EGG QUALITY AND FECUNDITY IN RAINBOW TROUT: THE DETERMINING FACTORS AND MECHANISMS OF CONTROL

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Submitted for the degree of PhD

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July 1985

To my mother and father

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SUMMARY

This thesis considers the factors involved in the determination of egg quality and fecundity in farmed stocks of rainbow trout (Salmo gairdneri R). Measurements of egg quality, ie. percentage survivals of eggs and fry, from the production batches of eggs of seven fish farms, showed mean survivals of 70% to eying but levels of only 35% to 4.5g fry (approx. 130 days post-fertilisation). Under optimum conditions survivals may reach 85% suggesting that husbandry methods exert significant influences on egg quality.

Chemical analyses of the protein, fat, vitellogenin, ash, amino acids, free fatty acid and mineral levels of eggs of varying quality and from parents of different strains showed compositional differences even between individuals of the same stock. However, none of these differences were correlated with egg quality. Egg size showed similar variations but, again under hatchery conditions there was no correlation with differences in egg quality. The only factor which has been shown to exert a significant influence on egg quality is the time of stripping after ovulation. At 10°C eggs should be removed from gravid females within ten days of ovulation to achieve optimum egg and fry survival.

Studies of egg production from approximately 10,000 broodstock revealed that total fecundity and egg size increased and relative fecundity decreased with increasing fish size. In general, most fish appeared to produce a constant volume of eggs. This is consistent with a hypothesis that egg size can only be increased by parallel reductions in fecundity.

Feeding broodstock at half-ration (0.35% body weight day^{-1}) did not affect egg quality but reduced total fecundity and egg size and increased relative fecundity when compared with eggs produced by fish on full-ration.

Comparisons of regressions of total fecundity against fish weight for three strains using ANOCO revealed that one strain was significantly more fecund than two other strains considered. Trout of the same strain maintained on different farms behaved similarly suggesting there was some reproducibility of strain characteristics.

Key words: Salmonid, Fecundity, Egg-quality, Husbandry, Fish-farming.

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PREFACE

The title "Egg quality and fecundity in rainbow trout: the determining factors and mechanisms of control" summarises the basic aims of this thesis. It will be seen that the project is subdivided into three main fields of investigation, namely; egg quality, secondly fecundity and lastly factors which may control these biological parameters. The approaches used, although focussing on a single theme, are necessarily diverse and it is considered that some mention of how the project was conceived would help to explain why the various approaches were chosen.

In 1978, Trehaven Fish Farms Ltd., part of the Trehaven Group, constructed a hatchery at Pewsey, Wiltshire (Avon Springs Hatchery). The farm was designed to produce up to 20 million eggs per annum for sale to other farms as eyed eggs or 4.5 g fry.

During the first four years of production it became clear that the egg and fry survivals were unacceptably low and furthermore there was little information relating to the merits of the three strains maintained on the farm as far as egg producing capabilities and egg quality were concerned. Consequently it was decided that an elucidation of the factors controlling egg quality and fecundity would be of considerable economic importance to the farm, especially bearing in mind their aims to produce the greatest number of the highest quality eggs from each tonne of broodstock. Similar lines of research were also being considered by The Fish Cultivation group at

Aston University and thus a project evolved in 1982 between Trehaven Fish Farms Ltd. and the Department of Biological Sciences to study egg quality and fecundity in domesticated stocks of rainbow trout considering both the determining factors and mechanisms of control.

This research was supported by equivalent grants from the Natural Environmental Research Council (N.E.R.C.) and from Trehaven Fish Farms Ltd. to Dr. Niall Bromage.

As a result of the joint industrial and Research Council support of this work it is designed to be read by both the scientist interested in the biological aspects of reproduction in the rainbow trout, and by the fish farmer concerned with the commercial advantages to be gained from a working knowledge of the factors involved in the determination of egg quality and fecundity in one of the most important species under intensive culture in the Northern Hemisphere.

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CHAPTER 1

General introduction

The artificial rearing of trout for recreational purposes, for stock enhancement and for food has been practised for at least half a century. Recently aquaculture has received attention as a way of producing high quality protein quickly and efficiently (Gall and Gross, 1978). The rapid expansion of salmonid farming over the last decade has led to an increase in research effort on many aspects of salmonid culture in particular on problems of nutrition and disease. However it is perhaps surprising that relatively little effort has been directed towards managing broodstock, with its far reaching effects on egg quality and fecundity. This imbalance of resource provision is perhaps a reflection of the relative value of eggs when compared with that of market size fish. However, considering that eggs represent the starting point of the industry this outlook appears very shortsighted (Springate and Bromage, 1983).

There has been very little research directed towards studying egg quality (Bromage *et al*, 1982; Craik and Harvey, 1984a) and although many studies of fecundity have been made the vast majority are concerned solely with wild stocks of salmonids (Rounsfell, 1957; Nicholls, 1958; Nomura, 1963; Gibson *et al*, 1976; Thorpe *et al*, 1984) where a wide variety of other factors may be influencing the results. This study differs in that it examines farmed stocks of rainbow trout where the environment

can be more closely controlled and there is a commercial requirement to produce the greatest number of high quality eggs.

In 1978 the U.K. rainbow trout industry required 40 million eggs (Purdom and Hill, 1978). However due to the expansion of the industry during recent years it has been estimated that there is currently a requirement for approximately 100 million eyed-eggs (Springate and Bromage, 1983). Some 100 million green eggs are produced by the 'home hatcheries' but after early losses only 70 million reach the eyed stage. Therefore the industry requires an additional 30 million eyed-eggs which it imports from all over the world, mainly from Denmark and the U.S.A. Disease monitoring of imported eggs is very difficult and it is uncertain if overseas supplies will continue to satisfy the essential disease certification procedures which are stipulated by the Ministry of Agriculture Fisheries and Food (M.A.F.F.) and the Scottish Department of Agriculture and Fisheries (D.A.F.S.). There are grave risks of bringing in diseases along with the imported eggs which are not endemic to this country, diseases such as the virus diseases: viral hemorrhagic septicema (V.H.S.) and infectious hematopoietic necrosis (I.H.N.) (Hill and Purdom, 1981).

The U.K. is more fortunate than many other countries who farm trout in having had its own disease control since 1937 in the form of the Disease of Fish Act. This single piece of legislation, which provides the legal powers to control the fish movements within the U.K. on disease grounds, has also prohibited the importation of live salmonids and demanded health certification for all imports of eyed ova. Although the health

certification requirements for imports of eyed-eggs can significantly reduce the risk of introducing disease they cannot provide absolute guarantees that such consignments are totally free from disease. There are several trout diseases which can be transmitted vertically, i.e. from parent to offspring via the egg and although the widely practised disinfection of eyed-ova before and after shipment may reduce the chance of disease transfer they are unable to completely remove every disease agent. The only way to remove the disease risk associated with imported eggs is to stop all imports. This can only sensibly be done when the U.K. industry can produce enough good quality eggs for its own requirements.

The availability of 'out of season' eggs and the provision of 'new blood' for breeding stocks are additional reasons for importing rainbow trout eggs. However, it is now possible to spawn U.K. broodstocks in every month of the year by photoperiodic manipulation (Bromage, 1982; Buss, 1982). A suitable range of genetic material is also available from different U.K. trout farms and from limited stocks maintained by the M.A.F.F. at their experimental fish farm (Purdom and Hill, 1978).

Clearly more eggs will be required to satisfy the growing demands of the U.K. industry. However, if we are to avoid the risks of introducing diseases we must seek to maximise supplies of high quality eggs from our own hatcheries.

There are four ways to be considered of achieving the goal of producing more eggs:-

1. Maintain more broodstock.

2. Increase the ratio of female to male broodstock kept.

3. Increase the yield of eggs from the existing broodstock.

4. Reduce the unacceptably high mortality rates currently experienced i.e. improve egg quality.

To simply maintain more broodstock would superficially appear to be the easiest method of increasing egg production. However it is the least cost effective method (Reay, 1984). Maintaining rainbow trout broodstock is a high cost, high risk but low profit operation. Consequently it would be better to increase the productivity of existing stocks.

One method of improving the egg producing capacity of a fixed tonnage of broodstock is to increase the proportion of females to males. This can now be achieved by using 'feminised males' to produce all female progeny (Bye and Lincoln, 1981).

It has been demonstrated that under ideal conditions the milt from one male (weighing one Kg.) can fertilise the eggs from more than 100 similar weight females (Billard, 1983). However the genetic implications of such manipulations must be

carefully considered and it is suggested that a ratio of 10 females to each male is a more advisable ratio for commercial broodstocks (V. Bye, personal communication, 1984).

Given that we can increase the proportion of females in a broodstock we are left with the problems of producing the maximum number of the highest quality eggs from the broodstock. In the present climate of economic stringency it is essential that all aspects of rainbow trout culture are made more cost effective and productive.

The aim of this research is to firstly define egg quality and fecundity and then investigate factors that may effect these parameters. This should provide a strategy which will enable egg production and egg quality to be optimised for a given weight of broodstock.

The work in this thesis is divided into three major sections: Part I considers egg quality. Initially an examination is made of the levels of mortality which are experienced under commercial conditions; this is followed by consideration of egg size and egg chemical composition; Part II of the thesis considers fecundity. Firstly, the levels that are experienced under commercial conditions, this is followed by an investigation of the factors which may affect fecundity such as the size, age and strain of broodstock; Finally in Part III investigations are made on the effects of the timing of

stripping on egg and fry survival and also of varying broodstock ration level on egg quality and fecundity.

CHAPTER 2

General materials and methods

Materials and methods having application to more than one section of this thesis are included in this chapter. Materials and methods relevant to a particular experiment will be discussed in the protocol for that experiment.

All experiments were carried out on domesticated stocks of rainbow trout (Salmo gairdneri R.). Fish of many different stocks and strains were used in this work, but care was taken to ensure that fish from the same source were used in related experiments. Details relating to the size, age and origin of fish are included in the materials and methods sections of the individual chapters.

Fish were fed on commercial dry pellet and at rates specified by the manufacturers, or by the experimental protocol.

2.1 Fish handling

2.1.1 Anaesthesia

All procedures requiring fish handling e.g. stripping, weighing, blood sampling etc. were performed under anaesthesia, using a 1:20,000 (v/v) solution of 2-phenoxy-ethanol (B.D.H. Poole, U.K.). Fish were starved for 24 hours prior to

anaesthesia. Mortalities caused by anaesthesia and handling were very rarely seen.

2.1.2 Fish measurements

All fish length measurements were made from the tip of the nose to the fork of the caudal fin (fork length). Measurements were made to the nearest 0.1 cm.

All weight determinations were made on anaesthetised, live wet fish and, unless stated to the contrary, were to the nearest 1 g.

2.1.3 Stripping

The eggs of captive salmonids are ovulated, that is they are released into the body cavity, but they are not oviposited and therefore the eggs must be manually forced from the fish. The process of forcing gametes from the mature trout of both sexes is commonly referred to as 'stripping'. Eggs can only be stripped from fish in which the eggs have been ovulated. Such fish are termed 'ripe' fish. External signs of ripeness in females include an extrusion of the anal papilla and reddening and a softening of the abdomen. In males ripeness is characterised by a dark skin colour and a well developed kype.

Ripe females were stripped by holding the anaesthetised fish dorsal side up with the spawner's left hand grasping the caudal fin and the right hand gently massaging the abdomen in such a

manner that the eggs were stroked from the fish into a dry bowl. It is essential during this process to avoid excessive water contamination of the eggs as this causes the micropyle to close before the sperm has the opportunity to fertilise the egg. During the whole spawning process the fish must be handled gently as internal damage, especially liver damage, can be caused by rough handling.

After stripping the eggs from each ripe female into separate bowls a sperm pool was made by collecting sperm from ripe males in the ratio of one male for three females (minimum two males). Sperm was stripped from ripe males in the same manner as eggs were forced from ripe females. Again, care was taken to avoid water contamination of the sperm as water prematurely activates the sperm. Sperm activity only lasts for approximately 30 seconds. Sperm from the pool was then added to the eggs in the concentration of 1 ml to 10,000 eggs and gently, but thoroughly mixed. The eggs were then left for 1 minute before washing in fresh water and leaving to water-harden (see Table 2.1 for full sequence of events).

In some experiments sperm was collected from 'masculinised' or sex-reversed females, to produce all female offspring.

2.1.4 Fish identification

Individual fish were tagged with small, plastic numbered tags (Charles Neal (Finchley) Ltd., East Finchley, U.K.). These

Table 2.1 Normal egg development and relevant methodology.

Time 10°C	Stage	Method and information
-24 to 0 hours	Stripping	Dry method. Ripe females stripped into separate bowls. Sperm pool collected from sufficient males to give adequate genetic mix.
0	Fertilisation	Sperm from pool thoroughly mixed with eggs. Ratio 1 ml sperm to 10,000 eggs.
¼ hour	Water-hardening	Excess sperm washed off and eggs left for 40 mins to water-harden.
¾ hour	Laying down	Eggs placed in tray or vertical incubator for incubation.
12 hours	Fertilisation rate can be determined	Sample of eggs placed in clearing soln. [acetic acid:methanol:water (1:1:1)].2 mins. later fertilization success estimated by observing the number of cells that had undergone division to the 4 cell stage. Test: occasionally equivocal.
7 days	Best time to determine fertilisation rate	Developing embryo can be clearly seen as a thick white line when placed in clearing soln. Not equivocal.
16 days	Eying	Eyes can be clearly seen through the shell.
19 days	Shocking	Infertile eggs are turned white by rupturing the yolk membrane. Usually achieved by siphoning from the incubation container into a bucket of water.
20 days	Picking	Infertile eggs are removed (picked) either manually or mechanically. Eying rate determined.
30 days	Hatching	Egg shells rupture and the larval fish (alevins) become free swimming.
50 days	Swim-up	Yolk sacs are absorbed and fish start feeding.
130 days approx.	100/lb. (Each fry 4.5g)	100 fish weigh 1 lb.

N.B. At temperatures above or below 10°C the stages of development will be reached at earlier or later times respectively

were attached by punching two plastic pins through manufactured holes, one at each end of the tag, and then through the muscle just below the dorsal fin. The device used for punching the pins was a gun used by the textiles industry, which accepts a cassette of plastic pins (Kimbal Systems Ltd., Leics., U.K.). Using this method in the Fish Culture Unit at Aston, very few tags were lost. However, under commercial conditions, repeated netting sometimes caused a high proportion of tags to be unfastened due to entanglement. Consequently different treatment groups which were maintained in the same tank had to be additionally marked by fin clipping or removal of the adipose fin. The exact marking regimes used in individual experiments will be outlined under the experimental protocol for that experiment.

2.1.5 Blood sampling

Blood samples were withdrawn from the Cuverian duct of the anaesthetised fish using 5 ml serum monovettes (Sarstedt, Leics., U.K.) fitted with either 1½ (21G) or 2 inch (19G) needles. The monovettes are essentially combined syringes and centrifuge tubes containing glass beads which create a greater surface area on which the clot can form. The blood obtained was allowed to stand for at least 15 minutes and then centrifuged at 2,500 r.p.m. for 20 minutes. The resultant serum was pipetted into clean plastic tubes (LP3, Luckham Ltd., Sussex, U.K.), stoppered and stored at -20°C for future analyses. Post-sampling haemorrhage or mortalities were rarely seen.

2.2 Egg measuring

All fecundity and egg size determinations were made on water-hardened eggs. Extreme care was taken not to rupture the egg membranes by rough handling.

2.2.1 Egg size

Egg size was determined by aligning the water-hardened eggs along a measuring groove of fixed length (either 120 mm or 300 mm). The number of eggs aligned along the groove were counted to the nearest $\frac{1}{4}$ of an egg. Mean egg diameter was then calculated as below:

$$\text{ova diameter (O.D.)} = \frac{\text{Length of groove}}{\text{No. of eggs along groove}}$$

This method is widely used by farmers and scientists (Vladykov, 1956; Buss and McCreary, 1960; Gibson et al, 1976; Ridelman, 1981; Sandes et al, 1984). The accuracy of this method was validated by comparisons with individual egg diameter measurements, made with calipers. The result of student 't' tests showed there were no significant differences at the 5 per cent level (see Table 2.2).

Table 2.2 Comparison of two methods of measuring egg diameter. Individual measurements with calipers vs. measuring trough (120 mm)

	Calipers			Measuring Trough				
	n	$\bar{x}$	SEM	n	$\bar{x}$	SEM	't'	SIG
Fish A	30	0.44	0.004	30	0.43	0.002	2.45	NS
Fish B	30	0.48	0.005	30	0.46	0.011	1.63	NS
Fish C	30	0.45	0.004	30	0.44	0.002	0.94	NS

2.2.2 Egg number

After measuring the egg diameter (O.D.) the volume of eggs produced female^{-1} was determined. The number of eggs produced female^{-1} can then be calculated using the formula below which was derived from the work of Von Bayer (1950) (in Leitritz and Lewis, 1976).

$$Y = -0.283\ X + 5.41$$

Total fecundity (T. F.) = Antilog Y* (Z/1000)

Y = Log 10 No. Eggs L^{-1}, X = O.D. (mm) and Z = vol. eggs produced (ml)

Relative fecundity = Total fecundity/ somatic weight (kgs)

The 'Von Bayer' method of egg number determination has been used by commercial fish farmers and scientists (Buss and McCreary, 1960: Satia et al, 1974; Baiz, 1978; Leitritz and Lewis, 1976; Roley, 1983). However, because of its wide usage, and therefore importance, in this thesis a thorough investigation was conducted to verify its accuracy against actual counts. There was a highly significant correlation ($r=0.998$, $P<0.001$) between actual counts and 'Von Bayer' determinations. This was confirmed by analysis of variance $F=8960$ d.f. 1,25 $P< 0.001$ (see Fig 2.1). The mean percentage error was 0.7% with a coefficient of variation of 6.6%.

2.3 Fish holding and egg incubation

Depending upon the location at which experiments were performed and the particular experimental design, brood fish and eggs were maintained in a variety of different systems. The particular system used will be specified in the individual experimental protocols, while the major systems used at Aston and Avon Springs are fully described below.

2.3.1 Fish culture unit, Aston

In certain experiments broodstock were maintained in an oxyder, a commercially available unit (Field Stream and Covert Ltd., Meriden, U.K.). This unit utilizes a through flow of

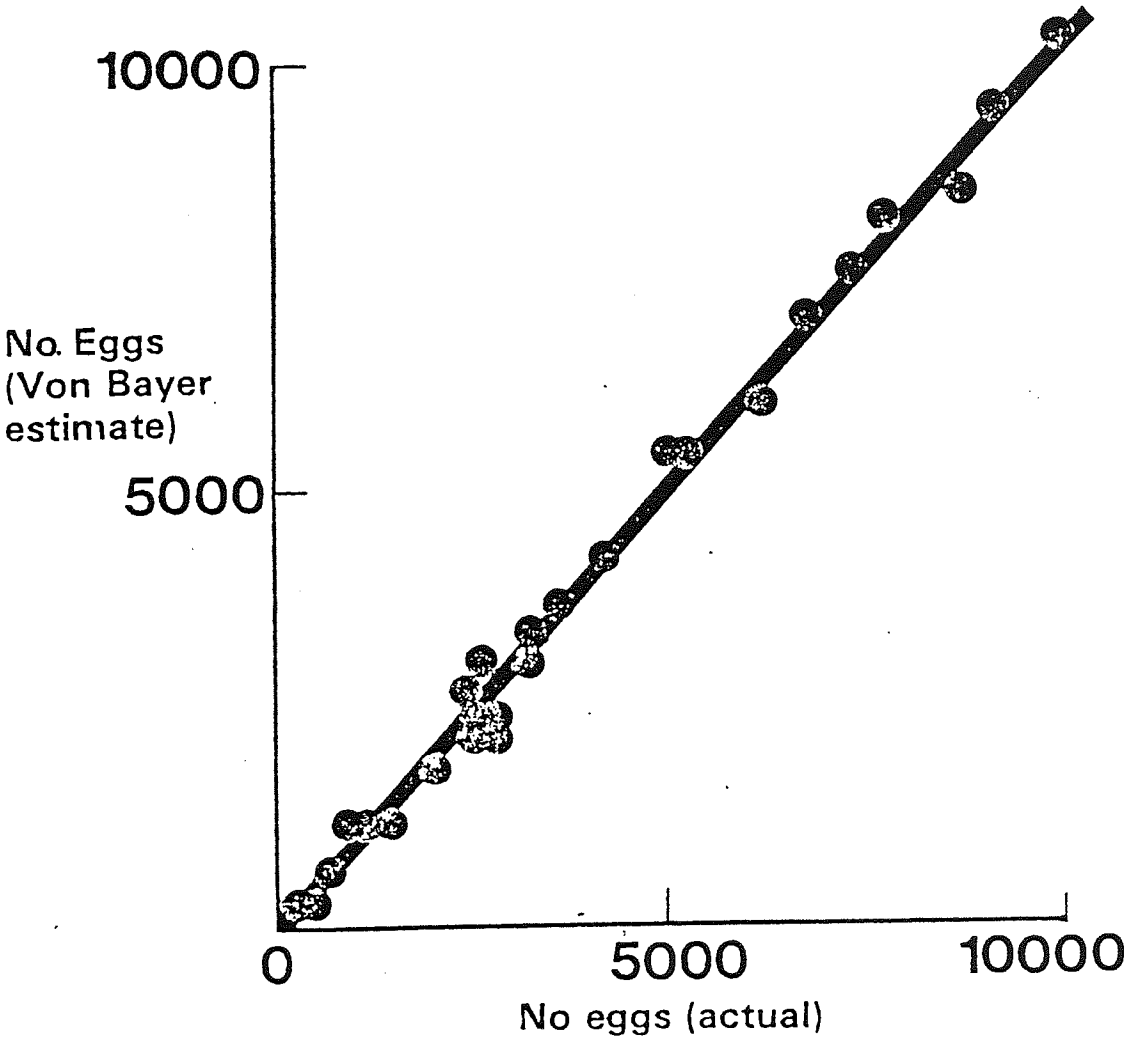


Fig 2.1 Regression of Von Bayer estimates vs actual counts of egg number.

mains water of approximately 12 litres minute^{-1}. Flow and oxygen levels were increased by means of a submersible pump drawing air down a pipe and through a venturi. The unit was drained by a pump protected by screens. A series of movable screens allowed different experimental groups to be segragated.

Eggs were incubated in a pumped recirculatory system, allowing the bulk of the water to be recirculated with only a small volume (approximately 5-10%) of fresh ('make-up') water to allow for spillage and evaporation. The system (Fig. 2.2) consisted of 3 standard hatchery troughs (only one in diagram), gravity fed from a header tank, at a flow rate of around 4 litres minute^{-1} to each tank. One trough was used for incubating relatively large numbers of eggs (up to 30,000) in horizontal hatchery trays. The second trough contained a small batch egg incubator system consisting of 92 x 150 ml round containers (Sterilin Ltd., Middlesex, U.K.) each with their own flow through water supply and capable of incubating 50 eggs in each. The third trough was used for the growing on of groups of hatched fry.

The three troughs emptied into a common solid trap, which allowed settlement of solids. The outflow from the solids trap fed a gravity filter bed, which also received an overflow from the header tank. An overflow pipe from the filter bed carried any surplus water to waste. Water from the base of the filter was pumped back to the header tank by means of an electric pump (Beresfords Ltd., Coventry, U.K.) protected by an in line filter.

Pumped recirculation system used for the incubation of small aliquots of rainbow trout eggs (Fig. 2.2)

Key

a	Header tank
b	Common feed pipe
c	150 ml egg incubation pots (92)
d	Common waste pipe
e	Faecal trap
f	Biological filter
g	Discharge for faecal trap
h	Overflow from filter
i	In line filter
j	Pump
k	Overflow from header tank

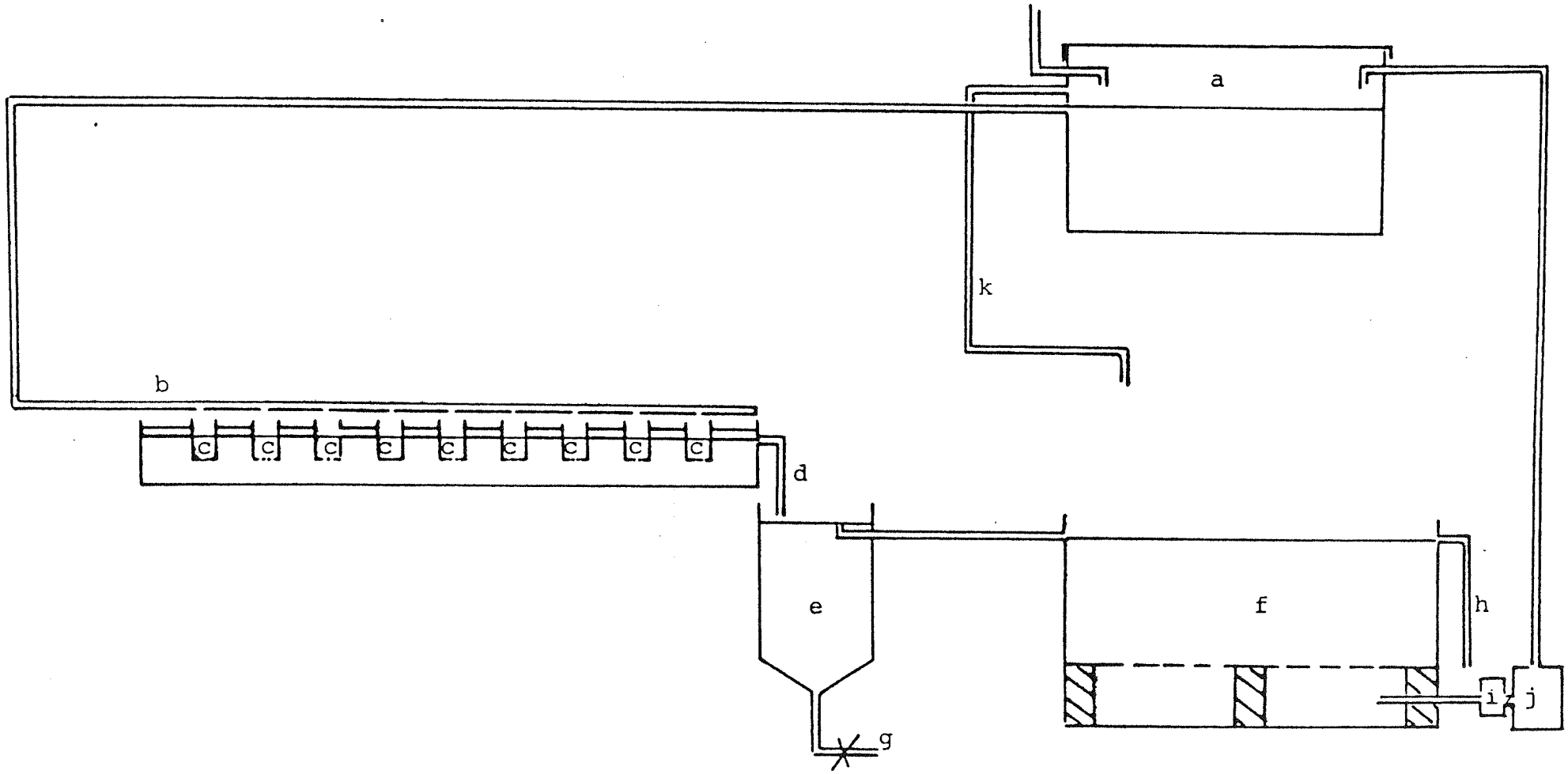


Fig. 2.2 Pumped recirculation system used for the incubation of small aliquots of rainbow trout eggs.

During the course of experiments, oxygen saturation, ammonia, nitrates, pH and temperature were monitored to allow the maintenance of suitable water quality. At weekly intervals, the solids trap was cleared; and bi-monthly, or when there was a build up of solids on the surface of the filter bed, the top 3 inches of gravel were dug over and washed.

2.3.2 Avon Springs Hatchery

Experimental fish were maintained in a variety of different tanks. However, all tanks were supplied with constant 10°C bore hole water at an appropriate flow rate. Hatched fry were reared in standard commercial hatchery troughs (Grice and Young, Purewell, Dorset, U.K.) until they reached approximately 2g in size after which they were moved to 2m diameter circular fry tanks.

Small numbers of broodstock (40) were maintained in 2m diameter tanks and greater numbers in 10m circular tanks, and also 40m x 20m earth ponds.

Eggs were incubated in flow through systems supplied with 10°C borehole water of the appropriate flow rate. Small groups of eggs were incubated in horizontal trays and a small batch system as at Aston. Large batches of eggs (up to 500,000) were incubated in upwelling vertical incubators.

2.3.3 Other farms

Broodstock fish were maintained in large brood lakes, earth ponds, concrete raceways and circular tanks made of concrete, fibre glass and corrugated iron of varying sizes. Some farms had a supply of constant temperature spring water whilst others were supplied with ambient temperature surface water.

2.4 Proximate analysis

Proximate analyses were carried out on fertilised water-hardened eggs by the methods below.

2.4.1 Water content

Ten eggs from each fish were individually weighed (nearest 0.1 mg), dried for 24 hours at 70°C and re-weighed. Water content was calculated by the equation:

$$\% \text{ water} = ((\text{weight wet} - \text{weight dry})/\text{weight wet})*100$$

2.4.2 Protein analysis

Protein was determined by the micro-kjeldahl method in which organic nitrogen in protein is converted to ammonium, which is then measured.

Digestion

Approximately 100 mg of ground dried eggs were carefully weighed and added to a micro-kjeldahl digestion flask, 1-5 ml of concentrated sulphuric acid and ½ of a tablet of kjeldahl catalyst were added to each flask. The flasks were placed on a digestion rack and heated. Heating was continued until the digest cleared. At the end of digestion, the flask was allowed to cool and the walls were washed down with a small volume of distilled water.

Distillation and titration

The digest was steam distilled in a Markham still. Steam from a steam generator was run through the apparatus prior to sample loading and the inner vessel washed by automatic syphoning of distilled water from the inner vessel to waste. This procedure was repeated between samples.

Once the apparatus had been washed, the cooled digest was introduced into the inner vessel of the still via the funnel. 10 ml of NaOH (40%) added. The flask and stopper were carefully washed out with distilled water into the inner chamber taking care not to break the liquid seal of the stopper, otherwise ammonia may have been lost.

Steam was passed through the reaction mixture with all valves closed and the distillate collected in a conical flask containing 10 ml saturated boric acid solution with a few drops

of mixed indicator (methyl red plus bromocresol green). As ammonia was driven into this flask the colour changed from a reddish brown to blue green. Distillation was carried out for another five minutes after this colour change.

The boric acid solution was titrated against 0.1 N HCl; the end point of the titration being the loss of the blue green colour and the appearance of a slight pink tinge.

Calculation

The titre of HCl obtained was substituted into the equation below to give the % protein:

$$\% \text{ Protein} = ((0.14 \times \text{titre (ml)} \times 6.25)/\text{Sample wt (g)})$$

2.4.3 Lipid content

Soxhlet extraction was used to assay the ether-extractable lipid fraction of the eggs. The sample of eggs was dried, ground in a pestle and mortar, weighed (approximately 100 - 150 mg) and placed in a porous paper extraction thimble (Fisons, Loughborough, U.K.). The thimble plus sample was then placed in the soxhlet apparatus which consisted of a condenser which prevented the ether (pet ether 40°C- 60°C) boiling out of the apparatus and delivered the ether to the sample. There was a sample holder which supported the condenser. The holder incorporated a syphon which returned the ether and lipids extracted from the sample to a pre-weighed flask (250 ml round

bottom). The flask was then placed on a heating mantle. The apparatus was set up with 150 ml of ether and run for 3 hours. The sample was removed and the ether collected in the sample holder before it could syphon back into the flask. The flask was removed containing only a small amount of ether and dried at 70°C. The sample in the flask was then placed in a desiccator to reach room temperature before weighing and lipid content was calculated as follows:

(Wt. flask + fat) - wt. flask = wt. fat

% fat = (wt. fat/ wt. sample) * 100

2.4.4 Ash content

A sample of eggs were dried at 70°C, weighed and placed into a pre-weighed crucible. The sample was heated in a muffle furnace at 550°C for 24 hours. Placed in a desiccator to reach room temperature and the crucible plus sample weighed.

% Ash = (Wt. ash/ wt. sample) * 100

2.5 Detailed egg composition

2.5.1 Amino Acids

Procedure for hydrolysation

1. Hydrolyse single dried egg in 2ml of 6M HCl at 110°C for twenty-four hours (sealed under vacuum)
2. Neutralize with 5.3ml of 2M NaOH
3. Dilute to 25ml H_2O
4. Centrifuge at 1500 rpm for fifteen minutes (removing precipitate)
5. Load 100 µl of supernatant onto column of Locarte Amino Acid Analyser for ion exchange chromatography

Calculations

Amino acid standards (25nM) were loaded onto the column and the peak areas calculated from the formula:

Peak area = height x ½ width at mid-height.

The unknowns were calculated by direct proportion.

2.5.2 Free fatty acids

Procedure

1. Extract single dried egg in 3mls hot isopropanol (80°C water bath) for fifteen minutes
2. Homogenize tissue (add more isopropanol if necessary)

3. Centrifuge at 1500 rpm for five minutes
4. Decant supernatant into 150 ml evaporating flask
5. Extract pellet twice with 5 mls isopropanol : chloroform (1:1) and finally with 2ml chloroform
6. Evaporate combined lipid extracts to near dryness on a rotary evaporator
7. Take up in 2ml of chloroform
8. Wash the extract three times against 1ml of 0.88 M KCl (folch wash), discard the aqueous layer after each wash
   Transfer extracts to 5ml pear shaped flasks, and reduce to a small volume on a rotary evaporator
10. Transfer lipid extracts to a small screw-top vial and reduce to dryness in a stream of nitrogen. Stored at -20°C before methylation

Methylation of fatty acids for Gas Liquid Chromatography (G.L.C.)

1. Add 1ml of methylation solution (25% boron trifloride : 20% Benzene : 55% Methanol) to dried lipid extracts
2. Heat in boiling water bath for forty-five minutes
3. Extract fatty acid methyl esters with 1ml H_2O followed by 2 mls of pentane and BUT (an anti-oxidant)
4. Pipette off top layer into a fresh screw-top vial, and reduce to dryness in a stream of nitrogen
5. For G.L.C. analysis, take samples up in 200 µl of pentane and inject 5 µl samples onto G.L.C. column

Gas Liquid Chromatography operating conditions

Samples were run on a Pye-Unicam 304 gas liquid chromatograph. Stationary phase was 10% D.E.G.S., injection temperature was 190°C, column temperature was 190°C, detector temperature was 240°C and the carrier gas was nitrogen (40 ml min^{-1}.).

Calculations

Retention times of known fatty acids were recorded and plotted as log. retention time vs free fatty acid. Retention times of the unknowns were then used to determine the fatty acid chain length, and number of double bonds. The concentration of free fatty acid unknown was determined by direct proportion to the peak area of the known standards.

2.5.3 Minerals

Procedure

1. Digest ten dried eggs in 25 ml of concentrated nitric acid: perchloric acid (5:1) (care).
2. Make up the volume to 50mls with deionized water. This solution was used for determinations of Zn, Fe, Cu, Mn, Na, Mg, Ca and K using Perkin Elmer 373 atomic absorption spectrophotometer

2.6 Statistics

All arithmetic means are expressed ± one standard error of the mean (SEM). The SEM was derived by dividing the standard deviation (SD) by the square root of the number of observations (n).

$$\text{i.e.} \qquad SEM = \frac{SD}{\sqrt{n}}$$

Differences between means were compared by students 't' test or analysis of variance (ANOVA). Regression analyses were carried out by the method of least squares analyses. ANOVA and regression analyses were carried out using a 'minitab' statistical package (Ryan et al, 1981) on a Harris mainframe computer.

2.7 List of fish species mentioned in the thesis

Rainbow trout	(Salmo gairdneri)
Sturgeon	(Acipenser Sp.)
Chum salmon	(Oncorhynchus keta)
Atlantic salmon	(Salmo salar)
Bream	(Abramis brama)
Chinook salmon	(Oncorhynchus tchawytscha)
Pink salmon	(Oncorhynchus gorbuscha)
Coho salmon	(Oncorhynchus kisutch)
Arctic char	(Salvelinus alpinus)
Brown trout	(Salmo trutta)
Herring	(Clupea harengus)
Carp	(Cyprinus carpio)
Catfish	(Clarias macrocephalus)
Ayu	(Plecoglossus altivelis)
Guppy	(Lebistes reticulatus)
Three spined stickleback	(Gasterosteus aculeatus)
Striped bass	(Roccus saxatilis)
Haddock	(Melanogrammus aeglefinus)
Plaice	(Pleuronectes platessa)
Norway pout	(Trisopterus esmarkii)
Roach	(Rutilus rutilus)
Convict cichlid	(Cichlasoma nigrofasciatum)
Winter flounder	(Pseudopleuronectes americanus)
Chubsucker	(Erimyzon oblongus)
Goby	(Gillichthys mirabilis)

PART I

Egg and fry quality

PART I

General introduction to egg and fry quality

In view of its great importance to commercial fish production, it is surprising that egg quality in teleosts has received little attention from biologists (Craik and Harvey, 1984a).

Considerable variation has been reported in the hatching rates of eggs from cultured rainbow trout, even between individuals of the same stock maintained in the same pond (Hirao et al, 1954). This observation has been supported by the recent work of Craik and Harvey (1984a, 1984b) who found that some batches of eggs, whilst appearing healthy and normal, suffered high mortalities during subsequent rearing. Large variations in the quality of rainbow trout eggs reared under commercial conditions have also been reported (Springate and Bromage, 1983). Under commercial conditions survivals from egg to 4.5g fish average 45%, although, in consultation with farmers, it is clear that there is an enormous range with some batches of eggs having survivals higher than 80-90% whilst in others only 20% or less survive, for little apparent reason (Springate and Bromage, 1983).

Fish farmers and fisheries biologists define good quality eggs as those which exhibit low levels of mortality and produce fast growing fish. However there is no general agreement of

what constitutes the absolute values of quality and this needs to be established.

The factors which may influence egg quality are the chemical composition and genetic make-up of the eggs together with their physical size. Egg size is likely to be important because large eggs contain proportionally more nutrients than smaller ones. This increase in stored nutrients may be an advantage in salmonids which have a long embryonic development and throughout this period the fry are entirely dependent on the yolk for their supplies of nutrient. Other factors which can have considerable effects on egg survival are hatchery and broodstock management techniques, e.g. frequency of stripping, methods of fertilisation, feeding of adults and environmental conditions. These important determinants of egg quality will be considered in Part III.

A fundamental question facing the hatchery manager is why do eggs from fish of the same stock, fed with the same diet, maintained under similar conditions and fertilised with sperm from the same sperm pool, produce eggs of varying quality? Surprisingly, given the commercial implications of this question and its scientific importance, very little research has been directed towards an answer. Often the significance of results from studies concerned with egg quality are obscured by large variation in the survivals of the control groups of eggs.

This part of the thesis considers egg quality and the possible reasons for its variability. It is evident that egg quality is synonymous with egg survival to both fisheries scientists and commercial fish farmers. Therefore, the initial aim of this part of the study was to investigate the levels of egg and fry survivals that are encountered under commercial and laboratory conditions. Once these levels of survival have been established consideration of the physical and chemical components of eggs can be made and related to egg quality as determined by egg survival.

CHAPTER 3

Egg and fry survival

3.1 Introduction

Under natural conditions the percentage of young fish that survive from eggs to maturity is extremely small. For the sturgeon it is only 0.1%, for the chum salmon 0.13 - 0.58%, for the Atlantic salmon 0.125% and for the bream 0.006 - 0.022% (Nikolsky, 1969). In contrast, under the more benign conditions prevalent in modern hatcheries, where the environment is closely controlled and predators are largely absent, survivals are considerably higher. However 100% survivals are not achieved, and this maybe due to higher pathogen levels and poorer water quality which are often experienced under conditions of intensive culture. Theoretically, under ideal laboratory conditions there is no reason why near 100% survival should not be achieved. However, such high levels are rarely obtained. Frequently groups with zero survivals (blanks) are recorded under experimental and commercial conditions.

The aims of this Chapter were to investigate variability in egg quality and to quantitatively define egg quality under commercial and experimental conditions.

There are little published data regarding egg and early fry survivals under commercial conditions. Small (1979) reported that only 60% of the eggs from two year old female rainbow trout

survived from eying to 11 months of growth whereas 88% of the eggs from five year old females survived over the same period. These findings were reported from farm data, where often commercial constraints, at best only allow crude estimates of survival to be made with blanks being omitted from records. Commercial data from several farms are closely examined in this Chapter.

Most of the figures published for egg survival in the scientific literature are confused by the use of different experimental conditions, and treatment of broodstocks. Few studies have been carried out solely to investigate egg survival and therefore one must examine control groups of other experimental treatments for information about levels of survival that are experienced under laboratory conditions.

In one such study of induced triploidy in rainbow trout, Lincoln and Scott (1983) published survival rates of 59% to hatching for one control group of eggs from three year old females. In contrast Craik and Harvey (1984b), investigating egg quality from two year olds of the same species recorded survivals of only 28% to the same stage. In a selection programme to improve the growth rate of rainbow trout, Kincaid et al (1977) measured survival of fry from fertilisation to 147 days post-fertilisation under 'standard hatchery conditions'. The overall mean survival for control groups over the seven years of the study was 40% with a range which extended from 17% in 1973 to 77% in 1974. Smith et al (1979) and Roley (1983) in studies on the effects of different broodstock diets reported

mean survivals of 79% and 54% respectively, up to eying, although the experimental diets exerted no adverse effects. In another study of the effects of different feeding strategies Ridelman et al (1984) found that survivals to hatching for the control and experimental regime were 43.8% and 42.9% respectively. Studying the relationship between chemical constituents of rainbow trout eggs and their eying survival Hirao et al (1954) recorded a mean eying rate of 72.8% for 10 batches of eggs with a range which extended from 37.5% to 94.0%. Pitman (1979) examined the effects of age of female on egg and early fry survial in rainbow trout and found an overall survival of 43% from eying to 186 days of growth for the eggs derived from two year old females and 83% for eggs from five year old females. All these studies indicate the usually poor survivals of rainbow trout eggs.

Generally better survivals are seen in other species of salmonid. Presented below are the mean survivals to eying for the control groups of eggs from different experiments on other species of salmonid: Chinook salmon 56% (Johnson, 1984) and 76% (Erdahl et al, 1984); pink salmon 96% (Wertheimer, 1984); coho salmon 91% (Fitzpatrick et al, 1984) and 97% (Wertheimer, 1984); Atlantic salmon 77% and 85% (Crim and Glebe, 1984); Arctic char 17% and 88% for cultured and wild stocks respectively (Papst and Hopky, 1984) and brown trout 76% (Erdahl et al, 1984). Although these results indicate a highly variable success rate the general trend is clearly towards higher survival rates for these eggs than those obtained for the eggs of rainbow trout.

Comparisons of the results from the above studies are difficult because they are concerned with many species of salmonids of different age and strain and maintained under a variety of different conditions. The survival rates of the eggs have also been measured at different developmental stages. However, these studies do indicate the enormous range in survivals which are present when egg quality is considered. This variation is usually so large that it masks any significant difference of experimental treatments on either the broodstock or their eggs.

In view of the possible effects of measuring survival rates at different developmental stages shown in other studies, consideration is made here of the relationship between survival to the different stages.

In order to investigate factors which might affect egg quality it was necessary to quantitatively define egg quality in the commercial and laboratory situation. With this in mind three investigations were carried out; the first involves measurement of survival characteristics of production data on commercial farms, the second to consider what levels of survival are possible under the carefully controlled, near optimum conditions in the laboratory and finally a further study conducted on a commercial farm to establish the variation due to different husbandry and methods of recording.

3.2 Production data

These data were collected from seven commercial fish farms and generally involved measurements made by the farm staff. The farms are distributed throughout the U.K. The aim of presenting these data was to indicate the ranges of survival that are encountered on commercial fish farms, and not to compare fish farms. The provision of these data were dependent on confidentiality and therefore the farms are letter coded (P-W).

3.2.1 Materials and methods

Under commercial conditions eggs from many individual females are pooled after fertilisation and incubated as batches. Each batch being comprised of the eggs from up to 250 fish. The farms surveyed all used mechanical egg picking machines which operate via a photoelectric cell which separates living, translucent eggs from dead, opaque ones. Batches of eggs were 'picked' at eying, after they had been shocked (Table 2.1). The proportions of living to dead eggs were estimated and from these figures an eying rate for each batch was calculated as follows:-

$$\frac{a}{a+b} \times 100 = \text{\% survival to eying}$$

a = number of living eggs; b = number of dead eggs

Eying is the only stage at which all the egg producers measured survival and is therefore used exclusively in this section.

3.2.2 Results

The highest survival of rainbow trout eggs to eying was 83.0±3.4% from the five year old females on Farm S. The poorest survivals were eggs from two year old females on Farm P, 46.5±6.8%. No zero survivals or blanks were recorded. The mean survivals for the eggs from the different age females at the seven farms were 57.9±4.5%, 75.3±2.2%, 74.9±1.0% and 74.3±9.8% for the two, three, four and five year old females respectively (Table 3.1, Fig. 3.1). Students 't' tests showed that survivals of the eggs from three, four and five year old females were significantly higher ($P < 0.01$) than the survival of the eggs from the two year old females for the combined data with 'n' as the number of farms.

The eggs from the brown trout (Farm W) had higher survivals to eying than any of the rainbow trout eggs, 92.1±1.9% and 92.6±1.0% for the three and four year olds respectively. The best rainbow trout eggs survival was approximately 10% poorer than those from the brown trout of comparable age.

Taking a mean survival for each farm indicates that Farm T has the highest overall survival (77.9%) and Farm W the lowest (68.0%). The combined mean figure for all seven farms was 72.7%. The mean survival for brown trout eggs (92.4%) far exceeds the highest rainbow trout eggs survival (Fig. 3.2)

Table 3.1 Survivals to eying of eggs from different age parental fish on seven commercial fish farms (production data)

FARM	Age of female												Mean [c]
	2			3			4			5			
	n[a]	$\bar{x}$	SEM	n[a]	$\bar{x}$	SEM	n[a]	$\bar{x}$	SEM	n[a]	$\bar{x}$	SEM	
P	16	46.5	6.8	46	76.1	1.9		-			-		68.5
Q	4	60.0	2.0	18	82.0	1.0	19	77.2	1.4	23	63.5	1.7	71.1
R		-			-		9	73.6	4.1		-		73.6
S		-		6	70.4	5.8	6	75.8	5.6	5	83.0	3.4	76.0
T	8	57.0	3.3	60	80.7	2.4		-			-		77.9
U		-		7	74.3	2.5	7	72.9	2.3		-		73.6
Wd	6	67.9	7.7	8	68.0	5.6		-			-		68.0
We	5	92.1	1.9	5	92.6	1.0		-			-		92.4
Mean	4[b]	57.9	4.5	6[b]	75.3	2.2	4[b]	74.9	1.0	2[b]	72.7	1.4	

- = no data available
a = number of batches
b = number of farms
c = mean weighted for number of batches
d = rainbow trout
e = brown trout

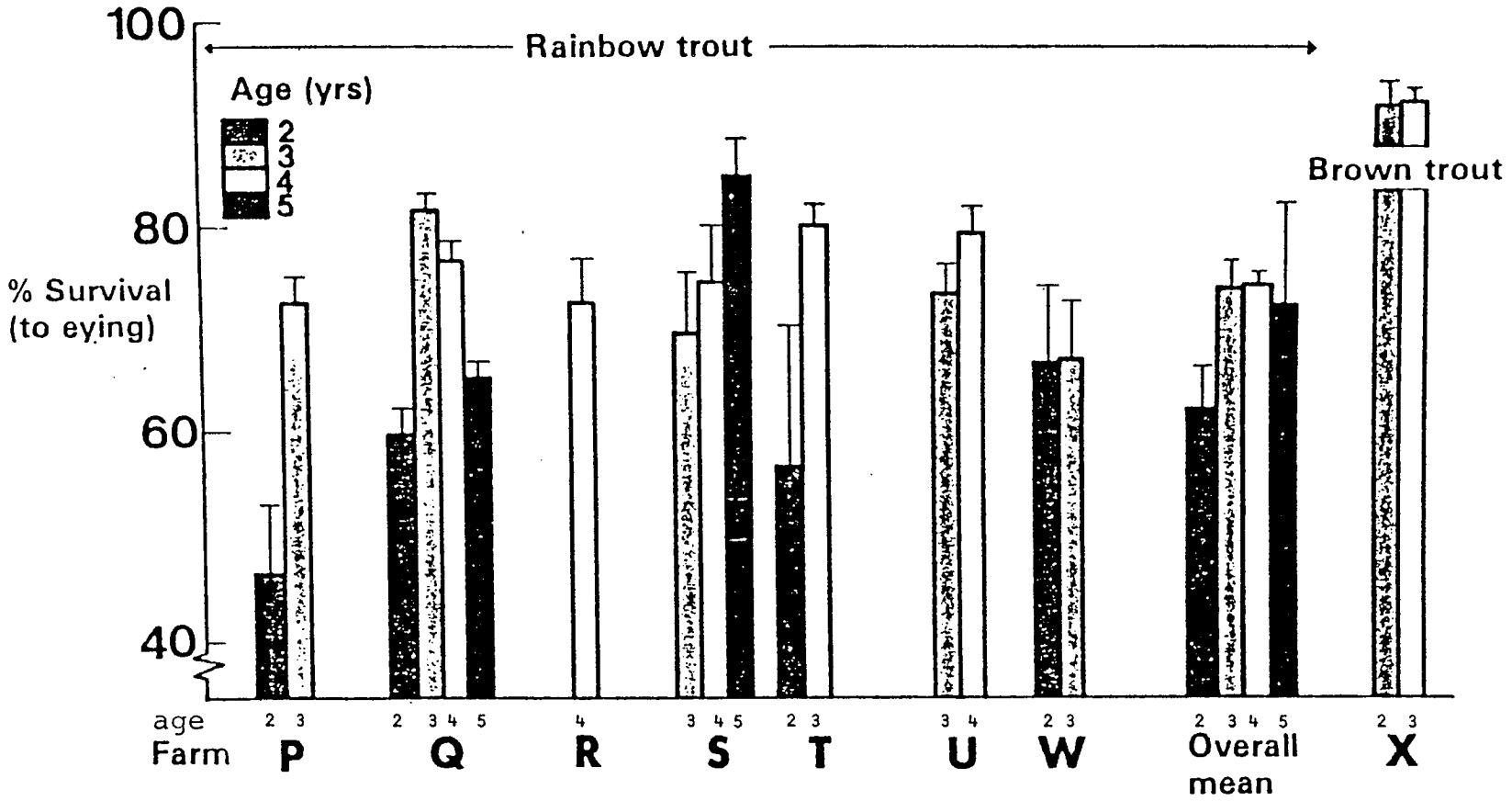


Fig. 3.1 Survival to eying of eggs from seven U.K. trout farms (Mean ± SEM) including the age of females.

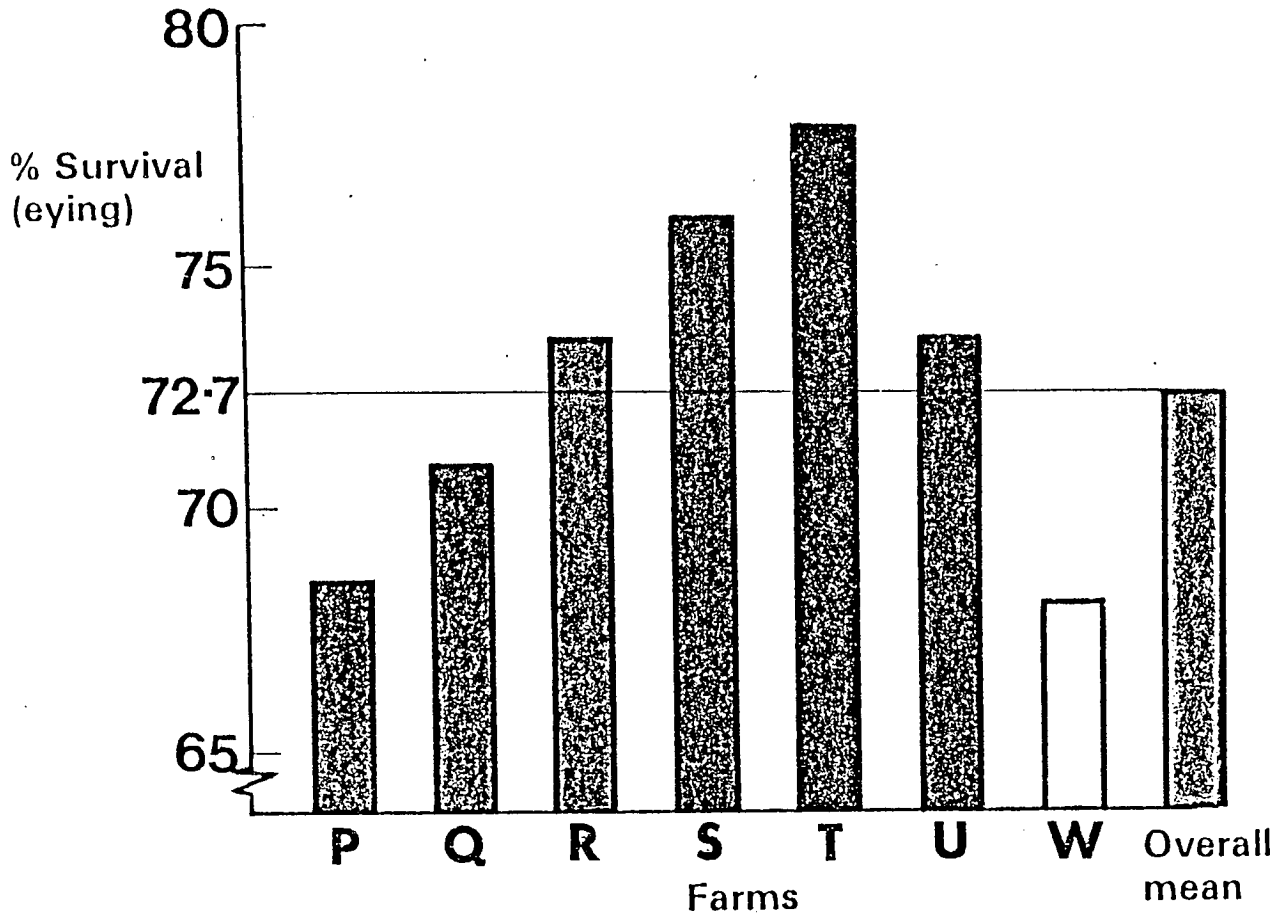


Fig. 3.2 Mean survival to eying for eggs from seven U.K. rainbow trout farms.

3.3 Experimental trial

The aim of this experiment was to measure the levels of mortality under the closely controlled, near optimum, conditions offered by the small batch incubation system (Fig. 2.2). Also to measure the replication between different aliquots of eggs from the same female maintained in separate containers.

3.3.1 Materials and methods

A group of gravid three year old females were randomly selected from a large pool of commercially reared broodstock. As the time of expected spawning approached the fish were examined every 10 days to ensure that the stripped eggs from each fish were at equivalent stages of ripeness. Sorting the broodstock with this periodicity avoids the problems of overripening which ovulated eggs undergo if retained in the abdominal cavity for periods of time longer than 10 days. (Nomura et al, 1974; Springate et al, 1984; see also Chapter 8).

The eggs from eight females were kept separate and fertilised with appropriate amounts of sperm from a sperm pool collected from five ripe two year old males. Approximately 200 eggs were taken at random from the total 'strip' from each female and incubated in duplicate in a small batch incubation system. Rates of fertilisation, eying and hatch were determined as described in the General Materials and Methods Chapter.

3.3.2 Results

Of the eight batches of eggs studied, three had fertilisation rates of 100%. The lowest fertilisation rate was 61%. The highest eying rate was 100% and the lowest 48% and the highest survival to hatching was 98% and the lowest 44%. There were no groups with 0% survival. The mean percentage fertilisation, eying and hatching rates were 90.3±5.1%, 86.1±7.2% and 79.3±9.3% respectively (Fig. 3.3). These survival rates were significantly higher than those for comparable rates under commercial conditions ('t' test, $P < 0.05$).

There were very good replications for the aliquots of eggs from the individual females. Mean coefficients of variation were 1.5% and 5.0% for the eying and hatching rates respectively.

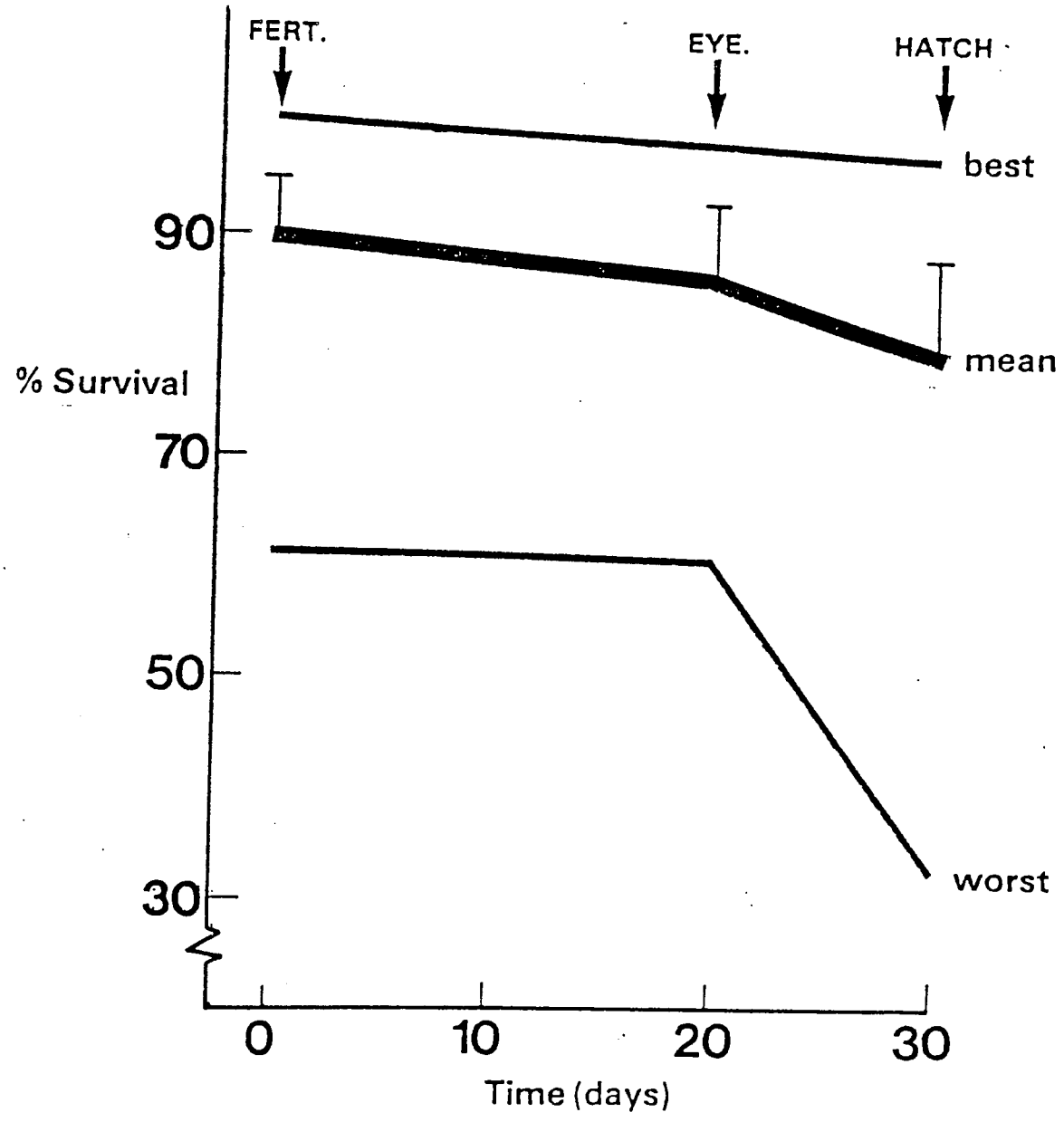


Fig. 3.3 Percentage survival of eggs at fertilisation, eying and hatch under carefully controlled experimental conditions. Mean ± SEM (n=8).

3.4 Commercial trial

The aim of this study was to examine survival rates under commercial conditions but with the survival rates determined by the author and not by the farm staff. The eggs from individual females were examined rather than data from large commercial batches of eggs comprised of the eggs from many females.

3.4.1 Materials and methods

A group of 500 two and three year old rainbow trout were maintained at a commercial hatchery (Farm P) in a 10m diameter tank supplied with bore-hole water at a constant 10°C. Care was taken to ensure that eggs were not overripe (See 3.3.1).

Two groups of 10 and 13 females were selected at random on two separate occasions. On each occasion the selected gravid females were hand-stripped and dry-fertilised using a common pool of sperm collected from five two year old males. After water-hardening the eggs from each female were incubated in identical, individual, horizontal, hatchery trays supplied with constant 10°C bore-hole water at a rate of 8L min^{-1}. At 12 hours post-fertilisation, approximately 100 eggs were randomly removed from each tray to assess the fertilisation rate. The rates of eying, hatch and swim-up were determined by total counts.

After one month of feeding the fry were removed from the hatchery troughs into separate 2m diameter fry tanks supplied with 10°C bore-hole water at a rate of 15 L min^{-1}. Overall survival rates to 120 days post-fertilisation were determined by total counts of the fry.

3.4.2 Results

The best batch of eggs had a survival of 90% to 120 days post-fertilisation whilst three groups had 0% survival to the eying stage. The mean survivals to fertilisation, eying, hatch, swim-up and 120 days post-fertilisation were 74.7±4.5%, 58.0±7.3%, 54.0±7.1%, 51.2±8.5% and 31.4±5.2% respectively (Fig. 3.4, Table 3.2).

Students 't' test showed there were no significant differences between the survivals of the eggs from the two and three year old parents (Table 3.3).

Analysis of variance of the data and calculation of the correlation coefficient showed that there is a strong positive correlation ($P < 0.01$) between percentage fertilisation and percentage eying, hatch, swim-up and survival to 120 days post-fertilisation (Table 3.4).

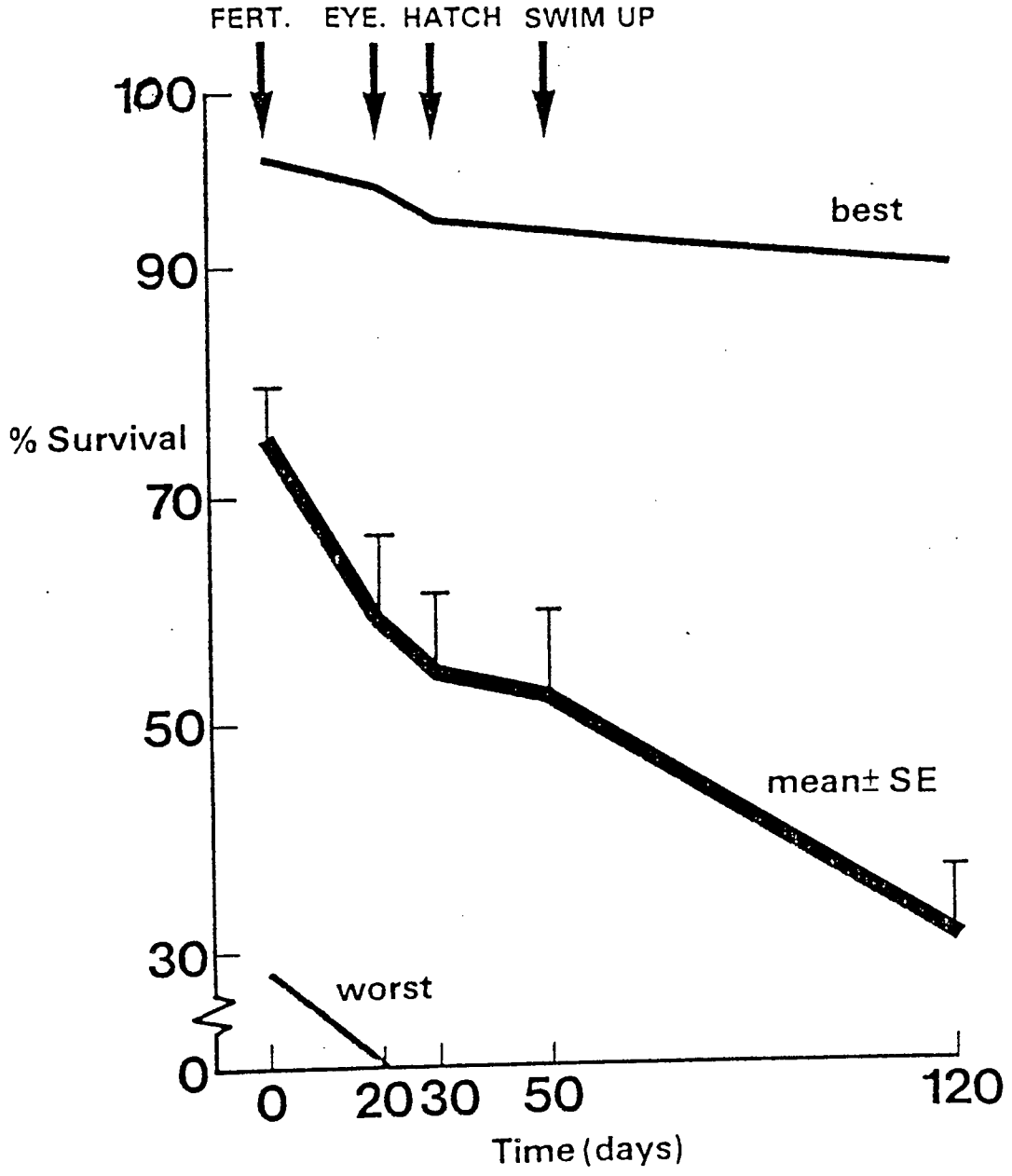


Fig. 3.4 Percentage survival of eggs at fertilisation, eying hatch, swim-up and to 120 days post-fertilisation under controlled commercial conditions (Mean ± SEM n=23.

Table 3.2 Egg and fry survival rates (%) from the commercial trial Pooled data from two and three year old females

	Development stage				
	% fert.	% eye	% hatch	% swim-up	survival 120 days
n	20	23	23	13	23
Mean	74.7	58.0	54.0	51.2	31.4
SEM	4.5	7.3	7.1	8.5	5.2
Max	99	98	97	93	90
Min	28	0	0	14[a]	0

[a] only measured for 13 fish

Table 3.3 Egg and fry survival rates (%) from the commercial trial. Data for two and three year old females presented separately

Two year old females

	Development stage				
	% fert.	% eye	% hatch	% swim-up	survival 120 days
n	4	7	7	6	7
Mean	70.5	65.9	60.4	57.3	26.3
SEM	10.9	11.2	10.7	12.6	5.3
Max	94	94	89	88	43
Min	44	18	17	14	8

Three year old females

n	16	16	16	7	16
Mean	75.7	54.6	51.2	45.9	33.6
SEM	5.0	9.4	9.2	11.9	7.1
Max	99	98	97	93	90
Min	28	0	0	15	0

Table 3.4 Regression equations between survival at the four developmental stages considered and fertilisation success

% Eying = 1.27 %fert. - 40.6	r=0.708, d.f. 18, P<0.001 F=18.05, d.f. 1, 18, P<0.001
% Hatch = 1.26 %fert. - 43.9	r=0.725, d.f. 18, P<0.001 F=19.98, d.f. 1, 18, P<0.001
% Swim-up = 1.21 %fert. - 37.1	r=0.863, d.f. 8, P<0.01 F=23.40, d.f. 1, 8, P<0.01
% Survival = 0.97 %fert. - 40.0 120 days	r=0.727, d.f. 18, P<0.001 F=20.20, d.f. 1, 18, P<0.001

3.5 Discussion

The most striking feature of all the data presented here is the variation in egg quality which exists amongst rainbow trout under both commercial and laboratory conditions. It is also clear that generally higher levels of mortality are experienced under commercial conditions than previously reported by Small (1979).

The results from the commercial trial and the small experimental system indicate that some groups of eggs from individual females had very high survivals (100%) whereas others had very low ones (0%) despite receiving apparently equivalent treatments. Similar, although less pronounced variabilities in survival were also reported by Johnson (1984) working with Chinook salmon who recorded that some of the eggs had survivals of 90% to eying whereas under equivalent conditions many females produced eggs with poor survivals. The paradox of apparently healthy and normal batches of eggs suffering high mortalities during subsequent rearing is well-known to fish farmers and fish breeders (Craig and Harvey, 1984a).

One difficulty with commercial trials is that it is only possible to collect batch data, as eggs from individual fish are never incubated separately. Consequently survival rates from different batches of eggs each of which may be derived from varying numbers of fish may be given equivalent importance. Batches with zero survivals will only rarely be represented for it would require eggs from many fish to show coincidental heavy

losses. There are other limitations which may relate to the varying topography, local climate, water temperature and chemistry, husbandry practices, strain and age of females etc. of different farms. It is difficult to draw too many conclusions from these data. What the results do show are that survivals of 70% to eying are all that can be expected on commercial fish farms. Batches of eggs with higher survivals than this should be designated good quality and those with lower survivals poor quality. Poor quality eggs not only lose revenue for the farmer but also increase the labour required to manage the incubation of such eggs. The advent of mechanical egg picking machines has reduced the work load involved in the manual picking of batches of eggs at eying but these machines are expensive and somewhat temperamental. Also poor batches of eggs, which would have been rejected by manual pickers, are salvaged by such machines. This approach may not be the most sensible as the strong correlation between the survival rates at the different developmental stages indicates that batches with poor survival at eying will perform badly up to 120 days of growth.

Indeed using the equations derived from the commercial trials (Table 3.4) on the production data one would predict survivals of 44 % of all eggs produced to become fish of 120 days old (approx. 4.5g at a constant 10°C). This is higher than the commercial trial result (31%) but this is almost certainly due to the somewhat optimistic eying survivals claimed by

commercial fish farmers. It is also possible that commercial farmers may not include all blanks or zero survivals.

The survivals under the conditions of the commercial trial agree well with the findings of Kincaid et al (1977) who quoted survivals of 39.7% from green eggs to fry 147 days old. In contrast Pitman (1979) reported survivals of 43% up to 168 days old for the progeny of two year old rainbow trout and 83% for those of five year old females over the same period.

The results from the carefully controlled experimental system show that survival to eying under near optimum conditions can exceed 85%. This survival is much higher than that under commercial conditions and from production data (Fig. 3.5). No zero survivals were recorded under these conditions.

The survival of the eggs incubated under commercial conditions, but carefully monitored, confirms that data collected from fish farms tend to portray higher survivals than are actually experienced. The survivals under commercial conditions were much lower than those recorded under the near optimum conditions of the small batch system. This suggests that other factors may be having a serious adverse effect on egg survival under commercial conditions and that more research effort should be directed towards determining the requirements of developing eggs in terms of water quality and disease prophylaxis.

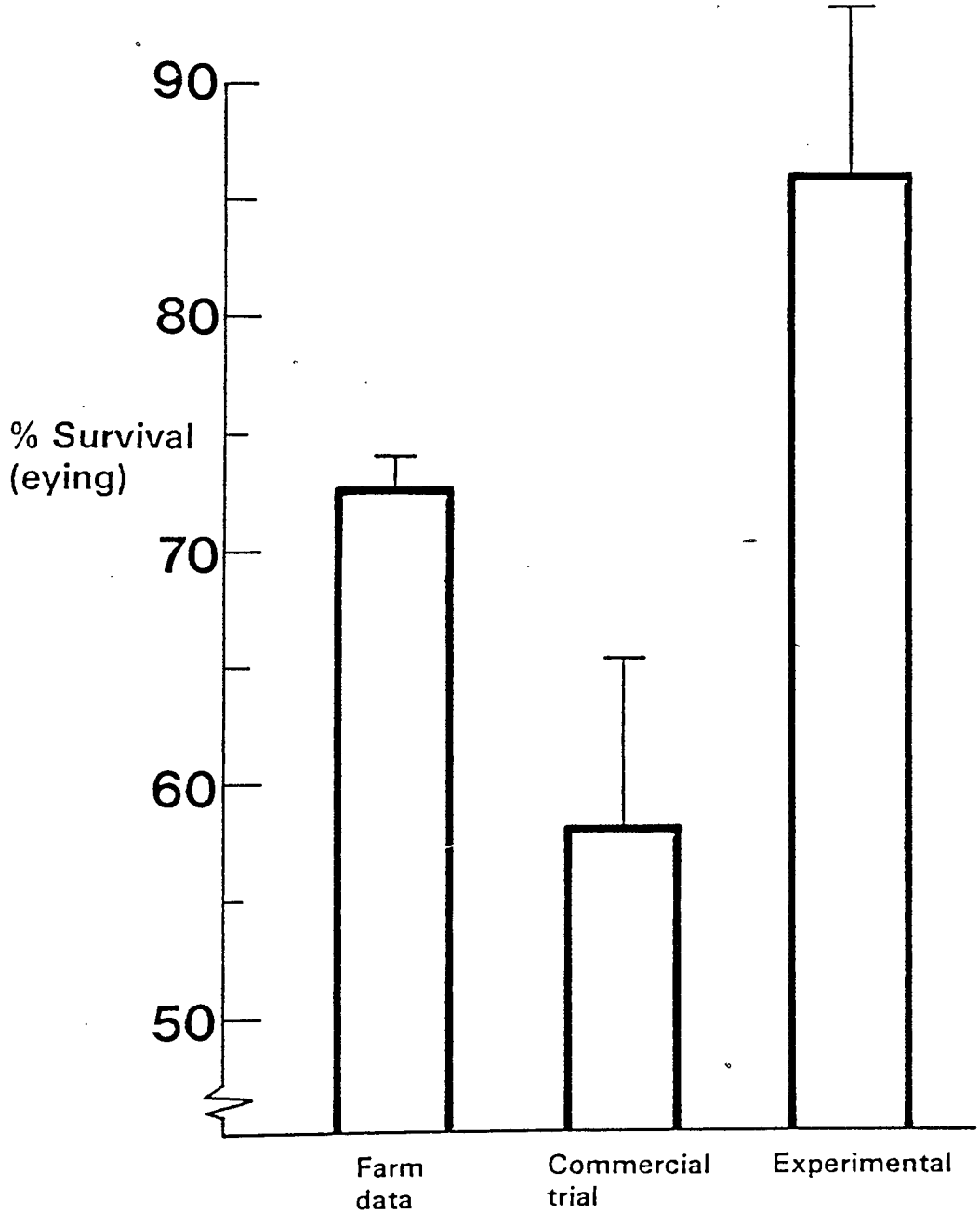


Fig. 3.5 Summary graph. Mean survivals (± SEM) to eying under farm, commercial trial and experimental conditions.

Possible reasons for the observed variations in egg quality under all conditions will be considered in the following two chapters and possible reasons why near 100% survivals are not consistently achieved discussed.

CHAPTER 4

Egg size and its relationships with egg survival and fry growth

4.1 Introduction

It is well-known that the size of eggs of fish shows considerable intra- and inter-specific variation (Bagenal, 1971). Even parental fish of the same strain, weight and length may have eggs of different size (Bagenal, 1969b). Individual fish, however, generally produce eggs which are more uniform in size (Bagenal, 1969b; Zonova, 1973; Larsson and Pickova, 1978). Although egg size is primarily determined by the genotype of the parental fish, it is also known to be affected by other factors including the age and size of the female parent. Thus Millenbach (1950), Buss and McCreary (1960), Gall (1974) and Smith et al (1979) have shown in studies of hatchery-reared trout that older and heavier females produce larger eggs than younger and smaller fish. The availability of food also affects egg size (Springate and Bromage, 1984b; Springate et al, 1985). Alterations in egg size also occur in batch-spawning fish as the season progresses (Bagenal, 1971) and in synchronous spawners as a result of photoperiodic modifications of the timing of maturation (Bromage et al, 1984).

Clearly, the differences in egg size between different fish species reflect alternative reproductive strategies for survival. The adaptive significance of differences in egg size shown between individuals of the same stock or strain is

however, less clear. Blaxter and Hempel (1963) and Bagenal (1969b) have both shown that without food the fry from larger eggs show better survival rates and suggest that the differences in size of egg and fry may be of selective advantage under the competitive conditions which prevail in wild stocks of fish. If such advantanges are shown to be present in hatchery-reared eggs and fry this would have considerable implications for the management of salmonid broodstocks.

In the past conclusions regarding the fundamental importance of egg size have been clouded, by conflicting results and, by the difficulties in partitioning effects of egg size on fry viability from other influences relating to the age, strain, and geographic location of the parental fish. Gall (1974) showed that egg size was positively correlated with both the survival of the egg to eying and the growth of the fry, although the results varied over the two years of the study and included different genetic stocks. Pitman (1979) observed that the progeny from the larger eggs of five year old fish were bigger and showed higher survivals than those derived from the smaller eggs of two year old parents. Glebe et al (1979) failed to show any relationship between egg size and fry survival for Atlantic salmon although this study used different strains for the comparisons of egg size. In contrast Fowler (1972) working with Chinook salmon reported that the larger fry and fingerlings from larger eggs sustained higher mortalities than the smaller fish from smaller eggs. Similarly, Smith et al (1979) found that the percentage of rainbow trout eggs that reached the eyed stage

tended to decrease as the age of the fish, and the size of the eggs, increased.

Despite the potentially conflicting influences of age and strain of the broodstock, generally, it would appear that larger eggs initially produce larger fry. However, it is not clear whether egg size per se confers any permanent or long-term advantages as far as growth and survival of the fry are concerned. Some authors report no lasting effects of egg size on subsequent development (Kincaid, 1972; Zonova, 1973; Reagan and Conley, 1977) whereas others suggest that these differential effects persist throughout the early life of the fish (Millenbach, 1950; Pitman, 1979).

There are two main aims of this Chapter. Firstly, to see if there is any relationship between egg size and egg quality and secondly to examine the performance of fry derived from eggs of different sizes.

4.2 Egg size and survival

This section examines the relationship between egg size and egg quality as determined by fertilisation success. Fertilisation rates are possibly the best index of egg quality as the determinations are made very early during development before other factors are likely to have had a significant effect.

4.2.1 Materials and methods

Groups of eggs from 105 females of different ages, sizes and strains were examined throughout this work. All eggs were stripped from fish within 10 days of ovulation to avoid overripening (see Chapter 8). Approximately 100 eggs from each ripe female were checked for fertilisation success, after 12 hours incubation at 10°C by visualization of early segmentation with a clearing solution (Table 2.1).

The mean egg sizes (ova diameter) were determined as described in the General Materials and Methods (Chapter 2).

4.2.2 Results

The percentage fertilisation of the different size eggs are plotted in a scatter plot (Fig. 4.1). There was no significant correlation between percentage fertilisation and ova diameter ($r = 0.06$, $P > 0.05$). Arcsine transformation of the percentages did not improve the relationships.

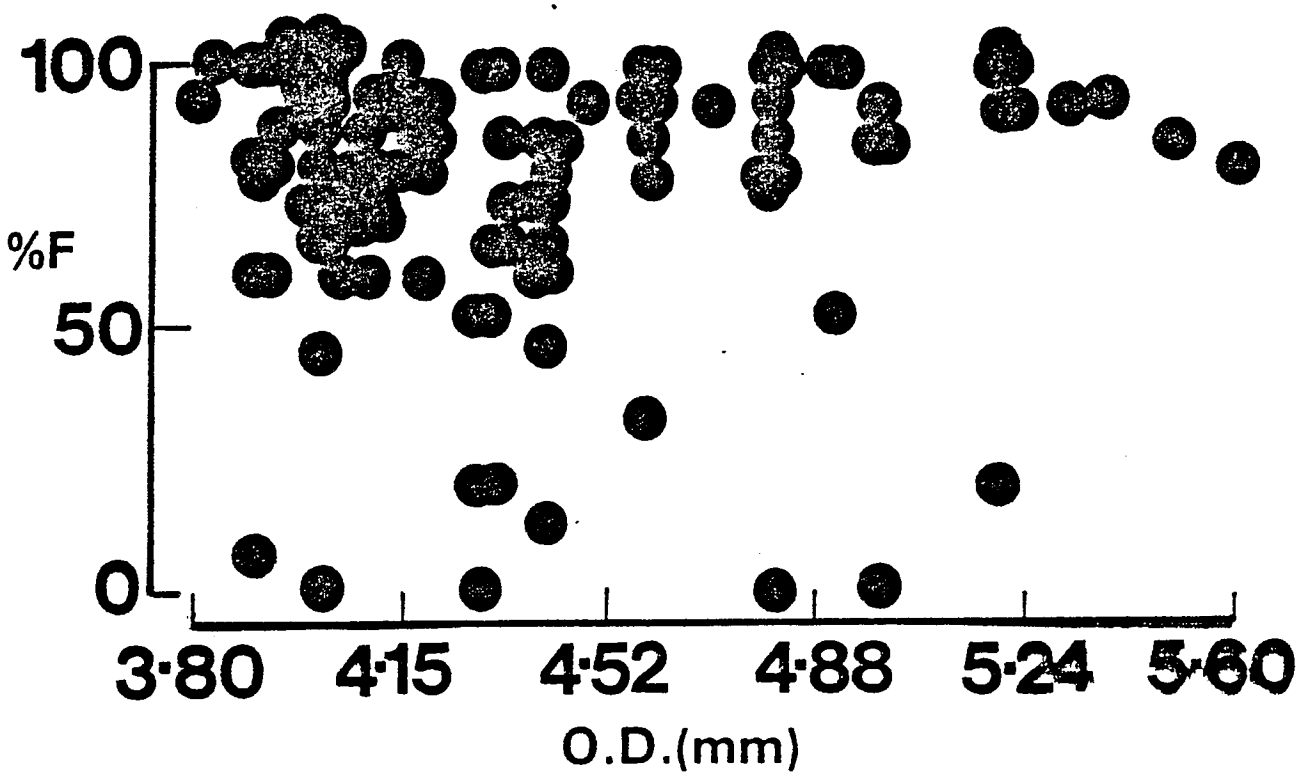


Fig. 4.1 Scatter plot of the % survival through fertilisation (%F) of eggs of varying diameter. (O.D.). (r=0.06; F=0.38, d.f.,1,103; NS).

4.3 Egg size and fry growth

This section examines the performance of fry derived from eggs of different sizes from two year old and three year old parents

4.3.1 Materials and methods

A group of 500 mixed two and three year old rainbow trout of the same Autumn-spawning strain were maintained at a commercial hatchery in a 10m diameter tank supplied by bore-hole water at a constant 10°C. As the time of expected spawning approached the fish were examined every 10 days to ensure that the stripped eggs from each fish were at equivalent stages of ripeness. Sorting of the broodstock with this periodicity avoids the problems of overripening which ovulated eggs undergo if retained unfertilised in the abdominal cavity for periods of time longer than 10 days (Chapter 8; Nomura *et al*, 1974; Springate *et al*, 1984). After approximately one-third of the broodstock had spawned, six two year old and seven three year old fish with mean weights of 1146g and 2266g respectively were randomly selected and their eggs separately maintained for more detailed examination.

The selected gravid females were hand-stripped and dry-fertilised using a common pool of sperm collected from five two year old male fish. After water-hardening the mean diameter of the eggs from each female was calculated by counting the number of eggs aligned along a 300mm measuring groove. The remainder of the eggs were incubated in thirteen identical horizontal

hatchery trays each fed with constant 10°C water at a flow rate of eight $L.min^{-1}$. The rates of fertilisation, eying, hatch and swim-up of each batch of eggs were determined using the methods of Springate et al (1984).

Just before the time at which the young fish were expected to feed, i.e. 60 days post-fertilisation, a sample of twenty alevins were randomly selected from each group and weighed. Further samples were taken every two weeks for the next eight weeks and for some groups up to 45 weeks post-fertilisation although commercial pressures did not allow all 14 batches to be separately maintained for the last two measurements. Specific growth rates were calculated using the following equation:-

$$SPGR = \left[\left(\frac{\text{Final wt}}{\text{Initial wt}} \right)^{\frac{1}{t(\text{days})}} - 1 \right] \times 100$$

Over the initial eight week period overall survival rates were determined for each group by counting mortalities and total numbers of fry.

4.3.2 Results

Figures 4.2, 4.3, 4.4 and 4.5 show the growth of the fry derived from the 13 groups of eggs. The growth was recorded from the first sample, taken just before the fry took their first feed, i.e. at eight week post-fertilisation, up to a maximum of 45 weeks post-fertilisation. The groups of fry were numbered from 1-13, number one being those derived from the smallest eggs etc.

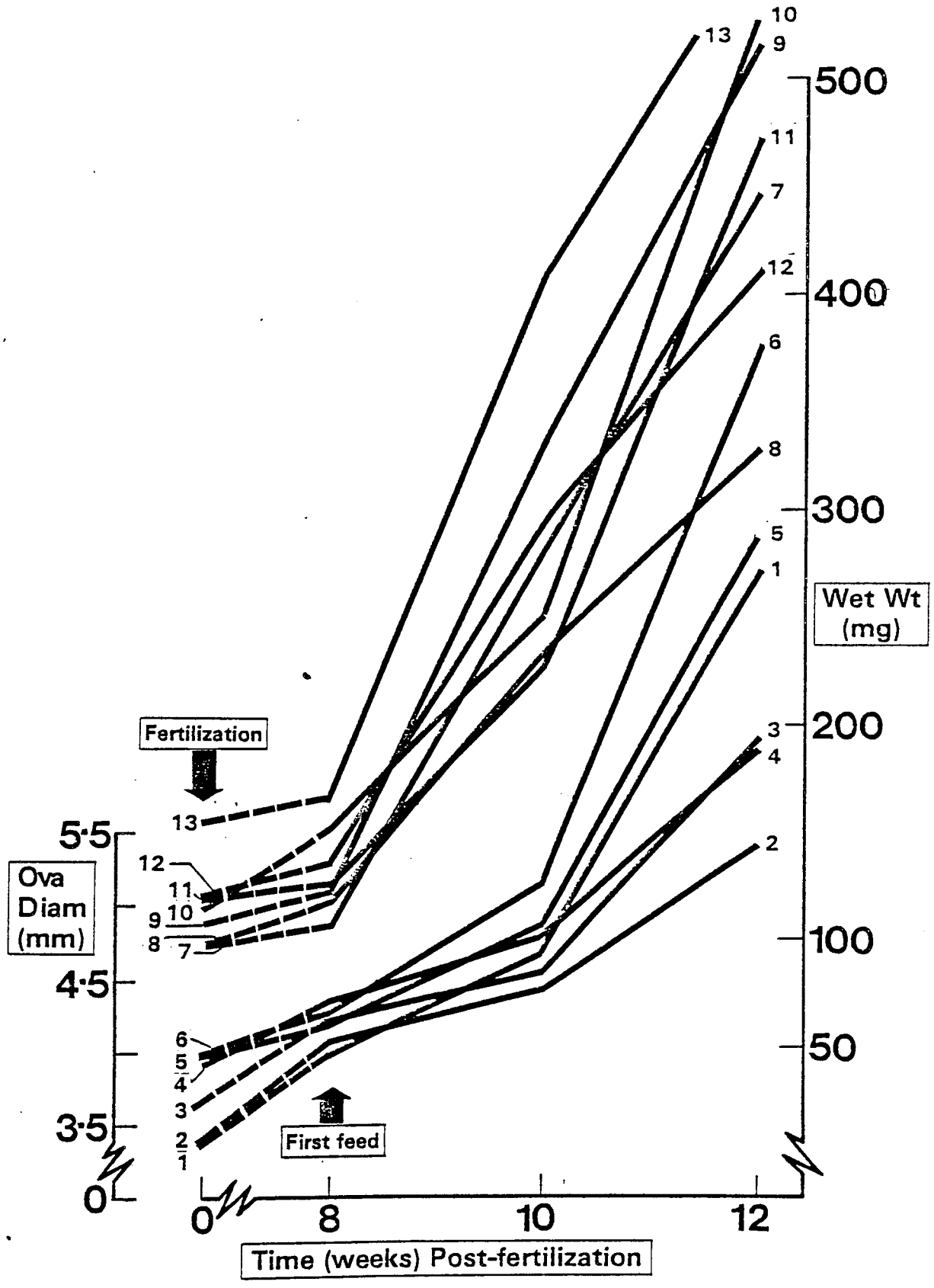


Fig.4.2 Egg size and growth of fry from 8-12 weeks post-fertilisation. Eggs and fry from 2 year old and 3 year old fish are labelled 1-6 and 7-13 respectively.

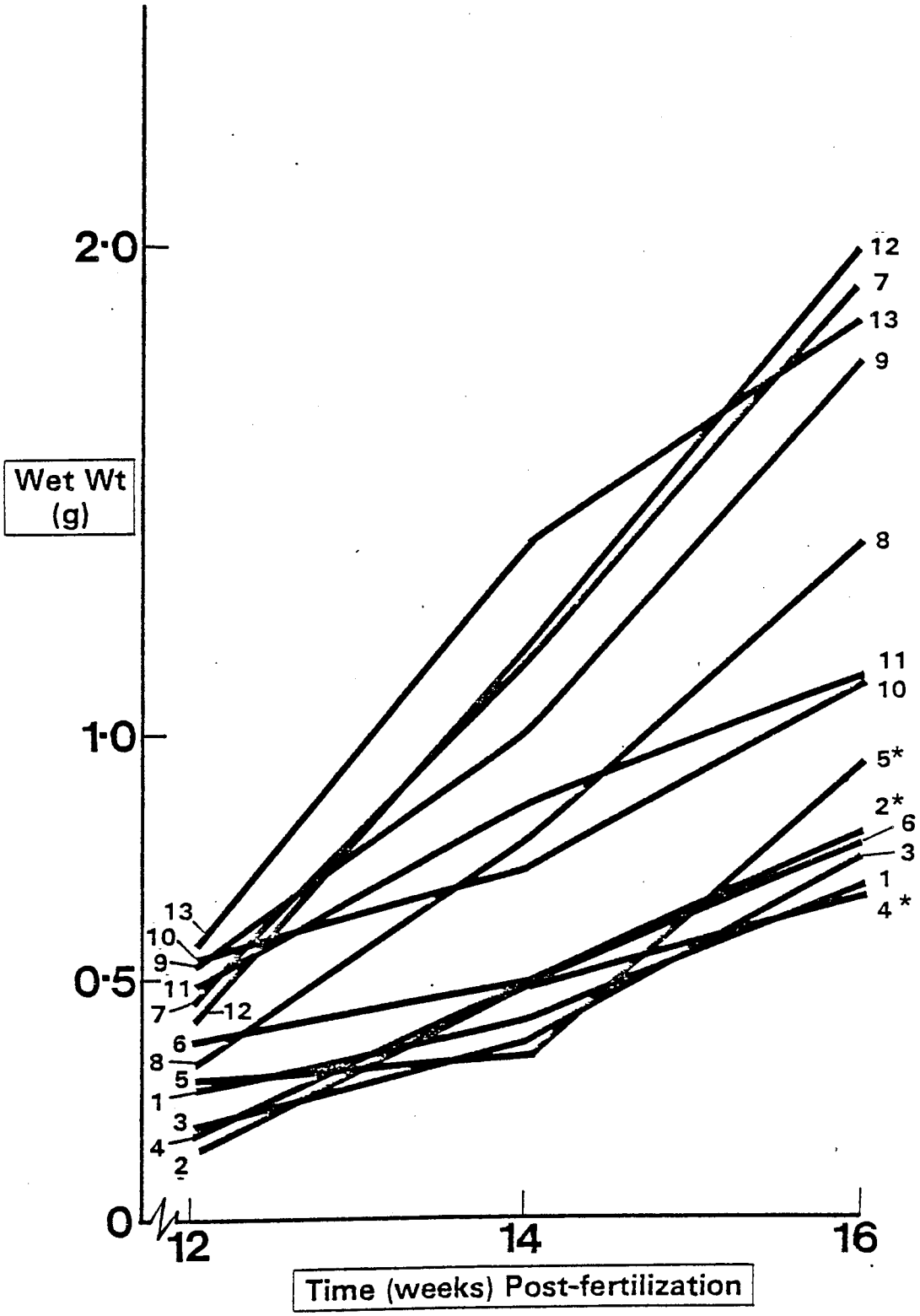


Fig.4.3 Growth of fry from 12-16 weeks post-fertilisation. This shows a continuation of the fry growth from Fig.4.2. Fish labelled as Fig.4.2. (* denotes group terminated.

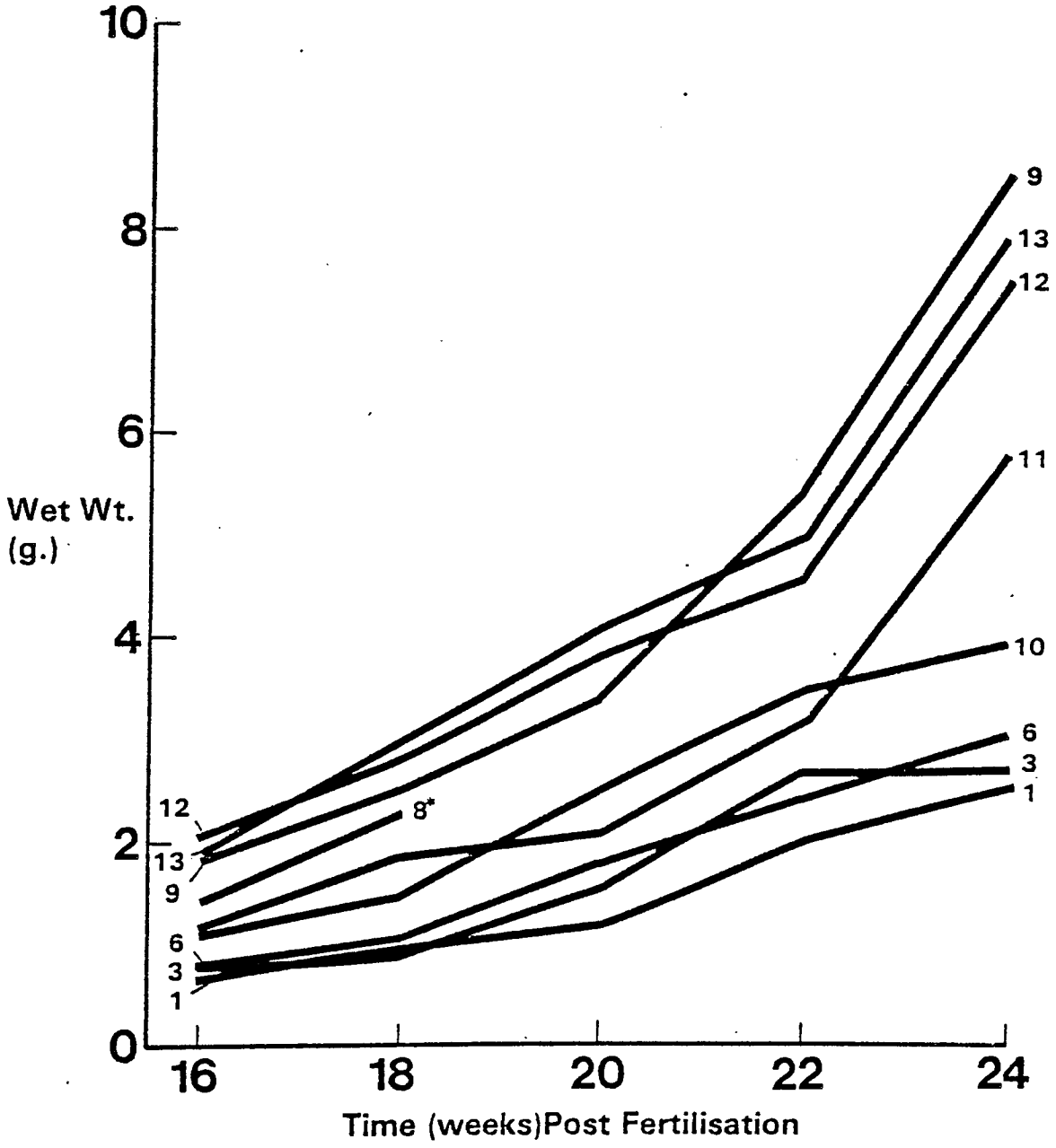


Fig 4.4 Growth of fry from 16-24 weeks post-fertilisation. This shows a continuation of the fry growth from Fig.4.3. Fish labelled as Fig.4.2. (* denotes group terminated).

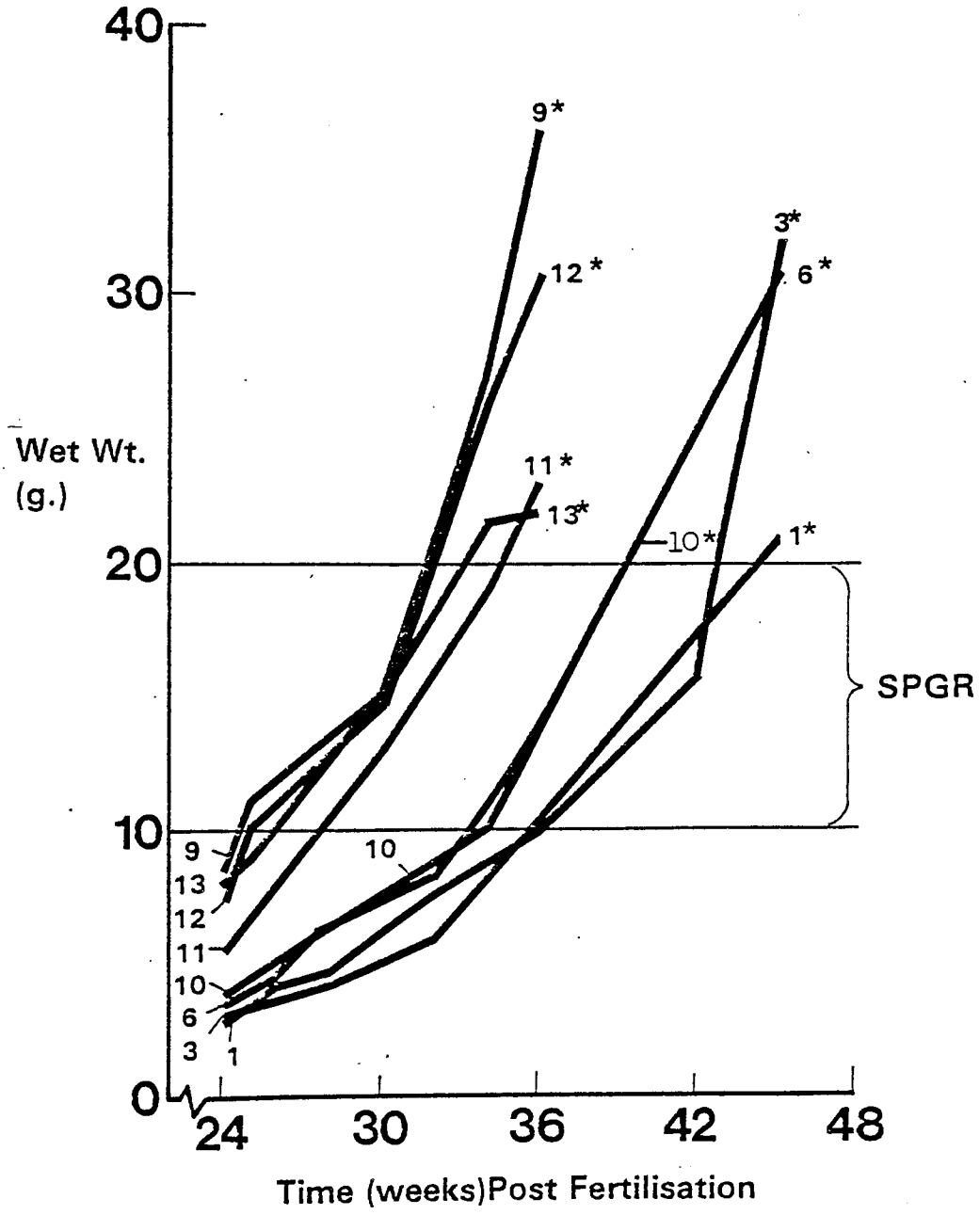


Fig.4.5 Growth of fry from 24-46 weeks post-fertilisation or until 20g.+ in size. This shows a continuation of the fry growth from Fig.4.4. Fish labelled as Fig.4.2. (*denotes group terminated).

The data (Tables 4.1a and b) were analysed with least squares regression by regressing mean fry body weight (n=20) on mean egg diamter (n=50). The correlation coefficients clearly show that at 8 weeks post-fertilisation fry wet weight was significantly correlated with egg diameter for fry from both two ($r=0.886$; 10 d.f. $P < 0.001$) and three year old ($r=0.849$; 12 d.f.; $P < 0.001$) females. However, 2 weeks after the onset of first feeding there were no significant correlations between egg size and fry weight ($P > 0.05$) in either of the two age groups of fish considered (Tables 4.2a and b).

Table 4.1 Weight and length of parental fish, egg diameter (OD) and mean weight of resultant fry from a) two year old and b) three year old females

a) Two year old fish

	Size			Mean fry weight (mg) 8-16 weeks post-fertilisation (n=20 for each fish				
FISH No.	WT (g)	LTH (cm)	OD (mm)	8WKS	10WKS	12WKS	14WKS	16WKS
1	1060	42.7	3.35	47	93	273	409	684
2	1190	46.0	3.37	52	77	141	490	797
3	1130	41.0	3.70	61	84	194	360	766
4	1270	45.4	3.91	70	102	189	483	684
5	1060	44.4	3.92	61	104	286	339	942
6	1170	46.4	4.04	64	125	375	489	784
Mean (±SEM)	1146 (±33)	44.3 (±0.9)	3.72 (±0.12)	59 (±4)	98 (±7)	244 (±35)	428 (±28)	776 (±39)

b) three year old Fish

7	1800	50.4	4.74	105	260	453	1115	1941
8	1500	50.3	4.77	115	230	328	779	1431
9	2315	58.4	4.90	120	330	529	1092	1798
10	2380	61.2	5.00	150	249	525	728	1122
11	2700	58.6	5.06	122	225	476	858	1171
12	1525	48.4	5.06	134	292	411	1184	2013
13	3545	63.1	5.63	161	409	571	1396	1874
Mean (±SEM)	2252 (±276)	55.8 (±2.2)	5.02 (±0.11)	130 (±0.8)	285 (±25)	470 (±31)	1021 (±91)	1621 (±141)

contd...

Table 4.1 (cont.) Mean weight of fry from a) two year old and (b) three year old females (g)

a) Two year old females

	18WKS	20WKS	22WKS	24WKS	26WKS
1	0.989	1.294	2.001	2.546	4.020
2	1.150a	*	*	*	*
3	0.888	1.614	2.650	2.661	-
4	1.394a	*	*	*	*
5	1.476	2.121a	*	*	*
6	1.129	1.856	2.297	3.091	4.519
Mean ±SEM	1.171 ±0.092	1.723 ±0.178	2.349 ±0.189	2.766 ±0.166	4.270 ±0.250

b) Three year old females

7	2.883a	*	*	*	*
8	2.152a	*	*	*	*
9	2.484	3.393	5.404	8.571	11.306
10	1.410	2.503	3.48	3.982	*
11	1.700	2.171	3.330	5.760	*
12	2.714	3.763	5.329	7.458	10.149
13	2.985	4.249	5.061	8.000	8.790
Mean (±SEM)	2.384 ±0.227	3.016 ±0.415	4.521 ±0.460	6.754 ±0.837	10.082 ±0.727

a = Too few fish surviving to continue trial

* = No data available

contd...

Table 4.1 (cont) Mean weight of fry from a) two year old and b) three year old females (g)

a) Two year old females

	28WKS	30WKS	32WKS	34WKS	36WKS	39WKS	42WKS	45WKS
	4.59	*	7.59	*	9.72	*	*	21.33[b]
	4.25	*	6.29	*	*	*	15.8	32.70[b]
	6.51	*	8.16	*	13.77	*	*	30.56[b]
Mean	5.12		7.35		11.75			28.19
(±SEM)	±0.71		±0.55		±2.02			±3.49

b) Three year old females

9	*	15.17	*	26.28	36.12[b]	
10	*	6.69	*	9.93	*	19.7[b]
11	*	11.38	*	19.02	23.05[b]	
12	*	14.42	*	26.42	30.83[b]	
13	*	15.17	*	21.79	21.7[b]	
Mean		12.57		20.69	29.93	
(+SEM)		± 1.63		±3.03	±3.39	

b = Denotes experiment ended

* = No data available

Table 4.2 The correlation coefficient (r) and computed F values from analyses of variance for regressions of mean fry wet weight of four successive two week intervals (from first feeding) on mean egg diameter for a) six groups of progeny from two year old females and b) seven groups of fry from three year old females.

a) Two year old fish

	Age post-fertilisation (weeks)				
ANALYSIS	8	10	12	14	16
r	0.886[b]	0.803	0.535	0.000	0.318
F	14.582[a]	7.295[b]	1.604	0.001	0.448

b) Three year old fish

	Age post-fertilisation (weeks)				
ANALYSIS	8	10	12	14	16
r	0.849[b]	0.760	0.623	0.602	0.148
F	12.937[a]	6.843[b]	3.172	2.842	0.112

[a] $P<0.01$, [b] $P<0.005$; Values for 12 - 16 weeks were not significantly different

The F values from the analyses of variance showed similar trends to the correlation coefficents. The variances due to the regression were initially significantly greater than the variances due to the residuals ($P < 0.01$) for both groups but these relationships were not significant from 12 weeks post-fertilisation onwards ($P > 0.05$) (Tables 4.2a and b).

There was no significant difference in the specific growth rate of the fry derived from each age group of parent ($t=0.22$, 11 d.f. $P > 0.05$). Over the eight week period the fry from the two and three year old females had mean specific growth rates of 4.71 ± 0.13 and 4.58 ± 0.21 respectively. There were also no significant differences in specific growth rates when determined over the same growth period for each age group of fish i.e. from 10-20g (Table 4.3). Egg size was not correlated with specific growth rate in either age group or in the data as a whole ($P > 0.05$).

Calculation of correlation coefficients revealed no sigificant difference between egg size and survival at any of the four developmental stages considered (eying, hatch, swim-up, and for the first 56 days of fry growth) ($P > 0.05$) for either of the two age groups of females or for the pooled data (Table 4.3).

Table 4.3 Survival of eggs and fry and specific growth rate (SPGR) of fry in relation to egg size (OD)

	OD	EYE*	HATCH	S-UPS*	SPGR* (8-16wks)	SPGR* (10-20gms)
	(mm)	(%)	(%)	(%)	(%)	(%)
1	3.36	91	88	87	4.90	1.22
2	3.37	33	30	29	4.57	-
3	3.70	85	75	73	5.00	1.59
4	3.91	18	17	14	4.14	-
5	3.92	64	53	53	4.98	-
6	4.04	94	89	88	4.64	1.66
7	4.74	21	18	17	5.33	-
8	4.77	18	16	15	4.48	-
9	4.90	50	45	43	4.59	1.40
10	5.00	96	94	93	4.95	1.52
11	5.06	93	90	85	4.93	1.79
12	5.06	55	44	43	4.11	1.42
13	5.63	34	27	25	3.65	1.40

* None of these measurements were correlated with egg size (OD) $P > 0.05$

4.4 Discussion

The present study confirms for the rainbow trout that fry hatched from larger eggs are initially bigger in size than those developed from smaller eggs. Similar results have also been recorded for the Atlantic salmon (Glebe et al , 1979; Kazakov, 1981; Thorpe et al, 1984) the Arctic char (Wallace and Aasjord, 1984) and herring (Blaxter and Hempel, 1963). Pitman (1979) reported equivalent findings for the rainbow trout although eggs from different aged fish were used for the size comparisons in this study. The use of parental fish of the same strain and geographic location, together with separate evaluations of two age groups, has avoided some of the difficulties of interpretation which were present in the earlier studies.

An important question which has remained in the literature is whether the initial size advantage of the fry, hatched from larger eggs, is maintained during the subsequent growth of the fry, fingerlings and underyearling stock. In the present work the significant correlation which existed between egg and fry sizes at hatching was lost four weeks after the time of first feeding. The reduction or disappearance later in development of the initial differences in fry weight, resulting from differences in egg size, have also been recorded for the Atlantic salmon (Hayes and Armstrong, 1942; Thorpe et al, 1984), carp (Kirpichnikov, 1966) and catfish (Reagan and Conley, 1977). Kincaid (1972) also suggested that differences in size which were initially present in rainbow trout fry were not important after 150 days of growth. In contrast Pitman (1979)

concluded that differences in fry size became pronounced as development proceeded in the Atlantic salmon although these data also showed that the percentage differences in fry size diminished over this period. Surprisingly, the same author also commented that the smaller fry were growing at a faster rate. Pitman's conclusions were based on cumulative increases in length and, as larger fry would be expected to show larger increments in size, when expressed in absolute terms, it might be more appropriate if such comparisons included assessments of specific growth rate. The use of such measurements in the present study revealed no significant egg size or parental age related changes in specific growth rate of the fry up to 26 weeks post-fertilisation. The ability of the smaller fry to grow at the same rate as the initially larger fry is of considerable commercial importance. Providing both groups have a similar potential for growth such differences in size would only involve an additional three-four weeks of growth in the hatchery before sale as 4.5g fry.

Potentially, of more importance is the finding that survival of the eggs and fry is not affected by the size of the egg or the age of the parental fish. This is in agreement with the studies of Glebe et al (1979) and Thorpe et al (1984) on the Atlantic salmon and Zonova (1973) and Tomita et al (1980) on the carp. Gall (1974) and Kirpinchnikov (1966) both found initial reductions in survival of eggs and fry from smaller eggs although in both investigations these effects disappeared later in development. In contrast Small (1979) and Pitman (1979)

reported long-term improvements and Fowler (1972) reduction in survival of fry hatched from larger eggs. Possibly, the variability in these results is due to the use of eggs at different stages of ripeness for this has been shown to be the major determinant of early egg and fry losses (Chapter 9; Springate et al, 1984). Certainly, the present work would indicate that small eggs do not have any inherent problems as far as survival is concerned, provided they are processed in an equivalent way to other eggs in the hatchery. Hatchery trays or incubators should never be filled on the basis of egg size because larger eggs differ from smaller ones mainly in the size of the yolk and water composition and not in the weight of respiring and developing embryonic tissue (Kazakov, 1981; Wallace and Aasjord, 1984). Increased problems with disease may also arise because the proportionally greater surface to volume ratio of smaller eggs offers greater areas for pathogen attack. Consequently, trays should be filled with the same numbers of eggs and each egg, irrespective of its size, should receive the same space and flow of water.

If as the present study shows smaller eggs have similar survival patterns to large eggs then there is no reason why such eggs should not be equally acceptable for general sale, particularly as hatcheries in many countries sell eggs by number rather than by volume. Alternatively, if there is still purchaser resistance to small eggs then they should be hatched and grown-on for sale as fry.

The production of smaller eggs may also provide some advantages for egg and fry producers as there would appear to be a 'trade off' between egg size and number, and reductions in egg size are inevitably associated with improvements in total fecundity (Chapter 6; Springate and Bromage, 1984b). Consequential alterations in egg size and numbers have also been produced by modifications of ration (Chapter 9 ; Springate and Bromage, 1984b; Springate et al, 1985), accelerations of spawning (Bromage et al, 1984) and the use of smaller broodstock (Springate and Bromage, 1984b). In each case relative fecundity, or the number of eggs per kilogram of somatic weight, is increased, allowing hatcheries to produce more eggs from the same total weight of broodstock. Although each farm presents a different set of conditions, it is suggested that consideration is given to the use of strains of rainbow trout with higher relative fecundities and a smaller size of egg for this might provide the optimum strategy for egg and fry production (Chapter 7).

CHAPTER 5

Egg chemical composition

5.1 Introduction

The total amounts of nutrients available and the proportions of the different constituents in the egg are likely to be of importance in determining egg quality (Springate and Bromage, 1984c). The nutrients in the newly fertilised egg have to support the egg and fry until further supplies are ingested from the external environment; this takes approximately 50 days at 10°C in the rainbow trout.

Many workers have examined the effects of varying dietary components on reproduction by deleting some essential component and recording the effects on egg quality. These investigations reveal some information regarding egg chemical composition and their quality.

The chemical composition of rainbow trout eggs is affected by broodstock diet (Dumas, 1961). Therefore in this study all experimental fish received the same commercial broodstock food (Ewos Baker Ltd.) (Table 5.1)

Satia (1973) compared two experimental and two commercial rainbow trout broodstock feeds and found that egg number and egg weight were not significantly changed in the different groups.

Table 5.1 Composition of commercial broodstock food used throughout this study (Ewos Baker Ltd.)

Protein	47%
Carbohydrate	22%
Ash	13%
Moisture	9%
Oil	7%
Fibre	2%
Canthaxanthin	40 ppm

However, the green eggs (unfertilised) and alevins showed significant variation in protein, lipid and water composition and Satia suggested that this affected egg survival. In contrast Ridelman (1981) using the same Washington broodstock as Satia failed to find a significant correlation between the proximate composition of eggs and their viability.

This investigation considers the composition of the major chemical components of rainbow trout eggs (protein, fat, vitellogenin and ash) and their constituent parts i.e. amino acids, free fatty acids and minerals; and correlates these results with egg quality.

Protein is the major component of the dry weight of salmonid eggs, and has been the subject of several nutritional studies relating to egg quality. Takeuchi et al (1981) maintained rainbow trout on different diets and examined the effects on

reproductive performance of low protein and high calorie diets. They found that eggs produced from the fish fed on the low protein diet with a high energy value gave eggs with a higher survival to eying and hatching compared with the eggs from the fish fed on a control commercial diet. Similarily Phillips et al (1964) working with brown trout found that the eggs from the fish fed on experimental diets low in protein and calories were the best quality. In contrast Satia et al (1974) reported a significant positive correlation between percentage protein in the ova and total embryonic survival to the yolk-sac absorption stage. They suggested that egg protein level was an important determinant of the success of embryonic development, although in all the diets used all the essential amino acids were present in greater amounts than those specified by the F.A.O. as the minimum levels required for human nutrition. However, Smith et al (1979) examined the effects of diets which were low, intermediate and high in protein and energy on egg quality in rainbow trout, and found no significant differences in egg quality among any of the groups. In a detailed study of the effects of feeding rainbow trout broodstock with isocaloric diets with four levels of protein (27%, 37%, 47% and 56%). Roley (1983) noted that there were no significant correlations between egg and embryo survival and dietary protein levels. Unfortunately, he did not measure the protein levels in the eggs from these females.

The results of the studies discussed above, which have fed experimental diets of varying protein content to broodstock trout and recorded their effects on egg quality, aside from giving conflicting results are often confused with other nutritional variables, mainly energy content.

Commercially available rainbow trout broodstock diets contain a very high proportion of protein (approximately 50%), and are supplemented with essential amino acids; therefore it is unlikely that free amino acids or protein are limiting factors for egg quality in domesticated stocks of rainbow trout.

Fat is the second largest component of the dry weight of rainbow trout eggs with a mean value from the literature of 19% (Table 5.2). However, there is considerable variation in the fat levels (coefficient of variation 36%) recorded in the literature.

Lipids are considered by some Russian authors (Kuznetsov, 1973; Kuznetsov and Khalitov, 1978) to be the most important constituent for determining egg quality although little definitive evidence is presented.

There is some ambiguity concerning the role of fat in energy production during different stages of development. Fat is undoubtedly used as a fuel, possibly 70-80% being consumed during egg development (Hayes, 1949). According to Hayes,

% composition dry wt			% dry wt of total wt	Species	Authors
Protein	Fat	Ash			
53.4	16.0	3.7	37.6	rainbow trout	Hirao et al, 1954
72.9	14.5	-	35.9	" "	ibid, 1955
67.0	15.3	4.3	29.4	" "	Satia & Donaldson, 1974*
71.3	11.4	3.9	33.8	" "	Satia et al, 1974
70.8	22.5	4.0	29.4	" "	Vuorela et al, 1979
70.5	28.2	-	40.7	" "	Takeuchi et al, 1981
71.2	24.9	-	45.0	" "	Craik & Harvey, 1984a
67.5	11.1	4.0	41.8	" "	Ridelman et al, 1984
59.6	23.9	8.7	23.0	brook trout	Phillips et al, 1956
69.6	8.2	3.8	38.0	brown trout	Phillips & Dumas, 1959
68.1	22.5	9.7	-	Atlantic salmon	Wood et al, 1960
52.2	36.1	2.8	36.0	" "	Hamor & Garside, 1977

* unpublished

Table 5.2 Proximate composition of salmonid eggs

energy requirements before hatching may be supplied by fat or by fats in combination with protein. Clearly, lipids are of major importance to developing fish, both as an energy source (primarily triglycerides) and as a structural component (primarily phospholipids) (Atchinson, 1975). The omega 3 fatty acids (n-3) are believed to perform an essential role in fish development and growth, with 22:6 being the key compound, especially for phospho-lipids (Lee et al, 1967; Hayes et al, 1973).

Many commercial diets are low in lipid content, scarcely exceeding 6-14% dry weight (Hilton and Slinger, 1981). Indeed Watanabe et al (1980), proposed that the optimum ratio of lipid to protein for rainbow trout growth was 1:2. Yu et al (1977) recommended increasing the energy level of the diet by lipid incorporation, and particularly the addition of omega 3 fatty acids, both of which improve the growth of rainbow trout. The effects of various dietary fat levels for broodstock rainbow trout and their effects on subsequent egg quality need to be fully appraised.

Large proportions of the fat and protein in rainbow trout eggs are contained within the yolk. Yolk is the main nutrient store in the egg, and yolk stores are the only source of food for the developing egg and alevins before the oesphagus opens and the first food is ingested (Escaffre and Bergot, 1984). The protein and fat components of yolk are in the form of phosvitin and lipovitin, which are components of the large molecule vitellogenin. Vitellogenin is a lipoglycophosphoprotein synthesised in the liver after induction with oestradiol and then released into the blood stream. It is then taken up by the maturing oocytes. Inside the egg it is currently believed that phosphitin, lipovitellin and vitellogenin are all present (Wallace et al, 1983). Vitellogenin levels in the egg have not previously been measured for any fish. As vitellogenin is such an important part of egg structure and function it is suggested that low levels could be indicative of poor quality eggs.

Over 20 mineral elements have been shown to fulfil significant structural or metabolic functions in the tissues of vertebrates (Underwood, 1971). In mammals and birds, post natal requirements are derived largely from the diet, although ingested water may contribute significantly. During ontogenesis the mammalian placenta serves as a nutrient conduit, through which certain mineral elements (and other small molecules) are delivered from the maternal circulation to that of the embryo or

foetus, and in birds, nutrients in the egg are transferred to the embryonating chick.

In teleosts, the external environment may contribute significantly to the mineral requirements of the adult and young. Minerals may be absorbed and become part of the tissue, or they may serve important functions in osmoregulation (Zeitoun et al, 1976). Considerable mineral translocation from the water environment to the developing ova of the rainbow trout has been demonstrated by Ogino and Yasuda (1962) and Zeitoun et al (1976).

In early investigations Hirao et al (1954, 1955) showed that rainbow trout eggs containing lower levels of iron and vitamin B_2 had reduced hatching rates. However in a more recent study of eggs, again from rainbow trout, Craik and Harvey (1984a) did not find a correlation between iron content and subsequent hatching success although Craik and Harvey (1984a) used first spawning fish, being mainly concerned with the differences between ripe and overripe eggs, whereas Hirao et al (1954, 1955) presumably only examined ripe eggs. Other Japanese workers (Takeuchi et al, 1981) fed rainbow trout on a diet deficient in trace elements. Eggs from the fish fed on a diet without trace element supplementation showed significantly lower values for both eying and hatchability. However, there were no marked differences in general composition of the eggs due to the

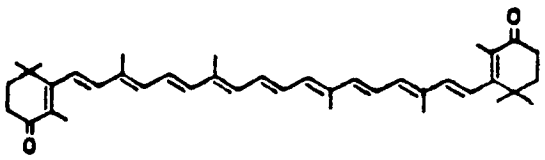
experimental diets although the contents of manganese was significantly lower in the eggs from the fish fed the trace element deficient diet compared with the control group.

Although the importance of minerals in the reproductive process has been implicated in experimental stocks of fish it is not clear whether the levels of minerals in commercial trout diets and their external environment exert any influence on egg quality. It is unlikely, for commercial rainbow trout diets are supplemented with trace elements and thus minerals are not thought to be limiting.

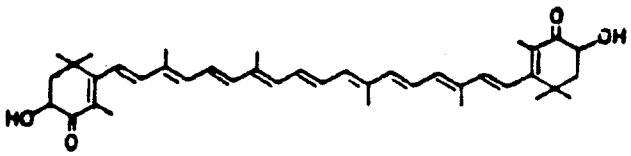
One of the minor components of rainbow trout eggs are the carotenoids. Rainbow trout cannot synthesise carotenoids and must therefore ingest them (Tacon, 1981; Torrissen, 1984). Most commercial broodstock food is pigmented with 4, 4'-diketo-β-carotene (trivial name canthaxanthin), whereas the main and presumably functionally active carotenoid found in wild stocks of fish is 3, 3'-dihydroxy-4, 4'-diketo-β-carotene (trivial name astaxanthin). These two have very similar structures (Fig. 5.1).

Fig. 5.1 Structure of canthaxanthin and astaxanthin

Canthaxanthin



Astaxanthin



Canthaxanthin in the diet gives the fish flesh a desirable pink colour, and for the egg producer it produces the deep orange colour, supposedly associated with good quality eggs. Despite the wide occurrence of carotenoids within fish ovaries and eggs, their role, other than a source of provitamin A in normal embryonic development remains obscure. Hartman et al (1947), showed that astaxanthin in the egg enhanced its chemotaxic attraction for sperm in the rainbow trout. However, Quantz (1980), stated that higher amounts of carotenoids in the diet, and prolonged feeding, did not cause any significant change in the chemotaxis of spermatozoa, or in the fertilisation rate in the same species. Bauerfeind (1976), noted that canthaxanthin in the diet increased both the fertilisation rate and the percentage of fish that spawned. Deufel (1965),

demonstrated improved growth, maturation and fecundity when the fish diet was supplemented with the same pigment. There have also been reports (reveiwed by Mikulin and Soin, 1975) on the effects of different levels of egg pigmentation on mortality during ontogenesis, especially with respect to their ability to improve tolerance to variation in a number of environmental factors including high temperature, high ammonia levels, harmful effects of light and low oxygen. There are however, few sound data to support all these claims. Consequently, other than a source of provitamin A, the role that carotenoids play in normal embryonic development remains obscure (Tacon, 1981). Indeed Torrissen (1984) found no relationship between carotenoid level and the survival of eggs of Atlantic salmon in darkness. Light sensitivity increased with increasing amounts of carotenoids in the eggs.

Monitoring astaxanthin and canthaxanthin in the diets and the eggs produced from broodstock fish and relating these with subsequent egg and fry development, as well as varying the levels of these pigments in the broodstock diet, should give valuable information as to their importance. Whether the extra cost involved in pigmenting food is worthwhile in terms of improving egg quality, fecundity and fry performance should also be evaluated, especially as canthaxathin is an artificial additive. Unfortunately the measurement of canthaxanthin and astaxanthin could not be achieved by GLC, and measurement by simple colorimentry does not separate these two closely related molecules. The measurement of these two pigments and possible

elucidation of their functional significance (if any) is an area for future research.

Cholesterol and carbohydrate are not considered to be energy sources of any significance in trout eggs as they form only 0.2% and 0.3% of the dry weight respectively (Smith, 1952).

The effects of ascorbic acid supplementation in broodstock feeds on the reproduction of rainbow trout were investigated by Sandnes et al (1984). Two experimental diets differing in supplementation of ascorbic acid and a third commercial diet used for comparison were fed to rainbow trout broodstock. The supplementation of ascorbic acid significantly increased the number of eggs which hatched compared to the eggs from fish without dietary ascorbic acid supplementation. However, fish fed on the commercial diet gave eggs of similar quality as those from the ascorbic acid supplemented experimental food.

Ridelman (1981) noted that broodstock rainbow trout fed diets which were fortified with vitamins C and E produced batches of eggs of higher quality than those from control groups.

It is clear that rainbow trout eggs are composed of many different components. Several components have been implicated as being involved in egg quality determinations but no firm conclusions have been made. This Chapter aims to a) examine the major components of rainbow trout eggs, and their constitutent parts, from four strains of rainbow trout fed on the same

commercial diet and b) study the correlations between levels of these components and egg survival.

5.2 Materials and methods

All analyses were performed on fertilised, water-hardened eggs which contain a water filled perivitelline space. The eggs were stripped from ripe two year old females maintained on a commercial fish farm in 10°C borehole water.

As the time of expected spawning approached the fish were examined every 10 days to ensure that the stripped eggs from each fish were at equivalent states of ripeness. Sorting the broodstock with this periodicity avoids the problems of overripening which ovulated eggs undergo if retained unfertilised in the abdominal cavity for periods of time longer than 10 days (Nomura et al, 1974: Springate et al, 1984). The fish were fed with a commercially available broodstock food (Table 5.1). During the peak spawning period for each strain 7 to 10 ripe fish were randomly selected and the eggs manually expressed from them. The eggs were fertilised and incubated according to standard procedures (Table 2.1). The eggs were fertilised immediately after stripping using milt from the same stock. In order to counter the possibility that a single male might be sterile or otherwise reproductively incompetent, on each date of stripping the milt from six males were mixed and appropriate amounts of the sperm pool were used for each egg batch. Freshly fertilised water-hardened eggs were stored at -20°C until analyses.

Gross levels of protein, fat and ash and their respective components were all determined as described in the General Materials and Methods Chapter.

All percentages were arcsine transformed prior to statistical analyses. All differences between means were tested for significance by the students 't' test and all regression analyses were performed by the method of least squares.

Vitellogenin levels in single eggs were measured by radioimmunoassay in conjunction with Dr. J. Sumpter of Brunel University. The antibody used cross reacted with both vitellogenin and lipovitellin.

Vitellogenin Radioimmunoassay (RIA)

R.I.A. Buffer	Phosphate buffered saline (0.05M NaP, 0.15 M NaCl) contain 1% egg albumin; 0.1% NaN_3 (pH 7.0)
Standard	Intact purified trout vitellogenin
1st Antibody	Anti-vitellogenin (R2B8) at 1:50,000 made up in 1:400 normal rabbit serum

Normal rabbit Serum (N.R.S.)	Diluted 1:400 in R.I.A. buffer
2nd Antibody	Anti rabbit gamma globulin, diluted 1:20 with R.I.A. buffer
Label	I^{125} vitellogenin sufficient to give approximately 25,000 c.p.m.

Procedure

1. prepare total counts in triplicate:- 50 µl buffer, mixed with 50µl label

2. Prepare non-specific binding tubes (NSB) in triplicate:- 50 µl buffer mixed with 50 µl N.R.S. and 50 µl label

3. Prepare maximum binding tubes in triplicate:- 50 µl buffer; 50 µl of 1st antibody mixed with 50 µl label.

4. Prepare standards in triplicate from 1000 $ng.ml^{-1}$ downwards by serial dilution in buffer.

5. To all unknown assay tubes add 50µl of unknown sample (one egg homogenized in 1ml of R.I.A. buffer and diluted to fit the standard range) in duplicate.

6. Add 50µl of the 1st antibody and 50µl label to all assay tubes (standards and unknowns).

7. Vortex mix and incubate for five hours at room temperature.

8. Add 50 µl of 2nd antibody.

9. Vortex mix and incubate for at least 18 hours at 4°C.

10. Centrifuge at 3000 x g for 30 minutes at 4°C

11. Aspirate supernatants - not total count tubes.

12. Count all precipitates - for 100 seconds to obtain C.P.M.

Calculations

Determine maximum binding i.e. mean of maximum binding tubes less mean of non-specific binding tubes. Correct all standards and unknowns for non-specific binding; determine mean values. Plot log. ng vitellogenin standard against % maximum binding. Read unknown samples from standard curve. Express results as % vitellogenin of wet weight of the egg.

5.3 Results

The analyses of the data revealed that there were no significant differences ($P > 0.05$) between the proximate analyses and egg quality for the three strains (Table 5.3). There were

no significant correlations ($P>0.05$) between egg survival and egg composition at the three developmental stages considered (fertilisation, eying and hatch) or eggs from individual fish of the same strain.

Table 5.3 Proximate analysis and survival rates of eggs from three strains of rainbow trout

	Caribou			Grampian			Whitebrook		
	mean	± SEM	n	mean	± SEM	n	mean	± SEM	n
Wet Wt (mg	36.7	1.3	10	43.5	2.4	10	44.1	2.9	7
Dry Wt (mg)	15.0	0.6	10	14.5	1.1	10	16.2	1.1	7
% Water	59.2	0.5	10	66.8	0.9	10	63.1	0.4	7
Ova Diam (mm)	4.05	0.03	10	4.10	0.06	10	4.15	0.07	7
Dry weight									
% Protein	65.8	2.1	7	66.6	1.7	8	69.6	1.2	8
% Fat	10.8	0.4	5	9.4	0.3	8	9.7	0.5	7
% Ash	3.6	0.2	3	3.6	0.3	3	3.7	0.2	3
Survival rates									
% Fert	80	10	10	86	4	10	80	11	8
% Eye	46	11	10	19	9	10	57	9	7
% Hatch	41	10	10	15	9	10	53	9	7
Parent size									
Weight (gms)	953	41	10	580	26	7	703	33	7
Length (cm)	43.6	0.5	10	39.5	0.5	7	39.5	0.5	7

There were significant differences ($P<0.005$) between 14 of the 16 free amino acids measured in eggs from the three strains of rainbow trout. There were no significant ($P>0.05$) differences between the levels of threonine and glycine (Table 5.4). Correlation analyses revealed no significant correlation ($P>0.05$) between amino acid content of the eggs and survival to fertilisation, eying and hatch from different strains of fish or between eggs from different fish of the same strain.

Table 5.4 Free amino acids (nmol g^{-1} dry wt egg x 10^3) of eggs from three strains of rainbow trout (Mean ± SEM)

	Caribou	Grampian	Danish
ASP	18.5 ± 0.2	20.4 ± 0.2	17.8 ± 0.1
THR	14.0 ± 0.4	13.1 ± 0.1	12.6 ± 1.2
SER	15.9 ± 0.1	16.8 ± 0.1	15.8 ± 0.1
GLU	22.3 ± 0.3	24.2 ± 0.1	22.4 ± 0.4
PRO	14.7 ± 0.7	15.8 ± 0.0	12.3 ± 0.8
GLY	12.4 ± 0.1	11.6 ± 0.3	13.1 ± 1.0
ALA	26.1 ± 0.1	25.9 ± 0.2	24.6 ± 0.3
VAL	17.6 ± 0.1	16.0 ± 0.3	16.3 ± 0.0
MET	5.5 ± 0.0	6.6 ± 0.2	5.6 ± 0.2
ILE	11.6 ± 0.2	12.8 ± 0.1	12.4 ± 0.1
LEU	18.3 ± 0.1	19.9 ± 0.4	18.0 ± 0.2
TYR	6.3 ± 0.0	7.7 ± 0.1	6.3 ± 0.0
PHE	8.5 ± 0.0	7.9 ± 0.3	8.4 ± 0.1
HIS	4.9 ± 0.0	5.1 ± 0.0	4.7 ± 0.1
LYS	15.6 ± 0.0	16.0 ± 0.1	16.3 ± 0.1
NH 3	20.1 ± 0.1	19.9 ± 0.1	18.8 ± 0.1
ARG	10.3 ± 0.0 (n = 6)	8.3 ± 0.1 (n = 6)	10.6 ± 0.1 (n = 6)

Analyses of yolk proteins (vitellogenin and lipovitellin) revealed no significant differences between strains or between eggs from fish of the same strain with different survival characteristics (Tables 5.5 and 5.6).

The mean vitellogenin content for the Grampian eggs (n=152) expressed as a percentage of the wet weight of the egg was 25.8% which was not significantly different from the sum of the protein and fat content of the eggs expressed as wet weight (22.2% protein + 3.1% fat = 25.3%) (See Tables 5.3 & 5.6).

There were differences between the vitellogenin levels in the two assays but this is part of the normal intra-assay variation (John Sumpter personal communication).

Table 5.5 Vitellogenin levels (% of wet weight for individual eggs; mean ± SEM)

Strain	Age	Vitellogenin Mean ± SEM
Grampian	2	49.9 ± 2.9
Cloan	5	43.5 ± 1.1
Winthrop	3	41.0 ± 6.0
Winthrop	2	46.2 ± 12.4
Whitebrook	2	47.2 ± 8.6
Caribou	2	52.9 ± 4.2

n = 5

Table 5.6 Vitellogenin levels (% vg of wet weight) for individual eggs; mean ± SEM

Strain	Fish No.	Vitellogenin Mean ± SEM	n
Grampian	339	24.7 ± 0.7	39
"	344	22.8 ± 1.1	38
"	341	22.3 ± 0.9	9
"	342	30.5 ± 2.3	10
"	343	21.8 ± 1.3	8
"	347	21.6 ± 1.2	9
"	340	29.3 ± 1.9	10
"	345	32.3 ± 3.5	10
"	338	33.1 ± 4.6	9
"	346	30.7 ± 1.6	10
Beulah	191	31.2 ± 5.2	10
"	277	25.2 ± 1.7	10
"	pool	34.6 ± 4.0	19

There were significant differences ($P<0.05$) between the free fatty acids 16:1, 18:2 and 20:5 between the two strains of rainbow trout considered (Table 5.7). Correlation analyses revealed no significant difference ($P>0.05$) between free fatty acid content of the eggs and egg survival from the two strains or between eggs from individual fish of the same strain

Table 5.7 Free fatty acids (relative percentages of total in two strains of rainbow trout; mean ± SEM)

Trivial Name	Chain Length: no double bonds	Position of double bond	Caribou	Grampian
MYRISTIC	14:0		2.4±0.0	2.2±0.2
PALMITOIC	16:1	n-7	9.3±0.1	7.2±0.7
STEARIC	18.0		4.0±0.1	4.6±0.2
OLEIC	18:1	n-9	26.5±1.0	25.4±0.9
LINOLEIC	18:2	n-6	7.7±0.1	8.1±0.2
LINOLENIC	18:3	n-3	0.8±0.1	0.7±0.1
GONDOIC	20:1	n-9	2.6±0.2	2.7±0.1
HOMOLINOLENIC	20:3	n-6	1.4±0.0	1.3±0.1
ARACHIDONIC	20:4	n-6	1.4±0.2	1.1±0.1
-	20:5	n-3	3.1±0.1	3.6±0.2
-	22:5	n-3	0.9±0.1	1.0±0.2
-	22:6	n-3	18.7±0.6	19.1±0.8
			n=6	n=6

There were significant differences between the mineral composition of the four strains of rainbow trout considered for the elements calcium, zinc, iron, copper ($P<0.001$) and potassium ($P<0.01$). There were no significant differences between the levels of magnesium and sodium ($P>0.05$). Manganese was only present in trace quantities (Table 5.8). However, correlation analyses revealed no significant correlation ($P>0.05$) between mineral content of the eggs from different strains of fish or between eggs from individual fish of the same strain and survival to fertilisation, eying and hatch.

Table 5.8 Mineral composition of eggs from four strains of rainbow trout (μg/mg.g $^{-1}$ dry weight; Mean ± SEM)

Element	Caribou	Whitebrook	Grampian	Winthrop*
K (mg g^{-1})	4.6±0.1	4.5±0.2	4.3±0.2	5.1±0.0
Ca (mg g^{-1})	1.2±0.0	1.0±0.1	1.1±0.0	1.0±0.0
Mg (mg g^{-1})	1.2±0.0	1.1±0.1	1.1±0.0	1.1±0.0
Na (mg g^{-1})	1.1±0.2	1.2±0.2	0.8±0.1	0.8±0.0
Zn (μg.g^{-1})	49.9±1.7	59.0±3.3	47.5±1.7	60.2±3.3
Fe (μg g^{-1})	35.2±1.2	28.9±1.0	29.6±1.8	32.7±1.7
Cu (μg g^{-1})	11.2±0.4	7.3±0.3	7.5±0.2	10.3±0.4
Mn (μg g^{-1})	Trace n=10	Trace n=11	Trace n=24	Trace n=11

* Fish from this strain on a different farm to other three

5.4 Discussion

The results of this study clearly show that there is variation between certain chemical components of rainbow trout eggs from fish of different strains and between individual fish of the same strain. The variation in gross biochemical composition appears to be a regular feature of salmonid eggs. These individual differences in egg composition are probably the biochemical counterpart of the well-documented variation between female teleosts in egg size and fecundity (Craik and Harvey, 1984b).

The heterogeneity in egg composition recorded in this study is unlikely to have been caused by differences in nutrition, because all the fish were fed with identical food at the same ration level. Commercial broodstock diets are formulated as a complete food and are supplemented with essential fatty acids, amino acids, trace elements, vitamins and carotenoids and therefore deficiency of one or more of these factors is unlikely to be the cause of the variation seen in egg quality. It is suggested that the variation in chemical composition between different batches of eggs must be attributed to experimental, genetic or environmental variation.

This work also shows that, within the stated experimental conditions, these variations in egg composition between females bear little significant correlation to the large variation in egg quality. These findings contrast with those of Hirao *et al* (1954, 1955) who in a similar series of experiments demonstrated

a positive correlation between hatching percentage and egg iron content, and Satia et al (1974) who found a positive correlation between levels of protein and egg survival.

The results agree well with the findings of Ridelman (1981), Craik and Harvey (1984b), and Torrissen (1984) who also found no significant correlation between egg survival and selected components of egg composition.

Craik and Harvey (1984b) concluded that the timing of stripping of eggs in relation to the date of ovulation is the major determinant of egg quality. However, in the present study all fish were sorted every 10 days to avoid the problems of overripening (Chapter 9; Nomura et al, 1974; Springate et al, 1984).

Possibly some minor component of rainbow trout eggs, as yet not fully investigated, may be responsible for variations in egg quality. These minor components need to be studied in great detail. The examination of the biochemical constituents carried out in this study will serve as a baseline for future work.

SUMMARY AND CONCLUSIONS TO PART I

The experiments in this Part of the thesis have examined egg quality under a number of different conditions. Egg quality has been shown to be highly variable in the laboratory and commercial situation with fertilisation success ranging from 0 to 100%, despite the maintenance of groups of broodstook and aliquots of eggs under broadly equivalent conditions. Mean survival to eying was found to be less than 60% under commercial conditions whilst under the more favourable condition of the laboratory, mean survivals to eying were in excess of 85%

Considering the large variation in egg quality an investigation was made of the relationships between egg quality and the size and composition of the eggs. Previous work has suggested that large eggs are of higher quality than smaller ones (Gall, 1974; Pitman, 1979). This was not confirmed in the present study, although large eggs did initially produce larger fry than smaller eggs. This size advantage was soon lost and furthermore no correlation between egg size and egg or fry survival was found. Similar findings were reported by Glebe et al (1979) and Thorpe et al (1984) both working with Atlantic salmon stocks.

In the final Chapter of Part I the chemical composition of rainbow trout eggs was described. Despite large variations in the composition of eggs from different strains and also from different individuals of the same strain, there were no significant correlations between the individual components of

the eggs and their survival. Similar results have also been reported by Ridelman (1981) and Craik and Harvey (1984b) both working with rainbow trout.

To conclude it would appear that the large variation observed in egg quality cannot be explained in terms of egg size or any of the major chemical components of the eggs. These findings agree well with those of Phillips and Dumas (1959) who conclude that egg size was not necessarily a criterion for egg quality, and that all other factors being equal, there are sufficient materials even in the smallest eggs to produce normal fry.

It is suggested that one of the minor components of rainbow trout eggs e.g. vitamins or specific enzymes may affect egg quality although there is little evidence to support this in the literature. Possibly of more significance are genetic, environmental and husbandry factors all of which may have profound effects on egg quality. Some aspects of rainbow trout husbandry will be considered in part III of this thesis.

PART II

Fecundity

PART II

General introduction to fecundity

Many studies have been made of the fecundity of wild stocks of salmonids, but surprisingly little attention has been paid to commercial broodstocks. This part of the thesis considers fecundity of domesticated stocks of female rainbow trout. Little is known about the factors which determine the number of eggs which will be produced, although only modest improvements in fecundity, for example 10%, would increase production figures for average size hatcheries by one to two million eggs per year. Possible factors which may significantly influence the fecundity of stocks of rainbow trout are strain, age and size of females and also environmental factors.

There are many definitions of fecundity. Total or absolute fecundity, is usually defined as the number of ripening eggs found in the female just prior to spawning. This contrasts with fertility, which is the number of eggs shed (Bagenal, 1978). Both are difficult to determine in commercial stocks of rainbow trout, because oviposition or natural release of eggs from the body does not occur and sacrifice of valuable broodstock before spawning is not feasible. In this study total fecundity is defined as the number of eggs manually stripped from a fish after ovulation. However, commercial pressures often did not allow the females to be 'second stripped' and consequently most of the fecundity measurements were derived from an estimation of the eggs from a single manual stripping. Therefore, it is to be

expected that some eggs were left in the ovaries (Nicholls, 1958; Pope et al, 1961; Bulkley, 1967), and therefore the figures obtained are likley to be somewhat lower than those which would be expected if the fish had been sacrificed and all the eggs removed. Although Bagenal (1978) reports that nearly all spent fish have some residual eggs after spawning and therefore it may not be unreasonable to estimate fecundity from a single manual stripping.

Total fecundity has been shown to increase with size and age of female rainbow trout (Rounsfell, 1957; Gall, 1974; Baiz, 1978) so in order to compare fecundities of fish of different size or different places, many authors calculate the relative fecundity, which is the number of eggs unit weight^{-1} (usually Kg). Although relative fecundity does change with the size and age of the parent it is the most useful commercial index of the reproductive capabilities of the broodstock as it provides a measure of the number of eggs that might be produced by the total weight of fish on the farm (Springate and Bromage, 1984b). The farmers ultimate goal must be to produce the maximum number of the highest quality eggs from each tonne of broodstock.

Values of relative fecundity have been obtained for a large number of species and have not surprisingly been found to be species dependent. Many marine fish for example produce 500,000 egg kg^{-1} body weight of fish, common carp approximately 200,000, pike 25,000 whereas salmonids produce only 1,000-3,000 depending on the species, age, size and strain of broodstock.

In addition to the wide range of fecundities there are highly significant differences in egg sizes between different species of fish. The volume of an average rainbow trout egg, for instance, is approximately 125 times greater than that of a carp egg (Springate and Bromage, 1984b).

The difference in size of eggs between individuals of the same species is of great importance because, aside from genetic influences, ultimately it is the food on which the parent fish is fed and her growth which determines egg size, and food, at 40% of total production costs, is the single most important item on the fish farmer's budget.

Egg size and fecundity, together with fish size and growth, comprise a complex of interrelated characters. Larger and older salmonids have higher total fecundities and produce larger eggs than younger and smaller fish (Rounsfell, 1957; Pope et al, 1961; Nomura, 1963; Bulkley, 1967; Gibson et al, 1976; Baiz, 1978; Thorpe et al, 1984). However, larger fish have lower relative fecundities, i.e. for a fixed ovarian biomass total fecundity has an inverse relationship with egg size (Miller, 1984).

Fecundity is usually found to be closely correlated with fish length (Bagenal, 1978). A great number of workers have plotted fecundity and fish length and have concluded that the relationship is of the form:-

$$T.F. = a\ L^b$$

where T.F. = total fecundity, L = fish length and a and b are constants derived from the data. A logarithmic (log.) transformation gives the straight line regression of log. total fecundity on log. length.

$$\log. T.F. = \log. a + b \log. L$$

The line may be fitted by the method of least squares, allowing the subsequent use of standard statistical procedures. The transformation of data tends to equalise the variance throughout the range of lengths thereby avoiding the problem that the fecundities of large fish are more variable than those of smaller ones (Pope et al, 1961).

It is often considered that since fish weight is connected with the condition of the fish, fecundity is more likely to be closely correlated with weight than with length (Bagenal, 1978). Indeed Bulkley (1967) studying steelhead trout found a better correlation for fecundity with weight (r = 0.81) than with length (r = 0.77) i.e. 65% of the variation in fecundity was associated with weight whereas only 59% was associated with length.

If fish weight is to be correlated with fecundity then somatic weight must be used because if total weight (somatic plus gonad) is used a spurious correlation may be obtained since the greater number of eggs in the more fecund fish will increase

the overall weight more than those of the less fecund fish. In addition because of the applied nature of the research programme, fish weight was considered as being the most important parameter as commercially it is the number of eggs tonne^{-1} of fish which is all important.

Older fish are usually, but not always, larger; hence egg number and age are not always so clearly correlated (Thorpe et al, 1984). It was difficult to study age effects on commercial farms because it is common practice to maintain broodstock of different ages in the same tanks or ponds. The age of these fish cannot be distinguished by the usual methods of scale observation because firstly the scales from the broodfish on farms with constant temperature, subterranean, water do not have distinct growth rings and secondly commercial broodstock are too valuable to damage by scale removal especially at spawning, when the fish are easily stressed. Age can be estimated by fish size but this is not reliable as there is usually some overlap in ranges of size of different age groups of fish. However, some data on different age groups were available where farmers maintained the different age classes separately and these data will be presented. Of particular interest are first spawning fish which have a very high relative fecundity but as a possible consequence their eggs are very small.

Fecundity studies which have included observations of individual fish of the same weight and length have demonstrated a large variation in fecundity of these fish (Nomura, 1963; Bagenal, 1973). The fecundity of individuals of comparable size

varying considerably from one locality to another has been demonstrated (Bulkley, 1967) and also year-to-year variation in fecundities of wild stocks has been shown (Gerking, 1959). These variations in fecundity are usually explained on the basis of dietary and genetic differences. The year-to-year variation of wild stocks has been attributed to differences in the availability of food although under commercial conditions feed rates remain constant from year-to-year. The variation in fecundity of fish of similar size but from different localities implies that heredity is very important in determining fecundity and that there may be 'strain' differences in fecundity. Both dietary and genetic differences undoubtedly account for the fluctuations in fecundity between individuals whose size is similar. The effects of ration on fecundity will be considered in Chapter 9 and the effects of strain of broodstock, and locality on fecundity in Chapter 7.

The internal mechanism which controls alterations in egg number in inadequately fed fish is probably follicular atresia. It has been shown that the nutritional status of the fish may directly affect the maturation and vitellogenesis of oocytes and diet restriction may result in atresia of oocytes (Scott, 1962; Wallace and Selman, 1981). Levels of atresia caused by known diet restriction will be discussed in Chapter 9 . Elliott (1982) suggested that atresia plays no significant role in the reduction of oocytes, i.e. the fecundity, at any stage of the reproductive cycle of rainbow trout, maintained on an adequate diet under farmed conditions.

In order to optimise egg production from rainbow trout broodstocks it is essential that the relationships between female age (size) and the number of eggs produced are fully understood, bearing in mind the changes in egg size which are associated with alterations in these variables. Presently wide ranges of size and age of broodstock are maintained with no apparent managerial strategy.

The first Chapter (Chapter 6) in this Part of the thesis considers the relationship between female size, fecundity and egg size, and the second Chapter (Chapter 7) investigates the fecundity levels of some of the strains of rainbow trout currently available in the U.K.

CHAPTER 6

The relationships between female age (size), fecundity and egg size in commercial stocks of rainbow trout

6.1 Introduction

It is essential that the reproduction indices (fecundity and egg size) and their relationships with broodstock size are fully investigated so that captive broodstocks can be managed with maximum efficiency.

6.2 Analyses of large batches of eggs from commercial farms

Data collected by commercial fish farmers on batches of eggs spawned from many fish are presented in this section.

6.2.2 Materials and methods

Analyses were made on large batches of eggs from three farms (P, Q and S). Each farm had its own methods of collecting production related data and therefore each farm is considered separately.

Farm P

All the fish were weighed individually to the nearest 10g after spawning. Fish lengths were not recorded. Egg diameter was determined by aligning water-hardened eggs along a 300mm

measuring trough. Total egg numbers were determined by counting into a one litre measuring cylinder using a two-hundred-hole egg counting board, and then by direct ratio to the total volume of eggs. This farm had five strains of rainbow trout broodstock. Fish of each strain were maintained up to their fourth spawning (five years of age). Unfortunately the eggs from the different strains and age groups of broodfish (except for the two year olds) were not separately maintained during development.

Farm S

The first 10 fish of each batch were weighed to the nearest 30g. Egg numbers were determined as for Farm P. Fish lengths and ova diameters were not recorded. The farm had only one strain of fish, but three age groups (three, four and five) of broodstock which were spawned and maintained separately. Fish did not spawn at this farm until their third year.

Farm Q

Egg numbers were determined as for Farm P. The individual weights and lengths of the fish at spawning were not recorded, although the average weight of the fish from the different groups was determined prior to spawning by batch weighings. The egg diameters were estimated from the number of eggs litre^{-1} using a derived form of the Von Bayer equation (see 2.2.2). The farm had three strains which were maintained and spawned in separate strain and age classes. However, the data are presented in age classes only.

6.2.3 Results

For all farm data 'n' refers to the number of production batches of eggs with each batch consisting of the eggs from many females (up to 250). Production records for Farms Q, S and P are summarised in Table 6.1. The highest total fecundity 7983±399 (mean±SEM) was from the largest (5.16±0.16Kg) and oldest (five years old) fish held on Farm Q. These were also the largest eggs (5.49±0.03mm). These fish had the lowest relative fecundity (1555±63). The groups of fish which had the lowest total fecundity (1957±103) were from the two year old fish on Farm P. These were the smallest fish (0.73±0.05Kg) and produced the smallest eggs (4.15±0.08mm). However these fish had the highest relative fecundity (2734±144) (Table 6.1). The results from Farm Q show that the general trend was for total fecundity and egg size to increase and relative fecundity to decrease with increasing age of female (Fig. 6.1).

Table 6.1 Weight, fecundity, egg size and survival data (mean ± SEM) from production batches of eggs

Age (years)	Female (n*)	Female (Weight) (kg)	Total Fecundity (no of eggs)	Relative Fecundity (no eggs Kg^{-1})	Oocyte Diameter (mm)
Farm Q					
2	(4)	1.47±0.17	3560±403	2200±214	4.23±0.19
3	(18)	2.51±0.02	4900±163	1991± 68	4.75±0.04
4	(19)	3.23±0.18	6037±435	1850± 55	5.04±0.05
5	(23)	5.16±0.16	7983±399	1555± 63	5.49±0.03
Farm S					
3	(6)	1.10±0.12	2293±227	2118±165	n.a.
4	(6)	2.06±0.16	3493±260	1738±154	n.a.
5	(5)	2.94±0.30	4676±254	1624±102	n.a.
Farm P					
2	(9)	0.73±0.05	1957±103	2734±144	4.15±0.08
3	(16)	2.60±0.10	5664±323	2203±121	4.86±0.08

* NB These are production batches and not individual fish

n.a. Data not available

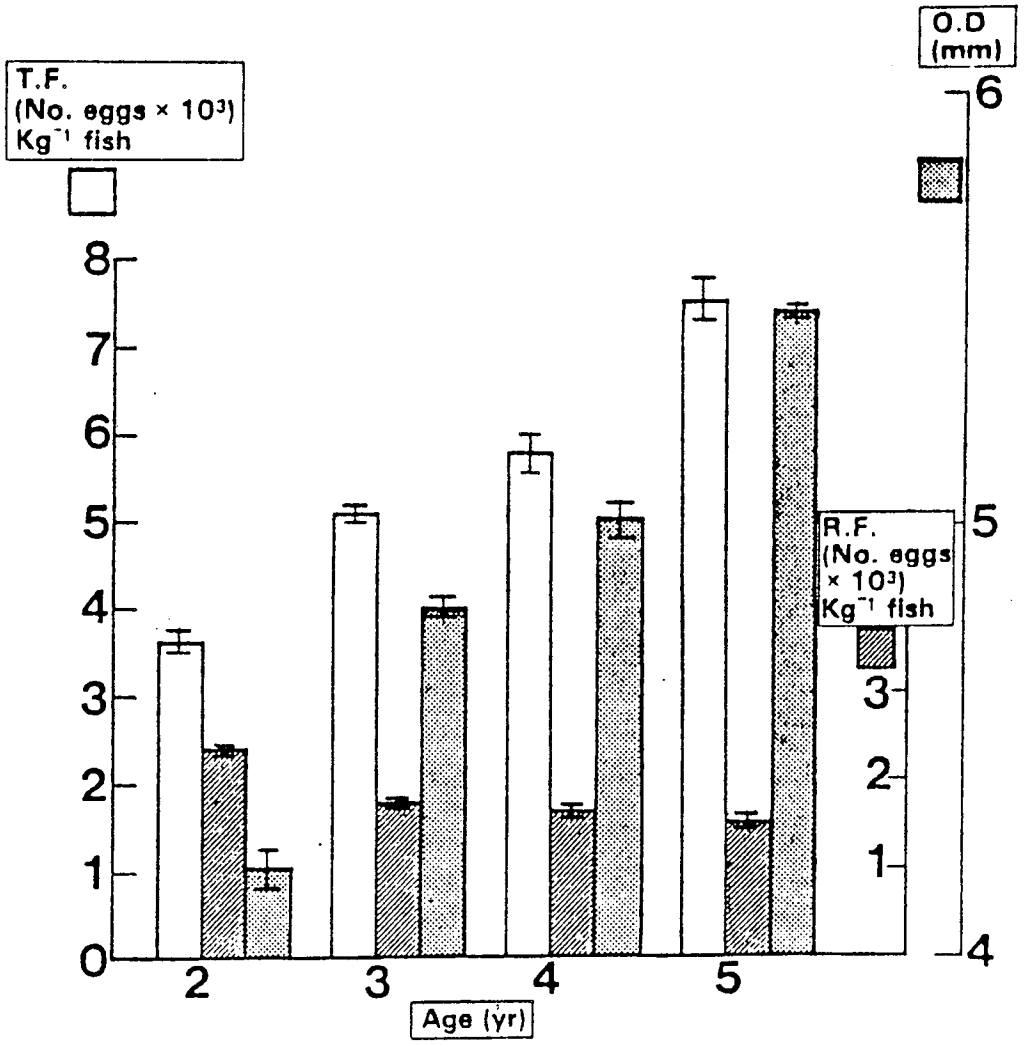


Fig. 6.1 Histogram showing the relationships between age, total fecundity (T.F.), relative fecundity (R.F.) and egg size (O.D.) for Farm Q. (Mean ± SEM).

6.3 Analyses of fecundity and egg size data from 56 individual fish

6.3.1 Introduction

Fecundity and egg size data were collected from individual females of a large size range.

6.3.2 Materials and methods

A group of 56 rainbow trout were selected at random from a broodstock (approx. 500 fish), maintained in a large lake (Farm R) until one month before expected ovulation, when they were moved to concrete raceways. The fish were three years old or older and of a single strain. Their mean post stripped weight was 4.37 Kg (range 0.95-7.70Kg) and mean length 66.2 cm (range 44.5-78.5 cm). Ripe fish were quietened by placing them in 'kicking baskets' (Chapter 8) before being stripped. All measurements of fish weight, length and egg diameter were performed as descirbed in the General Materials and Methods Chapter (Chapter 2). Regression lines were fitted by the method of least squares and the correlation coefficient (r) and variance ratio value (F) calculated for relationships between total and relative fecundity, total and relative volume of eggs, egg size and fish size. Total and relative volumes were calculated as follows:-

Total Volume (T.V.) = $(4/3\ \pi\ r^3)$ T.F.

Relative Volume (R.V.) = $(4/3\ \pi\ r^3)$ R.F.

r = ova diameter/2

6.3.3 Results

Logarithmic (log.) transformation equalizes the variance throughout the size range of broodstock examined. To illustrate this both transformed and non-transformed data are presented (Figs. 6.2.a, 6.2.b, 6.3.a, 6.3.b, 6.4, 6.5a, 6.5.b, 6.6.a, 6.6.b).

There was a significant positive correlation between total fecundity and fish weight ($r=0.45$, $P < 0.001$) (Fig. 6.2a). Logarithmic (log.) transformation slightly improved the relationships ($r=0.55$, $P < 0.001$) (Fig. 6.2b). There was a significant positive relationship between total fecundity and fish length ($r=0.47$, $P < 0.001$) (Fig. 6.3a) which was again slightly improved by log. transformation ($r=0.52$, $P < 0.001$) (Fig. 6.3b). There were significant positive correlations between egg size and fish weight both in non transformed ($r=0.53$, $P < 0.001$) (Fig. 6.4) and log. data ($r=0.58$, $P< 0.001$). There were significant negative correlations between relative fecundity and fish weight ($r=-0.50$, $P < 0.001$) (Fig. 6.5a), and after log. transformation ($r= -0.63$, $P < 0.001$) (Fig. 6.5b). The total volume of eggs produced per female was correlated with fish weight ($r=0.60$, $P < 0.001$) (Fig. 6.6a). Again this

relationship was improved by log. transformation ($r=0.74$, $P< 0.001$) (Fig. 6.6b).

There was no significant correlation between log. relative volume and log. fish weight ($r=0.08$, $P >0.05$).

Fig 6.2a

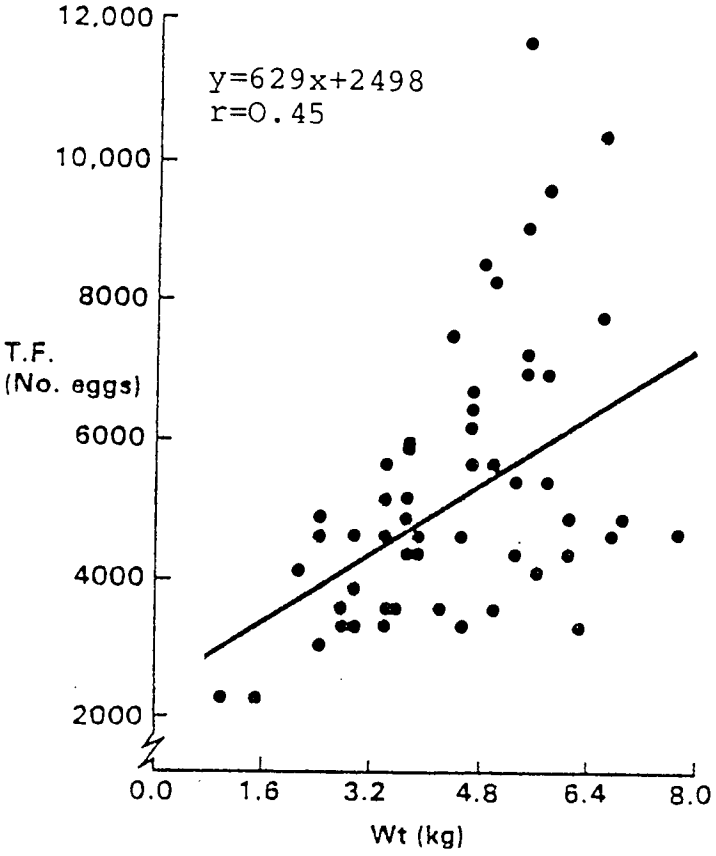
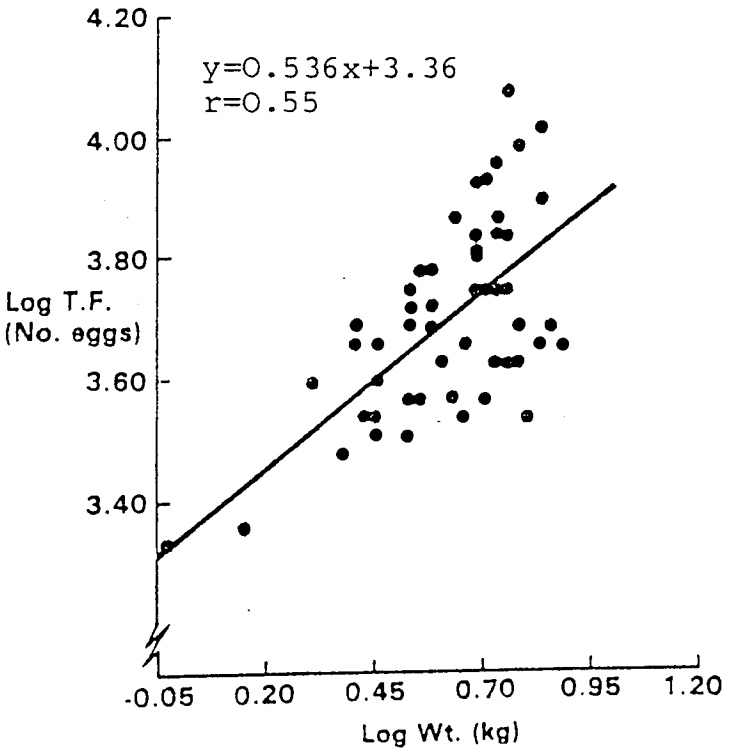


Fig. 6.2b



Figs. 6.2a & 6.2b Regressions of total fecundity (T.F.) on fish weight (Wt.). a) Raw data, b) log. transformed. n=56.

Fig. 6.3a

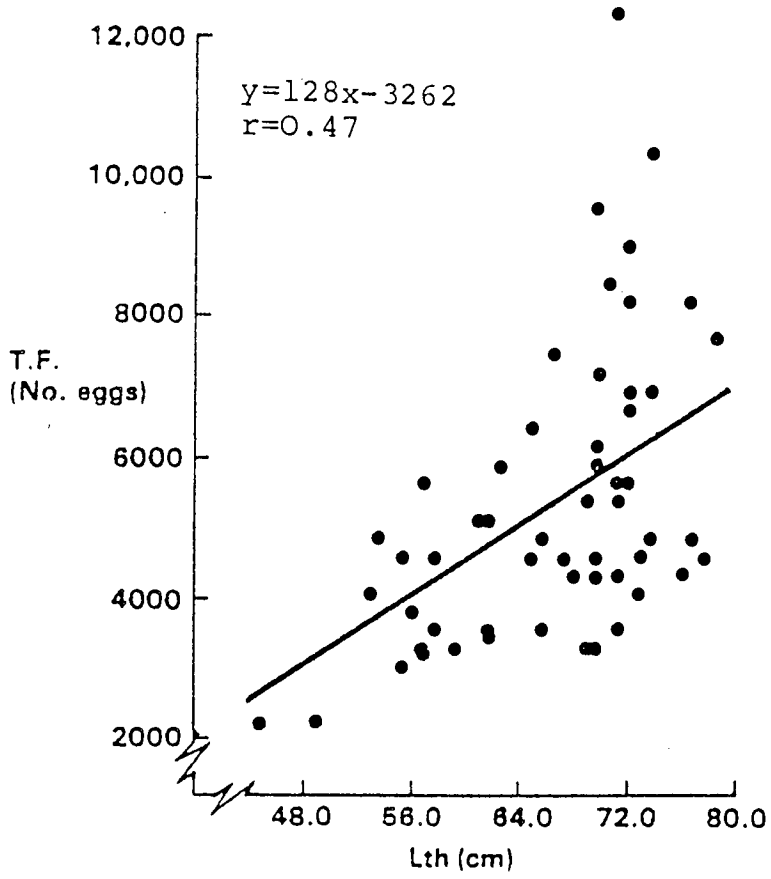
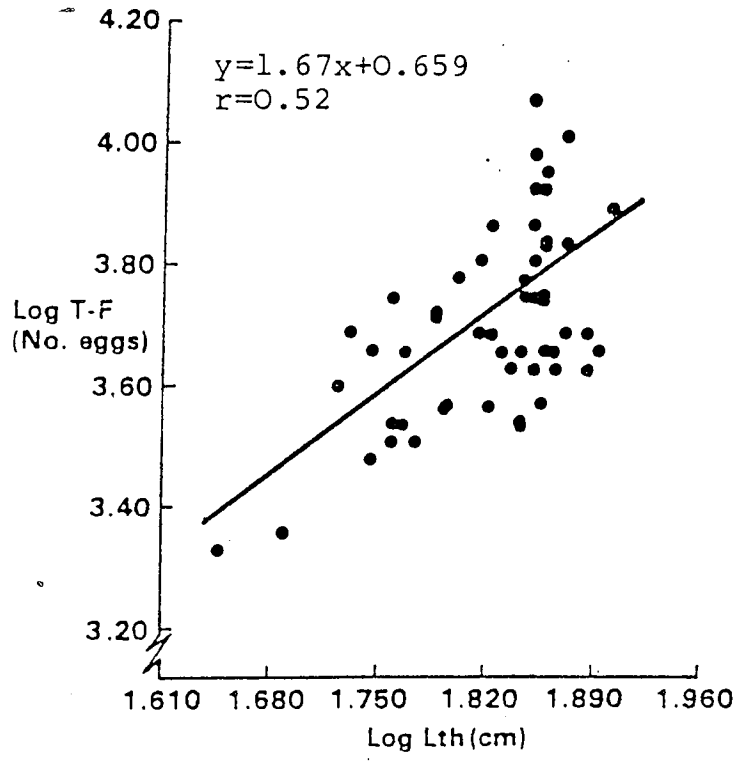


Fig. 6.3b



Figs 6.3a & 6.3b Regressions of total fecundity (T.F.) on fish length (Lth.). a) Raw data, b) log. transformed. n=56.

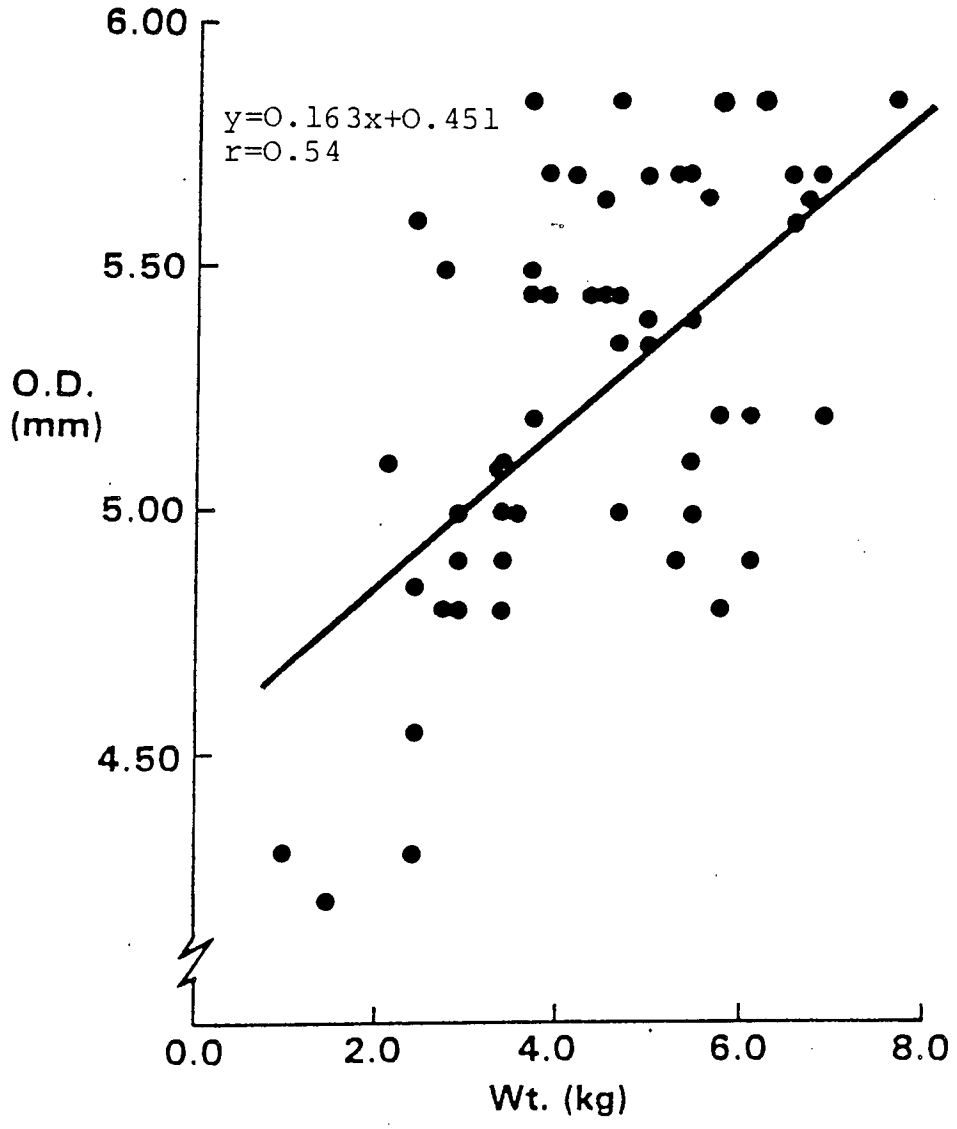


Fig 6.4 Regression of egg size (O.D.) on fish weight (Wt.).

n=56.

Fig. 6.5a

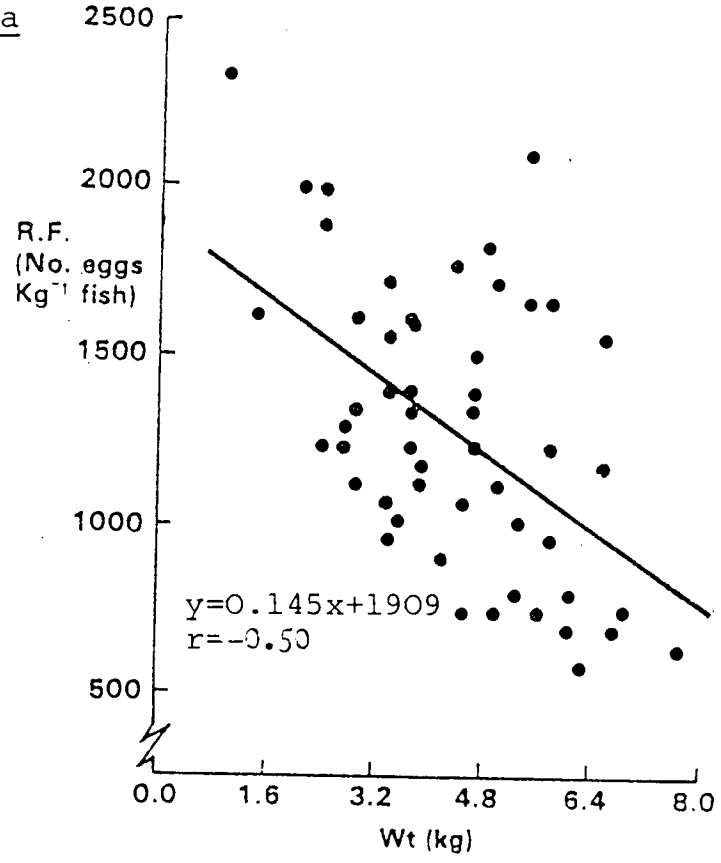
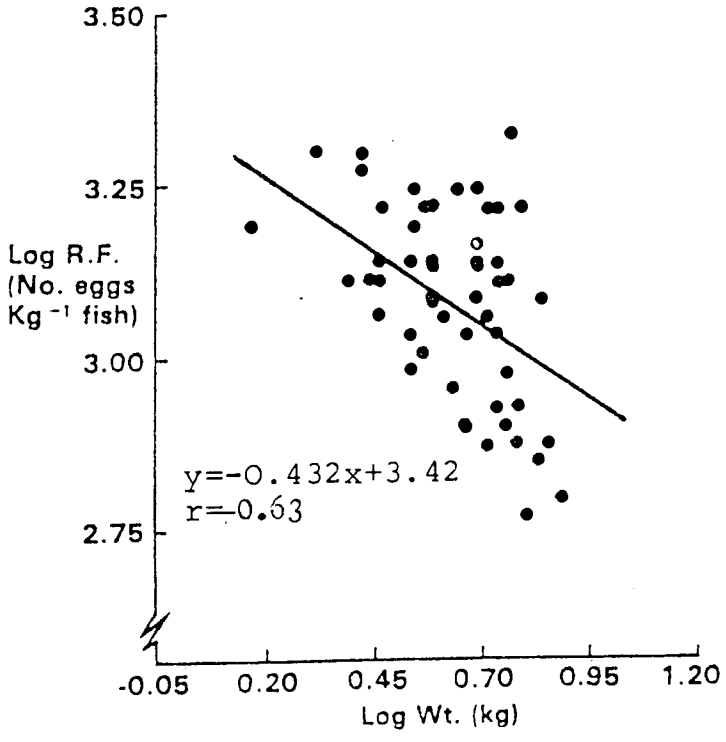


Fig 6.5b



Figs 6.5a & 6.5b Regressions of relative fecundity (R.F.) on fish weight (Wt.). a) Raw data, b) log. transformed. n=56.

Fig. 6.6a

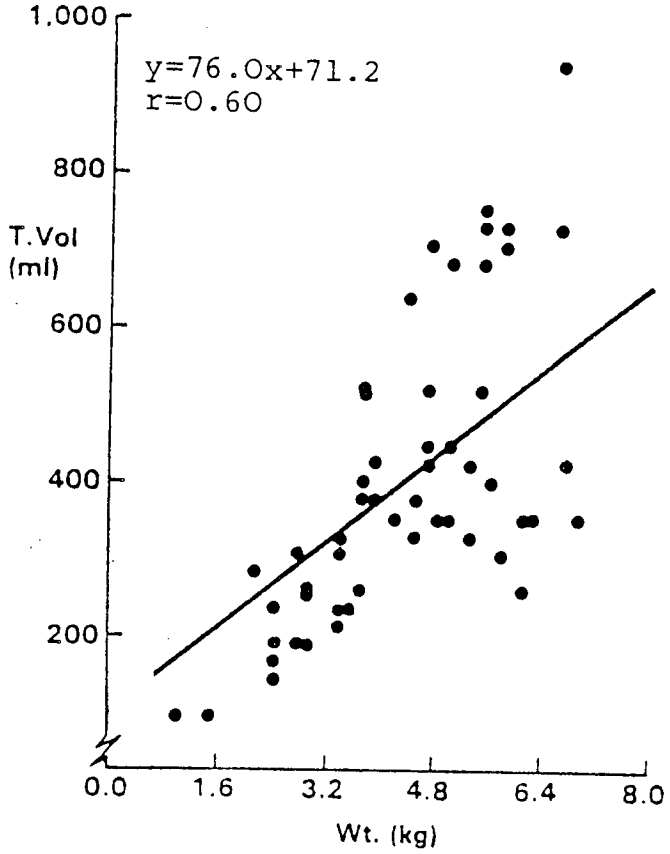
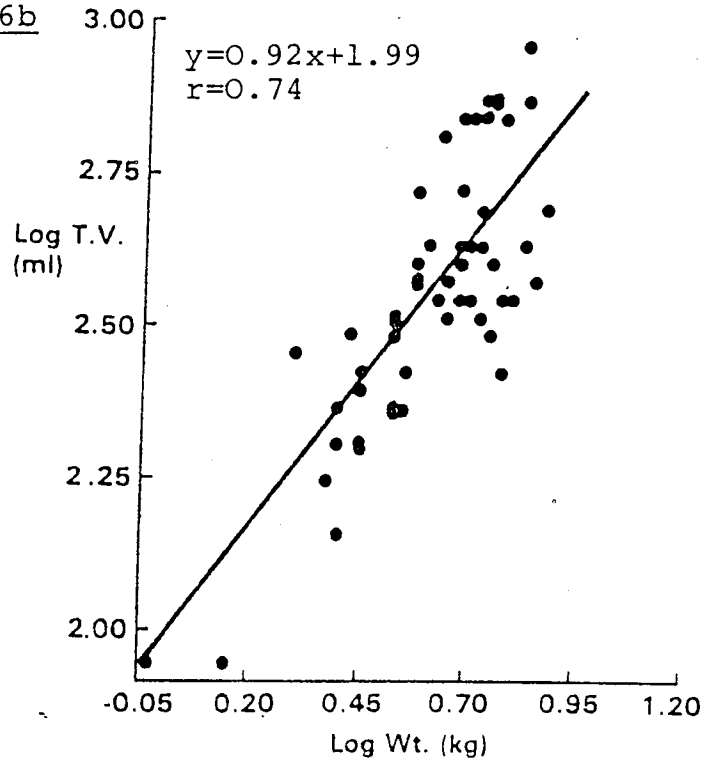


Fig. 6.6b



Figs. 6.6a & 6.6b Regressions of total egg volume (T.V.) on fish weight (Wt.). a) Raw data, b) log. transformed. n=56.

6.4 Analyses of fecundity and egg size data from 173 individual fish

6.4.1 Introduction

The aim of this study was to confirm the results of section 6.3 by considering a larger number of fish.

6.4.2 Materials and methods

Spawning data were collected from 173 fish on two farms P and R. The fish had a mean post-stripped weight of 2.93 Kg (range 0.64 - 7.70Kg) and mean length of 59.1 cm (range 39.6 - 88.0 cm). The fish were of unknown age and mixed strains. Fish were handled and measured, and the eggs measured as described in the General Materials and Methods Chapter (Chapter 2). Statistical analyses were performed as described in section 6.3.2.

6.4.3 Results

Only log. transformed data were considered because of the improvement of the fit of the regression line shown by the results of the last section on the transformed data. The results of the correlations between the log. transformed data are summarised in Table 6.2.

Table 6.2 Regression equations for fish weight and egg biometrics for 173 female rainbow trout

y		x	r	P
log. T.F.	vs	log.Wt.	0.67	<0.001
log. T.F.	vs	log.Lth.	0.64	<0.001
log. O.D.	vs	log.Wt.	0.62	<0.001
log. R.F.	vs	log.Wt.	-0.48	<0.001
log. T.V.	vs	log.Wt.	0.81	<0.001
log. R.V.	vs	log.Wt.	0.00	>0.05

See Figs. (6.7, 6.8, 6.9, 6.10 and 6.11) respectively.

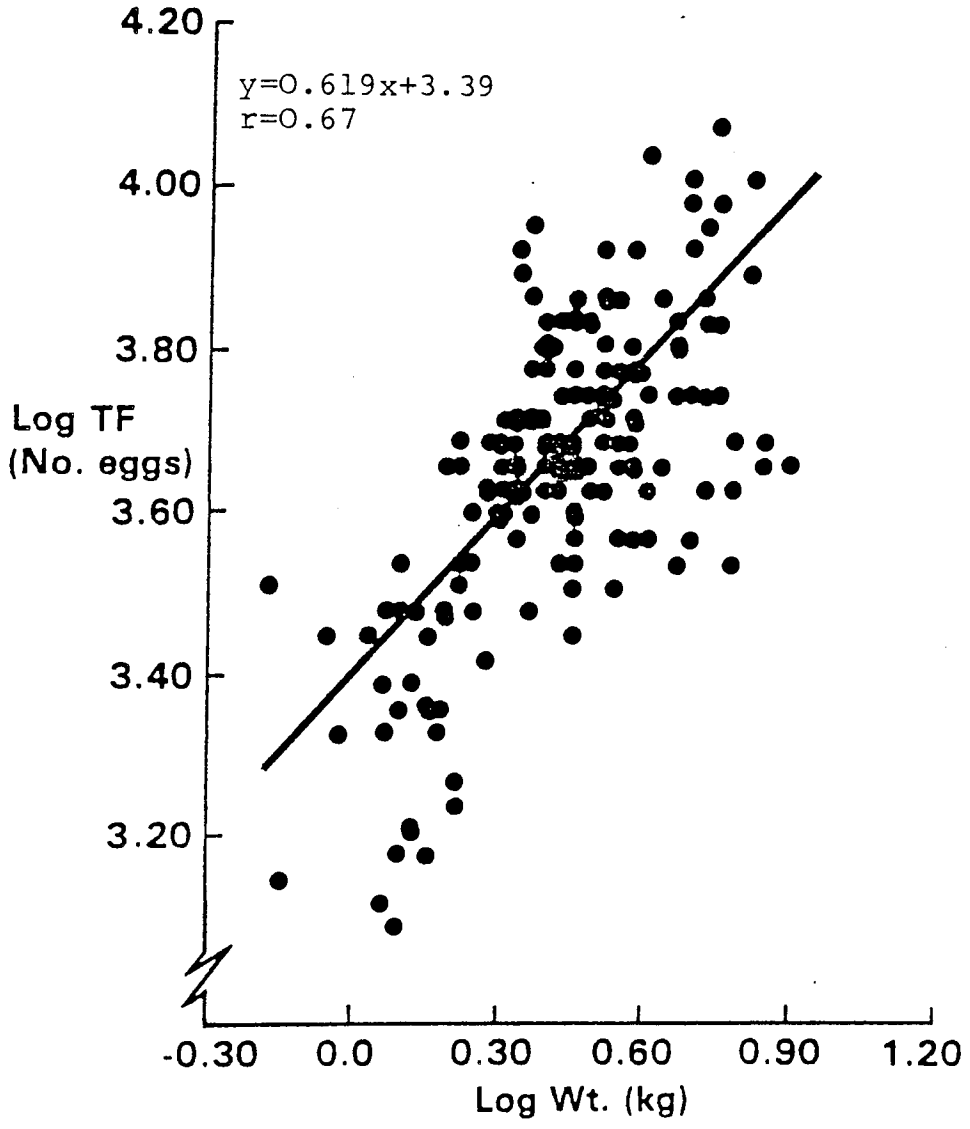


Fig. 6.7 Regression of log. total fecundity on log. fish weight (Wt.). n=173.

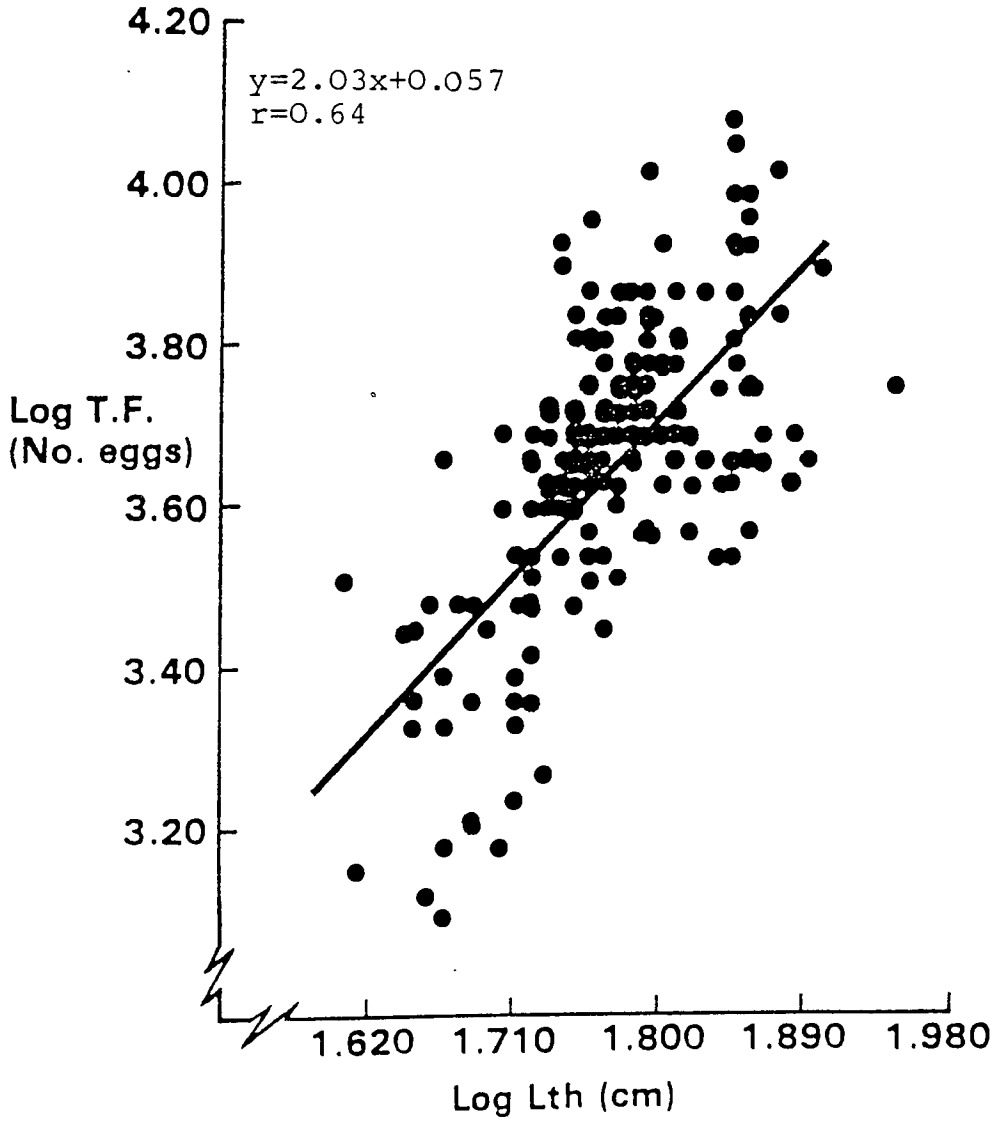


Fig. 6.8 Regression of log. total fecundity (T.F.) on log. fish length (Lth.). n=173.

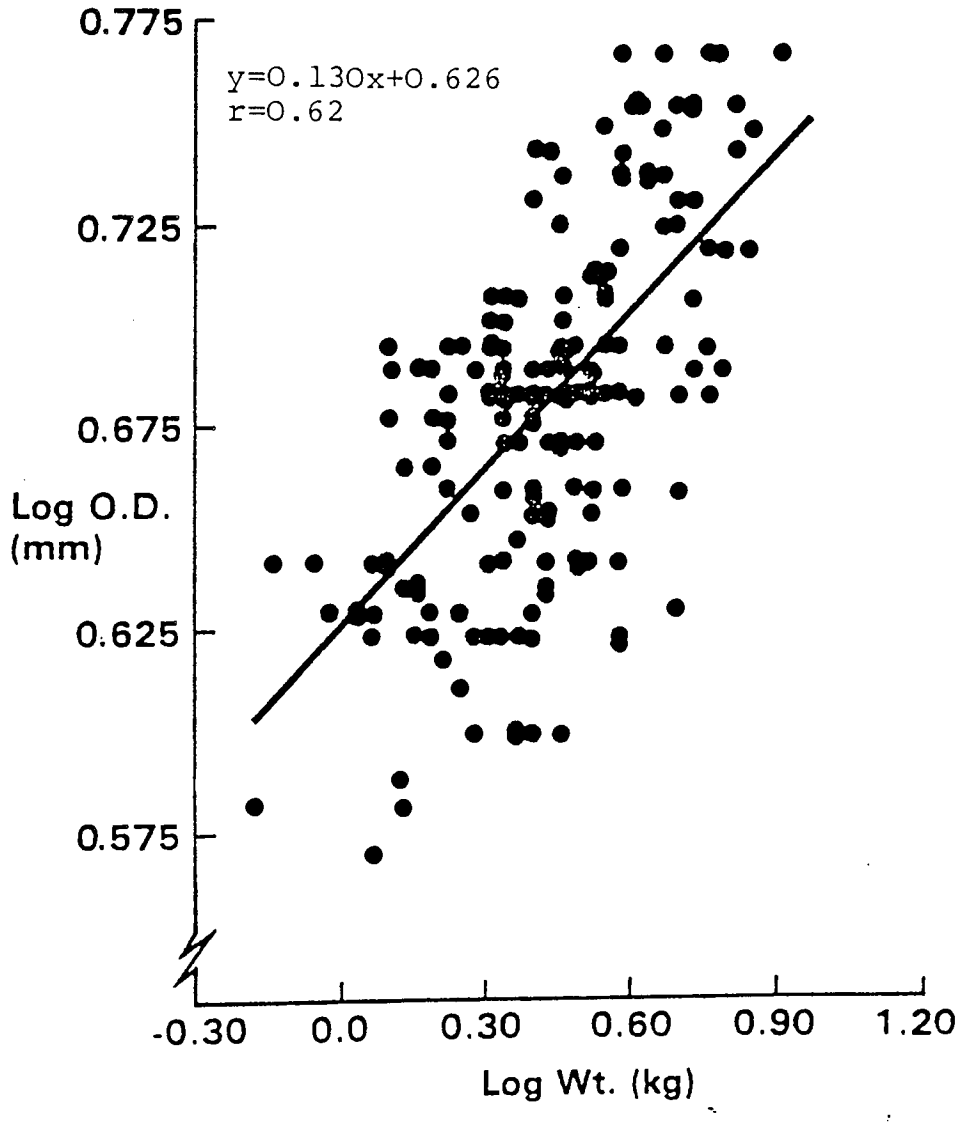


Fig. 6.9 Regression of log. egg size (O.D.) on log. fish weight (Wt.). n=173.

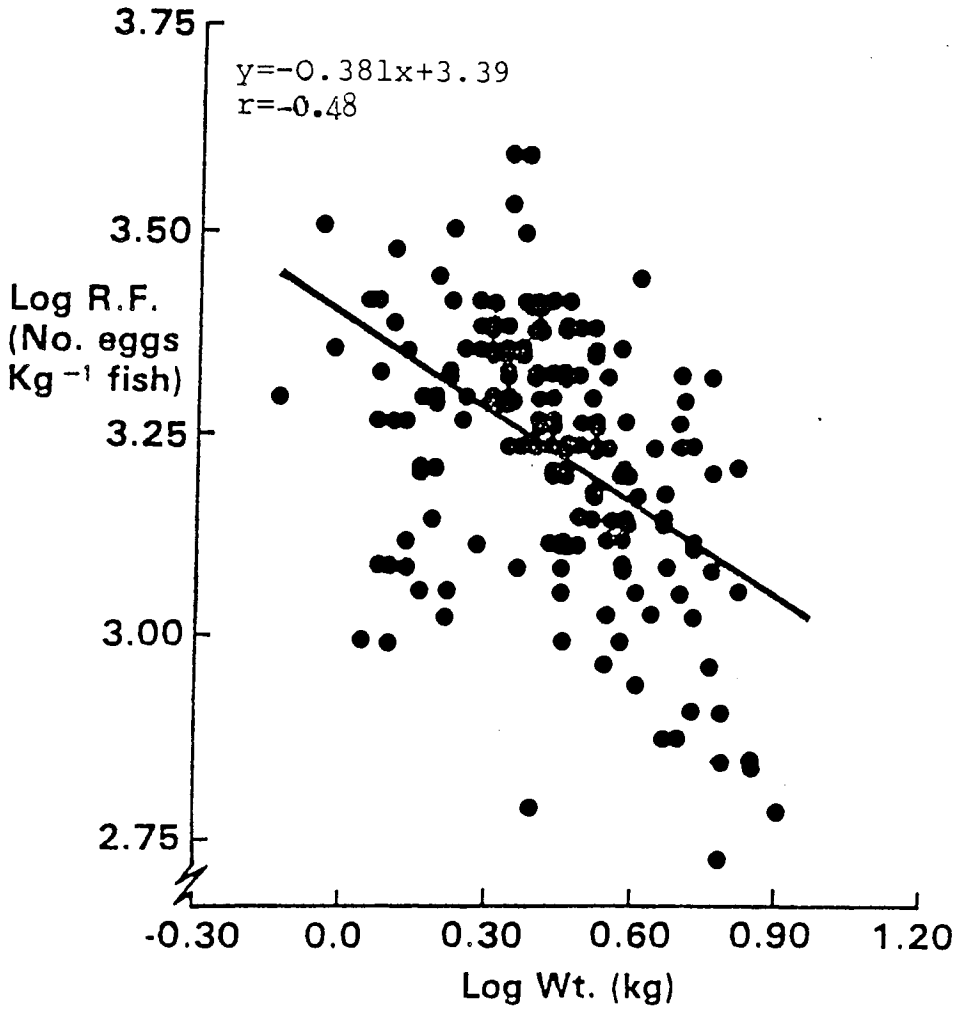


Fig. 6.10 Regression of log. relative fecundity (R.F.) on log. fish weight (Wt.). n=173.

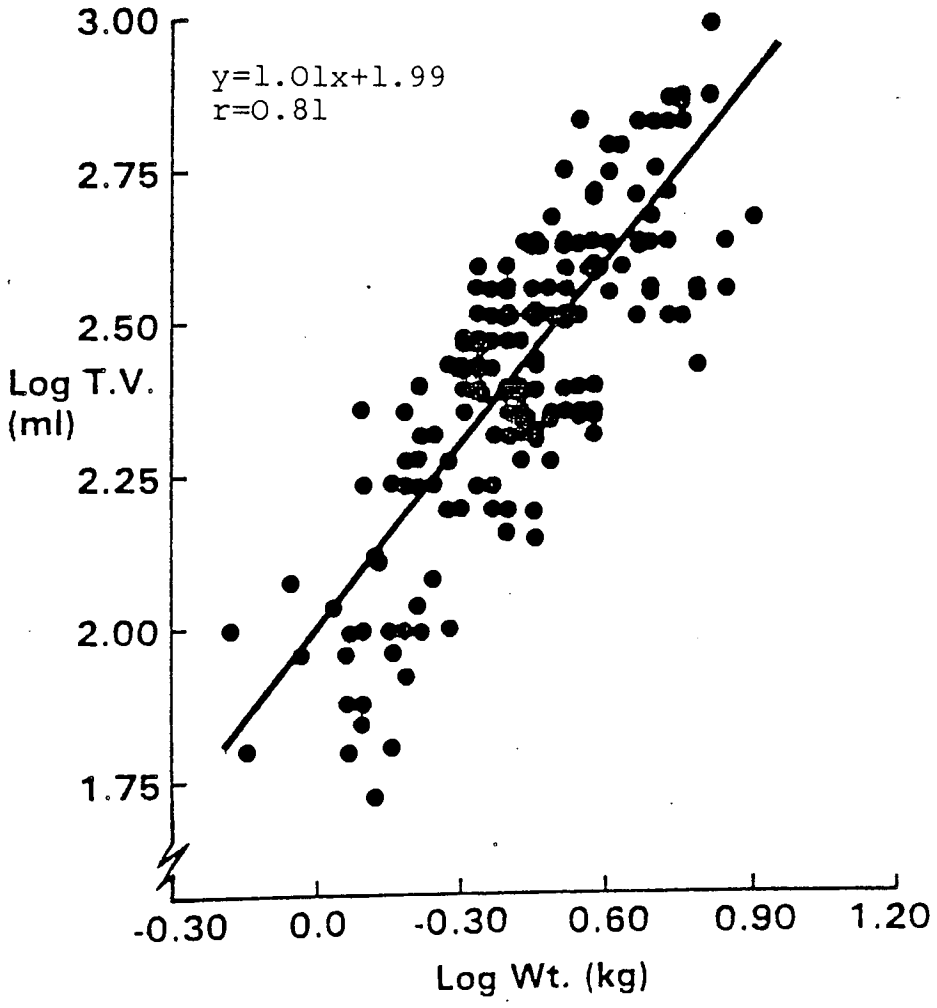


Fig. 6.11 Regression of log. total egg volume (T.V.) on log. fish weight (Wt.). n=173.

6.5 Discussion

The data collected in all three investigations show that total fecundity and egg size increases and relative fecundity decreases with age in commercial stocks of rainbow trout. These results agree well with those obtained by Baiz (1978) who also studied farmed rainbow trout. These findings also confirm other studies on wild stocks of salmonids (Rounsfell, 1957; Pope *et al*, 1961, Nomura, 1963; Bulkley, 1967; Gibson *et al*, 1976; Thorpe *et al*, 1984).

Aside from the relationships between fecundity and egg size and fish weight these indices may also be affected by the age of the fish. It is very difficult to partition age from size effects on fecundity of broodstock without dietary deprivation which has been shown to have profound effects on reproduction (Scott, 1962; Springate *et al*, 1984; Townshend and Wootton, 1984).

Possibly the most significant finding presented here is that each female broodfish produces a specific volume of eggs which is closely related to weight and this consistency of production is achieved by compensatory alterations in egg number and size. This relationship is clearly demonstrated by the absence of correlation between relative volume and fish weight. The relationship between egg size and total volume which results in a constant volume of eggs being produced has been termed a 'trade off' (Springate and Bromage, 1984b). This phenomenom has far reaching consequences for commercial fish farming. Rainbow

trout eggs are sold by number in the U.K., and most other countries, and therefore the greatest financial return is to be achieved by producing the maximum possible number of eggs from each tonne of fish kept. Therefore the most profitable broodstock management strategy, providing the reduction in egg size does not have any direct implications as far as egg quality or consumer acceptance is concerned, would appear to be to maintain only two year old broodstock as these fish have the highest relative fecundity. Although the results in Chapters 3 and 4, and Springate and Bromage (1984b), suggest that the small eggs from two year old fish can have equal survivals and produce fish with similar specific growth rates as larger eggs from older fish, generally there is consumer resistance against these small eggs. Indeed it has been reported that such eggs have poorer survivals than larger eggs (Gall, 1974; Pitman, 1979; Small, 1979). However eggs from three year old females are generally of an acceptable size, i.e. greater than 4.75 mm in diameter (Springate and Bromage, 1984b) and these fish have the second highest relative fecundity (Fig. 6.1). After three years of age the relative fecundity decreases at the expense of egg size, consequently a tonne of three year old females would produce more, albeit smaller eggs, than a tonne of older, larger fish. Therefore it is suggested that the optimum stategy for broodstock management, in order to produce the maximum number of eggs, is to only maintain broodstock up to, and including, their second spawning. After spawning for the second time it is suggested that the fish are allowed to re-gain condition and then sold for stocking purposes or for the table. Fish in their

fourth year are generally of a size (2-3 Kg) which is highly desirable for stocking purposes and also there is an increasing demand for larger trout for human consumption.

Unless there are changes in market demand and/or methods of selling eggs, i.e. by volume or weight as suggested by Purdom (1977) then it does not make economic sense to maintain broodstock after their third or, exceptionally, their fourth year.

CHAPTER 7

The relationships between female weight and total fecundity for three strains of rainbow trout

7.1 Introdution

Currently commercial fish farmers have the opportunity to stock their farms with a number of 'strains' of rainbow trout. These can be obtained as eggs or fry from commercial hatcheries in the U.K., from limited stocks held by the M.A.F.F. (Purdom and Hill, 1978) or as eyed eggs from any of the rainbow trout producing countries of the world, who are able to satisfy the disease certification required for importation of salmonid eggs.

A strain is probably best defined as a population of fish that exhibits reproducible physiological, morphological or cultural performance characteristics that are significantly different from other fish populations, or a broodstock derived from such a population and maintained thereafter as a pure breeding line stock (Kincaid, 1981).

This definition is based on the concept that a population living in a particular environment undergoes natural selection adapting it to survive and proliferate better in that environment. As this selection process continues, gene frequencies move towards an equilibrium that tends to be maintained. Strain differences are established when gene pools of two populations have been changed sufficiently to produce

significant reproducible differences in the performance of fish from the two populations. The time required to produce new strains shows considerable variation, varying widely from two to several hundred generations, depending on evolutionary or selection pressures involved (Kincaid, 1981).

A special problem arises as to when a fish population developed by man and having unique performance characteristics should be recognised as a new strain. Kincaid (1981) recognises populations as new strains if they are able to meet the following conditions:-

1.* The population has been separate from the original source or sources for at least two generations.

2. The population is sufficiently large to allow supplies of fertilised eggs to be commercially available.

3. The population has been shown to differ (significantly) in one or more performance traits from the original stock.

The term strain is often misused by fish farmers and fisheries scientists alike. Often the first two criteria are met but frequently the characteristics which might be present and may distinguish one population from another, and thus allow it to be termed a strain, are not documented.

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* This condition is not satisfied in this work as this would not allow comparisons to be made between the same strain maintained on different farms.

The application of selection techniques to various domestic animal and plant species has often proved to be effective. Specific approaches vary, but all involve the selection of superior individuals or families from the mating population for use as parents in the succeeding generation. Since 1930, tremendous improvements have been made and documented in numerous quantatitve traits (e.g. growth rate, fecundity and litter size) in populations of rats, mice, poultry, swine and cattle. However, the effective application of genetic selection principles in fish has not been widely practised and has been given little attention before 1960 (Kincaid et al, 1977).

Donaldson and Menasveta (1961) reported that one generation of selection for chinook salmon resulted in a one year advance in maturation, a higher return from the ocean and 5% higher fecundity, although the significance of these changes has been questioned. Since then many other intentional breeding programmes for salmonids have been conducted. The most common selected characteristics have been faster growth (Piper and Osborne, 1976; Kincaid et al, 1977; Reinitz et al, 1978; Gunnes and Gjedrem, 1978; Morkramer et al, 1985) earlier maturity (Vincent, 1960; Donaldson and Menasveta, 1961) disease resistance (Gjedrem, 1976) and tolerance to acid rain (Gjedrem, 1976; Robinson et al, 1976).

Of special interest here are the possible benefits to be gained by maintaining strains of broodstock with a high fecundity in order to increase commercial egg production. Of

equal interest is whether the reproduction performance of one strain is reproducible on another farm. With these aims in mind fecundity data were collected from two fish farms which maintained a total of five separate populations of rainbow trout, but only three strains, two of the strains being common to both farms.

7.2 Materials and methods

All measurements of fish weight (post-stripped), fecundity and egg size were performed as described in the General Material and Methods Chapter. Data were collected from two fish farms (P and R). Farm P maintained three strains designated P11, P12 and P13 and Farm R two strains designated R11 and R13. The letter (P or R) preceding the strain number refers to the farm at which the strain was maintained. Strain P13 originated from Strain R13 in 1978 and had been maintained separately since that time. Strains P11 and R11 were both obtained from a stock in North America in 1978, all were at least third generation descendants at the time of sampling.

In all data were collected from 130 randomly selected females; 28 strain P11, 22 strain P12, 21 strain P13, 35 strain R11 and 24 strain R13.

Comparisons of regression lines were made by analysis of cavariance (ANOCO) (Snedecor and Cochran, 1980). A computer programme was written to perform the lengthy computation of

ANOCO using the terminology of the minitab software package. The programme is reproduced in Appendix I.

7.3 Results

Analyses of variance showed there was a significant difference between post-stripped female weight ($F=16.93$ d.f. 4,125, $P < 0.001$) and egg size ($F=3.61$ d.f. 4,125, $P < 0.001$) for the five populations (Tables 7.1.a, 7.1.b). The mean post-stripped weight of the fish was 2.26 Kg. (range 1.02 to 5.20 Kg), mean total fecundity 4,425 (range 1,738 to 11,213) and mean egg diameter 4.85 mm range 4.00 to 5.39 mm (Table 7.1c).

For each of the five populations considered there was a significant ($P < 0.001$) positive relationship between log. total fecundity and log. fish weight. The regression relationship between log. total fecundity and log. fish weight for strain P12 accounted for the largest percentage of the variation (55%) and the relationship for Strain R11, the least (28%) (Table 7.2).

The homogeneity of the variances between the strains were not significantly different ($P > 0.05$) and therefore it was possible to test the differences between the strains with ANOCO. Between strains, fecundity increased with parent weight at the same rate (difference between slopes not significant, $P > 0.05$). However Strain P13 was more fecund at a given weight than Strains P11 and P12 (Table 7.3a, Fig. 7.1). Strains P11 and P12 could be considered as being the same for the weight and fecundity relationship and therefore data from these two strains

could be combined and compared with Strain P13. Strain P13 was significantly ($P < 0.001$) more fecund than Strain P11 and P12 combined (Table 7.3a, Fig. 7.2). There were no significant differences ($P > 0.05$) in the fecundity weight relationships between the two strains R11 and R13 maintained on Farm R (Table 7.3b, Fig. 7.3) or between the two pairs of strains P11 and R11; and P13 and R13 maintained on different farms (Table 7.3c, Figs. 7.4 and 7.5).

Table 7.1 Fecundity, egg size and fish weight relationships of three strains of rainbow trout on farms P and R

a) On Farm P

	STRAIN P11			STRAIN P12			STRAIN P13		
	Wt	TF	OD	Wt	TF	OD	Wt	TF	OD
n	28	28	28	22	22	22	21	21	21
Mean	1.51	2930	4.76	2.50	4640	4.97	2.02	4353	4.93
SEM	0.007	161	0.06	0.17	466	0.04	0.09	175	0.04
Max	2.35	4613	5.20	3.97	11213	5.39	2.90	6450	5.36
Min	1.02	1825	4.00	1.27	1738	4.71	1.21	3025	4.48

b) On Farm R

	STRAIN R11			STRAIN R13		
	Wt	TF	OD	Wt	TF	OD
n	35	35	35	24	24	24
Mean	2.62	5221	4.81	2.62	4874	4.86
SEM	0.09	222	0.04	0.18	352	0.04
Max	4.05	8765	5.39	5.20	9967	5.16
Min	1.72	2448	4.36	1.40	2091	4.53

c) Combined data for Farms P and R

	Wt	TF	OD
n	130	130	130
Mean	2.26	4425	4.85
SEM	0.07	145	0.02
Max	5.20	11213	5.39
Min	1.02	1738	4.00

Table 7.2 Regression equations of log. total fecundity on log. weight for Strains P11, P12 and P13 (Farm P) and Strains R11 and R13 (Farm R)

	STRAIN	EQUATION	r	F	sig.
Farm P	P11	Y = 0.79x + 3.32	0.66	20.5	P <0.001
	P12	Y = 1.06x + 3.22	0.74	24.9	"
	P13	Y = 0.52x + 3.48	0.62	11.7	"
	P11 andP12	Y = 0.89x + 3.29	0.78	76.5	"
Farm R	R11	Y = 0.69x + 3.42	0.53	12.7	"
	R13	Y = 0.72x + 3.38	0.69	20.3	"

y = log. total fecundity

x = log. fish weight

Table 7.3 Differences between regressions of log. egg number on log. fish weight, by strains (ANOCO) a) Farm P, b) Farm Q, and c) common strains on Farms P and R compared

a) Farm P

	Significance of difference between:-		
	Homogeneity	Slope	Elevation
P11 vs P13	NS	NS	$P < 0.01$
P11 vs P12	NS	NS	NS
P12 vs P13	NS	NS	$P < 0.05$
(P11 + P12) vs P13	NS	NS	$P < 0.01$

b) Farm R

	Significance of difference between:-		
	Homogeneity	Slope	Elevation
R11 vs R13	NS	NS	NS

c) Common strains on Farms P and R compared

	Significance of difference between:-		
	Homogeneity	Slope	Elevation
R11 vs P11	NS	NS	NS
R13 vs P13	NS	NS	NS

NS = Not Significant

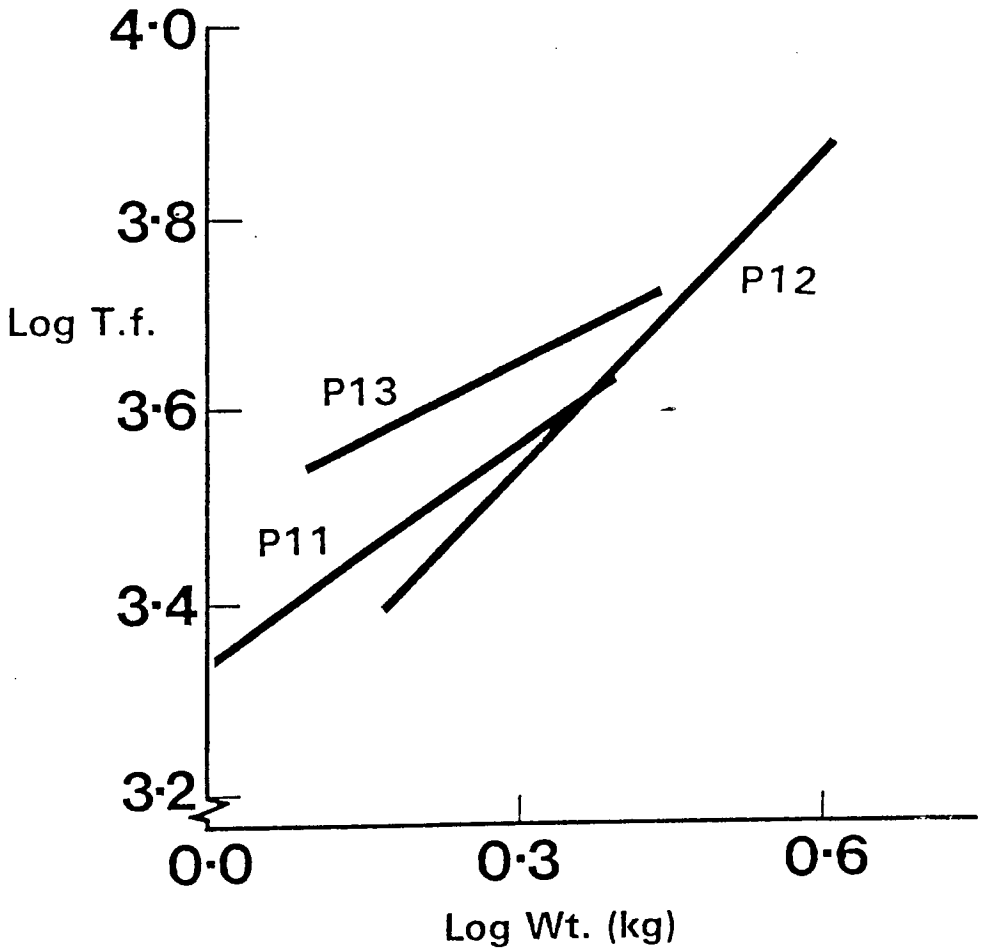

Fig. 7.1 Regressions of log. total fecundity (T.f.) on log. Fish Weight (Wt) for three strains of rainbow trout (P11,P12,P13) on Farm P.

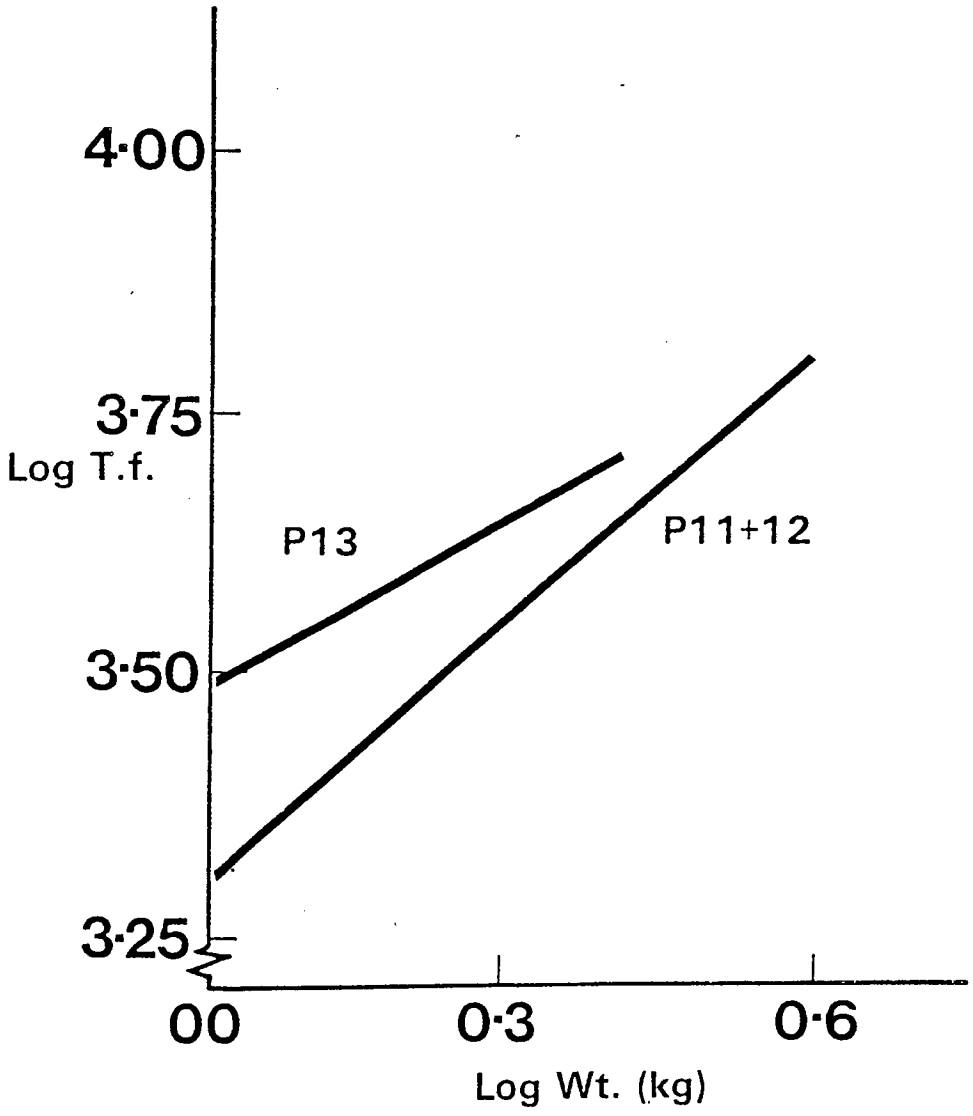

Fig.7.2 Regressions of log. total fecundity (T.f.) on log. fish weight (Wt.) for two strains combined (P11 and P12) & Strain P13, on Farm P

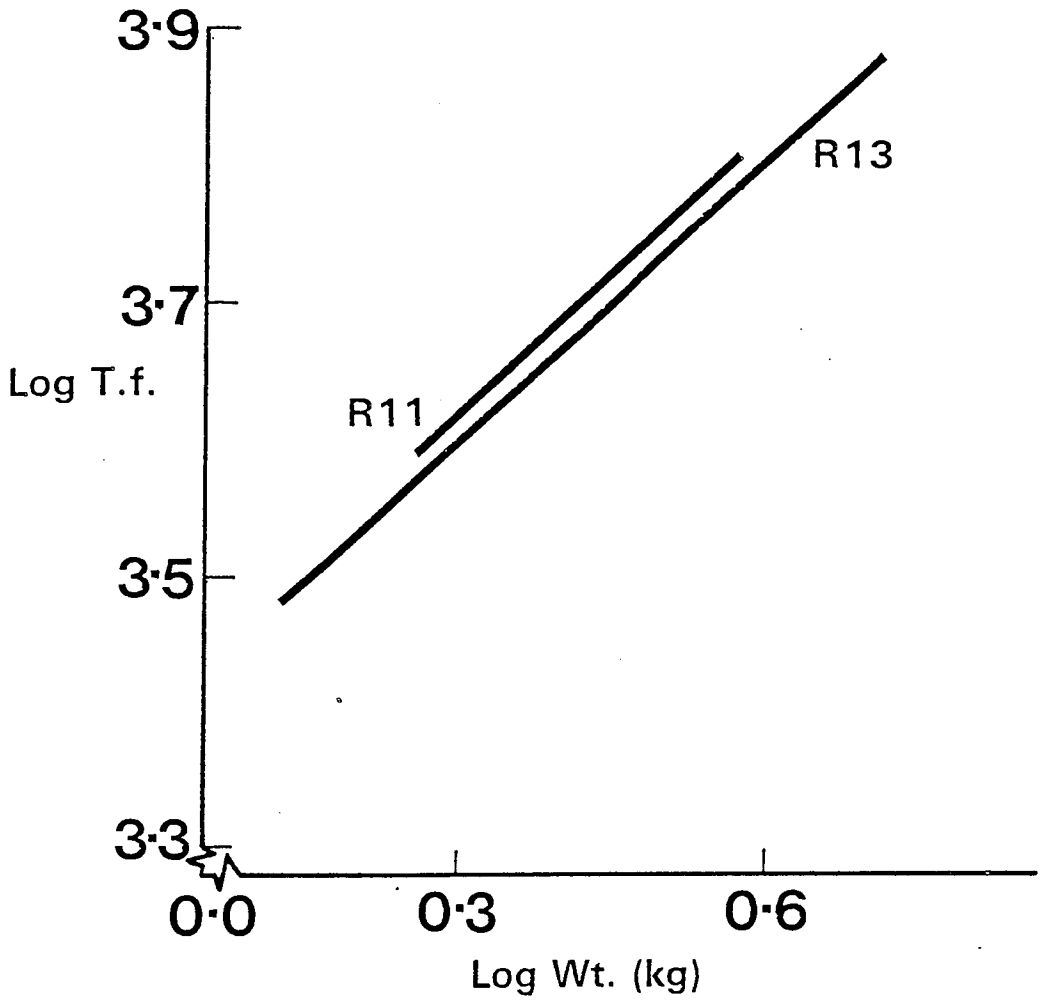

Fig.7.3 Regressions of log. total fecundity (T.f.) on log. fish weight (Wt.) for two strains of rainbow trout (R11 and R13) on Farm R.

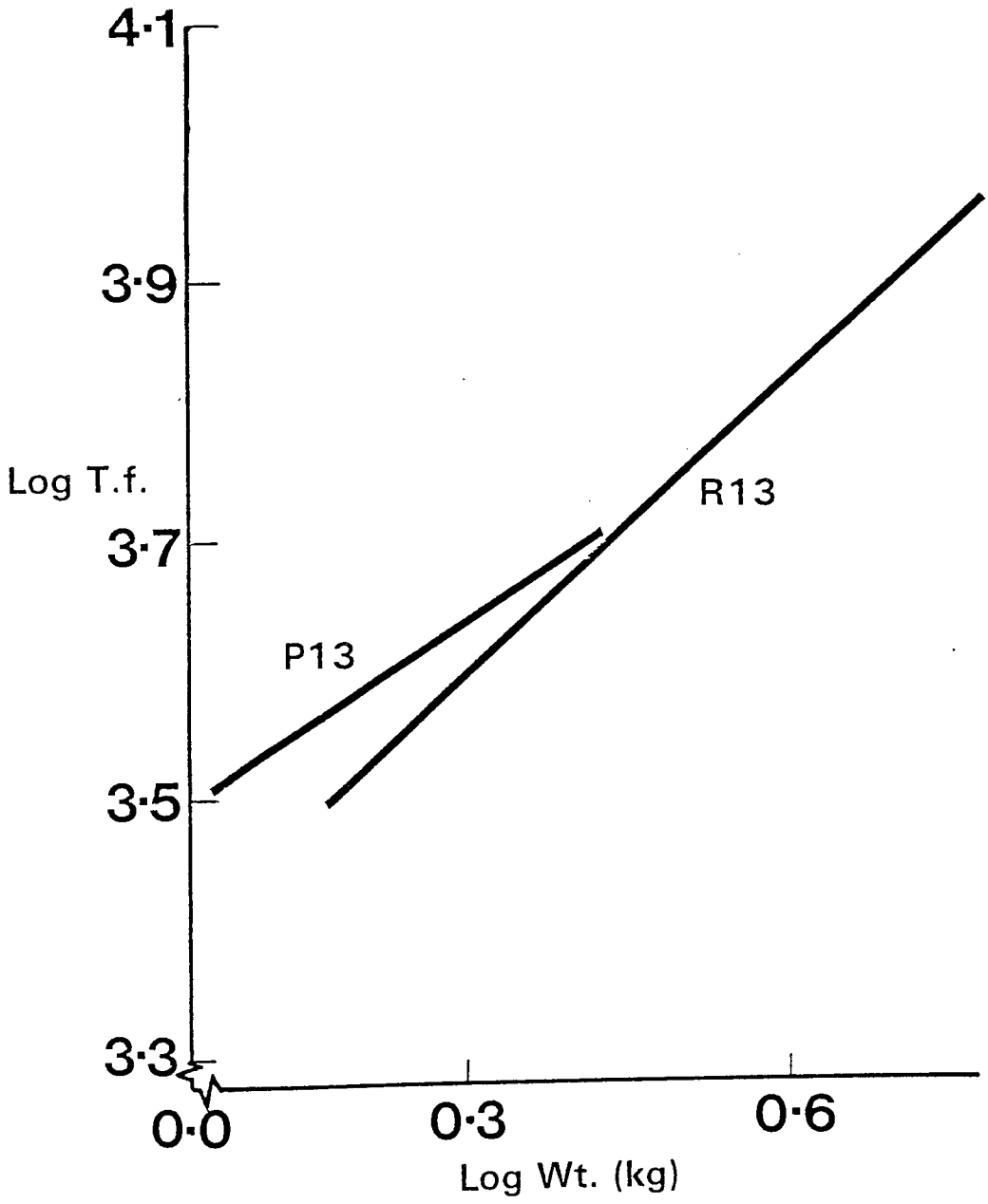

Fig. 7.4 Regressions of log total fecundity (T.f.) on log. fish weight (Wt.) for two strains of rainbow trout (R13 and P13). Strain R13 on Farm R and Strain P13 on Farm P.

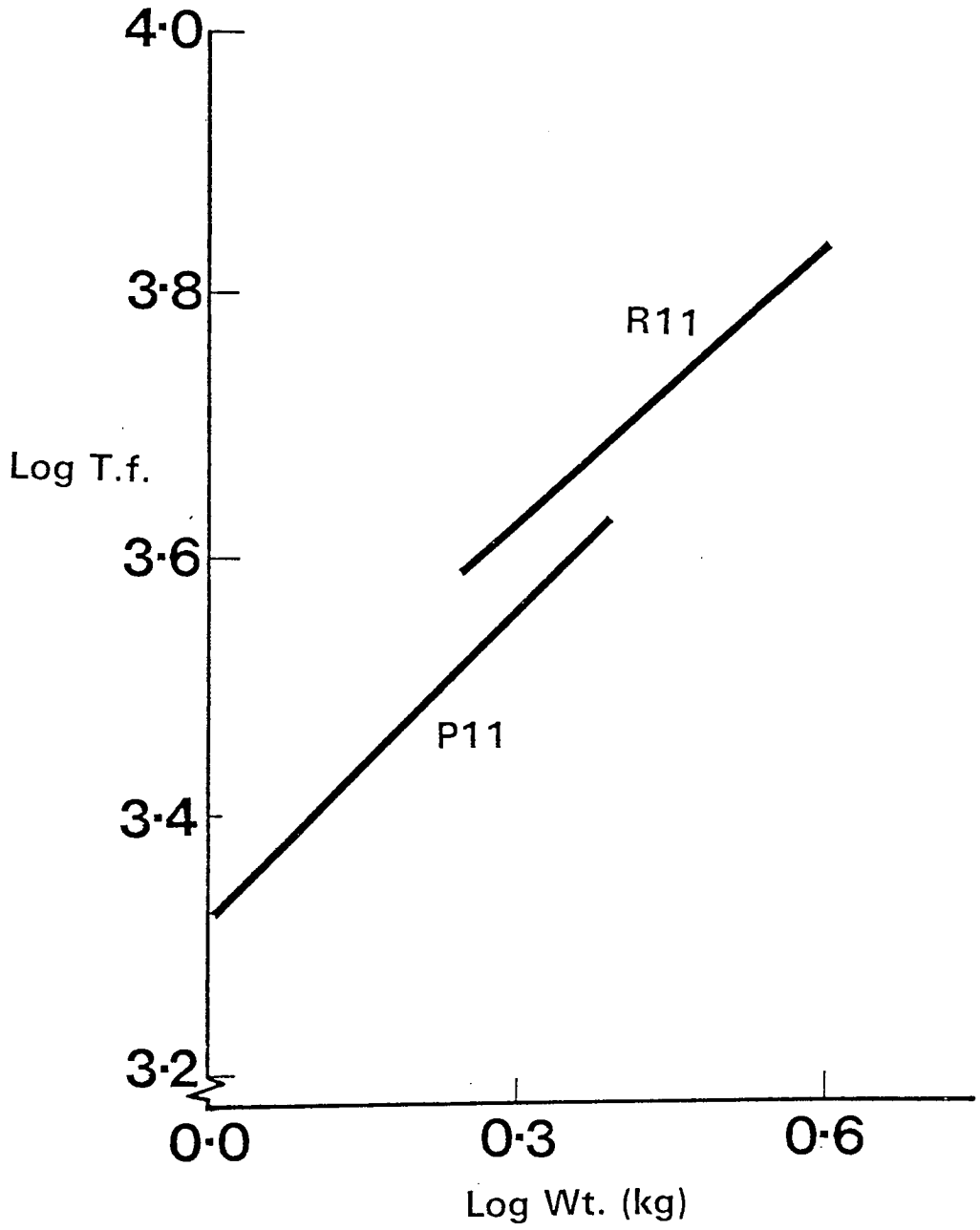

Fig. 7.5 Regressions of log. total fecundity (T.f.) on log. fish weight (Wt.) for two strains of rainbow trout (R11 and P11). Strain R11 on Farm R and Strain P11 on Farm P.

7.4 Discussion

The most important result of this study is that one strain (Strain P13) produced significantly more eggs than two other strains (P11 and P12) for a fixed weight on the same farm (Farm P). This could not be simply explained by the egg size relationship outlined in Chapter 6 as the eggs of the most fecund Strain P13 were on average 3.5% larger (egg diameter) than the eggs from the least fecund Strain P11. This result agrees well with the findings of Gall and Gross (1978) who evaluated reproductive performance in three Californian strains of rainbow trout. Strain differences occured in volume of eggs produced per female, egg size and total fecundity. Indeed the females of one strain produced 16% more eggs and these were 17% larger than females of another strain even though the parent fish weighed 15% less.

The two strains which were common to both farms, but maintained separately, showed no significant difference in fecundity when compared like with like. This suggests that there is some degree of reproducibility of reproductive traits.

However, the fecundity relationship between Strain R13 and R11 was not the same as the relationship for Strain P13 and Strain P11. Strain R13 and Strain R11 had the same fecundity for a given weight. There are many explanations for this result. One possible reason is that often strains are not managed, genetically, correctly on commercial farms. Genetic variability is one of the most precious characteristics of any

population of organisms (Gall, 1970) and this variation can be lost, and hence inbreeding can occur, if insufficient fish (males and females) are used to continue a strain. Currently 50 pairs of fish are the recommended minimum for strain continuation (Kincaid personal communication, 1983). Due to commercial constraints and widespread ignorance of genetics this criterion is seldom met, and therefore commercial strains tend to become inbred. The Donaldson strain in the U.S.A. is possibly the best documented case of inbreeding with an associated lack of reproductive performance (Satia, 1973; Roley, 1983).

The results of this study, and others (Donaldson and Menasveta, 1961; Gall and Cross, 1978), suggests that there are strain differences in reproductive performance. Many more strains need to be studied in order that a strain could be located that would be ideal for farming as an egg producing animal. However, to simply study strains would be very time consuming and perhaps not the best method. Possibly a better alternative would be to carry out a selection programme for desired traits. The high fecundity and large variation in reproductive traits of salmonids should make it possible to obtain much higher genetic gain per time units than in other farm animals.

Before a selection programme can be established some requirements have to be met. The objectives of a breeding programme have to be defined. Genetic and phenotypic parameters for economically important traits have to be known. In addition the economic values for each trait must be established.

The general principles in population genetics are well known, thanks to plant and animal population geneticists. Our knowledge of phenotypic and genetic parameters for ecomonic traits in salmonids is however, rather limited (Gjedrem , 1976). Therefore it is important to collect data in order to establish these parameters.

The degree of heritability for a trait is a very important part of the selection procedure. If heritability is high and close to 1.0, then most of the variation in a trait is heritable and selection will be very efficient. When the value is low and close to zero, environmental factors have caused most of the variation in the trait. Gall and Gross (1978) estimated heritabilities for performance characteristics for three broodstocks of rainbow trout (Table 7.4)

The results clearly indicate the enormous variability in all the traits considered for the three strains. However the authors concluded from the work that there seems to be little need to select for increased egg number whenever selection is practised for body size or growth rate.

Given the large variation in reproductive performance of rainbow trout strains, it is suggested that more detailed strain evaluation is conducted and that selection programmes are instigated. It is essential for fish farming that a more productive animal which is better adapted to captivity is developed (Gjedrem , 1976).

Table 7.4

Heritability estimates for performance characteristics for three rainbow trout broodstocks. A, Post-spawning body weight and fertility measured as number of eyed eggs. B, Egg production before adjustment for female weight. C, Egg production after regression on female post-spawning weight (Gall and Gross, 1978).

	Trait	Heritability (± SEM)			
		Strain 1	Strain 2	Strain 3	Average
A	Female weight	0.40±0.11	0.74±0.15	0.37±0.12	0.50
	Male weight	0.14±0.13	0.63±0.18	0.17±0.11	0.31
	Fertility	0.09±0.11	0.19±0.11	0.40±0.13	0.23
B	Egg volume	0.47±0.12	0.76±0.15	0.32±0.11	0.52
	Egg size	0.40±0.11	0.50±0.14	0.06±0.08	0.32
	Egg number	0.36±0.11	0.67±0.15	0.30±0.11	0.44
C	Egg volume	0.36±0.11	0.54±0.14	0.16±0.10	0.35
	Egg size	0.35±0.11	0.48±0.14	0.05±0.08	0.29
	Egg number	0.28±0.10	0.51±0.14	0.16±0.10	0.32

Summary and Conclusions to Part II

In this section of the thesis a study has been made of the fecundity of broodstock rainbow trout maintained under conditions of intensive culture. Total fecundity and egg size were found to increase with increasing size of female and at the same time relative fecundity decreased. It would appear that female trout produce a constant volume of eggs for each unit weight of fish and that increases in egg size can only be achieved by reductions in fecundity. This is in agreement with the 'trade off' hypothesis as proposed by Springate and Bromage (1984b).

Although not entirely clear from the present data it would appear that female size, and not age is the most important determinant of fecundity and egg size. This may have considerable commercial implications for the management of two year old, or first stripping, broodstock whose eggs are generally small and often considered to be of poor quality. If the egg size from these fish could be improved by increasing the weight of the fish then this would constitute a significant economic advance.

Commercial considerations relating to egg size and number and to the overall weight of broodstock maintained on the farm indicates that two year old broodstock were the most efficient and cost effective egg producers.

The most important result of the strain investigations was that one strain of rainbow trout produced significantly more eggs (13%) for a fixed weight of fish (1kg), than two other strains maintained on the same farm. Trout of the same strain maintained on different farms behaved similarly suggesting that there was some reproducibility of strain characteristics. The commercial implications of these results are far reaching and it is strongly recommended that a more extensive evaluation of strains is conducted and that selection programmes are initiated with the aim of isolating strains with the highest egg producing capabilities.

PART III

Husbandry

PART III

General introduction to husbandry

Different farms experience enormous variation in egg quality and fecundity. Under commercial conditions this variation may be due to the wide range of husbandry techniques which are in current use.

A detailed study was made on the timing of stripping after ovulation which has far reaching effects on egg quality (Chapter 8) and also considered in detail were the effects of feeding broodstock at two different ration levels, on egg quality and fecundity (Chapter 9).

CHAPTER 8

The timing of ovulation and stripping and their effects on egg and and early fry quality.

8.1 Introduction

Ovulated eggs of oviparous teleosts become overripe if retained in the body cavity and these eggs show a progressive reduction in viability (Sakai *et al*, 1975; Hirose *et al*, 1977; Lam *et al*, 1978; Mollah and Tan, 1983; McEvoy, 1984; Springate and Bromage, 1984d). During the period in which they became overripe, eggs have been shown to undergo a series of morphological changes (Nomura *et al*, 1974; Hirose *et al*, 1977). These changes are characterised by the aggregation and fusion of oil droplets and the migration of cortical alveoli to the animal pole in the rainbow trout (Nomura *et al*, 1974) and the ayu (Hirose *et al*, 1977) and by increases in water content and ova diameter in the three spined stickleback (Lam *et al*, 1978). It is not known whether the morphological changes *per se* are responsible for the reductions in viability of the eggs and fry. Whatever the cause of overripeness, it is of profound importance in fish culture (Lam *et al*, 1978). Farmed salmonids in particular are thought to suffer high losses amongst their eggs as a result of overripeness. This problem can only

be overcome by more frequent examination of the stock, as the eggs of most salmonids in culture can only be expressed by manual stripping of the fish, and this repeated handling, in itself, may lead to mortality in the broodstock.

Several authors have investigated the effects of the retention of ovulated eggs in the abdominal cavity and found that the duration for which the eggs remain viable varies with species (Mollah and Tan, 1983) and the ambient water temperature (Billard and Gillet, 1981). Sakai et al (1975) demonstrated that the mean percentage of eggs reaching the eyed stage exceeded 70% in rainbow trout eggs which had been stripped by the 10th day post-ovulation at 13°C. The percentage eyed values ranged from 0-100% even on the first day post-ovulation suggesting that some factor other than the timing of stripping was important in determining survival. Escaffre and Billard (1979), working with three populations of the rainbow trout noted that egg survivals determined 10 days after fertilisation remained higher than 90% for 8 days post-ovulation in one population and for 15 days in the other two. This experiment was conducted at a varying water temperature (6.5-13.0°C). Bry (1981) showed that eying rate of rainbow trout eggs decreased signficantly by the 9th day of retention in the female body cavity at the relatively high temperature of 13°C. Most authors have concerned themselves solely with eying rates in relation to

retention within the body cavity and have not considered viability at other stages of development. The present investigation extends these studies by considering the survival rates at fertilization, eying, hatching and swim-up of eggs and fry for varying periods of time up to 20 days post-ovulation.

8.2 Materials and methods

Twenty virgin spawning female rainbow trout weighing 790 ± 30g which on morphological grounds were considered to be nearing spawning, were maintained in 10 ± 1°C water with five two year old, sexually-mature male fish. Each day the female fish were examined under 2-phenoxyethanol anaesthesia (1: 20,000, v/v) until mature ova could be manually removed from them by applying gentle pressure on the abdomen in an anterior to posterior direction. The first day that eggs could be removed from the fish was considered to be the day of ovulation (Day 0). On alternate days from day 0 onwards up to 250 mature ova were stripped from each female fish into a dry 500 ml plastic bowl and immediately fertilised with the milt from 2 males. Surplus sperm was washed off 2 minutes later with successive water changes. Each batch of eggs was then left for 30 minutes to water-harden, before being sub-divided into 5 groups of 20-30 eggs. The first group was incubated for 12 hours and then checked for fertilisation rate by visualisation of early segmentation

with a solution of acetic acid: methanol: water (1:1:1,v/v). Two groups were separately placed into a small batch incubation system (Fig. 2.2) for determinations of eying, hatching and swim-up rates. Eggs from the remaining group were accurately weighed (to the nearest 0.0001g), dried to constant weight at 70°C, and subsequently reweighed for determination of dry weight and moisture content. The mean ova diameter was measured by aligning 25-30 of the remaining eggs on a 120 mm measuring trough.

Starting 2 weeks before expected ovulation, and continuing after ovulation blood samples were removed as described in the General Materials and Methods. After clotting, whole blood was centrifuged at 2,500 r.p.m. for 20 minutes and the serum decanted into clean test tubes and stored at -20°C for determination of 17α-hydroxy-20β-dihydroprogesterone (17α20β-P) and 17α -hydroxyprogesterone (17α-OH-P) by radioimmunoassay using the methods of Scott et al (1982).

8.3 Results

Mean fertilisation rose from 88±7% (mean ± SEM) for eggs taken on the day of ovulation to 100% by days 4 and 6 post-ovulation. There was then a steady decline to 68± 5% for the mature ova which had remained in the fish for 20 days post-ovulation (Fig 8.1a). Survival to the other

developmental stages followed similar patterns as fertilisation percentage relative to the time mature ova had remained in the fish. Eying increased from 78 ± 13% for eggs taken at day 0 to 100% by day 6, then declined to 56 ± 17% for eggs removed at day 20 (Fig 8.1b). Initial hatching rate increased from 76 ± 13% for eggs taken at day 0 to 96 ± 2% by day 4, and then fell to 48 ± 12% for eggs removed at day 20 (Fig 8.1c). Swim-up success rate increased from 70 ± 13% for eggs taken at day 0 to reach a maximum of 94 ± 2% by day 4, and then decreased to a low of 35 ± 5% for eggs removed at day 20 (Fig 8.1d). The similarity in patterns of survival for each developmental stage is confirmed by the strong positive correlation ($P < 0.001$) between fertilisation and the other three developmental stages. Correlation coefficients between fertilisation and survival to eying, hatching and swim-up were $r = 0.99$, 0.98, and 0.93 respectively (Fig. 8.2). Mean ova diameter ranged from 4.09 - 4.26 mm and mean water content from 59 - 65% over the 20 day post ovulation period (Table 8.1), although at no time were any of these alterations significantly different from each other (students 't' test).

Measurement of hormone levels showed that there were increases in 17α-OH-P five days pre-ovulation and in 17α20β-P 3 days pre-ovulation (Fig 8.3). 17αOH-P reached its highest level 3 days post-ovulation whereas 17α20β-P peaked on the day of ovulation. Both levels decreased

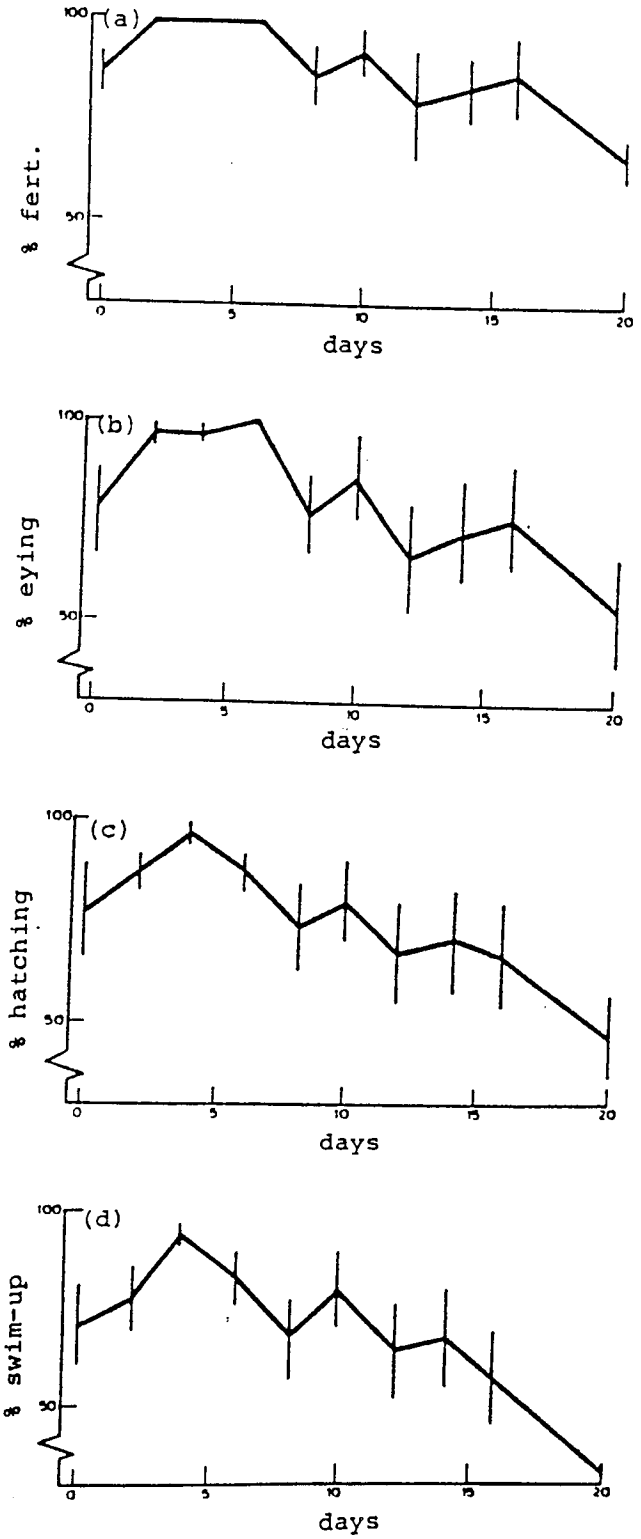

Fig. 8 .1 Changes in survival rates at the four developmental stages of fertilisation (a), eying (b), hatching (c) and swim-up (d). Mean ± SEM n=8.

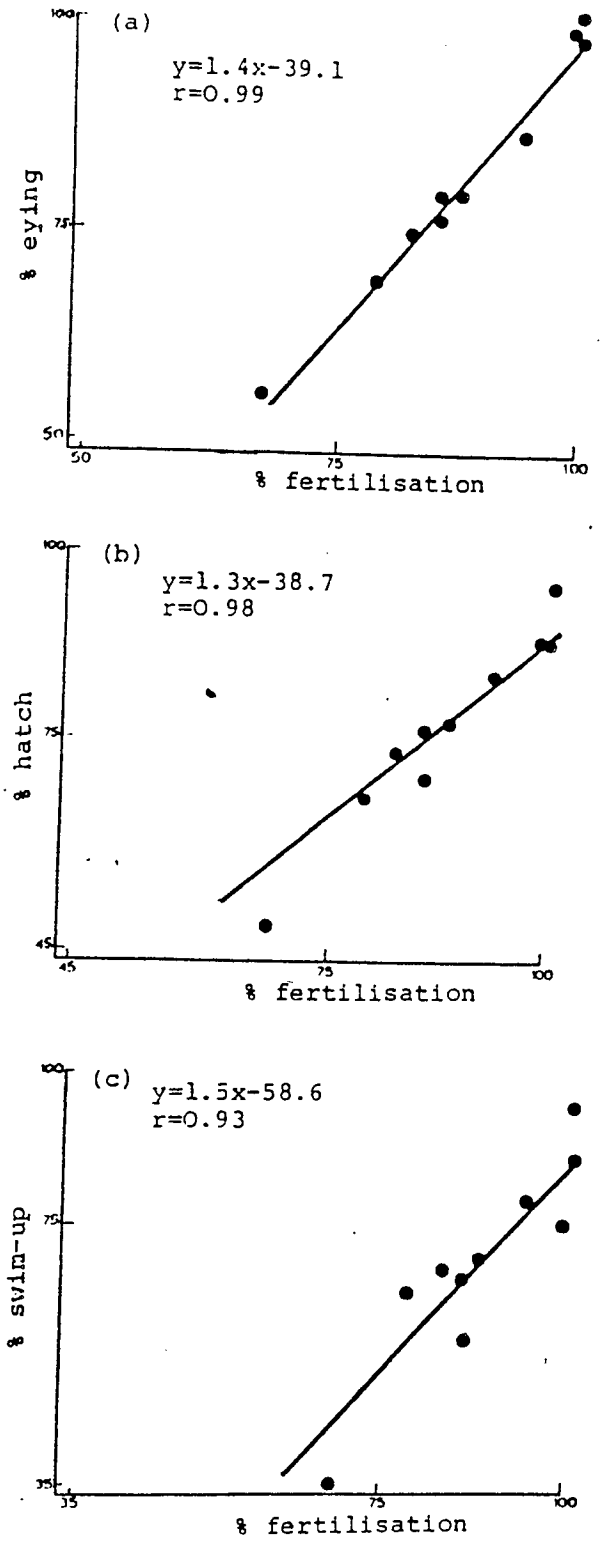

Fig. 8.2 Correlations between percentage fertilisation and eying (a), hatching (b) and swim-up (c). $P < 0.001$ for all correlations.

Table 8.1 Ova diameter and moisture content of eggs retained in the abdominal cavity for varying lengths of time post-ovulation (n = 8).

Days Post-Ovulation	Egg Diam(mm) Mean ± SEM	% water Mean ± SEM
0	4.18 ± 0.06	65 ± 2
2	4.21 ± 0.05	63 ± 1
4	4.26 ± 0.04	62 ± 1
6	4.23 ± 0.06	63 ± 1
8	4.17 ± 0.06	62 ± 1
10	4.18 ± 0.07	64 ± 1
12	4.09 ± 0.06	63 ± 2
14	4.18 ± 0.07	61 ± 1
16	4.35 ± 0.06	59 ± 3
20	4.24 ± 0.08	61 ± 1

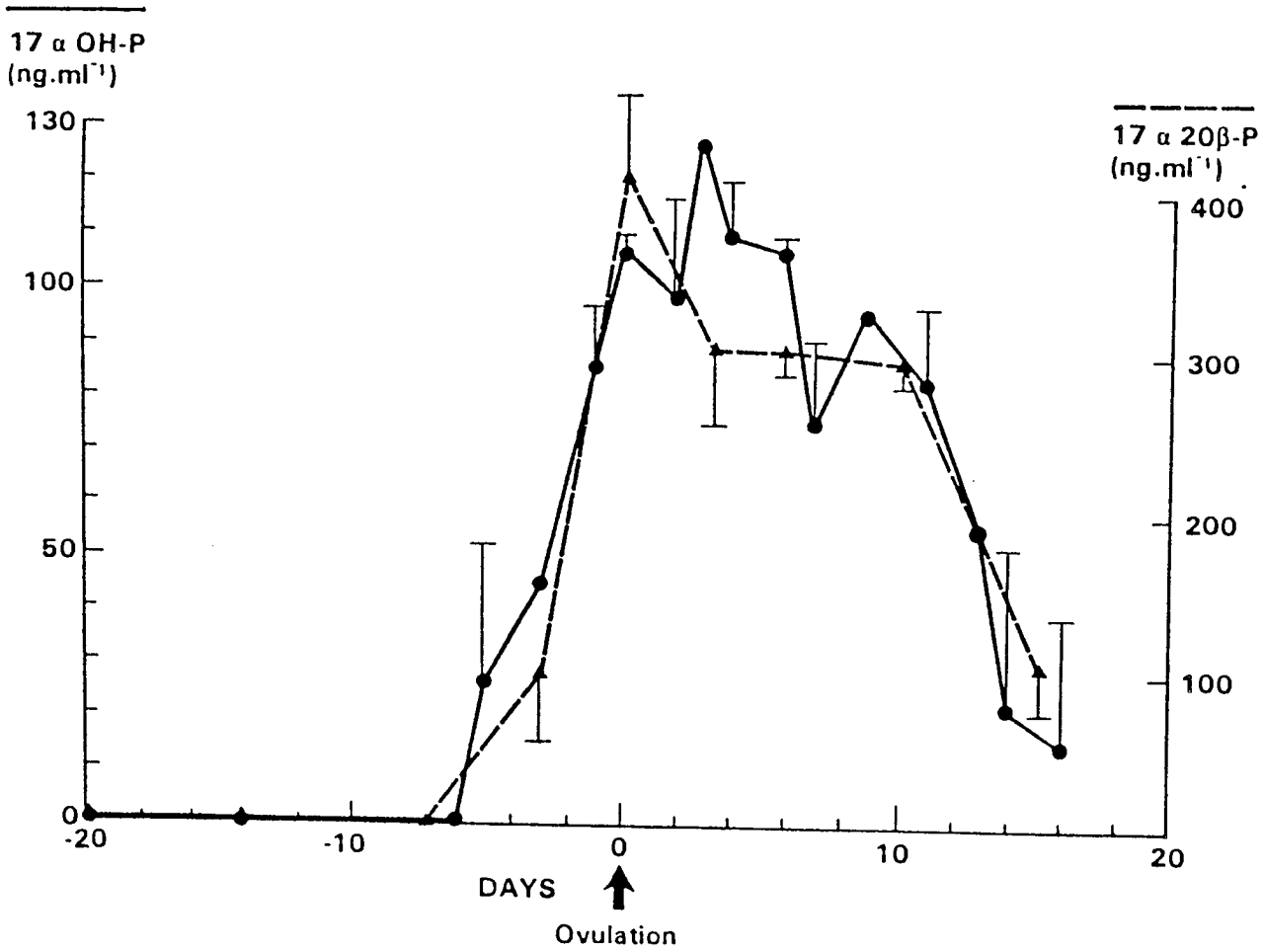

Fig. 8.3 Changes in serum levels of 17 α-hydroxyprogesterone and 17 α-hydroxy--20β-dihydroprogesterone of female trout around the time of ovulation Mean ± SEM.

steadily, for the 16 days post-ovulation that levels were monitored, without reaching basal levels.

8.4 Discussion

The present work shows that eggs retained in the body cavity of rainbow trout post-ovulation pass through a ripening process which has profound effects on the ability of the eggs to be fertilised and to develop further through the stages of eying, hatch and swim-up. The strong correlation between fertilisation rate and survival to these other stages clearly shows that the process of overripening as well as reducing fertilisation rate also produces additional mortalities at eying and early growth of the fry. These results agree well with the findings of Chapter 3. In this experiment maximum fertilisation rate was achieved 4-6 days post ovulation and after this time fertilisation rates of 100% could not be achieved. This agrees well with the results of Sakai *et al* (1975) who suggested that rainbow trout broodfish, maintained at 13°C, should be artificially stripped within 7 days post-ovulation.

In most fish examined there was an initial short period (4 days) when some of the eggs were possibly 'underripe' and this may have reduced fertilisation. This early 'underripe' phenomenon was also observed by Sakai *et al* (1975) and Hirose *et al* (1977). Whether these eggs had

not fully completed maturation is not known although it is reported that ovulation is synchronous in the rainbow trout (Wallace and Selman, 1978). This has been confirmed by the examination of ovaries of fish sacrificed immediately post-ovulation. Furthermore, the timing of the hormonal profiles of 17α0H-P and 17α20β-P agrees well with the results of Scott et al (1983) suggesting that the time of ovulation as determined by physical stripping is an accurate one. However, it is not known whether the maintenance of 17α0H-P and 17α20β-P at high levels after the initial ovulation is involved in any additional way with the presence of ovulated eggs in the body cavity.

It would appear that after 6 days some of the eggs were becoming 'overripe' and had lost their ability to undergo division. The cause of this loss is unclear although Stevens (1966) proposed that the eggs of striped bass lost viability as a result of hypoxia within the ovary after ovulation, caused by a change in the ovarian fluid. However, little evidence was presented for this hypothesis. During the period of overripening the ovulated eggs which are retained in the body cavity go through a series of morphological changes, and it has been suggested that these changes might be responsible for the decrease in egg viability which occurs during this period (Sakai et al, 1975; Hirose et al, 1977; Escaffre and Billard, 1979). Nomura et al (1974) divided the process of overripening into 4 stages; stage I including normal

ova immediately following ovulation; stage II eggs showed early stages of overripening; stage III and IV eggs were both overripe but the latter consisted of irregular shaped ova with marked reductions in material content. In Nomura et al's study the progressive formation of overripe eggs through stages II, III and IV began at about the 10th, 30th and 35th days following ovulation, respectively. The eggs in the present study were all in stages I and II of the above classification, however, some of the batches of eggs that had reached stage II (i.e. early stage of overripening according to Nomura et al 1974) were still capable of 100% fertilisation. Sakai et al (1975) also observed that some of the groups of eggs that had reached stage II were capable of developing to the eyed stage, whereas eggs which had reached stages III and IV were no longer capable of reaching this developmental stage. These findings suggest that factors other than those involving the morphological changes described by Nomura et al (1974) may be affecting egg survival.

Despite the appearance of morphological changes characteristic of stages I and II no significant changes in either water content or ova diameter of the eggs occurred over the 20 day post-ovulatory period considered in this study. Hirose et al (1977) also reported that there was no change in the moisture content of eggs of the ayu over a similar period. Similarly Escaffre and Billard (1979) recorded no change in mean weight of eggs

retained in the body cavity of rainbow trout for 30 days post-ovulation. In contrast, Lam et al (1978) demonstrated that overripe eggs of the stickleback were larger and had a higher water content. This suggests that there may be considerable differences between species with regards to the correlation of morphological changes with the overripening process.

In addition to species differences Mollah and Tan (1983) and Escaffre and Billard (1979) presented evidence that the viability of eggs taken at different times after ovulation may vary depending on the type or strain of fish used. Mollah and Tan (1983) demonstrated that there was a significant difference in the viability of eggs derived from two batches of fish of the same stock although in these experiments ovulation was artificially induced with human chorionic gonadotropin (HCG). Escaffre and Billard (1979) showed that the variation between the egg viability from three populations of rainbow trout was dependent on female body weight and on mean egg weight. In an earlier study the same authors reported that the eggs retained by larger females remained viable longer than eggs retained by smaller females (Escaffre et al, 1977). There was, however, no significant relationship between female size and duration of egg viability in the present study.

As far as relevance to commercial farms is concerned it is clear from the results of this investigation that

rainbow trout farmers should examine their broodstock at least every ten days if they are to achieve optimum survival rates from their eggs. Furthermore, for management purposes the quality of specific batches of eggs may be easily assessed by determination of fertilisation rates as this simple measurement provides a reliable prediction of subsequent egg and fry survivals.

CHAPTER 9

The effects of two different ration levels on fecundity and egg quality.

9.1 Introduction

In the wide literature relating to egg production and the population dynamics of fish it is often implied that the supplies of food are of prime importance in determining the reproductive potential of different fish stocks (Woodhead, 1960; Nicolsky , 1969; Bagenal, 1973). However, much of the evidence is circumstantial mainly because it is derived from records of wild stocks of fish where dietary influences are difficult to quantify and to separate from other environmental variables. Thus, McFadden et al (1965) reported that brown trout from oligotrophic streams had lower fecundities and reached sexual maturity later than similar sized fish from more fertile waters. Fry (1949) similarly suggested that the variation in fecundity of lake trout from year to year occurred as a result of differences in diet. Martin (1970) in an exhaustive study of the same salmonid species proposed that the increases in fecundity and egg size shown in the later years of a 21 year series were due to improved food supplies. Better feeding conditions were also said to advance the age of first spawning and increase fecundity in brook trout (Vladykov, 1956) although this finding was not substantiated in a similar study of the same species and area by Gibson et al (1976).

Working on non-salmonid species Hodder (1965), Bagenal (1966) and Raitt (1978) related variations in the fecundities of different stocks of haddock, plaice and Norway pout, respectively, to alterations in population density and/or food supplies. Various studies of the roach have indicated that diminished food supplies reduced fecundity (MacKay and Mann, 1969; Kuznetzov and Khalitov, 1978) delayed maturation (Mackay and Mann, 1969) but did not affect egg size (Kuznetzov and Khalitov, 1978).

Primarily, because of the inherent difficulties of associating cause and effect in the determination of fecundity and egg composition amongst wild stocks of fish, the data for wild stocks, at best, offers only supportive evidence for a direct relationship between nutrition and reproduction. Laboratory investigations of the effects of modified ration on egg size, number and quality (i.e. survival) would improve our understanding of this relationship but surprisingly, few such studies have been conducted. Scott (1962) in a three year investigation of the effects of varying ration on the rainbow trout showed that the proportion of fish which became mature was lower in fish fed on approximately half ration; these fish also produced fewer eggs although the eggs were unchanged in size. Some questions, however, remain regarding these findings as less than 5% of all the fish matured by the end of the experiment. Working with the brown trout Bagenal (1969a) showed that diet restriction reduced total

fecundity and delayed maturation. There were also alterations in the mean wet and dry weights of the eggs although these changes were probably due to the measurements being made on eggs from prespawned fish and these would have included oocytes at different stages of growth and final maturation. Baiz (1978) in a study of the fecundity of the rainbow trout also reported that a period of starvation reduced egg numbers but like Scott (1962), could find no effect on egg size. Hislop et al (1978) showed that egg production and feed levels were positively correlated in laboratory maintained haddock. There was also a suggestion that a lower proportion of the fish under the lowest ration matured and that their eggs were lighter on a dry weight basis. In contrast Kato (1975) and Ridelman et al (1984) reported that diet restriction for either 4 months or 40 days before spawning respectively produced no changes in either fecundity or egg size in the rainbow trout. In an extensive series of papers on the stickleback Wootton and co-workers (see review Wootton, 1982) have shown that high food levels increase both the percentage of fish maturing and their spawning frequency and, that by improving the growth of fish, fecundity was increased. Food level however had no effect on egg size. More recently Townshend and Wootton (1984) showed for the convict cichlid that both fecundity and egg size were positively correlated with ration and that fish size per se had negligible effects on these parameters. Clearly, the weight of evidence points to

direct effects of nutrition on varying aspects of reproduction but the details of such changes remain obscure.

It is also not clear how the possible alterations in fecundity and egg size are achieved. It has been suggested that food deprivation reduces oocyte numbers by inducing atresia (Vladykov, 1956) and/or by modifying the recruitment of oocytes into vitellogenesis (Robb, 1982). However, Henderson (1963) reported that the levels of atresia in the brook trout were too low to acount for these changes, and Townshend and Wootton (1984), although finding a reduction in oocyte recruitment in convict cichlids on reduced ration, concluded that the relative importance of this process and atresia in determining fecundity could not be ascertained.

In view of the wide range of reported influences of diet on fish reproduction and the unresolved questions relating to atresia and recruitment, the present study investigated the effects of altered ration on the timing of reproduction, and the number, size and quality of the eggs produced in the rainbow trout under carefully-controlled experimental conditions; and also by histological examination the ovarian changes which could mediate these changes.

9.2 Materials and methods

Groups of 40 female rainbow trout were maintained in 2m circular tanks and fed in replicate at either 0.35% (half-ration) or 0.70% (full-ration) body weight day^{-1} on a dry commercial trout pellet (Ewos-Baker: Pellets 7 and 8; Table 5.1). Water inflow to each tank was 40L.min^{-1} at a constant temperature of 10°C. The fish, which were of North American origin, but with an October/November spawning time under local conditions, were two years old at the beginning of the experiment on 13th October and weighed 0.581 ± 0.005 Kg (Mean ± SEM). Only fish which had spawned for the first time during the week before the experiment was begun were used.

The fish were individually tagged. At approximately monthly intervals fish were individually weighed and examined for signs of sexual maturation and the presence of ripe eggs. At the same time, blood samples were taken and, after separation, the serum was deep frozen at -20°C for later analyses of calcium as an index of vitellogenin and testosterone using the methods of Elliott *et al*, (1984).

Eggs were stripped from the gravid females and egg number and egg size determined as described in the General Materials and Methods. After measurement, the eggs were transferred to hatchery trays and the

fertilisation rates and subsequent mortalities at eying and as feeding fry recorded. Parallel groups of water-hardened, fertilised eggs from each female were taken for estimation of wet and dry weights and for analyses of their proximate composition as described in the General Materials and Methods Chapter.

After swim-up the fry were fed ad libitum on commercial fry diets (Ewos-Baker Ltd) and when they had reached a mean weight of approximately 1g (in March) they were transferred to 1.5m diameter fry tanks. Throughout this period, mortalities were recorded and at approximately 3-week intervals from the time of first feed individual fry weights were taken.

Ovarian samples were taken from the fish from each group approximately 14 days pre- and post-ovulation for histological examination after fixation in Bouin's fixative, alcohol dehydration and JB4 plastic (Polysciences) embedding. Sections were cut at approximately 1µm thickness. Parallel ovarian samples were also fixed and separated in modified Gilson's fluid for determination of the total numbers of each of the seven oocyte stages (van den Hurk and Peute, 1979) and of the levels of atresia.

9.3 Results

By the time of the first sampling in February the differences in dietary input were clearly shown by the significant difference in growth between the groups (Fig 9.1 $P < 0.05$). The growth divergence became more pronounced as the trial progressed and from May onwards the differences between means was highly significant ($P < 0.001$). At all times there was an overlap in the range of fish sizes in the two groups and at spawning the half-ration fish were 0.835 $\pm$ 0.020 (Mean $\pm$ SEM) Kg in weight (range 0.580-1.225) whereas the full-ration fish were 1.330 $\pm$ 0.040 Kg (range 0.500-2.000).

The first fish to be stripped were those under full-ration, 2-3 weeks earlier than those on half-ration (Fig. 9.2). No relationship could be seen between fish size and their spawning times. However, all the fish which had been fed full rations spawned, whereas 11% of the half-ration group failed to mature.

Determinations of fecundity showed that the full-ration fish produced significantly more eggs per fish than those on half-ration (Fig 9.3 $P < 0.001$). However, the eggs from the full-ration group were significantly larger (Fig 9.4 $P < 0.001$). The concomitant changes in fecundity and egg size in the two groups were reflected in a significantly higher relative fecundity in the fish

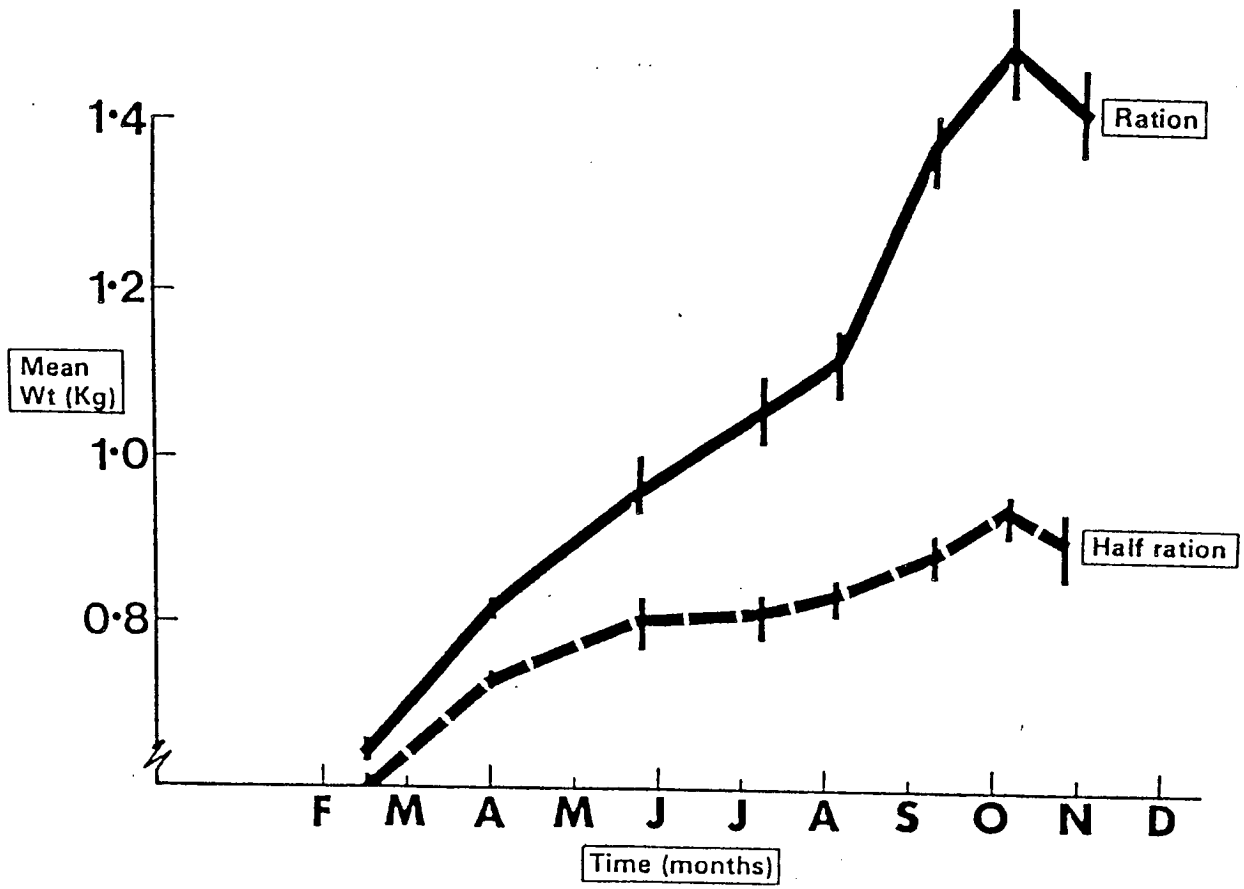

Fig. 9 .1 Growth of rainbow trout fed on 0.35% (half-ration) and 0.70% (full-ration) body weight day^{-1}.(Mean ± SEM).

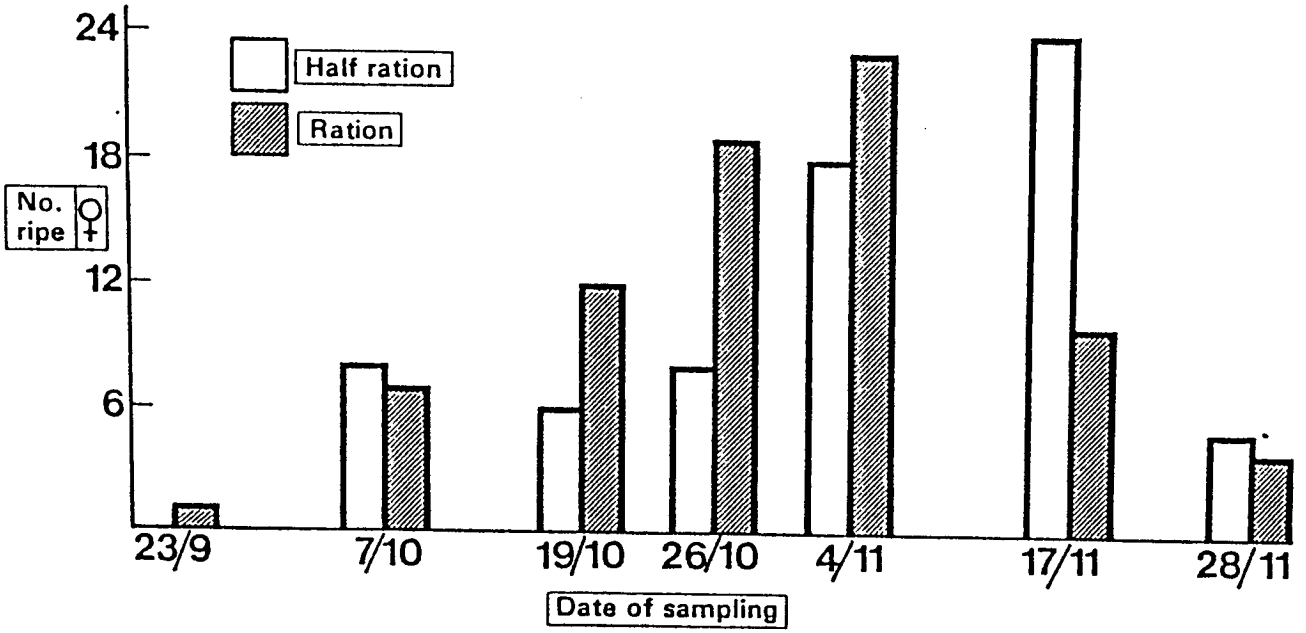

Fig 9.2 Spawning profile of the fish on half- and full-ration.

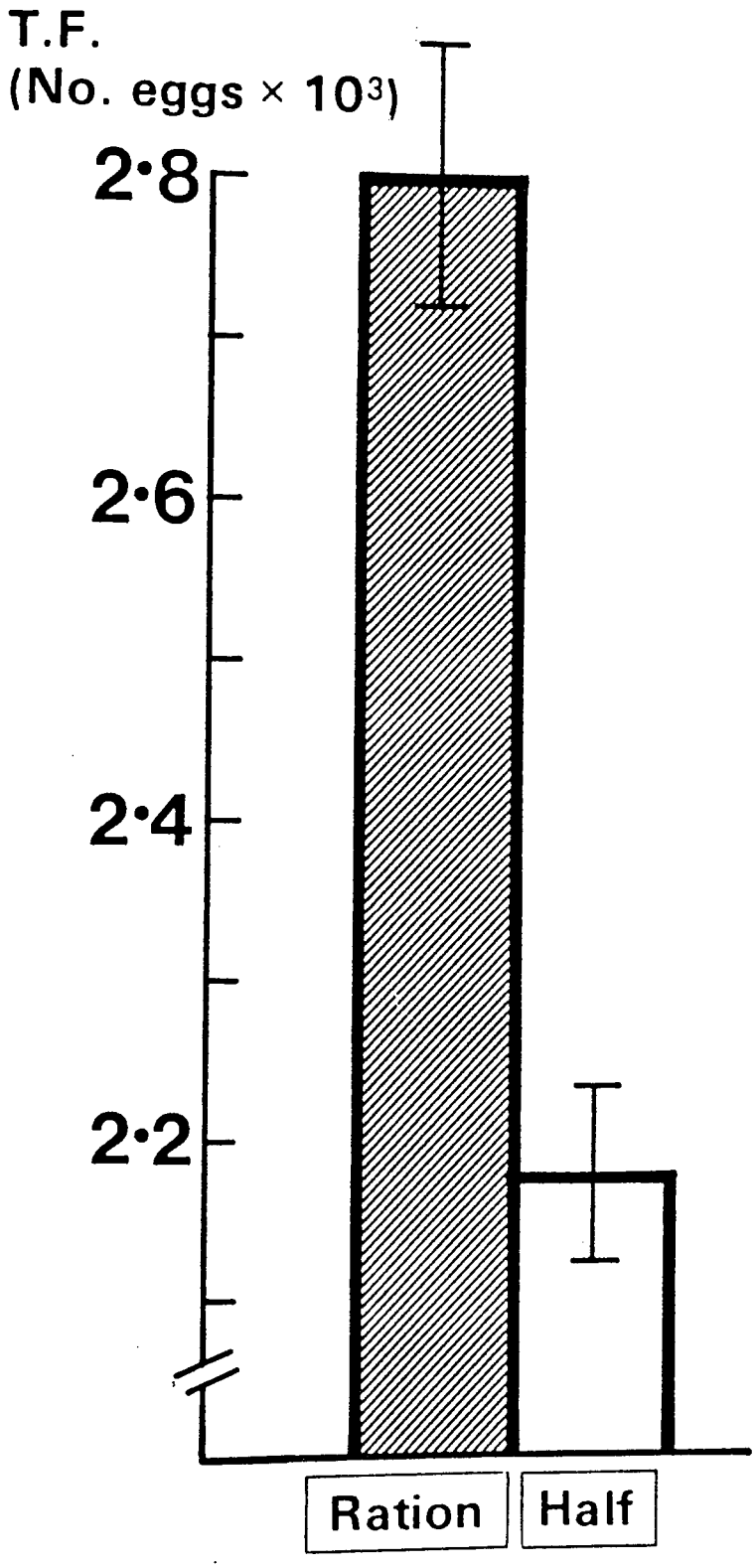

Fig. 9.3 Total fecundity (T.F.) of the fish on half- and full-ration (Mean ± SEM).

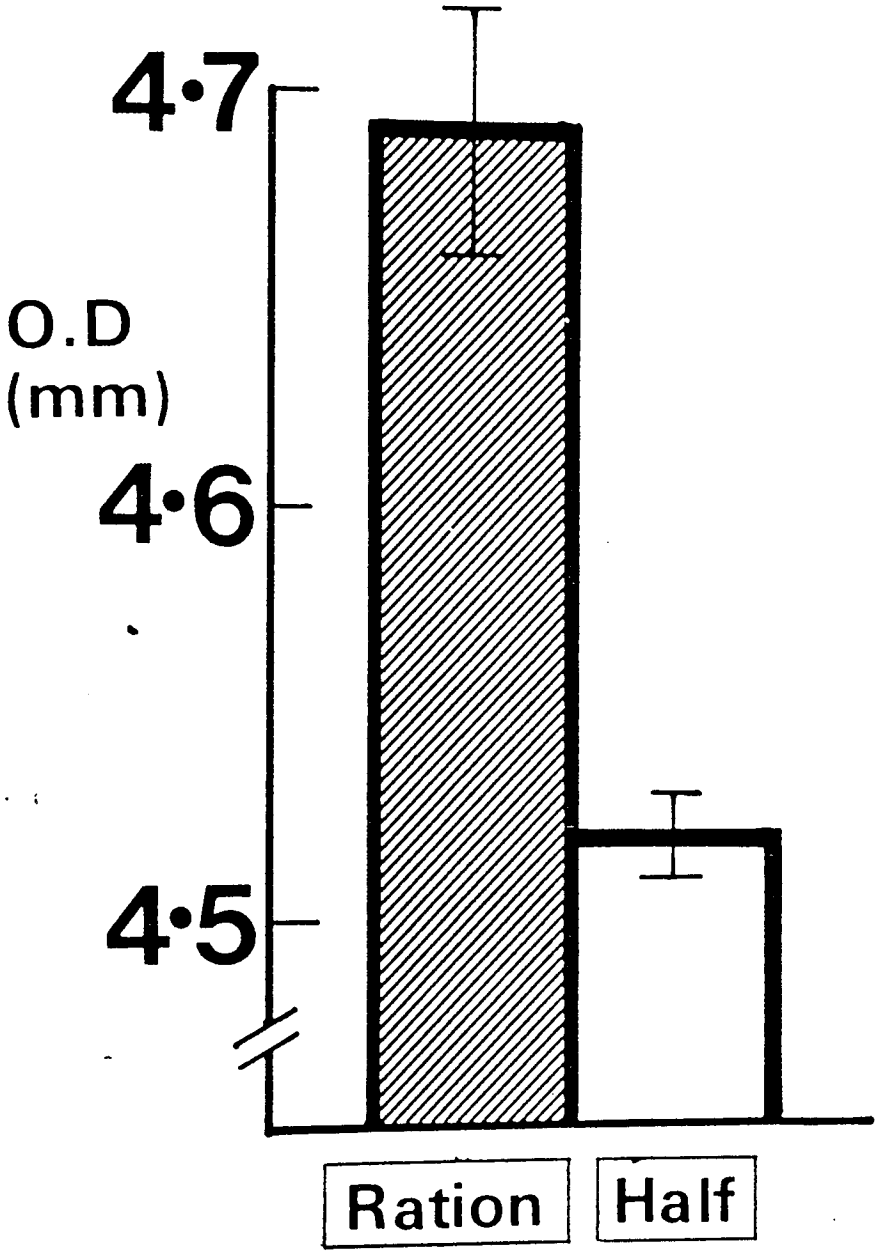

Fig. 9.4 Diameter (O.D) of the eggs from the half- and full-ration fish (Mean ± SEM).

fed with half-rations (Fig 9 .5 $P < 0.001$) when compared with the full-ration group.

Measurements of serum calcium (as an index of vitellogenin) and testosterone revealed similar profiles to those reported elsewhere (Elliott et al, 1984) with peak values occurring around the time of ovulation. The peak heights for both calcium and testosterone were reduced in the half-ration fish (Fig 9 .6).

The eggs of the full-ration fish were significantly heavier on a dry weight basis than those from fish on reduced ration (Fig 9..7 $P < 0.001$) although the proportion of water to dry matter was the same in the two groups (Table 9 .1).

The proximate composition of the water-hardened eggs from the two treatment groups are shown in Table 9 .1. Proximate analyses showed no changes in either total fat or protein levels. The amino acid profiles of the eggs from the full- and half-ration fish were similar, as were the concentrations of mineral ions, both expressed per unit weight of egg (Table 9.2 and 9 .3). There were few changes in free fatty acid composition although a moiety approximately described as 20:0 was consistently present in small amounts in the eggs from fish on half-ration but absent from the eggs of fish on full-ration (Table 9 .4).

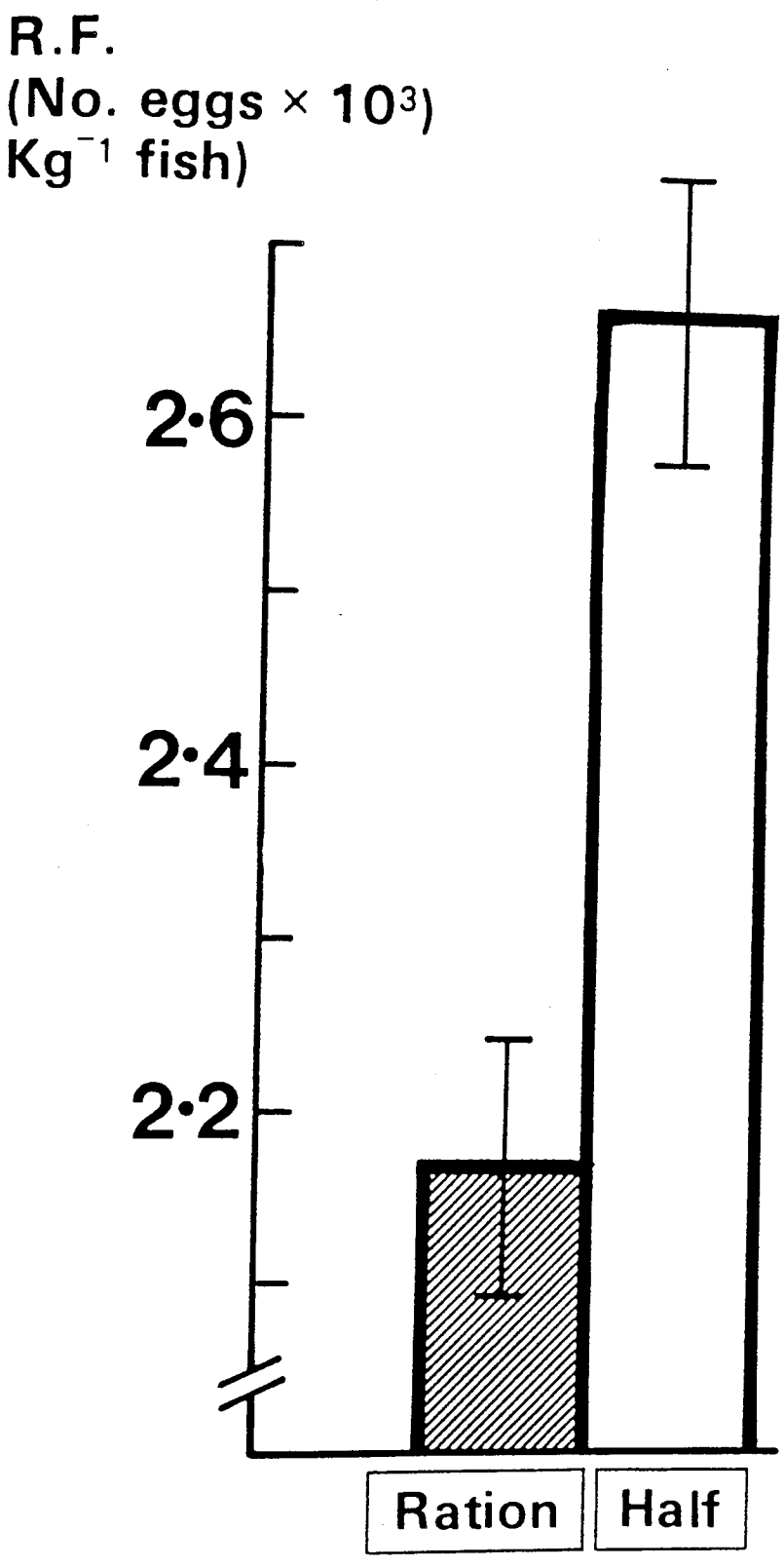

Fig. 9.5 Relative fecundity (R.F.) of the fish on half- and full-ration (Mean ± SEM).

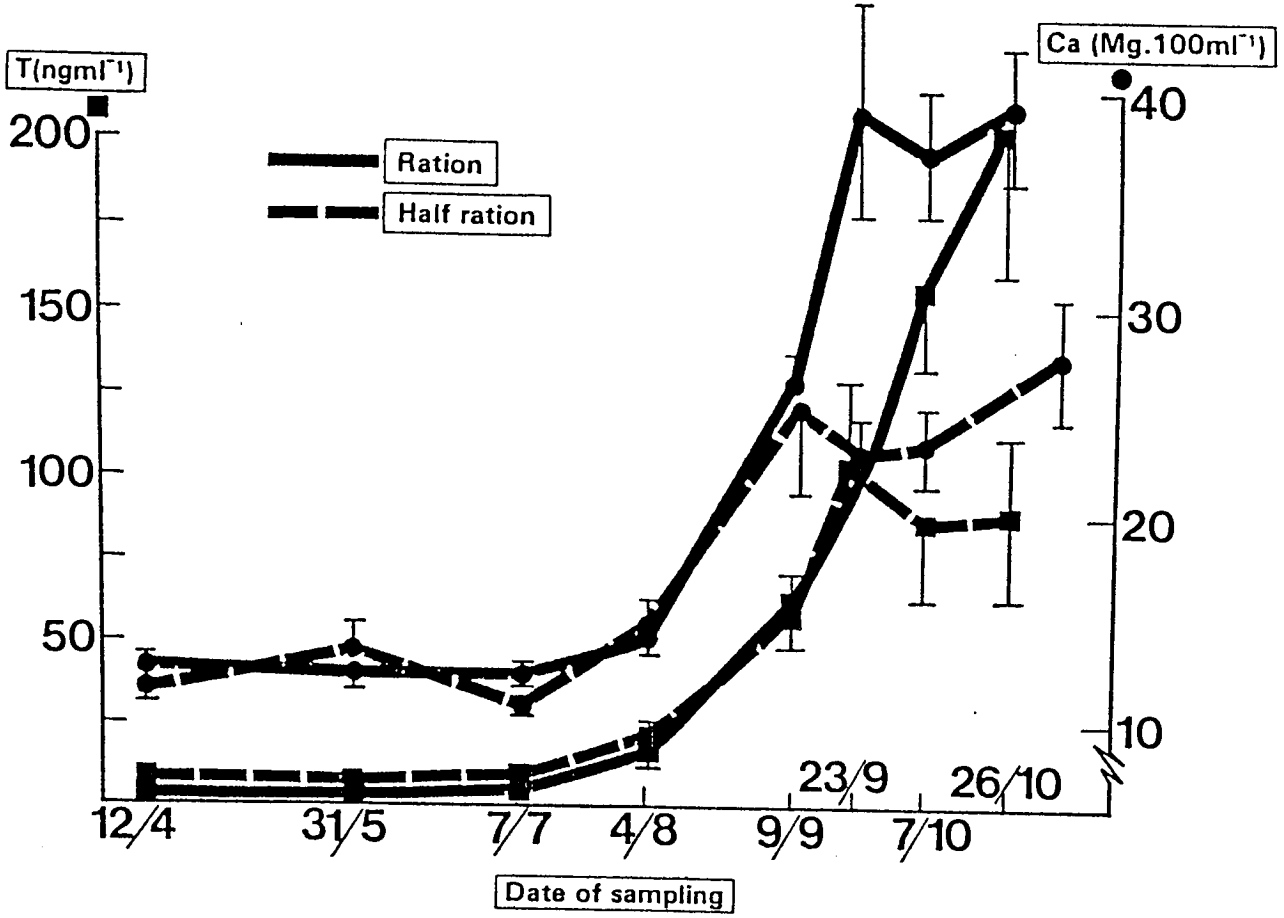

Fig 9.6 Serum levels of calcium (Ca) (as an index of vitellogenin) and testosterone (T) in the half- and full-ration fish.

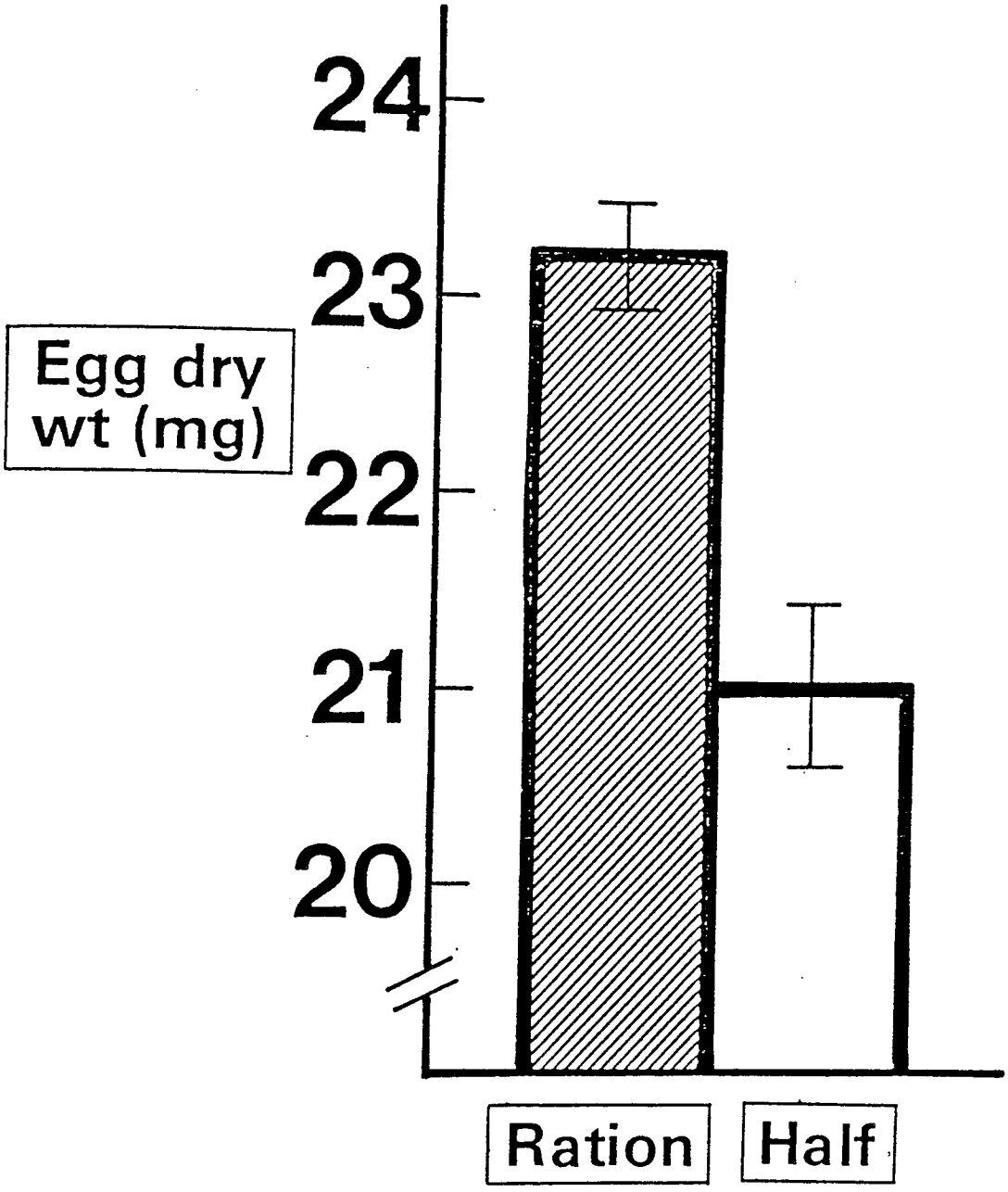

Fig. 9.7 Dry weight (mg) of the eggs from the half- and full-ration fish (Mean ± SEM).

Table 9.1 Proximate analyses of eggs from fish fed on full- and half-ration

	Half Ration			Full Ration			Sig.
	mean ± SEM		n	mean ± SEM		n	't'
Wet Wt. (mg)	47.4	0.7	50	51.0	0.8	50	**
Dry Wt. (mg)	21.0	0.4	44	23.2	0.4	49	***
% water	55.7	0.4	44	54.5	0.3	49	N.S.[a]
Ova Diam (mm)	4.54	0.02	70	4.69	0.03	76	***
Dry weight							
% Protein	69.8	2.0	10	69.1	3.3	10	N.S.[a]
% Fat	7.5	0.2	10	7.3	0.1	10	N.S.[a]
% Ash	3.6	0.1	10	3.7	0.2	10	N.S.[a]
Survival Rates							
% Fert.	92.0	1.9	9	86.3	5.9	9	N.S.[a]
% Eying	82.5	5.8	9	74.8	9.0	9	N.S.[a]
Parent Size (at spawning)							
Wt (kg)	0.835	0.020	70	1.330	0.040	76	***
Lth (cm)	41.6	0.3	70	46.0	0.5	76	***

[a] = with arcsine transformation.

*** = $P < 0.001$

Table 9.2 Levels of Free Amino Acids

(nmol g^{-1} dry wt egg x 10^3)

	Half-Ration		Full-Ration	
	Mean	± SEM	Mean	± SEM
Asp	17.3	0.2	15.6	1.1
Thr	12.4	0.4	12.8	0.4
Ser	15.8	1.1	15.7	0.8
Glu	21.2	0.4	21.3	0.5
Pro	17.3	0.5	17.2	0.7
Gly	10.4	0.5	11.1	0.3
Ala	20.1	0.2	20.0	0.3
Val	15.9	0.4	15.4	0.1
Met	6.5	0.1	6.5	0.1
Ile	11.8	0.2	11.5	0.2
Leu	14.9	0.2	16.2	0.4
Tyr	7.2	0.1	7.2	0.1
Phe	8.9	0.1	9.1	0.1
His	5.5	0.1	5.6	0.1
Lys	13.8	0.2	13.8	0.1
NH_3	16.1	0.2	14.9	0.5
Arg	11.7	0.3	8.9	0.2

Table 9.3 Mineral concentration (mg/µg.g^{-1} egg dry weight) n=6

Element	Half ration Mean ± SEM	Full ration Mean ± SEM
Na (mg.g^{-1})	1.7 ± 0.7	2.1 ± 0.7
K (mg.g^{-1})	4.7 ± 0.5	4.5 ± 0.2
Ca (mg.g^{-1})	2.0 ± 0.1	2.5 ± 0.2
Mg (mg.g^{-1})	1.9 ± 0.2	2.0 ± 0.1
Zn (g.g^{-1})	86.7 ± 7.1	108.0 ± 7.1
Fe (g.g^{-1})	60.4 ± 2.7	66.0 ± 4.4
Cu (g.g^{-1})	8.8 ± 3.6	11.3 ± 0.9

Table 9.4 Percentage composition of fatty acid in the total lipid extract (n = 6)

Free Fatty Acid	Half-ration Mean ± SEM	Full-ration Mean ± SEM
14 : 0	3.3 ± 0.2	3.5 ± 0.1
16 : 0	18.6 ± 0.9	17.4 ± 0.4
16 : 1	7.4 ± 0.4	7.7 ± 0.2
18 : 0	3.1 ± 0.2	2.5 ± 0.2
18 : 1	16.9 ± 2.5	16.4 ± 1.4
18 : 2	6.5 ± 0.4	4.9 ± 0.2
18 : 3	0.9 ± 0.04	0.9 ± 0.04
20 : 1	4.0 ± 0.2	4.2 ± 0.4
20 : 2	0.7 ± 0.04	0.7 ± 0.08
20 : 3	1.4 ± 0.1	1.5 ± 0.1
20 : 4	2.2 ± 0.3	2.6 ± 0.6
20 : 5	7.2 ± 1.0	7.2 ± 0.4
22 : 5	2.5 ± 0.1	2.5 ± 0.1
22 : 6	22.1 ± 1.8	27.5 ± 0.7

Similar survivals of the eggs were seen at fertilisation, and eying (Table 9.1), and in the fry and fingerlings up to six months of age there were overall survivals of 45% in both groups.

Initially, there were highly significant differences in the size of the fry (Fig 9.8 $P < 0.001$) and for the next 4 months this difference was maintained. Subsequently, the variance in growth within each group increased and difference between the means was not significant.

Histological examination of the preovulatory samples revealed that atresia was present in vitellogenic oocytes, Stages V-V11 (van den Hurk and Peute, 1979), of the ovaries of both full- and half-ration fish. However, the overall atresia levels of $6.9 \pm 2.1\%$ of the total numbers of vitellogenic oocytes in the full-ration fish were significantly different from the $21.9 \pm 1.2\%$ found in half-ration fish ($P < 0.05$). True atresia was not seen in Stages 1-1V oocytes in either treatment group although some Stage 1V oocytes particularly from the full-ration group were showing early signs of this process.

Total counts of the oocytes in the preovulatory ovaries of both half- and full-ration fish revealed similar numbers of vitellogenic oocytes to the numbers of fully-ripe eggs which would have been stripped at full

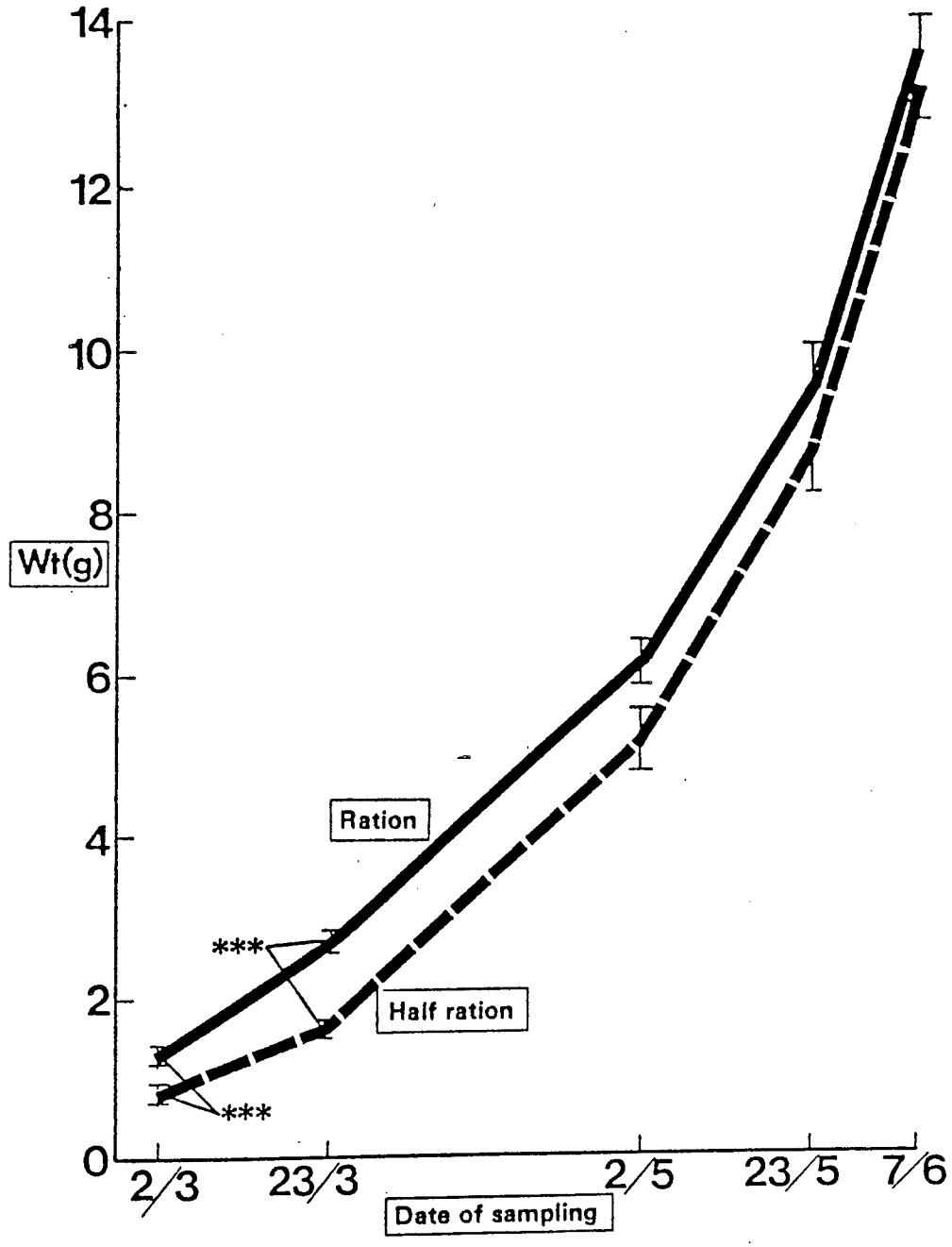

Fig. 9.8 Weight of fry (Mean ± SEM) derived from the eggs of half- and full-ration fish.

maturity. The total numbers of oocytes of all Stages were higher in the fish on full-ration as might have been predicted from the mean total fecundity of this group at spawning. There was also a delay in the development of vitellogenesis in the ovaries of the half-ration fish. At the time of sampling of the preovulatory fish most of the vitellogenic oocytes (i.e. Stage V-VII) had reached late Stage VI or early Stage VII whereas in the half-ration fish the majority of the vitellogenic oocytes were only in the early portion of Stage VI. No Stage V oocytes were present in either ration group. Examination of the post-ovulatory ovaries showed that in the full-ration fish only a small number of vitellogenic eggs remained unovulated. In contrast up to 7% of the same stages remained in the ovaries of half-ration fish although the significance of this change just failed to reach the $P<0.05$ probability level. Atresia levels were 11.9% and 17.5% respectively in the full and half-ration groups although this difference was not significant.

9.4 Discussion

The most significant effect of feeding a low ration to female rainbow trout was a 22% reduction in total fecundity, expressed as the number of ripe eggs produced per fish at stripping. Similar reductions in fecundity following experimental deprivation of food have also been reported for salmonids by other workers (Scott, 1962;

Bagenal, 1969a; Baiz, 1978). However, only Scott (1962) and Baiz (1978) determined fecundity from measurements of ripe fish. Furthermore, in Scott's study overall maturity levels in the experimental fish were very low probably because the fish on the highest ration only reached 150g in weight at three years of age. Non-salmonid species also experience reductions in fecundity following decreases in ration size (Wootton, 1973; Townshend and Wootton, 1984). Full interpretation of the results of the majority of these studies is, however, complicated by the effects of diet on somatic growth because fecundity is primarily determined by fish size (Bagenal, 1973; Wootton, 1982). Consequently, if one is aiming to determine the direct influence of ration on fecundity then the primary effects of diet on fish size must be first removed by appropriate experimental techniques (Wootton, 1982). Few studies are able to offer such differentiation and, even where such analyses have been possible, they have shown that there is considerable interspecific variation. Thus, in the stickleback, egg production is primarily a function of fish size whereas in the convict ciclid the reductions in fecundity produced by lower rations were greater than would be expected from the reductions in fish weight (Wootton, 1982; Townshend and Wootton, 1984). A direct correlation of fecundity with the amount of food received even after the size differences were taken into account was also shown for the brown trout (Bagenal, 1969a). However, there is little evidence for a direct effect of

ration on fecundity in the rainbow trout. The present work shows a significant correlation of size with fecundity in the full-ration fish ($r = 0.51$, $p<0.001$) but not for those on reduced ration, suggesting that diet may have had a direct influence in this group. However, fish of equivalent sizes in the two ration groups had similar fecundities.

In parallel with the change in fecundity both the diameter of the eggs and their mean dry weight were significantly reduced in the fish on half-ration. Hislop *et al* (1978) have also reported similar changes in laboratory-maintained haddock. In contrast Bagenal (1969a) reported that diet restriction in brown trout led to the production of larger eggs by dry weight. However Bagenal's measurements were conducted on preovulatory fish and, as the feeding regime also delayed maturity, the sampling procedure would have included eggs of varying stages of final ripeness and hydration. Generally, other investigations on salmonids have shown that egg size is not affected by reduced ration (Scott, 1962; Ridelman *et al*, 1984) or, as in the study of Baiz (1978), by a period of starvation enforced by disease problems in the experimental fish. Reductions in ration also failed to change egg size expressed as either wet or dry weight in the stickleback (Wootton, 1973) and the convict cichlid (Townshend and Wootton, 1984). However, both these species are multiple spawners and the length of the

interspawning interval, which is extended by decreased ration, may also have effects on egg size. Increases in egg size have also been reported to occur in the rainbow trout after a delay in spawning time (Bromage et al, 1984).

Although there were no differences in percentage wet and dry weights of the eggs, the absolute dry weight of the eggs was less in the fish on half-ration and closely paralleled the reduction of egg diameter in this group. Possibly, some of the varied effects of diet on egg size described above relate more to the changes in water relations experienced by the eggs during their final maturation rather than to ration per se. Such difficulties would be minimised by only comparing the sizes of water-hardened, ovulated eggs.

Proximate analyses of the gross protein, fat and ash constituents of the eggs revealed no differences between the ration groups nor were there any alterations in mineral or amino acid composition, if expressed in terms of concentration. Apart from relatively low levels of fat, which probably reflect the levels of lipid in the feed, the proximate composition of the eggs was similar to that reported by other workers (Takeuchi et al, 1981; Ridelman et al , 1984). The absence of any measurable difference between the eggs as far as proportional composition is concerned does not preclude a role for the

absolute amounts of these materials in each egg, because ultimately each hatched fry will be solely dependent on this nutrient supply for its development. Experimental reductions of either mineral or fat levels in broodstock feeds for rainbow trout have been shown to decrease the quality of the eggs produced (Takeuchi et al, 1981). Bearing in mind that the modest difference in egg diameter between the treatment groups represent a 10% difference in volume then it is possible that the additional amounts of material possessed by the larger eggs may be of selective advantage.

Certainly, like other workers (Bagenal, 1969b) it has been shown that larger eggs produce larger fry (Chapter 4) and it is possible that, under adverse conditions as for example those experienced in the wild, these larger progeny might show better survivals. However, despite clear differences in size of the eggs and hatched fry of the two ration groups there were no reductions in viability expressed on the basis of fertilisation and eying rates of the eggs and mortality levels of the fry and fingerlings. The overall survival levels were also similar to those reported for other rainbow trout stocks (Ridelman et al, 1984). The absence of reductions in viability of the developing eggs and hatched fry derived from small eggs confirms other work (Springate and Bromage, 1984b) and supports the conclusion reached by Phillips and Dumas (1959) in their study of the brown

trout that ample materials for viable embryo production exist even in the smallest egg.

In addition to the differences in egg number and size there was also a two week delay in spawning time in fish on half-ration. However the failure of 11% of the low ration group to achieve maturity is probably of more significance. Delays of spawning and extensions of the interspawning interval after diet restriction have been reported for some multiple spawners (Wootton, 1982; Townshend and Wootton, 1984) whereas salmonids stocks appear to respond to lowered ration by reducing the overall percentage of maturing fish (Scott, 1962; Bagenal, 1969a). In the present work the fish on low ration which failed to spawn had a lower mean weight (0.68±0.05Kg) than the spawning members of this group. Low fish size may have also been the reason for the very low levels of maturity described by Scott (1962) for fish on both high and low rations. Although, it is possible that a fish has to reach a certain weight before it can mature, from the present results, it would appear that size is not of importance in determining the timing of ovulation within the spawning spread of a stock. This is in contrast to the results of Kato (1975) who suggested that it was the larger fish amongst stocks of rainbow trout which spawned first.

Turning now to the possible mechanisms by which the alterations in fecundity and and/or egg size are achieved, it is apparent even from the most cursory examination of the ovary of fish throughout the year that enormous reductions in oocyte number occur during development. In the present study up to 50,000 oocytes were present in the ovary a few weeks after ovulation but only 5-6000 of these would have been expected to reach full maturity at the following spawning. Three mechanisms might account for these changes: the first involves atresia or the resorption of oocytes particularly those undergoing yolk incorporation; the second the recruitment of Stage 1-1V oocytes into vitellogenesis; and lastly the extent of oogonial multiplications.

Although the relative importance of these processes in determining fecundity and egg size may vary with different fish, in the rainbow trout the most significant change following a reduction in food was an increase in the level of atresia up to 22% of the total numbers of vitellogenic oocytes present in pre-spawning fish. Much lower levels were found in the fish on full-ration. Levels up to 27% were also reported by Scott (1962) even for the group of fish which were fed on the highest rations of food. However, closer examination of the final weights of these fish would suggest that all the experimental and control groups were being subjected to restricted rations. Vladykov (1956) described 80% and 45%

reductions in oocyte numbers up to 1mm and 3.5mm in diameter respectively in the brook trout and again suggested that this reduction was due to atresia. Levels of 27% atresia were also recorded for natural populations of the chubsucker although figures for individual fish ranged from 4-72% (Wagner and Cooper, 1963). In contrast Henderson (1963) found far fewer atretic oocytes in the brook trout although the levels, averaging 4.0%, were of the same order as those found in the fish on full-ration in the present experiment, probably because both groups were maintained under equivalent hatchery conditions and fed to full-ration according to feed manufacturers tables. It is clear that the levels of atresia reported here commonly occur in the rainbow trout in the months leading up to spawning. Furthermore, their alteration in response to reductions in ration suggests a physiological role for atresia in the determination of fecundity in the rainbow trout.

An effect of diet on the recruitment of oocytes is more difficult to assess from the present results primarily because the numbers of fish under experiment would not allow histological samples to be taken at more frequent intervals throughout the year and more specifically at the time of onset of vitellogenesis. Reductions in recruitment as a result of feeding low rations have been reported for the winter flounder (Tyler and Dunn, 1976), a cichlid (Townshend and Wootton, 1984)

and Poecilia an ovoviviparous fish (Hester, 1964). In contrast de Vlaming (1971) showed that starvation induced atresia in the yolky oocytes of the goby but did not affect recruitment. There was evidence from the present work that vitellogenesis was more advanced in the fish on full-ration. However, this apparent difference was probably due to an increase in the rate of maturation of these fish and the sampling of both groups on the same day. Vitellogenin and testosterone levels were significantly lower at spawning in the half-ration fish and although the reductions in size and number of eggs in these fish could be attributed to these serum changes, the relationship remains unclear. There were no changes in the proportion of eggs undergoing vitellogenesis in the full-ration fish despite the higher fecundities of this group and the presence in the pre-spawning ovaries of much higher numbers of yolky oocytes. One must conclude that effects on recruitment or the oogonial divisions, if present, must have occurred much earlier in the year-long cycle of ovarian development.

As a consequence of the parallel changes in total fecundity and egg size, there was an increase in relative fecundity i.e. the number of eggs per kilogram of body weight, in the fish on half-ration. Similar changes in relative fecundity are also seen with decreasing body weight in commercial stocks of rainbow trout (Springate and Bromage, 1984b). The decrease in relative fecundity

of the larger full-ration fish is partly explained by the increased size of their eggs. Decreases in relative fecundity and increases in egg size are also found with increasing body weight in rainbow trout maintained under normal hatchery conditions (Springate and Bromage, 1984b). Other workers have also reported that larger fish often produce fewer eggs than would be expected from the regression of fecundity and fish size (Rounsefell, 1957; Nicholls, 1958). A probable explanation is that the rainbow trout and possibly other salmonids, produce a volume of eggs at stripping which bear a constant relationship to body weight and that reductions in either fecundity or egg size, possibly caused by alterations in diet, will involve corresponding increases in the other parameters. A similar conclusion regarding a 'trade off' between egg size and number was also reached by Rounsefell (1957) in his extensive review of the fecundity of North American Salmonidae. Although the production by the trout of a weight-related volume of eggs would not preclude direct effects of ration or nutrient on fecundity and egg size it might provide a finite range of egg size and number on which subsequent alterations in atresia or recruitment induced by nutritional and other environmental changes might be superimposed.

The presence of a reciprocal relationship involving egg size and number which can be modified by dietary change, together with the decrease in relative fecundity

and increase in egg size with increased body weight are potentially of considerable commercial importance. Currently, salmonid eggs are sold by number and not by size and the potential production of a hatchery is determined by the total weight of broodstock which can be maintained on the farm. Under such constraints, an increase in the total numbers of eggs from the same weight of broodstock might be achieved by varying the feeding regime or by using more broodfish but of smaller size. Providing the consequent decreases in size of the eggs do not affect their viability or their acceptance for general sale such approaches may confer considerable economic advantage.

In summary the present results provide evidence for multiple effects on the fecundity, egg size and rate of maturation of rainbow trout. Further work is necessary to investigate the effects of varying nutrient composition in the diet and of different feeding rates at different times of the year.

Summary and Conclusions to Part III

This section of the thesis clearly demonstrates that good husbandry and management of the broodstock can significantly improve egg quality and fecundity. Possibly the most important determinant of egg quality is the timing of stripping after ovulation. The results indicate that maximum egg and fry survivals are achieved if the eggs are stripped 4 - 6 days after ovulation and that rainbow trout farmers should examine their broodstock at least every 10 days if they are to achieve the highest survival rates from their eggs and fry.

It is becoming increasingly more important for fish farmers to adopt specialist techniques for egg production and in order to take advantage of these techniques, broodstock and gamete management must be of the highest quality and involve a thorough understanding of the biology of rainbow trout reproduction. Only with this knowledge will improvements be made in the quality of commercially produced eggs.

Egg quality was not affected by the different ration levels fed to the broodstock. However at the end of the experiment the fish fed on the full-ration (0.70% body weight day^{-1}) were significantly bigger and produced more, larger, eggs than the fish that had been fed on half-ration (0.35% body weight day^{-1}), although the fish on

half-ration had a higher relative fecundity than those which had received full-ration. Consequently a tonne of fish fed at half-ration would produce more, albeit smaller, eggs than a tonne of fish fed on full-ration.

General Conclusions

1. Observations made on seven commercial rainbow trout farms revealed that egg and fry mortalities were unacceptably high. On average only 70% of eggs survived to the eyed stage (20 days post-fertilisation at 10°C) and only 35% survived to become 4.5g fry (approx 130 days post-fertilisation at 10°C).

2. In the laboratory, survival of eggs derived from the same commercial broodstock showed much higher survival (85% to the eyed stage). This suggests that environmental factors and husbandry techniques may be exerting major influences on egg quality and that improved management could substantially improve egg quality on many commercial farms. Indeed such improvements alone would avoid some of the requirement for importing rainbow trout eggs into the U.K.

3. It was confirmed that large eggs produce larger alevins than smaller ones. This size advantage was however lost four weeks after the time of the first feeding.

4. By following the growth and survival of fry derived from eggs of different size it was shown that there was no significant relationship between egg size and

egg quality at least up to 26 weeks of life. This has far reaching implications for the management of hatcheries and in particular the use of eggs from first spawning fish.

5. There was a large variation in the chemical composition of eggs from fish of different strains and also between eggs from individuals of the same strain. However none of the chemical components measured during this research (ie. protein, fat, vitellogenin, ash, amino acids, free fatty acids and minerals) could be correlated with egg quality.

6. Total fecundity and egg size increased, but relative fecundity decreased with increasing fish weight i.e. the fish produced a constant volume of eggs. This suggests that there is a 'trade off' between egg size and egg number and that increased egg size can only be achieved by a reduction in fecundity. As rainbow trout eggs are sold by number in this country one must conclude that it is better to produce the greatest number of eggs from each tonne of broodstock and that this can be best achieved by producing the smallest eggs which are acceptable for sale.

7. One strain of rainbow trout was significantly more fecund than two other strains all maintained on the

same farm. The difference between the strains could not be explained simply be alterations in egg size.

8. Trout of the same strain maintained on different farms behaved similarily suggesting that there was some reproducibility of strain characteristics.

9. The timing of stripping after ovulation was found to be a major determinant of egg quality. The optimum period for removing eggs from rainbow trout was found to be 4 - 6 days post-ovulation (at 10°C). It was recommended that commercial farms should examine their broodstock at least every 10 days if they are to achieve optimum survival rates from their eggs.

10. Fish fed on full-ration (0.70% body weight day^{-1}) were larger, produced bigger eggs and had higher total fecundities than fish fed on half-ration (0.35% body weight day^{-1}). However, the fish fed on half-ration had higher relative fecundities. Consequently a tonne of fish maintained on half-ration would produce more, albeit smaller, eggs than a tonne of fish maintained on full-ration. Much

more research effort is required to determine the optimum feeding strategy for broodstock rainbow trout.

Appendices

Appendix 1. Computer programme for comparison of regression by analysis of covariance (ANOCO). Written in the language of the 'minitab' software package.

C1=x_1 ; C2=y_1 ; C3=x_2 ; C4=y_2.

```
 1 MEAN C1 K1
 2 LET C5=C1-K1
 3 LET C6=C5*C5
 4 SUM C6 K2
 5 PRINT K2
 6 MEAN C2 K3
 7 LET C7=C2-K3
 8 LET C8=C7*C7
 9 SUM C8 K4
10 PRINT K4
11 LET C9=C5*C7
12 SUM C9 K5
13 PRINT K5
14 LET K6=K5*K5
15 LET K7=K4-(K6/K2)
16 PRINT K7
17 COUNT C1 K8
18 LET K8=K8-2
19 LET K9=K7/K8
20 PRINT K9
21 MEAN C3 K10
22 LET C10=C3-K10
23 LET C11=C10*C10
24 SUM C11 K11
25 PRINT K11
26 MEAN C4 K12
27 LET C12=C4-K12
28 LET C13=C12*C12
29 SUM C13 K13
30 PRINT K13
31 LET C14=C10*C12
32 SUM C14 K14
33 PRINT K14
34 LET K15=K14*K14
35 LET K16=K13-(K15/K11)
36 PRINT K16
37 COUNT C3 K17
38 LET K17=K17-2
39 LET K18=K16/K17
40 PRINT K18
41 LET K20=K17+K8
42 LET K21=K7+K16
43 LET K22=K21/K20
44 PRINT K22
45 LET K23=K2+K11
46 LET K24=K5+K14
47 LET K24=K24*K24
48 LET K25=K4+K13
49 LET K26=K8+K17+1
```

```
 50 LET K27=K25-(K24/K23)
 51 PRINT K27
 52 LET K28=K27/K26
 53 PRINT K28
 54 LET K29=K27-K21
 55 PRINT K29
 56 JOIN C1 C3,C14
 57 MEAN C14 K30
 58 LET C15=C14-K30
 59 LET C16=C15*C15
 60 SUM C16 K31
 61 PRINT K31
 62 JOIN C2 C4,C17
 63 MEAN C17 K32
 64 LET C18=C17-K32
 65 LET C18=C17-K32
 66 LET C19=C18*C18
 67 SUM C19 K33
 68 PRINT K33
 69 LET C20=C15*C18
 70 SUM C20 K34
 71 PRINT K34
 72 LET K35=K34*K34
 73 LET K36=K33-(K35/K31)
 74 PRINT K36
 75 LET K37=K36-K27
 76 PRINT K37
 77 OUTF 'AA'
 78 NOTE RESULTS
 79 NOTE HOMOGENEITY
 80 LET K38=K9/K18
 81 PRINT K38
 82 NOTE**********
 83 NOTE D.F FOR HOMOGENEITY
 84 PRINT K8
 85 PRINT K17
 86 NOTE  SLOPE
 87 LET K39=K29/K22
 88 PRINT K39
 89 NOTE*********
 90 LET K41=K8+K17
 91 NOTE D.F FOR SLOPE
 92 NOTE 1
 93 PRINT K41
 94 NOTE ELEVATION
 95 LET K40=K37/K28
 96 PRINT K40
 97 NOTE********
 98 LET K42=K8+K17+1
 99 NOTE D.F FOR ELEVATION
100 NOTE        1
101 PRINT K42
102 OUTF
103 END
```

Appendix 2. Research publication. Reprint of a paper concerning the experiment reported in Chapter 8 of this thesis.

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THE TIMING OF OVULATION AND STRIPPING AND THEIR EFFECTS ON THE RATES OF FERTILIZATION AND SURVIVAL TO EYING, HATCH AND SWIM-UP IN THE RAINBOW TROUT (*SALMO GAIRDNERI* R.)

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