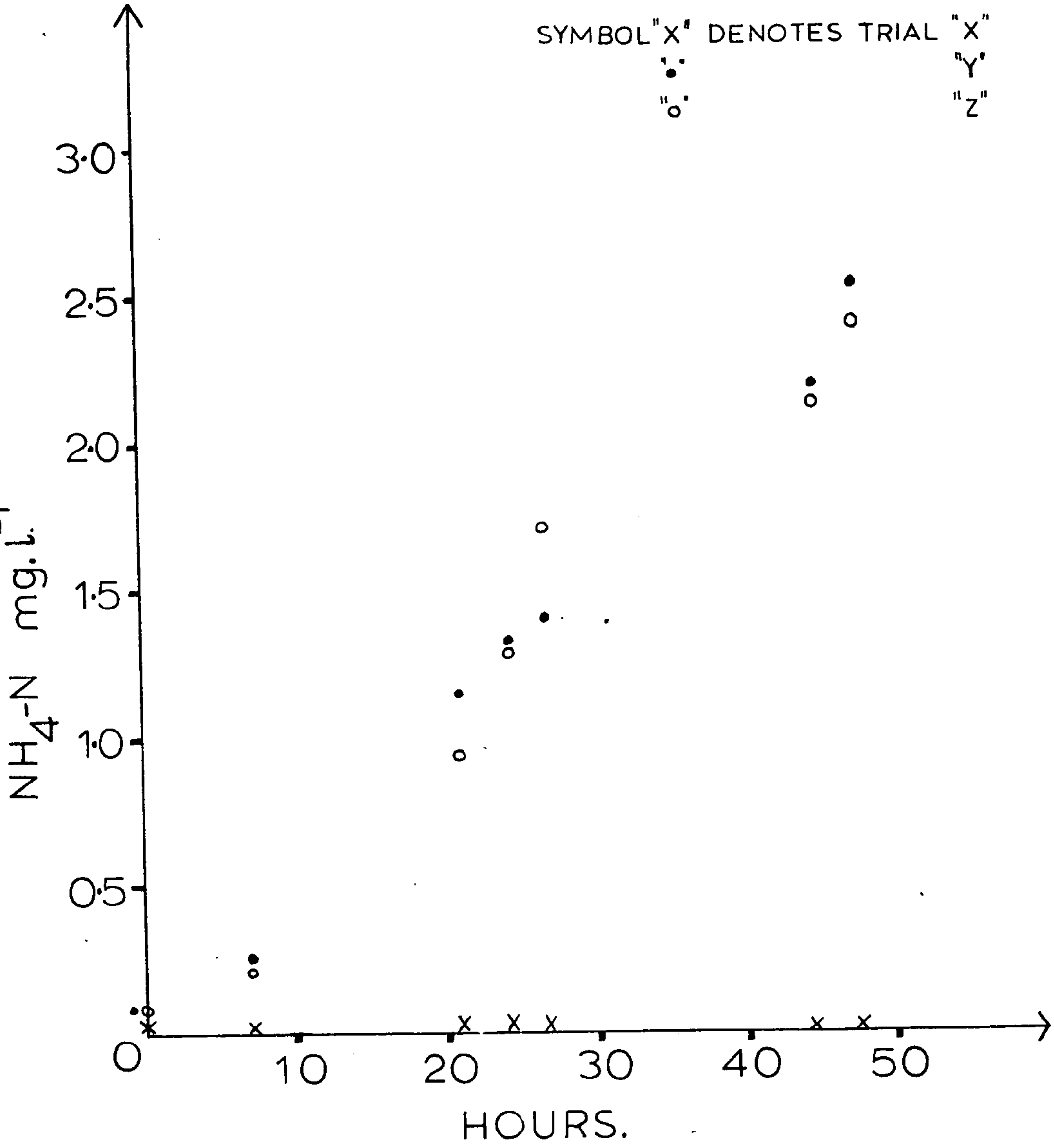


FIGURE 18.

of a narrow diameter so that much of the water flowing through a pipe is in contact with the walls of the pipe, and so the heat exchange is effective. In this method of regulating the water temperature, there would be a biological film covering the insides of the pipes, but there would be no loose, large lumps of it which might become lodged, in for example the pipe returning water to the fish tank. Also, there would be no additions of foul water to the fish tank.

### (vi) The flow meter.

After leaving the temperature control unit the water passes upwards, to the pipe which distributes the water over the surface of the biological filter. On the way, the flow rate is metered and is shown by a rubber bung rising to a particular level in a graduated glass tube. When the recirculation unit has been running for 4 - 6 weeks, this bung has been observed to "stick" at a false reading due to accumulations of biological growth. However, this can easily be remedied by routine cleaning of the flow meter, and it is important that this is kept working properly because when the bung falls due to too low a recirculation rate, (eg: if the pump fails), then a light beam passing through the graduated tube is broken; this is recorded by a photo-electric cell, and an alarm bell rings.

# CHAPTER 7

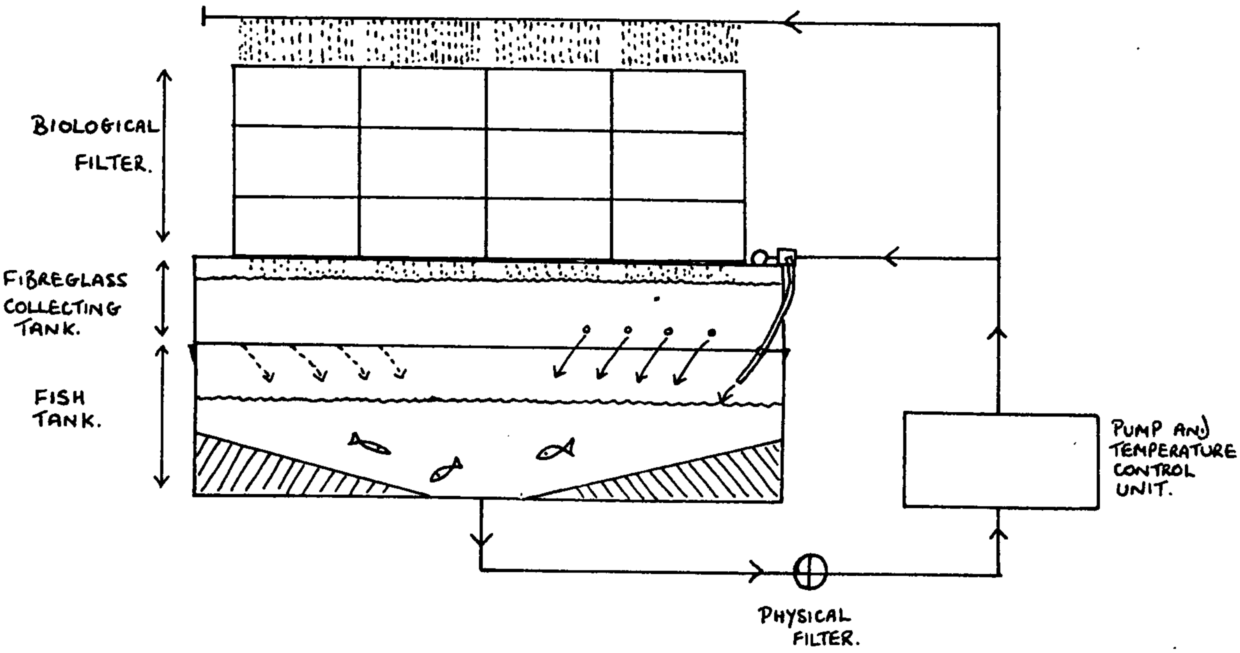
## The construction of biological filters to serve two of the fish tanks.

Permission was obtained to convert the filtration system on two of the tanks to biological filtration. However, a seriously limiting factor influencing the possible design of any such filtration system was the lack of space available in the building. Tanks 1 and 2 had the most space available, this being beside them, but this was an area only 2 ft 6" wide by 7 ft long. The other two tanks had practically no available space beside them due to the work bench area. So, as any filtration system should be designed so that each tank could be provided with an identical system, the only answer was to build the filter over the tank, in the 5 ft or so of space between the top of the tank and the roof.

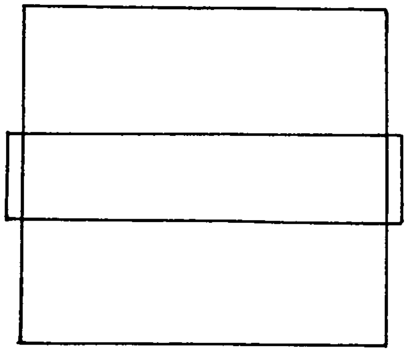
This idea of building the filter above the tank also had the advantage that only one pump wpuld be needed for the recirculation system. That is the water would drain out of the tank, be pumped up through the temperature control unit to the top of the filter, and then percolate down throught the filter draining back into the fish tank. If the filter had been situated on the ground, then unless the fish tank had been built at a high level, or alternatively had been sunk into the ground; the water would have drained out of the tank, been pumped up to the top of the filter, drained through the filter, and then have had to be pumped up again into the fish tank.

Figure 19

The design of the two biological filters which were built.



VIEW FROM ABOVE:



Biological filters were built for tanks 1 and 2, and the design is shown in figure 19. The splash tank was removed from the unit so that an unknown amount of water would not be added to the system as make-up, or lost from it by the splash tank overflowing, as the pump's efficiency changed. The small filter was kept as the sole method of physical filtration. It was appreciated that this filter was not completely efficient at removing solids and that as it became dirty the resistance across it would increase and so the recirculation rate would drop; but there was no possibility of constructing another physical filter. By far the best method of removing particulate matter from the water would be to pass the water through a settling tank as described on page 48, or a rapid sand filter. The advantage with the latter is that it is compact, but it does need frequent back-flushing for cleaning purposes. However, there could not conceivably be room for these in the building.

After passing through the temperature control unit, the water was piped upwards to a point where a proportion of it could be passed back into the fish tank untreated (see fig. 19), and the rest of it was sprayed onto the surface of the filter, through holes drilled in a length of plastic piping which stretched the full length of the filter.

The water then percolated through the filter and collected in a long fibreglass tank which supported in a wooden frame, itself supported the filter boxes. A head of water 15 inches deep was maintained in the fibreglass tank, and the water bound for the fish tank left through a series of small holes drilled, at an angle, towards the base. This water shot out of each hole in a jet, and upon striking the water in the fish tank, it both reoxygenated itself and helped to turn the body of water in a circular current.

This design for an improved recirculation unit was the result of a lengthy study and consideration of all the features that a filtration system, in this situation, would be required to provide and these features are listed below.

## The features that the biological filtration system would be required to provide.

(i) Efficient removal of metabolic products.

(ii) The water to be treated should be dispersed evenly over the surface of the filter.

(iii) There should be a good aeration of the water prior to entering the filter and after leaving the filter.

(iv) The filter must be compact.

(v) The quantity of water filtered every hour must be fixed, so that it can be known.

(vi) The filter size should be alterable.

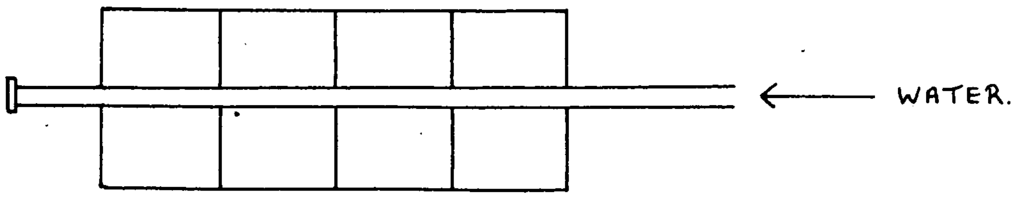
(vii) The media used in the filter should be light in weight.

## A discussion of the ways in which the listed features were incorporated into the filter design.

(i), (ii), (iii)and (iv) are all related to the efficiency of the performance of the filter. A good aeration of the water prior to entering the filter will help to ensure that the dissolved oxygen concentration of the water in the filter does not fall below 4 ppm; this is important because the nitrifying bacteria are not active below this dissolved oxygen level (EIKUM 1967). A good aeration of the water after it has left the filter is important because if a fish tank is to be stocked at it's maximum density for a particular recirculation rate, the water entering the fish tank should be saturated with air.

An even dispersion of the water to be treated, over the filter surface is essential so that all the media within the filter is brought into contact with the water, and all the water is brought into contact with the media. If the dispersion is not even, then the water tends to run through the filter bed in channels and the filter's performance is extremely inefficient. This feature of good water dispersion was one which caused alot of trouble in the design of the system, and the reason for this can be seen in the drawing below and the following discussion. Water must pass from the pipe, and be distributed evenly over the whole surface of the filter.

VIEW FROM ABOVE:-



FILTER BOXES. (RECTANGULAR)

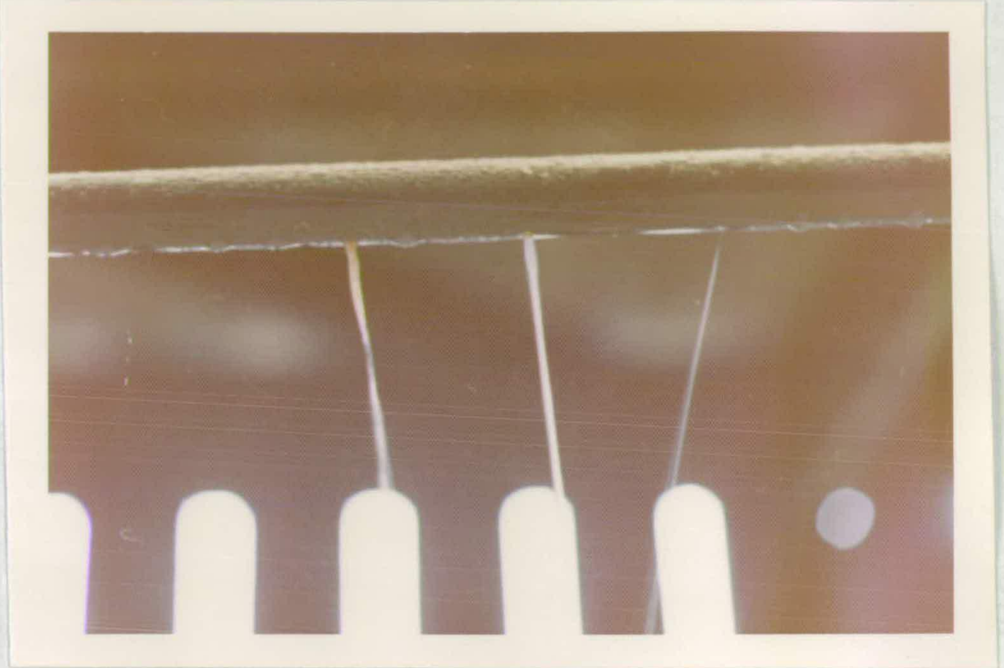
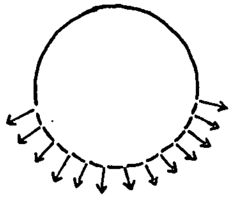


Figure 20

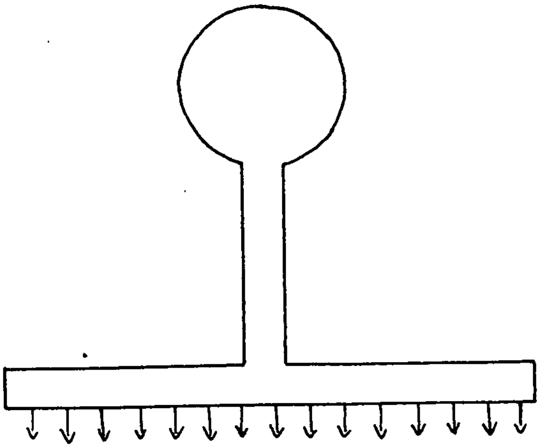
This picture shows clearly how the holes in the pipe distributing water over the surface of the biological filter become obstructed by biological growth.

Various methods of water dispersion were considered and/or tested, but no really satisfactory solution was found.

(a) The pipe was drilled with holes as shown below, in the hope that by spacing the holes correctly and by drilling them far enough up the side of the pipe, the water would be thrown out to the ends of the filter boxes in equalquantities as in the middle. However, testing showed that having so many holes in the pipe presented problems. Firstly, if there were so many holes in the pipe, then unless the holes were extremely fine (less than 1/16" diameter), much more than one quarter of the flow available would be distributed over the first filter box. Secondly, if the holes are fine then they quickly become partially blocked by biological growth (see fig. 20 ).



(b) Consideration was given to providing a modification to the main water pipe, which would serve each filter box as shown below:



The sub-pipe would run the full length of a filter box; but again, unless there were a considerable number of fine holes in the pipe, the distribution of water across the width of the filter box would be inefficient, and so the same problems apply as in case (a). Another problem with this method is that the extra pipe work over each filter box provides an added resistance to water flow, and for this reason the quantities of water distributed over each section of the filter may differ.

(c) The possibility of using a method of water dispersion similar to the mist producing system used on horticulture propogation benches was considered; but the water pressure available was not high enough, and in any case, due to the filter box being rectangular the water has to be dispersed further lengthways than widthways, and this would be a severe problem. Also the mist producing mechanism would be prone to becoming blocked by biological growth.

(d) A sheet of glass was fixed above one of the boxes, in the hope that the water from the main pipe would run over the top surface of the glass and then run across it's underside, dripping off evenly. Some sucess was had with this method in a laboratory test study, but the glass had to be in a precisely horizontal position or the method would not work, and in practice this was found to be too difficult.

(e) A $2\frac{1}{2}$" thick piece of foam rubber, the same length and width as a filter box, was placed on top of the media in the hope that it would absorb the water coming down from the main pipe, and then release it again from the underside, in a more even distribution. However, when the sponge became soaked, it was so heavy that it sagged considerably, thereby giving an uneven water distribution. If the sponge was supported from underneath in order to stop it sagging, then the water left the sponge chiefly at the points where it was being supported, again giving an uneven distribution. Of course the sponge would also be subject to blocking by biological growth.

(f) The best solution seemed to be to drill many holesin the bottom of a cut down empty filter box and then put this box on top of the top filter box. If the number of holes of a particular diameter is correct in relation to the flow of water passing into the box, then a steady head of water should be maintained in the box, ensuring that if the distribution of holes in the box is even, then so will be the distribution of water onto the top of the filter.

The problem with this method is that if the rate at which water arrives at the top of the filter drops, even slightly, then the head of water in the box will fall, and an uneven distribution of water will result. There is a provision made in the design of the new recirculation system, to counteract any decrease in recirculation rate due to a dirty filter, but the flow of water out of the pipe above the filter may still vary. Figure 20 shows how the holes in the overhead pipe become obstructed by biological growth thereby reducing the flow. Biological growth anywhere in the system may reduce the recirculation rate by increasing the friction in the pipes, or by growth sloughing off and becoming lodged in various places. Also, the holes in the bottom of the box will tend to become blocked with growth and so will have to be scrubbed.

So, there seems to be no easy ready solution to the efficient, even distribution of water over the filter surface, but method (f) represents the best one tested.

Other factors affecting the efficient removal of metabolic products include: the size of the media; the size of the filter; the ammonia loading; the retention time; the hydraulic loading; the pH of the water and the temperature of the water.

The size of the filter media used was a compromise between the demands of: (a) building a filter of a compact size in the strictly limited space available; and (b) choosing a media size sufficiently large so that the filter would not become blocked in a very short time. (It was suggested on page 47 that ideally the size of the media chosen for the filter should be large enough for the filter to run indefinitely without becoming blocked.) However, in this particular case, this advice could not possibly be applied and it was appreciated that the filter would tend to become clogged. This would mean that from time to time the filter would need to be cleaned by removing filter boxes from the filter, stirring the media by hand and hosing it down, in order to dislodge accumulated film and particulate matter. This would inevitably mean a reduction in the erformance of the cleaned box and so to compensate for this, the filter was built larger than should be needed. It was calculated from figures 6 and 7 of Speece (1973) that considering the maximum fish loading that was envisaged, and for the media size chosen, the filter would have to be four filter boxes long and three filter boxes deep. (7.8 cubic feet)

Infact the filter was built four boxes deep. It was considered that the top filter box would be the first to become clogged due to the fact that most of the particulate matter would become lodged there, and there tends to be a prolific growth of carbonaceous compound degrading micro-organisms towards the top of a filter profile (see page 46 ). So, when this top box had been removed and cleaned, all the other boxes would be moved up one place, and the clean box would then be placed at the bottom of the filter profile. While the new top box was becoming dirty, the clean bottom box would be being recolonised by nitrifying bacteria washed down from above. It was thought that this system would provide on average, the equivalent of three fully functional filter boxes in the filter profile.

The actual calculations involved in determining the size of biological filter needed for each fish tank, are explained in the section that follows.

## Calculations for determining the size of biological filter needed for each of the 6 foot diameter fish tanks.

The size of biological filter required for each 6 foot diameter fish tank was determined by following the procedures set out below. It should be borne in mind that these calculations were made after having spent only a short time on the project and so with hindsight, the inaccuracies and assumptions can be seen, but in basic principle the method remains sound.

(a) Calculate the volume of water present in the fish tank and so from this and the literature, calculate the maximum advisable density of fish of a particular size, solely from the density point of view. That is pounds of fish per cubic foot of water. If this maximum advisable density is exceeded, then the fish may suffer due to their being stressed.

(b) Calculate the rate of changeover of the tank's [illegible] water, and so from this and the literature, calculate the maximum advisable density of fish of a particular size that will be supported in the tank without reducing the water quality to such an extent that the fish will be adversely affected.

(c) The lower of the two densities recommended by (a) and (b) is the one which is chosen, because neither (a) nor (b) may be safely exceeded.

(d) As fish size and water temperature vary then so do: feeding rates (the percentage of body weight fed daily increasing with water temperature and decreasing with increasing fish size); oxygen consumption rates (pounds of oxygen used per hundred pounds of fish per day decreasing with increasing fish size, and pounds of oxygen used per pound of/food per day increasing with increasing fish size); ammonia production reates (pounds of ammonia produced per hundred pounds of fish per day decreasing with increasing fish size, and pounds of ammonia produced per pound of food per day increasing with increasing fish size).

So, it seemed to be most sensible to design the filter so that it could cope with the highest ammonia load that was ever likely to be present.

It was envisaged that any experiments would be run at 10°C and not above this level, and so as 10°C meant a higher ammonia production rate than a lower temperature, 10°C was used in the calculations. It is now felt that perhaps 21°C would have been a better temperature on which to base the calculations, bearing in mind Elliott's (1974) data on temperatures for optimum growth rate in trout.

The size of fish chosen when calculating the density advised in (b), matters little, because whether the fish is 1" long or 10" long, the density is adjusted so that the effluent is such that if the oxygen concentration fell any lower and/or the ammonia concentration rose any higher, then the fish would be adversely affected. So, for no particular reason, a fish size of 1" was selected.

(e) If the temperature of the water is known, and the number of 1" fish is known, then the ammonia production (pounds of ammonia produced per hundred pounds of fish per day) can be calculated from the literature.

(f) Speece (1973) (see figure 7 ) gives details of the specific surface area of nitrifying filter reouired for a certain weight of 1" fish, living in an environment at 10°C .

(g) All that is now left to do is to decide what media size to use in the filter, and then, following figure 6 of Speece (1973), the size of filter required can be calculated.

Now, steps (a) to (g) will be run through, using a temperature of 10°C and a fish size of 1".

A consideration of the density-stress interaction.

(a) The fish tanks are 6 feet long, 6 feet wide and 1½ feet deep, and it would seen reasonable to fill then with water to a depth of 1 foot, thereby filling them with 36 cubic feet of water (224.3 gallons). Now, Piper (1970, 1972) suggests that rainbow trout would not be stressed too greatly if they are not held at a higher density than: half their length in inches in poundsper cubic foot. (However, he now feels that this may be safely exceeded, but he is awaiting the results of further experiments.) So, at the moment, 1" fish should not be kept at a density greater than half a pound per cubic foot.

## A consideration of the effect of the allowable density of fish, bearing in mind their effect on the dissolved oxygen level of the fish tank water.

(b) The volume of water in the fish tank is 224.3 gallons, and the recirculation rate is approximately 200 gallons per hour. So, it seems reasonable to think in terms of the whole body of water in the tank being changed once every hour.

Haskell (1955) states that 1" brown trout can safely be held at 1.6 lb per cubic foot when the water temperature is 10°C. His results, as they stand are not very helpful, and so one has to look at other data in his paper, in order to adapt his figures o that they will be applicable to the system that is being designed. His rearing troughs are each of 11.8 cubic feet capacity, which is 88.3 U.S. gallons; the flow rate is 900 U.S. gallons per hour. So the water in the troughs is changed 12 times per hour. Now, if the loading density of 1.6 lb per cubic foot is divided by 12, then it can be seen that the density is: 0.13 lb per cubic foot per single water change per hour. But, Haskell's stocking density is the average for three ponds, which serially reuse the 900 U.S. gallons per hour and so in order to bring his results in line with those which might be found in a single pond, the loading density must be multiplied by three. Thus, the density is infact: 0.39 lb per cubic foot per single water changeover per hour. (This density may be a little too high due to some reaeration of the water occuring as the water flowed from one pond to the next.)

Piper (1972) offers the following equation for calculating the advisable loading of ponds:

$$W = D \times V \times L$$

Where: W is the allowable weight of fish in pounds.

D is the density index 0.5 ( suggested optimal value pending further investigation)

V is the volume of water in the pond in cubic feet.

L is the fish length in inches.

Thus, $W = 0.5 \times 36 \times 1$

$W = 18$ pounds.

So, the advised density of fish is 0.5 lb per cubic foot. Now, the changeover rate of the water in the tank is approximately once per hour, and so this density can be expressed as :

0.5 lb per cubic foot per single water changeover per hour.

Westers (1970) provides graphs relating the carrying capacity of ponds in pounds per cubic foot, to the number of times per hour that the pond water is changed. For a fish size of 1", water temperature of 10°C, and a water changeover rate of once per hour, his graphs advise a loading density of: 0.5 lb per cubic foot.

Burrows and Coombes (1968) give details of rearing 3" rainbow trout in ponds which are 75 feet long, 17 feet wide and 2.5 feet deep. These ponds provide 3187.5 cubic feet of water, or 23846 U.S. gallons; and the flow rate is 600 U.S. gallons per minute, or 36000 U.S. gallons per hour. The stocking rate of the 3" fish (90 to the pound) was 3000 per pond, which provided a density of 0.94 lb per cubic foot. So, we have 0.94 lb per cubic foot per 1.5 water changeovers per hour, which reduces to 0.63 lb per cubic foot persingle changeover per hour. Burrows and Coombes (1968) make no mention of the water temperature, but if it was about 10°C, then their practices agree quite well with those which Westers (1970) would advise for 3" fish.

Burrows and Chenoweth (1970) advise a stocking density of approximately 0.62 lb per cubic foot per single changeover of water per hour for 3" rainbow trout.

When the evidence discussed so far is considered, it seems that a loading density of around 0.5 lb per cubic foot per single water changeover per hour would be a good guide to the level which should not be exceeded for 1" fish at 10°C. Haskell's figure was somewhat below this, but that was to be expected because he was dealing with brown trout, which are known to be less tolerant of higher densities than rainbow trout. Burrows and Coombes (1968) figures and those of Burrows and Chenoweth (1970) were somewhat higher than the 0.5 figure, but this would be expected because they were dealing with 3" fish and not 1" fish.

The level of ammonia produced daily by the fish.

(e) As there are 36 cubic feet of water in the fish tank, and the loading density is 0.5 lb per cubic foot; this will mean that there will be 18 pounds of 1" fish in each tank. Speece (1973) shows that at 10°C, 100 pounds of 1" fish produce 0.24 lb $NH_4$-N per day; so 18 pounds of 1" fish produce 0.432 pounds of $NH_4$-N per day.

After the filter had been designed, the report of Kramer, Chin and Mayo (1972) arrived, and it was noticed that their figures for daily $NH_4$-N production from a given weight of rainbow trout were vastly different from those of Speece (1973). For example: for 3" fish, Kramer, Chin and Mayo's figures were $\frac{1}{3}$ - $\frac{1}{2}$ those of Speece, and for 1" fish their figures were 1/5 - 1/8 those of Speece. So, the accuracy of Speece's estimate of the specific surface area of nitrifying filter required per hundred pounds of fish, is now open to conjecture, because the report of Kramer, Chin and Mayo is well thought of, the findings being based on the results from many 'field trials'.

The specific surface area of filter needed, to treat a particular ammonia loading.

(f) Figure 7 of Speece (1973) shows that a nitrifying filter with a specific surface area of 2400 x 18/100 = 432 square feet is needed to remove the daily ammonia production mentioned in (e).

The media size used, and hence the size of the filter.

(g) The media chosen, had a diameter of 7 mm (0.28"). From figure 6 of Speece (1973), it can be seen that a media size of 7 mm yields a specific surface area of 165 square feet per cubic foot of media. Now, the specific surface area needed is 432 square feet, and so the filter must be 432/165 = 2.6 cubic feet in volume. In his calculation of the specific surface area of nitrifying filter required per hundred pounds of fish, Speece (1973) does not take into accout that there will be carbonaceous compounds in the waste, which will mean that a proportion of the filter surfaces will be occupied not by nitrifying micro-organisms, but by carbonaceous compound degrading micro-organisms. So, Speece's estimate for the size of filter required, will have to be increased. Forster (1974 personal comment) advised that as an approximate guide, the filter size might be doubled, and so the recommended filter volume is now 5.2 cubic feet.

The design of the filter is described on page 83, and it was stated that the filter was constructed of interstacking polypropylene boxes. These boxes were stacked to a depth of 3 boxes, the bottom layer being an extra layer to facilitate the constant performance of the filter after times of cleaning. So, the total filter size must be: $5.2 + \frac{5.2}{2} = 7.8$ cubic feet. Now, each filter box contains a volume of 0.75 cubic feet and so this means that the number of boxes required is: $\frac{7.8}{0.75} = 10.4$ boxes. Finally, as the boxes are arranged 3 deep, the total number of boxes must be 12.

## The hydraulic performance of the filters.

The recirculation rate was set (see page102) so that the filter received for treatment, 120 gallons of water per hour. This produced a hydraulic loading of 0.33 gallons per minute per square foot, which is very low; but the retention time, which cannot be controlled except by building a larger filter, was also very low at 0.3 minutes. N.B. The effective retention time for the purpose of examining how efficient the recirculation system is in removing ammonia from the fish tank water, is multiplied by; "the number of times during 24 hours that the whole body of water in the system is filtered". That is a low retention time can be compensated for by filtering the water more often.

## Conclusions.

Figure 8 of Kramer, Chin and Mayo (1972) shows clearly that at retention times which are as low as those provided by the newly built filter, only a very small percentage of the ammonia loading passed into the filter is removed.

So this study has shown the inaccuracy of Speece's (1973) suggestion that the only matter of importance when calculating the size of filter needed, is the specific surface area within the filter. Figure 7.1, taken from Speece (1973), advises the specific surface area of nitrifying filter needed per hundred pounds of fish (of a certain length), must apply to a filter with a particular retention time and particular hydraulic loading as well as a particular ammonia loading. He makes no mention of the hydraulic loading or the retention time, and the ammonia loading must be deduced from a graph of his which relates daily ammonia production to fish size. The omission of details of the hydraulic loading and the retention time, would seem to be serious mistakes, because Kramer, Chin and Mayo (1972), quite reasonably state that only when the intended hydraulic loading and the necessary retention time of a filter are known, can the necessary depth of the filter bed be determined. Burrows and Coombes (1968) also mention that a filter must be more than a certain minimum depth. Speece (1973) makes no reference to the depth of filter beds, which after all is important in eliminating hydraulic short circuiting as well as increasing retention time.

So with hindsight, it would seem that Speece's entire paper must be considered with the utmost caution.

The real test of whether a filter is designed adequately, is to check that it's capacity for removing ammonia is keeping pace with the fishs' rate of production of ammonia. If the filter cannot remove daily all the ammonia that is being produced, then the level of ammonia in the water will build up and will eventually be toxic to the fish. (N.B. It must also be remembered that usually 5% of the recirculating water is exchanged for fresh water every cycle and so this will help the filter in keeping down the ammonia level. However, it must be remembered that this is not the primary purpose of the water exchange.

(v) It is important that the quantity of water filtered every hour is fixed. This must be the case, so that all four tanks can be run as replicates. Also it is essential to know how often the water is being filtered so that some meaning can be attached to a reading taken of the ammonia concentration in a fish tank at any particular time.

The new recirculation system is designed so that the quantity of water being filtered every hour can be set at any level, from 0 gallons per hour up to 180 gallons per hour. (The latter was found to be the limit of the pump's capability.) The quantity of water being filtered every hour could be increased by increasing the number of holes drilled towards the base of the fibreglass collecting tank (see page 85 ), or alternatively the diameter of existing holes could be enlarged. If it was desired to decrease the quantity of water being filtered every hour, then one or more holes could be plugged up.

These relatively simple changes had the desired effect, because resting on top of the water in the fibreglass collecting tank was a ballcock mechanism which ensured that a steady head of water was maintained in the fibreglass tank. This ballcock mechanism determined how much of the water available for treatment went to the top of the filter, and how much was sent back into the fish tank untreated. For example, if more holes were drilled in the fibreglass tank, then the ballcock would tend to fall as the head of water in the collecting tank decreased. This falling of the ballcock would result in a greater proportion of the available water for treatment being directed up to the top of the filter, and less of it being sent back to the tank untreated. (See figure 19)

In practice, the system was never set so that all of the available water for treatment was directed to the top of the filter, because this would mean that as the physical sleeve filter became dirty during the day and so the pump's efficiency declined, then the rate at which water was biologically filtered would also fall. This would be contrary to the requirements made of the system, as mentioned earlier.

(vi) It was felt to be important that the filter size should be alterable. This would mean that if more fish were placed in the fish tank, then the size of the filter could be increased so that the extra metabolic wastes would be removed. It was also important to be able to vary the size of the filter, because the original size that was built was at best, only an approximation of the filter size needed.

The most promising solution to this problem of making the filter size alterable, was provided by using interstackable polypropylene boxes. These boxes were 18" long 12$\frac{3}{4}$" wide and 6$\frac{3}{4}$" deep, providing 0.75 cubic feet of holding capacity. They were stacked 3 boxes deep in the filter, and it was ensured that the water flowed freely and evenly from box to box, by drilling an excess of 2/8 " diameter holes all over the bottom of every box.

(vii) It was very important that the media in the filter should be light in weight. The interstacking boxes, even if made of heavy duty polyethylene would buckle under the weight of gravel, especially when there were other boxes of gravel resting on top of them. Apart from this, the whole filter weight was resting on the asbestos fish tank walls and the less weight placed on them, then the less stress these walls would be placed under. (The tank was already stressed from the weight of the concrete floor in it.)

The only small, light-weight media readily available appeared to be polystyrene beads, and as the Unilever laboratory at Findon had found a polystyrene bead media to be quite effective, an order was placed with a manufacturer to make polystyrene beads of 7 mm diameter. However, when the consignment arrived, there were many beads that were far too small and so because the beads clung to each other electrostatically, the small beads had to be laboriously separated by hosing them off through a 7 mm mesh sieve.

# CHAPTER 8

## Conclusions.

The discussion so far has revealed the shortcomings of the equipment, and how these might be overcome. The changes which have been suggested would be of value, vary in their importance, but the most fundamental essential change needed is to remove the large filters from the recirculation units and build a large biological filter which would serve all four tanks. It is suggested that one large filter should be built and not one small filter per tank for the following reasons:

(i) If there is one large filter, then the number of fish in a particular tank can be increased without worrying about whether the filter is adequate to cope with the increased ammonia loading. So, having one large filter makes for greater flexibility in the hatchery.

(ii) It should be much simpler to ensure an even water dispersion over a single large filter than to ensure an even water dispersion over four small filters. On a large filter it may even be an economic proposition to have a moving water distributor arm, as used on sewage works biological filters.

(iii) Cost is an important consideration, and it must surely be cheaper to build one large biological filter rather than four small ones. Also the maintenance on one large filter would be less than on small ones.

(iv) Most importantly, by mixing up the water for treatment from all four tanks, then filtering it and then redividing it, the chemical state of the water re-entering each tank would be practically the same. This would be most unlikely to occur if all the tanks had their own small filters, especially if one tank having more fish than another, was given a correspondingly larger filter. Although this larger filter would be designed to produce the same quality of effluent as a smaller filter serving a lower loading of fish; in practice this would seldom be achieved. So, if there was just one large filter, any difference in the chemical quality of the water in tank A compared to that of tank B, could be attributed solely to a difference between the fish populations of the two tanks and not to a difference in the performance of filter A compared to filter B.

If a biological filter was to be built, then there should be a reasonably effective way of removing particulate matter from the recirculating water before it reached the biological filter. This would seem to be best provided by a settlement trough rather than a polypropylene sleeve filter (for reasons already discussed). A rapid sand filter would have the disadvantage of needing a second pump to draw the water through the sand and to backflush it.

The temperature control facility could be tremendously improved by using a proper heat exchanger as has been explained; and really many smaller tanks would be of much more value than four 6 foot diameter tanks.

Even to build just the big biological filter would be expensive (probably in excess of £1,000), and in view of the other shortcomings of the equipment, this expenditure could not really be justified. It is important to realise that there has already been a considerable amount of work carried out in the United States in the field of water reuse, biological filters, and ammonia/oxygen toxicity in rainbow trout. Now, further development is taking place in this country, directed principally by Shearwater, a division of British Oxygen Company, and so there is no room for a research project to be carried out which can be seen from the outset, to be able to offer results of a very limited value, due to the shortcomings of the equipment.

# CHAPTER 9

## Suggestions for further experimental work.

During the work on the recirculating fish rearing systems, various ideas for experimental work were developed, and several of them could have been made into useful projects. The ones falling into this category are set out below. Unfortunately, the failure of the equipment has prevented the further development of any of them.

(i) The first experiment would have to be to put identical fish populations into all four tanks. The recirculation rate would be set at the same rate for all the tanks, and all the fish populations would be fed the same amount of food. So, as far as is possible all four systems would be made identical. The point of this experiment would be to test whether, after a suitable growing period, say two months, the four fish populations were the same. This would be expected if all four tanks were equally favourable for fish growth. Any significant differences between the populations would reflect a difference in the favourability of the tanks' environment for trout growth. Such differences should be documented in order that an accurate interpretation might be made of any differences that might arise between the trout populations of different tanks, during future experiments.

(ii) An experiment designed to disentangle the low oxygen high ammonia toxicity complex, described by Larmoyeux and Piper (1973) (see pages 13 to 23), would produce results of tremendous value to fish farming research. Larmoyeux and Piper (1973) describe their equipment as a series of seven troughs; fresh water entering the top trough and being serially reused until it is discarded after it flows out of the last trough. Each trough has the same density of fish.

As the water flows from trough to trough, the dissolved oxygen level of the water falls and the $NH_4$-N level rises. Larmoyeux and Piper (1973) describe how after a certain period of time the fish in trough five for example, exhibit certain symptoms, whereas the fish in trough six exhibit different symptoms etc. However, there is a problem in interpreting their findings because the water in each trough has a different value for the dissolved oxygen concentration and the $NH_4$-N concentration.

What is really needed is an experiment to be designed so that fish are kept in different tanks, each of which contains water maintained at the same dissolved oxygen concentration but different $NH_4$-N concentrations. This could best be done by putting identical fish populations in a series of tanks (the minimum size of each population necessary for a statistical analysis of the results would have to be determined). If the populations are the same, then the dissolved oxygen levels in all the tanks should be the same. The $NH_4$-N concentration could be made different in each tank, by drip-feeding an ammonium sulphate solution into the water flowing into each tank.

Further investigations on this theme could be carried out by having the same $NH_4$-N concentrations in the various tanks, but testing different constant dissolved oxygen levels. However, the engineering of these different dissolved oxygen levels would be more difficult.

Unfortunately, the four large fish tanks and their recirculation units, are not suited to tackle this fundamentally important research.

(iii) Probably, if the equipment was fully functional, then it would be best suited for an experiment involving feeding trout different daily ration sizes at different temperatures. It would be found that, at a particular temperature, if one fed above a certain ration size, then the conversion efficiency would fall off, from it's maximum value. When this particular ration size had been determined, varying the temperature would show that as the temperature rises, then the ration size which should be fed to give the maximum conversion efficiency, also increases. This change is associated with a rising temperature causing an increase in the activity and metabolic rate of the fish and so the fish expending more energy on what is termed it's "maintenance requirements". That is day to day energy expenditure, not directed towards growth. Varying the temperature would also provide some useful data on fish appetites and growth rates at different temperatures and ration sizes.

A deeper investigation into the work could involve calorimetry studies in order to look into the "energy budget" aspects of the observed changes.

(iv) The purpose of this experiment would be to determine how well rainbow trout feed at night, and to what extent the level of available light influences their efficiency at capturing the food available. At the moment, it is not common fish farming practice to feed fish at night; but if their stomachs are not full, and they are prepared to feed, then it would make good sense to feed them during this period, so that they would grow faster and thus a fuller use of the expensive fish farm ponds and equipment would be made. The idea of this experiment was prompted especially by the following two statements:

(a) Jenkins (1969), "Trout were found to feed or be in feeding readiness at nearly all hours of the night, at least in summer. But, they appeared to take a smaller percentage of the marked ants provided, than during the day." (Experiment on a small stream.)

(b) Jenkins, Feldmeth, Elliot (1970), "At night the food may have to pass between the fish and the water surface for the fish to be able to see it. Any food passing beneath the fish may not be visible against the background of the dark stream bed, and so at night less of the available food may be eaten."

The experimental regime would be as follows:

Tank 1 Bright light for 24 hours per day, so that the food could always clearly be seen.

Tank 2 Bright light for 12 hours per day, followed by a dim light for 12 hours.

THE VARIATIONS IN LENGTH OF MEMBERS OF A RAINBOW TROUT POPULATION.

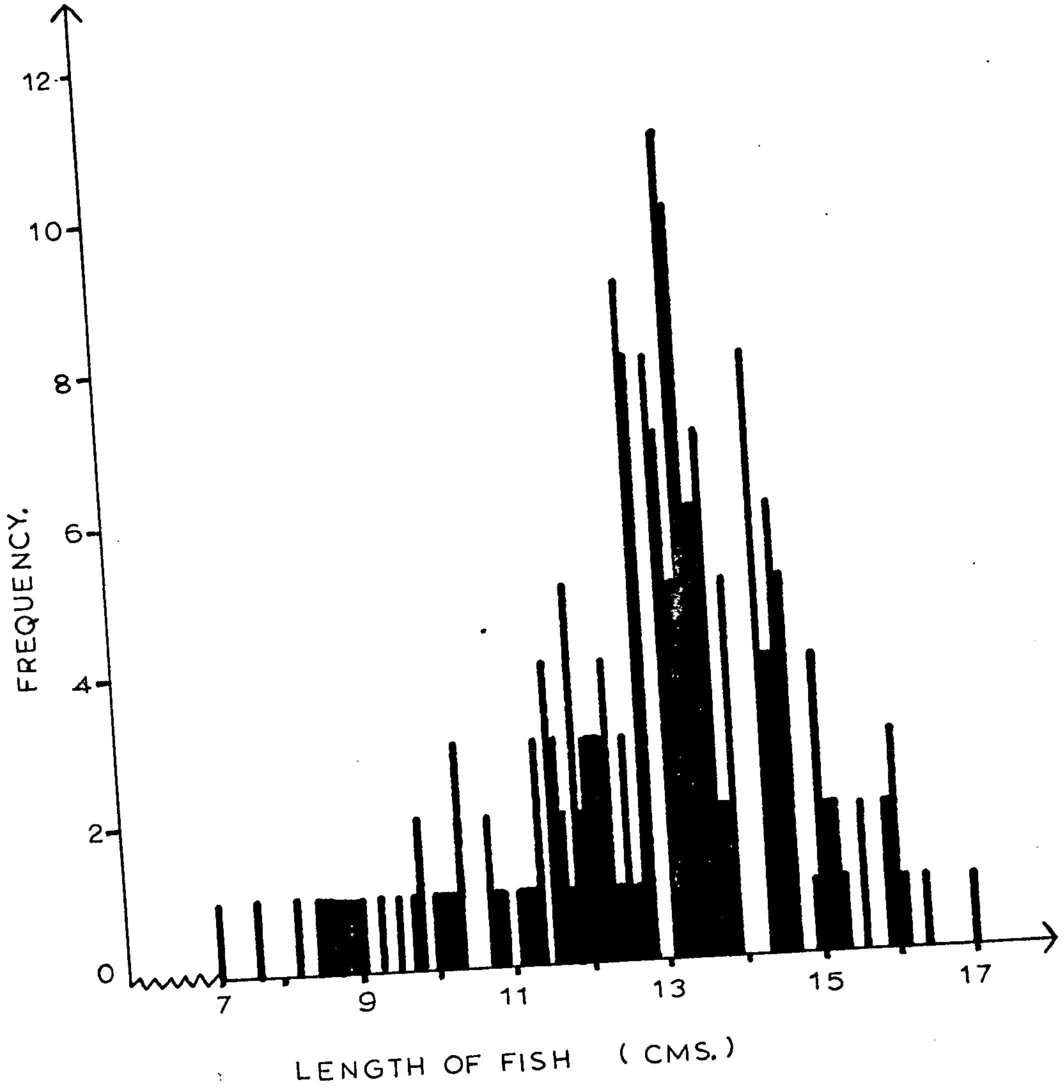


FIGURE 21.

Tank 3 Bright light for 12 hours, followed by a very dim light for 12 hours.

Tank 4 Bright light for 12 hours, followed by absolute darkness for 12 hours.

Other experimental details are that the tank floors would be painted a dark colour (probably black); the food fed to the fish should be a non-floating pellet; the tanks should be covered by a black polythene hood; and the lights should be controlled by an automatic time switch. Identical fish populations would be placed in all four tanks at the start of the experiment, and after the end of the experiment, in say 2 months, any differences in the populations would be attributed to the difference in experimental regime (after considering the results from experiment (i)).

This experiment runs into the problems of copying the natural light present on a dim night. To achieve this is important, if the results are going to be used to explain the night-time feeding behaviour of wild fish. However, even the difference between tank 1 and tank 4 would be interesting. N.B. The quantity of food fed during the 12 hours of light would correspond to that recommended by Cooper's nutrition in their feeding chart for one day.. Then during the succeeding 12 hours, the same quantity of food would be given.

(v) Figure 21 shows the size distribution of individual fish in a rainbow trout population which had been reared in the same tank, for the last six months of their eight months lives. The size range stretches from 7 cms. to 17 cms., with the average length being 13 - 14 cms.

It would be very interesting to know how the shape of this length-frequency curve might change at different fish densities. After all, it would seem reasonable to expect that a fish population living at a high density would be under greater stress than a population living at a low density. Fish in the former population mightwell be competing severely for living space and for the food which is entering the tank. Any such competition would surely tend to flatten the length-frequency curve of the fish population. Clearly there will always be runts, but an experiment which kept different population densities of fishin various tanks, would give an indication of how the growth of certain fish could be suppressed by competition. The different fish population densities reared, would in effect test different intensities of competition.

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# APPENDIX

## Determination of $NH_4$-N

This method can detect $NH_4$-N within the range of 0.05 mg/l to 1.0 mg/l.

## Reagents

(i) 8.5% (w/v) Sodium salicylate 0.06% Sodium nitroprusside

Dissolve 85 g Sodium salicylate and 0.6 g Sodium nitroprusside in 500 mls deionised water and dilute to 1000 mls with deionised water.

(ii) 0.25% Sodium dichloroisocyanurate in 0.3 N NaOH

Dissolve 0.625 g Sodium dichloroisocyanurate and 3 g Sodium hydroxide in 100 mls deionised water and dilute to 250 mls with deionised water. (To make 1 litre of solution, multiply the weight of the reagents by 4.)

## Standards

100 mg $NH_4$-Nper litre (stock)

Dissolve 0.4715 g $(NH_4)_2SO_4$ in deionised water and dilute to 1000 mls with deionised water.

Dilute stock standard to give 250 mls of a solution of concentration 2 mg /l and one of 1 mg /l.

## Standards (working)

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 0.1 mg/l | 5 mls | of | 1 mg/l | solution | to | 50 mls | (deionised | water) |
| 0.2 | 10 | " | " | " | " | " | " | " |
| 0.4 | 20 | " | " | " | " | " | " | " |
| 0.6 | 30 | " | " | " | " | " | " | " |
| 0.8 | 40 | " | " | " | " | " | " | " |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 1.0 mg/l | 25 | mls | of | 2 mg/l | solution | to | 50 mls | (deionised | water) |
| 1.2 | 30 | | " | " | " | " | " | " | " |
| 1.4 | 35 | | " | " | " | " | " | " | " |
| 1.6 | 40 | | " | " | " | " | " | " | " |
| 1.8 | 45 | | " | " | " | " | " | " | " |

## Method

To a 10 mls volumetric flask, add 5 mls of the standard and 2 mls of each reagent. Shake the solution and place in a water bath (37°C) for 10 minutes until the emerald green colout develops. Cool to room temperature and dilute to the mark with deionised water. The standards now have a value of half the original value i.e. 0.05 mg/l to 1.0 mg/l.

Shake and read absorbance, against a blank, at 667 mu, narrow slit in Beckman Spectrophotometer.

A plot of the concentration against absorption should give a straight line.

Samples are treated in the same way except that the dilution of the sample may vary to fit the absorbance within the standard range.

## Note.

Reagents are stable for approximately two days.

## Reference.

A modification of the method used in the following paper:

CROOKE, W.M. and SIMPSON, W.E. Determination of Ammonium in Kjeldahl digests of crops by our Automated Procedure.
J. Sc. Fd. Agric., 1971, Vol. 22, January.

# ABSTRACT OF THESIS

*Name of Candidate* Philip John Parkinson
*Address* 17 Morningside Gardens, Morningside, Edinburgh.
*Degree* Master of Philosophy *Date* June 1975
*Title of Thesis* A consideration of the problems involved in rearing rainbow trout in recirculating water.

This thesis examines the problems involved in rearing rainbow trout in recirculated water, with special reference to the particular problems encountered with the equipment at Edinburgh University.

There is a thorough review and discussion of the relevant literature. The primary problem encountered when fish are reared in recirculated water is that of the toxic effect of high free-ammonia levels on the fish and compounded with this is the toxic effect of a low oxygen concentration. The literature review looks closely at these problems and those of reconditioning the recirculating water, with the main emphasis being placed on a reconditioning of the water by biological filtration.

Following these topics, is a review of the recirculation based, fish rearing equipment available at Edinburgh University and there are details of the modifications which were made to this equipment and those which still should be made so that valuable research work could be facilitated.

Experimental results are included where appropriate and the thesis is concluded by suggestions for further experimental work.

*Use other side if necessary.*