THE CONTROL OF REPRODUCTION IN RAINBOW TROUT - COMMERCIAL AND TECHNICAL ASPECTS.

THESIS

Submitted for the degree of
Doctor of Philosophy
of the
University of Aston in Birmingham.

by

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June 1979.

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COLIN WHITEHEAD. PhD. 1979.

Summary.

The thesis provides a detailed study of the endocrine control of reproduction in the rainbow trout. By using photoperiod and temperature manipulations and hormonal treatments, designed to interfere with this natural control, possible solutions are provided to the two major problems related to reproduction in the farming of rainbow trout, i.e. the limited availability of eggs, and the regressive changes which accompany maturation amongst production stock.

During the normal spawning cycle, sequences of changes in serum levels of gonadotropin, oestradiol 17β, phosphoprotein phosphorus and total calcium in females, and gonadotropin and testosterone in males were observed. Under shortened seasonal photoperiod cycles, these changes occurred earlier in the year although the basic sequences remained unchanged. As a result, spawning was advanced by up to four and five months in the female and male fish respectively. Injection with oestradiol caused similar changes and thus established the importance of this hormone in the control of vitellogenesis.

At low temperatures, the egg incubation period was increased by 50 days, thus providing a further method of extending the availability of eggs.

To exploit a supply of eggs throughout the year, a computer model was formulated to predict growth under a variety of environmental conditions. This information could enable the trout farmer to design an optimum stocking programme.

Treatment of first - feeding fry with high doses of methyl testo - sterone and with oestradiol 17β produced sterilization and feminisation respectively without any adverse effects on growth and mortality. Pairing masculinised females with normal female fish demonstrated that the female rainbow trout is the homogametic sex and that by using such sex - reversed fish monosex populations could be produced.

Clearly, the production of eggs at any times as specified by computer assisted stocking schedules, together with the potential for producing sterilized or feminised stock is a joint approach which will be of considerable benefit to fish farming in the U.K.

Key words : trout, maturation, photoperiod, hormone, spawning.

i. PREFACE.

The title " The Control of Reproduction in Rainbow Trout - Commercial and Technical Aspects " summarises the basic theme followed during the development of this thesis. However, it will be seen that the project further subdivides into three main fields of investigation, namely, the control of reproduction to supply eggs throughout the year, the development of computer models to predict and optimise production under different farm conditions and the prevention of maturation among production stock. Hence, the approaches used, although focused on a single theme, are necessarily diverse. A brief description of how the project developed should serve to explain why the various approaches were chosen.

In 1974, Shearwater Fish Farming Ltd., part of the B.O.C. Ltd., New Venture Secretariat, the research and development section of the company, constructed a farm at Low Plains, Armathwaite, Cumbria, as an experimental development designed wholly to investigate, on a commercial scale, a high technology approach to fish farming.

During the development stages of this farm it became clear that the limited seasonal availability of eggs was an important constraint to the planning of all year round production of table fish. Additionally, as production at Low Plains has grown, the B.O.C. management, like those of other large farms have encountered increasing difficulty in optimising their forecasts of production in the face of differences in growth due to the environmental conditions, different possible feeding alternatives, etc. The provision of eggs throughout the year would only exacerbate these problems and to improve the long term planning of production it was thought that forecasting models could be developed, like in many other areas of industry

and agriculture,which would enable the full potential of the farm to be realised. Similar lines of research were also being considered at the University of Aston and thus a project evolved, between B.O.C. Ltd., and the Department of Biological Sciences through the Interdisciplinary Higher Degrees Scheme at the University, to investigate the control of reproduction in rainbow trout and assess the commercial advantages to be gained from such an approach.

The Interdisciplinary Higher Degrees Scheme is a distinctive approach combining the technical and managerial aspects of real commercial problems, hence providing an excellent training for graduates. The basic structure of projects involves a collaboration between a commercial company and the University. In this case, Shearwater Fish Farming Ltd., providing the practical context for investigation, including adapted facilities from their production units, and the University providing the necessary technical expertise and training.

The supervisory team throughout the project was administered by Dr. David van Rest (IHD Scheme Tutor), and consisted of:
Dr. Niall Bromage (Biological Sciences, Main Supervisor),
Dr. John Forster (Shearwater Fish Farming Ltd., Industrial Supervisor),
Mr. George Lindfield (Computer Centre, Associate Supervisor), and
Mr. Robert Harrison (formerly Management Centre, Associate Supervisor).

Financial support was provided jointly by a research studentship from the Joint Committee of the Science Research Council and the Social Science Research Council and Shearwater Fish Farming Ltd.

The thesis is designed to be read by both the scientist interested in the biological aspects of reproduction in salmonids, and the

fish farmer concerned with the commercial advantages to be gained from controlled reproduction. Hence, no apology is made for the occasional repetition to clarify a point for this wider readership.

ii. CONTENTS.

SUMMARY.

PREFACE.

1. GENERAL INTRODUCTION.

In most forms of animal husbandry, including fish, considerable advantage has been gained from the manipulation and optimisation of the natural sexual cycle of the species under cultivation. Although the culture of rainbow trout has been practised in Britain for the last hundred years, it is only recently, during the rapid growth of fish farming that attention has been focused, both on the control of the natural sexual cycle and on ways this knowledge can be used to improve trout culture. Two aspects of maturation continue to pose considerable difficulties for the fish farmer. Firstly the availability of eggs for ongrowing is at present seasonal, and this is an important constraint to the long term planning of all year round production of table fish. However, even if eggs were available at all times of the year, in order to optimise the capability of a given farm site, more information is required regarding the growth of trout under different environmental conditions. Once this information is available, it will be possible to determine at which times of the year to introduce eggs and hence stock the farm to the best advantage. Secondly, maturation among production stock, which in rainbow trout like all salmonids leads to diminished growth, loss of condition and thus reduced production of saleable fish flesh. Both aspects and their implications to the fish farmer are considered in more detail below.

1.1. Egg Availability.

All trout farms in this country aim to produce a constant supply of good quality table fish throughout the year. However, achievement of this ideal has partly been prevented by the availability of eggs over only a short period of the year.

This difficulty was confirmed in a survey conducted in late 1976 (Appendix 1.) which demonstrated that eggs are only available to U.K. fish farmers for a limited period during any one year. Replies from this survey suggested that farms in the U.K. fall into one of two groups. The majority carry only sufficient broodstock to meet their own requirements and are therefore independent of other suppliers; the remainder carry no broodstock at all and rely for their supplies on major hatcheries specialising in egg and fry production. The periods over which the larger hatchery facilities are able to supply eggs are shown in Fig.1.1. From these results it is evident that eggs are only available in Britain from November to March, and only the northern farms such as Grampian Fisheries appear to be able to extend this period, mainly as a consequence of colder water temperatures.

The restricted availability of eggs imparts a serious constraint on fish farm management, as at various times of the year differential loads are placed on each part of the farming system. Hence, during the spawning period, the hatchery and fry facilities are usually fully stocked, often overstocked, to meet production demands during the following year, whereas at other times of the year parts of the farm may be under-utilised or not used at all, as stocks of the requisite size are not available. Clearly, in situations where overstocking or understocking occur the whole system is inefficient in terms of proper usage of capital equipment and labour and consequently will be less profitable.

Until recently, an effective way of extending the availability of eggs in Britain and therefore, of partially overcoming these

HATCHERY.	EGG AVAILABILITY.
HOOKE SPRING TROUT FARM	NOVEMBER - MARCH
CAMBRIAN FISHERIES	NOVEMBER - MARCH
CLEARWATER (ISLE OF MAN)	DECEMBER - FEBRUARY
WYE VALLEY FISHERIES	DECEMBER - MARCH
WHITEBROOK FISHERIES	DECEMBER - MARCH
LAKE FISHERIES (ISLE OF MAN)	JANUARY - MARCH
HIGHLANDS AND ISLANDS DEVELOPMENT BOARD	JANUARY - MARCH
GRAMPIAN FISHERIES	JANUARY - JUNE
HOWIETOWN AND NORTHERN	FEBRUARY - APRIL

FIG.1.1. THE APPROXIMATE AVAILABILITY OF EGGS FROM LARGER U.K. HATCHERY FACILITIES (1976) - SEE APPENDIX 1.

problems,was to import eggs from abroad. Other countries have strains of rainbow trout which spawn at different times of the year to those in Britain, due to their adaptation to different environmental conditions. However, in an attempt to prevent any further introduction of salmonid disease, the Ministry of Agriculture, Fisheries and Food have quite rightly recently introduced very strict controls regarding the import of fish eggs (Min. DOF6,4,75). Although this is an important step to protect the long-term interests of fish farming in this country, it has greatly reduced the number of farms abroad from which eggs may be imported. It is probable that the Ministry would prefer to completely stop the import of eggs and to meet demand at present they are encouraging a scheme of certification amongst British suppliers to provide good quality disease free eggs from our own stocks. However, although this scheme will prevent the introduction and spread of disease, the farms will still only produce eggs during the normal spawning period of trout in the U.K.

These recent developments increase the urgency for the development of techniques to produce eggs throughout the year from stocks already present in Britain. A number of methods have been suggested, but few if any have been properly investigated, or their use to the practising fish farmer evaluated. The complete control of spawning is required.

1.1.1. The Control of Spawning: The overall process of maturation and spawning in salmonids is controlled by both internal (genetic) and external (environmental) factors, whose control is realised by modifications in the dynamics of the endocrine system. Consequently it is possible that maturation may be controlled by an approach from one or more of the areas of genetic, environmental or hormonal manipulation.

The improvement of stock by genetic manipulation has been carried out in all forms of plant and animal husbandry. In trout farming the usual practise is to select the best looking fish to be the parents of the next generation. However, very little systematic breeding work has been carried out with salmonids. One of the few examples is the work of Donaldson with both rainbow trout (Donaldson,1968; Limbach,1969) and salmon (Donaldson & Menaveta, 1961). Unfortunately, unselected controls were not maintained for comparison and it is possible that some of the reported gains in growth arose over the years from improved husbandry techniques rather than a modification of the genetic structure of the fish population.

The use of genetic selection on a commercial scale as a means of altering spawning time is at present questionable. Strains from Federal hatcheries in the U.S.A. are reputed to spawn at different times of the year (Fig.1.2). However, it is not clear whether the differences in spawning time are a true function of genetics, of different hatchery conditions or a combination of both. The regulations regarding the import of eggs practically prohibit the purchase of eggs from such strains, although some long-term studies are being carried out within the confines of M.A.F.F. at Lowestoft. Furthermore, Purdom (1978, personal communication) one of the few scientists working on the genetic improvement of fish in this country, has indicated that a selection programme designed to significantly alter the spawning time of rainbow trout would take at least fifteen years. Breeding experiments to alter spawning might also seriously complicate selection programmes for other factors such as increased growth rate, improved food conversion, survival, flesh quality, etc. In view of these difficulties and the requirement of the developing trout industry for more

STRAIN.	SPAWNING TIME.
WINTHROP	JANUARY - FEBRUARY
SHASTA	AUGUST - SEPTEMBER
ENNIS	NOVEMBER - DECEMBER
KAMLOOPS	MARCH - MAY

FIG. 1.2. SPAWNING TIMES OF STRAINS OF RAINBOW TROUT MAINTAINED IN FEDERAL HATCHERIES IN THE U.S.A.

immediate solutions, a genetic approach to alter spawning is not included in the present work.

In their natural environment, fish spawn at times of the year which give the greatest chance of survival for their young. In practice this means that fish must have the ability to predict the times of the year when conditions are most likely to be favourable for further development, possibly with regard to food availability or water supply. To achieve the correct spawning time, the maturing fish takes cues from the environment to synchronise the different stages of development with the appropriate time of the year. Of the various environmental factors implicated in the control of maturational events photoperiod, temperature and nutritional status appear to be the most important, with subsidiary influences imparted by rainfall, pheromones and salinity in some species (e.g. Donaldson, 1975).

In salmonids, photoperiod appears to be the major factor controlling reproduction, although temperature may have an effect if conditions are encountered which are outside the normal range. Of the factors mentioned, photoperiod is the only factor which is exactly the same on each day from year to year. In view of this, and of the information concerning the effects of different light regimes on reproduction already available in the literature (Hoover, 1937; Hoover & Hubbard, 1937; Alison, 1951; Hazard & Eddy, 1951; Corson, 1955; Combs et al, 1959; Nomura, 1962; Henderson, 1963; Carlson & Hale, 1973; Kunesh et al, 1974) it was decided that the manipulation of photoperiod not only offered the most likely method to achieve the desired alteration in spawning time, but also provides an approach which could easily be applied to the fish farm.

Unfortunately, the literature regarding the environmental control of spawning is confounded by the variety of experimental techniques, strains of fish and methods of evaluation (e.g. spawning time, gonad growth comparison, gonadosomic index or histological evaluation) used in these studies. It is very difficult to draw firm conclusions from the data for, as Pickford & Atz in 1957 stated, "One factor that makes the different experiments difficult to compare, as well as undoubtedly explaining some of the discrepancies in results obtained with the same species, is the difference in the response of fish to light during various periods of the annual cycles. The most probable existence of such differences indicates that experiments of longer duration, and covering all periods of the year are needed". Surprisingly, little has changed since that date and therefore several long-term trials are included in the present work to study the effects of different photoperiod regimes on spawning in both male and female rainbow trout. At the same time, an evaluation of changes in various blood parameters associated with maturation and spawning should give both an indication of the effect of each photoperiod regime on these processes, and suggest methods by which changes in photoperiod could affect the endocrine system and thus bring about changes in spawning time.

Both genetic and environmental factors act upon the hypothalamus, causing the secretion of releasing hormone which in turn initiates the release of gonadotropin(s) from the pituitary. The gonadotropin thus produced acts upon specific follicular receptors in the ovary to cause specific steroid biosynthesis. As well as acting directly to influence oocyte maturation within the follicle the steroids also produce effects on many other tissues of the body; these are sometimes referred to as

"whole body" maturation. One of the most important of these is that of oestrogen on the liver where it promotes the synthesis of egg proteins. Large amounts of calcium are also bound to the egg protein complex and once in the blood, this is transported to the ovary, where it is sequestered by the developing oocytes.

From this brief account it is clear that as well as providing insight into the mechanism of control of reproduction, the direct manipulation of hormone status also offers another possible approach to the overall control of maturation. For the last 50 years the hypophysation of fish by administration of crude pituitary extract has been used to promote gonadal maturation and induce spawning in fish that do not spawn readily under artificial conditions. More recently, such techniques have also been used to spawn Chinese and Indian carps (Varghese, 1975), flounder (Smigrelski, 1975) and Japanese and American eels (Ochiai et al, 1972, 1974; Yamamoto et al, 1974a, 1974b; Edel, 1975). However, such methods are unnecessary in salmonid culture where fish spawn readily under farm conditions although hand-stripping is required. The main application of hormones in this context appears to lie in their possible use in salmonid culture to increase or decrease the rate of maturation and so alter spawning time. Although some success has been reported by injection of various hormones, reviewed recently by Fontaine (1976), as expected, the response appears to vary depending on the stage of the reproductive cycle. Thus, before this approach can be applied to salmonids, a better understanding of the normal sequence of hormonal events which lead up to spawning is required. Experiments are therefore included here to study the effects of injected hormones on the normal sequence of events which take place during maturation.

The mechanisms of reproductive control described above ensure that eggs are produced at the correct time of year. In addition, the development of the fertilized eggs is also modified by the environment to ensure that hatching occurs when conditions are most likely to be favourable for further development. The rate of development of fish embryos is dependent primarily upon water temperature with subsidiary influences imparted by water flow and oxygenation. The time taken from fertilization to hatching is conveniently approximated in terms of "degree days", i.e. the temperature in degrees centigrade multiplied by the time period in days. For salmon and brown trout for example, this value is approximately 400 degree days. Thus if eggs were incubated at 10°C, the time taken for hatching would be around 40 days, whereas at 5°C, the incubation period would be around 80 days. Early work involving the incubation of eggs at low temperatures to extend their availability was unsuccessful due to high mortalities (e.g. Embody, 1934). However, recent research has provided more promising results (Combs, 1965; Maddock, 1974) and therefore experiments are included to study the commercial application of methods of low temperature egg incubation.

1.1.2. Production Planning: Clearly the managerial problems caused by limited egg availability would be adequately solved if fish could be induced to breed at any time during the year. However, to fully exploit the ability to introduce eggs throughout the year requires considerable detail regarding the growth of trout under the specific conditions of the farm in question. Once this information is known, then decisions can be taken to select the appropriate times of year in which to introduce eggs and in what quantity to optimise production.

The growth of trout is determined by a number of separate although often interacting factors. These include water temperature, feed level, conversion ratio, stocking density, growth variance, starting size, ultimate size required and mortality. As many of these factors are reasonably consistent from year to year, the growth of fish under a given set of conditions can be predicted. For example, consider a farm which is supplied with a borehole water at a constant temperature of 9°C, throughout the year. If factors such as stocking density, conversion ratio and appropriate feed level remain constant throughout the year, then an input of eggs at 3 monthly intervals would give an all-year-round supply of table fish and ensure that the farm was utilised to its optimum capacity throughout the year. Obviously, as a production unit this would compare most favourably with the situation at present where eggs are only available for a few months during the winter. As the temperature is constant, the calculation of growth curves (assuming a fixed conversion ratio) is a relatively simple operation as growth is consistent from week to week. However, the majority of farms in Britain do not have a constant water temperature and it is both laborious and complex to calculate growth curves especially when fry are required to be introduced into the system at different times of the year to maintain production.

In view of these difficulties the present study aims to develop and implement a computer model for planning fish production on a variety of farms with either fixed or fluctuating environmental conditions. This information would enable management decisions to be taken as to which months of the year eggs or fry are required and in what numbers, to give an all-year-round production schedule.

1.2. The Prevention of Maturation.

The second major problem area related to reproduction in salmonids is that of maturation among farm stock destined for table consumption. At present, the majority of trout farms produce portion-sized trout of around 250g, which on average takes approximately 18 months from hatching. In the main, female rainbow trout usually mature after three years growth and the majority of male fish mature in their second year. During the process of sexual maturation, particularly in the male fish, changes occur in skin pigmentation and flesh quality which seriously reduce market acceptability. Furthermore, there are associated losses in growth and conversion efficiency and an increased susceptibility to bacterial and fungal invasion. As many of the male fish in a population mature before they are of a sufficient size to be cropped and marketed, they represent a significant loss in production. There is also a market trend towards the production of larger fish, up to 3 kg. or more in weight, and on those farms the problem of maturation among production stock becomes more acute as most fish will mature before they have reached the requisite size. It is therefore necessary to develop methods by which sterile or all-female stocks of trout can be produced. These methods of necessity must be relatively easy for the fish farmer to use.

The same methods of approach, i.e. genetic, environmental, and hormonal, already discussed in relation to the control of spawning are also directly applicable to the prevention of maturation. The genetic approach to this problem again appears questionable. According to Wexelsen (1964), the general rule in plants is that the tetraploid (4n) plants produced artificially are usually fertile and give rise to

triploid (3n) progeny after fertilization by normal diploid (2n) plants. Several workers have attempted to apply this principle to fish, by using heat and cold shock treatment to induce polyploidy in the sturgeon (Vesetski,1967),stickleback (Swarup, 1951a, 1951b), and plaice and plaice/flounder hybrid (Purdom,1972). However, it appears that salmonid eggs are too large for successful heat/cold shock treatment, and these methods have so far failed in salmonids. The mitogen cytochalasin B has also been used in an attempt to produce polyploids (Refstie & Gjedrem, 1977), but mortalities were extremely high and the results unclear.

Hybrids between salmonid species may be sterile, but the culture of such hybrids could give rise to problems, as firstly they may require different husbandry techniques, and secondly problems may be encountered due to market resistance to a new product. In view of these difficulties and also the time required for such studies, a genetic approach to the prevention of maturation is not included in the present work.

The use of environmental control to prevent maturation among production stock is questionable because of the scale of environmental control that would be required. However, previous studies have shown some delay in maturation by environmental manipulation (e.g. Henderson, 1963), and thus experiments are included to determine the effects of photoperiod regimes designed to prevent or delay maturation, although their use on a commercial scale may be limited.

Clearly both genetic and environmental methods of controlling maturation have important difficulties and at present it would appear that the most effective way of overcoming the problems of maturation among production stock would be to produce either sterile or all-female

stocks by feeding steroid hormones during the first few months of feeding (Yamazaki, 1975; Johnstone et al. 1978). While this hormonal approach is reasonably understood under laboratory conditions, more work is required to study the effects of treatment on growth and survival before such methods are used on a commercial scale. A study was therefore carried out to determine the effect of various hormone treatments on the sex ratio, gonad development, growth and survival of fish in production units in a commercially operating fish farm.

1.3. Summary of Aims.

The overall aim of this study is to investigate the commercial and technical aspects of problems associated with maturation in rainbow trout. Throughout the study, emphasis is placed upon the endocrine control of maturation in relation to internal and external factors, as only knowledge of the exact mode of control of maturation in fish will lead to more efficient methods of manipulation.

For descriptive purposes, the scope of investigation falls conveniently into three subject areas. The primary aim is a study of the effects of altered photoperiod regimes on spawning in rainbow trout. This approach is intended to provide a commercially viable method for all-year-round egg production as well as providing information on the endocrine mediation of the response of maturing fish to altered photoperiod.

Providing eggs can be introduced into the farm at times outside the normal spawning period, a parallel aim is to investigate the use of computer models to forecast production under different farm conditions. The development of a suitable model to predict production and stocking under a variety of different environmental conditions

and stocking regimes should enable efficient management programs to be formulated which make full use of all-year-round supply of eggs.

Additionally, methods are evaluated, for the production on a commercial scale of sterile or all-female stocks of rainbow trout, thus preventing the altered skin pigmentation, loss in flesh quality, growth and conversion, which are associated with maturation among production stock.

Used in combination, these methods should enable the fish farmer to produce good quality table size or larger fish throughout the year, without any of the associated maturation problems. Furthermore, by introducing eggs and fry at times of the year specified by computer predictions, the farming system can be optimised in terms of the use of equipment, labour and food, and consequent return on investment.

2. GENERAL MATERIALS AND METHODS.

2.1. Fish Maintenance, Handling and Blood Sampling.

Rainbow trout (Salmo gairdneri) of both Danish and Californian origin were used as specified in the individual experiments. As the size and age of fish, size of tank used, stocking density, water flow and quality etc., varied according to the respective experimental proceedure, this information is given in the methods section of the relevant chapter.

In the experiments where individual identification of the fish was considered desirable, the fish were tagged with small plastic numbered tags (Charles Neal, Finchley Ltd., 3780 High Road, East Finchley, London, E2.). The tags were attached to the dorsal surface directly in front of the dorsal fin, with fine nickel wire. In other experiments, where only group identification was required, fin clipping was employed.

During the first year's experiments, the fish were fed with Edward Bakers trout diet, and in the second and subsequent years with B.P. Nutrition Mainstream diet. In all cases feeding was carried out as recommended from the appropriate feeding tables.

In all the controlled photoperiod experiments, the fish were examined monthly (when blood samples were being taken) to check for signs of maturity. In the male fish, a darkening in colour and the development of a kipe were taken as guidelines for assessment of the state of maturity; they were called ripe fish if sperm was emmitted from the anal papilla when gentle pressure was applied to the abdomen. In the female fish, the extension of the anal papilla, which becomes very red and swollen as maturity approaches, and the softening of the abdomen were taken as indices of the approximate state of maturity. The female fish were termed ripe when eggs were emmitted freely from

the anal papilla upon gentle pressure of the abdomen. Care was taken not to apply too much pressure, thus avoiding damaging the fish.

Hand-stripping was performed (under anaesthesia) when the fish were ripe. The eggs were gently squeezed from the females into a dry bowl and semen from two or more males added. The mixture was stirred gently with a small feather and water added immediately. The eggs were then allowed to stand for approximately 45 minutes to water harden, then placed in hatchery trays and incubated at 9°C, until hatching.

During all experiments handling of the fish was kept to a minimum to avoid scale damage and subsequent infection, and to keep stress to a minimum. Blood sampling was therefore always performed under anaesthesia, using Benzocaine (ethyl-4-amino-benzoate, BDH Chemicals Ltd., Poole, U.K.) at a concentration of 1:20,000. This gave "knock out" and recovery times of approximately two and five minutes respectively. Blood was removed from the Cuverian vein in all cases using a 21g needle. Post-sampling haemorrhage was seen on only very rare occasions and throughout the study, very few mortalities were encountered which could be directly attributed to handling or blood sampling. The blood obtained by this method was allowed to stand for 15 minutes, then centrifuged at 2500 rpm. for 10 minutes. The resultant serum was pipetted into clean plastic tubes (Luckham Ltd., Sussex, U.K.), stoppered and stored at -20°C, until future analysis.

All laboratory assays were carried out at Aston University, except the radioimmunoassay for serum gonadotropin, which was carried out by Dr.B. Breton, at the Laboratoire de Physiologie des Poissons, Institut National de Recherche Agronomique, 78350, Jouy en Josas, France. The transport of samples from Low Plains to Aston University was

performed using a large Dewar flask filled with dry ice. Samples were transferred to straws used for freezing bull semen, and packaged in dry ice, before transport by air to France.

2.2. Radioimmunoassay of Serum Oestradiol 17β.

Radioimmunoassay of serum oestradiol 17β was performed using a modification of the method of Leonard & Craig (1974) in which a serum sample of 200µl was used, and in the extraction phase, the sample was brought within the correct standard range by taking a 500µl aliquot of the original 5ml extraction.

The sheep anti-17β-oestradiol serum was supplied by Searle Diagnostic, Lane End Road, High Wycombe, Buckinghamshire, England. Each vial contained a freeze dried anti-17β-oestradiol-6-bovine serum albumin, selected as the antigen because it gives better specificity than conjugates linked in other positions (Leonard & Craig, 1974). When reconstituted and diluted to the volume shown on the vial, in the appropriate buffer, a working dilution of the antiserum was obtained which bound approximately 50% of a trace of tritiated 17β-oestradiol. Each tube required 200µl of this working dilution. An antibacterial (sodium azide) was added to the buffer, giving the reconstituted antisera a shelf life of six weeks at 4°C.

The standard curve on all occasions exhibited full parallelism with dilutions of extracted rainbow trout sera (Fig.2.1.). Cross-reaction data of the anti-oestradiol 17β serum with various other steroids, at the working dilution of the antiserum is shown in Fig.2.2. The sensitivity of the assay was approximately 50pg/ml and the overall precision of the assay was estimated by making six replicate determinations from a pool of female serum. The intra-assay coefficient of variation was 4.23% and the inter-assay coefficient of variation was 7.49%. The efficiency of extraction was 82.53 ± 2.38% over a total of ten assays.

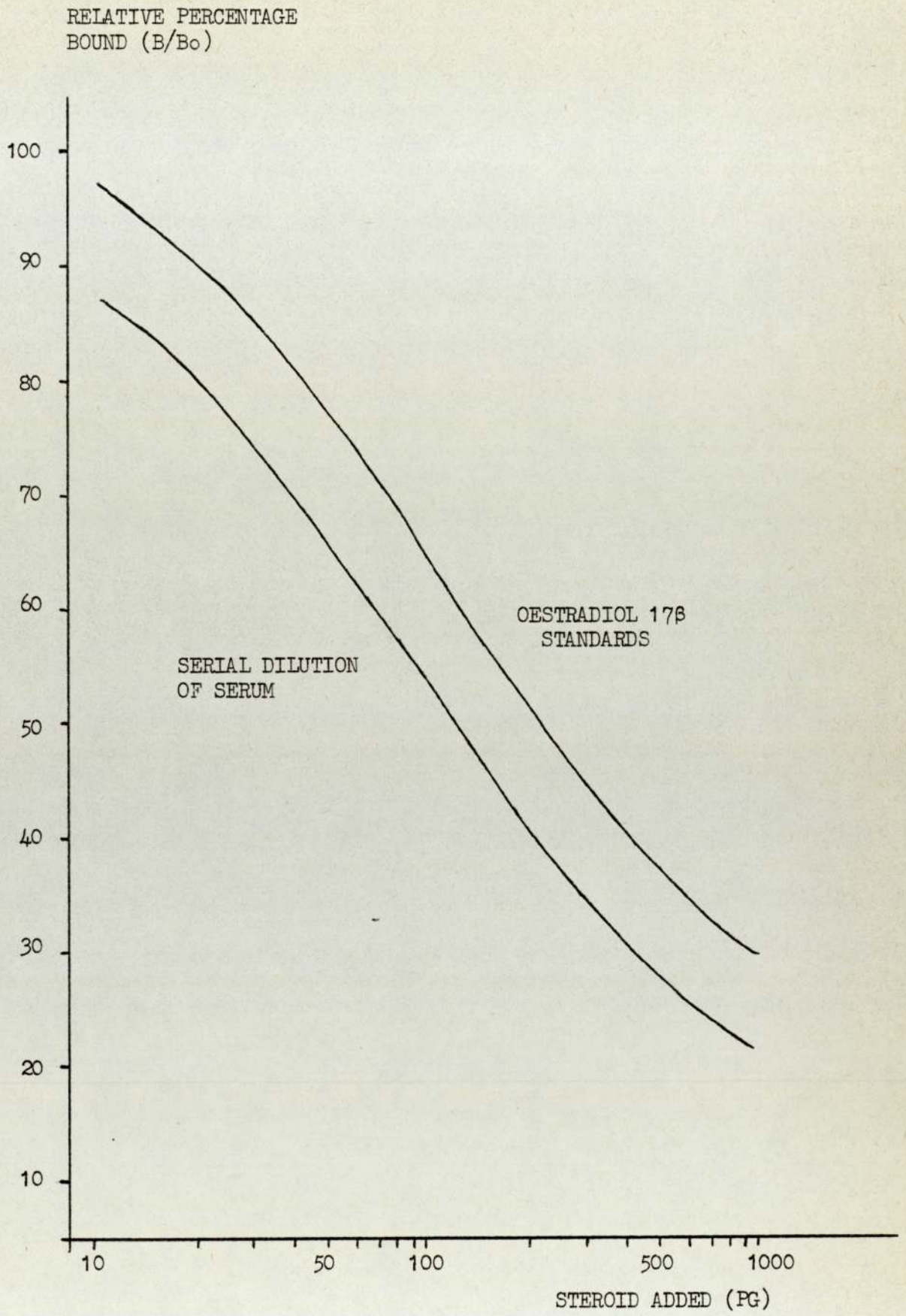


FIG. 2.1. PARALLELISM OF STANDARD CURVE FOR OESTRADIOL 17β WITH SERIAL DILUTIONS OF SERUM.

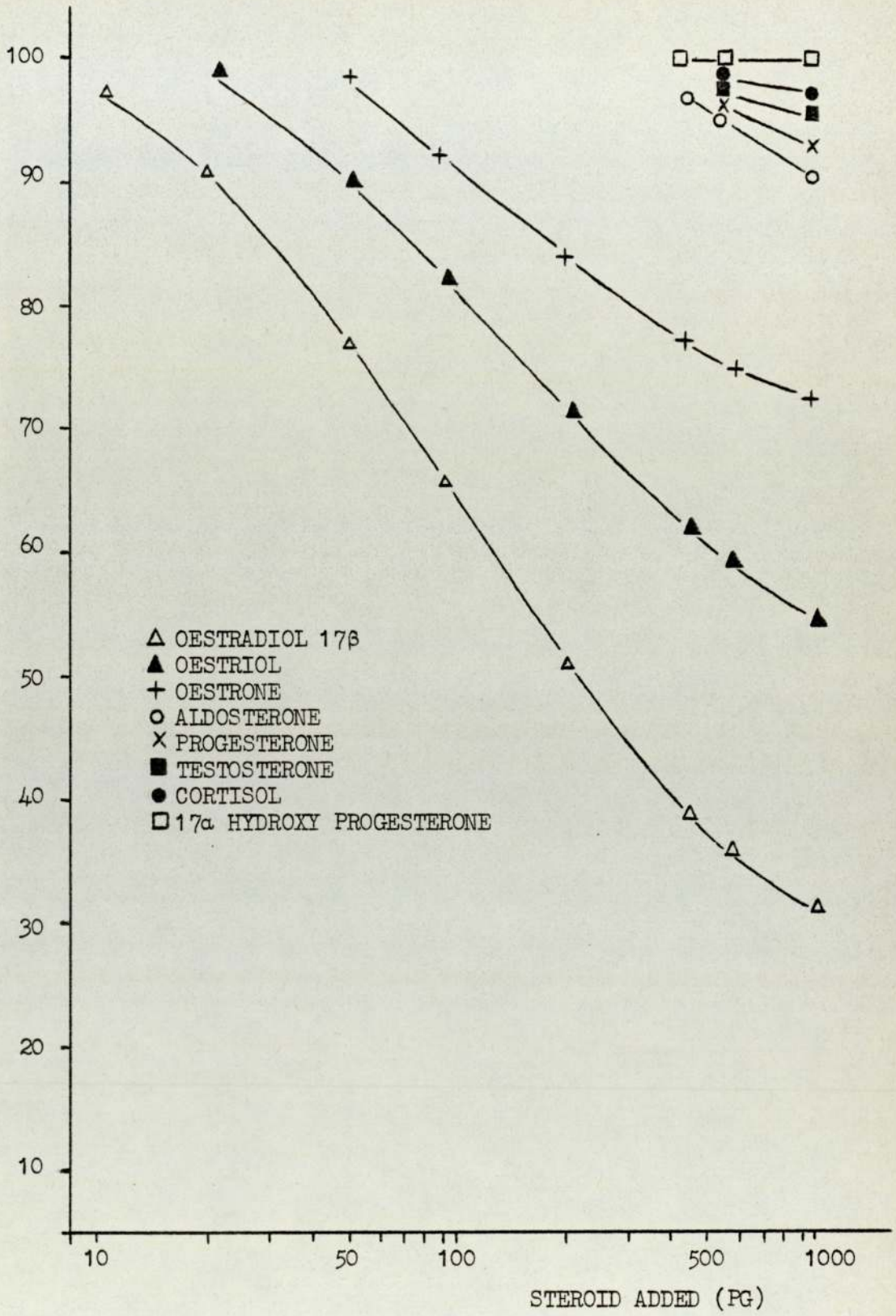


FIG. 2.2. CROSS REACTION DATA FOR ANTI - OESTRADIOL 17β SERUM WITH OTHER STEROIDS.

2.2.1. Materials.

(a) Dichloromethane with 1% methanol - frequently re-distilled.

(b) Distilled water.

(c) Buffer A (0.1M phosphate buffer, pH 6.8 - 7.0)

Disodium hydrogen phosphate ($Na_2HPO_4 12H_2O$)	21.82g.
Sodium dihydrogen phosphate ($NaH_2PO_4 2H_2O$)	6.08g.
Sodium chloride	9.00g.
Sodium azide	1.00g.
Bovine serum albumin (BSA)	1.00g.

(made up to one litre)

(d) Buffer B. As buffer A, but containing no BSA.

(e) Charcoal suspension.

250mg powdered charcoal (Norit OL) in 50ml buffer B.

(f) Dextran solution.

25mg Dextran (Pharmacia T40) in 50 ml buffer B.

(g) 6,7 - ^3H - oestradiol - 17β (Obtained from Radiochemical Centre, Amersham, England).

Specific activity	44Ci/mM
Stock solution	20μCi/mM
Working solution	4μCi/100ml buffer B.

(h) Scintillator solution.

20g. 2, 5 Diphenyloxazole in 2.5l toluene and 2.5l ethoxyethanol.

(i) Antiserum. Reconstituted as specified in buffer A.

(j) Standard oestradiol 17β solution.

0,5,10,20,50,100,200,500, and 1000 pg/100μl oestradiol in ethanol.

2.2.2. Assay Proceedure.

(a) Shake vigorously 200µl of serum with 5ml dichloromethane,(2 mins.).

(b) Discard serum phase.

(c) Wash extract with 2ml double distilled water and mix gently for 1.5 minutes.

(d) Centrifuge for 10 minutes at 2500 rpm. to separate emulsion and discard the aqueous phase.

(e) Set up water blanks by washing 5ml dichloromethane with 2ml double distilled water as in (c).

(f) Take 500µl aliquot of dichloromethane extract into a clean assay tube.

(g) Pipette 100µl of each standard into similar tubes.

(h) Take extracts and standards to dryness at 40°C under a stream of medical grade nitrogen.

(i) Add 200µl antiserum solution.

(j) Add 200µl working label solution, mix thoroughly, (vortex for 30 seconds).

(k) Incubate at 37°C for 1 hour.

(l) Transfer to an ice bath for 30 minutes (minimum time).

(m) Add 200µl of cooled dextran coated charcoal suspension, mix reagents (e) and (f) 1:1 v/v,not more than 30 minutes prior to use).

(n) Mix twice briefly using a vortex mixer - centrifuge for 10 minutes at 1500 rpm. (4°C).

(o) Transfer a 400µl aliquot to 10ml scintillation fluid in a counting vial.

(p) Count for 10 minutes or to 10,000 free counts.

(q) For total counts, count 200µl working label solution in 10ml scintillant.

2.2.3. Calculations.

(a) Correct free counts from 400µl aliquot to total free counts.
(i.e. multiply by 0.6/0.4).

(b) Calculate % free and hence % bound.
$B = 100 - (F/T \times 1.5 \times 100)\%$.

(c) Plot standard curve of % bound against pg oestradiol.

(d) Calculate water blank correction (i.e. y-x).

(e) Correct % bound of assay samples
$B' = B + (y-x)$

(f) Read off pg oestradiol from standard curve using B'.

(g) Correct for recovery.

(h) Correct results to pg/ml.

Explanation of symbols;

B = % bound	T = total counts
B' = corrected % bound	y = % bound in water blank
F = % free	x = % bound in 0 standard

2.3. Radioimmunoassay of Serum Testosterone.

Radioimmunoassay of serum testosterone was carried out using a modification of the method of Williams et al, (1974). A serum sample of 50µl was taken and 2 ml of a diethyl ether: petroleum spirit (1:4) mixture added, mixed on a vortex mixer for 15 seconds, allowed to stand for 15 minutes, mixed again for 15 seconds and allowed to stand for a further 15 minutes. Using this method of extraction, extraction efficiencies of 86 ± 5% were obtained. A second modification was applied in the separation of free testosterone from bound after the incubation phase. Separation was accomplished by using dextran - coated charcoal in preference to extracting with cold toluene scintillator solution as suggested in the original method.

The antiserum was supplied by Searle Diagnostic, Lane End Road, High Wycombe, Buckinghamshire, England. Each vial contained freeze-dried anti-testosterone serum prepared by immunisation of a rabbit with testosterone-3-bovine serum albumin. When reconstituted and diluted to the volume shown on the vial, in the appropriate buffer, a working dilution of the antiserum was obtained which bound approximately 50% of a trace of tritiated testosterone. Each tube required 200µl of this working dilution. An antibacterial (sodium azide) was incorporated in the buffer, giving the reconstituted antiserum a shelf life of six weeks.

A number of dilution studies were carried out to ensure that the dilution curves of the extracted serum were parallel to the standard curve (Fig. 2.3). The sensitivity of the assay was approximately 200pg/ml, and the overall precision of the assay was estimated by

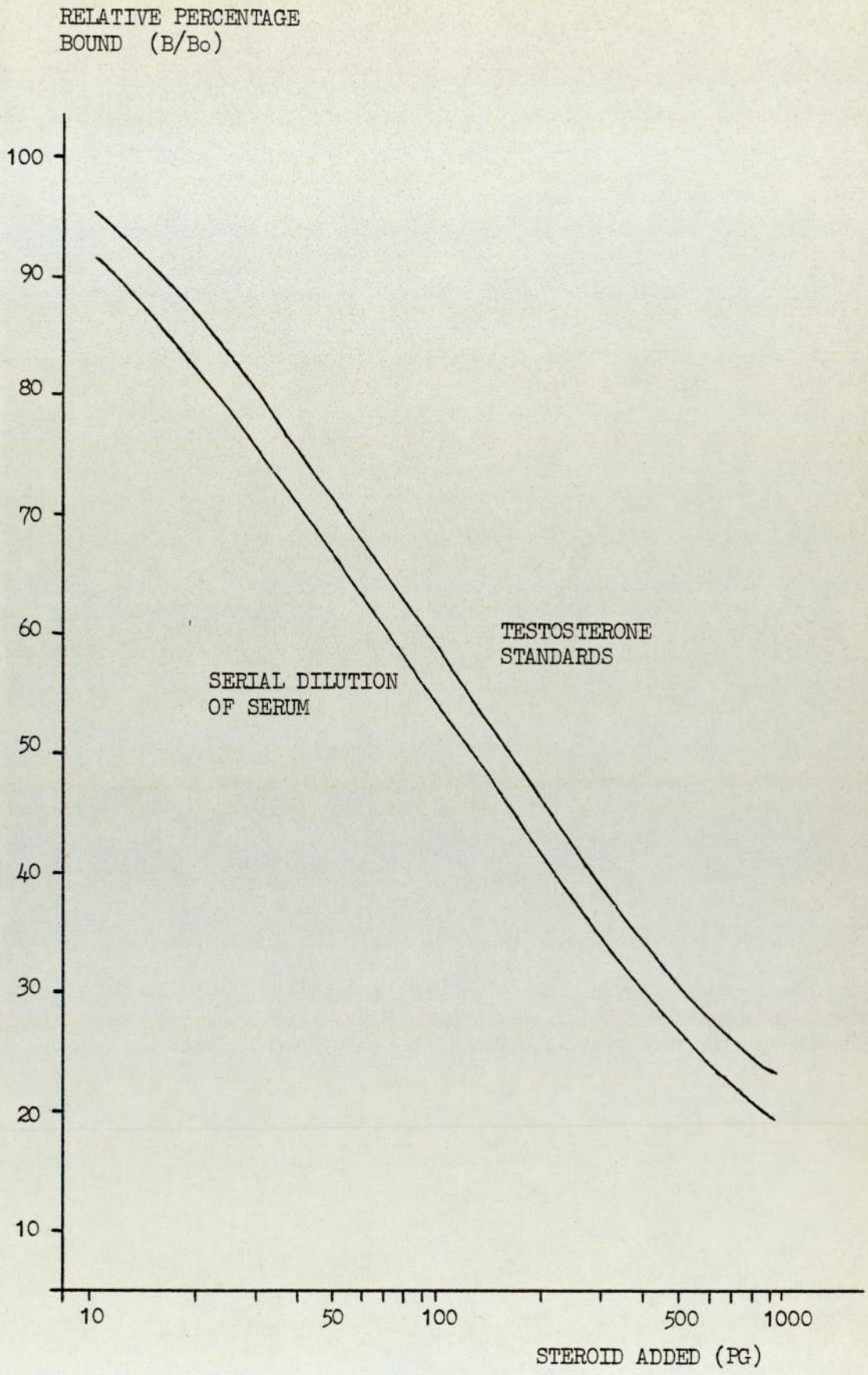


FIG. 2.3. PARALLELISM OF STANDARD CURVE FOR TESTOSTERONE WITH SERIAL DILUTIONS OF SERUM.

making five replicate determinations from a pool of male serum. The intra-assay coefficient of variation was 6.18%, and the inter-assay coefficient of variation was 8.23%. The cross reaction data of the anti-testosterone serum with other steroids at the working dilution of the antiserum is shown in Fig.2.4.

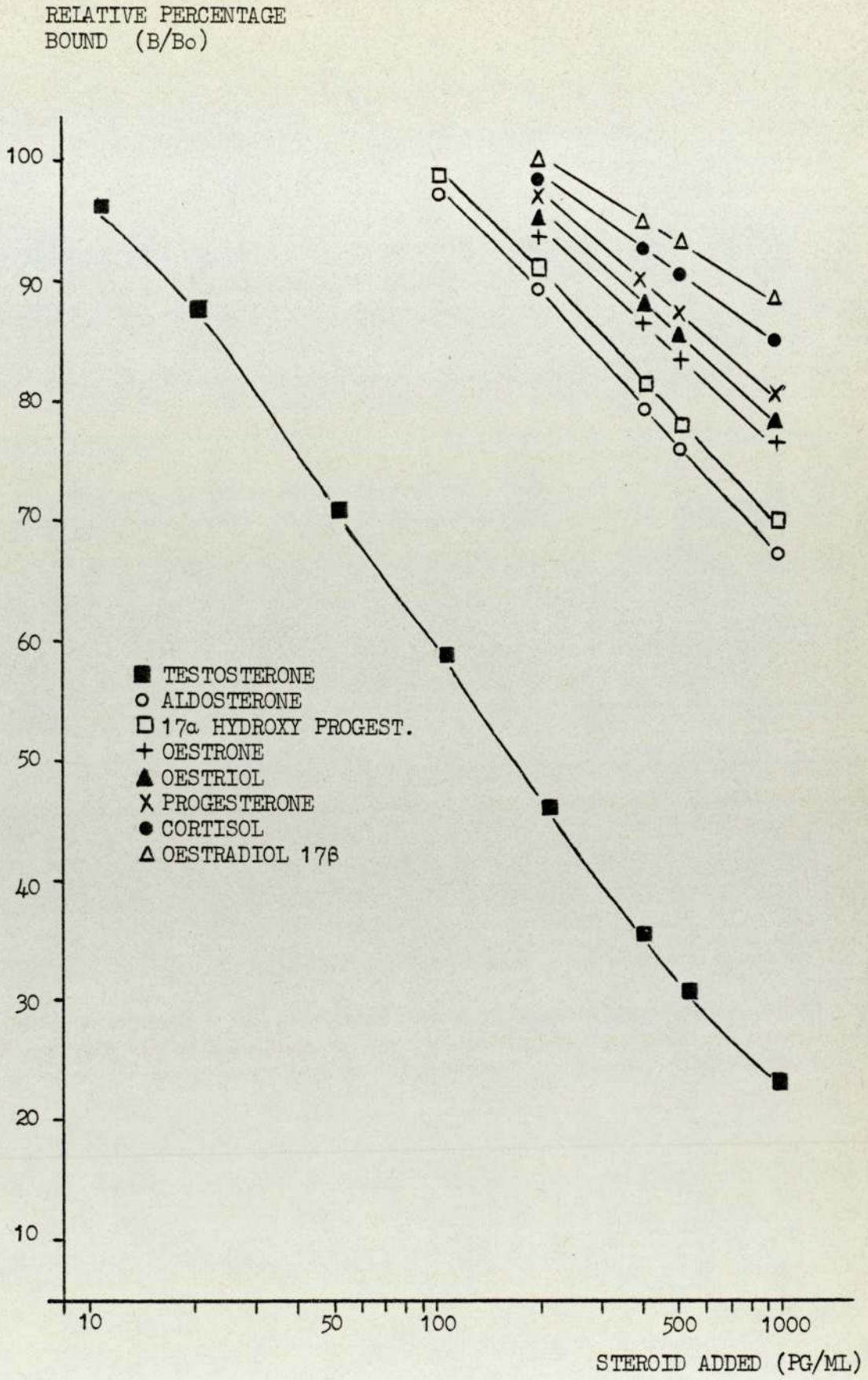


FIG. 2.4. CROSS REACTION DATA OF ANTI - TESTOSTERONE SERUM WITH OTHER STEROIDS.

2.3.1. Materials.

(a) Petroleum spirit (60-80°C).

(b) Diethyl ether.

(c) Distilled water.

(d) 1,2-^{3}H- Testosterone (Obtained from Radiochemical Centre, Amersham.).

Specific activity 50 Ci/mM

Working solution 0.02 μCi/ml

(e) Antiserum.

Made up as specified in buffer (f) below.

(f) Buffer for antiserum dilution (0.1M Tris buffer, pH8.0, containing 0.1% BSA).

50ml 0.1M Tris (12.114 g/l + 29.2ml 0.1M HCl, made up to 100ml with distilled water. 100mg BSA is dissolved in this solution.

(g) Charcoal suspension.

250mg powdered charcoal in 50ml buffer.

(h) Dextran solution.

25mg Dextran (Pharmacia T40) in 50ml buffer.

(i) Standard testosterone solution.

0,5,10,20,50,100,200,500, and 1000pg/100μl testosterone in ethanol.

(j) Scintillator solution.

20g 2,5 Diphenyloxazole in 2.5l toluene and 2.5l ethoxyethanol.

2.3.2. Assay Proceedure.

(a) To 50µl serum add 2ml diethyl ether : petroleum spirit (1:4).

(b) Vortex for 15 seconds, stand for 15 minutes.

(c) Repeat (b).

(d) Centrifuge at 1000rpm for 10 minutes to separate emulsion.

(e) Pipette 1ml extract into a clean assay tube,and add 100µl 0.02µCi/ml ^{3}H testosterone.

(f) Similarly add 100µl 0.02 µCi/ml ^{3}H testosterone to 100 µl of each standard.

(g) Take extracts and standards to dryness in a vacuum oven at 37°C.

(h) Resuspend extract and standards in 200µl buffer (tris).

(i) Add 200µl antiserum to extracts and standards and vortex for 10 seconds.

(j) Equilibrate for 2 hours at room temperature.

(k) Incubate for 1 hour at 0-4°C.

(l) Add 200µl cooled dextran coated charcoal suspension, (mix reagents (g) and (h) 1:1v/v, not more than 30 minutes prior to use, keep cool on ice).

(m) Mix briefly using a vortex mixer - centrifuge for 15 minutes at 1500rpm (4°C).

(n) Transfer a 400µl aliquot to 10ml scintillation fluid in a counting vial.

(o) Count for 10 minutes or to 10,000 free counts.

(p) For total counts, count 200µl working label solution in 10ml scintillant.

The calculation of the results is identical to that described for oestradiol 17β in section 2.2.3.

2.4. Radioimmunoassay of Serum Gonadotropin.

Serum gonadotropin was assayed by Dr. B. Breton at the Laboratoire de Physiologie des Poissons, Institut National de Recherche Agronomique, 78350, Jouy en Josas, France. The double antibody radioimmunoassay system for trout gonadotropin was carried out using a highly purified trout gonadotropin (Breton et al, 1976). The hormone was labelled using the chloramine T method with I^{125} (Greenwood et al, 1963) under the same conditions for carp gonadotropin (Breton et al, 1971). The antibody was raised in guinea pig by subcutaneous injection of 250µg GTH in 500µl 8% NaCl, mixed with complete Freunds adjuvant. A total of seven injections were found necessary to obtain an antibody binding 50% of the labelled hormone at $\frac{1}{2} \times 10^{-5}$ dilution. The sensitivity of the assay was 7 to 10pg.

2.4.1. Assay Proceedure: The assay was performed in 500µl plastic tubes, each containing 10,000cpm of labelled hormone, 20µl of unknown serum or standard solution and antibody at the final dilution of $\frac{1}{2} \times 10^{-5}$. The buffer was Veronal 0.025M pH 8.6 containing 2.5% of human serum albumin. The tubes were incubated at 3°C for 4 days. The separation of the free from bound hormone was accomplished by double immunoprecipitation using anti guinea pig γ globulins raised in rabbit. 50µl of rabbit sera were added to each tube and allowed to incubate for 1 hour at room temperature. Before centrifugation 1.5ml of cold incubation buffer without serum albumin was added to each tube. After centrifugation, (20 minutes, 4°C, 3600g), the supernatant was discarded. The results were analysed using logit-log transformation.

2.5. Assay of Serum Egg Protein (Vitellogenin).

Previous workers have used three methods to determine vitellogenin in serum. Radioimmunoassay was used by Redshaw & Follett (1972), immunoassay by Plack et al, (1971), and phosphoprotein determination by Laskowski (1936) and Wallace (1970). As the first two of these methods require the preparation of a specific antisera to purified yolk protein, which is a lengthy process, vitellogenin levels were estimated by the measurement of serum phosphoprotein (Boehringer Corporation Test Handbook, 1969). The method has been modified as suggested by Craik (personal communication). A typical standard curve for the assay is shown in Fig. 2.5. The levels of phosphoprotein phosphorus measured by this method may be converted to vitellogenin by multiplying by 71.43 (assuming vitellogenin contains 1.4% phosphoprotein phosphorus as in other vertebrates).

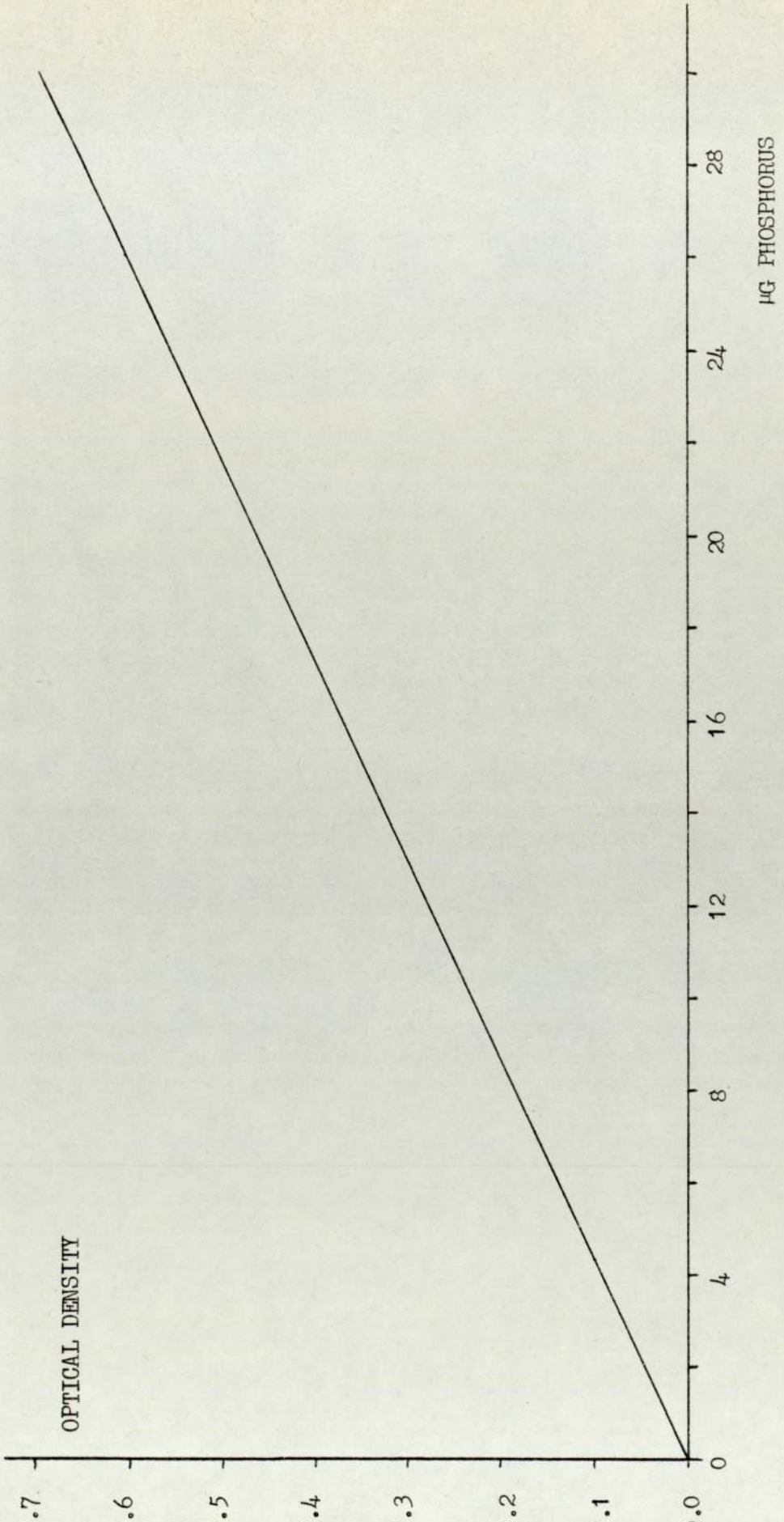


FIG. 2.5. TYPICAL STANDARD CURVE FOR PHOSPHOPROTEIN PHOSPHORUS ASSAY.

2.5.1. Materials.

(a) 20% Trichloroacetic acid.

(b) Ethanol.

(c) Chloroform : ether : ethanol (1:2:2).

(d) Acetone.

(e) Ether.

(f) 60% Perchloric acid (Analar).

(g) 100 vol Hydrogen peroxide (Analar).

(h) Molybdate reagent : 40 mM ammonium molybdate, 2.5N H_2SO_4.

(i.e. 49.44 g/l $(NH_4)_6 MO_7 O_{24} 4H_2O$ + 122.5 g/l conc. H_2SO_4).

(i) Vanadate reagent : 21mM ammonium vanadate, 0.28M HNO_3.

(i.e. 2.46 g/l $NH_4 VO_3$, + 17.6 g/l conc HNO_3).

(j) Standard solutions prepared from Na_2HPO_4 hydrate crystals, NaH_2PO_4 crystals or $K_2H_2PO_4$ crystals to contain 0, 25, 50, 75, and 100 µg of phosphorus / 100µl.

2.5.2. Assay Proceedure.

(a) Take 100µl serum.

(b) Precipitate proteins with 5ml 20% trichloroacetic acid.

(c) Centrifuge for 10 minutes at 2500 rpm to isolate precipitate.

(d) Wash with organic solvent to remove lipid.

i.e. wash successively with hot ethanol, chloroform : ether : ethanol (1:2:2), acetone and ether (Wallace, 1970).

(e) Dry protein pellet.

(f) To protein pellet, add 60% perchloric acid (500µl) and 100µl hydrogen peroxide.

(g) Mix well and stand for 12 hours.

(h) Heat at 180-200°C for 20 minutes. (If not completely colourless to eye and free of black solid, add further 100µl hydrogen peroxide and heat for a further 20 minutes).

(i) Add: 2ml H_2O

1ml molybdate reagent

1ml vanadate reagent

(j) Stand for 10 minutes and measure optical density at 400nm against a solution of:

2ml H_2O

1ml vanadate reagent

1ml molybdate reagent.

(k) Plot standard curve of µg phosphorus against optical density and read off samples.

2.6. Assay of Serum Calcium.

Total serum calcium was measured using the Corning Calcium analyser model 940. The complexiметric titration was first introduced by Schwarzenbach & Biedermann (1946). The method is based upon a measurement of the fluorescence of a dye when associated with calcium. The dye, calcein, is a fluorescein derivative, and forms an intensely fluorescent, un-dissociated complex with calcium ions in an alkaline medium. Calcein was first introduced by Diehl & Ellingboe (1956) and applied to the complexiметric titration of calcium.
The analytical proceedure incorporated in the model 940 is based upon the quenching of this fluorescence by chelating the calcium ions with the titrant EGTA. EGTA is preferable to EDTA, especially in the presence of magnesium ions (Schmidt & Reilley,1957). The sample size used was 100µl. The method of operation is described in the Corning model 940 instruction manual.

3. THE ENVIRONMENTAL CONTROL OF MATURATION AND SPAWNING.

3.1. Introduction.

Reproduction in fish like other vertebrates is a complex sequence of events involving meiosis resumption, oocyte maturation, ovulation and oviposition in females and spermiation and sperm release in males. It is now clear that environmental factors play a major role in the control of these stages of reproduction, although with only 51 of a total of over 20,000 species of fish examined, the results are inevitably contradictory (Htun - Han, 1977). It is also evident that the mediation of the effect of the environment is performed by the endocrine system, and although information is available on fish hormones, heterogeneity in many assays precludes unequivocal interpretation. Furthermore, very few workers have studied endocrine function in response to environmental change. The aim of the present chapter is therefore two-fold, firstly to investigate the effect of altered photoperiod regimes on spawning time with a view to achieving "off-season" spawning, and secondly to study the changes in endocrine dynamics which accompany this process. Only such an approach will provide the necessary information for the development of better methods of control.

In seasonally-breeding fish, the timing of specific phases of maturation to be coincident with favourable environmental conditions is of considerable adaptive significance. Many factors from both the internal and external environments are thought to act as cues for the initiation of the complex series of neuroendocrine changes which exert overall control of the processes of gonadal maturation and spawning, (de Vlaming, 1972; Donaldson, 1975). Amongst the large number of factors mentioned in the literature, photoperiod, temperature

and nutritional status appear to be the most important with subsidiary influences imparted by salinity, rainfall and pheromones, although the relative importance of each factor varies with different species.

Previous work has suggested that photoperiod exerts the major influence in salmonids. Thus Hoover,(1937) and Hoover & Hubbard, (1937) obtained eggs from brook trout four months ahead of the normal spawning time for the strain with accelerated light regimes. Similar results were also obtained in brook trout by Hazard & Eddy, (1951) and Corson, (1955). Allison, (1951) further demonstrated that extended photoperiods can delay spawning in brook trout, although by only six weeks in this particular study. In 1963, Henderson showed that the rate of gonadal development is the same at 16°C and 8.5°C provided the fish are exposed to natural daylengths. If the fish are subjected to either long or short photoperiods the gonadal response at 16°C is different to that at 8.5°C, although the full significance of this is not clear.

A high percentage of naturally-spawning fish can be produced within 15 months of egg fertilization when brook trout are exposed to a simulated natural photoperiod and an optimum water temperature regime for embryo incubation, alevin rearing and reproduction (Carlson & Hale, 1973). This particular work is in contrast with that of Henderson (1963) who stated that altered environmental regimes were without effect on fish that were undergoing gametogenesis for the first time, and suggested that the stimulatory effect of environmental factors is dependant upon the maturation of a hypothalamic - hypophyseal mechanism.

In the rainbow trout, accelerated light regimes have also produced spawning some 6 - 8 weeks earlier than the normal spawning time for the strain (Nomura, 1962; Kunesh et al, 1974) and similar effects have also

been demonstrated in salmon (MacQuarrie et al, 1978). However, in a number of the above studies, other environmental factors besides photoperiod varied during the course of the experiments, and this, together with the variety of different experimental techniques, strains of fish used and methods of evaluation, makes comparison very difficult.

Additionally, apart from Sundararaj & Sehgal (1970) and Breton & Billard (1977) with catfish and male rainbow trout respectively, few workers have studied the physiological and endocrine changes, occuring in response to the various environmental triggers, which initiate and control the onset and course of maturation and spawning.

Many reviews are available on the endocrine control of maturation (Pickford & Atz,1957; Dodd,1972; Hoar,1969; Barr,1968; Lofts,1968; Reinboth,1972; de Vlaming,1974; Jalabert,1976). It is thought that the environmental factors act via the exteroreceptors and hence through the central nervous system, hypothalamus, pituitary and gonad (Fig.3.1). Initiated by environmental changes, it is probable that in fish, like mammals, releasing hormones from the hypothalamus elicit the release of gonadotropin(s) from the pituitary (Weil et al, 1978). The influence of the pituitary on vitellogenesis has been demonstrated in several teleost species; hypophysectomy results in the degeneration of yolky oocytes and replacement therapy using pituitary extracts reinitiates vitellogenesis (de Vlaming, 1974). However, the number of pituitary hormones involved in the regulation of vitellogenesis in teleosts is still controversial. Thus, several workers have proposed the existence in fish of one gonadotropin (e.g. Burzawa-Gerard et al, 1975; Breton et al, 1976; Sumpter et al, 1978), whereas other evidence suggests that two gonadotropins are involved (e.g. Idler et al, 1975a, 1975b; Pierce et al, 1976; Farmer & Papkoff, 1977).

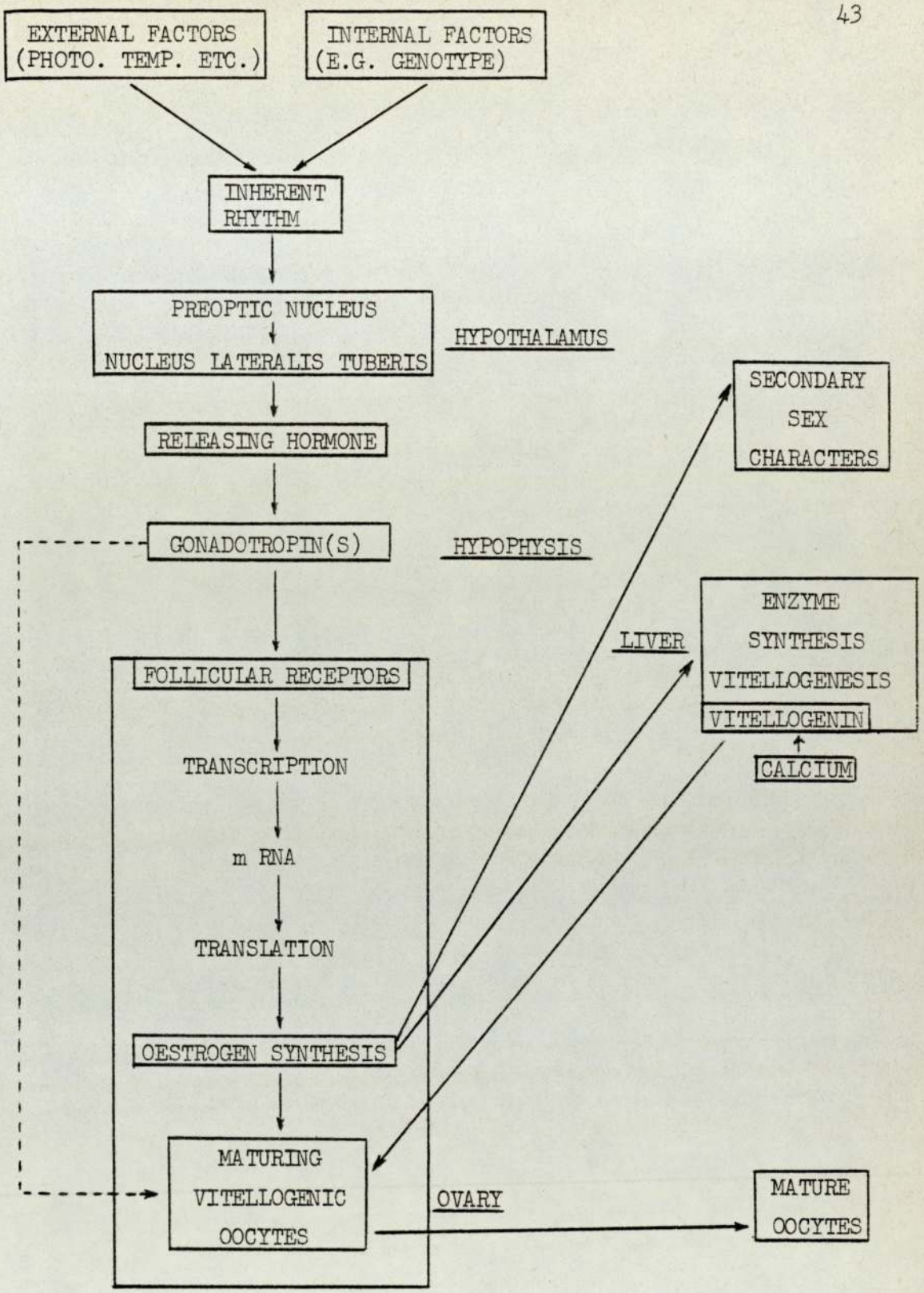


FIG. 3.1. MECHANISMS INVOLVED IN THE OVERALL CONTROL OF MATURATION. FEMALE FISH.

In female fish the gonadotropin(s) thus produced act in turn on specific follicular receptors within the ovary to initiate both vitellogenesis and the transcriptional and translational processes required for specific steroid biosynthesis within the follicle. The steroids produced under the influence of gonadotropin, as well as acting directly upon the oocyte receptors within the follicle, also have more far-reaching effects regarding sexual maturation, e.g. secondary sex characters. One of the more important of these effects is that of oestrogen on the liver, where it promotes the synthesis of egg proteins (hereafter vitellogenin) which are required in large amounts in oviparous animals.

Much of the work on the stepwise control of this process has been carried out in amphibians (Wallace & Bergink, 1974; Wallace & Jared, 1968, 1976) and the results from the few reported studies in teleosts (Urist & Schjeide, 1961; Ho & Vanstone, 1961; Plack et al, 1971; Arimante, 1972; Aida et al, 1973; Mugiya & Watabe, 1977; de Vlaming et al, 1977; Campbell, 1978) collectively suggest that gonadotropins and gonadal steroids are also both important in this group for the regulation of vitellogenin production.

Vitellogenin is composed of lipovitellin (2 peptides in 1:1 ratio, molecular weights 120,000 and 31,000) bound to a phosphoprotein of low molecular weight (35.000), known as the phosvitin (Wallace & Bergink, 1974). Wallace,(1970) has also shown that one atom of calcium appears to be associated with every protein-phosphate group. In amphibians, Zelson & Wittliff (1973) have found by immunochemical methods that vitellogenin appears in the serum 12 hours after the administration of oestradiol 17β. Since Wittliff & Kenney (1972) were unable to detect

vitellogenin in the liver by nine hours, but could by twelve hours, these data indicate that the secretion of vitellogenin into the blood stream occurs within three hours after it is synthesised in the liver (Wallace & Bergink,1974). Once in the serum, the vitellogenin complex is transported to the ovary where it is sequestered, specifically by the vitellogenic oocyte in a micropycnotic process. The uptake of vitellogenin, which is highly selective, is stimulated by gonadotropins and may involve attachment to receptors on the oocyte membrane (Jalabert, 1976; Campbell, 1978). Once incorporated into the oocyte, vitellogenin is converted within the yolk platelets into the yolk proteins lipovitellin and phosvitin, for which it seems to be the sole source. The conversion process appears to involve a proteolytic cleavage of the vitellogenin peptides into those which make up lipovitellin and phosvitin (Wallace & Bergink,1974). Thus as maturation proceeds, gross changes may be expected to occur in the serum levels of gonadotropin, oestradiol 17β, phosphoprotein phosphorus (as a measure of vitellogenin) and total calcium (bound specifically to the vitellogenin complex) in response to environmental change.

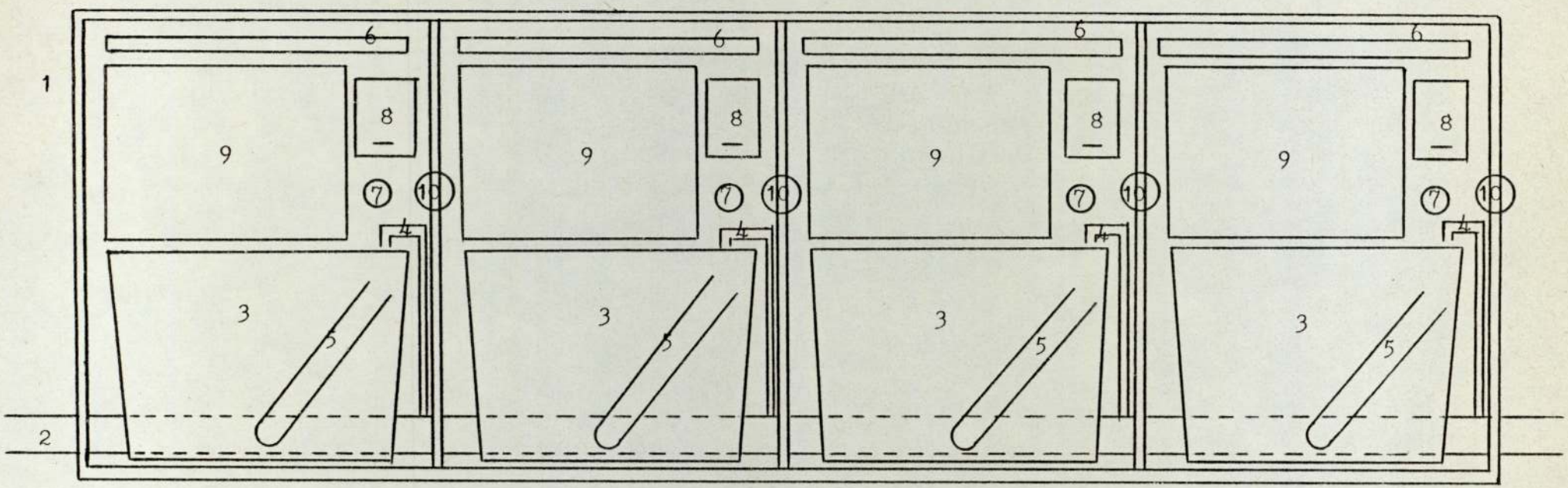
Although less documented than the female, similar neuroendocrine mechanisms involving both pituitary and gonadal hormones are thought to be concerned with the control of the processes of spermatogenesis, spermiation and sperm release in male fish. Thus, gonadotropin (e.g. Yamazaki & Donaldson, 1968; Crim et al, 1975; Breton & Billard, 1978) and the two major androgens in fish, 11 - keto testosterone and testosterone (Idler et al, 1960; Grajcer & Idler, 1961; Idler et al, 1971; Schreck et al, 1972; Billard, 1974; Schreck & Hopwood, 1974; Wingfield & Grimm, 1977), have been implicated in the control of maturation in male fish. As reproduction is also seasonal in the male,

it is probable that these hormones will also be affected by altered environmental regimes.

In the main, previous studies on the endocrine dynamics of maturation in teleosts have been on captured wild fish (Schmidt & Idler, 1962; Di Prisco et al, 1967; Katz & Eckstein, 1974;Wingfield, 1974; Crim et al, 1975; Wingfield & Grimm, 1976) because close environmental control over long periods of time is difficult to accomplish in the laboratory. In view of these difficulties, the present study, whilst maintaining close control of all other environmental variables, investigates firstly the effects of various photoperiod regimes on spawning time in the rainbow trout, and secondly the dynamic changes in serum levels of gonadotropin, oestradiol 17β, testosterone, total calcium and phosphoprotein (as a measure of vitellogenesis) which accompany these processes. The endocrine dynamics are measured for two reasons, firstly as a method of evaluation of the effectiveness of a given environmental regime, and secondly to provide information regarding the response of the endocrine system to altered environmental conditions. Using this information progress can then be made towards a more complete control of salmonid reproduction and the possibility of all year round spawning for the fish farmer.

3.2. General Materials and Methods.

The basic arrangements of the four controlled photoperiod chambers used throughout all series of experiments is shown in Fig. 3.2. The wooden framework was covered with heavy duty black polythene to provide the four light-proof chambers. Each chamber was accessed via a hinged inspection hatch and a main hatch constructed from marine



1. WOODEN FRAMEWORK.
2. WATER INFLOW (OXYGENATED).
3. 800 LITRE FRY TANK.
4. WATER FLOW CONTROL TAP.
5. WATER OUTFLOW AND LEVEL CONTROL.
6. 40 WATT FLUORESCENT TUBE.
7. FOOD INLET PIPE.
8. INSPECTION HATCH (HINGED).
9. MAIN ACCESS HATCH
10. ELECTRIC TIME CLOCK.

FIG. 3.2. ARRANGEMENT OF TANKS IN PHOTOPERIOD CONTROL SYSTEM USED THROUGHOUT ALL SERIES OF EXPERIMENTS.

plywood secured with wing nuts and captured bolts. Each tank (800 l. capacity) was supplied with an individual flow-through water supply (oxygenated in the inlet pipe) at a rate of 400 l./hour make up. The borehole source ensured that the water had a constant temperature of 9°C throughout the experimental periods, oxygenation maintained a dissolved oxygen content of 100% in the effluent and pH was constant at 6.6. The water level was maintained by an adjustable side arm, which also served to flush the tanks daily. Feeding was accomplished via a closed feeding pipe, to keep disturbance of the system to a minimum. The fish were fed at 0.5% body weight per day.

The light cycles were provided by individual 40 watt. fluorescent tubes suspended one metre above the water surface and controlled by electric time clocks adjusted once per week. Three series of experiments were performed in the period 1976 to 1978, using different light cycles and different strains of fish in each series, as described in the following sections.

3.3. Series 1. Experiments.

The aim of this series was to advance the time of spawning of broodstock fish using seasonal light cycles compressed into shorter periods of time than found under natural conditions. At the same time, monthly blood samples were taken and assayed for serum gonadotropin, oestradiol 17β, testosterone, vitellogenin and total calcium, in order to ascertain how the reproductive system responds to altered photoperiod regimes. Basal and maximum levels were compared in all cases using either the Student's t-test or analysis of variance.

3.3.1. Materials and Methods: The fish used during this series of experiments were all of Danish origin, and were known to spawn in January or early February under the conditions at Low Plains. Four groups of 20 immature male and female rainbow trout, each weighing approximately 1.3kg were placed in each tank at the start of the series of experiments. All these experiments were commenced in April and completed in February of the following year. The photoperiod regimes under investigation during this series are described below:

(a) Normal 1 year seasonal cycle (control) in which July 4th. was the longest day and December 20th. the shortest.
(b) Normal seasonal cycle compressed into 9 months in which June 4th. was the longest day and October 20th. the shortest.
(c) Normal seasonal cycle compressed into 6 months in which May 4th. was the longest day and August 8th. the shortest.
(d) Constant 12 hour light / 12 hour dark regime.

These photoperiod regimes are shown in more detail in Fig. 3.3.

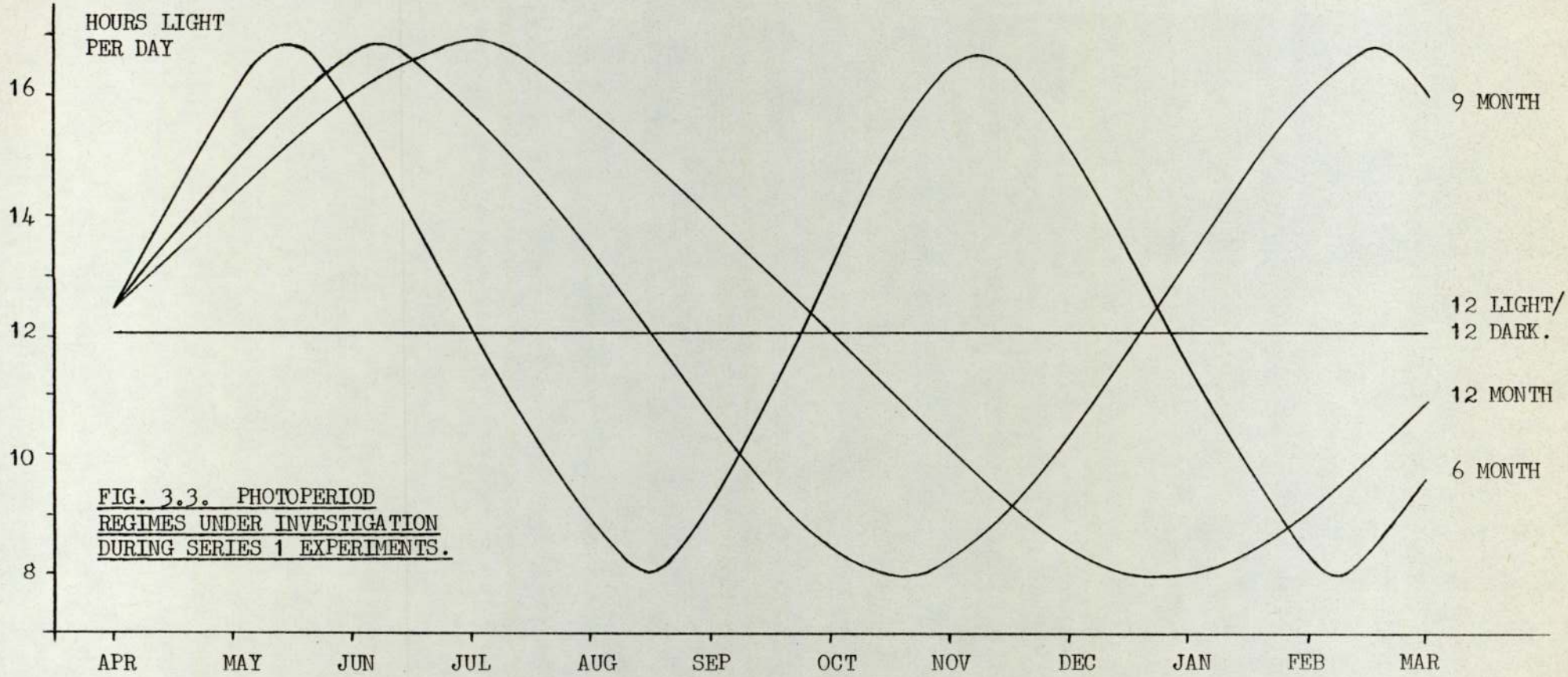


FIG. 3.3. PHOTOPERIOD REGIMES UNDER INVESTIGATION DURING SERIES 1 EXPERIMENTS.

At the start of the series, and each month thereafter, blood samples were taken under anaesthesia and the fish checked for signs of sexual maturity using methods already described (Chapter 2.1.). A close examination was also maintained on similar fish held in outside open ponds in order to determine any differences from the fish maintained in the experimental system.

3.3.2. Results: The various photoperiod regimes employed produced no adverse physiological or behavioural changes in either the male or female experimental fish, and apart from variations in spawning time, they were indistinguishable from similar fish of the same age maintained outside in open ponds under natural environmental conditions. The eggs obtained from each group were fertilized by hand-stripping and showed normal development at all stages through to the time when they were cropped. Egg size was fairly uniform irrespective of spawning time (4.7 ± 0.2mm) and there were no significant differences in fecundity (approx. 2000 eggs /kg body weight). In all groups survival to hatching was 75 - 80%. The food conversion ratio was stable between 1.3:1 and 1.6:1 until the fish approached the time of spawning, at which time, a slowing down in feeding and growth occurred, and preliminary data also suggests a loss of food conversion efficiency (see Chapter 7.1.).

As maturation progressed in each group, changes were seen in the time of spawning and the serum levels of gonadotropin, oestradiol 17β, phosphoprotein phosphorus and total calcium in the female fish, and serum gonadotropin and testosterone in the male fish. These changes were different under each photoperiod regime, and to aid comparison, the results from each experiment are grouped according to sex.

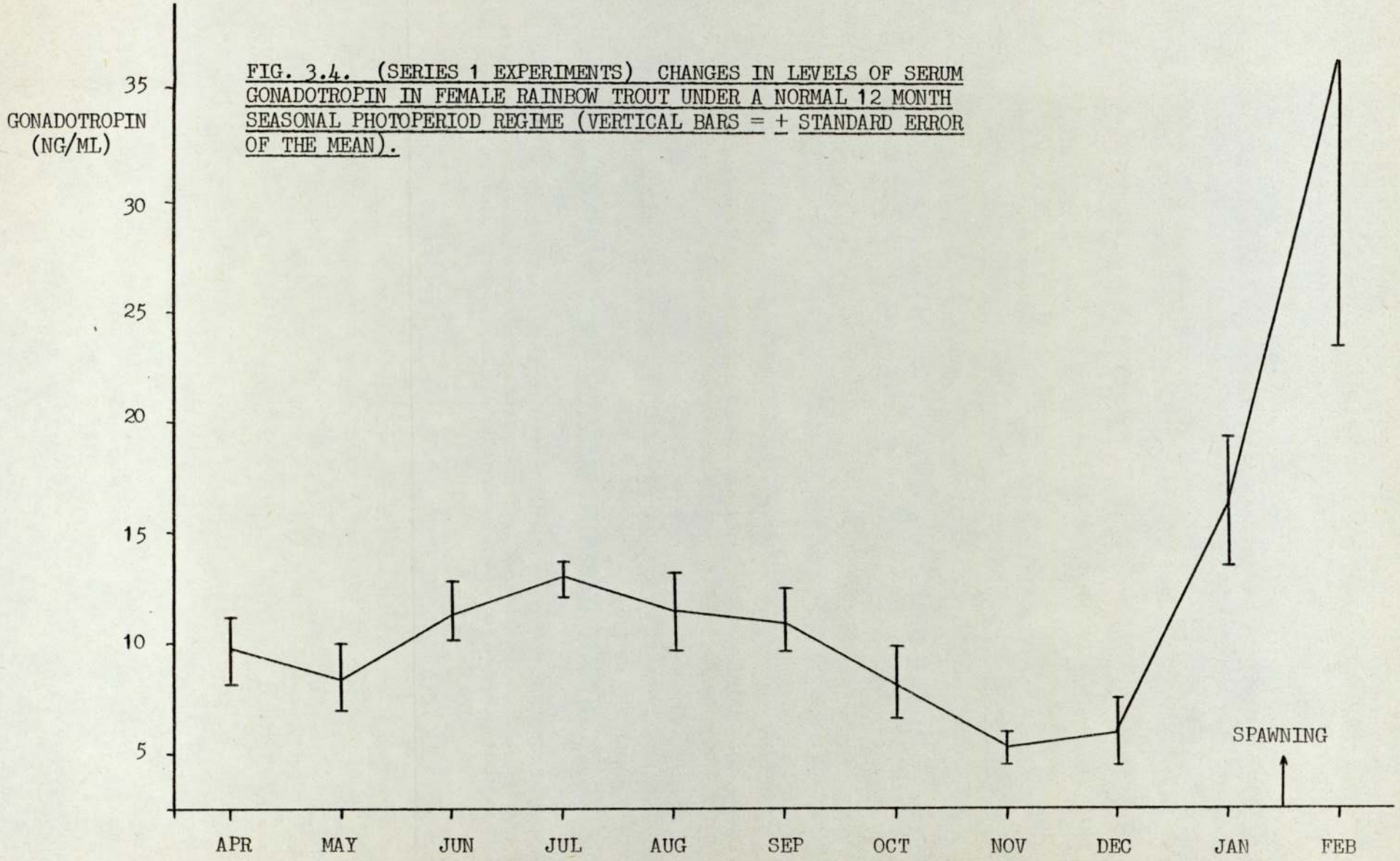
Female Fish: All fish, irrespective of photoperiod treatment, had similar basal serum levels of total calcium (12 ± 2mg%, i.e. mean ± standard error of the mean) and phosphoprotein phosphorus (25 ± 7µg/ml) during the months of April to July. Basal serum levels of oestradiol 17β (130 ± 15 pg/ml) and gonadotropin (9.0 ± 1.6ng/ml) were observed in April and May. The subsequent changes in these parameters under each photoperiod regime are descibed in more detail below.

Normal 1 year seasonal cycle (see Table 3.1): The female fish in this group spawned in mid-January, at exactly the same time as broodstock maintained outside in open ponds. Serum levels of gonadotropin rose to 13.2 ± 0.8ng/ml in July and gradually returned to basal by November. Levels in July were significantly different from those in November ($P<0.01$). Just prior to spawning in mid-January, levels rose rapidly to a peak of 37.1 ± 14.0ng/ml (Fig 3.4). Levels in February were also significantly different from those in November ($P<0.01$). Between June and July, serum oestradiol 17β levels increased in the female fish from basal levels to a peak of 4808 ± 1105pg/ml in October, and then returned to resting levels before the onset of spawning in mid-January (Fig.3.5). Levels in October were significantly different from those in April ($P<0.001$). By September, the serum levels of phosphoprotein phosphorus and total calcium in females had both begun to rise, reaching peaks of 400 ± 66µg/ml and 58 ± 5mg% respectively in January (Figs. 3.6 and 3.7). Both serum levels of phosphoprotein phosphorus and total calcium in January were significantly different from their respective levels in April ($P<0.001$).

	APR	MAY	JUN	JUL	AUG	SEP	OCT	NOV	DEC	JAN	FEB
GONADOTROPIN (NG/ML)	9.9	8.6	11.2	13.2	11.4	10.9	8.3	5.5	6.1	16.5	37.1
± STANDARD ERROR	1.8	1.4	1.3	0.8	1.8	1.4	1.8	0.6	1.4	2.8	14.0
OESTRADIOL 17β (PG/ML)	122	128	234	371	886	3471	4808	4107	2729	310	130
± STANDARD ERROR	11	11	29	50	126	549	1105	666	667	109	12
PHOSPHOPROTEIN PHOSPHORUS (μG/ML)	15.3	20.0	39.3	34.7	54.3	93.3	176.3	206.6	255.9	400.9	132.9
± STANDARD ERROR	5.4	2.0	8.0	6.6	7.5	14.9	25.7	38.8	55.3	66.9	23.1
TOTAL CALCIUM (MG%)	10.5	11.9	14.2	14.3	15.6	21.9	33.0	36.1	43.6	57.5	30.3
± STANDARD ERROR	0.8	0.2	0.2	0.5	0.5	1.0	3.5	5.0	7.2	5.4	2.8

TABLE 3.1. (SERIES 1 EXPERIMENTS) LEVELS OF GONADOTROPIN, OESTRADIOL 17β, PHOSPHOPROTEIN PHOSPHORUS, AND TOTAL CALCIUM IN SERUM OF FEMALE RAINBOW TROUT UNDER A NORMAL 12 MONTH SEASONAL PHOTOPERIOD REGIME (n = 7).

FIG. 3.4. (SERIES 1 EXPERIMENTS) CHANGES IN LEVELS OF SERUM GONADOTROPIN IN FEMALE RAINBOW TROUT UNDER A NORMAL 12 MONTH SEASONAL PHOTOPERIOD REGIME (VERTICAL BARS = ± STANDARD ERROR OF THE MEAN).



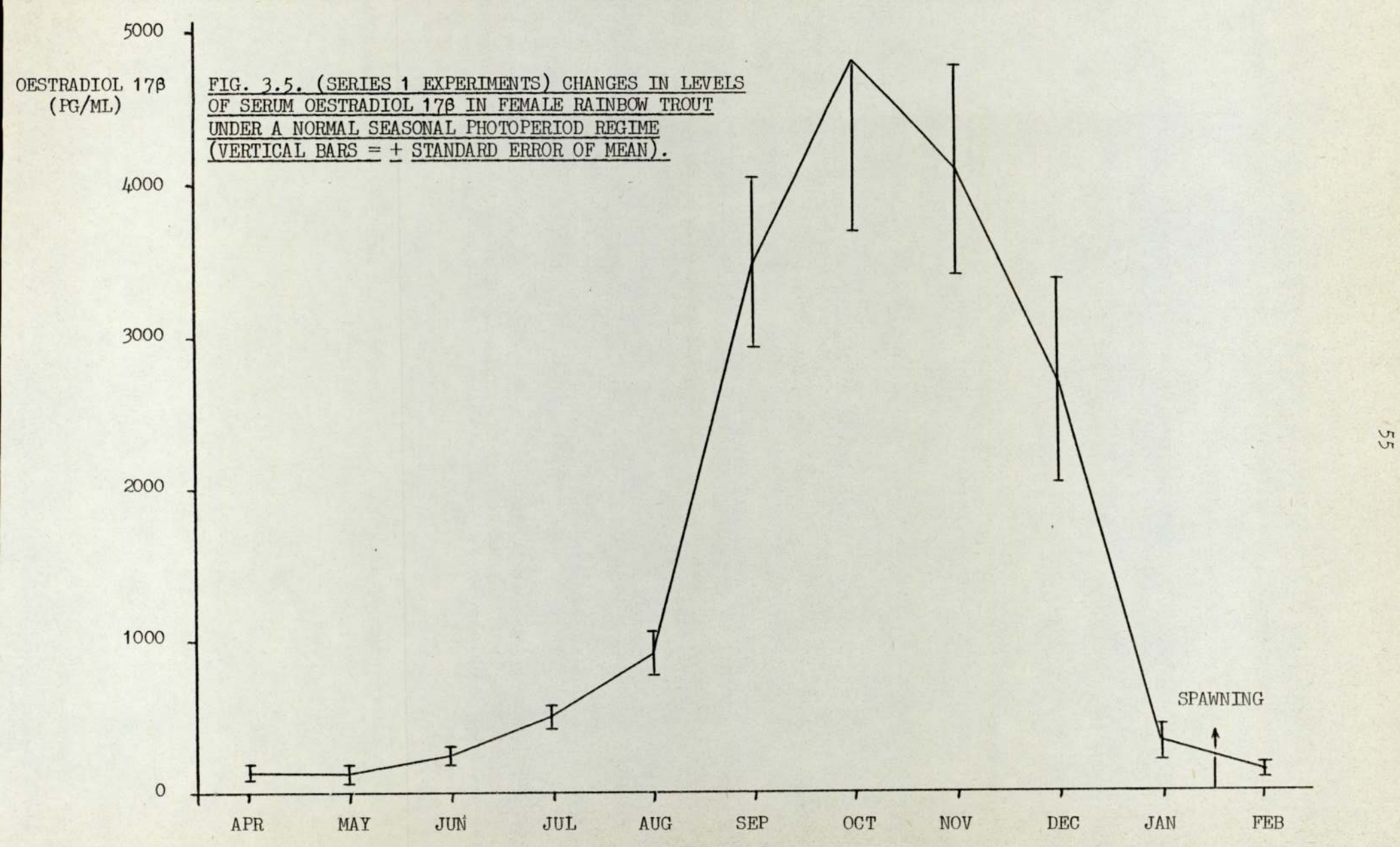


FIG. 3.5. (SERIES 1 EXPERIMENTS) CHANGES IN LEVELS OF SERUM OESTRADIOL 17β IN FEMALE RAINBOW TROUT UNDER A NORMAL SEASONAL PHOTOPERIOD REGIME (VERTICAL BARS = ± STANDARD ERROR OF MEAN).

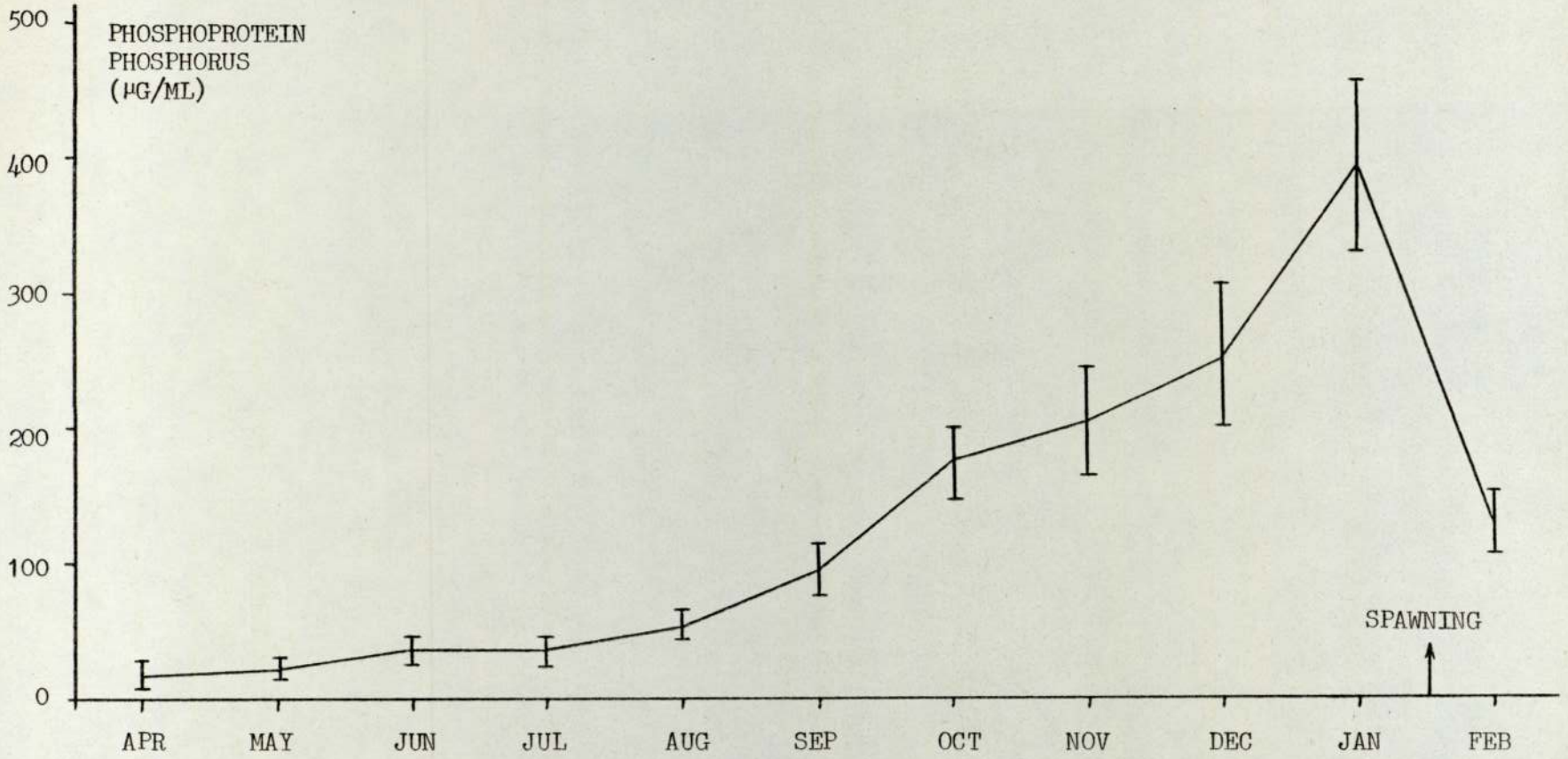


FIG. 3.6. (SERIES 1 EXPERIMENTS) CHANGES IN LEVELS OF SERUM PHOSPHOPROTEIN PHOSPHORUS IN FEMALE RAINBOW TROUT UNDER A NORMAL SEASONAL PHOTOPERIOD REGIME (VERTICAL BARS = ± STANDARD ERROR OF THE MEAN).

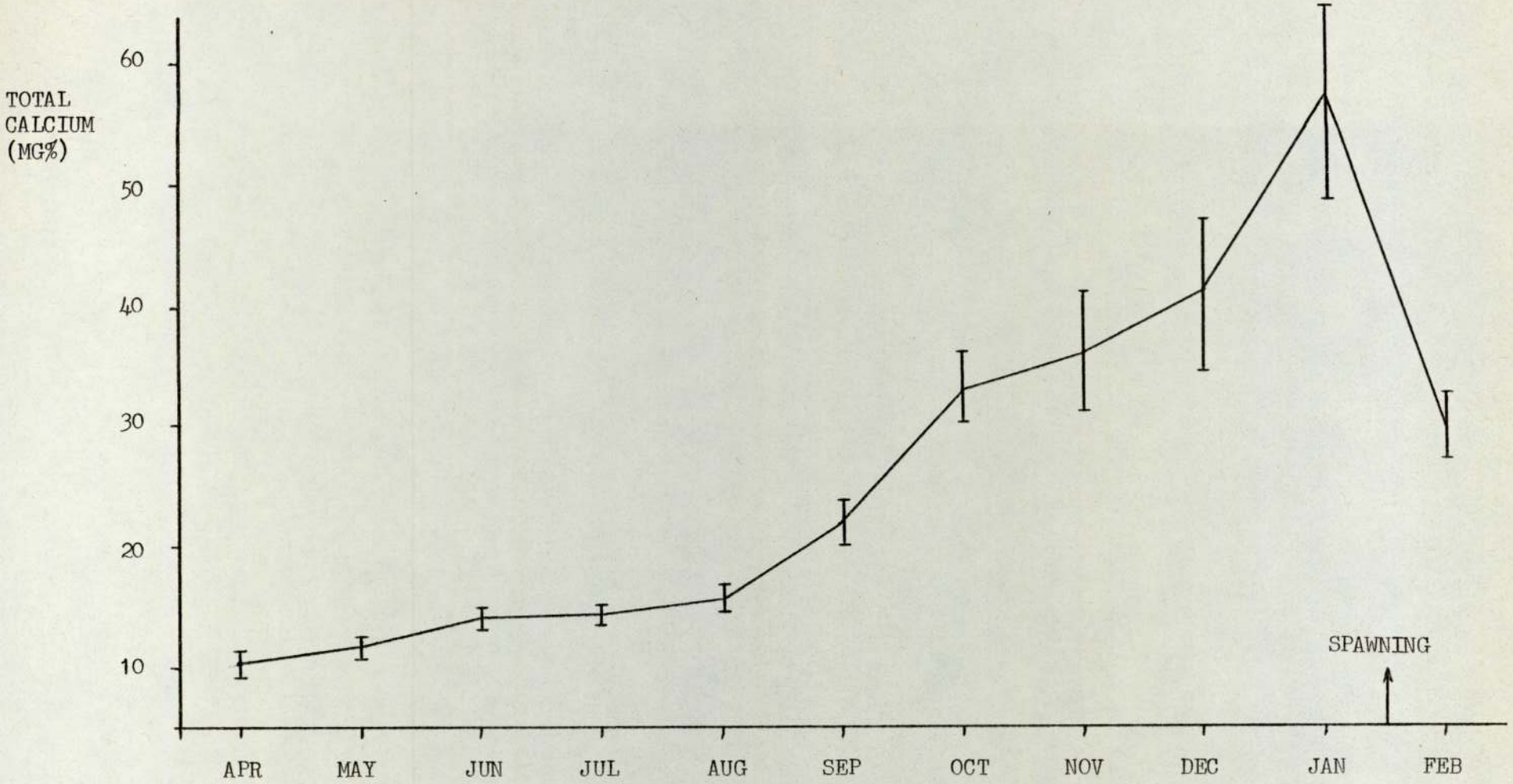


FIG. 3.7. (SERIES 1 EXPERIMENTS) CHANGES IN LEVELS OF TOTAL SERUM CALCIUM IN FEMALE RAINBOW TROUT UNDER A NORMAL SEASONAL PHOTOPERIOD REGIME (VERTICAL BARS = ± STANDARD ERROR OF THE MEAN).

Normal seasonal cycle compressed into 9 months. (see Table 3.2): Spawning among this group of females occurred in early December, 6 weeks earlier than in fish from either the control photoperiod group or from the outside open ponds exposed to natural environmental conditions. Serum levels of gonadotropin rose to 16.2 ± 1.5ng/ml by July, returned to basal by November, only to increase rapidly to a higher peak of 28.5 ± 12.4ng/ml in January (Fig.3.8). Levels in November were significantly different from those in July ($P<0.01$) and January ($P<0.01$). Between June and July, serum levels of oestradiol 17β in the female fish started to increase, reaching a peak of 3310 ± 429pg/ml in October, before returning to basal levels just prior to spawning in early December (Fig.3.9). Levels in October were significantly different from those in April ($P<0.001$). Serum levels of phosphoprotein phosphorus and total calcium in the female fish were both increased in August, reaching peaks of 602 ± 79μg/ml and 81 ± 11mg% respectively in December, just prior to spawning (Figs. 3.10 and 3.11). Levels of both serum phosphoprotein phosphorus and total calcium in December were significantly different from their respective levels in April ($P<0.001$).

	APR	MAY	JUN	JUL	AUG	SEP	OCT	NOV	DEC	JAN	FEB
GONADOTROPIN (NG/ML)	10.5	8.6	12.5	16.2	13.3	6.0	7.5	3.8	8.6	28.5	8.5
± STANDARD ERROR	1.6	0.8	1.8	1.5	1.5	0.8	1.6	0.4	1.6	12.4	1.8
OESTRADIOL 17β (PG/ML)	122	157	184	292	707	2690	3310	2380	639	107	153
± STANDARD ERROR	8	7	21	48	133	257	429	297	339	17	11
PHOSPHOPROTEIN PHOSPHORUS (μG/ML)	15.9	17.1	26.0	19.0	64.8	273.5	308.0	344.2	601.6	324.0	82.0
± STANDARD ERROR	9.6	5.0	4.7	3.0	14.9	78.9	25.9	47.8	79.0	90.3	33.0
TOTAL CALCIUM (MG%)	12.5	12.4	12.8	12.7	16.2	41.7	57.1	63.3	81.0	54.4	20.9
± STANDARD ERROR	0.2	0.3	0.6	0.7	1.5	9.4	12.3	13.8	10.9	9.9	7.0

TABLE 3.2. (SERIES 1 EXPERIMENTS) LEVELS OF GONADOTROPIN, OESTRADIOL 17β, PHOSPHOPROTEIN PHOSPHORUS, AND TOTAL CALCIUM IN SERUM OF FEMALE RAINBOW TROUT UNDER A NORMAL SEASONAL PHOTOPERIOD COMPRESSED INTO 9 MONTHS (n = 5).

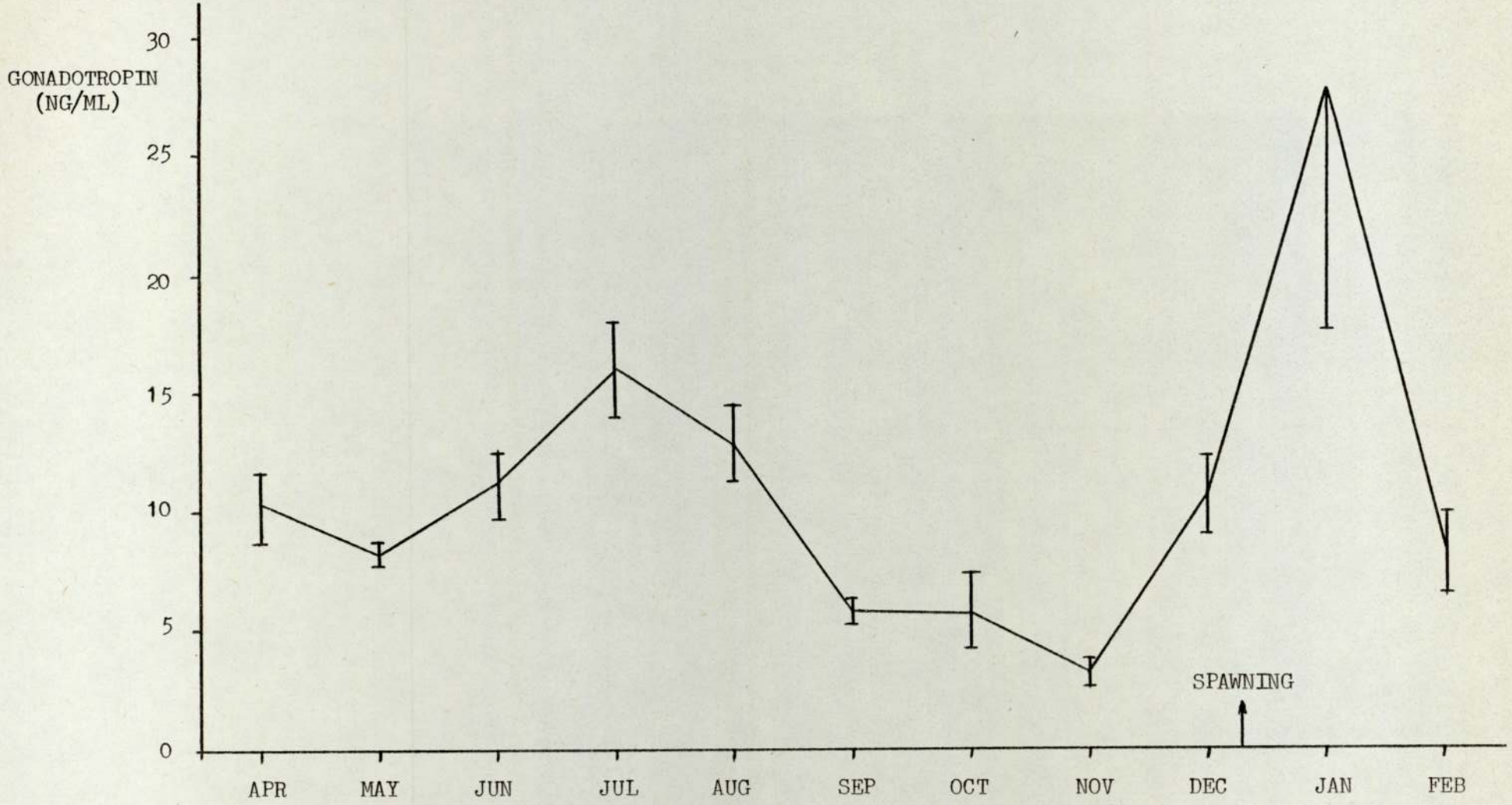


FIG. 3.8. (SERIES 1 EXPERIMENTS) CHANGES IN LEVELS OF SERUM GONADOTROPIN IN FEMALE RAINBOW TROUT UNDER A NORMAL SEASONAL PHOTOPERIOD COMPRESSED INTO 9 MONTHS (VERTICAL BARS = ± STANDARD ERROR OF THE MEAN).

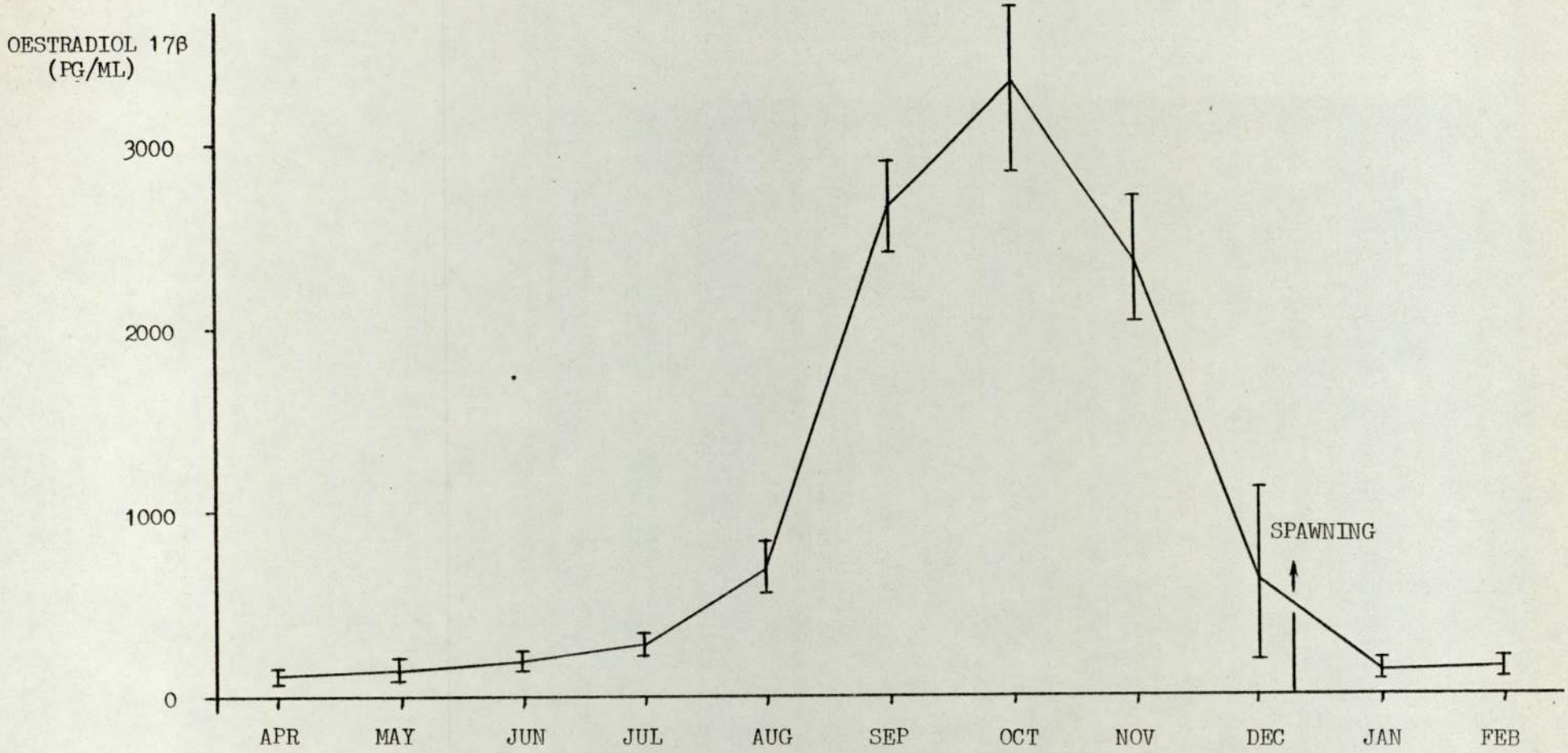


FIG. 3.9. (SERIES 1 EXPERIMENTS) CHANGES IN LEVELS OF SERUM OESTRADIOL 17β IN FEMALE RAINBOW TROUT UNDER A NORMAL SEASONAL PHOTOPERIOD COMPRESSED INTO 9 MONTHS (VERTICAL BARS = ± STANDARD ERROR OF THE MEAN).

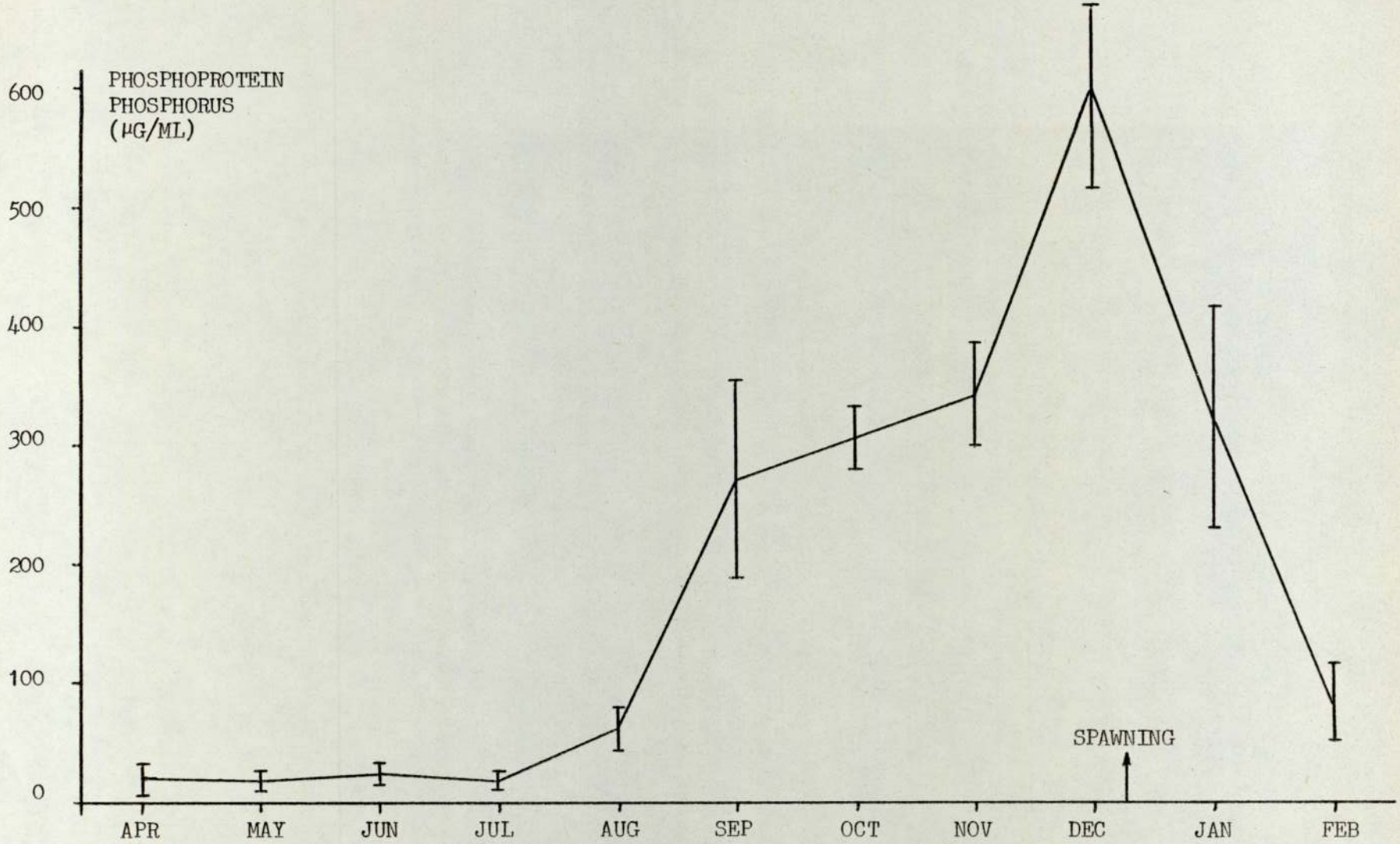


FIG. 3.10. (SERIES 1 EXPERIMENTS) CHANGES IN LEVELS OF SERUM PHOSPHOPROTEIN PHOSPHORUS IN FEMALE RAINBOW TROUT UNDER A NORMAL SEASONAL PHOTOPERIOD COMPRESSED INTO 9 MONTHS (VERTICAL BARS = ± STANDARD ERROR OF THE MEAN).

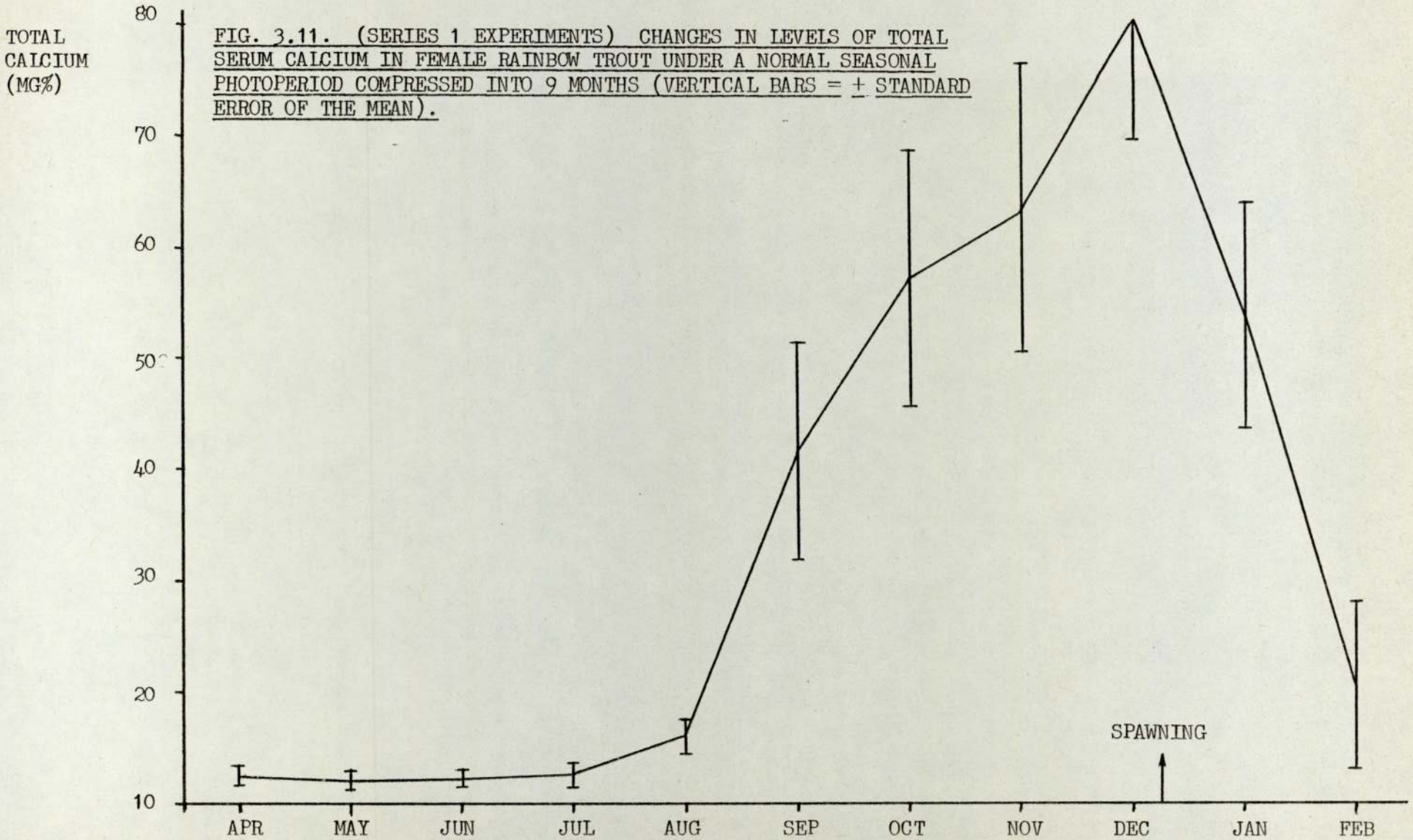


FIG. 3.11. (SERIES 1 EXPERIMENTS) CHANGES IN LEVELS OF TOTAL SERUM CALCIUM IN FEMALE RAINBOW TROUT UNDER A NORMAL SEASONAL PHOTOPERIOD COMPRESSED INTO 9 MONTHS (VERTICAL BARS = ± STANDARD ERROR OF THE MEAN).

Normal seasonal cycle compressed into 6 months. (see Table 3.3): In this group, spawning among the females occurred in mid-October, approximately 12 weeks earlier than in fish from either the control group or the outside ponds. Levels of serum gonadotropin rose to 26.5 $\pm$ 3.2ng/ml in July, returned to basal by October and reached a further peak of 30.0 $\pm$ 2.5ng/ml in December (Fig.3.12). Levels in October were significantly different from those in July ($P<0.01$) and December ($P<0.01$). Serum levels of oestradiol 17β in the females started to increase in July, reaching a peak of 2425 $\pm$ 589pg/ml in late September, but returning to basal levels in December (Fig.3.13). Levels in September were significantly different from those in April ($P<0.01$). By August, levels of serum phosphoprotein phosphorus and total calcium had increased, reaching peaks of 672 $\pm$ 77μg/ml and 90 $\pm$ 6mg% respectively in October just prior to spawning (Figs.3.14 and 3.15). Levels of both serum phosphoprotein phosphorus and total calcium in October were significantly different from their respective levels in April ($P<0.001$).

	APR	MAY	JUN	JUL	AUG	SEP	OCT	NOV	DEC	JAN	FEB
GONADOTROPIN (NG/ML)	9.9	11.1	15.5	26.5	19.2	11.9	8.4	12.8	30.0	15.7	10.7
± STANDARD ERROR	1.3	0.4	2.1	3.2	3.1	1.4	2.0	2.9	2.5	3.4	5.2
OESTRADIOL 17β (PG/ML)	129	158	229	320	1191	2425	2413	1170	108	172	178
± STANDARD ERROR	17	19	23	30	219	589	380	679	33	60	14
PHOSPHOPROTEIN PHOSPHORUS (μG/ML)	19.4	23.3	35.8	41.3	195.5	397.3	456.8	671.5	459.5	77.5	26.0
± STANDARD ERROR	10.5	4.6	7.9	5.5	47.7	47.8	20.5	76.0	113.0	29.3	6.6
TOTAL CALCIUM (MG%)	13.1	12.2	13.4	14.0	29.1	64.6	85.6	90.1	69.9	20.3	13.1
± STANDARD ERROR	0.4	0.6	0.7	1.3	4.1	7.5	6.7	6.2	15.1	5.5	0.7

TABLE 3.3. (SERIES 1 EXPERIMENTS) LEVELS OF GONADOTROPIN, OESTRADIOL 17β, PHOSPHOPROTEIN PHOSPHORUS AND TOTAL CALCIUM IN SERUM OF FEMALE RAINBOW TROUT UNDER A NORMAL SEASONAL PHOTOPERIOD COMPRESSED INTO 6 MONTHS (n = 4).

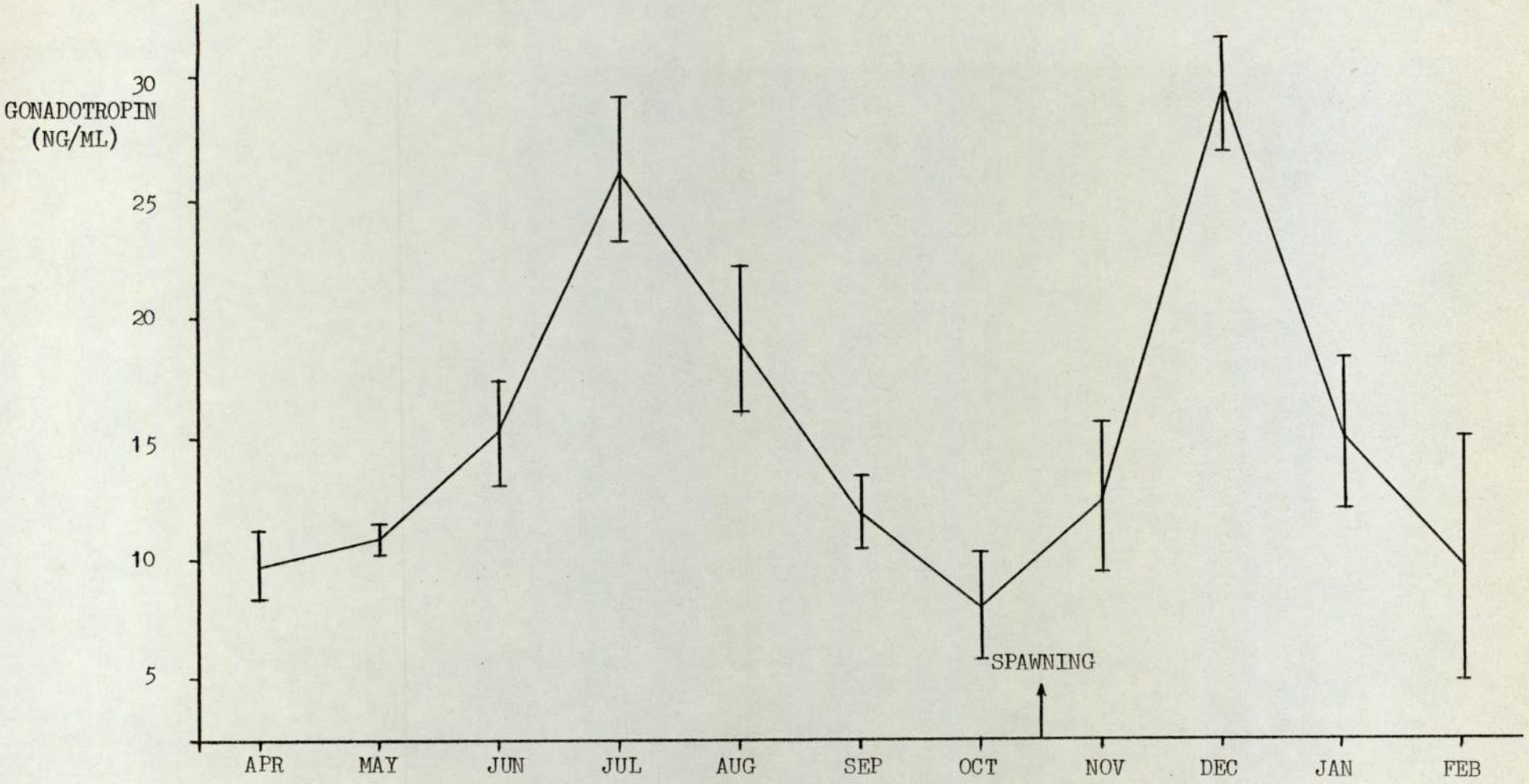


FIG. 3.12. (SERIES 1 EXPERIMENTS) CHANGES IN LEVELS OF SERUM GONADOTROPIN IN FEMALE RAINBOW TROUT UNDER A NORMAL SEASONAL PHOTOPERIOD COMPRESSED INTO 6 MONTHS (VERTICAL BARS = ± STANDARD ERROR OF THE MEAN).

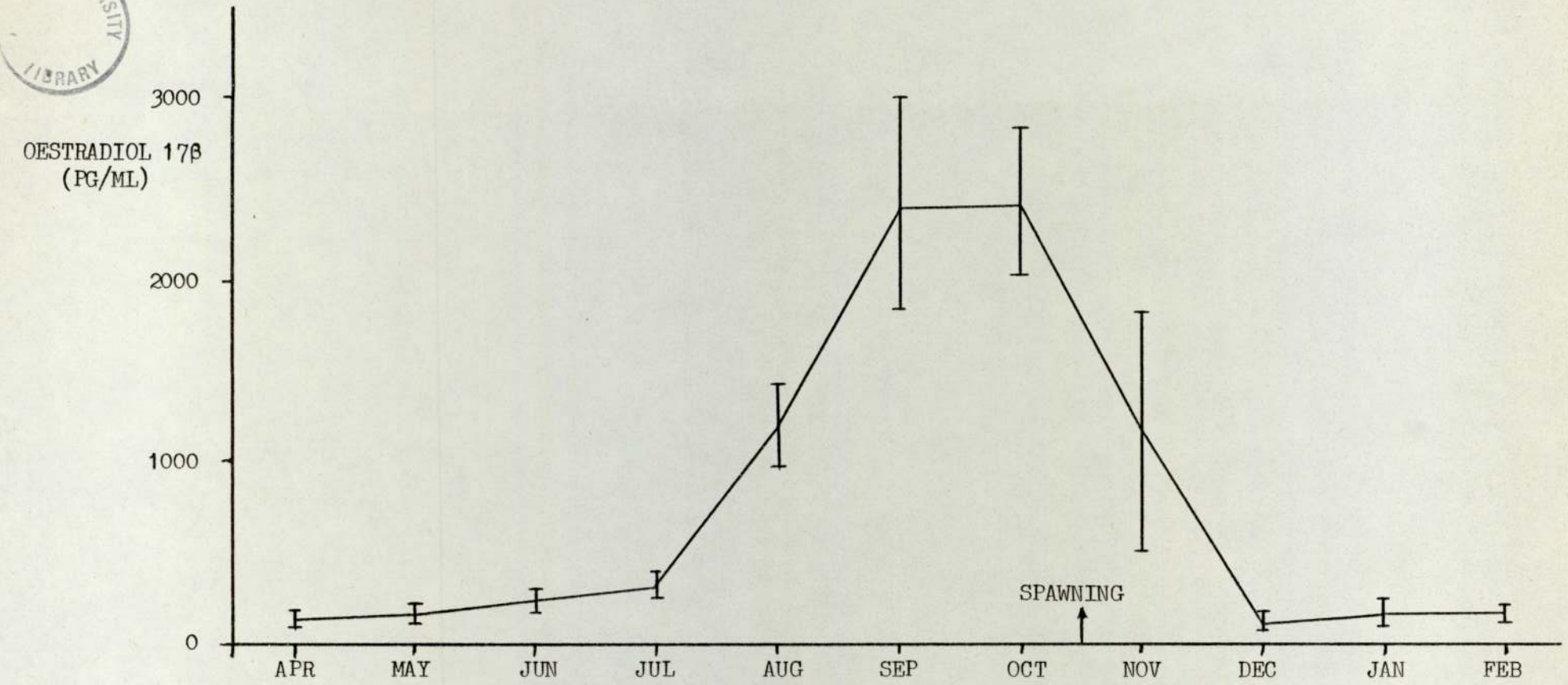


FIG. 3.13. (SERIES 1 EXPERIMENTS) CHANGES IN LEVELS OF SERUM OESTRADIOL 17β IN FEMALE RAINBOW TROUT UNDER A NORMAL SEASONAL PHOTOPERIOD COMPRESSED INTO 6 MONTHS (VERTICAL BARS = ± STANDARD ERROR OF THE MEAN).

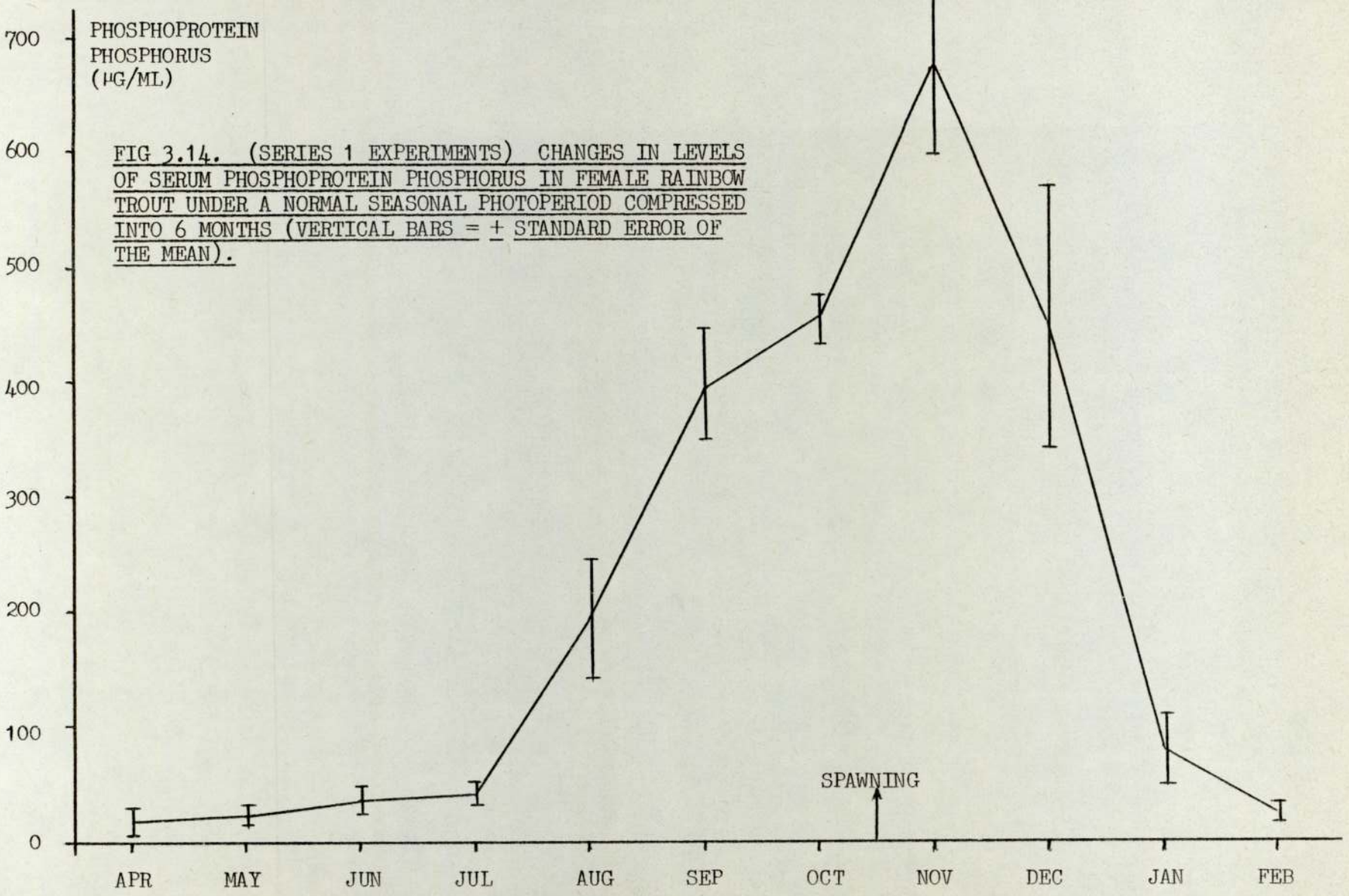


FIG 3.14. (SERIES 1 EXPERIMENTS) CHANGES IN LEVELS OF SERUM PHOSPHOPROTEIN PHOSPHORUS IN FEMALE RAINBOW TROUT UNDER A NORMAL SEASONAL PHOTOPERIOD COMPRESSED INTO 6 MONTHS (VERTICAL BARS = ± STANDARD ERROR OF THE MEAN).

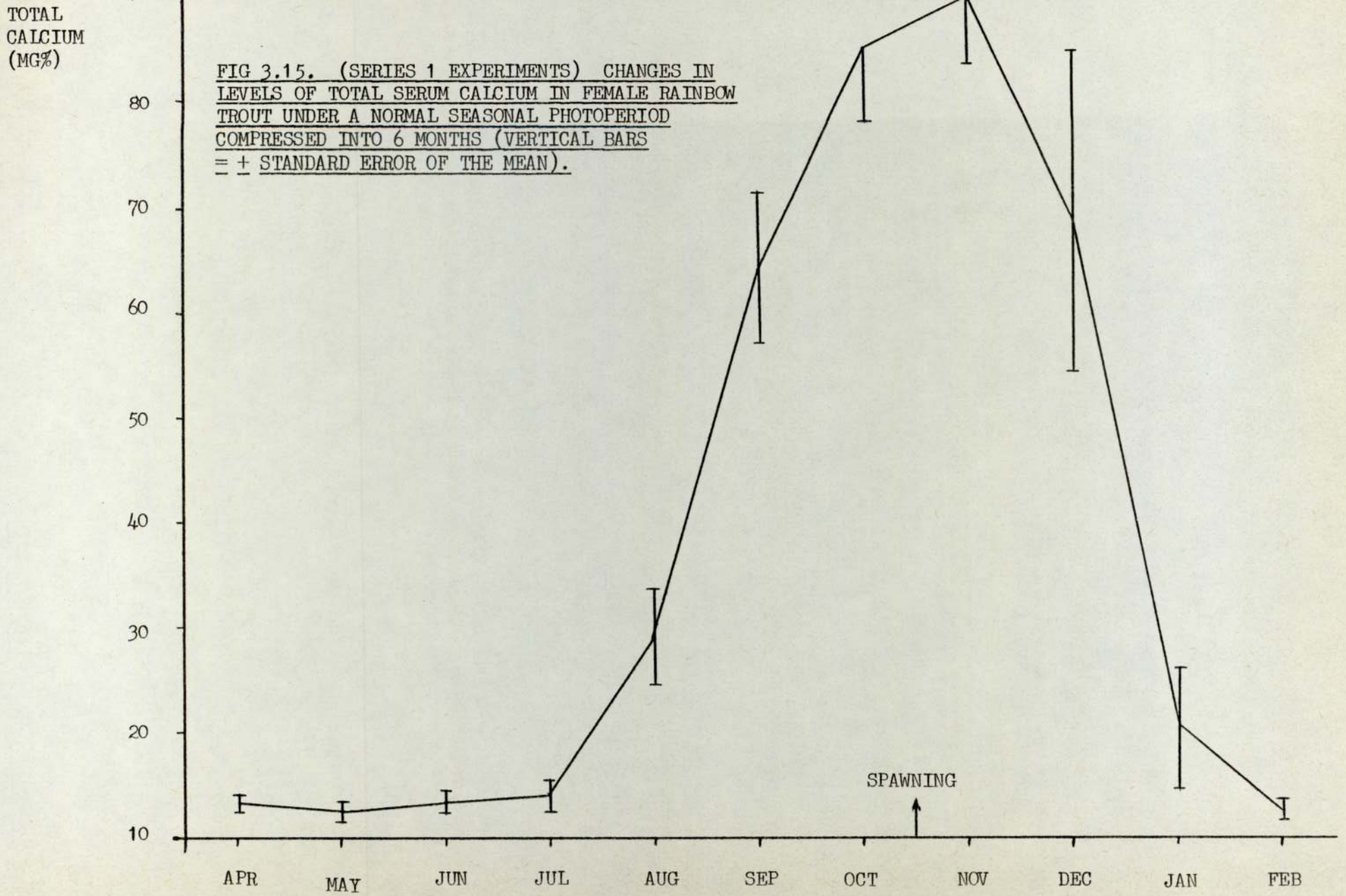


FIG 3.15. (SERIES 1 EXPERIMENTS) CHANGES IN LEVELS OF TOTAL SERUM CALCIUM IN FEMALE RAINBOW TROUT UNDER A NORMAL SEASONAL PHOTOPERIOD COMPRESSED INTO 6 MONTHS (VERTICAL BARS = ± STANDARD ERROR OF THE MEAN).

Constant 12 hour light / 12 hour dark regime. (see Table 3.4): Spawning among the females in this group occurred in January at approximately the same time as the control group and fish in the outside ponds. Levels of serum gonadotropin increased to 15.5 ± 2.6ng/ml in July, returned to basal by December, and reached a higher peak level of 24.7 ± 8.9ng/ml in January around the time of spawning (Fig.3.16). Levels in December were significantly different from those in July ($P<0.05$) and January ($P<0.01$). Serum levels of oestradiol 17β in the females started to increase in July, reaching a peak of 3725 ± 827pg/ml in October, before returning to basal levels in January at which time spawning occurred (Fig.3.17). Levels in October were significantly different from those in April ($P<0.001$). Both serum levels of phosphoprotein phosphorus and total calcium began to increase during August up to peaks of 481 ± 61µg/ml and 69 ± 10%mg respectively in December, just before spawning (Figs. 3.18 and 3.19). Levels of both phosphoprotein phosphorus and total calcium in December were significantly different from their respective levels in April ($P<0.001$).

	APR	MAY	JUN	JUL	AUG	SEP	OCT	NOV	DEC	JAN	FEB
GONADOTROPIN (NG/ML)	8.0	10.7	13.3	15.5	11.9	8.0	6.9	5.4	4.1	24.7	14.7
± STANDARD ERROR	1.2	2.4	1.9	2.6	2.2	1.6	1.2	2.6	1.5	8.9	2.6
OESTRADIOL 17β (PG/ML)	130	140	220	425	1105	3300	3725	2550	1162	82	147
± STANDARD ERROR	20	22	43	58	346	1153	827	496	181	9	22
PHOSPHOPROTEIN PHOSPHORUS (μG/ML)	15.6	14.4	32.2	24.0	52.5	107.0	337.0	345.3	481.3	241.5	60.3
± STANDARD ERROR	8.6	1.5	7.4	1.7	14.0	27.0	75.2	91.8	61.0	39.9	12.0
TOTAL CALCIUM (MG%)	12.0	13.0	13.3	13.2	15.8	30.6	57.0	55.6	69.2	46.1	18.1
± STANDARD ERROR	0.3	0.3	0.3	0.2	0.9	6.1	13.1	13.3	9.7	5.9	1.7

TABLE 3.4. (SERIES 1 EXPERIMENTS) LEVELS OF GONADOTROPIN, OESTRADIOL 17β, PHOSPHOPROTEIN PHOSPHORUS AND TOTAL CALCIUM IN SERUM OF FEMALE RAINBOW TROUT UNDER A CONSTANT 12 HOUR LIGHT / 12 HOUR DARK PHOTOPERIOD (n = 4).

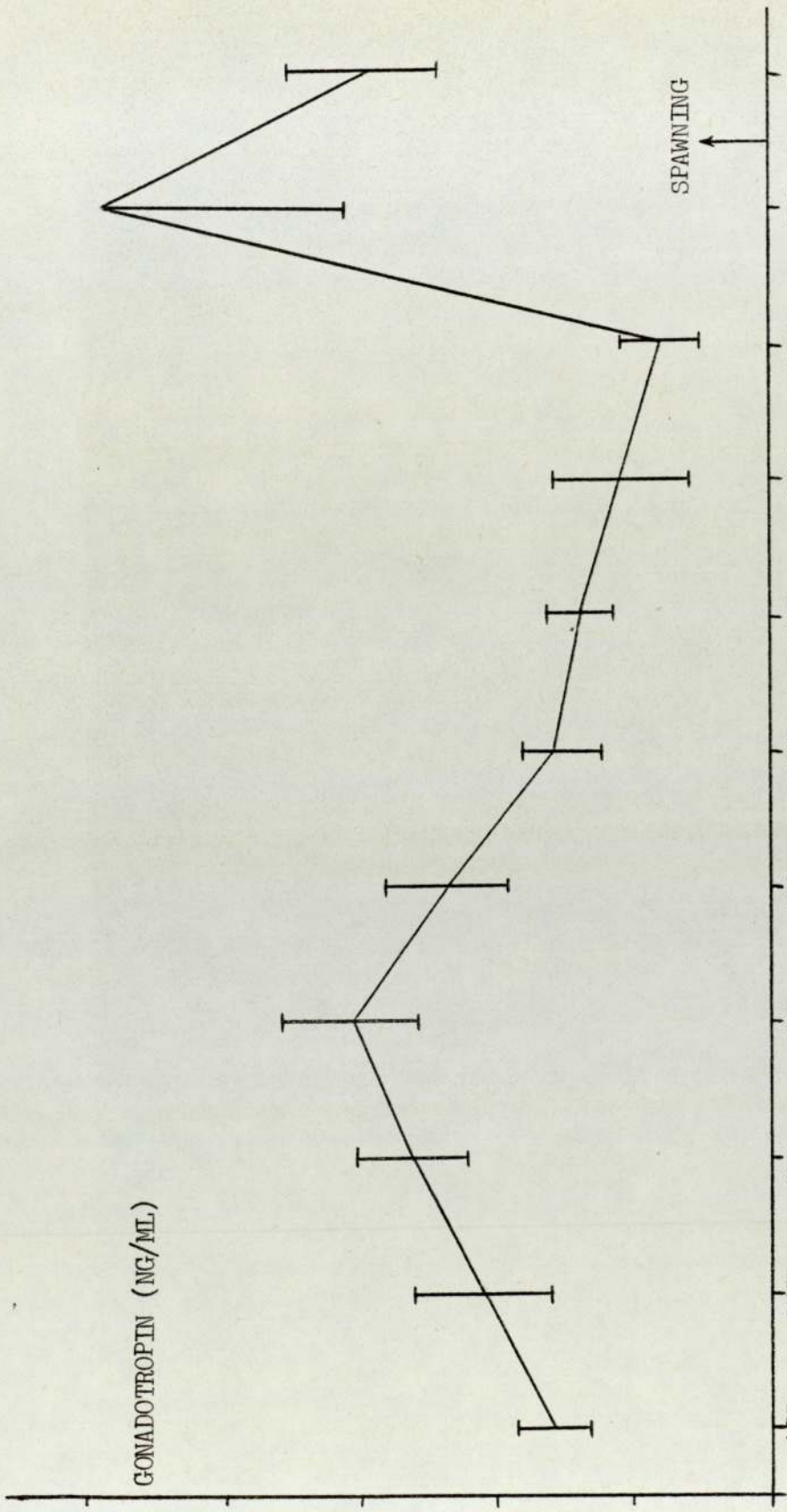


FIG. 3.16. (SERIES 1 EXPERIMENTS) CHANGES IN LEVELS OF SERUM GONADOTROPIN IN FEMALE RAINBOW TROUT UNDER A CONSTANT 12 HOUR LIGHT / 12 HOUR DARK PHOTOPERIOD REGIME. (VERTICAL BARS = $\pm$ STANDARD ERROR OF THE MEAN).

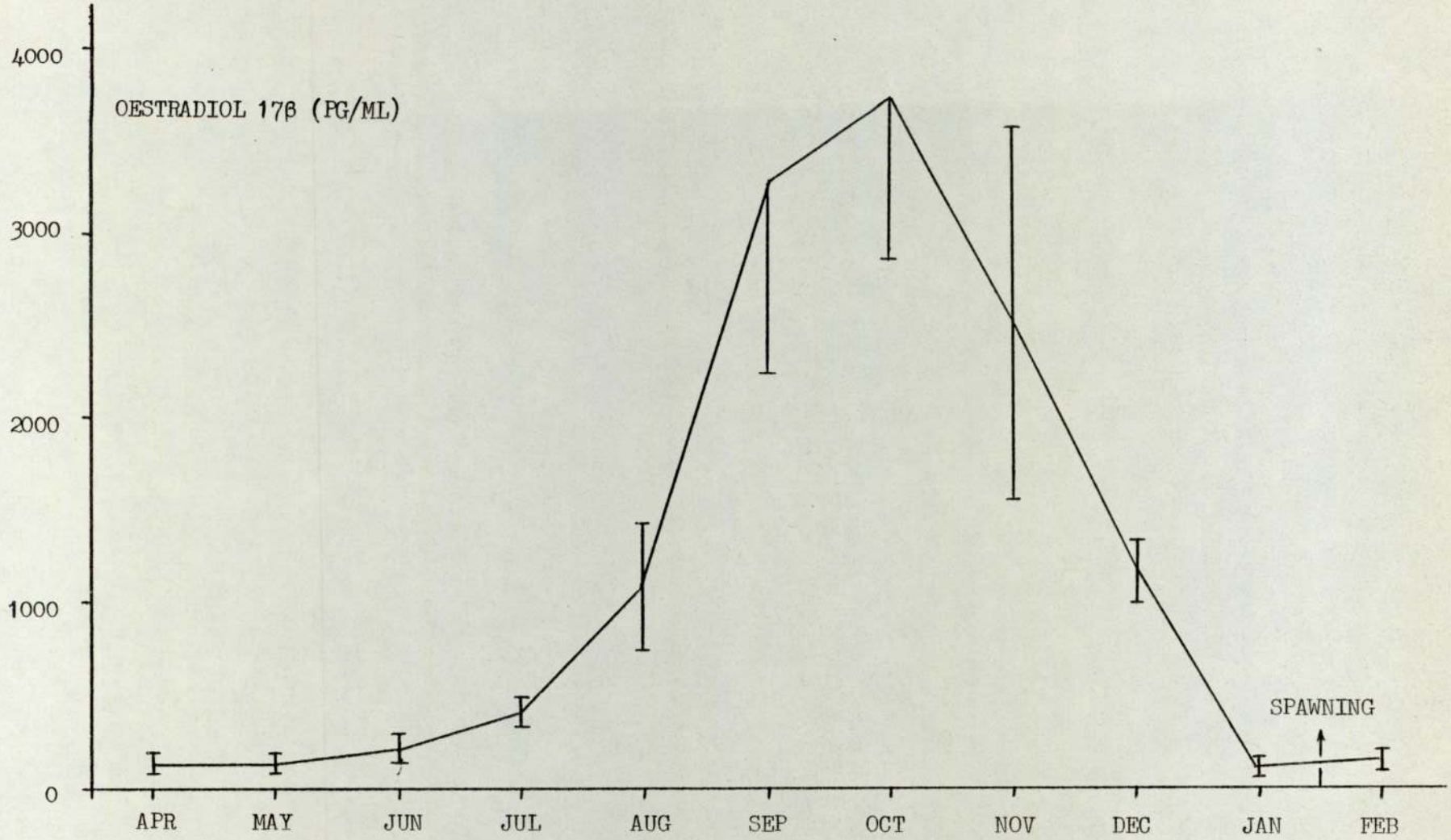


FIG. 3.17. (SERIES 1 EXPERIMENTS) CHANGES IN LEVELS OF OESTRADIOL 17β IN SERUM OF FEMALE RAINBOW TROUT UNDER A CONSTANT 12 HOUR LIGHT / 12 HOUR DARK PHOTOPERIOD REGIME. (VERTICAL BARS = ± STANDARD ERROR OF MEAN).

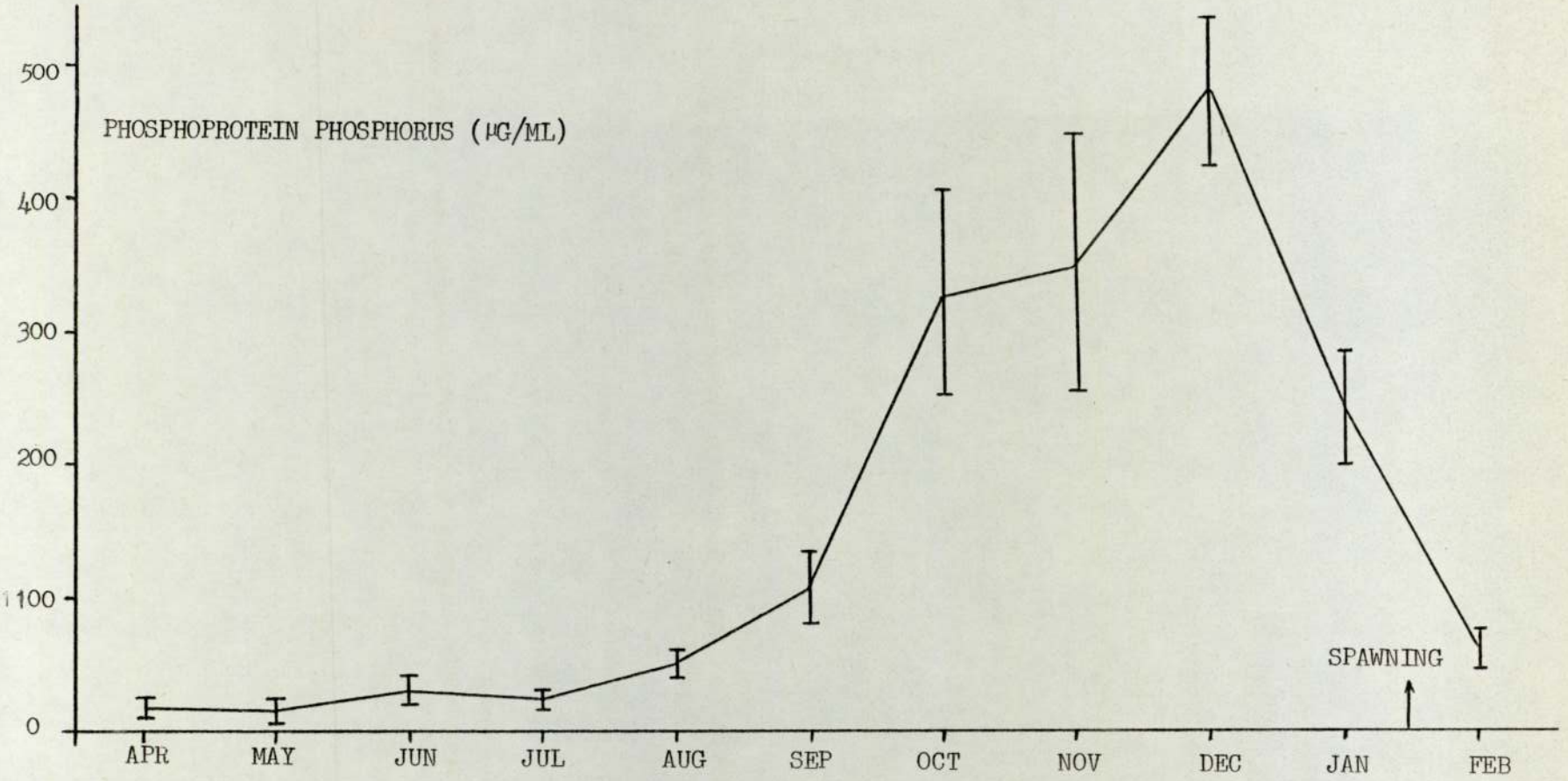


FIG. 3.18. (SERIES 1 EXPERIMENTS) CHANGES IN LEVELS OF PHOSPHOPROTEIN PHOSPHORUS IN SERUM OF FEMALE RAINBOW TROUT UNDER A 12 HOUR LIGHT / 12 HOUR DARK PHOTOPERIOD. (VERTICAL BARS = ± STANDARD ERROR OF THE MEAN).

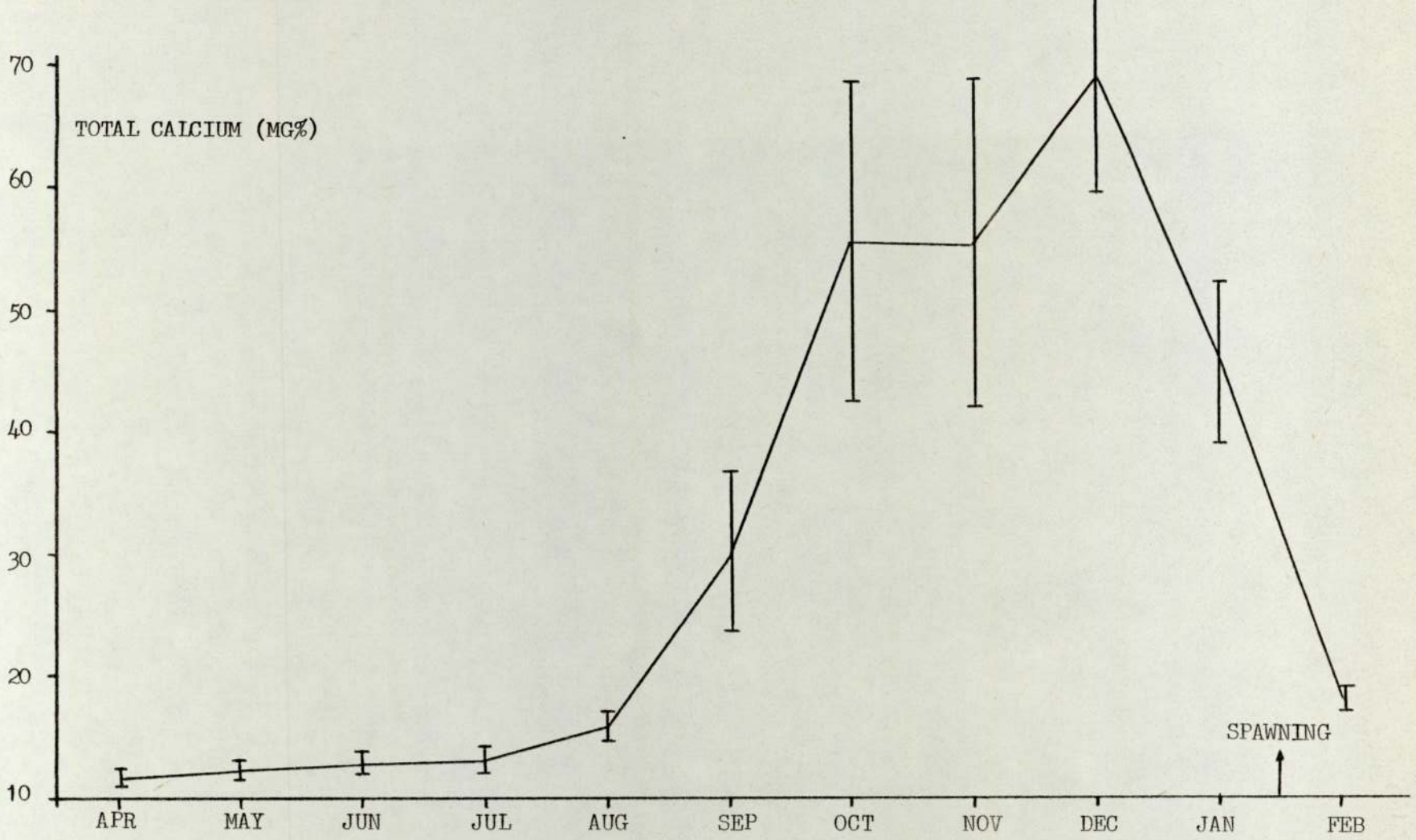


FIG. 3.19. (SERIES 1 EXPERIMENTS) CHANGES IN LEVELS OF TOTAL CALCIUM IN SERUM OF FEMALE RAINBOW TROUT UNDER A CONSTANT 12 HOUR LIGHT / 12 HOUR DARK PHOTOPERIOD REGIME. (VERTICAL BARS = ± STANDARD ERROR OF THE MEAN).

Male Fish: All male fish, irrespective of treatment, had similar basal levels of serum gonadotropin (5.6 ± 0.6ng/ml) and testosterone (4.2 ± 0.4ng/ml) in April at the start of the experiments. Basal levels of oestradiol 17β (130 ± 15pg/ml) phosphoprotein phosphorus (25 ± 7µg/ml) and total calcium (12 ± 2mg%) were found in the males, these values being similar to those in the female fish at the start of the experiments. However, in contrast to the females, no significant change in serum levels of oestradiol 17β, phosphoprotein phosphorus or total calcium from basal levels was observed in the male fish irrespective of photoperiod treatment (Tables 3.5 - 3.8). Changes were seen in the time of commencement of spermiation and in the serum levels of gonadotropin and testosterone, which varied according to photoperiod treatment, and are discussed in detail below.

	APR	MAY	JUN	JUL	AUG	SEP	OCT	NOV	DEC	JAN	FEB
OESTRADIOL 17β (PG/ML)	138	162	157	165	176	175	230	140	136	98	125
± STANDARD ERROR	16	19	57	55	23	13	95	31	24	19	11
PHOSPHOPROTEIN PHOSPHORUS (μG/ML)	8.3	26.1	26.4	17.8	30.0	24.0	25.7	28.0	21.7	27.7	21.0
± STANDARD ERROR	0.6	5.3	14.9	2.7	12.5	5.0	5.4	7.0	6.6	4.0	2.1
TOTAL CALCIUM (μG/ML)	11.2	12.7	12.7	12.3	13.1	14.5	14.5	13.5	12.8	16.9	11.8
± STANDARD ERROR	0.5	0.6	0.4	1.1	0.2	1.0	0.4	0.3	0.4	0.5	0.8

TABLE 3.5. (SERIES 1 EXPERIMENTS) LEVELS OF OESTRADIOL 17β, PHOSPHOPROTEIN PHOSPHORUS AND TOTAL CALCIUM IN SERUM OF MALE RAINBOW TROUT UNDER A NORMAL 12 MONTH SEASONAL PHOTOPERIOD REGIME (n = 3).

	APR	MAY	JUN	JUL	AUG	SEP	OCT	NOV	DEC	JAN	FEB
OESTRADIOL 17β (PG/ML)	115	139	133	221	131	139	204	143	95	129	124
± STANDARD ERROR	10	4	19	23	17	17	38	22	4	22	11
PHOSPHOPROTEIN PHOSPHORUS (μG/ML)	7.7	18.9	15.3	14.2	16.4	23.4	27.0	28.0	27.0	17.4	25.2
± STANDARD ERROR	6.5	4.4	4.7	2.2	1.9	4.1	4.3	6.1	3.0	4.4	5.1
TOTAL CALCIUM (MG%)	12.0	13.1	12.6	11.3	12.5	14.3	16.0	13.7	14.7	16.2	11.4
± STANDARD ERROR	0.4	0.2	0.2	0.2	0.5	0.2	0.4	0.9	1.1	1.8	0.6

TABLE 3.6. (SERIES 1 EXPERIMENTS) LEVELS OF OESTRADIOL 17β, PHOSPHOPROTEIN PHOSPHORUS AND TOTAL CALCIUM IN SERUM OF MALE RAINBOW TROUT UNDER A NORMAL SEASONAL PHOTOPERIOD COMPRESSED INTO 9 MONTHS (n = 5).

	APR	MAY	JUN	JUL	AUG	SEP	OCT	NOV	DEC	JAN	FEB
OESTRADIOL 17β (PG/ML)	111	129	152	169	156	150	136	108	94	121	138
± STANDARD ERROR	16	23	24	24	15	16	27	9	5	34	6
PHOSPHOPROTEIN PHOSPHORUS (μG/ML)	34.9	17.5	31.4	13.9	17.6	16.0	20.5	27.2	18.2	13.0	15.0
± STANDARD ERROR	3.0	3.4	5.0	1.7	5.1	3.1	3.0	5.3	4.6	3.2	2.0
TOTAL CALCIUM (MG%)	12.7	12.7	12.0	11.5	12.4	13.9	20.6	12.7	11.0	4.5	7.2
± STANDARD ERROR	0.3	0.2	0.2	0.5	0.3	1.0	7.4	0.8	0.5	0.9	0.5

TABLE 3.7. (SERIES 1 EXPERIMENTS) LEVELS OF OESTRADIOL 17β, PHOSPHOPROTEIN PHOSPHORUS AND TOTAL CALCIUM IN SERUM OF MALE RAINBOW TROUT UNDER A NORMAL SEASONAL PHOTOPERIOD COMPRESSED INTO 6 MONTHS (n = 6).

	APR	MAY	JUN	JUL	AUG	SEP	OCT	NOV	DEC	JAN	FEB
OESTRADIOL 17β (PG/ML)	140	150	187	249	140	158	193	126	95	124	157
± STANDARD ERROR	12	12	28	18	12	24	24	22	5	23	14
PHOSPHOPROTEIN PHOSPHORUS (μG/ML)	30.4	12.9	27.0	11.7	26.0	15.2	21.0	20.6	14.2	11.8	20.2
± STANDARD ERROR	11.9	3.1	3.5	2.8	9.1	1.7	0.6	3.3	1.7	3.2	3.0
TOTAL CALCIUM (MG%)	11.4	12.3	11.9	11.3	12.5	12.2	21.8	12.7	12.4	10.0	10.4
± STANDARD ERROR	0.3	0.3	0.3	0.2	0.4	0.4	9.2	0.5	0.5	1.0	0.3

TABLE 3.8. (SERIES 1 EXPERIMENTS) LEVELS OF OESTRADIOL 17β, PHOSPHOPROTEIN PHOSPHORUS AND TOTAL CALCIUM IN SERUM OF MALE RAINBOW TROUT UNDER A CONSTANT 12 HOUR LIGHT / 12 HOUR DARK PHOTOPERIOD (n = 6).

Normal 1 year seasonal cycle. (see Table 3.9): Spermiation among this group of males started in December, at exactly the same time as broodstock male fish maintained outside in open ponds. Levels of serum gonadotropin gradually increased from basal to reach a peak of 14.7 $\pm$ 0.2ng/ml in October (Fig. 3.20). Levels in October were significantly different from those in April ($P<0.05$). Serum levels of testosterone also gradually increased from the basal levels found in April, to reach a maximum of 20.4 $\pm$ 1.9ng/ml in January (Fig. 3.21). Levels in January were significantly different from those in April ($P<0.001$).

	APR	MAY	JUN	JUL	AUG	SEP	OCT	NOV	DEC	JAN	FEB
GONADOTROPIN (NG/ML)	5.3	6.0	9.5	10.2	10.6	9.9	14.7	8.5	6.3	7.8	8.4
± STANDARD ERROR	0.5	0.7	1.6	1.5	1.4	0.9	0.2	1.2	0.6	0.9	0.7
TESTOSTERONE (NG/ML)	3.9	4.5	8.1	8.3	8.9	13.6	14.6	15.1	19.3	20.4	19.5
± STANDARD ERROR	0.4	0.8	1.3	0.7	0.4	2.4	0.9	0.6	1.3	1.9	3.8

TABLE 3.9. (SERIES 1 EXPERIMENTS) LEVELS OF GONADOTROPIN AND TESTOSTERONE IN SERUM OF MALE RAINBOW TROUT UNDER A NORMAL 12 MONTH SEASONAL PHOTOPERIOD REGIME (n = 3).

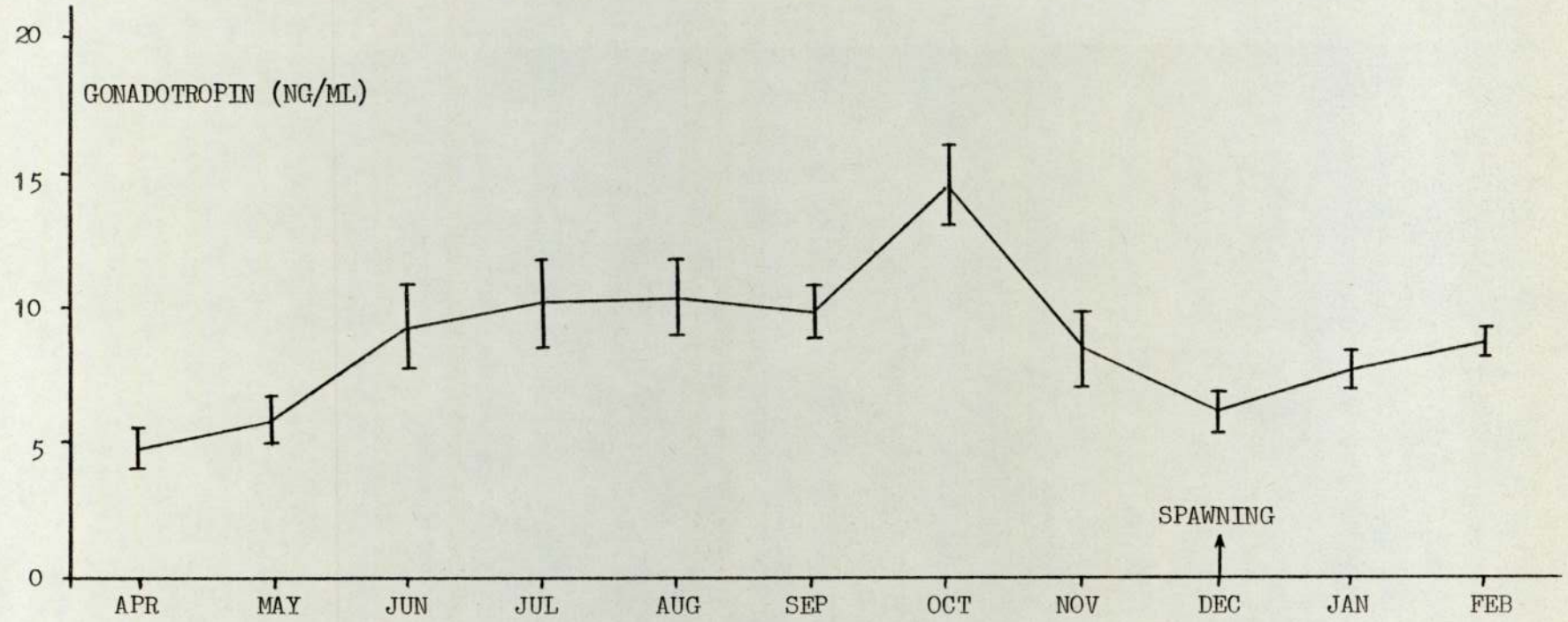


FIG. 3.20. (SERIES 1 EXPERIMENTS) CHANGES IN LEVELS OF GONADOTROPIN IN SERUM OF MALE RAINBOW TROUT UNDER A NORMAL SEASONAL PHOTOPERIOD REGIME. (VERTICAL BARS = ± STANDARD ERROR OF THE MEAN).

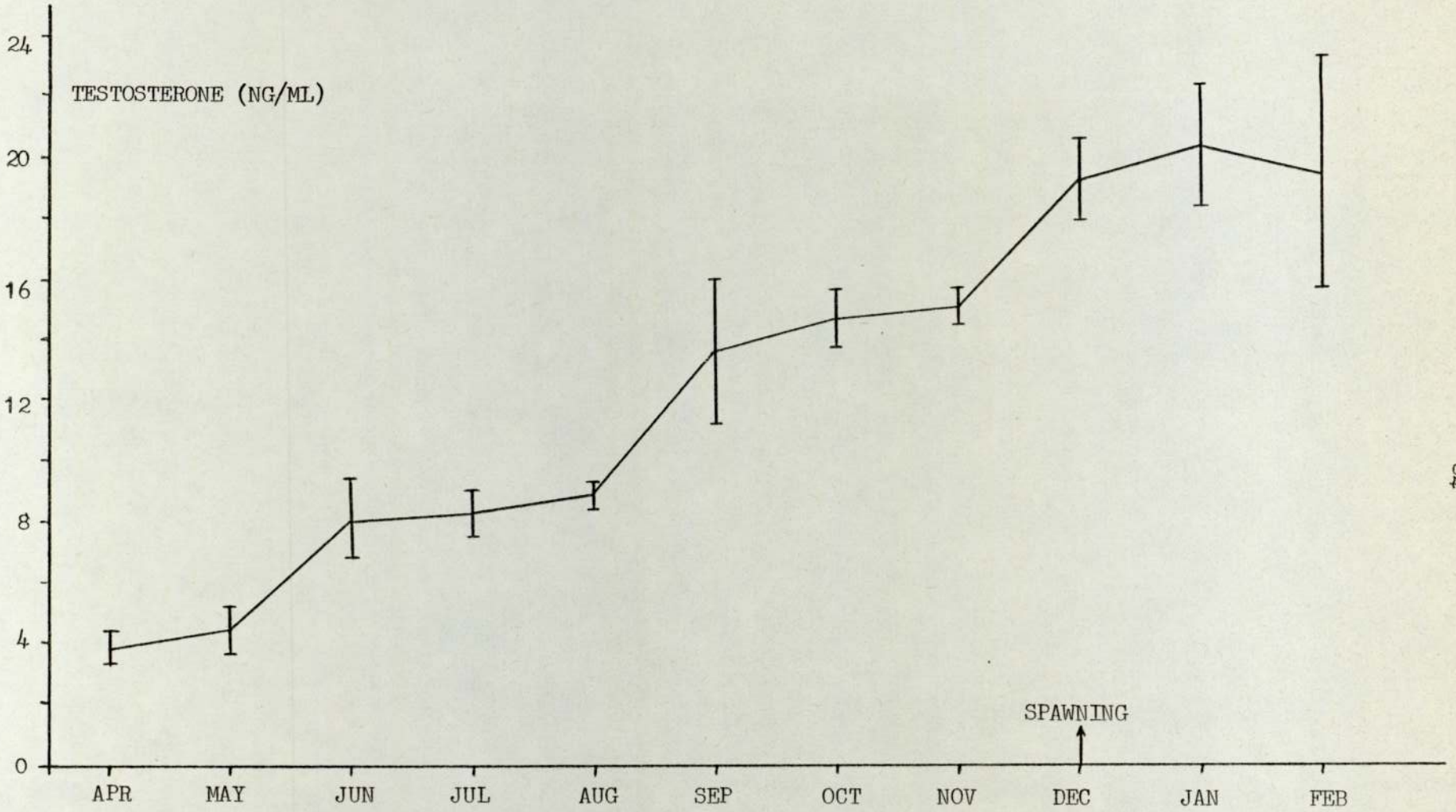


FIG. 3.21. (SERIES 1 EXPERIMENTS) CHANGES IN LEVELS OF TESTOSTERONE IN SERUM OF MALE RAINBOW TROUT UNDER A NORMAL SEASONAL PHOTOPERIOD REGIME. (VERTICAL BARS = ± STANDARD ERROR OF THE MEAN).

Normal seasonal cycle compressed into 9 months. (see Table 3.10): Spermiation among this group of males started in November, 4 weeks earlier than in male fish from either the control photoperiod, or the outside ponds. Serum levels of gonadotropin gradually increased to a peak of 12.3 $\pm$ 1.3ng/ml by September (Fig. 3.22). Levels in September were significantly different from those in April ($P<0.05$). Levels of testosterone in the serum also gradually increased from basal in April to reach a maximum of 21.6 $\pm$ 1.2ng/ml in December (Fig. 3.23). Levels in December were significantly different from those in April ($P<0.001$).

	APR	MAY	JUN	JUL	AUG	SEP	OCT	NOV	DEC	JAN	FEB
GONADOTROPIN (NG/ML)	5.6	5.8	8.2	8.1	9.1	12.3	12.2	6.2	7.7	8.7	5.9
± STANDARD ERROR	0.5	0.3	0.8	0.9	0.9	1.3	3.2	1.2	0.4	1.2	1.5
TESTOSTERONE (NG/ML)	4.4	6.1	6.0	7.3	10.4	15.8	18.7	19.9	21.6	19.8	19.6
± STANDARD ERROR	0.5	1.2	0.8	0.6	0.9	0.9	0.9	1.2	1.2	2.3	2.9

TABLE 3.10. (SERIES 1 EXPERIMENTS) LEVELS OF GONADOTROPIN AND TESTOSTERONE IN SERUM OF MALE RAINBOW TROUT UNDER A NORMAL SEASONAL PHOTOPERIOD COMPRESSED INTO 9 MONTHS (n = 5).

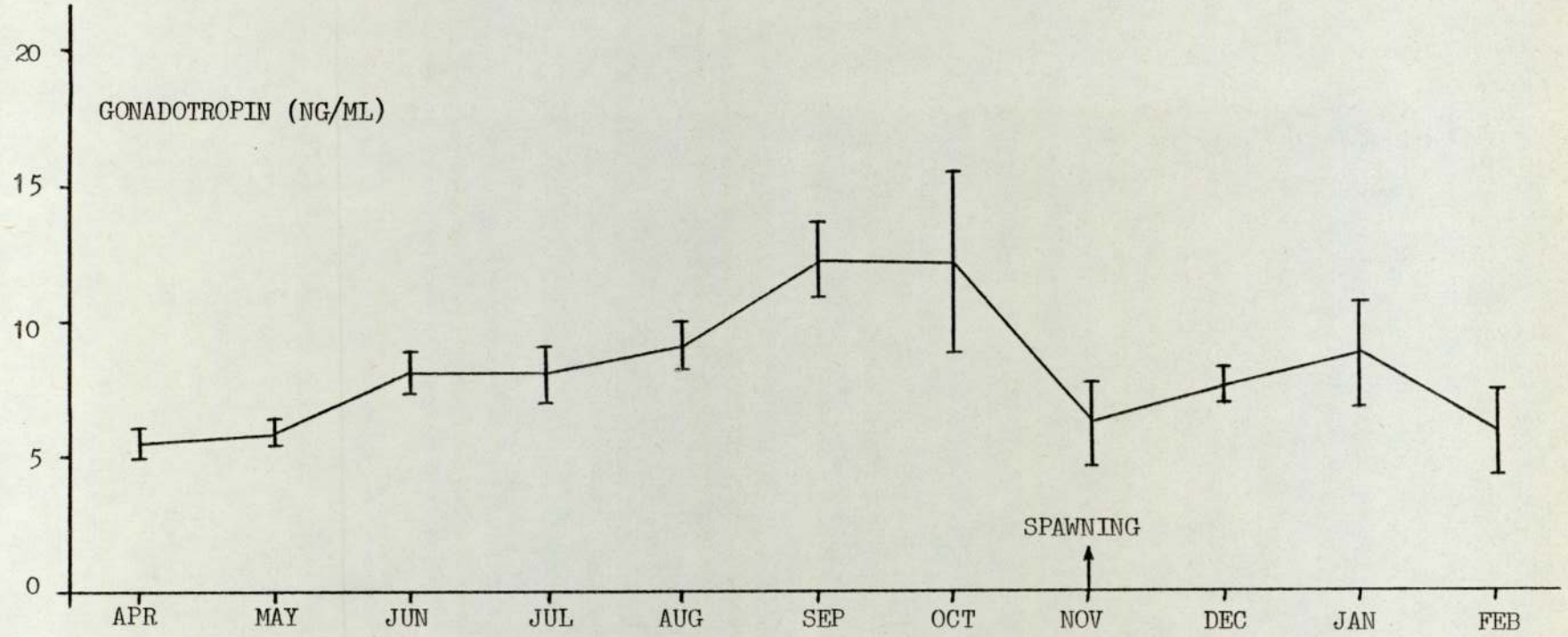


FIG 3.22. (SERIES 1 EXPERIMENTS) CHANGES IN LEVELS OF GONADOTROPIN IN SERUM OF MALE RAINBOW TROUT UNDER A NORMAL SEASONAL PHOTOPERIOD CYCLE COMPRESSED INTO 9 MONTHS. (VERTICAL BARS = ± STANDARD ERROR OF MEAN).

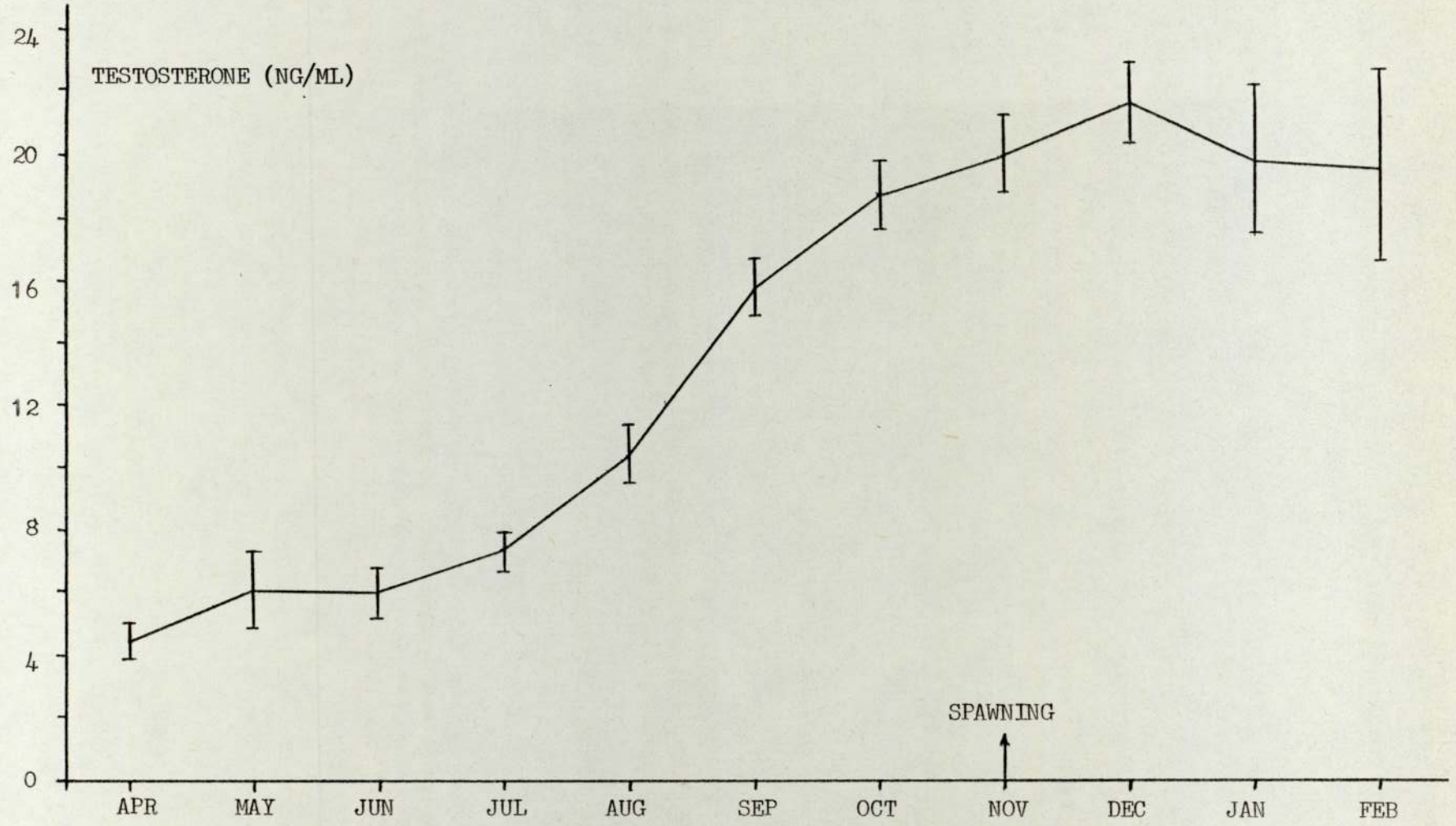


FIG. 3.23. (SERIES 1 EXPERIMENTS) CHANGES IN LEVELS OF TESTOSTERONE IN SERUM OF MALE RAINBOW TROUT UNDER A NORMAL SEASONAL PHOTOPERIOD CYCLE COMPRESSED INTO 9 MONTHS. (VERTICAL BARS = ± STANDARD ERROR OF MEAN).

Normal seasonal cycle compressed into 6 months. (see Table 3.11): In this group of males, spermiation commenced in October, 8 weeks earlier than in either the control photoperiod, or the fish maintained in the outside ponds. Serum levels of gonadotropin gradually increased from basal to reach a peak level of 13.3 $\pm$ 1.1ng/ml in August (Fig. 3.24). Levels in August were significantly different from those in April ($P<0.01$). Serum testosterone levels also gradually increased from basal levels in April, to a peak of 21.0 $\pm$ 1.8ng/ml in November (Fig. 3.25). Levels in November were significantly different from those in April ($P<0.001$). Under this photoperiod regime, the peak levels in both hormones therefore occurred 2 months earlier than under the control photoperiod.

	APR	MAY	JUN	JUL	AUG	SEP	OCT	NOV	DEC	JAN	FEB
GONADOTROPIN (NG/ML)	5.5	6.9	8.1	11.4	13.3	7.3	7.7	4.7	4.6	4.2	4.2
± STANDARD ERROR	0.7	1.6	1.3	1.2	1.1	1.8	1.4	0.7	0.8	0.9	1.2
TESTOSTERONE (NG/ML)	4.2	5.3	8.4	10.9	14.1	17.5	19.8	21.0	19.5	16.2	12.3
± STANDARD ERROR	0.2	0.3	0.2	1.6	0.6	0.9	0.8	1.8	1.2	2.0	1.4

TABLE 3.11. (SERIES 1 EXPERIMENTS) LEVELS OF GONADOTROPIN AND TESTOSTERONE IN SERUM OF MALE RAINBOW TROUT UNDER A NORMAL SEASONAL PHOTOPERIOD COMPRESSED INTO 6 MONTHS (n = 6).

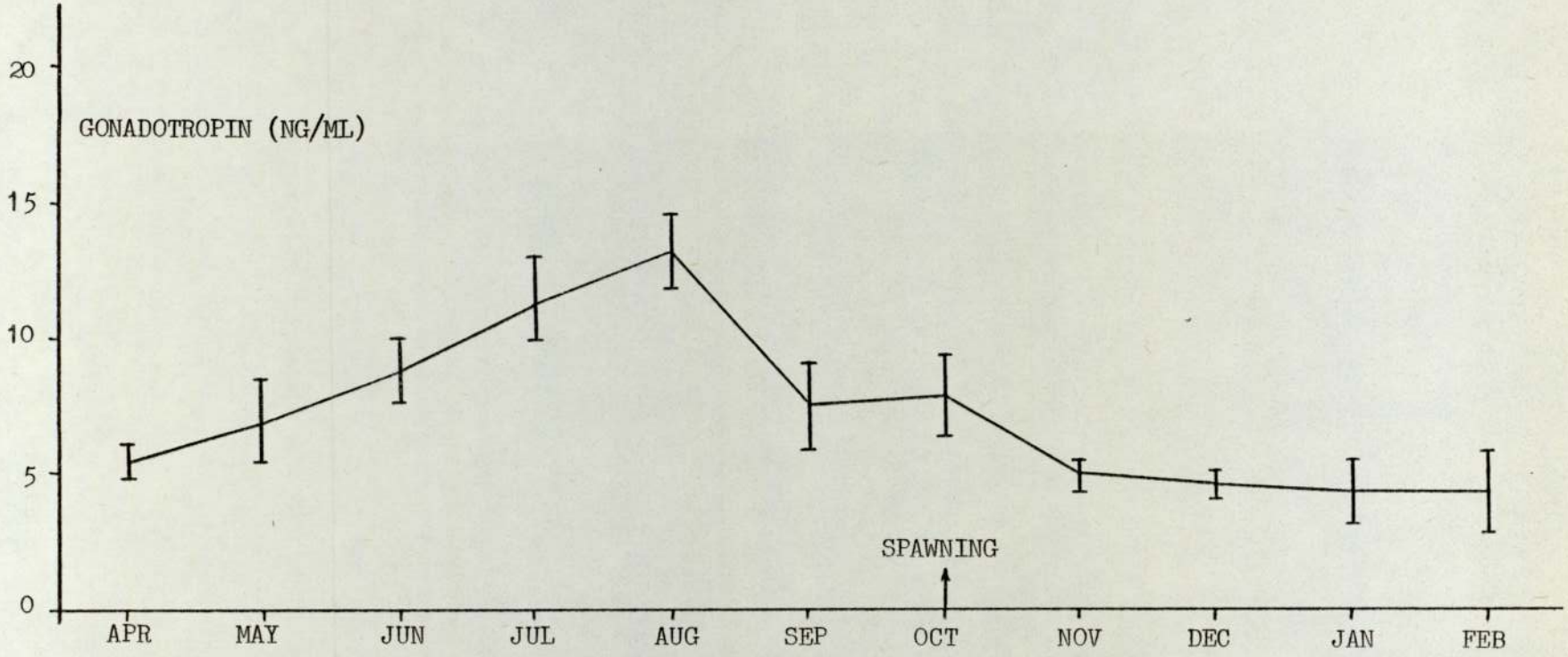


FIG. 3.24. (SERIES 1 EXPERIMENTS) CHANGES IN LEVELS OF GONADOTROPIN IN SERUM OF MALE RAINBOW TROUT UNDER A NORMAL SEASONAL PHOTOPERIOD CYCLE COMPRESSED INTO 6 MONTHS. (VERTICAL BARS = ± STANDARD ERROR OF THE MEAN).

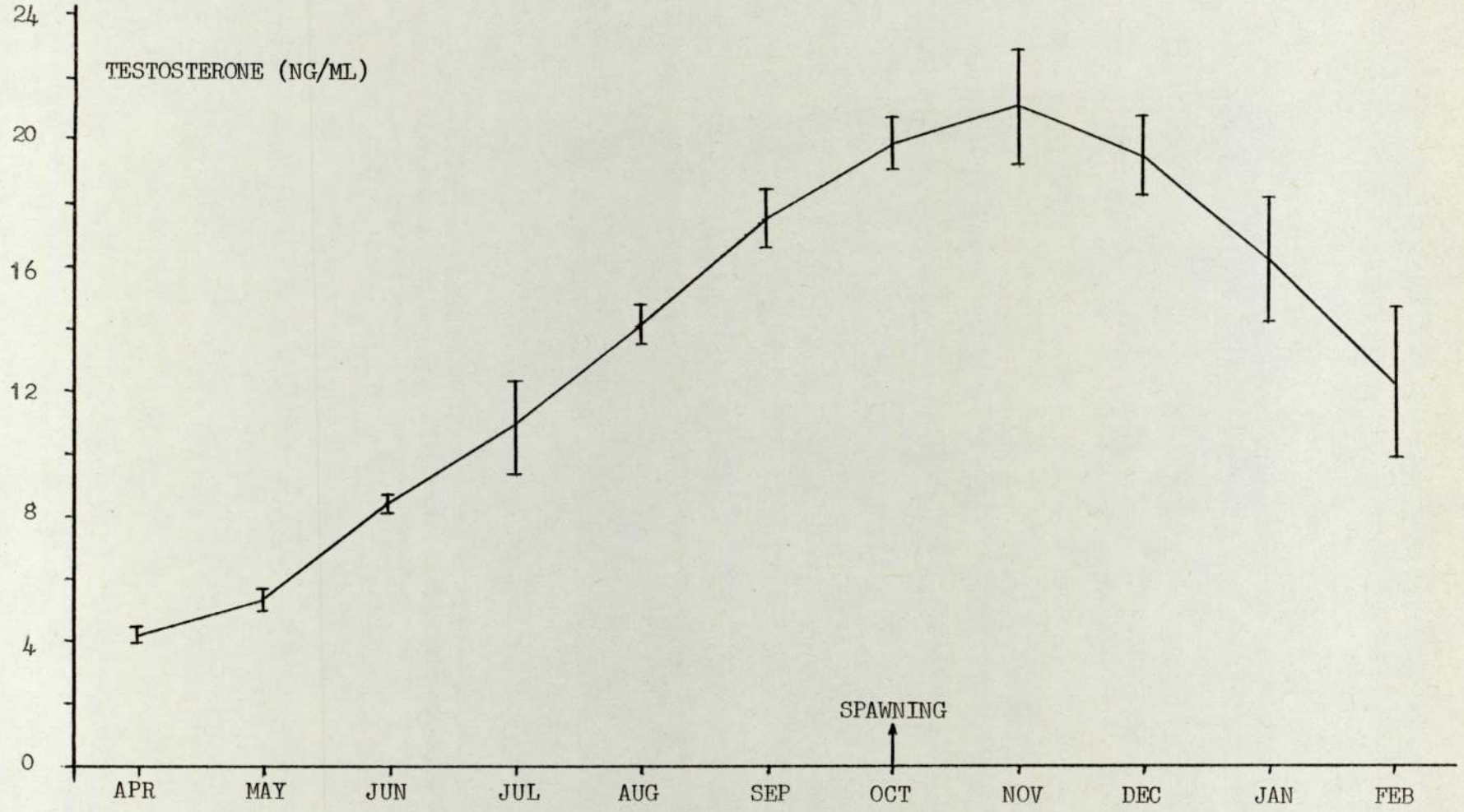


FIG. 3.25. (SERIES 1 EXPERIMENTS) CHANGES IN LEVELS OF TESTOSTERONE IN SERUM OF MALE RAINBOW TROUT UNDER A NORMAL SEASONAL PHOTOPERIOD CYCLE COMPRESSED INTO 6 MONTHS. (VERTICAL BARS = ± STANDARD ERROR OF THE MEAN).

Constant 12 hour light / 12 hour dark regime. (see Table 3.12): Spermiation in this group started in December, at approximately the same time as males from the control photoperiod regime and the outside ponds. Serum levels of gonadotropin increased gradually to a maximum level of 10.0 $\pm$ 0.7ng/ml in August, (Fig. 3.26). Levels in August were significantly different from those in November ($P<0.05$). Testosterone also gradually increased to a peak level of 20.2 $\pm$ 1.3ng/ml in December (Fig. 3.27). Levels in December were significantly different from those in April ($P<0.001$).

	APR	MAY	JUN	JUL	AUG	SEP	OCT	NOV	DEC	JAN	FEB
GONADOTROPIN (NG/ML)	5.9	6.0	6.0	7.4	10.0	8.2	6.7	4.6	6.7	5.7	5.1
± STANDARD ERROR	0.6	0.8	0.8	0.7	0.7	0.8	0.9	0.9	0.7	0.8	1.3
TESTOSTERONE (NG/ML)	4.2	4.9	7.0	7.8	9.5	14.6	16.3	17.4	20.0	19.9	19.5
± STANDARD ERROR	0.5	0.9	1.0	0.7	0.6	1.5	0.9	0.8	1.3	2.0	3.3

TABLE 3.12. (SERIES 1 EXPERIMENTS) LEVELS OF GONADOTROPIN AND TESTOSTERONE IN SERUM OF MALE RAINBOW TROUT UNDER A CONSTANT 12 HOUR LIGHT / 12 HOUR DARK PHOTOPERIOD (n = 6).

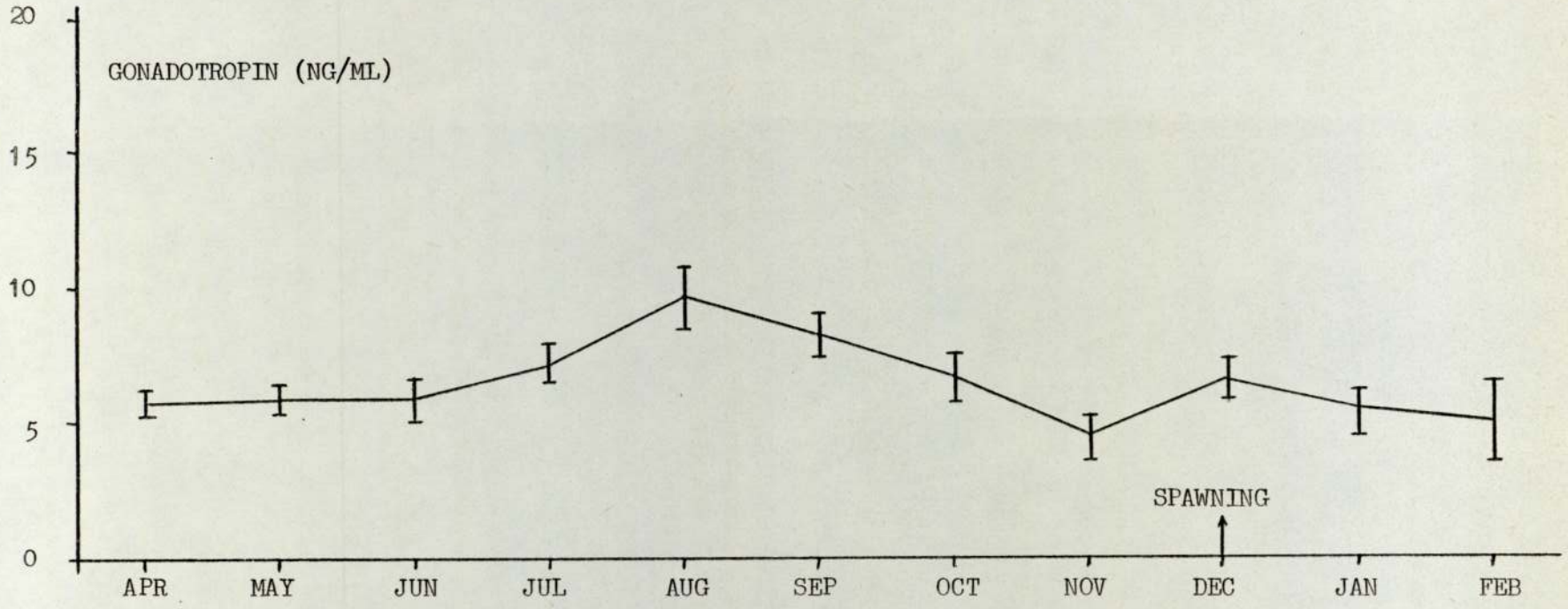


FIG. 3.26. (SERIES 1 EXPERIMENTS) CHANGES IN LEVELS OF GONADOTROPIN IN SERUM OF MALE RAINBOW TROUT UNDER A CONSTANT 12 HOUR LIGHT / 12 HOUR DARK PHOTOPERIOD. (VERTICAL BARS = ± STANDARD ERROR OF THE MEAN).

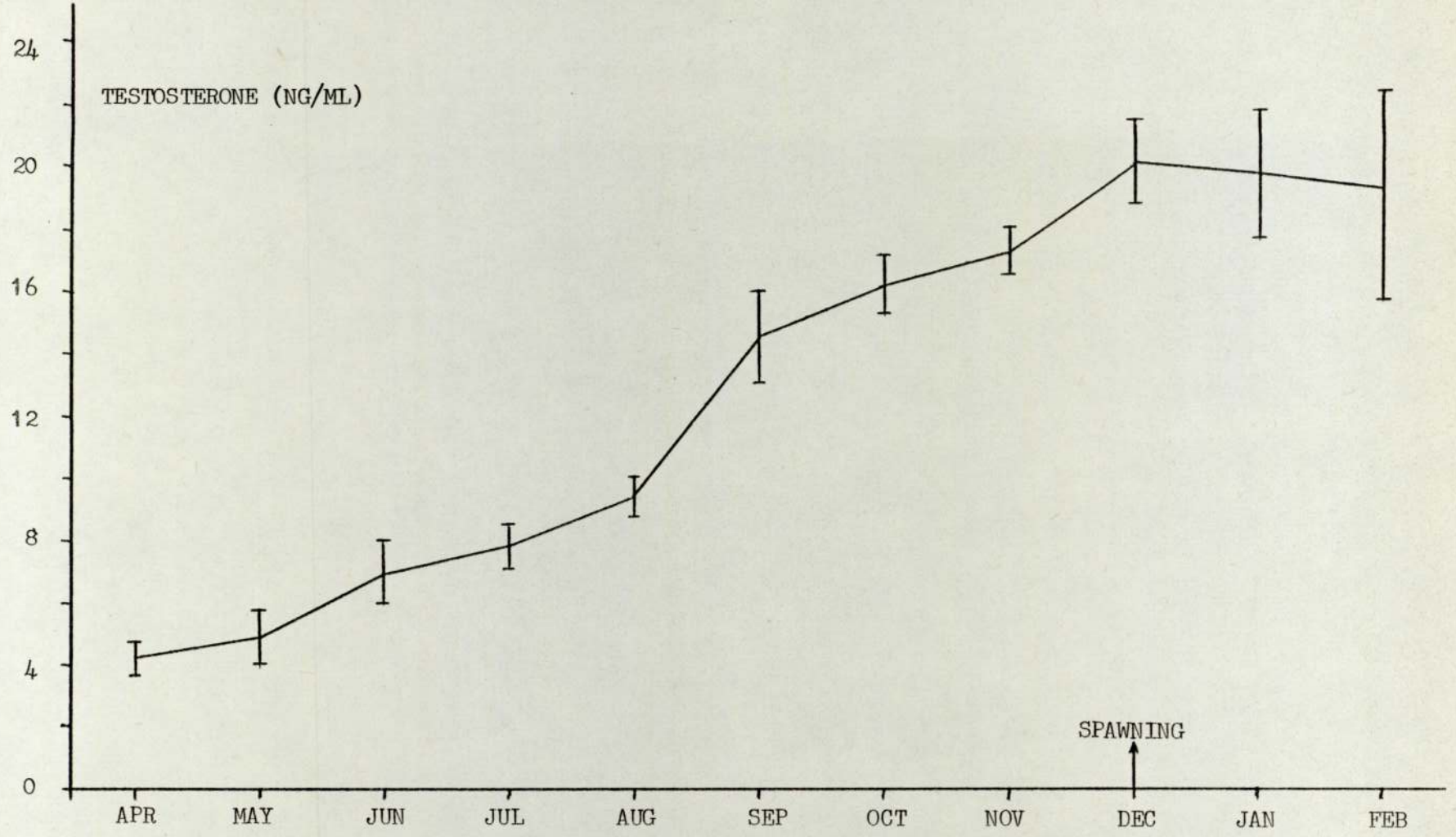


FIG. 3.27. (SERIES 1 EXPERIMENTS) CHANGES IN LEVELS OF TESTOSTERONE IN SERUM OF MALE RAINBOW TROUT UNDER A CONSTANT 12 HOUR LIGHT / 12 HOUR DARK PHOTOPERIOD. (VERTICAL BARS = ± STANDARD ERROR OF THE MEAN).

3.4. Series 2. Experiments.

During this series of experiments, the fish used were again of Danish origin, but in this case they had all spawned once previously (normal time, January-February) before being placed under experimental control (experiments a, b and c). Experiment (d) also contained Danish fish, but these fish had previously been exposed to either a 9 or 6 month photoperiod regime during series 1 experiments and hence had previously spawned earlier than normal. The latter experiment thus studies the effect of two years exposure to shortened light regimes. All experiments in series 2 were commenced in February and completed in February of the following year. By starting this series earlier in the year than series 1, the effect of moving the shortened cycles forward in the year was also investigated.

3.4.1. Materials and Methods: In experiments (a), (b) and (c), three groups of 20 male and female rainbow trout were used, having an average weight of 2.5kg at the start of the experiment. Experiment (d) contained 20 fish from the first series of experiments, having been previously exposed to either 9 or 6 month photoperiod regimes.

The light cycles under investigation during series 2 experiments were:

(a) Normal 1 year seasonal cycle, in which June 21st. was the longest day and December 21st. the shortest.

(b) Normal seasonal cycle compressed into 9 months, in which May 2nd. was the longest day and September 26th. the shortest.

(c) Normal seasonal cycle compressed into 6 months, in which April 4th. was the longest day and June 27th. the shortest.

(d) As experiment (c), but using fish which had already been exposed to shortened cycles during series 1.

These light cycles are shown in more detail in Fig. 3.28.

Blood samples were again taken monthly as in the previous series 1 experiment and the resultant serum assayed for oestradiol 17β, phosphoprotein phosphorus and total calcium using methods described in Chapter 2. The state of sexual maturity was also assessed each month.

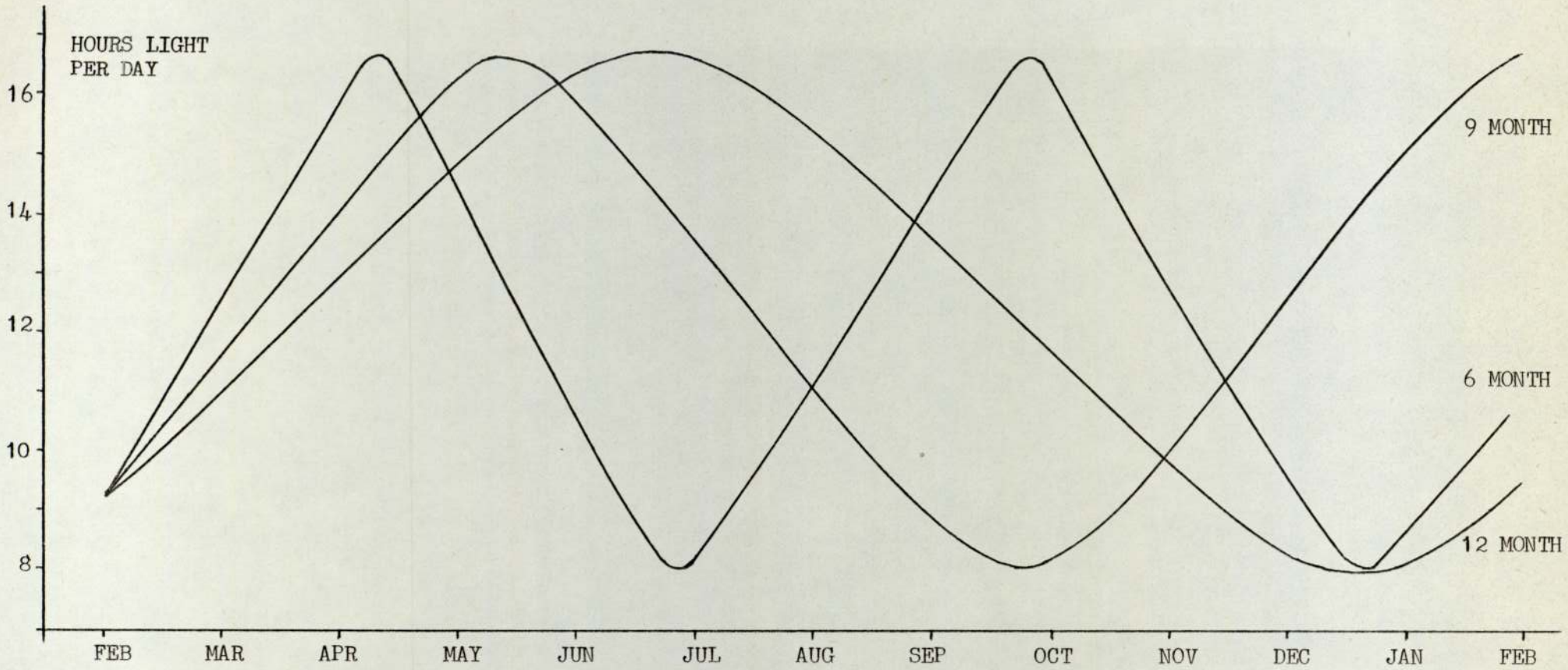


FIG. 3.28. PHOTOPERIOD REGIMES UNDER INVESTIGATION DURING SERIES 2 EXPERIMENTS.

3.4.2. Results.

Female Fish: All fish, irrespective of photoperiod treatment, had similar resting levels of oestradiol 17β (110 ± 25pg/ml) in February at the start of the experiments. At this time the levels of serum phosphoprotein phosphorus and total calcium were 120 ± 24μg/ml and 24 ± 4mg% respectively. By April the levels of serum phosphoprotein phosphorus and total calcium had fallen to basal (61 ± 5μg/ml and 12 ± 0.5mg% respectively). The subsequent changes in each of these parameters under each photoperiod regime are described in detail below.

Normal 1 year seasonal cycle. (see Table 3.13): The fish in this group spawned in January-February, at exactly the same time as broodstock fish maintained outside in open ponds. By August, serum levels of oestradiol 17β had started to increase, reaching a peak of 2556 ± 647pg/ml in November, then returning to basal by January just prior to spawning (Fig. 3.29). Levels in November were significantly different from those in February ($P<0.01$). Serum levels of phosphoprotein phosphorus and total calcium gradually increased from August, reaching maximum levels of 190 ± 50μg/ml and 29 ± 6mg% respectively at the time of spawning in January-February (Figs. 3.30 and 3.31). Levels of both serum phosphoprotein phosphorus and total calcium in January were not significantly different from those in April.

	FEB 18	MAR 17	APR 19	MAY 18	JUN 14	JUL 20	AUG 25	SEP 27	NOV 4	DEC 6	JAN 10	FEB 1
OESTRADIOL 17β (PG/ML)	73	364	158	241	244	222	976	2453	2556	1366	552	97
± STANDARD ERROR	11	93	63	60	109	37	296	531	647	353	103	17
PHOSPHOPROTEIN PHOSPHORUS (μG/ML)	178.5	68.0	67.0	79.3	83.0	134.6	107.6	168.3	151.6	186.0	108.0	190.0
± STANDARD ERROR	41.1	9.7	11.4	21.7	44.4	29.2	15.2	25.2	18.9	34.8	46.7	49.8
TOTAL CALCIUM (MG%)	29.8	15.0	12.9	14.7	17.2	15.6	16.9	22.0	22.4	26.4	29.1	26.1
± STANDARD ERROR	5.0	1.3	1.7	1.7	4.2	3.1	2.1	2.1	2.0	4.6	6.1	5.3

TABLE 3.13. (SERIES 2 EXPERIMENTS) LEVELS OF OESTRADIOL 17β, PHOSPHOPROTEIN PHOSPHORUS AND TOTAL CALCIUM IN SERUM OF FEMALE RAINBOW TROUT UNDER A NORMAL 12 MONTH SEASONAL PHOTOPERIOD REGIME (n = 8).

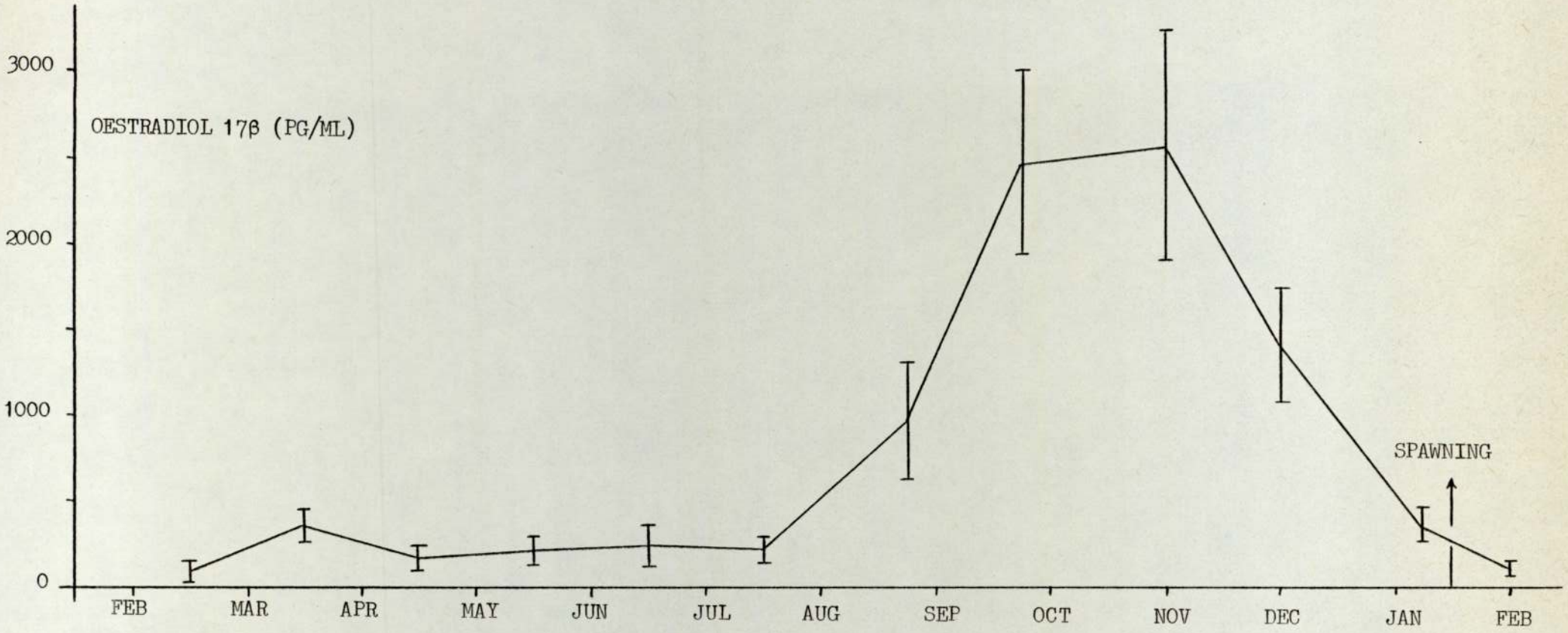


FIG. 3.29. (SERIES 2 EXPERIMENTS) CHANGES IN LEVELS OF SERUM OESTRADIOL 17β IN FEMALE RAINBOW TROUT UNDER A NORMAL 12 MONTH SEASONAL PHOTOPERIOD REGIME. (VERTICAL BARS = ± STANDARD ERROR OF THE MEAN).

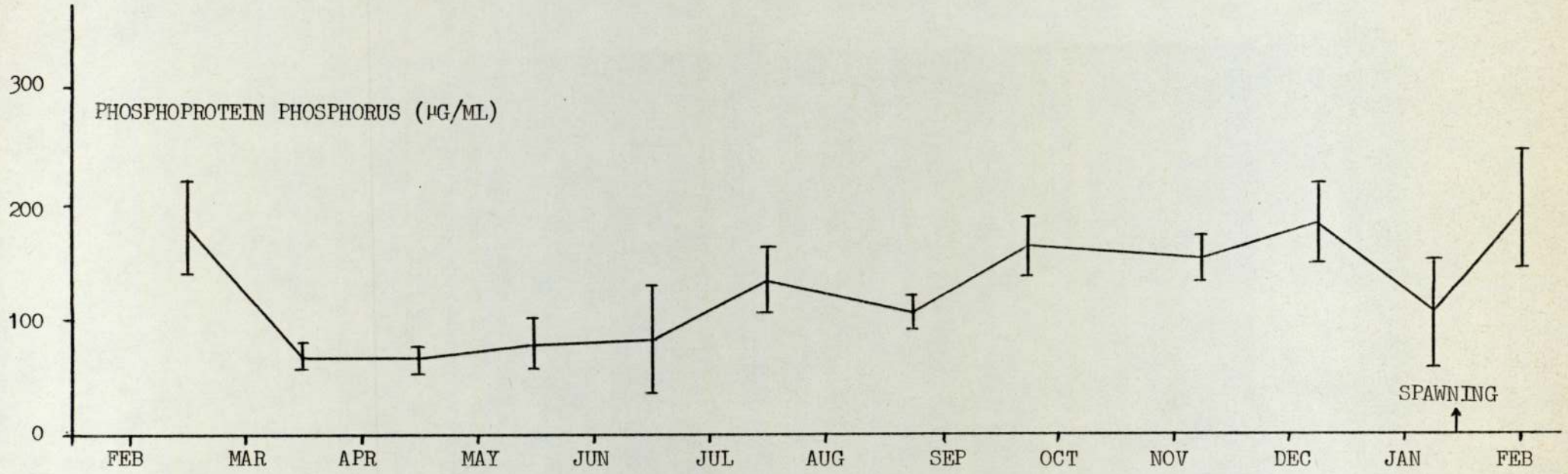


FIG. 3.30. (SERIES 2 EXPERIMENTS) CHANGES IN LEVELS OF SERUM PHOSPHOPROTEIN PHOSPHORUS IN FEMALE RAINBOW TROUT UNDER A NORMAL 12 MONTH SEASONAL PHOTOPERIOD REGIME. (VERTICAL BARS = ± STANDARD ERROR OF THE MEAN).

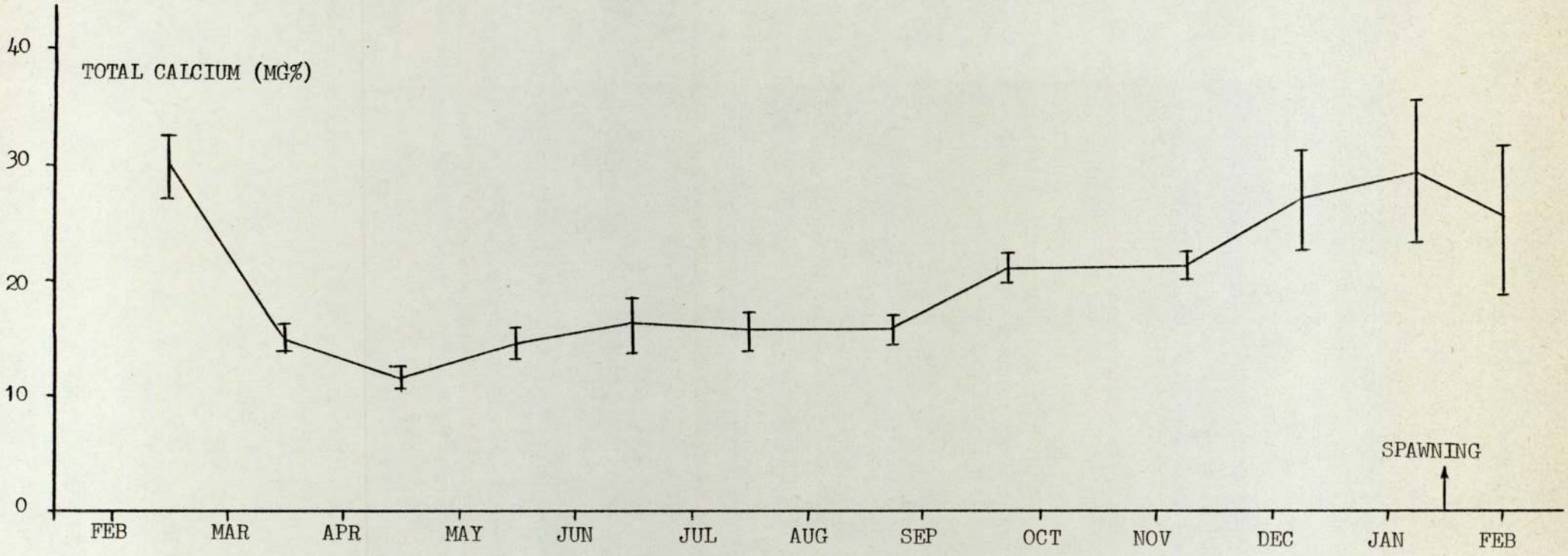


FIG. 3.31. (SERIES 2 EXPERIMENTS) CHANGES IN LEVELS OF TOTAL SERUM CALCIUM IN SERUM OF FEMALE RAINBOW TROUT UNDER A NORMAL 12 MONTH SEASONAL PHOTOPERIOD REGIME. (VERTICAL BARS = ± STANDARD ERROR OF THE MEAN).

Normal seasonal cycle compressed into 9 months. (see Table 3.14): Spawning among this group occurred in November, 8 weeks earlier than fish from either the control photoperiod or the outside ponds. By August, levels of serum oestradiol 17β had started to increase, reaching a maximum of 2975 ± 268pg/ml in late September. Levels in September were significantly different from those in February ($P<0.01$). At spawning time in November, levels had almost returned to basal (Fig. 3.32). Serum levels of phosphoprotein phosphorus and total calcium began to rise by August, reaching peak levels of 496 ± 131μg/ml in November, and 53 ± 8mg% in October respectively, again coinciding with the time of spawning (Figs. 3.33 and 3.34). Levels of serum phosphoprotein phosphorus in November were significantly different from those in April ($P<0.01$) and levels of serum total calcium in October were significantly different from those in April ($P<0.01$).

	FEB 18	MAR 17	APR 19	MAY 18	JUN 14	JUL 20	AUG 25	SEP 27	NOV 4	DEC 6	JAN 10	FEB 1
OESTRADIOL 17β (PG/ML)	108	179	145	276	201	508	2607	2975	790	56	71	85
± STANDARD ERROR	39	60	26	26	74	78	581	808	268	4	11	8
PHOSPHOPROTEIN PHOSPHORUS (μG/ML)	104.0	52.2	58.0	65.0	54.4	107.3	287.2	339.0	496.0	188.4	70.5	69.4
± STANDARD ERROR	22.4	5.3	3.0	12.9	11.5	22.9	38.3	63.1	130.8	55.8	18.3	9.2
TOTAL CALCIUM (MG%)	20.8	12.3	11.5	13.1	14.4	19.7	31.8	52.2	47.6	47.7	12.3	12.6
± STANDARD ERROR	3.3	0.4	0.3	0.8	1.1	2.3	4.1	8.1	10.7	12.9	2.3	1.4

TABLE 3.14. (SERIES 2 EXPERIMENTS) LEVELS OF OESTRADIOL 17β, PHOSPHOPROTEIN PHOSPHORUS AND TOTAL CALCIUM IN SERUM OF FEMALE RAINBOW TROUT UNDER A NORMAL SEASONAL PHOTOPERIOD COMPRESSED INTO 9 MONTHS (n = 7).

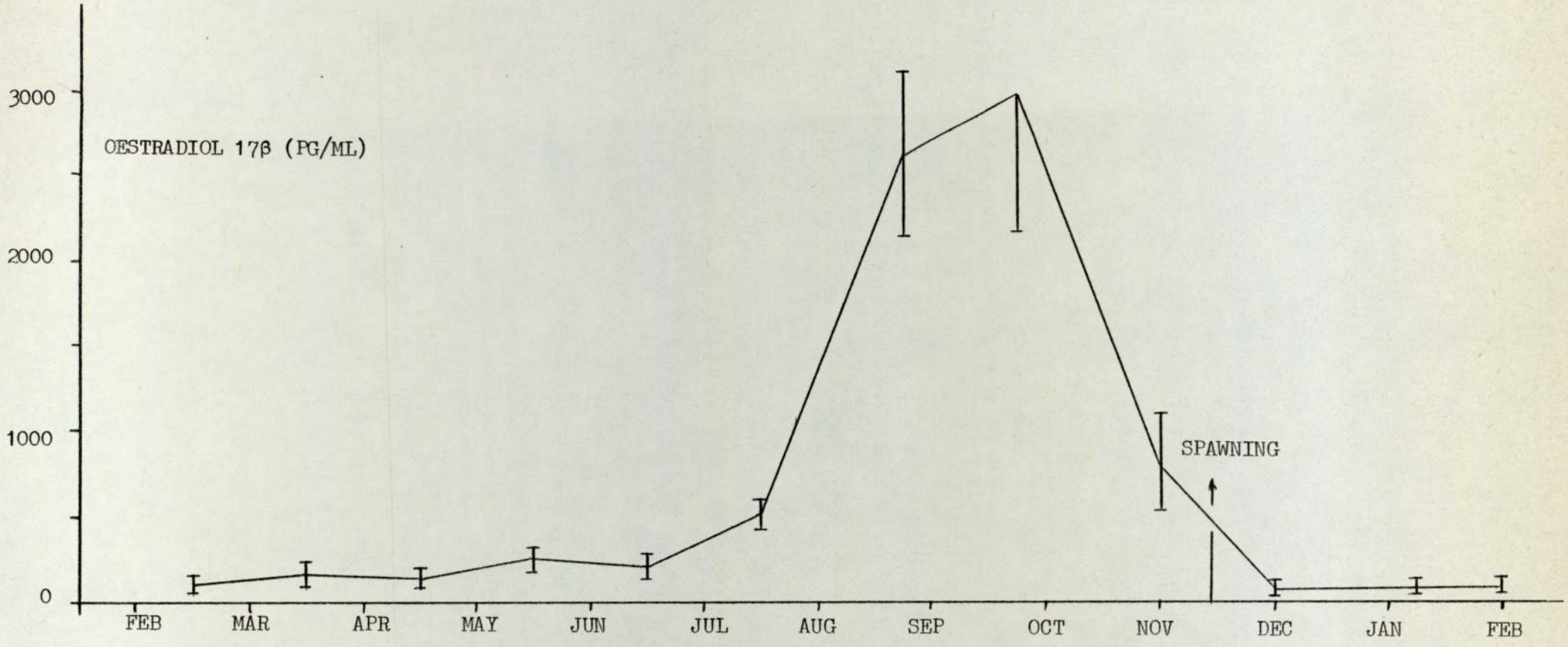


FIG. 3.32. (SERIES 2 EXPERIMENTS) CHANGES IN LEVELS OF OESTRADIOL 17β IN SERUM OF FEMALE RAINBOW TROUT UNDER A NORMAL SEASONAL PHOTOPERIOD COMPRESSED INTO 9 MONTHS. (VERTICAL BARS = ± STANDARD ERROR OF THE MEAN).

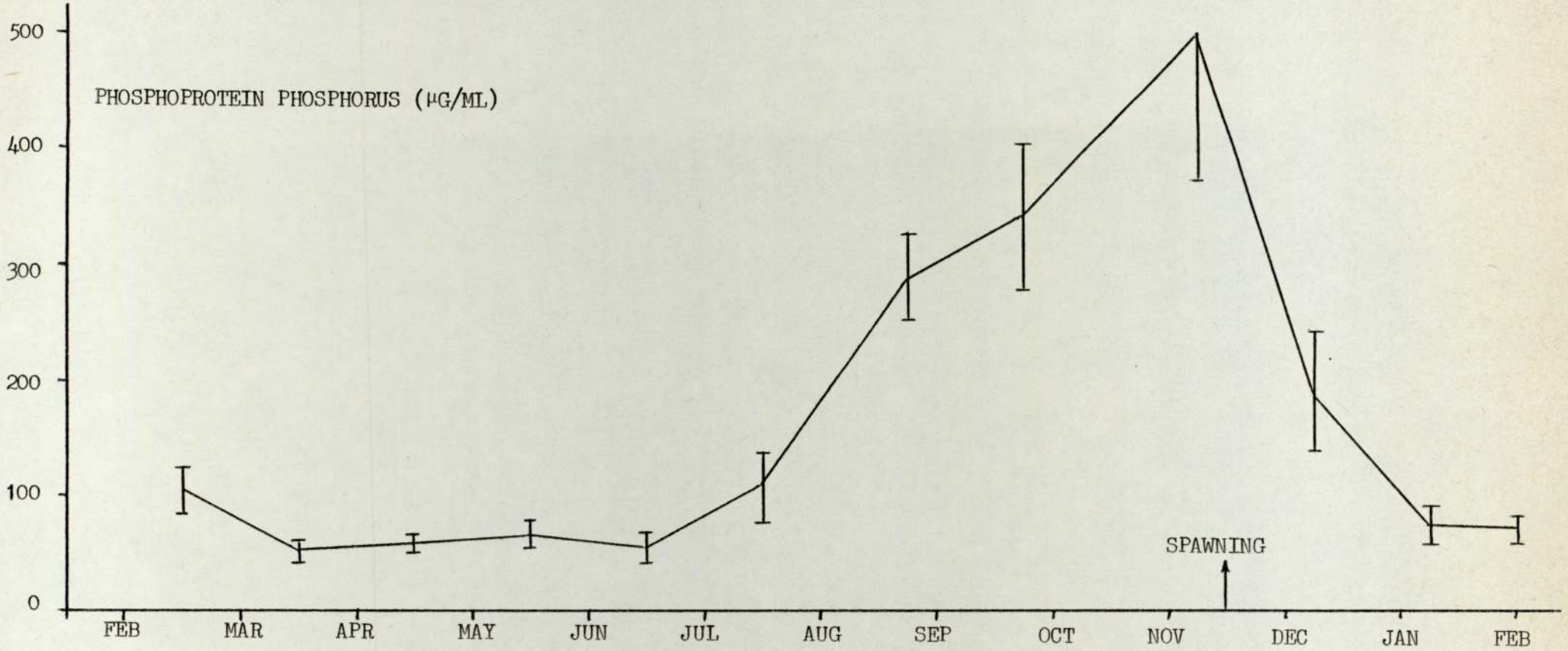


FIG. 3.33. (SERIES 2 EXPERIMENTS) CHANGES IN LEVELS OF PHOSPHOPROTEIN PHOSPHORUS IN SERUM OF FEMALE RAINBOW TROUT UNDER A NORMAL SEASONAL PHOTOPERIOD COMPRESSED INTO 9 MONTHS. (VERTICAL BARS = ± STANDARD ERROR OF THE MEAN).

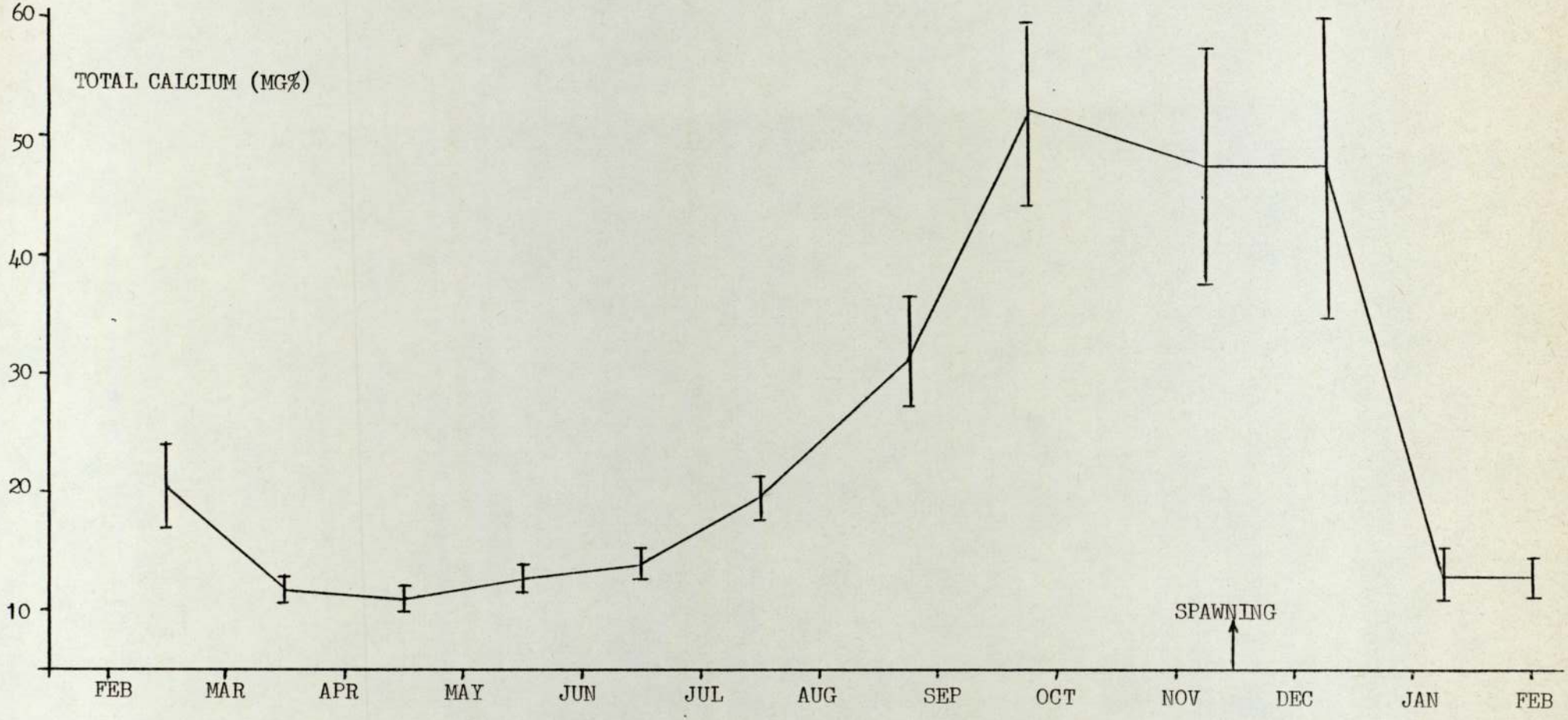


FIG. 3.34. (SERIES 2 EXPERIMENTS) CHANGES IN LEVELS OF TOTAL CALCIUM IN SERUM OF FEMALE RAINBOW TROUT UNDER A NORMAL SEASONAL PHOTOPERIOD COMPRESSED INTO 9 MONTHS. (VERTICAL BARS = ± STANDARD ERROR OF THE MEAN).

Normal seasonal cycle compressed into 6 months. (see Table 3.15): In this group of fish, spawning occurred in September, 16 weeks earlier than in fish from either the control photoperiod or outside ponds. Serum levels of oestradiol 17β were increased in mid-July and reached a peak of 2763 ± 611pg/ml in late August. At the time of spawning in September levels were returning towards basal (Fig. 3.35). Levels in mid-August were significantly different from those in February ($P<0.01$). Levels of phosphoprotein phosphorus and total calcium in the serum gradually increased from mid-June to reach peaks of 713 ± 104μg/ml and 77 ± 10mg% respectively, at spawning time in late September (Figs. 3.36 and 3.37). Levels of both serum phosphoprotein phosphorus and total calcium in late September were significantly different from their respective levels in April ($P<0.001$).

	FEB 18	MAR 17	APR 19	MAY 18	JUN 14	JUL 20	AUG 25	SEP 27	NOV 4	DEC 6	JAN 10	FEB 1
OESTRADIOL 17β (PG/ML)	108	244	154	323	548	448	2763	505	147	301	125	107
± STANDARD ERROR	25	69	43	40	160	48	661	241	36	77	23	11
PHOSPHOPROTEIN PHOSPHORUS (μG/ML)	139.4	54.8	58.8	62.6	117.0	400.8	288.3	713.6	175.6	63.1	187.7	393.1
± STANDARD ERROR	22.9	2.4	2.4	7.9	10.6	45.3	80.4	103.9	46.4	9.6	38.4	71.5
TOTAL CALCIUM (MG%)	24.7	13.2	11.5	13.5	20.0	48.4	66.5	77.3	31.7	17.5	26.1	37.5
± STANDARD ERROR	2.7	0.9	0.2	0.5	0.6	5.0	7.9	10.5	9.3	0.9	4.3	6.1

TABLE 3.15. (SERIES 2 EXPERIMENTS) LEVELS OF OESTRADIOL 17β, PHOSPHOPROTEIN PHOSPHORUS AND TOTAL CALCIUM IN SERUM OF FEMALE RAINBOW TROUT UNDER A NORMAL SEASONAL PHOTOPERIOD COMPRESSED INTO 6 MONTHS (n = 8).

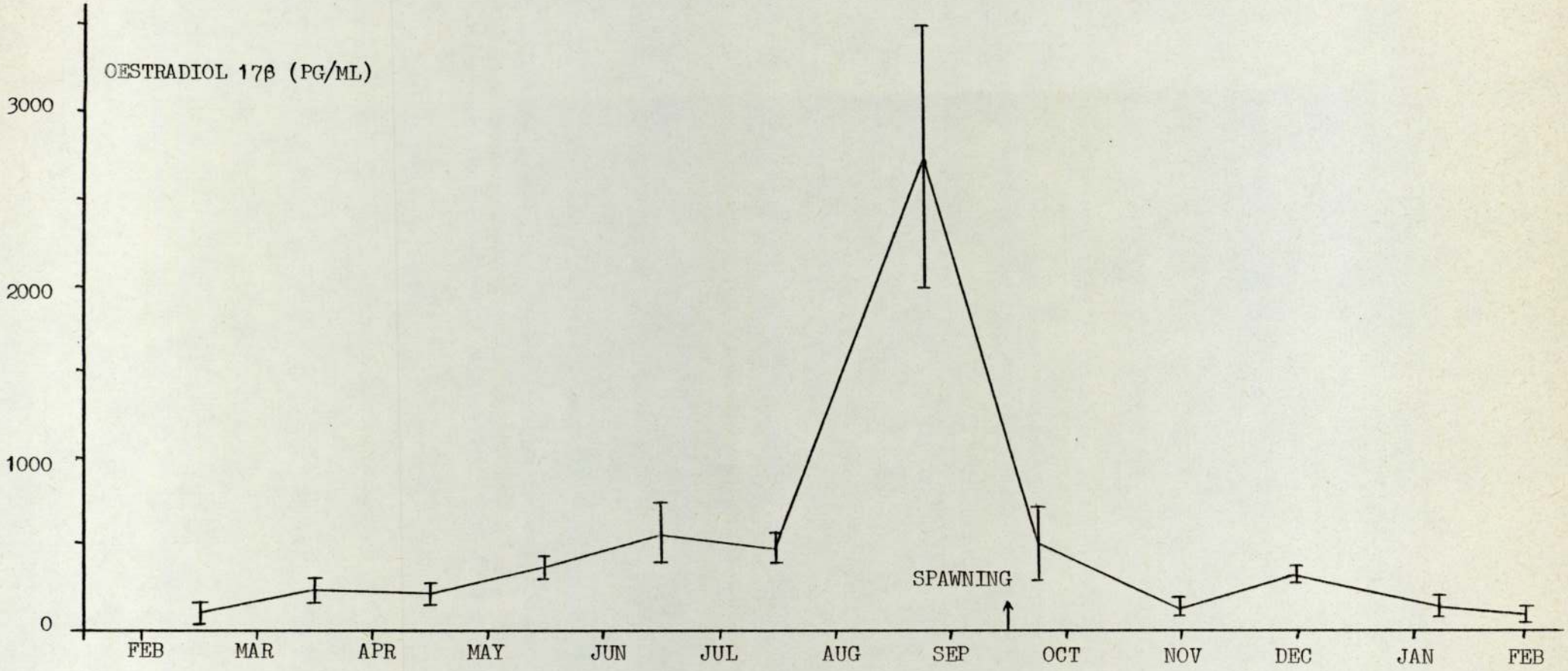


FIG. 3.35. (SERIES 2 EXPERIMENTS) CHANGES IN LEVELS OF OESTRADIOL 17β IN SERUM OF FEMALE RAINBOW TROUT UNDER A NORMAL SEASONAL PHOTOPERIOD COMPRESSED INTO 6 MONTHS. (VERTICAL BARS = ± STANDARD ERROR OF THE MEAN).

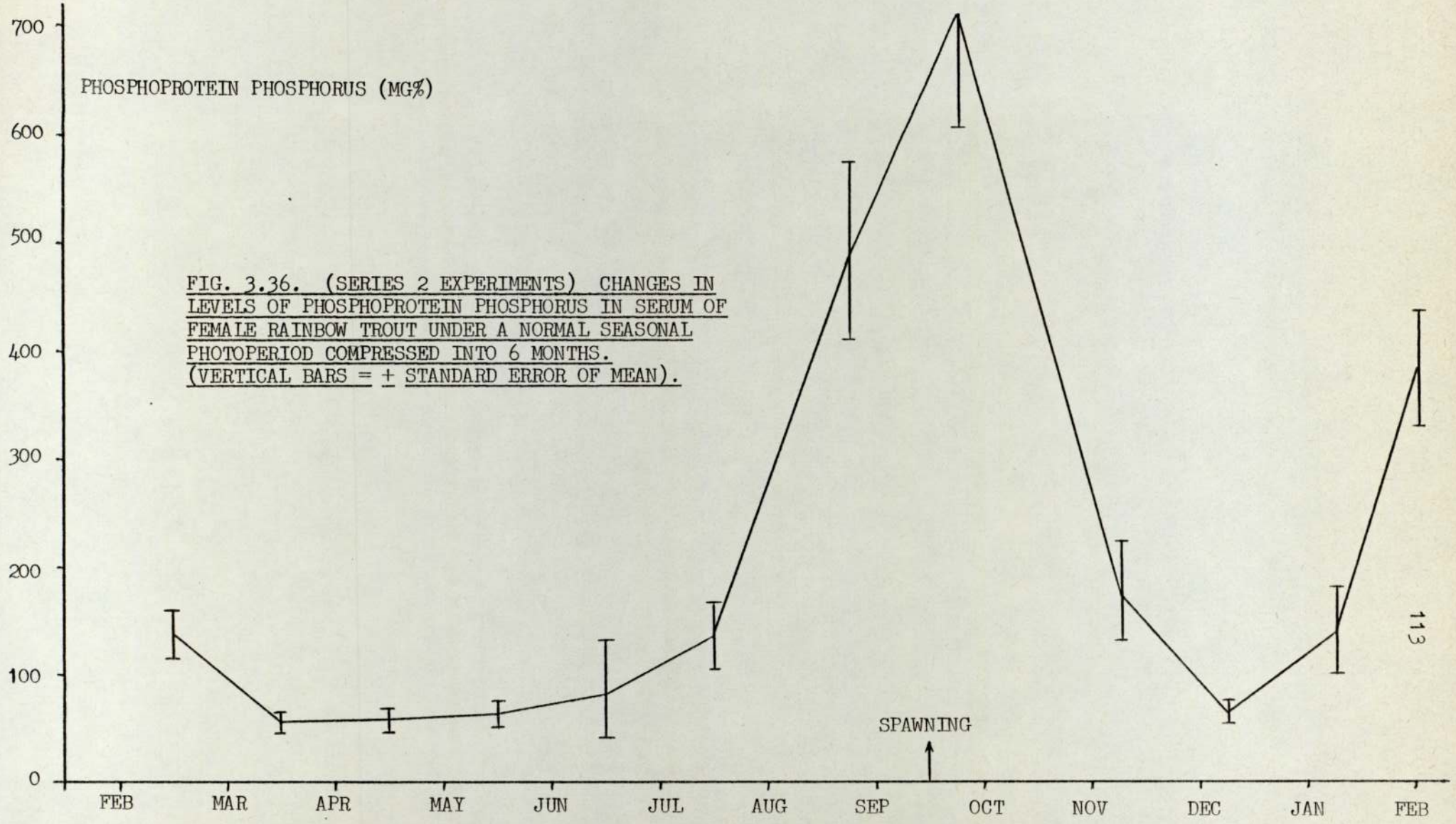


FIG. 3.36. (SERIES 2 EXPERIMENTS) CHANGES IN LEVELS OF PHOSPHOPROTEIN PHOSPHORUS IN SERUM OF FEMALE RAINBOW TROUT UNDER A NORMAL SEASONAL PHOTOPERIOD COMPRESSED INTO 6 MONTHS. (VERTICAL BARS = ± STANDARD ERROR OF MEAN).

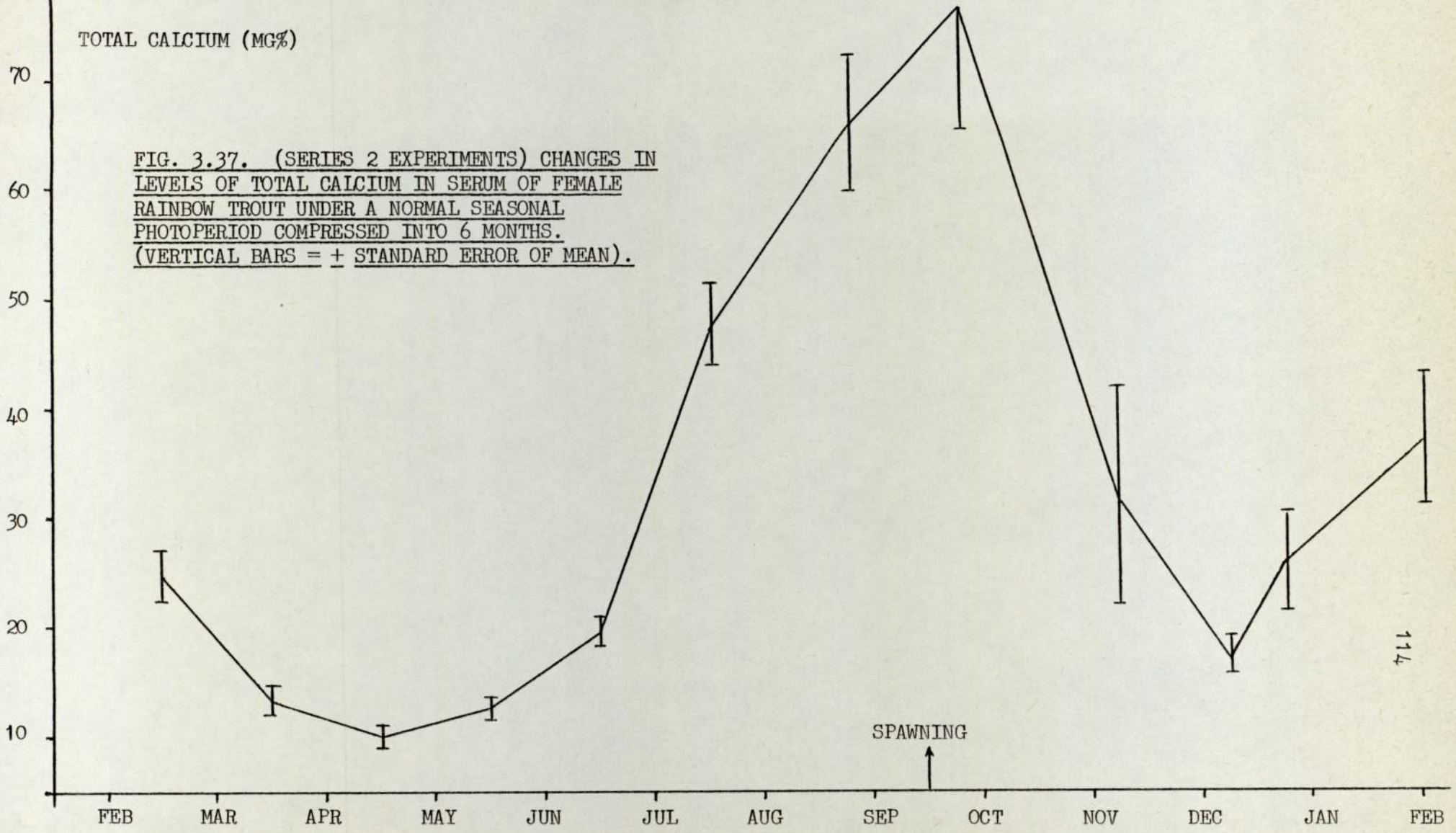


FIG. 3.37. (SERIES 2 EXPERIMENTS) CHANGES IN LEVELS OF TOTAL CALCIUM IN SERUM OF FEMALE RAINBOW TROUT UNDER A NORMAL SEASONAL PHOTOPERIOD COMPRESSED INTO 6 MONTHS. (VERTICAL BARS = ± STANDARD ERROR OF MEAN).

Normal seasonal cycle compressed into 6 months (containing fish which had previously been exposed to either 9 or 6 month photoperiod regimes during series 1 experiments, see Table 3.16): The fish in this group spawned in September, 16 weeks earlier than in fish under the control photoperiod or in the outside ponds. Oestradiol 17β levels in the serum were increased in May and by late August had reached a peak of 3031 ± 509pg/ml. At spawning time in September, levels had almost returned to basal (Fig.3.38). Levels in late August were significantly different from those in February ($P<0.01$). Levels of serum phosphoprotein phosphorus and total calcium increased from mid-June to reach peaks of 562 ± 105μg/ml and 70 ± 17mg% respectively in August-September at around spawning time (Figs. 3.39 and 3.40). Levels of both serum phosphoprotein phosphorus and total calcium in September were significantly different from their respective levels in April ($P<0.01$).

	FEB 18	MAR 17	APR 19	MAY 18	JUN 14	JUL 20	AUG 25	SEP 27	NOV 4	DEC 6	JAN 10	FEB 1
OESTRADIOL 17β, (PG/ML)	147	187	124	364	796	483	3031	413	147	301	125	107
± STANDARD ERROR	22	53	34	108	258	77	509	65	36	77	23	11
PHOSPHOPROTEIN PHOSPHORUS (μG/ML)	60.3	51.8	61.2	66.2	135.4	478.3	537.0	562.5	261.5	63.1	187.7	393.1
± STANDARD ERROR	12.1	2.2	2.4	12.1	28.4	98.7	135.9	104.7	54.0	9.6	38.4	71.5
TOTAL CALCIUM (MG%)	18.4	13.3	11.8	13.9	23.3	54.0	69.6	61.6	19.4	17.5	26.1	37.5
± STANDARD ERROR	3.4	0.5	0.3	0.7	3.1	9.3	12.1	8.2	2.5	0.9	4.3	6.1

TABLE 3.16. (SERIES 2 EXPERIMENTS) LEVELS OF OESTRADIOL 17β, PHOSPHOPROTEIN PHOSPHORUS AND TOTAL CALCIUM IN SERUM OF FEMALE RAINBOW TROUT UNDER A NORMAL SEASONAL PHOTOPERIOD COMPRESSED INTO 6 MONTHS, HAVING BEEN PREVIOUSLY EXPOSED TO EITHER A 9 OR 6 MONTH PHOTOPERIOD REGIME DURING SERIES 1 EXPERIMENTS (n = 8).

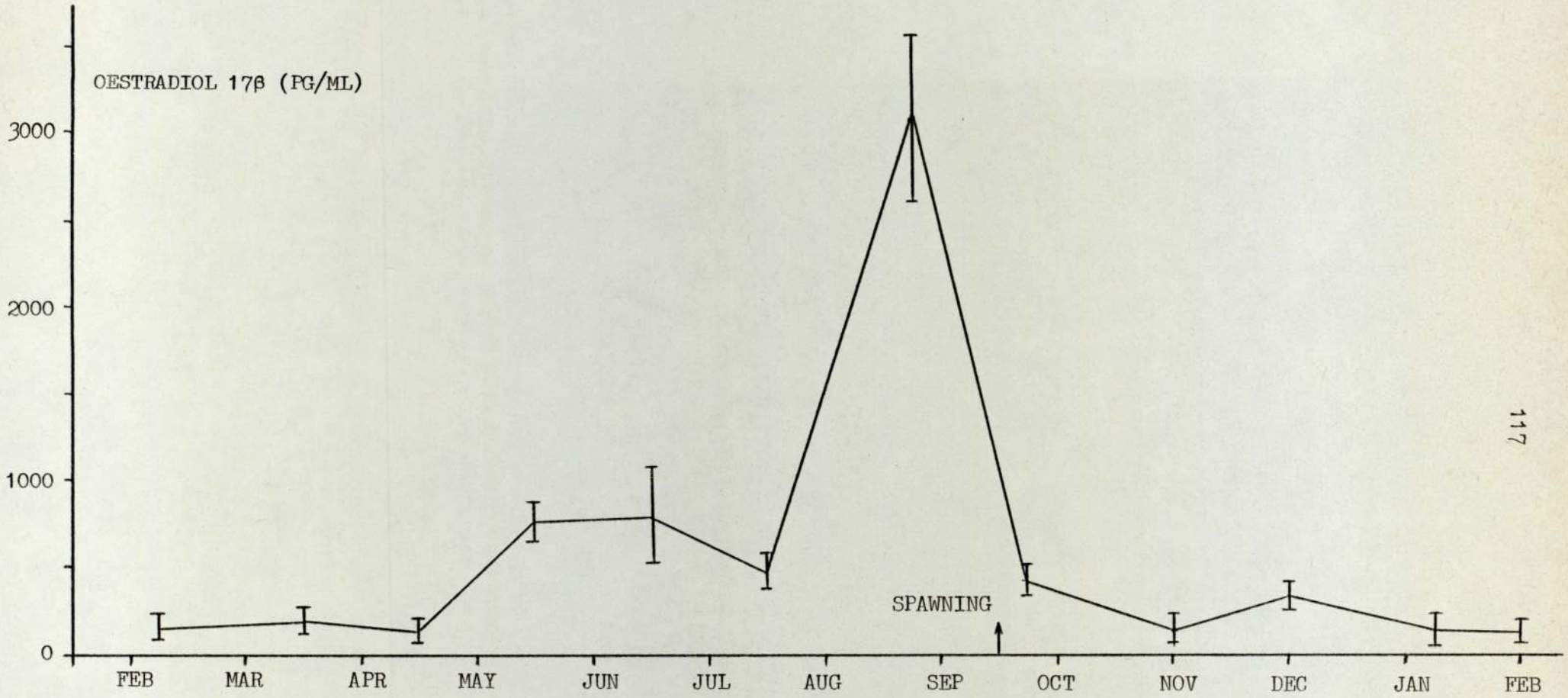


FIG. 3.38. (SERIES 2 EXPERIMENTS) CHANGES IN LEVELS OF OESTRADIOL 17β IN SERUM OF FEMALE RAINBOW TROUT UNDER A NORMAL SEASONAL PHOTOPERIOD COMPRESSED INTO 6 MONTHS, THE FISH HAVING BEEN PREVIOUSLY EXPOSED TO EITHER A 9 OR 6 MONTH PHOTOPERIOD REGIME DURING SERIES 1 EXPERIMENTS. (VERTICAL BARS = ± STANDARD ERROR OF THE MEAN).

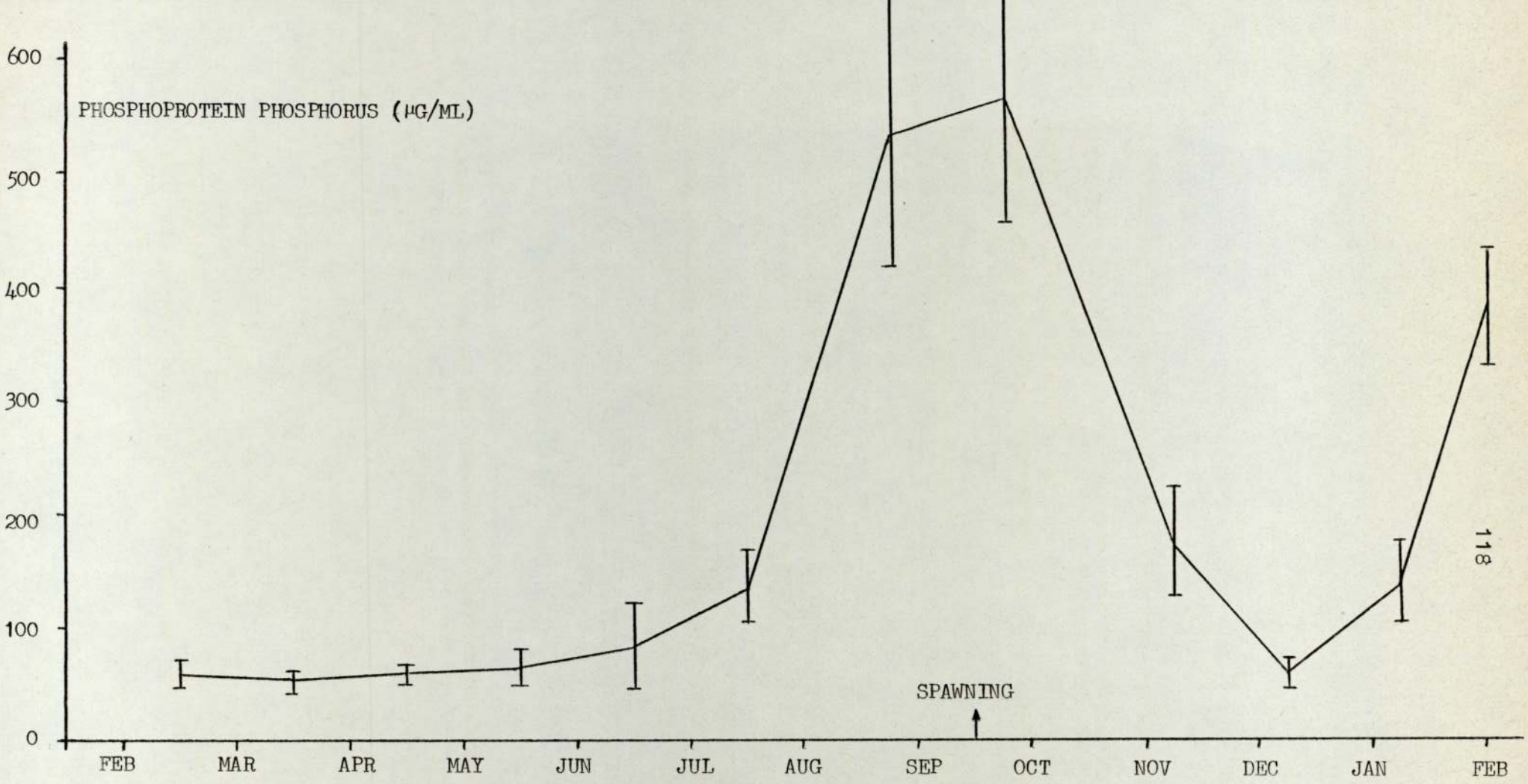


FIG. 3.39. (SERIES 2 EXPERIMENTS)CHANGES IN LEVELS OF PHOSPHOPROTEIN PHOSPHORUS IN SERUM OF FEMALE RAINBOW TROUT UNDER A NORMAL SEASONAL PHOTOPERIOD COMPRESSED INTO 6 MONTHS, THE FISH HAVING BEEN PREVIOUSLY EXPOSED TO EITHER A 9 OR 6 MONTH PHOTOPERIOD REGIME DURING SERIES 1 EXPERIMENTS. (VERTICAL BARS = ± STANDARD ERROR OF THE MEAN).

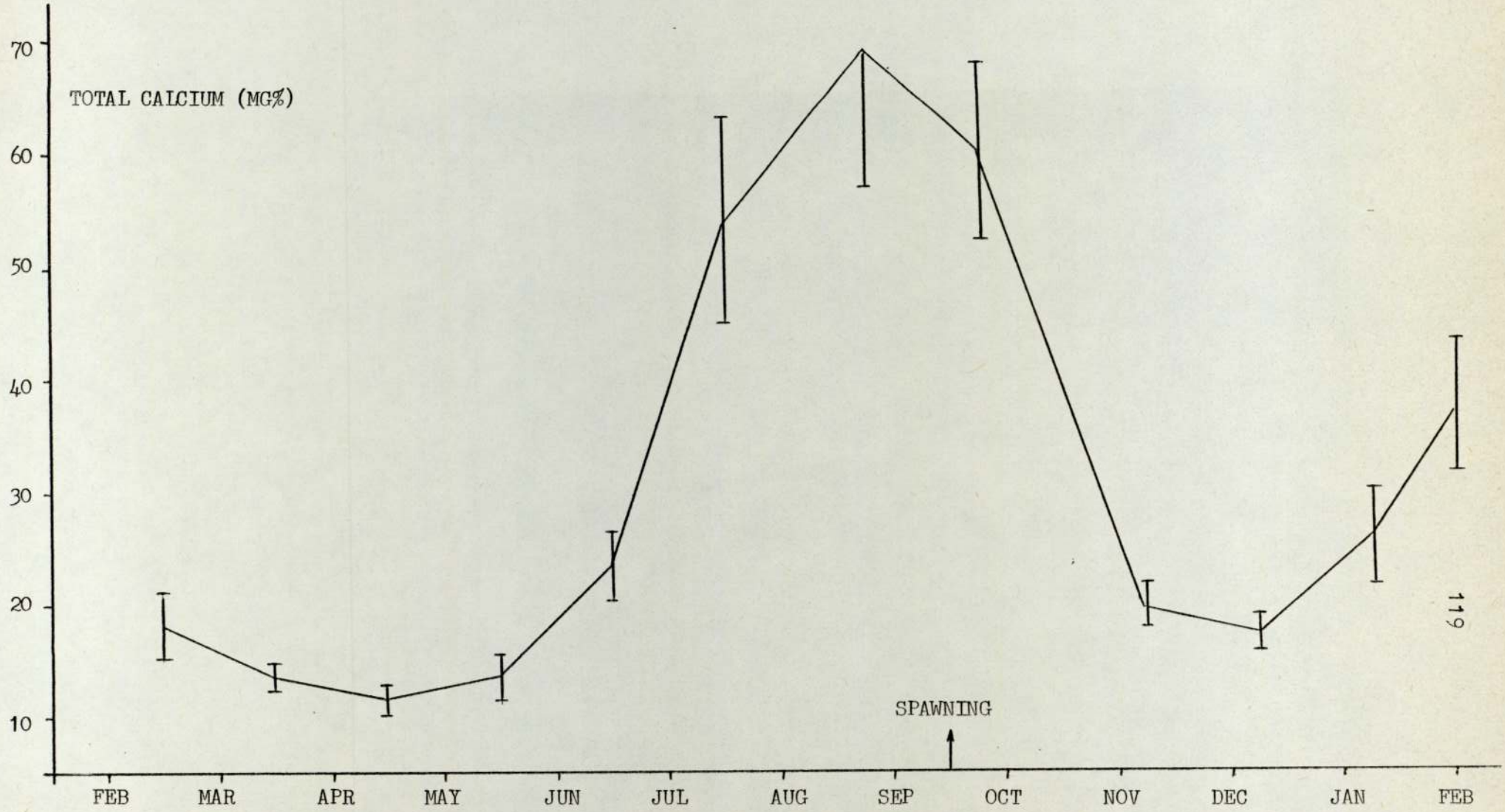


FIG. 3.40.(SERIES 2 EXPERIMENTS) CHANGES IN LEVELS OF TOTAL CALCIUM IN SERUM OF FEMALE RAINBOW TROUT UNDER A NORMAL SEASONAL PHOTOPERIOD COMPRESSED INTO 6 MONTHS, THE FISH HAVING BEEN PREVIOUSLY EXPOSED TO EITHER A 9 OR 6 MONTH PHOTOPERIOD REGIME DURING SERIES 1 EXPERIMENTS. (VERTICAL BARS = ± STANDARD ERROR OF THE MEAN).

Male Fish: At the start of this series of experiments in February, all the male fish under the 12, 9 and 6 month photoperiod regimes had similar levels of serum testosterone (21.5 ± 2.4ng/ml) and were still ripe and running with milt from the previous spawning period. The male fish which had previously been exposed to 1 year of shortened cycles, now under a 6 month regime had levels of 12.3 ± 1.4ng/ml serum testosterone at this time. By April levels had fallen to around 7.5 ± 1.5ng/ml in all groups. Subsequent to this, changes were observed in the time of the commencement of spermiation and in the serum levels of gonadotropin and testosterone but these varied according to photoperiod treatment. The changes under each photoperiod regime are described in detail below.

Normal 1 year seasonal cycle. (see Table 3.17): Spermiation in this group of males commenced in December, at exactly the same time as in similar males maintained in outside open ponds. Levels of serum testosterone started to increase in May, gradually rising to a peak of 27.2 ± 4.2ng/ml in January (Fig. 3.41). Levels in January were significantly different from those in May ($P<0.01$).

	FEB 18	MAR 19	APR 19	MAY 18	JUN 14	JUL 20	AUG 25	SEP 27	NOV 4	DEC 6	JAN 10	FEB 1
TESTOSTERONE (NG/ML)	24.4	17.8	10.4	9.3	13.3	13.5	14.1	17.1	22.9	26.9	27.2	25.2
± STANDARD ERROR	1.8	3.3	2.2	1.6	3.0	2.2	2.5	3.3	4.1	3.7	4.2	2.9

TABLE 3.17. (SERIES 2 EXPERIMENTS) LEVELS OF TESTOSTERONE IN SERUM OF MALE RAINBOW TROUT UNDER A NORMAL SEASONAL PHOTOPERIOD REGIME. (n = 5).

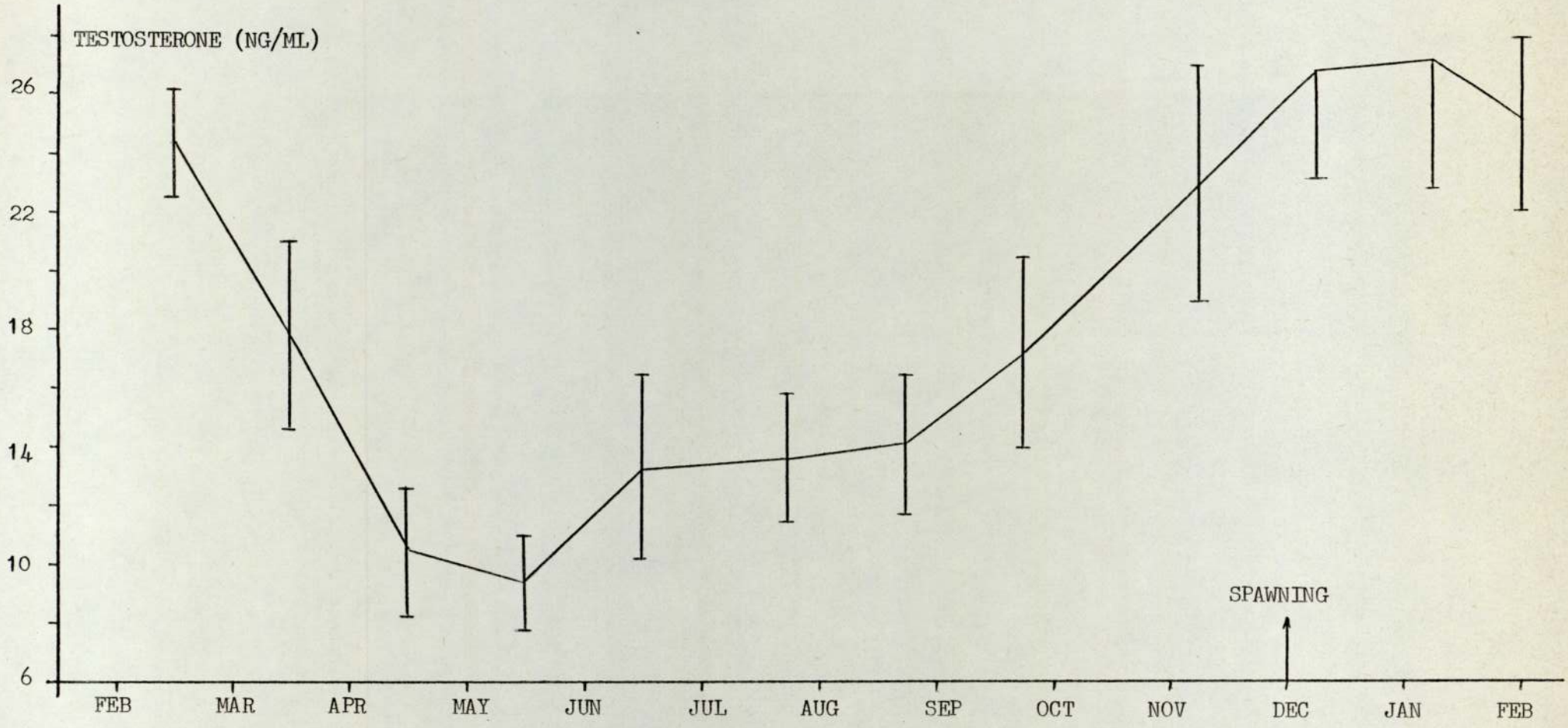


FIG. 3.41. (SERIES 2 EXPERIMENTS) CHANGES IN LEVELS OF TESTOSTERONE IN SERUM OF MALE RAINBOW TROUT UNDER A NORMAL 12 MONTH SEASONAL PHOTOPERIOD REGIME. (VERTICAL BARS = ± STANDARD ERROR OF THE MEAN).

Normal seasonal cycle compressed into 9 months. (see Table 3.18): Spermiation among this group of males commenced in October, 8 weeks earlier than in the control fish or fish from the outside ponds. Serum levels of testosterone gradually increased from April to reach a peak of 31.7 $\pm$ 3.0ng/ml in December, 4 weeks earlier than under the control photoperiod (Fig. 3.42). Levels in December were significantly different from those in April ($P<0.01$).

	FEB 18	MAR 17	APR 18	MAY 18	JUN 14	JUL 20	AUG 25	SEP 27	NOV 4	DEC 6	JAN 10	FEB 1
TESTOSTERONE (NG/ML)	20.8	17.1	8.3	9.7	13.3	19.7	24.0	27.7	29.2	31.7	17.2	14.3
± STANDARD ERROR	3.9	1.3	1.9	1.8	2.5	2.9	4.1	3.3	3.0	3.0	1.9	0.8

TABLE 3.18. (SERIES 2 EXPERIMENTS) LEVELS OF TESTOSTERONE IN SERUM OF MALE RAINBOW TROUT UNDER A NORMAL SEASONAL PHOTOPERIOD COMPRESSED INTO 9 MONTHS. (n = 6).

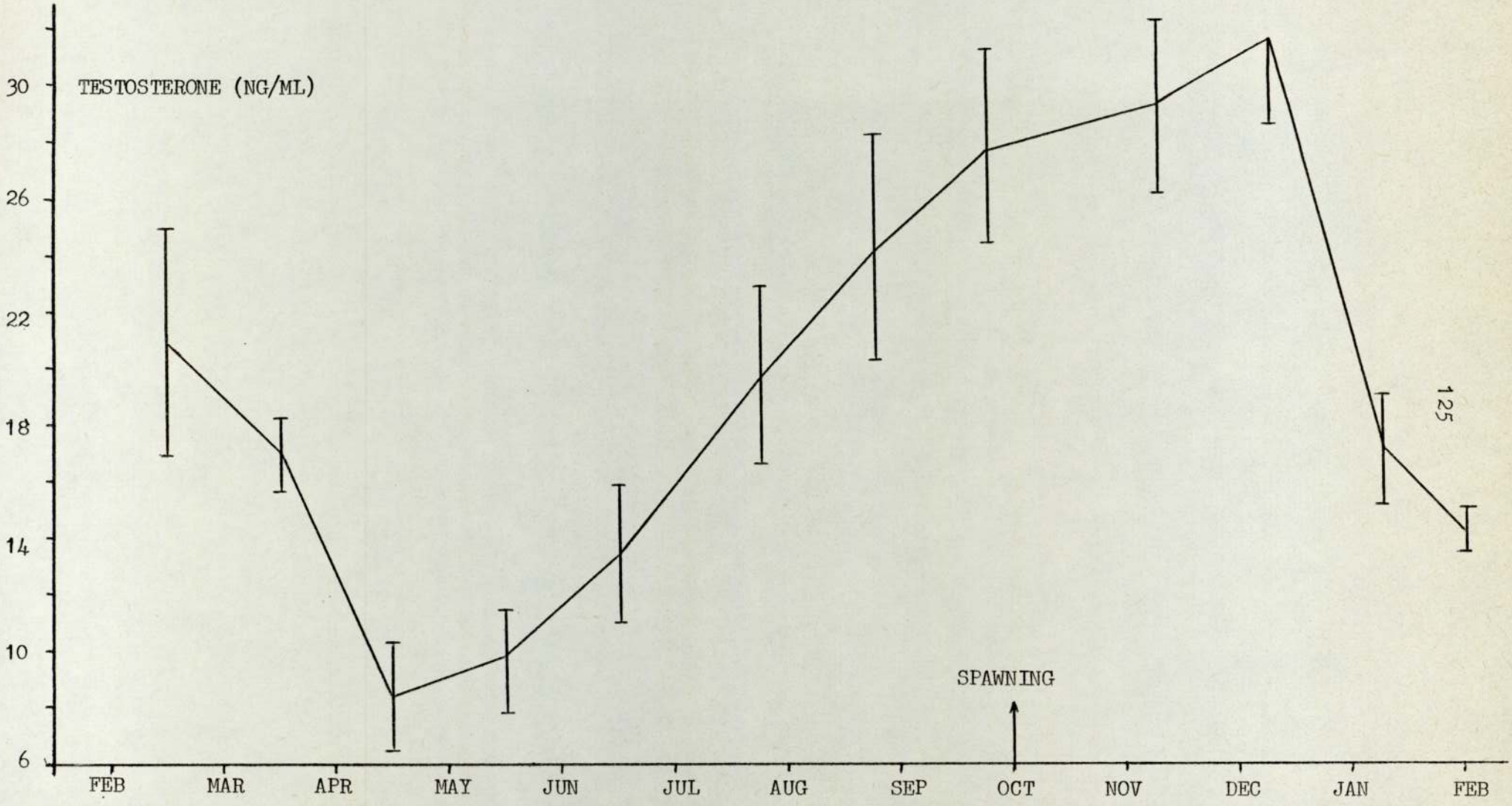


FIG. 3.42. (SERIES 2 EXPERIMENTS) CHANGES IN LEVELS OF TESTOSTERONE IN SERUM OF MALE RAINBOW TROUT UNDER A NORMAL SEASONAL PHOTOPERIOD COMPRESSED INTO 9 MONTHS. (VERTICAL BARS = ± STANDARD ERROR OF THE MEAN).

Normal seasonal cycle compressed into 6 months. (see Table 3.19): In this group, spermiation commenced in August, some 16 weeks earlier than in fish under the control photoperiod or in the outside ponds. Serum levels of testosterone increased rapidly from May to reach a peak level of 20.1 $\pm$ 4.8ng/ml in June-July, approximately 20 weeks earlier than the peak level observed in fish under the control photoperiod (Fig.3.43). Levels in June were significantly different from those in April ($P<0.05$).

	FEB 18	MAR 17	APR 19	MAY 18	JUN 14	JUL 20	AUG 25	SEP 27	NOV 4	DEC 6	JAN 10	FEB 1
TESTOSTERONE (NG/ML)	19.5	8.3	5.9	7.2	20.1	19.8	16.1	12.5	10.3	8.4	8.9	10.3
± STANDARD ERROR	2.0	1.5	1.2	1.1	4.8	8.6	2.8	3.1	3.0	1.9	2.4	1.5

TABLE 3.19. (SERIES 2 EXPERIMENTS) LEVELS OF TESTOSTERONE IN SERUM OF MALE RAINBOW TROUT UNDER A NORMAL SEASONAL PHOTOPERIOD COMPRESSED INTO 6 MONTHS. (n = 6)

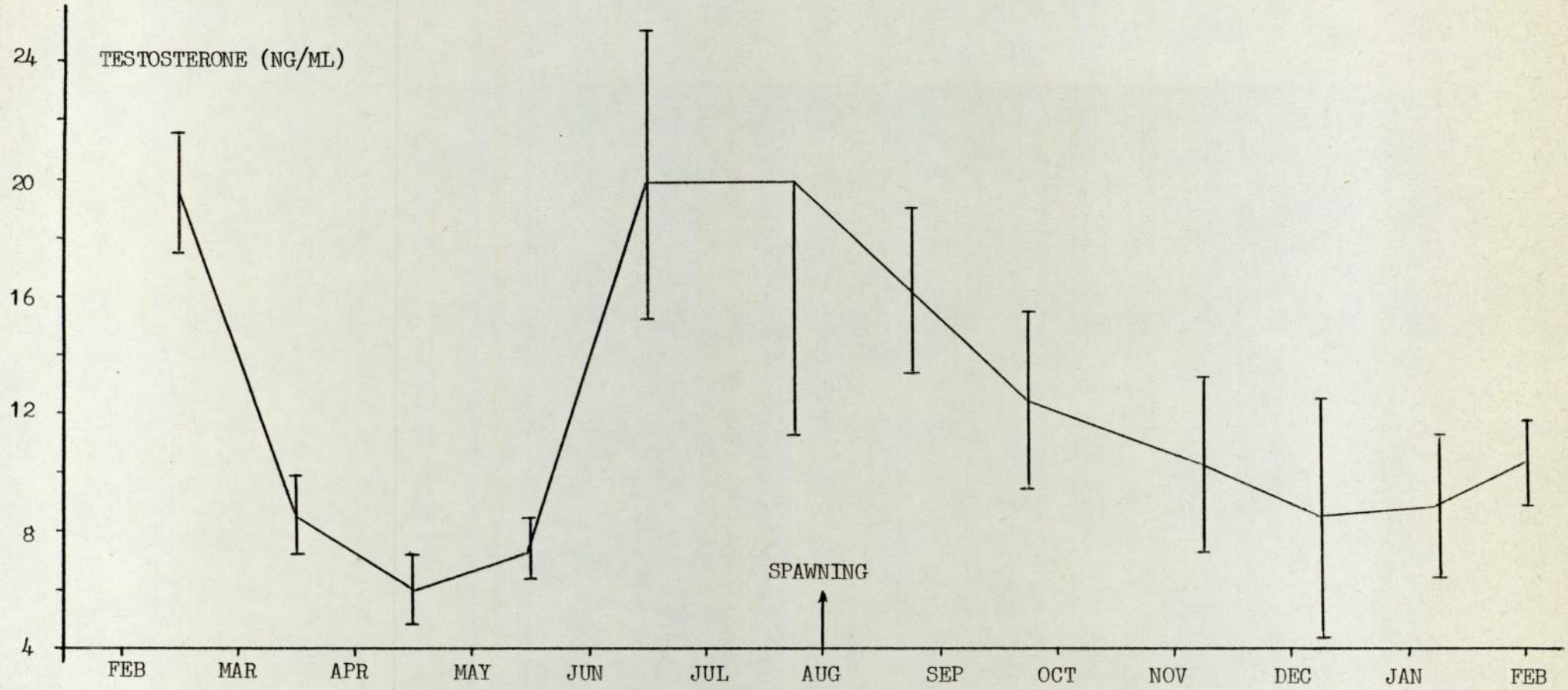


FIG. 3.43. (SERIES 2 EXPERIMENTS) CHANGES IN LEVELS OF TESTOSTERONE IN SERUM OF MALE RAINBOW TROUT UNDER A NORMAL SEASONAL PHOTOPERIOD COMPRESSED INTO 6 MONTHS. (VERTICAL BARS = ± STANDARD ERROR OF THE MEAN).

Normal seasonal photoperiod compressed into 6 months - the fish having been previously exposed to either 9 or 6 month regimes during series 1 experiments (see Table 3.20): Spermiation in this group of males commenced in August, 16 weeks earlier than in fish under the control photoperiod or in outside ponds. Serum levels of testosterone rose rapidly from May to reach a peak level of 24.4 ± 1.6ng/ml in August (Fig. 3.44), 16 weeks earlier than under the normal photoperiod. Levels in August were significantly different from those in April ($P<0.001$).

	FEB 18	MAR 17	APR 19	MAY 18	JUN 14	JUL 20	AUG 25	SEP 27	NOV 4	DEC 6	JAN 10	FEB 1
TESTOSTERONE (NG/ML)	12.3	5.5	5.7	8.9	17.1	22.0	24.4	13.9	8.5	5.0	4.4	4.2
± STANDARD ERROR	1.4	1.0	1.0	1.9	4.0	4.7	1.6	3.1	2.0	0.7	0.4	1.1

TABLE 3.20. (SERIES 2 EXPERIMENTS) LEVELS OF TESTOSTERONE IN SERUM OF MALE RAINBOW TROUT UNDER A NORMAL SEASONAL PHOTOPERIOD COMPRESSED INTO 6 MONTHS HAVING BEEN PREVIOUSLY EXPOSED TO EITHER A 9 OR 6 MONTH PHOTOPERIOD REGIME DURING SERIES 1 EXPERIMENTS. (n = 5)

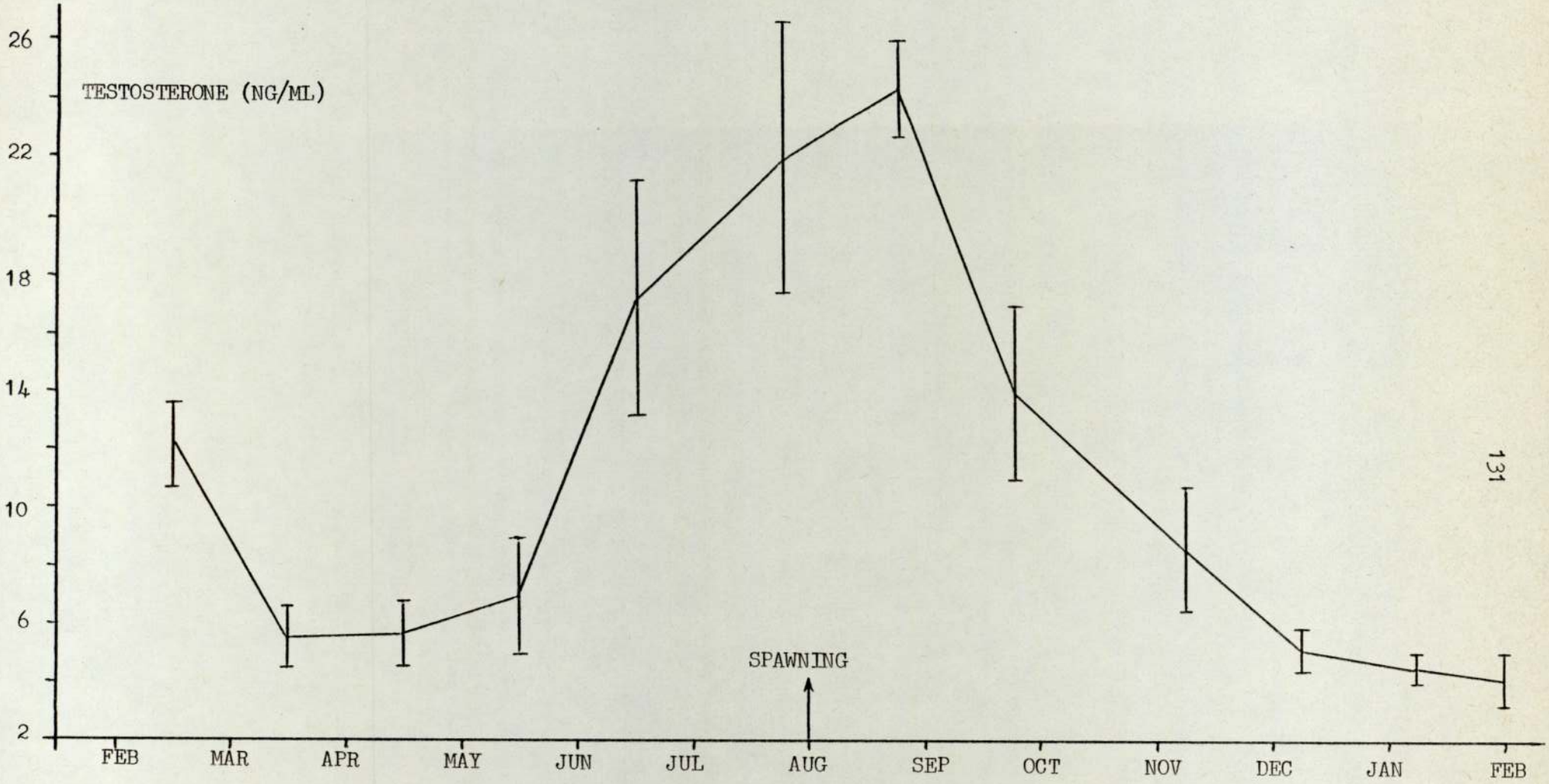


FIG. 3.44. (SERIES 2 EXPERIMENTS) CHANGES IN LEVELS OF TESTOSTERONE IN SERUM OF MALE RAINBOW TROUT UNDER A NORMAL SEASONAL PHOTOPERIOD COMPRESSED INTO 6 MONTHS, THE FISH HAVING BEEN PREVIOUSLY EXPOSED TO EITHER A 9 OR 6 MONTH PHOTOPERIOD REGIME DURING SERIES 1 EXPERIMENTS. (VERTICAL BARS = ± STANDARD ERROR OF THE MEAN).

3.5. Series 3 Experiments.

3.5.1. Materials and Methods: The fish used during this experiment were of Californian origin; these were found to spawn normally in November-December under the conditions at Low Plains. The fish had spawned once prior to exposure to experimental photoperiods. One group of 20 male and female rainbow trout, average weight 2.0kg were placed under a normal seasonal photoperiod cycle compressed into 6 months, in which February 26th. was the longest day and May 28th. the shortest (Fig. 3.45). As the experiment was commenced in November and completed in September of the following year, the effect of applying shortened regimes earlier in the year was further investigated.

At the start of the experiment, and approximately each month thereafter, blood samples were taken under anaesthesia and the fish checked for external signs of maturity using methods already described (Chapter 2.1.) The serum obtained was assayed for oestradiol 17β, total calcium and phosphoprotein phosphorus using the methods already described in Chapter 2.

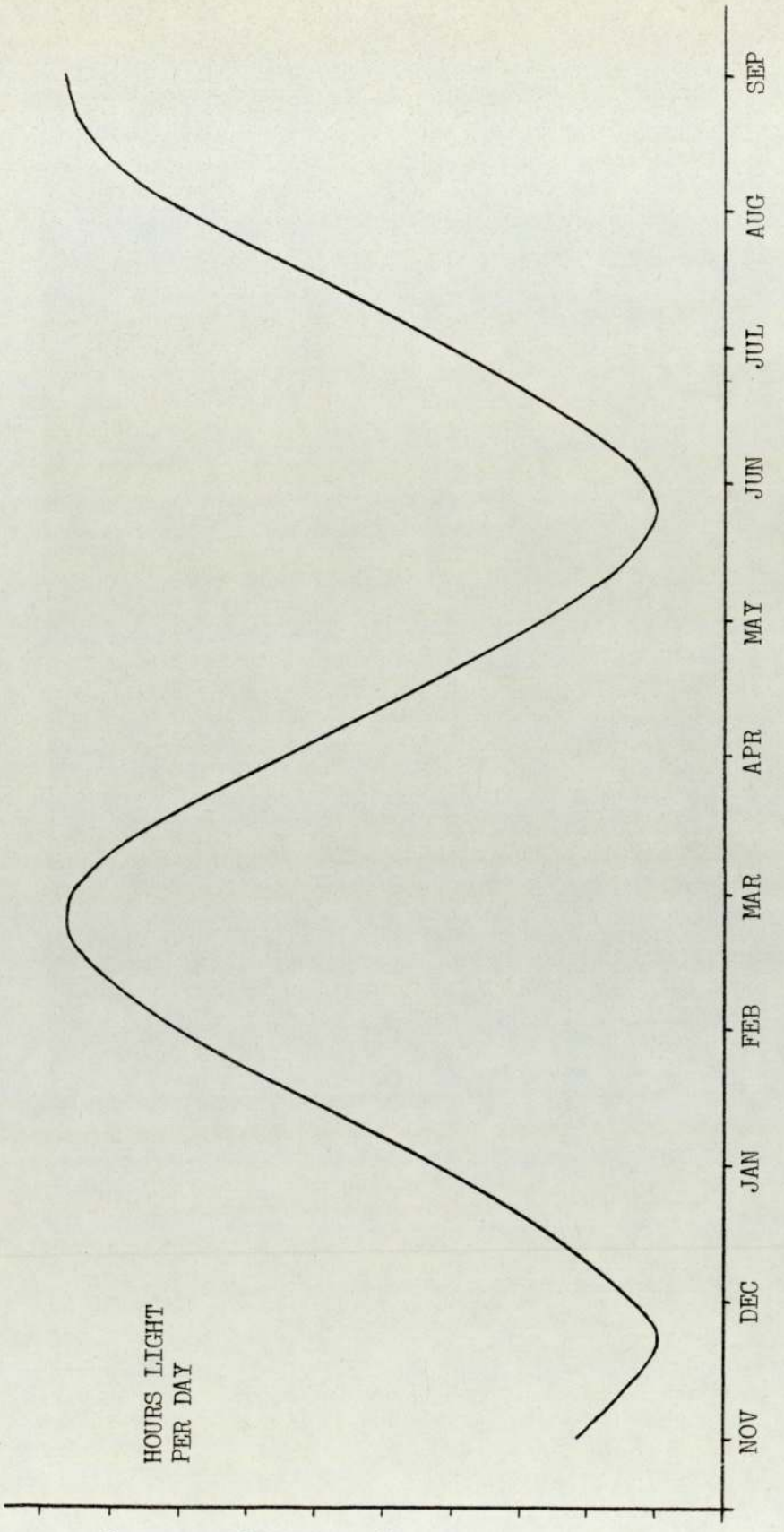


FIG. 3.45. PHOTOPERIOD REGIME UNDER INVESTIGATION DURING SERIES 3 EXPERIMENTS.

3.5.2. Results (see Table 3.21): Spawning in this group of females occurred in late July, 16 weeks earlier than in similar Californian stock maintained outside in open ponds, and almost 24 weeks earlier than in the Danish females maintained in the same pond.
In November, at the start of the experiment, levels of 76 ± 9pg/ml serum oestradiol 17β were observed. In late February levels had risen to 1194 ± 426pg/ml, and continued to rise, reaching a maximum of 7486 ± 1959pg/ml in late May. At the time of spawning in late July, serum levels of oestradiol 17β had returned towards basal, falling to 2478 ± 470pg/ml by September (Fig. 3.46). Levels in May were significantly different from those in November ($P<0.001$).

Serum levels of 261 ± 54µg/ml and 56 ± 10mg% phosphoprotein phosphorus and total calcium were seen in November. By January, levels had returned to basal, and then started to increase in April, reaching a maximum serum level of 794 ± 80µg/ml for phosphoprotein phosphorus and 88 ± 9mg% for total calcium at approximately the time of spawning (Figs. 3.47 and 3.48). Levels of both serum phosphoprotein phosphorus and total calcium in August were significantly different from their respective levels in January ($P<0.001$).

Unfortunately, high mortality was encountered among the male fish during this experiment, and no data is reported here.

	NOV 4	DEC 6	JAN 10	FEB 28	APR 3	MAY 4	MAY 25	JUL 4	AUG 1	SEP 7
OESTRADIOL 17β (PG/ML)	76	58	63	1194	1281	2970	7486	6902	2718	2478
± STANDARD ERROR	9	5	4	426	515	841	1959	1154	791	470
PHOSPHOPROTEIN PHOSPHORUS (μG/ML)	261.5	93.3	47.1	83.6	45.5	94.9	198.7	687.3	794.0	207.3
± STANDARD ERROR	54.0	25.4	2.9	23.3	6.3	9.2	24.2	62.6	79.6	52.4
TOTAL CALCIUM (MG%)	56.3	17.3	13.1	12.8	12.8	16.8	25.9	77.2	88.2	35.6
± STANDARD ERROR	9.7	2.2	1.6	0.4	0.5	1.0	2.4	8.0	8.8	7.6

TABLE 3.21. (SERIES 3 EXPERIMENTS). LEVELS OF OESTRADIOL 17β, PHOSPHOPROTEIN PHOSPHORUS AND TOTAL CALCIUM IN SERUM OF FEMALE RAINBOW TROUT UNDER A NORMAL SEASONAL PHOTOPERIOD COMPRESSED INTO 6 MONTHS. (n = 9).

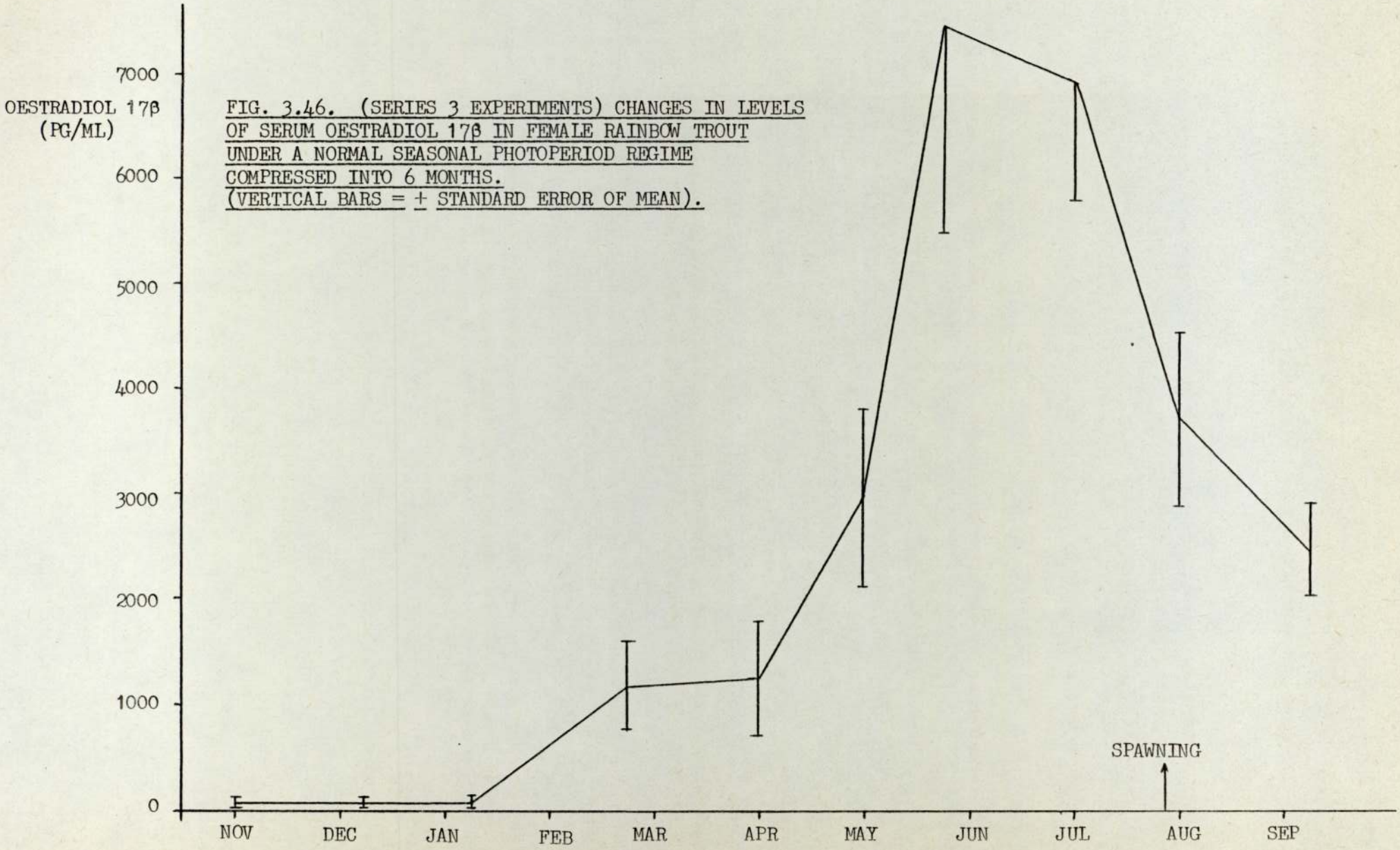


FIG. 3.46. (SERIES 3 EXPERIMENTS) CHANGES IN LEVELS OF SERUM OESTRADIOL 17β IN FEMALE RAINBOW TROUT UNDER A NORMAL SEASONAL PHOTOPERIOD REGIME COMPRESSED INTO 6 MONTHS.
(VERTICAL BARS = ± STANDARD ERROR OF MEAN).

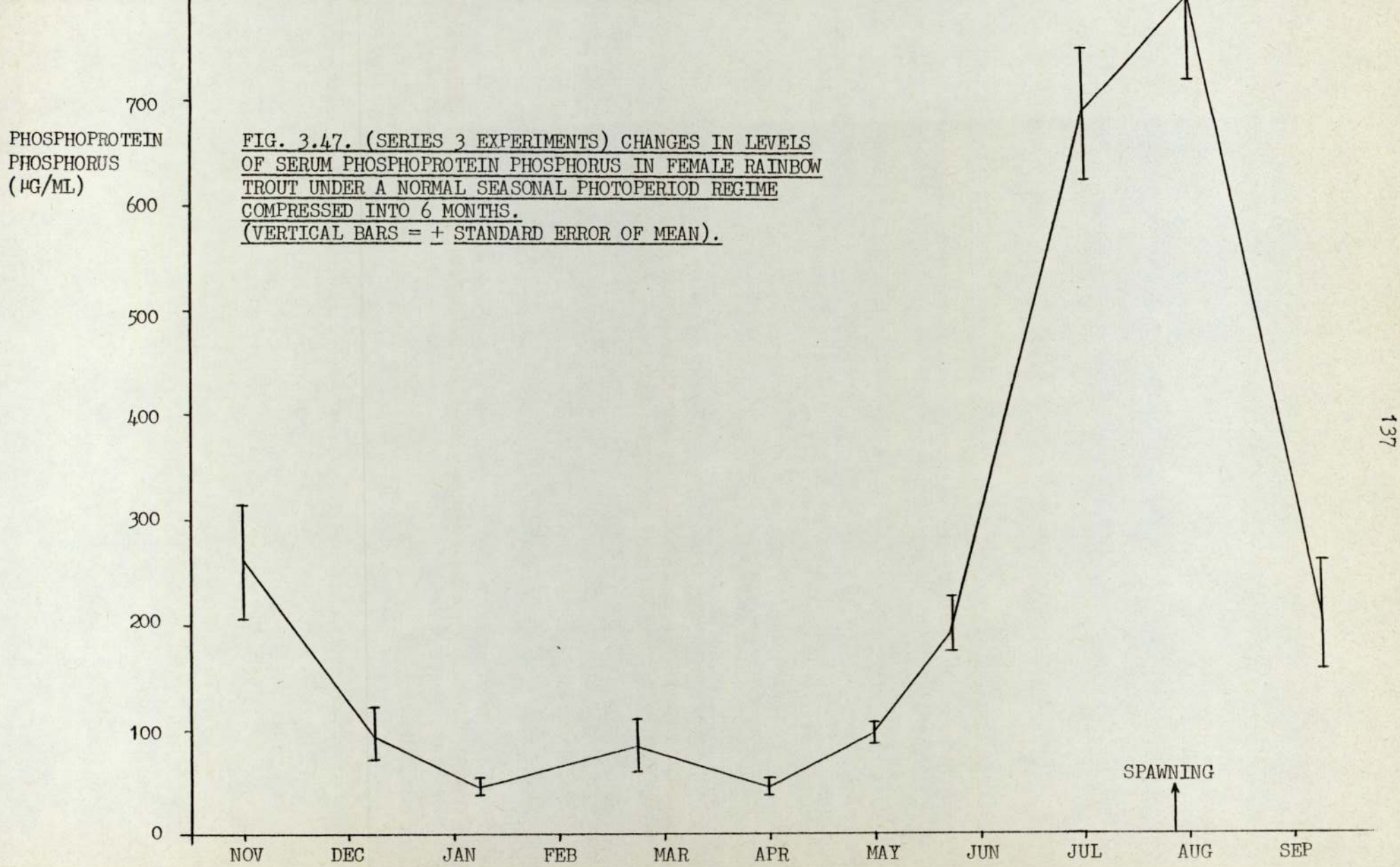


FIG. 3.47. (SERIES 3 EXPERIMENTS) CHANGES IN LEVELS OF SERUM PHOSPHOPROTEIN PHOSPHORUS IN FEMALE RAINBOW TROUT UNDER A NORMAL SEASONAL PHOTOPERIOD REGIME COMPRESSED INTO 6 MONTHS.
(VERTICAL BARS = ± STANDARD ERROR OF MEAN).

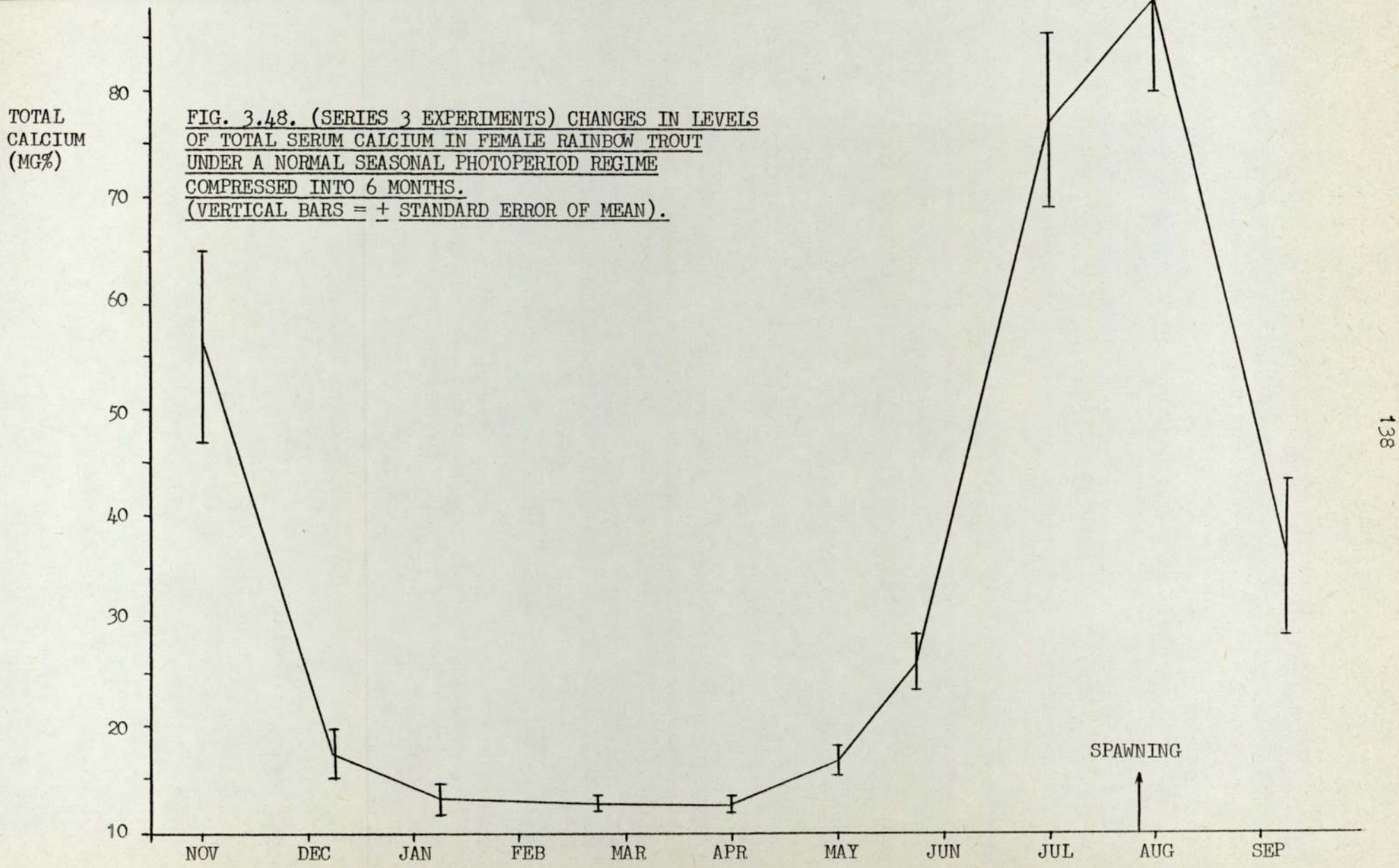


FIG. 3.48. (SERIES 3 EXPERIMENTS) CHANGES IN LEVELS OF TOTAL SERUM CALCIUM IN FEMALE RAINBOW TROUT UNDER A NORMAL SEASONAL PHOTOPERIOD REGIME COMPRESSED INTO 6 MONTHS.
(VERTICAL BARS = ± STANDARD ERROR OF MEAN).

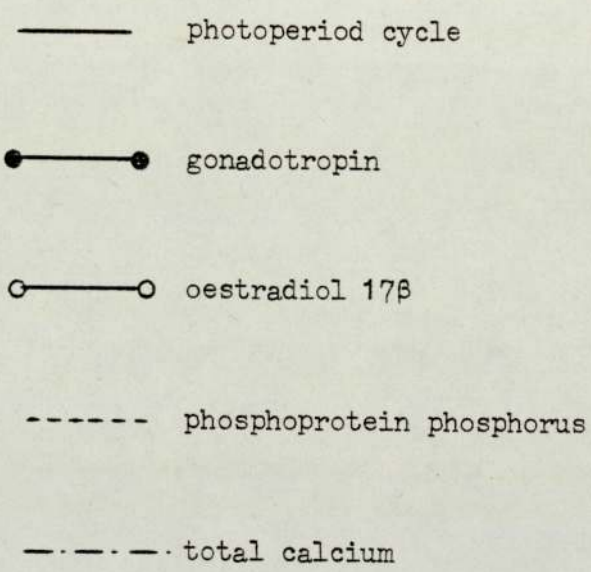
3.6. Discussion.

As maturation proceeds, significant changes occur in reproductive structure and function in preparation for spawning. Although clearly synchronised by environmental change, the formulation of a generalised hypothesis to account for the coordination of events leading up to spawning has until recently been hampered by the paucity of the data and the failure of many studies to closely control all environmental variables. The present study clearly demonstrates that the timing of the sequence of changes of pituitary and gonadal hormones and the egg protein complex in the female is all important in the control of reproduction in the rainbow trout, and that these changes are initiated primarily by changes in daylength. Shortened seasonal photoperiod regimes advanced spawning by a modification of the timing and levels of these serum components although the sequence was unchanged.

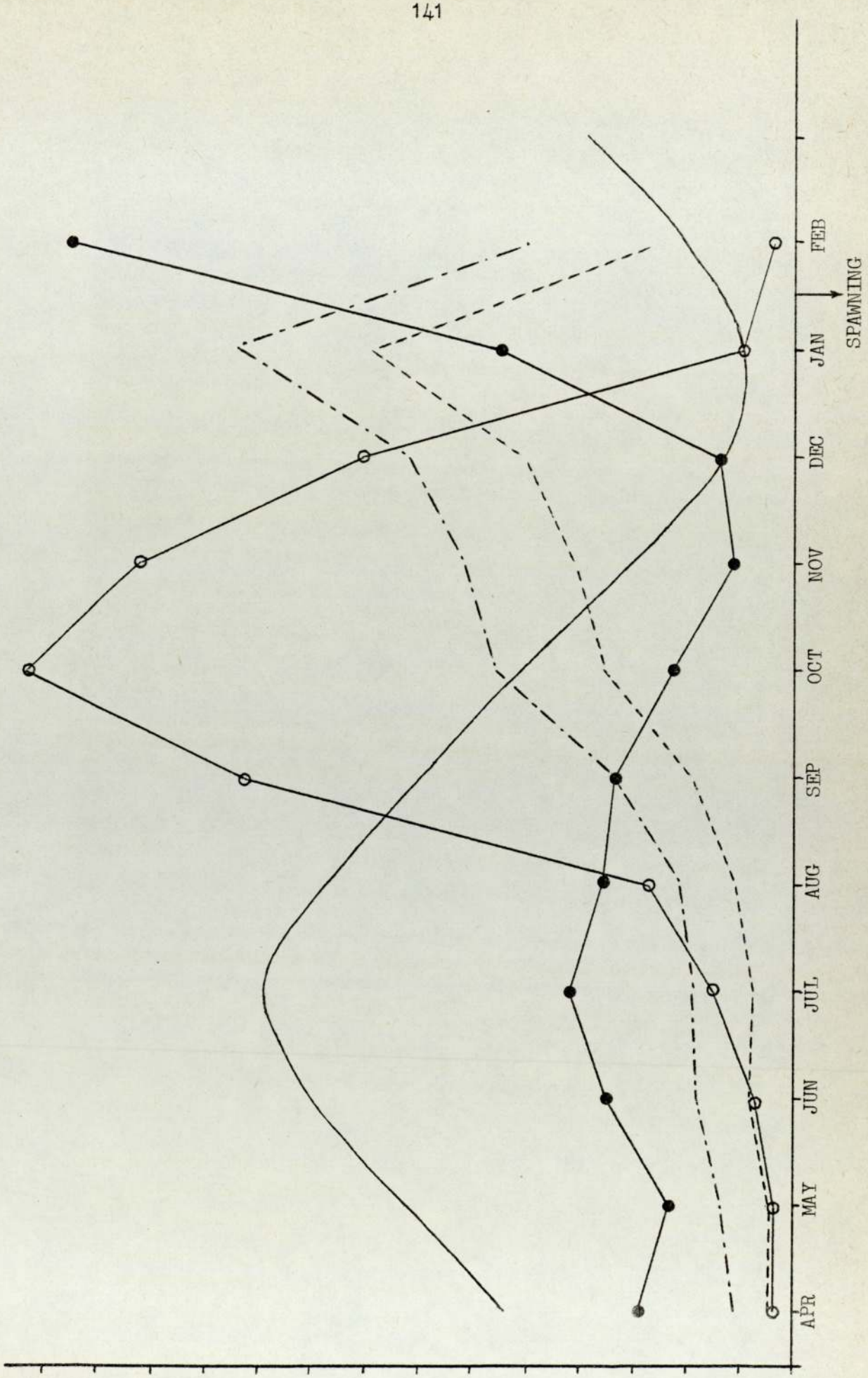
The results are important for two reasons, firstly they clearly demonstrate the endocrine mediation of photoperiod-induced spawning and secondly they provide information which might enable "off-season spawning" to be used by commercial fish farmers. Although both aspects are closely linked, they are considered separately in the following discussion.

3.6.1. The Endocrine Mediation of Photoperiod-Induced Spawning: Under the control photoperiod, the female fish showed a sequence of changes in serum levels of gonadotropin, oestradiol 17β, phosphoprotein phosphorus and total calcium. The temporal relationship of these changes is shown more clearly in Fig.3.49. The initial event here was an increase in serum levels of gonadotropin. Levels were raised

Fig. 3.49. (Series 1 Experiments). The Sequential Changes in Serum Levels of Gonadotropin, Oestradiol 17β, Phosphoprotein Phosphorus and Total Calcium in Female Rainbow Trout Under A Normal 12 Month Seasonal Photoperiod Cycle.



The vertical scale is composite, the graphs being drawn to the same scale as previously. Spawning in this group of female fish occurred in mid-January.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SPAWNING

early in the year, after which they fell to a basal value. Just before spawning levels rose rapidly to reach a much higher value presumably at the time of ovulation. In a similar study, Breton & Billard, (1977) have shown that under a decreasing photoperiod, one of the primary events is an increase in plasma gonadotropin. Crim et al, (1973) found undetectable (<6.0ng/ml) or low levels (6.0 - 17.5ng/ml) of plasma gonadotropin during gonadal recrudescence. However, plasma gonadotropin levels were much higher in ovulated fish (74.9ng/ml). In a later study, using a more sensitive radioimmunoassay, Crim et al, (1975) showed that levels of plasma gonadotropin were undetectable in immature brook trout and in female fish during the early stages of gonadal recrudescence, but were significantly higher in ovulated fish. The same study also showed levels of 3.3ng/ml during preliminary vitellogenesis in female sockeye salmon rising to 9.4ng/ml during advanced vitellogenesis and to 300ng/ml in ovulated fish. Fostier et al, (1978) have also observed high levels of gonadotropin in rainbow trout during ovulation although they only investigated the very last stages of the reproductive cycle. Increased serum gonadotropin levels have also been found at ovulation in the tench (Breton et al, 1975) and goldfish (Breton et al, 1972). Thus, it is clear that raised gonadotropin levels are associated with both the early stages of oocyte maturation and also ovulation in trout as well as in other species of teleost fish.

Following the initial increase of serum gonadotropin in control fish reported in this work, serum levels of oestradiol 17β started to increase, reached a peak and returned to basal levels just prior to spawning. It therefore seems likely that the observed increases in serum oestradiol 17β are initiated by gonadotropin stimulation,

supporting the hypothesis of a photoperiod control of maturation via the brain and pituitary, with the subsequent gonadotropin-induced release of steroids from the gonad. The fall in serum levels of oestradiol 17β may, by the means of diminished negative feedback on the pituitary, provide a signal for the rapid increase in levels of gonadotropin observed just prior to spawning, presumably at the time of ovulation. A similar cycle in levels of oestradiol 17β has also been demonstrated by Lambert et al, (1978) in the rainbow trout, where levels of 1.3ng/ml were observed during previtellogenesis, rising at the beginning of exogenous vitellogenesis to reach a maximum of 16.9ng/ml at the end of vitellogenesis some weeks before spawning. Fostier et al, (1978) observing the process of ovulation in more detail confirmed that oestradiol 17β dropped to a low level before oocyte maturation at a time when plasma gonadotropin was high. A similar pattern of changes in plasma oestradiol 17β and gonadotropin was observed in the brown trout by Crim & Idler, (1978) although this study was not continued to the completion of the spawning cycle. High levels of oestradiol 17β during vitellogenesis, and low levels during spawning have also been reported in the catfish (Eleftheriou et al, 1966) and plaice (Wingfield & Grimm, 1977). Thus, in rainbow trout, like other teleosts oestradiol 17β levels are raised, probably as a result of an earlier increase in gonadotropin, at a time when the developing oocytes are actively incorporating yolk.

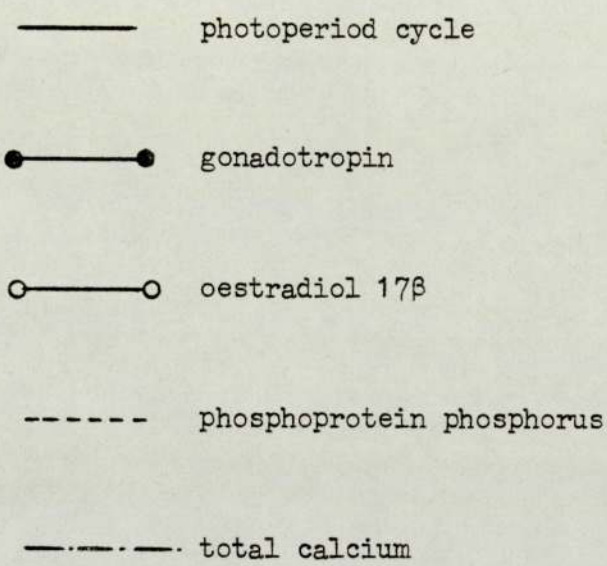
Following the increase in serum oestradiol 17β in control fish, levels of phosphoprotein phosphorus were raised from an initial basal level to a peak just prior to spawning. An identical pattern was found in the total serum calcium levels. Similar changes in both blood phosphorus and calcium levels were first reported by Miescher (1897)

and Hess et al, (1928) respectively although in both studies the techniques available to these authors were relatively insensitive. Since that time, seasonal variations in serum calcium and phosphoprotein phosphorus have also been demonstrated in the Atlantic salmon, Salmo salar (Fontaine et al, 1950), Tilapia esculenta (Garrod & Newell, 1958), Fundulus kansae (Fleming et al, 1964) and cod, Gadus morhua (Woodhead, 1968), with the highest levels occurring during spawning.

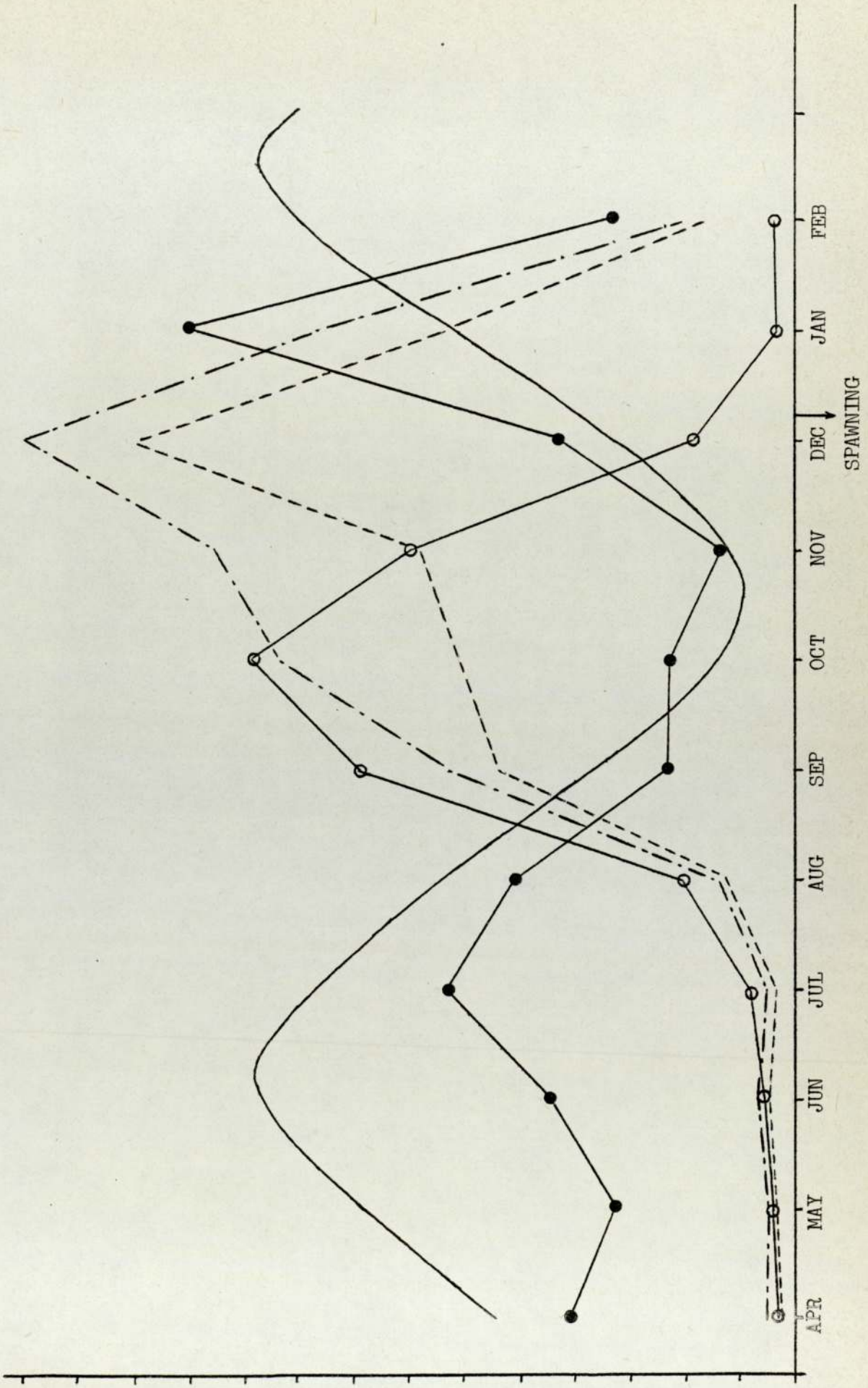
In summary therefore, under the natural seasonal photoperiod, the initial event in the control of reproduction was an increase in the serum levels of gonadotropin. Following this increase, oestradiol 17β levels in the serum began to rise, and this in turn initiated increases in serum levels of phosphoprotein phosphorus and total calcium which were at their highest levels before and during spawning when yolk proteins are required in large amounts by the developing oocytes. At the time of spawning, gonadotropin levels rapidly increased, after a fall in oestradiol levels.

Under all shortened seasonal photoperiod regimes, a similar sequence of changes in serum levels of gonadotropin, oestradiol 17β, phosphoprotein phosphorus and total calcium was observed. This similarity is more evident when one examines the serum changes in fish under one of these shortened seasonal photoperiods, for example the seasonal regime compressed into 9 months (see Fig.3.50.). Clearly, the sequence of changes is the same as that observed under the control photoperiod, but the timing of the changes, and the levels of hormones and the vitellogenin complex were modified under the shortened photoperiod regimes. The alterations produced earlier spawning times

Fig. 3.50. (Series 1 Experiments). The Sequential Changes in Serum Levels of Gonadotropin, Oestradiol 17β, Phosphoprotein Phosphorus and Total Calcium in Female Rainbow Trout Under A Normal Seasonal Photoperiod Cycle Compressed into 9 Months.



The vertical scale is composite, the graphs being drawn to the same scales as previously. Spawning in this group of female fish occurred in early December, 6 weeks earlier than the control group.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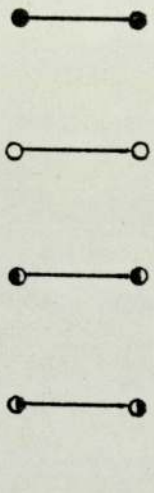
in these groups of fish. Thus, under the 9 month regimes, spawning was advanced by up to 8 weeks, and under the 6 month regimes by up to 16 weeks, relative to the respective control groups of fish.

When one compares in turn the effects of shortened photoperiod regimes on gonadotropin, oestradiol 17β, phosphoprotein phosphorus and total calcium levels, the advancing effect of photoperiod is emphasised. Thus the levels of serum gonadotropin were higher in July under the 6 month photoperiod than under the 9 month or control photoperiods (Fig.3.51.), and the rapid increase in levels of gonadotropin just prior to spawning occurred earlier under each of the shortened regimes. Fig.3.52. demonstrates that the fall in levels of oestradiol 17β also occurred at an earlier time under the shortened photoperiod regimes and was clearly correlated with the earlier spawning time in each group. Similarly, Figs. 3.53. and 3.54. clearly demonstrate the earlier appearances of increases in levels of both serum phosphoprotein phosphorus and total calcium under each of the shortened photoperiod regimes. Furthermore, the rates of increase of serum calcium and phosphoprotein phosphorus and the levels achieved before spawning were far greater in the groups under the 6 and 9 month photoperiod regimes than in the controls, probably reflecting an increased rate of vitellogenesis in preparation for early spawning in these groups. Thus, the earlier times of spawning observed under each of the shortened seasonal photoperiod regimes result from an advancement of the sequence of changes in serum levels of gonadotropin, oestradiol 17β, phosphoprotein phosphorus and total calcium.

Although similar to series 1, series 2 and 3 experiments were conducted to investigate the effect of previous maturity on

Fig. 3.51. (Series 1 Experiments). The Sequential Changes in Levels of Serum Gonadotropin in Female Rainbow Trout Under Each of the Photoperiod Regimes Under Investigation.



Control 12 month seasonal cycle

Seasonal cycle compressed into 9 months

Seasonal cycle compressed into 6 months

Constant 12 hour light / 12 hour dark photoperiod

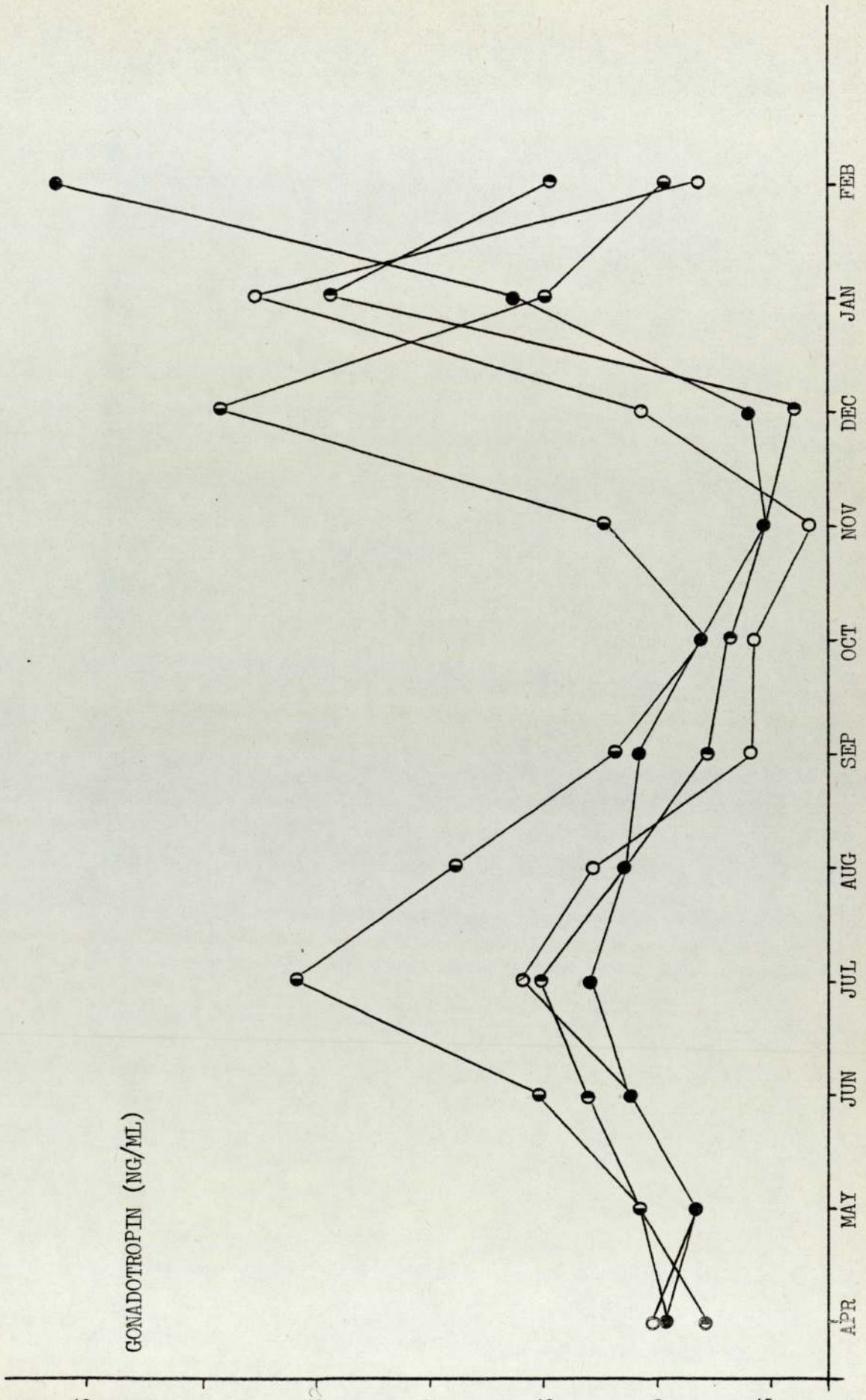

GONADOTROPIN (NG/ML)
35
30
25
20
15
10
5
APR
MAY
JUN
JUL
AUG
SEP
OCT
NOV
DEC
JAN
FEB

Fig. 3.52. (Series 1 Experiments). The Sequential Changes in Levels of Serum Oestradiol 17β in Female Rainbow Trout Under Each of the Photoperiod Regimes Under Investigation.

●——● Control 12 month seasonal cycle

○——○ Seasonal cycle compressed into 9 months

◐——◐ Seasonal cycle compressed into 6 months

◐——◐ Constant 12 hour light / 12 hour dark photoperiod

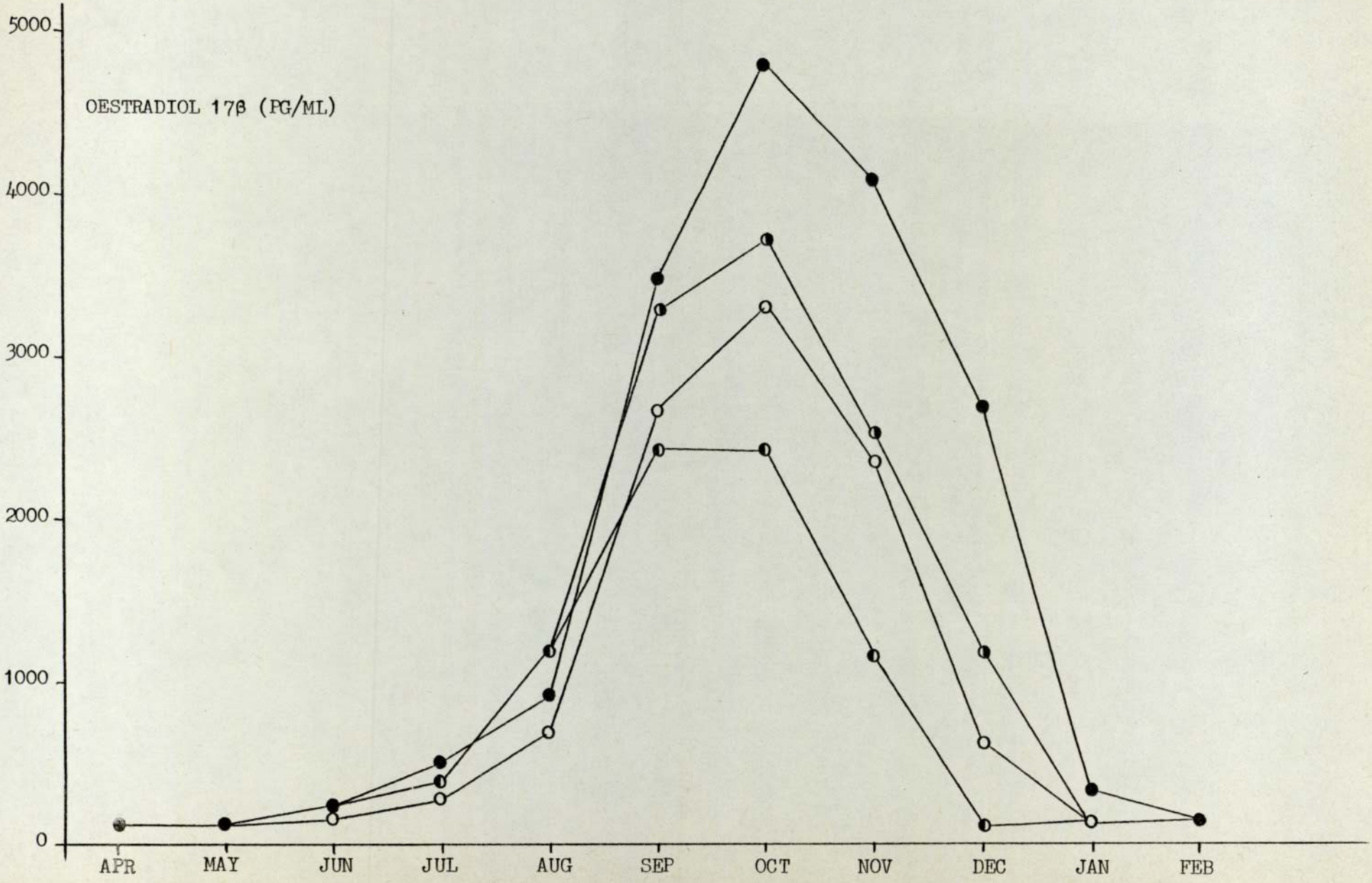
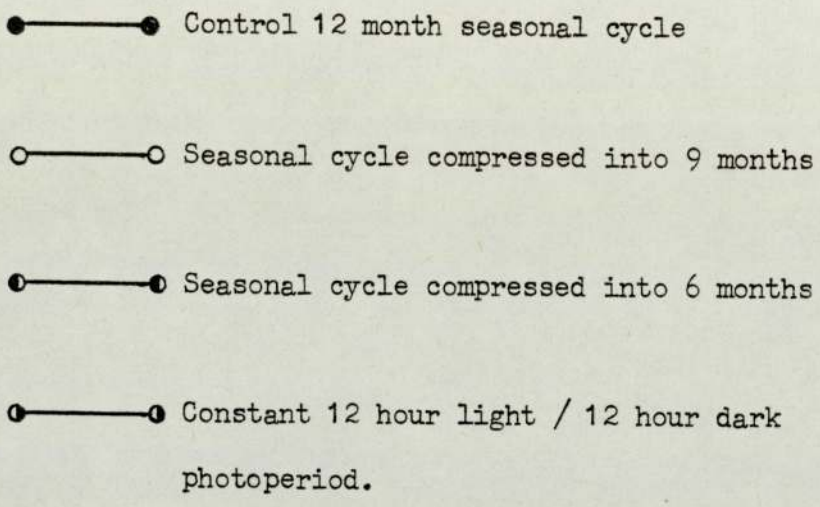

OESTRADIOL 17β (PG/ML)
5000
4000
3000
2000
1000
0
APR
MAY
JUN
JUL
AUG
SEP
OCT
NOV
DEC
JAN
FEB

Fig. 3.53. (Series 1 Experiments). The Sequential Changes in Levels of Serum Phosphoprotein Phosphorus in Female Rainbow Trout Under Each of the Photoperiod Regimes Under Investigation.



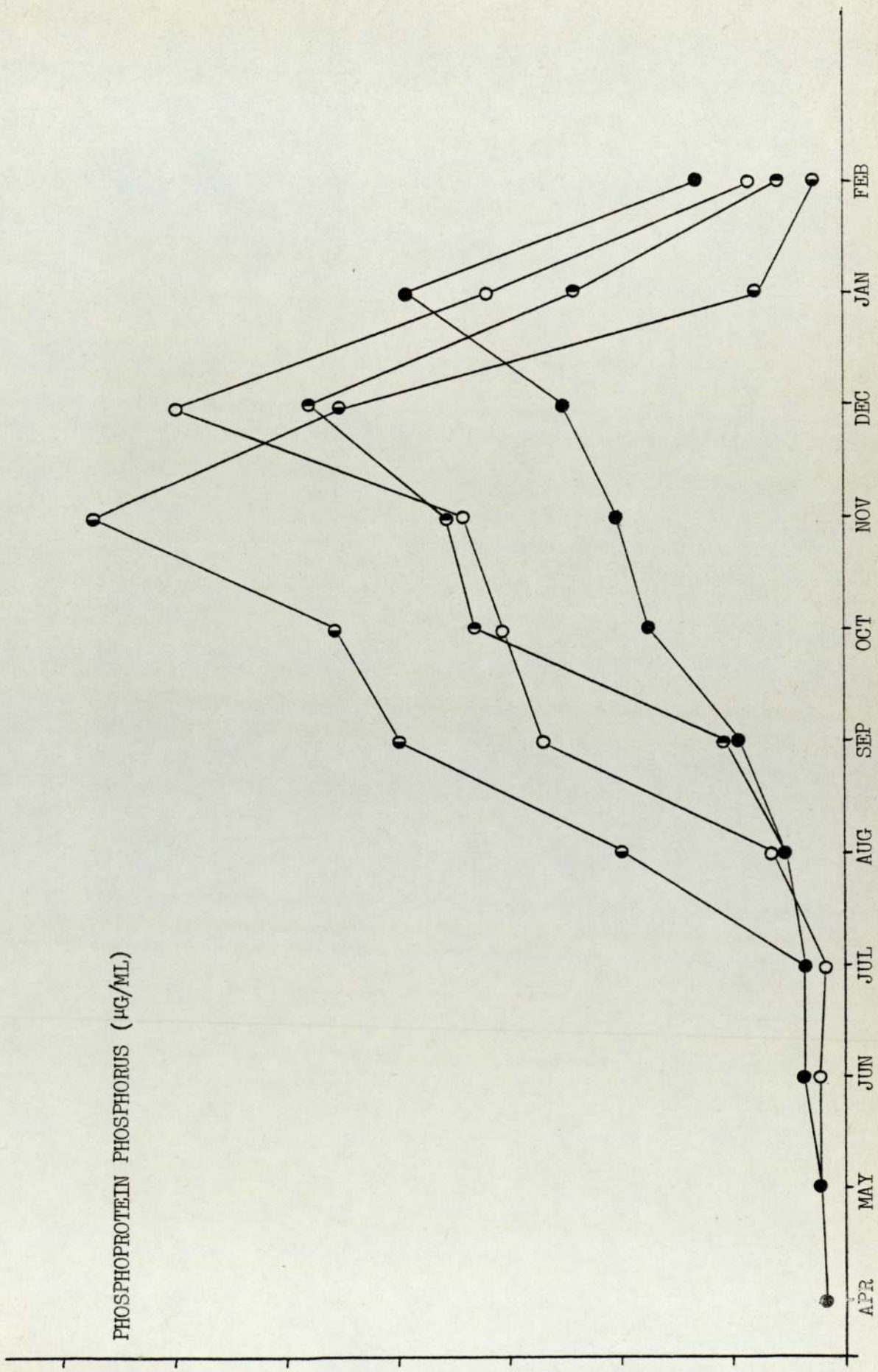

PHOSPHOPROTEIN PHOSPHORUS (µG/ML)
700
600
500
400
300
200
100
0
APR
MAY
JUN
JUL
AUG
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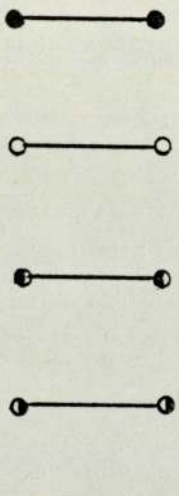
Fig. 3.54. (Series 1 Experiments). The Sequential Changes in Levels of Total Serum Calcium in Female Rainbow Trout Under Each of the Photoperiod Regimes Under Investigation.

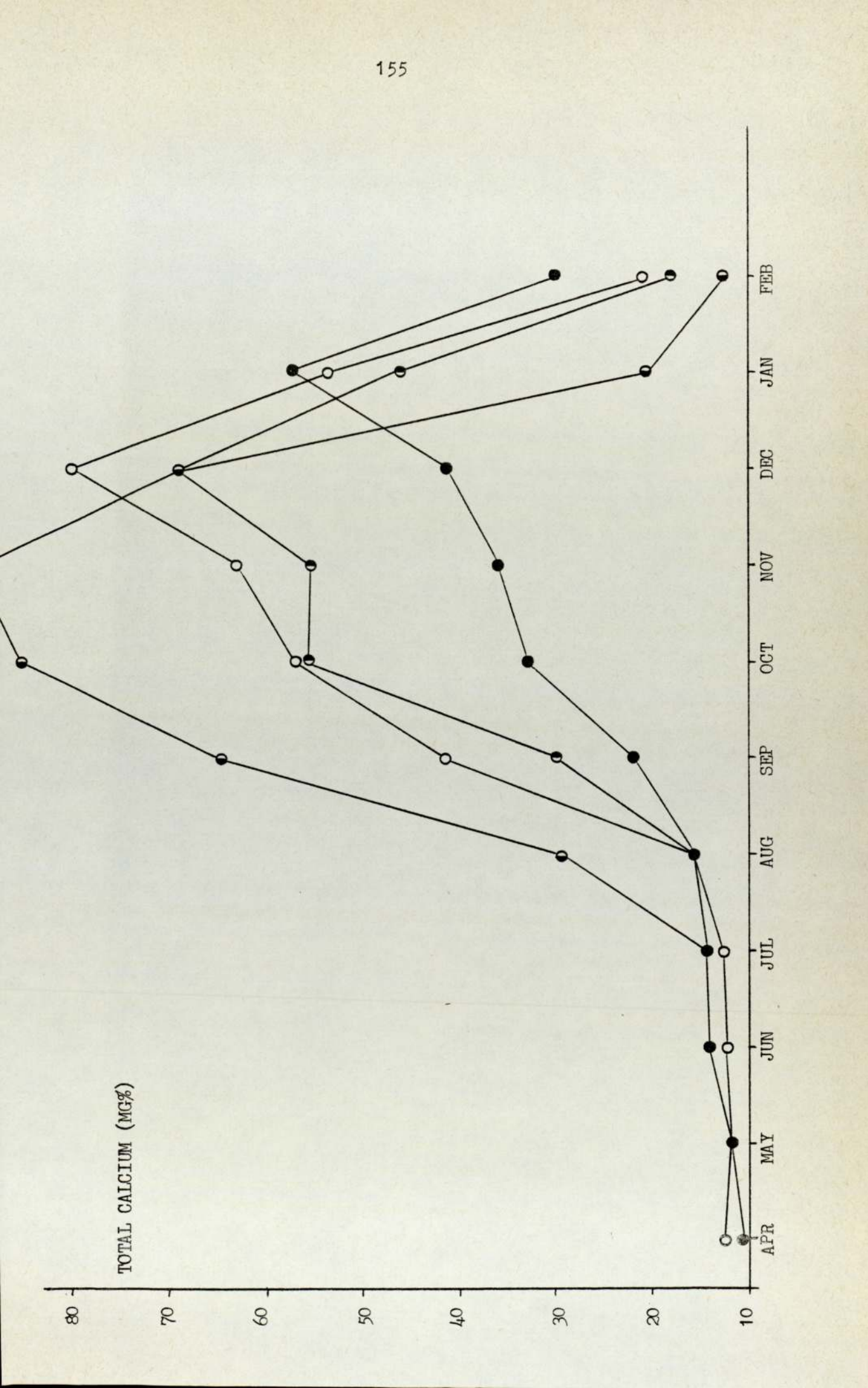


Control 12 month seasonal cycle

Seasonal cycle compressed into 9 months

Seasonal cycle compressed into 6 months

Constant 12 hour light / 12 hour dark photoperiod


TOTAL CALCIUM (MG%)
80
70
60
50
40
30
20
10
APR
MAY
JUN
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photoperiod-induced spawning, and also of moving the shortened cycles further forward in the year. Hence, in series 2 experiments, the maximum hours light per day occurred in May and the minimum in September (c.f. June and October in series 1) under the 9 month regime; under the 6 month regime in series 2, the maximum was in April and the minimum in June (c.f. May and August in series 1). Under both these photoperiod manipulations, the sequence of changes in serum parameters was the same as in the series 1 experiments although the changes initially occurred earlier in the year and advanced spawning by 8 weeks under the 9 month regime and 16 weeks under the 6 month regime (c.f. 6 and 12 weeks during series 1). Further advancement of the shortened 6 month cycle during series 3 experiments again produced essentially similar results.

Clearly, previous maturity in these groups was not affecting subsequent photoperiod-induced early spawning, as both immature (series 1 experiments) and previously spawned fish (series 2 and 3 experiments) responded in an identical way to photoperiod manipulation. This finding is at variance with the work of Henderson, (1963) in brook trout, who stated that only previously spawned fish responded to photoperiod changes.

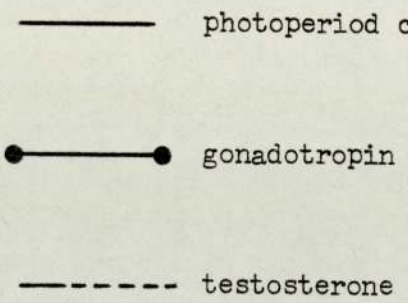
In summary, the study clearly demonstrates that in female rainbow trout a similar sequence of changes in serum levels of gonadotropin, oestradiol 17β, phosphoprotein phosphorus and total calcium occurs under a variety of different photoperiods but in all cases the timing of spawning would appear to be determined by the relative position and onset of these changes.

The endocrine system is similarly important in the mediation of the effects of photoperiod on reproduction in male fish. Thus, under the normal seasonal photoperiod cycle, changes were observed firstly in the levels of serum gonadotropin and then testosterone. These changes which eventually lead to spermiation are shown in more detail in Fig.3.55.

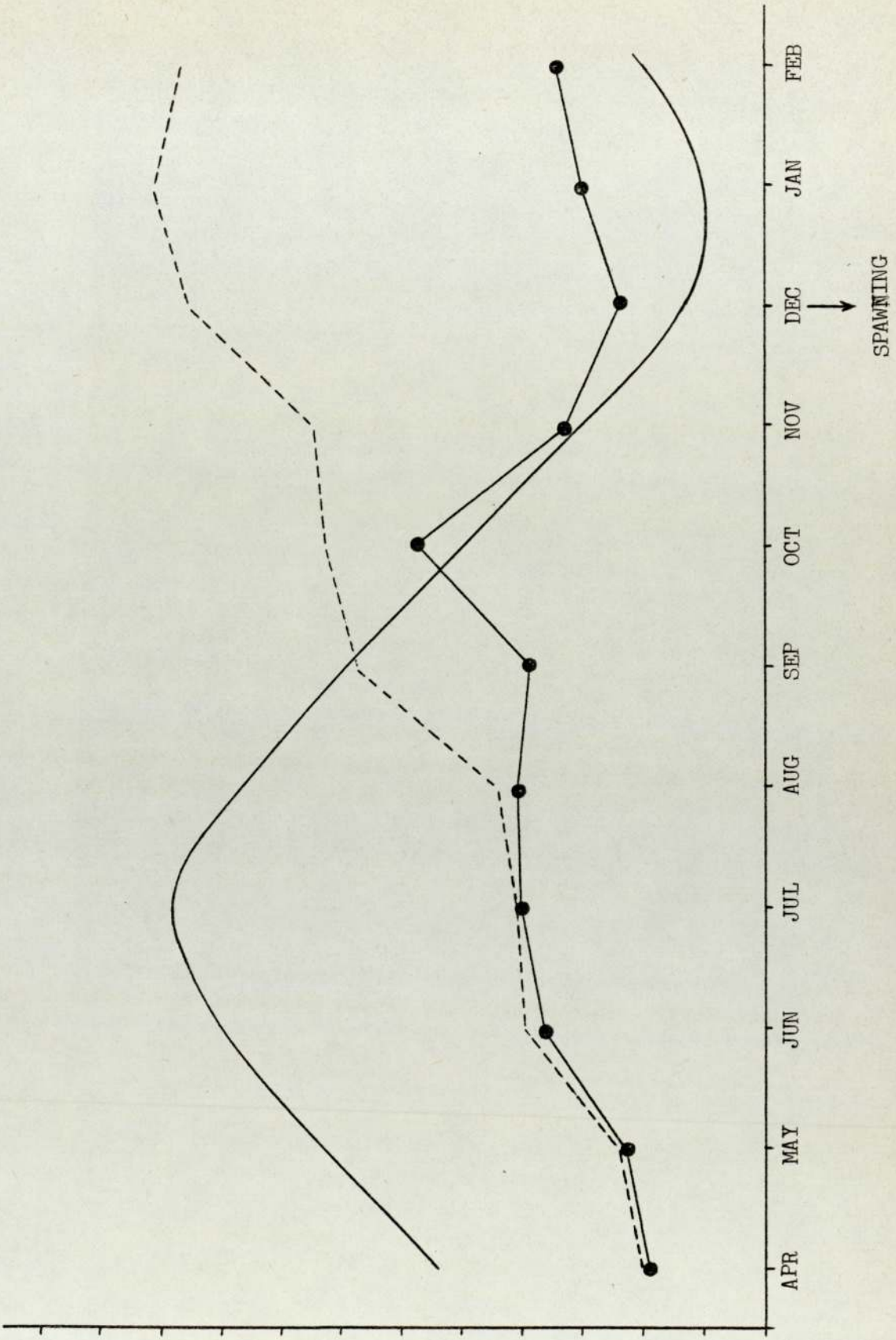
Levels of serum gonadotropin increased gradually from basal to reach a maximum prior to the commencement of spermiation. As in female fish, Crim et al, (1975) found undetectable levels of gonadotropin in male brook trout that were still immature, or in the early stages of gonadal recrudescence, whereas higher levels were found in both this species and Atlantic salmon with spermatozoa present in the testis. Spermiation in Atlantic salmon, but not in brook trout, was also associated with significantly higher plasma gonadotropin levels. In parallel studies on male sockeye salmon, gonadotropin levels were below the sensitivity of the assay in immature fish. However, gonadotropin levels increased as the males progressed through gonadal recrudescence to sperm development, and were highest in spermiating fish. Breton et al, (1975) have made similar observations in the male rainbow trout and in a more recent study, Crim & Evans, (1978) related increased gonadotropin levels to precocious sexual development in male Atlantic salmon parr. Although the results from these studies were not related to environmental change, gonadotropin is clearly implicated in the endocrine mediation of reproductive function in the male rainbow trout.

There is little evidence in the literature concerning spermiation and its precise hormonal control. However, Sanchez-Rodriguez et al, (1978) studied the hormonal changes, sperm production and variation

Fig. 3.55. (Series 1 Experiments). The Sequential Changes in Levels of Serum Gonadotropin and Testosterone in Male Rainbow Trout Under a Normal Seasonal Photoperiod Cycle.



The vertical scale is composite, the graphs being drawn to the same scales as previously. Spermiation in this group of males commenced in December, at the same time as similar male broodstock maintained outside in open ponds.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in seminal fluid composition in male rainbow trout during the 12 weeks following the onset of spermiation. They showed that when sperm release is first detected, small amounts of sperm are produced and high levels of gonadotropin are found in the plasma. Significant sperm production only occurred several weeks later when plasma gonadotropin had decreased and androgens had reached a maximum level. At the onset of spermiation, the relationship between gonadotropin, androgen and sperm production was not clear although this may be due to the involvement of gonadotropin in the initiation of spermiation and in producing the increase in circulating androgens which itself might then reduce gonadotropin secretion by negative feedback. At a later stage, from 6 to 12 weeks after the onset of spermiation, androgen levels were at a maximum. These results agree with those reported in the present study, where a high level of gonadotropin was observed prior to the commencement of spermiation, and this gradually fell towards basal as spermiation progressed and androgen production increased.

Under the normal seasonal photoperiod, levels of serum testosterone also began to rise, after the increase in gonadotropin, from their basal levels to a maximum approximately 1 month after the onset of spermiation. Similar seasonal changes in the levels of circulating androgens, with increasing levels towards the time of spawning have been reported in male rainbow trout by Schreck et al, (1972), Atlantic and sockeye salmon, (Idler et al, 1960; Grajcer & Idler, 1961; Schmidt & Idler, 1962; Idler et al, 1971), goldfish (Schreck & Hopwood, 1974) and plaice (Wingfield & Grimm, 1977) although in each case these observations were not related to environmental change.

As with gonadotropin, the precise role played by androgens in the hormonal control of spermatogenesis in teleosts is presently unclear. For example, testosterone effects on spermatogenesis and spermiation have only been reported at high dose levels (Billard, 1974). Also, although the increased levels of testosterone correlates with the onset of spawning in the present work, the situation is further complicated by the finding that in some species, the 11-oxygenated metabolites of testosterone, i.e. 11-ketotestosterone and 11-hydroxytestosterone may also be functionally important in maturation.

Since Idler et al, (1960) first isolated 11-ketotestosterone from the peripheral plasma of sockeye salmon, it has been demonstrated in the plasma of Atlantic salmon (Idler et al, 1964), winter flounder (Campbell et al, 1976) and rainbow trout (Simpson & Wright, 1977). Plasma levels of 11-ketotestosterone increased at the approach of sexual maturity in Atlantic salmon (Schmidt & Idler, 1962; Idler et al, 1971) and winter flounder (Campbell et al, 1976). In the rainbow trout, 11-ketotestosterone in the plasma rose sharply with the onset of maturation (Simpson & Wright, 1977). Both testosterone and 11-ketotestosterone are thus found at high levels in mature fish. 11-ketotestosterone has been shown to have approximately 10 times the potency of testosterone in promoting the appearance of male secondary sex characters in the medaka (Arai, 1967; Hishida & Kawamoto, 1970). In other species, however, 11-hydroxytestosterone appears to be a major circulating androgen (Idler et al, 1976).

Although the results from the present study strongly suggest that testosterone plays an important role in the control of maturation in male salmonids, clearly further work is required to elucidate the

relative importance of testosterone and 11-ketotestosterone in this process.

Thus, the present study demonstrates that pituitary gonadotropin is important in the mediation of spawning in the male rainbow trout under a normal seasonal photoperiod, most probably via a direct action on testosterone, although the role played by 11-ketotestosterone must also be considered.

Under all the shortened photoperiod regimes, the same sequence of changes in serum levels of gonadotropin and testosterone was observed although there were variations in both the onset and extent of these changes which depended on the particular photoperiod employed. One such cycle, the normal seasonal cycle compressed into 6 months is shown in Fig.3.56. From this it is clear that the same sequence occurred but they were advanced in the year in order to produce an earlier time of spermiation. Thus, under the 6 month regimes spermiation was advanced by up to 20 weeks, and under the 9 month regimes by up to 8 weeks, in every case as a result of an advancement in the timing of the changes in serum levels of gonadotropin and testosterone.

The alterations in timing become more evident when one compares in turn the effects of shortened photoperiod regimes on gonadotropin and testosterone levels (see Figs.3.57. and 3.58). In each case the initial increases and subsequent changes of these hormones was advanced by similar periods of time to the spermiations. Clearly, we are seeing a similar functional link between hormone levels and spermiation in male fish to that already described for oocyte maturation and ovulation in female fish.

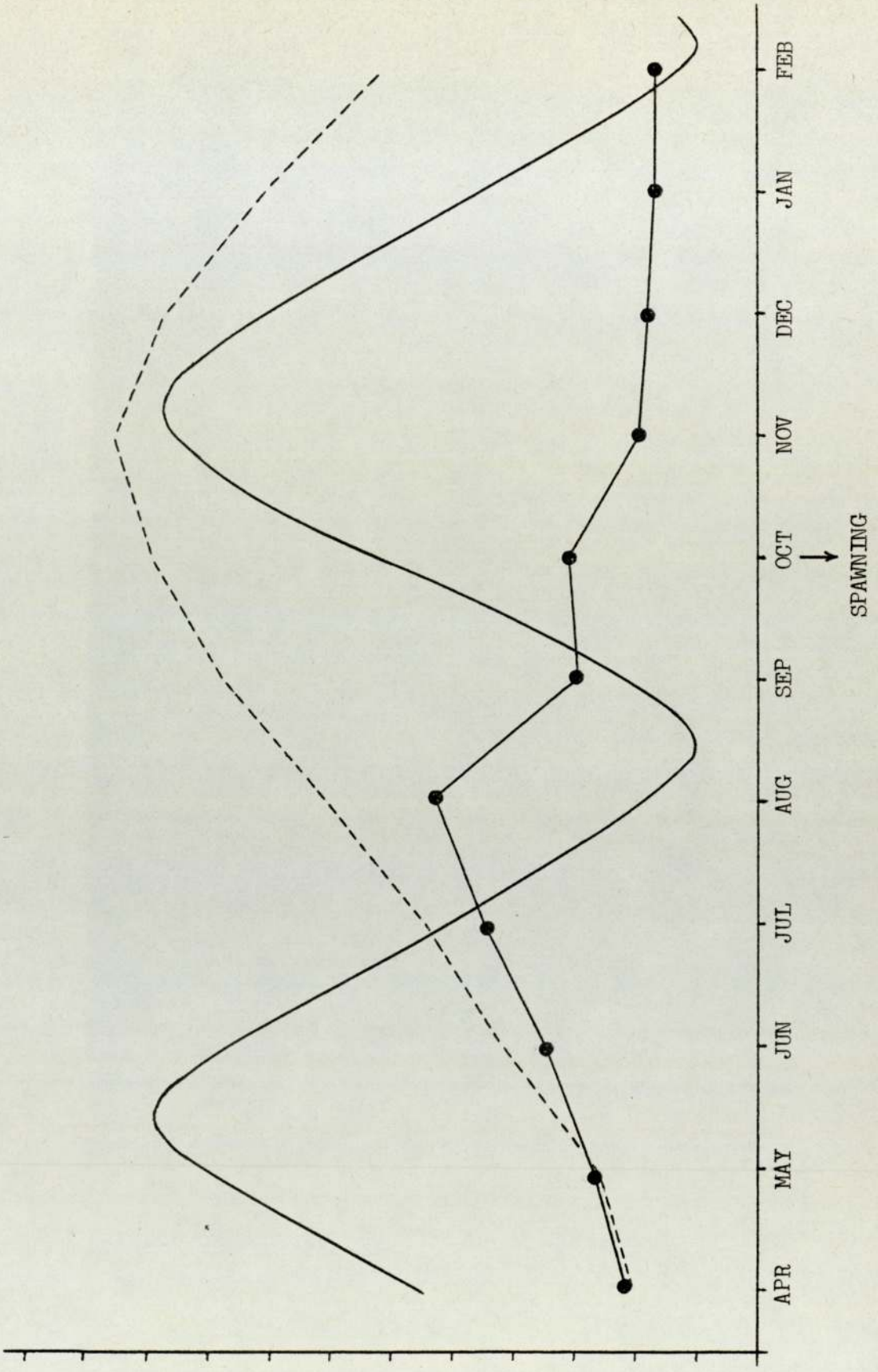
Fig. 3.56. (Series 1 Experiments). The Sequential Changes in Levels of Serum Gonadotropin and Testosterone in Male Rainbow Trout Under a Normal Seasonal Photoperiod Compressed into 6 months.

——— photoperiod cycle

●——● gonadotropin

- - - - - - testosterone

The vertical scale is composite, the graphs being drawn to the same scale as previously. Spermiation in this group of males commenced in October, 8 weeks earlier than in the control group.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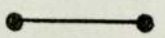
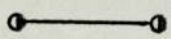
SPAWNING

Fig. 3.57. (Series 1 Experiments). The Sequential Changes in Levels of Serum Gonadotropin in Male Rainbow Trout Under Each of the Photoperiod Regimes Under Investigation.

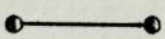


Control 12 month seasonal cycle

Seasonal cycle compressed into 9 months



Seasonal cycle compressed into 6 months



Constant 12 hour light / 12 hour dark photoperiod.

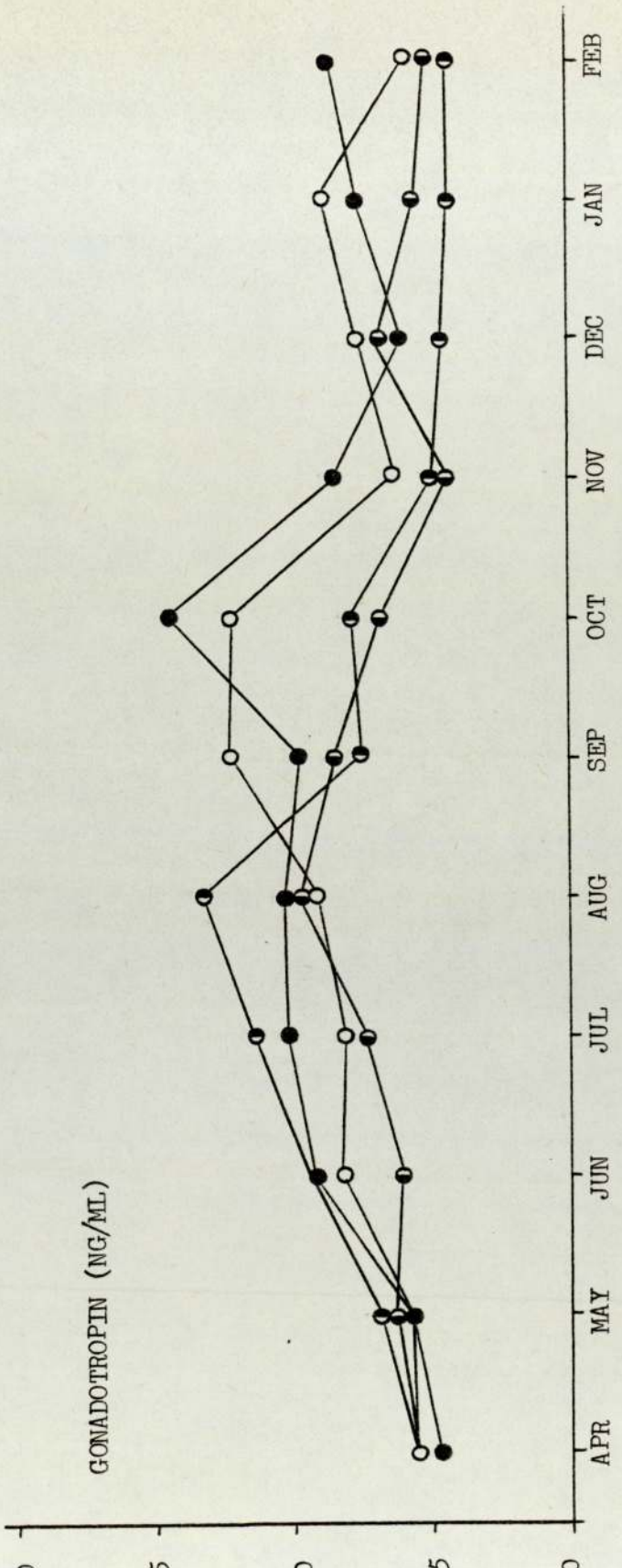
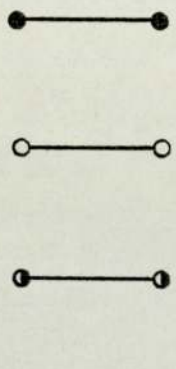

GONADOTROPIN (NG/ML)
20
15
10
5
0
APR
MAY
JUN
JUL
AUG
SEP
OCT
NOV
DEC
JAN
FEB

Fig. 3.58. (Series 1 Experiments). The Sequential Changes in Levels of Serum Testosterone in the Male Rainbow Trout Under Each of the Photoperiod Regimes Under Investigation.

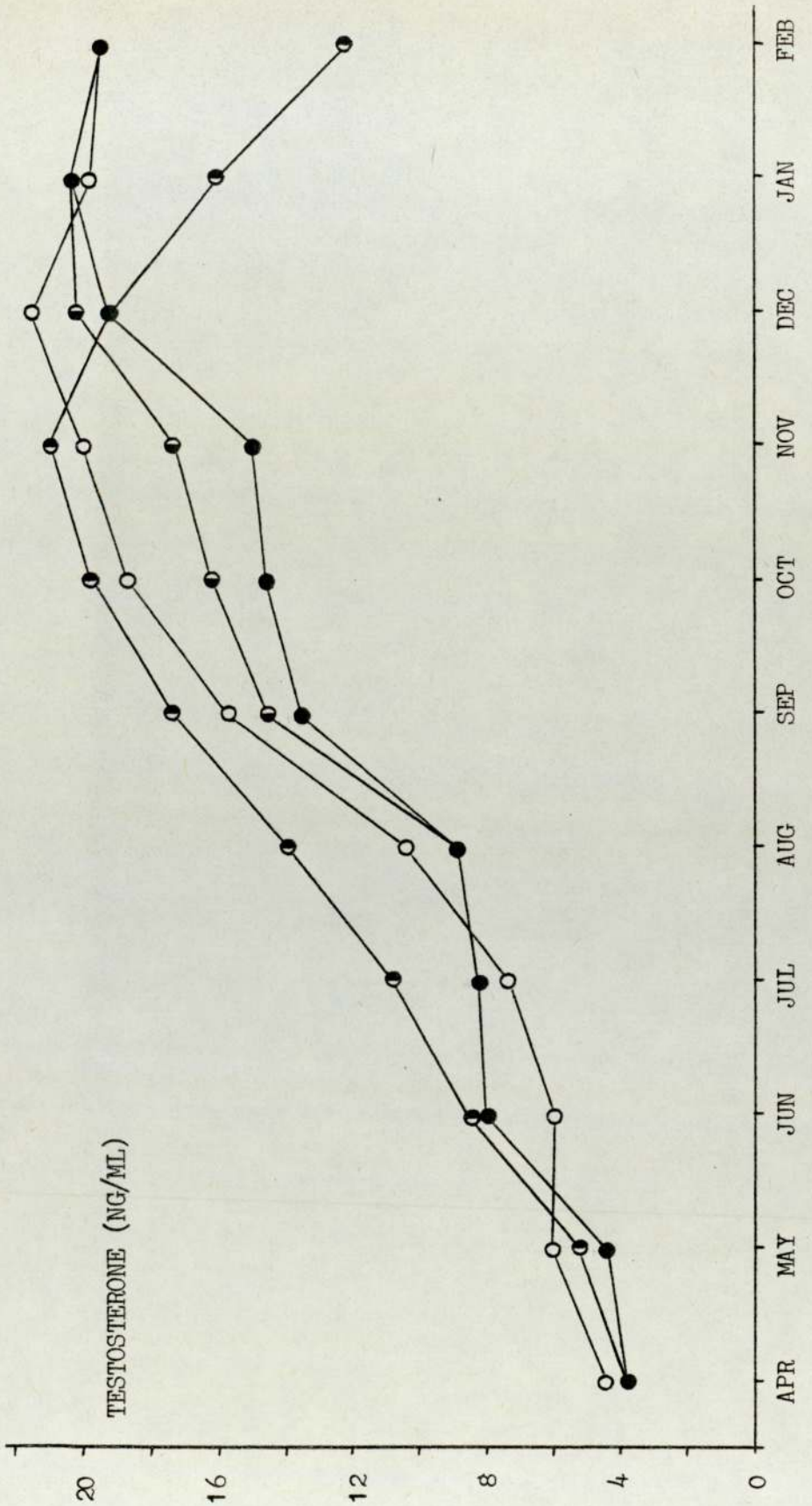


Control 12 month seasonal cycle

Seasonal cycle compressed into 9 months

Seasonal cycle compressed into 6 months

Constant 12 hour light / 12 hour dark photoperiod.


TESTOSTERONE (NG/ML)
20
16
12
8
4
0
APR
MAY
JUN
JUL
AUG
SEP
OCT
NOV
DEC
JAN
FEB

In the series 2 experiments the shortened photoperiod cycles were brought forward in the year and this produced advancements in both the timing of the sequence of changes in levels of serum gonadotropin and testosterone, and as a result of this, the onset of spermiation. Clearly previous maturation is unimportant in male as well as female fish as far as photoperiod stimulation is concerned.

In summary, the study clearly demonstrates that the sequential changes in serum levels of gonadotropin and testosterone seen under the normal 12 month seasonal cycle in the male rainbow trout occur at an earlier time under seasonal cycles compressed into shorter periods of time, resulting in an earlier time of spermiation.

The alterations in spawning time in both male and female fish reported here corresponds well with the observations of other workers already discussed (Hoover, 1937; Hoover & Hubbard, 1937; Hazard & Eddy, 1951; Corson, 1955; Carlson & Hale, 1973, Henderson, 1963; Nomura, 1962; Kunesh et al, 1974; MacQuarrie et al, 1978), although none of these studies related the changed spawning time to its endocrine control.

As Breton & Billard (1977) have suggested that a decreasing photoperiod is necessary for gonadotropin release in the rainbow trout, it is surprising that under the constant 12 hour light / 12 hour dark photoperiod regimes, both females and males spawned at approximately the same time as fish under a normal 12 month seasonal photoperiod. Under this constant photoperiod there were also similar changes in serum levels of gonadotropin, oestradiol 17β, phosphoprotein phosphorus and total calcium in the females, and gonadotropin and testosterone in the males. These changes apparently occurred in the absence of a

cue from a seasonally fluctuating photoperiod, thus suggesting that rainbow trout, like many other seasonally breeding vertebrates may have an endogenous rhythm of reproductive function which is modified under the influence of photoperiod. However, it is possible that the 12 hour light / 12 hour dark photoperiod contributed enough light at the appropriate time to stimulate the gonadotropin release necessary for spawning.

The results from the groups of males and females exposed to 2 years of shortened 6 month cycles also pose a number of important questions. Over the 2 years, the fish had actually undergone 4 cycles of increasing and decreasing photoperiod (2 x 6 month cycles in both series 1 and series 2 experiments). However, the fish had only responded to the 1st. and 3rd. photoperiod cycles, failing to spawn under the influence of the 2nd. cycle during series 1. This would suggest that, following the spawning induced by the 1st. cycle, there was a refractory period during which time the fish were not responsive to the particular light cycle applied at that time. After this period, the fish responded to the 3rd. cycle and spawned again. The implications of a refractory period are discussed in more detail in Chapter 4.

The foregoing account thus demonstrates that in both male and female rainbow trout, spawning is initiated by photoperiod changes mediated by the endocrine system. In order to respond to environmental changes the rainbow trout must have evolved a mechanism for precise measurement of the length of the daily photoperiod. While this study was primarily designed to study the endocrine changes in response to altered photoperiod the results obtained, especially concerning the

constant photoperiod and the repeat 6 month cycles already briefly discussed, pose important questions regarding the mechanism of photoperiod time measurement. Although several hypotheses exist to account for this mechanism, certain anomalies still unresolved preclude a definite interpretation at the present time.

Photoperiodism has been studied in a wide variety of species but probably our knowledge is most advanced with regard to birds, where several excellent reviews already exist (Farner, 1959, 1961, 1964; Farner & Follett, 1966; Follett, 1973a) and although a complete review of photoperiodic time measurement would not be appropriate at the current stage of understanding of teleost periodicity, comparison of the present results with established findings in birds should provide insight into the mechanism of photoperiodic time measurement in the rainbow trout.

Two major hypotheses are relevent to this discussion. The first is based on an "hour glass" or "interval timer" principle and suggests that the photoperiod effect results from a light-dependent reaction culminating in the formation of an essential compound. The accumulation, duration of physiological activity, or release from the site of production of this essential compound may be a function of the daily cycle of light and dark (Farner & Follett, 1966; Wolfson, 1959, 1960). Hence, if sufficient of the essential compound accumulates as a result of being exposed to specific stimulatory periods of light and dark, then a threshold is exceeded and a physiological process initiated. The important principle here is the requirement for a critical duration of light and dark in each cycle.

It would appear that this principle is sufficient to account for photoperiod response in many insects (Lees, 1966, 1971; Beck, 1968). Furthermore, Lees (1973) has demonstrated that the duration of the dark period may be as, if not more, important than the duration of the light period in the photoperiod response of the aphid (Megoura viciae). A modification of this hypothesis has been used to explain early experiments on photoperiodism in birds, but as a result of information from more recent experiments, a second hypothesis now seems more appropriate.

In 1936, Bunning originally suggested a hypothesis that living organisms measured the length of the daily photoperiod by using an endogenous circadian rhythm of sensitivity to light (Bunning, 1936, 1960, 1969). He envisaged a circadian rhythm of cellular function consisting of 2 halves per cycle. During the first half of the cycle (of approximately 12 hours duration), known as the photophil phase, the organism is light insensitive. During the second half of the cycle (again of approximately 12 hours duration), known as the scotophil phase, the organism is sensitive to light. Hence, the photoperiod induction of a process requiring long days only occurs when the duration of the natural daylength extends into the sensitive phase of the cycle (the scotophil phase). For example, in winter, the daylength is too short to stimulate the sensitive phase, but as daylength extends into the spring, light progressively engages the sensitive phase of the cycle and stimulates the release of gonadotropin (Bunning, 1969; Follett, 1973a). Whether the circadian rhythm in sensitivity to light is a single biochemical system, or one composed of several different and independent reactions is as yet unknown.

A modified version of the Bunning hypothesis has been developed by Pittendrigh (1966) and Pittendrigh & Minnis (1964, 1971). These authors suggested that light performs a dual function, acting as both an entraining agent (or Zeitgeber) for the circadian rhythm and also as a photoperiodic inducer. At the same time they also emphasised that the scotophil phase may not last for a full 12 hour half-cycle and suggested the term "photo-inducible phase". It now appears that the Bunning hypothesis in some form can be used to account for photoperiodism in a wide variety of birds (Lofts et al, 1970; Follett, 1973a). An interesting feature of the work in birds is that under the same photoperiod regime the response of the gonads of closely related species (Lofts et al, 1967) or of races within the same species (Lofts & Murton, 1968) may be different, suggesting a very precise timing mechanism.

In birds, the environmental photoperiod is thought to act via specialised receptors linked to the central nervous system, which pass nervous impulses to the hypothalamus. In response to these impulses, neuroendocrine cells produce neurohumoral secretions which are transported via the hypophyseal portal system to the pituitary where they regulate gonadotropin secretion (reviewed by Lofts et al, 1970; Assenmacher, 1973; Follett, 1973b; Lofts & Lam, 1973). In the male quail, under a stimulatory long day photoperiod, the discharge of gonadotropin releasing hormone between approximately 16 and 19.5 hours after dawn has been correlated with the pituitary release of gonadotropin (Follett & Sharp, 1969). The timing of the secretion agrees with the duration of the photo-inducible phase in this species, and suggests the immediate secretion of gonadotropin induced by light engaging the photo-inducible phase (Lofts et al, 1970).

The results reported from this study do not, unfortunately, provide an immediate solution to the question of the mechanism of photoperiodic time measurement in the rainbow trout, although they do demonstrate clearly the endocrine effects resulting from this process. However, several interesting points do emerge. Firstly, the gradually decreasing photoperiod previously thought important for gonadotropin release in rainbow trout (Breton & Billard, 1977) does not appear to be important, as maturation occurred at the same time in fish maintained on a constant 12 hour light / 12 hour dark photoperiod as those under a normal seasonal cycle. Results from recent experiments not reported here also demonstrate that fish maintained on a constant long day photoperiod (17 hours light / 7 hours dark) also matured normally, but some 10 weeks earlier than control fish under a normal seasonal photoperiod. This may have occurred as a result of the 17 hours light / day photoperiod engaging the photo-inducible phase of the circadian rhythm at an earlier time in the year than the 12 hours light / day photoperiod. Furthermore, in fish exposed to a 17 hour light photoperiod from February to June, then reduced directly to 8 hours light / day, spawning occurred 14 weeks earlier than in the control group. While the immediate implications of these results are as yet not clear, they demonstrate that a seasonally fluctuating photoperiod is not necessary for normal maturation and spawning, and suggest a complex mechanism for photoperiodic time measurement.

Furthermore, if the experiments during series 1, for example, are considered, the female fish under the control photoperiod spawned after having received a total of 3840 hours of experimental illumination. The females under the 9 month regime spawned after receiving 3350 hours and under the 6 month regime after only 2730

hours. Females under the constant 12 hours light / 12 hours dark photoperiod spawned after a total of 3610 hours illumination. It would therefore appear that a simple "hour glass" mechanism of summing up the total amount of light received is not sufficient to account for photoperiodic time measurement in the rainbow trout, but rather where the light falls relative to a circadian rhythm in sensitivity.

In other species of fish, it appears that some form of the Bunning hypothesis can also be applied. A circadian rhythm in sensitivity to light in the three-spined-stickleback, Gasterosteus aculeatus has been demonstrated by Baggerman (1969, 1972) and may explain the photosexual response in this species, where long day photoperiods and high temperatures cause the completion of gametogenesis and consequently, nest building and oviposition (Baggerman, 1957, 1959). Using a similar proceedure to the skeleton photoperiods employed in birds, she exposed fish to an initial photoperiod of 6 hours + 2 hours light at different times in the 18 hour dark period, after previous acclimation to short day photoperiod (8 hours light + 16 hours dark). The experiments were conducted at 20 C. Maximal sexual maturation occurred in fish exposed to 2 hours of light between 14 and 16 hours after the onset of the 6 hour photoperiod. Hence, it appears that in this species at least it is not the quantity of light which is important, but where it falls relative to the photo-inducible phase of the endogenous circadian rhythm.

Similar results have been reported for the catfish (Heteropneustes fossilis) by Sundararaj & Vasal (1976). Fish were exposed to the following photoperiod regimes: (12L + 12D), (9L + 15D), (14L + 10D) and regimes which interrupted the dark period by 1 hour light: (9L + 7D + 1L + 7D), (14L + 2D + 1L + 7D), (1L + 23D).

Gonadal recrudescence was significantly greater in all groups exposed to the photoperiods in which the dark periods were interrupted by 1 hour light.

In further experiments, Sundararaj & Vasal (1976) showed more closely the position of the photo-inducible phase in catfish, where a non-stimulatory 6 hour photoperiod was followed by 1 hour light pulses at various times during the dark period. They showed that the response was bimodal in that light falling between 16 and 17 hours, and 20 and 21 hours after the onset of the primary 6 hour photoperiod caused significant ovarian recrudescence, whereas the same light pulse given between 18 and 19 hours, 14 and 15 hours, 12 and 13 hours, 10 and 11 hours, and 6 and 7 hours after the onset of the primary 6 hour photoperiod did not stimulate the ovaries. A similar bimodal response in gonadal recrudescence has been shown in the Hong Kong tree sparrow, Passer montanus (Lofts & Lam, 1973) and thus raises the question of entrainment of the position of the photo-inducible phase by the daily photoperiod (Lofts et al, 1970; Follett & Sharp, 1969). It is therefore possible that under certain skeleton photoperiods, misinterpretation of the light interruption may occur, being read as the primary photoperiod and causing re-entrainment of the photo - inducible phase (see Follett, 1973a).

From this discussion, it would appear that the endocrine effects which are elicited by alterations in photoperiod cannot readily be accounted for by a simple "hour glass" mechanism. The Bunning hypothesis in some form at least would seem to provide a solution which more closely fits the results reported here. This is to be investigated in more detail in the near future.

3.6.2. The Commercial Application of "Off - Season" Spawning: For the fish farmer the most important implication of the photoperiod experiments is that rainbow trout may be induced to spawn up to 4 months ahead of their usual spawning time. This effect was produced in both Californian and Danish strains of fish, each of which has its own natural spawning time.

To examine the commercial potential in more detail, a real situation, such as the one at Low Plains, is considered. The broodstock at this farm is composed of both Californian and Danish strains of fish. Californian broodstock females were shown to have a normal spawning time of November to December at Low Plains. Under the same conditions, females of Danish origin spawned in January and February. With a large broodstock, these periods would probably be extended by a few weeks due to the slight natural variation in spawning time within a given strain. Hence, without any photoperiod manipulation, eggs could be obtained in the months from October to March.

To extend this period on a commercial scale the following proceedure could be adopted. Eggs could be obtained in the months of June and July by placing the earliest spawning fish among the Californian fraction on a natural seasonal cycle compressed into 6 months. Similarly eggs would be available in August and September if the earliest spawning fish among the Danish fraction were placed under a similar photoperiod regime. Consequently, April and May would be the only months in which eggs were not available, a considerable improvement on the existing situation. Photoperiod manipulation over a further year should enable these months also to be covered.

The practical implications of this approach to the commercial egg producer become more clear when the example is examined in more detail. Consider the farm carrying sufficient broodstock to produce 6 million green eggs / year which allowing for mortalities under good hatchery conditions should comfortably produce 5 million eyed eggs / year. If it is assumed that 2000 eggs/kg body weight can be obtained, 3000kg of female broodstock would be required. 1800kg of male broodstock should be sufficient for breeding purposes, bringing the total weight to 4800kg.

The broodstock would thus be composed of 2400kg of Danish origin fish and 2400kg of Californian origin fish, thus giving a spread of spawning over the months of October to March. If half of the Californian and Danish groups were placed under 6 month photoperiod regimes, eggs would be obtained in the months of June to September and clearly, only half of the broodstock would be under photoperiod control. This could easily be achieved by maintaining each group in a production tank at Low Plains, or in a small pond on a more conventional farm.

The cost of photoperiod manipulation will vary depending on the type of farm. However, a simple light-proof cover over the tank or pond, provided with fluorescent lights and controlled by an electric time clock should prove relatively inexpensive. Furthermore, the cost would be offset by the higher market price that "off - season" eggs would command, and the subsequent advantages to be gained by their production.

Clearly, the input of eggs at specific times of the year would be the easiest way to spread production and eliminate the underutilisation

of facilities during certain periods of the year. A second advantage would be the ability to replace stock immediately after mortalities following accidents, diseases etc., rather than having to wait for the next spawning season.

When used in conjunction with a production schedule formulated with the aid of computer modelling (see Chapter 6), eggs could be produced at the most appropriate times of the year to give continuous production under a variety of environmental conditions. Collectively, these improvements to stock management should be of considerable benefit to trout farming in the U.K.

4. THE HORMONAL CONTROL OF MATURATION AND SPAWNING.

4.1. Introduction.

In teleosts and all other vertebrates, gonad maturation and spawning are controlled by the endocrine system and thus manipulation of the hormone dynamics of fish offers a further possible approach for alteration of the time of spawning.

The important role played by gonadotropins and steroids in vitellogenesis has already been discussed. In addition, these hormones also have a direct effect on oocyte maturation in the female, and spermatogenesis and spermiation in the male, although the precise mechanisms by which each phase is controlled are by no means certain at the present time.

Much of the work on female fish has been carried out in vitro. From such work Jalabert, (1976) has suggested that oocyte development may be seperated into two distinct phases: oocyte maturation and ovulation. Oocyte maturation includes the structural and biochemical modifications which develop simultaneously with the resumption of meiosis whereas ovulation is the emission of the denuded oocyte from within the follicle.

Follicular maturation is ultimately under the control of pituitary gonadotropin which induces the follicle to synthesise specific steroids; these steroids in turn act upon the oocyte to promote maturation. Evidence has shown that in trout, 17α - hydroxy - 20β - dihydroprogesterone (17α - 20β Pg) seems to be the strongest inducer of oocyte maturation in vitro (Fostier et al, 1973).This is also true of the pike (Jalabert & Breton, 1973) and goldfish (Jalabert, 1976). The action of 17α - 20β Pg may be modified by circulating levels of

other steroids, in particular corticosteroids which tend to enhance the oocyte's sensitivity to 17α - 20β Pg (Jalabert, 1976). These corticosteroids are probably produced by the interrenal, although Colombo et al, (1973) have demonstrated their production in the ovaries of some fish species. Jalabert (1976) has suggested that the action of corticosteroids on the maturation of the oocyte may be indirect, and to some extent independent of gonadotropin control.

Ovulation in trout may be triggered in vitro by prostaglandins, particularly PGF2α, (Jalabert, 1976) probably involving activation of α - adrenergic receptor sites. In mammals, the participation of prostaglandins in the ovulation process is now well established (for reviews, see Stacey & Pandey, 1975; Jalabert & Szollosi, 1975). Jalabert, (1976) has proposed that the relay linking the end of maturation and ovulation could be the sympathetic nervous system receiving an " end of maturation " signal and then inducing the stimulation of α - adrenergic receptor sites in the ovary and follicle. These would then trigger synthesis and/or release of prostaglandins causing the contraction of follicular envelopes.

Clearly, the control of some of these stages in development using hormone manipulation offers great potential to the fish farmer. Previous work using hormone administration techniques has tended to be concentrated on the later stages of development or the time of ovulation. Over the past 50 years the hypophysation of fish by the administration of crude pituitary material has been used successfully to promote maturation of the gonads and induce spawning in species which do not spawn readily in captivity. Thus, Smigrelski, (1975) spawned summer flounders by injecting freeze-dried carp pituitary

powder (approx. 10mg/kg fish); the fish would otherwise not have spawned under laboratory conditions. Similarly, in the culture of Indian and Chinese carps it has been common practice to inject fish pituitary glands to induce spawning (Varghese, 1975). However, not every batch of pituitaries in a given species exhibits the same activity, and moreover, besides gonadotropin pituitary powder contains a number of other hormones which may not always be appropriate to the physiology of the fish or improve the quality of the genital products (Fontaine, 1976).

The development of technology to fractionate and purify fish pituitary material (Donaldson et al, 1972; Idler et al, 1975) has overcome some of the problems of injecting whole pituitary material. Thus, Sinha, (1971) injected carp with 3 different fractions of carp pituitary and found that only the second fraction contained gonadotropin activity and induced spawning. Many workers have also used mammalian hormones such as FSH,LH, mixed mammalian anterior pituitary and chorionic hormones,ACTH, and posterior pituitary hormones but these have often failed to induce courtship behaviour and ovulation due to the well - recognised species specificity to the peptide hormones. However, two species of carp, Labes robita and Cirrhina mrigala were successfully spawned by administration of pituitary extracts from 2 species of marine catfish, Tachysurus thalassus and T. jella (Varghese, 1975). Purified carp gonadotropin has also evoked a dose dependent maturation and spawning response in hypophysectomised gravid catfish (Sundararaj et al, 1976). Spawning has been induced in the female Japanese eel using salmon pituitaries or synahorin which is a purified gonadotropin (Ochiai et al,1972, 1974; Yamamoto et al, 1974a, 1974b).

Female American eels have also been induced to mature by injecting the silver migratory forms with powdered carp pituitaries (Edel, 1975). Synthetic LH - RH at a relatively high dose induced ovulation in the goldfish (Lam et al, 1976). Human chorionic gonadotropin has been successfully used to induce spawning of captive grey mullet (Kuo et al, 1973) and goldfish (Yamamoto et al, 1966). Although some of the results from the above examples are contradictory, they demonstrate that maturation and spawning can be modified by hormonal treatments. Pickford & Atz, (1957) concluded that injection of pituitary material brings about the release of mature sex products rather than the promotion of their development. Hence in the above examples, only the terminal stages of the reproductive process are being controlled. Obviously, the commercial fish farmer requires a more complete control, and for this to be achieved, techniques must be developed which control the earlier as well as the later stages of maturation.

Therefore, in salmonid culture, the main application of hormones would appear to lie in their possible use at various stages of development to increase or decrease the rate of maturation in order to obtain an altered spawning time. Thus, Funk et al, (1973) accelerated ovarian maturation in immature pink salmon with injections of purified chinook salmon gonadotropin alone, and in combination with oestradiol 17β. Oocytes containing yolk globules were evident in fish treated 3 times per week with 1.0ng/g body weight salmon gonadotropin in combination with 1.5ng/g body weight oestradiol 17β for 126 days. After 168 days they were also seen in salmon treated with the same dosage of gonadotropin alone. Schmidt et al, (1965) showed that the development of gonads in intact immature male rainbow trout was

stimulated by extracts of pituitary glands from adult Pacific salmon, the increase in testis weight being proportional to the dose. The gonads of immature female rainbow trout were stimulated to a lesser degree. Similarly, female blueback salmon injected with 6 chum salmon pituitaries spawned 1 month before the uninjected controls (Combs & Burrows, 1959). The eggs, however, were about half normal size and showed very high mortality during development.

Studies in vitro, however, have shown that salmon gonadotropin failed to induce ovulation of catfish oocytes at concentrations of 1.4ng/ml (Sundararaj et al, 1972). In a further study the Indian workers showed that C_{18} oestrogenic steroids (oestradiol 17β and 17α) were also ineffective in inducing in vitro oocyte maturation in the catfish, whilst androgenic steroids (19-nortestosterone and 11-ketotestosterone) were only marginally effective. Significant maturational ability was confined to the C_{21} steroids, the most potent being 11-deoxycortisol, 11-deoxycorticosterone and 21-deoxycortisol. Ovulation has also been induced in vitro in trout follicles by adding PGF 2α at doses of 1 and 5μg/ml (Jalabert & Szollosi, 1975). Clearly, the effects will vary depending on the stage of development at the time of treatment. The timing of hormone administration is thus important not only in relation to effectiveness, but also with regard to the quality of the mature sex products.

Some stages of reproductive function can also be advanced or delayed by synthetic drugs which act to modify the secretion of gonadotropin from the pituitary. For example, clomiphene citrate at a dose of 1 and 10μg/g body weight produced ovulation in the gravid goldfish (Pandey & Hoar, 1972), probably by competing with the natural oestrogen

for receptor binding sites in the hypothalamo-hypophyseal axis and thus effectively displacing it from receptor sites. In this way, clomiphene could mitigate the inhibitory effect of oestrogens on the hypothalamo-hypophyseal axis and thereby trigger the release of gonadotropins. This gonadotropin surge induced by clomiphene may be responsible for the induction of ovulation (Pandey et al, 1973; Pandey & Stacey, 1975). A similar mode of action has been suggested for clomiphene in mammals (Roy et al, 1964; Igarashi et al, 1967; Kato et al, 1968; Kahwanago et al, 1970; Schally et al, 1970). In man it is as yet unclear whether the mode of action of clomiphene is a direct one on the ovary, (Smith, 1966; Papanicolaou et al, 1970) or an indirect one through the hypothalamo-hypophyseal axis (Jacobson et al, 1968; Gambrell et al, 1971).

The inhibitory effect of methallibure on pituitary gonadotropin secretion has been used to block the initiation of spermatogenesis in juvenile guppies and also for inhibiting spermatogenesis in adults (Martin & Bromage, 1970; Pandey & Leatherland, 1970). This effect has also been observed in the goldfish, stickleback and sea perch (Hoar et al, 1967). However, methallibure is known to affect the thyroid and the gonads directly and both may complicate treatment. Generally, the precise effects of such compounds in fish are unclear although their modification of reproductive function may be important to the commercial fish farmer.

A number of authors have realised the commercial potential for the application of drugs and hormones which modify maturation. These have been reviewed recently by Fontaine (1976). However, as expected, the response appears to depend upon the stage of the reproductive

cycle when the drug or hormone is administered. Furthermore, the repeated administration of hormones over extended periods of time as carried out by Funk et al, (1973), is likely to be far too laborious and expensive to be adopted commercially. Methods must be perfected which require minimal fish treatment at low cost. These will only be developed when a more complete understanding of the exact endocrinological processes that take place during maturation is achieved. The present work therefore examines the effects of the injection of oestradiol 17β on serum levels of vitellogenin and total calcium in order to gain a better understanding of the timing of the changes associated with maturation. The experiments described in this chapter were performed in collaboration with Mr. J.A.K. Elliot, an undergraduate project student at Aston University.

4.2. Materials and Methods.

4.2.1. Fish Maintenance and Handling: The system used to maintain the fish during these experiments was constructed in the Fish Culture Unit, Aston University, and was almost entirely recirculating, " make up " water being sufficient only to account for spillage and evaporation. The system consisted of 3 circular tanks 1m in diameter, gravity fed with water from a header tank at a flow rate of 4l/min through each tank. The depth of water in each tank was 35cm, this level being maintained by a single central outlet pipe in each tank, which in turn connected to a single waste pipe emptying into a faecal trap. The faeces and other waste collected in the bottom of the faecal trap and were removed daily. The outflow from the top of the faecal trap fed directly onto a gravel filter bed. The unfiltered water was washed through the filter with water from the overflow of the header tank. An overflow from the gravel filter bed took any surplus water to waste. A diagram of the system is shown in Fig.4.1. Routine measurements of oxygen, ammonia, nitrates, pH and temperature ensured that water quality was maintained consistently throughout all experiments.

The handling, tagging, weighing, injection and blood sampling were carried out under anaesthesia using methods already described in Chapter 2.1. Post-anaesthetised fish were placed in a recovery tank before being returned to the system. Fish to be anaesthetised were not fed for 24 hrs. beforehand.

4.2.2.: Experimental Proceedures: Three separate experiments were conducted as detailed below.

Experiment 1: The aim of this experiment was to observe the timing of

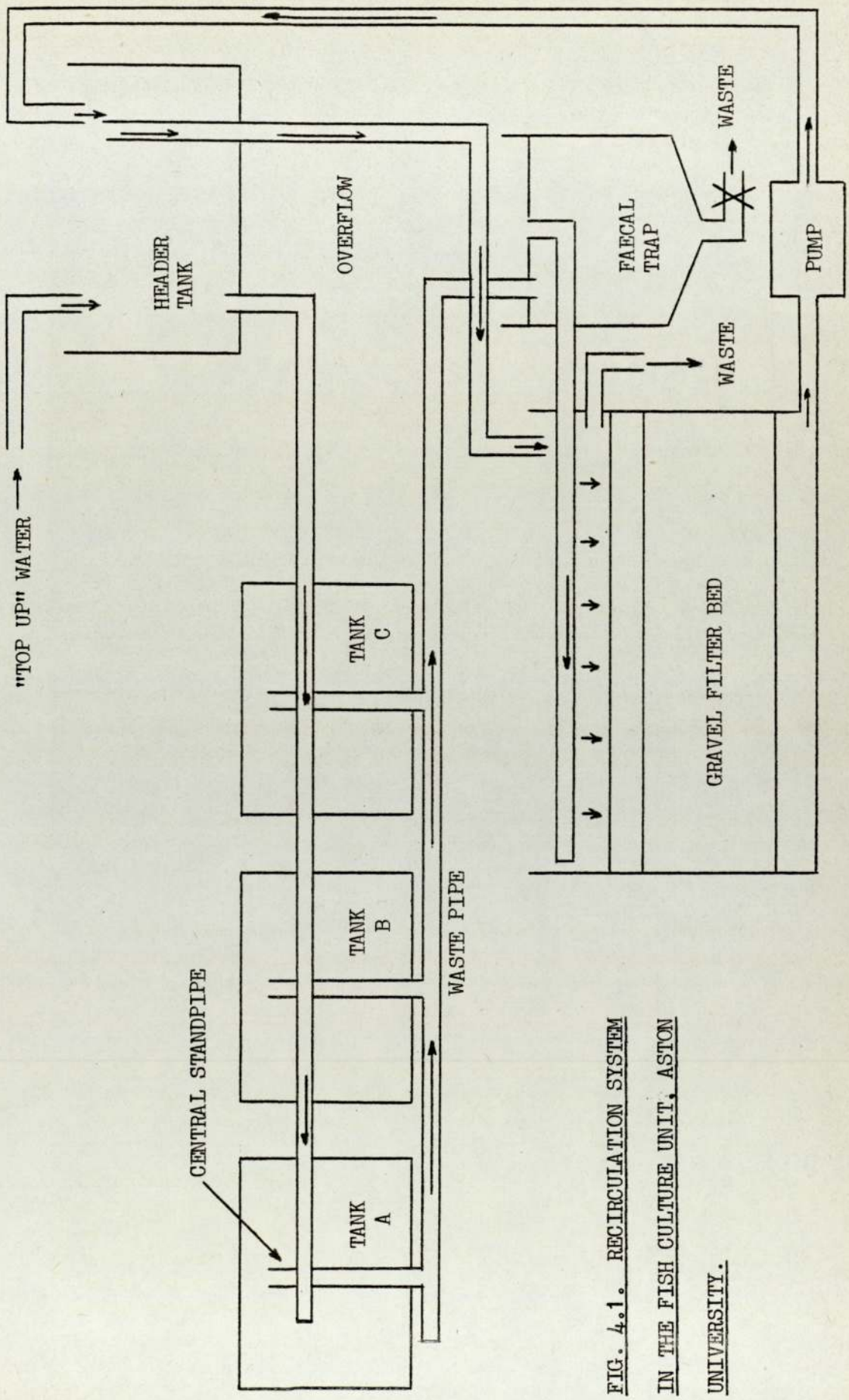


FIG. 4.1. RECIRCULATION SYSTEM IN THE FISH CULTURE UNIT, ASTON UNIVERSITY.

changes in serum levels of vitellogenin and total calcium after single and multiple injections of oestradiol 17β.

Towards the end of October, 1977, 50 immature fish were selected with weights between 160 and 260g. 5 were taken at random and bled to obtain base line values of vitellogenin and total calcium in the serum. The fish were then divided at random into 3 tanks and allowed to acclimatise for 6 days.

Following the acclimatisation period, 25 fish were divided into 5 groups (A - E) and injected intraperitoneally with oestradiol 17β. The oestradiol 17β was first dissolved in a minimum quantity of ethanol (1.5ml) and then made up to an emulsion with Arachis oil (8.5ml). The dose given was arranged so that each fish received 5μg/g body weight in a final injection volume of 200μl. The remaining 25 fish, injected with 200μl Arachis oil/ethanol emulsion in the same ratio, served as controls. Each group was identified by fin clipping, and the control and treated fish mixed and distributed between 2 tanks. The protocol for the experiment is shown in Fig. 4.2. Fish in group A were sampled 12 hours after injection of oestradiol or carrier and those in group B after 24 hours. Both groups were subsequently given daily injections of either oestradiol or carrier for the next 3 days. Groups C, D and E were bled at days 2, 3 and 4 respectively and subsequently on the days specified in Fig. 4.2. The fish in groups A and B suffered high mortality,and bleeding of these fish was discontinued after day 4. The serum samples obtained were stored at -20 °C until assayed for the serum oestradiol 17β, total calcium and vitellogenin using the methods described in Chapter 2.

TIME (DAYS)	GROUP A	GROUP B	GROUP C	GROUP D	GROUP E
0	I	I	I	I	I
0.5	S				
1	I	I+S			
2	I	I	S		
3	I	I		IS	
4	I+S	I+S			S
5					
6					
7					
9			S		
11				S	
14					S
17			S		
21				S	
25					S
46			S		
65				S	
85					S
112			S		

I = FISH INJECTED WITH OESTRADIOL 17β AT 5 μG/G BODY WEIGHT.

S = BLOOD SAMPLING.

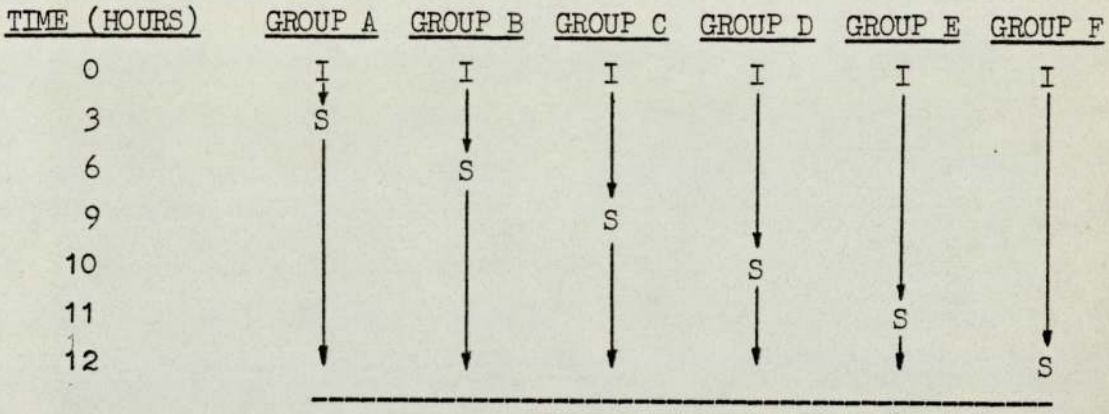
FIG. 4.2. EXPERIMENTAL PROCEEDURE FOLLOWED DURING EXPERIMENT 1.

Experiment 2: Preliminary results from experiment 1 showed a 3-fold increase in the total serum calcium, and a 30-fold increase in phosphoprotein phosphorus after only 12 hours. The aim of this experiment was therefore to observe in more detail, the changes in these blood parameters over the first 12 hours following a single injection of oestradiol 17β.

At the end of November, 1977, a further 48 immature fish in the 150 - 200g range were selected. 5 were bled to obtain base line values for oestradiol 17β, total calcium and phosphoprotein phosphorus in the serum. The fish were divided equally between 2 tanks and allowed to acclimatise for 7 days. Following this period, 24 fish were injected with oestradiol 17β at a dose rate of 5μg/g body weight, as in the previous experiment. A further 24 fish injected with the same volume of Arachis oil/ethanol suspension served as controls. The fish were fin-clipped, mixed and distributed equally between 2 tanks.

The proceedure following the start of the experiment is shown in Fig. 4.3. 3 hours after injection 8 fish (4 treated and 4 controls) were removed, blood samples taken and the serum frozen. A further 4 treated and 4 control fish were removed and bled at 6, 9, 10, 11 and 12 hours after injection. After blood sampling, the fish were held in a separate tank to prevent individual fish from being bled twice during the experiment.

Experiment 3: The aim of this experiment was to investigate the response of the vitellogenic processes of the liver to varying doses of oestradiol 17β. In January, 1978, 31 immature rainbow trout were anaesthetised and tagged. Blood samples were taken from each



I = FISH INJECTED WITH OESTRADIOL 17β AT 5 μG/G BODY WEIGHT.

S = BLOOD SAMPLING.

FIG 4.3. EXPERIMENTAL PROCEEDURE FOLLOWED DURING EXPERIMENT 2.

individual fish to obtain base line values for oestradiol 17β, total calcium and phosphoprotein phosphorus in the serum before the commencement of treatment. A period of 7 days was then allowed for the fish to acclimate. The experimental proceedure followed after this period is shown in Fig. 4.4. 4 groups (A, B, C and D) were injected intraperitoneally with 0.005, 0.05, 1.0 and 20.0μg/g body weight respectively. In all cases adjustments were made so that a final injection volume of 200μl could be administered. The remaining 7 fish, (group E) were injected with 200μl Arachis oil/ethanol emulsion to serve as controls.

All the fish were anaesthetised and blood samples taken 48 hours after the injections and the serum thus obtained assayed for oestradiol 17β, total calcium and phosphoprotein phosphorus. Surprisingly, no increase in either serum phosphoprotein phosphorus or total calcium occurred (reported in detail later). In view of these results further experiments were carried out to investigate the effects that maturity, varying weight and previous oestradiol 17β treatment might have on the response of serum levels of total calcium and phosphoprotein phosphorus to a standard injection of oestradiol 17β. The experiments were performed as follows:-

(i) At the beginning of March, 1978, 8 immature fish were selected in the weight range 50 - 250g and allowed to acclimate for 4 days. All the fish were bled to obtain base line values for oestradiol 17β, total calcium and phosphoprotein phosphorus in the serum. An intraperitoneal injection of oestradiol 17β at a dose of 5μg/g body weight was administered. The oestradiol 17β was prepared for injection as in the previous experiments. Blood samples were taken 48 hours after injection

TIME (HOURS)	A	B	C	D	E
	(0.005 µg/g)	(0.05 µg/g)	(1.0 µg/g)	(20.0 µg/g)	(CONTROL)
0	I	I	I	I	I
	↓	↓	↓	↓	↓
48	S	S	S	S	S

I = FISH INJECTED WITH STATED DOSE OF OESTRADIOL 17β.

S = BLOOD SAMPLING.

FIG. 4.4. EXPERIMENTAL PROCEEDURE FOLLOWED DURING EXPERIMENT 3.

and the resulting serum assayed.

(ii) In April, 1978, 4 recently spawned females, together with 4 immature control fish and 4 previously treated with oestradiol 17β in October, 1977, were bled to obtain base line values. 1 week later all the fish were given an intraperitoneal injection of oestradiol 17β at a dose rate of 2.5μg/g body weight, prepared as previously. All 12 fish were held in the same tank for the duration of the experiment and bled after 48 hours. The serum thus obtained was assayed for serum phosphoprotein phosphorus and total calcium.

4.3. Results.

Experiment 1: In all fish, similar basal levels of serum oestradiol 17β (472 ± 212pg/ml), total calcium (11 ± 1mg%) and phosphoprotein phosphorus (21 ± 5μg/ml) were observed prior to the start of the experiment. In the oestradiol 17β treated group gross changes were observed in all three parameters during the course of the experiment.

The serum levels of total calcium increased significantly after 12 hours to 41mg%, ($P<0.01$) rising to a maximum level of 75mg% 21 days after injection ($P<0.001$). By 112 days following injection the serum levels of total calcium had almost returned to basal, falling to 22mg% (Table 4.1, Fig. 4.5). In the control fish, there was no significant change from basal levels throughout the experiment.

The changes observed in serum levels of phosphoprotein phosphorus followed a similar pattern to those for total serum calcium. After 12 hours, the serum levels had increased significantly ($P<0.001$) from basal to reach 307μg/ml, then continued to increase to reach a maximum level of 832μg/ml after 21 days ($P<0.001$). Levels then began to fall gradually and by 112 days were returning to basal at 107μg/ml (Table 4.2, Fig. 4.6). In the control group, no significant change was observed in the serum levels of phosphoprotein phosphorus throughout the course of the experiment.

The serum oestradiol 17β profile differed markedly from those for total serum calcium and serum phosphoprotein phosphorus (Table 4.3, Fig. 4.7). There was a significant increase in the levels of serum oestradiol 17β, after 12 hours to 10,875pg/ml ($P<0.05$), rising to a maximum value of 38,000pg/ml 2 days after injection ($P<0.001$).

TIME(DAYS)	0	0.5	1	2	3	4	9	11	14	17	21	25	46	65	85	112
TREATED	11.0	41.1	34.2	38.1	49.6	41.9	44.5	59.7	52.3	53.3	75.2	72.3	51.8	54.1	47.7	22.5
± S.E.	1.0	2.9	5.9	7.5	2.1	10.2	8.3	4.9	10.2	8.4	6.0	4.9	8.6	6.7	15.1	2.4
CONTROL	11.0	11.8	11.6	11.4	11.7	11.1	12.6	13.3	14.7	14.1	14.9	15.1	16.3	13.9	15.4	13.1
± S.E.	1.0	0.3	0.7	0.4	0.3	0.4	0.8	0.7	0.7	0.6	1.3	0.9	1.3	0.6	0.4	0.7
P VALUE	——	<.01	<.01	<.01	<.001	<.01	<.01	<.001	<.001	<.01	<.001	<.001	<.01	<.001	<.01	<.01

TABLE 4.1. MEAN TOTAL SERUM CALCIUM LEVELS DURING EXPERIMENT 1. (ALL VALUES IN MG%).

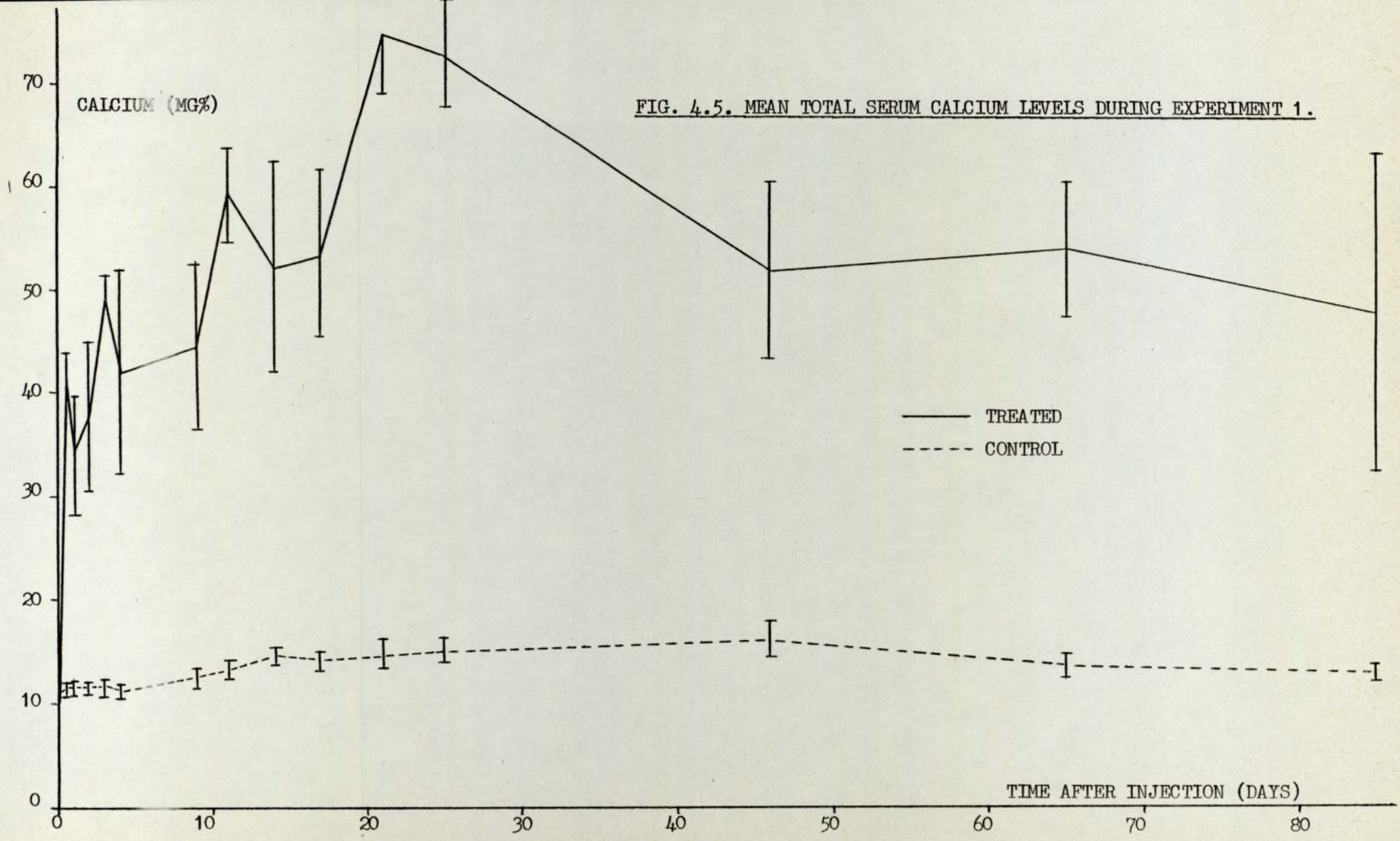


FIG. 4.5. MEAN TOTAL SERUM CALCIUM LEVELS DURING EXPERIMENT 1.

TIME(DAYS)	0	0.5	1	2	3	4	9	11	14	17	21	25	46	65	85	112
TREATED	21.0	307.0	308.0	351.0	438.0	292.0	316.0	543.0	449.0	364.0	832.0	745.0	436.0	385.0	400.0	107.0
± S.E.	5.0	23.0	76.0	77.0	32.4	106.6	109.6	56.7	117.0	104.0	95.2	54.4	77.1	132.8	85.7	18.8
CONTROL	21.0	14.0	10.2	23.2	7.4	10.2	36.8	43.2	25.2	47.5	110.2	116.1	76.2	23.0	24.3	17.5
± S.E.	5.0	4.6	2.6	6.3	5.4	2.5	12.2	16.3	8.6	16.7	24.8	22.0	14.8	8.8	7.6	3.4
P VALUE	----	<.001	<.01	<.01	<.001	<.01	<.025	<.001	<.001	<.025	<.001	<.001	<.001	<.025	<.025	<.05

TABLE 4.2. MEAN SERUM PHOSPHOPROTEIN PHOSPHORUS LEVELS DURING EXPERIMENT 1. (ALL VALUES IN µG/ML).

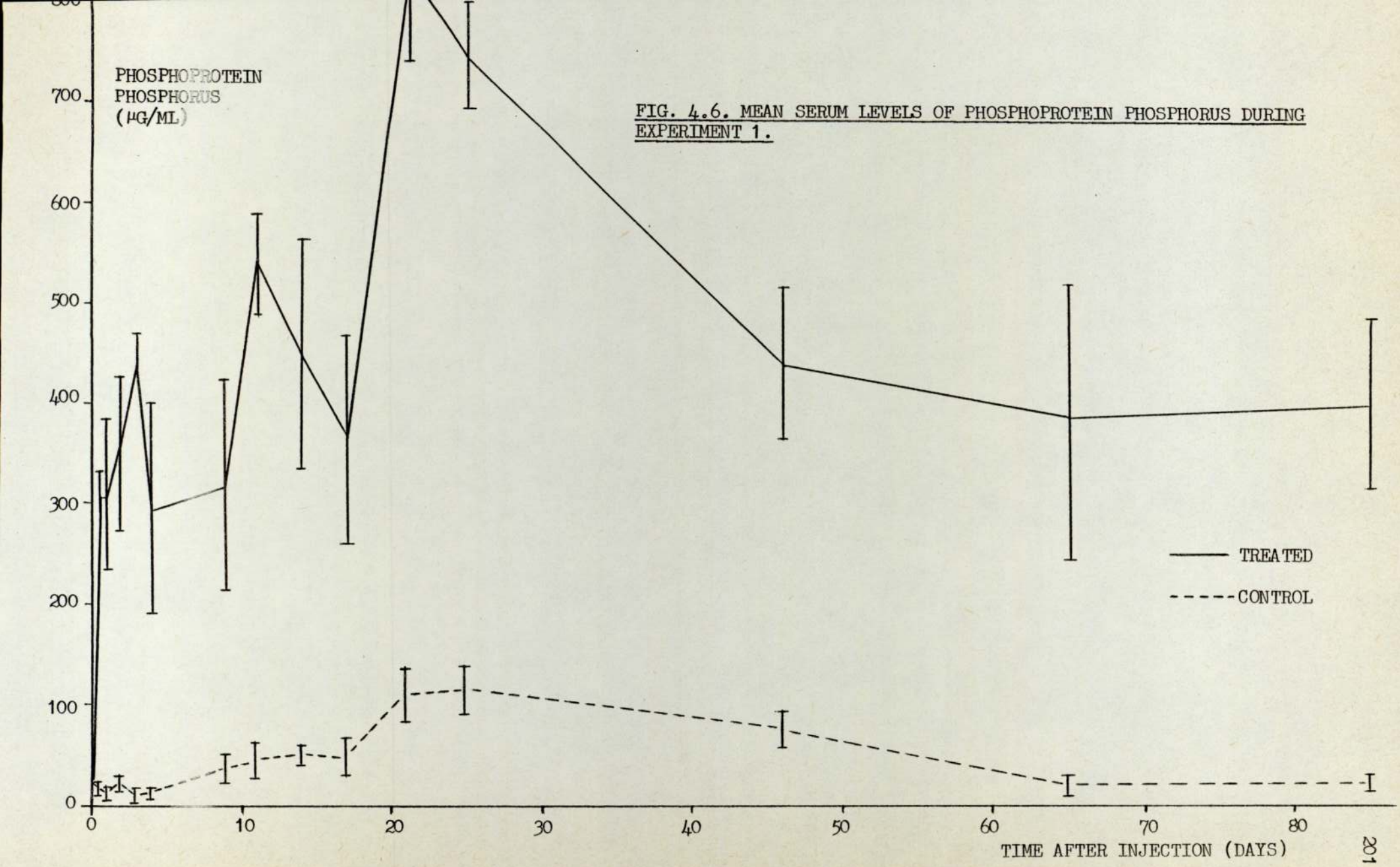


FIG. 4.6. MEAN SERUM LEVELS OF PHOSPHOPROTEIN PHOSPHORUS DURING EXPERIMENT 1.

TIME(DAYS)	0	0.5	1	2	3	4	9	11	14	17	21	25	46	65	85
TREATED	472	10875	5375	38000	4750	6733	1637	850	805	855	620	535	249	305	328
± S.E.	212	5026	125	5612	1376	1649	192	206	458	323	238	180	130	20	87
CONTROL	472	445	370	600	708	863	405	833	638	450	413	275	257	392	480
± S.E.	212	185	161	291	282	334	147	268	115	98	91	117	39	130	91
P VALUE	----	<.05	<.01	<.001	<.01	<.05	<.05	----	----	----	----	----	----	----	----

TABLE 4.3. MEAN SERUM LEVELS OF OESTRADIOL 17β DURING EXPERIMENT 1. (ALL VALUES IN PG/ML).

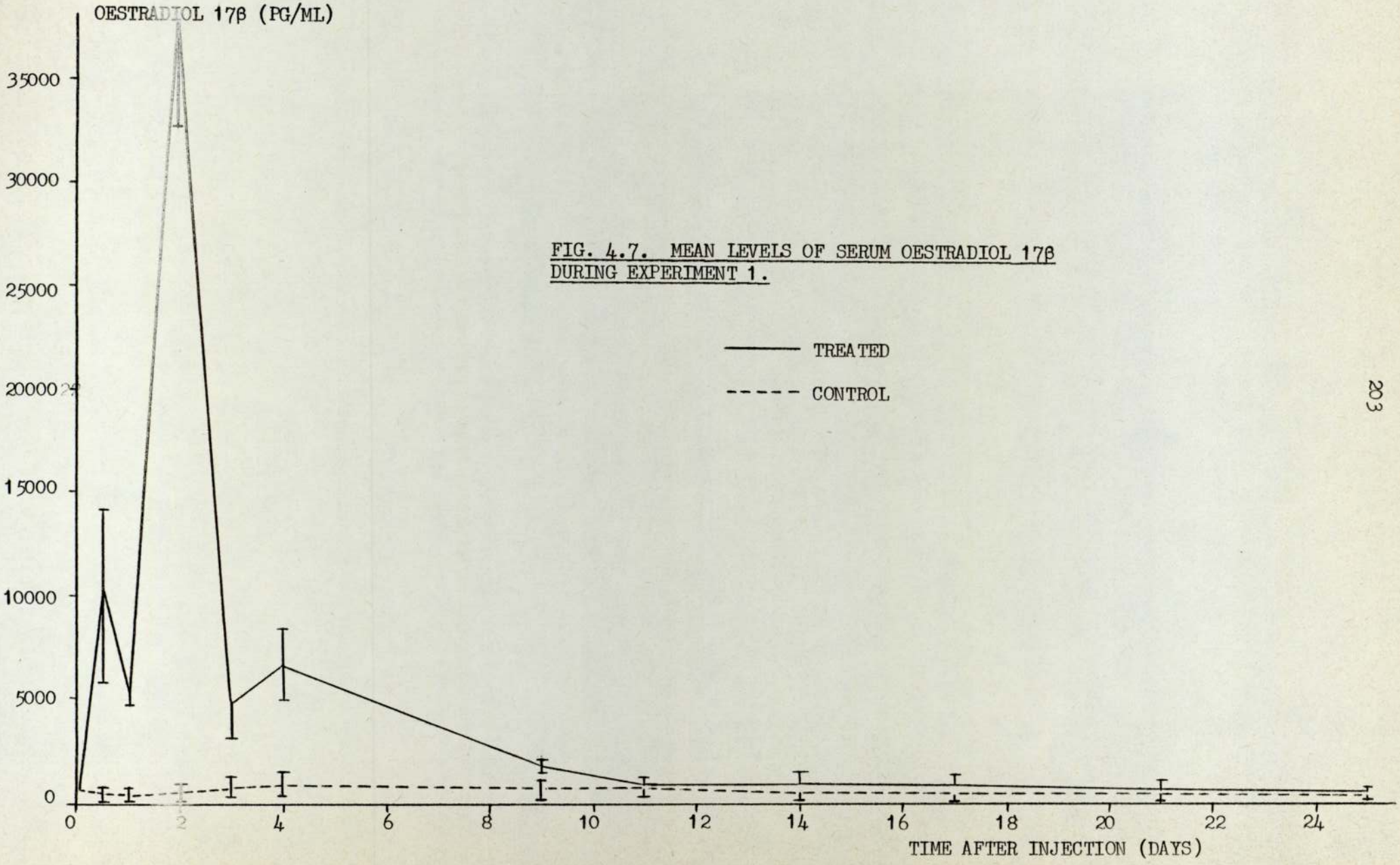


FIG. 4.7. MEAN LEVELS OF SERUM OESTRADIOL 17β DURING EXPERIMENT 1.

By 11 days following injection the levels had returned to basal. Throughout the course of the experiment no significant change was observed in the serum levels of oestradiol 17β in the control fish.

Experiment 2: Before treatment, basal levels of total serum calcium (9.58 ± 0.6mg%), phosphoprotein phosphorus (16.4 ± 4.4µg/ml), and serum oestradiol 17β (452 ± 145pg/ml) were established. Over the course of 12 hours, significant changes were observed in the serum levels of total calcium and phosphoprotein phosphorus following the single injection of oestradiol 17β.

After 12 hours, serum levels of total calcium had increased significantly to reach 33 ± 2mg% ($P<0.001$). No such change was observed in the control fish (Table 4.4 , Fig. 4.8).

Similarly, after 12 hours, serum levels of phosphoprotein phosphorus had increased significantly in the treated fish to reach 323 ± 52µg/ml ($P<0.001$). Again, no significant change from basal levels was observed in the control fish throughout the duration of the experiment (Table 4.5, Fig.4.9). A significant increase in serum levels of oestradiol 17β ($P<0.05$) was seen in the treated fish after 3 hours (Table 4.6, Fig. 4.10). Levels increased to reach a maximum of 10,000pg/ml 12 hours after injection ($P<0.001$). The levels in the control fish showed no significant alteration throughout the experiment.

TIME (HOURS)	0	3	6	9	10	11	12
TREATED	9.58	11.42	11.44	12.33	12.80	15.30	33.2
± SE	0.6	0.5	0.4	1.23	1.9	0.7	2.0
CONTROL	9.58	11.01	11.04	11.60	11.4	11.3	11.4
± SE	0.6	0.4	0.4	0.7	0.7	0.7	0.7
P.VALUE	-	-	-	-	-	-	<.001

TABLE 4.4. MEAN TOTAL SERUM CALCIUM LEVELS DURING EXPERIMENT 2. (ALL VALUES IN MG%).

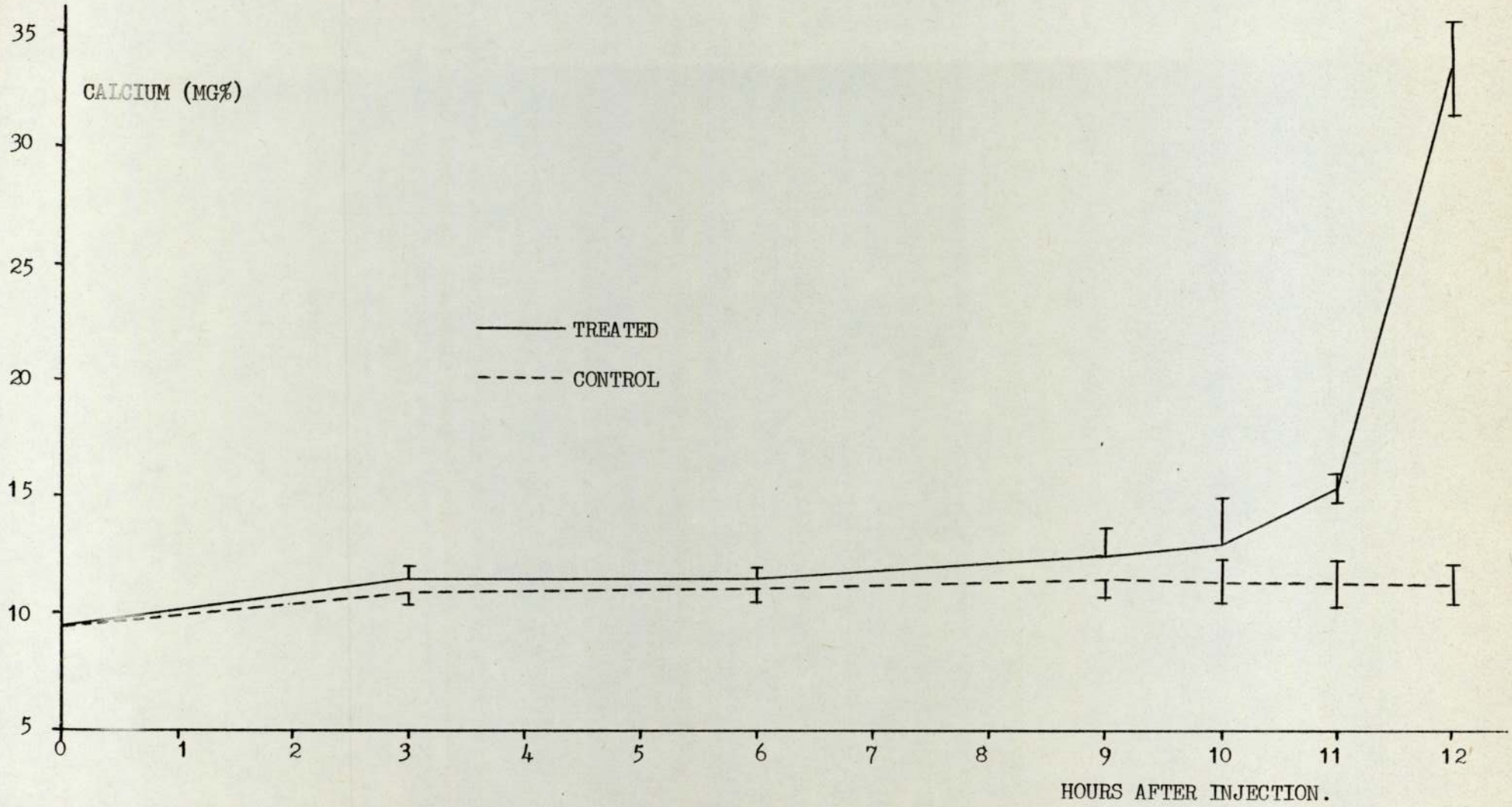


FIG. 4.8. MEAN TOTAL SERUM CALCIUM LEVELS DURING EXPERIMENT 1.

TIME (HOURS)	0	3	6	9	10	11	12
TREATED	16.4	26.0	20.0	17.1	21.5	59.5	323.0
± SE	4.4	9.3	12.0	28.7	19.8	20.0	52.0
CONTROL	16.4	16.0	16.3	18.1	21.8	8.9	9.0
± SE	4.4.	5.4	12.0	8.5	13.7	5.6	3.5
P.VALUE	-	-	-	-	-	-	<.001

TABLE 4.5. MEAN LEVELS OF PHOSPHOPROTEIN PHOSPHORUS IN SERUM DURING EXPERIMENT 2.(ALL VALUES IN µG/ML).

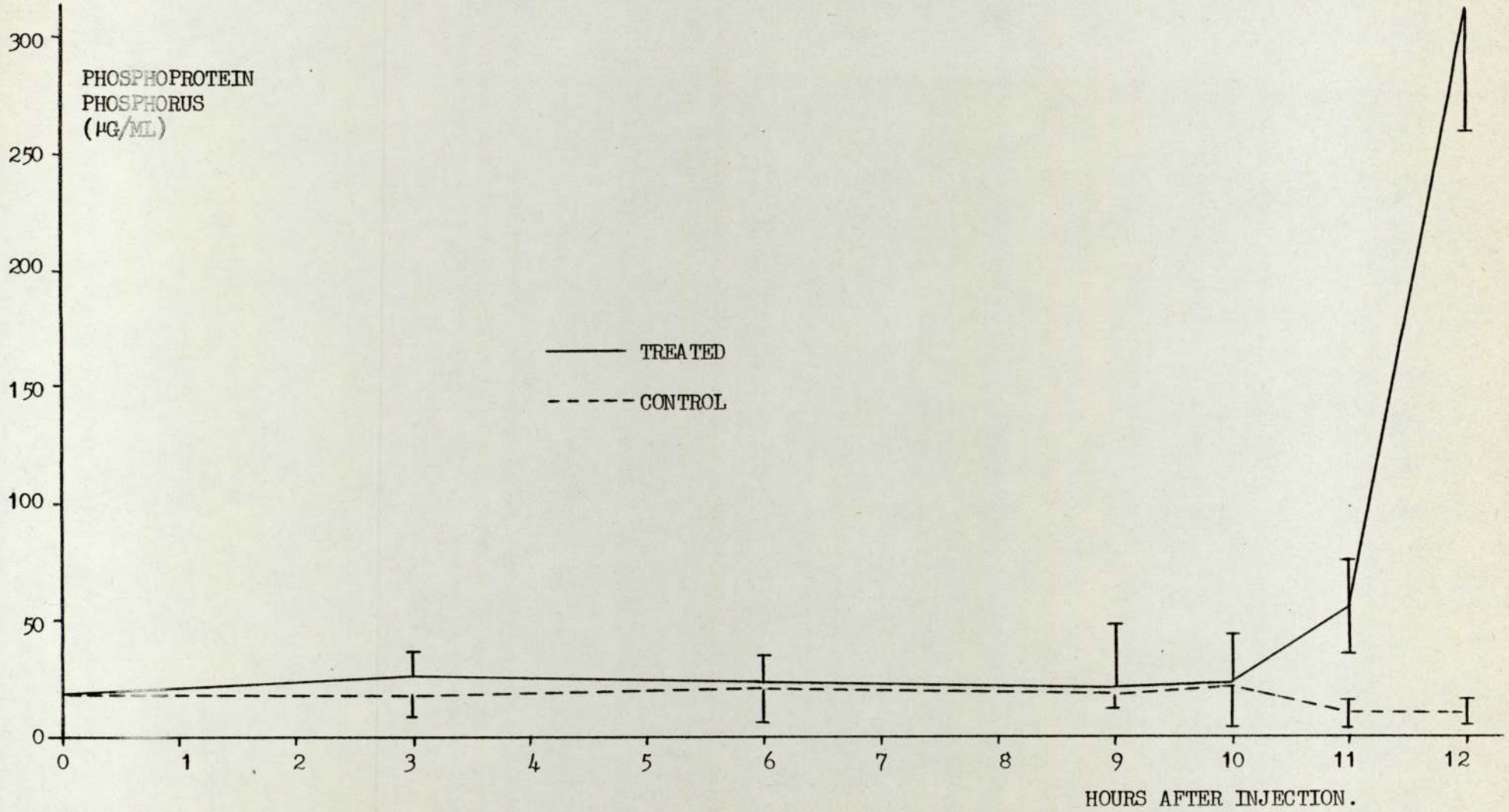


FIG. 4.9. MEAN SERUM PHOSPHOPROTEIN LEVELS DURING EXPERIMENT 2.

TIME (HOURS)	0	3	6	9	10	11	12
TREATED	452	2225	5100	4933	9188	5300	10250
± SE	145	729	839	2579	4516	2128	433
CONTROL	452	322	538	658	368	385	457
± SE	145	37	363	98	130	50	89
P.VALUE	-	<.05	<.05	-	<.05	-	<.001

TABLE 4.6. MEAN SERUM LEVELS OF OESTRADIOL 17β DURING EXPERIMENT 2. (ALL VALUES IN PG/ML).

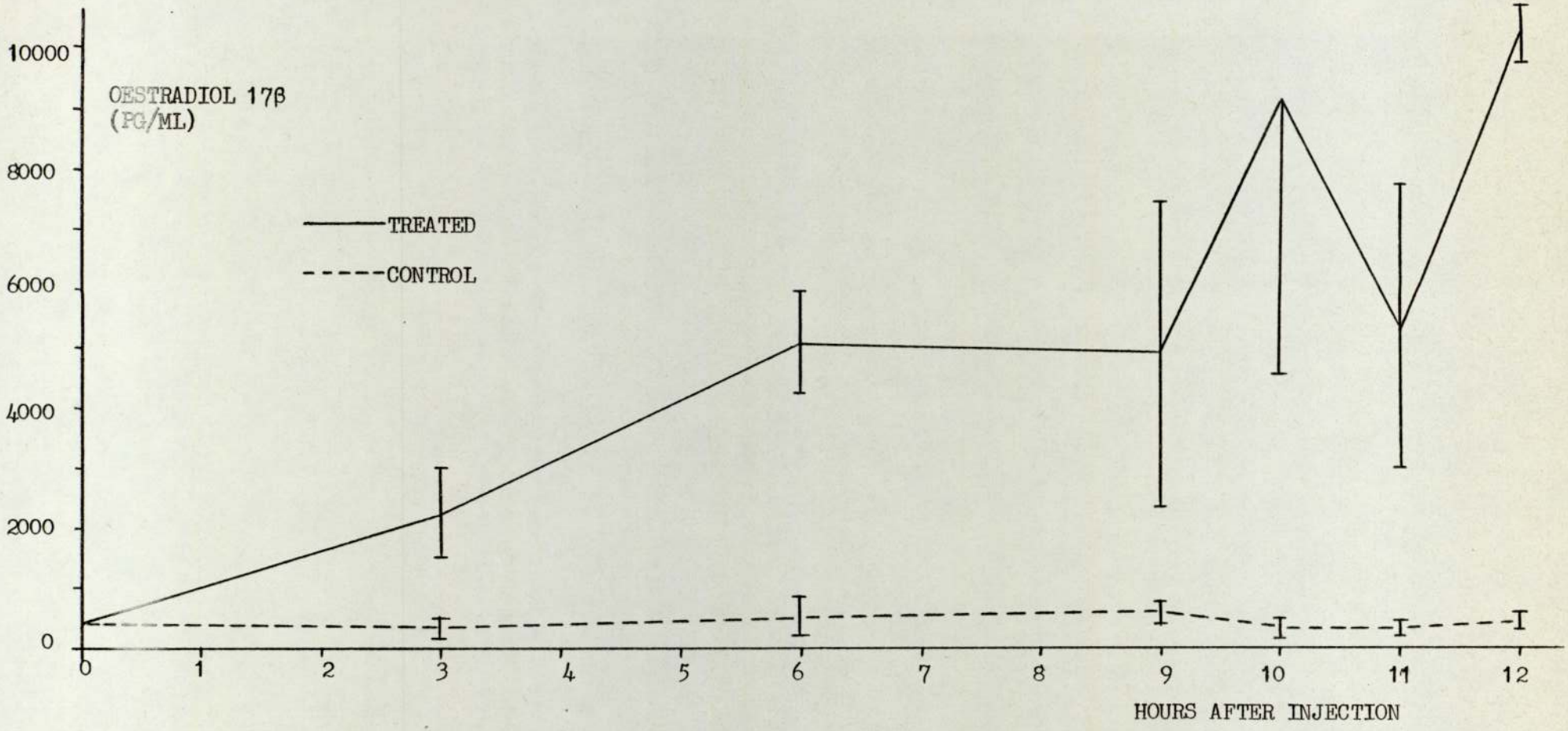


FIG. 4.10. MEAN SERUM LEVELS OF OESTRADIOL 17β DURING EXPERIMENT 2.

Experiment 3: The serum levels of oestradiol 17β in the treated fish showed a significant increase after 48 hours in all 4 groups (Table 4.7, Fig. 4.11). Those fish injected with 20.0μg/g body weight oestradiol 17β showed a value of 38,000pg/ml at 48 hours ($P<0.001$). Those fish injected with 1.0, 0.05, and 0.005μg/g body weight oestradiol 17β showed mean 48 hour values of 4963pg/ml ($P<0.01$), 1360pg/ml ($P<0.05$) and 967pg/ml ($P<0.05$) respectively. No significant change was observed in the control group during the same period.

However, in all cases, no change in the serum levels of total calcium or phosphoprotein phosphorus was observed (Table 4.7). This result is in contrast with those from the previous 2 experiments.

Effect of Fish Size on Response to Oestradiol: Despite the significant increase in serum oestradiol 17β no treated fish, irrespective of weight, showed any change in either total serum calcium or phosphoprotein phosphorus after 2 days, the values being similar to those obtained prior to treatment.(Table 4.8).

Effect of Maturation on Response to Oestradiol: Again there were no changes in serum total calcium and phosphoprotein phosphorus levels after 30 hours in any of the post-spawned, previous oestradiol treated or untreated groups. This result is again in contrast to the findings from experiments 1 and 2, where injection of oestradiol 17β caused significant increases in these serum parameters.(See Tables 4.9. and 4.10.).

DOSE	TIME (HOURS)	CALCIUM (MG%)	PHOSPHOPROTEIN PHOSPHORUS (μG/ML)	OESTRADIOL 17β (PG/ML)
CONTROL	0	9.70 ± 0.54	15.57 ± 4.23	392.0 ± 87.7
	48	9.90 ± 0.30	13.80 ± 3.30	514.0 ± 79.4
	P.VALUE	-	-	-
0.005μG/G	0	10.47 ± 0.20	18.00 ± 2.90	498.8 ± 68.9
	48	9.98 ± 0.49	21.33 ± 5.58	966.7 ± 122.0
	P.VALUE	-	-	(<.05)
0.05μG/G	0	10.49 ± 0.37	16.80 ± 2.20	409.0 ± 71.0
	48	9.98 ± 0.29	17.70 ± 2.49	1360.0 ± 237.0
	P.VALUE	-	-	(<.05)
1.0μG/G	0	9.87 ± 0.44	16.40 ± 2.30	432.50 ± 122.5
	48	9.52 ± 0.18	14.0 ± 2.62	4962.5 ± 1621.4
	P.VALUE	-	-	(<.01)
20.0μG/G	0	9.69 ± 0.37	11.33 ± 3.38	546.0 ± 53.3
	48	9.63 ± 0.23	14.36 ± 2.87	38,330 ± 7378
	P.VALUE	-	-	(<.001)

TABLE 4.7. THE EFFECT OF VARIOUS DOSES OF OESTRADIOL 17β ON SERUM LEVELS OF PHOSPHOPROTEIN PHOSPHORUS AND TOTAL CALCIUM DURING EXPERIMENT 3.

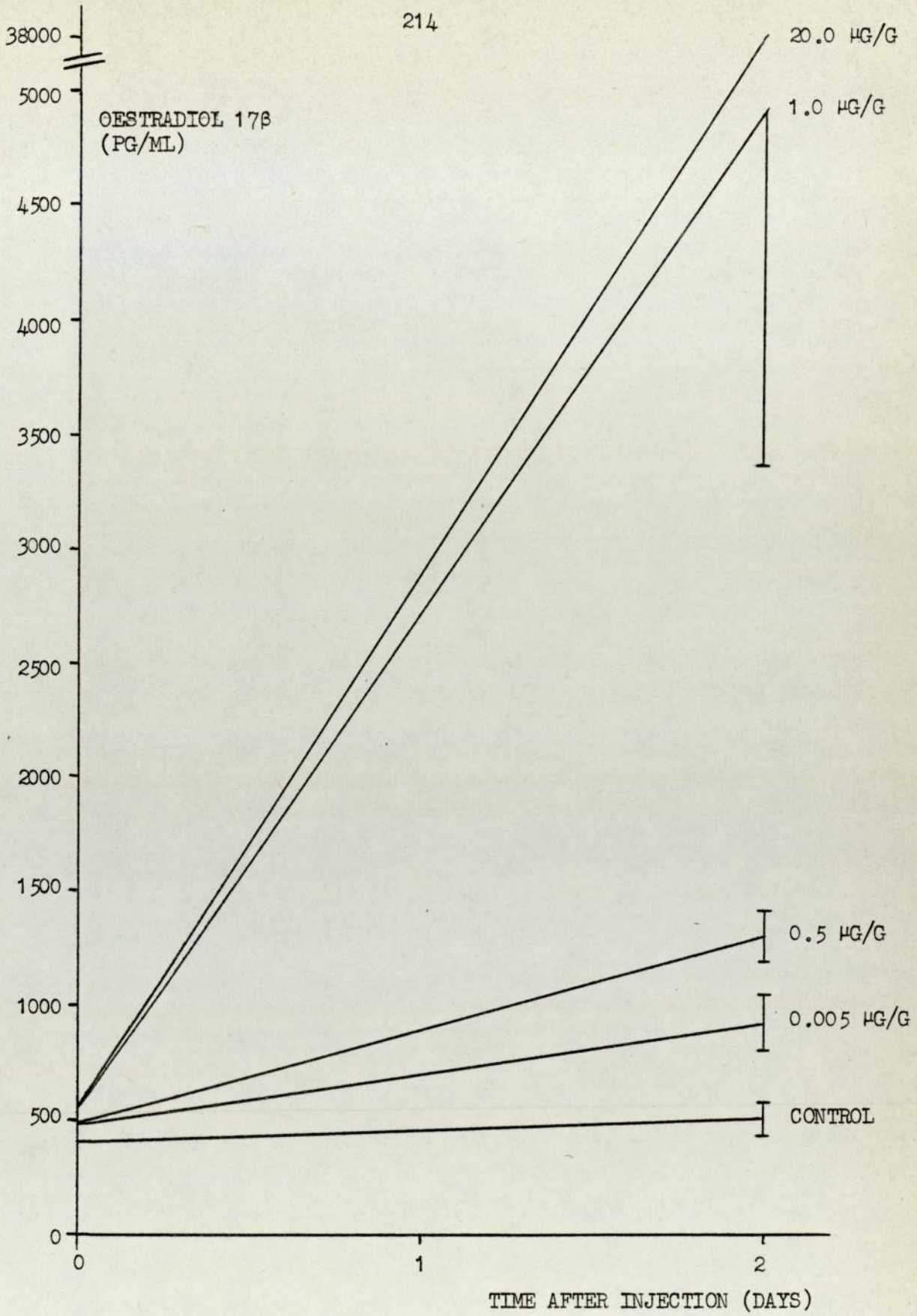


FIG. 4. 11. 2 DAY SERUM LEVELS OF OESTRADIOL 17β AFTER DIFFERENT INJECTED DOSES.

FISH WEIGHT (G)	SERUM CALCIUM (MG%)		PHOSPHOPROTEIN (µG/ML) PHOSPHORUS	
	DAY 0	DAY 2	DAY 0	DAY 2
60	9.16	10.83	15.32	10.77
70	7.51	8.01	18.46	14.38
80	9.94	10.82	12.44	15.38
90	8.96	9.03	17.34	18.10
120	8.74	9.68	13.47	21.20
130	10.36	9.82	16.43	16.70
230	16.34	14.87	21.61	18.72
250	16.42	14.05	27.30	16.51

TABLE 4.8. THE EFFECT OF FISH WEIGHT ON THE RESPONSE OF SERUM LEVELS OF PHOSPHOPROTEIN PHOSPHORUS AND TOTAL CALCIUM TO A SINGLE INJECTION OF OESTRADIOL 17β.

FISH WEIGHT (G)	TOTAL CALCIUM (MG/%)		PHOSPHOPROTEIN (µG/ML) PHOSPHORUS	
	0 HOURS	30 HOURS	0 HOURS	30 HOURS
(a) POST-SPAWNED:				
620	11.97	10.65	21.80	10.20
570	13.80	10.16	14.00	17.50
580	12.43	10.93	16.00	23.20
590	9.33	8.95	16.30	18.90
(b) PREVIOUS TREATED:				
350	14.35	14.78	22.00	15.50
260	15.10	14.92	14.75	13.40
240	12.55	12.84	12.67	13.65
300	12.89	14.67	18.35	18.30
(c) PREVIOUS CONTROL:				
330	9.81	10.44	13.00	12.12
320	14.92	14.53	16.00	14.78
280	11.71	10.98	6.00	15.20
270	10.63	10.27	14.00	17.80

TABLE 4.9. THE EFFECT OF MATURITY AND PREVIOUS TREATMENT ON THE RESPONSE OF SERUM LEVELS OF PHOSPHOPROTEIN PHOSPHORUS AND TOTAL CALCIUM TO A SINGLE INJECTION OF OESTRADIOL 17β.

GROUP	TOTAL CALCIUM (MG%)			PHOSPHOPROTEIN (µG/ML) PHOSPHORUS		
	0 HOURS	30 HOURS	P	0 HOURS	30 HOURS	P
(a)	11.88	10.17	-	17.00	17.40	-
± SE	0.94	0.44		1.06	1.13	
(b)	13.72	14.30	-	16.60	15.2	-
± SE	0.60	0.49		2.10	1.48	
(c)	11.77	11.60	-	12.20	14.60	-
± SE	1.12	1.00		2.54	0.88	

TABLE 4. 10. MEAN SERUM LEVELS OF TOTAL CALCIUM AND PHOSPHOPROTEIN PHOSPHORUS SHOWN IN TABLE 4. 9.

4.4. Discussion.

The results clearly demonstrate that from the beginning of October to December single injections of oestradiol 17β were able to induce increases in the serum levels of vitellogenin and total calcium which were similar to those observed in older fish during the course of normal maturation (see Chapter 3).

During the first 48 hours following injection, the release of oestradiol 17β into the circulation would appear to be linear. After this period, the serum levels fell rapidly and reached near-control values 11 days after treatment. This rapid decline in serum levels of oestradiol 17β following the linear increase correlates closely with the data of Johnstone et al, (1978) who showed that the half-life of oestradiol 17β in trout serum is approximately 12 hours.

In immature rainbow trout, during the months of October, November and December, vitellogenin appears in significant quantities in the serum 12 hours after a single injection of oestradiol 17β. Similar results have also been reported in amphibians where vitellogenin appeared in the serum between 9 and 12 hours after a single injection of oestradiol 17β (Wittliff & Kenney, 1973; Zelson & Wittliff, 1973). Since, in the present work, significantly raised serum levels of oestradiol 17β did not occur until 3 hours following injection, it would appear that the enzymes necessary for the biosynthesis of the vitellogenic components in the liver are activated very soon after stimulation by oestradiol 17β.

Although the increased serum levels of oestradiol 17β are short-lived, returning to basal values 11 days following injection,

the highest levels of total serum calcium and phosphoprotein phosphorus did not occur until 21 days following treatment and did not return to control values until 135 days. It appears therefore, that a single injection of oestradiol 17β is sufficient to initiate a long-lasting synthesis of vitellogenin by the liver although it is exposed to oestrogen for only a short period of time. A similar phenomenon was observed during the normal spawning cycle (see Chapter 3) where the maximum level of oestradiol 17β occurred in October, whereas serum levels of total calcium and phosphoprotein phosphorus continued to rise long after this time, reaching their highest levels just prior to spawning in January at a time when serum oestradiol 17β levels had returned to control values. Thus, increased serum levels of vitellogenin are maintained following stimulation even though serum levels of oestradiol 17β are low.

The serum levels of total calcium and phosphoprotein phosphorus observed during these experiments were higher following treatment than those observed during the normal course of maturation. However, comparison of these levels is difficult because firstly these studies were carried out using immature fish, in which no vitellogenin is sequestered by the ovary as in normal maturation and secondly, during the normal course of maturation the liver is exposed to oestrogen stimulation for much longer periods of time. Although not demonstrated here, a dose dependent response of the liver to varying levels of oestrogen has been reported in amphibians and other species of fish (Aida et al, 1973; Follett & Redshaw, 1968; de Vlaming et al, 1977).

In the later experiments from January to April, similar oestradiol treatments failed to produce any increase in the serum levels of

total calcium and phosphoprotein phosphorus. Although all four dose rates employed produced significant increases in the serum levels of oestradiol 17β, in one case far in excess of physiological levels, the serum levels of phosphoprotein phosphorus and total calcium remained unchanged. Furthermore, the response to oestradiol 17β was absent throughout this period in all fish irrespective of age or weight.

The duration of this non-responsiveness would appear to be in excess of 4 months as a response to oestradiol 17β administration was observed in October, November and December. Preliminary results not reported here suggest that from the middle of January to mid-April, no response occurs. Whether the fish will respond at other times of the year is as yet unknown. However, the non-responsiveness occurred approximately at a time when mature fish of this particular strain would have been post-spawned. It is therefore likely that the lack of response to oestrogen may be the cause of the post-spawning refractory period of the gonad referred to by some authors (de Vlaming, 1972; Sundararaj & Vasal, 1976; Breton & Billard, 1977). Furthermore, as the fish used throughout the experiments were all immature, it is possible that this refractory period is part of an inherent rhythm which controls unseasonal gonad maturation and normally allows a period of recovery following spawning.

Although the precise mechanism by which this control occurs is as yet unknown it is possible that other endocrine processes may be involved. For example, it is well established that thyroid hormones can depress plasma gonadotropin levels. In birds this may be an important mechanism in the regulation of unseasonal gonadal growth (Chandola et al, 1974) and promotion of gonadal regression after

breeding (Jallageas et al, 1974). Although in teleosts annual cycles of thyroid activity have been correlated with gonadal maturation, their precise action on the liver is as yet unknown (Hurlburt, 1977). However, in a recent study, White & Henderson (1977) observed an annual variation in circulating levels of the thyroid hormones, triiodothyronine (T_3) and thyroxine (T_4) in the brook trout. They showed that the minimal values of T_3 and T_4 in the serum occurred in November at the time of spawning, and the maximal values in mid-spring. Since it has been suggested that a negative feedback of T_4 on gonadotropin activity occurs in teleosts (Sage & Bromage, 1970), the varying levels of T_3 and T_4 found during the year may contribute to the changes of gonadotropin and steroid levels found during the reproductive cycle in salmonids. They may also be involved with the failure of oestrogen to induce vitellogenin production in the liver from January to April.

Similarly, corticosteroidogenesis is also believed to be important in the processes of maturation and ovulation in fish, although it is not clear whether this is of ovarian or interrenal origin (Jalabert, 1976; Colombo et al, 1973). Colombo et al, (1978) have shown that the enzymes necessary for the synthesis of corticosteroids are present in the mature teleostean ovary, for which gonadotropin may provide the hormonal control. It is also believed that gonadotropin and adrenocorticotropic hormone may act on the interrenal to stimulate the synthesis and release of corticosteroids (Sundararaj et al, 1972). It may be that high serum levels of corticosteroids are involved in desensitising the liver to oestrogen following spawning.

Clearly, the summation of all the individual processes involved

in the precise hormonal control of reproduction ultimately uncovers a very complex system which requires much further investigation. The results discussed in this chapter demonstrate that a single injection of oestradiol 17β is able to reproduce part of this natural control if administered at the correct time of the year. For the fish farmer, these results have 2 main implications. Firstly, it would appear that the natural process of maturation and spawning can be elicited at other times of the year by hormone administration providing the timing of administration is appropriate to the stage of ovarian development. Secondly, a more detailed study of the endocrine mechanisms involved in the post-spawning non-responsiveness to oestrogen may enable broodstock to be "held" by hormone manipulation at an intermediate stage of development equivalent to the post-spawning state. From such a population of fish in an arrested state of maturation, groups could be induced to spawn when required, by further manipulation of hormone status, thus providing the farmer with a potential supply of eggs throughout the year.

5. THE EXTENSION OF EGG AVAILABILITY BY THE DELAY OF INCUBATION TIME.

5.1. Introduction.

Salmonid eggs undergo continuous development from the time of fertilization to the time of hatching (see Fig.5.1). Throughout this process, the rate of development is dependent upon a number of environmental factors, of which the most critical appears to be water temperature, with subsidiary influences imparted by oxygen concentration and water flow.

Higher temperatures increase, and lower temperatures decrease the rate at which the embryos develop, probably as a consequence of altered metabolic rate. This controlling influence of temperature can be modified by the level of dissolved oxygen. Thus, at any given temperature reduced oxygen concentration produces a retarded rate of development (Alderdice et al, 1958; Garside, 1959, 1966). Conversely, a high level of oxygen produces an accelerated rate of development (Kinne & Kinne, 1962). If development is at an advanced stage, a reduction in oxygen concentration can induce earlier hatching, (Alderdice et al, 1958; Kotlyarevskaya, 1967). The ability to co-ordinate the rate of development with environmental factors ensures that hatching occurs in nature only when conditions are favourable, thus imparting a greater chance of survival to the newly hatched fry.

Clearly, the artificial manipulation of water temperature during the incubation period provides a further approach by which the availability of eggs may be extended, although early experiments were contradictory and accompanied by high mortalities. Thus, Embody (1934) demonstrated that the incubation period required by the eggs of brown trout was extended to 148 days when carried out at 1.7°C, compared

DAY.	
0	FERTILIZATION
4	NEURAL KEEL FORMATION
5	OPTIC ANLAGEN
7	BLASTOPORE CLOSES
10	GILL BUDS FORMED
12	DEVELOPMENT OF PIGMENTED EYES
13 - 22	DEVELOPMENT MAINLY AN INCREASE IN SIZE
23	HATCHING
39	YOLK SAC ALMOST ABSORBED AND FIRST FEEDING

FIG. 5.1. STAGES OF DEVELOPMENT OF RAINBOW TROUT EGGS AT A CONSTANT TEMPERATURE OF 12 C. (KNIGHT, 1963).

with 41 days at 10°C, but losses were much higher than normal. Embody concluded that if the eggs became eyed at temperatures above 4.4°C, development could be safely completed at extremely low temperatures, without high mortalities. Combs & Burrows (1957) found that in the chinook and pink salmon, egg mortalities at 1.7°C were extremely high unless an initial incubation temperature of 5.6°C was maintained for one month after fertilization. Combs (1965), furthering this work, demonstrated that normal temperatures should be maintained for 7 days after fertilization in chinook salmon if heavy mortalities are to be avoided.

More recently, Maddock (1974), has extended the work of Embody and successfully prolonged the incubation period of brown trout eggs to 139 days at 1.4°C after an initial development period of 12 days at 7.6°C. The most significant feature of this study is that by applying the normal earlier incubation period, a 95% hatch was obtained, which is above the normal figure for most commercial hatcheries. However, it should be pointed out that all these studies were performed in the laboratory, presumably under better conditions than those found in the commercial hatcheries.

In view of this success, an investigation was carried out to examine the possibility of extending the incubation period of rainbow trout eggs by applying lower water temperatures. Special attention was paid to survival and development, subsequent to hatching, in order to assess the commercial applicability of this approach.

5.2. Materials and Methods.

The cooled incubation unit consisted of four circular hatching trays arranged vertically. Water was recirculated from a 110l reservoir, cooled by a refrigeration unit. The arrangement of the apparatus is shown in Fig.5.2. Eggs in the control group were incubated in a similar hatchery tray in a larger recirculation unit.

On the 11th. January, 1977 (day 1), eggs were hand-stripped from two females, fertilized, and divided into two groups of 3000 each. One of these groups (group A) was placed in a hatchery tray in the control system to serve as controls at a constant temperature of 9°C throughout the experiment. The second group (group B) was placed in a hatchery tray in the temperature controlled unit at 9°C. On three subsequent days (days 2, 3 and 4) eggs were stripped, fertilized and also placed in the temperature controlled unit, (groups C, D and E respectively).

All groups were maintained at 9°C until day 14, at which time the temperature was gradually reduced over a period of 4 days to 2°C. The protocol for the experiment is shown in Fig. 5.3. In this way a normal incubation temperature of 9°C was maintained for 10, 11, 12 and 13 days in groups B, C, D and E respectively prior to applying the low temperature period. The incubation temperature was then maintained at 2°C until day 76, at which time the temperature was gradually raised to 9°C over a period of 2 days.

Throughout the course of the experiment, the eggs were periodically checked for mortalities, and the dead eggs removed and recorded. After hatching, the subsequent performance of the four experimental groups was monitored.

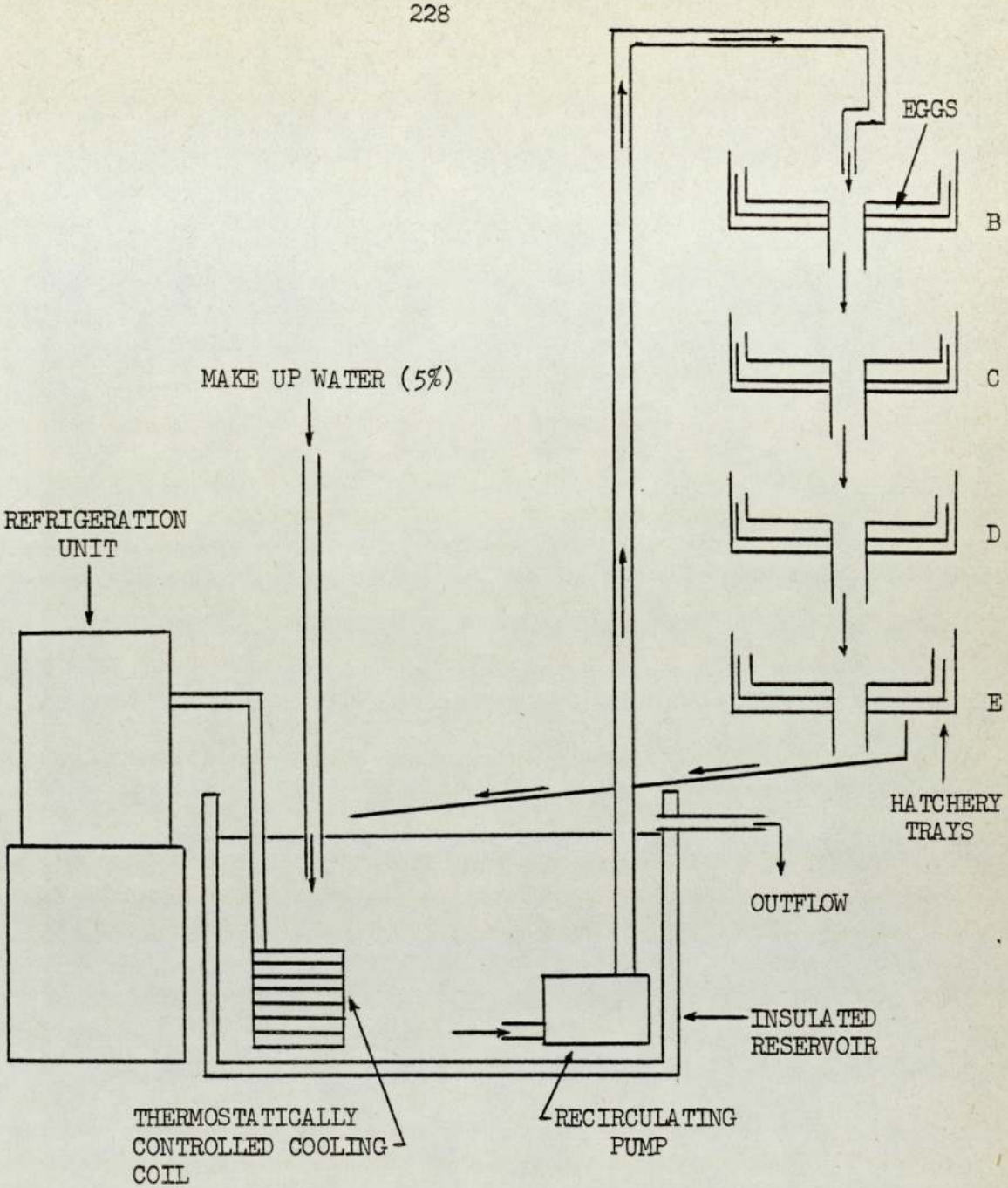


FIG. 5.2. ARRANGEMENT OF THE APPARATUS IN THE COOLED INCUBATION SYSTEM USED TO EXTEND THE HATCHING TIME OF RAINBOW TROUT EGGS.

DAY.	TEMPERATURE (°C).	PROCEEDURE.
1	9	INTRODUCE GROUP B
2	9	INTRODUCE GROUP C
3	9	INTRODUCE GROUP D
4	9	INTRODUCE GROUP E
5 - 13	9	
14	9	DECREASE TEMPERATURE FROM 9 - 2°C OVER A PERIOD OF 2 DAYS.
16	2	
17 - 76	2	
77	2	INCREASE TEMPERATURE FROM 2 -9°C OVER A PERIOD OF 2 DAYS.
79	9	
80 ONWARDS	9	

THROUGHOUT THE EXPERIMENT, CONTROL GROUP A WAS MAINTAINED AT A CONSTANT TEMPERATURE OF 9°C. FROM DAY 1 ONWARDS.

FIG. 5.3. FLOW DIAGRAM OF EXPERIMENTAL PROCEEDURE DURING LOW TEMPERATURE INCUBATION.

5.3. Results.

Under the experimental regime employed, all four groups (B, C, D and E) started to hatch at day 86, 50 days after the control group (group A), where hatching began at day 36. On hatching, the yolk sac alveins from all the experimental groups were slightly smaller than those from the control group, presumably a reflection of the greater amount of energy channelled into maintenance of the embryo, rather than growth over the extended incubation period. After the "swim up" stage, the four experimental groups were pooled in one fry tank. Unfortunately, due to pressures of the fry system at a time of the year when the loading is high, it was not possible to pursue the experiment for a period of more than three months after first feeding.

At all stages of development, higher mortalities were sustained in the groups maintained at the lower temperature. Groups B, D and E encountered losses 5-10% higher than the control group, and in group C almost 50% higher mortalities were observed. Apart from group C, these increased mortalities were not high enough to be of significant concern, especially when consideration is given to the fact that the eggs used in all groups (including controls) were "first stripping" eggs, which are invariably of poorer quality than eggs from the second or subsequent years of stripping. The mortalities encountered in each group throughout the incubation are shown in Fig.5.4. At the conclusion of the experiment (three months after first feeding) the mortalities among the pooled experimental groups were 66%, some 28% higher than those among the control group at a similar stage of development, although growth was comparable.

DAY.

GROUP.		0	14	28	39	52	57	65	69	95	TOTAL %
(CONTROL).	A	0	232	307	285						27.4
	B	0	110	106	114	300	26	23	36	400	37.2
	C	0	87	123	213	225	223	229	113	450	55.4
(EXPERIMENTAL)											
	D	0	31	60	10	47	19	32	47	700	31.5
	E	0	35	126	96	150	43	59	53	550	37.1

FIG. 5.4. MORTALITIES ENCOUNTERED DURING LOW TEMPERATURE EGG INCUBATION.

5.4. Discussion.

The low temperature regime increased the incubation period from 36 days (9°C) to 86 days, thus producing an extension of 50 days which is of considerable commercial significance. The results also demonstrate that the "critical period" for which normal temperatures should be maintained is less than 10days. In all of the low temperature groups, however, the mortalities during incubation were slightly higher than in the control group. This result is in contrast with the work of Hamor & Garside (1976), who demonstrated no difference in mortality between groups of Atlantic salmon eggs maintained at 5°C and 10°C, and Maddock (1974), who obtained a 95% hatch after maintaining brown trout eggs at 1.4°C after an initial period at 7.6°C. However, it should be pointed out that these were laboratory investigations on small numbers of eggs, and there are a number of reasons which might account for the increased mortalities in this pilot scale experiment. Throughout the experiment, the regular examination and removal of the dead eggs to prevent fungal growth involved a degree of mechanical shock. It is therefore possible that the handling of the eggs over the extended incubation period was a major factor in the increased mortalities. This may be improved by the use of a fungicide such as malachite green, although this was not possible in the completely closed sytem used here. It was further discovered that after the first feeding stage, the experimental fry had suffered a systemic infection of Saprolegnia which probably contributed to the increased mortality among this group. The possibility that the experimental proceedure increased the susceptibility to infection cannot however be discounted.

In summary, the present work clearly demonstrates that egg development up to hatching can be delayed by at least 50 days by lowering the temperature of the circulating water. Although higher mortalities were encountered amongst the experimental fry, it is probable that these losses could be reduced by improvement of the proceedure used. The extension in development time reported here would enable eggs to be available some two months after the end of the normal spawning season.

6. THE USE OF COMPUTER MODELS TO PREDICT TROUT FARM PRODUCTION.

6.1. Introduction. .

We have seen in the general introduction that the greatest return on investment will only be achieved if production from a given site is efficiently maintained at its highest level. To optimise production from a given site, it is clear that facilities need to be fully utilised, without any under-stocking or over-stocking. In practice, this means that the farm must be managed in such a way, that the size and composition of the stock at any given time are adapted to the seasonal fluctuations of the external environment.

Water temperature is known to have profound effects on both the metabolic activities of the fish and the physical characteristics of the water supply (e.g. oxygen carrying capacity). Hence in situations where a seasonal change in water temperature is encountered it would be necessary to decrease the farm stock during the higher summer temperatures, as feeding would be high and oxygen carrying capacity low. Conversely, it would be possible to increase the stock held during the low winter temperatures, as feeding is lower and oxygen carrying capacity higher. As temperature also affects the growth rate, more fish are available for cropping in the summer than during the winter months. In addition, the management of fish stocks is at present made more difficult by the availability of eggs and fry for only a part of the year and also by the absence of reliable growth models which take into account the major factors affecting growth. It has been demonstrated in the preceding chapters that it is now possible to produce eggs and fry most of the year, an improvement which will ameliorate some of management's difficulties. In the present chapter a computerised model is formulated which will allow the

estimation of growth, stocking and cropping schedules under varying farm conditions and thus exploit the wider availability of eggs and fry.

The difficulties involved in formulating accurate production forecasts arise from the need to solve the following problem. The future weight of a fish has to be predicted on a given date, given a certain starting size, a fixed feeding regime, and given a seasonal variation in water temperature. Several authors have suggested solutions to this question based largely on the principles of fish energetics, postulated originally by Hathaway (1927) and Pentelow (1939). In 1956, Winburg produced a simple relationship between food consumption, metabolism and growth which implicated both water temperature and fish size, and Paloheimo & Dickie (1965, 1966a, b) re-stimulated interest in the formulation of mathematical models for growth in fish. These bioenergetic considerations were extended by Warren & Davies (1967) to include a whole series of factors: food consumption, faeces, urine, excretion through the gills and skin, growth and metabolism. This study, and similar investigations by Brett et al, (1969) and Gerking (1971) have indicated that the models of Paloheimo & Dickie (1965, 1966a, b) can only be used for a limited size range of fish, fed at a relatively high ration level. Staples & Nomura (1976) concluded that detailed analyses of the energy content of the fish body, especially in relation to body size are required.

Stauffer, (1973) developed a growth model for salmonids based on energy flow principles. In this study it was stated that the following factors might influence growth: husbandry, diet, disease,

sexual maturity, photoperiod, ration and feeding frequency, social hierarchy, species and race, swimming activity and exercise, fish size, age and temperature. Feed level, fish size and temperature were considered to be the most important factors in the formulation of any mathematical growth model. More recently, both From & Rasmussen (1978) and Sparre (1976) have also developed models based on factors of anabolism and catabolism. The field of fish bioenergetics upon which all these models are based has been recently reviewed by Beamish et al, (1975).

While studies on the energy relationships in fish, and growth models based upon these relationships, are important, at the present time the data or models available are either too incomplete or complex to enable growth models to be more fully used by the fish farmer. As energy relationships in fish are difficult to formulate accurately, there is therefore a requirement to develop less complex models of growth in fish farms based on factors which are more readily determined, and preferably factors which the fish farmer himself can ascertain and understand. Furthermore, while a complete production or optimisation program may be desirable, there are often unpredictable circumstances which may interfere with the production plan. Factors such as disease outbreaks, power cuts, water shortage, etc., which are inevitable even under the best conditions, can easily invalidate the most complex program.

Thus, the aim of the present study is therefore not to produce a complete optimisation program, but to develop a model for predicting growth, stocking and cropping, under varying temperature conditions, from which decisions on stock management can be made. The flexibility

arising from the simplicity of this model will enable the program to be easily modified or extended either when new information or other factors emerge.

6.2. Description of the Model.

In the present model, temperature, fish size and feed level are considered to be the primary factors influencing growth in salmonids (assuming a reasonably constant stocking density). This is in agreement with the conclusions of Stauffer (1973), Sparre (1976) and From & Rasmussen (1978).

The food conversion ratio has been used by most fish culturists in the calculation of carrying capacities and yield within a given system. It is calculated as a function of the food fed over a given time interval divided by the increase in net weight over the same period, (Phillips, 1970).

i.e. food conversion ratio = $\frac{\text{feed level during week 1}}{\text{weight at week 1 - weight at week 0}}$

Brauhn et al,(1976) have suggested that the food conversion ratio is dependent upon the stocking density, being higher at increased densities. Thus, from laboratory experiments, they predicted that at a stocking density of 3.0kg of fish /l/ min flow the conversion ratio would be approximately 40% higher than at a stocking density of 1.0kg of fish /l/ min flow. It is also likely that the conversion ratio will depend upon the feed level. However, From & Rasmussen, (1978) have demonstrated that the food conversion ratio is largely independent of fish size, and thus for the purpose of the model, conversion ratio is assumed to be constant for a given set of conditions. This assumption has been supported by data from Low Plains (Forster, 1978 - personal communication) although clearly experiments are required to establish the effects of stocking density on the conversion ratio under commercial conditions.

Once available this information could readily be incorporated into the model to improve its accuracy.

As temperature is probably the major factor affecting growth, a fundamental requirement of the program is the provision of information concerning the yearly temperature cycle of the water supplying the farm. In this model, an average temperature regime was calculated from weekly means based on information from previous years.

The feed level for any given week was fixed by the recommended feed tables supplied by B.P. Nutrition (U.K.) Ltd., Speciality Division, Witham, Essex. In these tables the feed level is determined by the water temperature and fish size. Thus, if the water temperature is 10°C, and the fish has a weight of 40g, then the feed level from the table is 1.7% body weight per day. During the execution of the growth equation, feed levels were calculated as a percentage of body weight on a weekly basis.

Accepting the above limitations in the use of the conversion ratio as a factor, the basic growth equation can now be formulated. Consider a fish of a given size, fed a given diet at a level recommended from the feed tables, under a given seasonal temperature regime. Assuming a fixed conversion ratio, the growth equation can be expressed as:

$$WTKI = WTK + \frac{(WTK \cdot FL)}{CR}$$

where:

WTKI = weight at week 1

WTK = weight at week 0

FL = feed level for week 1

CR = conversion ratio

Temperature and fish size are also taken into account as they are used in fixing the feed level. Thus, at week 0, fish of 5g, at a temperature of 8°C, require a feed level of 19.6% body weight per week. If one assumes a conversion ratio of 1.6:1,

$$WTKI = WTK + \frac{(WTK \cdot FL)}{CR}$$

becomes $$WTKI = 5.00 + \frac{(5.00 \cdot 0.196)}{1.6}$$

$$= \underline{5.61g}$$

Therefore, at the end of one week's growth the fish has attained a weight of 5.61g. For the second week, if the temperature increases to 9°C for example, the feed level from tables under these conditions becomes 21.7% body weight per week.

$$\therefore \quad WTK2 = 5.61 + \frac{(5.61 \cdot 0.217)}{1.6}$$

$$= \underline{6.37g}$$

Therefore, at the end of the second week, the fish has reached a weight of 6.37g. By repeating this proceedure, a growth curve for any number of weeks can be calculated.

The growth equation described is based on the growth of one fish. It is well known however, that fish of the same size do not grow at an equal rate. In fish farming practice, all the decisions on feed level, stocking, etc., are taken from information regarding the mean weight of the population in question. Fish are also graded at intervals to maintain a consistancy of size within a given tank or pond. For predicting production therefore, the distribution of

fish size which arises in a population grown from a given starting size is of little consequence until the population reaches a size such that part of the distribution can be cropped. To illustrate this more clearly, consider an example where a population of 91,000 (the number of fish required to give 25 tons of fish at an average cropping size of 275g), are grown from a starting size of 5g under a constant temperature of 9°C. Experience at Low Plains has shown that the cropping period for such a group would extend over approximately 16 weeks (Harman & Forster, 1978 - personal communications). From this information, the first fish would be cropped from the population when the mean weight of the population is 200g. The likely distribution around the 200g mean at this point is shown in Fig.6.1. Sparre (1976) stated that such an assumed distribution was valid as "a certain lawfulness in randomness" is found and there is for instance practically nil probability that all the fish will grow at a minimum rate at the same time. There is therefore, a sound basis for the application of specific distributions in appropriate circumstances.

Thus, the growth equation is executed based on the mean weight of a given population. Once the fish have reached a mean weight of 200g as calculated from the basic growth equation, the distribution of weights is taken into account by dividing the population into 29 groups at intervals of 5g from 130g to 270g (Fig.6.1). This number of groups was chosen because there will always be an upper limit for the number of growth expressions any computer will be able to evaluate within a reasonable time. For example, the number of times the growth expression is evaluated during a complete run under a temperature regime of 7-15°C would be approximately 3250. This number will vary

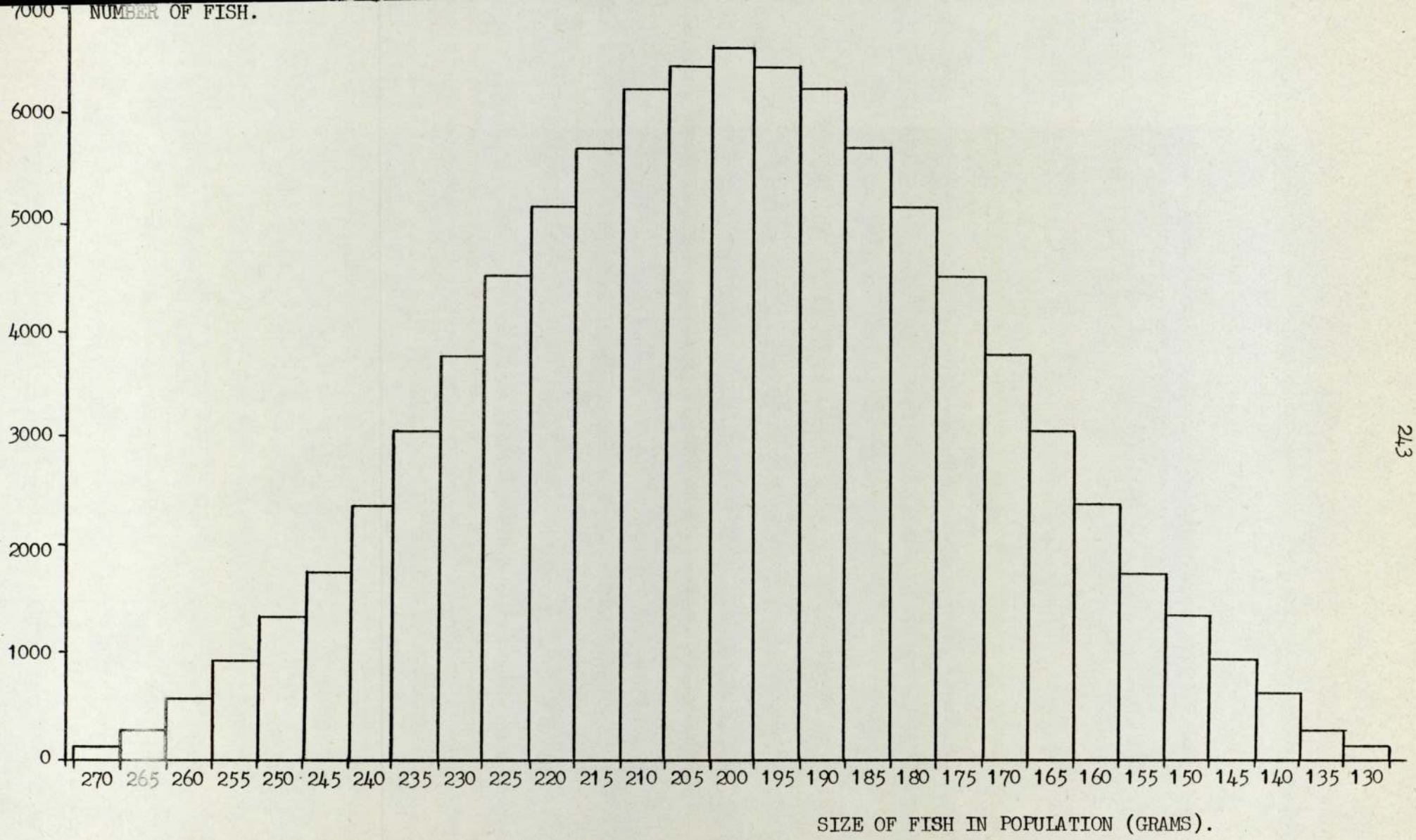


FIG. 6.1. ASSUMED NORMAL DISTRIBUTION OF FISH AROUND A 200 GRAM MEAN.

under different temperature regimes tested. Clearly, the choice of the number of groups is a compromise between having many small groups which produce a good approximation but many calculations, and fewer groups which produce a slightly less accurate approximation but fewer calculations.

Having divided the population into size groups at this point, it is then possible to apply the growth expression individually to each group and calculate the time at which each group would be cropped at 275g. By applying this proceedure, it is now possible to calculate the growth of a given population of fish (taking into account the size distribution), the time at which each group within the population would be cropped, and the amount of crop.

The stock loading due to any one population of fish is easily calculated by multiplying the mean weight of the population by the number of fish in that population. The total stock loading on the farm is then the sum of the individual loadings due to each population present at any given time.

In order to enable decisions to be taken on stocking, the carrying capacity of the farm needs to be calculated. Ultimately the capacity of the farm will be determined by water quality parameters. Under the conditions at Low Plains, which has a water flow of approximately one million gallons per day, it has been found that adverse water quality conditions develop, mainly as a result of increased suspended solids, when more than 0.5 tons of food are fed per day (Forster, 1978 -personal communication). Therefore it is necessary to base the upper limit of carrying capacity on the number and sizes of fish that could

be supported by 3.5 tons of food per week (0.5 ton/day).

Mortality will obviously have an effect on overall stocking decisions, and again it would be reasonable to assume a fixed mortality rate for a given size range of fish. However, although mortality needs to be considered in terms of fish numbers introduced at any one time, the overall growth and stocking data from the program will be little changed, as mortality is greatest when the fish are small and hence feed/fish is small. Thus when the fish are large, mortality is low enough to make only a small difference to the overall feed/week required. Although it is intended to include mortality in the program in the future, it is sufficient at present to modify the stocking slightly to account for mortality after major decisions have been made.

In summary, the model calculates the growth of a given population of fish under a specified temperature regime, the time at which a particular size range of fish within the population will be cropped and the total crop produced. The total stock load and the amount of food required to support each population each week are also calculated. The program computes these parameters, starting from the introduction of fry every four weeks throughout the year. By selecting the appropriate growth and stocking data at the different starting times it is now possible to make decisions on how best to stock the farm under a given set of conditions. How these decisions can be made is described in the next section. The Fortran program is described in appendix 2. The computer used throughout the study was the ICL 1904S at the Computer Centre, Aston University.

6.3. Use of the Program.

In order to discuss the model and its application in detail, three different theoretical farm sites are considered. It is assumed that all three farms are identical in construction for the purpose of comparison, and each is designed to produce around 100 tons of fish per year at an average weight of 275g per fish. Each of the farms is assumed to be supplied with one million gallons of water per day, which enables a maximum of 3.5 tons of food to be used each week. It is also assumed that the farms are located at different sites and hence are subject to different seasonal water temperatures. Farm A is based on the conditions encountered at Low Plains, i.e. a borehole water supply at a constant temperature of 9°C throughout the year (Fig.6.2). Farm B is based on a theoretical Southern Britain farm whose temperature varies between 7°C and 15°C (Fig.6.3), and Farm C is based on a typical Northern Britain temperature regime, with a seasonal variation of 3°C to 15°C (Fig.6.4). Thus, although the yearly temperature means of farms A, B, and C are similar, i.e. 9, 10 and 8°C respectively, the seasonal variations are quite different.

In each case, a solution is required to the question of how to stock the farm throughout each year to yield maximum fish production. In practice this means that the 3.5 tons of feed per week limit needs to be approached as closely as possible. In solving the problem, it is assumed that 5g fry are available for introduction into the farm at intervals of 12 weeks, which was considered to be reasonable from a management point of view. The feed level for all cases is fixed (according to the fish size and water temperature) from the recommended feed tables.

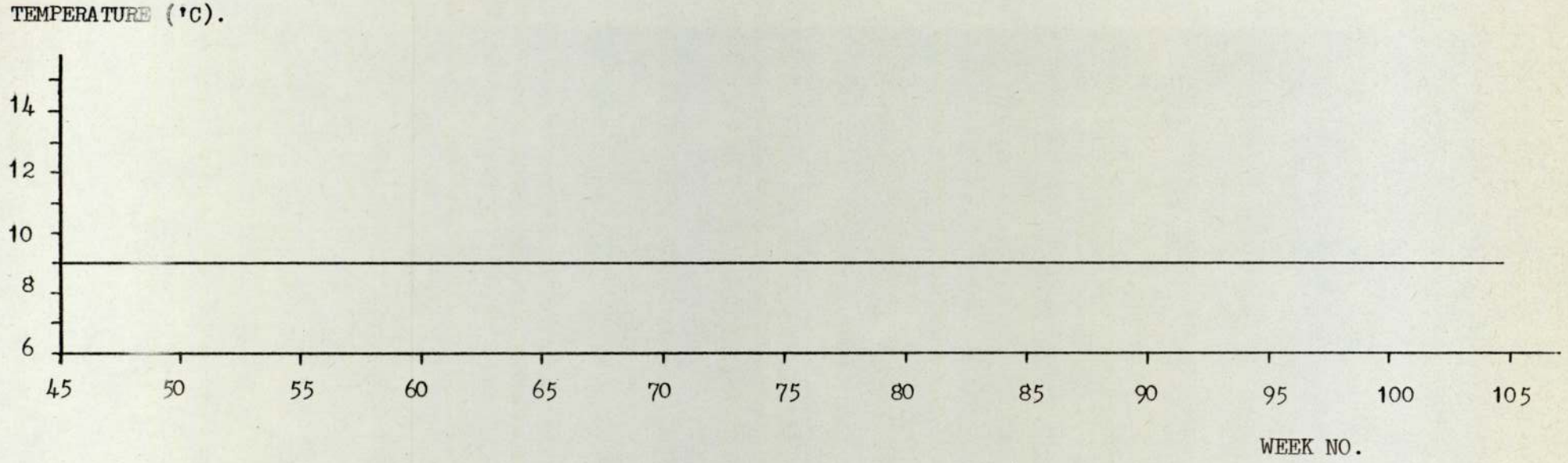


FIG. 6.2. PREDICTED TEMPERATURE REGIME FOR THE WATER SUPPLYING FARM A DURING THE WEEKS UNDER CONSIDERATION.

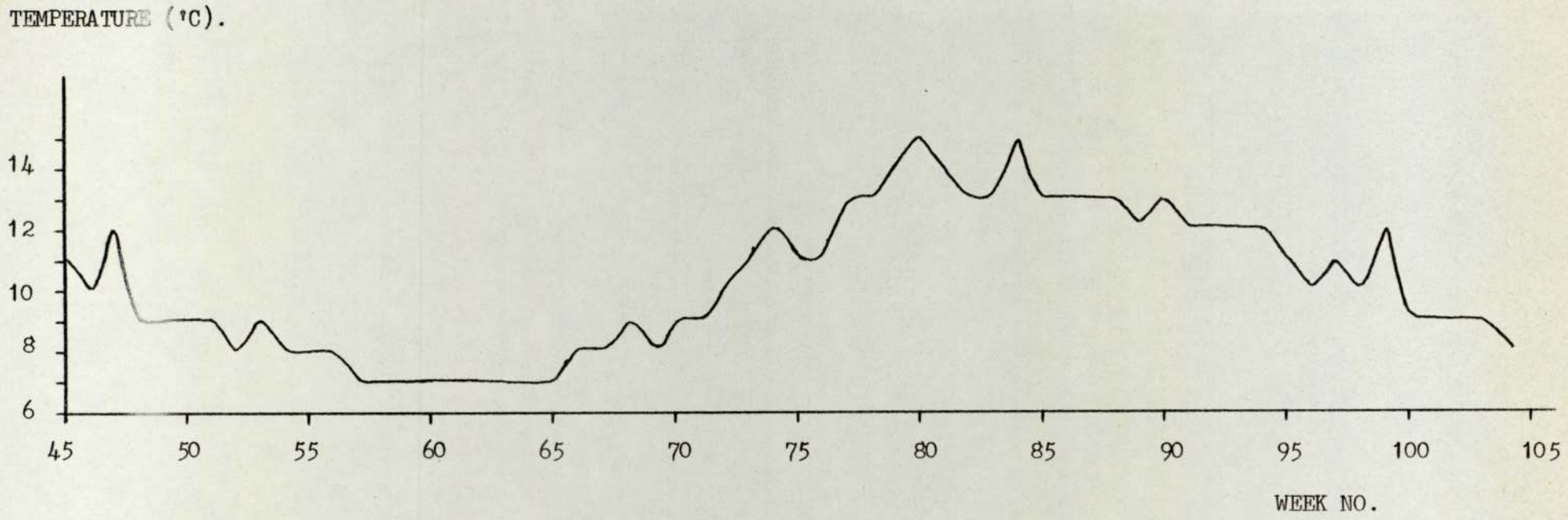


FIG. 6.3. PREDICTED TEMPERATURE REGIME FOR THE WATER SUPPLYING FARM B DURING THE WEEKS UNDER CONSIDERATION.

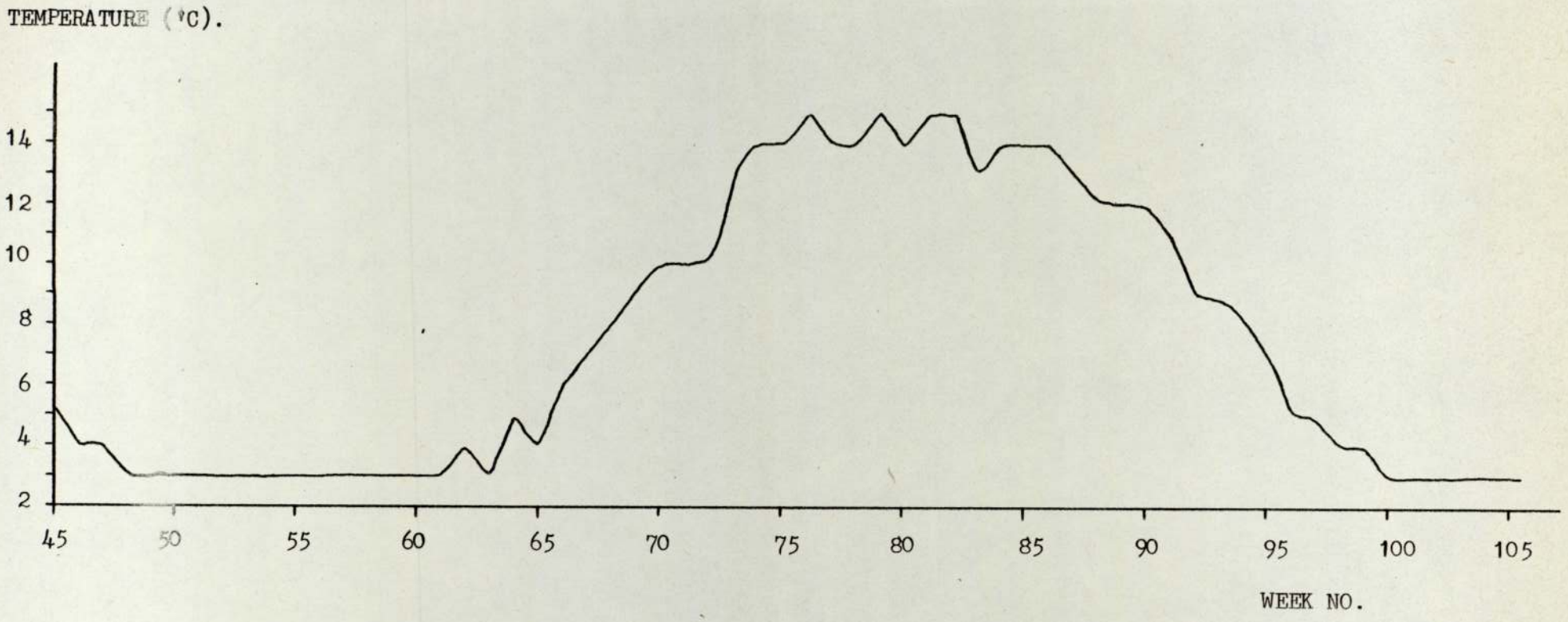


FIG. 6.4. PREDICTED TEMPERATURE REGIME FOR THE WATER SUPPLYING FARM C DURING THE WEEKS UNDER CONSIDERATION.

In order to construct a stocking schedule for each of the different sites, it is assumed that each farm is being stocked from zero (week 0) during the first year, and consideration is given to the production from the farm during its second year (weeks 52 - 104) when the fish are of marketable size. This is done solely to enable comparison of the three sites, and to highlight differences. In an identical manner, production schedules for subsequent years (i.e. when the farms are already fully-stocked) could be easily formulated.

In summary therefore, the following constraints apply to each farm site:

1) The maximum food level should not exceed around 3.5 tons per week.
2) Stocking should be such that feeding can be performed according to tables throughout the year, thus obtaining maximum growth efficiency.
3) 5g fry are available to be introduced into the farm every twelve weeks.

If we now consider each site individually, suitable stocking schedules can be developed. All the tables created from data output during the computer runs and used in the calculations described, are grouped together for easy reference in appendix 3.

1) Farm A: This site is based upon conditions at Low Plains, i.e. a borehole supply of water at a constant temperature of 9°C throughout the year. In order to produce 100 tons of fish at an average individual fish weight of 275g, approximately 364,000 fish are required each year. As fry are being introduced every 12 weeks, each batch needs to contain approximately 91,000 fry at 5g at the time of introduction,

not accounting for mortality. To stock the farm over the first two years, populations of 91,000 5g fry would be introduced at weeks 0, 12, 24, 36, 48, 60, 72, 84, and 96. From the computer output produced, the individual mean weight, stock, cropping and tons of feed required to support each population can be determined at any given time. The total stock, cropping and tons of feed required to support all the populations present at any given time is therefore the sum of the values for each population present at that time. For example, at week 60, the total amount of food required to support all the populations present would be the sum of the individual amounts required to support the populations originally introduced at weeks 0, 12, 24, 36, and 48 (which is 0.383 + 1.275 + 0.726 + 0.443 + 0.227 = 3.054 tons). These total feed values can be calculated for each week under consideration (Table 6.1, Fig. 6.5). From these results we can see that over the weeks 52 - 104 (i.e. from when the farm is fully stocked for the first time) the amount of food introduced into the system each week varies between 3.1 and 3.7 tons, and does not seriously exceed the approximate maximum of 3.5 tons per week. As the water temperature is constant throughout the year, this cycle of feeding becomes repetitive. From this information it is clear that stocking the farm with 91,000 fry at 5g every three months (not accounting for mortality) closely approximates to the maximum feeding capacity of the farm. Similarly, the total amount of stock held on the farm at any one time is the sum of the stock loads due to each individual population present at that time. For example, at week 60, the total stock on the farm is the sum of the stock loading due to the populations originally introduced at weeks 0, 12, 24, 36 and 48 (which is 4.565 + 15.181 + 7.981 + 3.960 + 1.621 = 33.308 tons). Table 6.2. demonstrates the total stock on the

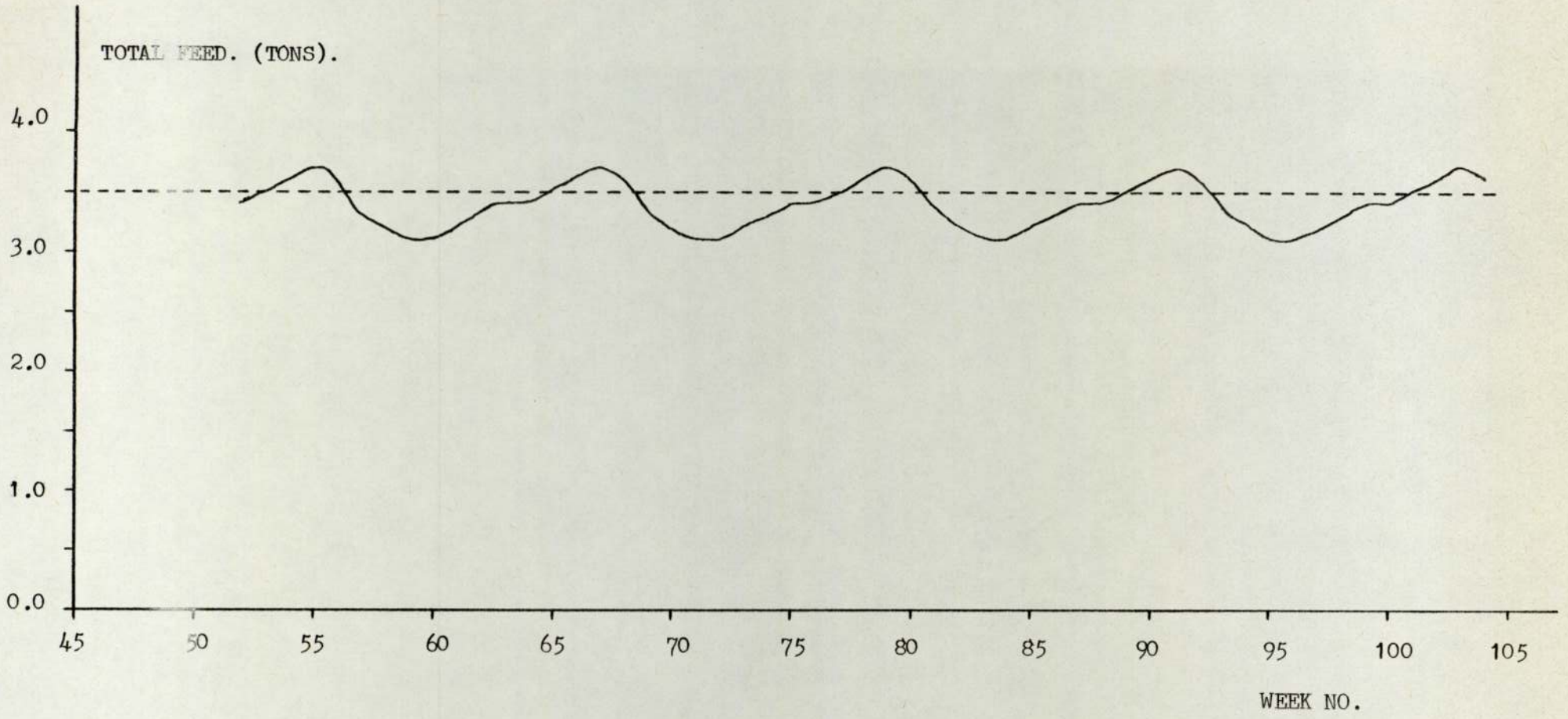


FIG 6.5. THE TOTAL AMOUNT OF FOOD REQUIRED PER WEEK (TONS) TO SUPPORT POPULATIONS OF 91,000 FISH INTRODUCED AT WEEKS 0,12,24,36,48,60,72,84, AND 96 UNDER THE CONDITIONS AT FARM A. ----- = APPROXIMATE 3.5 TONS/WEEK MAXIMUM.

farm each week between weeks 52 and 104. These results are plotted in Fig.6.6, from which it can be seen that the stock varies between 33 and 40 tons over the year. In an identical way, the crop available per week is calculated (Table 6.3, Fig. 6.7). In practice, it is unlikely that over four tons would be cropped in one week, and thus the cropping distribution shown in Fig.6.7 would tend to even out at around two tons per week, providing a relatively constant all-year-round production schedule.

Conclusions regarding Farm A: All the above information regarding stock held, cropping and feeding from week to week fits very closely to the actual situation encountered at Low Plains. In summary, therefore, under the conditions at Farm A, a suitable stocking schedule over the first two years would be:

week No:	No. of 5g fry introduced	
0	91,000	
12	91,000	
24	91,000	
36	91,000	(N.B. mortality is
48	91,000	not accounted for).
60	91.000	
72	91,000	
84	91,000	
96	91,000	

This schedule produces a reasonably even cropping throughout the year, and the total production over the weeks 52 - 104 would be 106 tons, at a cropping average of 275g per fish. As conditions in this case are constant, this production regime would be similar from

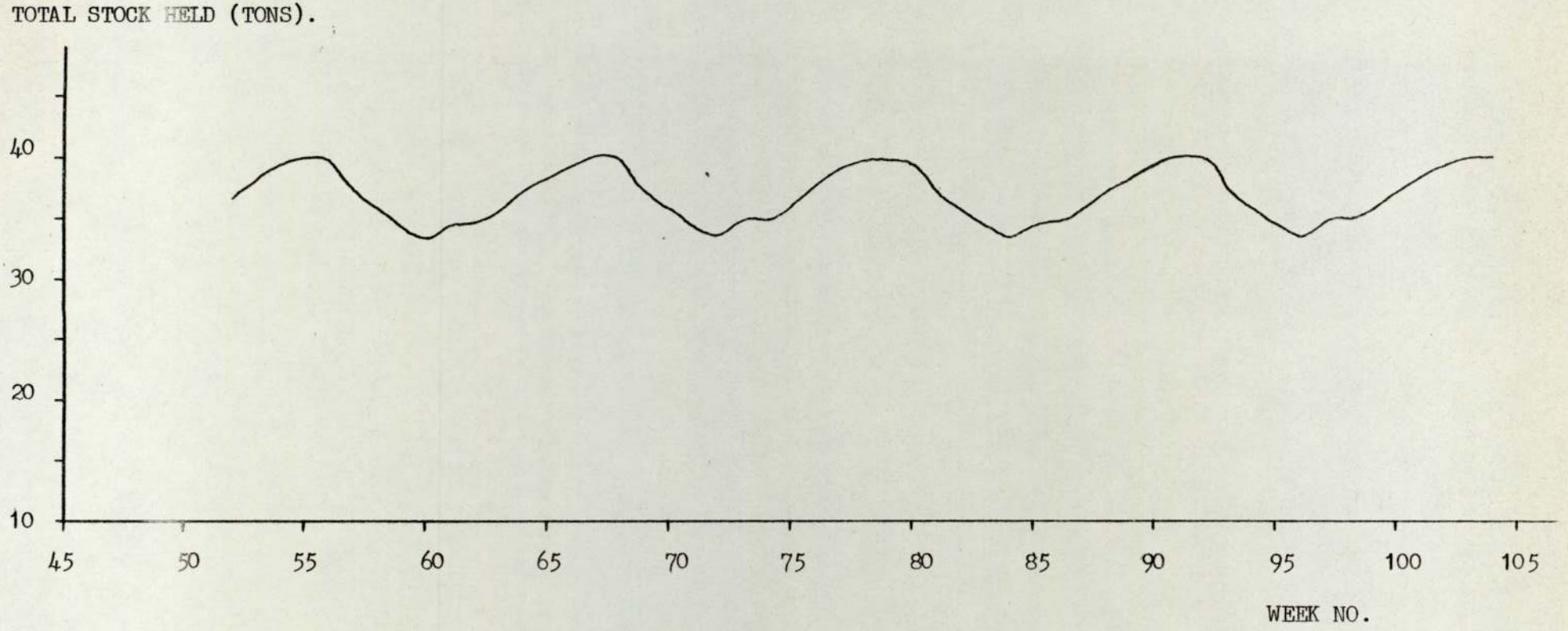


FIG. 6.6. THE TOTAL STOCK HELD (TONS) PER WEEK HAVING INTRODUCED POPULATIONS OF 91,000 FISH AT WEEKS 0,12,24,36,48, 60,72,84 AND 96 UNDER THE CONDITIONS AT FARM A.

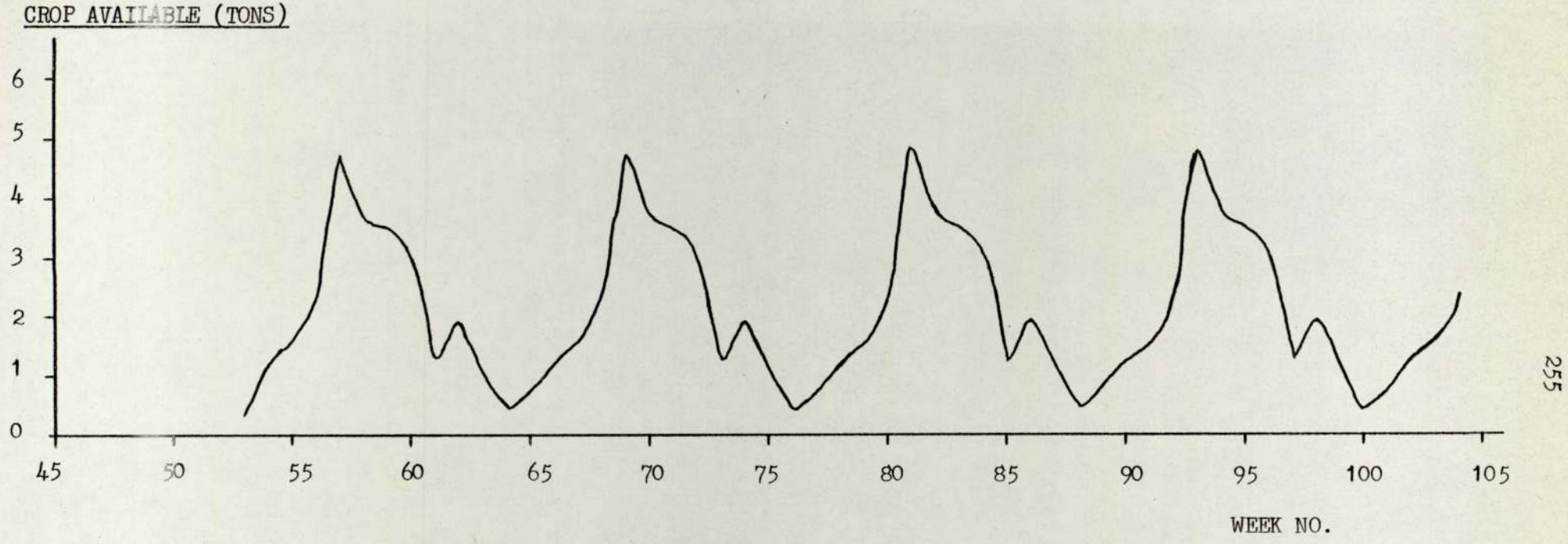


FIG. 6.7. CROP AVAILABLE PER WEEK UNDER THE STOCKING SCHEDULE EMPLOYED AT FARM A.

year to year. It should be emphasised however, that mortality is not taken into account, and in practice therefore the numbers of fish introduced in each population should be increased by factor sufficient to allow for mortality. Mortality should be able to be predicted for a given site, and as explained previously, increasing the initial numbers of fish introduced will not seriously affect the overall results.

2) Farm B: This farm is based on a theoretical Southern Britain temperature regime which varies between 7°C and 15°C as shown in Fig.6.3. If the same considerations are applied to Farm B, a suitable stocking schedule can be formulated.

Assuming the same availability of fry as in the first example (Farm A), 5g fry can be introduced at weeks 0, 12, 24, 36, 48, 60, 72, 84, and 96. The feed that would be required to support populations of 91,000 5g fry introduced at these times is shown in Table 6.4 and Fig.6.8. From these results it is apparent that the feeding regime that would be required is quite different from that in the previous example, although the introduction of fry is the same. There are two periods (weeks 52 - 72 and 102 - 130) where feeding is well below the capacity of the farm, and two periods (weeks 83 - 100 and 131 - 150) where more feed is required than the farm would be able to support. Clearly, the stocking schedule that was suitable for the previous case cannot be operated here. By adjusting the number of fish in a group at the time of introduction, it is possible to alter the feed required at each week and bring the feeding regime closer to the maximum of around 3.5 tons per week. In this way, the periods of over-feeding and under-feeding in terms of the total

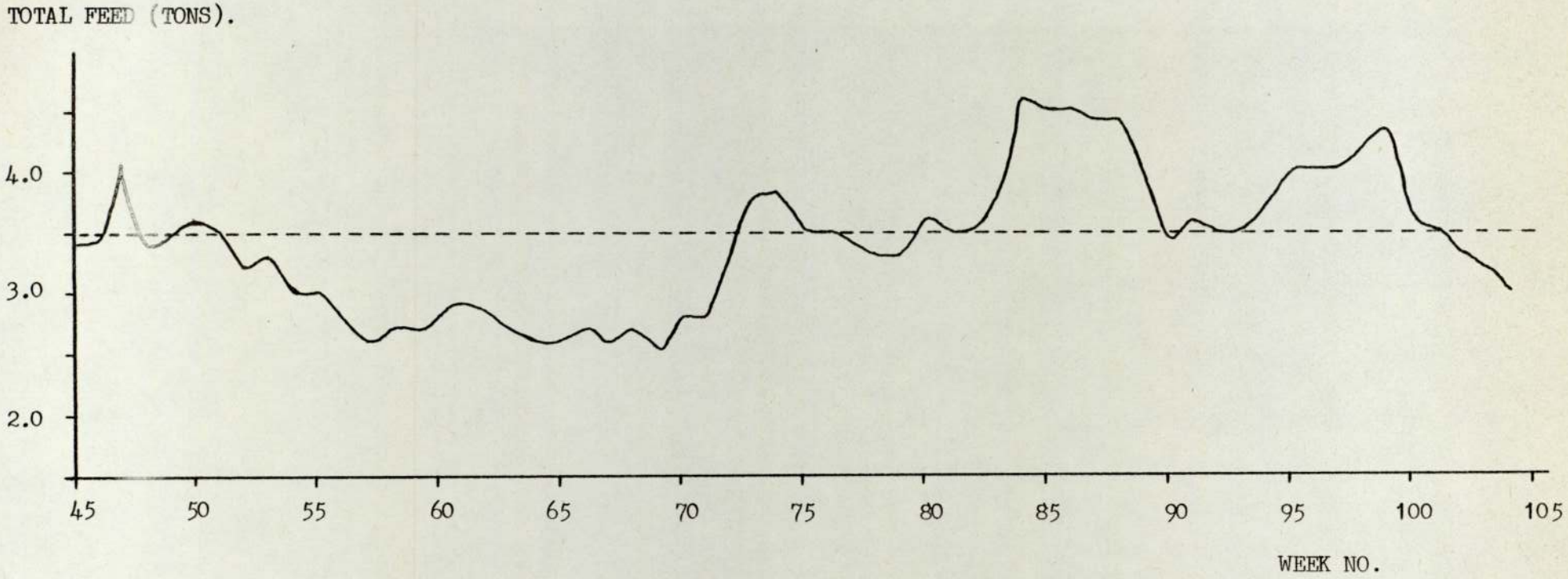


FIG. 6.8. THE TOTAL AMOUNT OF FOOD THAT WOULD BE REQUIRED TO SUPPORT POPULATIONS OF 91,000 FISH INTRODUCED AT WEEKS 0, 12, 24, 36, 48, 60, 72, 84 AND 96 UNDER THE CONDITIONS AT FARM B. ----- = APPROXIMATE 3.5 TONS/WEEK MAXIMUM.

(CONTINUED OVERLEAF).

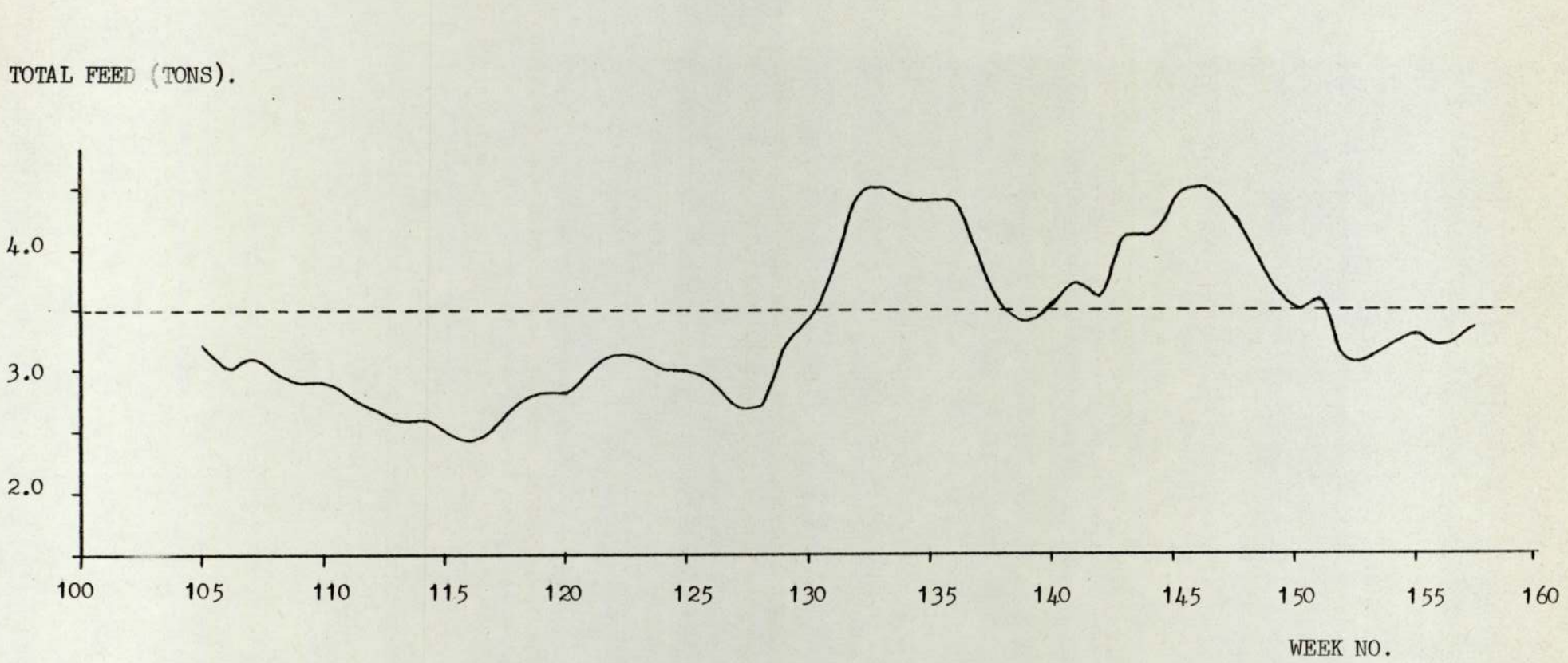


FIG. 6.8. (CONTINUED).

capacity of the farm can be reduced to a minimum. For example, at week 84, the amount of food required is 4.601 tons, which is 1.101 tons above the maximum feed capacity. At week 84, the largest amount of food being fed to the population originally introduced at week 36 (Table 6.4). By reducing the number of fish in this population by a factor sufficient to reduce the feed required by 1.101 tons, the total amount of food that would be required at week 84 would be 3.5 tons, which is within the capacity of the farm. This means reducing the population introduced at week 36 by 0.520, in other words, stocking at week 36 with 48,000 5g fry instead of 91,000.

If we now consider week 61, the feed required is 2.877 tons, which is below the total capacity of the farm. At this time, the majority of the food is being fed to the population originally introduced at week 12. If this group is increased by a factor to increase the total food required to 3.5 tons at week 61, the under-feeding at this time would be eliminated. This would require increasing the population introduced at week 12 by 1.559, or stocking with 142,000 5g fry at week 12 instead of 91,000.

By applying this proceedure to all the populations, and taking into account that changes in the size of population introduced alters the total feed required at all the weeks those populations are present, the modifications required would be as follows:

week No.	No. of fry introduced at 5g
0	91,000 x 0.683 = 63,000
12	91,000 x 1.559 = 142,000
24	91,000 x 1.222 = 112,000
36	91,000 x 0.520 = 48,000

week No.	No. of fry introduced at 5g
48	91,000 x 0.629 = 58,000
60	91,000 x 1.179 = 108,000
72	91,000 x 1.429 = 130,000
84	91,000 x 0.592 = 54,000
96	91,000 x 0.596 = 55,000

Having adjusted the introduction of fry in this way, the feed regime required over the first year of cropping (weeks 52 - 104) now becomes closer to the 3.5 tons per week maximum, as shown in Table 6.5 and Fig. 6.9. Although periods still exist where feeding under total capacity occurs (weeks 63 - 72 and 75 - 84) they are much shorter than under the original regime, and feeding over capacity has now been practically eliminated.

The total stock held on the farm at any given time over weeks 52 - 104 is shown in Table 6.6 and Fig. 6.10. In this case the stock held varies between 47 and 17 tons over the year, but more important, it can be seen that as the temperature increases at the start of the summer, the stock decreases. Hence stocking is low when the water temperature is high and vice versa.

The cropping available under this stocking regime is shown in Table 6.7 and Fig. 6.11. Although the cropping periods are reasonably consistent, the weight of fish cropped in each period varies. Hence, during the weeks 60 - 80, cropping is higher due to the need to decrease stock held over the high summer temperatures, and later in the year, cropping is lower as a result of the decreased stock held over the high summer temperatures.

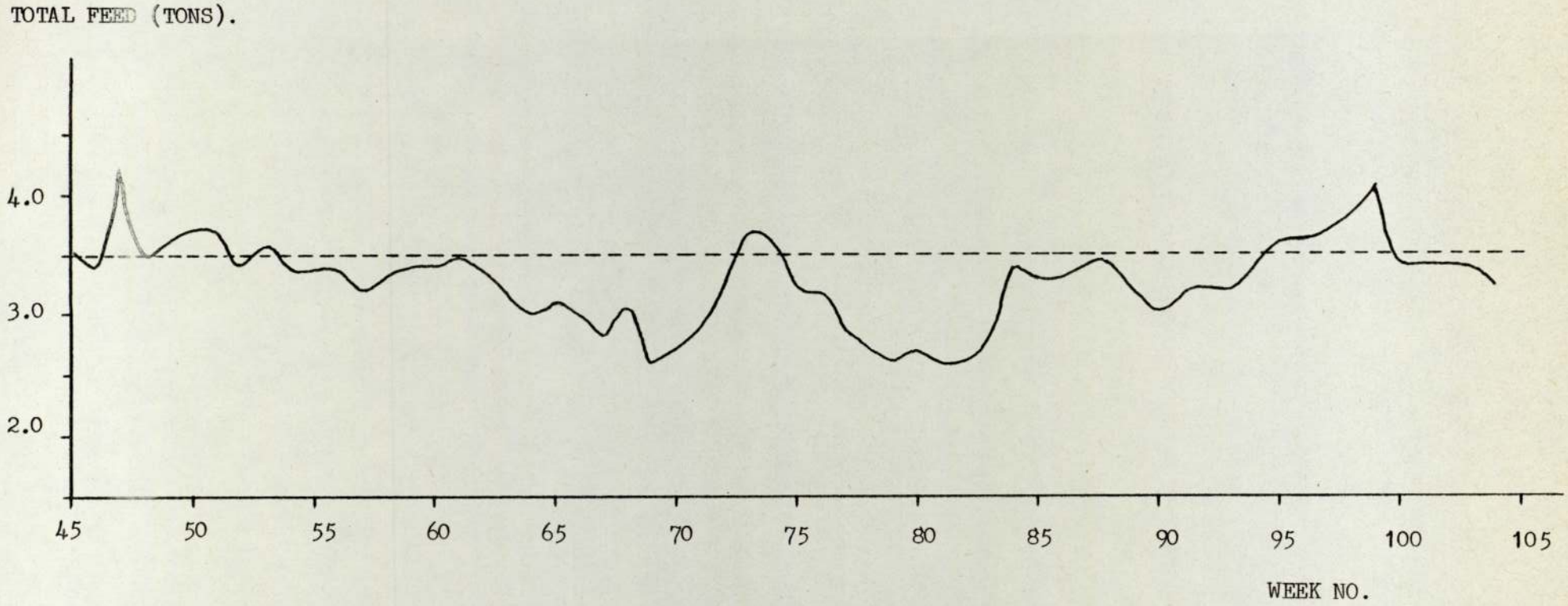


FIG. 6.9. THE TOTAL AMOUNT OF FOOD REQUIRED PER WEEK TO SUPPORT THE SUGGESTED MODIFIED STOCKING SCHEDULE UNDER THE CONDITIONS AT FARM B. ----- = APPROXIMATE 3.5 TONS/WEEK MAXIMUM.

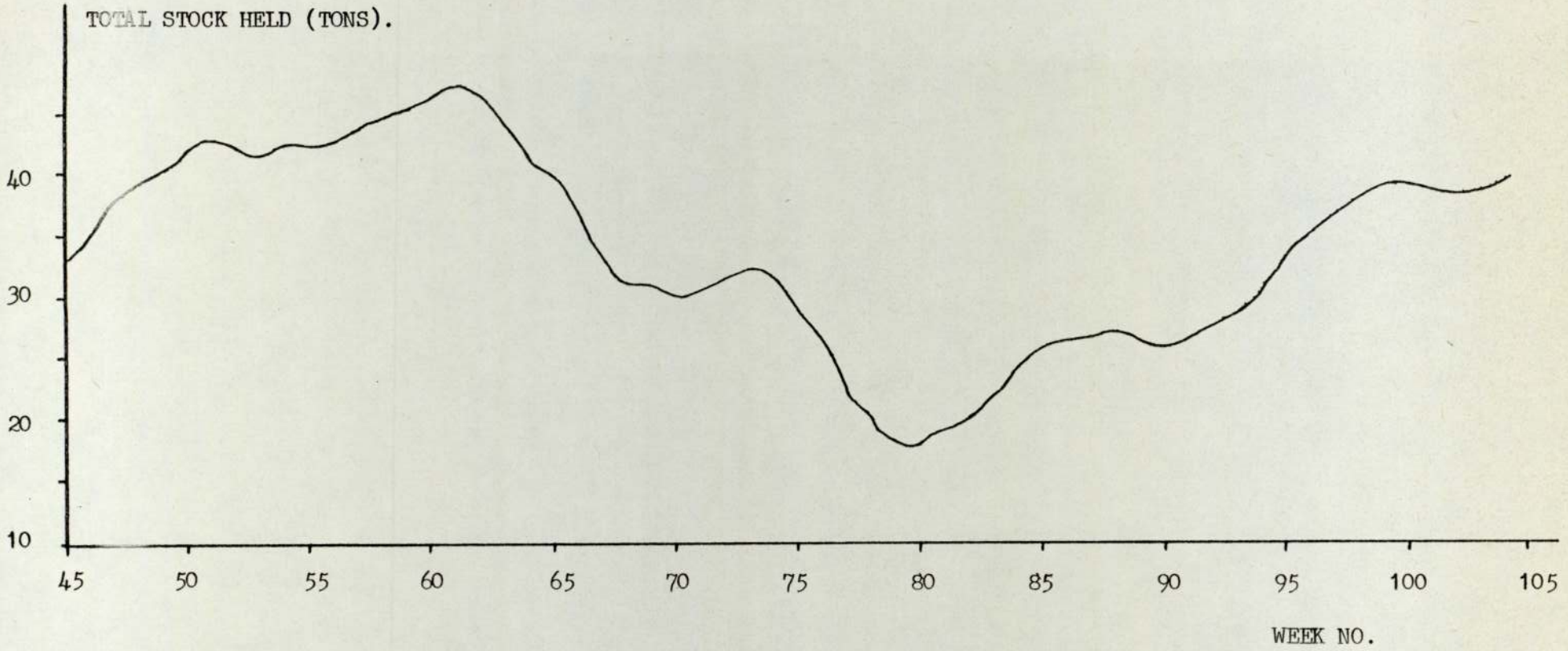


FIG. 6.10. THE TOTAL STOCK HELD (TONS) PER WEEK DURING THE SUGGESTED MODIFIED STOCKING SCHEDULE UNDER THE CONDITIONS AT FARM B.

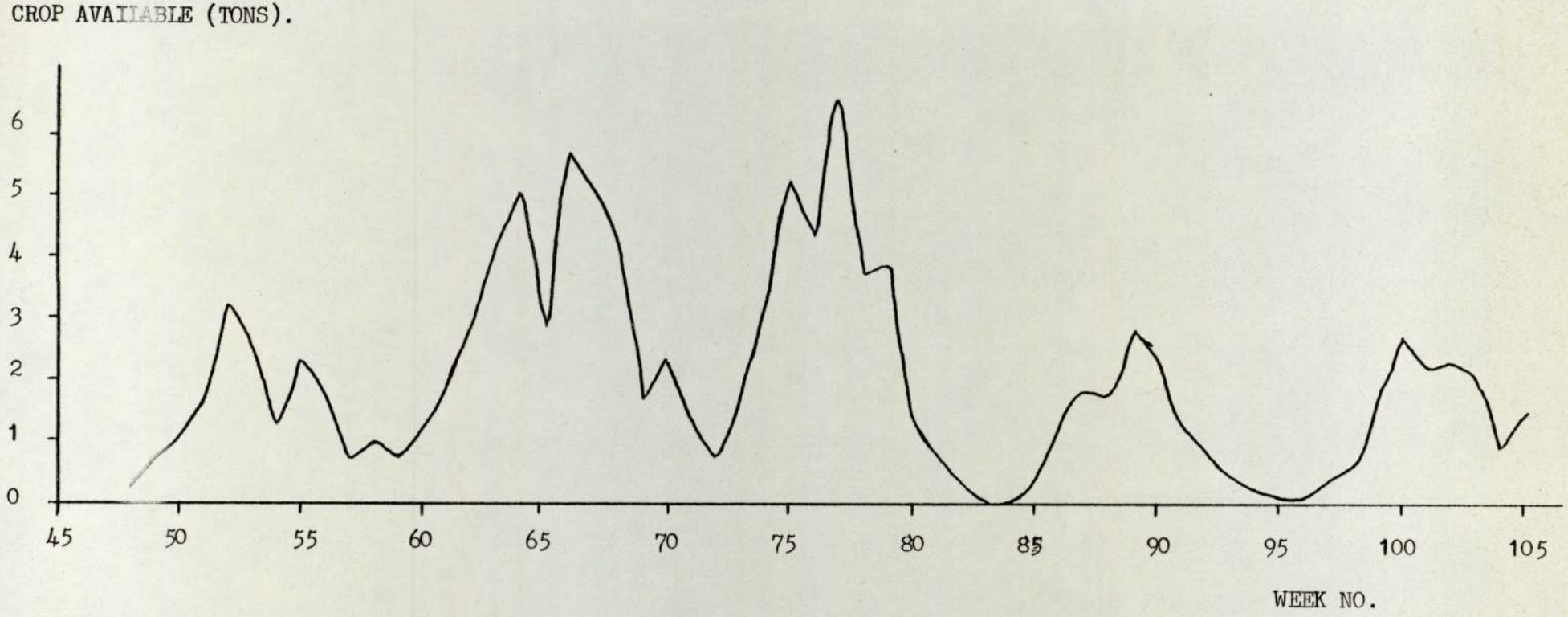


FIG. 6.11. CROP AVAILABLE PER WEEK (TONS) FROM THE SUGGESTED MODIFIED STOCKING SCHEDULE UNDER THE CONDITIONS AT FARM B. (WEEKS 48-104).

The second year of cropping under this stocking schedule (weeks 105 - 156) is shown in Table 6.8 and Fig. 6.12. Again cropping is highest as the summer approaches, but the cropping periods are still reasonably consistent. The stocking schedule thus formulated provides all-year-round production and maximises the use of the farm. It is interesting to note however, that during the first year of cropping (weeks 52 - 104) the total crop produced is 109 tons, whereas in the second year of cropping, the total crop produced is 100 tons. This is a reflection of being able to increase the stock in the early groups during the initial stocking of the farm and thus this increased production could not be repeated.

Conclusions regarding Farm B: In summary therefore, the stocking over the first two years to suit the temperature regime would be:

week No:	No. of 5g fry introduced
0	63,000
12	142,000
24	112,000
36	48,000
48	58,000
60	108,000
72	130,000
84	54,000
96	55,000

This schedule produces an all-year-round cropping regime, although the amounts do vary. Production over the first year of cropping would be 109 tons, and in the second and subsequent years 100 tons. Furthermore, stocking is such that at high water temperature stock is low, and at low temperature stock is high.

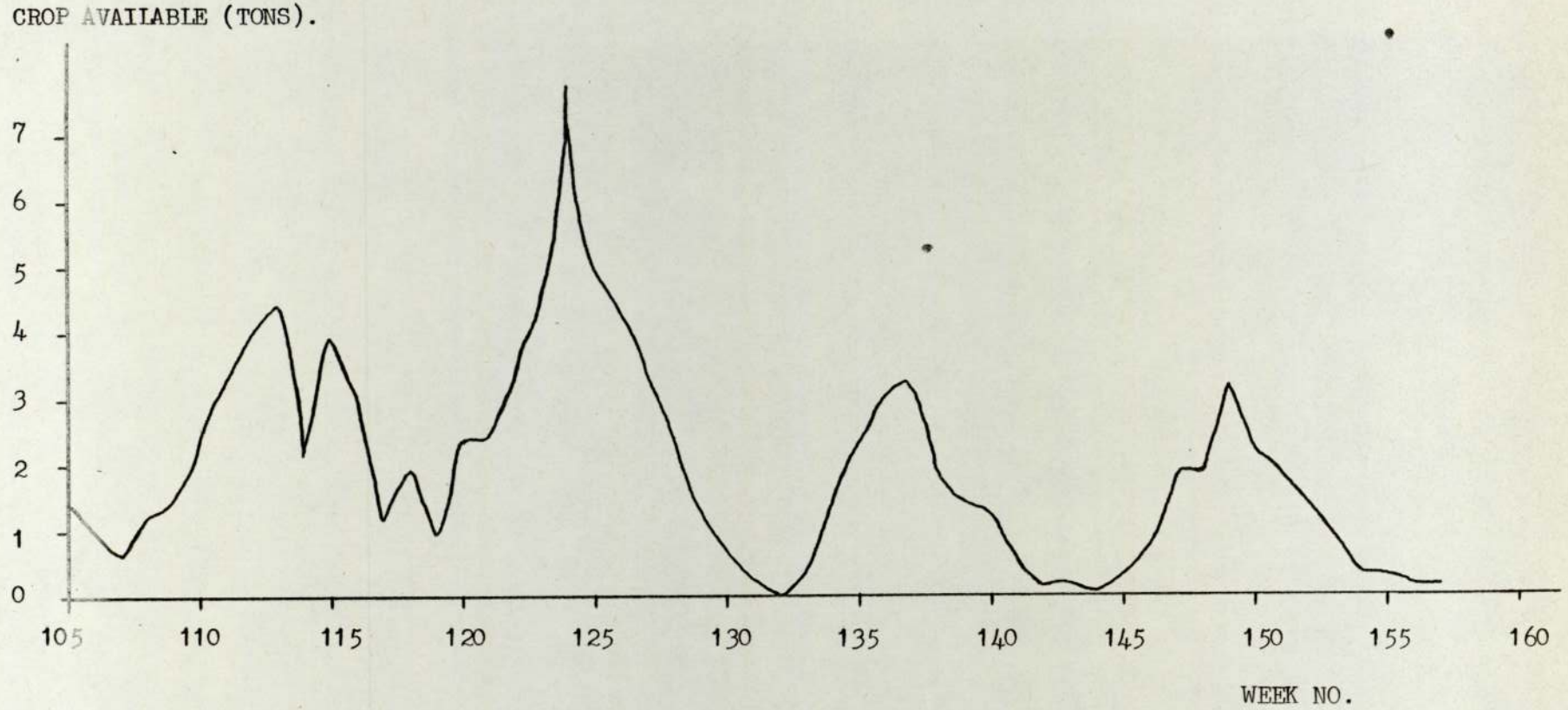


FIG. 6.12. CROP AVAILABLE PER WEEK (TONS) FROM THE SUGGESTED MODIFIED STOCKING SCHEDULE UNDER THE CONDITIONS AT FARM B. (WEEKS 105 - 156).

3) Farm C: This farm is based on a theoretical Northern Britain temperature regime, the water temperature varying between 3°C and 15°C, throughout the year (Fig. 6.4). In formulating a stocking schedule to suit these conditions, the same availability of 5g fry as in the two previous examples is assumed, and thus they are again introduced at weeks 0, 12, 24, 36, 48, 60, 72, 84, and 96 during the first two years of operation. The total amount of food required to support populations of 91,000 fish introduced at these times is shown in Table 6.9 and Fig. 6.13. Two periods occur where the feed that would be required to support such a stocking schedule exceeds the maximum capacity of the farm (weeks 69 - 94 and 121 - 144). Similarly between weeks 94 and 121 there is a period when feeding would be well below capacity. Once more, the stocking schedule needs to be modified before it can be applied to this site. By adjusting the numbers of fish in each group in the way shown in the previous example, and hence altering the feed required, the total amount of food fed per week can be brought closer to the 3.5 tons per week maximum. Hence the following changes would be required:

week No.	No. of 5g fry introduced.
0	91,000 x 2.560 = 233,000
12	91.000 x 1.117 = 102,000
24	91,000 x 0.650 = 60,000
36	91,000 x 0.552 = 51,000
48	91.000 x 0.671 = 62,000
60	91,000 x 1.410 = 128,000
72	91,000 x 0.610 = 56,000
84	91,000 x 0.565 = 52,000
96	91,000 x 1.000 = 91,000

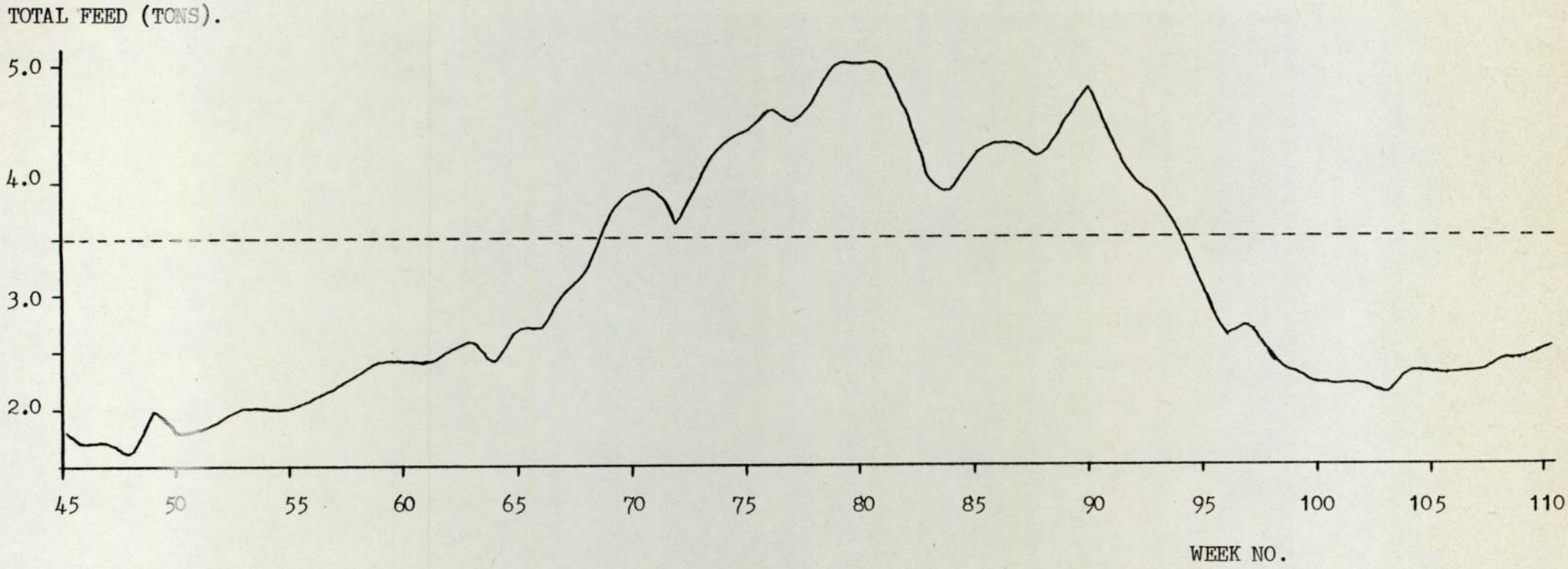


FIG. 6.13. THE TOTAL AMOUNT OF FOOD THAT WOULD BE REQUIRED TO SUPPORT POPULATIONS OF 91,000 FISH INTRODUCED AT WEEKS 0,12,24,36,48,60,72,84 AND 96 UNDER THE CONDITIONS AT FARM C. ----- = APPROXIMATE 3.5 TONS/WEEK MAXIMUM.

(CONTINUED OVERLEAF).

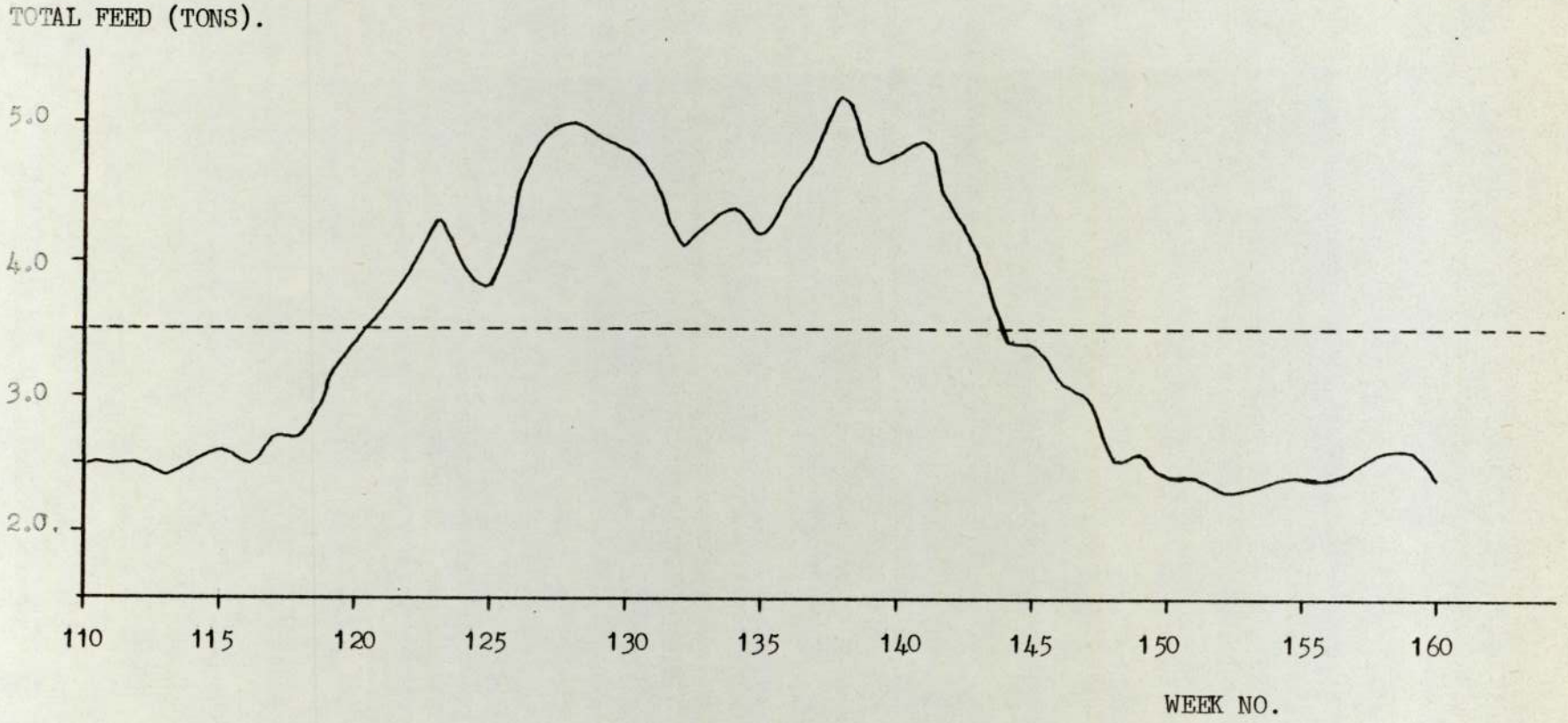


FIG. 6.13. (CONTINUED).

Having adjusted the introduction of fry in this way the amount of food required per week now becomes closer to the 3.5 tons per week maximum, as shown in Table 6.10. and Fig. 6.14. From these results, it is clear that for the majority of the year, the feed regime is very close to the maximum, and although periods are still present when under-feeding occurs, they are reduced to a minimum. These periods of under-feeding could not be further reduced, as if more feed was input into the system at this time, problems would be created by the necessity to feed larger amounts, and hence exceed the limit at a later date.

The total stock held under this schedule, at any given time is shown in Table 6.11. and Fig. 6.15. In this case the stock varies between 71 and 22 tons over the first year. The high stock over the early part of the year is again due to the ability to stock the farm with more fish during the initial stocking. This high of 71 tons would not be repeated in the second and subsequent years, when it is unlikely that the stock held will exceed 40 - 45 tons at maximum. Furthermore, in this particular case, while a stock holding of 71 tons is theoretically possible, it may well be decided that it is too high to be manageable, and thus the initial introduction of fry would have to be reduced.

The cropping available under this regime is shown in Table 6.12. and Fig. 6.16. As in the previous case, the majority of crop is available at the start of the summer, as the stock is reduced in time for the approach of the higher water temperatures. The total crop produced during the first year of cropping (week 52 - 104) would be 117 tons, unless of course, it was decided not to maintain such a

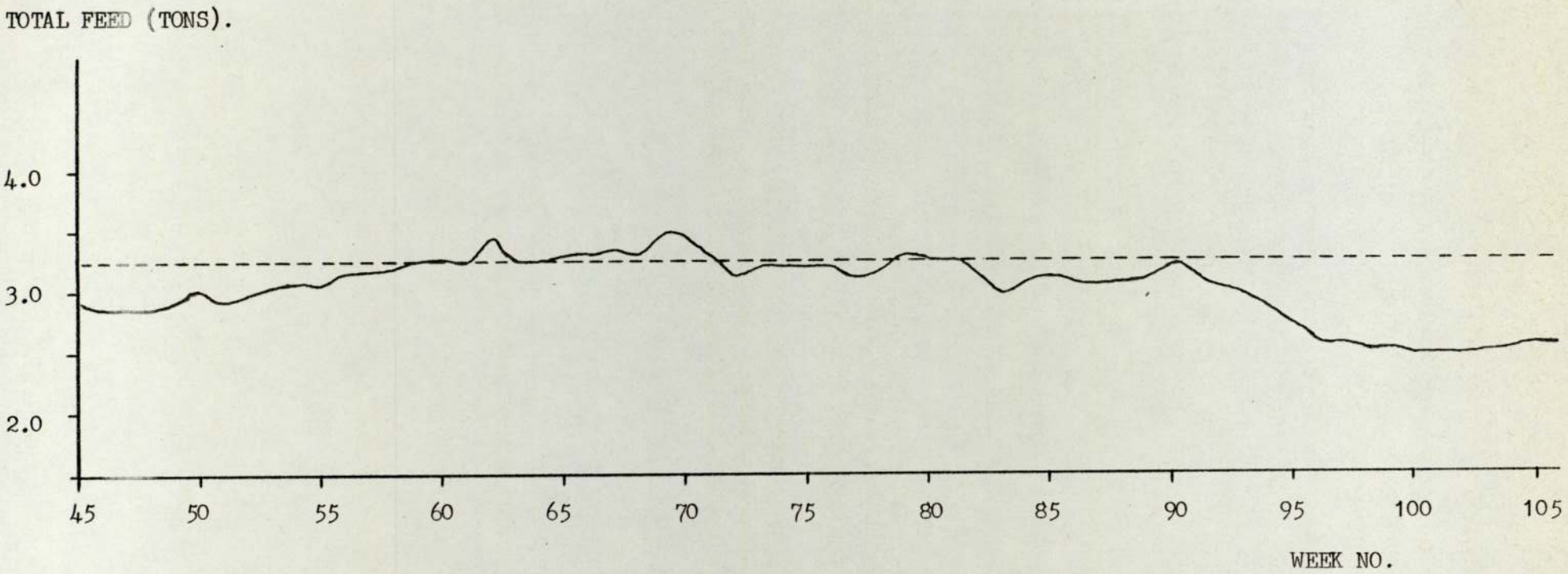


FIG. 6.14. THE TOTAL AMOUNT OF FOOD REQUIRED PER WEEK TO SUPPORT THE SUGGESTED MODIFIED STOCKING SCHEDULE UNDER THE CONDITIONS AT FARM C. ----- = APPROXIMATE 3.5 TONS/WEEK MAXIMUM.

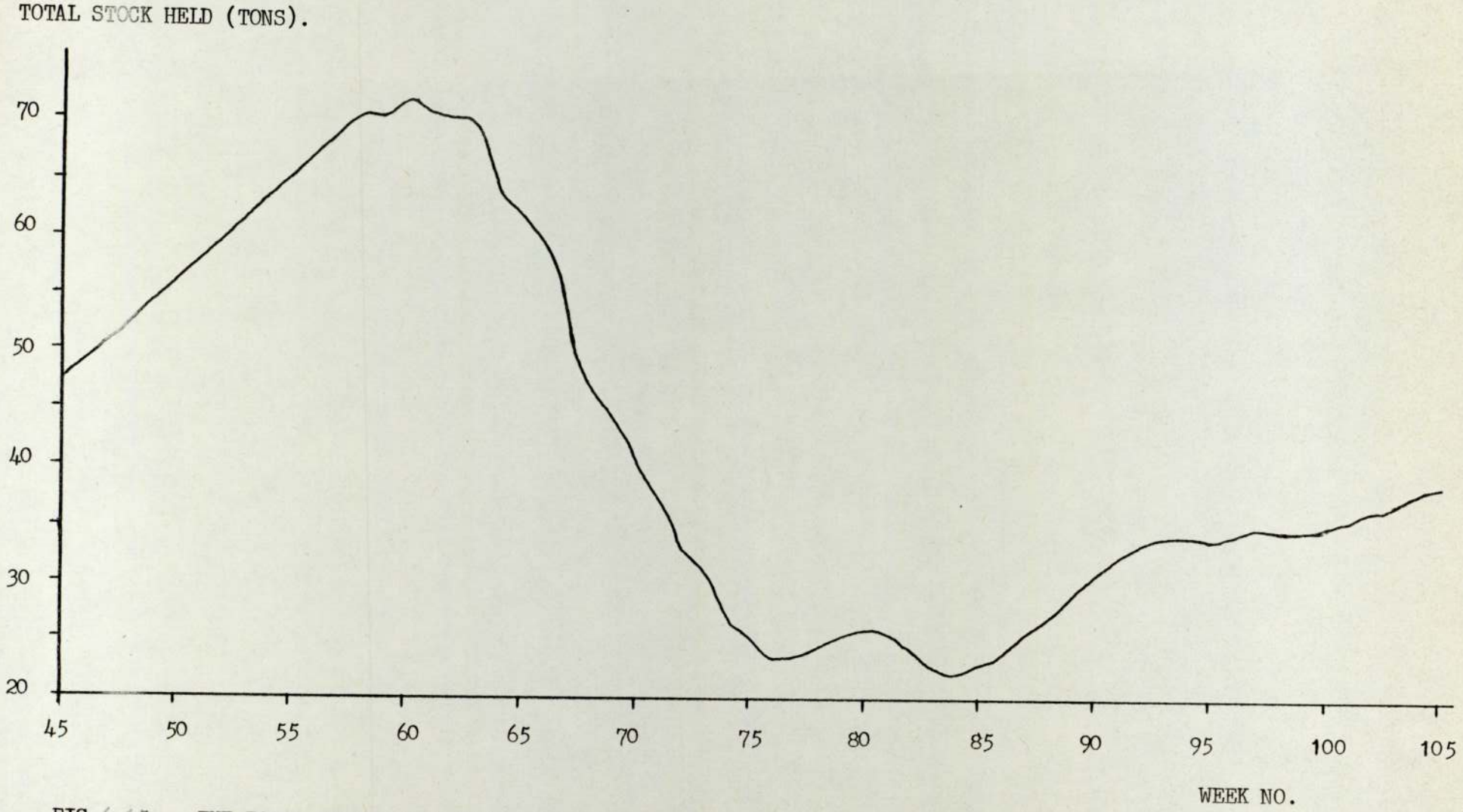


FIG 6.15. THE TOTAL STOCK HELD (TONS) PER WEEK DURING THE SUGGESTED MODIFIED STOCKING SCHEDULE UNDER THE CONDITIONS AT FARM C.

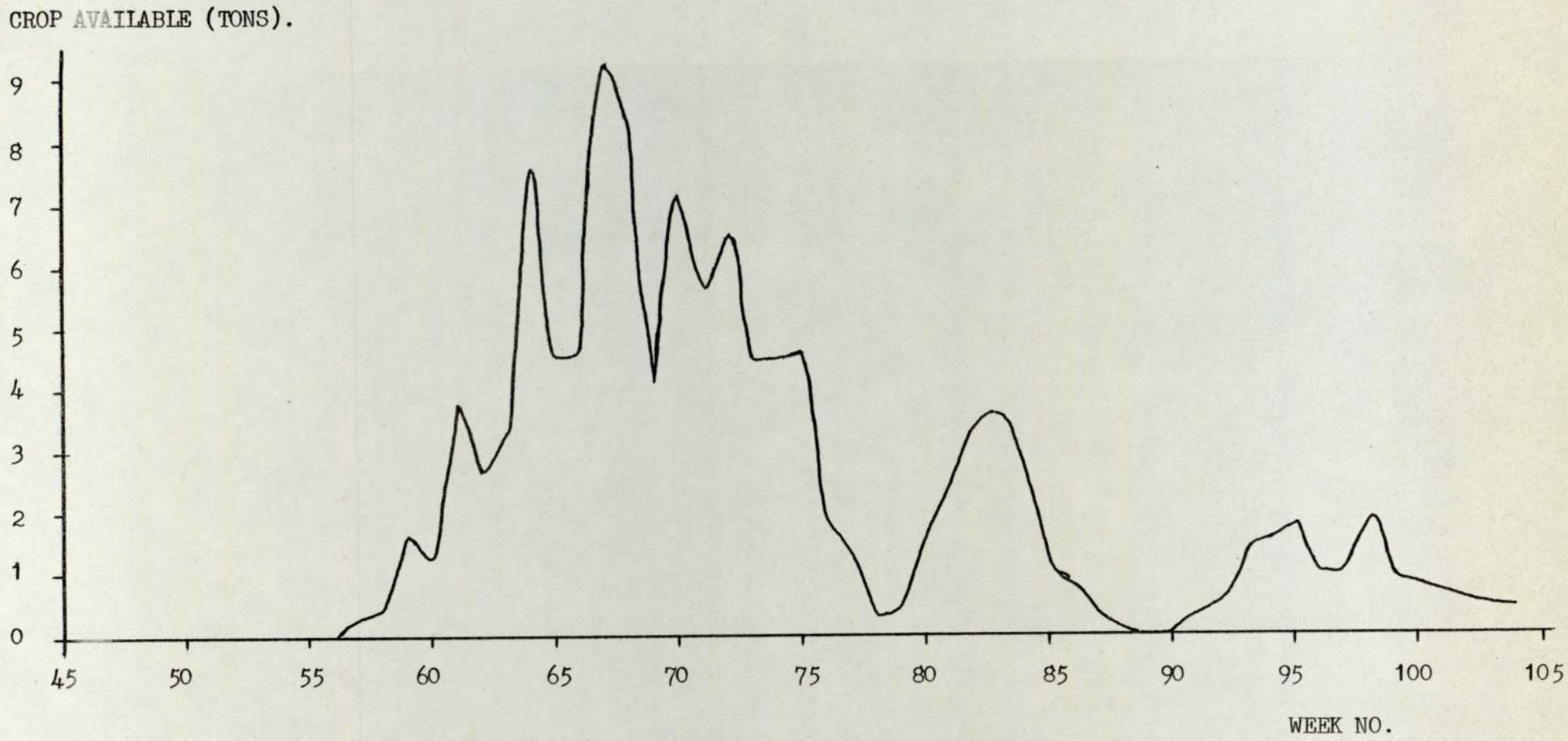


FIG. 6.16. CROP AVAILABLE PER WEEK (TONS) FROM THE SUGGESTED MODIFIED STOCKING SCHEDULE UNDER THE CONDITIONS AT FARM C. (WEEKS 52 - 104).

high stock load as 71 tons early in the first year, in which case the crop available between weeks 60 and 75 would be reduced. The second year of cropping (weeks 105 - 156) under this stocking schedule is shown in Table 6.13 and Fig. 6.17. Once more, the cropping is highest as the summer temperatures approach, but the cropping periods are relatively consistent, thus giving all-year-round production. The total crop produced during the second and subsequent years of cropping would be 86 tons. This reduced production compared with the previous two examples (100 tons in the second and subsequent years) is a result of the longer periods encountered at very low water temperatures, hence producing a much slower growth rate.

Conclusions regarding Farm C: In summary, the stocking schedule over the first two years to suit the temperature regime would be:

week No.	No. of 5g fry introduced.
0	233,000
12	102,000
24	60,000
36	51,000
48	62,000
60	128,000
72	56,000
84	52,000
96	91,000

Again mortality is not taken into account at this stage and the numbers introduced would have to be increased accordingly. However, as previously explained, this does not seriously affect the overall results. This schedule produces an all-year-round cropping regime,

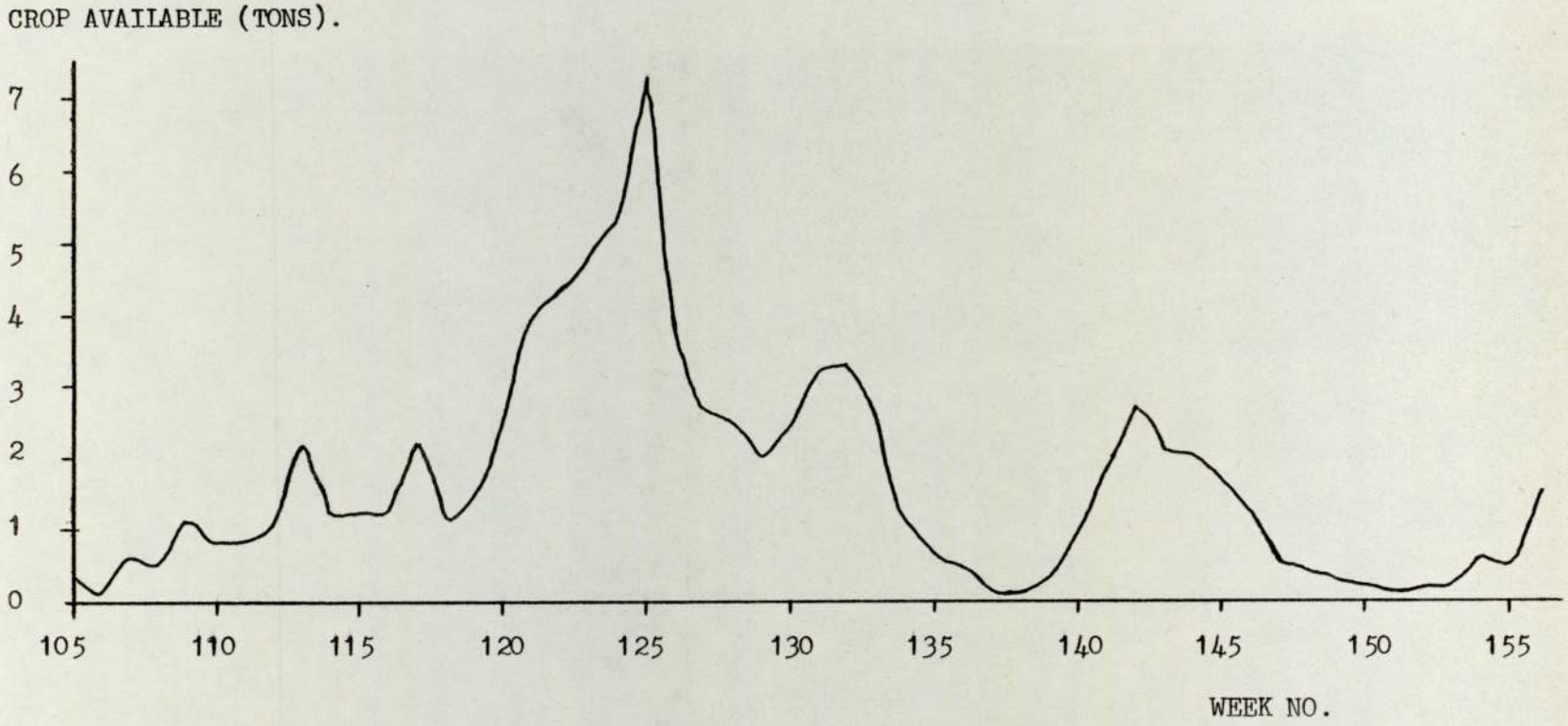


FIG. 6.17. CROP AVAILABLE PER WEEK (TONS) FROM THE SUGGESTED MODIFIED STOCKING SCHEDULE UNDER THE CONDITIONS AT FARM C. (WEEKS 105 - 156).

although again the amount available for cropping per week varies over the year. Production in the first year of cropping could be a possible 117 tons (although perhaps unlikely), and more important, during the second and subsequent years of cropping, production would be around 86 tons per year.

6.4. Discussion.

The three farms considered here clearly demonstrate that different sites require quite different approaches to stocking in order to maintain efficient production. However, the program provides for each of the different farms, all the necessary information to enable stocking to be optimised for any given set of conditions, constraints and fry availability.

Although a full and more complex program could be developed to produce stocking alternatives directly, there will always be a limit to the number of alternatives available for stock management, until fish farmers are able to control spawning on a scale sufficient to produce large quantities of eggs at any time of the year. Thus there is little point in producing a complete optimisation program at this stage, as it is probable that some of the alternatives suggested may currently be difficult or impossible to implement. With the development of large scale controlled spawning the more alternatives will be available, and once identified, it will be possible to build further optimisation proceedures into the program.

The results from this model and their subsequent manipulation demonstrate the stocking regime that would be required under given conditions, based on optimal feeding and growth in terms of efficient food utilisation. However, the results may be further modified at certain times of the year to increase production when there is available stocking capacity. For example, in the case of Farm C, cropping could be increased in the period of weeks 100 - 120 by increasing the numbers of fish in the population originally introduced at week 36, but feeding them below the value recommended from tables

during the latter part of the first year. Although the numbers of fish in that population are increased as they are being fed less, the capacity of the farm would still not be exceeded during weeks 60 - 90 (Fig. 6.14). During weeks 100 - 120, as there would now be more fish in this population, the amount of food fed would be increased. This total amount could be maximised by also feeding above the value recommended from tables. The period of feeding below capacity from week 90 to 120 would thus be eliminated and production from the population originally introduced at week 36 increased. However, in this case, growth efficiency is not as high as in other groups, due to the divergence from feeding tables. Thus, gaps in production can be filled and this example further illustrates the type of decisions possible, once the basic information on how the stock will respond to certain conditions is available.

The results obtained for Farm A (based on the conditions at Low Plains) appear to fit very closely with the actual situation and suggest that the assumptions used in the control model are valid. Up to the present time, it has not been possible to fully determine the accuracy of the model under varying temperature conditions. However, as the progress of stocks is constantly monitored on all Shearwater farms, it will be a relatively simple task to re-assess the situation periodically and modify the program or adjust the stocking schedule should the need arise. This process of periodic comparison between the predicted and obtained results, will produce a more accurate model and a better understanding of the behavior of stock under different conditions.

Most disease states adversely affect feeding, conversion ratio

and hence growth. Consequently it is possible that the model could be used to assess the situation during or following a disease outbreak, to identify the problems caused in terms of lost production and to provide solutions for subsequent management to re-optimise stocking.

Although the examples considered apply only to a given set of constraints, exactly the same proceedure can be used for different alternatives, such as a larger site designed to produce far more than 100 tons per year, or the stocking schedule that would be required if fish of a different cropping size were to be produced. Similarly the effects of stocking with fry at 10g instead of 5g could be evaluated, or the stocking required if the conversion ratio was either higher or lower than the 1.6:1 used in this model.

In summary, the model is based on a simple growth equation calculated from factors that can be readily determined and evaluated by the fish farmer. Although complete optimisation of the program has intentionally not been formulated at this stage, the results produced with reference to growth, stocking, cropping and feeding required, provide the fish farmer with sufficient information to enable basic decisions to be taken on stocking to maximise the use of his farm and hence production. The suggested stocking proceedures provided, still leave sufficient scope for the farm manager to make day to day decisions on cropping, feeding, etc., which without such background predictions could not be taken accurately. The model therefore has great potential in suggesting solutions to the following problems:

a) How to stock the farm with fry of a certain size at different times of the year under a given set of conditions.

b) The effects of stocking with fry of different sizes.

c) The changes in stocking that would be required if fish of a different cropping size were to be produced.

d) Evaluating the effects of disease outbreaks on subsequent production, and the modifications required to offset the problem.

e) The effects of different conversion ratio on the required stocking schedule.

f) By periodically comparing the actual growth and production with the predicted values, and making any necessary adjustments, production each week should be able to be calculated with a reasonably high degree of accuracy.

g) The model could be used as a simulation system to enable future managers to gain a better understanding of the problems likely to be encountered during the management of variable temperature sites.

The answers to the above problems will enable the farm manager to make better day to day decisions concerning the overall management of the farm, and predict production more accurately. Furthermore, as new sites are being planned, an evaluation of the likely yearly production from a potential site, the size of site required, water requirements, etc., would prove an invaluable tool in assessing the suitability of a certain site, and provides a yardstick by which to compare several

potential sites.

In conclusion the use of the model is therefore two-fold. It will provide basic information required to assess the suitability of new sites, and once in operation, provides information on how to stock a farm site under a given set of conditions. By using a more accurate and predictive approach to fish farming, better overall management decisions on selection of sites and stocking, will therefore be possible.

7. THE PREVENTION OF MATURATION AMONG PRODUCTION STOCK.

7.1. Introduction.

The second major problem concerning reproduction in salmonids is maturation of farm stock before they are of a suitable size for table consumption. Generally, female rainbow trout mature after three years growth whereas the majority of male fish mature one year earlier. During the process of sexual maturation, particularly among the male fish, changes occur in skin pigmentation due to the formation of secondary sex characters, which seriously reduce the market acceptability. Furthermore there is an increased susceptability to bacterial and fungal invasion, and also of importance, associated losses in growth, flesh quality and food conversion efficiency. Although this phenomenon is well known amongst fish farmers there are few records in the scientific literature.

The changes in growth and food conversion efficiency that occur as maturation takes place are shown in Figs. 7.1 and 7.2. When the time of spawning is advanced under the shortened photoperiod regimes, these changes occur earlier, thus demonstrating that the changes are closely related to maturation and spawning. The food conversion ratio increases as maturity approaches (Fig. 7.3), and as the fish begin to spawn, the weight loss encountered means that no conversion ratio can be calculated. It is not known whether this is due to an alteration in food intake, or food utilisation, or a combination of both. Similar changes have been demonstrated by Purdom et al, (unpublished data), who showed a significant difference in the weights of mature and immature rainbow trout during and after the spawning season (Fig. 7.4).

As the majority of farms at present aim to market fish of around

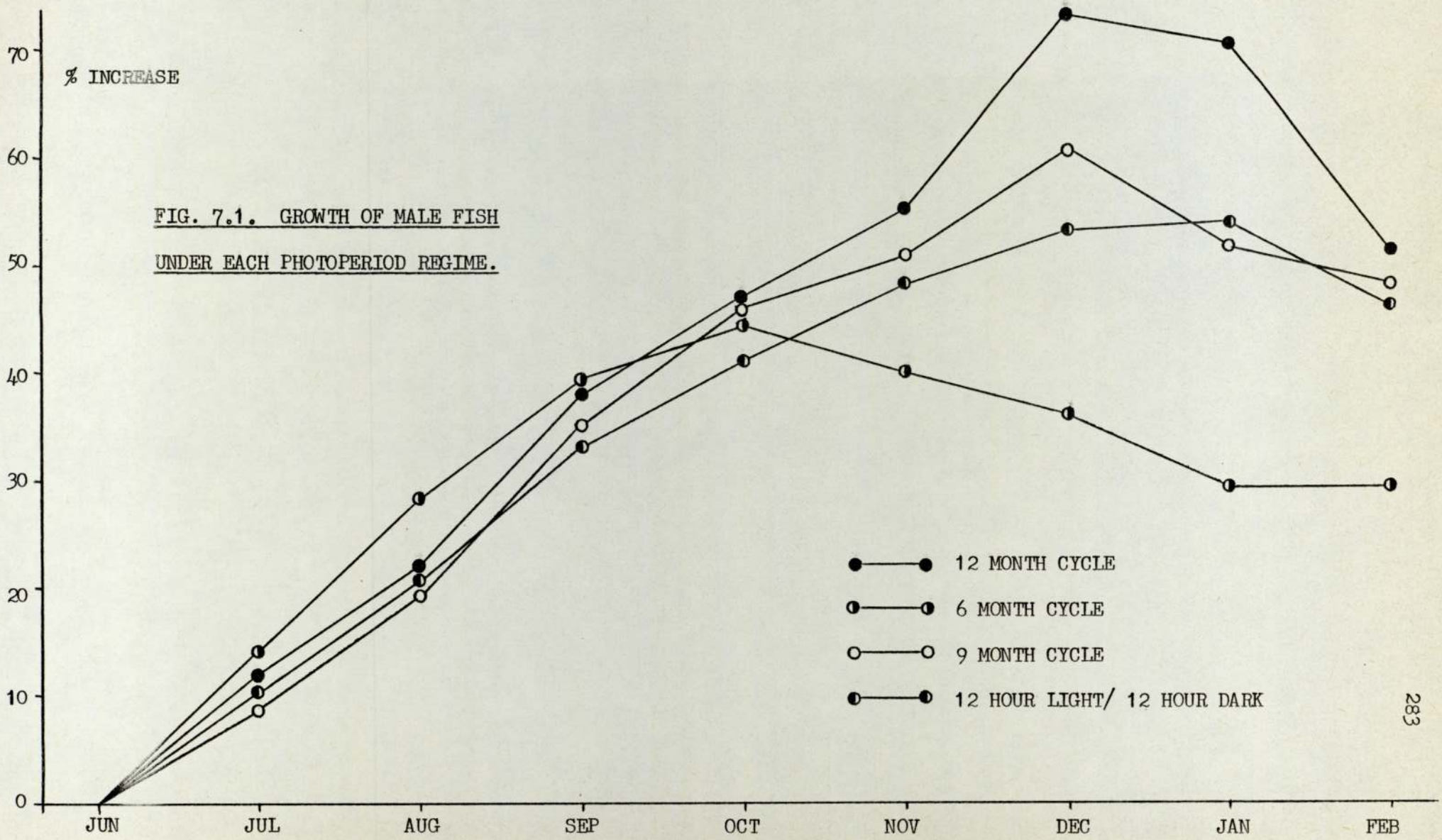


FIG. 7.1. GROWTH OF MALE FISH UNDER EACH PHOTOPERIOD REGIME.

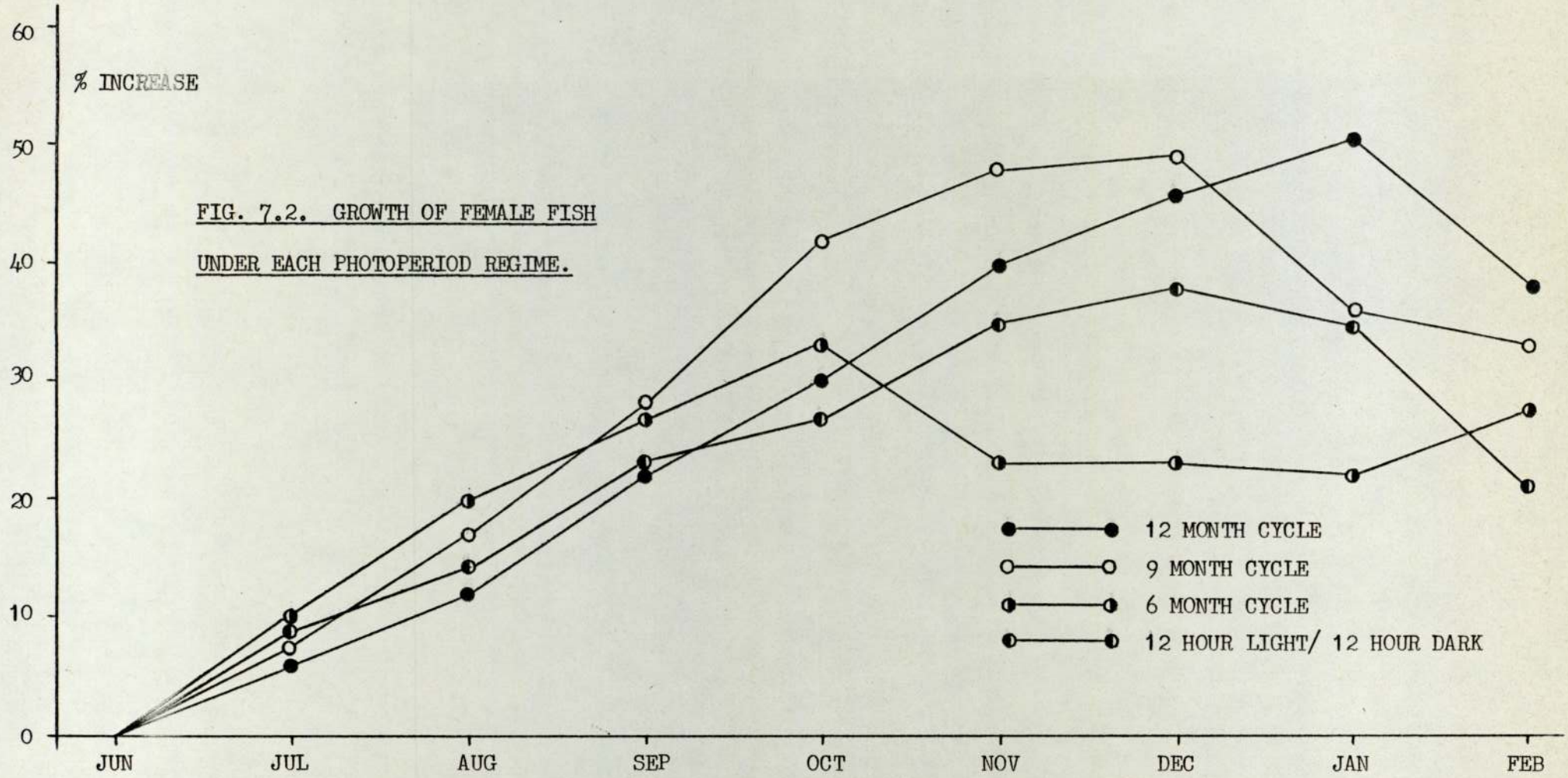


FIG. 7.2. GROWTH OF FEMALE FISH UNDER EACH PHOTOPERIOD REGIME.

MONTH.	PHOTOPERIOD CYCLE.			
	12 MONTH	9 MONTH	6 MONTH	12/12 CONSTANT
JULY	1.86	1.48	1.97	1.06
AUGUST	1.42	1.51	1.45	2.03
SEPTEMBER	1.47	1.51	1.49	1.78
OCTOBER	1.32	1.41	2.56	1.65
NOVEMBER	1.39	2.09	+	2.25
DECEMBER	2.01	2.35	+	2.94
JANUARY	7.81	+	+	+

+ INDICATES WEIGHT LOSS DURING MONTH .'. NO CONVERSION RATIO AVAILABLE.

FIG. 7.3. FOOD CONVERSION RATIO DURING SERIES 1 PHOTOPERIOD EXPERIMENTS (SEE CHAPTER 3).

MATURE MALES.

MONTH OF SAMPLING.	NO. OF FISH.	MEAN LENGTH(CM.).	MEAN WEIGHT(G.).
DECEMBER	20	28.3	351.2
JANUARY	20	28.4	346.3
APRIL	9	29.8	399.9

IMMATURE FISH.

MONTH OF SAMPLING.	NO. OF FISH.	MEAN LENGTH(CM.).	MEAN WEIGHT(G.).
DECEMBER	81	29.9	394.9
JANUARY	80	31.7	491.3
APRIL	67	35.6	748.7

FIG. 7.4. THE MEAN LENGTH AND WEIGHT OF MATURE AND IMMATURE RAINBOW TROUT DURING AND AFTER THE SPAWNING PERIOD. (PURDOM ET.AL. - UNPUBLISHED DATA).

250g (i.e. approximately 18 months from hatching, depending upon local conditions), the problem is largely confined to the male fish present in the population. However, there is an increasing interest in the production of larger fish particularly in salt water culture. In this case, the difficulties caused by the maturation of males becomes more acute, and furthermore maturation amongst females becomes a problem, as a result of the diversion of the female's resources into the production of eggs. Clearly, in the production of 3+ year old fish all fish will be expected to mature. These difficulties would be eliminated if stocks of rainbow trout could be produced which were sterile, or at least reduced if all-female stocks could be obtained.

The processes of sexual maturation, including the formation of secondary sex characters, is ultimately under the control of the endocrine system. Thus, gonadotropin (e.g. Yamazaki & Donaldson, 1968; Crim et al, 1975; Breton & Billard, 1978) and the two major androgens in fish, 11-ketotestosterone and testosterone (Idler et al, 1960; Gracjer & Idler, 1961; Idler et al, 1971; Schreck et al, 1972; Billard, 1974; Schreck & Hopwood, 1974; Wingfield & Grimm, 1977) have been implicated in the control of maturation in the male fish. The endocrine control of maturation in the female fish has already been discussed. Hence, solutions to the problem must eventually revolve around the disruption, either directly or indirectly, of the hormonal mechanisms controlling maturation and the formation of secondary sex characters. The important hormones in this context are gonadotropin and gonadal steroids although adrenocorticotropic hormone and melanocyte stimulating hormone may also be involved.

A number of methods of preventing or slowing maturation would appear to be available to the fish culturist and generally these involve similar areas of investigation to those which have been discussed with regard to the alteration of spawning time. Most of these methods have only been studied on a relatively small scale in the research laboratory, and few investigations have combined these studies with field-scale trials. Additionally, a variety of species have been studied, and not surprisingly the treatments have yielded variable results. The various methods available are discussed in more detail below and an appraisal made of their possible application to rainbow trout culture.

7.1.1. Surgical Castration: The most obvious approach is to surgically remove the gonad. Castration removes not only the gamete producing cells but also the endocrine cells responsible for the production of the sex steroids. This is important because it is the sex steroids which are thought to elicit the changes in flesh, colour and condition which accompany gonadal maturation.

7.1.2. Irradiation: Radiation is known to have a sterilising action in many groups of animals and plants. In vertebrates the sex cells which produce the sperms and eggs are far more vulnerable than the cells responsible for producing the gonadal hormones, and thus high doses of radiation have to be used if both types of cells are to be destroyed.

7.1.3. Environmental Control: As environmental factors, in particular photoperiod, are known to have profound effects on reproduction, it is probable that altered light regimes might be used successfully to

delay or prevent maturation. Several studies are available in the literature although some of the results are contradictory. Hoover, (1937) and Allison (1951) produced a delay in spawning of the brook trout by altering the photoperiod, although some of their data is open to criticism. However, Pyle, (1967) kept brook trout in constant light and constant darkness and although a better growth rate was demonstrated in fish maintained in constant light, both groups matured and spawned at approximately the same time as control fish. Similar results were also seen in the present work where a constant 12 hour light/ 12 hour dark photoperiod regime had little effect on maturation and spawning time.

7.1.4. Genetic Manipulation: Three separate approaches to the problem are available from within the field of genetics: selection, hybridisation and the production of polyploid fish.

(i) Selection: Improvements of stock by genetic selection have been carried out in all forms of plant and animal farming. In trout farming, the usual practice is to select the best looking and often largest fish as parents for the next generation but unfortunately other components of their genotype, for example age at sexual maturity, may not be considered. The simple selection of fish stocks which spawn naturally in their third year rather than in their second would reduce the problems associated with the production of 250g fish, but would not help farmers concerned with the production of larger fish. It is unlikely that selection could produce fish that spawn later than in their third year.

(ii) Hybridisation: In nature, interspecific and occasionally intergeneric hybrids between closely related species of fish are sometimes encountered. Since the earliest development of fish culture, interest has been shown in the artificial production of hybrids between different species of salmonids (Day, 1884; 1887: Hofer, 1909). This interest has increased with the further growth and development of fish culture (e.g. Foerster, 1935; Jones, 1947; Winge & Ditlevsen, 1948; Stokel, 1949; Stenton, 1950, 1952). More recently, attention has been turned to the possibility of producing hybrids which may be sterile, thus eliminating the problems caused by maturation. However, although a wealth of information exists, comparison is difficult as a result of the numerous combinations which are available and the different characters used for evaluation. For example, in 1954, Alm demonstrated that of the combinations available from salmon (Salmo salar), sea trout (Salmo trutta trutta) and brown trout (Salmo trutta) crosses, only those between female Salmo trutta trutta and male Salmo salar produced offspring which were still found to be immature even after six summers growth. However, in contrast Piggins (1965) reported fertile offspring with a normal rate of maturation in crosses between Salmo salar and Salmo trutta trutta.

Recently, in a more comprehensive study, Suzuki (1976) has shown that intergeneric hybrid crosses between species belonging to Salvelinus, Salmo and Onchorynchus produced offspring of which only a small percentage matured. Hence, crosses between female Salvelinus fontinalis and male Salmo trutta produced sterile offspring. Similarly, offspring produced from crosses between female Salvelinus fontinalis and male Onchorynchus nerka, and female Salmo trutta and

male Onchorynchus nerka were also sterile. Conversely, interspecific crosses within the genus Salvelinus or Onchorynchus produced offsprings which showed a similar percentage maturity to that of the parents. Hence, this particular study inferred that generally, intergeneric hybrids produced mainly sterile offspring, whereas interspecific crosses within a particular genus produced offspring which mature similarly to the parents.

(iii) Induced Polyploidy: According to Wexelsen (1964), tetraploid (4n) plants produced artificially are usually fertile and give rise to sterile triploid (3n) progeny after fertilization with normal diploid (2n) plants. This sterility is due to the mechanical disturbances produced during pairing in meiosis by the unequal sets of chromosomes received from each parent. This principle has been applied to many domesticated species of plants, but only recently has the induction of polyploidy been applied to vertebrates, and in particular to fish culture.

In vertebrates, polyploidy does not occur commonly outside the amphibia, but triploid salmonids have been reported in nature (Svardson, 1945). In the laboratory, Vesetski (1967) has produced triploidy in sturgeon larvae, Valenti (1975) induced polyploidy in Tilapia aurea, and triploidy has been induced in Gasterosteus aculeatus (Swarup, 1951a, b), by application of temperature shocks to eggs at different stages of development. In the flatfish, Purdom (1972) has also successfully induced polyploidy in the plaice and the plaice-flounder hybrid, using a similar experimental approach.

7.1.5. Autoimmune Rejection of Testis: Recently, Laird et al, (1978) have suggested that autoimmune rejection of testis occurs in Atlantic salmon after injection with testis homogenate suspended in complete Freunds adjuvant, although as they admit, their work is based on few experimental animals. The autoimmune destruction of the testis has been demonstrated in several species, including guinea pig (Bishop & Carlson, 1965), rat (Levine & Savinski, 1970), mouse (Malkiel & Hargis, 1970), monkey (Andrada et al, 1969), bull (Parsonson et al, 1971), rabbit (Yantorno et al, 1971), bird (Wentworth & Mellen, 1964) and man (Mancini et al, 1965), but previously not in the lower vertebrates. The field of autoimmunity to spermatozoa has been reviewed recently by Rumke & Hekman (1975), and although the mechanism by which testicular damage occurs is not completely understood, immunological factors appear to be the most important cause of aspermatogenesis.

Although immunisation with testis extract would appear to produce aspermatogenesis, there is little evidence in the literature for an effect on hormone production, which as discussed earlier would appear to be the cause of the problems associated with male maturation. Studies on the metabolism of testosterone precursors (mainly ^{14}C-progesterone) have demonstrated a reduced production of testosterone during autoimmune aspermatogenesis in rabbits and guinea pigs (Sananez et al, 1971; Becker, et al, 1966) although in other studies, no indication of hormonal failure during autoimmune aspermatogenesis has been detected (Freund et al, 1955; Katsh & Bishop, 1958; Mancini et al, 1965).

7.1.6. Prevention of Maturation by Hormone Administration: It is possible that hormone treatment offers the greatest potential for preventing, or reducing the incidence of maturation in salmonid fish culture. Currently three methods are available as follows.

(i) Sterilization by feeding large doses of methyl testosterone.

(ii) Direct feminisation by feeding oestradiol 17β.

(iii) The production of functional phenotypic males with a female genotype (by feeding low doses of methyl testosterone) and subsequently crossing these males with normal females.

These methods are considered in more detail below. The experiments described later in this chapter, involving the feeding of hormones to juvenile fish were carried out in collaboration with the D.A.F.F.S. laboratory, Victoria Road, Torry, Aberdeen. Some of the growth results reported here were published as a part of a study by Johnstone et al, (1978).

(i) Sterilization with Large Doses of Methyl Testosterone: In 1958, Yamamoto reported that medaka, (Oryzias latipes) treated with methyl testosterone at a dose of more than 30mg/kg diet, during the juvenile period (starting from first feeding) eventually became sterile at the full grown stage, irrespective of sex. More recently, Takahashi (1977) has demonstrated that juvenile guppies (Poecilia reticulata) fed methyl testosterone at a dose of 1-2g/kg diet were completely sterilized providing the treatment continued for 70 days after birth. In the rainbow trout (Salmo gairdneri), Jalabert et al, (1975) stated that oral administration of androgen at dosage levels of 15-60mg/kg diet to juvenile fish for five months produced some sterilized adults,

although mortalities were very high. Similarly, Yamazaki (1976) showed that the administration of methyl testosterone at a concentration of 50mg/kg diet inhibited gonadal development in rainbow trout if the treatment was initiated shortly after hatching and was continued for a period of five months. Even at three years of age, the gonads of the experimental fish (although only three remained at this stage) were small and it was not possible to determine the sex of the fish by gross autopsy. Subsequent histology showed that two were male fish containing spermatogonial cysts, the third being female with a thread like ovary containing a small number of oocytes at the yolkless stage. The gonads of control fish of the same age, of both sexes were fully mature.

(ii) Direct Feminisation by Oestradiol 17β Administration: At present, the majority of trout farms aim to market fish which are less than two years old. For the reasons outlined previously this means that the problems associated with maturation are largely confined to the male fish, and would be eliminated if stocks of all-female fish could be produced.

Functional sex reversal has been performed in a number of species by administering steroid hormones in the food before normal differentiation of the gonad begins. In the medaka, sex reversal has been demonstrated from male to female (Yamamoto, 1958) and from female to male (Yamamoto, 1953; Yamamoto et al, 1968; Hishida & Kawamoto, 1970). Sex reversal has also been carried out in both directions in the goldfish using methyl testosterone and oestrone (Yamamoto & Kajishima, 1968). In addition, unisexual broods have been produced in Poecilia reticulata (Dzwillo, 1962), Tilapia mossambica (Clemens &

Inslee, 1968) and Tilapia nilotica (Jalabert et al, 1974) using methyl testosterone.

Earlier work on sex reversal by steroid administration in salmonids was mainly unsuccessful (Padoa, 1937, 1939; Ashby, 1957, 1965). Jalabert et al, (1975) produced more successful results using oestrone, but as the treated fish suffered high mortalities, these results are difficult to interpret. Johnstone et al, (1978) have produced 100% female populations in both trout and salmon by administering oestradiol 17β orally to juvenile fish for periods of 0-30 and 0-21 days respectively following first feeding.

(iii) Indirect Feminisation using Masculinised Females: While the sex determining mechanism in fish is not fully understood, evidence is accumulating mainly from karyotype studies, to suggest that in the rainbow trout at least, an XX-XY mechanism is present with the female being the homogametic sex (Thorgaard, 1976, 1977). Thus, a further method of producing all-female stocks would be to reverse the sex of normal females to give functional phenotype males (with a female genotype) which could then be paired with normal females (Fig. 7.5). In essence this means that two female genotypes (XX x XX) would be paired, and thus produce all-female offspring. This approach has been successful in Oryzias latipes (Yamamoto, 1958) and Poecilia reticulata (Takahashi, 1975) where normal female fish were paired with functional phenotypic males produced by treatment with methyl testosterone. A similar claim by Okada (quoted by Yamazaki, 1976) for the rainbow trout, does not appear to have been confirmed.

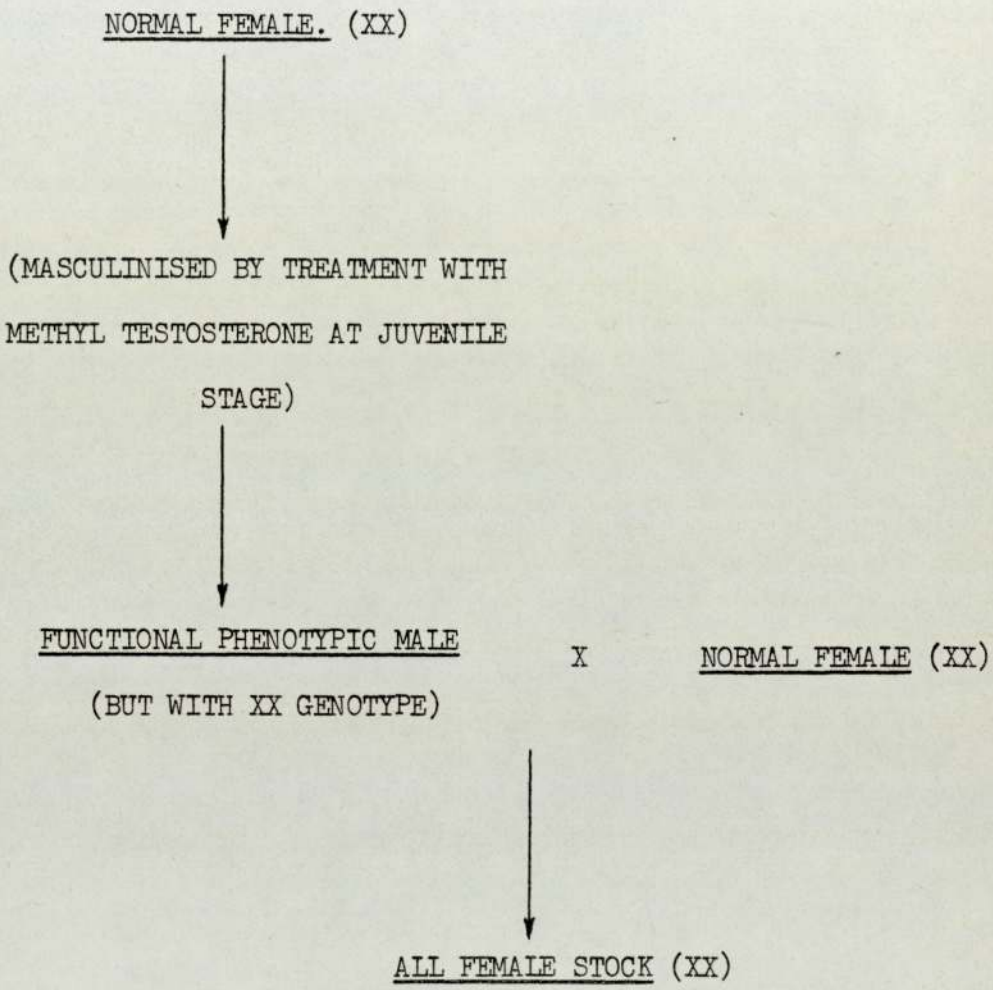


FIG. 7.5. THE PRODUCTION OF ALL-FEMALE STOCKS USING MASCULINISED FEMALE FISH.

7.1.7. Appraisal of the Application of the Methods of Sterilization and Sex Reversal to Rainbow Trout Culture: Surgical castration has generally been performed under sterile laboratory conditions and not under the very different conditions encountered on the farm. Although it has recently been claimed that 6 of these operations could be performed per hour, even at this rate almost 17,000 man-hours would be required to castrate a batch of 100,000 fish. Hence, it is generally considered that manual castration will be too laborious and expensive to be of much commercial value. Additionally there will almost certainly be some post-operative problems, due to infection by bacteria or fungi or to internal or external haemorrhage. Failure to remove all the gonad may lead to regeneration and the continued production of sex steroids and gametes.

Similarly, the commercial application of irradiation to produce sterile fish is questionable. The techniques used have been relatively crude, often using whole body irradiation, and this has inevitably produced reductions in growth and the activities of other mitotic/ dividing cells (Srivastava & Rathi, 1966). No long-term studies have been carried out and further work is required to decide whether the improvements in technology which are now available medically would enable the radiation to be strictly localised in the area of the gonad, and what capital expenditure for equipment would be required.

The genetic approaches available also pose certain problems. As stated earlier, simple selection would not produce an ideal answer, as it is unlikely that fish maturing later than three years old could be produced by this method. Furthermore, a selection programme to produce late spawning fish would seriously complicate the process of

selection for other factors, such as improved growth, disease resistance, etc.

Great care is required in interpreting the potential use of hybridisation in fish culture for the production of sterile fish. Hybrid fish would have to be sold under their own name and would require a completely new market, with all its associated marketing problems. Furthermore, the various hybrids produced appear to show quite different characteristics in terms of growth, survival, disease resistance, etc., (Alm, 1954; Buss, 1956; Crossmann & Buss, 1966; Gould, 1966; Suzuki & Fukuda, 1971a,b; Chevassus & Petit, 1975; Gjedrem, 1975; Suzuki, 1976), and therefore much research would be required to determine the suitability of the different hybrids for intensive fish culture.

Induced polyploidy in salmonids using temperature shock at different stages of egg development has to date been unsuccessful, as a result of the large size of salmonid eggs. Although Refstie & Gjedrem (1977) have attempted to induce polyploidy with the mitogen Cytochalasin B, mortalities were extremely high and the results unclear. Hence, although induced polyploidy is of value in some species, the technology presently available would not appear to be applicable to salmonid culture.

Because of the close involvement of gonadal steroids with the problems caused by sexual maturation, more research is required both on the hormonal and testicular changes that take place following the induction of autoimmune rejection of the testis before the method can be reliably applied to commercial fish culture. There is insufficient evidence available at present to ensure that the aspermatogenesis

produced is accompanied by hormonal failure. If this is not the case, then although the fish do not produce gametes, secondary sex characters may still be produced under the influence of sex steroids still present.

While some of the above methods of control may be of commercial value in the future, at present they are not at a sufficient stage of development to provide immediate solutions for the fish farmer. Of the remainder, the use of environmental factors to prevent maturation will obviously be limited by the type of farm and tank or pond design which is in operation. Although altered environmental regimes can be successfully used to advance spawning (Chapter 3), it is by no means clear whether environmental manipulation will delay or prevent maturation altogether. The results presented in chapter 3 clearly demonstrate the relationship between changing photoperiod and the hormone dynamics which control reproduction, and from these and similar studies it should be possible to contruct an environmental regime which will prevent maturation. This approach is further investigated in the present work.

The second area of study related to the production of the problems caused by maturation is that of hormone administration. With the present technology, it would appear that sterilization by feeding high doses of methyl testosterone in the diet to fry from first feeding for a period of 100 days is a promising approach to the prevention of maturation on a commercial scale. Previously this form of treatment has only been carried out in the laboratory (Yamamoto, 1969), and clearly, more information is required regarding the growth, survival, etc., of fish following treatment with methyl testosterone

on a commercial scale. Furthermore, it is considered that the doses of methyl testosterone used by previous workers were unnecessarily high for such a potent androgen. A study is therefore included on the effects of feeding 30mg/kg diet methyl testosterone to fry for a period of 110 days following first feeding. Growth, mortality and gonad development were monitored throughout the course of the experiment to provide the necessary information regarding the commercial applicability of this approach.

As the majority of farms currently market fish which are less than two years old, the problems associated with maturation are largely confined to male fish, and would be eliminated if stocks of all-female fish could be produced. Again, apart from the studies of Johnstone et al, (1978) most investigations have been on a laboratory scale with little regard given to commercial operations. Hence, a study is included to determine the effects of feeding 20mg/kg diet oestradiol 17β to fry for a period of 80 and 110 days following first feeding. Again growth, mortality and sex ratio were monitored during the experiment, to assess the commercial viability of such an approach.

The final study included in this context is to determine the sex ratio of offspring produced from crosses between sex-reversed phenotypic female fish and normal males. The production of all-female stocks by this method has the advantage that steroids are not administered to fish destined for human consumption, although Johnstone et al, (1978) have clearly demonstrated that this is not a danger. However, great care must be exercised as treatment would be limited to only a relatively small number of broodstock, and the possibility of inbreeding depression is likely to increase.

This experiment should also provide further information regarding the sex determining mechanism of rainbow trout.

7.1.8. Summary: It is clear from the foregoing review that a number of the suggested methods to prevent the problems associated with maturation cannot be carried out on a commercial scale until further research is completed. Each method has its associated advantages and disadvantages, but a general problem common to all of them is the lack of basic scientific data from commercial scale trials. Experiments were therefore conducted on a commercial scale to investigate the possibilities of using (a) environmental control, (b) direct sterilization using methyl testosterone, (c) direct feminisation using oestradiol 17β and (d) the production of all-female stocks from sex-reversed phenotypic female fish. These methods are at a sufficient stage of development to be of immediate use to the fish farmer, and although other methods should not be discounted, it is considered that further work is required before they can be applied to commercial fish culture.

7.2. Sterilization and Sex Reversal by Hormone Treatment.

7.2.1. Materials and Methods: The steroids used during all the trials were added to the food as follows: A 10% portion of the food was taken and defatted as it is possible that the steroids may suffer degradation as a result of coupled oxidation in the presence of dietary fats and oils (Johnstone et al, 1978). The removal of the fats was achieved by placing 750g of food in a 60cm x 10cm column, terminated by a sintered disc, and passing 2l of ethanol slowly through the column of food. The steroids used, either oestradiol 17β or 17α methyl testosterone (Sigma Ltd.,) were incorporated in ethanolic solution into the defatted portion by rotary evaporation in vacuo. At the start of evaporation, sufficient ethanol was placed in the flask to cover the food completely, thus ensuring an even mix. The amounts of steroid incorporated into the defatted portion of the food were carefully calculated to give the required steroid level (see below) when remixed with the remaining 90% portion of the normal food. The steroid loaded food was stored in the dark at 4°C and was mixed with normal food at intervals of around 10 days. Defatted portions containing no steroid were also produced to serve as a control diet.

The first feeding fry used during the experiments were reared in shallow 400l fry tanks and fed treated food daily between the hours of 9 am. and 5 pm. The food was administered at the rate recommended from feeding tables (B.P. Nutrition) for the size of the fish, at a constant temperature of 9°C. Mortalities were removed daily and recorded, from both treated and control fish.

The proportion of sexual types in each population was also determined when the fish had reached a size sufficient to enable sexing by macroscopic examination (approx. 5g +). This was achieved by opening the abdomen, removing the head, tail and viscera and fixing the remaining trunk containing the gonads in Bouin's fluid; the gonads were then examined under a low power binocular microscope. Subsequently, 5μ sections were cut from the wax embedded samples and stained with Harris's haematoxylin and eosin for later light microscopic examination.

The experimental proceedure for each trial was generally similar, but differed in detail as described below.

(i) Sterilization with High Doses of Methyl Testosterone: 5,000 swim up fry were placed in a shallow 400l fry tank and fed with a diet loaded with 30mg 17α methyl testosterone/kg food. The period of feeding extended from first feeding for 110 days. After this time the fry were fed with normal untreated food.

(ii) Direct Feminisation by Oestradiol 17β Administration: 75,000 swim up fry were distributed equally between 3 x 400l shallow fry tanks and fed with a diet containing 20mg oestradiol 17β/kg food for a period of 80 days following first feeding; the fry were then fed with normal untreated food.

7.2.2. Results: Both steroid treatments had profound effects on gonad development in the treated fish, discussed in detail below.

(i) Direct Sterilization: 3 months after the end of steroid treatment, examination of the gonads of the treated group showed that all fish

showed abnormal gonad development. Under the binocular microscope (x12) all of the 80 fish examined exhibited gonads of male type, although in all cases the appearance was very filiform when compared to the normal gonads in control fish of the same size and age. Histologically, the filiform gonads were very different from those of control fish with a germinal epithelium which was totally absent in most fish and reduced in the remainder. There was also an increase in connective tissue and vascularisation.

The growth observed during the experiment is shown in Fig.7.6. A significant depression in growth ($P<0.001$) was observed throughout the treatment period and at day 185 this was still apparent, the controls attaining a weight of 8.93 ± 0.21g and a length of 90.89 ± 0.73mm compared with a weight and length of 6.30 ± 0.12g and 81.10 ± 0.66mm respectively in the treated group (Fig.7.6). Mortality throughout the treatment period was similar in both control (22.0%) and treated (21.1%) groups.

Unfortunately, due to an error while the fish were being transferred from the fry sheds, the experimental group was accidentally mixed with a large number of untreated fish. Hence, no further evaluation was possible.

(ii) Direct Feminisation: At an average weight of 5g, macroscopic examination of the gonads of the treated groups revealed a female to male ratio of 2.5:1. Subsequent sectioning from wax embedded samples confirmed this result. Hence the treatment had increased the proportion of females in this group from the normal 1:1 ratio. Ovarian development at this stage was macroscopically normal and

TREATMENT	MEAN WEIGHT(G) ± S.E.	NUMBER IN SAMPLE	VARIANCE (S^2)	MEAN FORK LENGTH(MM) ± S.E.	NUMBER IN SAMPLE	VARIANCE (S^2)
CONTROLS (DAY 185)	8.93 ± 0.21	205	9.08	90.89 ± 0.73	205	109.1
METHYL TESTOSTERONE (DAY 185)	6.30 ± 0.12	225	3.03	81.10 ± 0.66	225	98.4

FIG. 7.6. THE EFFECT OF 30 MG METHYL TESTOSTERONE / KG FOOD (0 - 110 DAYS AFTER FIRST FEEDING) ON THE GROWTH OF RAINBOW TROUT FRY.

histologically indistinguishable from that of female control fish sampled at the same time.

During the period of steroid administration, oestradiol 17β slightly depressed body weight ($P<0.05$) and length ($P<0.05$) gains. However, at 185 days, although the mean fork length of the treated fish was approximately 2% lower than that of the controls, similar differences in mean length were seen in the replicate tanks of both control and treated fish. At this stage the mean weight of the treated fish was not statistically different from the controls (Fig.7.7). Mortality throughout treatment was similar in both control (16.1%) and treated (16.8%) groups.

TREATMENT	MEAN WEIGHT(G) ± S.E.	NUMBER IN SAMPLE	VARIANCE (S^2)	MEAN FORK LENGTH(MM) ± S.E.	NUMBER IN SAMPLE	VARIANCE (S^2)
CONTROLS (DAY 185)	5.31 ± 0.28	233	4.75	76.9 ± 1.3	233	100.6
				78.1 ± 0.8	500	78.0
OESTRADIOL 17 β (DAY 185)	5.43 ± 0.25	250	3.97	77.0 ± 0.9	375	77.8
				75.1 ± 0.8	393	69.1
				75.1 ± 0.9	366	69.1

FIG 7.7. THE EFFECT OF 20 MG OESTRADIOL 17β / KG FOOD (0 - 80 DAYS AFTER FIRST FEEDING) ON THE GROWTH OF RAINBOW TROUT FRY.

7.3. Indirect Feminisation by 17α Methyl Testosterone Administration to Produce Functional Phenotypic Males with a Female Genotype, and Subsequently Crossing with Normal Females.

7.3.1. Materials and Methods: Functional sex-reversed phenotypic "male" fish were produced at the Department of Fisheries for Scotland, Victoria Road, Aberdeen, by R. Johnstone. They were produced by the administration of 17α methyl testosterone to first feeding fry at a level of 3mg/kg food for a period of 30 days (Johnstone et al, 1978). In 1977, 40 of these fish were selected as potential sires using the presence of secondary sex characters as an index of maturity, and were transferred from Aberdeen to Low Plains to be grown to full maturity. Similarly, 20 functional sex-reversed female fish (Johnstone et al, 1978) were also transferred to Low Plains.

In February, 1978, it was discovered that of the 31 "male" fish remaining, only 7 emitted semen upon gentle pressure of the abdomen, although all the "male" fish at this time appeared to be normal mature male fish exhibiting full secondary sex characters. An autopsy of several of the "non producing" males showed that mature gonad elements of varying size were present in all fish and although they all contained spermatozoa, the sperm ducts were incomplete, adhered to the body wall and did not extend the full length to the anal papilla. As normal emission of the sperm present was not possible, semen was removed surgically from these "males" and used to fertilize normal eggs. Other eggs were also fertilized by the "males" which produced semen normally. In both cases the following fertilization and hatchery proceedure was adopted. Eggs were taken by hand stripping and divided into 10 groups of approximately 2,000. 6 of these groups

were then fertilized by sperm taken from the males that produced semen normally and the remaining 4 groups with semen taken by surgery. The eggs were incubated in hatchery trays until the eyed stage, at which time 6 of the groups were transferred to Aberdeen for further development, the remaining 4 being hatched at Low Plains. Subsequent to hatching, swimming up and first feeding, the groups were all maintained separately under identical conditions, and the proportion of sexual types in each population determined when individual fish weighed approximately 5g.

At the same time, all the functional sex-reversed "females" produced eggs and were hand-stripped, fertilized with semen from normal males and 5 crosses raised to 5g. This approach gave a further test to determine which sex is homogametic.

7.3.2. Results: Differences in the proportion of sexual types in both groups were observed as described below.

(i) The Progeny of Hormonally Masculinised Fish: The numbers of males and females in the populations derived from the pairing of normal female fish with the 10 "males" produced by androgen addition to the diet during the period of gonadal differentiation are shown in Fig.7.8. In 9 of these pairings, the sex ratios observed were not significantly different from unity. The offspring from 1 pairing (parent no. 006) were all female, indicating that both parents in this particular pairing had been homogametic.

(ii) The Progeny of Hormonally Feminised Fish: The numbers of males and females observed in the populations arising from the fertilization of ova from the 5 "females" produced by dietary treatment of

PARENT NO.	NO. OF FEMALES.	NO.OF MALES.	SEX RATIO ($\frac{♂}{♀}$)	SIGNIFICANCE OF DIFFERENCE OF RATIO FROM UNITY.
+ 003	122	110	0.9	NS
+ 004	96	107	1.1	NS
+ 005	106	97	0.9	NS
+ 006	124	0	0	P<0.001
585	75	69	0.9	NS
587	122	91	0.75	NS
588	49	57	1.2	NS
590	46	56	1.2	NS
591	117	119	1.0	NS
592	60	62	1.0	NS

+ INDICATES MILT REMOVED SURGICALLY.

FIG. 7.8. SEX RATIOS OBSERVED IN SAMPLES TAKEN FROM THE PROGENY OF "MALE" 17α METHYL TESTOSTERONE TREATED RAINBOW TROUT.

oestradiol with mixed sperm from normal male fish are shown in Fig.7.9. The results show that although the ratios of males to females in the progeny of 3 "female" fish were not significantly different from unity, the ratios of the progeny of the remaining 2 fish were 2.0:1 and 2.4:1 male/female respectively.

PARENT NO.	NO. OF FEMALES.	NO. OF MALES.	SEX RATIO ($\frac{♂}{♀}$)	SIGNIFICANCE OF DIFFERENCE OF RATIO FROM		
				1.0	2.0	3.0
677	43	34	0.8	NS	P<0.001	P<0.001
595	39	39	1.0	NS	P<0.001	P<0.001
661	39	38	1.0	NS	P<0.001	P<0.001
665	30	73	2.4	P<0.001	NS	NS
671	43	88	2.0	P<0.001	NS	P<0.001

FIG. 7.9. SEX RATIOS OBSERVED IN SAMPLES TAKEN FROM THE PROGENY OF "FEMALE" OESTRADIOL 17β TREATED RAINBOW TROUT.

7.4. Photoperiod Control of Fish in Production Tanks.

7.4.1. Materials and Methods: To test the effect of a constant long day on sexual maturity in fish, one of the 20 circular production tanks at Low Plains was placed under photoperiod control on the 8th. July, 1976. This was achieved by fixing 3 x 40 watt fluorescent tubes approximately 1m above the water surface. The constant photoperiod was fixed at 17 hours light/7 hours dark per day (on at 04-30, off at 21-30) and controlled automatically by an electric time clock. A second identical tank was used as a control tank, being exposed to natural seasonal daylengths. At the start of the experiment both tanks contained approximately 1.5 tons of fish each with an average initial weight of 70g drawn from an identical stock. Both tanks of fish were treated as normal production stock with regard to feeding and husbandry throughout the duration of the experiment. On the 1st. December, 1976, cropping from both tanks was commenced and during processing the fish were checked for sexual maturity, particular attention being given to the males and the gonads weighed from each group.

7.4.2. Results: A total of 1400 fish were examined from the tank subjected to a constant long day (17 hours light/7 hours dark) photoperiod. The mean weight of the fish at the time of cropping was 230g. 717 fish, both male and female, showed negligible gonad development being typically immature at this stage. 306 fish were found to be female, showing slight signs of ovarian development although gonad growth at this stage was very small. The remaining 377 fish were male with varying degrees of testicular development. The The mean weight of testis from this group was 10.87g/fish.

At the same time, 1200 fish were cropped from the control tank, having an identical mean weight of 230g. Of these, 419 fish showed negligible gonad development irrespective of sex. 498 were found to be female showing only slight signs of ovarian development and the remaining 283 fish were male exhibiting varying degrees of testicular development. The mean weight of testis from this group was 8.49g/fish. Thus, the constant long day photoperiod had no significant effect in retarding gonad development over the period under investigation. In fact, the reverse was true, more males in the treated group were beginning to mature and had attained a greater testis weight than in the control fish.

7.5. Discussion.

The present work suggests that hormonal additions to the diet during the stage of gonadal differentiation offers the most likely approach to avoiding the problems associated with maturation in rainbow trout, when all the alternatives currently available are taken into account.

The histological data from the experiments using 17α methyl testosterone at 30mg/kg diet suggest that this could be used for the direct sterilization of rainbow trout. These results are in agreement with those of Yamamoto (1958), working with the medaka and Takahashi (1977) with the guppy. Some sterilized adult fish have also been reported by Jalabert et al, (1975), Yamazaki (1976) and Johnstone et al, (1978) in rainbow trout following treatment with 17α methyl testosterone at high dose levels.

The growth data demonstrates a clear suppression of growth during the period of treatment. However, for commercial purposes, the slightly reduced growth during the early stages is a very small price to pay for the advantages to be gained later in the production cycle from having completely sterilized stocks of production fish. Mortality throughout the treatment was also acceptably low.

It should be pointed out that for this or any of the other methods to be of value to the fish farmer it must eliminate not only spermatogonial development but also the endocrine cells in the testis which produce the steroids thought to control the development of secondary sex characters. Whether this approach will fulfill these criteria is currently being investigated by taking serum samples from

treated fish as they grow towards the expected time of maturity, and assaying for circulating androgens.

Currently it would appear that direct feminisation is the most reliable and well documented approach to the problem of maturation, as long as only table fish of around 250g are to be produced. The results from the direct feminisation experiments show that oestradiol 17β treatment produced an increase in the ratio of females to males from 1:1 to 2.5:1. Using similar techniques, Johnstone et al, (1978) have produced populations of fish which were 100% female, in both laboratory and field experiments. There does not appear to be an immediate explanation as to why 100% female stocks were not produced during the course of these experiments. The answer probably lies in the rate at which the food, and hence the steroid was fed to the fish. These experiments were carried out at 9°C, whereas those of Johnstone et al, (1978) were performed at 14-20°C. The difference in feeding rate required at these different temperatures could be sufficient to account for the differing results. Different daily feeding regimes at different sites could also play a significant role. The same reasons could also explain in part why earlier work on sex reversal in rainbow trout (Padoa, 1937, 1939; Ashby, 1957, 1965) was substantially unsuccessful.

Although growth was significantly depressed during the period of steroid administration, after the cessation of treatment, growth improved in the treated groups and at 6 months of age, no differences could be detected suggesting that the smaller fish grew faster after treatment. Johnstone et al, (1978) have further suggested that these observed differences in growth are less discernible at higher stocking

densities. The changes in growth observed here and also the mortality which occurred during and after steroid treatment are both at levels which would not cause concern to the fish farmer.

One problem common to both direct feminisation and direct sterilization is that the steroids are administered directly to fish destined for human consumption. Although studies by Johnstone et al, (1978) and in this thesis (see chapter 4) have shown conclusively that because of the rapid metabolism of these hormones there is absolutely no danger to the consumer, in the U.K. the provisions of the Medicines Act, 1969, apply to the commercial use of hormone treatment. Veterinary supervision or product licenses are therefore required for the administration of such steroid loaded diets.

While these difficulties can be overcome, the production of all-female stocks from sex-reversed broodstock eliminates the requirement to feed hormones directly to fish destined for human consumption, the treatment being confined only to broodstock. The results from the progeny produced from the pairing of sex-reversed parents imply that in rainbow trout the female is homogametic and treatment with 17α methyl testosterone during the period of gonadal differentiation results in a reversal to a phenotypically male state. These results are in agreement with the claim by Okada (quoted by Yamazaki, 1976, but as yet apparently uncompleted) that all-female stocks resulted from the pairing of some androgenised rainbow trout with normal females.

It was to have been expected that approximately half of the "male" fish selected for pairing in these experiments would have a female

genotype (originating from a 1:1 male to female ratio, the other half being normal genetic males) and should therefore have produced all-female offspring. The fact that only 1 of these pairings gave all-female progeny was probably caused by a non-random selection of "males" at 18 months of age, when only those fish showing signs of maturation at that time were selected as potential sires. While this selection seemed reasonable at the time, in retrospect the fact that such a non-random selection should have led to a reduction in the number of "males" with a female genotype is not entirely unexpected. 17α methyl testosterone causes considerable atrophy of the germinal tissues (Jalabert et al, 1978) and it is likely to exert a more profound effect on the primordia of the female gonad than the corresponding tissues of males since these normally have a natural androgen secretory function.

The present finding that the female rainbow trout is homogametic as far as the sex chromosomes are concerned is in agreement with the cytological observations of Thorgaard (1977). It implies that the crossing of a normal male with a functional "female" obtained by feminisation involves 2 heterogametic individuals. In cases where the Y chromosome is male determining, the anticipated ratio of males to females in the progeny of an XY x XY cross is 3.0:1 when, as is commonly the case, the YY constitution is non-viable this ratio is reduced to 2.0:1.

Of the 5 crosses made, the progeny from 3 "female" fish were not significantly different from unity, the ratios in the progeny of the 2 remaining "females" were respectively 2.0 and 2.4:1. These ratios are unlikely to have arisen ($P<0.001$) from the random selection of

specimens from a population in which the actual sex ratio was unity. In the progeny of 1 "female" fish but not the other the sex ratio in the offspring was unlikely to have arisen ($P<0.001$) had the actual ratio been 3.0:1. Combining these results from the 2 fish is justified if it is assumed that the sex determining mechanisms operated the same in both pairings. The combined male:female ratio of 2.2:1 was significantly different ($P<0.001$) from 3.0:1. These results confirm male heterogamety in the rainbow trout, and although they suggest that the YY constitution is not wholly viable, they are insufficient to permit any firm conclusions as to the extent of this impairment.

Hence, as the female is homogametic, the production of all-female stocks from crosses between androgenised "males" produced by 17α methyl testosterone treatment and normal females eliminates the requirement to feed steroids directly to fish destined for the table. However, in any group of androgenised "males", only one half will have the female genotype, the remainder being normal males. At the present time, the only way in which fish can be reliably identified as belonging to 1 class or the other is by the determination of the sex ratio of their offspring. It is therefore inherent in the method that brood fish capable of producing all-female stocks are only available in subsequent seasons of maturity after they have been identified as being homogametic for sex by progeny testing in an earlier year. This is a lengthy process and is likely to be made more difficult if, as in the present study, malformation of the testes necessitates the surgical removal of semen.

These difficulties may be overcome as follows. The fry resulting from the individual pairings of masculinised fish with normal females

may be androgenised at "swim up" by treatment with 17α methyl testosterone, a small sub-group only being untreated and retained for the determination of the sex ratio. It follows that where the derived sub-group is composed of entirely females, the corresponding androgenised groups of "male" fish will be of the female genotype. The major advantage of such a modified approach is that from an initial stock of "male" fish which has been identified as being of the female genotype it should be possible to produce repeated generations of homogametic fish without further progeny testing.

The results from the experiment involving environmental control were less clear. While at the time it seemed likely that a constant long day photoperiod would inhibit gonadotropin release and hence prevent maturation (Breton & Billard, 1977), in the light of the results described in Chapter 3 and results from very recent data, the failure of the constant long day photoperiod to prevent maturation is not entirely unexpected. Although the long day photoperiod was only applied during a portion of the year, more recent data not reported in detail here, has shown that broodstock maintained on a constant 17 hours light/7 hours dark photoperiod not only matured normally, but spawned some 10 weeks in advance of fish maintained under a normal seasonal photoperiod. It therefore seems likely that if environmental control is to be of use in preventing or delaying maturation, the photoperiod employed would need to be short day (probably at the most 8 hours) rather than long day. This is being investigated further at the present time.

Hence, at the moment, the use of environmental control to retard maturation is open to question. A further consideration is also the

practical difficulties of providing photoperiod control to all the farm stock. However, these problems would be overcome if a non-stimulatory photoperiod is found which need only be applied for a short period during the production cycle.

In conclusion, the results described show that certainly all female, and probably sterile stocks can be produced on a commercial scale. Although each approach has its inherent problems, they are not insurmountable and the rewards to be gained more than warrant further investigation.

GENERAL SUMMARY AND CONCLUSIONS.

1. There are two important constraints to the growth of rainbow trout culture in the U.K. Firstly, the restricted supply of eggs to only a few months of the year and secondly, the regressive changes and mortalities which accompany maturation amongst production stock.

2. Manipulation of the environmental factors which affect gonadal and embryonic development and also hormonal treatments which alter gene expression and the normal processes of maturation and spawning are amongst the more promising approaches to these problems.

3. Throughout the thesis the efficacy of these methods was studied by detailed investigation of the endocrine and other changes which accompany and control reproduction. Only a thorough understanding of these processes in the normal cycle allows such assessments to be made.

4. During the annual spawning cycle of the female rainbow trout, the primary event was an increase in serum levels of gonadotropin. Following this, levels of oestradiol 17β began to rise producing consequent increases in phosphoprotein phosphorus and total calcium. Gonadotropin levels were highest during spawning, presumably at the time of ovulation, when oestradiol 17β levels had returned to basal; phosphoprotein phosphorus and total calcium also showed peak values at this time.

5. In the male fish, the serum levels of gonadotropin were again the first to rise and this was followed by an increase in levels of testosterone. Levels of gonadotropin were highest prior to the commencement of spermiation, whereas the maximum levels of testosterone were observed approximately one month after the start of spermiation. Thus, in both female and male rainbow trout, there exists a specific sequence of endocrine and other changes which ultimately control the process of reproduction.

6. Under shortened seasonal photoperiod regimes, these sequences of

changes occurred at an earlier time of the year in both male and female fish although the basic sequences remained unchanged.

7. Spawning was advanced by up to four months under these shortened regimes relative to the normal control cycle. By applying photoperiod control to broodstock, fish could be induced to spawn at almost any time of the year. Photoperiod manipulation would thus provide a relatively inexpensive method of providing eggs throughout the year from stocks already present in this country.

8. Low temperature incubation of eggs extended the time taken to hatch by 50 days. This extension could play an important role in perhaps acting as a " fine tuner " to eggs already produced by altered photoperiod regimes, enabling fry to be produced on demand, or to retain eggs into April and May, currently the months for which it is difficult to produce eggs by photoperiod manipulation.

9. Treatment with oestradiol 17β demonstrated a causal relationship between the increases in oestradiol 17β and vitellogenin. Similar changes were also seen during the course of maturation in all female fish irrespective of photoperiod treatment. Vitellogenin appeared in the serum 12 hours after injection of oestradiol 17β, and reached a peak 21 days later even though oestradiol 17β had returned to control levels after 11 days. Levels of vitellogenin in the serum did not return to basal until 135 days following injection. In this respect, these observations closely resemble those seen during the normal course of maturation.

10. The computer model formulated provides a solution to the problems of how best to stock farms under a variety of environmental conditions, and thus optimise production. The main advantage of this type of model is that it is based on factors which are easily determined and understood

by the fish farmer. Clearly, photoperiod manipulation of broodstock to provide eggs at times of the year specified by computer assisted stocking schedules is a joint approach which would enable better management decisions to be taken in the overall running of a given farm site.

11. Treatment with methyl testosterone at a dose of 30 mg / kg food for a period of 110 days following first feeding, produced sterilization. Further long - term trials are currently in progress to determine the full potential of this approach.

12. Treatment with oestradiol 17β at a dose of 20 mg / kg food for a period of 80 days following first feeding produced feminisation. For the trout farmer concerned with the production of portion - sized fish at around 250 g, direct feminisation is probably the most appropriate technique at the present state of our understanding.

13. The hormone treatments applied had no serious effects on growth and survival. Both aspects are of prime importance and concern if these methods are to be used by the commercial fish farmer.

14. Results from the pairings of sex - reversed fish imply that the female is the homogametic sex. The production of all - female stocks by crossing sex - reversed " males " with normal females, although obviating the need to feed hormones directly to fish eventually destined for human consumption, is a long - term approach which clearly requires careful planning to avoid genetic inbreeding.

In summary, the thesis provides scientific information which will assist the fish farmer to solve the problems related to reproduction in in the rainbow trout. The transition from scientific investigation to commercial application is always tedious and not without its inherent

problems. However, used in combination, the approaches developed during the formulation of this thesis should bring solutions to these problems one step closer. With further commercial enterprise, it would not be difficult to envisage a farm site producing supplies of fry which could be guaranteed either all - female or sterile, at any times of the year as specified by the requirements of a computer formulated stocking schedule.

ACKNOWLEDGEMENTS.

Acknowledgements.

Due to the nature of the project, the locations of the various experiments, and the travelling involved, expertise training and assistance has been drawn from numerous sources, without which the work would not have been possible.

My thanks go firstly to the supervisory team. I am indebted to Dr. Niall Bromage (Main Supervisor) for all his assistance and encouragement with all aspects of the project, to Dr. John Forster (Industrial Supervisor) for assistance and facilities at Shearwater Fish Farm, Low Plains, Armathwaite, Cumbria, to Mr. George Lindfield (Associate Supervisor) for assistance with the computer aspects, to Mr. Robert Harrison (Associate Supervisor) for an insight into marketing management, and to Dr. David van Rest (I.H.D. Tutor) and staff in the I.H.D. scheme for administration of the above team and integration of the project.

I would also like to thank Professor Alan Matty and staff for facilities within the Department of Biological Sciences and the staff at Shearwater Fish Farm, Low Plains, especially Mr. John Ralph for assistance with all the work conducted there.

I am also indebted to Dr. Tom Simpson, Mr. Ray Johnstone and staff at the Marine Laboratory, Department of Fisheries for Scotland, Aberdeen, for their collaboration with the experiments involving sex control and sterility, to Dr. Bernard Breton and staff at the Institute for Agricultural Research, Jouy en Josas, France, for their collaboration in determining the serum gonadotropin levels, and to Dr. Clive Craik, formerly of Bangor University, for his suggestions

in the modification of the vitellogenin assay.

Finally, I would like to thank my wife, Sue, for her patience throughout the project and assistance with the preparation of the manuscript, and my mother for typing the final draft.

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APPENDIX 1.

SURVEY OF BRITISH EGG PRODUCTION - QUESTIONNAIRE USED.

Shearwater
Fish Farming Limited
Low Plains Armathwaite
Carlisle CA4 9TT
Cumbria.

Ref. IHD/CW/GS
15th. October, 1975.

Dear Sir,

I am writing to you in the hope that you will be able to supply me with certain specific information. I am an IHD research student undertaking a co-operate Ph.D. with Aston University, and Shearwater Fish Farm. At the present time I am in the process of developing a strategy for buying rainbow trout eggs for Shearwater Fish Farm for a period over the next five years. This work is taking the form of a world-wide survey from which I hope to compile information concerning rainbow trout egg availability throughout the world as an integral part of my Ph.D. thesis. In the very near future I shall also be looking at various biological aspects of trout reproduction.

My prime interest is with particular regard to the times of year at which eggs may be available. If you expect to be in a position to supply rainbow trout eggs over either the whole or part of the next five years, I would be extremely grateful if you would complete and return the attached short questionnaire. It is designed to give a concise account of your facilities.

Please return the form to me at Shearwater Fish Farm, Armathwaite, the complete address for which is given above.

Thanking you in advance for your co-operation.

Yours faithfully,

Colin Whitehead.

1. In which of the next five years do you expect to be in a position to supply eggs ?

1976	1977	1978	1979	1980
___	___	___	___	___
___	___	___	___	___

2. During which months of the year would these eggs be available ?

January	___	July	___
February	___	August	___
March	___	September	___
April	___	October	___
May	___	November	___
June	___	December	___

3. Please indicate the numbers of eggs you would expect to be able to supply at the times you have specified above.

4. What is your approximate intended yearly egg production over the next five years ?

1976	___
1977	___
1978	___
1979	___
1980	___

5. Please estimate the cost of your eggs over the next year.

6. Please give a short history of your broodstock (origin, age, numbers, etc.,).

7. Please give a short account of any disease history with special reference to IPN, VHS, IHN, IAD(IDC), whirling disease and furunculosis.

8. Please give details of previous health certification and inspection.

9. Please give a brief description of your farm (location, utilisation of surface, spring or borehole water, fish numbers, planned expansion, etc.).

10. Has your stock been selected for any special characteristics ?

11. Would a visit to your farm be possible in the near future ?

12. Have you any further information you think might be useful ?

APPENDIX 2.

FORTRAN PROGRAM.

FOR GROWTH MODELLING.

```
      MASTER FISHCROP
C     PROGRAM TO CALCULATE GROWTH AND CROPPING OF 30 TONS
C     OF TROUT STARTING AT 5 GRAMS AT ONE MONTHLY INTERVALS DURING
C     A VARYING TEMPERATURE REGIME. FEED AS PER MAINSTREAM TABLES
C     WITH NO FEEDING AT 1 & 2 DEGREES. MORTALITY IS NOT ACCOUNTED FOR.
C     FCR = 1.6, CROPPING AVERAGE = 250G.
      DIMENSION FTAB(20,13),WTCATLEV(13),ICENT(140),IHOT(30),EAT(30)
      DIMENSION NOFISH(29),AVWT(29),S(30),STORE(30),ITIME(30)
      READ(1,10)((FTAB(I,J),J=1,13),I=1,20)
   10 FORMAT(13F6.3)
      READ(1,20)(WTCATLEV(K),K=1,13)
   20 FORMAT(7F8.2)
      READ(1,30)(ICENT(M),M=1,140)
   30 FORMAT(20I4)
      READ(1,40)(NOFISH(N),N=1,29)
   40 FORMAT(10I7)
      READ(1,50)(AVWT(L),L=1,29)
   50 FORMAT(10F8.2)
C     LOOP 1 : SETS INFORMATION REQUIRED AT ONE MONTHLY INTERVALS
      DO 190 IEXP=1,13
      IEXPA=4*(IEXP-1)
      K=3
      IWCAT=3
      WTK=5.00
      WRITE(2,60)IEXPA
   60 FORMAT(33H        GROWTH STARTING AT WEEK -,I3)
      WRITE(2,70)
   70 FORMAT(53H WEEK TEMP FEED/WEEK MEAN WT CROPPING STOCK TONS FEED)
C     CALCULATE WEEKLY GROWTH UP TO 180 GRAMS.
      DO 90 I=1,90
      M=I+IEXPA
      ITEMP=ICENT(M)
      FL=FTAB(ITEMP,IWCAT)
      WTK1=WTK+(FL*WTK)/1.6
      WTK=WTK1
      STOCK=WTK*0.12
      TFED=STOCK*FL
      WRITE(2,80)M,ITEMP,FL,WTK,STOCK,TFED
   80 FORMAT(1X,I3,I5,F9.3,F9.2,F16.3,F8.3)
      IF(WTK.GE.180.00)GOTO 100
      IF(WTK.LT.WTCATLEV(K))GOTO 90
      K=K+1
      IWCAT=IWCAT+1
   90 CONTINUE
C     LOOP 3 : ASSUMES NORMAL DISTRIBUTION ABOUT 180 GRAM MEAN,
C     FROM 110 TO 250 GRAMS AND CALCULATES WEEK WHEN EACH GROUP
C     IS CROPPED.
  100 IGP=1
      WTCROP=0.000
      DO 135 NO=1,30
  135 S(NO)=0.000
      IWKPREV=1
      DO 170 L=1,29
      IWK=0
      WTK=AVWT(IGP)
      N=M+1
C     CALCULATES GROWTH OF EACH INDIVIDUAL GROUP UP TO 245.00
C     GRAMS AND WRITES TIME OF CROPPING AND TONS CROPPED.
```

```
      DO 140 J=1,30
      ITEMP=ICENT(N)
      FL=FTAB(ITEMP,IWCAT)
      WTK1=WTK+(FL*WTK)/1.6
      WTK=WTK1
      IWK=IWK+1
      S(IWK)=WTK*NOFISH(IGP)/1000000+S(IWK)
      IF(WTK.GE.245.00)GOTO 150
      N=N+1
140   CONTINUE
150   IF(IWK.LE.IWKPREV)GOTO 155
      ITIME(IWKPREV)=N-1
      STORE(IWKPREV)=WTCROP
      IHOT(IWKPREV)=ITEMP
      EAT(IWKPREV)=FL
      WTCROP=0.000
155   WTCROP=WTCROP+WTK*NOFISH(IGP)/1000000
      IGP=IGP+1
      IWKPREV=IWK
170   CONTINUE
      STORE(IWK)=WTCROP
      ITIME(IWK)=N
      IHOT(IWK)=ITEMP
      EAT(IWK)=FL
      DO 175 I=1,IWK
      STOCK=S(I)-STORE(I)
      TFED=STOCK*EAT(I)
175   WRITE(2,177)ITIME(I),IHOT(I),EAT(I),STORE(I),STOCK,TFED
177   FORMAT(1X,I3,I5,F9.3,F17.3,F8.3,F8.3)
      WRITE(2,180)
180   FORMAT(52H XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX)
190   CONTINUE
      STOP
      END
      FINISH
```

APPENDIX 3.

TABLES USED IN THE CALCULATION OF THE FOOD REQUIRED, STOCK HELD AND CROPPING AVAILABLE UNDER THE STOCKING SCHEDULES EMPLOYED AT FARMS A, B AND C.

INFORMATION TAKEN FROM THESE TABLES FORMS THE GRAPHS SHOWN IN CHAPTER 6.

		WEEK NO.													
		52	53	54	55	56	57	58	59	60	61	62	63	64	65
WEEK AT WHICH	0	1.565	1.586	1.577	1.534	1.422	1.098	0.852	0.603	0.383	0.299	0.155	0.068	0.041	0.009
EACH GROUP WAS	12	0.906	0.958	1.012	1.070	1.039	1.094	1.151	1.212	1.275	1.342	1.414	1.487	1.565	1.586
INTRODUCED.	24	0.467	0.493	0.521	0.551	0.582	0.615	0.650	0.687	0.726	0.768	0.811	0.857	0.906	0.958
	36	0.281	0.303	0.327	0.353	0.381	0.362	0.387	0.414	0.443	0.475	0.508	0.543	0.467	0.493
	48	0.151	0.170	0.170	0.189	0.300	0.176	0.192	0.209	0.227	0.247	0.268	0.261	0.281	0.303
	60										0.112	0.127	0.134	0.150	0.070
TOTAL FEED/WEEK.		3.370	3.510	3.607	3.697	3.635	3.345	3.232	3.125	3.054	3.243	3.282	3.350	3.411	3.519

		WEEK NO.													
		66	67	68	69	70	71	72	73	74	75	76	77	78	79
WEEK AT WHICH	0	0.003	0.000												
EACH GROUP WAS	12	1.577	1.534	1.422	1.098	0.852	0.603	0.383	0.299	0.155	0.068	0.041	0.009	0.003	0.000
INTRODUCED.	24	1.012	1.070	1.039	1.094	1.050	1.212	1.275	1.342	1.413	1.487	1.565	1.586	1.577	0.534
	36	0.521	0.551	0.582	0.615	0.651	0.687	0.726	0.768	0.811	0.857	0.906	0.958	1.012	1.070
	48	0.327	0.353	0.381	0.362	0.387	0.414	0.443	0.475	0.508	0.543	0.467	0.493	0.521	0.551
	60	0.170	0.189	0.211	1.076	0.192	0.209	0.227	0.247	0.268	0.261	0.281	0.303	0.327	0.353
	72								0.112	0.127	0.134	0.151	0.170	0.170	0.189
TOTAL FEED/WEEK.		3.610	3.697	3.635	3.345	3.232	3.125	3.054	3.243	3.282	3.350	3.411	3.519	3.610	3.697

TABLE 6.1. CALCULATION OF THE TOTAL AMOUNT OF FOOD REQUIRED PER WEEK (TONS) TO SUPPORT POPULATIONS OF 91,000 FISH INTRODUCED AT WEEKS 0,12,24,36,48,60,72,84 AND 96 UNDER THE CONDITIONS AT FARM A. (CONTINUED OVERLEAF).

		WEEK NO.													
		80	81	82	83	84	85	86	87	88	89	90	91	92	93
	24	1.422	1.098	1.852	0.603	0.383	0.299	0.155	0.068	0.041	0.009	0.003	0.000		
WEEK AT WHICH	36	1.039	1.094	1.151	1.212	1.275	1.342	1.413	1.487	1.565	1.586	1.577	1.534	1.422	1.098
EACH GROUP WAS	48	0.582	0.615	0.650	0.687	0.726	0.768	0.811	0.857	0.906	0.958	1.012	1.070	1.039	1.094
INTRODUCED.	60	0.381	0.362	0.387	0.484	0.443	0.475	0.508	0.543	0.467	0.493	0.521	0.551	0.582	0.615
	72	0.211	0.176	0.192	0.209	0.227	0.247	0.268	0.261	0.281	0.303	0.327	0.353	0.381	0.362
	84						0.112	0.127	0.134	0.150	0.170	0.170	0.189	0.211	0.176
TOTAL FEED/WEEK.		3.635	3.345	3.232	3.125	3.054	3.243	3.282	3.350	3.411	3.519	3.610	3.697	3.635	3.345

		WEEK NO.										
		94	95	96	97	98	99	100	101	102	103	104
	36	0.852	0.603	0.383	0.299	0.155	0.068	0.041	0.009	0.003	0.000	
WEEK AT WHICH	48	1.151	1.212	1.275	1.343	1.413	1.487	1.565	1.586	1.577	1.534	1.422
EACH GROUP WAS	60	0.650	0.687	0.726	0.768	0.811	0.857	0.906	0.958	1.012	1.070	1.039
INTRODUCED.	72	0.387	0.414	0.443	0.475	0.508	0.543	0.467	0.493	0.521	0.551	0.582
	84	0.192	0.209	0.227	0.247	0.268	0.261	0.281	0.303	0.327	0.352	0.381
	96				0.112	0.127	0.134	0.151	0.170	0.170	0.189	0.211
TOTAL FEED/WEEK.		3.232	3.025	3.054	3.243	3.282	3.350	3.411	3.519	3.610	2.697	3.635

TABLE 6.1. (CONTINUED).

		WEEK NO.													
		52	53	54	55	56	57	58	59	60	61	62	63	64	65
	0	18.629	18.879	18.774	18.257	16.294	13.073	10.148	7.176	4.565	3.555	1.850	0.808	0.484	0.103
WEEK AT WHICH	12	9.957	10.523	11.122	11.757	12.372	13.021	13.705	14.424	15.081	15.978	16.817	17.700	18.629	18.879
EACH GROUP WAS	24	5.127	5.418	5.727	6.052	6.396	6.760	7.145	7.551	7.981	8.435	8.914	9.421	9.957	10.523.
INTRODUCED.	36	2.231	2.406	2.596	2.800	3.021	3.232	3.459	3.701	3.960	4.237	4.534	4.851	5.127	5.418
	48	0.744	0.839	0.934	1.041	1.159	1.371	1.260	1.490	1.621	1.763	1.917	2.068	2.231	2.046
	60										0.516	0.586	0.661	0.744	0.839
TOTAL STOCK.		36.668	38.065	39.153	39.904	39.872	37.346	35.828	34.342	33.308	34.484	34.618	35.509	37.172	38.068

		WEEK NO.													
		66	67	68	69	70	71	72	73	74	75	76	77	78	79
	0	0.035	0.000												
WEEK AT WHICH	12	18.774	18.257	16.924	13.073	10.148	7.176	4.565	3.555	1.850	0.808	0.484	0.103	0.035	0.000
EACH GROUP WAS	24	11.122	11.754	12.372	13.021	13.705	14.424	15.181	15.978	16.817	17.700	18.629	18.879	18.774	18.257
INTRODUCED.	36	5.727	6.052	6.396	6.760	7.145	7.551	7.981	8.435	8.918	9.420	9.957	10.523	11.112	11.754
	48	2.596	2.800	3.021	3.232	3.459	3.701	3.960	4.237	4.534	4.851	5.127	5.418	5.727	6.052
	60	0.934	1.041	1.159	1.260	1.371	1.490	1.621	1.763	1.917	2.063	2.230	2.406	2.596	2.800
	72								0.516	0.586	0.661	0.744	0.839	0.934	1.041
TOTAL STOCK.		39.188	39.904	39.872	37.346	35.828	34.342	33.308	34.484	34.618	35.509	37.172	38.168	39.088	39.904

TABLE 6.2. CALCULATION OF THE TOTAL STOCK HELD (TONS) PER WEEK HAVING INTRODUCED POPULATIONS OF 91,000 FISH AT WEEKS 0,12,24,36,48,60,72,84 AND 96 UNDER THE CONDITIONS AT FARM A. (CONTINUED OVERLEAF).

		WEEK NO.													
		80	81	82	83	84	85	86	87	88	89	90	91	92	93
	24	16.924	13.073	10.148	7.176	4.585	3.555	1.850	0.808	0.484	0.103	0.035	0.000		
WEEK AT WHICH	36	12.372	13.021	13.705	14.424	15.181	15.978	16.817	17.700	18.629	18.879	18.774	18.257	16.924	13.073
EACH GROUP WAS	48	6.396	6.760	7.145	7.550	7.981	8.435	8.914	9.421	9.957	10.523	11.122	11.754	12.372	13.021
INTRODUCED.	60	3.021	3.232	3.459	3.071	3.960	4.237	4.534	4.851	5.127	5.418	5.727	6.052	6.396	6.761
	72	1.159	1.260	1.371	1.490	1.621	1.763	1.917	2.068	2.231	2.406	2.596	2.800	3.021	3.232
	84						0.516	0.586	0.661	0.744	0.839	0.934	1.041	1.159	1.260
TOTAL STOCK.		39.872	37.246	35.828	34.342	33.308	34.484	34.618	35.509	37.172	38.168	39.188	39.903	39.872	37.346

		WEEK NO.										
		94	95	96	97	98	99	1100	101	102	103	104
	36	10.148	7.176	4.565	3.555	1.350	0.808	0.484	0.103	0.035	0.000	
WEEK AT WHICH	48	13.705	14.424	18.181	15.978	16.817	17.700	18.629	18.879	18.774	18.257	16.924
EACH GROUP WAS	60	7.145	7.551	7.981	8.435	8.917	9.421	9.957	10.523	11.122	11.757	12.373
INTRODUCED.	72	3.459	3.701	3.960	4.237	4.534	4.851	5.127	5.418	5.727	6.052	6.396
	84	1.371	1.490	1.621	1.763	1.917	2.068	2.231	2.406	2.596	2.800	3.021
	96				0.516	0.586	0.661	0.744	0.839	0.934	1.041	1.159
TOTAL STOCK.		35.828	34.342	33.308	34.484	34.618	35.509	37.172	38.168	39.188	39.904	39.872

TABLE 6.2. (CONTINUED).

		WEEK NO.													
		52	53	54	55	56	57	58	59	60	61	62	63	64	65
WEEK AT WHICH EACH GROUP WAS INTRODUCED.	0	0.000	0.257	1.096	1.502	2.291	4.740	3.611	3.505	2.988	1.249	1.891	1.139	0.367	0.406
	12														0.257
TOTAL CROP/WEEK.		0.000	0.257	1.096	1.502	2.291	4.740	3.611	3.505	2.988	1.249	1.891	1.139	0.367	0.663

		WEEK NO.													
		66	67	68	69	70	71	72	73	74	75	76	77	78	79
WEEK AT WHICH EACH GROUP WAS INTRODUCED.	0	0.073	0.037												
	12	1.096	1.502	2.291	4.470	3.611	3.505	2.988	1.249	1.891	1.139	0.367	0.406	0.073	0.037
	24												0.257	1.096	1.502
TOTAL CROP/WEEK.		1.169	1.534	2.291	4.470	3.611	3.505	2.988	1.249	1.891	1.139	0.367	0.663	1.169	1.539

TABLE 6.3. CALCULATION OF THE TOTAL AMOUNT OF CROP AVAILABLE PER WEEK (TONS) HAVING INTRODUCED POPULATIONS OF 91,000 FISH AT WEEKS 0,12,36,48,60,72,84 AND 96 UNDER THE CONDITIONS AT FARM A. (CONTINUED OVERLEAF).

		WEEK NO.													
		80	81	82	83	84	85	86	87	88	89	90	91	92	93
WEEK AT WHICH EACH GROUP WAS INTRODUCED.	24	2.291	4.740	3.611	3.505	2.988	1.249	1.891	1.139	0.367	0.406	0.073	0.037		
	36										0.257	1.096	1.502	2.291	4.740
TOTAL CROP/WEEK.		2.291	4.740	3.611	3.505	2.988	1.249	1.891	1.139	0.367	0.663	1.169	1.539	2.291	4.740

		WEEK NO.										
		94	95	96	97	98	99	100	101	102	103	104
WEEK AT WHICH EACH GROUP WAS INTRODUCED.	36	3.611	3.505	2.988	1.249	1.891	1.139	0.367	0.406	0.073	0.037	
	48								0.257	1.096	1.502	2.291
TOTAL CROP/WEEK.		3.611	3.505	2.988	1.249	1.891	1.139	0.367	0.663	1.169	1.539	2.291

TABLE 6.3. (CONTINUED).

		WEEK NO.													
		45	46	47	48	49	50	51	52	53	54	55	56	57	58
	0	1.638	1.607	1.976	1.586	1.577	1.534	1.303	1.094	0.778	0.667	0.454	0.245	0.184	0.086
WEEK AT WHICH	12	1.013	1.003	1.227	1.054	1.024	1.077	1.134	1.089	1.251	1.202	1.260	1.320	1.253	1.307
EACH GROUP WAS	24	0.517	0.512	0.627	0.538	0.569	0.601	0.635	0.617	0.707	0.686	0.722	0.760	0.731	0.766
INTRODUCED.	36	0.235	0.235	0.296	0.269	0.261	0.281	0.303	0.289	0.350	0.333	0.356	0.331	0.325	0.343
	48					0.112	0.127	0.134	0.134	0.168	0.147	0.162	0.178	0.141	0.152
TOTAL FEED/WEEK.		3.403	3.357	4.126	3.447	3.523	3.620	3.509	3.223	3.254	3.045	2.954	2.843	2.634	2.654

		WEEK NO.													
		59	60	61	62	63	64	65	66	67	68	69	70	71	72
	0	0.057	0.034	0.007	0.002	0.000									
WEEK AT WHICH	12	1.311	1.325	1.303	1.230	1.097	0.916	0.913	0.675	0.493	0.269	0.211	0.105	0.043	0.024
EACH GROUP WAS	24	0.803	0.841	0.882	0.837	0.873	0.911	0.951	1.097	1.150	1.320	1.268	1.456	1.533	1.858
INTRODUCED.	36	0.363	0.384	0.405	0.428	0.380	0.398	0.417	0.479	0.504	0.577	0.561	0.642	0.679	0.776
	48	0.163	0.175	0.188	0.202	0.217	0.234	0.230	0.251	0.268	0.325	0.309	0.376	0.405	0.411
	60			0.092	0.103	0.105	0.116	0.128	0.154	0.150	0.189	0.184	0.174	0.189	0.217
TOTAL FEED/WEEK.		2.697	2.759	2.877	2.803	2.672	2.575	2.629	2.656	2.565	2.680	2.543	2.753	2.849	3.286

TABLE 6.4. CALCULATION OF THE TOTAL AMOUNT OF FOOD REQUIRED PER WEEK (TONS) TO SUPPORT POPULATIONS OF 91,000 FISH INTRODUCED AT WEEKS 0,12,24,36,48,60,72,84,96,108,120,132 AND 144 UNDER THE CONDITIONS AT FARM B.

(CONTINUED OVERLEAF).

		WEEK NO.													
		73	74	75	76	77	78	79	80	81	82	83	84	85	86
	12	0.004	0.000												
WEEK AT WHICH	24	1.996	1.734	1.421	1.325	0.809	0.556	0.231	0.098	0.027	0.004	0.000			
EACH GROUP WAS	36	0.886	1.011	1.010	1.076	1.311	1.408	1.513	1.728	1.753	1.786	1.889	2.293	2.130	2.016
INTRODUCED.	48	0.470	0.537	0.549	0.592	0.601	0.645	0.737	0.843	0.861	0.874	0.939	1.136	1.161	1.178
	60	0.262	0.302	0.318	0.301	0.399	0.441	0.509	0.615	0.549	0.573	0.625	0.754	0.636	0.646
	72	0.133	0.168	0.163	0.186	0.250	0.257	0.315	0.308	0.313	0.323	0.360	0.418	0.429	0.455
	84													0.171	0.189
TOTAL FEED/WEEK.		3.751	3.752	3.461	3.480	3.370	3.307	3.305	3.593	3.503	3.540	3.813	4.601	4.527	4.484

		WEEK NO.													
		87	88	89	90	91	92	93	94	95	96	97	98	99	100
	36	1.743	1.492	0.917	0.509	0.272	0.131	0.050	0.011	0.004	0.000				
WEEK AT WHICH	48	1.266	1.360	1.370	1.369	1.562	1.560	1.668	1.772	1.888	1.870	1.709	1.996	1.486	1.208
EACH GROUP WAS	60	0.694	0.745	0.801	0.806	0.920	0.927	0.992	1.061	1.136	1.134	1.209	1.107	1.362	1.146
INTRODUCED.	72	0.503	0.556	0.531	0.521	0.628	0.616	0.555	0.594	0.635	0.635	0.676	0.670	0.819	0.703
	84	0.200	0.233	0.239	0.248	0.232	0.236	0.267	0.288	0.319	0.336	0.318	0.327	0.415	0.367
	96											0.133	0.139	0.170	0.159
TOTAL FEED/WEEK.		4.406	4.386	3.858	3.452	3.614	3.470	3.521	3.726	4.022	3.975	4.045	4.239	4.252	3.583

TABLE 6.4. (CONTINUED).

		WEEK NO.													
		101	102	103	104	105	106	107	108	109	110	111	112	113	114
	48	0.978	0.726	0.447	0.391	0.201	0.095	0.663	0.017	0.007	0.002	0.000			
WEEK AT WHICH	60	1.207	1.270	1.337	1.284	1.474	1.417	1.448	1.336	1.314	1.240	1.107	0.924	0.711	0.616
EACH GROUP WAS	72	0.743	0.786	0.830	0.807	0.924	0.897	0.944	0.994	0.865	0.902	0.942	0.983	1.026	1.071
INTRODUCED.	84	0.395	0.276	0.402	0.374	0.457	0.424	0.450	0.406	0.390	0.409	0.429	0.449	0.471	0.494
	96	0.179	0.179	0.199	0.149	0.180	0.174	0.188	0.203	0.206	0.221	0.238	0.223	0.238	0.254
	108									0.092	0.103	0.105	0.116	0.128	0.141
TOTAL FEED/WEEK.		3.502	3.337	3.215	3.005	3.236	3.007	3.093	2.956	2.874	2.877	2.821	2.695	2.574	2.576

		WEEK NO.													
		115	116	117	118	119	120	121	122	123	124	125	126	127	128
	60	0.411	0.243	0.200	0.095	0.069	0.018	0.009	0.003	0.000					
WEEK AT WHICH	72	1.118	1.167	1.218	1.404	1.579	1.440	1.521	1.410	1.332	0.990	0.757	0.455	0.257	0.140
EACH GROUP WAS	84	0.517	0.542	0.568	0.653	0.687	0.787	0.764	0.875	0.925	1.057	1.207	1.286	1.274	1.352
INTRODUCED.	96	0.270	0.288	0.307	0.350	0.325	0.398	0.370	0.452	0.484	0.552	0.519	0.592	0.592	0.631
	108	0.142	0.156	0.171	0.151	0.162	0.196	0.190	0.230	0.250	0.287	0.297	0.358	0.354	0.385
	120							0.100	0.126	0.133	0.161	0.196	0.209	0.229	0.193
TOTAL FEED/WEEK.		2.458	2.396	2.464	2.653	2.882	2.839	2.954	3.096	3.124	3.097	2.976	2.900	2.706	2.701

TABLE 6.4. (CONTINUED).

		WEEK NO.													
		129	130	131	132	133	134	135	136	137	138	139	140	141	142
	72	0.054	0.012	0.000											
WEEK AT WHICH	84	1.653	1.768	2.019	2.306	2.130	2.016	1.961	1.388	0.801	0.518	0.295	0.090	0.025	0.012
EACH GROUP WAS	96	0.768	0.825	0.942	1.007	1.101	1.117	1.200	1.452	1.485	1.412	1.511	1.617	1.730	1.729
INTRODUCED.	108	0.511	0.488	0.561	0.645	0.676	0.705	0.613	0.742	0.759	0.770	0.827	0.889	0.955	0.961
	120	0.243	0.271	0.327	0.412	0.417	0.395	0.437	0.550	0.564	0.518	0.565	0.617	0.674	0.569
	132					0.171	0.189	0.200	0.283	0.262	0.284	0.242	0.270	0.301	0.307
TOTAL FEED/WEEK.		3.229	3.364	3.849	4.440	4.495	4.422	4.411	4.416	3.871	3.502	3.440	3.483	3.685	3.558

		WEEK NO.													
		143	144	145	146	147	148	149	150	151	152	153	154	155	156
	84	0.000													
WEEK AT WHICH	96	1.973	1.971	1.981	1.952	1.636	1.438	0.926	0.759	0.391	0.219	0.104	0.069	0.018	0.009
EACH GROUP WAS	108	1.097	1.105	1.183	1.265	1.354	1.257	1.334	1.309	1.610	1.356	1.427	1.502	1.581	1.579
INTRODUCED.	120	0.627	0.632	0.676	0.723	0.774	0.773	0.824	0.816	0.998	0.857	0.905	0.957	1.011	0.983
	132	0.370	0.343	0.376	0.412	0.452	0.447	0.434	0.440	0.533	0.480	0.504	0.440	0.466	0.453
	144			0.145	0.170	0.176	0.190	0.195	0.205	0.257	0.180	0.196	0.213	0.232	0.225
TOTAL FEED/WEEK.		4.067	4.052	4.361	4.522	4.392	4.105	3.713	3.529	3.789	3.092	3.146	3.182	3.308	3.249

TABLE 6.4. (CONTINUED).

		WEEK NO.													
		45	46	47	48	49	50	51	52	53	54	55	56	57	58
	0	1.118	1.097	1.349	1.083	1.077	1.047	0.889	0.747	0.531	0.462	0.370	0.167	0.125	0.058
WEEK AT WHICH	12	1.589	1.564	1.913	1.643	1.596	1.679	1.768	1.698	1.950	1.874	1.964	2.058	1.953	2.083
EACH GROUP WAS	24	0.631	0.624	0.765	0.656	0.694	0.773	0.775	0.753	0.863	0.837	0.882	0.928	0.893	0.993
INTRODUCED.	36	0.112	0.112	0.153	0.139	0.135	0.146	0.157	0.150	0.182	0.173	0.185	0.172	0.169	0.178
	48					0.077	0.080	0.084	0.084	0.106	0.093	0.102	0.112	0.089	0.096
TOTAL FEED/WEEK.		3.450	3.407	4.180	3.521	3.573	3.685	3.673	3.432	3.632	3.439	3.443	3.437	3.229	3.305

		WEEK NO.													
		59	60	61	62	63	64	65	66	67	68	69	70	71	72
	0	0.038	0.023	0.004	0.001	0.000									
WEEK AT WHICH	12	2.044	2.066	2.031	1.918	1.710	1.428	1.423	1.502	0.769	0.419	0.345	0.164	0.067	0.037
EACH GROUP WAS	24	0.981	1.027	1.076	1.022	1.066	1.113	1.162	1.339	1.404	1.612	1.548	1.779	1.873	2.270
INTRODUCED.	36	0.188	0.199	0.210	0.222	0.197	0.206	0.216	0.249	0.262	0.300	0.291	0.333	0.353	0.403
	48	0.103	0.110	0.118	0.127	0.137	0.147	0.139	0.158	0.169	0.205	0.195	0.237	0.225	0.259
	60			0.108	0.121	0.124	0.137	0.151	0.182	0.177	0.223	0.217	0.205	0.223	0.256
TOTAL FEED/WEEK.		3.354	3.425	3.457	3.411	3.234	3.031	3.091	2.980	2.781	3.119	2.596	2.718	2.771	3.185

TABLE 6.5. CALCULATION OF THE TOTAL AMOUNT OF FOOD REQUIRED PER WEEK (TONS) TO SUPPORT THE SUGGESTED MODIFIED STOCKING SCHEDULE UNDER THE CONDITIONS AT FARM B. (CONTINUED OVERLEAF).

		WEEK NO.													
		73	74	75	76	77	78	79	80	81	82	83	84	85	86
	12	0.006	0.000												
WEEK AT WHICH	24	2.438	2.118	1.736	1.618	0.988	0.679	0.281	0.119	0.032	0.004	0.000			
EACH GROUP WAS	36	0:460	0.525	0.525	0.559	0.681	0.732	0.786	0.898	0.911	0.918	0.982	1.192	1.107	1.048
INTRODUCED.	48	0.296	0.338	0.346	0.373	0.379	0.406	0.464	0.531	0.542	0.551	0.592	0.716	0.731	0.742
	60	0.309	0.356	0.375	0.335	0.470	0.520	0.600	0.725	0.647	0.676	0.737	0.889	0.750	0.762
	72	0.190	0.240	0.233	0.266	0.357	0.367	0.450	0.440	0.447	0.462	0.514	0.597	0.613	0.650
	84													0.103	0.112
TOTAL FEED/WEEK.		3.699	3.577	3.215	3.171	2.875	2.704	2.381	2.713	2.597	2.611	2.825	3.394	3.304	3.314

		WEEK NO.													
		87	88	89	90	91	92	93	94	95	96	97	98	99	100
	36	0.906	0.775	0.476	0.264	0.141	0.068	0.026	0.005	0.002	0.000				
WEEK AT WHICH	48	0.796	0.357	0.863	0.862	0.984	0.983	1.047	1.116	1.189	1.178	1.077	1.257	0.936	0.761
EACH GROUP WAS	60	0.818	0.878	0.944	0.950	1.084	1.093	1.170	1.251	1.339	1.337	1.425	1.305	1.606	1.351
INTRODUCED.	72	0.719	0.795	0.759	0.745	0.897	0.880	0.793	0.849	0.907	0.907	0.966	0.957	1.170	1.005
	84	0.188	0.138	0.141	0.147	0.137	0.140	0.155	0.130	0.189	0.199	0.188	0.194	0.246	0.217
	96											0.079	0.082	0.101	0.094
TOTAL FEED/WEEK.		3.357	3.443	3.183	2.968	3.243	3.164	3.191	3.391	3.626	3.621	3.735	3.795	4.059	3.428

TABLE 6.5. (CONTINUED).

		WEEK NO.													
		45	46	47	48	49	50	51	52	53	54	55	56	57	58
	0	11.412	12.062	12.854	12.894	12.822	12.469	11.559	8.891	6.902	6.006	4.023	2.387	1.791	0.843
WEEK AT WHICH	12	15.039	15.961	17.077	18.048	18.996	19.994	21.043	22.056	23.213	24.331	25.502	26.729	27.898	29.119
EACH GROUP WAS	24	6.020	6.389	6.877	7.225	7.636	8.070	8.529	8.978	9.487	9.986	10.150	11.064	11.594	12.152
INTRODUCED.	36	0.760	0.830	0.917	0.997	1.076	1.161	1.252	1.340	1.445	1.547	1.655	1.756	1.856	1.961
	48					0.324	0.368	0.415	0.462	0.521	0.573	0.631	0.695	0.747	0.802
TOTAL STOCK.		33.231	35.242	37.685	39.164	40.834	42.062	42.798	41.727	41.568	42.443	42.321	42.629	43.886	44.877

		WEEK NO.													
		59	60	61	62	63	64	65	66	67	68	69	70	71	72
	0	0.557	0.330	0.069	0.023	0.000									
WEEK AT WHICH	12	29.187	29.500	29.029	27.393	24.438	20.402	18.494	13.669	9.156	5.356	4.109	1.951	0.738	0.380
EACH GROUP WAS	24	12.736	13.350	13.993	14.605	15.224	15.920	16.606	17.406	18.344	19.210	20.126	21.182	22.294	23.165
INTRODUCED.	36	2.073	2.191	2.316	2.448	2.565	2.689	2.818	2.966	3.122	3.299	3.473	3.670	3.879	4.116
	48	0.862	0.926	0.995	1.069	1.148	1.234	1.315	1.407	1.505	1.624	1.737	1.875	2.022	2.173
	60			0.596	0.664	0.734	0.811	0.897	0.998	1.100	1.224	1.347	1.465	1.594	1.740
TOTAL STOCK.		45.415	46.297	46.997	46.202	44.129	41.046	40.130	36.446	33.487	30.813	30.972	30.147	30.527	31.574

TABLE 6.6. CALCULATION OF THE TOTAL STOCK HELD (TONS) DURING THE SUGGESTED MODIFIED STOCKING SCHEDULE UNDER THE CONDITIONS AT FARM B. (CONTINUED OVER LEAF).

		WEEK NO.													
		73	74	75	76	77	78	79	80	81	82	83	84	85	86
	12	0.054	0.000												
WEEK AT WHICH	24	23.227	21.623	17.716	14.459	8.827	5.709	2.243	1.004	0.298	0.043	0.000			
EACH GROUP WAS	36	4.387	4.694	5.002	5.330	5.727	6.153	6.610	7.131	7.661	8.198	8.771	9.462	9.888	9.360
INTRODUCED.	48	2.344	2.539	2.739	2.954	3.174	3.410	3.697	3.984	4.298	4.618	4.963	5.374	5.797	6.228
	60	1.915	2.116	2.329	2.533	2.798	3.093	3.432	3.837	4.206	4.593	5.014	5.518	5.953	6.397
	72	0.751	0.880	1.007	1.153	1.344	1.544	1.787	2.029	2.277	2.537	2.826	3.159	3.505	3.874
	84													0.332	0.381
TOTAL STOCK.		32.678	31.852	28.793	26.429	21.870	19.909	17.751	17.985	18.740	19.989	21.573	23.513	25.465	26.240

		WEEK NO.													
		87	88	89	90	91	92	93	94	95	96	97	98	99	100
	36	8.094	6.927	4.539	2.365	1.346	0.647	0.248	0.053	0.018	0.000				
WEEK AT WHICH	48	6.692	7.190	7.693	8.198	8.772	9.347	9.960	10.615	11.311	12.204	11.815	11.956	11.130	9.043
EACH GROUP WAS	60	6.873	7.384	7.933	8.488	9.119	9.758	10.441	11.172	11.953	12.739	13.575	14.346	15.288	16.090
INTRODUCED.	72	4.281	4.729	5.164	5.594	6.107	6.616	7.079	7.593	8.671	8.636	9.202	9.767	10.450	11.044
	84	0.444	0.518	0.595	0.676	0.753	0.832	0.919	1.016	1.123	1.236	1.344	1.456	1.596	1.722
	96											0.313	0.358	0.413	0.466
TOTAL STOCK.		26.383	26.748	25.924	25.321	26.097	27.200	28.647	30.429	33.106	34.614	36.249	37.883	38.877	38.365

TABLE 6.6. (CONTINUED).

		WEEK NO.													
		48	49	50	51	52	53	54	55	56	57	58	59	60	61
WEEK AT WHICH EACH GROUP WAS INTRODUCED.	0	0.175	0.748	1.025	1.564	3.223	2.486	1.227	2.272	1.829	0.700	1.025	0.323	0.251	0.275
	12												0.397	0.963	1.763
TOTAL CROP/WEEK.		0.175	0.748	1.025	1.564	3.223	2.486	1.227	2.272	1.829	0.700	1.025	0.720	1.214	2.038

		WEEK NO.													
		62	63	64	65	66	67	68	69	70	71	72	73	74	75
WEEK AT WHICH EACH GROUP WAS INTRODUCED.	0	0.049	0.024												
	12	2.904	4.153	5.105	2.801	5.715	5.171	4.181	1.608	2.374	1.315	0.400	0.347	0.057	0.000
	24											0.315	1.356	3.128	5.232
TOTAL CROP/WEEK.		2.953	4.177	5.105	2.801	5.715	5.171	4.181	1.608	2.374	1.315	0.715	1.703	3.185	5.232

TABLE 6.7. CALCULATION OF THE TOTAL AMOUNT OF CROP AVAILABLE PER WEEK (TONS) FROM THE SUGGESTED MODIFIED STOCKING SCHEDULE UNDER THE CONDITIONS AT FARM B : WEEKS 48-104. (CONTINUED OVERLEAF).

		WEEK NO.													
		76	77	78	79	80	81	82	83	84	85	86	87	88	89
WEEK AT WHICH EACH GROUP WAS INTRODUCED.	24	4.341	6.644	3.736	3.889	1.416	0.780	0.276	0.046	0.000					
	36										0.268	1.220	1.765	1.733	2.872
TOTAL CROP/WEEK.		4.341	6.644	3.736	3.889	1.416	0.780	0.276	0.046	0.000	0.268	1.220	1.765	1.733	2.872

		WEEK NO.													
		90	91	92	93	94	95	96	97	98	99	100	101	102	103
WEEK AT WHICH EACH GROUP WAS INTRODUCED.	36	2.472	1.185	0.787	0.441	0.210	0.038	0.019							
	48								0.320	0.531	1.610	2.670	2.197	2.271	2.064
TOTAL CROP/WEEK.		2.472	1.185	0.787	0.441	0.210	0.038	0.019	0.320	0.531	1.610	2.670	2.197	2.271	2.064

TABLE 6.7. (CONTINUED).

		WEEK NO.													
		105	106	107	108	109	110	111	112	113	114	115	116	117	118
WEEK AT WHICH EACH GROUP WAS INTRODUCED.	48	1.444	0.943	0.298	0.390	0.090	0.045	0.023	0.000						
	60			0.301	0.735	1.344	2.215	3.166	3.893	4.262	2.121	3.910	3.134	1.201	1.764
TOTAL CROP/WEEK.		1.444	0.943	0.599	1.125	1.434	2.260	3.189	3.893	4.262	2.121	3.910	3.134	1.201	1.764

		WEEK NO.													
		119	120	121	122	123	124	125	126	127	128	129	130	131	132
WEEK AT WHICH EACH GROUP WAS INTRODUCED.	60	0.558	0.733	0.170	0.087	0.043	0.000								
	72	0.365	1.560	2.129	3.246	4.322	7.688	5.028	4.341	3.295	2.179	1.227	0.588	0.161	0.000
TOTAL CROP/WEEK.		0.923	2.293	2.299	3.333	4.365	7.688	5.028	4.341	3.295	2.179	1.227	0.588	0.161	0.000

TABLE 6.8. CALCULATION OF THE TOTAL AMOUNT OF CROP AVAILABLE PER WEEK (TONS) FROM THE SUGGESTED MODIFIED STOCKING SCHEDULE UNDER THE CONDITIONS AT FARM B : WEEKS 105-157. (CONTINUED OVERLEAF).

		WEEK NO.													
		133	134	135	136	137	138	139	140	141	142	143	144	145	146
WEEK AT WHICH EACH GROUP WAS INTRODUCED.	84	0.305	1.390	2.187	3.035	3.185	1.791	1.371	1.190	1.368	0.087	0.666	0.000		
	96													0.305	0.879
TOTAL CROP/WEEK.		0.305	1.390	2.187	3.035	3.185	1.791	1.371	1.190	1.368	0.087	0.666	0.000	0.305	0.879

		WEEK NO.										
		147	148	149	150	151	152	153	154	155	156	157
WEEK AT WHICH EACH GROUP WAS INTRODUCED.	96	1.863	1.807	3.220	2.102	1.815	1.366	0.896	0.284	0.374	0.087	0.065
TOTAL CROP/WEEK.		1.863	1.807	3.220	2.102	1.815	1.366	1.896	0.284	0.374	0.087	0.065

TABLE 6.8. (CONTINUED).

		WEEK NO.													
		45	46	47	48	49	50	51	52	53	54	55	56	57	58
	0	0.739	0.723	0.747	0.712	0.733	0.756	0.730	0.751	0.773	0.795	0.818	0.841	0.855	0.872
WEEK AT WHICH	12	0.554	0.509	0.527	0.515	0.532	0.550	0.568	0.587	0.607	0.518	0.596	0.614	0.633	0.662
EACH GROUP WAS	24	0.341	0.324	0.268	0.277	0.287	0.297	0.308	0.318	0.330	0.341	0.353	0.365	0.378	0.391
INTRODUCED.	36	0.130	0.129	0.137	0.134	0.420	0.150	0.159	0.169	0.177	0.172	0.180	0.191	0.201	0.211
	48					0.069	0.075	0.082	0.079	0.086	0.082	0.100	0.108	0.109	0.117
TOTAL FEED/WEEK.		1.764	1.685	1.679	1.638	2.041	1.828	1.847	1.903	1.972	1.978	2.048	2.119	2.176	2.244

		WEEK NO.													
		59	60	61	62	63	64	65	66	67	68	69	70	71	72
	0	0.870	0.873	0.830	0.930	0.777	0.806	0.700	0.746	0.608	0.446	0.390	0.236	0.113	0.043
WEEK AT WHICH	12	0.672	0.693	0.714	0.736	0.822	0.734	0.925	0.904	1.117	1.296	1.579	1.701	1.661	1.419
EACH GROUP WAS	24	0.405	0.419	0.434	0.424	0.464	0.454	0.560	0.516	0.673	0.776	0.891	1.020	1.165	1.144
INTRODUCED.	36	0.222	0.234	0.246	0.259	0.225	0.235	0.271	0.257	0.325	0.372	0.425	0.520	0.483	0.513
	48	0.126	0.135	0.109	0.116	0.132	0.130	0.160	0.158	0.193	0.221	0.252	0.272	0.311	0.337
	60			0.069	0.075	0.086	0.080	0.096	0.099	0.126	0.138	0.159	0.200	0.176	0.193
TOTAL FEED/WEEK.		2.295	2.354	2.402	2.540	2.566	2.439	2.712	2.680	3.042	3.249	3.696	3.949	3.909	3.649

TABLE 6.9. CALCULATION OF THE TOTAL AMOUNT OF FOOD REQUIRED PER WEEK (TONS) TO SUPPORT POPULATIONS OF 91,000 FISH INTRODUCED AT WEEKS 0,12,24,36,48,60,72,84,96,108,120,132 AND 144 UNDER THE CONDITIONS AT FARM C.

(CONTINUED OVERLEAF)

		WEEK NO.													
		73	74	75	76	77	78	79	80	81	82	83	84	85	86
	0	0.027	0.004	0.000											
WEEK AT WHICH	12	1.476	1.028	0.747	0.310	0.150	0.030	0.004	0.000						
EACH GROUP WAS	24	1.209	1.592	1.817	2.076	2.106	2.263	2.272	2.287	1.994	1.328	0.861	0.414	0.220	0.092
INTRODUCED.	36	0.554	0.710	0.811	0.927	0.947	1.022	1.168	1.194	1.365	1.478	1.421	1.527	1.640	1.762
	48	0.364	0.509	0.511	0.588	0.617	0.676	0.778	0.657	0.751	0.813	0.782	0.893	0.963	1.039
	60	0.210	0.290	0.350	0.441	0.394	0.437	0.528	0.542	0.549	0.605	0.603	0.692	0.759	0.670
	72	0.120	0.181	0.208	0.274	0.254	0.293	0.287	0.291	0.366	0.415	0.355	0.411	0.456	0.505
	84													0.171	0.205
TOTAL FEED/WEEK.		3.950	4.314	4.444	4.616	4.468	4.721	5.037	4.971	5.025	4.639	4.002	3.937	4.209	4.273

		WEEK NO.													
		87	88	89	90	91	92	93	94	95	96	97	98	99	100
	24	0.026	0.004	0.000											
WEEK AT WHICH	36	1.775	1.773	1.889	2.013	1.578	1.590	1.340	1.092	0.729	0.655	0.545	0.378	0.268	0.211
EACH GROUP WAS	48	1.055	1.062	1.136	1.216	1.215	1.023	1.077	1.034	0.981	0.813	0.841	0.822	0.850	0.759
INTRODUCED.	60	0.680	0.684	0.732	0.784	0.783	0.717	0.758	0.736	0.707	0.601	0.625	0.575	0.595	0.582
	72	0.536	0.460	0.498	0.539	0.551	0.524	0.450	0.437	0.420	0.357	0.371	0.342	0.354	0.366
	84	0.202	0.215	0.216	0.245	0.200	0.189	0.206	0.200	0.203	0.191	0.203	0.171	0.180	0.189
	96											0.077	0.080	0.087	0.081
TOTAL FEED/WEEK.		4.274	4.198	4.471	4.798	4.327	4.043	3.831	3.499	3.040	2.617	2.662	2.368	2.334	2.188

TABLE 6.9. (CONTINUED).

		WEEK NO.													
		101	102	103	104	105	106	107	108	109	110	111	112	113	114
	36	0.161	0.119	0.084	0.057	0.024	0.002	0.011	0.005	0.002	0.000				
WEEK AT WHICH	48	0.780	0.803	0.826	0.850	0.855	0.873	0.870	0.873	0.830	0.807	0.774	0.730	0.601	0.619
EACH GROUP WAS	60	0.601	0.621	0.592	0.610	0.629	0.648	0.668	0.688	0.709	0.731	0.754	0.728	0.749	0.770
INTRODUCED.	72	0.379	0.392	0.406	0.420	0.435	0.425	0.439	0.454	0.469	0.484	0.500	0.517	0.534	0.552
	84	0.119	0.210	0.221	0.233	0.245	0.258	0.224	0.234	0.244	0.255	0.266	0.278	0.290	0.302
	96	0.087	0.094	0.101	0.109	0.111	0.119	0.128	0.138	0.111	0.118	0.124	0.131	0.139	0.147
	108									0.069	0.075	0.082	0.079	0.086	0.092
TOTAL FEED/WEEK.		2.207	2.239	2.139	2.279	2.299	2.345	2.340	2.392	2.434	2.470	2.500	2.463	2.399	2.482

		WEEK NO.													
		115	116	117	118	119	120	121	122	123	124	125	126	127	128
	48	0.471	0.490	0.307	0.288	0.246	0.140	0.105	0.043	0.022	0.003	0.000			
WEEK AT WHICH	60	0.917	0.819	1.031	1.076	1.331	1.463	1.461	1.434	1.204	0.938	0.648	0.417	0.234	0.056
EACH GROUP WAS	72	0.603	0.590	0.729	0.633	0.783	0.908	1.046	1.202	1.376	1.454	1.537	2.024	2.310	2.272
INTRODUCED.	84	0.316	0.329	0.308	0.283	0.370	0.436	0.490	0.560	0.641	0.680	0.761	0.941	1.075	1.229
	96	0.168	0.164	0.202	0.170	0.211	0.241	0.275	0.333	0.381	0.413	0.397	0.535	0.615	0.707
	108	0.106	0.108	0.117	0.118	0.151	0.182	0.161	0.195	0.223	0.244	0.226	0.367	0.391	0.472
	120							0.100	0.126	0.130	0.162	0.184	0.232	0.284	0.278
TOTAL FEED/WEEK.		2.581	2.500	2.694	2.668	3.092	3.360	3.638	3.883	3.977	3.894	3.753	4.516	4.909	5.014

TABLE 6.9. (CONTINUED).

		WEEK NO.													
		129	130	131	132	133	134	135	136	137	138	139	140	141	142
	60	0.012	0.000												
WEEK AT WHICH	72	2.160	1.985	1.405	0.912	0.440	0.209	0.098	0.012	0.004	0.000				
EACH GROUP WAS	84	1.256	1.355	1.549	1.489	1.701	1.835	1.745	1.992	2.140	2.300	1.989	1.960	1.760	1.284
INTRODUCED.	96	0.742	0.655	0.749	0.765	0.875	0.947	0.911	1.040	1.122	1.210	1.228	1.237	1.323	1.322
	108	0.485	0.538	0.545	0.572	0.658	0.724	0.722	0.667	0.720	0.777	0.788	0.793	0.849	0.908
	120	0.282	0.316	0.398	0.403	0.435	0.486	0.478	0.552	0.533	0.584	0.608	0.596	0.646	0.582
	132					0.194	0.209	0.206	0.262	0.266	0.308	0.248	0.253	0.279	0.308
TOTAL FEED/WEEK.		4.937	4.849	4.646	4.141	4.303	4.410	4.160	4.525	4.785	5.179	4.861	4.839	4.857	4.404

		WEEK NO.													
		143	144	145	146	147	148	149	150	151	152	153	154	155	156
	84	0.862	0.609	0.355	0.180	0.103	0.070	0.044	0.026	0.011	0.005	0.002	0.000		
WEEK AT WHICH	96	1.310	1.181	1.243	1.195	1.134	0.939	0.971	0.950	0.981	0.855	0.873	0.870	0.873	0.830
EACH GROUP WAS	108	0.908	0.831	0.879	0.854	0.820	0.697	0.725	0.630	0.651	0.620	0.639	0.657	0.679	0.700
INTRODUCED.	120	0.581	0.532	0.563	0.547	0.525	0.447	0.464	0.427	0.442	0.432	0.447	0.461	0.477	0.492
	132	0.325	0.275	0.296	0.282	0.282	0.258	0.273	0.265	0.230	0.240	0.251	0.262	0.273	0.285
	144			0.112	0.114	0.108	0.097	0.106	0.109	0.105	0.113	0.121	0.130	0.105	0.111
TOTAL FEED/WEEK.		3.986	3.428	3.448	3.172	2.972	2.508	2.583	2.407	2.420	2.265	2.333	2.382	2.407	2.418

TABLE 6.9. (CONTINUED).

		WEEK NO.													
		45	46	47	48	49	50	51	52	53	54	55	56	57	58
	0	1.891	1.850	1.912	1.822	1.876	1.935	1.868	1.922	1.978	2.035	2.094	2.152	2.188	2.234
WEEK AT WHICH	12	0.618	0.568	0.588	0.575	0.594	0.614	0.634	0.655	0.678	0.645	0.665	0.685	0.707	0.728
EACH GROUP WAS	24	0.221	0.210	0.174	0.180	0.186	0.193	0.200	0.206	0.214	0.221	0.229	0.237	0.245	0.254
INTRODUCED.	36	0.071	0.071	0.075	0.073	0.078	0.082	0.087	0.092	0.097	0.094	0.099	0.105	0.110	0.116
	48					0.046	0.050	0.055	0.053	0.057	0.061	0.067	0.072	0.073	0.078
TOTAL FEED/WEEK.		2.801	2.699	2.749	2.650	2.780	2.974	2.844	2.928	3.024	3.025	3.149	3.251	3.323	3.410

		WEEK NO.													
		59	60	61	62	63	64	65	66	67	68	69	70	71	72
	0	2.227	2.234	2.124	2.380	1.989	2.063	1.972	1.909	1.556	1.141	0.998	0.604	0.289	0.110
WEEK AT WHICH	12	0.750	0.774	0.797	0.882	0.918	0.819	1.033	1.009	1.247	1.447	1.763	1.900	1.855	1.585
EACH GROUP WAS	24	0.263	0.272	0.282	0.275	0.301	0.295	0.364	0.335	0.437	0.504	0.579	0.663	0.757	0.743
INTRODUCED.	36	0.122	0.129	0.135	0.142	0.124	0.129	0.149	0.141	0.179	0.205	0.234	0.287	0.266	0.283
	48	0.084	0.090	0.073	0.077	0.088	0.087	0.107	0.106	0.129	0.148	0.169	0.182	0.208	0.226
	60			0.097	0.103	0.121	0.112	0.135	0.139	0.177	0.194	0.224	0.282	0.248	0.272
TOTAL FEED/WEEK.		3.446	3.499	3.508	3.861	3.541	3.505	3.580	3.639	3.725	3.639	3.967	3.918	3.623	3.219

TABLE 6.10. CALCULATION OF THE TOTAL AMOUNT OF FOOD REQUIRED PER WEEK (TONS) TO SUPPORT THE SUGGESTED MODIFIED STOCKING SCHEDULE UNDER THE CONDITIONS AT FARM C. (CONTINUED OVERLEAF).

		WEEK NO.													
		73	74	75	76	77	78	79	80	81	82	83	84	85	86
	0	0.069	0.010	0.000											
WEEK AT WHICH	12	1.648	1.148	0.834	0.346	0.167	0.033	0.004	0.000						
EACH GROUP WAS	24	0.785	1.034	1.181	1.349	1.368	1.470	1.476	1.486	1.296	0.863	0.559	0.269	0.143	0.059
INTRODUCED.	36	0.300	0.391	0.447	0.571	0.522	0.564	0.644	0.659	0.753	0.815	0.784	0.842	0.905	0.972
	48	0.244	0.341	0.342	0.394	0.414	0.453	0.552	0.440	0.503	0.545	0.524	0.599	0.646	0.697
	60	0.296	0.408	0.493	0.621	0.555	0.616	0.744	0.764	0.774	0.853	0.850	0.975	1.070	0.944
	72	0.073	0.110	0.126	0.167	0.154	0.178	0.175	0.177	0.223	0.253	0.216	0.250	0.278	0.308
	84												0.046	0.115	0.114
TOTAL FEED/WEEK.		3.415	3.442	3.423	3.388	3.180	3.314	3.595	3.526	3.548	3.329	2.933	3.031	3.157	3.094

		WEEK NO.													
		87	88	89	90	91	92	93	94	95	96	97	98	99	100
	24	0.016	0.002	0.000											
WEEK AT WHICH	36	0.979	0.978	1.042	1.111	0.871	0.877	0.739	0.602	0.402	0.361	0.300	0.208	0.147	0.116
EACH GROUP WAS	48	0.707	0.712	0.762	0.815	0.815	0.686	0.722	0.693	0.658	0.545	0.564	0.551	0.570	0.504
INTRODUCED.	60	0.958	0.964	1.032	1.105	1.104	1.010	1.068	1.037	0.996	0.847	0.881	0.810	0.838	0.820
	72	0.326	0.280	0.303	0.328	0.336	0.319	0.274	0.266	0.256	0.217	0.226	0.218	0.215	0.223
	84	0.121	0.122	0.138	0.113	0.106	0.116	0.113	0.114	0.107	0.114	0.096	0.101	0.106	0.112
	96											0.077	0.080	0.081	0.081
TOTAL FEED/WEEK.		3.107	3.058	3.277	3.472	3.232	3.008	2.966	2.712	2.419	2.084	2.144	1.958	1.963	1.861

TABLE 6.10. (CONTINUED).

		WEEK NO.													
		45	46	47	48	49	50	51	52	53	54	55	56	57	58
	0	33.794	34.915	36.072	37.176	38.315	39.488	40.624	41.792	42.992	44.229	45.501	46.809	47.603	48.596
WEEK AT WHICH	12	9.813	10.156	10.512	10.860	11.220	11.592	11.976	12.373	12.782	13.173	13.578	13.993	14.422	14.863
EACH GROUP WAS	24	2.880	3.006	3.111	3.220	3.333	3.449	3.570	3.695	3.824	3.958	4.096	4.240	4.388	4.452
INTRODUCED.	36	0.684	0.726	0.771	0.814	0.861	0.910	0.962	1.016	1.074	1.131	1.190	1.253	1.318	1.387
	48					0.331	0.360	0.392	0.423	0.456	0.492	0.531	0.573	0.615	0.661
TOTAL STOCK.		47.171	48.803	50.466	52.070	54.070	55.799	57.524	59.299	61.124	62.983	64.896	66.868	68.346	70.049

		WEEK NO.													
		59	60	61	62	63	64	65	66	67	68	69	70	71	72
	0	48.412	48.593	46.190	44.928	43.238	36.864	33.786	30.315	22.220	14.822	11.886	6.653	3.174	1.205
WEEK AT WHICH	12	15.318	15.788	16.271	16.770	17.325	17.823	18.447	19.045	19.805	20.675	20.999	20.883	20.393	17.417
EACH GROUP WAS	24	4.233	4.865	5.036	5.203	5.385	5.564	5.783	5.985	6.247	6.548	6.891	7.283	7.729	8.169
INTRODUCED.	36	1.460	1.537	1.617	1.702	1.777	1.855	1.944	2.229	2.136	2.257	2.395	2.563	2.720	2.887
	48	0.710	0.763	0.807	0.852	0.905	0.956	1.019	1.081	1.157	1.243	1.341	1.447	1.567	1.697
	60			0.696	0.758	0.827	0.892	0.931	1.051	1.153	1.263	1.390	1.549	1.692	1.847
TOTAL STOCK.		70.133	71.456	70.617	70.213	69.457	63.954	61.950	59.519	52.721	46.808	44.902	40.378	37.275	33.222

TABLE 6.11. CALCULATION OF THE TOTAL STOCK HELD (TONS) DURING THE SUGGESTED MODIFIED STOCKING SCHEDULE UNDER THE CONDITIONS AT FARM C. (CONTINUED OVERLEAF).

		WEEK NO.													
		73	74	75	76	77	78	79	80	81	82	83	84	85	86
	0	0.619	0.089												
WEEK AT WHICH	12	14.723	9.648	6.624	2.907	1.409	0.264	0.039							
EACH GROUP WAS	24	8.633	9.238	9.925	10.706	11.503	12.369	12.411	11.796	10.284	7.706	4.704	2.260	1.200	0.535
INTRODUCED.	36	3.064	3.292	3.551	3.846	4.149	4.476	4.848	5.330	5.664	6.135	6.591	7.082	7.608	8.175
	48	1.838	2.031	2.227	2.451	2.687	2.946	3.242	3.497	3.789	4.103	4.409	4.756	5.131	5.575
	60	2.016	2.246	2.521	2.863	3.176	3.523	3.959	4.371	4.810	5.294	5.781	6.337	6.947	7.494
	72	0.317	0.375	0.442	0.530	0.613	0.710	0.806	0.905	1.028	1.167	1.290	1.431	1.588	1.361
	84													0.307	0.367
TOTAL STOCK.		31.210	26.919	25.290	23.303	23.357	24.278	25.285	25.799	25.575	24.405	22.375	21.866	22.781	23.867

		WEEK NO.													
		87	88	89	90	91	92	93	94	95	96	97	98	99	100
	24	0.158	0.022												
WEEK AT WHICH	36	8.746	9.321	9.932	10.584	10.369	10.449	9.607	8.607	7.185	6.458	5.677	3.940	3.215	2.531
EACH GROUP WAS	48	5.946	6.363	6.807	7.285	7.762	8.170	8.599	9.012	9.407	9.736	10.077	10.411	10.756	11.065
INTRODUCED.	60	8.052	8.615	9.218	9.864	10.511	11.109	11.741	12.357	12.952	13.462	13.992	14.482	14.988	15.484
	72	1.947	2.108	2.283	2.473	2.668	2.855	3.017	3.176	3.329	3.459	3.596	3.772	3.852	3.987
	84	0.428	0.494	0.561	0.637	0.702	0.763	0.830	0.896	0.962	1.025	1.093	1.150	1.210	1.274
	96											0.498	0.544	0.594	0.691
TOTAL STOCK.		25.277	26.923	28.801	30.843	32.012	33.346	33.794	34.948	33.835	34.140	34.933	34.299	34.615	34.982

TABLE 6.11. (CONTINUED).

		WEEK NO.													
		57	58	59	60	61	62	63	64	65	66	67	68	69	70
WEEK AT WHICH EACH GROUP WAS INTRODUCED.	0	0.279	0.373	1.582	1.210	3.801	2.590	3.176	7.618	4.480	4.590	9.290	8.371	3.648	5.857
	12													0.310	1.285
TOTAL CROP/WEEK.		0.279	0.373	1.582	1.210	3.801	2.590	3.176	7.618	4.480	4.590	9.290	8.371	3.958	7.144

		WEEK NO.													
		71	72	73	74	75	76	77	78	79	80	81	82	83	84
WEEK AT WHICH EACH GROUP WAS INTRODUCED.	0	3.857	2.147	0.655	0.573	0.097	0.064	0.000							
	12	1.767	4.357	3.882	3.942	4.465	1.806	1.317	0.260	0.043	0.000				
	24									0.337	1.538	2.441	3.387	3.554	2.794
TOTAL CROP/WEEK.		5.624	6.504	4.537	4.515	4.562	1.870	1.317	0.260	0.380	1.538	2.441	3.387	3.554	2.794

TABLE 6.12. CALCULATION OF THE TOTAL AMOUNT OF CROP AVAILABLE PER WEEK (TONS) FROM THE SUGGESTED MODIFIED STOCKING SCHEDULE UNDER THE CONDITIONS AT FARM C : WEEKS 57-104. (CONTINUED OVERLEAF)

	WEEK NO.													
	85	86	87	88	89	90	91	92	93	94	95	96	97	98
WEEK AT WHICH 24 EACH GROUP WAS 36 INTRODUCED.	1.227	0.754	0.414	0.145	0.024	0.000	0.281	0.464	1.389	1.462	1.798	0.978	1.007	1.924
TOTAL CROP/WEEK.	1.227	0.734	0.414	0.145	0.024	0.000	0.281	0.464	1.389	1.462	1.798	0.978	1.007	1.924

	WEEK NO.							
	99	100	101	102	103	104	105	106
WEEK AT WHICH 36 EACH GROUP WAS INTRODUCED.	0.855	0.776	0.677	0.557	0.457	0.357	0.000	0.000
TOTAL CROP/WEEK.	0.855	0.776	0.677	0.557	0.457	0.357	0.000	0.000

TABLE 6.12. (CONTINUED).

		WEEK NO.													
		105	106	107	108	109	110	111	112	113	114	115	116	117	118
WEEK AT WHICH	36	0.257	0.020	0.202	0.140	0.080	0.094	0.020	0.000						
EACH GROUP WAS	48	0.071	0.097	0.414	0.317	0.996	0.679	0.829	0.956	2.193	1.186	1.219	1.199	2.192	0.942
INTRODUCED.	60														0.151
TOTAL CROP/WEEK.		0.328	0.117	0.616	0.457	1.076	0.773	0.849	0.956	2.193	1.186	1.219	1.199	2.192	1.093

		WEEK NO.													
		119	120	121	122	123	124	125	126	127	128	129	130	131	132
WEEK AT WHICH	48	0.829	1.245	0.439	0.564	0.172	0.148	0.024	0.000						
EACH GROUP WAS	60	0.556	1.190	3.547	3.749	4.667	5.173	7.210	3.784	2.681	2.162	0.572	0.156	0.000	
INTRODUCED.	72										0.315	1.443	2.282	3.166	3.323
TOTAL CROP/WEEK.		1.385	2.435	3.986	4.313	4.879	5.321	7.234	3.784	2.681	2.477	2.015	2.438	3.166	3.323

TABLE 6.13. CALCULATION OF THE TOTAL AMOUNT OF CROP AVAILABLE PER WEEK (TONS) FROM THE SUGGESTED MODIFIED STOCKING SCHEDULE UNDER THE CONDITIONS AT FARM C : WEEKS 105-156. (CONTINUED OVERLEAF).

		WEEK NO.													
		133	134	135	136	137	138	139	140	141	142	143	144	145	146
WEEK AT WHICH EACH GROUP WAS INTRODUCED.	72	2.633	1.161	0.711	0.479	0.045	0.023	0.000							
	84							0.290	0.854	1.773	2.684	2.062	2.001	1.706	1.279
TOTAL CROP/WEEK.		2.633	1.161	0.711	0.479	0.045	0.023	0.290	0.854	1.773	2.684	2.062	2.001	1.706	1.279

		WEEK NO.									
		147	148	149	150	151	152	153	154	155	156
WEEK AT WHICH EACH GROUP WAS INTRODUCED.	84	0.472	0.371	0.268	0.206	0.144	0.081	0.040	0.021	0.020	0.000
	96						0.109	0.146	0.618	0.473	1.485
TOTAL CROP/WEEK.		0.472	0.371	0.268	0.206	0.144	0.190	0.186	0.639	0.493	1.485

TABLE 6.13. (CONTINUED).